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Hyperparasitic *Stagonospora* sp. on *Botryosphaeria stevensii*

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Summary

An unknown fungus, *Stagonospora* sp., has been found on and in stromata of *Botryosphaeria stevensii* Shoemaker anamorph (*Diplodia mutila* Fr. & Mont.) and *Diatrypella quercina* (Persoon) Nitschke. Microscopic investigations indicated that the fungus might be a hyperparasite of some ascomycetous fungi, necrotrophs or weak parasites on sessile oak [*Quercus petraea*] and turkey oak (*Quercus cerris*). Dual culture studies carried out with monoconidial isolates of *Stagonospora* sp. and *B. stevensii* have demonstrated that *Stagonospora* sp. is a necrotrophic mycoparasite which might suppress, to some extent, the natural population of *B. stevensii*. *Botryosphaeria stevensii* is one of the biotic factors causing oak decline. Morphology of the fungus and symptoms of mycoparasitic interaction are described. *Stagonospora* sp. found in Hungary is assumed to be identical with hyperparasitic *Stagonospora* sp. reported from Germany and Austria as parasite of *Ascodichaena rugosa* and *Ascodichaena mexicana* in Mexico. This is the first record of hyperparasitic *Stagonospora* sp. of *B. stevensii* and *D. quercina*.

1 Introduction

In recent years (1996–2001) we have studied mycobiota of *Quercus* spp. in connection with oak decline phenomenon. Forest surveys were conducted and samples were collected in the north-eastern region of Hungary. This is the region where we found the *Botryosphaeria stevensii* anamorph (*Diplodia mutila*) for the first time (1982) in Hungary and described it as one of the biotic factors of dieback of sessile oak (Vajna 1986). In many samples of dying and dead branches and twigs of sessile and turkey oak near Budapest we found a great number of young stromata of *D. mutila,* the anamorph of *B. stevensii* and ascomata of *Diatrypella quercina*. These two fungi occurred often together. The first one is known as a necrotrophic pathogen, and the second as a colonizer of weakened, dying branches. During the first microscopic investigations we found intra- and intercellular hyphae in the stromata of *D. mutila* and *D. quercina*. These hyphae penetrated cell walls and colonized loculi in the stromata and also young, hyaline and mature, brown, two-celled conidia of *D. mutila*. Formation of pycnidia and conidial sporulation of this unknown fungus have been observed on the external parts of the stromata and in those immersed in the host tissues. The aim of this work was to identify the unknown fungus and to determine its relationship to the host fungi, especially *B. stevensii*, the necrotrophic parasite of several oak species, apple and grapevine.

2 Materials and methods

Field surveys were carried out in autumn (September–November) 1996 in the forest sites of Buda Hills (near Vadaspark). A total of 270 samples of cankered, dying or dead branches of turkey and sessile oak trees were randomly collected. Later, in July 2001, 16 branch samples were taken again at the same location. The number of all the collected and investigated samples was 286.

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Characterization and identification of the fungi colonizing the cankered branches were carried out with light microscopy using hand made 10–30 μm thin sections in sterile water or stained with cotton blue in lactophenol. Fungi were isolated on potato dextrose agar (PDA) and cultured at 20°C. Later monoconidial cultures were prepared. Possible mycoparasitic activity of the *Stagonospora* sp. (isolate no. 184) on *B. stevensii* (isolate no. 187) was studied in dual cultures on cellophane according to ELAD et al. (1983). The medium was PDA, pH 7.0, and incubation occurred at 18°C. The plates were inoculated with mycelial agar plugs of each fungus. The distance between plugs was 30 mm. Plates were incubated in growth chamber with 12 h daily fluorescent light (TL-D 18w). Samples for microscopic investigations under the transmission light microscope were taken from the zone of contact of the two colonies when they initially touched and again, between 24 and 48 h later. Samples of about 1 cm^2 containing the interacting mycelium of the two colonies were cut out and removed for microscopic investigation.

3 Results and discussion

3.1 Microscopic observations

Stromata of *B. stevensii* and *Diatrypella quercina* were frequently observed (21 and 37%, respectively) on the 286 bark, branch and twig samples collected in 1996 and in 2001. On the bark surface of branches and twigs around and on the stromata of these two fungi small pycnidia were observed. In many cases a white mass of conidia appeared on their surface. Vertical and horizontal sections made from the stromata of *B. stevensii* and *Diatrypella quercina* produced in the cortex of naturally infected branches also showed great numbers of pycnidia around, on, and in the stromata of the these two fungi (Fig. 1). At the same time loculi in the stromata of *D. mutila* were colonized with dense mycelium (Fig. 2). Young, newly developing conidia of *D. mutila* were parasitized and destroyed by hyphae of an unknown fungus (Fig. 3). Hyphae penetrating conidia of *D. mutila* were about 0.5–2.0 μm thick with many small side branches. Penetration holes of about 1.0 μm diameter were seen on the surface of the conidia. In thin sections made from *B. stevensii*, hyphae of an unknown fungus were observed penetrating the brown cells of the *B. stevensii* stromata and penetration holes could also be seen. Incidence of the putative mycoparasitic fungus was high. On 12 samples with *B. stevensii* all the examined hundreds of stromata were parasitized. Pycnidia of the supposed hyperparasitic fungus were in close association with the stromata of *B. stevensii* and *D. quercina* in each sample examined. Pycnidia were not found separately in the cortex tissue of the examined samples of *Quercus* spp.

3.2 Morphology of the fungus

Hyphae on PDA medium are hyaline, later pale brown, and are 2.5–3.5 μm wide. The brown pycnidia are unilocular, globose or a little suppressed, without ornamentation. Their shape may be irregular when growing inside the stromata of the host fungi. The diameter on PDA plates was 530–780 μm, on the natural substrate it was 112–275 μm. The ostiole is single, circular, central and papillate, and colour is brown (Fig. 4). Pycnidia are arranged separately or sometimes aggregated. The walls are thick (15–20 μm), dark brown on the outside and hyaline on the inside, textura angularis. Conidiophores are absent. Conidiogenous cells are holoblastic, discrete, doliiform, hyalin, and formed from the inner cells of the pycnidial wall (Fig. 5). Conidia are hyalin, are smooth, typically with 3 (1–6) transverse eusepta, cylindrical, straight or slightly curved. The average size is 25.2 × 7.3 μm (range: 18–32 × 6–9 μm). Conidia, after eruption on the bark surface, turn to pale brown (Fig. 6). Presence of the teleomorph of this fungus was not observed.

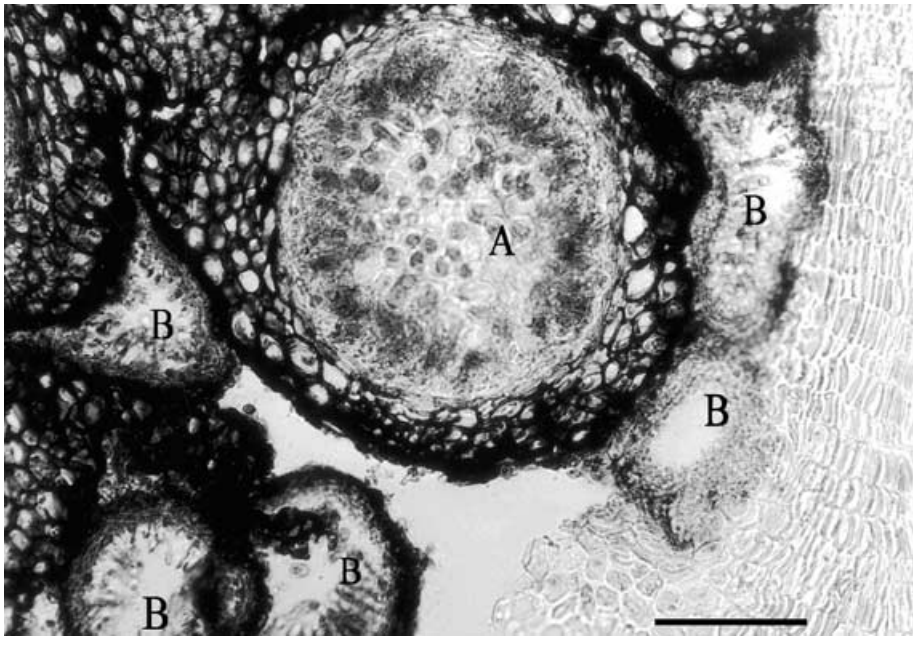

Fig. 1. Horizontal section of the stromata of *Botryosphaeria stevensii* (A) with several pycnidia of *Stagonospora* sp. (B) (bar = 200 μm)

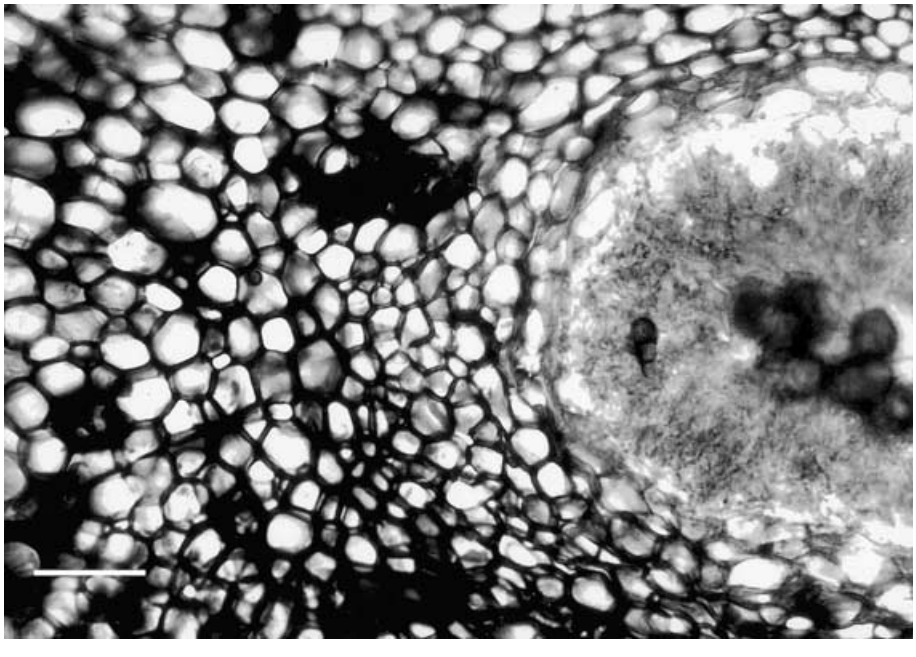

Fig. 2. Dense mycelium of the mycoparatic *Stagonospora* sp. in a locus of the anamorph of *Botryosphaeria stevensii* (bar = 20 μm)

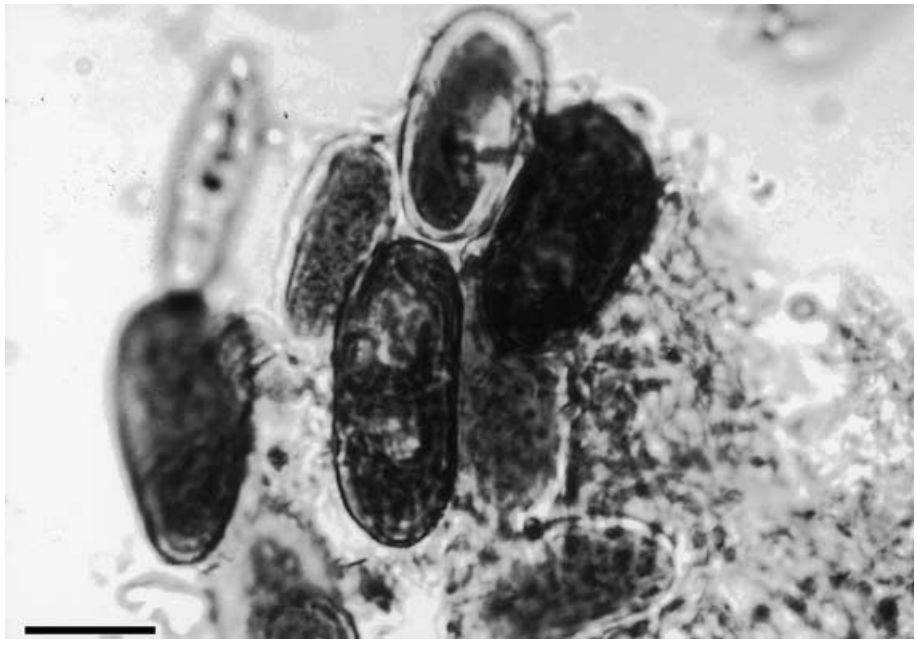

Fig. 3. Parasitized and destroyed conidia of *Diplodia mutila* (bar = 10 μm)

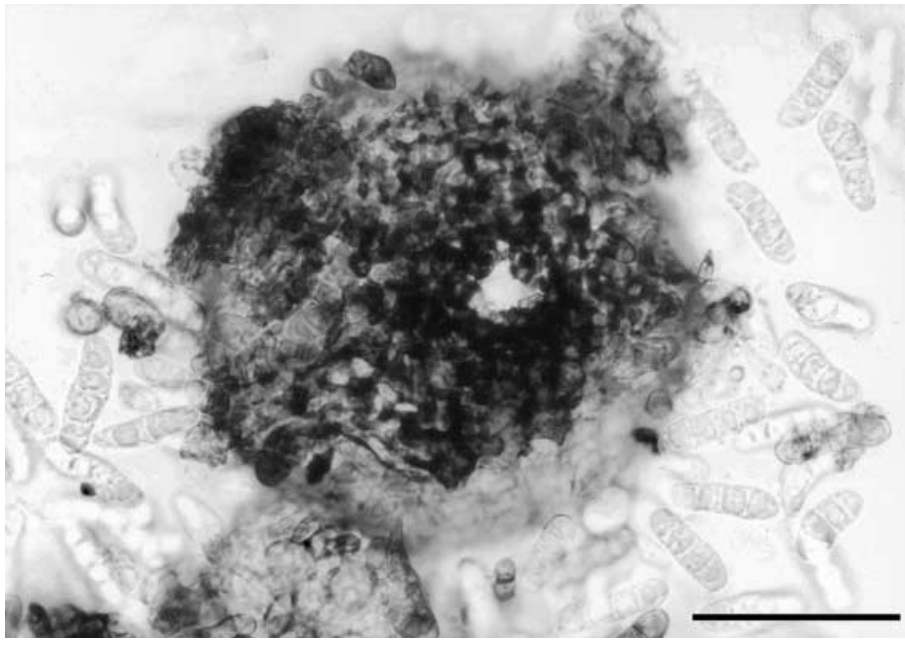

Fig. 4. Globose pycnidium of *Stagonospora* sp. (bar = 200 μm)

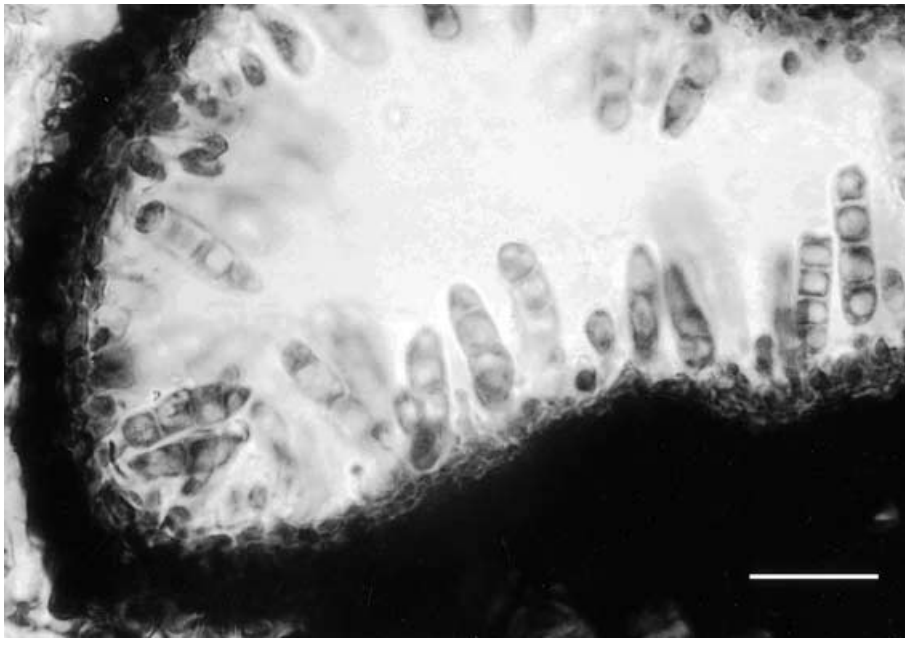

Fig. 5. Developing conidia of *Stagonospora* sp. (stained with cotton blue in lactophenol) (bar = 20 μm)

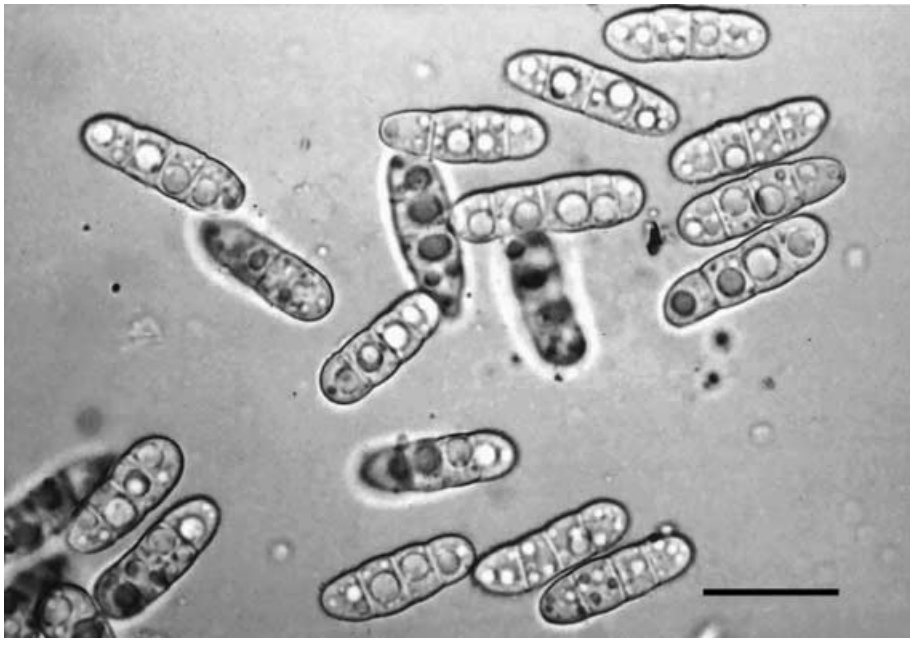

Fig. 6. Conidia of *Stagonospora* sp. typically have three transverse eusepta (bar = 20 μm)

Colonies on PDA are circular, hyalin at the margin, pale to dark brown at the centre. Pycnidium formation begins the fifth day. The optimum temperature is 22–24°C. The daily growth rate of colonies is 3.5 mm on PDA, at 18°C, with 12 h photoperiod in the growth chamber.

3.3 *In vitro* mycoparasitism

Observations on interactions between *Stagonospora* sp. and *B. stevensii* were carried out between 24 and 48 h after contact of the two colonies. *Stagonospora* sp. produced dense mycelium at the contact zone. This zone enlarged when the fungus advanced, overgrowing the colony of *B. stevensii*. Hyphae of *Stagonospora* sp. coiled around aerial hyphae of *B. stevensii* (Fig. 7). Penetration sites of up to 6–8 per cell were clearly seen. Infection branches of thin 1–2 μm wide hyphae could be observed on the surface of the host hyphae. Penetrating infection hyphae frequently were localized at the point of penetration by a brown protective sheath, which is known as a non-specific defence reaction in many host–parasite interactions (VAJNA 1985). The containment of the penetrating hyphae by a wall-like material was usually ineffective and temporary. Thin infection hyphae, breaking through these sheaths, grew rapidly within the host hyphae (Fig. 8). Additional new septae developed in the cells of the host hyphae, perhaps as a defence reaction. Multiple infections resulted in the formation of an extensive internal network of parasitic mycelium of *Stagonospora* sp. and desintegration of the host hyphal cells. Conidiomata (wall-cells, chambers, and newly forming young conidia) of *B. stevensii* were similarly colonized and penetrated by the hyphae of *Stagonospora* sp.

Fig. 7. In vitro parasitism: hyphae of *Stagonospora* sp. coiling around the hyphae of *Diplodia mutila* (bar = 10 μm)

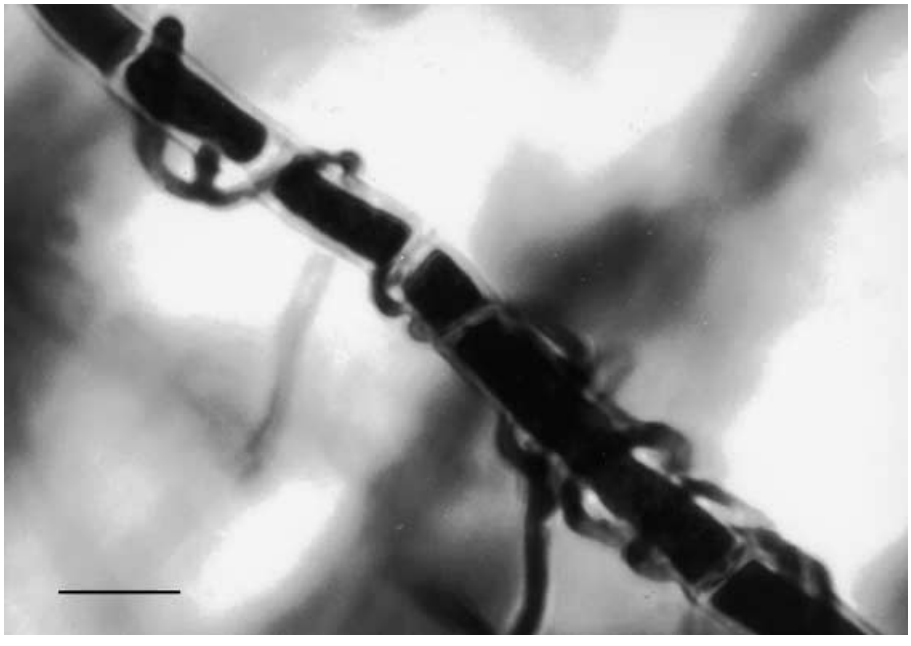

Fig. 8. Hyphae of *Stagonospora* sp. coiling around and penetrating *Diplodia mutila* hyphae (bar = 20 μm)

3.4 Identification

The fungus was identified as a species belonging to the genus *Stagonospora* according to the monography of Sutton (1980). A *Stagonospora* species registered as mycoparasite of *Ascodichaena rugosa* Butin – a bark-pathogenic fungus of *Quercus* and *Fagus* spp. – was described by Butin (1979). Morphology of a parasitic fungus found by Butin in samples of *A. rugosa* from Germany and Austria is very similar to those found by us in Hungary. Later, Butin and Marmolejo (1990) published *Ascodichaena mexicana* (Butin & Marm.) sp. nov. This bark-pathogen fungus infects several *Quercus* species in north-eastern Mexico. According to their data, this fungus may also be parasitized by a *Stagonospora* sp. hyperparasite. Until now only six mycoparasitic *Stagonospora* species have been described on fungi belonging to Ascomycetes, and these species were reported from the tropics (Hawksworth 1981). In addition, some other hyperparasitic species were recorded on *Botryosphaeria* spp., e.g. *Naohidea sebacea* (Berk. & Broome) Oberw. on *B. quercinum* (McNabb 1965), and on *B. dothidea* (Piątek 2001).

Based on the close similarity of the morphology of hyperparasitic *Stagonospora* sp. from *Ascodichaena rugosa* (Butin 1979), and from *Ascodichaena mexicana*, (Butin and Marmolejo 1990) the hyperparasitic *Stagonospora* sp. (isolate no. 184) found by us on *B. stevensii* and *D. quercina* (on *Q. cerris* and *Q. petraea* in Hungary), we concluded that the two *Stagonospora* species are conspecific. Description of this fungus – as a new species – needs further comparative studies.

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Résumé

Stagonospora sp. hyperparasite de Botryosphaeria stevensii

Un champignon inconnu, *Stagonospora* sp, a été trouvé sur et dans les stromas de *Botryosphaeria stevensii* Shoemaker (anamorphe *Diplodia mutila* Fr. & Mont.) et *Diatrypella quercina* (Persoon) Nitschke. D'après les études microscopiques, le champignon pourrait être un hyperparasite de champignons ascomycètes, nécrotrophes ou parasites de faiblesse du chêne sessile [*Q. petraea*] et du chêne chevelu (*Quercus cerris* L.). Des confrontations en culture d'isolats monoconidiens de *Stagonospora* sp. et *Botryosphaeria stevensii* montrent que *Stagonospora* sp. est un mycoparasite nécrotrophe qui pourrait limiter dans une certaine mesure les populations naturelles de *Botryosphaeria stevensii. Botryosphaeria stevensii* est un des facteurs biotiques associés au dépérissement des chênes. La morphologie du champignon et les symptômes de l'interaction mycoparasitaire sont décrits. L'espèce de *Stagonospora* trouvée en Hongrie est supposée identique au *Stagonospora* décrit en Allemagne et Autriche comme parasite de *Ascodichaena rugosa* et au Mexique sur *Ascodichaena mexicana*. Cette étude constitue la première mention de *Stagonospora* sp. hyperparasite de *B. stevensii* et *D. quercina.*

Zusammenfassung

Nachweis einer hyperparasitischen Art von Stagonospora auf Botryosphaeria stevensii

Eine bisher unbekannte Art von *Stagonospora* wurde in und auf Stromata von *Diatrypella quercina* (Persoon) Nitschke sowie der Anamorphe (*Diplodia mutila* Fr. & Mont.) von *Botryosphaeria stevensii* Shoemaker nachgewiesen. Mikroskopische Beobachtungen legen eine hyperparasitische Lebensweise auf zu den Ascomyceten gehörenden, nekrotrophen Parasiten und Schwächeparasiten von Trauben- (*Q. petraea*) und Zerreiche (*Quercus cerris*) nahe. Dualkulturen von Monokonidien-Isolaten von *Stagonospora* sp. und *B. stevensii* zeigten, dass es sich bei der *Stagonospora*-Art um einen nekrotrophen Mycoparasiten handelt, der möglicherweise unter gewissen Umständen die Entwicklung natürlicher Populationen von *B. stevensii* zu hemmen vermag. *B. stevensii* ist einer der biotischen Faktoren des „Eichensterbens". Die in Ungarn nachgewiesene Art dürfte mit derjenigen *Stagonospora*-Art identisch sein, welche *Ascodichaena rugosa* in Deutschland und Österreich sowie *Ascodichaena mexicana* in Mexico parasitiert. Bei der vorliegenden Arbeit handelt es sich um den ersten Nachweis einer hyperparasitischen *Stagonospora*-Art auf *B. stevensii* und *D. quercina*.

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