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ORIGINAL PAPER

Assessing the virulence of the wound-associated species of the *Ophiostomatales* to hardwood seedlings

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ABSTRACT

The *Ophiostomatales* (*Ascomycota*) is a diverse and important group of fungi to forest ecosystems in Europe. A survey of ophiostomatalean species associated with wounds on mature hardwood trees in Poland was conducted from 2015 to 2017. Among others, 10 ophiostomatalean species were isolated. We hypothesized that some of the wound-associated *Ophiostomatales* may interfere with callus tissue formation. Therefore, to better understand virulence of these fungi, artificial inoculations of seedlings of six hardwood tree species were conducted. Two-year-old seedlings were inoculated with ten ophiostomatalean species isolated from wounds on mature hardwood trees. After 11 weeks after inoculation, all fungal species produced visible dark-brown lesions on both the bark and the xylem of inoculated hardwood seedlings. Lesion lengths varied depending on the fungal species. *Leptographium vulnerum* was the only fungus that caused lesion lengths that did not significantly differ from the control. Lesions associated with *Ophiostoma quercus* were among the longest, especially on *Acer pseudoplatanus*, *Betula pendula* and *Carpinus betulus*. The largest lesions were generated on stems of *Quercus robur* and *B. pendula* seedlings although the lesion lengths varied across tree species. All fungal species, except *O. quercus* on *A. pseudoplatanus* caused very minor mortality on inoculated hardwoods seedlings. In general, the fungi were found to be mildly pathogenic and are probably involved, to a lesser extent in inhibiting callus tissue formation.

KEY WORDS

Deciduous trees, *Leptographium*, Lesion, *Ophiostoma*, Ophiostomatalean fungi, Pathogenicity, *Sporothrix*

Introduction

The order *Ophiostomatales* (*Ascomycota*) includes numerous well known fungal species. This group includes the genera *Ophiostoma*, *Ceratocystiopsis*, *Graphilbum*, *Grosmannia*, *Raffaelea*, *Fragosphaeria*, and *Leptographium*. A common morphological trait of these fungi is globose ascomata with usually elongated necks that give rise to masses of sticky spores at their tips, an entomochoric adaptation to allow spore dispersal via arthropods. In addition, fungi in the order *Ophiostomatales* produce hylaorhinocladiella-, pesotum-, leptographium- or sporothrix-like asexual morphs (De Beer and Wingfield, 2013; De Beer *et al.*, 2013).

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Ophiostomatales is one of the largest groups of tree-or wood-infecting fungi, with currently several hundred recognized species (De Beer *et al*., 2013). They can colonize a wide diversity of hosts tree species, including angiosperms and gymnosperms. The members of *Ophiostomatales* are commonly associated with bark- and wood-dwelling beetles and their mites. Most of them cause the economically important blue-stain in freshly exposed sapwood of softwood species (Seifert, 1993; Uzunović and Byrne, 2013). Some of these species are important tree pathogens that have dramatically re-shaped natural forests and caused major losses to the forestry industry (Wingfield *et.al*., 2017). For example, *Ophiostoma novo-ulmi* Brasier is a highly virulent tree pathogen that is responsible for Dutch elm disease (Brasier, 1991).

Ophiostomatalean fungi are known to infect trees via wounds, which are commonly caused by animals, wind, frost, silvicultural practices (pruning and harvesting) and various arthropods (e.g. Gibbs, 1993; Geldenhuis *et al*., 2004; Kamgan Nkuekam *et al*., 2008, 2010, 2011, 2012; Musvuugwa *et al*., 2016; De Errasti *et al*., 2016; Osorio *et al*., 2016; Kwaśna *et al*., 2021). Recently studies have provided evidence that a large diversity of species that belong to the *Ophiostomatales* infect hardwoods in Poland, including species of *Graphilbum*, *Leptographium sensu lato*, *Ophiostoma sensu lato* and *Sporothrix* (Jankowiak *et al*., 2019a).

Very little is known regarding the pathogenicity of the wound-associated *Ophiostomatales* in Poland. A recent study by Jankowiak *et al*. (2019a) showed that some ophiostomatalean fungi may interfere with the callus tissue formation within wounds on tree stems. Previous research (Geldenhuis *et al*., 2004; Kamgan Nkuekam *et al*., 2011) had shown that the virulence of wound-associated *Ophiostomatales* is low. Inoculations on young *Schizolobium parahybum* (Vell.) S.F. Blake trees with *Graphium penicillioides* Corda, *Ophiostoma quercus* (Georgev.) Nannf. and the *Pesotum* sp. in Ecuador resulted in very small lesions (Geldenhuis *et al*., 2004). Similar results were obtained in Australia, where *O. quercus*, *O. tsotsi* Grobbel., Z.W. De Beer & M.J. Wingf., *O. tasmaniense* Kamgan, Jol. Roux & Z.W. de Beer and *O. undulatum* Kamgan, M.J. Wingf. & Jol. Roux produced very small lesions on inoculated *Eucalyptus grandis* W. Hill trees (Kamgan Nkuekam *et al*., 2011).

A survey of ophiostomatalean species associated with wounds on mature hardwood trees in Poland was conducted from 2015 to 2017. Among others, 10 species belonging to the *Ophiostomatales* were isolated from wounds of various hardwood tree species. We suspect that some of the wound-associated *Ophiostomatales* may interfere with callus tissue formation. In this study, we tested the hypothesis that some wound-associated species of the *Ophiostomatales* have the pathogenic ability to damage their host trees. The objective of the present study was to evaluate the pathogenicity of ten ophiostomatalean species on 2 year old hardwood trees: *Acer pseudoplatanus* L., *Betula pendula* Roth, *Carpinus betulus* L., *Fagus sylvatica* L., *Quercus robur* L. and *Tilia cordata* Mill. seedlings.

Material and methods

Pathogenicity tests were conducted on 2-year-old *A. pseudoplatanus*, *B. pendula*, *C. betulus*, *F. sylvatica*, *Q. robur* and *T. cordata* seedlings in currently unused farmland. One strain from each of the ten fungal species identified in the previously study (Jankowiak *et al*., 2019a) were used in the inoculation experiment (Table 1). These seedlings of six tree species were selected because they have been shown to be susceptible to colonization by wound-associated species of the *Ophiostomatales* in previous research. The fungal strains were isolated from wounds formed on stems of different tree species, and tree age ranged from 60 to 120 years (Jankowiak *et al*., 2019a). Each fungus was inoculated onto twenty seedlings of each species for a total of 1200

Table 1.

Fungal isolates used in the pathogenicity test. All isolates were isolated from fresh wound on hardwood trees in Poland between 2015-2017 (Jankowiak et al., 2019a)

Fungal species	Isolate no.	Isolate date	Site	Host
Grosmannia grandifoliae (R.W. Davidson) et al.	KFL36516NLRJ	June 2016	Muszyna	*Tilia cordata*
Leptographium flavum R. Jankowiak & A. Ostafińska	KFL6NDB15RJ	October 2015	Wierzchosławice	*Quercus robur*
Leptographium vulnerum R. Jankowiak & A. Ostafińska	KFL277NGB16RJ	May 2016	Babimost	*Carpinus betulus*
Ophiostoma pseudokarelicum T. Aas, H. Solheim & R. Jankowiak	KFL268NBRZ16RJb	May 2016	Babimost	*Betula pendula*
Ophiostoma quercus (Georgev.) Nannf.	KFL278NBK16AO	June 2016	Dąbrówka	*Fagus sylvatica*
Ophiostoma sparsiannulatum Zanzot, ZW. De Beer & M.J. Wingf.	KFL33JS15AO	June 2015	Dylągowa	*Fraxinus excelsior*
Sporothrix dentifunda (Aghayeva & M.J. Wingf.) et al.	KFL307NDB16AO	September 2016	Wybrzeże	*Quercus robur*
Sporothrix prolifera (T. Kowalski & Butin) et al.	KFL218N16TARAO	June 2016	Dylągowa	*Prunus spinosa*
*Sporothrix undulata** R. Jankowiak & A. Ostafińska	KFL221NBK16RJ	May 2016	Czajowice	*Fagus sylvatica*
Sporothrix sp. 16	KFL129NCZE15AO	October 2015	Siedliska	*Cerasus avium*

*Isolate identified during previous surveys in Poland as Sporothrix sp. 12 in the study of Jankowiak *et al.* (2019a)

Table 2.

Percentage of dead hardwood seedlings inoculated with ten fungal species

Fungal species	*Acer pseudoplatanus*	*Betula pendula*	*Carpinus betulus*	*Fagus sylvatica*	*Quercus robur*	*Tilia cordata*
Grosmannia grandifoliae	0aA	0aA	0aA	0aA	0aA	0aA
Leptographium flavum	5aA	5aA	0aA	20aA	0aA	0aA
Leptographium vulnerum	0aA	0aA	0aA	0aA	0aA	5aA
Ophiostoma pseudokarelicum	5aA	0aA	0aA	0aA	0aA	0aA
Ophiostoma quercus	40aA	5abA	5abA	0bA	0bA	0bA
Ophiostoma sparsiannulatum	0aA	5aA	0aA	0aA	0aA	0aA
Sporothrix dentifunda	0aA	10aA	0aA	0aA	0aA	0aA
Sporothix prolifera	0aA	5aA	0aA	0aA	0aA	0aA
Sporothrix undulata	5aA	0aA	0aA	0aA	0aA	0aA
Sporothrix sp. 16	0aA	5aA	0aA	5aA	0aA	5aA
Control	0aA	0aA	0aA	0aA	0aA	0aA

Seedling mortality with the same letter in column (capital letter) or row (small letter) were not significantly different by (χ^2) test inoculated seedlings. Twenty additional plants of each species were inoculated with sterile agar discs to serve as controls following the technique described by Jankowiak (2013), for a combined total of 1320 seedlings.

On July 10, 2017, the seedlings were superficially wounded by removal with a sterile scalpel of a bark flap (4×10 mm), 11-12 cm above the root collar. To inoculate the trees, 3 mm diameter discs were cut from the margins of 14-day-old culture of fungi that were grown on 2% MEA at 22°C. These discs were placed on the wounds, covered by the bark flaps, and wrapped in Parafilm®. Stem diameters at the inoculation site ranged from 4.3 to 7.6 mm (mean 5.5 mm).

Plants were examined at weekly intervals for mortality over 11 weeks. A seedling was considered dead when the whole stem had died. After 11 weeks, all plants were harvested, and the bark around the inoculation site was removed. The total lengths of the necrotic lesions on the sapwood surfaces were measured. Stem sections were then cut above and below the inoculation point and the depth of any visible sapwood stain was measured using a binocular microscope. Re-isolations were made from the lesions by removing small sapwood samples near the points of inoculation and incubating on 2% MEA at 22°C to meet the requirements of Koch's postulates.

Depth of sapwood blue-stain, lesion length and seedling mortality were used to assess fungal virulence. Differences in the depths of sapwood blue-stain and lesion lengths between both fungal isolates and tree species were analyzed using the Kruskal-Wallis test, followed by a nonparametric multiple comparison of mean ranks. These statistical calculations were performed using STATISTICA software, version 12 (Stat-Soft, Inc., Tulsa, USA). The chi-square (χ^2) test was performed to evaluate differences among the proportions of death seedlings, and this was followed by use of the Marascuilo procedure to determine differences in plant mortality caused by the tested fungal isolates. These tests were carried out using the StatTools.net software (www.statstodo.com <http://www.statstodo.com>).

Results

There was very little mortality induced by the 10 fungal species on the inoculated seedlings. Out of the 1200 seedlings, only 26 (2.2%) plants died. No mortality was in the 2-year-old hardwood seedlings inoculated with *Grosmannia grandifoliae*. In addition, no *Q. robur* seedling died. *Ophiostoma quercus* killed 40% of the two-year-old *A. pseudoplatanus* seedlings, while *Leptographium flavum* killed 20% of the *F. sylvatica* plants (Table 2). The other fungal species each contributed to the death of a single seedling (Table 2). The first signs of seedlings dieback (i.e., yellowing leaves and a darkened discolored stem) were observed 8 weeks after inoculation. No control plants died, and the callus tissue closed the wounds of the inoculated control plants (Figs. 1, 2; Table 2).

All fungal species used for inoculation produced dark-brown sunken lesions on both the bark and the xylem of inoculated hardwood seedlings (Fig. 1). The lengths of lesions ranged from 26.3 to 185.7 mm, depending on both the fungal species used for inoculation and the tree species that was inoculated (Table 3). The largest lesions were generated on stems of *Q. robur* and *B. pendula* seedlings (Table 3). *Leptographium flavum*, and *L. vulnerum* gave rise to the smallest lesions on most plant hosts, but both *Leptographium* species generated the longest lesions on stems of *Q. robur* and *B. pendula* (Table 3). Relatively small lesions were induced by *G. grandifoliae*, although this fungus was able to induce large lesions on *A. pseudoplatanus*, *C. betulus* and *Q. robur* (Table 3). *Ophiostoma quercus* induced the longest necrotic lesions on *B. pendula*, *A. pseudoplatanus* and *C. betulus* seedlings, but the lesions were not significantly longer compared to those produced by the other *Ophiostoma* and *Sporothrix* species tested (Table 3). The longest lesions on

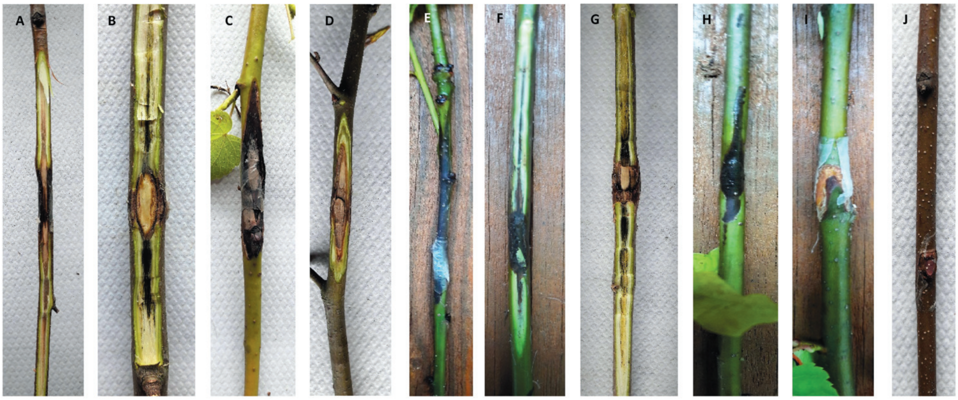

Fig. 1.

Lesions on the stem surfaces of seedlings caused by *Ophiostoma quercus* on *Betula pendula* (A), *Acer pseudoplatanus* (B), *Tilia cordata* (C), *Carpinus betulus* (D), *Quercus robur* (E); *Leptographium flavum* on *Q. robur* (F); *O. pseudokarelicum* on *A. pseudoplatanus* (G); *Sporothrix undulata* on *Q. robur* (H); Negative control on *T. cordata* (I) and *B. pendula* (J)

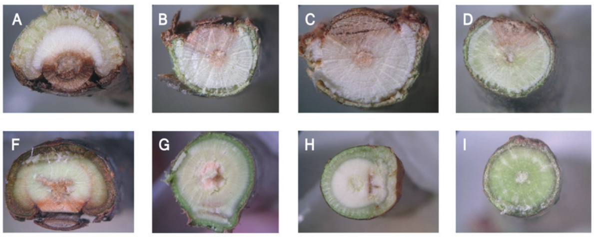

Fig. 2.

Cross section through seedling stems at the points of inoculation: *Ophiostoma quercus* on *Tilia cordata* (A), *Fagus sylvatica* (B); *Sporothrix undulata* on *F. sylvatica* (C); *Ophiostoma pseudokarelicum* on *Carpinus betulus* (D); *Leptographium flavum* on *F. sylvatica* (F); Negative control on *Acer pseudoplatanus* (G), *T. cordata*. (H), *Carpinus betulus* (I)

Q. robur stems were produced by *Sporothrix* sp. 16, although they were not significantly different from the other six fungal species (Table 3). *Sporothrix undulata* induced the longest lesions on *F. sylvatica* stems although *O. quercus*, *Sporothrix dentifunda* and *S. prolifera* generated only slightly smaller lesions. *Sporothrix dentifunda* produced the longest lesions on *T. cordata*. However, no significant differences were observed between this fungus and five other fungi that were tested (Table 3). Only *L. vulnerum* induced lesions that were not significantly different from those of controls on all tested tree species. Similar results were seen for *L. flavum* inoculated on *A. pseudoplatanus*, *C. betulus* and *T. cordata*, and by *G. grandifoliae* on *B. pendula* and *F. sylvatica* (Table 3).

All fungal species caused cambial and xylem necrotic discoloration of similar depth, independent on fungal and tree species, ranging from 1.24 to 1.51 mm (Fig. 2; Table 4). All species were successfully re-isolated from the lesions. Control inoculations were covered with callus tissue when the experiment was terminated and no inoculated fungi were isolated from them (Figs. 1, 2).

Discussion

The inoculations on the six tree species showed that members of the *Ophiostomatales* isolated from wounds on mature trees in hardwood forests were able to induce lesions on artificially

Table 3.
Mean lesion length (mm) on inoculated 2-year-old hardwoods seedlings

Fungal species	*Acer pseudoplatanus*	*Betula pendula*	*Carpinus betulus*	*Fagus sylvatica*	*Quercus robur*	*Tilia cordata*
Grosmannia grandifoliae	59.27bcABC	53.70bcCDE	83.92abAB	30.68cBC	146.55aAB	61.25bBCD
Leptographium flavum	26.3cCD	87.38aBCD	31.42cCD	51.66abcB	99.29abBC	35.22bcDE
Leptographium vulnerum	36.28abBCD	41.46abDE	28.92abCD	26.52bBC	44.32aCD	37.32aCDE
Ophiostoma pseudokarelicum	59. 40cAB	100.55abABCD	60. 60cBC	56.33cAB	161.25aAB	75.45abABC
Ophiostoma quercus	92.93bA	179.30aA	113.11abA	94.00bA	123.63abAB	87.96bAB
Ophiostoma sparsiannulatum	84.51abcA	103.94abABC	67.25cABC	77.65bcA	124.93aAB	56.91cBCD
Sporothrix dentifunda	52.99cAB	130.29abAB	92.47bcAB	94.01bcA	152.88aAB	119.13abA
Sporothix prolifera	61.37bAB	135.83aAB	67.94bAB	92.79abA	119.46aBC	95.46abAB
Sporothrix undulata	48.19cABC	160.65aAB	65.55bcABC	94.37bA	152.63aAB	68.67bcBCD
Sporothrix sp. 16	78.13bAB	110.37bABC	80.08bAB	87.89bA	185.66aA	68.72bABCD
Control	0aD	0aE	0aD	0aC	0aD	0aE

Lesion length with the same letter in column (capital letter) or row (small letter) were not significantly different at P=0.05 by non-parametric Kruskal-Wallis test

Table 4.
Mean depth of sapwood blue-stain (mm) on inoculated 2-year-old hardwoods seedlings

Fungal species	*Acer pseudoplatanus*	*Betula pendula*	*Carpinus betulus*	*Fagus sylvatica*	*Quercus robur*	*Tilia cordata*
Grosmannia grandifoliae	1.25aA	1.35aA	1.4aA	1.29aA	1.39aA	1.46aA
Leptographium flavum	1.38aA	1.33aA	1.33aA	1.21aA	1.3aA	1.26aA
Leptographium vulnerum	1.3aA	1.35aA	1.4aA	1.29aA	1.39aA	1.46aA
Ophiostoma pseudokarelicum	1.41aA	1.37aA	1.32aA	1.29aA	1.42aA	1.32aA
Ophiostoma quercus	1.25aA	1.42aA	1.38aA	1.35aA	1.34aA	1.41aA
Ophiostoma sparsiannulatum	1.31aA	1.24aA	1.43aA	1.32aA	1.28aA	1.24aA
Sporothrix dentifunda	1.44aA	1.45aA	1.3aA	1.36aA	1.35aA	1.4aA
Sporothix prolifera	1.32aA	1.35aA	1.43aA	1.41aA	1.30aA	1.36aA
Sporothrix undulata	1.54aA	1.24bA	1.5aA	1.51aA	1.47abA	1.41abA
Sporothrix sp. 16	1.3aA	1.28aA	1.4aA	1.33aA	1.38aA	1.41aA
Control	0aB	0aB	0aB	0aB	0aB	0aB

Depth of sapwood blue-stain with the same letter in column (capital letter) or row (small letter) were not significantly different at P=0.05 by non-parametric Kruskal-Wallis test inoculated seedlings, but without serious symptoms of die-back. In addition, these fungi were able to penetrate deeply into the sapwood of the seedlings. This suggested that those fungi are mild or moderate pathogens. Our results are consistent with previous studies that have reported that three *Ophiostoma* species associated with declining of *Q. robur* trees are non- or mild pathogenic (Simonin *et al*., 1993; Delatour *et al*., 1994; Selochnik *et al*., 2015). Similar results were obtained in pathogenicity tests in Australia on *E. grandis* trees (Kamgan Nkuekam *et al*., 2011). Inoculations of *S. parahybum* with *G. penicillioides*, *O. quercus* and *Pesotum* sp. in Ecuador also resulted in very small lesions (Geldenhuis *et al*., 2004).

The ophiostomatalean fungi tested in this study exhibited differences in pathogenicity depending on tree species. The tissues of *B. pendula* and *Q. robur* exhibited the most damage when inoculated with the *Ophiostomatales* species examined in this study. These findings confirm the results of a previous study in Poland (Jankowiak *et al*., 2019a) that determined that *Q. robur* appeared to be most vulnerable to infection by members of the *Ophiostomatales*. The wounds on oaks were associated with the greatest number of ophiostomatalean species, which occurred in 74% of the wounds (Jankowiak *et al*., 2019a).

Lesion lengths on both the bark and xylem of inoculated hardwood trees varied depending on the fungal species. Lesions associated with *O. quercus* were among the longest, especially in *A. pseudoplatanus*, *B. pendula* and *C. betulus*. *Ophiostoam quercus* is the most frequent ophiostomatalean species on hardwood trees in Poland, found in association with numerous different anthropods and tree species (Jankowiak *et al*., 2019ab). The results of our study are partly in agreement with the previous studies that have reported that *O. quercus* causes lesions when artificially inoculated on *Q. robur* trees, but without symptoms of die-back (Simonin *et al*., 1993; Delatour *et al*., 1994). Although *O. quercus* caused significantly larger lesions than the controls, no symptoms of die-back were observed on inoculated *Q. robur* seedlings confirming its weak pathogenic status on *Q. robur* (Taerum *et al*., 2018). However, our preliminary results suggest that pathogenicity of *O. quercus* varies across tree species. This fungus seems to be more pathogenic on *A. pseudoplatanus* because it caused die-back of 40% inoculated seedlings. *Ophiostoma quercus* is probably involved in the prevention of wound healing on sycamore. Different levels of *O. quercus* virulence may be the result of high genetic variability in populations of this species (Taerum *et al*., 2018). Since a relatively small number of isolates of each inoculated species were used in this study, it is not possible to derive comprehensive conclusions regarding population--level variation in the pathogenicity of the tested fungi.

The other *Ophiostoma* species (*Ophiostoma pseudokarelicum* and *O. sparsiannulatum*) caused relatively small lesions, suggesting that the species are very weak pathogens of hardwood trees. In this study, the relatively large lesions on stem seedlings were associated with four *Sporothrix* species, suggesting that these species may play similar roles to *O. quercus*, and may interfere with the healing process of wounds. Interestingly, all *Sporothrix* species exhibited similar level of virulence. Our results contradicted the hypothesis that *S. undulata* is highly virulent to hardwood trees (Jankowiak *et al*., 2019a). Jankowiak *et al*. (2019a) suspected that this fungus may play an important role in the reduction of callus activity of the injured area of *Q. robur*. According to Jankowiak *et al*. (2019a), *L. flavum* was predicted to be similarly pathogenic on *Q. robur* trees. However, our results did not support this hypothesis because this fungus generated relatively small lesions and was not capable of causing oak seedling mortality. Unexpectedly, seedlings of *F. sylvatica* inoculated with *L. flavum* developed serious symptoms of seedling die-back, suggesting that each wound-associated *Ophiostomatales* species may have different levels of virulence depending on the tree host.

Inoculations with *L. vulnerum* resulted in small lesions that were not statistically different to those of the controls, suggesting that the species is non-pathogenic despite being able to penetrate the sapwood of the seedlings. Two other members of *Leptographium sensu lato* also showed very small ability to cause diseases on the seedlings, often generating the smallest lesions. *Leptographium flavum* seems to be non-pathogenic to *A. pseudoplatanus*, *C. betulus* and *T. cordata*, while *G. grandifoliae* was not pathogenic to *B. pendula* and *F. sylvatica*.

Conclusions

The results of this study provide preliminary data regarding the potential pathogenicity of the fungal species found in wound on mature hardwood trees in Poland. Most of these fungi appear to be mild pathogens, and probably are not involved in the inhibiting of wound healing. Although it cannot be excluded that some of these may contribute to the slowing down of wound healing on hardwood trees. Our preliminary results showed that the pathogenicity of the wound-associated *Ophiostomatales* depends on the tree species. Additional research is needed to test the virulence of these fungi on more mature trees.

Authors' contributions

A.O. – research concept, fieldwork, data analyses, manuscript preparation; R.J. – research concept, manuscript preparation.

Conflict of interest

The authors declare the absence of potential conflicts of interest.

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STRESZCZENIE

Ocena wirulencji grzybów z rzędu *Ophiostomatales* związanych z ranami w stosunku do sadzonek drzew liściastych

Grzyby workowe należące do rzędu *Ophiostomatales* to niezwykle zróżnicowana i ważna gospodarczo grupa grzybów. Większość z nich powoduje zasinienie drewna, które znacząco obniża wartość handlową surowca. Niektóre gatunki, dzięki swojej wysokiej patogeniczności, mogą powodować groźne choroby drzew leśnych, jak np. *Ophiostoma novo-ulmi*, który jest sprawcą holenderskiej choroby wiązu. Znane są też symbiotyczne powiązania tych grzybów z różnymi gatunkami korników. Grzyby należące do rzędu *Ophiostomatales* powszechnie infekują także

rany na pniach drzew liściastych, jednak niewiele wiadomo o ich potencjalnej patogeniczności. W trakcie badań prowadzonych w latach 2015-2017 dotyczących występowania grzybów z rzędu *Ophiostomatales* w ranach na pniach różnych gatunków drzew liściastych rosnących na terenie Polski zidentyfikowano 32 gatunki grzybów należących do rzędu *Ophiostomatales*. Podejrzewa się, że niektórzy przedstawiciele rzędu *Ophiostomatales* związani z ranami na pniach drzew mogą zakłócać proces gojenia tych ran. W celu poznania wirulencji tych grzybów przeprowadzono sztuczne inokulacje na dwuletnich sadzonkach: *Acer pseudoplatanus*, *Betula pendula*, *Carpinus betulus*, *Fagus sylvatica*, *Quercus robur* i *Tilia cordata*. Badano 10 gatunków grzybów związanych z ranami na dojrzałych drzewach liściastych: *Grosmannia grandifoliae*, *Leptographium flavum*, *L. vulnerum*, *Ophiostoma pseudokarelicum*, *O. quercus O. sparsiannulatum*, *Sporothrix dentifunda*, *S. prolifera*, *S. undulata* i *Sporothrix* sp. 16 (tab. 1). Dwuletnie sadzonki rosnące na nieużytkowanym gruncie rolnym były inokulowane dwutygodniową grzybnią wyrosłą na 2-procentowej pożywce agarowo-maltozowej. Inokulacje przeprowadzono na 20 sadzonkach każdego gatunku drzewa. Kontrolę stanowiło 20 sadzonek inokulowanych sterylną pożywką. Łącznie przebadano 1200 sadzonek inokulowanych i 120 sadzonek kontrolnych. Sadzonki zakażano poprzez nacięcie strzałki pędu i wprowadzenie inokulum w postaci krążka pożywki z grzybnią. Po upływie 11 tygodni mierzono długość nekroz i głębokość przebarwień wytworzonych na strzałce pędu oraz ustalono śmiertelność inokulowanych sadzonek. Śmiertelność sadzonek z powodu sztucznych zakażeń była bardzo niewielka, gdyż obumarło jedynie 26 (2,2%) roślin (tab. 2). Po 11 tygodniach od inokulacji izolat *O. quercus* spowodował zamarcie 40% sadzonek *A. pseudoplatanus*, zaś izolat grzyba *L. flavum* – 20% sadzonek *F. sylvatica*. Inne gatunki doprowadziły do zamarcia jedynie pojedynczych sadzonek (tab. 2). Wszystkie gatunki grzybów spowodowały na inokulowanych strzałkach pędów powstanie ciemnobrunatnych nekroz (ryc. 1, 2). Wśród nich *L. vulnerum* był jedynym gatunkiem generującym nekrozy, których długość nie różniła się istotnie od kombinacji kontrolnej (tab. 3). Długość nekroz wytworzonych na strzałkach pędów była różna i zależała od gatunku drzewa. Największe nekrozy powstały na strzałkach pędów sadzonek *Q. robur* i *B. pendula* (tab. 3). Wszystkie gatunki grzybów spowodowały na inokulowanych strzałkach pędów powstanie przebarwień widocznych w drewnie bielastym. Głębokość tych przebarwień mieściła się w zakresie od 1,24 do 1,52 mm (tab. 4). Na żadnej sadzonce kontrolnej nie zaobserwowano objawów chorobowych, a w punktach inokulacyjnych wytworzyła się tkanka kalusowa, która całkowicie lub częściowo zabliźniła rany (ryc. 1, 2; tab. 3, 4). Wyniki testu patogeniczności wykazały, że grzyby z rzędu *Ophiostomatales* są prawdopodobnie słabymi patogenami drzew liściastych i odgrywają niewielką rolę w procesie zabliźniania się ran na pniach drzew liściastych.