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Pētniecības projekts Nr. 9 «Bioloģiskā preparāta izstrāde sakņu trupes izraisīto zaudējumu samazināšanai skuju koku audzēs»

Starpposma rezultāta atskaite

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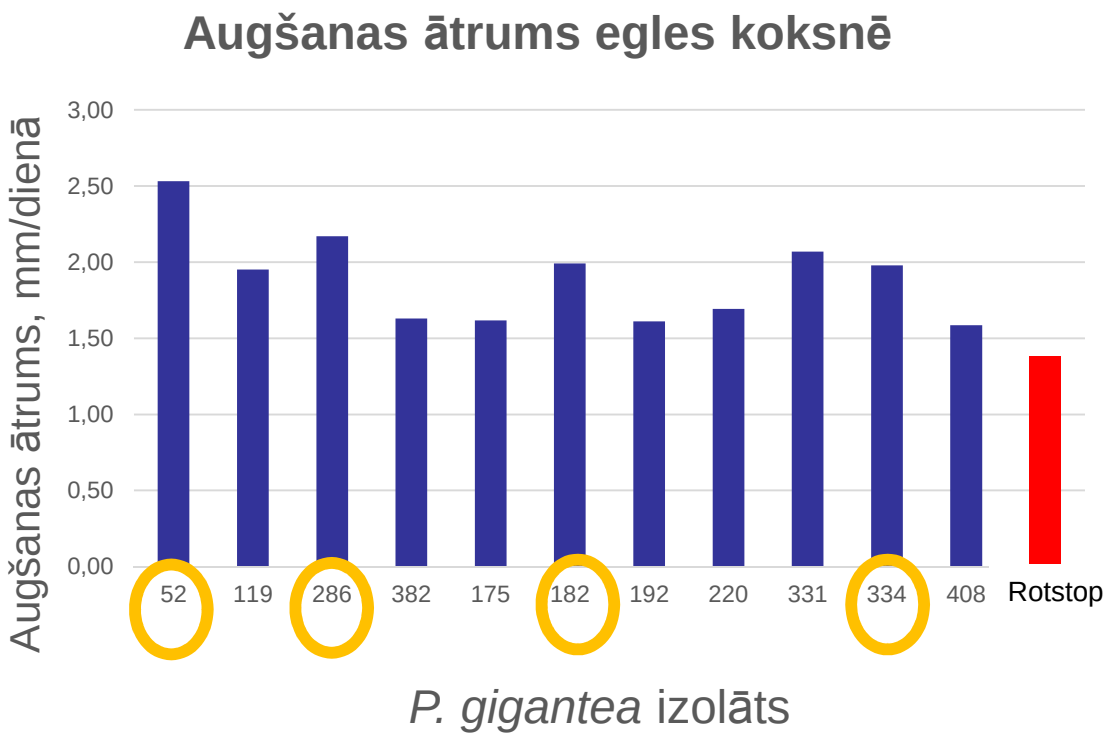
2020. gada septembrī

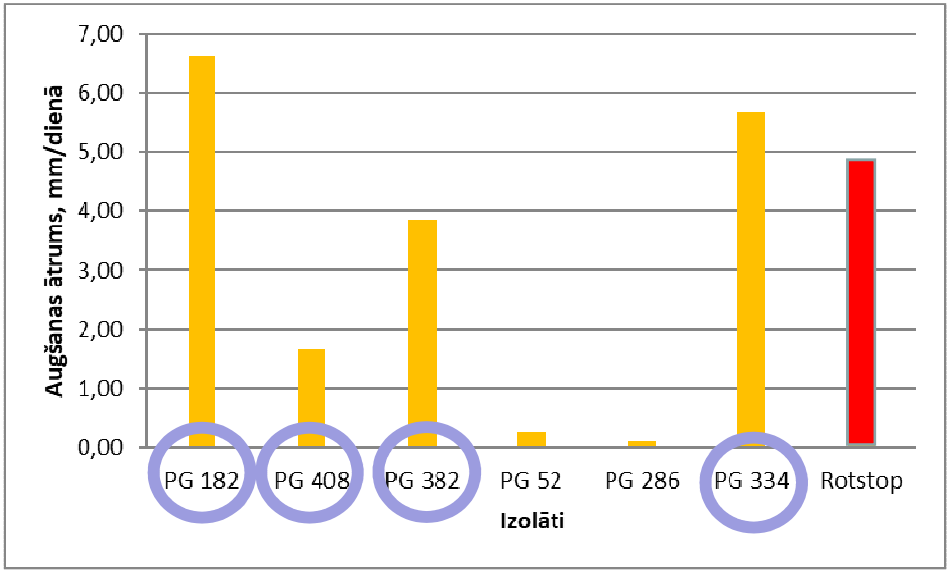
Meža nozares kompetences centrs
Forest Sector Competence Centre of Latvia
NACIONĀLAIS ATTĪSTĪBAS PLĀNS 2020
EIROPAS SAVIENĪBA
Eiropas Reģionālās attīstības fonds
IEGULDĪJUMS TAVĀ NĀKOTNĒ

3. Starpposma rezultāts: P*. gigantea* izolātu kombināciju sastāvu pārbaude labaratorijā un lauka apstākļos, publikācijas sagatavošana

- Starprezultāta īss apraksts:
  - atkārtoti ierīkots eksperiments, lai pārbaudīta atsevišķu izolātu attīstību priedes koksnes bluķīšos augšanas kamerā, atlasīti labākie izolāti un izveidoti 2 *P. gigantea* izolātu kombināciju sastāvi;
  - ierīkots eksperiments, lai 5 atšķirīgu suspensiju efektivitāti pārbaudītu lauka apstākļos 56 koksnes bluķīšos;
  - publikācijas «Efficacy of Phlebiopsis gigantea against Heterobasidion conidiospore and basidiospore infection in spruce wood »sagatavošana.

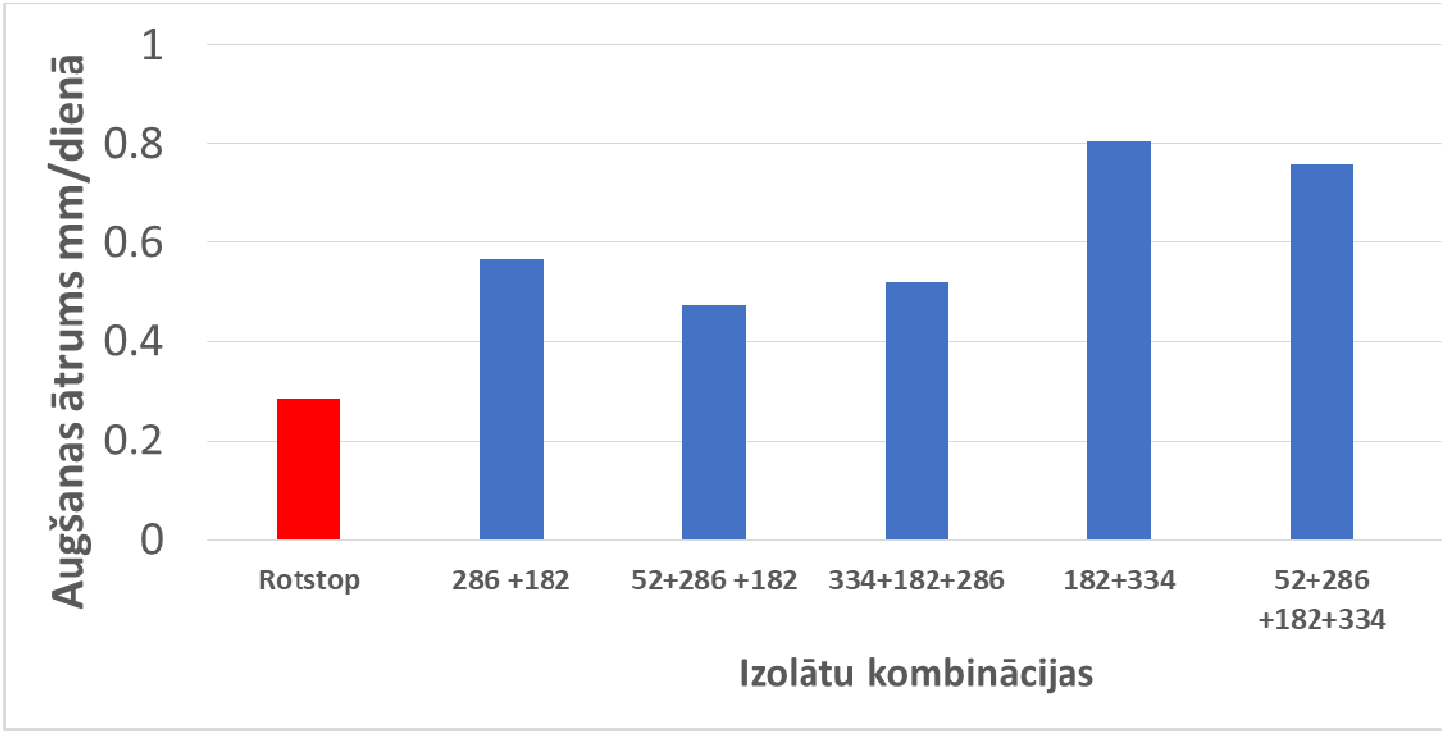
3. Starpposma rezultāts: laboratorijā priedes koksnē atkārtoti pārbaudīti labākie P*. gigantea* izolāti

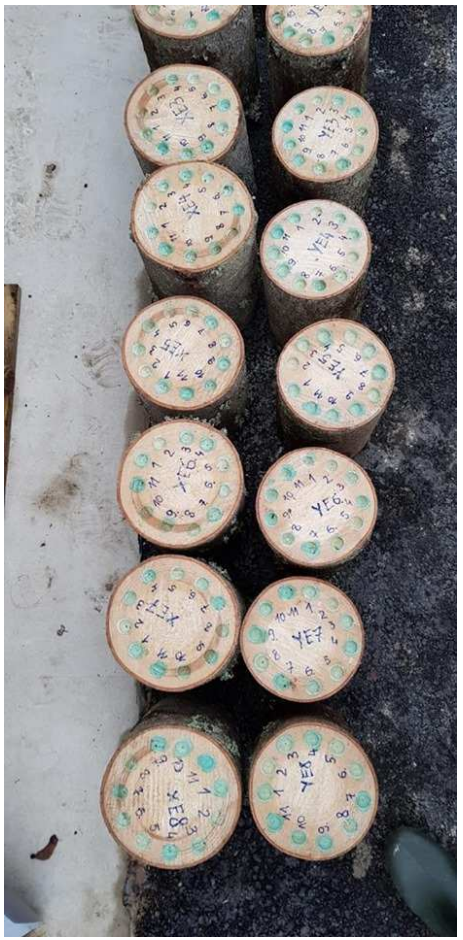




Attēlā parādīti izolāti, kuri egles un priedes koksnē attīstījās labāk nekā Rotstop; izolātu atlasi nosaka arī citas to īpašības: antagonisms un sporu veidošanās potenciāls. Atzīmētie izolāti izmantoti, lai izveidotu labākos izolātu kombināciju sastāvus

3. Starpposma rezultāts: laboratorijā atlasīti *P. gigantea* izolātu kombināciju sastāvi





Konstatēts, ka egles koksnē ievērojami labāk attīstās suspensijas, kuru sastāvā ir izolāti 182 un 334.
Priedes un egles koksnē labāk attīstījās izolāti (un to suspensijas), kuri veidoja mazāk sporas.

3. Starpposma rezultāts: ierīkots lauka eksperiments

Eksperiments sniegs informāciju par 2 *P. gigantea* izolātu kombināciju sastāvu efektivitāti pret sakņu piepes bazīdijsporu infekciju. Papildus ierīkots eksperiments, lai iegūtos datus varētu salīdzināt ar Rotstop un citiem koksnē lēnāk augošiem izolātiem (arī suspensijās, ko veido vairāki izolāti).

Eksperiments tiks pabeigts 2020. gada oktobrī. Iegūtie rezultāti sniegs informāciju, lai izveidotu tehnoloģijas prototipu un pārbaudītu tehnoloģijas prototipu reālā darbības vidē.

3. Starpposma rezultāts: sagatavota publikācija

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Efficacy of *Phlebiopsis gigantea* against *Heterobasidion* conidiospore and basidiospore infection in spruce wood

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Treatment of freshly cut stumps with biological control agents containing *Phlebiopsis gigantea* spores effectively restricts the spread of new *Heterobasidion* infections in conifer forests. To test the control efficacy of different *P. gigantea* strains, conifer stumps or billets cut from tree stems can be artificially infected with asexual *Heterobasidion* conidiospores or sexual basidiospores or left for natural basidiospore infection. Currently, no information is available about whether the control efficiency of *P. gigantea* in Norway spruce wood is affected by *Heterobasidion* spore type. In the present study, the impact of four *P. gigantea* strains (including the commercial product Rotstop®) on initiation and development of *Heterobasidion* basidiospore and conidiospore infections as well as the relationship between the area occupied by *P. gigantea* and control efficacy were analysed in spruce billets. The mean size of the area occupied by *P. gigantea* was larger, and the efficacy of *P. gigantea* against *Heterobasidion* was significantly higher in billets left for natural basidiospore infection compared to treatment with *Heterobasidion* conidiospore suspension. The control efficacy against *Heterobasidion* infection was high, although only a small area of the billet surface was occupied by *P. gigantea* and even when there was no visible discoloration caused by *P. gigantea* infection on wood surfaces.

Keywords: ***Picea abies*, Billets, Conidiospores, Basidiospores**

Introduction

Infection of freshly cut stump surfaces by airborne spores is the most common way of *Heterobasidion* species establishment into previously uninfected conifer stands. From infected stump roots, the fungus spreads to adjacent healthy trees causing root and butt rot in the residual stand (Redfern & Stenlid 1998, Witzell et al. 2011). An effective way to restrict *Heterobasidion* spore infections of conifer stumps is to treat the stump surfaces with biological or chemical control agents (Holdenrieder & Greig 1998, Pratt et al. 1998). Biological preparations containing asexual spores of *Phlebiopsis gigantea* – an antagonist of *Heterobasidion* species – are very effective in pine stumps but can be less effective in spruce stumps (Sun et al. 2009 and literature therein). However, good control efficiency can also be achieved in spruce wood when the spore concentration of treatment suspension is high, *i.e.*, 5 million spores L^{-1} (Korhonen 2003). A commercial preparation of *P. gigantea*, Rotstop®, was developed almost 30 years ago in Finland and is the most commonly used biological control agent against *Heterobasidion* root rot in Europe (Korhonen et al. 1994, Thor et al. 2006). However, the use of a genetically homogenous preparation may negatively affect fungal communities, therefore different studies were carried out to evaluate the impact of the biological control agent on below ground and stump colonizing fungal communities (Holdenrieder & Greig 1998, Vainio et al. 2001, Roy et al. 2003, Vasiliauskas et al. 2004, Vasiliauskas et al. 2005, Menkis et al. 2012, Terhonen et al. 2013). Previous studies indicate that local *P. gigantea* strains can be as effective, or

Field testing of the efficiency of *P. gigantea* strains is usually done in stumps at sites with natural *Heterobasidion* spore infection (Korhonen et al. 1994, La Porta et al. 2003, Berglund & Rönnberg 2004, Annesi et al. 2005, Berglund et al. 2005, Nicolotti & Gonthier 2005, Rönnberg et al. 2006, Covert et al. 2013, Kenigsvalde et al. 2016). However, under *in vivo* conditions, the results can be influenced by several factors such as erratic densities of airborne *Heterobasidion* spores, as well as variable background levels of natural *P. gigantea* spore load (Berglund & Rönnberg 2004, Gaitnieks et al. 2018). Laborious and long-lasting field experiments may even be inconclusive due to a lack of *Heterobasidion* infections in control stumps (Korhonen et al. 1994). The growth rate and efficiency of *P. gigantea* against *Heterobasidion* infection is also strongly dependent on the characteristics of individual trees (Sun et al. 2009). In several infection experiments, billets cut from tree stems have been used instead of stumps, or billets are used in conjunction with stumps (Holdenrieder 1984, Korhonen et al. 1994, Korhonen 2003, La Porta et al. 2003, Roy et al. 2003, Annesi et al. 2005, Sun et al. 2009). Cutting several billets were exposed near *H. parviporum* fruit bodies (unpublished data). Therefore, the majority of basidiospores causing natural infection were probably derived from *H. parviporum*. Inoculation experiments carried out by Gunulf et al. (2012) showed the competitive advantage of *H. parviporum* over *H. annosum*: *H. parviporum* totally replaced *H. annosum* in Norway spruce billets inoculated with a mixture of homokaryotic conidiospores of both species. Moreover, as also demonstrated in a study by Gunulf et al. (2012), *H. parviporum* grows successfully deeper in spruce wood compared to *H. annosum*. Dominance of *H. parviporum* may partly explain the result of our study indicating that infections caused by basidiospores were larger in area in the lower part of the control sector in billets.

In our experiment, we used billets cut from three individual trees instead of stumps in order to decrease variability due to differences between wood characteristics and of the individual trees. Despite maximizing the homogeneity of wood material, the variation in fungal colonization was high. The area occupied by *Heterobasidion* varied greatly both after conidiospore (4%-81%) and natural basidiospore (3%-71%) infections. A similar range in area occupied by *Heterobasidion* in Sitka spruce stumps after infection with *Heterobasidion* basidiospores (0.02%-56.6%), was found in a study by Morrison & Redfern (1994). In our study with spruce billets, the area occupied by Rotstop® was on average 22.8%. Whereas, a study in Sweden showed that area occupied by *P. gigantea* after treatment with Rotstop® in spruce stumps at depths of 2-12 cm was 5.9% (Berglund & Rönnberg 2004). Stumps can remain alive at least for 10 years after cutting if they have root contact with neighbouring trees (Redfern 1993). Unlike *Heterobasidion*, *P. gigantea* colonizes only deadwood, thus growth of *P. gigantea* is more likely to be inhibited in stumps than in billets (Vainio et al. 2001 and literature therein, Tubby et al. 2008). Thus, our results indicate that *P. gigantea* grows faster and may be more efficient against *Heterobasidion* infections in Norway spruce billets than in Norway spruce stumps.

In several studies *P. gigantea* efficacy is related to its occupied area (Korhonen 2003, Berglund & Rönnberg 2004, Tubby et al. 2008). An earlier Latvian study indicated that when the area occupied by *P. gigantea* exceeds 10% of stump surface area, occurrence of *Heterobasidion* is significantly decreased (Kenigsvalde et al. 2016). Interest-

P. gigantea appears only after longer period of incubation (K. Korhonen personal communication). This is supported by the results obtained by Oliva et al. (2017). By quantifying the biomass of *H. annosum* and *P. gigantea* in Norway spruce stumps, they reported that visual assessment after incubation may be a poor measure of presence or absence of both fungi. In Rotstop® treated stumps, no differences in biomass of *P. gigantea* could be found between areas with visual presence or absence of *P. gigantea* after incubation. For *Heterobasidion*, a significant difference in *Heterobasidion* biomass between areas with or without growth of *Heterobasidion* after incubation was found in stumps artificially inoculated with conidia suspension but not in naturally infected stumps (Oliva et al. 2017).

In several studies, the efficacy of the biological control agent Rotstop® varies from 50 to 100% (Korhonen 2003, Berglund & Rönnberg 2004, Berglund et al. 2005, Nicolotti & Gonthier 2005, Rönnberg et al. 2006, Cech et al. 2008, Kenigsvalde et al. 2016). In our study, the average efficacy of Rotstop® was 55% to 96 %. The average area occupied by the three Latvian *P. gigantea* strains after treatment with *Heterobasidion* conidiospores was smaller than after treatment with Rotstop®. However, indigenous *P. gigantea* strains showed slightly higher occupation areas at a depth of 8 cm after *Heterobasidion* basidiospore infection. Efficacy of the local *P. gigantea* strain G1 used in our experiment has also been demonstrated in previous studies (Kenigsvalde et al. 2016). To limit the spread of *Heterobasidion* in the long term (*via* secondary infection) at a stand level, it is critical to restrict the growth of *Heterobasidion* mycelium deeper in the wood (Korhonen et al. 1994, Pettersson et al. 2003, Berglund & Rönnberg 2004). Therefore, the obtained results demonstrate the potential of local *P. gigantea* strains to limit *Heterobasidion* infection in long term. Although in our experiment with *Heterobasidion* conidiospores local *P. gigantea* strains showed lower efficacy than Rotstop®, with high ambient *Heterobasidion* natural basidiospore level, efficacy of the same *P. gigantea* strains was high.

Conclusions

Efficacy of *P. gigantea* treatment in a representative site was lower in Norway spruce billets after inoculation with conidiospores from four *Heterobasidion* strains belonging to two species than after natural infection through *Heterobasidion* basid-

strains used in the study. However, local *P. gigantea* strains have the potential to effectively limit advance of *Heterobasidion* infection deeper in the wood, thereby decreasing vegetative spread of *Heterobasidion*.

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3. Starpposma rezultāts: laboratorijā egles koksnē pārbaudīti un atlasīti 10 labākie *P. gigantea* izolāti un izveidoti 2 *P. gigantea* izolātu kombināciju sastāvi, sagatavota publikācija, ierīkots lauka eksperiments

- Papildus informācija par sasniegto rezultātu t.sk.:
  - iegūtais rezultāts pilnībā atbilst plānotajam;
  - iegūtais rezultāts ir nozīmīgs, jo no tā atkarīga turpmākā projekta īstenošana;
  - uzsākts eksperiments, lai labāko *P. gigantea* izolātu kombināciju sastāvu un atsevišķu izolātu efektivitāti pārbaudītu lauka apstākļos koksnes bluķīšos, eksperimenta rezultātā tiks izveidos tehnoloģijas prototips;
  - projekta gaitā šobrīd nav paredzētas izmaiņas.

Iespējamo risku izvērtējums

- Meteoroloģiskie faktori:
  - var ietekmēt projekta 4. starpposma rezultātus;
  - nevēlamu meteoroloģisko faktoru ietekmi ir iespējams samazināt, ierīkojot eksperimentu, vadoties no ilgtermiņa meteoroloģiskajām prognozēm.
- Nesankcionētās darbības mežā:
  - 4. starpposma rezultātus var ietekmēt apstrādāto celmu virsmas destrukcija, apstrādājamā materiāla aizvākšana;
  - lai samazinātu nesankcionētu darbību potenciālos riskus, rūpīgi izvēlēta eksperimenta ierīkošanas vieta.

Prognozes par tālāko pētījuma gaitu, ņemot vērā līdz šim sasniegto

Optimistiskais scenārijs

- Izdalītie sēņu izolāti izmantoti efektīva bioloģiskā celmu apstrādes līdzekļa izstrādē.

Pesimistiskais scenārijs

- Iegūtie dati izmantoti publikāciju sagatavošanā, kā arī prezentēti konferencēs un semināros.

Paldies par uzmanību!