BALTIC FORESTRY

http://www.balticforestry.mi.lt
ISSN 1392-1355
eISSN 2029-9230

Baltic Forestry 2022 28(1): 2–10
Category: Research article
https://doi.org/10.46490/BF637

In vitro screening of Latvian isolates of *Bjerkandera adusta* and *Sistotrema brinkmannii* as potential biocontrol agents against *Heterobasidion* root and butt rots

NATĀLIJA BURŅEVIČA [1]*, DĀRTA KĻAVIŅA [1], GUGLIELMO LIONE [2], MARTINA PELLICCIARO [2], LAUMA SILBAUMA [1], ASTRA ZAĻUMA [1], VIZMA NIKOLAJEVA [3] AND PAOLO GONTHIER [2]

[1] *Latvian State Forest Research Institute "Silava", Rigas str. 111, Salaspils, LV-2169, Latvia*
[2] *University of Torino, Department of Agricultural, Forest and Food Sciences (DISAFA), Largo Paolo Braccini str. 2, I-10095 Grugliasco, Italy*
[3] *University of Latvia, Faculty of Biology, Department of Microbiology and Biotechnology, Jelgavas str. 1, Riga, LV-1004, Latvia*

** Corresponding author: natalija.burnevica@silava.lv; phone: +371 25951553*

Burņeviča, N., Kļaviņa, D., Lione, G., Pellicciaro, M., Silbauma, L., Zaļuma, A., Nikolajeva, V. and Gonthier, P. 2022. In vitro screening of Latvian isolates of *Bjerkandera adusta* and *Sistotrema brinkmannii* as potential biocontrol agents against *Heterobasidion* root and butt rots. *Baltic Forestry* 28(1): 2–10. https://doi.org/10.46490/BF637.

Received 21 February 2022 **Revised** 28 April 2022 **Accepted** 29 April 2022

Abstract

Root and butt rots caused by *Heterobasidion* spp. are destructive diseases of conifer forest stands that may be controlled by spraying freshly cut stumps with chemical or biological treatments, including the effective and widely used biological control agent *Phlebiopsis gigantea*. In this study, we tested *in vitro* different Latvian isolates of the fungal species *Bjerkandera adusta* and *Sistotrema brinkmannii* for their antagonistic activity against both *Heterobasidion* species present in Northern Europe and Baltic countries, namely *H. annosum* and *H. parviporum,* using Latvian isolates of *P. gigantea* and the Rotstop® strain as controls. The growth rate of isolates on agar media and their ability to produce oidia or conidia were also assessed. In general, *B. adusta* performed better than *S. brinkmannii*. Some isolates of *B. adusta* ranked similarly to those of *P. gigantea* in terms of oidia production, growth rate and ability to overgrow the mycelium of both *H. annosum* and *H. parviporum*. These isolates will be further tested in field conditions.

Keywords: biological control, antagonistic fungi, fungal isolates

Introduction

Heterobasidion root and butt rots are some of the most significant conifer diseases in the Northern Hemisphere, causing losses of approximately 800 million euros to the European forestry annually (Woodward et al. 1998). These fungal diseases are caused by basidiomycetes included in the *Heterobasidion annosum* species complex, namely *H. abietinum*, *H. annosum sensu stricto*, hereafter referred to as *H. annosum*, and *H. parviporum* in Eurasia, and *H. irregulare* and *H. occidentale* in North America (Garbelotto and Gonthier 2013). In Northern Europe and the Baltic States, particularly in Latvia, both *H. annosum* and *H. parviporum* are present and abundant, the former mainly associated with Scots pine (*Pinus sylvestris*) and the latter with Norway spruce (*Picea abies*) (Korhonen et al. 1992, Korhonen and Piri 1994, Gaitnieks et al. 2021).

Heterobasidion species spread in two ways: 1) by airborne basidiospores produced in fruit bodies and infecting freshly cut conifer stumps or tree wounds; 2) by mycelium spreading through root contacts to adjacent trees (Korhonen and Stenlid 1998, Gonthier and Thor 2013). To prevent spore infection of freshly cut stumps, stump treatment using biological or chemical control agents is widely used (Nicolotti and Gonthier 2005, Thor and Stenlid 2005, Gonthier and Thor 2013). If a forest stand is already severely infested, stump removal or planting tree species resistant or less susceptible to the disease could be considered (Vasaitis et al. 2008, Garbelotto and Gonthier 2013).

The most effective biological control agent (BCA) for stump treatments against *Heterobasidion* spp. is the basidiomycete *Phlebiopsis gigantea* (Pratt et al. 2000, Garbelotto and Gonthier 2013), although the bacterium *Pseudomo-*

nas protegens has recently been proved as effective both *in vitro* and in forest conditions (Pellicciaro 2021a, b). In Europe, the biological product based on *P. gigantea* most widely employed in practical forestry is Rotstop® (Thor 2003). In the Baltic countries, Rotstop® is registered for use in Estonia and Latvia since 2004 and 2007, respectively (Drenkhan et al. 2008, Kenigsvalde et al. 2016). Rotstop® is extensively used by Latvia's State Forests: each year it is applied to an area of more than 5,000 hectares using at least 300 thousand litres of prepared suspension for stump treatment (J. Mālakalns, personal communication). Rotstop® was developed decades ago and it contains a single *P. gigantea* strain native to Finland (Korhonen et al. 1994). In the long term, such a large-scale application of one isolate could negatively affect populations of native *P. gigantea* and of other stump colonizing fungi, as previously suggested (Varese et al. 2001, Vasiliauskas et al. 2004). Although the abundance of the Rotstop® strain of *P. gigantea* in Scots pine and Norway spruce stumps has been reported to decrease within a period of three to six years after treatments (Vainio et al. 2001, Vasiliauskas et al. 2005), from a biological diversity perspective it would be beneficial to use also other BCAs, including native *P. gigantea* strains and other fungi. Moreover, *P. gigantea* is better adapted to Scots pine stumps than to Norway spruce stumps (Korhonen 2003, Webber and Thorpe 2003) making treatments with this BCA more effective when applied on stumps of the former tree species than on stumps of the latter one (Korhonen et al. 1994, Berglund and Rönnberg 2004, Rönnberg et al. 2006). For instance, data from Latvia showed that the efficacy of Rotstop® was on average 82% on Scots pine stumps but only 64% on Norway spruce stumps (Kenigsvalde et al. 2016). Altogether the above findings point to the opportunity of identifying additional antagonistic fungi effective against *Heterobasidion* spp., especially on Norway spruce stumps. Various saprotrophic fungi have been tested against *Heterobasidion* spp. in laboratory and field conditions (Hyppel 1968, Rishbeth 1970, Aufsess 1976b, Lundborg and Unestam 1980, Holdenrieder 1982, 1984, Negrutskii 1986, Vasiliauskas 1989, Capretti and Mugnai 1989a, b, 1993, Hanso and Hanso 1985, 1992, Holmer and Stenlid 1993, 1996, Negrutskii 1993, Anselmi and Nicolotti 1997, Nicolotti et al. 1999, Roy et al. 2003, Woods et al. 2005, Hamberg et al. 2012). Among them, the basidiomycete *Bjerkandera adusta* was identified as one of the most promising species after *P. gigantea* (Holdenrieder and Greig 1998). *Bjerkandera adusta* is common in wood of deciduous trees (Holdenrieder and Greig 1998), but several studies indicate that it is also associated with Norway spruce stumps (Pechmann and Aufsess 1971, Kallio and Hallaksela 1979, Vasiliauskas et al. 2002). Interestingly, this fungus has been reported to be able to replace other fungi both *in vitro* and in stem sections of *Platanus* sp. (Holdenrieder and Greig 1998 and literature therein). Another fungal species showing the ability to replace other fungi both in laboratory and field experiments is *Sistotrema brinkmannii* (Holdenrieder and Greig 1998 and literature therein), a fungus reported to colonize wood both at the early and at the final stage of decay (Morrison and Johnson 1978, Vasiliauskas et al. 2005, Stokland and Larsson 2011). *Sistotrema brinkmannii* frequently colonizes wood already decayed by *P. gigantea* (Vasiliauskas et al. 2005).

In Latvia, analyses of wood samples collected from 1182 decayed Norway spruce stumps revealed that *H. parviporum* and *Stereum sanguinolentum* are the most common stump-inhabiting basidiomycetes (Arhipova et al. 2011a). The next most abundant basidiomycetes were right *B. adusta* and *S. brinkmannii*, showing approximately the same isolation frequency (Arhipova et al. 2011a).

While the antagonism of *B. adusta* and *S. brinkmannii* towards wood decay fungi, including *Heterobasidion* spp., *Armillaria* spp. and *S. sanguinolentum* has been documented (Rishbeth 1970, Aufsess 1976a, b, Kallio and Hallaksela 1979, Capretti and Mugnai 1989a, b, Nicolotti et al. 1993), as noted by Holdenrieder and Greig (1998) studies on the efficacy of these two fungi against *Heterobasidion* spp. are still scarce to determine their level of antagonistic potential.

From a biotechnological perspective, oidia production by a fungus is an important feature for the production of a biological product (Pratt et al. 2000). Similarly, to *P. gigantea*, *B. adusta* produces oidia, although little is known about intraspecific variation of the oidia production between different *B. adusta* isolates. *Sistotrema brinkmannii*, to our knowledge, can produce both basidiospores and Ptychogaster-like conidia, but information about this production are scarce (Carris and Glawe 1989).

The aim of this study was to screen *B. adusta* and *S. brinkmannii* isolates *in vitro* for their antagonistic potential against both *H. annosum* and *H. parviporum*, using *P. gigantea* as a control. The specific aims were: i) to assess the mycelial growth of *B. adusta* and *S. brinkmannii* isolates on malt agar media; ii) to evaluate their level of antagonism against *H. annosum* and *H. parviporum* using dual culture assays; iii) to appraise the oidia production potential of isolates of *B. adusta*. The results will be used for selecting the most promising fungal isolates for further field experiments.

Material and methods

Fungal isolates

In total, 11 isolates of *B. adusta*, six isolates of *S. brinkmannii*, four isolates of *P. gigantea*, two isolates of *H. annosum*, and two isolates of *H. parviporum* were used in this study (Table 1). Isolates of *B. adusta, S. brinkmannii* and *P. gigantea* were collected from different substrates during the years 2005 to 2016 in different regions of Latvia. Fungal isolates were obtained from decayed Scots pine, Norway spruce and alder (*Alnus* sp.) wood. The isolation procedure was described in Arhipova et al. 2011a, b. Brief-

Table 1. Fungal isolates used in this study

Fungal species	Isolate ID	GenBank accession number	Coordinates of the site of origin	Host	Isolation source
Bjerkandera adusta	B25	-	56°33'00N 23°01'54E	*Picea abies*	Stump
	B50	FJ903311	57°00'00N 23°01'54E	*Picea abies*	Stump
	E67	-	57°18'51N 22°50'42E	*Picea abies*	Stem
	E182	-	57°21'23N 25°11'49E	*Picea abies*	Stem
	I216	-	56°50'39N 22°22'13E	*Alnus incana*	Stem
	I345	GU062301	56°41'43N 25°55'24E	*Alnus incana*	Stem
	L38A	-	56°44'50N 23°55'57E	*Picea abies*	Stem
	L38B	-	56°44'50N 23°55'57E	*Picea abies*	Stem
	M59	JF340266	56°41'59N 25°54'26E	*Alnus glutinosa*	Stem
	M108	-	56°41'59N 25°54'26E	*Alnus glutinosa*	Stem
	M111	-	56°41'59N 25°54'26E	*Alnus glutinosa*	Stem
Sistotrema brinkmannii	B5	FJ903297	56°59'29N 23°10'38E	*Picea abies*	Stump
	E136	-	56°59'35N 23°10'11E	*Picea abies*	Stem
	I390	GU062313	57°00'30N 23°07'22E	*Alnus incana*	Stem
	I409	-	56°41'43N 25°55'24E	*Alnus incana*	Stump
	P31	-	57°00'23N 23°13'02E	*Pinus sylvestris*	Stem
	S73	MK911637	57°06'28N 25°07'09E	*Picea abies*	Root
Phlebiopsis gigantea	Rotstop®	-	-	*Picea abies*	Stem
	PG382	-	56°50'57N 24°48'18E	*Picea abies*	Stem
	PG182	-	56°37'30N 25°50'58E	*Pinus sylvestris*	Stem
	PG408	-	56°40'53N 25°53'24E	*Pinus sylvestris*	Stem
Heterobasidion annosum	HAZ	-	56°37'49N 24°27'24E	*Pinus contorta*	Stump
	HAU	-	57°15'43N 22°00'35E	*Pinus contorta*	Stump
Heterobasidion parviporum	HPN	-	57°06'28N 25°07'09E	*Picea abies*	Root
	HPO	-	56°45'42N 24°45'57E	*Picea abies*	Root

ly, wood samples were taken by using a Pressler borer. In the laboratory, small pieces of decayed wood were placed on Hagem agar in Petri plates. Fungal cultures that were obtained were identified as follows. DNA was extracted, PCR reactions were run using the primer combination ITS1F and ITS4, Sanger sequenced, and BLAST search were performed using NCBI (National Center for Biotechnology Information) fungal database (NCBI 2022) (Arhipova et al. 2011a, b, Arhipova et al. 2012, Burņeviča et al. 2021). Selected sequences were deposited in the NCBI database. For the identification of *Heterobasidion* species, intersterility tests were performed (Korhonen 1978) using homokaryotic test cultures 03013 and 03015 (*H. annosum*), and 96119 and 98036 (*H. parviporum*) (courtesy of Dr. Kari Korhonen, LUKE, Finland) (Zaļuma et al. 2019, Burņeviča et al. 2021). Latvian *P. gigantea* isolates as well as the Finnish one of the fungi contained in Rotstop® were used as controls in this study for the mycelial growth rate *in vitro*, oidia production and antagonism of *B. adusta* and *S. brinkmannii* against *H. annosum* and *H. parviporum*.

Assessment of fungal mycelium growth rate

For the assessment of the mycelial growth *in vitro*, circle-shaped agar blocks (Ø 0.6 cm) were cut from actively growing fungal cultures and placed at the edge of a Ø 9 cm Petri plate containing malt-agar medium (7 g malt extract, 6 g agar per 1 L water). The Petri plates were incubated in the dark at 20°C. The fungal growth was determined by measuring the radial growth along the agar surface and then tracing the leading edge of the growing colony with a marker. The growth rate was calculated every 48 hours at approximately the same time for 8 days. The average growth rate of three replicates was calculated for each fungal isolate.

Assessment of antagonistic activity against H. annosum and H. parviporum

For the assessment of antagonistic activity of the fungal isolates against *Heterobasidion* spp., the dual culture assay technique was used (Holdenrieder and Greig 1998). Circle-shaped agar blocs (Ø 0.6 cm) of each pure fungal culture was transferred to a sterile malt-agar Petri plate, using a flame sterilized inoculation needle. A similar agar block (Ø 0.6 cm) of either *H. annosum* or *H. parviporum* was transferred to the same Petri plate at a distance of 5–6 cm from the test cultures. All the Petri plates were incubated in the dark at 20°C and the width of the inhibition zone between the two fungal isolates was measured after 14 days. For the evaluation of fungal interaction, a modified classification system developed by Holdenrieder (1984a) was used: ++ strong antagonism, antagonist overgrows *Heterobasidion* sp.; + moderate antagonism, formation of mycelium-free inhibition zone between antagonist and *Heterobasidion* sp.; +– sporadical antagonism, antagonist creates a mycelium-free inhibition zone only with one *Heterobasidion* sp. isolate; – antagonistic activity absent, *Heterobasidion* sp. overgrows the antagonist.

Assessment of oidia production

Oidia suspensions were prepared from each fungal isolate of *B. adusta* and *P. gigantea* by washing the agar media surface of 12-day old pure cultures with 0.5 ml of sterile water, gently shaking the mycelium with a flame sterilized scalpel. Using a 0.3 ml Eppendorf automatic pipette, oidia suspension was transferred to a sterile 1.5 ml Eppendorf tube. A similar approach was used to collect conidia of *S. brinkmannii*.

The number of oidia/conidia in the suspensions was counted in a Goryaev chamber (a variation of a hemocytometer) (Muntyan et al. 2012). The cover glass was placed on the middle grid of the chamber and rubbed so that the Newton's rings could be observed (to ensure the depth of the chamber). Using an Eppendorf automatic pipette, 100 µl of suspension was transferred to the edge of the cover glass in the Goryaev chamber. The oidia/conidia were counted in 30 small grids of the chamber at 100× magnification using a *Leica* DM 4000B microscope. On the upper left corner of the grid, oidia/conidia were counted in the diagonally selected four smaller squares. Following the same principle, oidia/conidia were counted in all the squares by moving the camera to the right and finding the next large square. Oidia/conidia located at the edge with at least half of the cell inside the square were considered on the left and lower line. The average number of oidia/conidia in one square and in 1 ml of suspension was calculated, considering the area of the square.

Data analysis

Data was organized and analyzed using Microsoft Excel spreadsheet. The concentration of oidia/conidia in 1 ml of suspension was calculated using the following formula:

$$C_{oc} = 16N_{ss} \cdot N_{oc1} \cdot 25 \cdot 10^4 , \quad (1)$$

where:

N_{ss} is the number of small squares;

N_{oc1} is the number of oidia/conidia in one small square.

For data analysis, *t*-test was run at $\alpha = 0.05$. Correlation between mycelial growth rate and oidia production was calculated using the Pearson correlation (Liepa 1974).

Results

Mycelial growth rate of B. adusta, S. brinkmannii and P. gigantea isolates

The average mycelial growth rate of each isolate is summarized in Table 2. The results show that the average mycelial growth of the fungal isolates is uneven and differs not only between fungal species, but also between isolates of the same species. For *B. adusta*, the highest average growth rate was observed for isolates E182 (0.88 cm day^{-1}) and I345 (0.84 cm day^{-1}), while the lowest one was observed for L38A (0.42 cm day^{-1}). For *S. brinkmannii*, the highest growth rate was observed for isolates B5 (0.46 cm day^{-1}) and P31 (0.42 cm day^{-1}), while the lowest one for E136 (0.18 cm day^{-1}) and I409 (0.16 cm day^{-1}). The highest average growth rate among *P. gigantea* isolates was observed for the isolate from Rotstop® (0.73 cm day^{-1}) and for isolate PG382 (0.70 cm day^{-1}). The average growth rate of *B. adusta* isolates (0.73 ± 0.11 cm day^{-1}) was slightly higher than the average growth rate of *P. gigantea* isolates (0.69 ± 0.03 cm day^{-1}) although differences were not significant ($p = 0.31$). However, five *B. adusta* isolates showed higher average growth rate than that of *P. gigantea*: B25 (0.77 ± 0.15 cm day^{-1}), E182 (0.88 ± 0.04 cm day^{-1}), I216 (0.76 ± 0.09 cm day^{-1}), I345 (0.84 ± 0.09 cm day^{-1}) and M111 (0.78 ± 0.18 cm day^{-1}). The average growth rate of *S. brinkmannii* isolates was much lower (0.32 ± 0.11 cm day^{-1}) compared to *B. adusta* and *P. gigantea* ($p < 0.01$). The growth rate of *Heterobasidion* spp. varied between 0.3–0.7 cm day^{-1} with an average of 0.5 cm day^{-1} for *H. annosum*, and 0.52–0.62 cm day^{-1} with an average of 0.6 cm day^{-1} for *H. parviporum*. Although the mycelium of *Heterobasidion* spp. had a lower growth rate in comparison with *B. adusta* and *P. gigantea*, the difference was not significant ($p > 0.05$).

Table 2. Mycelial growth rate, antagonism against *Heterobasidion* spp. and oidia/conidia production of different *B. adusta*, *S. brinkmannii* and *P. gigantea* isolates

Fungal species	Fungal isolate	Average mycelial growth rate on malt agar media, cm per day (± SD)	Antagonism against *H. annosum*/ *H. parviporum* *	Average number of oidia/ conidia in 1 mL of suspension and SD × 10^6
Bjerkandera adusta	B25	0.77 ± 0.15	++/++	0.17 ± 0.08
	B50	0.72 ± 0.15	–/+	0.16 ± 0.01
	E67	0.74 ± 0.11	–/+	0.36 ± 0.10
	E182	0.88 ± 0.04	+/–	0.20 ± 0.06
	I216	0.76 ± 0.09	–/+	0.26 ± 0.06
	I345	0.84 ± 0.09	–/+	0.16 ± 0.07
	L38A	0.42 ± 0.08	+/+	0.57 ± 0.52
	L38B	0.73 ± 0.17	–/–	0.07 ± 0.01
	M59	0.69 ± 0.25	++/++	0.29 ± 0.33
	M108	0.70 ± 0.17	+–/+–	0.14 ± 0.01
	M111	0.78 ± 0.18	–/+	0.27 ± 0.08
Sistotrema brinkmannii	B5	0.46 ± 0.09	+/+	–
	E136	0.18 ± 0.07	+/+	0.03 ± 0.01
	I390	0.34 ± 0.11	–/–	0.06 ± 0.01
	I409	0.16 ± 0.05	–/–	–
	P31	0.42 ± 0.12	++/++	0.08 ± 0.02
	S73	0.34 ± 0.10	+–/+–	0.06 ± 0.08
Phlebiopsis gigantea	Rotstop®	0.73 ± 0.21	++/++	1.44 ± 0.03
	PG408	0.64 ± 0.13	++/++	1.26 ± 0.03
	PG382	0.70 ± 0.17	++/++	1.05 ± 0.01
	PG182	0.68 ± 0.22	++/++	1.50 ± 0.02
Heterobasidion annosum	HAZ	0.50 ± 0.07	–	–
	HAU	0.50 ± 0.14	–	–
Heterobasidion parviporum	HPN	0.52 ± 0.18	–	–
	HPO	0.62 ± 0.13	–	–

Notes: * ++ strong antagonism; + moderate antagonism; +– sporadic antagonism; – no antagonism.

Antagonistic activity against Heterobasidion spp.

All *P. gigantea* isolates showed high antagonistic activity against both *Heterobasidion* species, whereas the antagonistic activity of *B. adusta* and *S. brinkmannii* varied depending on isolates (Table 2). Three *B. adusta* isolates – B25 (Figure 1), L38A and M59 – showed a distinct antagonism against both *Heterobasidion* species. Similarly, to all the analyzed *P. gigantea* isolates, including that from Rotstop®, the previously mentioned *B. adusta* isolates were able to overgrow *Heterobasidion* spp. mycelia. The other

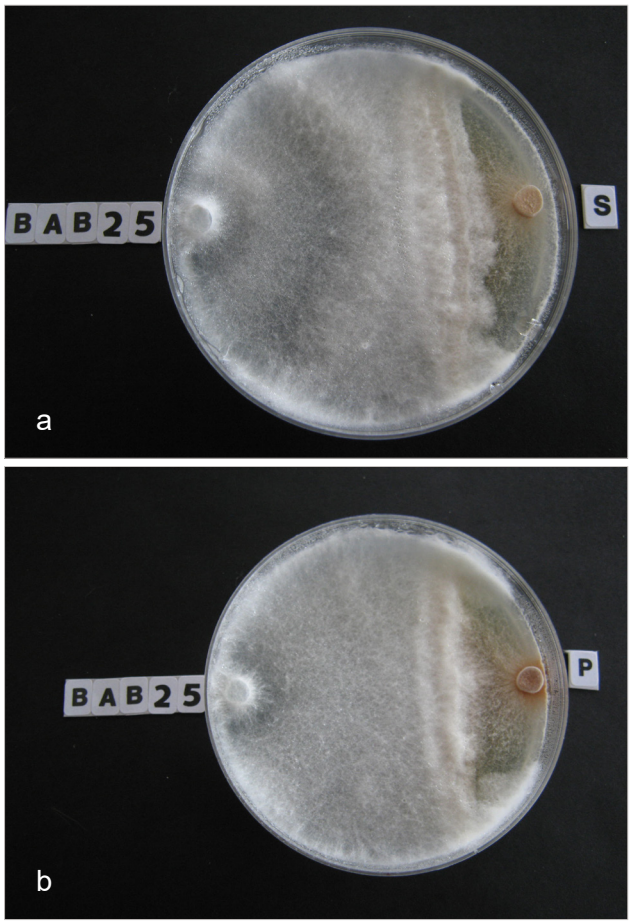

Figure 1. Strong antagonistic reaction of B. adusta isolate B25 (BAB25) against: a) *H. parviporum* (S); b) *H. annosum* (P)

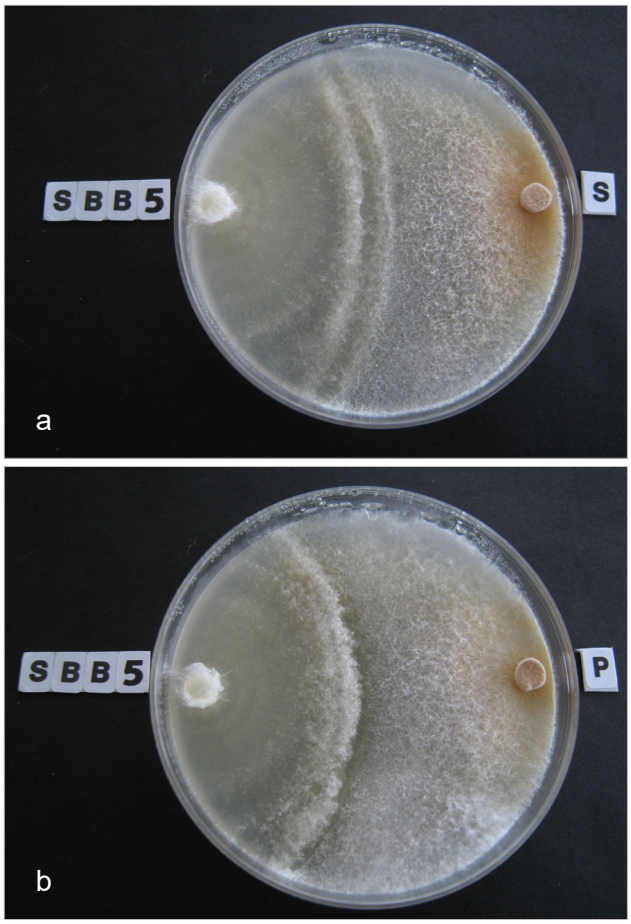

Figure 2. Moderate antagonistic reaction of *S. brinkmannii* isolate B5 (SBB5) against: a) *H. parviporum* (S); b) *H. annosum* (P)

investigated *B. adusta* isolates either showed weak antagonistic activity against some isolates of *Heterobasidion* spp. or did not inhibit the growth of the pathogen at all.

Three *S. brinkmannii* isolates – B5 (Figure 2), E136 and P31 – showed an antagonistic reaction against both *Heterobasidion* species. The remaining *S. brinkmannii* isolates showed either a slight inhibition of the growth of some isolates of *Heterobasidion* spp. or a lack of antagonistic activity. As for *B. adusta*, most of the *S. brinkmannii* isolates had a stronger antagonistic activity against *H. parviporum* than against *H. annosum* isolates.

Oidia/conidia production

The average number of oidia/conidia of *B. adusta*, S. *brinkmannii* and *P. gigantea* is summarized in Table 2.

Among the *B. adusta* isolates, the highest oidia production was observed for isolate L38A (0.57×10^6 oidia per 1 mL suspension), which was characterized by a slow mycelial growth rate. The remaining *B. adusta* isolates had lower oidia production (0.07×10^6–0.36×10^6 oidia per 1 mL suspension). A significant negative correlation was observed between mycelium growth rate of *B. adusta* and oidia production ($r = –0.7$; $p < 0.01$). The highest rate of conidia production of *S. brinkmannii* was observed for isolate P31 (0.08×10^6 conidia per 1 mL suspension); the other isolates had slightly lower conidia production ranging from 0.03 to 0.06×10^6 oidia per 1 mL suspension. Two isolates (B5 and I409) did not produce conidia on agar media. The were no significant correlation observed between mycelium growth rate of *S. brinkmannii* and conidia production ($r = 0.3$; $p > 0.05$).

The number of oidia of *P. gigantea* in 1 mL of suspension was comprised between 1.05×10^6 and 1.50×10^6 depending on isolates. No correlation was observed between the mycelial growth rate and oidia production for *P. gigantea* ($r = 0.1$; $p > 0.05$).

Discussion

The results obtained in this study highlighted that *B. adusta* and *S. brinkmannii* show high intraspecific variability of the parameters investigated under laboratory conditions. *Sistotrema brinkmannii* isolates showed great variation in mycelial growth rate while *B. adusta* displayed a relevant variability in oidia production. Oidia production of *B. adusta* isolates was lower compared to that of *P. gigantea* isolates used as controls. Our results are consistent with those of Holdenrieder (1984), who reported that only

two out of the three tested isolates of *B. adusta* produced oidia and that the oidia production of this species was scarce. However, Holdenrieder (1984) suggested that sporulation of *B. adusta* can be stimulated by changing nutrient content or using UV light.

As previously mentioned, the ability of producing oidia/conidia is an important parameter for screening and assessing fungal isolates as potential biocontrol agents (Pratt et al. 2000, Sun et al. 2009). The isolates of *B. adusta* investigated in this study differed by more than 10 times in terms of ability to produce oidia, and this points to the possibility of identifying the most promising ones for further experiments.

Interestingly, the analyzed *P. gigantea* isolates, including the Rotstop® one, showed small variability in the oidia production although other authors have reported large variability of oidia production for *P. gigantea* isolates (Sun et al. 2009, Zaļuma et al. 2019). Reduced variability among isolates of this species in our study could be related to the low number of *P. gigantea* strains tested (three Latvian isolates and the Rotstop® one).

Bjerkandera adusta isolates showed a growth rate similar to the *P. gigantea* isolates used as controls, on average 0.73 and 0.69 cm per day for the former and the latter species, respectively. The observed growth rate of *P. gigantea* is consistent with that reported in other studies (Zaļuma et al. 2019). A study from Finland reported a negative correlation between mycelial growth rate in wood and oidia production of *P. gigantea* as well as a positive correlation between growth rate of isolates on agar medium and in wood (Sun et al. 2009). While we could not confirm the above correlations for *P. gigantea*, possibly because of the limited number of isolates included in the experiments, our results showed that the growth rate on agar medium is negatively correlated with oidia production for *B. adusta* isolates.

However, the most important parameter to evaluate *in vitro* for the selection of potential BCA against *Heterobasidion* spp. is the performance of candidates in dual culture tests (Pratt et al. 2000). For this purpose, malt agar media and mixtures with sawdust as well as conifer wood blocks or discs are used (Rishbeth 1970, Aufsess 1976a, Lundborg and Unestam 1980, Woods et al. 2005, Drenkhan et al. 2008, Pellicciaro et al. 2021a).

Our results show that all the analyzed *P. gigantea* isolates are strong antagonists against *Heterobasidion* spp. under laboratory conditions, supporting the results of previous experiments (Negrutskii 1986, Vasiliauskas 1989, Hanso and Hanso 1992, Annesi et al. 2005, Drenkhan et al. 2008, Sun et al. 2009, Mgbeahuruike et al. 2011, Zaļuma et al. 2019). Two of the tested *B. adusta* isolates and one *S. brinkmannii* isolate also showed explicit antagonism against both *H. annosum* and *H. parviporum*. In a similar study from Italy evaluating the antagonism of various fungal species against *Heterobasidion* spp. *in vitro* including *P. gigantea* used as a control, the authors concluded that *P. gigantea* performs better than *S. brinkmannii* (Capretti and Mugnai 1989a, b). Antagonism in our study was assessed after 14 days although it was previously reported that fungal antagonism can differ significantly if evaluated after 5 or 30 days (Negrutskii 1986). The temperature of incubation of dual culture plates could also influence the outcomes of the interaction, as noted in previous studies (Capretti and Mugnai 1989a, Hanso and Hanso 1992, Drenkhan et al. 2008, Mgbeahuruike et al. 2011). In our study, plates were incubated at 20°C. *In vitro* studies (Holdenrieder 1984, Capretti and Mugnai 1989a) revealed that some *P. gigantea* isolates in dual cultures display greater antagonistic activity against *Heterobasidion* spp. at 11–12°C than at 20–21°C. This aspect is very important since in Fennoscandia and the Baltic countries active sporulation of *Heterobasidion* spp. has been reported in September–October (Brandtberg 1996, Bruna et al. 2021) but sporulation of *P. gigantea* decreases starting from late summer (Kallio and Hallaksela 1979, Vasiliauskas et al. 2005). Therefore, the possible impact of temperature should be considered when assessing potential BCAs. In contrast to *P. gigantea, S. brinkmannii* antagonism against *Heterobasidion* spp. did not differ at 12 and 20°C (Capretti and Mugnai 1989a). However, it is worth noting that several studies have emphasized that results obtained under laboratory conditions need to be confirmed in field experiments (Holdenrieder and Greig 1998, Mgbeahuruike et al. 2011).

Our findings confirm that *B. adusta* is a very promising antagonist of *Heterobasidion* spp. Some isolates of this species originating from Latvia, i.e. B25 and M59, ranked similarly to *P. gigantea* in terms of oidia production, growth rate and ability to overgrowth the mycelium of both *H. annosum* and *H. parviporum*. These isolates, along with *S. brinkmannii* isolate P31 showing the ability of producing conidia and high antagonistic activity against *Heterobasidion* spp. in dual cultures, will be further tested in conifer wood alone but also in combination with *P. gigantea*. In fact, it was previously demonstrated that mixed suspensions of *P. gigantea* with other wood decay fungi like *Chondrostereum purpureum* may improve the performances of *P. gigantea* against *Heterobasidion* spp. (Hamberg et al. 2012).

Acknowledgements

The study was financially supported by European Regional Development fund project (No 1.1.1.1/20/A/095) and Latvian Forest Research Institute "Silava".

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