Chondrostereum purpureum-based Control of Stump Sprouting of Seven Hardwood Species in Lithuania

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Abstract

The ability of *Chondrostereum purpureum* preparates to control sprouting of seven hardwood species in Lithuania one year following applications was tested: native *Betula pendula, Alnus incana*, *Populus tremula* and *Salix caprea*, and invasive *Acer negundo, Hippophae rhamnoides* and *Robinia pseudoacacia* were inoculated with *C. purpureum*. The experiments were performed twice: in July and October 2010. Each time, twenty trees of each species were cut and inoculated with four Lithuanian strains of *C. purpureum* using two formulations: i) mycelial water suspension mixed with 'AgroAquaGel'®, and ii) mycelial water suspension mixed with xanthan gum and glycerine. Two different types of negative controls were used: a blank formulation control and a slash control. Herbicide 'Roundup® BIO' was used as a positive control.

All four inoculated strains of *C. purpureum* persisted in treated stumps of *B. pendula*, *A. incana*, *P. tremula*, *S. caprea* and *A. negundo*, formed abundant fruitbodies, and showed similar effect in preventing sprout formation. Compared to negative controls, mycoherbicide treatment in summer had significantly reduced percentage of living stumps and sprouts on *B. pendula* and *A. negundo* (the effect was as good as of chemical herbicide) as well as mean height of the tallest living sprout of these species. A slight effect of *C. purpureum* treatments on stump and sprout mortality was observed for *A. incana*, *P. tremula* and *S. caprea*, although fruitbody formation occurred after treatment. No mycoherbicide effect was observed on *R. pseudoacacia* and *H. rhamnoides* which are either resistant to *C. purpureum* infection or symptom development is delayed relative to the other species tested. Summer treatments gave significantly better results than the autumn treatments in terms of stump and sprout mortality: 75.9% and 38.5% of the stumps, and 83.6% and 46.0% of the sprouts were dead in summer and autumn treatments, respectively (pooled data from *B. pendula, A. incana*, *P. tremula, S. caprea* and *A. negundo* plots). There was no seasonal treatment effect on fruitbody formation. In conclusion, local strains of *C. purpureum* can be successfully applied to control stump sprouting of *B. pendula* and *A. negundo* in Lithuania if performed during a period of active tree growth.

Key words: biological control, deciduous species, hardwood weeds, mycoherbicides, stump treatment, vegetation management

Introduction

Fast growing hardwoods cause problems in young forest plantations, under power lines, alongside roads and railways, in urban settings (parks, banks of various water bodies) and other sites where tree vegetation is undesired. Among those hardwoods, several species of invasive alien plants as e.g., black locust (*Robinia pseudoacacia* L.), box elder (*Acer negundo* L.) and sea buckthorn (*Hippophae rhamnoides* L.) are of great concern in Europe (Binggeli et al. 1992, Isermann and Cordes 1992, Isermann et al. 2007, Hulme et al. 2008, Isermann 2008, Pyšek et al. 2008) and urgent control measures are to be taken. Manual removal (cutting) of undesired deciduous trees is often short term because of regrowth of stump sprouts that considerably reduces the effect of such treatment. Chemical herbicides, such as glyphosate and triclopyr, have shown their effectiveness in controlling competition and thus leading to maximum yields in forest plantations (Wagner et al. 2006). Herbicides can aid in controlling undesired vegetation in many cases (e.g., Liegel et al. 1984, Howard and Parker 1995, Jobidon 1998, Pitt et al. 1999), however, impact on the environment (pollution), increasing public opposition to these products and scarcity of suitable registered chemicals for some

weed control problems have led to limitations in their applicability. In Lithuania, the use of herbicides is restricted in certain protected areas, urban settlements, in the vicinity of open water bodies or other sensitive ecosystems, thus mechanical cutting is currently the most common method to control stump sprouting. It has to be carried out periodically due to continuous regrowth, and each re-treatment increases cost. This critical situation has resulted in the search for effective and environmentally-friendly alternatives.

To provide an alternative solution, the potential of a white-rot fungus *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar as a biocontrol agent has been investigated. Dutch (Scheepens and Hoogerbrugge 1989, de Jong et al. 1990, 1991, de Jong 2000), Canadian (Wall 1990, 1994, 1997, Wall et al. 1996, Gosselin 1996, Dumas et al. 1997, Jobidon 1998, Shamoun and Hintz 1998a, b, Becker et al. 1999, Gosselin et al. 1999, Harper et al. 1999, Pitt et al. 1999, Becker et al. 2005), South African (Morris et al. 1998) and Finnish (Vartiamäki et al. 2007, 2008, 2009, Hamberg et al. 2011a, b) researchers have shown that *C. purpureum* is a promising bioherbicide for controlling stump sprouting of many broad-leaved trees by placing fungal mycelium on freshly cut stumps. Several commercial preparates (so-called mycoherbicides) have been developed on the basis of this fungus: 'BioChon®' was registered and applied for sprouting control in the Netherlands (de Jong 2000), 'Myco-Tech™' paste and 'Chontrol Peat Paste'® were invented and used in Canada (Vandenbroucke et al. 2005, de la Bastide and Hintz 2007, Shamoun 2008, Roy et al. 2010).

As a potential biocontrol agent, *C. purpureum* is attractive because it has a broad host range and is globally distributed (Rayner and Boddy 1986, Peace 1962). It is an early colonizer of fresh wounds on many broad-leaved trees, logging slash and stored logs (e.g., Brooks and Moore 1926, Mazelaitis 1976, Rayner 1977, Spiers and Hopcroft 1988). *C. purpureum* is also known as the pathogen responsible for silver-leaf disease of many fruit trees (Brooks and Moore 1926). It causes the occlusion of vessels and foliar damage that may lead to tree mortality (Spiers et al. 1987, Spiers and Hopcroft 1988, Wall 1991, de Jong 2000). A biological control strategy using *C. purpureum* seems to have a low likelihood of soil or water contamination and minimal risk to non-target plant species (Scheepens and Hoogerbrugge 1989, de Jong et al. 1990, 1996, Gosselin et al. 1999, Becker et al. 2005). Susceptibility of trees to *C. purpureum* has been shown to vary throughout the year (Wall 1991, 1994, Spiers et al. 1998) and the most suitable time for stump treatment has been found to be between late spring and early autumn (Vartiamäki et al. 2009). Also, it has been shown that there are differences in efficacy of single strains of the fungus in preventing sprouting (Jobidon 1998, Harper et al. 1999, Pitt et al. 1999, Vartiamäki et al. 2008) and their pathogenicity toward different broad-leaved species (Harper et al. 1999, Pitt et al. 1999).

The fungus is also widespread in Lithuanian forests and fruit orchards, both as a saprophyte and the cause of silver leaf disease (Mazelaitis 1976), however the potential of *C. purpureum* as a biocontrol agent in Lithuania has been tested only once. The authors of the present paper have established experiments of birch sprout control in 2003–2004 (unpublished). Mycoherbicide treatment has proved to be effective following summer inoculations, but the results have yet to be summarized.

The native fast-growing hardwoods (silver birch (*Betula pendula* Roth), grey alder (*Alnus incana* (L.) Moench), common aspen (*Populus tremula* L.) and goat willow (*Salix caprea* L.)) are in most cases undesired in young forest plantations of conifers or hardwoods, under power lines and in rights-of-way; sometimes their regeneration causes problems on abandoned agricultural land and urban settings. All of these species produce abundant stump sprouts which could be to a greater or lesser extent successfully controlled by *C. purpureum* preparates (e.g., Vartiamäki et al. 2007, 2009, Hamberg et al. 2011a). The invasive *A. negundo*, *R. pseudoacacia* and *H. rhamnoides* have yet to be tested with mycoherbicides. All three alien tree species produce abundant stump sprouts and are able to occupy large territories through clonal spread and efficient seed dispersal (e.g., Pearson and Rogers 1962, Maeglin and Ohmann 1973, Boring and Swank 1984, Binggeli et al. 1992, Krízsik and Körmöczi 2000).

The main aims of this study were to: i) determine the potential of local *C. purpureum* strains, in comparison to a commercial herbicide 'Roundup® BIO', to prevent stump sprouting of seven weedy hardwoods in Lithuania, including the problematic invasive species – *A. negundo*, *R. pseudoacacia* and *H. rhamnoides*; ii) test the effect of the application time, summer vs. autumn treatments, and two different preparate formulations on the efficacy of mycoherbicide treatment and formation of *C. purpureum* fruitbodies; and iii) determine the differences in efficacy of different *C. purpureum* strains.

Materials and methods

Preparation of the inoculum

Four strains of *C. purpureum* (isolates P2-2, P2-8, P3-12 and P4-17) were obtained following isolation of the fungus from four birch stumps found in a pre-commercial felling site in Palanga Forestry District,

Kretinga State Forest Enterprise (55°56'N, 21°06'E). Isolations of pure fungal cultures on Petri dishes containing Hagem agar (Modess 1941) media were made according to Vasiliauskas and Stenlid (1998).

Inoculum for the field applications was prepared in 1000-ml Erlenmeyer flasks containing 500 ml of autoclaved liquid Hagem media amended with 0.5 g/l antibiotic chloramphenicol (BioChemica, AppliChemGmbH, Darmstadt, Germany). For every *C. purpureum* strain, each flask was inoculated with twenty 6-mm agar plugs overgrown by fungal mycelium taken from the edge of a two-week-old culture. The flasks were stoppered with cotton plugs and incubated for three weeks at 20 ºC in the dark. To ensure even distribution of the mycelium, the flasks were occasionally shaken by hand by rotating.

Selection of materials for inoculum formulations was based on their potential ability to prevent drying-out of the fungal inoculum. First, we tested a water suspension of 'AgroAquaGel'® ('Artagro' Sp. Z. o. o., Kraków, Poland) which is usually applied to seedling roots before their outplanting in the field. This material is based on poly-acrylic acid potassium salt cross-linked superabsorbent polymer. The formulation was prepared following manufacturer's instructions: 30 g of 'AgroAquaGel'® powder was diluted in 200 ml of distilled water in 500-ml glass jars. The second formulation type was the modified protocol of Dumas et al. (1997): 170 ml of distilled water was mixed with 30 ml of glycerin and 20 g of xanthan gum (Dalsa UAB, Vilnius, Lithuania) in 500-ml glass jars. The jars with both prepared formulations were covered with metal lids, autoclaved and stored under sterile conditions for maximum of one week until the day of field treatments.

On the morning of field applications, ca. 15 g (fresh weight) of *C. purpureum* culture was taken aseptically from each flask inoculated with different fungal strains, strained off by a sterilized metal mesh and transferred to respective glass jars containing prepared formulations (four fungal strains × two formulations = eight jars). The inoculum was homogenized using a 'Turrax T-18 Basic' apparatus (IKA®-Werke GmbH and Co. KG, Staufen, Germany) for about 1 min. Blank formulation (formulation control) was prepared by adding 15 ml of sterile liquid Hagem media to the jars containing each of the two formulations. The ready-made preparates were applied within 10 hours after their preparation. Concentration, viability and purity of the fungal inoculum were confirmed before and after application in the field with the most probable number method (MPN) (Harris and Sommers 1968) to be sure that the concentration of the inocula of each strain was approximately the same and that the fungus was viable.

Study sites, experimental design, and field treatments

Experimental sites for control of *B. pendula* (54°47'N, 25°00' E), *P. tremula* (54°48'N, 25°00' E), *A. incana* (54°47'N, 25°00' E), *S. caprea* (54°47'N, 24°59' E), *A. negundo* (54°47'N, 24°54' E) and *H. rhamnoides* (54°48'N, 24°54'E) stump sprouting were selected about 25 km west of Vilnius in Dūkštos Forest District (Vilnius' State Forest Enterprise). For autumn treatments, additional *A. negundo* plots were selected in Vilnius city (54°41'N, 25°18' E). Plots of *R. pseudoacacia* were selected in a post-fire forest site on sandy dunes in Smiltynė Forest District (Kuršių Nerija National Park), located at the Curonian Spit peninsula in the Baltic Sea, western Lithuania (55º39'N, 21º07'E). Mean age of the saplings in all stands was about 5-7 years and mean diameter at stump height (ca. 20 cm above ground) was about 3-4 cm (ranged between 1 and 12 cm). Study plots were circular and variable in diameter and a minimum of a 2-m-wide buffer zone separated the plots from each other.

Within each plot, twenty healthy-looking trees were selected, cut with a chain saw 15–25 cm above ground level and were either: i) inoculated with one of the four *C. purpureum* strains using two formulations – water suspension of 'AgroAquaGel'® (10 stumps) and xanthan gum in water+glycerine (10 stumps); ii) treated with blank inoculum, composed of the above-mentioned formulations without added mycelium (10 stumps per each formulation per plot); iii) left untreated ("slash control", 20 stumps); or iv) left untreated until the next growing season to allow formation of vigorous sprouts. Two months before scoring the results (the end of June, 2011), the stumps and emerged sprouts were sprayed with a glyphosate-based chemical herbicide 'Roundup® BIO' (Monsanto Crop Science Danmark A/S, Denmark) which served as a positive control treatment ("Roundup control", 20 stumps) and was applied by a 'Solo' hand sprayer following manufacturer's instructions (15 ml of herbicide in 1000 ml of water).

For each tree species, four plots were *C. purpureum*-treated, each with one of the four strains of *C. purpureum*, with 20 stumps per strain, one plot was assigned for the formulation control (20 stumps), one plot – for the slash control (20 stumps), and one plot – for the Roundup treatment (20 stumps) (Table 1). To assess the effect of the application season, stump inoculations were conducted twice – in summer (28th of July) and in autumn (15th of October), 2010. For the autumn treatment, new plots were selected in the vicinity of summer plots. In this way, a total of 980 stumps were treated in each season (7 tree species × 7 plots × 20 trees, Table 1).

Table 1. The number of *B. pendula, A. incana*, *P. tremula, S. caprea*, *A. negundo, H. rhamnoides* and *R. pseudoacacia* stumps cut in summer and in autumn and treated with four different strains of *Chondrostereum purpureum* and three types of controls

Tree species	Treatment[a]							
	C. purpureum P2-2	*C. purpureum* P2-8	*C. purpureum* P3-12	*C. purpureum* P4-17	Formulation control	Slash control	Roundup control[b]	All treatments
	stumps cut in summer (28th of July, 2010)							
B. pendula	20	20	20	20	20	20	20	140
A. incana	20	20	20	20	20	20	20	140
P. tremula	20	20	20	20	20	20	20	140
S. caprea	20	20	20	20	20	20	20	140
A. negundo	20	20	20	20	20	20	20	140
R. pseudoacacia	20	20	20	20	20	20	20	140
H. rhamnoides	20	20	20	20	20	20	20	140
All tree species	140	140	140	140	140	140	140	980
	stumps cut in autumn (15th of October, 2010)							
B. pendula	20	20	20	20	20	20	20	140
A. incana	20	20	20	20	20	20	20	140
P. tremula	20	20	20	20	20	20	20	140
S. caprea	20	20	20	20	20	20	20	140
A. negundo	20	20	20	20	20	20	20	140
R. pseudoacacia	20	20	20	20	20	20	20	140
H. rhamnoides	20	20	20	20	20	20	20	140
All tree species	140	140	140	140	140	140	140	980

[a] for more information on applied treatments see Materials and Methods section.
[b] stumps were cut and left untreated until formation of vigorous sprouts in the next growing season. In all cases, herbicide 'Roundup® BIO' was sprayed directly on these stumps and emerged sprouts two months before scoring the results (the end of June, 2011).

The treatments were applied manually using a paintbrush to the exposed stump surfaces within 5–10 minutes after felling. Following treatments, the stumps were immediately covered with aluminium foil caps to prevent drying or washing out of the inoculum and contamination by undesired microorganisms (Dr. Meindert de Jong, personal communication). Non-treated stumps (including those assigned for Roundup application) were not covered with the aluminium foil caps. Summer inoculations were conducted between 10:00 a.m. and 8:00 p.m. on a warm, sunny day (T_{min} = 21°C, T_{max} = 30°C), while autumn inoculations were conducted between 10:00 a.m. and 7:00 p.m. on a cool cloudy day (T_{min} = 3°C, T_{max} = 6°C). Each treated stump was marked with a fluorescent spray; exact geographic coordinates of all plots were captured with a Magellan® Triton™ 500 GPS receiver.

Re-isolation of C. purpureum from inoculated stumps

In October 2011, five stumps of *B. pendula*, *A. incana*, *P. tremula*, *S. caprea* and *A. negundo* bearing *C. purpureum* fruitbodies from plots initially inoculated with four strains of the fungus were assigned for wood sampling and fungal isolations (5 tree species × 4 strains × 5 stumps). Stumps of *R. pseudoacacia* and *H. rhamnoides* were not sampled because of absence of the fruitbodies. The sampling was performed by removing a small piece of wood from underneath the fruitbodies. In the laboratory, five tissue samples were aseptically taken from each woody piece using a sterile scalpel, sterilized by flaming for 1-2 seconds and placed on Hagem agar with added 0.5 g/l antibiotic chloramphenicol. Fungal cultures with morphology similar to *C. purpureum* were selected to test their compatibility to the applied strains P2-2, P2-8, P3-12 and P4-17. This was done with the aid of somatic incompatibility tests. The fungal isolates from the inoculated stumps were paired in all possible combinations, including reference strains and self-pairings as controls, on 9-cm Petri dishes containing Hagem agar media (Stenlid 1985, Lygis et al. 2004). If the reactions were not clear, the pairings were repeated. Interactions between two mycelia were regarded as compatible and the strains were classed as genetically identical when a continuous mycelial mat was formed between the isolates, corresponding to that of self-pairing controls (Korhonen and Stenlid 1998).

Result scoring and statistical analyses

During the September 2011, the occurrence and number of living sprouts per stump, height of the tallest living sprout, number of dead sprouts per stump, and occurrence of *C. purpureum* fruitbodies were recorded for every treated stump. No difference in performance of both formulations has been observed af-

ter the first vegetation season (Table 4) so the stumps were pooled together to simplify the statistical interpretation of complex data. In fungus treatments, both formulations are further referred to as "*C. purpureum* treatment", and in treatments with blank formulations – as "formulation control".

The differences in sprouting potential, mortality of sprouts (a proportion of dead sprouts on a given stump) and stumps, and fruitbody formation among treatments, tested *C. purpureum* isolates and different seasons of application were analysed by one-way analysis of variance (ANOVA) and applying Tukey's HSD test for significance (Fowler et al. 1998). The statistics were computed using SPSS Statistical Software (v. 17.0, SPSS Inc., IBM, Chicago, IL, USA).

Results

Field observation of the treated stumps and somatic compatibility tests performed with strains of *C. purpureum* re-isolated from the infected stumps confirmed that inoculated strains persisted during the first post-treatment year and formed abundant fruitbodies. Stumps of *B. pendula, A. incana*, *P. tremula* and *S. caprea* were extremely well colonized: 71.4–100.0%, 76.2–100.0%, 38.1–100.0% and 80.0–100.0% of the stumps of respective tree species bore *C. purpureum* fruitbodies (up to 40 pieces could be counted per stump in separate cases).

Table 2 summarizes the statistical results of stump sprouting control experiments, providing information on how different tree species have reacted to different treatments conducted in summer and in autumn. The best performance of the biocontrol agent was observed on *B. pendula* (Fig. 1) and *A. negundo* stumps (summer treatments): stump and sprout mortality and formation of *C. purpureum* fruitbodies was significantly (at $p \leq 0.05$) higher than in negative (formulation and slash) controls. As compared to positive (Roundup) control treatment, the results of stump (75.0–100.0%) and sprout (77.3–100.0%) mortality for both tree species (summer treatments) were very similar, although the formation of *C. purpureum* fruitbodies was far better on fungus-treated stumps: 95.0–100.0% of *B. pendula* and 25.0–66.7% of *A. negundo C. purpureum*-treated stumps bore fruitbodies which emerged on 0.0–20.0% of the Roundup-treated stumps. Excellent mycoherbicide performance was observed in preventing sprouting of *B. pendula*: on average, 97.6% and 63.0% of the stumps, and 99.3% and 77.3% of the sprouts were dead following summer and autumn treatments, respectively (pooled results from all plots treated with different *C. purpureum* strains) which in general was as good as Roundup treatment (Fig. 1, Table 2).

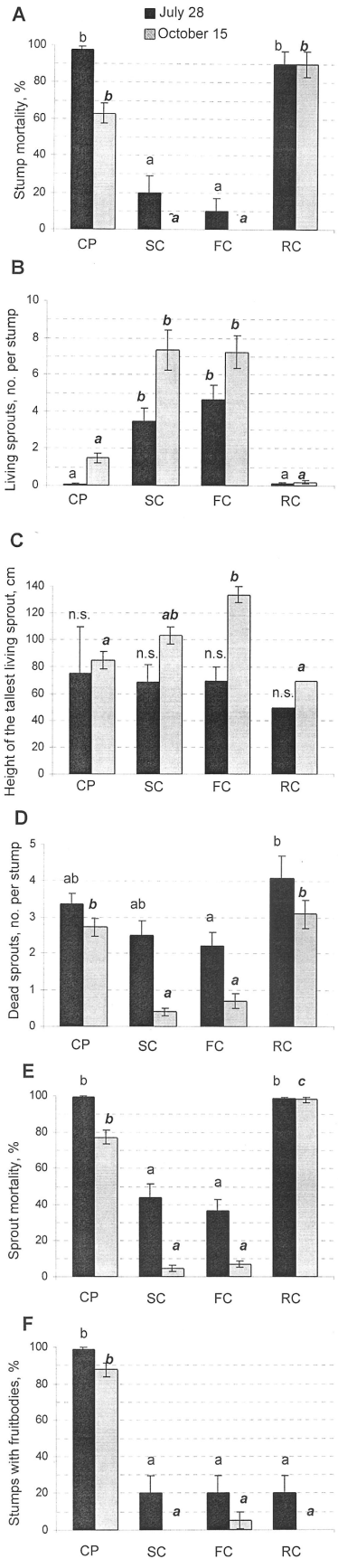

Figure 1. The effect of *Chondrostereum purpureum* (CP, pooled results from four applied *C. purpureum* strains), slash control (SC), formulation control (FC) and Roundup control (RC) treatments on sprouting of *Betula pendula* stumps (**A-E**) and formation of *C. purpureum* fruitbodies (**F**). For more information on applied treatments see Materials and Methods section. Bars are means ± standard error. Bars labeled with different letters are significantly different from each other at $p \leq 0.05$; n.s. – no significant difference at $p \leq 0.05$. Labels for autumn treatments (October 15) are given in boldface

No effect of biological control was measured on stumps of *R. pseudoacacia* and *H. rhamnoides* either in summer or in autumn treatments (Table 2). Not a single fruitbody of the inoculated fungus was observed on their stumps. In some cases, relatively high stump mortality of *H. rhamnoides* both in *C. purpureum* treatments (up to 90.5%) and negative controls (up to 65.0%) was likely the result of adverse site micro-conditions that are not yet completely understood. Roundup treatment gave high rate of stump and sprout mortality in *H. rhamnoides* compared to negative controls, although this effect was notable only for stumps cut in summer (Table 2). Alternatively, a large proportion of *R. pseudoacacia* stumps showed proliferant sprouting regardless of treatment (sprout mortality was as low as 18.9–42.2%). Roundup treatment has significantly (at $p \leq 0.05$) increased stump and sprout mortality in *R. pseudoacacia* compared to negative controls (Table 2).

Reaction to mycoherbicide treatments on the other three tree species – *A. incana*, *P. tremula* and *S. caprea* was not statistically different from the negative controls, although formation of *C. purpureum* fruitbodies was observed (71.4–100.0% of *C. purpureum*-treated stumps bore fruitbodies). Stumps of *P. tremula* showed few sprouts (0.0–0.5 of living sprouts per stump on average) both in mycoherbicide and control treatments making interpretation of the results prob-

Table 2. The effect of different treatments on sprouting of *B. pendula, A. incana*, *P. tremula, S. caprea*, *A. negundo, H. rhamnoides* and *R. pseudoacacia* stumps and formation of *C. purpureum* fruitbodies. Presented are mean values of respective variables ± standard error. Grouped by tree species, values labelled with the different letters are significantly different at $p \leq 0.05$; n.s. – no significant difference at $p \leq 0.05$; n.t. – not tested (missing or too few values)

	summer (28th of July, 2010)/autumn (15th of October, 2010) treatment[a]						
	C. purpureum P2-2	*C. purpureum* P2-8	*C. purpureum* P3-12	*C. purpureum* P4-17	Formulation control	Slash control	Roundup control[b]
				B. pendula			
Stump mortality, %	100.0±0.0a/	100.0±0.0a/	100.0±0.0a/	90.0±6.9a/	10.0±6.9b/	20.0±9.2b/	90.0±6.9a/
	76.2±9.5a	20.0±9.2b	80.0±9.2a	75.0±9.9a	0.0b	0.0b	90.0±6.9a
Living sprouts (no.	0.0a/	0.0a/	0.0a/	0.3±0.2a/	4.7±0.8b/	3.5±0.7b/	0.1±0.1a/
per stump)	0.7±0.3a	3.6±0.7b	0.8±0.4a	1.0±0.4ab	7.3±0.9c	7.4±1.1c	0.2±0.1a
Height of the tallest	- n.t./	- n.t./	- n.t./	75±35.0 n.t./	70±10.0 n.t./	69±12.4 n.t./	50±0.0 n.t./
living sprout, cm	80±15.8ab	78±8.7a	120±24.2ab	88±11.3ab	134±6.0b	103±6.3ab	70±0.0a
Dead sprouts (no.	4.1±0.8 n.s./	3.1±0.4 n.s./	2.6±0.4 n.s./	3.7±0.5 n.s./	2.2±0.4 n.s./	2.5±0.4 n.s./	4.1±0.6 n.s./
per stump)	2.3±0.4ab	3.5±0.5a	1.9±0.3abc	3.2±0.7a	0.7±0.2bc	0.4±0.1c	3.1±0.4a
Sprout mortality, %	100.0±0.0a/	100.0±0.0a/	100.0±0.0a/	97.1±2.0a/	36.2±6.6b/	43.8±7.5b/	98.9±0.8a/
	78.7±8.4a	54.8±7.3b	84.3±7.1a	86.7±5.3a	6.9±1.6c	4.6±1.6c	98.0±1.4a
Stumps with	100.0±0.0a/	100.0±0.0a/	95.0±5.0a/	100.0±0.0a/	20.0±9.2b/	20.0±9.2b/	20.0±9.2b/
fruitbodies, %	71.4±10.1b	90.0±6.9ab	100.0±0.0a	90.0±6.9ab	5.0±5.0c	0.0c	0.0c
				A. incana			
Stump mortality, %	23.8±9.5a/	65.0±10.9ab/	52.4±11.2ab/	65.0±10.9ab/	50.0±10.9ab/	40.9±10.7ab/	75.0±9.0b/
	10.0±6.9a	28.6±10.1ab	20.0±9.2ab	19.1±8.8ab	10.0±6.9a	15.0±8.2ab	50.0±11.5b
Living sprouts (no.	3.9±0.8b/	1.4±0.5a/	1.4±0.4a/	1.5±0.6a/	1.6±0.5a/	2.0±0.5ab/	0.7±0.3a/
per stump)	3.9±0.6ab	2.5±0.5a	2.6±0.5a	2.1±0.5a	5.5±1.1b	3.9±0.7ab	1.3±0.4a
Height of the tallest	79±7.2ab/	96±23.8ab/	70±10.2ab/	71±10.6ab/	103±13.2b/	74±15.4ab/	40±3.7a/
living sprout, cm	68±9.4ab	80±10.1b	72±8.1ab	50±4.8ab	77±7.7b	58±10.5ab	36±5.4a
Dead sprouts (no.	3.0±0.5 n.s./	3.8±0.5 n.s./	3.1±0.3 n.s./	4.3±0.7 n.s./	4.2±0.7 n.s./	2.9±0.5 n.s./	3.0±0.6 n.s./
per stump)	0.5±0.3a	0.9±0.2a	0.9±0.3a	1.3±0.3a	1.0±0.3a	0.7±0.2a	5.1±0.9b
Sprout mortality, %	52.6±7.3 n.s./	82.9±6.1 n.s./	78.9±5.7 n.s./	78.1±7.0 n.s./	71.7±7.7 n.s./	64.0±7.8 n.s/	80.0±8.9 n.s./
	7.7±3.4a	37.7±9.2b	28.9±8.4ab	44.3±8.4b	15.8±3.9ab	16.7±5.7ab	81.0±6.8c
Stumps with	95.2±4.8a/	100.0±0.0a/	95.2±4.8a/	100.0±0.0a/	4.5±4.5b/	4.5±4.5b/	8.3±5.8b/
fruitbodies, %	80.0±9.2a	76.2±9.5a	95.0±5.0a	85.7±7.8a	0.0b	5.0±5.0b	10.0±6.9b
				P. tremula			
Stump mortality, %	100.0±0.0a/	95.2±4.8ab/	100.0±0.0a/	100.0±0.0a/	100.0±0.0a/	75.0±9.9b/	80.0±9.2ab/
	95.0±5.0a	90.5±6.6ab	100.0±0.0a	100.0±0.0a	85.7±7.8ab	70.0±10.5b	100.0±0.0a
Living sprouts (no.	0.0 n.s./	0.1±0.0 n.s./	0.0 n.s./	0.0 n.s./	0.0 n.s./	0.5±0.2 n.s./	0.4±0.2 n.s./
per stump)	0.2±0.2 n.s.	0.1±0.1 n.s.	0.0 n.s.	0.0 n.s.	0.3±0.2 n.s.	0.3±0.1 n.s.	0.0 n.s.
Height of the tallest	- n.t./	50±0.0 n.t./	- n.t./	- n.t./	- n.t./	28±15.6 n.t./	10±0.0 n.t./
living sprout, cm	30±0.0 n.t.	50±10.0 n.t.	- n.t.	- n.t.	13±3.3 n.t.	34±9.0 n.t.	- n.t.
Dead sprouts (no.	2.4±0.4a/	1.4±0.3ab/	1.2±0.2b/	0.7±0.2b/	0.6±0.2b/	0.7±0.2b/	1.1±0.4b/
per stump)	0.5±0.2 n.s.	0.5±0.1 n.s.	0.2±0.1 n.s.	0.2±0.1 n.s.	0.4±0.2 n.s.	0.4±0.2 n.s.	0.6±0.2 n.s.
Sprout mortality, %	100.0±0.0a/	96.7±3.3a/	100.0±0.0a/	100.0±0.0a/	100.0±0.0a/	72.3±9.4ab/	66.7±14.2b/
	85.0±15.0 n.s.	80.0±13.3 n.s.	100.0±0.0 n.s.	100.0±0.0 n.s.	66.7±16.7 n.s.	37.5±15.7 n.s.	100.0±0.0 n.s.
Stumps with	70.0±10.5a/	38.1±10.9a/	63.6±10.5a/	47.6±11.2a/	0.0b/	0.0b/	0.0b/
fruitbodies, %	85.0±8.2ab	71.4±10.1b	100.0±0.0a	85.7±7.8ab	0.0c	0.0c	0.0c

lematic. Frequency of sprouting of *P. tremula* stumps, and lower stump and sprout mortality, was observed in the slash control (Table 2).

Roundup treatment showed generally good performance in sprouting control of all seven tree species with stump mortality reached as high as 100.0% in separate cases; however, efficacy of Roundup treatment in *A. incana* (50.0%), *A. negundo* (50.0%) and *H. rhamnoides* (60.0%) plots felled in autumn, and in *S. caprea* (66.7%) plot felled in summer was low (Table 2).

In general, the summer mycoherbicide treatments gave better results than the autumn treatments in terms of stump and sprout mortality (Table 2). Effect of treatment time on stump sprouting of *B. pendula, A. incana*, *P. tremula, S. caprea* and *A. negundo* (pooled results of all five species) is summarized in Table 3. *R. pseudoacacia* and *H. rhamnoides* were not included into these calculations as these species did not show any response to the mycoherbicide treatments (Table 2). Summer treatments showed significantly (at

Table 2 (continued). The effect of different treatments on sprouting of *B. pendula, A. incana*, *P. tremula, S. caprea*, *A. negundo, H. rhamnoides* and *R. pseudoacacia* stumps and formation of *C. purpureum* fruitbodies. Presented are mean values of respective variables ± standard error. Grouped by tree species, values labelled with the different letters are significantly different at $p \leq 0.05$; n.s. – no significant difference at $p \leq 0.05$; n.t. – not tested (missing or too few values)

	summer (28th of July, 2010)/autumn (15th of October, 2010) treatment[a]						
	C. purpureum P2-2	*C. purpureum* P2-8	*C. purpureum* P3-12	*C. purpureum* P4-17	Formulation control	Slash control	Roundup control[b]
			S. caprea				
Stump mortality, %	40.0±11.2ab/ 5.0±5.0a	20.0±9.2a/ 5.0±5.0a	57.1±11.1ab/ 0.0a	52.2±10.7ab/ 30.0±10.5ab	21.7±8.8a/ 71.4±10.1cd	22.7±9.1a/ 45.0±11.4bc	66.7±9.8b/ 100.0±0.0d
Living sprouts (no. per stump)	4.6±1.1d/ 4.2±0.5ab	3.5±0.7bcd/ 5.5±0.9a	1.1±0.3ab/ 5.3±0.5a	1.6±0.4abc/ 3.7±0.8abc	4.2±0.7cd/ 1.2±0.5cd	2.6±0.6abc/ 2.5±0.7bcd	0.3±0.1a/ 0.0d
Height of the tallest living sprout, cm	50±9.2a/ 99±9.2 n.t.	77±11.5ab/ 142±7.1 n.t.	97±12.1ab/ 82±5.4 n.t.	70±13.6ab/ 91±9.9 n.t.	105±8.4b/ 88±12.2 n.t.	94±9.7ab/ 80±10.7 n.t.	58±9.4ab/ - n.t.
Dead sprouts (no. per stump)	9.4±1.5c/ 1.6±0.3ab	5.7±1.5b/ 3.1±0.6a	1.0±0.3a/ 2.3±0.5ab	1.0±0.2a/ 2.8±0.8a	1.5±0.3a/ 0.5±0.3b	1.6±0.3a/ 1.0±0.4ab	4.0±0.8ab/ 2.7±0.5a
Sprout mortality, %	72.3±6.3bc/ 27.3±5.7a	57.3±7.5ab/ 38.3±5.2a	55.7±10.5ab/ 26.5±3.8a	47.6±9.1ab/ 44.9±8.2a	35.2±7.4a/ 36.2±13.0a	46.6±7.2ab/ 26.4±8.3a	93.3±2.0c/ 100.0±0.0b
Stumps with fruitbodies, %	80.0±9.2a/ 85.0±8.2a	85.0±8.2a/ 95.0±5.0a	90.5±6.6a/ 100.0±0.0a	91.3±6.0a/ 95.0±5.0a	8.7±6.0b/ 4.8±4.8b	22.7±9.1b/ 15.0±8.2b	16.7±7.8b/ 0.0b
			A. negundo				
Stump mortality, %	95.2±4.8a/ 0.0a	75.0±9.9a/ 0.0a	90.5±6.6a/ 5.0±5.0a	95.0±5.0a/ 5.0±5.0a	19.1±8.8b/ 0.0a	33.3±10.5b/ 0.0a	81.8±8.4a/ 50.0±11.5b
Living sprouts (no. per stump)	0.3±0.3a/ 8.7±1.3a	2.3±1.0ab/ 12.4±2.2ab	0.5±0.4a/ 13.3±2.0ab	0.2±0.2a/ 12.2±2.3ab	7.2±1.2c/ 19.7±2.8b	4.2±0.9b/ 18.4±2.0b	0.2±0.1a/ 5.1±1.4a
Height of the tallest living sprout, cm	70±0.0 n.t./ 186±8.6a	108±9.7 n.t./ 210±16.7ab	65±25.0 n.t./ 213±10.8ab	140±0.0 n.t./ 202±15.7ab	111±8.4 n.t./ 243±11.0b	91±7.7 n.t./ 206±11.0ab	40±5.8 n.t./ 198±9.3ab
Dead sprouts (no. per stump)	2.8±0.6 n.s./ 4.7±0.8a	3.2±0.5 n.s./ 4.6±0.8a	2.5±0.5 n.s./ 5.1±0.9a	3.4±0.6 n.s./ 6.4±0.9ab	2.7±0.5 n.s./ 9.1±1.1bc	3.1±0.5 n.s./ 4.9±0.7a	3.2±0.6 n.s./ 12.8±1.1c
Sprout mortality, %	95.8±4.2a/ 37.0±4.2a	77.3±9.0ab/ 28.5±2.8a	92.2±6.3a/ 31.0±5.4a	96.1±3.9a/ 37.6±5.8a	32.9±7.2c/ 34.3±2.7a	53.6±7.1bc/ 21.2±1.3a	98.0±0.9a/ 77.8±5.5b
Stumps with fruitbodies, %	66.7±10.5a/ 35.0±10.9a	25.0±9.9bc/ 35.0±10.9a	57.1±11.1ab/ 25.0±9.9ab	60.0±11.2ab/ 35.0±10.9a	0.0c/ 0.0b	9.5±6.6c/ 0.0b	0.0c/ 0.0b
			R. pseudoacacia				
Stump mortality, %	6.0±2.6a/ 1.2±1.2a	2.4±1.7a/ 0.0a	2.5±1.7a/ 2.5±1.7a	7.1±2.8a/ 1.2±1.2a	1.2±1.2a/ 2.5±1.7a	1.5±1.5a/ 1.5±1.5a	84.0±4.3b/ 72.0±6.4b
Living sprouts (no. per stump)	4.9±0.3a/ 3.1±0.2b	3.4±0.3bc/ 4.7±0.3cd	4.9±0.4a/ 4.0±0.3bc	2.7±0.2c/ 5.2±0.4cd	4.7±0.5ab/ 4.2±0.4bc	4.4±0.4ab/ 5.8±0.5d	0.2±0.1d/ 0.5±0.1a
Height of the tallest living sprout, cm	147±6.6 n.s./ 150±6.2 n.s.	174±6.8 n.s./ 146±6.1 n.s.	155±8.1 n.s./ 137±6.5 n.s.	152±7.3 n.s./ 149±7.0 n.s.	143±5.7 n.s./ 154±5.7 n.s.	151±7.7 n.s./ 156±7.6 n.s.	143±3.9 n.s./ 144±8.1 n.s.
Dead sprouts (no. per stump)	3.6±0.3c/ 1.0±0.1a	2.0±0.2ab/ 1.1±0.1a	2.6±0.2bc/ 1.0±0.1a	1.2±0.1a/ 1.4±0.2a	3.3±0.3c/ 1.3±0.1a	2.8±0.3bc/ 1.8±0.2a	7.0±0.5d/ 6.2±0.5b
Sprout mortality, %	42.2±2.5c/ 23.3±2.2a	31.3±2.8ab/ 19.0±2.2a	37.0±2.1abc/ 19.2±2.3a	29.7±3.0a/ 18.9±2.3a	40.3±2.2bc/ 25.6±2.5a	36.3±2.4abc/ 22.9±2.4a	97.0±0.8d/ 95.5±1.1b
Stumps with fruitbodies, %	0.0 n.s./ 0.0 n.s.	0.0 n.s./ 0.0 n.s.	0.0 n.s./ 0.0 n.s.	0.0 n.s./ 0.0 n.s.	0.0 n.s./ 0.0 n.s.	0.0 n.s./ 0.0 n.s.	0.0 n.s./ 0.0 n.s.

[a] for more information on applied treatments see Materials and Methods section.
[b] stumps were cut either in summer (28th of July, 2010) or in autumn (15th of October, 2010) and were left untreated until formation of vigorous sprouts in the next growing season. In all cases, herbicide 'Roundup® BIO' was sprayed directly on these stumps and emerged sprouts two months before scoring the results (the end of June, 2011).

$p \leq 0.0005$) higher stump and sprout mortality and lower mean height of the tallest living sprout than the autumn treatments, although there was little effect of the felling season on formation of *C. purpureum* fruitbodies (non-significant differences at $p \leq 0.05$): 78.0% and 76.8% of summer and autumn treated stumps bore fruitbodies, respectively (Table 3). Interestingly, a similar trend was observed not only for *C. purpureum*-treated stumps, but also for negative controls, where mean number of living sprouts per stump, mean height of the tallest living sprout were significantly (at $p \leq 0.0005$) higher and sprout mortality was significantly (at $p \leq 0.0005$) lower in autumn than in summer treatments. Yet, significantly more fruitbodies (at $p \leq 0.05$) were formed on the negative control stumps cut in summer than in autumn, although the percentages were generally low (9.0% vs. 3.0%). Felling time had little effect, in terms of stump and sprout mortality, on Roundup treatments (Table 3) and this is not surprising since treatment of both stump categories (summer and autumn) was performed during the next growing season at basically the same time. This allowed pooling of the data from the plots treated with both formulations to simplify statistical interpretation of the results (Tables 2, 3 and 5).

Comparison of the results of the two negative (formulation and slash) controls showed no statistically significant differences (at $p \leq 0.05$) in stump or sprout mortality, mean number of dead sprouts per stump and fruitbody formation both in summer treatments and summer+autumn (pooled data) treatments. However, pooling of the results from both negative controls could not be performed due to statistically significant differences in mean number of living sprouts per stump and mean height of the tallest living sprout: more numerous and higher sprouts have been produced by stumps treated with blank formulations than by non-treated ones (Table 4). Slight (yet non-significant at $p \leq 0.05$) effect was also recorded for fruitbody formation: nearly two times more non-treated stumps without aluminium foil caps bore *C. purpureum* fruitbodies than those treated with blank formulations and covered with the foil.

Table 3. The effect of treatment time on stump sprouting of five hardwood species (pooled data from *B. pendula, A. incana*, *P. tremula, S. caprea* and *A. negundo* plots) and formation of *C. purpureum* fruitbodies. Presented are mean values of respective variables ± standard error. *R. pseudoacacia* and *H. rhamnoides* were not included as these species did not show any response to the mycoherbicide treatments (Table 2)

	treatments[a]					
	C. purpureum[b]		Negative controls (formulation + slash control)		Positive (Roundup) control	
	summer[c]	autumn[d]	summer[c]	autumn[d]	summer[e]	autumn[f]
Stump mortality, %	75.9±2.1**	38.5±2.4**	38.9±3.4 n.s.	30.2±3.2 n.s.	78.2±5.6 n.s.	78.0±5.9 n.s.
Living sprouts (no. per stump)	1.1±0.1**	4.1±0.3**	3.1±0.3**	6.6±0.6**	0.4±0.1*	1.3±0.5*
Height of the tallest living sprout, cm	77±4.1**	122±4.6**	87±3.9**	126±6.5**	42±6.7*	113±25.7*
Dead sprouts (no. per stump)	3.1±0.2**	2.3±0.1**	2.2±0.2 n.s.	1.9±0.2 n.s.	3.1±0.4*	4.9±0.7*
Sprout mortality, %	83.6±1.6**	46.0±2.0**	51.9±2.7**	22.3±2.2**	89.3±4.1 n.s.	89.7±3.3 n.s.
Stumps with fruitbodies, %	78.0±2.0 n.s.	76.8±2.1 n.s.	9.0±2.0*	3.0±1.2*	9.1±3.9 n.s.	2.0±2.0 n.s.

[a] for more information on applied treatments see *Materials and methods* section.
[b] pooled results from all plots treated with different *C. purpureum* strains.
[c] treated on the 28th of July, 2010.
[d] treated on the 15th of October, 2010.
[e] stumps cut on the 28th of July, 2010 and treated with 'Roundup® BIO' in the end of June, 2011.
[f] stumps cut on the 15th of October, 2010 and treated with 'Roundup® BIO' in the end of June, 2011.
* within lines of a certain treatment values are significantly different at $p \leq 0.05$;
** within lines of a certain treatment values are significantly different at $p \leq 0.0005$; n.s. – no significant difference at $p \leq 0.05$

The effect of different treatments (formulations) on stump sprouting and formation of *C. purpureum* fruitbodies is compared in Table 4. The data show that there was no difference in performance of the two mycoherbicide formulations: water suspension of 'AgroAquaGel'® and water+glycerine suspension of xanthan gum resulted in similar effects in summer treatments and summer+autumn (pooled data) treatments.

As demonstrated in Table 2 and Table 5 (summarized results for five tree species), different strains of *C. purpureum* have different efficacy, although in many cases the differences were not significant at $p \leq 0.05$. In general, strain P2-8 resulted in the worst performance in terms of stump mortality and fruitbody formation both in summer and autumn treatments (Table 5), however it was one of the best in controlling sprout-

Table 4. Pair-wise comparison of the effect of different treatments (formulations) on stump sprouting of five hardwood species (pooled data from *B. pendula, A. incana*, *P. tremula, S. caprea* and *A. negundo* plots) and formation of *C. purpureum* fruitbodies. Presented are mean values of respective variables ± standard error. *R. pseudoacacia* and *H. rhamnoides* were not included as these species did not show any response to the mycoherbicide treatments (Table 2)

	treatments[a]							
	C. purpureum		*C. purpureum*		negative control		negative control	
	Agro-AquaGel[b], summer[d]	xanthan gum[c], summer[d]	Agro-AquaGel[b], summer[d]+ autumn[e]	xanthan gum[c], summer[d]+ autumn[e]	formulation, summer[d]	slash, summer[d]	formulation, summer[d]+ autumn[e]	slash, summer[d]+ autumn[e]
Stump mortality, %	74.8±3.0 n.s.	77.3±2.9 n.s.	55.5±2.5 n.s.	59.4±2.4 n.s.	39.6±4.8 n.s.	38.1±4.8 n.s.	37.0±3.4 n.s.	32.2±3.3 n.s.
Living sprouts (no. per stump)	1.2±0.2 n.s.	1.0±0.2 n.s.	2.7±0.2 n.s.	2.5±0.2 n.s.	3.6±0.4*	2.6±0.3*	5.1±0.5 n.s.	4.5±0.4 n.s.
Height of the tallest living sprout, cm	79±5.7 n.s.	74±5.9 n.s.	109±4.8 n.s.	109±5.6 n.s.	96±5.2*	78±5.7*	119±6.0**	96±5.4**
Dead sprouts (no. per stump)	3.3±0.3 n.s.	2.8±0.2 n.s.	2.9±0.2 n.s.	2.5±0.1 n.s.	2.3±0.2 n.s.	2.2±0.2 n.s.	2.3±0.2 n.s.	1.8±0.2 n.s.
Sprout mortality, %	83.6±2.1 n.s.	84.0±2.3 n.s.	65.0±2.0 n.s.	65.7±2.1 n.s.	49.3±4.0 n.s.	54.5±3.5 n.s.	39.1±2.8 n.s.	37.8±2.7 n.s.
Stumps with fruitbodies, %	80.1±2.8 n.s.	76.3±3.0 n.s.	76.4±2.1 n.s.	78.6±2.0 n.s.	6.6±2.4 n.s.	11.4±3.1 n.s.	4.3±1.4 n.s.	7.8±1.9 n.s.

[a] for more information on applied treatments see *Materials and methods* section.
[b] *C. purpureum* preparate in water suspension of 'AgroAquaGel®'.
[c] *C. purpureum* preparate in water+glycerine suspension of xanthan gum.
[d] treated on the 28th of July, 2010.
[e] treated on the 15th of October, 2010.
* within lines of a certain treatment values are significantly different at $p \leq 0.05$;
** within lines of a certain treatment values are significantly different at $p \leq 0.005$; n.s. – no significant difference at $p \leq 0.05$

ing of *A. incana* (yet the differences from the other strains were not significant at $p \leq 0.05$, Table 2). The other fungal strains (P2-2, P3-12 and P4-17) provided similar results (Table 5), although for different tree species this wasn't always true: for example, strain P2-2 has performed significantly worse than the others in controlling sprouting of *A. incana*. Therefore, the most consistent results were provided by strains P3-12 and P4-17.

Discussion

Results of the present study showed that the efficacy of biological control using basidiomycete *C. purpureum* varied greatly amongst different tree species and was largely dependant on application season. As regards birch, our results correspond well with findings of Finnish researchers (Vartiamäki et al. 2008, 2009) who found that mycoherbicide treatment per-

Table 5. Performance of different *C. purpureum* strains in controlling stump sprouting of five hardwood species (pooled data from *B. pendula, A. incana*, *P. tremula, S. caprea* and *A. negundo* plots) and formation of fruitbodies. *R. pseudoacacia* and *H. rhamnoides* were not included as these species did not show any response to the fungus treatment (Table 2).
Presented are mean values of respective variables ± standard error. Within lines of summer treatment or summer + autumn (pooled data) treatments, values labelled with different letters are significantly different at $p \leq 0.05$; n.s. – no significant difference at $p \leq 0.05$

	C. purpureum treatments, strain nos.							
	summer[a]				summer[a] + autumn[b]			
	P2-2	P2-8	P3-12	P4-17	P2-2	P2-8	P3-12	P4-17
Stump mortality, %	76.6±3.8ab	64.2±5.4a	80.0±3.9b	79.8±4.0b	59.1±3.3b	44.8±3.7a	61.0±3.4b	63.1±3.4b
Living sprouts (no. per stump)	1.4±0.3ab	1.8±0.4a	0.6±0.1b	0.7±0.2b	2.4±0.3 n.s.	3.4±0.4 n.s.	2.4±0.3 n.s.	2.2±0.4 n.s.
Height of the tallest living sprout, cm	66±6.1 n.s.	86±8.8 n.s.	81±7.7 n.s.	74±9.2 n.s.	100±6.3 n.s.	118±7.0 n.s.	112±7.5 n.s.	103±8.2 n.s.
Dead sprouts (no. per stump)	4.1±0.4a	3.5±0.4ab	2.0±0.2c	2.5±0.3bc	3.1±0.2b	2.9±0.2b	2.0±0.2a	2.6±0.2ab
Sprout mortality, %	86.2±2.5 n.s.	77.6±3.8 n.s.	85.0±3.2 n.s.	84.0±3.2 n.s.	67.2±2.8 n.s.	59.1±2.9 n.s.	64.9±3.1 n.s.	69.5±2.9 n.s.
Stumps with fruitbodies, %	85.5±3.2b	61.7±5.4a	80.0±3.9b	79.8±4.0b	79.1±2.7b	68.3±3.4a	82.0±2.7b	79.1±2.8b

[a] treated on the 28th of July, 2010.
[b] treated on the 15th of October, 2010.

formed in summer (especially – in June and July) significantly increased stump and sprout mortality, and the frequency of stumps with fruitbodies, as compared to negative controls, one year following treatments. During our earlier experiment on *C. purpureum*-based biocontrol of *B. pendula* sprouting (unpublished), stump mortality following fungus treatments was somehow lower (reached up to 76.5%) than observed during the present study, although the conditions under which the earlier experiment was performed were far from optimal. In Canada, a strong potential of *C. purpureum* for stump sprouting control of paper birch (*B. papyrifera* Marsh.) and yellow birch (*B. alleghaniensis* Britton) was also demonstrated one year after the treatments by Jobidon (1998) and Wall (1990). Although different techniques of mycoherbicide application have been used in these studies (different mycoherbicide formulations, methods of application and protection of the treated stumps) the results obtained by us and by other researchers confirm the tendency noted in the literature that family *Betulaceae* is one of the most likely to be affected by application of C. *purpureum* (Wall 1990, 1996, 1997, Dumas et al. 1997, Jobidon 1998, Harper et al. 1999, Pitt et al. 1999, Vartiamäki et al. 2007, Roy et al. 2010).

This is the first study to assess biocontrol of *A. negundo* with *C. purpureum*. However, stump sprouting control experiments have included other members of the genus *Acer*: red maple (*Acer rubrum* L.) (Wall 1997, Pitt et al. 1999, Roy et al. 2010), bigleaf maple (*A. macrophyllum* Pursh) (Wall 1996, Shamoun and Hintz 2002), sugar maple (*A. saccharum* Marsh.) and striped maple (*A. pensylvanicum* L.) (Wall 1997), and the results of these studies indicate that trees of this genus are only moderately susceptible to C. *purpureum*. For example, while investigating the effect of commercial mycoherbicide 'Myco-Tech™' paste, Roy et al. (2010) found that mortality of red maple was negligible one year after release, while two years after release it reached only about 20%. This difference from our results showing a high susceptibility of *A. negundo* might be explained by high inter-specific variation in susceptibility to *C. purpureum* within the genus *Acer*.

Sprouting of *A. incana* and *S. caprea* stumps was slightly controlled by the mycoherbicide treatments in our study, although more time is needed to obtain reliable results. In Finland, a similar situation was reported with *A. incana* and *Salix* spp. (Vartiamäki et al. 2007). Although about 60% of the treated alder stumps and about 90% of the willows bore fruitbodies and the percentage of non-sprouting stumps was increased and the number of living sprouts per stump was reduced by the application of *C. purpureum*, the difference between treated and control stumps was not significant one year after the treatments. Usually the best-expressed biocontrol results are observed during the second year after the treatment (Wall 1994, Pitt et al. 1999, Becker et al. 2005, Vartiamäki et al. 2008, 2009), so we also expect better results in the second post-treatment year. In general, the performance of *C. purpureum* in controlling stump sprouts of various alder species has proved to be good: for example, in Canada, more than 90% of red alder (*Alnus rubra* Bong.) stumps treated with *C. purpureum* died in the first year and 100% died in the second year after treatment (Becker et al. 2005); similar results were also obtained with Sitka alder (*A. viridis* ssp. *sinuata* (Regel) Á. Löve & D. Löve) by Harper et al. (1999). Again, this indicates high inter-specific variation in susceptibility to *C. purpureum* infections within a tree genus.

Results for *P. tremula* are less conclusive. Generally poor sprouting did not allow reliable comparison among different treatments and reasons for this are difficult to explain. The same target species (*P. tremula*) was investigated by Vartiamäki et al. (2007) and Hamberg et al. (2011a) who found little effect of *C. purpureum* treatment on sprout formation, however the results were summarized one year after the treatments which may have underestimated the true effect. In stump treatment experiments on trembling (*P. tremuloides* Michx.) and largetooth aspen (*P. grandidentata* Michx.), Dumas et al. (1997) and Becker et al. (1999) also observed little effect of the mycoherbicide treatments. Wall (1990) reported after the first post-treatment year that there was initially little evidence of infection of *P. tremuloides* and, as a result, little evidence of sprout reduction. Investigations by Wall (1990, 1996, 1997) and Becker et al. (1999) showed that *Populus* is noticeably more resistant than *Alnus* or *Betula*. Moreover, aspen produces numerous root suckers that emerge at various distances from a tree or a fresh stump (Jobling 1990, Worrell 1995) and the efficacy of mycoherbicides in controlling stump sprouting might be difficult to assess. However, a rather high proportion of *P. tremula* stumps with *C. purpureum* fruitbodies observed during our present study (Table 2) suggests that aspen in Lithuania may be more susceptible to the fungus.

Absence of biological control effect on stump sprouting of *R. pseudoacacia* and *H. rhamnoides* in summer or in autumn treatments, and absence of fruitbodies of the inoculated fungus on stumps of either species suggest that these species are either resistant to *C. purpureum*, or that the effect will be observed later (2-3 years after the treatments) than for the other tested tree species. Similar conclusions have been

drawn by Wall (1990, 1997) and Roy et al. (2010) who investigated efficacy of mycoherbicides on pin cherry (*Prunus pensylvanica* L.f.) in Canada and claimed that some hardwood tree species may be completely resistant to the pathogen. As we were not able to find any information on the *C. purpureum*-based stump sprouting control for *R. pseudoacacia* and *H. rhamnoides*, further surveys of the experimental sites are necessary.

The results of our study have shown that in certain cases, such as summer treatments of *B. pendula* and *A. negundo* stumps, *C. purpureum* can have an efficacy comparable to that of a glyphosate-based herbicide one year after application. This has also been reported from biocontrol field trials of *A. rubra* (Becker et al. 2005), black cherry (*Prunus serotina* Ehrh.) (Scheepens and Hoogerbrugge 1989), *B. papyrifera* (Jobidon 1998), and *A. viridis* ssp. *sinuata* and *P. tremuloides* (Harper et al. 1999). Although the effect of Roundup treatment caused more mortality than that of *C. purpureum* and the applied chemical herbicide proved to be the only effective measure against sprouting of *R. pseudoacacia* and *H. rhamnoides* during first post-treatment year, the abundant fruitbody formation on treated stumps of *B. pendula*, *A. incana*, *P. tremula*, *S. caprea* and *A. negundo* might point to much higher potential of the applied fungus in the next years performance. Occurrence of *C. purpureum* fruitbodies on some of the Roundup-treated stumps indicate that interpretation of the results obtained during the forthcoming surveys could be complicated due to natural *C. purpureum* infections which may result in an overlapped effect of the fungal activity and the chemical herbicide.

In general, summer mycoherbicide treatments gave better results than the autumn treatments in terms of stump and sprout mortality and mean height of the tallest living sprout, although there was little effect of the felling season on formation of *C. purpureum* fruitbodies. It has been demonstrated by Finnish (Vartiamäki et al. 2009) and Canadian (Wall 1994) researchers as well as by our earlier experiment (unpublished) that *C. purpureum* treatment is the most effective when performed during active tree growth during summer, and loses its efficacy toward the end of the growing season. This is in accordance with earlier assumptions that resistance of certain tree species (*Betula alleghaniensis* and *Fagus grandifolia* Ehrh.) against the pathogen is greatest in spring and decreases toward midsummer, after which it increases again (Wall 1991). Alternatively, in southern Canada Dumas et al. (1997) found little seasonal effect of biocontrol on sprouting of trembling and largetooth aspen stumps, however the trials were established during the period of likely active tree growth (from late June till late September with rather high air temperature during the September treatment), which is not comparable to the results obtained by us and Finnish researchers (Vartiamäki et al. 2009) in September-October in Eastern and Northern Europe.

Dumas et al. (1997) compared the formulation control with the slash control and found that application of the blank formulation to the freshly cut stumps of *P. tremuloides* and *P. grandidentata* did not significantly affect the ability of stumps to resprout, the number of sprouts per stump, sprout mortality, or the height of the tallest sprout. Comparing both types of negative control, Vartiamäki et al. (2009) also found no significant differences in stump mortality (or frequency of stumps with living sprouts), in number of living sprouts per stump, in the mean height of the tallest living sprout, or in frequency of stumps with *C. purpureum* fruitbodies. Additionally, blank formulations had a positive effect on sprouting of both speckled alder (*A. rugosa* (Du Roi) Spreng) and *A. viridis* ssp. *sinuata*, indicating that the formulation alone may have acted as a "wound dressing," preventing moisture loss and allowing greater nutrient allocation to sprout regeneration (Pitt et al. 1999). Comparison of the effects of both negative controls was not possible in our study as stumps treated with the blank formulation were covered with aluminium foil caps while non-treated stumps were not, and this likely had an impact on stump sprouting and fruitbody formation. Significantly more numerous and higher sprouts were produced by stumps treated with the formulation control than by non-treated stumps indicating that covering stumps with the foil possibly prevented drying out, thus stumps remained alive for the longer time. The stump covering could also prevent aerial infections by pathogenic microorganisms, including *C. purpureum*.

Covering stumps treated with mycoherbicide preparates may have a positive effect on initial sprout formation although "greenhouse conditions" for the inoculated fungus likely facilitated its establishment and subsequent sprout infections. This could also explain the efficient stump colonization and excellent sprouting control of the most susceptible tree species. Among the negative control treatments, open stumps (slash control) were significantly more acceptive to natural infections by *C. purpureum*. The occurrence of its fruitbodies on control stumps indicated that felling hardwoods and exposing their stumps alone might occasionally result in sufficient natural infection to achieve sprouting control in non-treated stumps.

Our experiments provided no evidence that both formulation types, water suspension of

'AgroAquaGel'® or water+glycerine suspension of xanthan gum, differentially influenced mycoherbicide efficacy. These formulations have likely created suitable conditions for growth and establishment of *C. purpureum*; although the even stronger positive effect of the aluminium foil caps that could somehow equalize the effect of both formulations should not be neglected. Pitt et al. (1999) and Jobidon (1998) suggested that fungal isolate is more important than formulation in determining efficacy success; however, we are in agreement with a suggestion by Harper et al. (1999) that formulation may also enhance efficacy.

Across our experiments, the most efficacious *C. purpureum* treatments consistently contained strains P3-12 and P4-17, although the performance of all four tested strains was in general good (Table 5) indicating that well-performing isolates of the fungus, at least for sprouting control in *B. pendula* and *A. negundo*, can easily be found within local (Lithuanian) populations of *C. purpureum*. The results of our earlier experiment on biocontrol of birch sprouting (unpublished) showed significant differences of the applied *C. purpureum* strains in the ability to prevent sprouting (stump mortality ranged between 32.7% for the least-efficient strain and 76.5 % for the most-efficient one). This is also supported by Jobidon (1998), Pitt et al. (1999), Harper et al. (1999) and Vartiamäki et al. (2008) who suggested that virulence could vary among isolates of *C. purpureum*, particularly for less susceptible tree species. While all fungal strains tested in the present study showed high virulence and potential for biocontrol applications, at least for sprouting control in *B. pendula* and *A. negundo*, further isolate selection may lead to future efficacy enhancements, especially against more resistant tree species. More research is needed to assess the variation in virulence within a large population of Lithuanian *C. purpureum* strains on a number of host species and to develop accurate tools for fast strain selection for use in biological control.

Conclusions

One year after treatment, the best performance of *C. purpureum* preparates, which was equivalent to that of applied chemical herbicide 'Roundup® BIO', in preventing stump sprouting was observed on *B. pendula* and *A. negundo* treated during the summer: stump and sprout mortality and formation of *C. purpureum* fruitbodies was significantly (at $p \leq 0.05$) higher than in negative (formulation and slash) controls.

Mycoherbicide effect on sprouting of *A. incana*, *P. tremula* and *S. caprea* was negligible one year after the treatments, although abundant formation of *C. purpureum* fruitbodies on fungus-treated stumps may indicate enhanced sprout control in the future.

Absence of mycoherbicide effect on sprouting and fruitbody formation on stumps of *R. pseudoacacia* and *H. rhamnoides* suggested that these species are either resistant to *C. purpureum*, or the effect will be delayed relative to the other tested species.

In Lithuanian conditions, *C. purpureum*-based biocontrol treatments should be performed during a period of intense tree growth as mycoherbicide efficacy decreased toward the end of the growing season; although the effect of the felling season on formation of *C. purpureum* fruitbodies is negligible.

Both applied mycoherbicide formulations are fungus-friendly, easy to prepare and to use, and were able to prevent fungal inoculum from drying-out; both showed equal effect in the field experiments.

Across the experiments, the most successful *C. purpureum* treatments consistently contained strains P3-12 and P4-17, although the performance of all four tested strains was in general similar, indicating that well-performing isolates of the fungus can be easily found in local Lithuanian populations of *C. purpureum*.

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ИСПОЛЬЗОВАНИЕ *CHONDROSTEREUM PURPUREUM* ДЛЯ КОНТРОЛЯ ВОЗОБНОВЛЕНИЯ ПОРОСЛИ СЕМИ ВИДОВ ЛИСТВЕННЫХ ПОРОД В ЛИТВЕ

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Резюме

Нами были протестированы опытные препараты класса микогербицидов на основе гриба *Chondrostereum purpureum* для изучения контроля прорастания семи видов лиственных пород, произрастающих в Литве (автохтонные *Betula pendula, Alnus incana*, *Populus tremula* и *Salix caprea*, и инвазионные *Acer negundo, Hippophae rhamnoides* и *Robinia pseudoacacia*). Исследования проводились в июле и октябре 2010 года. В каждом случае двадцать деревьев каждого вида обрезали и инокулировали четырьмя литовскими штаммами *C. purpureum* двумя способами (эффект обоих оказался одинаковым): 1) с использованием водной суспензии «AgroAquaGel ®»; 2) с использованием водно-глицеринового раствора ксантановой камеди. В качестве отрицательного контроля мы использовали оба способа инокуляции деревьев, но без добавления штамма гриба. Для положительного контроля мы применяли гербицид «Roundup® BIO».

Через год после инокуляции все четыре штаммы *C. purpureum* были обнаружены в соответственно обработанных пнях *B. pendula*, *A. incana*, *P. tremula*, *S. caprea* и *A. negundo*; они образовали многочисленные плодовые тела и все показали похожий эффект против прорастания поросли. По сравнению с негативным контролем, применение микогербицидов в течение лета значительно снизило количество порослевых пней и образования веток, а также значительно уменьшила среднюю высоту живой поросли *B. pendula* и *A. negundo.* В данном случае высокая гербицидная активность *C. purpureum* была сравнима с воздействием химического гербицида. Незначительное влияние *C. purpureum* наблюдалось на *A. incana*, *P. tremula* и *S. caprea*, хотя обильное образование плодовых тел на опытных пнях этих пород указывает на несомненное воздействие препаратов. При применении микогербицидов на пнях *R. pseudoacacia* и *H. rhamnoides* не наблюдалось никакого эффекта. Возможно, эти виды более устойчивы к *C. purpureum*, или необходим более длительный период для развития симптомов инфекции. В общем, летний вариант проведения опыта показал значительно лучшие результаты, чем проведение опыта осенью: гибель летом и осенью составляла 75,9% и 38,5% для пней и 83,6% и 46,0% для поросли соответственно (данные опытов с *B. pendula, A. incana*, *P. tremula, S. caprea* и *A. negundo*), хотя влияния времени рубки на образование плодовых тел *C. purpureum* незамечено. Так, применение местных штаммов *C. purpureum* против прорастания поросли *B. pendula* и *A. negundo* может дать отличные результаты при проведении обработки пней в период активной вегетации дерева.

Ключевые слова: биологический контроль, лиственные породы, нежелательная растительность, микогербициды, обработка пней