Susceptibility of Silver Birch Pruning Wounds to Infection by White-Rot Fungus (*Chondrostereum purpureum*), a Potential Bioherbicide

Henna Vartiamäki, Jarkko Hantula and Antti Uotila

We artificially inoculated pruning wounds of silver birch (*Betula pendula* Roth) to study seasonal variation in their vulnerability to infection by the fungal decomposer *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar. This information is critical to the assessment of incidental infection risks in areas where *C. purpureum* may be used as a bioherbicide. On seven monthly occasions between April and October 2005, 30 birch trees were pruned to yield a total of 210 experimental trees. On each occasion, 10 trees were inoculated immediately with *C. purpureum* mycelium, 10 were inoculated with blank inoculum and 10 were only pruned. In the summer of 2007, a survey of 129 experimental trees showed that pruning wounds were most susceptible to infection during May. Treatment with *C. purpureum* at other times during the growing season also increased the extent of discoloration or decay but the effect was considerably less.

Keywords biological control, vegetation management, fungal decay, pruning, birch

Addresses Vartiamäki and Hantula, Finnish Forest Research Institute, Vantaa Research Unit, P.O. Box 18, FI-01301 Vantaa, Finland; Uotila, University of Helsinki, Hyytiälä Forestry Field Station, Hyytiäläntie 124, FI-35500 Korkeakoski, Finland

E-mail henna.vartiamaki@metla.fi

Received 28 January 2009 Revised 24 March 2009 Accepted 10 June 2009

Available at http://www.metla.fi/silvafennica/full/sf43/sf434537.pdf
1 Introduction

Fast growing broad-leaved trees cause problems in young coniferous stands, under power lines and alongside roads and railways. Because the use of herbicides is discouraged or illegal, most situations where broad-leaves are a problem are mechanically cut. Cutting is a temporary and increasingly expensive management tool once begun, and the search for effective and environmentally neutral alternative is ongoing priority. Recently, use of white-rot fungus (Chondrostereum purpureum (Pers. ex Fr.) Pouzar) as a biocontrol agent has been investigated. Dutch (Scheepens and Hoogerbrugge 1989, De Jong 2000), Canadian (Wall 1990, Wall 1994, Gosselin 1996, Dumas et al. 1997, Jobidon 1998, Shamoun and Hintz 1998, Harper et al. 1999, Pitt et al. 1999) and Finnish (Vartiamäki et al. 2008, Vartiamäki et al. 2009) researchers have collectively shown that C. purpureum can be used to control sprouting in many broad-leaves by placing fungal mycelium on freshly cut stumps.

As a potential biocontrol agent, C. purpureum is attractive because it has a broad host range and is globally distributed (Rayner and Boddy 1986). Ideally, a good biocontrol agent has a low risk of incidental infection in the treatment area. The use of C. purpureum mycelium as a biocontrol agent has a low risk of incidental infection (Becker et al. 1999a, b) but basidiospores released by mature sporophores would represent a source of infective material to nearby non-target trees (De Jong et al. 1990a, b, De Jong 1992, Gosselin et al. 1999, Wall 1994, Wall 1997, Vartiamäki et al. 2008, Vartiamäki et al. 2009). Chondrostereum purpureum can establish sporophores within a few months and fructificate continuously for up to 2 years (Setliff and Wade 1973, Dye 1974, Wall 1997, Vartiamäki et al. 2008, Vartiamäki et al. 2009). However, given that most basidiospores are disseminated locally and their dispersal over long distances is unlikely, it has been suggested that C. purpureum poses little threat to non-target, unwounded trees and shrubs more than 500m from the treatment area (De Jong et al. 1990a, b).

Although undamaged trees are relatively safe from infection, wounded trees are vulnerable to basidiospores. Storm damage as well as pruning programs can result in non-target trees having exposed tissue during the treatment period or soon thereafter. Silver birch (Betula pendula Roth) is the most important broad-leaved cultivar in Finland (Niemistö et al. 2008). In low-density birch plantations (ca. 1600 seedlings per hectare), stem quality is typically poor due to the branchiness of resulting trees (Niemistö 1995). To reduce branchiness, forest managers have pruned birches in Finland since the 1930s (Lappi-Seppälä 1934, Laitakari 1937, Lehonkoski 1949). Given this practice, a biocontrol program using C. purpureum adjacent to a pruned plantation might represent the worst-case scenario with respect to incidental infection risk and commercial loss.

Pruning live sapwood damages xylem vessels and exposes easily infected tissues. Trees have developed defense mechanisms against desiccation as well as microbial infection. Wounding stimulates local parenchyma cells to form a barrier to protect the living tissue. This suberization process begins in the radial and axial parenchyma cells near the wound, and damaged vessels eventually become filled with fibril plugs and fibers to form a protective layer (Schmitt and Liese 1990, Schmitt and Liese 1991, Schmitt and Liese 1993). Pruning live wood can reduce timber quality and the risk is directly related to the size of the wound (Heiskanen 1958, Vuokila 1982, Rintala 1995). However, pruning dead branches does not appear to affect health or timber quality in birch, if it is done without damaging the stem bark.

Because physiological state and vulnerability to infection are known to vary during the year, question remain concerning the optimal pruning period when target trees can be effectively controlled but the risk of incidental infection is minimized. Many studies have shown that pruning should be avoided during peak periods of sap transportation (i.e., in spring) when phloem release more easily (Schöning 1935, Heikinheimo 1936, Heiskanen 1958, Vuokila 1976, Vuokila 1982). In order to minimize infection risk, several authors (Vuokila 1976, Verkasalo and Rintala 1998) recommended pruning birch during the early-mid summer when wounds heal faster (Biggs, 1987). If pruning takes place in the late summer or fall, potential pathogens enjoy longer infection periods because callus formation is slower (Vuokila 1976, Verkasalo and Rintala 1998).

Mechanical damages by wounding and subse-
quent microbial attack can be seen as a discolouration or decay. Shigo and Marx (1977) and Shigo (1984) formulated the Compartmentalisation of Decay in Trees (CODIT) hypothesis in which discolouration following wounding was interpreted as barrier tissue to confine the spread of micro-organisms. Because wood decay is a gradual and continuous process, discoloration and the early stages of hard rot are difficult to discriminate by visual inspection (Rayner and Boddy 1988).

This study aims to determine the period(s) during the growing season when birch pruning wounds are most susceptible to infection by the decay fungus *C. purpureum*. This information will enable the risk of incidental infection to be evaluated and the efficacy of a biocontrol program using *C. purpureum* to be maximized. Results will provide the first exploration of seasonal vulnerability of birch pruning wounds to infection by decay fungi and identify the safest time for pruning in general.

2 Material and Methods

2.1 Fungal Isolate and Inoculum for the Field Experiments

The dikaryotic isolate (P3) of *C. purpureum* used in the field experiment was originally isolated from silver birch in Vantaa, southern Finland. It was isolated in 2005 from a piece of basidiocarp on potato dextrose agar (PDA) Petri plate. P3 was selected for the field experiment because of its fast growth rate on different nutrient agar media and under various temperatures (unpublished data). The fungal inoculum for the field experiments consisted of 12 g of potato dextrose broth (Becton Dickinson) and 10 g Sipernat 22s (Evonik Degussa) added to 500 ml of distilled water and autoclaved at 121 °C for 15 min. The medium was inoculated with *C. purpureum* mycelia taken from the edge of a 7-day-old culture growing on a PDA cellophane plate, and subsequently incubated for 8–10 days in the dark at 20 °C on a rotation shaker (100 rpm). The inoculum was homogenized using an Ultra Turrax apparatus and diluted 1:10 with tap water immediately before application. Viability and purity of the inoculum was confirmed before and after application with the most probable number method (MPN) (Harris and Sommers 1968).

2.2 Pruning Stand and Field Treatments

The pruning stand was located in Ruovesi, central Finland, about 200 km north of Helsinki. It was a 20-year-old silver birch plantation and classified as Oxalis-Myrtillus type in the Finnish forest site classification system (Cajander 1949). A total of 210 experimental trees were treated on seven monthly occasions between April 14 and October 12 in 2005. On each of these occasions, 30 trees that were free of existing wounds and with at least five live and five dead branches were pruned and marked with numbered aluminum tape. Branches were pruned with a pruning saw as close to the stem as possible without damaging the branch collar. The border of dead and live branches was marked by cutting off the lowest live branch to leave a long stub. The pruning wounds of ten trees were immediately inoculated with a suspension of *C. purpureum* mycelium delivered via a spray bottle (approximately 1 ml containing an average of $3.4 \times 10^6$ CFU/ml), ten trees were inoculated with blank inoculum (formulation control) and the remaining ten trees were only pruned (control).

2.3 Analyses of the Experimental Trees

In June 2007 (two years after inoculation), 129 experimental trees were felled and the effects of test and control treatments were evaluated. The remaining experimental trees (81) were left standing for the future analysis of long-term effects. Before trees were felled, the trunk diameter at breast height was measured. Total height, pruning height and the height of the border of dead and live branches were all measured after felling. Felled trees were divided into two different groups depending on their subsequent analysis as either stem sample trees or branch sample trees. Proportion of discolored or decayed area in stem cross-section was investigated from the stem sample trees and spread of the discoloration/decay through the pruning wounds was investigated from the branch sample trees.
2.3.1 Stem Sample Trees

A total of 87 stem sample trees were felled in June 2007: four trees per each treatment on each pruning dates, except five trees for *C. purpureum* treatments in September and October and the formulation control treatment in October. From the trunk of each felled tree, discs were cut at 50 cm intervals (maximum height of 350 cm) and immediately debarked, uniquely labeled and stored individually in plastic bags at 4 °C until laboratory analysis. In the laboratory, discs were scored for their stage of decay using the following classification: 0 = no decay, 1 = discoloration or hard rot, 2 = soft rot. The total area of the disc and the proportion of discolored or decayed area was calculated with a Planimeter Tamaya Digitizing Area-Line Meter PLANIX10S “Marble” (Tamaya Technics Inc.).

At least four separate samples of *C. purpureum* were isolated from the discolored/decayed area of each disc. Approximately 0.5 cm² thin slices of wood were taken from the discolored/decayed area and placed on a 90 mm Petri plate containing 1% PDA (Becton Dickinson) culture medium. Plates were incubated at 25 °C in the dark for at least 48 hours before microscopically determining the presence of *C. purpureum* according to Eriksson and Ryvander (1976).

2.3.2 Branch Sample Trees

A total of 42 branch sample trees were felled in June 2007: two trees of each treatment on each pruning date. Depending on treatment and pruning date, 14–19 discs per stem were cut from pruning wounds. Stem height and whether the disc originated from a pruning wound in a dead or live branch was recorded in the field. Discs were debarked, uniquely labeled and stored individually in plastic bags at 4 °C until laboratory analysis. In the laboratory, the diameter of pruned branches was measured and the spread of the discoloration/decay through the pruning wounds was scored as follows: 1 = decay had not spread through the wound; 2 = decay had spread to the core of the pruned branch and to the surrounding living tissue.

2.4 Statistical Analyses

Data were exposed to an analysis of variance (ANOVA) in SPSS (version 16.0). In stem sample trees, the effects of treatment and pruning date on the proportion of discolored/decayed discs and the discolored/decayed area of discs was tested by a two factorial ANOVA. In branch sample trees, the effects of the treatments, pruning date and whether the pruned branch was dead or live on the spreading of discoloration/decay through the pruning wounds to surrounding living tissue were similarly explored. If the treatment had a significant effect (p < 0.05) it was broken down into two single orthogonal contrasts: formulation control vs. control, and *C. purpureum* treatment vs. no *C. purpureum* treatment (average of both type of controls). Significant differences (p < 0.05) between different combinations of treatments and pruning dates were exposed to Tukey’s post hoc test.

3 Results

3.1 Description of Examined Trees

The average height of the felled trees was 9.7 m (6.4–13.2 m) and the average breast height diameter was 10.0 cm (6.3–15.9 cm). The average pruning height was 2.8 m (1.8–4.3 m) and the height of the border of dead and live branches averaged at 1.6 m (0.3 m–2.6 m). For stem sample trees, decayed tissue was nearly always classified as discolored wood or hard rot. Soft rot was only found on a single disc resulting from a formulation control treatment in October.

3.2 Stem Sample Trees

3.2.1 Proportion of Discs with Discolouration/Decay

Table 1 shows the proportion (%) of discs in which discoloration or decay was observed. Occurrence of discoloration/decay was scored in three different classes: 1) no decay; 2) less than 5% of disk area was discolored/decay; 3) more than 5% of the disc area was discolored/decay.

![Image](image.png)
The proportion of discs at least 5% discolored/decayed varied significantly between treatments \((p<0.001)\) but not among dates \((p=0.218)\). No significant interaction was detected between treatments and pruning dates \((p=0.134)\) or when controls were compared with each other \((p=0.783)\). Consequently, the control data were pooled. When trees inoculated with *C. purpureum* were compared to the pooled controls, a significant difference \((p<0.001–0.027)\) was found between the proportion of discs that were at least 5% discolored/decayed \((33.3–92.7\% \text{ in inoculated trees vs. } 0–25\% \text{ in controls, depending on date; see Table 1})\).

### 3.2.2 Proportion of Discolored/Decayed Area in Stem Cross-Section

The average proportion of discolored/decayed area in stem cross-sections varied significantly between different treatments \((p<0.001)\) and pruning dates \((p<0.001)\) (Fig 1.). The interaction \((p=0.008)\) between treatments and pruning dates was also detected. Controls did not differ significantly \((p=0.354)\) and were pooled. The average proportion of discolored/decayed area in stem cross-section was significantly different \((p<0.001)\) between trees inoculated with *C. purpureum* \((5–33\%\), depending on date; see Fig. 1) and trees in the pooled controls \((0.1–2.9\%\). Inoculated and control trees differed statistically significantly from each other in May, July and September.

### 3.3 Branch Sample Trees

#### 3.3.1 Spreading of the Discoloration/Decay through the Pruning Wound

Spreading of the discoloration/decay through the pruning wounds varied statistically significantly between treatments \((p<0.001)\) and pruning dates \((p<0.001)\). Spread of the discoloration/decay through dead or live branches did not differ significantly \((p=0.564)\), and the interaction between test variables was not significant \((p=0.127–0.912)\). No significant difference was found \((p=0.679)\) between controls, so they were pooled and found to differ significantly \((p<0.001)\) from trees inoculated with *C. purpureum*.

The mean diameter of pruned branches was 10.0 mm \((\text{S.E. 0.6 mm})\). When using pruned branch diameter as a covariate of discoloration/decay spread through the pruning wound, the effect was not statistically significant \((p=0.522)\).
Fig. 1. The average proportion (%) of discolored/decayed area in discs cut from experimental trees (treatment and control). Treatments were conducted on seven different dates during the growing season (April–October). Values are means ± standard error of means. Bars with different letters are significantly different from each other at the p<0.05 level.

Fig. 2. Spread of the discoloration/decay from the pruning wounds to surrounding living tissue (proportion (%) of pruning wounds). Treatments were conducted on seven different dates during the growing season (April–October). Values are means ± standard error of means. Bars with different letters are significantly different from each other at the p<0.05 level.
Although infection had spread through the wound in most trees inoculated in April, May or July (Fig. 2), only the treatment in May differed significantly from other treatment dates (see Fig. 3 for examples). Inoculated trees differed significantly from control trees on all pruning dates. Although discolouration/decay was observed, no fruiting bodies of *C. purpureum* were found on the branch sample trees.

**4 Discussion**

This study shows that pruned silver birches are most susceptible to the infection by *C. purpureum* in spring. Decay was most often found and the proportion of discolored or decayed area in stem cross-section was highest when trees were pruned and inoculated in May. Additionally, the spread of discoloration/decay from the pruning wounds to the surrounding living tissue was highest for trees inoculated in May. These results agree with those of Brooks and Moore (1926), who infected twigs of *Prunus* sp. more easily in late winter and early spring than in summer. In contrast to our results on silver birch, the resistance of yellow birch to fungal infection was found to be greatest in spring and decreasing towards mid-summer, after which it appeared to increase again (Wall 1991). Earlier, Wall (1986) had not observed any differences in the size of cankers on yellow birch produced from mid-summer as opposed to late-summer. Many studies have shown that pruning of birch should be avoided during the time when sap transportation is the most intense, i.e. in spring (Schöning 1935, Heikinheimo 1936, Heiskanen 1958, Vuokila 1976, Vuokila 1982). It has been shown that the wound healing is slower in early spring than later in summer (Schmitt and Liese 1990).

*Chondrostereum purpureum*, like other fungi, thrives in moist environments and the humid conditions of spring may explain its enhanced performance in May. However, rainfall at the field site was consistent and high during summer 2005 and no prolonged droughts were observed. Moreover, a brief period of drought following *C. purpureum* treatment did not appear to affect its infectivity (Vartiamäki et al. 2009). So, if available moisture is an important factor in the infectivity and spread of white-rot fungus, season still plays a significant role.

We successfully isolated *C. purpureum* from
79% of inoculated trees. Although they showed signs of infection, we were unable to isolate the fungus from the remaining 21%. Stem discoloration is not always caused by fungi. It may also be caused by the trees’ defense mechanisms and bacterial infections (Shigo 1965, Shortle et al. 1978, Hallaksela and Niemistö 1998), so it is possible that some trial inoculations were unsuccessful but trees were infected by other pathogens in the environment. According to Hallaksela and Niemistö (1998), the most common microbial community found in discolored xylem includes primary invaders such as non-decaying fungi (e.g., *Phialophora fastigiata*, *Phialemonia* spp.) yeast like fungi, *Enterobacter* and *Pseudomonas* type bacteria. These microbes turn woody tissue into a more favorable substrate for the invasion of decomposers such as *Fomes fomentarius* (L.:Fr.) Fr., *Phellinus igniarius* (L.:Fr.) Quélet, *Piptoporus betulinus* (Bull.: Fr.) P. Karst. and *Inonotus obliquus* (Fr.) Pilát (Niemelä 1975, Roll-Hansen and Roll-Hansen 1993, Niemelä and Kotiranta 1983, Niemelä et al. 1995). Unfortunately, in this study, we did not have the possibility to study other naturally occurring wood decay fungi or bacteria that had colonized the trees.

It has been shown that the size of the pruning wound is directly related to the risk of stem infection (Verkasalo and Rintala 1998). This is due to the fact that the protective layer is more easily breached in large diameter branches (Schmitt and Liese 1990). With respect to live branches, Heikinheimo (1953) recommended pruning only those less than 1.5 cm in diameter while Heiskanen (1958) relaxed this restriction to 3 cm. In our study, the mean diameter of pruned living branches was 1 cm and we did not find any significant correlation between the diameter of pruned branches and spread of the infection. Furthermore, only wounds inoculated in May showed any sign of remarkable infection two years after pruning. Had we only pruned branches greater than 3 cm in diameter, we might well have seen more pronounced results over all treatment dates.

When evaluating the potential risks of incidental infection one must bear in mind that this study relied on artificial rather than natural inoculation and wounds had no time to heal before exposure to the pathogen (Schmitt and Liese 1990). Placing live mycelia directly on freshly wounded tissue greatly increases the chance of infection compared to natural inoculation by airborne basidiospores. Furthermore, using a mycelial suspension to inoculate trees produces an infection that penetrates deeper into host tissues (Spiers and Hopcroft 1988). As such, the risk of incidental inoculation via basidiospore seems rather minor and any non-target infections would suffer milder symptoms than those described here.

One might consider the risk of incidental infection during application of the inoculum under different weather conditions, e.g., windy versus calm. We used spray bottle and if the wind was strong during the treatment time, inoculum could have been spread to nearby non-target (control) trees. However, similar to Becker et al. (1999a, b), we found the proportion of discoloration or decay to be quite low in control trees, suggesting a low risk of incidental inoculation in treatment areas.

The role of *C. purpureum* in stem discoloration or decay is poorly understood. The fungus is a common primary stem pathogen of woody plants in temperate regions and is present during early (Brooks and Moore 1926, Rayner 1977) and later stages of the decay process (Terho et al. 2007). At our field site, we left 81 of 210 experimental trees intact in order to study the decay process in inoculated and control trees over a longer period of time.

Our study has shows that pruning wounds of silver birches are susceptible to infection by *C. purpureum* and that this susceptibility varies during the growing season, peaking in May. In areas where *C. purpureum* has been applied, there might be an increased risk of incidental infection for non-target birches for at least two years. However, the artificial inoculation used in this study is presumably much more effective than the natural means of infection by basidiospores. Nevertheless, a comprehensive investigation of spore dispersal and infectivity of *C. purpureum* treatment sites should be completed before this risk is evaluated explicitly.
Acknowledgements

We thank Larissa Marx and Suvi Niemi for their help in field work. The study was financially supported by the Foundation for Research of Natural Resources, Niemi-foundation and Finnish Cultural Foundation.

References


and correlation of these characteristics with their capability to prevent sprouting of birch in field. Biological Control 47: 46–54.


*Total of 58 references*