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Frequency of stump infections by *Heterobasidion annosum* s.l. and benefits from urea treatments vary with tree species and season in European Alpine forests

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Abstract

The destructive forest pathogen *Heterobasidion annosum sensu lato* (s.l.) may be controlled either by timing logging operations in periods of the year characterised by a low risk of airborne stump infection or by treating stump surfaces immediately after felling. 1616 stumps of four of the most widely-distributed European host tree species, either untreated or manually and operationally treated with urea, were sampled from 29 pure and mixed forest stands of the western Italian Alps and analysed for the presence of the pathogen. The aims of the study were to (i) compare the overall and seasonal frequency of stump infection of different tree species; (ii) assess the efficacy of treatments on different tree species; (iii) explore if the efficacy of treatment is affected by the season of application, and (iv) estimate the benefits resulting from treatments at different time periods. Overall, the frequency of infection of untreated stumps was significantly higher ($P < 0.05$) in Norway spruce (*Picea abies*) than in European larch (*Larix decidua*), silver fir (*Abies alba*) and Scots pine (*Pinus sylvestris*). In general, the frequency of stump infection of each tree species increased throughout the year, reaching a maximum in autumn, possibly in relation to the higher availability of airborne inoculum in that season. Manual operational stump treatment with urea reduced the frequency of infected stumps compared to controls both overall and for every tree species, although the reduction was significant only in the overall analysis and in the analysis performed separately for Norway spruce and larch. Binary logistic regression models point to the general efficacy of treatments regardless of the season of application, although the frequency of infection of treated stumps is predicted to increase slightly in autumn. Despite the efficacy of treatments being slightly reduced in autumn, the predicted reduction of stump infection probability due to treatments, hence the benefits provided by treatments, is expected to vary in magnitude depending on the species but to increase similarly according to a roughly sigmoid curve throughout the year, approaching a maximum in autumn. The information provided by this paper may help forest managers to design profitable and/or sustainable tactics to control the disease.

Keywords

Alps, conifers, control, epidemiology, fungal disease, host susceptibility, operational treatments, root and butt rots, spores, treatment efficacy.

1. Introduction

Root and butt rots caused by the fungal species complex *Heterobasidion annosum* (Fr.) Bref. *sensu lato* (s.l.) are among the most destructive diseases of conifers worldwide (Garbelotto and Gonthier, 2013). In Europe, the species complex comprises three native species, each characterised by a distinct host preference: *H. annosum sensu stricto* (s.s.) on pines (*Pinus* spp.) and other conifers and even broadleaves, *H. abietinum* Niemelä & Korhonen on firs (*Abies* spp.), including the widely distributed silver fir (*A. alba* Miller), and *H. parviporum* Niemelä & Korhonen on Norway spruce (*Picea abies* (L.) Karsten) (reviewed by Garbelotto and Gonthier, 2013). In addition, the North American *H. irregulare* Garbel. & Otrosina is also present as an alien invasive species in coastal pine and oak stands of central Italy (Gonthier et al., 2007; Gonthier et al., 2012b; Gonthier et al., 2014).

Regardless of the *Heterobasidion* species involved, infections occur by means of meiospores through freshly cut stumps or wounds (primary infection). Once established, *H. annosum* s.l. may infect uninjured neighbouring trees by growth of the mycelium through root contacts or grafts (secondary infection) (reviewed by Garbelotto and Gonthier, 2013). Due to its peculiar infection biology and its ability to exploit the presence of recently created stumps, the fungus is particularly destructive in intensively managed forests, especially in monoculture plantations established on former agricultural land (Korhonen et al., 1998). *H. annosum* s.l. is deemed to play a subordinate role in more natural forest ecosystems (Shaw et al., 1994). However, high levels of disease incidence have also been reported in mixed and naturally regenerated forest stands. For instance, disease incidence as high as 71% was reported in Norway spruce and European larch (*Larix decidua* Miller) subalpine mixed forest stands of the western Italian Alps (Gonthier et al., 2012a). In this area, where all three European native species of *H. annosum* s.l. are present (Gonthier et al., 2001), disease incidence has been reported to differ significantly from stand to stand depending on the size of the trees and on the host species, Norway spruce being the most severely affected host (44.2% of infected trees), followed by silver fir (17.6%), larch (12.0%) and Scots pine (*P. sylvestris* L.) (5.6%) (Gonthier et al., 2012a). While susceptibility to the disease has been reported to vary among tree species as a consequence of secondary infections (Vollbrecht et al., 1995; Greig et al., 2001; Gonthier et al., 2012a), the susceptibility of stumps of different species to primary infection has not yet been definitively determined, as all efforts carried out so far by inoculating the pathogen have given rise to variable and sometimes conflicting results probably reflecting differences in methodology, the availability of natural airborne inoculum and interactions with environmental factors at the time of inoculation (reviewed by Redfern and Stenlid, 1998). In addition, by relying on natural airborne infections, the comparative analysis of infection frequency would require stumps of different species either to co-occur in the same mixed forest stands or, at least, to be present in separate stands yet located in the same area consistently infested by the pathogen, a rather uncommon condition to be met. It should be noted that good knowledge of the susceptibility of stumps to airborne infections might allow for tactics to be implemented to control the disease in mixed stands.

Root and butt rots caused by *H. annosum* s.l. may be controlled in several ways (reviewed by Gonthier and Thor, 2013). In general, satisfactory levels of disease control may be achieved either by timing logging operations (e.g. cutting or thinning) in periods of the year characterised by a low risk of stump infection due to limited spore loads or by protecting stump surfaces immediately after felling with chemical or biological treatments (Gonthier and Thor, 2013). For instance, based on the seasonal availability of airborne inoculum or on the probability of stump infections, winter thinnings and winter or spring thinnings are recommended in northern European countries and in the Alps, respectively, to minimise the risk of stump infection (Brandtberg et al., 1996; Gonthier et al., 2005). Several products have been developed to treat pine or spruce stumps, including the biological control agent *Phlebiopsis gigantea* (Fr.) Jülich, sodium tetraborate decahydrate (borax), and urea (Holdenrieder and Greig, 1998; Pratt et al., 1998; Gonthier and Thor, 2013). When tested on Norway spruce stumps in the Italian Alps, all of the above treatments performed equally well (Nicolotti and Gonthier, 2005). Nevertheless, 30% urea solution was recommended for subsequent large scale operational treatments (Nicolotti and Gonthier, 2005) due to its long history of stump treatment in Europe (reviewed by Pratt et al., 1998) and its effects on non-target organisms inhabiting stumps that are moderate and only transient (Varese et al., 2003; Vasiliauskas et al., 2004). While the efficacy of urea on Norway spruce stumps is supported by a large body of literature (Brandtberg et al., 1996; Pratt et al., 1998 and references therein; Nicolotti and Gonthier, 2005; Thor and Stenlid, 2005) and is well-documented even in long-term experiments (Oliva et al., 2008), its treatment efficacy on stumps of other tree species has been poorly investigated, except for pine species, Sitka spruce (*Picea sitchensis* (Bong.) Carrière), hybrid larch (*Larix* × *eurolepis*) and Cilician fir (*Abies cilicica* (Antoine & Kotschy) Carrière) (Pratt et al., 1998; Lehtijärvi et al., 2011; Wang et al., 2012).

Another poorly understood aspect is whether treatment efficacy may be affected by the season of application. It has previously been shown that the performance of borates on artificially inoculated Sitka spruce stumps depended on the inoculum density of *H. annosum* s.l. (Pratt, 2000). As the availability of natural airborne inoculum has been reported to vary significantly throughout the year in the Alps (Gonthier et al., 2005), treatments performed in different seasons may differ in their efficacy levels. However, if and to what extent this may happen still requires elucidation.

An efficient and standardised application method is always crucial for the final outcome when performing treatments against *H. annosum* s.l., and this is particularly true for manual stump treatment programmes (Gonthier and Thor, 2013). While experimental efficacy trials are generally performed by researchers with the maximum standardisation and the highest stringency, the question of whether operational stump treatments, i.e. treatments performed with commercial products directly by workers, may be effective in reducing *H. annosum* s.l. primary infections has been determined only for mechanised stump treatments (Thor and Stenlid, 2005), but not yet for manual stump treatments.

This study was carried out in pure and mixed forest stands of the Aosta Valley Region, in the western Italian Alps, an area reported as consistently infested by *H. annosum* s.l. (Nicolotti and Gonthier, 2005; Gonthier et al., 2012a) and where manual operational stump treatments with 30% urea solution had been performed. The specific goals were: (i) to compare the overall and seasonal frequency of primary infection of stumps of different tree species native to the Alpine range; (ii) to assess the efficacy of operational stump treatment with urea on different tree species; (iii) to explore if and to what extent the treatment efficacy is affected by the season of application, and (iv) to determine, on a seasonal basis, the predicted reduction of stump infection probability due to the treatment so as to obtain an estimate of the benefits resulting from treatments at different time periods and depending on the species.

2. Materials and methods

The study was conducted over the period 2004-2007 during thinning operations in 29 naturally regenerated pure and mixed forest stands in the Aosta Valley, in the western Italian Alps. The main characteristics of forest stands and the periods of thinning and treatment are reported in Table 1. The treatments were performed manually, on different dates from early spring to late autumn depending on the stand (Table 1), with garden sprayers by forestry workers of the Aosta Valley Autonomous Region who had previously completed a specific training course based on the methods described by Nicolotti and Gonthier (2005). The 30% urea aqueous solution was prepared on the day of treatment using granular urea (F.lli Fissore, Carmagnola, TO). Immediately after felling, at least 20 randomly selected stumps per species per stand that did not show symptoms of incipient or advanced decay on their surface were uniformly sprayed and covered with an approximately 1 mm thick layer of urea solution, which corresponded to a dosage rate of approximately 1 l m^{-2} (Nicolotti and Gonthier, 2005). At least another 20 stumps per species per stand with the same characteristics as those indicated above were left untreated to serve as a control. To be recognisable for subsequent sampling, the treated and control stumps were labelled.

Two years after the treatments, the treated and control stumps were sampled by cutting a 2-3 cm thick disc 15-20 cm below the top surface of each stump as described by Nicolotti et al. (1999). To avoid the computation of possible infections originating from roots, discs from stumps displaying visible symptoms of decay on a cross-section at the root collar were discarded. The discs were transferred to the laboratory and incubated at room temperature for about two weeks in polythene bags containing wet blotting paper to promote the development of *H. annosum* s.l. in its anamorphic form (Garbelotto and Gonthier, 2013). After incubation, the discs were inspected under a dissecting microscope for the presence of the easily-recognisable conidiophores of the pathogen, as previously described (Nicolotti and Gonthier, 2005).

In order to gather information on the presence and airspora composition of *H. annosum* s.l. species during treatments, 10 healthy Norway spruce wood discs were exposed for 24 hours on one of the treatment days in each forest stand, in accordance with the wood disc exposure method as described by Gonthier et al. (2001). The discs, 10-13 cm in diameter and 1 cm thick, were prepared the day before exposure by spraying them, after bark removal, with 65% ethanol and placing them onto wet filter paper in Petri dishes. Open Petri dishes were placed on the ground at random sites within each forest stand. After exposure, the discs were sprayed with a benomyl solution and incubated in moist conditions for approximately 10 days at 20°C as described by Gonthier et al. (2001). Subsequently, the wood discs were inspected for the presence of conidiophores of the pathogen as described above. At least 5 randomly selected colonies per disc were isolated and identified at species level by using the polymerase chain reaction (PCR)-based methods described by Gonthier et al. (2003).

The frequency of infected stumps (%) was calculated as the ratio between the number of infected stumps, i.e. stumps bearing *H. annosum* s.l. conidiophores on the wood disc taken for analyses, and the number of stumps sampled for each i) host species, ii) timeframe, iii) host species within timeframe, iv) treatment, v) treatment within timeframe, vi) treatment within host species, and vii) treatment within host species and timeframe, this latter only for Norway spruce and larch. The timeframes were as follows: A- before 31 May, B- from 1 June to 31 July, C- from 1 August to 30 September, and D- after 1 October. The 95% confidence interval associated with the frequency of infected stumps was calculated as reported in Blaker (2000).

The frequency of stumps primarily infected by spores of *H. annosum* s.l. was compared among host species, among host species within timeframe and among timeframes within host species by contrasting the frequency of infected stumps with either χ^2 tests (with Yates' correction applied to 2 x 2 contingency tables) or Fisher's exact test depending on whether or not the assumptions for a reliable χ^2 asymptotic approximation were met (Agresti, 2001; Crawley, 2013). When multiple comparisons were performed, the resulting P-values were adjusted with the Benjamini and Hochberg correction (Benjamini and Hochberg, 1995).

The treatment efficacy was tested as described above by comparing the frequency of infection between all treated and control stumps, and between treated and control stumps within timeframe, host species or both. Data from sites where no infection was detected in both treated and control stumps were excluded from the analyses targeting treatment efficacy.

The probability of stump infection depending on the time of treatment was assessed by fitting to the frequency of infected stumps observed at each treatment time (expressed in number of days since 1 January) a binary logistic regression model (Hosmer and Lemeshow, 1989). Separate models were fitted to treated and control stumps, gathering the maximum likelihood estimates of coefficient β associated with the treatment time and of the intercept β_0, whose significance was assessed with the Wald's test (Hosmer and Lemeshow, 1989; Crawley 2013). The binary logistic models were compared to the corresponding null model by contrasting the associated Akaike information criterion (AIC) and AIC weight (AICw), while the whole model significance was assessed with the likelihood ratio test (Hosmer and Lemeshow, 1989; Wagenmakers and Farrell, 2004; Grueber et al., 2011; Crawley, 2013). The 95% confidence interval for the average predicted probability of stump infection was calculated after the models fitting (Crawley, 2013). The predicted reduction of stump infection probability due to treatment was assessed as a function of treatment time by calculating the difference between the infection probability modelled by the binary logistic regression equations fitted to the control and treated stumps. Binary logistic models were fitted to data from all species, and from Norway spruce and larch separately, excluding those sites where no infection was detected for both treated and control stumps.

All statistical analyses were carried out on R version 3.2.3 (R Core Team, 2015) with a significance threshold set to 5% (Crawley, 2013).

3. Results

A total of 1616 stumps were sampled; of these, 804 were treated stumps and 812 were control stumps. Regardless of their treatment status, 737 were of larch, 558 of Norway spruce, 203 of Scots pine, and 118 of silver fir. Isolates of *H. annosum* s.l. were obtained from wood discs exposed in 26 out of the 29 forest stands (Table 2). Based on the outcomes of the diagnostic PCR-based assay performed on those isolates, *H. parviporum* was present either alone or in co-occurrence with other species in 22 forest stands, *H. annosum* s.s. in 13 and *H. abietinum* in 5 (Table 2).

Detailed data on the infection frequency of treated and untreated control stumps in each forest stand are reported in Mendeley Data repository. Overall, the infection frequency of untreated control stumps was significantly higher in Norway spruce (23.1% infected stumps, i.e. stumps carrying *H. annosum* s.l. as a result of primary infection) than in the other tree species (Fig. 1). The second most infected species was larch (6.6%), followed by silver fir (3.4%) and Scots pine (1.9%). However, these last three species did not differ significantly from each other in terms of infection frequency (Fig. 1).

Norway spruce control stumps displayed higher infection frequency compared to the other tree species within all timeframes, although the differences were significant only in the last timeframe, i.e. from 1 October (Fig. 2). In general, the infection frequency of stumps of each tree species increased throughout the first to the last timeframe, although a substantial and significant increase was observed only in the last timeframe and exclusively for Norway spruce and larch (Fig. 2).

The treatment with urea substantially reduced the frequency of infected stumps compared to the controls overall (2.6% vs. 16.8%) and for every tree species (3.7% vs. 29.4% for Norway spruce, 2.9% vs. 12.3% for larch, 0% vs. 5% for Scots pine, 0% vs. 10% for silver fir) (Fig. 3). The reduction was significant in the overall analysis and in the analysis performed separately for Norway spruce and larch, while it was not significant for Scots pine and silver fir (Fig. 3).

Binary logistic regressions modelling the probability of stump infection (%) as a function of the treatment time in treated and control stumps are reported in Table 3. Overall, the probability of stump infection was significantly lower in treated stumps compared to control stumps within all timeframes (Fig. 4a). The probability of stump infection increased substantially and significantly in control stumps passing from the first two timeframes (less than 10.4% infected stumps) to the third (20.0% infected stumps), and to the final one (31.2% infected stumps), while in treated stumps a significant, although not substantial, increase was observed only when comparing the first two timeframes (less than 0.4% infected stumps) with the last one (6.6% infected stumps) (Fig. 4a). For Norway spruce, the probability of stump infection was also significantly lower in treated stumps compared to control stumps within all timeframes (Fig. 4b). While a substantial and mostly significant increase in the probability of stump infection through time was observed for the control stumps (from 11.4% to 52.6% infected stumps in the first and last timeframe, respectively), such increase was neither substantial (from 0.4% to 9.8% infected stumps), nor significant, for treated stumps (Fig. 4b). For larch, the probability of stump infection was significantly lower in treated stumps compared to control stumps only from 1 August to the end of the year (Fig. 4c). The probability of stump infection increased through time, although not significantly, both in control stumps and in treated stumps (Fig. 4c).

The predicted reduction of stump infection probability due to treatment ranged, depending on the period, from 5.4% to 24.6%, from 11.0% to 42.8%, and from 2.7% to 13.7% for all species, for Norway spruce and for larch, respectively, increasing up to a maximum that was reached during the last timeframe in all cases (Fig. 5a,b,c).

4. Discussion

In this study, conducted in the context of ordinarily programmed thinning of forest stands in the western Italian Alps, I have shown that, regardless of the host species, the frequency of stump infection by *H. annosum* s.l. increased throughout the host growing season, and mostly significantly in the last time period, with Norway spruce displaying significantly higher rates of infection compared to the other investigated tree species native to the Alpine range. Furthermore, I have shown that manual operational stump treatment with urea is effective on all species regardless of the season of application, with the benefits resulting from treatments being maximised at the end of the growing season and being greater for Norway spruce.

Most, if not all, studies previously conducted in the Alps on stump infection and control of *H. annosum* s.l. referred to Norway spruce (Nicolotti et al., 1999; Nicolotti and Gonthier, 2005). A very high frequency of infection of untreated stumps, often reaching 100%, was reported in those studies, which were conducted in summer in individual or in a few forest stands. The average infection frequency of untreated control stumps of Norway spruce during the same time period was lower in this study, suggesting that the infection frequency may vary from stand to stand and possibly from year to year. However, spores of *H. annosum* s.l. were successfully collected from wood discs exposed in the large majority of the investigated forest stands (26 out of 29, i.e. 90% of the investigated stands), supporting the notion that separate stands in the area are consistently infested by the pathogen. This finding concurs with previous reports on the occurrence of the disease in the area (Gonthier et al., 2012a; Vezzola et al., 2018). The widespread distribution of *H. annosum* s.l. in forest stands of the Aosta Valley may be due to soil factors and environmental conditions, previously reported as highly conducive to infections (Nicolotti and Gonthier, 2005). The large clearcuttings carried out during the 17^{th} and 18^{th} centuries in support of mining activities, as well as the selective cuttings commonly performed until recently over most of the forest surface (Nicco, 1997), may also have influenced the widespread distribution and consistent occurrence of the disease in the area, as previously suggested (Nicolotti and Gonthier, 2005; Gonthier, 2010).

The data presented here clearly show that Norway spruce stumps are significantly more frequently infected than stumps of larch, silver fir and Scots pine, in that order, and this was particularly evident starting from June. Interestingly, a similar ranking of susceptibility, with Norway spruce and Scots pine being, respectively, the most and least susceptible species, was documented in relation to active infection of trees from roots through secondary root-to-root contagion (Gonthier et al., 2012a). This finding may suggest that the host tree species' susceptibility to *H. annosum* s.l. infections may be driven by wood characters differentiating tree species (e.g. presence and relative abundance of extractives), the degree of vitality of host tissues playing either a subordinate role or a similar role for all the investigated host species.

Data on the frequency of stump infection at different time periods in this study clearly show that compared to northern European countries, where the probability of stump infection follows a bell shaped curve reaching a maximum in the summer (Brandtberg et al., 1996), in the Alps, the highest frequencies of stump infection are shifted to the end of the growing season. Such an infection pattern, which is evident for Norway spruce and larch, although not recognisable for silver fir and Scots pine - possibly due to the limited number of sampled stumps for these last two species - is fully consistent and overall overlaps with the seasonal availability of airborne inoculum of *H. annosum* s.l. previously described in the area (Gonthier et al., 2005). An analysis of environmental factors associated with spore deposition in the area revealed that the availability of airborne inoculum is correlated to the minimum air temperature rather than to the maximum air temperature or rainfall, this last parameter reaching approximately 700 mm per year (Gonthier et al., 2005). Spore deposition was reported to stop in autumn at a minimum monthly air temperature below +5°C and to resume the following year in late winter or spring when temperature exceeds +4°C (Gonthier et al., 2005). The minimum air temperature could also affect the rate of stump infection, although caution is needed with this claim as solid conclusions on the role played by environmental factors on stump infections by *H. annosum* s.l. would require specific experiments. Notwithstanding, findings reported in this paper strongly support the notion that the most risky periods for stump infections by *H. annosum* s.l. are those at the end of the growing season. Such a temporal pattern of stump infections may be common in the Alps, and possibly in areas of continental Europe, as previously suggested based on the availability of airborne inoculum (Gonthier and Thor, 2013).

A few practical guidelines to minimise the risk of stump infection may be drawn from the above observations. Firstly, cutting should be allocated in winter or in spring rather than at the end of the growing season. Secondly, cutting should preferably target tree species other than Norway spruce in mixed stands. While these recommendations may be inapplicable or impractical depending on the forest management system and objectives or because of the general climate of the area and/or technical constraints (e.g. snowy winters), they may have beneficial effects in lowering infections by *H. annosum* s.l. and hence they should be adopted whenever possible.

In this study, all the analyses on the frequency of stump infections were conducted at the species complex level (i.e. *H. annosum* s.l.). However, data on the pathogen's airspora composition during treatments clearly showed that, overall, all three European species of the pathogen were present in the study sites, either alone or in co-occurrence with other species, depending on the forest. It should be noted that the co-occurrence of different *H. annosum* s.l. species in the same site may have some epidemiological implications as it might boost fruiting, and hence sporulation potential of either species (Giordano et al., 2018). Notwithstanding, the overall data on the presence and pathogen's airspora composition in this study was not unexpected based on previous information on the occurrence of *H. annosum* s.l. species in the Alps (Gonthier et al., 2001). Since it has been reported that the different *Heterobasidion* species are characterised by different temporal patterns of spore deposition in the Alps (Gonthier et al., 2005) and that they may show different saprotrophic abilities in colonising wood, at least of Norway spruce (Daniel et al., 1998), stump infection and colonisation may be largely dependent on the *Heterobasidion* species present as airspora at the time of cutting in a given site. Further research is needed to address this issue, and this should include isolation and molecular typing of isolates from stumps at different times. The outcomes of this research may be pivotal to implementing practical guidelines more sophisticated than those reported in this paper, accounting for the *Heterobasidion* species involved rather than for the species complex.

Any comparison between the results of this study and those of other studies on the efficacy of urea against *H. annosum* s.l. (Brandtberg et al., 1996, Soutrenon et al., 2000; Nicolotti and Gonthier, 2005; Pratt and Redfern, 2001; Thor and Stenlid, 2005; Lehtijärvi et al., 2011; Wang et al., 2012) is to some extent hampered by differences in host species and in the treatment application methods, being operational in this study, and in the rate of infection of control stumps, not reaching 100% in this study. While the assessment of whether the efficacy of operational stump treatment may be reduced compared to that performed by researchers would require a specific experimentation, the data here presented clearly support the notion that manual operational stump treatments with urea perform rather well. In fact, they significantly reduced the infection rates compared to controls not only overall, i.e. considering cumulatively stumps of all tree species, and on Norway spruce, but also on European larch. This is the first report on the efficacy of urea treatments on European larch. Previously, treatment efficacy has only been demonstrated for hybrid larch (Wang et al., 2012). The treatment also reduced, although not significantly, infection rates on Scots pine and silver fir stumps compared to the controls. The failure in detecting significance is probably attributable to the low number of stumps treated for these species. To the best of my knowledge, this is one of the first reports on the use of urea against *H. annosum* s.l. on silver fir stumps. Taken together, these results concur with the general efficacy of urea treatments on stumps of the most widespread coniferous tree species native to Europe.

The probability of infection of treated stumps was not constant across the different time periods. In fact, the average infection rates slightly increased starting from August. This was true for Norway spruce, for larch and for all tree species, although significance was only detected in the comparison between stumps treated before August and after 1 October in the overall analysis. Thus, the probability of infection of treated stumps was greatest in the period of the year characterised by the highest probability of infection of the control stumps, suggesting that the efficacy of urea treatments may be slightly reduced in the presence of high levels of *H. annosum* s.l. airborne inoculum, notably occurring in autumn in the area (Gonthier et al., 2005). This interpretation is consistent with the previously reported reduction of efficacy of borates in Sitka spruce stumps experimentally inoculated with increasing inoculum concentrations of *H. annosum* s.l. (Pratt, 2000). The mechanisms of action of urea against these pathogens have been elucidated (Johansson et al., 2002): an increase in pH resulting in the inhibition of spore germination occurs soon after treatments, when urea is decomposed by host urease naturally present on stump surfaces. Therefore, it cannot be excluded that the reduction of efficacy of urea observed in this study at the end of the growing season could also be due to shifts in the urease production by the host and/or to the temperature. It should be noted that the pH increase after treatment was found to be strongly dependent on temperature (Johansson et al., 2002).

Despite the efficacy of treatments being slightly reduced in autumn, the predicted reduction of stump infection probability due to treatments, hence the benefits provided by treatments, are expected to increase according to a roughly sigmoid curve throughout the growing season, approaching a maximum in autumn, as a result of the concomitant increase in the probability of stump infection in the absence of treatments. This was true both in the overall analysis and in the analyses conducted separately for Norway spruce and larch. While the curves show similar trends regardless of the species, the curve-associated values differ, as they are much higher for Norway spruce than for larch, pointing towards greater benefits from treatments on the former than on the latter species. The information summarised by the curves, including this last observation, may have some practical significance, especially for production forests. In fact, depending on the quality of wood and the cost of treatment, it may help in defining time periods when treatments are not only effective, but also profitable and justified from an economic perspective.

5. Conclusions

The co-occurrence of four of the most important and widespread European coniferous tree species in pure or mixed forests relatively close to each other as well as of the three native *H. annosum* s.l. species make the Alpine area ideal for comparative analyses of stumps' susceptibility to pathogen airborne infections as well as of the performances of stump treatments. This paper provides evidence that stumps of Norway spruce are significantly more infected than stumps of the other tree species and that the frequency of stump infection increases throughout the growing season, reaching a maximum in autumn, possibly linked to the higher availability of airborne inoculum in that season. In addition, the results indicate the general efficacy of manual operational stump treatments with urea regardless of the tree species and season of application, although the infection frequency of treated stumps is predicted to increase slightly in autumn. The information provided by this paper, including that supporting the claim that the benefits resulting from treatments vary in magnitude depending on the species but increase similarly throughout the growing season, may help forest managers to design profitable and/or sustainable tactics to control the disease.

6. Data availability statement

All data used in this study have been made available in the article text, and Mendeley Data repository.

7. Competing interests statement

I declare no competing interests.

8. Acknowledgments

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Table 1- Main features of the forest stands hosting the treatment experiments against *Heterobasidion annosum* s.l. Treatments were performed in 2004-2005 and samplings to determine their efficacy two years later.

Stand code	Location	Forest parcel code	Forest parcel area (ha)	Longitude (m[1])	Latitude (m[1])	Elevation (m a.s.l.)	Average slope (°)	Average aspect (°[2])	Day[3] and year of cutting and treatment	Mean stand diameter at breast height (cm)	Tree species composition (%)
1	Arvier	Arpettaz p. 5	22.1	355375	5060217	1499	30	107	186, 2004	27	Norway spruce 28.0, larch 11.4%, silver fir 60.6%
2	Brusson	Estoul p. 69, 70	33.4	404365	5068448	1896	13	262	237, 2004	24	Norway spruce 10.0%, larch 85.0%, other species 5.0%
3	Challand St. Anselme	Col Tsecore p. 15	25.1	400199	5063996	1583	25	296	157, 2004	30	Norway spruce 19.5%, larch 36.6%, Scots pine 43.9%
4	Charvensod	Usellieres 9	15.1	369943	5062439	1606	28	123	307, 2004	30	Norway spruce 56.0%, larch 37.4%, Scots pine 2.6%, silver fir 1%, other species 3.0%
5	Courmayeur	Plan Cereux p. 90-91	67.2	342287	5075687	1536	32	190	296, 2004	36	Norway spruce 51.0%, larch 46.0%, other species 3.0%
6	Doues	Belvedere p. 18	11.5	368037	5077427	1828	17	344	231, 2004	32	Norway spruce 56.0%, larch 44.0%
7	Emarèse	Ru de Joux p. 3	17.2	398859	5063046	1085	14	216	225, 2004	28	Larch 60.0%, Scots pine 40.0%
8	Gignod	Ru Collet p. 4	13.2	363923	5072436	2008	19	30	203, 2004	31	Norway spruce 36.4%, larch 63.6%
9	La Salle	Planaval p. 117	10.9	350185	5070796	1719	25	158	162, 2004	35	Norway spruce 81.4%, larch 14.9%, Scots pine 0.1%, silver fir 3.6%

10	Pontboset	Bois de Fournier p. 17	7.3	396447	5050898	1319	40	298	171, 2004	28	Larch 90.0%, other species 10.0%
11	Pontey	Eaux Noires p. 6-7	38.5	389928	5064305	1421	32	83	275, 2004	31	Norway spruce 11.9%, larch 19.7%, Scots pine 6.5%, silver fir 42.4%, other species 19.5%
12	Pré St. Didier	Bois de Revers p. 16	34.2	341186	5065723	1686	30	78	184, 2004	35	Norway spruce 66.4%, larch 27.2%, silver fir 5.7%, other species 0.7%
13	Saint Vincent	Bois de Sapé p. 28-29	26.8	395818	5069389	1122	33	280	120, 2004	30	Norway spruce 0.4%, larch 5.7%, Scots pine 91.0%, other species 3.0%
14	Torgnon	Ru de Torgnon 33	14.8	388683	5075762	1960	25	357	257, 2004	31	Norway spruce 1.8%, larch 94.5%, other species 3.7%
15	Valtournanche	Promindoz 9	2.6	390284	5078814	2202	22	346	299, 2004	39	Norway spruce 80.0%, silver fir 20.0%
16	Aymavilles	Ozein p.11	8.3	363041	5060689	1294	27	122	111, 2005	24	Norway spruce 54.3%, larch 13.9%, Scots pine 31.7%, other species 0.1%
17	Challand Saint Anselme	Fontaine p.22	19.0	399442	5062169	1191	26	281	108, 2005	26	Norway spruce 1.1%, larch 25.8%, Scots pine 71.7%, other species 1.4%
18	Challand Saint Victor	Chiosun p. 12	4.2	403266	5061116	1823	42	91	200, 2005	35	Larch 100.0%
19	Cogne	Crétaz p. 21	8.2	370730	5051861	1761	28	44	185, 2005	26	Norway spruce 76.9%, larch 23.1%
20	Doues	Belvedere p. 19	10.9	368010	5077025	1700	31	271	145, 2005	33	Norway spruce 90.9%, larch 9.1%
21	Etroubles	Bioley p. 17	5.5	363402	5073626	1534	21	97	233, 2005	30	Norway spruce 87.4%, larch 8.4%, other species 4.2%

22	Montjovet	Gettaz p. 1	16.4	395854	5063240	858	42	341	258, 2005	28	Larch 2.5%, Scots pine 22.5%, other species 75.0%
23	Morgex	Arpy p. 30	12.7	345231	5067280	1894	29	130	232, 2005	38	Norway spruce 74.0%, larch 25.1%, silver fir 0.7%, other species 0.2%
24	Pila	San Grato p. 16-17-18	56.0	369920	5061036	1971	27	133	252, 2005	30	Norway spruce 42.4%, larch 30.1%, Scots pine 0.5%, other species 27.0%
25	Pontey	Eau Noire p. 3	15.0	389054	5064322	1452	28	69	292, 2005	27	Norway spruce 3.4%, larch 14.2%, Scots pine 4.2%, silver fir 70.0%, other species 8.2%
26	Saint Oyen	Plantaluc p. 5	16.5	360181	5076263	1550	31	288	123, 2005	36	Norway spruce 23.4%, larch 76.6%
27	Saint Vincent	Fremies p. 16-17	41.7	397911	5068962	1622	22	234	147, 2005	32	Norway spruce 69.2%, larch 8.9%, Scots pine 21.6%, other species 0.4%
28	Torgnon	Bois de Maisonette p. 15	5.4	388324	5071545	1681	31	67	306, 2005	28	Norway spruce 58.2%, larch 37.1%, Scots pine 3.7%, other species 1.0%
29	Verrayes	Col Bornel p. 10	26.1	385849	5071875	1861	31	291	228, 2005	37	Norway spruce 27.5%, larch 47.9%, Scots pine 24.6%

[1] easting and northing are expressed in m based on UTM WGS84 zone 32N
[2] ° cunterclockwise angle from East assumed as origin 0°
[3] for each year, day counted from 1 January

Table 2- Presence and airspora composition of *H. annosum* s.l. species during cutting and stump treatment in 29 forest stands of the western Alps. For stand codes, refer to table 1.

Stand code	Successfully isolated *H. annosum* s.l. colonies	Isolates identified as *H. abietium*	Isolates identified as *H. annosum* s.s.	Isolates identified as *H. parviporum*
1	0			
2	5			5
3	4		2	2
4	25		7	18
5	7			7
6	22		3	19
7	3		3	
8	17			17
9	7	1		6
10	3		1	2
11	4	2		2
12	3	2		1
13	6		6	
14	5		4	1
15	27	4		23
16	3			3
17	5		5	
18	0			
19	8			8
20	4		1	3
21	7		2	5
22	3		3	
23	2			2
24	9		2	7
25	32	19		13
26	2			2
27	4		2	2
28	7			7
29	0			

Table 3- Binary logistic regressions modelling the probability of stump infection (%) as a function of the treatment time in control (C) and treated (T) stumps. The probability of stump infection was modeled for all species (Overall), Norway spruce (NS) and Larch (L). Within each treatment, the null model and the model including the treatment time as independent variable are reported along with: the maximum likelihood estimates of the intercept (β_0) and of the treatment time coefficient (β); the coefficients standard errors (SE); the Wald's test *P* value; the model Akaike information criterion (AIC) and AIC weight (AIC_w); and the likelihood ratio test (LRT) P value. Asterisks next to the β or β_0 values indicate significant ($P < 0.05$) Wald tests outcomes, while asterisks next to the model label "treatment time" indicate significant ($P < 0.05$) likelihood ratio tests.

Species	**Treatment**	**Model**	β_0	**SE(β_0)**	***P*(β_0)**	β	**SE(β)**	***P*(β)**	**AIC**	**AIC_W**	***P*(LRT)**
Overall	C	null	-1.599*	$1.12\cdot10^{-1}$	<0.001	-	-	-	160.62	0	<0.001
		treatment time*	-4.494*	$4.90\cdot10^{-1}$	<0.001	$1.273\cdot10^{-2}$*	$1.96\cdot10^{-3}$	<0.001	114.08	1	
	T	null	-3.607*	$2.71\cdot10^{-1}$	<0.001	-	-	-	57.17	0	<0.001
		treatment time*	-10.252*	2.23	<0.001	$2.607\cdot10^{-2}$*	$7.81\cdot10^{-3}$	<0.001	38.03	1	
NS	C	null	-0.876*	$1.45\cdot10^{-1}$	<0.001	-	-	-	90.74	0	<0.001
		treatment time*	-3.979*	$6.09\cdot10^{-1}$	<0.001	$1.409\cdot10^{-2}$*	$2.56\cdot10^{-3}$	<0.001	58.38	1	
	T	null	-3.272*	$3.60\cdot10^{-1}$	<0.001	-	-	-	31.21	$1.30\cdot10^{-2}$	<0.001
		treatment time*	-8.696*	2.26	<0.001	$2.227\cdot10^{-2}$*	$8.08\cdot10^{-3}$	$5.868\cdot10^{-3}$	21.60	$9.87\cdot10^{-1}$	
L	C	null	-1.963*	$2.14\cdot10^{-1}$	<0.001	-	-	-	47.52	$3.00\cdot10^{-2}$	$1.416\cdot10^{-3}$
		treatment time*	-5.748*	1.40	<0.001	$1.484\cdot10^{-2}$*	$5.16\cdot10^{-3}$	$4.02\cdot10^{-3}$	39.33	$9.70\cdot10^{-1}$	
	T	null	-3.502 *	$4.14\cdot10^{-1}$	<0.001	-	-	-	25.64	$6.30\cdot10^{-2}$	$3.309\cdot10^{-3}$
		treatment time*	-13.809*	5.36	$9.960\cdot10^{-3}$	$3.757\cdot10^{-2}$*	$1.82\cdot10^{-2}$	$3.903\cdot10^{-2}$	19.01	$9.37\cdot10^{-1}$	

Figure Captions

Fig. 1. Frequency of stumps of different tree species native to the Alpine range primarily infected by spores of *H. annosum* s.l. For each host species, the dotchart shows the observed frequency of infected stumps (% - black dots) along with its associated 95% confidence interval (lateral whiskers). Different letters indicate significant ($P < 0.05$) differences among frequency values.

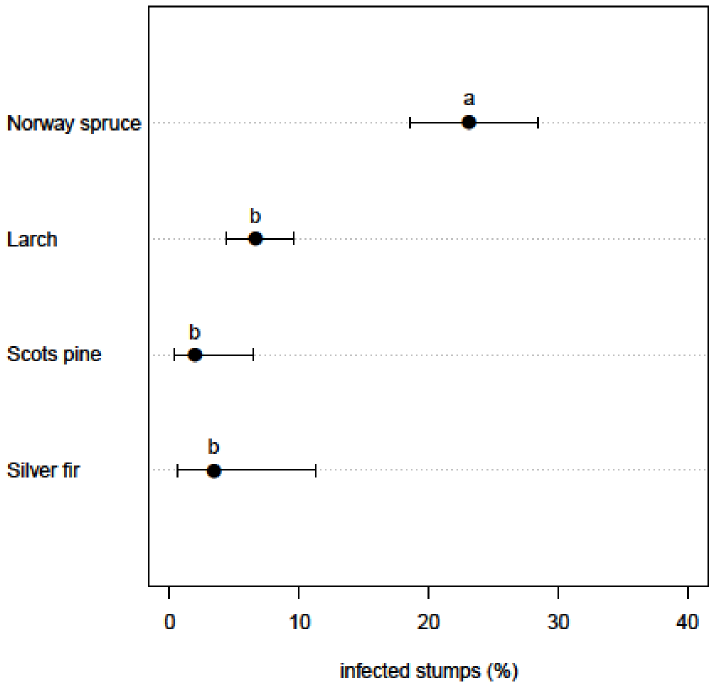
Fig. 2. Frequency of stumps of different tree species primarily infected by spores of *H. annosum* s.l. at different timeframes. For each host species and timeframe, the barplots show the observed frequency of infected stumps (% - vertical bars) along with its associated 95% confidence interval (vertical whiskers). Different letters indicate significant ($P < 0.05$) differences of the frequency values among host species within timeframe, while different integers point out significant ($P < 0.05$) differences detected among timeframes within host species. Capital letters above each barplot indicate the timeframes (A- before 31 May, B- from 1 June to 31 July, C- from 1 August to 30 September, and D- after 1 October). NA indicates data not available.

Fig. 3. Efficacy of stump treatment with urea against *H. annosum* s.l. on different tree species. The barplots show the frequency of infection (% - vertical bars) along with its associated 95% confidence interval (vertical whiskers) comparing treated and control stumps overall and for each tree species. Different letters indicate significant ($P < 0.05$) differences between infection frequency attained by treated and control stumps.

Fig. 4. Observed frequency/average probability of stump infection (%) depending on the timeframe/time of the treatment. The overall frequency of infection observed (% - dots) along with its associated 95% confidence interval (vertical whiskers) is reported for each timeframe (A- before 31 May, B- from 1 June to 31 July, C- from 1 August to 30 September, and D- after 1 October). Different letters indicate significant ($P < 0.05$) differences between the frequency of infection attained by control and treated stumps within each timeframe, while different one-digit integers point out significant ($P < 0.05$) differences detected among timeframes within treatment. The ascending curves plot the probability of stump infection for treated and control stumps as a function of the time of the treatment (expressed in number of days since January 1st), according to their associated binary logistic regression models. The region comprised between the contours above and below each curve delimits the 95% confidence interval of the predicted probability. Logistic curves are plotted for all species (a) and separately for Norway spruce (b) and larch (c).

Fig. 5. Predicted reduction of stump infection probability (%) due to the treatment as a function of the treatment time (expressed in number of days since 1 January). Capital letters indicate their associated timeframe (A- before 31 May, B- from 1 June to 31 July, C- from 1 August to 30 September, and D- after 1 October). The curves are plotted for all species (a) and separately for Norway spruce (b) and larch (c).

Figure 1



Norway spruce
Larch
Scots pine
Silver fir
a
b
b
b
0
10
20
30
40
infected stumps (%)

Figure 2

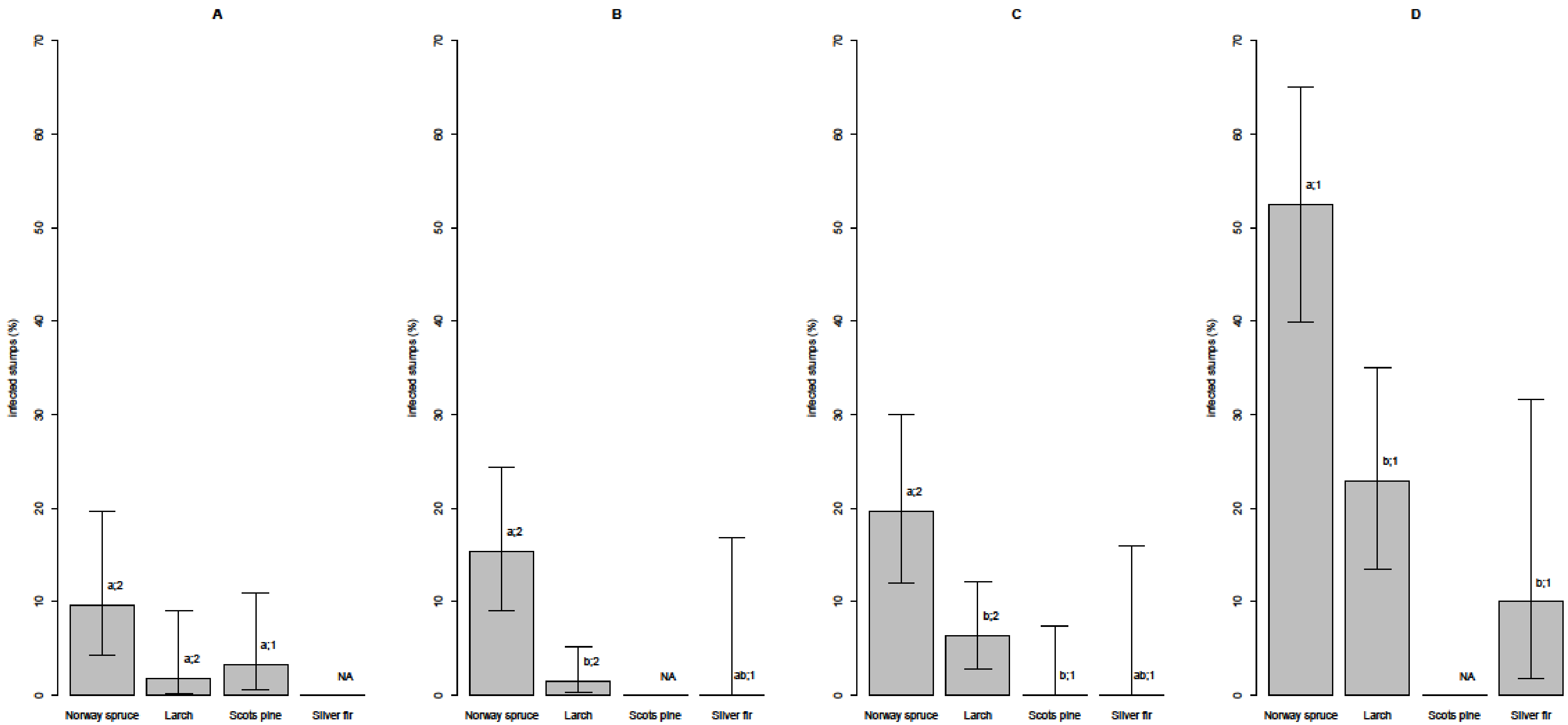
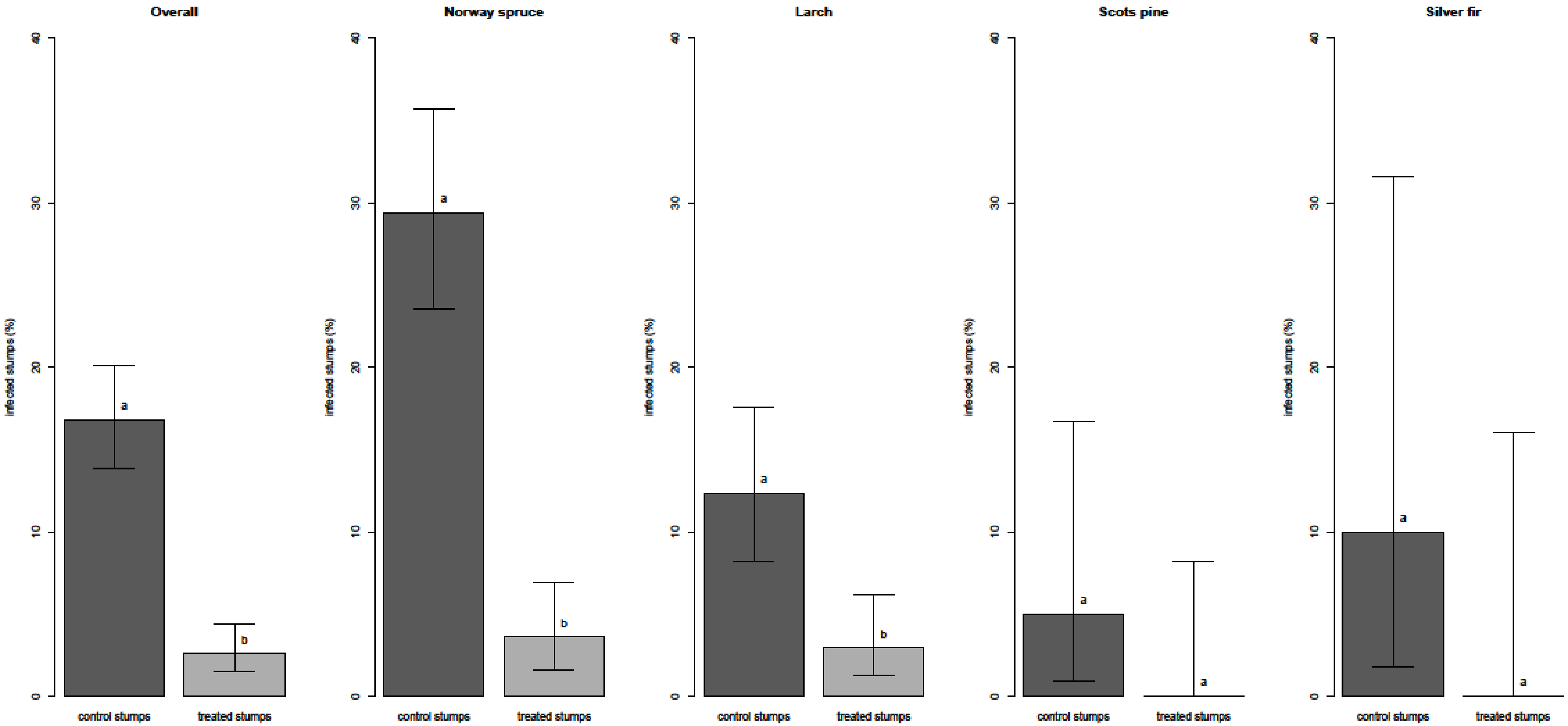
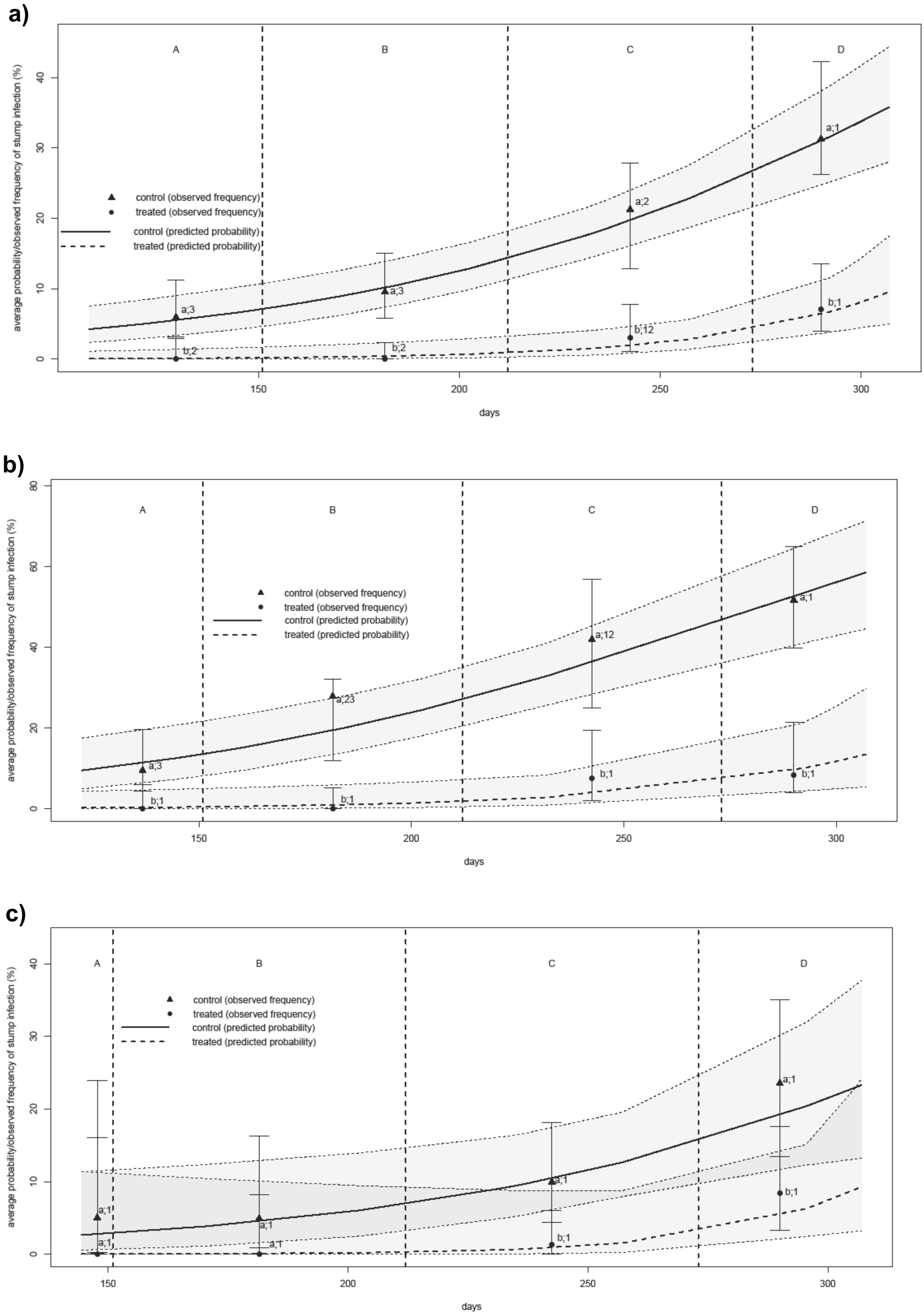

A
Infected stumps (%)
0
10
20
30
40
50
60
70
a;2
a;2
a;1
NA
Norway spruce
Larch
Scots pine
Silver fir
B
Infected stumps (%)
a;2
b;2
NA
ab;1
Norway spruce
Larch
Scots pine
Silver fir
C
Infected stumps (%)
a;2
b;2
b;1
ab;1
Norway spruce
Larch
Scots pine
Silver fir
D
Infected stumps (%)
a;1
b;1
NA
b;1
Norway spruce
Larch
Scots pine
Silver fir

Figure 3



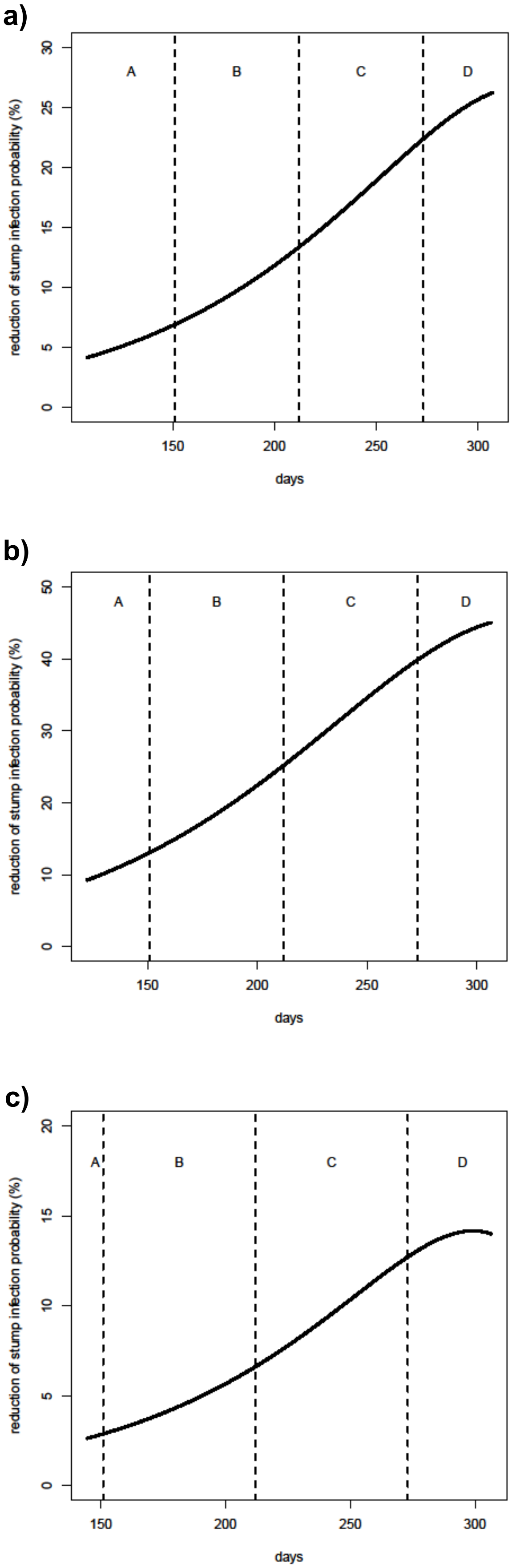
Overall
Norway spruce
Larch
Scots pine
Silver fir
infected stumps (%)
0
10
20
30
40
a
b
control stumps
treated stumps

Figure 4



a)
A
B
C
D
control (observed frequency)
treated (observed frequency)
control (predicted probability)
treated (predicted probability)
average probability/observed frequency of stump infection (%)
days
b)
A
B
C
D
control (observed frequency)
treated (observed frequency)
control (predicted probability)
treated (predicted probability)
average probability/observed frequency of stump infection (%)
days
c)
A
B
C
D
control (observed frequency)
treated (observed frequency)
control (predicted probability)
treated (predicted probability)
average probability/observed frequency of stump infection (%)
days

Figure 5



a)
A
B
C
D
reduction of stump infection probability (%)
0
5
10
15
20
25
30
150
200
250
300
days
b)
A
B
C
D
reduction of stump infection probability (%)
0
10
20
30
40
50
150
200
250
300
days
c)
A
B
C
D
reduction of stump infection probability (%)
0
5
10
15
20
150
200
250
300
days