ACTA AGROBOTANICA
Vol. 66 (2), 2013: 85–94
DOI: 10.5586/aa.2013.026

ASSESSING THE VIRULENCE OF OPHIOSTOMATOID FUNGI ASSOCIATED WITH THE PINE-INFESTING WEEVILS TO SCOTS PINE *Pinus sylvestris* L. SEEDLINGS

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Received: 19.11.2012

Abstract

The pine-infesting weevils are known to be effective vectors of ophiostomatoid fungi. To understand more about fungal virulence of these fungi, inoculation studies were conducted on Scots pine (*Pinus sylvestris* L.). Two-year-old seedlings were wound-inoculated with one of eleven ophiostomatoid fungi associated with pine-infesting weevils. After 11 weeks, a darkened lesion, extending from the point of inoculation, was observed in all species, except for *Ophiostoma* cf. *abietinum* Marm. & Butin, *Ophiostoma quercus* (Georgev.) Nannf., and *Sporothrix inflata* de Hoog. Seedling mortality was observed in seedlings inoculated with *Leptographium truncatum* (M.J. Wingf. & Marasas) M.J. Wingf., *Leptographium lundbergii* Lagerb. & Melin, *Leptographium procerum* (W.B. Kendr.) M.J. Wingf., *Grosmannia radiaticola* (J.J. Kim, Seifert & G.H. Kim) Zipfel, Z.W. de Beer & M.J. Wingf., *Ophiostoma floccosum* Math.-Käärik, *Ophiostoma minus* (Hedgc.) Syd. & P. Syd., and *Ophiostoma piliferum* (Fr.) Syd. & P. Syd. *Ophiostoma minus* and *L. truncatum* caused the largest lesions and sapwood blue-stain in Scots pine. *Grosmannia radiaticola, Ophiostoma piceae* (Münch) Syd. & P. Syd., *O. floccosum*, *O. piliferum, L. lundbergii*, and *L. procerum* produced significantly smaller lesions and sapwood blue-stain than *O. minus* and *L. truncatum*, while *O.* cf. *abietinum*, *O. quercus* and *S. inflata* did not cause any lesions.

Key words: blue-stain fungi, bark weevils, pathogenicity, *Pinus sylvestris,* regeneration weevils

INTRODUCTION

The pine-infesting weevils, including *Hylobius abietis* (L.), *Pissodes castaneus* (De Geer), *Pissodes piniphilus* (Herbst) and *Pissodes pini* (L.), are considered to be an important forest pest on Scots pine (*Pinus sylvestris* L.) in Poland (Kolk and Starzyk, 1996). These weevils occupy a variety of ecological niches. *Pissodes castaneus* is known to have a significant economic impact on regenerating stands and prefers the root neck of young (4–15-year-old) pine trees. *Pissodes piniphilus* and *P. pini* infest the aboveground parts of older trees; the former of these is mainly found on 30–40-year-old pine trees, while the latter colonises older trees (Day et al. 2004). Of the four species mentioned, *H. abietis* seemed to be the most important pest in forest, because adults prefer to feed on bark of young seedlings causing growth reduction or plant death (Wallertz et al. 2005).

Ophiostomatoid fungi are known to be associated with many weevils in North America (Wingfield, 1983; Nevill and Alexander, 1992; Jacobs and Wingfield, 2001; Eckhardt et al. 2007; Zanzot et al. 2010), however the interactions between ophiostomatoid fungi and weevils have been poorly studied in Europe (Piou, 1993; Lévieux et al. 1994). In France, *H. abietis* has been found carrying *Leptographium procerum* (W.B. Kendr.) M.J. Wingf., *Leptographium wingfieldii* M. Morelet, *Ophiostoma canum* (Münch) Syd. & P. Syd. and *Ophiostoma piliferum* (Fr.) Syd. & P. Syd. (Piou, 1993), while *Ophiostoma minus* (Hedgc.) Syd. & P. Syd., *Ophiostoma piceae* (Münch) Syd.& P. Syd., *Ophiostoma pluriannulatum* (Hedgc.) Syd. & P. Syd., *Ophiostoma stenoceras* (Robak) Nannf. and *Ceratocystiopsis minuta* (Siemaszko) H.P. Upadhayay & W.B. Kendr. were occasionally found in association with *P. pini* (Mathiensen-Käärik, 1953, 1960). In contrast to their previous studies, Jankowiak and Bilański (2013a) recently showed that considerably more diverse fungal species were associated with *H. abietis*. In Poland, *H. abietis* has been found to be infested mainly with *Leptographium lundbergii* Lagerb. & Melin, *L. procerum*, *Ophiostoma quercus* (Georgev.) Nannf., *Ophiostoma floccosum* Math.-Käärik, *O. piliferum*, *Sporothrix inflata* de Hoog, and ten other ophiostomatoid species. In this study, *H. abietis* also acted as an effective vector carrying ophiostomatoid species to *P. sylvestris* seedlings, especially in the case of *L. procerum* and *S. inflata*. In a recent study in Poland, *P. castaneus* has been found carrying mainly *L. procerum* and *S. inflata*, while *Ophiostoma* cf. *rectangulosporium* Ohtaka, Masuya & Yamaoka, *O. minus* and *O. quercus* have often been found in association with *P. piniphilus* and *P. pini* (Jankowiak and Bilański, 2013b). The data of Jankowiak and Bilański (2013ab) showed that the community of ophiostomatoid fungi associated with pine-infesting weevils was generally similar to the spectrum of fungi reported from different species of bark beetles in Europe (Kirisits, 2004; Linnakoski et al. 2012). The pathogenicity of these fungal associates has been studied in artificial inoculation trials using mature trees and seedlings (Kirisits, 2004). The majority of the ophiostomatoid species reported in these studies are capable of destroying plant cells and even some of them, e.g. *O. minus* or *L. wingfieldii*, demonstrated a high level of virulence to Scots pine (Långström et al. 1993; Solheim et al. 1993; Lieutier et al. 2004). However, only the pathogenicity of the most common fungal associates has been studied in artificial inoculation trials. Moreover, these inoculation experiments have also been carried out on various *Pinus* species. Little is, however, known regarding the potential pathogenicity other ophiostomatoid fungi, especially those associated with weevils occurring on Scots pine. This is especially important, because *H. abietis* adults introduced spores of ophiostmatoid fungi to pine seedling during maturation feeding (Jankowiak and Bilański, 2013a). Certainly, the presence of virulent fungal species in seedlings damaged by *H. abietis* feeding may affect plant health. Knowledge about virulence of ophiostomatoid fungi is also very important because several ophiostomatoid fungi of the *Leptographium* species, including *Leptographium terebrantis* S.J. Barras & T.J. Perry, *Leptographium serpens* (Goid.) M.J. Wing., *L. procerum* and *Grosmannia huntii* (Rob-Jeffr.) Zipfel Z.W. de Beer & M.J. Wingf. which have been isolated from the roots of diseased pines, and root-feeding insects vectors in the USA (Jacobs and Wingfield, 2001) have demonstrated a high level of virulence to pine (Harrington and Cobb, 1988).

The objective of the present study was to evaluate the pathogenicity of eleven ophiostomatoid species associated with pine-infesting weevils by inoculating 2-year-old Scots pine seedlings.

MATERIALS AND METHODS

To investigate the impact of the fungal associates of weevils on Scots pine, infection experiments were conducted on 2-year-old containerized *Pinus sylvestris* seedlings obtained from a nursery. Seedlings were grown in containers with a mixture of peat : perlite (8.5 : 1.5). The plants were maintained outdoor under ambient conditions and watered as required. Stem diameters at the inoculation site ranged from 3.1 to 6.8 mm (mean 4.48 mm).

Eleven species of ophiostomatoid fungi associated with *Hylobius abietis*, *Pissodes castanesus*, *Pissodes piniphilus,* and *Pissodes pini* were used for inoculation (Table 1). *Ophiostoma minus*, a known pathogen of *P. sylvestris*, was used as a positive control to estimate fungal virulence in this experiment. All isolates used in this study were obtained during a survey of ophiostomatoid fungi associated with weevils attacking Scots pine in Poland and were identified based on morphology, DNA sequence comparison for two gene regions and phylogenetic analysis (Jankowiak and Bilański, 2013ab). One random isolates of each fungus was used. On the 4–5th of April 2012, 330 seedlings were inoculated with selected isolates (30 seedlings for each isolate). Thirty plants were inoculated with sterile MEA as negative controls. Inoculations were made by cutting out a bark flap (4 x 8 mm) with a sterile scalpel, placing inoculum on the exposed sapwood surface and covering it up with the bark flap and a Parafilm® M strip, as described by Krokene and Solheim (1998). The wounds were made on the first-year shoot of plants, four cm above the root collar. The inoculum consisted of a 3 mm disc of fungus growing on 2% MEA or sterile 2% MEA. The inoculum was taken from the margin of 12-day-old cultures grown at 22^{o}C.

Observations of plant mortality were performed at weekly intervals for 11 weeks. A seedling was considered dead when the stem and needles above the inoculation site were brown discoloured. After 11 weeks, all plants were harvested and the bark was removed around the inoculation site. The length of the necrotic lesion on the sapwood surface and the depth of any sapwood blue-stain were measured. Data on the depth of sapwood blue-stain and lesion length were subjected to ANOVA, using a general linear model (GLM) in Statistica statistical software (STATISTICA® 10.0 (StatSoft, Inc., Tulsa, USA). Where significant treatment effects occurred ($p<0.05$), means were separated using Scheffe's test at $p=0.05$. For fungal isolates, 2x2 tables and Chi-square test were used to determine differences in plant mortality.

Re-isolations of the inoculated fungi from each seedling were attempted by removing two small sapwood pieces (1x1 mm) above and below the points of inoculation at a distance of 0.5–1 cm and placing them onto 2% MEA. In total, 720 plant pieces were used for re-isolations of fungi. Plates were incubated for 4 weeks at 22°C and then checked for the presence of the inoculated fungi.

Table 1
Fungal isolates used in the pathogenicity test

Species	Isolate numbers	Origin	Insect vector
Grosmannia radiaticola	535RJ	Koszęcin, beetle	*H. abietis*
Leptographium lundbergii	1314RJP	Przedbórz, larvae	*P. pini*
Leptographium procerum	630RJ	Jarosław, beetle	*H. abietis*
Leptographium truncatum	599RJP	Pateraki, beetle	*P. castaneus*
Ophiostoma cf. *abietinum*	764RJ	Czajkowa, beetle	*H. abietis*
Ophiostoma floccosum	1545RJP	Jędrzejów, gallery	*P. piniphilus*
Ophiostoma minus	1160RJp	Jędrzejów, gallery	*P. piniphilus*
Ophiostoma piceae	601RJ	Jarosław, beetle	*H. abietis*
Ophiostoma piliferum	1526RJP	Jędrzejów, larvae	*P. pini*
Ophiostoma quercus	619bRJ	Jarosław, beetle	*H. abietis*
Sporothrix inflata	297ARJP	Jędrzejów, gallery	*P. castaneus*

RESULTS

Mortality was observed throughout this experiment in the 2-year-old seedlings inoculated with *Grosmannia radiaticola* (J.J. Kim, Seifert & G.H. Kim) Zipfel, Z.W. de Beer & M.J. Wingf., *Leptographium truncatum* (M.J. Wingf. & Marasas) M.J. Wingf., *Leptographium lundbergii, Leptographium procerum, Ophiostoma floccosum, Ophiostoma minus*, and *Ophiostoma piliferum.* The isolates of *L. truncatum* and *L. lundbergii* killed 23–33% of the plants, while *L. procerum*, *G. radiaticola, O. floccosum* and *O. minus* caused the death of 10–17% of the seedlings. However, seedling survival was not significantly different between these fungal species. *Ophiostoma piliferum* contributed to the death of one seedling (Table 2). In the case of plants inoculated with these fungal isolates, the first mortality symptoms were observed 6–8 weeks after inoculation. Dying seedlings were characterized by the symptom of wilting of new shoots of the current year's growth and by yellow-brownish needles. No control plants or plants inoculated with isolates of *Ophiostoma* cf. *abietinum* Marm. & Butin, *Ophiostoma piceae*, *Ophiostoma quercus* and *Sporothrix inflata* died (Table 2).

All fungal species, except *O.* cf. *abietinum*, *O. quercus* and *S. inflata*, caused dark-brown sunken lesions in pine seedlings. The lesions were generally covered with crystallized resin and extended vertically from the wounded area (Fig. 1 A–L). *Ophiostoma minus* and *L. truncatum* induced significantly larger necrotic lesions than the other fungi tested in comparison to the control seedlings. The lesions associated with *L. lundbergii* infection were also large and significantly different from the other species (Table 2). In seedlings inoculated with these species, the callus tissue was considerably damaged (Fig. 1 B, D, G). Four other ophiostomatoid species, including *G. radiaticola*, *L. procerum*, *O. floccosum* and *O. piliferum*, generated significantly smaller necrosis with a range of 8.30 mm to 8.72 mm (Table 2). In seedlings treated with these fungi, the wounds were partially healed (Fig. 1 A, C, F, I). *Ophiostoma piceae* caused necrosis of the smallest mean length (5.47 mm). Eleven weeks after inoculation, the callus tissue enclosed the wounds of all control plants and plants inoculated with *O.* cf. *abietinum*, *O. quercus* and *S. inflata* (Fig. 1 E, J, K).

All the fungi concerned, except *O.* cf. *abietinum*, *O. quercus* and *S. inflata*, caused sapwood blue-stain in the 2-year-old seedlings (Fig. 2 A–L). Similar to lesion length, *O. minus* and *L. truncatum* caused the largest sapwood blue-stain. *Leptographium procerum* also caused sapwood blue-stain of similar depth, however significantly smaller than *O. minus* (Table 2). *Grosmannia radiaticola*, *L. lundbergii* and *O. piliferum* generated the second largest zones of sapwood blue-stain ranging from 0.47 mm to 0.88 mm (Table 2). The depth of sapwood blue-stain was significantly smallest in pine seedlings treated with *O. floccosum* and *O. piceae* (Table 2).

Re-isolation of fungal species from inoculated seedlings confirmed infection. All fungal species were successfully re-isolated from the inoculated pines. *Grosmannia radiaticola*, *L. procerum*, *O. floccosum*, *O. minus*, *O. piceae*, *O. quercus* were successfully re-isolated from 100% of the plants, while *L. lundbergii*, *L. truncatum*, *O.* cf. *abietinum*, *O. piliferum* and *S. inflata* were re-isolated from 53–86% of the plants (Table 2).

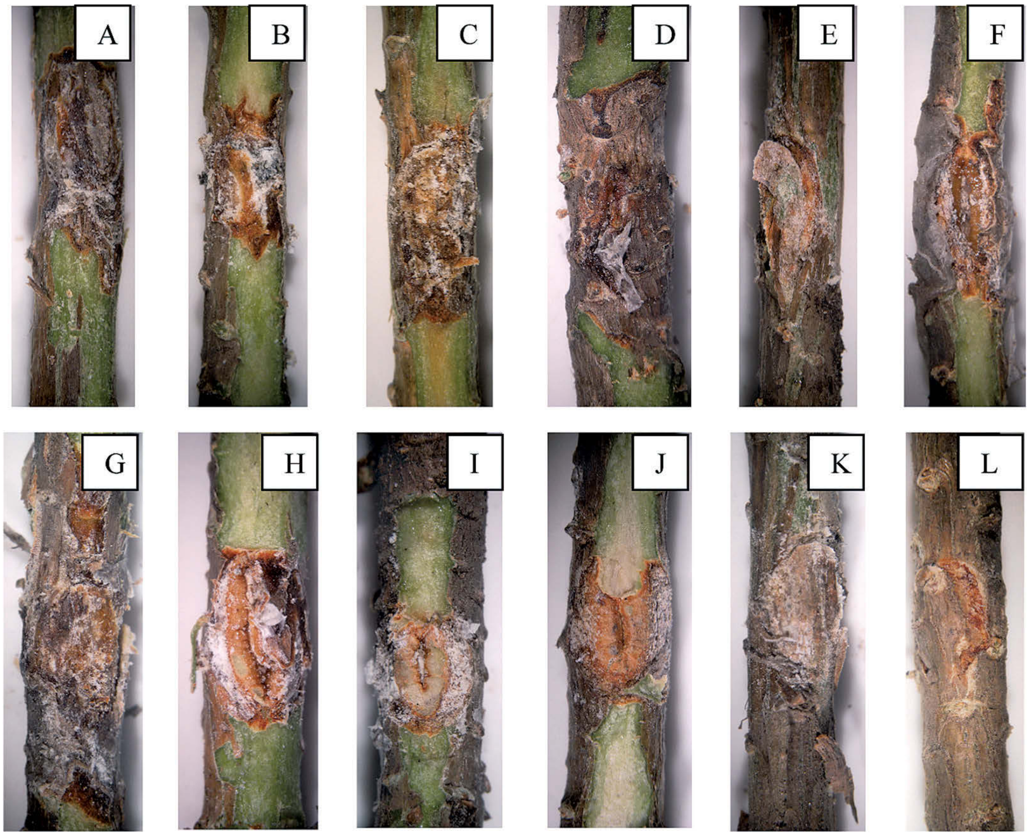

Fig. 1. Lesions on the surface of a pine stem caused by A – *Grosmannia radiaticola*, B – *Leptographium lundbergii*, C – *Leptographium procerum*, D – *Leptographium truncatum*, E – *Ophiostoma* cf. *abietinum*, F – *Ophiostoma floccosum*, G – *Ophiostoma minus*, H – *Ophiostoma piceae*, I – *Ophiostoma piliferum*, J – *Ophiostoma quercus*, K – *Sporothrix inflata*, L – control (photo by R. Jankowiak).

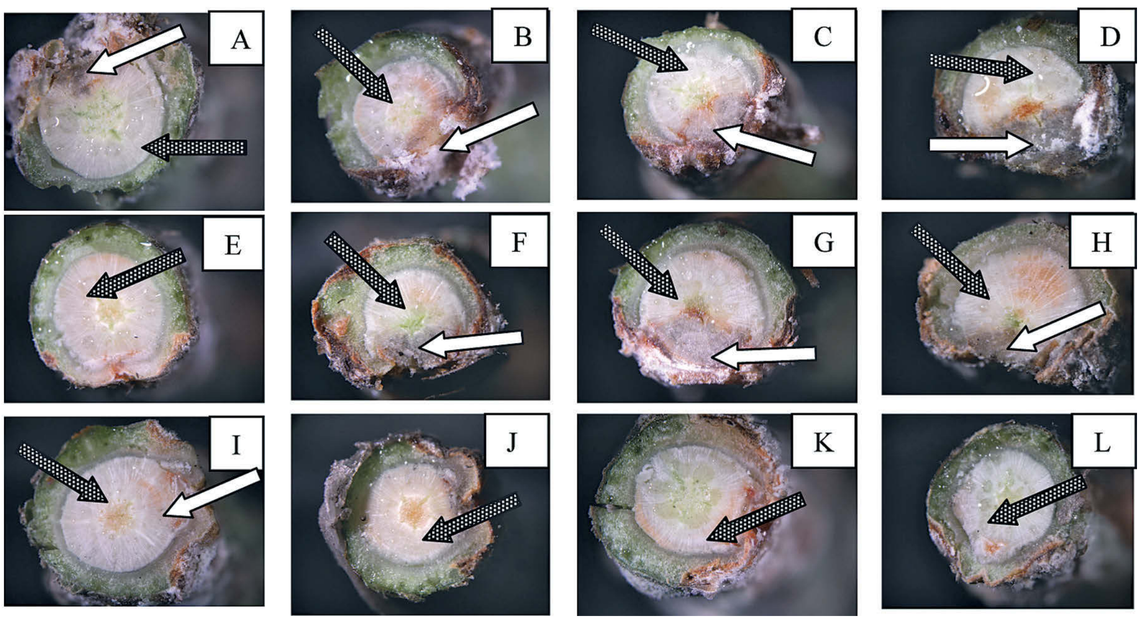

Fig. 2. Cross section through a pine stem at the points of inoculation: A – *Grosmannia radiaticola*, B – *Leptographium lundbergii*, C – *Leptographium procerum*, D – *Leptographium truncatum*, E – *Ophiostoma* cf. *abietinum*, F – *Ophiostoma floccosum*, G – *Ophiostoma minus*, H – *Ophiostoma piceae*, I – *Ophiostoma piliferum*, J – *Ophiostoma quercus*, K – *Sporothrix inflata*, L – control. The white arrow indicates dead parenchyma and sapwood blue stain, while the dark arrow indicates healthy sapwood (photo by R. Jankowiak).

Table 2
Effects of inoculation of 2-year-old Scots pine seedlings with ophiostomatoid fungi associated with pine-infesting weevils

Species	Mean depth of sapwood blue-stain (mm)*	Mean lesion length (mm)*	% dead plants*	Re-isolation (%)
Grosmannia radiaticola	0.68^{CD}	8.56^{C}	13^{ABC}	100
Leptographium lundbergii	0.88^{CD}	11.78^{B}	23^{AB}	67
Leptographium procerum	1.01^{BC}	8.72^{C}	17^{ABC}	100
Leptographium truncatum	1.29^{AB}	15.01^{A}	33^{A}	60
Ophiostoma cf. *abietinum*	0^{F}	0^{E}	0^{C}	86
Ophiostoma floccosum	0.18^{EF}	8.50^{C}	10^{ABC}	100
Ophiostoma minus	1.64^{A}	15.87^{A}	10^{ABC}	100
Ophiostoma piceae	0.24^{EF}	5.47^{D}	0^{C}	100
Ophiostoma piliferum	0.47^{DE}	8.30^{C}	3^{BC}	53
Ophiostoma quercus	0^{F}	0^{E}	0^{C}	100
Sporothrix inflata	0^{F}	0^{E}	0^{C}	80
Control	0^{F}	0^{E}	0^{C}	

* Depth of sapwood blue-stain, lesion length and seedling mortality with the same letter were not significantly different.

DISCUSSION

All ophiostomatoid species examined, except *Ophiostoma* cf. *abietinum*, *Ophiostoma quercus* and *Sporothrix inflata*, were capable of successful infection and symptom development in Scots pine seedlings. Lesions surrounding the point of inoculation in seedlings were observed and they extended beyond the wound beneath the surface of the epidermis. Similar lesion morphology and occurrence have been observed in previous pine seedling inoculation studies with many of the ophiostomatoid species (Nevill et al. 1995; Krokene and Solheim, 1998; Eckhardt et al. 2004; Jankowiak, 2006; Matusick and Eckhardt, 2010) or without the introduction of fungi (Wingfield, 1986; Klepzig et al. 1995; Eckhardt et al. 2004). These studies are the first to confirm lesion development and sapwood penetration associated with artificial inoculation with *Grosmannia radiaticola, Leptographium truncatum, Ophiostoma floccosum, O.* cf. *abietinum, O. quercus*, and *S. inflata.*

The eleven ophiostomatoid species tested in this study differed in their virulence to Scots pine. The seedling mortality as well as necrosis length and depth of sapwood blue-stain indicate that *L truncatum* and *Ophiostoma minus* were the most virulent species in this study. The high virulence of *O. minus* is in accordance with the results of other investigations (Lieutier et al. 1989; Solheim and Långström, 1991; Solheim et al. 1993; Solheim et al. 2001; Jankowiak et al. 2007). However, previous studies have shown higher virulence of *O. minus* to Scots pine seedlings (Jankowiak, 2006; Jankowiak, 2011; Jankowiak, 2012) when compared to the present study. This variation might be caused by differences in the virulence among isolates. Such variation has been reported for isolates of *Leptographium wingfieldii*, which showed high individual variability in growth characteristics and virulence to Scots pine (Lieutier et al. 2004). Of the eleven species tested, *O. minus* showed the largest radial spreading suggesting its high virulence to Scots pine. Radial movement is most characteristic of highly virulent ophiostomatoid fungi such as *Leptographium wageneri* (W.B. Kendr.) M.J. Wingf. (Cobb, 1988). These results indicate that this fungus may contribute to the acceleration of tree dieback after attack of *Pissodes pini* and *Pissodes piniphilus* in Poland. The data of Jankowiak and Bilański (2013b) could support this statement because these weevils transmitted *O. minus* with high frequency. According to the pathogenicity tests (Solheim and Långström, 1991; Solheim et al. 1993; Solheim et al. 2001; Jankowiak et al. 2007), *O. minus* at high inoculation densities demonstrated high virulence.

The virulence of *L. truncatum* to pine seedlings was surprisingly high, although several species of *Leptographium* spp. are known to be a primary pathogen of conifers (Jacobs and Wingfield, 2001). This fungus was capable of killing a large part of seedlings and similarly to *O. minus* penetrated deeper into the sapwood than other species. This is in agreement with the studies of Jankowiak (2012) that showed that a genetically similar species – *L.* cf. *truncatum* isolated from *Ips sexdenatus* (Börn.) – was pathogenic to Scots pine. Despite the relatively high pathogenicity of *L. truncatum* to *Pinus sylvestris* seedlings, its role in the dieback of trees after attack of weevils seems to be less important, because this fungus is relatively rarely isolated from weevils (Jankowiak and Bilański, 2013ab). However, this fungus may play an important role in increasing seedling mortality during maturation feeding of *Hylastes ater* (Paykull). Adults of *H. ater* feed on the stem of young pine seedlings, often leading to their death in Poland (Michalski and Mazur, 1999). *Leptographium truncatum* was recently recorded from Poland mainly in association with this beetle species (Jankowiak and Bilański, 2013c). Reay et al. (2002, 2005) observed a strong relationship between the severity of feeding damage by *H. ater* and the presence and number of *Ophiostoma* species that colonised the seedlings. To fully explain the level of virulence of *L. truncatum*, further pathogenicity studies of the same fungal isolates should be conducted using roots of older Scots pine trees, although the studies by Krokene and Solheim (1998) give clear evidence that inoculation of seedlings is a suitable method for determining the virulence of bark beetle-associated blue-stain fungi. However, results from seedling inoculations must be carefully interpreted because young seedlings do not have a well-developed defence system found in older trees (Sandnes and Solheim, 2002).

Consistent with previous results (Zhou et al. 2002), *Leptographium lundbergii* also had pathogenic capability because it generated relatively long lesions and caused the death of Scots pine seedlings. However, *L. lundbergii* gave rise to significantly shorter lesions and to shallower sapwood blue-stain than those associated with *L. truncatum* and *O. minus*, suggesting that this species is not highly pathogenic to Scots pine. The results of this study also indicate that *Leptographium procerum* has similar pathogenicity characteristics. The low virulence of *L. procerum* in this study is in accordance with previous observations using the same host tree in Poland (Jankowiak, 2006; Jankowiak et al. 2007) and various *Pinus* species (Wingfield, 1986; Harrington and Cobb, 1988). It seems that this fungus is highly virulent on *Pinus strobus* L. (Alexander et al. 1988). Due to the close relationship between *L. procerum* and regeneration weevils (Jankowiak and Bilański, 2013ab), the lack of high virulence of this fungus is very important information. This fungus is the predominant fungal organism in many seedlings damaged by maturation feeding of *Hylobius abietis* adults (Jankowiak and Bilański, 2013a) and in the galleries of *Pissodes castaneus* (Jankowiak and Bilański, 2013b). The low virulence of *L. procerum* obtained in this study indicated that this fungus rather do not accelerate the dieback of trees attacked by *P. castaneus* on reforestation sites in Poland. The mortality of seedlings destroyed by *H. abietis* also appeared to be a result of severe feeding damage by the beetles, rather than the presence of *L. procerum* in seedlings.

Inoculations with *G. radiaticola*, *Ophiostoma piliferum*, *Ophiostoma piceae,* and *O. floccosum* resulted in very small lesions. These fungi were also not able to penetrate deeply into the sapwood of the seedlings. This new inoculation trial supports previous findings that *O. piliferum* and *O. piceae* are mild pathogens to *P. sylvestris* (Harrington and Cobb, 1983; Jankowiak, 2006; Jankowiak et al. 2007). The present study is also the first to confirm lesion development associated with artificial inoculation with *G. radiaticola* and *O. floccosum*. The former species was the most dominant fungal taxa in the community of ophiostomatoid fungi recorded in association with root-feeding bark beetles (Jankowiak and Bilański, 2013c) and was also found in association with *H. abietis* (Jankowiak and Bilański, 2013a) in Poland. In turn, *O. floccosum* is found occasionally on various bark beetles and weevils infesting pine (Jankowiak and Bilański, 2013ab; Linnakoski et al. 2012). The present study indicated that these two species should be considered as very weak pathogens of Scots pine.

This study showed that isolates of *Ophiostoma* cf. *abietinum*, *O. quercus* and *S. inflata* appeared to be non-pathogenic to Scots pine. These fungi are frequently associated with pine-infesting weevils in Poland (Jankowiak and Bilański, 2013ab). In addition, recently *S. inflata* was also found as a frequent root colonizer of dying and dead young *P. sylvestris* trees (Jankowiak et al. 2012). This inoculation test confirmed the supposition of Jankowiak et al. (2012) that *S. inflata* is a saprobe and probably does not play an important role in destroying roots of Scots pine trees. *O.* cf. *abietinum* and *O. quercus* probably show the same saprotrophic behaviour in relation to Scots pine.

CONCLUSIONS

1. Inoculation of Scots pine seedlings demonstrated that pine-infesting weevils were associated with ophiostomatoid fungi differing in virulence to Scots pine seedlings.
2. This study showed that *Ophiostoma minus* and *Leptographium truncatum* were more pathogenic than other pine weevil-associated fungi. Of these species, *O. minus* was more consistently associated with *Pissodes pini* and *Pissodes piniphilus* than *L. truncatum*, and it presumably plays a more important role in accelerating the dieback of Scots pine after attack of weevils.
3. The other ophiostomatoid species were non-pathogenic (*Ophiostoma* cf. *abietinum*, *Ophiostoma*

quercus, and *Sporothrix inflata*) or weakly pathogenic (*Grosmannia radiaticola, Leptographium lundbergii, Leptographium procerum, Ophiostoma floccosum, Ophiostoma piceae*, and *Ophiostoma piliferum*) to Scots pine. The low virulence of *L. procerum* may suggest that the abundant presence of this fungus in seedlings damaged by *Hylobius abietis* feeding rather does not increase the mortality of seedlings.

Acknowledgments

The work was supported by grants from the Polish Ministry of Education and Science N N309 049037.

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Ocena wirulencji grzybów ofiostomatoidalnych związanych z ryjkowcami w stosunku do sadzonek sosny zwyczajnej (*Pinus sylvestris* L.)

Streszczenie

Dwuletnie sosny rosnące w kontenerach z podłożem torfowo-perlitowym były inokulowane dwutygodniową grzybnią wyrosłą na 2-procentowej pożywce agarowo-maltozowej. Kontrolę stanowiło 30 sadzonek inokulowanych sterylną pożywką. Badano 11 gatunków grzybów związanych z owadami z rodziny ryjkowcowatych (Curculionidae) żerującymi na sośnie zwyczajnej. *Leptographium truncatum* (M.J. Wingf. & Marasas) M.J. Wingf., *L. lundbergii* Lagerb. & Melin, *L. procerum* (W.B. Kendr.) M.J. Wingf., *Grosmannia radiaticola* (J.J. Kim, Seifert & G.H. Kim) Zipfel, Z.W. de Beer & M.J. Wingf., *Ophiostoma* cf. *abietinum* Marm. & Butin, *O. floccosum* Math.--Käärik, *minus* (Hedgc.) Syd. & P. Syd., *O. piliferum*

(Fr.) Syd. & P. Syd., *piceae* Münch) Syd.& P. Syd., *O. quercus* (Georgev.) Nannf. i *Sporothrix inflata* de Hoog. Sadzonki zakażano poprzez nacięcie strzałki pędu i wprowadzenie inokulum w postaci krążka pożywki z grzybnią.

Izolaty *L. truncatum* i *L. lundbergii* spowodowały zamarcie 23–33% roślin, zaś izolaty *L. procerum*, *G. radiaticola, O. floccosum* i *O. minus* przyczyniły się do śmierci 10–17% sadzonek sosny. *Ophiostoma piliferum* spowodował zamarcie jednej sadzonki. U żadnej sadzonki kontrolnej ani sadzonki zakażanej grzybami *O.* cf. *abietinum*, *O. piceae*, *O. quercus* i *S. inflata* nie zaobserwowano objawów chorobowych. Wszystkie gatunki grzybów, z wyjątkiem *O.* cf. *abietinum*, *O. quercus* i *S. inflata* spowodowały na strzałce pędu powstanie ciemnobrunatnych nekroz. *Ophiostoma minus* i *L. truncatum* indukowały wyraźnie dłuższe nekrozy niż inne grzyby i niż te występujące na sadzonkach kontrolnych. Wszystkie gatunki grzybów, z wyjątkiem *O.* cf. *abietinum*, *O. quercus* i *S. inflata*, spowodowały siniznę drewna bielastego u inokulowanych sadzonek. Podobnie jak w przypadku nekroz, także *O. minus* i *L. truncatum* spowodowały najgłębsze przebarwienie drewna bielastego sadzonek sosny.