

Article

Inferences on the Susceptibility of Wood of Different Tree Species to *Heterobasidion annosum* Sensu Lato Primary Infections and on the Range of Pathogen Spores Dispersal

Lauma Brūna ^{1,*} , Guglielmo Lione ² , Kristīne Kenigšvalde ¹, Natālija Burneviča ¹, Astra Zaļuma ¹, Dārta Kļaviņa ¹ , Tālis Gaitnieks ¹ and Paolo Gonthier ² 

¹ Latvian State Forest Research Institute Silava, 111 Rīgas Street, LV-2169 Salaspils, Latvia; kristine.kenigšvalde@silava.lv (K.K.); natalija.burnevica@silava.lv (N.B.); astrazaluma@silava.lv (A.Z.); darta.klavina@silava.lv (D.K.); talis.gaitnieks@silava.lv (T.G.)

² Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy; guglielmo.lione@unito.it (G.L.); paolo.gonthier@unito.it (P.G.)

* Correspondence: lauma.bruna@silava.lv



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Abstract: Stumps play a pivotal role in the epidemiology of the fungal forest pathogens *Heterobasidion* spp. because they are the main courts of primary airborne infections. The aims of this study were (i) to determine the susceptibility of seven tree species (i.e., *Larix sibirica*, *Picea abies*, *Picea sitchensis*, *Pinus contorta*, *Pinus strobus*, *Pinus sylvestris* and *Pseudotsuga menziesii*) to primary infection by *H. annosum* and *H. parviporum* through comparative inoculation experiments of conidia on wood discs in controlled conditions; (ii) to compare the susceptibility of wood discs of the same tree species to natural airborne infections in two Latvian Norway spruce forest stands infested either by *H. annosum* or *H. parviporum*; (iii) to explore the rates of infection of wood discs at increasing distances from spore sources in these two forests to make inferences on the range of spores dispersal. Results obtained by spraying wood discs with conidial suspensions in controlled conditions are in agreement with those obtained by exposing wood discs to the natural airborne inoculum in the forests, as clearly supported by the significant correlation ($r = 0.79$; $p < 0.05$) between the two sets of data. Susceptibility was highest in *Pinus* species, followed by *P. abies* and *P. sitchensis*. Susceptibility was lowest for *L. sibirica* and *P. menziesii*. The area colonized by *Heterobasidion* spp. in the sapwood of wood discs was much greater than that colonized in the heartwood. A sharp decrease in the rate of infection of wood discs with distance from spore sources (i.e., fruiting bodies) was observed, further confirming the importance of local spore sources in the epidemiology of *Heterobasidion* spp. Taken together, these findings could help designing tactics to manage these fungal forest pathogens.

Keywords: basidiospores; conidia; *Heterobasidion* spp.; spore dispersal; susceptibility; wood discs

1. Introduction

Heterobasidion is a genus of basidiomycetes in the family of Bondarzewiaceae, including several species, two of which, i.e., *H. annosum* (Fr.) Bref. and *H. insulare* (Murrill) Ryvarden, have been reported to be species complexes [1]. While most *Heterobasidion* are saprotrophs, those included in the *H. annosum* species complex, hereafter referred to as *H. annosum* sensu lato (s.l.), are pathogenic and may be particularly damaging, especially in managed coniferous stands [1,2]. Within *H. annosum* s.l., three native species occur in Europe: *H. abietinum*, *H. annosum* sensu stricto (s.s.), hereafter referred to as *H. annosum*, and *H. parviporum*. While the former is found mainly in central and southern Europe in association with its main host, silver fir (*Abies alba* Mill.), *H. annosum* and *H. parviporum* are more widespread throughout Europe, including the Baltic States [2–4]. *Heterobasidion annosum* and *H. parviporum* display different host preferences: *H. parviporum* mainly infects Norway spruce (*Picea abies* (L.) H. Karst.), whereas *H. annosum* is commonly associated

with pines (*Pinus* spp.), especially Scots pine (*Pinus sylvestris* L.), although it can also infect Norway spruce and some deciduous trees [5–7]. In addition, the North American invasive *H. irregulare* has been reported in Italy on Italian stone pine (*Pinus pinea* L.) [8–10].

Heterobasidion annosum s.l. spreads by means of spores infecting either freshly cut stump surfaces or trees through tree wounds [1]. Once the fungus has colonized the root systems of stumps or trees, it can spread to neighboring healthy trees by the propagation of the mycelium, provided that root contacts are present [1]. Infection through spores and by the propagation of the mycelium are known as primary and secondary infections, respectively. Primary infection is mainly caused by basidiospores (sexual spores), which are produced in large amounts by fruiting bodies. The fungus also produces conidia (asexual spores), although the role of conidia in the infection process in nature seems to be negligible [11].

The susceptibility of different tree species to species of *H. annosum* s.l. has been extensively analyzed by determining the disease incidence in secondarily infected trees in naturally infested stands [12–17]. On the contrary, the susceptibility of stumps of different species to primary infection by *H. annosum* s.l. has received much less attention, and only a few studies have been conducted on this topic. In the Alps, Norway spruce was reported as significantly more susceptible to airborne stump infection by *H. annosum* s.l. than European larch (*Larix decidua* Mill.), silver fir and Scots pine, in that order [18]. Studies carried out in Latvia also showed that Norway spruce stumps are more susceptible to *H. annosum* s.l. airborne infection compared to Scots pine stumps [19].

As previously postulated [18], the comparison of levels of susceptibility of stumps of different tree species to primary infection by *H. annosum* s.l. is complicated by the fact that such analyses would require stumps of different species to co-occur in the same forests, which is not always the case. However, referring to *H. annosum* s.l., epidemiological traits related to spore release and deposition have been successfully revealed by exposing in the forest wood discs of conifers simulating stumps [17,18,20–26]. For those purposes, wood discs of Norway spruce, less often Scots pine, silver fir and larch have been employed. In addition, wood discs of Norway spruce, Italian stone pine and silver fir have been recently and successfully used to test the efficacy of biological and chemical treatments against species of *H. annosum* s.l. [27]. Only a few studies explored the susceptibility to artificial or natural spore infection by *H. annosum* s.l. of wood discs of different tree species at the same time. Redfern [28] analyzed the germination of *H. annosum* basidiospores inoculated on stem sections of Scots pine, lodgepole pine (*Pinus contorta* Douglas) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.), whereas Thomsen and Jacobsen [29] investigated the growth of formerly inoculated *H. annosum* mycelium in discs of Norway spruce, Scots pine, hybrid larch (*Larix × eurolepis* Henry), Sitka spruce, and Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco). Dimitri et al. [30] determined the number of *H. annosum* s.l. spores deposited on *P. abies* and *P. sylvestris* wood discs exposed in Norway spruce stands, while Wang et al. [26] made the same by exposing wood discs of *Larix × eurolepis*, *P. abies* and *P. sylvestris* in hybrid larch stands. To date, no studies have compared the levels of susceptibility of wood discs of different tree species to the infection by spores or conidia of *H. annosum* s.l. using wood discs originating from the same trees.

All the analyses on the susceptibility of stumps to airborne infections by *H. annosum* s.l. have been made by considering the species complex level; therefore, there is little knowledge on whether the susceptibility of stumps of different tree species may differ depending on the *Heterobasidion* species involved, as previously postulated [18]. The assessment of the risk of primary infection by *H. annosum* s.l. would be particularly important to predict the likelihood of establishment of the pathogen but also to modulate the intensity of thinnings on a given tree species in order to minimize the risk of outbreaks.

In Latvia, forests occupy 52% of the country's territory, and the area covered by Norway spruce and Scots pine is 46% of the total forest area [31], which underlines the importance of these dominant tree species in Latvian forestry. However, in the context of climate change, the proportion of other non-native conifer tree species may increase [32], re-

quiring investigation of their susceptibility to native *H. annosum* s.l. species, i.e., *H. annosum* and *H. parviporum*.

Primary infection by *H. annosum* s.l. on stumps depends on the levels of spore loads, which have been reported to vary depending on the season and on the distance from spore sources (i.e., fruiting bodies) [1]. Studies conducted in Sweden showed that spore deposition rate and the risk of stump infection reach a maximum in May to August [33], while in the Alps, spore deposition rate and risk of stump infection peak at the end of summer and autumn [18,24]. Most of *H. annosum* s.l. spores land within a few meters from fruiting bodies, and only a minority can travel 100 m and further [34,35]. Other studies indicate that a considerable amount of spores can spread 100 m from spore sources [21,36]. Most of the information on the range of spore dispersal of *H. annosum* s.l. refers to Norway spruce stands of Northern Europe [21,25] or to mixed oak–Italian stone pine stands of the Mediterranean Region [36]. There is a complete lack of knowledge on the levels of spore loads and on the range of spore dispersal in Norway spruce stands of the Baltic Region.

The aims of this work were (i) to determine the susceptibility of seven tree species to primary infection by *H. annosum* and *H. parviporum* through comparative inoculation experiments of conidia on wood discs in controlled conditions; (ii) to compare the susceptibility of wood discs of the same tree species to natural airborne infection in two Latvian Norway spruce forest stands infested either by *H. annosum* or *H. parviporum*; (iii) to explore the rates of infection of wood discs at increasing distances from spore sources in these two forests to make inferences on the range of spore dispersal.

2. Materials and Methods

2.1. Comparative Susceptibility of Wood Discs of Seven Coniferous Tree Species to Inoculation with *H. annosum* and *H. parviporum* Conidial Suspensions in Controlled Conditions

Two healthy looking trees with no signs of root rot or other diseases were selected in forest stands of central and eastern Latvia for each of the following tree species: Siberian larch (*Larix sibirica* Ledeb.), *Picea abies*, *Picea sitchensis*, *Pinus contorta*, Weymouth pine (*Pinus strobus* L.), *Pinus sylvestris* and *Pseudotsuga menziesii* [32]. All selected trees were without visible decay at stump level. Moreover, as *Heterobasidion* mycelium can be found 30–60 cm in advance of the discoloration [37,38], all discs used in the experiment were cut at least 1 m upward the stem basis. The age of trees was between 23 and 44 years. Wood discs were prepared in an area distant from forests by cutting the basal portion of stems (up to 2 m height from the root collar) into slices of 2–3 cm thick. Wood discs, 10.7–16.5 cm in diameter, were kept at -5°C until the day prior to the experiment when they were thawed at room temperature.

Conidial suspensions for the experiment were obtained by mixing conidia of three isolates, each of *H. annosum* (No. 93186, No. 98040, No. 03058, Finland) and *H. parviporum* (No. 98036, No. 03129, No. 05029, Finland). All isolates were cultured in Petri dishes on malt extract agar medium for 3 weeks at 20°C . Conidial suspensions for each *Heterobasidion* species were prepared in 0.5 L of tap water by washing the conidia several times from three Petri dishes. The number of conidia in the suspension was calculated by counting conidia within 30 sight fields per Petri dish in Hagem agar medium in two repetitions under a microscope (magnification $100\times$) [39]. Conidial concentration in the suspensions was 42.16×10^3 conidia/mL of water for *H. annosum* and 45.83×10^3 conidia/mL of water for *H. parviporum*.

The experiment was performed on 26 August 2008. The surface of each wood disc was divided into four sectors. Sectors were numbered clockwise (No. 1–4), and sector No. 1 and sector No. 3 (opposite sectors) were treated with conidial suspensions of either *H. annosum* or *H. parviporum* (Figure 1) by using a sprayer. The other sectors were covered with a sheet of paper during treatment. Three wood discs for each tree species were treated with conidial suspensions of both fungal pathogens.

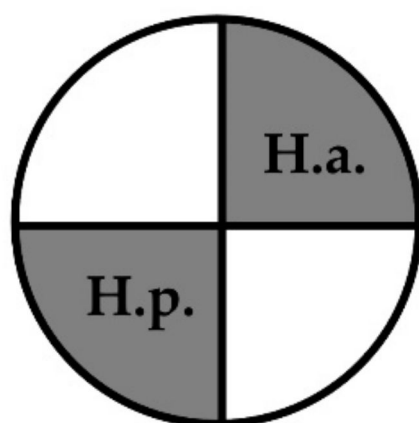


Figure 1. Spraying scheme of wood discs. Abbreviations: H.a.—area treated with *Heterobasidion annosum* conidial suspension; H.p.—area treated with *H. parviporum* conidial suspension.

After treatments, each disc was placed individually in a loosely closed plastic bag and incubated for 7 days in the dark at room temperature. After incubation, discs were inspected for the presence of typical conidiophores of *Heterobasidion* spp. by using a dissecting microscope with 20–30 magnification, as previously described [24]. The area colonized by *Heterobasidion* spp. was marked. Afterwards, discs were scanned, and areas were measured using a planimeter (PLANIX 10S ‘Marble’, Tamaya, Japan). Borders between the sapwood and heartwood in each disc were visually determined by the same person.

2.2. Comparative Susceptibility of Wood Discs of Seven Coniferous Tree Species to *Heterobasidion* spp. Natural Airborne Infections and Rates of Infection at Increasing Distance from Spore Sources

2.2.1. Study Areas and Characterization of *Heterobasidion annosum* s.l. Disease Centres

Experiments were established in two sample plots: Kalsnava and Tireli. The Kalsnava plot was located in the eastern part of Latvia (56°41′9″ N, 25°55′40″ E). It represented a 76-year-old Norway spruce stand on drained peat soil (forest type *Oxalidosa* turf. mel.) with an admixture of Scots pine, silver birch (*Betula pendula* Roth) and black alder (*Alnus glutinosa* (L.) Gaertn.). The Tireli sample plot (56°50′35″ N, 23°47′51″ E) was located in a 30-year-old Norway spruce stand with an admixture of *P. sylvestris* on drained mineral soil (forest type *Oxalidosa*).

The Kalsnava sample plot was heavily infested by *H. annosum* s.l. At the center of the sample plot, in a radius of four meters, there was a windthrown Norway spruce tree with *H. annosum* s.l. fruiting bodies with a total area of hymenophores of 1431 cm², two infected Norway spruce trees with fruiting bodies on the upper roots and a partly uplifted birch stump with *H. annosum* s.l. fruiting bodies under the roots. Some fruiting bodies were found at a distance of 20–40 m from the infection center. The total area of hymenophores of *H. annosum* s.l. fruiting bodies found in the Kalsnava sample plot was 2048 cm². To identify the fungus at the species level, wood samples were taken with a sterilized increment borer or an axe from the windthrown Norway spruce, birch stump and from the roots of the infected Norway spruce.

In the Tireli sample plot, the *H. annosum* s.l. infection center was a gap of 8 m radius containing six Scots pine stumps. At the margin of the gap, there were five Scots pines with symptoms of *H. annosum* s.l. infection (thin crown). At the center of the gap, in a 3 m radius, four Scots pine stumps were located, two of them partly uplifted, with *H. annosum* s.l. fruiting bodies on the roots showing hymenophores with a total area of 485 cm². Wood samples were collected from these two Scots pine stumps according to the method described above for the Kalsnava sample plot.

Isolations from wood samples were made according to Arhipova et al. [40]. Isolates were identified at the species level by assessing their ability to heterokaryotize the homokaryotic tester strains No. 05247 (*H. annosum*) and 05146 (*H. parviporum*) [41], kindly provided by Kari Korhonen. All isolates obtained from Tireli belonged to *H. annosum*, and all those obtained from Kalsnava belonged to *H. parviporum*.

2.2.2. Samplings to Determine the Susceptibility of Wood Discs to *Heterobasidion annosum* s.l. Natural Airborne Infections and the Rates of Spore Deposition at Increasing Distance from Spore Sources

In the Kalsnava sample plot, *H. annosum* s.l. spore deposition on wood discs was assessed along two transects in the four directions: north (N), south (S), east (E) and west (W) (Figure 2). The cross point of transects was established one meter from the largest *Heterobasidion* fruiting body group. The Tireli sample plot was located 20 m from a forest road, and therefore, spore deposition on wood discs was assessed along a single transect oriented south-east (SE) and north-west (NW) (Figure 2).

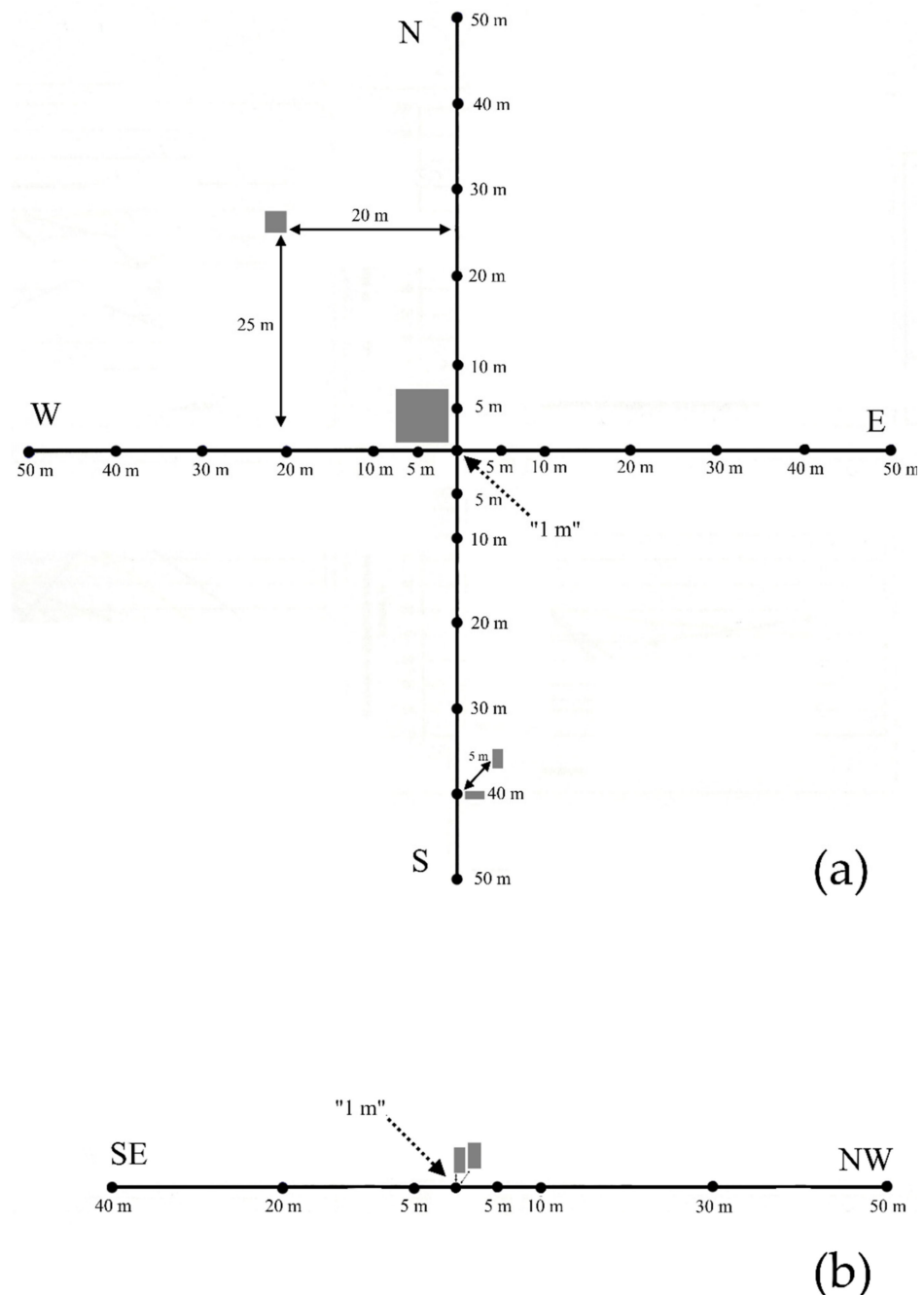


Figure 2. Location of sampling points and groups of *Heterobasidion annosum* s.l. fruiting bodies: (a) the Kalsnava sample plot and (b) the Tireli sample plot). Relative area of *H. annosum* s.l. fruiting body hymenophores; dashed line with arrow shows the cross point of transects located 1 m from the largest group of *H. annosum* s.l. fruiting bodies.

Four and two sampling campaigns were conducted at different time periods in Kalsnava and Tireli sample plots, respectively (Table 1). Ten wood discs prepared as described above were placed in each sampling period at each sampling point (two discs of *P. sitchensis*, two discs of *P. menziesii*, one disc of *P. abies*, one disc of *P. sylvestris*, two discs of *P. contorta*, one disc of *P. strobus* and one disc of *L. sibirica*) as summarized in Figure 2. Two discs of some species instead of one were used because of their smaller diameter. In total, 520 discs were exposed in the Kalsnava sample plot and 159 discs in the Tireli sample plot. At all sampling points, discs were placed on transparent plastic sheets during exposition to avoid any contamination originating from the ground. In all experiments, wood discs were exposed for approximately 17 h (4 p.m.–9 a.m.) (Table 1).

Table 1. Main features and experimental conditions of sampling campaigns of airborne *Heterobasidion annosum* s.l. spores in Kalsnava and Tireli.

Sample Plot	Date of Experiment in 2008	Analyzed Directions	Distance from the Group of Fruit Bodies, m	Air Temperature, °C	Air Humidity, %	Speed (m/s) and Direction of Wind
Kalsnava	15.07–16.07	N, S, E, W	1 *, 5, 10, 20, 30, 40, 50	16	80	1–3, SW
	03.08–04.08	N, S, E, W	1, 10, 30	18	80	1–4, SW, W
	11.08–12.08	N, S, E, W	1, 10, 30	17	89	1–3, S, SW
	21.08–22.08	N, S, E, W	1, 20, 40	15	89	3–4, SW
Tireli	30.08–31.08	NW SE	1, 5, 10, 30 10, 20, 40	12	86, heavy rain	1–4, NW
	11.09–12.09	NW SE	1, 5, 10, 30 10, 20, 40	8	72	3–4, NE

*: the transect cross point at the distance of 1 m from the largest *H. annosum* s.l. fruiting body group.

After exposure, each disc was placed individually in a plastic bag and brought to the laboratory. Discs were incubated for 7 days in loosely closed plastic bags at room temperature. Discs were inspected by using a dissecting microscope for the presence of *H. annosum* s.l. as described above.

Additionally, in order to explore the levels of spore deposition at increasing distance from spore sources in a way unbiased by the woody substrate, in the Kalsnava sample plot, a Petri dish filled with malt extract agar was placed directly under the largest *H. annosum* s.l. fruiting body so that the margin of the Petri dish gently touched its hymenophore. Additional Petri dishes were placed on the ground at 1 m distance from the largest *H. annosum* s.l. fruiting body group and in four directions (N, S, E, W) at a distance of 10 m and 30 m. Petri dishes were exposed during the daytime on 7 and 14 October 2008 for 1, 2, 4 and 8 min. Air temperature during exposure in these two days was 8 °C and 10 °C, respectively. The following day, spore counts were performed using light microscopy in 30 random sight fields in the Petri dish on Hagem agar medium.

2.3. Calculations and Statistics

In the experiment carried out under controlled conditions, the area colonized by either *H. annosum* or *H. parviporum* on the total surface of discs sprayed with conidial suspensions of either species (%) was determined. The sapwood area was calculated by subtraction of the heartwood area from the total area of the disc. Areas colonized by *Heterobasidion* spp. in sapwood and heartwood of wood discs (treated sectors) of different tree species were compared using non-parametric Mann–Whitney tests.

In the experiments carried out in the field, infection frequency expressed as the percentage of infected discs on the total number of discs exposed was compared between tree species in each sample plot using a chi-square test. The mean area occupied by *H. annosum* s.l. (percentage of the total area of the disc) in discs of each tree species, hereafter referred to as infection rate, was calculated for all discs in each sample plot (Kalsnava and Tireli). The correlation between the mean area occupied by *Heterobasidion* in

different tree species and distances from the largest *H. annosum* s.l. fruiting body group was explored using Spearman's rank-order correlation test.

The correlation between the mean proportion of area occupied by *H. annosum* s.l. on wood discs of different tree species after treating discs with conidial suspensions (data from *H. annosum* and *H. parviporum* pooled together) and by exposing discs in the field (data from Kalsnava and Tireli pooled together) was determined by using Spearman's rank-order correlation test.

H. annosum s.l. basidiospores deposition in Petri dishes was calculated taking into account the number of spores, the area of sight field, the area of the Petri dish and the exposure time. Spore deposition was expressed per m² in one hour (h). Data analyses were performed by using R 3.6.1 [42].

3. Results

3.1. Comparative Susceptibility of Wood Discs of Seven Coniferous Tree Species to *H. annosum* and *H. parviporum* Conidial Suspensions in Controlled Conditions

Heterobasidion annosum colonized a larger area than *H. parviporum* on wood discs of all the tested tree species except *P. contorta*, although differences were significant only for wood discs of *P. abies* ($p < 0.05$). The area occupied by *H. parviporum* was significantly larger in *P. contorta* compared to *P. sitchensis* and *L. sibirica* ($p < 0.05$) (Figure 3). The area occupied by *H. parviporum* in *P. menziesii* was significantly smaller compared to *P. contorta*, *P. sylvestris*, *P. abies* and *P. strobus* ($p < 0.05$).

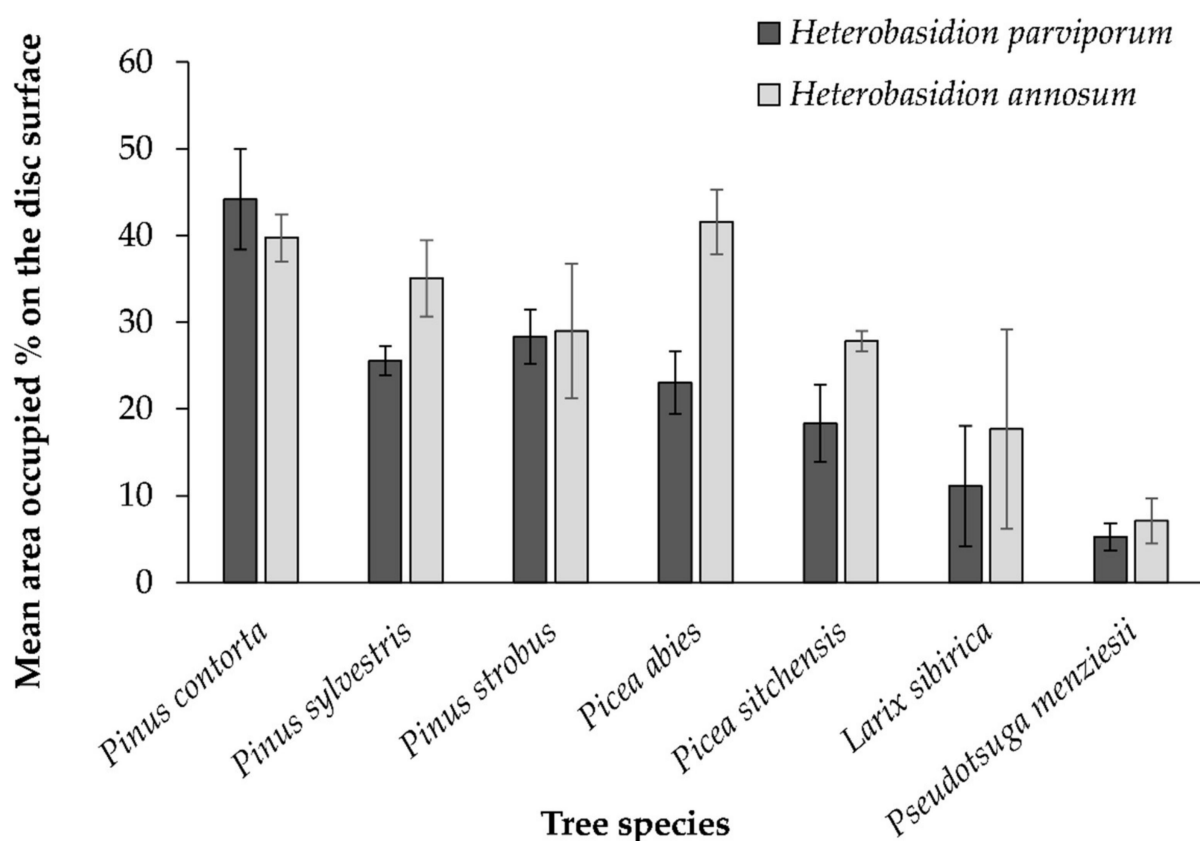


Figure 3. The mean area occupied by *Heterobasidion annosum* and *H. parviporum* on wood discs of seven coniferous tree species inoculated with conidial suspensions.

The area occupied by *H. annosum* in *P. sitchensis* was significantly smaller compared to that in *P. contorta* ($p < 0.05$). The area occupied by *H. annosum* in *P. menziesii* was significantly smaller than that in *P. contorta*, *P. sylvestris*, *P. abies* and *P. sitchensis* ($p < 0.05$).

The overall areas colonized by either *H. annosum* or *H. parviporum* on wood discs of different tree species were correlated ($r = 0.75$, $p = 0.052$). In the discs of all the tree species and for both *Heterobasidion* species, the area colonized by the fungi in sapwood was much greater than that colonized in heartwood (Table 2).

Table 2. The percentage of the area occupied by *Heterobasidion annosum* and *H. parviporum* in the sapwood and heartwood of the discs of seven tree species.

		Mean Area (%) Occupied by <i>Heterobasidion</i> spp.						
		<i>Larix sibirica</i>	<i>Picea abies</i>	<i>Picea sitchensis</i>	<i>Pinus contorta</i>	<i>Pinus strobus</i>	<i>Pinus sylvestris</i>	<i>Pseudotsuga menziesii</i>
<i>H. parviporum</i>	Sapwood	24.9	43.4	25.8	48.4	71.5	67.8	11.0
	Heartwood	0.1	2.1	4.7	0.0	0.2	0.0	0.1
<i>H. annosum</i>	Sapwood	40.8	71.4	40.6	44.4	68.0	80.6	18.0
	Heartwood	0.5	6.3	3.0	0.5	1.8	0.01	0.1

3.2. Comparative Susceptibility of Wood Discs of Seven Coniferous Tree Species to *H. annosum* s.l. Natural Airborne Infections

At Kalsnava, more discs were infected in the first and second experiment compared to the third and fourth experiment: 97% and 98% compared to 77% and 84%, respectively. Most frequently, *P. strobus*, *P. sylvestris* and *P. abies* discs were infected: 100%, 96% and 89%, respectively. The proportion of infected *L. sibirica*, *P. contorta*, *P. menziesii* and *P. sitchensis* discs was comprised between 83% and 87% (Table 3).

Table 3. The *Heterobasidion annosum* s.l. infection frequency (%) of wood discs of different tree species in the Kalsnava and Tireli study sites.

Tree Species	Kalsnava					Tireli		
	1 *	2	3	4	Average	1	2	Average
<i>Larix sibirica</i>	96	100	67	67	83	88	50	69
<i>Picea abies</i>	100	100	67	89	89	88	88	88
<i>Picea sitchensis</i>	98	100	72	78	87	100	69	85
<i>Pinus contorta</i>	92	100	78	72	86	94	69	82
<i>Pinus strobus</i>	100	100	100	100	100	75	100	88
<i>Pinus sylvestris</i>	96	100	89	100	96	100	100	100
<i>Pseudotsuga menziesii</i>	100	89	67	83	85	69	50	60
Average	97	98	77	84	89	88	75	82

*: Experiment number 1 to 4.

At Tireli, the average infection frequency of wood discs was 88% and 75% in the first and second experiment, respectively. The wood discs of *P. sylvestris* were the most frequently infected (100%), whereas only 60% of wood discs of *P. menziesii* and 69% of those of *L. sibirica* were infected by *H. annosum* s.l. The frequency of infected *P. abies*, *P. sitchensis*, *P. contorta* and *P. strobus* wood discs was comprised between 82% and 88% (Table 3).

The area occupied by *H. annosum* s.l. on discs exposed in the Kalsnava sample plot was significantly greater ($p < 0.05$) than that on discs exposed in the Tireli sample plot for all tree species (Figure 4). In both Kalsnava and Tireli, the mean area occupied by *H. annosum* s.l. on wood discs was greater for *P. contorta* and *P. sylvestris* and lower for *P. menziesii*.

At Kalsnava, the area occupied by *H. annosum* s.l. in wood discs of *P. sylvestris* was significantly larger compared to that occupied in wood discs of *P. abies*, *L. sibirica* and *P. menziesii* ($p < 0.05$). The area occupied by *H. annosum* s.l. in wood discs of *P. menziesii* was significantly smaller compared to that in wood discs of *P. strobus* and *P. contorta* ($p < 0.05$). The area occupied by *H. annosum* s.l. in heartwood was small—the average for the analyzed tree species ranged from 0.01% to 0.55%, and there were no significant differences between tree species ($p > 0.05$).

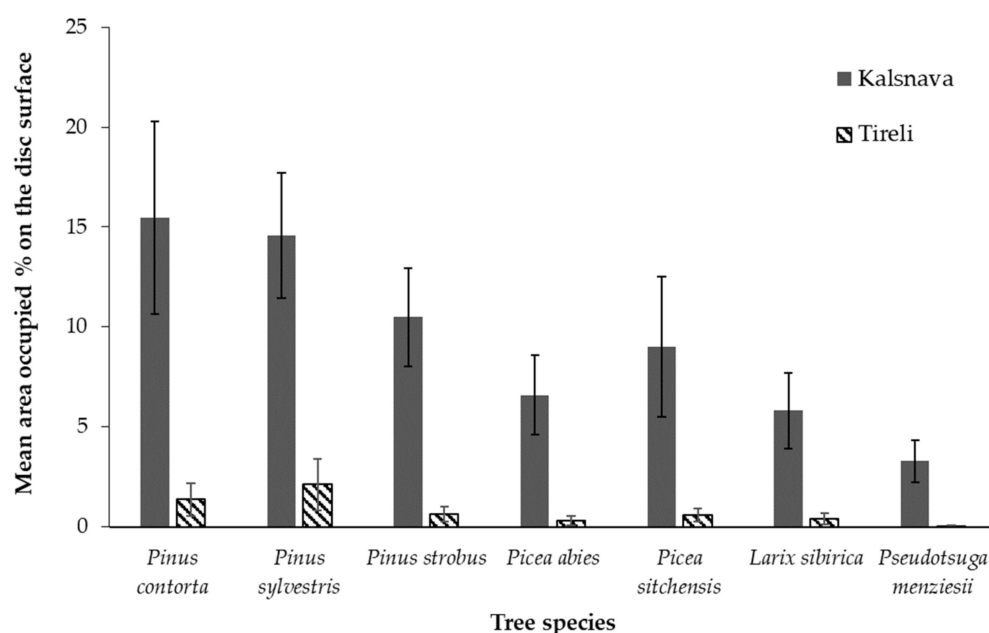


Figure 4. The mean area occupied by *Heterobasidion annosum* s.l. on wood discs of different tree species exposed in the forest plots of Kalsnava and Tireli.

At Tireli, the largest area occupied by *H. annosum* s.l. was in discs of *P. sylvestris* and *P. contorta*: 2.12% and 1.38% of the total surface of discs, respectively. The smallest occupied area was for *P. menziesii*—0.05%. The mean area occupied by *H. annosum* s.l. in discs of the other tree species ranged from 0.31% to 0.65%. At Tireli, no *H. annosum* s.l. infection was detected in the heartwood of any wood disc.

A positive and significant correlation was found between areas colonized by *H. annosum* s.l. on wood discs of different tree species in Kalsnava and Tireli sample plots ($r = 0.90$, $p < 0.05$). A positive and significant correlation was also found between the mean area occupied by *H. annosum* s.l. on discs treated with conidial suspensions in controlled conditions and on discs exposed in the forest plots of Kalsnava and Tireli ($r = 0.79$; $p < 0.05$).

3.3. Rates of Infection at Increasing Distance from Spore Sources in the Field

By analyzing the rates of infection of wood discs at different distances from the largest *H. annosum* s.l. fruiting body group at Kalsnava, a rapid decrease in the rate of infection of wood discs with distance was observed for most of the analyzed tree species and as an average (Figure 5). In the W direction, *P. sylvestris* and *P. contorta* showed higher rates of infection at a distance of 20 m compared to the other tree species. At 40 m distance in the S direction, a peak in rates of infection was observed for all tree species.

In Tireli, the highest infection rate in the first experiment was observed in the NW direction 5 m far from *H. annosum* s.l. fruiting bodies. In the second experiment, the highest infection rate was also observed in the NW direction at a distance of 1 m from *H. annosum* s.l. fruit bodies, and at a distance of 5 m. The infection rate decreased rapidly with distance from *H. annosum* s.l. fruiting bodies.

The levels of spore deposition under fruiting bodies of *H. parviporum* in Kalsnava determined by using Petri dishes was 87.17×10^6 spores per m^2h^{-1} on 7 October and 12.85×10^6 spores per m^2h^{-1} on 14 October (average 50.01×10^6 spores m^2h^{-1}). On 7 October, spore deposition attained more than one million spores per m^2h^{-1} at a distance of 1 m from spore sources, and 82,250 and 61,688 spores m^2h^{-1} at distances of 10 and 30 m from spore sources, respectively. On 14 October, spore deposition was more than 400,000 spores m^2h^{-1} at a 1-m distance from spore sources and varied between 4113 and 41,125 spores m^2h^{-1} at a distance of 10 m from the spore sources. At a distance of 30 m from spore sources, no spores were detected in any direction on 14 October.

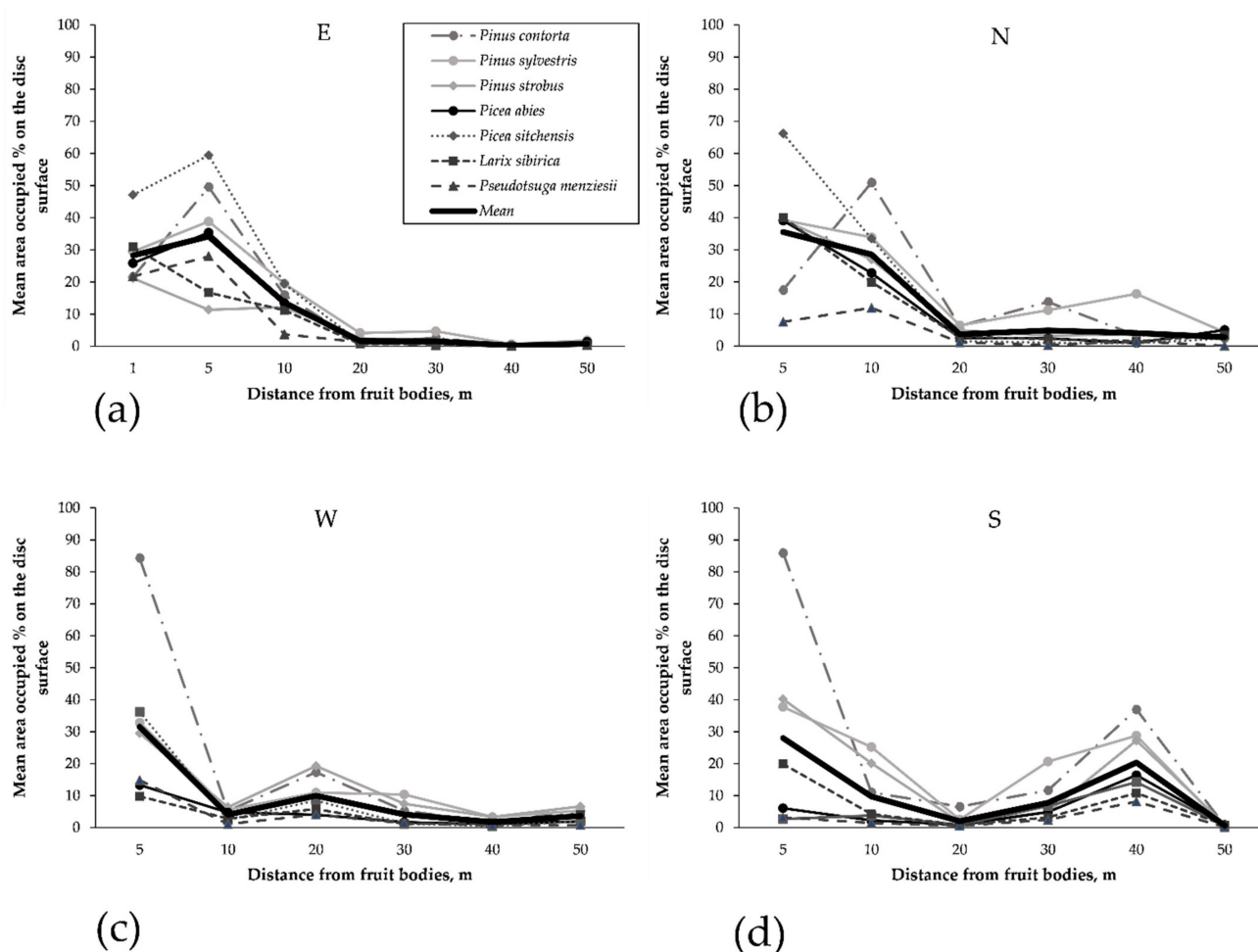


Figure 5. The rates of infection of wood discs of seven coniferous tree species by *Heterobasidion annosum* s.l. in the Kalsnava sample plot at increasing distances from spore sources. Disc exposition in different directions—(a) east, (b) north, (c) west and (d) south; only in figure (a) the area occupied by *Heterobasidion annosum* s.l. at a distance “1 m” from the largest group of fruiting bodies is shown; the same numbers refer to other directions.

4. Discussion

Our data shows variation in susceptibility of wood discs of different tree species to primary infection by *H. annosum* s.l., and, in this regard, results obtained by exposing discs to airborne infections in the forest are fully consistent with those of artificial inoculations in controlled conditions using conidial suspensions.

In comparative inoculation experiments on wood discs in controlled conditions using conidial suspensions, larger surfaces were colonized by *H. annosum* s.l. on wood discs of *P. sylvestris*, *P. strobus* and *P. abies*, and smaller in wood discs of *P. menziesii*. These findings support previous reports by Thomsen and Jacobsen [29], who observed higher growth rate of the fungus following artificial inoculation on wood discs of Scots pine and Norway spruce, followed by Douglas fir and hybrid larch. Nevertheless, in that study, the slowest growth rate was observed on Sitka spruce. Interestingly, on wood discs of Norway spruce, a significantly larger area was occupied by *H. annosum* compared to *H. parviporum*, despite many studies suggested that *H. parviporum* is better adapted to Norway spruce wood than *H. annosum* [43–46]. However, it should be noted that the development of *Heterobasidion* spp. on wood may vary depending on whether trees are alive or dead. In an experiment conducted in Latvia by inoculating Norway spruce billets with the same two *Heterobasidion* species [32], a slower growth rate was found for *H. parviporum* compared to *H. annosum*. In addition, a higher rate of isolation of *H. annosum* compared to *H. parviporum*

was observed following primary infection on Norway spruce stumps in subalpine stands heavily infested by *H. parviporum* [47], supporting the hypothesis that *H. annosum* may be a better saprobe than *H. parviporum*, as previously documented on the wood blocks in laboratory conditions [48].

When exposing wood discs in the forest at Kalsnava and Tireli to *H. annosum* s.l. natural airborne infections, the fungus colonized the largest areas on wood discs of pine species, followed by *P. sitchensis*, *P. abies* and *L. sibirica*. Again, the smallest areas colonized by the fungus were found on wood discs of *P. menziesii*. Thus, our findings are in agreement with data obtained by Dimitri et al. [30] and Wang et al. [26], who reported higher susceptibility to *H. annosum* s.l. airborne infections of pine discs compared to Norway spruce discs. However, this is not consistent with the results of studies conducted in the Alps reporting higher infection frequencies in Norway spruce stumps compared to pine stumps [18]. More infected Norway spruce stumps compared to pine stumps have also been found in mixed spruce–pine stands in Latvia [19], as well as in small diameter stumps after pre-commercial thinning [49,50]. The above inconsistencies could be related to the substrate that was analyzed—discs vs. stumps. Discs are more prone to drying, and this may have accounted for the inconsistencies. In addition, the discs analyzed in this study had different diameters, and therefore, they could have been subjected to different degrees of drying.

In our study, the area occupied by *H. annosum* s.l. on wood discs of *P. contorta* was larger compared to that occupied by the fungus on wood discs of *P. sitchensis*, which is consistent with data provided by Redfern [28] using stumps as a substrate. At the same time, our results pointing to a lower susceptibility of wood discs of Douglas fir to primary infections by *H. annosum* s.l. corroborate previous observations by Morrison and Johnson [51], who found less infection of Douglas fir stumps compared to Sitka spruce stumps after precommercial thinning.

Rönnerberg et al. [52] concluded that in pine stumps *H. annosum* s.l. spore infection was mainly found in sapwood, whereas Oliva et al. [53] indicated that on Norway spruce stumps, the fungus develops both in sapwood and heartwood. In our study, wood discs of all coniferous tree species analyzed showed very little heartwood infection. Referring to *P. sylvestris* and *P. abies*, our observations are in agreement with those of other studies showing that *H. annosum* s.l. spore infection mainly occurs in sapwood [52,54–58]. Conversely, other studies documented higher infection of heartwood compared to sapwood in Sitka spruce [59,60]. This might be due to the different methodology we used, particularly the storage of the discs at a temperature below 0 °C. Although this methodology (even using wood discs 6–9 cm in diameter) had already been used in other studies [21,25], it is possible that the conditions during storage could have modified the properties of heartwood in some tree species.

The results obtained by spraying wood discs with conidial suspensions in controlled conditions are in good agreement with those obtained by exposing wood discs to the natural airborne inoculum in the forests, as clearly supported by the significant correlations between the two sets of data. In both cases, these data suggest higher susceptibility to primary infections by *H. annosum* s.l. for *P. contorta* and other *Pinus* and *Picea* species, followed by *L. sibirica*, and lower susceptibility for *P. menziesii*.

The results obtained in this study, both through the comparative inoculation experiments of conidia on wood discs in controlled conditions and by exposing wood discs to *H. annosum* s.l. natural airborne infections in the two Norway spruce stands are in agreement with the results obtained by Zaluma et al. [32], who investigated the development of *H. annosum* s.l. mycelia in the wood of different tree species. In this latter study, a relevant *H. annosum* s.l. mycelium development deep in the wood was found with *P. sylvestris*, *P. strobus* and *P. abies*, an intermediate with *P. contorta* and *P. sitchensis*, and a limited development with *L. sibirica* and *P. menziensisii*. It should be noted that the similarity of results between our study and the study by Zaluma et al. [32], who also used conidial suspensions of both *H. annosum* and *H. parviporum*, was more pronounced for the

inoculation experiment with conidia of *H. parviporum* in controlled conditions and for discs exposed in the Kalsnava plot where, incidentally, all fruiting bodies sampled were identified as *H. parviporum*. Taken together, these findings suggest that it could be desirable to test the susceptibility of different *Larix* species to *H. annosum* s.l. in Latvian conditions. *Larix sibirica* is of particular interest because of a relatively limited development of *H. annosum* s.l. mycelia were observed in the wood of this tree species but, in contrast to *P. menziesii*, the development of *Phlebiopsis gigantea*—a well-known and effective antagonistic fungus against *H. annosum* s.l.—has been reported to occur in the wood of *L. sibirica* [32]. Other studies have also reported *Larix* spp. to be less susceptible to *H. annosum* s.l. compared to Norway spruce [17,18,61].

In our study, the highest rate of infection of most wood discs was identified at a distance of 1 m from the infection center, but at 5 and 10 m, the wood discs' infection rates significantly decreased. This finding supports the results of investigations conducted in Fennoscandia [34,35], indicating that the maximum number of spores is released up to 10 m from the fruiting bodies. The exception to the above gradient in our study was the southern direction, where a significant increase in the infection rate was observed at a distance of 40 m. However, it should be noted that after additional surveys, initially undetected *H. annosum* s.l. fruiting bodies were found on rotten Norway spruce root fragments under the moss close to the points where discs were exposed. This confirms the importance of local spore sources in the prevalence of *H. annosum* s.l. infection in forest stands, as pointed out by several studies [20,23,35,36,62–64].

In this study, the spore load of *H. annosum* s.l. in a heavily infested Norway spruce stand was also analyzed using Petri dishes with agar medium. The number of spores observed in our study under the *H. parviporum* fruiting body was lower than that reported by Möykkynen et al. [21]. However, it should be noted that in this latter study conducted in Finland, the number of spores below the fruiting body was analyzed in June/August, at the peak of *H. annosum* s.l. sporulation in northern Europe [33], while in our study, samplings were conducted in October/November when sporulation is expected to be lower. In our study, the number of spores detected per m² in different directions at a distance of 10–30 m from the largest group of fruiting bodies varied widely from 0 to 131600 spores. Other studies have also reported a relatively large variation in spores' deposition levels in Norway spruce stands: 0–447 spores m² h^{−1} [25] and 40–1500 spores m² h^{−1} [30].

In conclusion, this study provides evidence that woods of different tree species differ in their susceptibility to primary infection by both *H. annosum* and *H. parviporum*. This study also supports the notion of a limited dispersal range of *H. annosum* s.l. basidiospores, pointing to the importance of local spore sources in the epidemiology of these pathogens.

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