

Phytoparasitica

First Report of an Emerging and Destructive Powdery Mildew Agent *Erysiphe corylacearum* in Hazelnut in Turkey

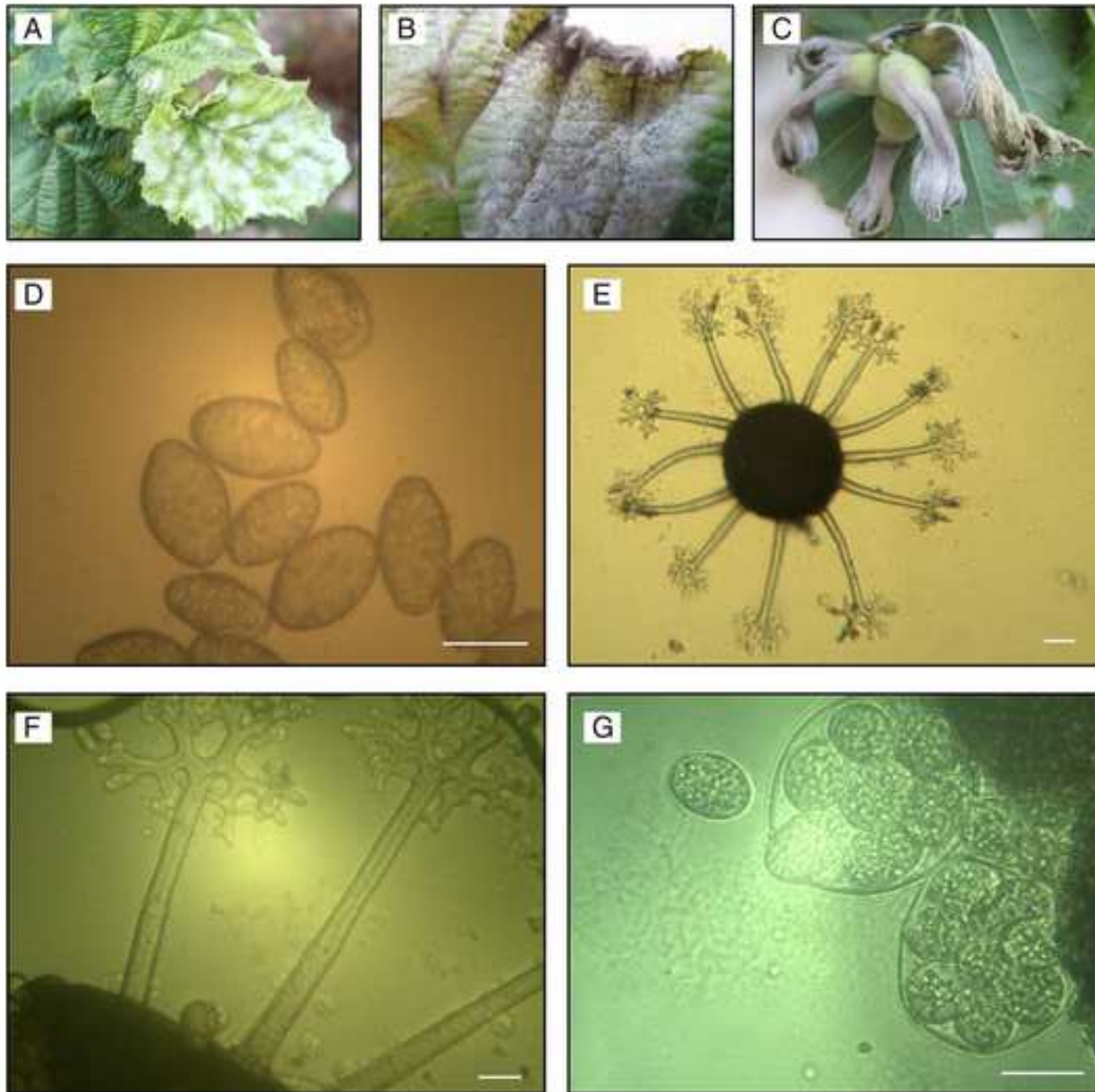
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Abstract:	<p>Hazelnut (<i>Corylus avellana</i> L.) is Turkey's most valuable agricultural export, and an essential source of income for many families in the Black Sea Region. In spring 2013, hazelnut leaves, fruit clusters and shoots showing powdery mildew infection symptoms different from those observed previously were discovered in Giresun, Ordu and Trabzon provinces of Turkey. The disease has become epidemic throughout all hazelnut production areas spreading from east to west of the Black Sea Region over the subsequent. This new and highly destructive powdery mildew agent has been identified as <i>Erysiphe corylacearum</i> U. Braun & S. Takam. based on its morphological characteristics and DNA sequence of the internal transcribed spacer (ITS) and 28S regions of the ribosomal DNA. Its pathogenicity to this species has been examined in an infection test and proven for the first time. To our knowledge, this is the first report of <i>E. corylacearum</i> parasitization of <i>Corylus avellana</i> worldwide.</p>
Suggested Reviewers:	Uwe Braun, Prof. Martin-Luther-Universität Halle-Wittenberg uwe.braun@botanik.uni-halle.de He has a distinguished career in mycology, fungal systematic research, especially about on powdery mildews, known worldwide
	Gürsel Karaca, Prof Suleyman Demirel Üniversitesi Ziraat Fakültesi gurselkaraca@sdu.edu.tr She works on phytopathology, mycology and have some studies on hazelnut fungal diseases.
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Online Resource 1. Multiple sequence alignment of ITS region of isolate ASezer (KY082910) with other *E. corylacearum* ITS sequences found in GenBank, listed by their Accession numbers. Only variant bases are shown, with identical bases indicated by dots and gaps by dashes

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KY082910.1   TCA TTA CAG AGT GTG AGG CTC ACT CGT GGC ATC TGC TGC GGG CTG GGC CGA CCC TCC CAC
LC009928.1   --- --- ... ..C ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   --. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   CCG TGT CGA TTT ATA TCT TGT TGC TTT GGC GGG CCG GGT TGT GTC GTC GCT GCC CGC AAG
LC009928.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   GAC ATG CGT TGG CCA CCC ACC GGC TTC GGC TGG AGC GCG TCC GCC AAA GAC CTA TAC CAA
LC009928.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   AAC TCA TGT TGT CTT TGC AGT CTA AGC TTT ATT ATT GAA TTG ATA AAA CTT TCA ACA ACG
LC009928.1   ... .. ... .. ... .. T. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   GAT CTC TTG GCT CTG GCA TCG ATG AAG AAC GCA GCG AAA TGC GAT AAG TAA TGT GAA TTG
LC009928.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ..A ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ..A ... .. ... .. ... .. ... .. ... ..

KY082910.1   CAG AAT TTA GTG AAT CAT CGA ATC TTT GAA CGC ACA TTG CGC CCC TTG GTA TTC CGA GGG
LC009928.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   GCA TGC CTG TTC GAG CGT CAT AAC ACC CCC TCC AGC TGC CTT TGT GTG GTT GCG GTG TTG
LC009928.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   GGG CAC GTC GCG GTG CGG CGG CCC TTA AAG ACA GTG GCG GTC CCG GCG TGG GCT CTA CGC
LC009928.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   GTA GTA ACT TGC TTC TCG CGA CAG AGT GAC GAC GGT GGC TTG CCA AAA GCC CTT TTG CTC
LC009928.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
JX880086.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..
KR048061.1   ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... .. ... ..

KY082910.1   CAG TCA CAT GGA T-C ACA GGT TGA CCT CGA ATC AGG TAG GAA TAC CCG CTG AAC TTA A--
LC009928.1   ... .. ... .. ... .. .- ... .. ... .. ... .. ... .. ... .. ... .. ... .. .GC
JX880086.1   ... .. ... .. ... .. .T. ... .. ... .. ... .. ... .. ... .. ... .. ... .. .GC
KR048061.1   ... .. ... .. ... .. .T. ... .. ... .. ... .. ... .. ... .. ... .. ... .. .GC

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7 **First Report of an Emerging and Destructive Powdery Mildew Agent *Erysiphe corylacearum* in Hazelnut**

8 **in Turkey**

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31 **Abstract**

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33 Hazelnut (*Corylus avellana* L.) is Turkey's most valuable agricultural export, and an essential source of income
34 for many families in the Black Sea Region. In spring 2013, hazelnut leaves, fruit clusters and shoots showing
35 powdery mildew infection symptoms different from those observed previously were discovered in Giresun, Ordu
36 and Trabzon provinces of Turkey. The disease has become epidemic throughout all hazelnut production areas
37 spreading from east to west of the Black Sea Region over the subsequent. This new and highly destructive
38 powdery mildew agent has been identified as *Erysiphe corylacearum* U. Braun & S. Takam. based on its
39 morphological characteristics and DNA sequence of the internal transcribed spacer (ITS) and 28S regions of the
40 ribosomal DNA. Its pathogenicity to this species has been examined in an infection test and proven for the first
41 time. To our knowledge, this is the first report of *E. corylacearum* parasitization of *Corylus avellana* worldwide.

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43 Key words: Hazelnut, powdery mildew, *Erysiphe corylacearum*, Black Sea Region

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61 **Introduction**

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63 Hazelnut (*Corylus avellana*) is one of the most important tree nut crops in Turkey. As well as having 80% of the
64 world production area, Turkey has the leading position in production and export of hazelnuts (Anonymous,
65 2017a). Hazelnut production extends along the Black Sea region, from the Georgia border in the east, to Istanbul
66 in the west. Although 33 provinces have hazelnut growing areas, only 16 of them are licensed for growing
67 hazelnut. Almost all production (90 %) is carried out in six provinces: Ordu, Giresun, Trabzon, Samsun, Sakarya
68 and Düzce (Anonymous, 2017b).

69 For hazelnut, powdery mildew disease is usually the result of *Phyllactinia guttata* (Wallr.: Fr) Lév. infection
70 (Hartney et al., 2005). This is also the case in Turkey, where Bremer (1948) noted the important role played by
71 disease in hazel cultivation, and identified *P. suffulta* (a synonym for *P. guttata*) as the causative agent of
72 powdery mildew. Previous surveys of powdery mildew infection caused by *P. guttata* have found it to be
73 widespread, while in the western part of the Black Sea Region it was found to be the most common disease, with
74 up to 70% infection in hazelnut groves (Yürüt et al., 1994). In spite of powdery mildew being the most
75 widespread disease on Turkish hazelnut production areas, treating it is often regarded as unnecessary because the
76 fungus does not directly affect the nut crop; it causes white powdery growth on the undersides of leaves only late
77 in the season.

78 However, over the last three years much more serious powdery mildew disease has been observed on cultivated
79 *C. avellana* in the Black Sea Region, with much greater impact on the infected trees. Observations in 2014-2015
80 showed that this more severe powdery mildew infection is found in a high proportion of hazelnut cultivation
81 areas throughout the eastern Black Sea region and the disease has caused significant damage. The disease was
82 observed in all 16 provinces licensed for hazelnut production in 2016 and its prevalence was 100% in most of
83 them. Here, we report that the causative agent of this disease has been identified as *Erysiphe corylacearum*, a
84 member of the Erysiphaceae family distinct from known powdery mildew agent *Phyllactinia guttata* in hazelnut.

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91 **Materials and Methods**

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93 **Observations of disease symptoms and fungal materials** Disease symptoms of powdery mildew were observed
94 in different stages and powdery mildew samples were collected from hazelnut leaves and fruit clusters naturally
95 infected with powdery mildew in hazelnut orchards in 2013-2015 at Black Sea Region (Ordu, Giresun, Trabzon,
96 Samsun, Rize, Artvin, Sakarya and Düzce provinces) of Turkey. Infected fresh materials were used for
97 microscopic observation and DNA extraction.

98

99 **Morphological observations of the pathogen** Sporulating fungal structures were dissected from plant tissues and
100 mounted in distilled water and examined using light microscopy with a camera attachment (Bel-Photonics,
101 STMPRO-T, P.C.R). One hundred of all structures including size of chasmothecia, appendages, asci, ascospores,
102 conidia, peridial cells were measured. Asci numbers / chasmothecia, ascospores numbers /ascus and appendages
103 branching style were also determined and photographed (Bacigalova and Markova 2006). Results were
104 compared with the species descriptions in Braun (1982) and Braun (1987).

105

106 **DNA extraction and PCR amplification** To confirm the morphological identification, sequence analysis of the
107 barcode regions of the fungal ribosomal DNA (rDNA) was carried out. Conidia and chasmothecia of the
108 pathogen were scraped from the surface of infected leaves, and DNA was extracted from 50 µg of fungal tissue
109 using the Nucleospin Plant II kit (Macherey Nagel, Düren, Germany) following the manufacturer's protocol for
110 fungal material. Two different regions of the rDNA, the internal transcribed spacer (ITS) and the variable region
111 of the 28S rRNA gene were amplified by polymerase chain reaction (PCR). In order to ensure specific
112 amplification of fungal DNA rather than any contaminating hazelnut tissue, nested PCR was carried out in both
113 cases using the primers listed in Table 1. PCR amplification was carried out using Maximo Taq polymerase
114 (GeneON, Ludwigshafen am Rhein, Germany) in a Techne TC-Plus thermocycler (Cole-Parmer, Stone, UK)
115 using the following amplification conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of 15s at 95°C,
116 25s at 52°C, 45s at 72°C; final extension for 7 min at 72°C. PCR products were analyzed by 1.2% agarose gel
117 electrophoresis in 0.5x TBE buffer (45 mM Tris-borate, 1 mM EDTA). For the PMITS1-PMITS2 primer pair,
118 some non-specific amplification was also observed, so the major PCR product was excised from the gel and
119 purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA, USA) before proceeding

120 with the second step of the nested PCR. Final PCR products were purified using the MinElute PCR Purification
121 Kit (Qiagen, Düsseldorf, Germany).

122

123 **DNA sequencing and data analysis** Purified PCR products were sent to Macrogen Europe (Amsterdam, The
124 Netherlands) for Sanger sequencing. The primers used for the inner amplification step from each nested PCR
125 (Table 1) were also used as sequencing primers for their PCR products. Sequencing chromatograms were
126 visualized, low quality bases trimmed, and forward and reverse reads combined using SeqTrace v0.9.0 (Stucky,
127 2012). Sequences were compared with known fungal DNA sequences in Genbank using the NCBI Nucleotide
128 BLAST server (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>; Zhang et al. 2000). Multiple sequence alignment and
129 phylogenetic tree construction was carried out using MEGA6 software (Tamura et al. 2013).

130

131 **Pathogenicity test** Pathogenicity tests were conducted on vigorous hazelnut suckers having young leaves in an
132 orchard where the disease was not observed. Healthy five leaves of each five suckers were inoculated by gently
133 pressing symptomatic leaves loaded with conidia onto them. Five non-inoculated suckers away from inoculated
134 ones served as a control (Erper et al., 2010). All suckers were covered with transparent plastic bags for two days.
135 The suckers were checked for powdery mildew symptoms occurrence.

136

137 **Results**

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139 The powdery mildew disease caused by the emerging agent develops comparatively early in the spring, with
140 symptoms being observed on leaves, young shoots and immature nut clusters. Initially circular to irregular white
141 patches of mycelium and conidia develop on the both side of leaves. If the colonies first develop on the
142 underside of leaves, it is seen mottled lightening and yellowing of the upper surface of leaves (Fig. 1A). In time
143 the leaves become tarnished, the lesions turn brown in colour and brown-black fungal chasmothecia can be
144 readily observed projecting from them (Fig. 1B) Infected leaves dry out, curl up and fall early. Similar symptoms
145 are observed on the husk of nut clusters (Fig. 1C), and in relatively sensitive varieties they dry out and fall early,
146 leading to crop losses.

147 Regarding identification, mycelium on leaves were amphigenous, white, persistent, dense, patches. Conidia were
148 produced singly on the conidiophores and measured $32.4 \pm 0.4 \mu\text{m} \times 20.2 \pm 0.3 \mu\text{m}$ (Fig. 1D). Chasmothecia
149 were scattered to gregarious, $88.0 \pm 0.9 \mu\text{m}$ in diam (Fig. 1E). Peridial cells were polygonal to rounded, $13.3 \pm$

150 0.3 µm in diam. Appendages were 6-15 in number, equatorial, stiff, straight, 0.75 to 1.34 times as long as the
151 chasmothecial diam., stalk aseptate, smooth to rough, hyaline, thin in the upper half, thick towards the base, apex
152 3-5 times closely and regularly branched, tips recurved (Fig. 1F). Each chasmothecium contained 3-5 asci that
153 were mostly sessile, $49.2 \pm 0.6 \mu\text{m} \times 37.7 \pm 0.6 \mu\text{m}$, and contained 6-8 ellipsoid-ovoid ascospores, each $19.4 \pm$
154 $0.3 \mu\text{m} \times 12.1 \pm 0.2 \mu\text{m}$ (Fig. 1G) The structures and measurements were in agreement with the descriptions of
155 *Erysiphe corylacearum* U. Braun & S. Takam. (Braun 1982, 1987, 2002).

156 Morphological identification was confirmed by PCR amplification and sequencing of the internal transcribed
157 spacer (ITS) and 28S rRNA regions of the rDNA gene from a field isolate. DNA isolation, PCR amplification
158 and sequencing were carried out in duplicate using different samples from the same infected tree. The duplicates
159 yielded identical results, giving a 623 bp sequence from the ITS and 843 bp from the 28S rRNA, both of which
160 were deposited in GenBank (Accession Nos. KY082910 & KX279887 respectively). Sequence similarity
161 searches revealed that the closest match, with >99% sequence identity in both regions, was the previously
162 published rDNA sequence from *E. corylacearum* isolate MUMH 0199 (Accession No: LY009928), which was
163 collected from Japanese hazel (*C. sieboldiana*, Takamatsu et al. 2015). In the 28S region, only 2/843 bases
164 differed from the previous sequence. For the ITS region 2 further *E. corylacearum* isolates collected from Asian
165 hazel (*C. heterophylla*, unpublished) were present in GenBank, and multiple alignment of the 4 sequences
166 revealed a total of 7 variant sites (Online Resource 1).

167 Inoculated hazelnut suckers developed typical powdery mildew symptoms after 10 days, whereas the controls
168 remained symptomless. The fungus present on the inoculated suckers was identical morphologically to that
169 originally observed on diseased plants.

170

171 **Discussion**

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173 For hazelnut, powdery mildew disease is usually the result of *Phyllactinia guttata* (Wallr.: Fr) Lév. infection
174 (Hartney et al., 2005). This is also the case in Turkey, where Bremer (1948) noted the important role played by
175 disease in hazel cultivation, and identified *P. suffulta* (a synonym for *P. guttata*) as the causative agent of
176 powdery mildew. This fungus can infect a diverse range of hard-shelled fruit bearing trees (Pscheidt et al. 2002)
177 in addition to *C. avellana*, and also attacks a very broad range of deciduous trees (Braun 1987). For commercial
178 hazelnut orchards the disease is regarded as not serious enough to warrant control (Hartney et al., 2005, Pscheidt
179 et al. 2002). Regarding the much more serious powdery mildew observed in recent years, the causal agent was

180 identified here as *Erysiphe corylacearum*, which has been observed to parasitize various *Corylus* species in the
181 world (Farr and Rossman, 2016). However, this taxon has not been reported to infect *C. avellana* before.
182 Based on rDNA ITS sequences, new scanning electron microscope (SEM) examinations and other
183 morphological data, Braun & Takamatsu (2000) reassessed the whole complex of powdery mildew genera with
184 Pseudoidium anamorphs and introduced a new circumscription of the genus *Erysiphe*. Braun (2002) made some
185 additional corrections including *Erysiphe corylacearum* U. Braun & S. Takam. nom. nov. (\equiv *Microsphaera*
186 *hommae* U. Braun, \equiv *Erysiphe hommae* (U. Braun) U. Braun & S. Takam.). Both the morphological and
187 molecular analysis of the powdery mildew pathogen identified in Turkey confirmed that is an example of *E.*
188 *corylacearum*, although there were a small number of single nucleotide sequence variations compared to
189 previously reported sequences. These differences may indicate that the strain reported here is distinct from those
190 found on Asian and Japanese hazel, and may also be useful as molecular markers for pathogenicity. Future
191 studies will survey samples of the pathogen from across the Black Sea region, to test for diversity within this
192 strain.

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197 of Turkey and Sabancı University.

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210 **References**

211

212 Anonymous. (2017a). Food and Agriculture Organization of the United Nations.
213 <http://faostat3.fao.org/download/Q/QC/E>. Accessed 3rd January 2017).

214

215 Anonymous. (2017b). Turkish Statistical Institute. www.tuik.gov.tr. Accessed 3rd January 2017.

216

217 Bacigalova, K. & Markova, J. (2006): *Erysiphe azaleae* (Erysiphales) – a new species of powdery mildew for
218 Slovakia and further records from the Czech Republic. Czech Mycol., 58 (3-4): 189-199.

219

220 Braun, U. (1982). Descriptions of new species and combinations in *Microsphaera* and *Erysiphe*. Mycotaxon
221 14(1): 369-374.

222

223 Braun, U. (1987). A monograph of the Erysiphales (powdery mildews). Beihefte zur Nova Hedwigia 89: 1-700.

224

225 Braun, U., & Takamatsu, S. (2000). Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysipheae) and
226 *Cystotheca*, *Podosphaera*, *Sphaerotheca* (Cystothecaceae) inferred from rDNA ITS sequences – some taxonomic
227 consequences. Schlechtendalia 4: 1-33.

228

229 Braun, U. (2002). Miscellaneous notes on some micromycetes (II). Schlechtendalia 8: 33-38.

230

231 Bremer, H. (1948). Phytopathology of Turkey. Special Issue. Ministry of Agriculture Publication, No: 657, 237
232 pp (in Turkish).

233

234 Cunnington, J. H., Takamatsu, S., Lawrie, A. C., & Pascoe, I. G. (2003). Molecular identification of anamorphic
235 powdery mildews (*Erysiphales*). Australas. Pl. Pathol. 32:421-428.

236

237 Erper, I., Karaca G. H., & M. Türkkan M. (2010). First report of *Phyllactinia fraxini* causing powdery mildew
238 on ash in Turkey. Plant Pathology, 59: 1168. doi:10.1111/j.1365-3059.2010.02295.x

239

240 Farr, D. F., & Rossman, A. Y. (2016). Fungal Databases, Syst. Mycol. Microbiol. Lab., ARS, USDA.
241 <http://nt.ars-grin.gov/fungaldatabases/>. Accessed 2nd January 2016.
242

243 Hartney, S., Glawe, D. A., Dugan, F., & Ammirati, J. (2005). First report of powdery mildew on *Corylus*
244 *avellana* caused by *Phyllactinia guttata* in Washington State. Online. Plant Health Progress doi:10.1094/PHP-
245 2005-1121-01-BR.
246

247 Mori, Y., Sato, Y. & Takamatsu, S. (2000). Evolutionary analysis of the powdery mildew fungi using nucleotide
248 sequences of the nuclear ribosomal DNA. Mycologia 92: 74–93.
249

250 Pscheidt, J. W., Brenneman, T.B., Doster, M.A. & Michailides, T.J. (2002). Powdery Mildew. Page 6 in
251 Compendium of Nut Crop Diseases in Temperate Zones. B. L. Teviotdale, T. J. Michailides, and J. W. Pscheidt,
252 eds. American Phytopathological Society, St. Paul, MN. Compendium of Nut Crop Diseases in Temperate Zones.
253 American Phytopathological Society, St. Paul, MN.
254

255 Stucky, B. J. (2012). SeqTrace: A Graphical Tool for Rapidly Processing DNA Chromatograms. J Biomol Tech
256 23: 90-93.
257

258 Takamatsu, S. & Kano, Y. (2001). PCR primers useful for nucleotide sequencing of rDNA of the powdery
259 mildew fungi. Mycoscience 42: 135-139.
260

261 Takamatsu, S., Arakawa, H., Shiroya, Y., Kiss, L., & Heluta, V. (2015). First comprehensive phylogenetic
262 analysis of the genus Erysiphe (Erysiphales, Erysiphaceae) I. The Microsphaera lineage. Mycologia 107: 475-
263 489.
264

265 Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary
266 Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725-2729.
267

268 White, T. J., Bruns, T. D., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal
269 genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (eds), PCR Protocols:

270 A guide to methods and applications. Academic Press, San Diego, pp. 315–322.

271

272 Yürüt, H.A., Erkal Ü., & Gürer, M. (1994). Hazelnut diseases in Bolu, Düzce and Bartın. 9th Congress of the
273 Mediterranean Phytopathological Union, Kuşadası, Aydın, Türkiye: Turkish Phytopathological Society
274 Publications No: 7, pp. 417-419.

275

276 Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences.
277 Journal of Computational Biology, 7: 203-214.

278

279 **Table 1** Primer pairs used in nested PCR amplification of fungal rDNA

280

281 **Figure 1** Symptoms of powdery mildew on hazelnut leaves (A and B) and fruit cluster (C); conidia (D), bar = 20
282 µm; chasmothecium (E), bar = 20 µm; appendages (F), bar = 10 µm; asci and ascospores of *Erysiphe*
283 *corylacearum* (G), bar = 20 µm

284

285 **Online Resource 1** Multiple sequence alignment of ITS region of isolate ASezer (KY082910) with other *E.*
286 *corylacearum* ITS sequences found in GenBank, listed by their Accession numbers. Only variant bases are
287 shown, with identical bases indicated by dots and gaps by dashes.

Amplified region	Primer name	Primer sequence	Reference
ITS (Outer)	PMITS1	5'-TCGGACTGGCCCAGGGAGA-3'	Cunnington et al. 2003
	PMITS2	5'-TCACTCGCCGTTACTGAGGT-3'	
ITS (Inner)	ITS5	5'-GGAAGTAAAAGTCGTAACAAGG-3'	White et al. 1990
	ITS4	5'-TCCTCCGCTTATTGATATGC-3'	
28S (Outer)	PM3*	5'-GKGCTYTMCGCGTAGT-3'	Takamatsu & Kano, 2001
	TW14	See below	
28S (Inner)	NL1	5'-AGTAACGGCGAGTGAAGCGG-3'	Mori et al. 2000
	TW14	5'-GCTATCCTGAGGGAAACTTC-3'	

* Degenerate primer. Variable bases are shown using IUPAC ambiguity codes.