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RECORDS OF PHYTOPATHOGENIC FUNGAL SPECIES ON NATIVE PLANTS NEW TO CROATIA

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In the period 2007-2012, the occurrence of phytopathogenic fungi on native plant species in natural ecosystems and urban environments was investigated in different locations of Croatia (national parks, nature parks etc.). Identification of fungal plant pathogenic species was performed on the basis of morphological charactericsof their spore-bearing structures and spores. A relatively great number of plant pathogenic fungal species was determined (over 150 species), out of which 12 species were determined for the first time in Croatia. These were the following: *Cercospora bolleana, Cercospora violae, Cercospora scandens, Coniothyrina agaves, Erysiphe aquilegiae, Erysiphe sedi, Erysiphe buhrii, Hainesia lythri, Microsphaeropsis hellebore, Pseudocercospora ceratoniae, Septoria cyclaminis* and *Septoria primulicola.* Considering the relatively limited data on the occurrence of phytopathogenic fungi in Croatia, these findings present a significant contribution to the knowledge of the biodiversity of fungi in Croatia. Some of the plant species like *Helleborus odorus* and *H. atrorubens* are recorded as new host plants for certain species of fungi.

Key words: first records, phytopathogenic fungi, host plants, mycoses, native plants

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U razdoblju od 2007. do 2012. godine praćena je pojava fitopatogenih vrsta gljiva na nekim samoniklim biljnim vrstama u prirodnim ekosustavima i urbanim područjima Hrvatske (nacionalni parkovi, parkovi prirode i dr.). Identificirane su na temelju morfoloških karakteristika njihovih sporulirajućih struktura (plodna tijela, konidiofori i dr.) i spora. Identificiran je relativno veliki broj fitopatogenih vrsta gljiva (preko 150 vrsta) od kojih je za 12 vrsta prvi put potvrđen njihov nalaz u Hrvatskoj. To su bile sljedeće vrste: *Cercospora bolleana, Cercospora violae, Cercospora scandens, Coniothyrina agaves, Erysiphe aquilegiae, Erysiphe sedi, Erysiphe buhrii, Hainesia lythri, Microsphaeropsis hellebori, Pseudocercospora ceratoniae, Septoria cyclaminis* i *Septoria primulicola*. Neke biljne vrste kao *Helleborus odorus* i *H. atrorubens* utvrđene su po prvi put kao novi domaćini fitopatogenih gljiva.

Ključne riječi: biljke domaćini, samonikle biljke, fitopatogene gljive, mikoze, prvi nalazi

INTRODUCTION

Phytopathogenic fungi cause various diseases or mycoses on cultivated plant species in agroecosystems, as well as on native plant taxa in natural ecosystems. Generally, all

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causal agents of plant diseases, among which plant pathogenic fungi are the most numerous group, are responsible for various changes in natural host plant populations and evolutionary changes through the selection of resistant genotypes (Gilbert, 2002). Today the direct role of plant pathogens in natural plant communities is better recognized than in previous times, although the nuances of their interactions and the cascade of ramifications that can flow through changing biotic and abiotic effects are only now gaining recognition (Burdon *et al*., 1993; 2006). According to Gilbert (2002) plant pathogens cause mortality, reduce fecundity and fitness of individual plants, drive host plant population dynamics and affect the structure and composition of natural plant communities. The occurrence of phytopathogenic fungi on native plant taxa in natural ecosystems in Croatia has not been investigated well enough. It is presumed that numerous currently undescribed or relatively unknown plant pathogenic fungi parasitize on these plants. In all, 97 330 species of fungi were recorded and described worldwide by Kirk *et al*. (2008), but it is presumed that their number could as high as 1.5 million species (Hawksworт, 2001). According to recent data (Tkalčec *et al*., 2008), there are around 4,500 species of fungi presently known in Croatia, but it is presumed there might be around 20 000 species. The exact number of plant pathogenic fungal species in Croatia is unknown. For more than 80 years in Croatia, there have been no scientific studies concerned with the occurrence of phytopathogenic fungi on native plant taxa. However, there are a large number of papers from recognized European and Croatian mycologists from the end of the 19th and from the 20th century that give descriptions of fungi from different parts of Croatia, most of them being phytopathogenic and are mostly connected with native plant taxa. One of the first mycological papers (Schuzler, 1857) to give an overview of fungi on Croatian territory, was by a much admired mycologist, Stephan Schuzler Müggenburg (1802-1892), and referred to the region of Slavonia. Several years later, the same author (Schuzler *et al*., 1866), mentions as many as 555 fungal species for Slavonia, most of which were phytopathogenic fungi. Schuzler also mentions a large number of phytopathogenic fungal species in his papers (Schuzler & Saccardo, 1884) that he co-authored with P.A. Saccardo, a celebrated mycologist of the period. For the region of Istria, the first and the most important mycological papers were from Bolle & Thümen (1878, 1880, 1885), in which 462 fungal species are mentioned, most of them again being phytopathogenic. Several years later other papers relating to Istria were published, such as that by Solla (1891) in which the author gives a list of 13 phytopathogenic species of fungi; then there was a paper by Sydow & Sydow (1903), in which 94, mostly phytopathogenic, fungal species were mentioned; and lastly a paper by Bubak (1914) where 26 species of fungi, also mostly phytopathogenic, were mentioned. A large number of phytopathogenic species of fungi mentioned in these papers referred to above were new species, like for example *Puccinia istriana* (Sydow & Sydow, 1903) on *Teucrium polium*. For the region of Dalmatia, one of the first mycological papers was one by the German naturalist Jaap (1916), in which 510 species of fungi were mentioned, almost all of them beingphytopathogenic; as many as 50 species were new species, described for the first time. In that paper, the author, when describing new phytopathogenic fungal species, named some of the new species after Croatian localities, places or regions of discovery asuch as *Diploida ragusina*, *Heterosporium dalmaticum*, *Phoma ragusea*, *Phoma dalmatina*, *Septoria dalmatica*, *Septoria lapadensis* and others. It should be said that the species described in that paper do not only refer to the present day region of Dalmatia that is a part of Croatia, for a few of the described species of fungi also refer to Boka Kotorska in present day Montenegro, which was considered by the author to be part of Dalmatia at the time. A paper by the Czech mycologist Baudyš (1914) also partly refers

to Dalmatia; he listed 25 species of fungi, all phytopathogenic, and described some new taxa like *Cercospora radiata* var. *dalmatica*, *Septoria anthyllidis* and others. Mycological papers, that refer to the wider area of the present day Croatia and that include a complete overview of phytopathogenic species of fungi known at the time, were published by Škorić (1928), who described around 400 species. Two years earlier, the same author (Škorić, 1926) had offered an overview of species of phytopathogenic fungi from the family Erysiphaceae (powdery mildews), listing 31 species. Also in those years, a paper was published by Picbauer (1928), partially including a description of some phytopathogenic fungi from Croatian territory. The latest review paper concerning phytopathogenic fungi, which mostly refers to cultivated plant species, but also in some smaller part to native plant taxa, was published by Kišpatić (1948).

MATERIALS AND METHODS

From 2007 to 2012 surveys were conducted in different locations in Croatia, in order to determine the presence of plant pathogenic fungi and mycoses on various native plant taxa in natural ecosystems. Surveys were conducted in Paklenica National Park, Northern Velebit National Park, Risnjak National Park, Velebit Nature Park, Biokovo Nature Park, Žumberak-Samoborsko gorje Nature Park, Medvednica Nature Park, Vrgorac region (Dalmatia), Island of Čiovo (Dalmatia), Island of Brač (Dalmatia), Island of Lokrum (Dubrovnik), Klek Mountain and Čabar region (Gorski Kotar), Maksimir Park (Zagreb) and Botanical Garden (Faculty of Science, University of Zagreb). The presence of plant mycoses was investigated principally on endemic plant taxa, protected plant taxa, threatened plant taxa and on wild relatives of cultivated plants. Current names of plant taxa were coordinated with Flora Croatica Database (http://hirc.botanic.hr/fcd/). Plants or plant parts with symptoms or signs of mycoses were collected and examined in the laboratory. Samples of plant tissue with symptoms of mycoses were examined using stereomicroscope and microscope. The presence of spores or sporulating structures on symptomatic plant tissue was searched for, followed by examination of sporulating structures and spore colour, shape and size. If sporulation was not observed, samples of symptomatic material were incubated in a moist chamber at 20°C and analysed when spores appeared. All fungi except powdery mildews (Erysiphaceae) were isolated and cultivated on MEA in darkness at 22°C. Colour, shape, growing rate and sporulation of pure cultures was determined. Fungal species were identified according to Braun (1995) for Erysiphales (powdery mildews), Ellis (1976) for *Cercospora* species, Perrotta *et al*. (1998) for *Pseudocercospora ceratoniae*, Brandenburger (1985) for *Septoria* species, Sutton & Gibson (1977) for *Hainesia lythri*, Petrak & Sydow (1927) for *Coniothyrina agaves* and van der Aa & Vanev (2002) for *Microsphaeropsis hellebori*. Current names of fungal taxa were coordinated with Index Fungorum Database (http://www.indexfungorum.org/Names/Names.asp). Host plants of some of the determined fungi are quoted from the Fungal Database (Farr & Rossman, 2009). Fungal species presented in this paper are restricted to those that, to our knowledge, have been recorded for the first time in Croatia, and those for which a new host plant is recorded.

RESULTS

A relatively great number of plant pathogenic fungal species on native plant taxa was determined during a six-year period of investigation (over 150 species). These included various species from Ascomycota (mostly Erysiphaceae) and Deuteromycota (anamor-

phic species). In all, 12 fungal species were determined for the first time in Croatia. Two plant taxa *Helleborus odorus* and *Heleborus atrorubens* are recorded as new host plants for certain fungi. It must be mentioned that plant pathogenic species from Chromista and Protozoa, which were traditionally considered fungi, were not included in this paper, because these species are not considered as a part of the kingdom Fungi according to new classifications (Kirk *et al*., 2008).

1. Fungal species from fam. Erysiphaceae (Ascomycota) new to Croatia

Erysiphe aquilegiae DC., in De Candolle & Lamarck, Fl. franç. Edn. 3 (Paris) 6: 105 (1815)

Specimen examined: On leaves of *Aquilegia kitaibelii* Schott, Northern Velebit, Croatia, June 2007 (PZF 05), and on leaves of *Aquilegia vulgaris* L., Risnjak and Žumberak, Croatia, July 2008 (PFZ 08)

Host plants: Ranunculaceae (Braun 1995). Liberato & Cunnington (2006) found *E. aquilegiae* on *Catharanthus roseus* (Apocynaceae), which is so far the only host record outside the Ranunculaceae

Comments: *E. aquilegiae* is a causal agent of powdery mildew on various taxa from Ranunculaceae (Braun, 1995). Superficial mycelium with sparse sporulation was found on both sides of the leaf. No cleistothecial state was found on the specimen examined and the fungus was determined according to the host plant and morphology of conidiophores and conidia. Škorić (1927) described powdery mildew on *Aquilegia vulgaris* L. and various Ranunculaceae, and recorded it as *Erysiphe polygoni*. *E. polygoni* attacks only Polygonaceae (Braun, 1995), and the record of Škorić (1927) can be considered as referring to *E. aquilegiae*.

Erysiphe sedi R.Y. Zheng & G.O. Chen, Sydowia 34: 253 (1981) (Nom. illegit., Art. 53.1)

= *Erysiphe sedi* U. Braun, Feddes Repert. Spec. Nov. Regni Veg. 92 (7-8): 502 (1981)

Specimen examined: On leaves of *Sedum maximum* (L.) Suter., Botanical garden Zagreb, Croatia, August 2009 (PZF 11).

Host plants: Crassulaceae (Braun, 1995)

Comments: *E. sedi* is a causal agent of powdery mildew on various taxa from Crassulaceae (Braun, 1995). Superficial mycelium with sparse sporulation was found on both sides of the leaf. No cleistothecial state was found on the specimen examined and the fungus was determined according to the host plant and morphology of conidiophores and conidia.

Erysiphe buhrii U. Braun, Česká Mykol. 32 (2): 80 (1978)

= *Erysiphe pisi* var. *buhrii* (U. Braun) Ialongo, Mycotaxon 44 (1): 255 (1992)

Anamorph: *Oidium dianthi* Jacz., Taschenbestimmb. f. Pilze 2, Erysiphaceen (1926).

Specimen examined: On leaves of *Silene alba* (Mill.) Krause, Botanical garden Zagreb, Croatia, July 2008 (PZF 12)

Host plants: Caryophyllaceae (Braun, 1995)

Comments: *E. buhrii* is a causal agent of powdery mildew on various taxa from Caryophyllaceae (Braun, 1995). Superficial mycelium with abundant sporulation was

found on both sides of the leaf. No cleistothecial state was found on the specimen examined and the fungus was determined according to the host plant and morphology of conidiophores and conidia. Braun (1995) reports that *E. buhrii* was recorded in former Yugoslavia on *S. alba* and *Myosoton aquaticum* (L.) Moench. To our knowledge, *E. buhrii* or its former synonyms was not previously recorded in Croatia.

2. Anamorphic fungal species (Deuteromycota) new to Croatia

***Cercospora bolleana* (Thüm) Speg., Michelia 1 (5): 475 (1879)**

Teleomorph: *Mycosphaerella bolleana* B.B. Higgins, Am. J. Bot. 7 (10): 443 (1921)

Specimen examined: On leaves of *Ficus carica* L. var. *caprificus* (Risso) Tschirch & Ravasini, Vrgorac (Dalmatia), Croatia, September 2008 (CSZF 01)

Host plants: *Ficus* spp. (Farr & Rossman, 2009)

Comments: *C. bolleana* is the causal agent of fig leaf spot (Belisario *et al*., 2002) Abundant sporulation of the fungus was detected on leaf spots on symptomatic leaves. Only conidial state was detected. According to Belisario *et al*. (2002), epidemic occurrence of *C. bolleana* can lead to fig tree defoliation. Such severe symptoms were not observed at the investigated location. Observations of wild and cultivated fig trees across the rest of Dalmatia revealed no symptoms similar to those caused by *C. bolleana*, which suggests that this fig disease could be rare in Croatia. To our knowledge, it has not been yet reported on cultivated figs in our county.

***Cercospora violae* Sacc., Nuovo Giorn. Bot. Ital. 8: 187 (1876)**

Teleomorph: Not known.

Specimen examined: On leaves of *Viola* sp., Botanical garden Zagreb, Croatia, July 2009 (Cer 07)

Host plants: *Viola* spp. (Ellis, 1976)

Comments: *C. violae* is the causal agent of viola leaf spot. Abundant sporulation of the fungus was detected on leaf spots on symptomatic leaves.

***Cercospora scandens* Sacc. & G. Winter, Hedwigia 22: 14 (1883)**

Teleomorph: Not known.

Specimen examined: On leaves of *Tamus communis* L., Botanical garden Zagreb, Croatia, July 2009 (Cer 08) Host plants: Dioscoreaceae (Ellis, 1976)

Comments: *C. scandens* is the causal agent of tamus leaf spot. Abundant sporulation of the fungus was detected on leaf spots on symptomatic leaves.

***Coniothyrina agaves* (Durieu & Mont.) Petr. & Syd., Repert. Spec. Nov. Regni Veg. Beih. 42 (1): 322 (1927)**

Synonym: *Coniothyrium agaves* (Durieu & Mont.) Sacc., Syll. fung. (Abellini) 3: 318 (1884)

Teleomorph: Not known.

Specimen examined: On leaves of *Agave americana* L., Čiovo, Croatia, May 2008 (CyZF 05)

Host plants: *Agave* spp. (Farr & Rossman, 2009)

Comments: *C. agaves* is the causal agent of anthrachnose of agaves. Abundant sporulation of the fungus was found on lesions on the upper side of the leaves.

***Hainesia lythri* (Desm.) Höhn., Sber. Akad. Wiss. Wien., Math.-naturw. Kl., Abt. 115: 687 (1906)**

Teleomorph: *Discohainesia oenotherae* (Cooke & Ellis) Nannf., Nova Acta R. Soc. Scient. upsal., Ser. 48 (1.2): 88 (1932)

Specimen examined: On leaves of *Fragaria vesca* Coville, Zagreb, Croatia, August 2007 (HlZF 01)

Host plants: A number of genera from different plant families (Sutton & Gibson, 1977)

Comments: *H. lythri* is a pathogen of strawberry (Sutton & Gibson, 1977). Acervuli of *H. lythri* were found on necrotic lesions mostly on the underside of the leaves of *F. vesca*. Only conidial state of the fungus was detected. To our knowledge, until now *H. lythri* has not been reported on cultivated strawberries in our country.

***Microsphaeropsis hellebori* (Cooke & Massee) Aa, in van der Aa & Vanev, A Revision of the Species Described in *Phyllosticta* (Utrecht): 91 (2002)**

Synonym: *Coniothyrium hellebori* Cooke & Massee, Grevillea 15 (no. 76): 108 (1887)

Teleomorph: Not known.

Specimen examined: On leaves of *Helleborus odorus* L., Botanical garden Zagreb, Croatia, June 2008 (ChZF 01), on leaves of *Helleborus niger* L., Žumberak-Samoborsko gorje, Croatia, June 2007 (ChZF 02), and on leaves of *Helleborus atrorubens* Waldst. & Kit., Zumberak-Samborsko gorje and Risnjak, Croatia, July 2008 (ChZF 03, ChZF 04)

Host plants: *Helleborus* spp. and *Consolida* sp. (Farr & Rossman, 2009)

Comments: *M. hellebori* is the causal agent of anthrachnose of hellebores. Abundant sporulation in form of dark conidial droplets oozing from pycnidia was detected after a few days of incubation in a moist chamber. Pycnidia were scattered over dark gray lesions mostly on the margins of symptomatic leaves. *H. odorus* is recorded as a new host plant for *M. hellebori*, as well as for the Croatian endemic species *H. atrorubens*. These host plants are not mentioned by Farr & Rossman (2009) in the *M. hellebori* host-fungal list.

***Pseudocercospora ceratoniae* (Pat. & Trab.) Deighton, Mycol. Pap. 140: 141 (1976)**

Teleomorph: Not known.

Specimen examined: On leaves of *Ceratonia siliqua* L., Dol (Island of Brač), Croatia, July 2008 (Cer 03)

Host plants: *Ceratonia siliqua* (Farr & Rossman, 2009)

Comments: *P. ceratoniae* is the causal agent of carob leaf spot (Perrotta *et al*., 1998) According to Perrotta *et al*. (1998), epidemic occurrence of *P. ceratoniae* can lead to carob tree defoliation. Such severe symptoms were not observed at the investigated location.

***Septoria cyclaminis* Durieu & Mont., Syll. Gen. Sp. Crypt. (Paris): 279 (1856)**

Teleomorph: Not known.

Specimen examined: On leaves of *Cyclamen hederifolium* Aiton, Biokovo, Croatia, July 2007 (ScZF 01)

Host plants: *Cyclamen* spp. (Brandenburger, 1985)

Comments: *S. cyclaminis* is the causal agent of cyclamen leaf spot (Raabe, 1971). Abundant sporulation in the form of whitish cirri oozing from pycnidia was detected after

few days of incubation in a moist chamber. There were few necrotic spots on the leaves, and only few plants at the investigated location were found to be affected. Observations of cyclamen plants in other locations revealed no symptoms of leaf spot, which suggest that *S. cyclaminis* is rare in Croatia.

***Septoria primulicola* Rostr., Bot. Tidsskr. 26: 312 (1905)**

Teleomorph: Not known.

Specimen examined: On leaves of *Primula vulgaris* Huds., Botanical garden Zagreb, Croatia, July 2009 (SjZF 01)

Host plants: *Primula* spp. (Brandenburger, 1985)

Comments: *S. primulae* is the causal agent of primula leaf spot. Many sporulating pycnidia were found in leaf spots on the upper side of symptomatic leaves. Only conidial state of the fungus was detected.

DISCUSSION AND CONCLUSIONS

The occurrence of plant pathogenic fungi on native plant taxa in natural ecosystems in Croatia has not been sufficiently investigated. This is the case with fungi in Croatia in general, except in the case of phytopathogenic fungi on cultivated plant taxa, which are rather well investigated since these fungi are causal agents of diseases on plants of huge economic importance. Presently we are aware of only a few phytopathogenic species of fungi on native plant taxa, and they come from mycological papers from the late 19th and the first half of the 20th century. For more than 80 years, since the papers of V. Škorić in 1926 and 1927, there have been almost no new studies of that sort in Croatia. Considering a rather very large number of vascular plant taxa in Croatian flora (5347 taxa from 1084 genera, Nikolić & Topić, 2005), it is certain that the number of phytopathogenic species of fungi is much larger than presently known in Croatia (around 800 species, according to our knowledge). Taking into account the large number of plant taxa in Croatian flora, we presume that the number of phytopathogenic species in Croatia could be between 4000 and 5000. Chorology, epidemiology and disease incidence and severity of phytopathogenic fungi can be particularly important for endemic, protected and threatened plant taxa, because occurrence of phytopathogenic fungi can in some instances cause considerable changes in their populations. Information about plant pathogens and their accurate management on these plant taxa is one of the protection measures proposed in IUCN documents (International Union for the Conservation of Nature). During six years of research presented in this paper, a large number of phytopathogenic fungal species (over 150 species) were determined, among which, to our knowledge, 12 were reported for the first time in Croatia. Three fungal species, *Erysiphe aquilegiae*, *Erysiphe sedi* and *Erysiphe buhrii* from the family Erysiphaceae (powdery mildews) were determined for the first time in Croatia, and that can be considered a relatively small number considering the large number of species in this family (over 500 species are currently described in the world) and their ubiquity, but that was expected since Erysiphaceae is one of the rare groups of phytopathogenic fungi that has been very well studied in Croatia (Škorić, 1926). Most of the fungal species determined in this study were from the division Deuteromycota or anamorphic fungi, because a large number of phytopathogenic fungi on their host plants occur mostly as anamorphs or asexual stages, although their teleomorphs or sexual stages belong mostly to the division Ascomycota. Since their teleomorphs either do not occur at all, or were not found

during this study, those species were presented as anamorphic fungi (Deuteromycota). Since classical mycological classification and taxonomy is based mostly on the teleomorphic stage they in fact belong to Ascomycota and therefore the name of teleomorph characteristics, the name of the teleomorph in this paper was stated next to quotation of individual species. A similar number of fungi were found from the former classes Hyphomycetes (*Cercospora bolleana, Cercospora violae, Cercospora scandens* and *Pseudocercospora ceratoniae*) and Coelomycetes (*Coniothyrina agaves*, *Hainesia lythri, Microsphaeropsis hellebori, Septoria cyclaminis* and *Septoria primulicola*). From the large number of host plant taxa analyzed during the course of this study two plant species were determined to be new host plants, not previously recorded in the world. *Helleborus odorus* and *Helleborus atrorubens* were determined as new host plants for the fungus *Microsphaeropsis hellebore.* The disease incidence and disease severity of plant pathogenic fungi on these plant species should be monitored. Since for some of these protected and endemic plant species, as a measure of protection, ex situ cultivation is recommended, the problem of occurrence of phytopathogenic fungi could then become more important than it is when those species are growing *in situ* in their natural habitats, where the natural balance is in place. All this speaks in favor of the importance of knowing the chorology of phytopathogenic fungi on native plant taxa in Croatian flora, not only for the sake of knowledge of their biodiversity and the development of Croatian mycology as a science, but also due to the national and economic importance that their host plants have.

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