Biological Sprout Control with *Chondrostereum purpureum* – Preliminary Results from Field Trials in Finland

Henna VARTIAMÄKI[a*] − Antti UOTILA[b] − Jarkko HANTULA[a] − Pekka MAIJALA[c]

[a] Vantaa Research Unit, Finnish Forest Research Institute (Metla), Finland
[b] Hyytiälä Forestry Field Station, Helsinki University, Finland
[c] Department of Applied Chemistry and Microbiology, Helsinki University, Finland

Abstract − The aim of our ongoing project is to test the efficiency of the indigenous decay fungus, *C. purpureum*, as a biocontrol agent against stump sprouting in Finland. *Chondrostereum purpureum* was applied to freshly cut birch (*Betula pendula, B. pubescens*) stumps at 12 different time points during the growing season. The most effective treatment time seemed to be the early summer, at least on birch. Efficacy of *C. purpureum* on aspen (*Populus tremula*), grey alder (*Alnus incana*) and willows (*Salix spp*.) was also tested. Treatment was done in July. One year after the treatments *C. purpureum* seemed to have a slight reductive effect on sprouting on all these tested tree species. According to the preliminary results high enzymatic activity of the fungus and good growth ability on wood chips in laboratory did not necessarily guarantee the good ability to prevent sprouting in the field. However, there were differences in the ability of different isolates of *C. purpureum* to prevent sprouting and it is worth to try to find more aggressive isolates in the future for biocontrol purposes. Preliminary results showed that the use of *C. purpureum* is a promising method for biological sprout control in Finland.

Basidiomycetes/ Vegetation management/ Mycoherbicide

Kivonat – Biológiai sarjadzásgátlás *Chondrostereum purpureum*-mal – a szabadföldi kísérletek előzetes eredményei Finnországban. Projektünk célja a *Chondrostereum purpureum* őshonos farontó gomba hatásának kimutatása a tuskósarjak visszaszorításában, Finnországban. A *C. purpureum*-ot friss nyírfa tuskókon alkalmaztuk a vegetációs időszak 12 különböző időpontjában. A leghatásosabb kezelési időpont a kora nyár volt, legalábbis a nyír esetében. A *C. purpureum* hatásosságát rezgőnyáron (*Populus tremula*), hamvas égeren (*Alnus incana*) és füzeken (*Salix* spp.) is kipróbáltuk. A kezeléseket júliusban végeztük. Egy év elteltével a *C. purpureum* gyenge visszaszorító hatását tapasztaltuk e fafajok sarjképzésére. Előzetes eredményeink szerint a gomba magas enzim-aktivitása és laboratóriumi jó növekedési képessége nem garantálja szükségszerűen a szabadföldi jó sarj-visszaszorító képességet. Különbségeket észleltünk az egyes izolátumok sarjadzásgátló képessége között, ezért érdemes megpróbálni agresszívebb izolátumokat találni a jövőbeni biokontrol céljaira. Előzetes eredményeink azt mutatták, hogy a *C. purpureum* felhasználása ígéretes módszer a sarjadzás biológiai gátlására, Finnországban.

Bazídiumos gombák / vegetáció kezelés / mikoherbicid

[*] Corresponding author: Henna.Vartiamaki@metla.fi; Jokiniemenkuja 1, FIN-01301 Vantaa, FINLAND

1 INTRODUCTION

Sprouting of fast growing broadleaved trees cause problems in newly planted and young coniferous stands, where undesirable vegetation contend for space, light, water and nutrients with valuable conifer trees. Sprouting is a problem also under power transmission lines, at roadsides and railways. Use of chemicals to prevent sprouting has nowadays awakened environmental concern. Hence, the most common method to prevent sprouting is mechanical cutting, but it is ineffective for many species as they can resprout vigorously without further treatment.

Researchers in the Netherlands and in Canada have demonstrated the potential of white rot fungus, *Chondrostereum purpureum* (Pers. ex. Fr.) Pouzar, to successfully control stump sprouting of many hardwood species (Scheepens – Hoogerbrugge 1989, De Jong 2000, Wall 1990, Wall 1994, Dumas et al. 1997, Jobidon 1998, Becker et al. 1999, Harper et al. 1999, Pitt et al. 1999). *C. purpureum* is a basidiomycete commonly found throughout the temperate regions of the world. It is an early colonizer of fresh wounds on many broadleaved trees, logging slash and stored logs (Brooks – Moore 1926, Rayner 1977, Spiers – Hopcroft 1988). *C. purpureum* is also known as a pathogen responsible for silver-leaf disease of many fruit trees (Brooks – Moore 1926).

We report here the preliminary results of biological sprout control with *C. purpureum* in Finland. We tested if the application time has an effect on the efficacy of the *Chondrosteum purpureum* treatment on birch and investigated the usefulness of *C. purpureum* as an inhibitor of stumps sprouting on aspen (*Populus tremulae*), grey alder (*Alnus incana*) and willows (*Salix* spp.). In addition, we studied if the activity of several secreted hydrolytic and oxidative enzymes and growth ability on wood chips *in vitro* correlates with the ability of the fungal isolates to prevent growth of new sprouts in the field.

2 MATERIALS AND METHODS

2.1 Effect of the application time of the stump treatment on birch

The experimental site located in Orivesi, Central Finland, about 200 km north of Helsinki. The site was a 15-year-old spruce stand with birches (*Betula pendula, B. pubescens*) as a mixed forest. Plots were established in 12 different time points, with intervals of two weeks, from the 2th of May to 12th of October in year 2005. Birches were manually cut with brush saw and the entire surface of the cut stems was treated with inoculum. The inoculum contained active mycelium of *C. purpureum* pre-grown on potato dextrose liquid medium containing 24 g/l potato dextrose broth and 20 g/l silica powder in distilled water. Control stumps were left untreated (control 1) or they were treated with a blank inoculum (control 2). Plots were circular and variable in diameter. Each plot contained at least twenty cut stems and four replicate plots were established for each treatment. At least three meters untreated buffer zones were left between plots. Plots were examined for the first time in late summer 2006, about one year after experiments were established. Twenty identified stumps per plot were observed. The amount of sphorosphores of *C. purpureum* on stumps was defined, the number of living sprouts per stump was counted and the height of the tallest living sprout was measured.

2.2 Efficacy of *C. purpureum* on aspen (*Populus tremula*), grey alder (*Alnus incana*) and willows (*Salix spp.*)

The experimental sites located in Parikkala, eastern Finland, about 350 km northeast of Helsinki. Field trials were established in July 2005. Trees were cut by brush saw and sprayed immediately with the mycelium of *C. purpureum* growing in liquid culture as described previously. Control stumps were treated with the blank substrate or left untreated after cutting. Plots were circular and variable in diameter. One plot contained at least twenty cut stems and four replicate plots were established for each treatment. Plots were examined first time in late summer 2006, one year after the experiment was established. The amount of sphorosphores of *C. purpureum* on each stump was defined and the number of living sprouts per stump was counted. In addition the height of the tallest living sprout was measured.

2.3 Production of wood decaying enzymes and fungal biomass production *in vitro*

We tested 21 isolates of *C. purpureum* in the laboratory to find out if the activity of several secreted hydrolytic and oxidative enzymes and growth ability on birch wood chips correlated with the ability of the fungal isolate to prevent sprouting. Activity of pectinases, proteases, cellulases, laccases and peroxidases was tested. Agar plates for the detection of pectinases, proteases, and cellulases were modified from Hagerman et al. 1985. ABTS and Poly R-plates used to test the ligninolytic enzyme activities (laccases, peroxidases) were modified from Steffen et al. 2000. Each screening plate was inoculated with a 6-mm diameter agar plug of a precultured mycelium. Two replicate plates of each strain/substrate combination were used. Cultures were incubated at 25 ºC and visually observed. Intensity of color reactions on plates was classified by minus and plus marks. If there was not any observable color reaction, minus mark was given. Three plus marks indicated a strong color reaction. General enzymatic activity value for each isolate was defined as the sum of plus marks given.

Fungal growth in wood chips was investigated using fluorescein diacetate (FDA) method (Boyle – Kropp, 1992). FDA is a fluorogenic substrate that becomes fluorescent upon enzymatic cleavage by a number of nonspecific enzymes of living cells and it has been used previously as a test of fungal viability (Söderström 1977, Schnürer – Rosswall 1984, Barak – Chet 1986). The product of this enzymatic conversion is fluorescein, which can be quantified by a spectrophotometer. The fresh birch (*Betula pendula*) wood chips (particle size 1 mm) used in this experiment were sawed and chipped in Ruotsinkylä, Finland. Wood chips in 20-ml glass vials were inoculated with the mycelium of *C. purpureum* growing in liquid culture and cultivated at 25 ºC. The amount of growing mycelium on wood chips was measured at four different time points: 3, 7, 10 and 14 days after inoculation.

Strain selection for the field was done based on tests made *in vitro*. Different isolates were put in order based on their general enzymatic activity value and FDA absorbance value. Totally 8 isolates of *C. purpureum* were chosen for the field trial. Four isolates with good ability to grow in woodchips and high general enzyme activity and four isolates with lower ability to grow in woodchips and with lower general enzyme activity were selected.

Field trial was established in Juupajoki, Central Finland, about 200 km north of Helsinki. Experimental site was a 13-year-old spruce stand with birches as a mixed forest. The plots were established in the middle of June 2006. A total of 40 treatment plots were established, consisting of four replicates of each treatment. Plots included control stumps, which were cut but treated with the blank inoculum or left untreated. Plots were circular and variable in diameter. Diameter was determined by the area required to locate at least 30 birch stems in each plot for each treatment. A minimum of a 2-m-wide buffer zone separated the plots from each other. Birches were manually cut using a brush saw and the entire surface of the cut stems was immediately treated with one of the eight selected isolates. Plots were examined

first time in late summer 2006, 14 weeks after the experiment was established. The amount of sphorosphores of *C. purpureum* on each stump was defined, the number of living sprouts per stump was counted and the height of the tallest living sprout was measured.

3 RESULTS

3.1 Effect of the application time for the birch stump treatment

One year after the treatments, the treated birch stumps had far more fruiting bodies than control stumps, irrespective of treatment time. The amount of fruiting bodies on treated stumps varied according to treatment time. The highest number of fruiting bodies was found from stumps, which were treated between early May and late June; 60-85% of treated stumps had fruiting bodies. Also control stumps from that time had the highest number of fruiting bodies; 0-14,5% of control stumps had fruiting bodies.

The percentage of sprouting stumps was decreased by the application of the fungus in each treatment time. The biggest difference between treated and control stumps was observed in the middle of July; only 20 % of treated stumps were resprouting whereas 90 % of control stumps put out new sprouts.

Number of living sprouts per stump decreased due to the application of the fungus. The reduction of the living sprouts per stump varied according to the time of cutting. Reduction was 60-80% when the fungus was applied to the stumps between the 4th of May and the 14th of July. When fungus was applied to the stumps between 29th of July and 15th of September the reduction of the number of living sprouts per stump was only 30-50% compared to control stumps. Significant reduction of living sprouts per stump could not be found in treatments done in late September and October. Although the number of living sprouts per stump decreased because of *C. purpureum* treatment, it had no effect on the maximum height of new sprouts in any treatment times.

3.2 Efficacy of *C. purpureum* on aspen (*Populus tremula*), grey alder (*Alnus incana*) and willows (*Salix spp.*)

Fruiting bodies of *C. purpureum* were found from every observed tree species one year after treatments. Control stumps had far less fruiting bodies than treated stumps on every tree species. Fructification was most abundant in willows; about 90% of treated stumps had fruiting bodies. About 60% and 30% of treated alder and aspen stumps had fruiting bodies, respectively. Only 0-2,5% of control stumps had naturally occurring fruiting bodies depending on tree species.

One year after the treatments, the percentage of non-sprouting stumps was increased and the number of living sprouts per stump was reduced by the application of *C. purpureum* on all tested tree species. However, the difference between treated and control stumps was not very clear and statistical significance between treated and control stumps could not be found in any observed tree species one year after the treatments. The maximum height of the living sprouts did not differ between treatments in any observed tree species.

3.3 Correlation of *in vitro* characteristics to inhibition of sprouting of birch in the field

Laccase activity was present in all isolates of *C. purpureum*, but there were clear differences between the isolates. Only four isolates bleached Poly-R, indicating manganese peroxidase (MnP) activity. Pectinase activity was scarce in *C. purpureum*; only two isolates produced detectable amounts of pectinases. Activity of proteases, lipases and cellulases was present in all isolates, although there was a lot of variation in the production levels of these enzymes.

There were clear differences in biomass production of mycelium in wood chips between different *C. purpureum* isolates. The amount of growing mycelium on wood chips was measured in four different time points, 3, 7, 10 and 14 days after inoculation, and differences were seen clearly between different isolates just until 10 days after inoculations.

Assessments conducted in the field 14 weeks after the treatments revealed that there were differences between different *C. purpureum* isolates in their ability to prevent sprouting on birch. However, differences seemed not to correlate with high or low enzymatic activity on agar plates or growth rate in the laboratory tests.

4 DISCUSSION

Preliminary results showed that *C. purpureum* is a promising method in biological sprout control in Finland. On birch it seems to reduce sprouting quite well and the most effective treatment time seems to be in the early summer.

C. purpureum treatment seemed also to have a slight effect on sprouting also on aspen, alder and willows. On these tree species the effect of the treatment seemed not to be as effective as on birch. However, one must remember that these are just preliminary results one year after treatments and the situation can still change in the following years.

According to the first year's results high enzymatic activity and good growth ability on wood chips in the laboratory do not seem to correlate with the good ability to prevent sprouting in the field. The hydrolytic enzymes tested here appeared not to be crucial for the biocontrol efficiency. Virulence of *C. purpureum* is most likely dependent on several factors, including phytotoxins, which were not analyzed in our study. However, there are differences in the ability of different isolates of *C. purpureum* to prevent sprouting and it is worth to try to find more aggressive isolates in the future for biocontrol purposes. Other characteristics, which could affect to the ability to prevent sprouting, could also exist.

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