Tests with Wood-Decay Fungi to Control Sprouting from Cut Stumps Infected by Dutch Elm Disease

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Menkis, A., Vasaitis, R., Östbrant, I.-L., Pliūra, A. and Stenlid, J. 2017. Tests with Wood-Decay Fungi to Control Sprouting from Cut Stumps Infected by Dutch Elm Disease. *Baltic Forestry* 23(1): 270-273.

Abstract

The Dutch elm disease pathogen *Ophiostoma novo-ulmi* in year 2005 invaded the Swedish island of Gotland, which possesses large and valuable population of elms. The control of the disease is accomplished when infected elms are harvested and destroyed, and stumps are treated with the glyphosate herbicide to kill the stumps and the root systems, and prevent further spread of the disease to the neighbouring trees via root contacts. The aim of the present study was to test an alternative method to control the stump sprouting by deploying two species of saprotrophic wood-decay fungi as biological control agents. The study was carried out during three consecutive years 2014, 2015 and 2016. Fungal inoculum of *Chondrostereum purpureum* and *Stereum hirsutum* was prepared by cultivating vegetative mycelia in liquid nutrient medium and then formulating into a gel. Each year, the inoculum was applied at the beginning of the growing season on the surface of fresh stumps. In total, 250 stumps were treated with saprotrophic wood-decay fungi and 250 stumps were left as non-treated control. Assessment of stump mortality was carried out ones after each growing season. Results showed that the mortality of stumps (without living sprouts) was low in each treatment and year (between 4.1% and 25.6%) and did not differ significantly from those in the control. In conclusion, the method tested had very limited or no effect on the mortality of elm stumps, and thus, appears to be unsuitable to control the spread of Dutch elm disease via root contacts and sprouts.

Keywords: biological control, Dutch elm disease, *Ophiostoma*, saprotrophic wood-decay fungi, sprouting control, *Chondrostereum*, *Stereum*

Introduction

The Swedish island of Gotland has large and highly valuable populations of elms (mainly *Ulmus minor*), which currently is threatened by the invasive Dutch elm disease (DED) (Menkis et al. 2016b). DED is an aggressive vascular wilt disease, which during the last hundred years have destroyed billions of elm trees worldwide (Phillips and Burdekin 1982). Despite the DED was present in Europe for decades (Brasier et al. 2004), it invaded Gotland only in 2005 (Menkis et al. 2016b). Geographical isolation of the island in the Baltic Sea was suggested to be the limiting factor preventing natural arrival of the disease, which was probably brought to the island with DED-infected elm wood (Menkis et al. 2016b). DED is caused by the pathogenic *Ophiostoma* (Ascomycota) fungi, which are vectored by *Scolytus* bark beetles (Ploetz et al. 2013, Menkis et al. 2016a). However, the disease also possesses the ability for secondary spread from tree to tree via root grafts (Santini and Faccoli 2015). The DED fungi reproduce by a yeast-like budding process, and the bud spores are distributed in the sap stream and spread rapidly throughout the current xylem. The fungi cause wilting and death, both by the plugging of the conducting system and by the production of toxins. A typical internal symptom of the disease is the formation of a brown ring in the infected sapwood resulting

from the formation of tyloses and gels in the xylem vessels (Santini and Faccoli 2015). In Gotland, the control of the disease is accomplished when infected elms are identified, harvested and destroyed, and stumps are treated with the glyphosate herbicide to kill the stumps and the root systems, and prevent further spread of the disease to the neighbouring trees via root contacts (Menkis et al. 2016b).

However, the use of synthetic herbicides involves the potential hazards due to toxicological and environmental risks. Besides, the use of synthetic herbicides may be restricted in certain areas. Therefore, alternative methods to control sprouting are desirable and would allow limit the input of synthetic herbicides. The deployment of indigenous saprotrophic fungi to control stump sprouting can be seen as environmentally friendly approach (Dumas et al. 1997, de Jong 2000). For example, a saprotrophic fungus *Chondrostereum purpureum* was shown to possess a considerable potential as a biocontrol agent to control stump sprouting in several deciduous tree species by applying vegetative mycelium on freshly cut stumps (Lygis et al. 2012 and references therein). *C. purpureum* aggressively colonises the cambium in an injured area causing mortality of stump sprouts (de Jong et al. 1990). Another saprotrophic fungus, *Stereum hirsutum*, was also shown to be an aggressive and common colonised of wounds in deciduous trees (Vasiliauskas 1998). It was also found in association with unusual decline of tanoak sprouts (McDonald et al. 1988), and therefore, may possess the capacity to control stump sprouting. Despite the numerous field tests and the development of some saprotrophic fungi into the commercial products to control stump sprouting (de Jong 2000), the information on the efficacy of such fungi to control sprouting from elm stumps is scarce.

The aim of the present study was to test an alternative method to control sprouting from elm stumps by deploying saprotrophic wood-decay fungi *C. purpureum* and *S. hirsutum* as potential biological control agents.

Materials and Methods

Study sites

The study was carried out at Ganthem (year 2014), Vallstena (2015) and Endre (2016) sites situated in the mid-eastern part of Gotland island, Sweden (Table 1).

All sites were in close proximity (between ca. 9 and 11 km from each other) and characterised by similar climatic conditions. However, the site at Vallstena included a number of open areas, while sites at Ganthem and Endre were less exposed. Forest stands were dominated by *Ulmus* spp. with *Pinus sylvestris* L., *Picea abies* (L.) Karst., *Betula pendula* Roth, and *Alnus* spp. in admixture. The sites were characteristic to Gotland in terms of landscape and trees species composition, and were in the areas characterised by a high incidence of DED.

Table 1. Sites in Gotland and the number of fresh elm stumps treated during 2014-2016 with saprotrophic wood-decay fungi. The geographical positions of the study sites at Ganthem, Vallstena and Endre are shown in parenthesis.

Treatment	Site			All
	Ganthem (N57°30' E18°37') 2014	Vallstena (N57°36' E18°39') 2015	Endre (N57°35' E18°30') 2016	
Chondrostereum purpureum	48	43	34	125
Stereum hirsutum	49	42	34	125
Control	97	85	68	250
Total	194	170	136	500

DED-diseased elms were identified based on external and internal disease symptoms (Menkis et al. 2016a, Menkis et al. 2016b) during the surveys that were carried out by the Swedish Forest Agency in each preceding growing season. After identification, DED-diseased elms were felled during the dormancy period (November to April), transported to the local power plant, chipped and burned. Stumps of felled trees were treated with the herbicide glyphosate in order to kill root systems and to prevent further spread of the DED via roots and sprouts (Menkis et al. 2016b). However, the stumps in protected areas such as Natura 2000 sites and on land of organic farming were not treated with the herbicide due to legislation requirements. Instead, these stumps were used for the treatment with selected saprotrophic wood-decay fungi native to Gotland. In total, there were 194 such stumps in 2014, 170 in 2015 and 136 in 2016, resulting in 500 stumps altogether. Each year, half of stumps were treated with mycelia of saprotrophic wood-decay fungi and half were left as non-treated control (Table 1). The stumps used for the treatment and non-treated control stumps were selected randomly resulting in intermixed distribution at each site.

Preparation of fungal inoculum and inoculation

Fungal cultures of *Chondrostereum purpureum* strains P2.2 and *Stereum hirsutum* strain S8 were obtained from the culture collection of the Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala. Inoculum of each fungal species was produced separately. Firstly, the fungi were sub-cultured on Hagem agar medium (Stenlid 1985) in order to check viability and sterility of the cultures. Sub-cultured fungi were maintained in 9 cm diameter Petri dishes at room temperature (ca. 21°C) in the dark. For the production of fungal inoculum, which comprised vegetative mycelia, 5 l Erlenmeyer flasks were used each containing 2 l of liquid Hagem media. Ten agar plugs 0.5 × 0.5 cm in size with established fungal mycelia from an actively grow-

ing colony were aseptically inoculated in each flask and incubated at room temperature in the dark for four weeks.

Fungal inoculum was prepared one day before application in the field. Mycelium of each fungal species was harvested from liquid cultures through filtering, placed in a Moulinex Q50 blender (SEB, Ecully, France) and homogenised for two minutes. In order to prevent rapid desiccation of fungal mycelia following application on the stumps, homogenised mycelia was formulated into a gel by mixing at a ration 1:10 with a water suspension of Agrisan gel (Flügel GmbH, Osterode am Harz, Germany), which was prepared following recommendations of the producer. Agrisan gel is based on the poly-acrylic acid potassium salt crosslinked superabsorbent polymer and is commonly applied to plant roots at their outplanting. Prepared fungal inoculum was stored at 4°C until used in the field.

Each year, inoculation was done ones in the middle of May by applying prepared fungal inoculum on the entire surface of fresh stumps using a trowel. Each fungal species was inoculated separately. The control stumps were treated in the same way only by applying a water suspension of Agrisan gel alone. The treatments were applied each year on new stumps. The number of stumps treated with saprotrophic wood-decay fungi and non-treated controls in each year are shown in Table 1. To check vitality of the inocula, after the application in the field was completed remaining inoculum was brought back to the laboratory and from each treatment the respective fungus was re-isolated onto Hagem medium.

Assessment of stumps

In order to evaluate the effect of different treatments on mortality of stumps, the presence or absence of sprouting from the stumps was used as a measurement. In the absence of living sprouts the stumps were scored as dead. Each year, the assessment was done in the middle of September by visual inspection of all treated and non-treated control stumps. The assessment for production of fungal sporocarps and the detection of fungi in wood samples was not carried out.

Statistical analyses

The impact of each treatment on stump mortality was evaluated within each year by comparing the actual observations (dead/living stump data) in different treatments and a non-inoculated control using chi-square tests (Mead and Curnow 1983). Similarly, the impact of site/year was evaluated by comparing the actual observations of the same treatment among different sites/years. As each of the datasets was subjected to multiple comparisons, confidence limits for *p*-values of chi-square tests were reduced corresponding number of times as required by the Bonferroni correction (Sokal and Rohlf 1995).

Results

The mortality of stumps at Ganthem site after the growing seasons 2014 was 6.3% in *C. purpureum* treatment, 4.1% in *S. hirsutum* treatment and 6.2% in non-inoculated control (Figure 1). Comparison by chi-square test showed that different treatments and a control did not differ significantly from each other. The mortality of stumps at Vallstena site after the growing seasons 2015 was 25.6% in *C. purpureum* treatment, 21.4% in *S. hirsutum* treatment and 23.5% in non-inoculated control (Figure 1), thereby all of these did differ significantly from each other. The mortality of stumps at Endre site after the growing seasons 2016 was 11.8% in *C. purpureum* treatment, 14.7% in *S. hirsutum* treatment and 13.2% in non-inoculated control (Figure 1), and chi-square test showed that different treatments and a control did differ significantly from each other.

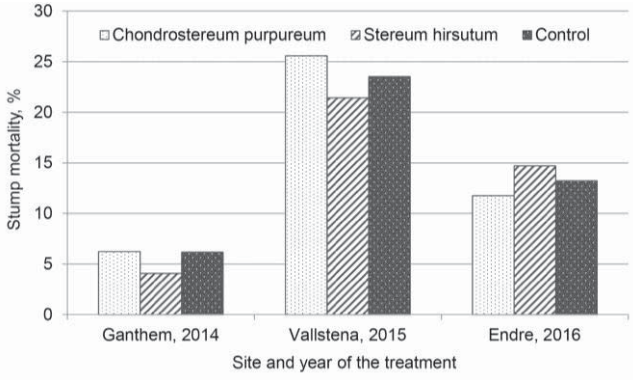

Figure 1. Mortality of elm stumps after the first growing season following inoculation with saprotrophic wood-decay fungi *Chondrostereum purpureum* and *Stereum hirsutum*.

Assessment of stump mortality after the second growing season following application of the treatments showed that the number of dead stumps remained largely unchanged (data not shown). Within each treatment (*C. purpureum*, *S. hirsutum* or control), comparison among different years showed that stump mortality was significantly lower in 2014 vs. 2015 ($p<0.04$), but did not differ significantly when compared 2014 vs. 2016 or 2015 vs. 2016.

Discussion

The results showed that the mortality of elm stumps was relatively low in each treatment and year (Figure 1). Moreover, within each year, the stump mortality in inoculation treatments was similar to those in the control (Figure 1), demonstrating that the method tested was largely unsuitable to control the stump sprouting, and thus, unsuitable to control the spread of Dutch elm disease via root and sprouts. By contrast, the glyphosate herbicide treatment is known to be highly efficient, thereby resulting in high rates

of stump mortality. The observed variation in stump mortality among different sites/years (Figure 1) was likely due to particular environmental conditions of each site. It was noted that the majority of dead stumps were in direct exposer to the sun while the stumps with sprouting were often in the shadow of the living trees. Consequently, the higher stump mortality at Vallstena (year 2015) (Figure 1) was likely due to their higher exposer to the sun as compared to those stumps at Endre (year 2016) and in particular at Ganthem (year 2014) sites. The latter suggests that environmental conditions and in particular sun radiation, but not the stump inoculation with saprotrophic wood-decay fungi, were largely responsible for the observed levels of stump mortality in different treatments and years.

Previously it was shown that the stump treatment with *C. purpureum* as compared to controls resulted in significantly higher levels of stump mortality in *Populus tremuloides* and *P. grandidentata* (Dumas et al. 1997), *Betula pendula* and *Acer negundo* (Lygis et al. 2012), *Alnus rubra* (Becker et al. 2005), *Sorbus aucuparia* (Hamberg et al. 2011) and *Prunus serotina* (de Jong 2000). In many cases the effect of this biological control treatment on mortality of stumps was as high as of synthetic herbicides. However, such positive effect of the stump treatment with *C. purpureum* appears to be host specific. For example, no effect of the treatment was observed on *Robinia pseudoacacia* and *Hippophae rhamnoides*, which was suggested to be due to either resistance of these trees to *C. purpureum* infection or significantly delayed response (Lygis et al. 2012). Taken together, the above demonstrates that the stump treatment with *C. purpureum* is not equally efficient on different tree species. In agreement, the result of the present study confirmed the latter observation at the same time showing that elm as a tree species exhibits little or no response to the stump treatment with tested wood-decay fungi.

In conclusion, the method tested had very limited or no effect on the mortality of elm stumps, and thus, appears to be unsuitable to control the spread of Dutch elm disease via root contacts and sprouts.

Acknowledgements

The financial support is gratefully acknowledged to the Foundation Oscar and Lili Lamms Minne, the Carl Tryggers Foundation and the EU Life+ Nature Elmias (LIFE12 NAT/SE/001139).

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