



# Article Survey Study Reveals High Prevalence of Heterobasidion Root Rot Infection in Scots Pine (*Pinus sylvestris*) Stands Established on Seemingly Low-Risk Sites

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**Abstract:** *Heterobasidion* spp. are among the most destructive root rot pathogens, causing severe economic losses to conifer forestry. High infection frequency has been observed in Scots pine stands growing on dry sandy soils with low organic matter or former agricultural soils. In this study, we investigated the incidence of *Heterobasidion* spp. infection in Scots pine forests established on low-risk sites where the trees looked healthy and unlikely to be infected. In total, 135 healthy-looking pine trees from nine different stands were examined for *Heterobasidion* spp. presence. *Heterobasidion* spp. was detected in six stands and infection frequency was 13%–33%. There was a significant correlation between site index and infection frequency, which was higher in pine stands established on more fertile soils. There was no correlation between disease incidence and defoliation level, diameter of tree at breast height, root diameter, tree volume, or stand age. Overall, our results showed that, regardless of the soil type, Scots pine can be intensively infected by Heterobasidion pathogens while showing no outward signs. Therefore, the risk of Heterobasidion disease should be taken into consideration in management of pine forests growing on both low- and high-risk sites for more productive and sustainable forests.

**Keywords:** root rot disease; *Heterobasidion annosum*; Scots pine; low-risk site; pine forest management; crown defoliation; stump treatment; thinning

# 1. Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most distributed pine species in the world [1]. In Sweden, Scots pine is the second most common and economically important tree species after spruce with 39% of the standing volume on productive forest land, and it is present all over the country [2]. The wood is used for pulp, sawn timber, and bio-energy and the forests offer recreational as well as invaluable biodiversity values [3]. However, these forests are increasingly threatened by root and butt rot caused by the fungal pathogen *Heterobasidion annosum*, which is among the most frequent and severe fungal diseases of conifers in the northern hemisphere. In Europe, losses due to Heterobasidion root rot have been estimated at EUR 800 million per year [4]. In Sweden, two species of *Heterobasidion* are present: (1) *H. annosum* sensu stricto (Fr.), which mainly attacks pines, but also infects other conifers such as Norway spruce and broadleaved trees [5–7], and (2) *H. parviporum* Niemelä & Korhonen, which mainly attacks Norway spruce, but can also infect Scots pine seedlings, Siberian larch, and silver birch [5,6,8].

The primary Heterobasidion root rot infection is caused by basidiospores dispersed from fruiting bodies that are formed at the base of infected stumps or diseased trees [9] when the temperature is above 0 °C [10]. Basidiospores land on newly created stump surfaces or wounds of living trees and produce mycelia [11,12] which colonize the host



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). tissues and grow into the root systems, subsequently infecting neighboring healthy trees via root contacts or grafts [9,13].

The types of damage caused by *Heterobasidion* depend on the host species. For instance, it causes stem decay which grows up to 4.8 m and 12 m in spruce and larch trees, respectively [14,15]. However, in Scots pine, the infection typically remains in the roots, causing root tissue decay and increasing the risk of wind throw. Additionally, the infection can reduce the volume growth and productivity of a stand by up to 10% annually [16]. Further, infected pine trees have been observed to have shorter needles and increased crown transparency [17]. The infection eventually leads to trees' mortality [18–20].

The probability of *Heterobasidion* infection in Scots pine is affected by complex and interacting factors, such as host resistance, intensity of stump infection, and environmental conditions affecting both the pathogen and the host [9]. Previous studies have shown that high incidence of *Heterobasidion* disease is associated with site conditions, such as sandy soil with low organic matter content [9,21–23], coarse soil texture [21–23], soil with high pH [9,22,23], and former agricultural soils [9]. These characteristics are therefore used in identifying sites with a high risk of trees becoming infected; conversely, their absence is taken as a sign of a low-risk site. A soil risk rating scale developed by Morris and Frazier (1966) [24] for potential *H. annosum* infection in loblolly pine stands classifies sands, loamy sands, and sandy loams as high-risk soils; loams and silt loams as intermediate-risk soils; and clays and clay loams as low-risk soils. A similar risk rating system was later developed in the United Kingdom [25]. Because these systems rely only on soil type to distinguish high and low risk sites, they may not be directly applicable for evaluation of risk levels in other countries and regions where other biological and management-related factors influence Heterobasidion infection. Such a risk-rating system is still lacking for Swedish forests [26].

In pine forests, *Heterobasidion* infection is usually difficult to detect because of the lack of clear symptoms, especially in the early stages of the infection. Wang et al. (2014) [16] found that 87.5% of trees in a high-risk Scots pine forest in southern Sweden were infected by Heterobasidion without above-ground symptoms. Rönnberg et al. (2006) [20] assessed trees' crown condition and *Heterobasidion* incidence in pine forests established on former agricultural soils. They found *Heterobasidion* spp. to be present in fourteen of fifteen sites. Although 73% of sampled trees were infected, 45% of infected trees were assessed as healthy. In addition, infection was more frequent among trees with defoliated crowns. In another study, Kurkela (2002) [17] found that the average crown condition of Scots pine was correlated with Heterobasidion disease incidence when dead trees were included, whereas the correlation was not significant without dead trees. In other words, based on crown condition, infected trees could not be distinguished from healthy trees. The difficulty of identifying clear visual signs of infection hinders efforts to manage the disease in pine stands [27]. All of these studies were performed on sites considered to be high risk. The external signs of infection on trees on low-risk sites may be different; the risk may not actually be lower, but the disease expression may be different. Regardless, the use of external symptoms such as crown defoliation may not be very useful for assessing infection levels in a stand. The situation on low-risk sites is not well investigated and may be different due to different soil and other conditions.

In Sweden, biological control using an antagonistic fungus, *Phlebiopsis gigantea* (Fr.) Jülich, is done mainly during the thinning of Norway spruce (*Picea abies* (L.)) stands. However, it is possible that *Heterobasidion* can also become established in Scots pine stands thinned during the summer if stump treatment is not applied. Several earlier studies have also shown the efficacy of stump treatment with *P. gigantea* for preventing *Heterobasidion* infection in pines. Rishbeth (1963) [28] inoculated pine stumps with *P. gigantea* spores and found a good result against *Heterobasidion*. More recently, Pellicciaro et al. (2021) [29] showed that treatment of Scots pine stumps with *P. gigantea* MUT 6212 completely prevented colonization by *H. annosum*. To support informed management decisions regarding stump treatments in pine stands, more evidence about infection risks is needed, especially on sites where the risk has traditionally been assumed to be low.

The aim of this study was to produce new knowledge about *Heterobasidion* root rot prevalence on low-risk sites by: (1) investigating the present prevalence of *Heterobasidion* root rot infection in Scots pine forests growing on low-risk sites, (2) analyzing the relationship between infection prevalence and stand characteristics (age, site index (the total height to which dominant trees of a given species will grow on a given site at 100 years), and tree growth measurements (volume, tree root diameters)), and (3) assessing the correlation between *Heterobasidion* infection and visually estimated crown defoliation of Scots pine trees on the same sites.

#### 2. Materials and Methods

# 2.1. Study Sites

Data for this study was collected from nine Scots pine stands in southern Sweden between 2018 and 2021 (Figure 1, Table 1). The stands were selected based on three main criteria. First, the stands should not be on sandy soils and should not have previously been used as pasture or for agriculture. Second, the trees had to be  $\geq$ 80% Scots pine. Third, the stand must have been thinned at least once, preferably during the summer or late spring, i.e., when spores of *Heterobasidion* spp. are expected to be abundant.



**Figure 1.** Location of the study sites in southern Sweden. The figures adjacent to open circles on the map correspond to the stand id, as indicated in Table 1.

#### 2.2. Sample Collection and Identification of Infected Trees

On each site, fifteen trees were selected by the respective forest manager following their regular thinning regime. To collect samples from pine roots, the selected trees were uprooted using a single-grip forest harvester machine. When the trees were down, stem diameter at breast height (DBH), bark thickness, height, and stem length to the first living branch were measured. Diameter was measured by cross-calipering at 1.30 m above ground level. Bark thickness was measured at DBH height at three points around the stem using a bark gauge (Haglof Barktax Bark Gauge, Hammerdal, Sweden). A measuring tape was

used to measure stem length (height) from ground level to the base of the first living branch as well as the total height of the tree. Tree volume was calculated using the following formula [30]:

$$V = 0.1193D^{2} + 0.02574D^{2}H + 0.004054DH^{2} + 0.007262D^{2}K - 0.003112DHB$$

where V = tree volume above cutting  $(dm^3)$ , D = stem diameter at breast height (cm), H = total tree height from the ground (m), K = stem length (height) from ground level to the base of the first living branch (m), and B = double bark thickness (mm).

The root system of each tree was cleaned using a garden spade. From each tree, five roots were randomly selected and haphazardly sampled at a 0–75 cm distance from the root collar [16]. The sampling area was cleaned with a brush and sprayed with 70% ethanol. Discs 2–5 cm thick were cut with a Japanese hand saw and put immediately into a labelled plastic bag and transferred to the lab for microscopy analysis. To prevent cross-contamination between samples, the saw was thoroughly cleaned by removing any dust and then sprayed with 70% ethanol between cuts. The diameter of root samples was measured by calculating the average of the perpendicular diameters of the root discs, and the samples were assigned to five classes according to their diameters.

#### 2.3. Microscopy Analysis

From nine different Scots pine stands, a total of 135 Scots pine trees and 675 root samples were examined for the presence of *Heterobasidion*. The root samples were first incubated at room temperature (20 °C) in darkness for 10 days. After that, samples were checked in a random order for presence of *Heterobasidion* spp. conidiophores by using a stereo microscope. When conidiophores were found, the sample was recorded as infected. The species of Heterobasidion were not identified because Scots pine is primarily infected by *Heterobasidion annosum* [5–7].

#### 2.4. Defoliation Assessment

A local reference tree was selected in each stand to compare with other selected trees. The reference tree was representative of typical crown morphology in the stand and had  $\leq 10\%$  defoliation. Crown defoliation level was visually evaluated on a scale of 0 to 4 (0—healthy trees, defoliation 0%–10%; 1—slightly defoliated trees, defoliation 11%–25%; 2—moderately damaged trees, defoliation 26%–60%; 3—severely damaged trees, defoliation 61%–99%; and 4—dead trees, defoliation 100%) [31]. The defoliation assessment was conducted while the trees were standing. Since the trees in stand six were felled prior sample collection, the defoliation assessment is not provided. To exclude defoliation caused by other foliage diseases or insects attack, the reference and sampled trees were checked for the presence of these stressors; no marked symptoms or signs of them were observed in any of the sampled stands.

#### 2.5. Statistical Analysis

The correlation between Heterobasidion root rot disease incidence and defoliation level, root diameter, and stand characteristics was tested using linear mixed models in the R package "lme4" with stand as a random effect. The null hypothesis was lack of an association between defoliation level, root size, and Heterobasidion root rot disease incidence. All analyses were performed using R version 4.1.3 (R Core Team, 2022).

#### 3. Results

#### 3.1. The Prevalence of Heterobasidion Infection on Low-Risk Sites

Heterobasidion root rot infection was recorded in six out of nine sites. In total, 20 trees (14.8%) and 28 root samples were infected. The frequency of infection across all sites was 0%–33% (Table 1).

Stand Id	Site Name	Location	Stand Age (yr)	Site Index	Soil Type	Previous Tree Species	Number of Thinnings	Number of Uprooted Trees	Number of Infected Trees	Infection Frequency (%)
1	Svenljunga	57°28′42.0″ N 12°52′27.0″ E	38	T25	Moraine	Scots pine + Norway spruce	3	15	0	0
2	Västervik	57°38′15.3″ N 16°37′00.1″ E	52	T18	Moraine	- *	_ **	15	0	0
3	Västervik	57° 37′ 24.2″ N 16° 38′ 03.8″ E	47	T21	Moraine	_ *	_ **	15	0	0
4	Norrköping	58°45′48.3″ N 16°15′22.4″ E	42	T29	Silty clay	_ *	1	15	5	33.33
5	Skövde	58°26′53.2″ N 13°56′35.3″ E	37	T24	Glacial sediments	Scots pine	1	15	2	13.33
6	Österbymo	57°48′05.4″ N 15°19′48.4″ E	62	T25	Moraine	Scots pine	2	15	2	13.33
7	Boxholm	58°09'40.4" N 15°04'27.3" E	45	T27	Moraine	Norway spruce	1	15	3	20
8	London Grytfall	58°07′29.8″ N 15°18′09.8″ E	58	T26	Moraine	Scots pine	2	15	3	20
9	Simonstorp	58°48′51.4″ N 16°06′05.7″ E	36	T28	Moraine	Scots pine	1	15	5	33.33
total								135	20	14.8

**Table 1.** Details of nine study sites with expected low risk of infection of *Heterobasidion* spp. in southern Sweden including actual infection rates.

\* Documentation about previous tree species is not available. \*\* Although thinnings have been carried out, there is a lack of documentation containing historic data, but they are confirmed by forest managers to be old forest sites.

# 3.2. Relation between Infection Frequency and Stand Characters (Site Index, Stand Age)

A significant positive correlation was found between site index and infection frequency (p = 0.004, t = 4.1946, df = 7; Table 1). The average site index of infected stands (i.e., stands with presence of *Heterobasidion* spp. infection) (T26.2) was higher than for healthy stands (T21.3) There was no significant correlation between infection frequency and age of the stand (p = 0.072). (Table 1).

# 3.3. Relationship between Infection Frequency and Tree Characteristics (Tree Volume, Root Diameter, Crown Defoliation)

There was no correlation between infection incidence and volume of the trees (p = 0.45). At the stand level, the differences in volume between infected and non-infected (healthy) trees were also not significant in all cases (Figure 2A).

In total, 28 of the 675 examined root disc samples were infected by *Heterobasidion* spp. (i.e., 4%) The diameter of sampled roots ranged from 1.85 to 12.55 cm (Figure 2B). A linear mixed model showed no correlation between root diameter and infection (p = 0.81, chi-square = 0.059, df = 1).

The visual assessment of crown status showed that about 62% of the investigated trees showed signs of defoliation while 38% had healthy crowns. Among the trees showing signs of defoliation, 18.5% were infected and 81.5% were not infected (Figure 3A).

In infected stands, 40% of trees had healthy crowns and 4.2% of them were infected. About 40% of trees showing defoliation belonged to defoliation class 1 and 34.6% of these were infected (Figure 3B). Defoliation level trended higher in trees infected by *Heterobasidion* (p = 0.072).



**Figure 2.** *Heterobasidion* spp. infection frequency and tree characteristics (tree volume, root diameter) in the analyzed stands. (**A**) Volume of infected and non-infected trees in each sampled stand). (**B**) Diameter of infected and non-infected (healthy) root discs. The boxes show the 25% and 75% quantiles, with the thick central line at 50%. The whiskers show the range of values no more than 1.5 times the interquartile range from the extremes of the boxes; the open points are observed values beyond this range.



Figure 3. (A) Tree defoliation status in all stands. (B) Defoliation level of healthy and infected trees in infected stands.

# 4. Discussion

Although the incidence and distribution of *Heterobasidion* root rot infection has been studied intensively in Scots pine forests growing on high-risk sites, this is the first study to investigate the prevalence of *Heterobasidion* infection in Scots pine forests established on sites thought to have a low risk of *Heterobasidion* infection. Six of nine stands had at least one tree infected by *Heterobasidion* spp., which demonstrates the ability of *Heterobasidion* spp. to establish and persist even in low-risk sites. None of the study stands showed any

characteristic symptoms for *Heterobasidion* spp. such as groups of dying pines, fruiting bodies, and windthrown pines with decayed roots. With an infection incidence above 30% of trees on some sites, it seems warranted to consider the risk for *Heterobasidion* infection when planning silvicultural activities on all sites. Furthermore, due to climate change, higher infection incidence by *Heterobasidion* spp. may be expected as the pathogen produces and releases more basidiospores during longer growing seasons and warmer temperatures [32]. Warmer temperatures also mean a lower proportion of thinning operations will be conducted during a shorter cold winter period [33]. Therefore, risk assessment systems based only on soil type are likely to underestimate the risk of *Heterobasidion* disease. Our results indicate that a hazard risk assessment system should consider soil type, stand management history (first generation or previous agricultural or pasture areas), and presence of *Heterobasidion* infection in the current and previous plantations. If one or more of the above-mentioned factors is present in a pine stand, prevention methods such as stump treatment and winter cutting must be implemented promptly, especially considering the potential increased planting of Scots pine as an adaptation to climate change.

In the Swedish classification system, higher site index reflects higher productivity, i.e., higher soil fertility [34,35]. We found infection incidence to be higher in more fertile stands (Table 1), which agrees with results of earlier studies [36,37]. Soil fertility may have a positive influence on pine trees' growth, but Rishbeth (1957) [38] reported a tendency for fast-growing trees to suffer more serious *Heterobasidion* damage than slow-growing ones. Even if there seems to be a relationship between the site index and the infection frequency, it is challenging to define clear thresholds for low or high risk. Nevertheless, stump treatment or winter cutting are likely to reduce the risk of *Heterobasidion* root rot infection in pine stands regardless of site index.

Once a tree is infected by *Heterobasidion* spp., some portion of the nutrients that are necessary for tree growth will be allocated to induce different defence mechanisms [39,40]. Over time, the fungus colonizes more root tissues, leading to dysfunction of roots and their capacity for water and nutrient uptake [41,42] and, consequently, declining tree growth. Wang et al. (2014) [16] found that the annual reduction in volume production of Scots pine stands infected by *Heterobasidion* spp. was approximately 10%. Likewise, Burdekin (1972) [19] has shown that the total loss of volume of Scots pine stands infected by Heterobasidion annosum was represented not only by volume of dead trees but also by a reduction in volume of the live, infected trees. However, our comparison between volumes of infected and non-infected trees in each stand did not reveal significant differences. This may have two main causes: first, the lower number of infected trees compared to noninfected ones (Figure 2A), and second, trees were sampled as part of ordinary thinning operations that first aimed to remove weak, malformed, or slow-growing trees; thus, the thinned non-infected trees may be of lower quality than the remaining non-thinned trees. Nevertheless, while the trees infected by *Heterobasidion* may survive for a long time without showing any symptoms, their overall productivity is likely to decline, which can significantly reduce the revenues from a plantation [26].

*Heterobasidion* infection spreads vegetatively from the roots of infected stumps to neighboring healthy trees via root contacts or grafting. The infection usually develops from smaller roots up into the primary root and then the stem of the tree [43]. Despite the lack of strong correlation between root diameter and disease incidence in this study, the infection was mainly observed in roots of diameter  $\leq 6$  cm; small-diameter roots have thinner bark which may become infected more easily. This result is in agreement with Wang et al. (2014) [16], who found that Scots pine roots with a diameter between 1–4 cm had the largest percentage of detected *Heterobasidion* infections. Morrison and Redfern (1994) [44] found that the diameter of Sitka spruce roots infected with *H. annosum* was less than 6 cm, and the site of the infection was at least 1 m from the root collar. Taken together, these findings suggest that in early stages of infection, the smaller roots are infected trees reallocate energy to block fungal growth. This result should also be considered when

planning sampling strategies for *Heterobasidion* infections; detection frequency could be improved by focusing sampling efforts on smaller roots.

Crowns of infected trees in this study had a weak trend toward slight or moderate defoliation levels, but no significant change in crown length. This indicates that visual assessment of crown condition or defoliation alone are not reliable infection indicators, in agreement with previous studies from high-risk sites [16,17,20,45]. Thus, foresters lack a practical method for detecting infection. New aerial monitoring methods based on multispectral or hyperspectral bands from UAV imagery [46] may provide efficient and affordable detection of cryptic *Heterobasidion* infections in the near future.

Our sample sizes were limited for several reasons. The most important is the cost of using heavy machinery to uproot trees. This problem was compounded by the severe drought in summer 2018 when no such machinery was allowed in forests due to fire risk. Further, a subsequent outbreak of spruce bark beetle in Sweden meant all machinery was occupied in cleanup operations, further limiting access to suitable pine forests. However, even with such limitations, the overall distribution of sampled stands was appropriate.

This study's results are important from a practical point of view, since stands on assumed low-risk sites are currently not managed to prevent *Heterobasidion* spp. infection, such as through application of stump treatment during summer thinnings. Treatment with P. gigantea combats primary infection by *Heterobasidion* spp. on Scots pine stumps [47,48] and may improve site productivity. However, this treatment is costly. Applying *P. gigantea* [49] to Scots pine stands in central Sweden with a site index of 25 during a typical rotation (two thinnings at 33 and 48 years and a final felling at 73 years) is estimated to cost SEK 2738 ha<sup>-1</sup> (ca. EUR 267), a value equivalent to 10 m<sup>3</sup> of pulpwood (SEK 270 m<sup>-3</sup> SUB, solid under bark) [50]. If calculated using a 3% discount rate back to the first thinning [16], the corresponding cost would be SEK 1341  $ha^{-1}$  (ca. EUR 131) at age 33. This value corresponds to 5.0 m<sup>3</sup> of timber. The estimated annual volume growth reduction due to Heterobasidion infection in the Wang et al. (2014) [16] study site cannot be compared to our results. The 5.0 m<sup>3</sup> figure can, however, be compared with the number of trees this volume would correspond to at this site index. At final felling, this is about ten trees, or around 1.4% of the trees in a hectare. In this study the overall infection frequency is 14.8%. It is therefore very likely that stump treatment would yield economic benefits.

## 5. Conclusions

The results of the present study confirm that *Heterobasidion* root rot disease can be present in pine roots, even in areas previously thought to pose a low risk. These findings suggest that the problem of *Heterobasidion* disease may be underestimated in Sweden and elsewhere, and that there is a significant risk of its spread and prevalence in pine forests due to climate warming and intensifying thinning actions, which are increasingly being conducted during the spore deposition period. To ensure sustainable forest management, prevention measures such as stump treatment are recommended to control *Heterobasidion* root rot in Scots pine forests.

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