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ORIGINAL RESEARCH PAPERS

Micromycetes on highbush blueberry *Vaccinium corymbosum* L. leaves

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Abstract

The study aimed at the identification of micromycetes found on the leaves of highbush blueberries *Vaccinium corymbosum*. The study yielded 23 fungal species (261 colonies) isolated from living and fallen leaves with symptoms of disease (necrosis and spots). The dominant species were *Pestalotiopsis sydowiana*, *Alternaria alternata*, *Epicoccum nigrum*, *Trichoderma viride*, *T. harzianum*, *Pestalotia* spp., *Penicillium expansum*, *P. jensenii*, *Mortierella* spp., *Truncatella* spp., and *Dichotomopilus* spp.

Keywords

Ericaceae; microscopic fungi; pathogenic fungi; necrosis of leaves

1. Introduction

Highbush blueberry *Vaccinium corymbosum* L. is wild, growing on bogs and peat bogs in the eastern part of North America, reaching up to two m high. It is also commonly cultivated due to the tasty dessert fruits. Every year the crop of this plant takes on importance. According to data of the National Research Institute of Agricultural and Food Economics in Poland, the area of blueberry cultivation in 2018 was 11.8 thousand hectares.

For many years this species was considered free of diseases and pests. According to Kukuła et al. (2017), several pathogens (viruses, fungi, and bacteria) and pests of this plant species have been identified and characterized in the cultivated highbush blueberry Poland.

Worldwide, in commercial orchards, fungi are the main causative agent of diseases of *V. corymbosum*. To the main diseases belong gray mold (caused by *Botrytis cinerea* Pers.), anthracnose (*Colletotrichum acutatum* J.H. Simmonds, *C. gloeosporioides* (Penz.) Penz. & Sacc.), leaf blight of highbush blueberry (*Valdensinia heterodoxa* Peyronel) and canker of highbush blueberry (*Godronia cassandrae* Peck) (Bryk, 2013; Hildebrand & Renderos, 2007; Meszka & Bielenin, 2012; Pszczółkowska et al., 2016; Shivas & Tan, 2009). Also, species of the genera: *Curvularia*, *Fusarium*, *Humicola*, *Phoma*, *Phomopsis*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, *Stemphyllium* (Wright et al., 2004, 2005), *Chrysomyxa* (*Ch. nagodhii* P.E. Crane, *Ch. neoglandulosi* P.E. Crane, *Ch. vaccini* (Ziller) P.E. Crane, *Ch. reticulata* P.E. Crane) (Crane, 2001), *Erysiphe* (=*Microsphaera*) (Braun et al., 2003), *Alternaria* (Luan et al., 2007; Schilder et al., 2006) and *Penicillium* (Greco et al., 2012) were detected on the leaves with symptoms of necrosis and isolated from infected tissues of leaves and fruits.

The study aimed was to determine the quantitative composition of micromycetes inhabiting leaves of highbush blueberries in two localities in Poland: (1) the Botanic Garden of the Jagiellonian University in Cracow, and (2) the Rogów Arboretum of the Warsaw University of Life Sciences (WULS-SGGW) in Rogów.

2. Materials and methods

Observations of the highbush blueberry (*V. corymbosum*) health status were conducted in the years 2012–2014 on the collection of ericaceous plants of the Botanic Garden of the Jagiellonian University and the Rogów Arboretum of the WULS-SGGW. Infected green leaves (showing necrotic symptoms) and fallen leaves were sampled three times a year (25th May, 25th July, and 25th September). Twenty leaves (10 living and 10 fallen) were collected from each plant at each site. Leaf fragments were cut out from the border of healthy and necrotic tissues from single spots. They were surface sterilized in 70% ethanol for one minute, then thoroughly rinsed three times for one minute in sterile water before placing on a Petri dish with a 2% PDA medium. Cultures were incubated for seven days at 21–22 °C. Subsequently,

young fungal colonies were transferred on slant agar with a 2% PDA medium. Macro- and microscopic comparisons of fungal colonies were made, and representative colonies were selected. The microscope used for observation was a Delta Optical microscope (Evolution 300). The species identification was based on the mycological keys and monographs (Domsch et al., 1980; Ellis & Ellis, 1987; Guba, 1961; Sutton, 1980). The taxonomic system proposed by Kirk and co-workers (2008) was adopted, while the names of taxa follow the Index Fungorum database accessed in July 2020. Participation of individual species in the total number of species was defined as the dominants (constituting >5% of the entire community), influents (1–5%), and accessory taxa (<1%). The coefficient of similarity S_o (Sörensen number) was calculated according to Kowalik (1993).

$$S_o = 100\frac{2c}{a+b}$$

where:

S_o - the coefficient of similarity (Sörensen number),

a - number of fungal species on the leaves of *V. corymbosum* in the Botanic Garden of the Jagiellonian University in 2012–2014,

b - number of fungal species on the leaves of *V. corymbosum* in the Rogow Arboretum of the WULS-SGGW in 2012–2014,

c - number of fungal species recorded at both research sites.

3. Results

As the results of the mycological analysis of plant material 261 fungi colonies belonging to the genera: *Alternaria*, *Ascochyta*, *Coleophoma*, *Dichotomopilus*, *Epicoccum*, *Fusarium*, *Gilmaniella*, *Humicola*, *Juxtiphoma*, *Mortierella*, *Nigrospora*, *Penicillium*, *Pestalotia*, *Pestalotiopsis*, *Phialophora*, *Trichoderma*, *Truncatella* and *Wardomyces* were obtained. The community of fungi isolated from green and fallen leaves with spots and necroses from highbush blueberries grown in the Botanic Garden and the RogowArboretum was different in colony number and species diversity (Table 1, Table 2).

From affected green and fallen leaves of highbush blueberry in the Botanic Garden, 134 colonies representing 16 species (14 genera) were isolated. 27% of the total number of colonies represented *Pestalotiopsis sydowiana*, referred to as the dominant species. The dominant role of *Alternaria alternata* and *Epicoccum nigrum* (together over 34% of all isolates) in inhabiting leaves of highbush blueberry was observed. Species of the genera: *Trichoderma* (*T. viride*, *T. harzianum*), *Pestalotia*, *Penicillium* (*P. expansum*, *P. jensenii*), *Mortierella*, *Truncatella,* and *Dichotomopilus* occurred with lower abundance and were classified as influents. The accessory group, accounting for less than 1% of the isolated colonies is constituted by *Coleophoma rhododendri*, *Fusarium chlamydosporum*, *Nigrospora oryzae* (syn. *Khuskia oryzae*), and *Phialophora cinerescens*.

From total green and fallen leaves of highbush blueberry in the Rogów Arboretum of the WULS-SGGW in Rogów, 127 colonies representing 17 species (14 genera) were isolated. The dominant role of *Pestalotiopsis sydowiana* (over 36%), *Alternaria alternata* (almost 12%), *Truncatella truncata* (over 10%), and *Trichoderma viride* (almost 8%) in colonizing affected leaves was observed. Species: *Penicillium lanosum*, *P. jensenii*, *E. nigrum*, *Phialophora cinerescens*, *Ascochyta medicaginicola* (syn. *Phoma medicaginis*), *Juxtiphoma eupyrena* (syn. *Ph. euprena*), *Humicola fuscoatra*, *Trichoderma koningii*, *Pestalotia rhododendri* and *Wardomyces anomalus* occurred with lower abundance and were classified as influents. Other species belonging to the genus *Coleophoma*, *Fusarium,* and *Gilmaniella* were included in the accessory group.

From affected green leaves of highbush blueberry in the Botanic Garden, 59 colonies representing 14 species were isolated. Over 35% of the total number of colonies represented *A. alternata*, referred to as the dominant species. Genera: *Pestalotiopsis*, *Pestalotia*, *Epicoccum*, and *Penicillium*, with the predominance of *P. sydowiana*, *P. rhododendri*, *E. nigrum*, *P. expansum,* and *P. jensenii* were also inhabited by affected highbush blueberry leaves.

The living leaves of *V. corymbosum* in the Rogów Arboretum were inhabited by 12 species of micromycetes. Almost 55% of the total number of colonies represented *P. sydowiana*, referred to as the dominant species. Genera: *Penicillium*, *Alternaria*, and *Humicola* were also numerous, accounting for more than 28% of all isolates.

From the necrotic tissues of fallen leaves in the Botanic Garden, nine species representing eight genera were isolated. Species *P. sydowiana* and *E. nigrum* were the dominant species. Genera *Gilmaniella* and *Trichoderma*, with the predominance of *G. humicola*, *T. harzianum*, and *T. viride* also inhabited the fallen leaves in this location. Species: *A. alternata*, *P. expansum*, *T. truncate*, and *M. parvispora* have sporadically colonized leaves, with small frequency.

From the fallen leaves in the Arboretum, 74 colonies of fungi were isolated. The dead tissue of the plant was dominated by *P. sydowiana* (almost 23%). The fungi belonging to the genera *Trichoderma*, *Alternaria*, *Truncatella*, *Juxtiphoma,* and *Ascochyta* with the predominance of *T. koningii*, *T. harzianum*, *A. alternata*, *T. truncata*, *J. eupyrena* (syn. *Ph. eupyrena*) and *A. medicaginocola* (syn. *Ph. medicaginis*), also inhabiting to fallen leaves. Also, other filamentous fungus complexes with the advantage of *E. nigrum* and *Ph. cinerescens* caused spots on the leaves.

Six species: *A. alternata*, *C. rhododendri*, *G. humicola*, *P. jensenii*, *P. sydowiana,* and *T. truncata* were common species that inhabited green leaves of highbush blueberries in the Botanic Garden in Cracow and the Rogów Arboretum. The main species living on fallen and dead leaves in these locations were: *A. alternata*, *E. nigrum*, *P. sydowiana*, *T. viride,* and *T. truncata*.

For the calculation of similarity coefficients (S_o) of micromycetes on leaves of *V. corymbosum* in the Botanic Garden and Arboretum, total values for the years 2012–2014 were used. For the micromycetes community on alive leaves in Botanic Garden and Arboretum, the highest similarity coefficient calculated was in 2012 (75%). The lower similarity coefficient was found in 2013 and 2014 (33.33% and 15.38%).

For the micromycetes community on fallen leaves in 2012 the similarity coefficient was calculated at 100%. The lower (58.82%) similarity coefficient was found in 2013. The lowest similarity coefficient (44.44%) was found in 2014.

Table 1 Micromycetes on leaves of *Vaccinium corymbosum* in the Botanic Garden of the Jagiellonian University in Cracow.

Species	Fungal frequency						Total [no.]	Percentage [%]
	Alive leaves			Fallen leaves				
	2012	2013	2014	2012	2013	2014		
Alternaria alternata (Fr.) Keissl.	10	9	2	3	2	-	26	19.4
Coleophoma rhododendri Syd.	-	-	1	-	-	-	1	0.75
Dichotomopilus funicola (Cooke) X.Wei Wang & Samson	2	-	-	-	-	-	2	1.49
Epicoccum nigrum Link	-	5	-	-	9	6	20	14.93
Fusarium chlamydosporum Wollenw. & Reinking	-	-	1	-	-	-	1	0.75
Gilmaniella humicola G. L. Barron	-	-	4	-	-	9	13	9.7
Mortierella parvispora Linnem.	-	-	-	-	-	3	3	2.24
Nigrospora oryzae (Berk. & Broome) Petch	-	-	1	-	-	-	1	0.75
Penicillium expansum Link	1	-	-	-	4	-	5	3.73
Penicillium jensenii K. W. Zaleski	-	2	2	-	-	-	4	2.99
Pestalotia rhododendri (D. Sacc.) Guba	-	-	6	-	-	-	6	4.48
Pestalotiopsis sydowiana (Bres.) B. Sutton	6	3	-	13	8	6	36	26.87
Phialophora cinerescens (Wollenw.) J. F. H. Beyma	-	1	-	-	-	-	1	0.75
Trichoderma harzianum Rifai	-	-	-	-	3	-	3	2.24
Trichoderma viride Pers.	-	-	1	-	5	-	6	4.48
Truncatella truncata (Lév.) Steyaert	2	-	-	3	1	-	6	4.48
Total	21	20	18	19	32	24	134	100

4. Discussion

261 colonies of micromycetes belonging to 23 species were identified from the green and fallen leaves of highbush blueberries in the Botanic Garden of the Jagiellonian University in Cracow and the Rogów Arboretum of the WULS-SGGWs. Among them, species from the genera *Botrytis*, *Colletotrichum*, *Valdensina,* and *Godronia,* which, according to numerous sources (Dzięcioł, 2008; Dzięcioł et al., 2014; Kukuła et al., 2017; Łabanowski et al., 2001; Wright et al., 2004, 2005; Shivas & Tan, 2009), are the main causative agents of highbush blueberry diseases in commodity crops, were not found.

Pestalotiopsis sydowiana and *Alternaria alternata* predominated in the total number of isolates. Both species were also earlier isolated from *Ericaceae* plants in the Botanic Garden of the Jagiellonian University in Cracow and the Rogów Arboretum of the WULS-SGGW by Duda and Bonio (2014) and Drzewiecka et al. (2016). The frequent occurrence of *P. sydowiana* on the leaves of the heath plants was also recorded by Keith et al. (2006) and Kowalik et al. (2011), who attributed to this species a causal role of numerous discolorations and spots. Hopkins and McQuilken (2000) reported that *P. sydowiana* and *Pestalotia* species can cause spots and necrosis of the heath plants, leading to their deaths, while Łabanowski et al. (2001) ascertained the fungi of these species do not have a pathogen attribute.

According to Greco et al. (2012), *Alternaria alternata* was isolated from round or irregular, greyish spots with a red rim, occurring on leaves, fruits, and other organs of highbush blueberry. Kowalik et al. (2010) reported that this toxin-producing species plays a dominant role in the development of disease lesions and increased leaf defoliation and fall. In the current research, this species was one of the dominants and accounted for almost 16% of the total number of isolates.

Fungi belonging to the *Fusarium* genus were isolated from necrosis and spotted leaves. Species of this genus were isolated by Wright et al. (2004, 2005) from the same blueberry host in Argentina. According to Werner et al. (1998), species of the

Table 2 Micromycetes on leaves of *Vaccinium corymbosum* in the Rogów Arboretum of the Warsaw University of Life Sciences – SGGW.

Species	Fungal frequency						Total [no.]	Percentage [%]
	Alive leaves			Fallen leaves				
	2012	2013	2014	2012	2013	2014		
Alternaria alternata (Fr.) Keissl.	4	-	-	2	1	8	15	11.81
Ascochyta medicaginicola Qian Chen & L. Cai	-	-	1	-	4	-	5	3.94
Coleophoma rhododendri Syd.	-	1	-	-	-	-	1	0.79
Epicoccum nigrum Link	-	-	-	-	1	4	5	3.94
Fusarium flocciferum Corda	-	-	1	-	-	-	1	0.79
Gilmaniella humicola G. L. Barron	-	-	1	-	-	-	1	0.79
Humicola fuscoatra Traaen	-	4	-	-	-	-	4	3.15
Juxtiphoma eupyrena (Sacc.) Valenz.-Lopez, Crous, Stchigel, Guarro & Cano	-	1	-	-	-	3	4	3.15
Penicillium jensenii K. M. Zaleski	-	3	-	-	1	-	4	3.15
Penicillium lanosum Westling	-	4	-	-	2	-	6	4.72
Pestalotia rhododendri (D. Sacc.) Guba	-	-	-	-	-	2	2	1.57
Pestalotiopsis sydowiana (Bres.) B. Sutton	11	8	10	6	2	9	46	36.22
Phialophora cinerescens (Wollenw.) J. F. H. Beyma	-	-	-	-	5	-	5	3.94
Trichoderma koningii Oudem.	-	-	-	-	3	-	3	2.36
Trichoderma viride Pers.	-	-	-	-	10	-	10	7.87
Truncatella truncata (Lév.) Steyaert	1	1	-	7	4	-	13	10.24
Wardomyces anomalus F. T. Brooks & Hansf.	-	-	2	-	-	-	2	1.57
Total	16	22	15	15	33	26	127	100

genus *Fusarium* and *Gibberella* infect the roots and the collar-root (*F. avenaceum* (Fr.) Sacc.) and destroy the fiber-vascular bundles (*F. oxysporum* Schltdl.), which leads to the gradual dieback of the shrubs. Kuzdraliński et al. (2014) reported that the symptoms of infestations by *Fusarium* species include: not fully developed inflorescences and flower petals, yellowing and falling leaves, which may lead to a decrease in not only the decorative value of plants but also the yield.

In the studies of Kowalik et al. (2011) and Drzewiecka et al. (2016) carried out in the Botanic Garden in Cracow and the Rogów Arboretum predominated *Phoma chrysanthemicola* Hollós (syn. *Paraphoma chrysanthemicola* (Hollós) Gruyter, Aveskamp & Verkley), *Ph. exigua* Desm. (syn. *Boeremia exigua* (Desm.) Aveskamp, Gruyter & Verkley), *Ph. herbarum* Westend., *Ph. leveillei* Boerema & G.J. Bollen, *Ph. macrostoma* Mont. (syn. *Didymella macrostoma* (Mont.) Qian Chen & L. Cai) and *Ph. pomorum* Thüm. (syn. *Didymella pomorum* (Thüm.) Qian Chen & L. Cai). They caused necrosis of stem rot (shoot necrosis and large, oval, necrotic, and dark-edged spots with visible pycnidia on the leaves). The current study confirmed the occurrence of *Phoma* species on highbush blueberry leaves having the above-mentioned disease symptoms. From tissues with spots and necroses species *Humicola* and *Penicillium* were also isolated, as in the research of Wright et al. (2004, 2005) and Greco et al. (2012).

In addition, the species of the following fungal genera were isolated for the first time: *Coleophoma*, *Dichotomopilus*, *Epicoccum*, *Gilmaniella*, *Nigrospora*, *Mortierella*, *Phialophora*, *Trichoderma,* and *Wardomyces*. They were isolated from leaves with discolorations, spots, and necrosis. Species from the genera were recorded in samples of atmospheric air and leaves of *Ericaceae* plants in both the Botanic Garden of the Jagiellonian University in Cracow and the Rogów Arboretum of the WULS-SGGW (Bonio & Drzewiecka, 2016; Bonio & Duda, 2014; Kowalik et al., 2014a, 2014b; Kowalik et al., 2015).

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