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Persistence and infectivity of *Heterobasidion parviporum* in Norway spruce root residuals following stump harvesting

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ABSTRACT

In boreal forests, stumps of Norway spruce are removed after clear cutting to gather wood for energy and, additionally, to reduce damage caused by the root rot fungus, *Heterobasidion parviporum*. In order to understand the behavior of *H. parviporum* at stump-harvested Norway spruce sites, the survival of *Heterobasidion* mycelia in buried root fragments and their ability to infect nearby spruce seedlings was investigated in southern Finland. The longevity of *Heterobasidion* mycelium in investigated root pieces exceeded the follow-up period of 83 months. After six years, 18% of the root fragments yielded living *Heterobasidion* mycelia. The survival of *H. parviporum* in root fragments was highest in sandy soils and increased with increasing volume of root fragment. The probability of occurrence of the fungus was highest when at least 60% of the root volume was originally colonized by the fungus, irrespective of the state of decay. It took 4.5 years until roots of the first seedling reached the buried root fragment and the seedling became infected by *H. parviporum* mycelia. After six years, 8% of the seedlings were found to be infected. In addition, the number of root residuals, i.e., the potential infection sources of the next tree generation, was determined at five stump-harvested Norway spruce sites. At these slightly infested spruce sites, on average 1500 potential disease sources ha^{-1} (in the form of decayed root fragments $\geqslant$ 1.5 cm in diameter) were found in the upper soil layer after stump removal. Thus, our results indicate that stump harvesting procedure aimed at gathering wood for energy may not be an efficient way to remove infected wood material from forest soils. Furthermore, our survey on stored stumps next to clear-cut areas showed that the formation of *Heterobasidion* fruiting bodies may be abundant in decayed stumps in the lowest parts of the storage pile. In order to avoid increased risks of spore infection in nearby forests, decayed spruce stumps, including the lowest muddy stumps, should be transported within two years from forest storage to the end-user.

1. Introduction

Norway spruce (*Picea abies* L. Karst.) is an economically important tree species in boreal forests in Europe. The high susceptibility of Norway spruce to Heterobasidion root and butt rot is one of the most demanding challenges in spruce forest management. It has been estimated that each year losses of ca. 800 million euros are caused by Heterobasidion root and butt rot in Europe (Woodward et al., 1998), and at least 44 million euros in Finland (Bendz-Hellgren et al., 1998). Rot in Norway spruce in Finland is primarily caused by *Heterobasidion parviporum* Niemelä & Korhonen (Korhonen and Piri, 1994). The other *Heterobasidion* species occurring in Northern Europe, *H. annosum* (Fr.) Bref. sensu stricto, causes mortality in pine stands and occasionally root and butt rot in spruce stands (Niemelä and Korhonen, 1998). Both fungi belong to the *Heterobasidion* species complex, *Heterobasidion annosum* sensu lato.

Heterobasidion infections are initiated by airborne spores, when they infect freshly exposed wood tissues, i.e., stump surfaces or logging wounds. After landing on the exposed tissue, spores germinate and produce mycelia, which colonize the roots of the host stump or tree. The fungus spreads to adjacent healthy trees by mycelium growing across root contacts or grafts (Korhonen and Stenlid, 1998). Under Nordic conditions, basidiospore-producing fruiting bodies usually occur in the cavities of old stumps and on the roots of windthrown trees (Laine, 1976). After final cutting of an infected stand, *H. parviporum* can remain viable in spruce stumps for more than 40 years (Piri, 1996). The fungus is able to spread from one forest generation to the next through root contacts and may cause high incidence root and butt rot in the new spruce generation (Yde-Andersen, 1978; Stenlid, 1987; Piri, 1996).

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Due to recent needs to increase the use of renewable energy, stumps are increasingly being used as a source of bioenergy in Nordic countries. Although the main reason for stump harvesting is to gather wood for bioenergy production, stump harvesting has also been proposed as an option to control Heterobasidion root rot and continue the growth of Norway spruce at infested sites (Lipponen, 2007; Vasaitis et al., 2008; Cleary et al., 2013).

However, the epidemiology of *H. parviporum* at spruce sites where stumps have been removed is poorly known. Even though stump removal eliminates most inoculum from a site, infected roots broken down during extraction remain in the soil (Uri et al., 2015). In terms of disease control it seems desirable to collect as much infected woody debris as possible to prevent the infection of seedlings of the next stand generation (Yde-Andersen, 1970). It has been assumed that broken roots less than 5 cm in diameter will decay rapidly and do not pose a significant threat of root rot to the next stand generation (Omdal et al., 2001). Moreover, it has been suggested that broken ends and removed bark of root residuals would facilitate the establishment of competing microorganisms, which could displace or deadlock *Heterobasidion* species (Rishbeth, 1951; Gibbs, 1967a). Nevertheless, several examples have shown that despite the careful removal of inoculum involving stump lifting followed by root raking to remove broken root pieces thicker than 5 mm from the soil, the next spruce generation may not be completely protected from Heterobasidion root rot (Stenlid, 1987; Cleary et al., 2013). In order to achieve maximum benefit from stump removal in terms of disease control, more information is needed particularly about characteristics of the inoculum capable of spreading disease into the next spruce generation and the quantity of such inoculum remaining in forest soils after stump removal.

Furthermore, the potential risks posed by long-term storage of spruce stumps colonized by *H. parviporum* need to be investigated. After lifting, the stumps are first piled up in the harvested area for a few weeks, after which they are hauled to a roadside landing area. Because stumps have a high and long-lasting heating value (Laurila and Lauhanen, 2010) they may be kept in roadside storage for several years. According to Uri et al. (2015) at least a two-year storage period is necessary to increase the heating value of harvested spruce stumps. However, in spite of long storage, moisture content in the lower parts of the pile may remain high and conditions may be favorable for the development of *Heterobasidion* fruiting bodies.

The aim of this study was to collect more detailed information about the behavior of *H. parviporum* at stump-harvested Norway spruce sites. For this purpose, we determined the survival of *H. parviporum* in naturally infected root fragments buried in forest soils and the ability of the fungus to spread to adjacent spruce seedlings. In addition, a survey to assess the amount of residual root wood remaining in the soil after stump harvesting was conducted. In order to find out the potential risks related to the storage of infected stumps, we also examined the occurrence of fruiting bodies of *H. parviporum* in decayed spruce stumps kept in roadside storage for three to four years.

2. Materials and methods

2.1. Preparation of root fragments for the inoculation experiment

Roots were collected from 155 freshly cut Norway spruce stumps at five clear cutting areas in southern Finland (in Läyliäinen, Ruotsinkylä and Latokartano), in the same region where the inoculation experiments were established (see Table 1 below). Each stump was colonized by different *H. parviporum* genotypes. Depending on the distribution of decay in the stump roots, 1–33 root sections were sawn from each stump. A disc 1–2 cm thick was cut from both ends of each sampled root section. The discs were washed with tap water, and incubated in plastic bags for 5–10 days. On each disc, the area occupied by *H. parviporum* (recognized by the production of characteristic conidiophores) was checked under a dissection microscope. The outline of decay was traced on transparent sheets (with grid lines 1×1 mm), and the area and location of decay as well as total cross-section area (inside bark at both ends of the root) were calculated to determine the proportion of decay in percentages. Only roots with living mycelia of *H. parviporum* were used in inoculation experiments. The final lengths of root fragments (after cutting the sample discs) varied from 4 to 42 cm (mean 16.5 cm) and the diameter from 1.5 to 19.5 cm (mean 5.6 cm). The root fragments were classified into three categories based on decay stage: (i) incipient decay (structurally intact stained wood with firm texture), (ii) intermediate decay (dark-shaded discoloration and minor changes in the wood texture), and (iii) advanced decay (soft and fibrous wood texture). The proportion of root fragments with incipient, intermediate and advanced stages of decay was 36.1%, 29.4% and 34.5%, respectively. Moreover, roots were classified into the following groups according to the location of decay on the cross-section: (i) decay in heartwood (18.4% of all roots), (ii) in sapwood (3.7%), (iii) in both heart- and sapwood (7.6%), and (iv) in the whole cross-section extending from pith to bark (70.3%). The proportion of wood decayed by *H. parviporum* was calculated for each root fragment and was, on average 61.8% (range 1.0–100.0%).

To get pure *Heterobasidion* cultures from root fragments, conidiophores from the decayed area were transferred to Petri dishes with malt extract agar (MEA; 2% w/v malt extract, 1.5% w/v agar) using tweezers that were dipped in ethanol and flame sterilized. Mating tests confirmed that all isolates belonged to *H. parviporum* (Korhonen, 1978). The genotypes were identified by somatic compatibility (Stenlid, 1985). The pure cultures were stored on MEA in test tubes in a fridge at ca. 4 °C for later use to confirm that the genotypes of *Heterobasidion* isolated from buried root fragments and infected seedlings were the original ones.

2.2. Establishment of inoculation experiments

At twelve clear-cut sites in southern Finland, altogether 401 root fragments colonized by *H. parviporum* were buried in old forest soils (at least two successive tree generations existed before the experiments), 5–10 cm below the soil surface (Table 1). Nine of the sites had recently been planted with 2-year-old Norway spruce seedlings. At these planted sites, the root fragments (307 in total) were placed close to spruce seedlings, i.e., about 10 cm from the stem base. Thus, the same number of spruce seedlings (307) was included in the study. In each experiment, root pieces were randomly placed next to the seedlings. The rest, 94 roots, were buried in three unplanted sites. A seedling/inoculum map was prepared for each site. The soil type of each site was determined from soil profiles using the soil particle size classification by Aaltonen (1941). Four soil samples per site were taken 10–15 cm below the original soil surface and the pH (in water) of each sample was determined in the laboratory.

2.3. Harvesting of buried root fragments

The first root fragments were excavated after 12 months (Läyliäinen 1) and the last fragments after 83 months (Latokartano 2, see Table 1). In the laboratory, soil from the root fragments were gently brushed and washed away. Roots were cut transversally into 5 cm thick discs with a handsaw. Each disc was washed under tap water, dried with a soft tissue and placed in a separate plastic bag. Discs were incubated for 5–15 days at