Journal of Environmental Management 171 (2016) 217–224

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

The efficacy of six elite isolates of the fungus *Chondrostereum purpureum* against the sprouting of European aspen

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ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form
4 February 2016
Accepted 8 February 2016
Available online 18 February 2016

Keywords:
Forest management
Decay fungus
Biological control agent
Sprouting

ABSTRACT

The sprouting of broad-leaved trees after cutting is problematic in forest regeneration areas, along roads and railways, under electric power and above gas pipe lines. In Finland, one of the most difficult species to control in these areas is the European aspen (*Populus tremula*), which produces both stump sprouts and root suckers after saplings have been cut. In this study, we investigated whether a decay fungus of broad-leaved trees, *Chondrostereum purpureum*, could be used as a biological control agent against aspen sprouting. The efficacy of six elite strains of *C. purpureum* (improved earlier in a breeding process) was investigated on aspen for three years. The most efficient *C. purpureum* strain, $R5_3$, tested earlier on birch (*Betula pendula* and *B. pubescens*), was efficient in causing mortality of aspen stumps and preventing the development of root suckers. With this strain, stump mortality was 78%, while significantly lower in control stumps which were cut only (47%). Aspen trees in the vicinity of the treatments (within a 10 m radius around each sapling) decreased the efficacy of *C. purpureum*. This study shows that the decay fungus *C. purpureum* can successfully be used in the sprout control of aspen saplings.

1. Introduction

The control of broad-leaved saplings is a challenge in forest areas where mature trees have been cut and young conifer trees are forming a new tree generation. Sprout control is also needed in the maintenance of roads, railways, and electric power and gas pipe lines due to the ability of broad-leaved saplings to produce new sprouts and form thickets after mechanical cutting. Biological control of broad-leaved tree sprouting with the decay fungus *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar is a promising method to supplement mechanical cutting (Hamberg et al., 2015), and as an alternative to the use of chemicals, which can have adverse effects for the environment (Bellgard et al., 2014; Benachour and Séralini, 2009; Graymore et al., 2001).

When the mycelia of a natural decayer of broad-leaved trees, such as *C. purpureum*, is applied to freshly cut stumps the fungus penetrates to wood and decays it, thus preventing the development of new stump sprouts (Butler and Jones, 1949; Hamberg et al., 2015; Lygis et al., 2012; Roy et al., 2010; Vartiamäki et al., 2009; Wall, 1990). When the hyphae of *C. purpureum* spread within a stump, tree vessels are occluded (Butler and Jones, 1949). Induced dehydration combined with fungal toxins strengthens the adverse effects of the fungus in preventing the resprouting of stumps (Butler and Jones, 1949; Strunz et al., 1997).

C. purpureum is a basidiomycetous decay fungus with a broad host range within boreal and temperate vegetation zones (Butler and Jones, 1949; Lygis et al., 2012; Pitt et al., 1999; Setliff, 2002). This fungus causes also silver-leaf disease on fruit and ornamental trees (Butler and Jones, 1949). *C. purpureum* is common in injured broad-leaved trees, but is not hazardous to conifers or unwounded broad-leaved trees (Butler and Jones, 1949; Rayner, 1977; Wall, 1990). Furthermore, the fungus is not harmful to wildlife or humans as it grows only on wood (Vandenbroucke et al., 2005).

C. purpureum produces wind-dispersed spores, which are abundant during autumn when stumps are covered by basidiomes (fruiting bodies) (Butler and Jones, 1949). Spores land on wounded wood where they grow and pair with other spores forming heterokaryotic mycelia that penetrate deeper into the wood. The fungus consumes carbohydrates, and during this process also breaks up lignin (Kirk and Farrell, 1987; Leatham, 1986; ten Have and Teunissen, 2001). When the decay process has penetrated deep enough into the wood, the ability of a stump to produce new sprouts diminishes and the stump dies. On stumps, *C. purpureum* is quickly replaced by other fungi (Rayner, 1977); therefore increased

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http://dx.doi.org/10.1016/j.jenvman.2016.02.016
0301-4797/

spore loads from inoculated stumps and possible risks to non-target species are only temporary. In fact, it has been shown that by applying *C. purpureum* inoculum to stumps and subsequent spore release does not substantially increase the risk of *C. purpureum* infection via spores because natural infections are frequent (Gosselin et al., 1999a).

C. purpureum is easily grown under artificial conditions, which helps in mass production for commercial use (Pitt et al., 1999). Furthermore, no specialization of *C. purpureum* has been found in a specific ecological region or tree species (Gosselin et al., 1999b; Vartiamäki et al., 2008b). This reduces concerns related to the application of *C. purpureum* in target areas (Pitt et al., 1999).

The efficacy in preventing sprouting of broad-leaved trees varies among different *C. purpureum* strains (Hamberg et al., 2015; Harper et al., 1999; Pitt et al., 1999; Vartiamäki et al., 2008a; Wall et al., 1996). However, efficacy is not purely associated with origin, as fungal isolates originating from the same *C. purpureum* basidiome may include both effective and ineffective individuals (Wall et al., 1996).

Thus, a field experiment is the only reliable way to investigate sprout control efficacies of different *C. purpureum* strains (Hamberg et al., 2015). Moreover, the reduction of sprouting after *C. purpureum* inoculation varies among broad-leaved tree species (Wall, 1990). The best performance of this biocontrol agent has been observed for birch species, e.g., *Betula pendula* Roth, *Betula pubescens* Ehrh., *Betula papyrifera* Marsh. and *Betula alleghaniensis* Britt (Hamberg et al., 2015; Lygis et al., 2012; Roy et al., 2010; Vartiamäki et al., 2008a, 2009; Wall, 1990), but promising results have also been observed for other broad-leaved trees (Hamberg et al., 2014; Wall, 1990).

In young Finnish forests, one of the most common and problematic species to control is the European aspen (*Populus tremula* L.) (Finnish Forest Research Institute, 1995). This species is distributed in boreal and temperate ecosystems in Eurasia (Myking et al., 2011). European aspen is a pioneer tree species, but old aspen trees can also be found in old-growth forests (Suvanto and Latva-Karjanmaa, 2005). European aspen reproduces both sexually and asexually (Myking et al., 2011 and references therein). Recent studies have shown that aspen may regenerate by seeds although root suckering is considered the main regeneration form in aspen in areas where it has been established (Farmer, 1963; Myking et al., 2011). When a mature aspen tree is cut, it can produce vast numbers of root suckers from underground stems (Farmer, 1963; Johansson, 1993; Johansson and Lundh, 1988; Worrell, 1995). Separate stems, having the same genotype, can form large clones up to ca. 1000 m^2 in area (Suvanto and Latva-Karjanmaa, 2005). High light and soil temperature promote root sucker development (Farmer, 1963; Johansson and Lundh, 1988; Man et al., 2008). Aspen is tolerant to both dry and wet conditions, but growth is optimal on fertile and well-drained mineral soils (Götmark et al., 2005; Myking et al., 2011; Possen et al., 2011 and references therein; Worrell, 1995).

Earlier studies have shown that more efficient *C. purpureum* strains to control aspen sprouting are needed because almost no significant differences have been found between treatments with the fungus and without it, and biocontrol efficacy has been quite low (Becker et al., 1999; Dumas et al., 1997; Hamberg et al., 2011, 2014; Roy et al., 2010; Wall, 1990). However, Pitt et al. (1999) reports promising results in trembling aspen (*Populus tremuloides* Michx.). In order to develop more efficient strains, a short breeding program of *C. purpureum* successfully improved the sprout control of birches, *B. pendula* and *B. pubescens* (Hamberg et al., 2015). In this study, five different strains of *C. purpureum*, developed on birch trees (see Hamberg et al., 2015), and the best original parent strain were tested on aspen in order to evaluate their biocontrol success on another tree species. Here we hypothesized that (i) the efficacy of these *C. purpureum* strains is better in the control of European aspen sprouting than mechanical cutting without the fungus, (ii) the most efficient strain developed for birch is superior also on aspen, and (iii) stump mortality on aspen is as high as was found earlier on birch (see Hamberg et al., 2015).

2. Materials and methods

2.1. Fungal strains

Efficacies of the previously described *C. purpureum* parent strain $HY4_1$ (first generation), two second generation strains $V1_2$ and $V2_2$ (progenies of $HY4_1$), and three third generation strains $R3_3$, $R5_3$ and $R9_3$ (progenies of $V1_2$ and $V2_2$) in preventing sprouting were investigated in aspen. The parent strain $HY4_1$ was originally collected from a birch stump (either on *B. pendula* or *B. pubescens*) in Juupajoki, Finland in 2003 (Vartiamäki et al., 2008a). Subsequent strains emerged through breeding, which aimed at increasing efficacy of the *C. purpureum* strain on birch (see Hamberg et al., 2015 for details). These six strains were selected to investigate their effects on European aspen. The efficacy of different *C. purpureum* strains were compared to a control treatment in which the inoculum medium without the fungal mycelium was spread on freshly cut aspen stumps (hereafter referred to as the liquid control).

2.2. Inoculum medium

Each fungal strain was grown for 7 d on potato dextrose agar (PDA: 24 g potato dextrose broth and 15 g agar with 1000 ml deionized water; Becton, Dickinson and Company). An agar plug with hyphae from the edge of the *C. purpureum* culture was transferred to a PDA cellophane plate, and was allowed to grow for seven more days. The inoculum medium for the field experiment was prepared using 24 g potato dextrose broth (Becton, Dickinson and Company) and 20 g Sipernat 22S (Evonik Degussa) per 1000 ml deionized water, and autoclaved in an Agarmatik machine at 121 °C for 15 min. Cooled autoclaved inoculum medium was added to autoclaved Erlenmayer flasks, 150 ml per bottle, with *C. purpureum* mycelia, ca. 0.167 ± 0.018 g (mean ± SD) per fungal strain. The mycelia were transferred from one PDA cellophane plate to the flask with a sterilized scalpel. The inoculum was incubated in the dark at 20 °C for 10 d in a rotary shaker (100 rpm), homogenized with an Ultra Turrax apparatus for 1.5 min, and diluted 1:10 with tap water before treatment in the field.

2.3. Field experiment

Five young forest sites were selected in Lapinjärvi, southern Finland (Table 1). Sites were dominated by European aspen (*P. tremula*) saplings ca. 1.2 ± 0.4 cm (mean ± SD) in basal diameter. At each site we established one sample plot for each fungal strain and the liquid control. One large site (Lapinjärvi 121) allowed for the establishment of two sets of sample plots. At these sites, ca. 18 saplings per treatment were investigated within each plot. Nine additional saplings were selected from two other sites (Lapinjärvi 67 and 349) in order to have enough saplings for each treatment. A total of 501 aspen saplings were investigated.

Treatment sample plots were placed randomly within each site. Saplings were cut from 10–13 May 2011 with a brush saw at a height of ca. 15 cm above ground, and the fungal inoculum (or inoculum without the fungus) was applied to the stumps immediately after cutting with plastic squirt bottles. The weather was sunny with a temperature of 14–21 °C. Viability of the fungal strains was verified by squirting the inoculum on PDA Petri plates