

## Short communication. A review on the efficacy tests and risk analyses conducted on *Chondrostereum purpureum*, a potential biocontrol agent, in Finland

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### Abstract

Hardwood sprouting is a problem in forest regeneration areas, under electric lines, on roadsides and railways. In Finland, isolates of *Chondrostereum purpureum* were screened by field experiments for their efficiency to control sprouting. The proportion of dead stumps with the best isolates exceeded 80% on birch (*Betula pendula* and *B. pubescens*), and *C. purpureum* was also found to affect the sprouting of aspen (*Populus tremula*) and rowan (*Sorbus aucuparia*). The risks of *C. purpureum* based biocontrol were evaluated by population genetic analysis. It showed that *C. purpureum* is a geographically undifferentiated species that does not reproduce clonally. The risk of infection of non-target trees was found to be highest in early spring. These findings suggest that the risks of using *C. purpureum* in biocontrol are small.

**Key words:** biocontrol; *Chondrostereum purpureum*; sprout control; risk analysis.

### Resumen

**Comunicación corta. Revisión de las pruebas de eficacia y análisis de riesgo llevados a cabo en *Chondrostereum purpureum*, un agente de control biológico potencial, en Finlandia**

El rebrote en las frondosas es un problema en las áreas de regeneración bajo las líneas eléctricas, en bordes de caminos y en vías férreas. En Finlandia, se seleccionaron aislados de *Chondrostereum purpureum* mediante ensayos de campo por su eficacia para controlar el rebrote. La proporción de cepas muertas con los mejores aislados superó el 80% en abedul (*Betula pendula* y *B. pubescens*). También se observó que *C. purpureum* afecta el rebrote de álamo temblón (*Populus tremula*) y del serbal (*Sorbus aucuparia*). Los riesgos del control biológico basado en *C. purpureum* se han evaluado por análisis de genética poblacional. Se demostró que *C. purpureum* es una especie geográficamente diferenciada que no se reproduce por clonación. El riesgo de infección de árboles que no fuesen el objetivo fue más altos a principios de la primavera. Estos hallazgos sugieren que el riesgo del uso de *C. purpureum* en el control biológico es pequeño.

**Palabras clave:** biocontrol; *Chondrostereum purpureum*; control de rebrote; análisis de riesgo.

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### Introduction

Fast and abundant stump sprouting of broadleaved trees causes problems in conifer regeneration areas, where undesirable vegetation competes for light, water, nutrients, and space with more valuable conifers. Hardwood sprouting also causes problems under power transmission lines, as well as at roadsides and railways.

Downy and silver birches (*Betula pubescens* Ehrh. and *Betula pendula* Roth.) are the most common hardwood species in Finland, and both of them regenerate vegetatively and sprout rapidly after cuttings.

*Chondrostereum purpureum* (Pers. ex Fr.) Pouzar is the causative agent of the silver-leaf disease on fruit and ornamental trees (Brooks and Moore, 1926; Peace, 1962; Setliff and Wade, 1973). In addition, as an eco-

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Received: 27-09-11. Accepted: 04-04-12.

onomically important pathogen, the fungus attacks many deciduous trees, shrubs and fruit trees throughout the temperate zone, and causes white rot on them (Rayner and Boddy, 1986).

*C. purpureum* is a rather common pathogen also on hardwoods in Fennoscandia, where it is found e.g. on *Betula*, *Populus* and *Alnus* species (Mazelaitis, 1976; Kauppila and Niemelä, 1986). The basidiospores of *C. purpureum* infect only fresh wounds (Spiers and Hopcroft, 1988) and no infection occurs on unwounded trees.

Studies on *C. purpureum* as a biocontrol agent for unwanted broadleaved trees and shrubs have been carried out in Canada and Netherlands during the recent decades, and commercial preparations are already available (Scheepens and Hoogerbrugge, 1989; Wall, 1990; Wall 1994; Gosselin, 1996; Dumas *et al.*, 1997; Jobidon, 1998; Shamoun and Hintz, 1998; Harper *et al.*, 1999; Pitt *et al.*, 1999; De Jong, 2000). The use of these preparations in Finland makes the risk of introducing alien pathogens; hence investigations on local strains of *C. purpureum* were started. The purpose of this review is to describe the developments in analyzing the potential of *C. purpureum* as a mycoherbicide in Finland.

## Search for an efficient biocontrol strain

The first task in developing any biocontrol product is to find an efficient isolate to be used in commercial product. For satisfactory control of hardwood sprouting, reduction of at least 80% of sprouts are needed as their density may be extremely high.

Vartiamäki *et al.* (2008a) screened 21 *C. purpureum* strains for several enzymatic activities and biomass production ability. Based on these analyses, two groups of isolates were selected for field trials. The first group included isolates with high enzymatic activity and biomass production whereas the isolates of the second group showed lower enzymatic activity and biomass production. In field trials considerable differences were observed between the isolates. The best isolates killed ca. 90% of birch stumps during the first two years after the treatment, and reduced the mean number of sprouts by more than 80%. The above-described grouping of isolates did not predict their performance in the field, but some enzymatic activities, the laccase activity in particular, seemed to correlate with the biocontrol efficacy (Vartiamäki *et al.* 2008a).

Mean diameter of the treated stumps was relatively large, about 30 mm (Vartiamäki *et al.* (2008a, 2009a). It is, however, known that stumps of older birch trees sprout less vigorously than stumps of younger trees. Johansson (1992) reported that 40-65% of stumps with diameter of 81-131 mm sprouted in mixed stands of downy and silver birches five years after cutting, and Andersson (1966) reported that 30-80% living stumps of 25-50-year-old downy birch sprouted two years after cutting. On the other hand, stump-sprouting ability of *B. papyrifera* has been shown to increase with the size of the stump up to an intermediate diameter (Perala and Alm, 1990). Therefore, it would be important to test further the efficacy of *C. purpureum* treatment on birch stumps with very small diameter.

In Finland birch is by far the most important tree species to be controlled. However, also other hardwood species cause problems locally. Therefore, it would be desirable if the control agent would be efficient also against them. It is known that aspen (*Populus tremula* L.), for example, is able to inhibit the growth of *C. purpureum* to a greater degree than some other broadleaved tree species (Wall, 1990; Becker *et al.*, 1999; Harper *et al.*, 1999; Pitt *et al.*, 1999). Hamberg *et al.* (2011a,b) tested the efficacy of *C. purpureum* against rowan (*Sorbus aucuparia* L.) and aspen. Both of these species seemed to be sensitive to the treatment, although to a lesser extent than the birches. However, it should be noted, that the treated saplings were smaller in diameter (ca. 17 and 18 mm respectively), and that the *C. purpureum* isolate used in these experiments was relatively weak, and is not among the potential strains to be selected for the commercial preparation.

## Timing of control

The second question in using a biocontrol product is to find the best time for controlling. This was tested by cutting birch seedlings and treating them with a biocontrol product throughout a summer. The results showed that the best control level in Finnish conditions was obtained when the treatment was conducted in late May, June or July (Vartiamäki *et al.*, 2009a). The treatment was less effective towards the end of the growing season. This is in accordance with the view that the resistance of birch against *C. purpureum* is greatest in the spring and decreases towards the midsummer, after which it increases again (Wall 1991). Also Dumas *et*

*al.* (1997) demonstrated on aspen, that a higher number of sprouts were observed after cuttings in late September than in late August.

## Biosafety aspects

As already stated above, the use of pathogenic organisms is potentially dangerous. Therefore a good body of information about the population biology of the control fungus should be collected before its spread to nature. This information was gathered by conducting a population study on the genetic diversity of *C. purpureum*.

The populations showed only a very low degree of geographical differentiation (Vartiamäki *et al.*, 2008b). Therefore, either a gene flow occurs naturally throughout Finland and the Baltic countries, or the fungus has such a high population size that the frequencies of selectively neutral markers studied change extremely slowly. Therefore, distributing any local genotype of *C. purpureum* as a biocontrol agent should not lead to introduction of novel genes or genotypes in Finland, and the use of any native genotype as a biocontrol agent should therefore be safe.

The population analysis conducted by Vartiamäki *et al.* (2008b) also suggested that *C. purpureum* has no asexual reproduction, a result in accordance with previous studies showing no support for clonal reproduction in *C. purpureum* (Gosselin *et al.* 1996, 1999, Ramsfield *et al.* 1999). Thus, in the lack of clonal propagation the genes of the biocontrol strain will mix with those of other individuals in the same population as soon as basidiospores are produced, and the original genotype will not spread outside the treated areas.

However, if the high pathogenicity of the control isolate is due to a single allele, the frequency of this allele might increase. Thus, if the sprout control by *C. purpureum* becomes a common practice, it would be important to figure out the genetics of the pathogenicity of *C. purpureum*, and follow the development of genetic diversity of this fungus also in the future.

Another problem might be the infection of non-target trees. Vartiamäki *et al.* (2009b) tested this risk by pruning and inoculating birches in one month intervals from spring to autumn, and showed that pruning causes highest risk for *C. purpureum* infection in early spring. However, the infection in neighboring trees in control plots was very low throughout the experiment. This indicates that the risk is not very high, but it cannot be ruled out that the basidiospores in favorable environmental conditions might infect

freshly wounded non-target trees, although a long distance spread of spores has been shown to be unlikely (De Jong *et al.* 1990a). De Jong *et al.* (1990b) concluded that there is a little risk that added *C. purpureum* would significantly affect non-target, unwounded forest and horticultural trees further than 500 m from the target area. In Canada, where commercial products (Chontrol® and Myco-Tech™ Paste) are already available, no buffer zone around treated trees is required (Anonymous, 2007).

## Practical issues

When an efficient biocontrol strain is available, many practical questions arise. The most important of them relates to the production and form of the commercial product. This problem has not been solved in Finland. The growth of *C. purpureum* on artificial medium is, however, relatively good, and this fungus has already been commercialized in Canada. Therefore, we do not see big practical problems in this issue.

Also the question of dispersal machinery should be solved, as it would be cost-efficient only to spread the control agent at the same time as the trees/sprouts are cut. This issue might be solved by using the same device as already applied for the biocontrol of Heterobasidion root rot with *Phlebiopsis gigantea* (Korhonen *et al.* 1994, Pratt *et al.* 2000). Trace amounts of *P. gigantea* in *C. purpureum* suspension, and *vice versa*, do not reduce the biocontrol efficiency (Hamberg *et al.*, unpublished).

## Conclusion

The use of *Chondrostereum purpureum* in biocontrol of hardwood sprouting in forest regeneration areas, under electric lines and at the road sides looks promising in Finland. Highly efficient isolates are available and the possible risks have been evaluated. The next step will be to solve the practical issues: the development of a commercial preparation and finding out cost-efficient methods for its application to stumps.

## Acknowledgments

We thank UPM-Kymmene forests, Metsäteho Ltd and Fingrid Ltd for their financial support.

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