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Complete List of Authors:	Zaluma, Astra; Latvian State Forest Research Institute Silava, Muižnieks, Indriķis; Latvijas Universitāte Gaitnieks, Talis; Latvian State Forest Research Institute Silava, Burgeviča, Natālija; Latvian State Forest Research Institute Silava Jansons, Aris; Latvian State Forest Research Institute „Silava, Jansons, Jurgis; Latvian State Forest Research Institute Silava Stenlid, Jan; Swedish University of Agricultural Sciences, Department of Forest Mycology and Pathology Vasaitis, Rimvydas; Swedish University of Agricultural Sciences
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**Infection and spread of root rot caused by *Heterobasidion* spp. in *Pinus contorta* plantations
in Northern Europe: three case studies**

A. Zaļuma¹, I. Muižnieks², T. Gaitnieks¹, N. Burņeviča¹, Ā. Jansons¹, J. Jansons¹, J. Stenlid³,
R. Vasaitis³

¹Latvian State Forest Research Institute Silava, Rigas 111, Salaspils, Latvia, LV 2169;

²Department of Microbiology and Biotechnology University, of Latvia 19 Raina Blvd., Riga
Latvia, LV- 1586,

³Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural
Sciences, P.O. Box 7026, SE – 750 07 Uppsala, Sweden;
E-mail: astra.zaluma@silava.lv (for correspondence).

Abstract

This study investigated the origins and spread patterns of *Heterobasidion* root disease in three *P. contorta* plantations established on forest and agricultural land, and subjected to three different management scenarios. Trees with decline symptoms, and stumps remaining from the previous rotation were sampled for fungal isolations. Ten isolates of *H. parviporum* and 425 of *H. annosum* were tested for clonality through somatic compatibility tests. Conclusions: i) *P. contorta* is highly susceptible to *H. annosum* and *H. parviporum* and both pathogens cause dieback of *P. contorta*; ii) *H. annosum* from previous rotation *P. sylvestris* stumps can effectively transfer to *P. contorta*; iii) the pathogens may form constantly expanding territorial clones; iv) basidiospores of both pathogens colonise stumps of *P. contorta* (primary infections); v) *H. parviporum* clones expanded more slowly than clones of *H. annosum*; vi) clonal spread proceeded more quickly from stumps with established secondary infections than from stumps with primary infections; vii) *H. annosum* can persist in pine stumps for at least 26 years; viii) stump treatment should be considered in order to control *Heterobasidion* primary infections.

Key words: Lodgepole pine, *Heterobasidion*, primary infection, secondary infection

1. Introduction

Since the 1950s, the introduced lodgepole pine, *Pinus contorta* Dougl. ex Loudon, has become increasingly important in forestry in Northern Europe, and so far hundreds of thousands of hectares have been planted with this species e.g. in the UK (Redfern 1982) and Scandinavia (Hagner 1983; Karlman 2001). Moreover, in Sweden tree breeding programmes for *P. contorta* have been developed (Hayatgheibi 2018). *P. contorta* is susceptible to root rot caused by fungi from the genus *Heterobasidion* (Redfern 1982; Vollbrecht *et al.*, 1995; Piri 1996; Woodward *et al.* 1998, and references therein; Greig *et al.* 2001; Rönnerberg and Svensson 2013) – the most economically important pathogens of conifers in the Northern Hemisphere (Woodward *et al.* 1998). In Northern Europe, there are two species of *Heterobasidion* that cause the damage: *H. parviporum* Niemelä & Korhonen and *H. annosum* (Fr.) Bref. (Niemelä and Korhonen 1998). Primary infections of the pathogens are established by airborne basidiospores. After landing on surfaces of freshly cut conifer stumps (in Northern Europe, mainly *Picea abies* (L.) H. Karst and *Pinus sylvestris* L.), the spores germinate and hyphae grow over the stump surface, and penetrate down into the inner part of stump, finally colonising lateral roots (Redfern and Stenlid 1998 and references therein; Swedjemark and Stenlid 2001, Garbelotto and Gonthier 2013). Secondary infections occur when hyphae of the pathogens spread to root systems of adjacent trees via root contacts, which can produce expanding, territorially defined disease centres (Stenlid and Redfern 1998; Garbelotto and Gonthier 2013). Moreover, *Heterobasidion* spp. effectively invades roots of trees replanted on infested forest clear-cuts creating inter-generational secondary infections (Stenlid 1987; Vasiliauskas and Stenlid 1998; Lygis *et al.* 2004). As a result, established disease centres on forest land have potentially the capacity for indefinite longevity; although the

complete arrest of disease centres expansion was documented in North America (Rizzo *et al.* 2000).

Surprisingly, *P. contorta* as a *Heterobasidion*-susceptible species has not been mentioned in more recent syntheses on *Heterobasidion*, in which the data on tentative susceptibility of numerous tree species (including nine *Pinus* species from Europe and North America) to five biological species of the pathogen were summarised (Garbelotto and Gonthier 2013; Gonthier and Thor 2013). In fact, the first, and to date the only work demonstrating the susceptibility of *P. contorta* to natural *Heterobasidion* sp. infections, was carried out in Finland in the early 1990s. Piri (1996) investigated infections of the disease in two *P. contorta* plantations (eight and 14 years old) established on clear-cut *P. abies* sites. It was found that 18.4 % and 5.8 % (9 and 11 trees) of the *P. contorta* were infected in the respective plantations, and in most of cases (7 and 10 trees) the infections were traced to stumps of the previous generation of *P. abies*. All of the detected infections proved to be due to *H. parviporum*, thus providing evidence that *H. parviporum* on *P. abies* can be transferred from infected stumps to the next generation of *P. contorta* (Piri 1996).

To the best of our knowledge there are no studies of *Heterobasidion* spp. (*H. annosum*, *H. parviporum*, *H. irregulare* (Underw.) Garbel. & Orosina) infectiousness, establishment, ability to transfer over forest generation and rate of spread from tree to tree via root contacts within stands of *P. contorta*. The aim of this work was to investigate the spread patterns of *Heterobasidion* spp. in *P. contorta* plantations established on forest and former agricultural land under three different management scenarios.

2. Materials and methods

2.1. Field work

The study was performed in three *P. contorta* plantations in Latvia (Fig. 1). Characteristics of the investigated plantations are presented in the Table 1. The management scenarios were: i) **Stand 1** (56°40'1.9"N, 25°49'59"E), a 26-year-old unmanaged plantation (established on a *Heterobasidion*-infested clear-cut site of approx. 100 year-old *P. sylvestris*); ii) **Stand 2** (56°41'06"N, 24°27'42"E) a 27-year-old unmanaged plantation (established on a *Heterobasidion*-infected clear-cut site of 100 years-old *P. sylvestris*), which was thinned directly after the first sampling and iii) **Stand 3** (57°03'47"N, 21°57'11"E), a 28-year-old plantation established on former agricultural land (assumed to be free of *Heterobasidion* infections), thinned 16 years earlier. **Stand 1** and **Stand 3** were sampled once, and **Stand 2** was sampled twice: before thinning and four years after thinning. Thinnings in **Stand 2** and **Stand 3** were conducted during September – October and stumps were not treated with control agents (neither urea nor Rotstop®) to prevent new (primary) infections by *Heterobasidion* spp. to freshly cut stumps.

In each stand, a visual evaluation of tree health condition allocated the trees to three categories: 1) crowns visually healthy; 2) crowns with dieback characteristic of *Heterobasidion* symptoms i.e. chlorotic foliage, resin flow, reduced growth (Piri, 1996); 3) dead or windthrown. All trees and previous generation stumps were examined for fruit bodies of *Heterobasidion*. To detect the pathogen, Category 2 and 3 trees and all trees with fruit bodies were first cut, and 3 cm thick wood discs were taken from stump surfaces. Previous generation stumps could not be sampled

by taking discs, as they were heavily decomposed. Consequently, decomposed parts of stumps were removed with an axe, and wood samples were cut from solid parts of the stumps / roots. All the wood discs taken from the felled trees and wood samples taken from old stumps were individually placed in plastic bags and brought to the laboratory. The numbers of surveyed, sampled trees and previous generation stumps are shown in Table 2. In each plot, the sampled trees and stumps were numbered and mapped.

2.2. Isolation and identification of *Heterobasidion* genotypes

In the laboratory, the discs were debarked and washed with a stiff brush under tap water (the brush was washed in the tap water immediately after each sample brushing). Discs were incubated for 5 – 8 days at room temperature in open plastic bags. Using a dissection microscope (at 20-30 times magnification), the presence of *Heterobasidion* spp. was confirmed by observing its characteristic asexual sporulation (conidiophores) on disc surfaces. The *Heterobasidion* colonies were sampled with sterilised surgical forceps and individually placed on malt extract agar (MEA) in Petri dishes. Outgrowing *Heterobasidion* mycelia were subcultured. Wood samples taken from stumps were cut with a knife to an approximate size of 1 × 0.5 cm, flame surface sterilised and incubated for 7 days at room temperature on MEA in Petri dishes. Outgrowing *Heterobasidion* mycelia were isolated. All the obtained isolates were individually stored on MEA in test tubes at 4 °C until used in subsequent pairing tests for somatic compatibility.

Isolates originating from the same sample plot were subjected to somatic compatibility tests by pairwise confronting their mycelia on MEA in 9 cm Petri dishes in all combinations. The genets were identified either by recording the line of demarcation in the contact zone of confrontation (demonstrating that the genotypes are different), or observing free fusion of the mycelia (implying that the genotypes were identical) (Stenlid 1985). Based on this data, delineation of genets (or territorial clones when comprised of at least two isolates) was accomplished by transferring their boundaries onto the constructed field maps. The species of each genet was determined by mating tests with *H. annosum* and *H. parviporum* single spore tester strains (Korhonen 1978).

2.3. Data analysis

Figures 2, 3 and 4 were created using geospatial tool QGIS 2.18.3 and the area of genets was measured using "Measure Area" tool. The Wilcoxon test (unpaired/paired samples) was used to compare width and area of genets in each plantation, and their rates of expansion between **Stands 2 and 3**. R version 3.4.3 for calculations was used (R Core Team 2017).

3. Results

3.1. Incidence of dieback

In plantations established on former forest land (clear-felled sites of 100-year-old *P. sylvestris*), the incidence of trees showing crown dieback symptoms was lower (29% in **Stand 1** and 11% in **Stand 2**) (Table 2), than in the plantation established on *Heterobasidion*-free former agricultural land (50 % in **Stand 3**), which had been thinned 16 years previously (Table 2; Figs. 3 and 4). In **Stand 3** 115 trees (52 % of declining/dead trees) were wind thrown (storm in January of 2005) and 70 trees were wounded by large game animals (red deer and moose).

Despite the removal of all trees with dieback in **Stand 2**, symptoms developed in 120 (11%) of the retained trees during the subsequent four-year post thinning, and the proportion of trees with dieback symptoms after four years was similar to that initially observed (Table 2). The additional decline observed in residual trees of **Stand 2** four years after first sampling increased the proportion of total observed diseased trees in **Stand 2** from 11% (1st survey) to 17% (pooled 1st and 2nd surveys) (Table 2).

3.2. Observed infections of *Heterobasidion*

Although *Heterobasidion* root disease was suspected as the primary cause of the disease symptoms observed in the sampled trees, the presence of the pathogen was not confirmed in all cases. After incubation of stem discs, conidiophores were observed on 66% of the samples from

Stand 1, 75-80% from **Stand 2**, and 33% from **Stand 3** (Table 2). As with dieback, the incidence of trees in which *Heterobasidion* infections were observed on cut discs from stump surfaces also differed between the two plantations established on former forest land (19 % and 9 %, in **Stand 1** and **Stand 2** - 1st sampling, unthinned, respectively). Thinning, and removal of symptomatic trees did not prevent the spread of disease to retained trees. The baseline (i.e. pre-thinning) incidence of disease was 9% (192 infected trees) and a subsequent survey four years later found once again that 9% (an additional 96 trees) of the retained trees were diseased. Thus the cumulative incidence of disease in the stand increased to 13% (288 trees). In **Stand 3**, the plantation established on former agricultural land the incidence of infected trees (conidia observed in 16 %) was similar to that in **Stand 1** and **Stand 2**. A total of 150 trees with *Heterobasidion* fruit bodies were observed, 36, 96 and 18 in each respective stand (Table 2). *Heterobasidion* fruitbody were observed on one (0.3 %) stump out of 311 inspected previous generation stumps.

3.3. Isolates and genets of *Heterobasidion*

Culturing success from stem discs bearing *Heterobasidion* conidiophores was high. In total, 432 isolates from *Heterobasidion* spp. infected trees were obtained: 83, 280 and 69 (95.4 %, 96.8 %, and 93.2 %) from **Stand 1**, **Stand 2** and **Stand 3**, respectively (Table 3); 422 of those were identified as *H. annosum* and 10 as *H. parviporum* (the latter only in **Stand 3**, Fig. 4). Spatial distributions of *Heterobasidion* genets in the plantations are shown in the Figures 2, 3 and 4. A total of 98 *Heterobasidion* genets were detected. In the unthinned **Stand 1** (former forest land), 4 genets (31 %) occurred only in single trees, while 9 (69 %) were detected in two or more standing trees. In **Stand 2**, single-tree genets were detected in 7 (20 %) *P. contorta* prior to thinning and in

15 (33 %) *P. contorta* four years after the thinning, while the respective numbers of territorial genets increased from 28 to 30. Somatic compatibility tests revealed that the proportions of single-tree vs. territorial genets were at least two times lower on former forest land sites (**Stand 1** and **2**) than on the former agricultural land site (**Stand 3**). In **Stand 3**, 28 of 40 genets (70 %) were single tree occurrences. The mean number of trees per genet was 3-4 times greater on former forest land than on former agricultural land, respectively, 6.4 trees per genet in **Stand 1**, 5.2 in **Stand 2** (increased to 6.2 in the 4-year period after thinning) and 1.7 in **Stand 3** (Table 3).

3.4. Territorial clones (including > 1 tree): forest vs. agricultural land (Stand 1 & 2 vs. 3)

The maximum number of trees comprising a territorial genet was 24 (mean 8.8) in **Stand 1**, 29 (mean 6.3 trees) in **Stand 2**, which increased to 49 (mean 8.8 trees) after thinning, and only 8 (mean 3.4 trees) in **Stand 3**. Thus, the extent of clonality on the former agricultural land was 2-3 times less than on forest land.

The mean width of territorial genets was about 8 – 14 m (the maximum width 30 – 40 m) on former forest land (**Stand 1** and **2**), compared to mean width 5 m (the maximum width 12 m) on former agricultural land (**Stand 3**) (Table 3); thus, in the latter stand the territorial genets were 1.5- to 3 smaller on a width on the latter (not significant). The number of *H. parviporum* territorial clones was limited (2 clones) and the maximum width reached 2.8 m (Fig. 4). Comparisons of mean clone width prior to and after thinning in **Stand 2** demonstrated that the width increased significantly ($p < 0.001$) during 4 years after thinning from 7.8 to 10.3 m (Table 3).

207
208 The maximum rate of territorial clone active expansion per year in width, calculated from the
209 expansion of clones between the first and the second sampling in **Stand 2** (the maximum
210 distance of red dotted lines in Fig. 3), was almost nine times greater than that on agricultural land
211 in **Stand 3** (calculated by dividing the distance between the two furthest trees of a clone by 16
212 years and by 2 (infection centre assumed to be in the middle): 3.5 vs 0.4 m, respectively. Also
213 the mean rate of active expansion (0.9 vs. 0.15 m year⁻¹, respectively in **Stand 2** vs. **Stand 3**)
214 calculated from expansion of territorial clones on former forest land (mean of all red-dotted lines
215 in Fig. 3) was significantly ($p=0.0015$) higher (by approx. 6-fold) than on former agricultural
216 land (mean of all distances between trees comprising territorial clones, Fig. 4).

217
218 The maximum area of the territorial clones on the former forest land sites was ~7-8 times greater
219 (190 m² in **Stand 1**; 210 m² in **Stand 2**) than on the former agricultural land site, **Stand 3** (27
220 m²) (Table 3). The mean area of territorial clones was also larger (not significant; $p>0.05$) on
221 former forest land (~ 21 m² and 43 m²) than on former agricultural land (~ 11 m²), thus differing
222 by ~ 2 to 4-fold (Table 3). Significant differences were not found between the mean widths and
223 areas of territorial clones **Stand 1** and **Stand 3**, which may have been due to the low number of
224 observations (9 and 12, respectively), and high variation in the datasets (Table 3).

225 In **Stand 2**, the mean area occupied by genets increased significantly, from 30 to 41 m²
226 ($p=0.0016$) during the four years between the first and the second surveys (Table 3). The
227 maximum expansion rate in area from already established clones on forest land in the **Stand 2**
228 was ~ 16 times greater than that on the former agricultural land of **Stand 3**: 13.5 vs 0.8 m² year⁻¹,
229 respectively. The average area expansion rate in **Stand 2** (forest land, 2.3 m² year⁻¹) was

significantly ($p=0.0001$) greater (by ~8 times) than that in the **Stand 3** plantation on former agricultural land ($0.3 \text{ m}^2 \text{ year}^{-1}$).

3.5. *Pinus sylvestris*

A total of 170 *P. sylvestris* trees were surveyed in the study. The incidence of those showing dieback symptoms differed among the plots: 16 %, 0 % (2 %) and 50 % for **Stand 1**, **Stand 2** and **Stand 3**, respectively (Table 2). *Heterobasidion* conidiophores were observed and pathogen was isolated from five symptomatic *P. sylvestris* (Table 2, Figs. 2, 3 and 4). Of the 311 wood samples taken from *P. sylvestris* stumps of the previous generation and subjected to fungal isolations, only three (1%) of them yielded pure cultures of *Heterobasidion* (Table 2), while *Trichoderma* spp. and *Ascogoryne* spp. were frequently isolated (168 and 86 isolates, respectively). Each genet from previous generation stumps has been isolated also from next generation *P. contorta* (Figs. 2, 3).

4. Discussion

This study presents the most extensive data on *H. annosum* and *H. parviporum* root rot disease in plantations of *P. contorta* to date. For the first time, comparative investigations on population structures and expansion of *Heterobasidion* spp. were conducted in *P. contorta* stands.

4.1. Susceptibility of *P. contorta* to *Heterobasidion* and sources of new infections

This study provides new evidence that airborne basidiospores of both *H. annosum* and *H. parviporum* are able to colonise cut stumps of *P. contorta* (primary infections), and that following primary infection, the pathogens spread to adjacent trees via root contacts (secondary infections) (Figs. 2, 3 and 4). Numerous studies had reported that only freshly cut stumps unoccupied by other decay fungi are susceptible to colonisation by *Heterobasidion* spp., and that after 4 – 8 weeks they become non-accessible to the pathogen (Redfern and Stenlid 1998, and references therein). Consequently, our work provides new evidence, that *H. annosum* from diseased stumps of previous generation *P. sylvestris* can transfer to planted trees of the next generation of *P. contorta* (Figs. 2 and 3). As the previous generation *P. sylvestris* stumps were cut at least 26 years previous to investigations (according to age of replanted next generation, Table 1), our work demonstrates that *H. annosum* can persist and remain viable in stumps of cut mature *P. sylvestris* at least 26 years. This finding is comparable with earlier studies, which reported the longevity of *H. annosum* in conifer stumps to be 35 – 68 years (Greig and Pratt 1976; Piri 1996, and references therein).

4.2. Implications for silviculture

The findings of this study confirm the susceptibility of *P. contorta* to both *H. annosum* and *H. parviporum*, via both primary and secondary infections. These findings have silvicultural implications, for example, when planning reforestation on an infected site, scheduling a thinning (during high or low risk times of the year) or considering an option for stump treatment to prevent primary infections. For example, our results (Table 2) demonstrate that the disease can occur in plantations established on infection free areas. On former agricultural land (Stand 3), we

observed that 50% of trees had dieback symptoms, and 16 years after thinning 16% of all trees had *Heterobasidion* infection on the root collar. This was a higher incidence rate than on already infested forest land (**Stand 1** and **2** had 29 % and 17% trees with symptoms, 19 % and 13 % of all trees were confirmed to be infected, respectively). **Stand 3** had been thinned during the autumn, which is a high risk season for *Heterobasidion* infection in Latvia (Brauners *et al.* 2014), and stumps were not treated with preventive substances like urea or a spore suspension of the biological control agent *P. gigantea*.

4.3. Probable infections by *Heterobasidion*

In all **Stand**s, there was a large proportion of declining and dead pines in which the pathogen was not detected (Table 2). Therefore, the possibility cannot be excluded that a certain proportion of declining/dead trees, were dying due to other causes than *Heterobasidion*. Other possible causes of their decline have not been investigated. However, in the Stand 3, a large proportion of declining/dead trees were wind thrown (115 / 220 or 52%), which indirectly indicates that their root systems might have been damaged by *Heterobasidion* during the 16 years since thinning, but that pathogen has not yet reached the stump surfaces.

Fruitbody of *Heterobasidion* has been observed only on one out of 311 inspected (0.3%) previous generation stumps. In fact, this provides the evidence for low vitality of the pathogen in those stumps and explains low frequency of pure culture isolations. By contrast, fruitbodies of *Heterobasidion* were observed on 150 dead / declining trees (5 %, Table 2), constituting

comparatively fresh woody substrates. This indicates that environmental conditions on investigated sites were otherwise suitable for fruiting of *Heterobasidion*.

4.4. Territorial clones, size and rates of expansion

As *Heterobasidion* fungi possess a sexual reproductive system, each individual is genetically unique. Thus, each territorial clone must originate from a single fungal individual established within a single stump. Therefore, the current study provides evidence that the territorial clones and each from a single stump. The sizes of the clones (tens of meters in width) were comparable to those observed in previous studies on pre-infected forest sites, in plantations established on clear-cuts of both *P. abies* and *P. sylvestris*. It is also interesting to note that in a number of these studies transfer of the pathogen to different tree species was observed (Stenlid 1985; Lygis et al., 2004; Piri 1996; Piri and Korhonen 2001; Vasiliauskas and Stenlid 1998). Moreover, the data from **Stand 2** provides evidence of territorial expansion of *H. annosum* over the period of four years after a thinning (Fig. 3). The observation that newly expanded areas of infection were occupied by the same genotypes as those detected during the first sampling provides proof that these infections originated from old stumps of the previous generation of *P. sylvestris*, either directly, or by transfer from infected root systems of thinned *P. contorta*. The present work did not estimate the exact borders and size of *Heterobasidion* genets (for this thorough analyses of excavated root systems would be required), but approximated the extent of their occupied area and rates of spread. As only the stems have been sampled, our work generates minimum estimates of size and expansion rates of the observed genets, as shown in the Table 3 and Figures 2, 3 and 4.

Eight of ten new genets detected in **Stand 2** during the second sampling (Table 3) were confined to a single tree (Fig. 3), and thus might have originated from new primary infections from nearby cut stumps of thinned *P. contorta*, from which it was later transferred by root contacts. However, it is most likely that genets originated from the previous *P. sylvestris* rotation and were present in host tree, but were not detected during the first sampling. Such a scenario was previously described by Rönnerberg *et al.* (2006) and Piri and Korhonen (2008). In this regard, it has been reported that development of territorial clonality following primary infections of *Heterobasidion* takes at least 7 – 8 years (Vasilias 1989; Swedjemark and Stenlid 1993; Stenlid and Redfern 1998). In our study, the yearly territorial expansion on former forest land (**Stand 2**) was in agreement with estimations by Hodges (1969) and Rizzo *et al.* (2000), who reported spread of about 2 m year⁻¹ in forests of southern pines and cedar in USA. According to Bendz-Hellgren *et al.* (1999) the average growth rate is 25 cm year⁻¹ in spruce stump roots and 9 cm year⁻¹ in living tree roots, which is comparable with *Heterobasidion* spp. expansion rate in the **Stand 3**. Consequently, the four year time period monitored in this study was insufficient for development of territorial clonality of a new pathogen for more than 2 m. However, high planting densities in the **Stand 1, 2 and 3** could have enhanced expansion of *Heterobasidion* territorial clones.

The situation observed in **Stand 3** (established on infection-free former agricultural land) was markedly different, as the spread originated exclusively from primary infections (Fig. 4). In this **Stand 3** the area occupied by the clones was much smaller, trees encompassed by the clones were fewer in number, and the rate of expansion was significantly slower than was observed on the pre-infected forest sites of **Stand 1 and 2** (Fig. 4 and Table 3). Although earlier studies

indicate that spread of *Heterobasidion* spp. should be faster on previous agricultural land (Woodward *et al.* 1998, and references therein), our study found that the pathogen spread more quickly from stumps with established secondary infections, than from freshly cut stumps with primary airborne infection. The importance of infected previous generation stumps on infection transfer has been noted in earlier-studies (Stenlid 1987; Woodward *et al.* 1998; Bendz-Hellgren *et al.* 1999; Greig *et al.* 2001; Piri and Korhonen 2008, and references therein). In **Stand 2** the proportion of new trees with dieback and infection four years after thinning was once again equal to what was observed before thinning, therefore our results support earlier observations that spruce stumps left after thinning transmit root rot to-residual trees (Pettersson *et al.* 2003; Piri and Korhonen 2008). In general, lower numbers of trees in clones occupying a smaller stand area indicate that these clones are relatively younger than those comprised of more trees and covering a larger area, which corresponds well with history of the plantations included in this study (Swedjemarm and Stenlid 1993).

4.5. Transfer to next forest generation and to different tree species

This study demonstrates effective transfer of *H. annosum* from *P. sylvestris* to *P. contorta* (**Stand 1** and **2**). Previous studies on local population structure and territorial clones of biological species of *Heterobasidion* mainly focused on stands of *P. abies* previously infected by *H. parviporum*. In pioneering investigation conducted in Sweden, Stenlid (1985) conducted a study in a 120-year-old *P. abies* stand thinned approx. 30 and 15 years previously, and detected a number of territorial clones reaching up to 30 m in diameter, all of which belonged to *H. parviporum*. Similarly, up to approx. 40 m long territorial clones were observed in an

intensively thinned *P. abies* stands in Serbia, and all of them also consisted of *H. parviporum* (Keča and Keča 2013). In Finland, related studies investigated spread of *Heterobasidion* from infested *P. abies* stands to trees of the next forest generation (Piri 1996; Piri and Korhonen 2001), in which efficient transfer of the disease from the previous forest generation to subsequently established 10 – 53 year-old trees was observed. In that study maximal length of territorial clones in each study was varied between approx. 10 – 30 m and *H. parviporum* was isolated from over 98% of infected trees, and the rest were *H. annosum*.

Two studies on *H. annosum* investigated its interspecific transfer from previously grown tree species to another species of the subsequent forest generation. Vasiliauskas and Stenlid (1998) reported that 43.7% of all *Heterobasidion* isolates (38) obtained in a *P. abies* stand self-established on a clear-cut of *P. sylvestris* belonged to *H. annosum*, while the others to *H. parviporum*. Lygis et al. (2004) provided evidence for *H. annosum* transfer from an infested and clear-cut *P. sylvestris* to the next generation of planted *Betula pendula* Roth. In the first study, maximal diameter of a territorial clone of *H. annosum* was up to 20 m, while in the second its maximal diameter was 48 m.

5. Conclusions

In this study we examined 444 *Heterobasidion*-infected *P. contorta* trees in three Latvian *P. contorta* plantations subjected to different management regimes and, for the first time: detected 51 territorial clones of the pathogen; demonstrated secondary tree-to-tree infections by *H. annosum*; traced the infections to a previous generation of *P. sylvestris*; reported observations

of 40 disease centres initiated by primary airborne infections of both *H. annosum* and *H. parviporum*. The study generated new data on the ecology and patterns of spread of *Heterobasidion* spp. in *P. contorta* plantations. From this body of novel data we conclude that:

- 1) *Pinus contorta* is susceptible to root rot and dieback caused by *H. annosum* and *H. parviporum*;
- 2) airborne basidiospores of both pathogens colonise cut stumps of *P. contorta*, establishing primary infections;
- 3) following establishment in stumps and root systems through primary infections, secondary spread to adjacent trees results via root contacts;
- 4) *H. annosum* from diseased stumps of a previous generation of *P. sylvestris* can transfer to planted next-generation *P. contorta*;
- 5) *H. annosum* can persist and remain viable in stumps of cut mature *P. sylvestris* for at least 26 years;
- 6) infections by both *H. annosum* and *H. parviporum* can produce large (up to 263 m²) territorial clones causing extensive tree dieback and mortality;
- 7) development of territorial clones of *H. parviporum* proceeds at a slower rate than that of *H. annosum*;
- 8) when planning thinning of *P. contorta* plantations established on non-infested areas (e.g. agricultural land), stump treatment with agents that prevent primary infection by *Heterobasidion* (particularly urea) should be considered, as it was also more recently pointed out by Gonthier, (2019).

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Table 1. Description of investigated 26 – 31 year-old plantations of *Pinus contorta* (+ < 5% admixture of *Pinus sylvestris*).

Plantation / establishment & management	Est. on clear- cut ^a , unthinned: Stand 1	Est. on clear-cut ^a , unthinned before first sampling (2 nd sampling done 4 years after thinning) ^b : Stand 2	Est. on agricultural land, thinned 16 years previously, Stand 3
Location	57°06 N, 21°95E	56°68 N, 24°46E	56°60 N, 25°49E
Area, ha	0.2	0.8	0.2
Age, years	26	27 (31)	28
Tree DBH, cm	12	12 (15)	14
Planting density, ha ⁻¹	5000	5000	5000
Density at sampling, ha ⁻¹	2290	2986 (1471)	2350

^a Previous forest generation in **Stand 1** and **Stand 2** (established on clear-cuts) consisted of approx. 100 year-old *P. sylvestris*.

^b In this column, numbers in brackets are the data reflecting the second sampling in the **Stand 2**, where the first sampling was done in unthinned plantation, and second sampling was done four years after thinning.

Table 2. Tree dieback and infections by *Heterobasidion* spp. in 26 – 31 year-old plantations of *Pinus contorta* (+ < 5% admixture of *Pinus sylvestris*).

Plantation / establishment & management	Est. on clear-cut, unthinned: Stand 1	Est. on clear-cut, unthinned (2 nd sampling done 4 years after thinning) ^a : Stand 2	Est. on agricultural land, thinned 16 years previously: Stand 3
<i>Pinus contorta</i> trees			
Visually surveyed, total no.	439	2270 (1124)	438
Dieback/windthrown, sampled, no. / %	128 / 29	257/11 (120/11)	220 / 50
- 1 st & 2 nd sampling pooled	-	(377/17 C)	-
<i>Heterobasidion</i> -infected ^b , no. / %	84 / 19	192 / 9 (96 / 9)	72 / 16
- 1 st & 2 nd sampling pooled	-	(288 / 13)	-
- dieback symptoms and infected %	66	75 (80)	33
- 1 st & 2 nd sampling pooled	-	(76)	-
<i>Pinus sylvestris</i> trees			
Visually surveyed, total no.	19	119 (53)	32
Dieback symptoms, sampled, no. / %	3 / 16	0 / 0 (1 / 2)	16 / 50
<i>Heterobasidion</i> -infected ^b , no. / %	3 / 16	0 / 0 (1 / 2)	2 / 6
<i>Heterobasidion</i> fruitbodies, tree no. / %	36 / 8	96 / 4	18 / 4
<i>Pinus sylvestris</i> stumps from previous generation			
- sampled, no.	67	244	-
- <i>Heterobasidion</i> isolated, no. / %	2 / 3	1 / 0.4	-

^a In this column, numbers in brackets are the data reflecting the second sampling in the **Stand 2**, where the first sampling was done in unthinned plantation, and second sampling was done four years after it has been thinned.

^b Conidia of *Heterobasidion* observed on stem cross-section surface.

Table 3. Isolates, genets and territorial clones of *Heterobasidion* spp. in 26 – 31 year-old plantations of *Pinus contorta* (+ < 5% admixture of *Pinus sylvestris*).

Isolates obtained	Est. on clear-cut, unthinned: Stand 1	Est. on clear-cut, unthinned (2 nd sampling done 4 years after thinning) ^a : Stand 2	Est. on agricultural land, thinned 16 years previously: Stand 3
Total, no. (including previous generation stumps)	85	184 (97)	69
Isolations from infected trees, no. / %	83 / 95.4	183 / 95.3 (97 / 100)	69 / 93.2
Genets detected			
In a single tree, no. / no. ha ⁻¹	4 / 20	7 / 9 (15 / 19)	28 / 140
In 2 or more trees: territorial clones, no. / no. ha ⁻¹	9 / 45	28 / 35 (30 / 38)	12 / 60
Total no. / no. ha ⁻¹	13 / 65	35 / 44 (45 / 57)	40 (200)
Trees per genet, no.	6.4	5.2 (6.2)	1.7
Characteristics of territorial clones ^b			
Max. no. of trees / stumps per clone	24	29 (49)	8
Average no. of trees per clone	8.8	6.3 (8.8)	3.4
Max. width, m	39.9	29.5 (35.1)	11.7
Average width ^c , mean ± SD, m	13.5 ± 13.9 AB	7.8 ± 6.4 A (10.3 ± 8.0) B	4.9 ± 2.5 A
Max rate of expansion ^{c, d} , m year ⁻¹ / average, m year ⁻¹	-	3.5 / 0.9 ± 0.6 A	0.4 / 0.2 ± 0.1 B
Max. area, m ²	191	209 (263)	27
Average area ^c , mean ± SD, m ²	43 ± 63 ABC	21 ± 40 B (29 ± 50) C	11 ± 8 ABC
Max. rate of expansion ^c , m ² / average rate, m ² year ⁻¹		13.5 / 2.3 ± 3.0 A	0.8 / 0.3 ± 0.2 B

^a In this column, numbers in brackets are the data reflecting the second sampling in the **Stand 2**, where the first sampling was done in unthinned plantation, and second sampling was done four years after thinning.

^b approximate estimates on the extent of their occupied area and rates of spread.

^c Values followed by different letters indicate that the differences between the means are statistically significant (*Wilcoxon* signed-rank test, $p < 0.05$).

^d For the **Stand 2**, calculations are based on lengths of red dotted lines from Figure 2 divided by 4 years (since thinning), and in **Stand 3**, based on the distances between the trees of territorial clones in Figure 3 divided by 2 and by 16 years (since thinning).

Figure 1. Location of study sites.

Figure 2. Distribution of *Heterobasidion annosum* genotypes in the **Stand 1**.

Figure 3. Distribution of *Heterobasidion annosum* genotypes in the **Stand 2**.

Figure 4. Distribution of *Heterobasidion* spp. genotypes in the **Stand 3**.

Figure captions

Figure 1. Location of study sites.

Figure 2. Distribution of *Heterobasidion annosum* genotypes in the **Stand 1**.

H. annosum isolated from: ● – *P. contorta*, ▲ – *P. sylvestris*, – previous generation

P. sylvestris stumps (in online version coloured green).

Heterobasidion not isolated from: × – *P. contorta* with dieback symptoms and *Heterobasidion* conidiophores at stump, ○ – *P. contorta* with dieback symptoms.

The lines connect trees / stumps from which somatically compatible *H. annosum* strains were isolated. Two ellipsoid areas, shadowed grey, cover approximate areas of two *H. annosum* clones, origin of which has been traced to previous generation stumps. Note, that (for technical reasons in drawing ellipses) larger clone encircles also six trees with dieback symptoms from which the pathogen has not been isolated. For similar reason, the smaller clone encircles one tree with *H. annosum* belonging to adjacent clone.

Figure 3. Distribution of *Heterobasidion annosum* genotypes in the **Stand 2**.

H. annosum isolated from: ● – *P. contorta* at 1st sampling, ■ – *P. contorta*, at 2nd sampling (4 years since thinning), ▲ – current generation *P. sylvestris* at 2nd sampling, – previous generation *P. sylvestris* stump (in online version coloured green).

Heterobasidion not isolated from: × – *P. contorta* with dieback symptoms and *Heterobasidion* conidiophores at stump, *P. contorta* with dieback symptoms, ○ – at 1st sampling, □ – at 2nd sampling.

Continuous lines connect trees / stump from which somatically compatible *H. annosum* strains were isolated in 1st sampling, and the dash-dotted lines connect trees / stump from which somatically compatible *H. annosum* strains were isolated in 2nd sampling (in online version all symbols and lines from 2nd sampling coloured red).

Ellipsoid area, shadowed grey, covers approximate area of *H. annosum* clone, origin of which has been traced to the previous generation stump. Note, that (for technical reasons in drawing ellipse) the clone encircles also two trees with dieback symptoms from which the pathogen has not been isolated, and excludes one tree with *H. annosum* belonging to the encircled clone.

Figure 4. Distribution of *Heterobasidion* spp. genotypes in the **Stand 3**.

H. annosum isolated from: ● – *P. contorta*, ▲ – *P. sylvestris*.

H. parviporum isolated from: – ★ – *P. contorta*.

Heterobasidion not isolated: × – *P. contorta* with dieback symptoms and *Heterobasidion* conidiophores at stump, ○ – *P. contorta* with dieback symptoms.

The lines connect trees / stumps from which somatically compatible *H. annosum* or *H. parviporum* strains were isolated.