Production of healthy Norway spruce seeds: host specificity, pathogenicity and infection biology of *Thekopsora areolata*

Final report to Kungl. Skogs- och Lantbruksakademien

Grant number: TFV 2018-0001

Grant amount: 2 000 000 SEK

Period: 2019-2020

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1. Background

The rust fungus *Thekopsora areolata* is widely distributed in Europe and Asia. It causes the disease cherry spruce rust ("grankotterost", ''kuusentuomiruoste'') on spruce trees (*Picea* spp.). The Norway spruce *P. abies* is the most susceptible spruce species, and it is one of the most important tree species in Fennoscandian forestry. Cherry spruce rust is not lethal to Norway spruce, but infected cones produce no fertile seeds or seeds with a highly reduced germination rate. The replanting of Norway spruce in Sweden and Finland relies on the high-quality seeds produced from seed orchards, and the supply of seeds is often in shortage because Norway spruce only produce abundant cones and seeds every six to seven years. Therefore, cherry spruce rust poses great risks to the Norway spruce seed production and sustainable forest production. For example, epidemics of cherry spruce rust with high yield losses were reported in Swedish and Finnish seed orchards in 2000 and 2006.

Thekopsora areolata is a heteroecious rust pathogen that requires two unrealated host plants to complete it´s two years life cycle. Infection of Norway spruce take place by basidiospores that develop on germinating telia on overwintering leaves of the main alternate host wild bird cherry (*Prunus padus*) (Gäumann 1959). The infection results in spermogonia, which are fertilized by spermatia, and aecia of the fungus develop in the cone scales in July-September. During the following year, aeciospores are released and infect the young leaves of the wild bird cherry. The release of spores from old cones may continue for several years (Kaitera & Tillman-Sutela 2014). The magnitude of infection multiplies through asexual reproduction by urediniospores on the alternate host during summer. *Thekopsora areolata* overwinters as telia on wild bird cherry leaves and form basidiospores in the spring and the two-year disease cycle is completed. Although rust epidemics have been reported repeatedly before, the infection cycle, fruiting stages and spore production of *T. areolata* are poorly studied.

In 2019 and 2020, it was predicted that Norway spruce trees would produce a large number of cones in Sweden and Finland. Therefore, there was a great opportunity to study the epidemiology, alternate host biology and population genetics of *T. areolata*. Field experiments and laboratory experiments were executed in Sweden and Finland in 2019 and 2020.

2. Research activities

2.1 Spruce cone development and susceptibility, *T. areolata* basidiospore dissemination modeling

From 2019 May to September, cone bagging experiments were performed in the seed orchards Ålbrunna, Sweden, and Suhola, Finland (Fig 1). Cones were bagged to inhibit the *T. areolata* infection by basidiospores, and the bags from different treatment groups were removed every two weeks, and therefore, the cones were only exposed to the basidiospores during a certain period. At each location, we bagged 125 cones. However, due to the low general infection rate/disease incidence in both Sweden and Finland in that year, we were not able to collect enough infected cones for statistical analysis.

Figure 1. Cone bagging of Norway spruce in 2019.

Furthermore, we developed a simple spore trap design to quantify *T. areolata* DNA, as well as aeciospores and urediniospores (Fig. 2). The method enables the inference of *T. areolata* basidiospore loads from the discrepancy between DNA quantity and urediniospores and aeciospore numbers. From April to June in 2019, we validated the feasibility of this method in a pilot study in Ultuna, Sweden. From April to June in 2020, the field experiments were performed in Ultuna, Sweden, and in Joutsa, Finland. We deployed twenty-one spore traps at each experiment site to investigate the temporal and spatial dynamics of *T. areolata* basidiospores, aeciospores, and urediniospores.

Figure 2. Spore traps from Ultuna, Uppsala, Sweden, in 2019.

We identified the different temporal peaks of the three types of spores in the Finnish and the Swedish sites (Table 1). The results showed that urediniospores were mainly distributed within ten meters from the bird cherry trees. Basidiospore production peaks of one to two weeks coincided with multiple rain events. The germination of *T. areolata* teliospores and the morphology of basidiospores were observed and documented in the lab as well.

Table 1. Average daily temperature, degree-day accumulation, number of rainy days, and total precipitation during each sampling interval in Sweden and Finland.

Location	Collection date	Average daily temperature (ºC)[a]	Total degree-day accumulation (ºC)	Number of rainy days[a]	Total precipitation (mm)[a]	Aeciospore peak	Basidiospore peak	Urediniospore peak	Pollen peak
Ultuna, Sweden	Apr/20	7.0	390.2	2	1.2				
	Apr/27	8.2	447.9	1	4.5				
	May/4	5.3	484.7	4	14.5	++	+++		
	May/11	8.1	541.2	1	11.6	+++			
	May/18	5.6	580.5	6	9.6	+++	+++		+
	May/25	10.0	650.5	1	3.4				+
	Jun/1	14.0	748.2	1	7.1				+++
	Jun/8	14.3	848.5	2	11.8				+++
	Jun/15	17.3	969.5	0	0				+++
	Jun/22	18.4	1098.3	2	28.5			+++	+
Joutsa, Finland	May/4	4.8	199.4	3	2.7[c]				
	May/11	6.8	246.7	3	19.9[c]				
	May/18	4.3	277.1	4	18.9	+++			
	May/25	9.8	345.6	2	1.6	+++			
	Jun/1	13.8	442.0	0	0				++
	Jun/8	13.6	537.5	4	18.2	+++	+++		+++
	Jun/15	17.4	659.3	1	4.6				+++
	Jun/22	19.1	793.2	1	5.1				++
	Jun/29	21.4	943.3	3	15.4			+++	+

[a] Data was calculated based on the daily temperature or precipitation within 7 days.

The results of this research activity are documented in Publication [2]. The research output will provide background information to develop efficient cherry spruce rust disease management strategies in Norway spruce seed orchards.

2.2 Screening of additional alternate hosts of *T. areolata*

In the summer of 2019 and 2020, we tested 21 native Fennoscandian plant species and 18 *Prunus* spp. by *T. areolata* aeciospore inoculation experiments in incubators or greenhouse. The plant materials were collected in both Finland and Sweden, and the experiments were conducted in Finland. In 2020 summer, we surveyed 41 trees of 25 *Prunus* spp. in the Uppsala Botanical Garden for their susceptibility to *T. areolata*. No new susceptible species were found in Sweden or Finland, but a new record of *P. grayana* with low susceptibility to *T. areolata* was found in Sweden (Fig. 3). Additionally, we updated the list of currently confirmed alternate hosts of *T. areolata* according to historical literature, field observations and inoculation results, This list provides information for practical application in forestry. We conclude that *Prunus padus*, *P. serotina*, and their hybrids, as well as the subspecies of *P. padus* are highly susceptible, while *P. depressa*, *P. grayana*, *P. spinosa*, and P. *tenella* are considered slightly susceptible. The results of this research activity are documented in Publication [1]. The seed orchard owners and other stakeholders will need information from this research to manage the potential alternate hosts around or within the seed orchards, the information is also valuable for policymakers.

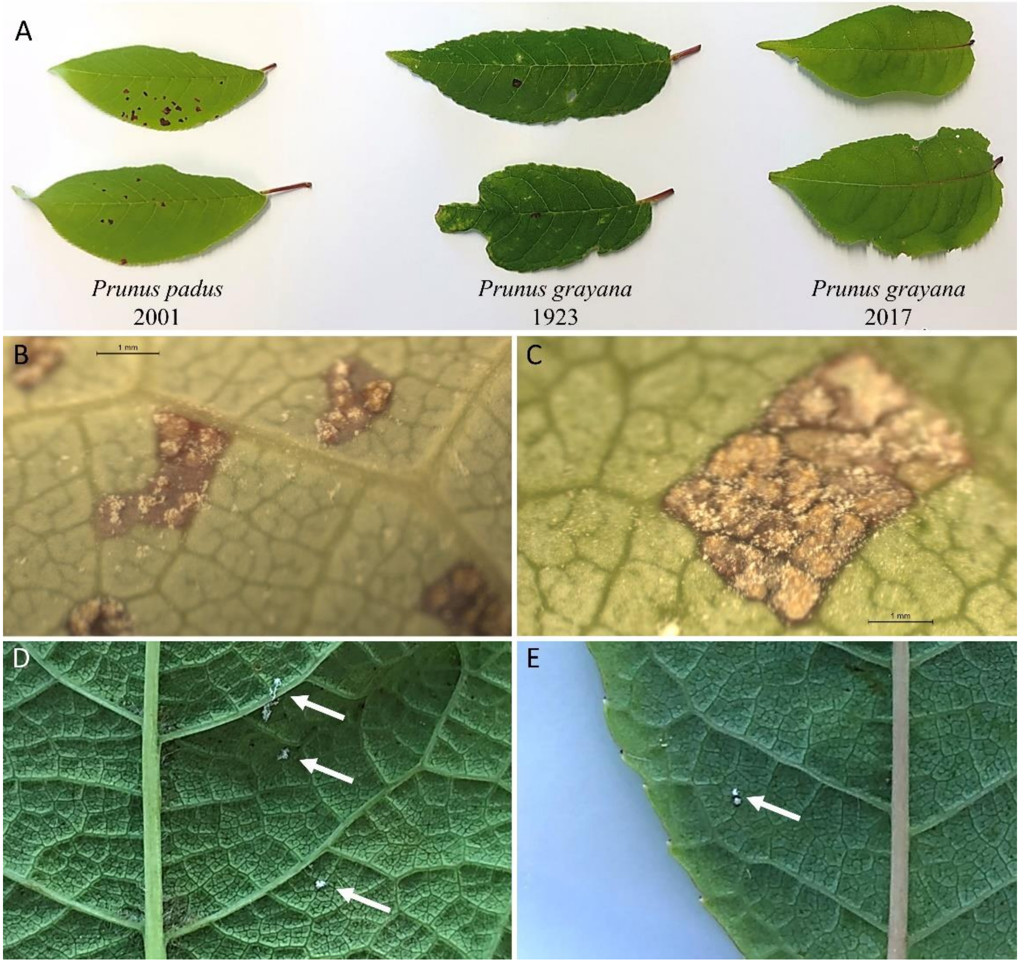

Figure 1. Thekopsora areolata infections on *Prunus padus* and *P. grayana* leaves in the Uppsala Botanical Garden and in detached leaf inoculation test. A, symptomatic *P. padus* leaves, symptomatic *P. grayana* (1923) leaves, and asymptomatic *P. grayana* (2017) leaves collected from the botanical garden. B, abaxial side of *P. padus* leaf with uredinia under dissecting microscope. C, abaxial side of *P. grayana* leaf with uredinia under dissecting microscope. D, urediniospore production on aeciospore inoculated *P. padus* leaf, arrows indicated urediniospore clusters. C, urediniospore production on aeciospore inoculated *P. grayana* leaf, arrow indicated urediniospore clusters.

2.3 Population diversity of *T. areolata* in Swedish and Finnish seed orchards

In 2019, May to September, developing spruce cones were bagged in the seed orchards Rörby, Sweden, and Suhola, Finland. In 2020, from May to September, developing spruce cones were bagged in the seed orchards Söregärde, Sweden, and Vuolenkoski, Finland. Samples from 55 cones in total were collected from 2015, 2019, and 2020 to investigate the genetic diversity and infection, sexual reproduction, and fructification in bagged and exposed cones. In addition, we developed ten new polymorphic simple sequence repeat markers based on the *T. areolata* genome to improve the resolution in population genetic analysis. The genotyping and data analysis of 574 samples with 18 markers was done in Sweden. We conclude that the *T. areolata* population in 2015 had the highest genetic diversity when high disease incidence occurred in the seed orchard. Cones collected in 2019 and 2020 were more frequently dominated by one multilocus genotype. The genetic diversity of *T. areolata* in the youngest seed orchard was exceptionally low. Cones that were isolated in bags produced either mature aecia with low diversity or homogenous aecial primordia, and the morphology of aecial primordia was recorded. The importance of outer spermatization for aecia formation was shown. The results of this research activity are documented in Publication [3]. This research provided new resources for future *T. areolata* population genetic studies. Improved understanding of the population genetics, infection, reproduction and fructification of the pathogen is important for efficient disease management.

2.4. Project challenges

In 2019 and 2020, abundant female flowers were produced in the seed orchards studied in this project. However, the cherry spruce rust disease incidence was much lower than in the previous years. As a result, a limited number of cones were infected among bagged cones and exposed cones. Our original experiment design to investigate the susceptibility stages of spruce cones and insect involvement in *T. areolata* sexual reproduction was unobtainable. Instead of abandon the field experiments entirely, we utilized available resources and added to our research objective to investigate the differences in population genetics and sexual reproduction of *T. areolata* between the year with high disease incidence and the year with low disease incidence. The role of outer spermatization is clear.

The travel and bilateral collaboration were fulfilled as planned in 2019, and field work and bench work were finished in both Sweden and Finland. However, due to the COVID-19 pandemic, travel restrictions were enforced in both countries in 2020. The postdoc could not travel from Sweden to Finland, and therefore field experiments in Finland were established, managed and samples collected by the Finnish collaborator.The samples were delivered to Sweden by postal service and analysed in Sweden. The research progress was communicated in time via channels such as emails and online meetings.

3. Publication list

This project results in three original research articles that have been published or will be published with open access:

[1] Zhang, K., Olson, Å., Samils, B., Kaitera, J. 2021. Alternate host screening of *Thekopsora areolata* in Scandinavia: A new record on *Prunus grayana*. Botany. *Accepted.* doi: 10.1139/cjb-2021-0023

[2] Zhang, K., Kaitera, J. Samils, B., Olson, Å. 2021. Temporal and spatial distribution of *Thekopsora areolata* basidiospores, aeciospores, and urediniospores. Plant pathology. *Under review.*

[3] Zhang, K., Kaitera, J. Samils, B., Olson, Å. 2021. Bagging of *Picea abies* cones during spermatization reduces genotypic diversity of *Thekopsora areolata*. *In preparation.*

4. Research impact

4.1. Contribution to the long-term collaboration between Finland and Sweden.

Finland and Sweden have 75% and 69%, respectively, of the land area covered by forests, and Norway spruce is one of the most important trees. Sustainable forestry is critical for the environment and economics in both countries. The Finnish and Swedish forest industry are both facing the challenges of Norway spruce seeds supply shortage, which is further increased by the cherry spruce rust disease. This project, which is financially supported and made possible by KSLA, brings intellectual resources from the two countries to work on the same issue. Output from this project will be beneficial to stakeholders in both countries. Besides the target pathogen in this project, the forestry in Finland and Sweden across Scandinavia often face the same challenges such as pests and climate change. Even though this research project is already finished, the communication channel has been established. The established close collaboration between Finland and Sweden will increase the communication and understanding in academia; create opportunities for future cooperation to solve emerging problems in forestry.

4.2. Strengthened research field at the departments in Finland and Sweden

The Natural Resources Institute Finland (Luke) focuses on the bioeconomy and sustainable use of natural resources, and forest damage is an important section within the research focus of Luke. Dr. Juha Kaitera has extensive expertise in tree rust diseases including cherry spruce rust. The Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences (SLU) perform both fundamental and applied research on forest diseases. Dr. Åke Olson and Dr. Berit Samils's expertise is in molecular biology and population genetics of forest pathogens. In this project, we performed different section of the study in different departments in consideration of each other's strength, such as alternate host screening was performed in Luke and genotyping was performed in SLU. The communication of expertise and equipment from the departments enabled research that would not have been feasible before. The execution of the project strengthened the techniques of forest disease researches in both departments. The research results have been, or will be published in open access journals, thus, the impact of forest disease studies from both departments will be increased.

4.3 Contribution to strengthening the forest sector in Finland and in Sweden

The forest sectors in Finland and Sweden have the same values and often suffer the same challengers such as plant pathogens. The disease cherry spruce rust does not post fatal risk in Norway spruce trees, but it can severely impair the seed supply and regeneration of the forest. The aims of this project were to find the potential alternate hosts, investigate the spore dissemination, and understand the insect involvement in reproduction of the pathogen. The output of this project includes practical information for stakeholders that can guide the development of efficient disease management strategies, as well as theoretical information for the forest research community to have a better understanding of the biology of rust disease.

5. Communication with stakeholders and endusers

Plant breeders and producers get new information about rust resistant southern *Prunus* spp. that can be cultivated north from their current cultivation latitudes along climate warming in the future in Finland. This information is launched out in seminars, newspaper articles and via internet pages. List of susceptible species indicate which species pose a threat to spread cherry-spruce rust to Norway spruce seed orchards. Information about the alternate host range and spore dispersal of *T. areolata* fulfills the information received from projects (MESIKE and SITKE) financed by the Finnish Ministry of Agriculture and Forestry, which provide new means of rust control in Finland. The seed orchard managers have a direct contact with the researchers to receive this new information and to adopt it directly to seed orchard managing strategies. Importance of outer spermatization in sexual reproduction of the rust serves direct information for insect researchers and seed orchard managers about the role of insects in rust reproduction and importanace of insect control in seed orchards.

The project and associated information on the subject have been presented to stakeholders and end users at several meeting. For example, both Åke Olson and Juha Kaitera, participated in a three-day meeting with Seed Ochard Managers from companies in Sweden and Finland in September 2019 in Jyväskylä, Finland. The project plan and preliminary data were also presented at the IUFRO wourld congress in Curitiba 2019. Furthermore, in January 2021 the project was presented in an online workshop with stakeholder participation in Sweden.

6. Financial accounting.

The total cost of the project have been 2 090 000 SEK for the complete period of 24 month. The costa have been devided according to the Table 2. Due to the travel restriction during 2020 and 2021 because of COVID-19 situation less money than budgeted have been spent on travels and accommodation. The resources were reallocated and used for payment of salary for the postdoc. The project had some changes in sub-projects which resulted in some minor reallocation of cost between specific categories. Overall the project have followed the time plan and budget with just small changes.

Table 2. Financial accounting for the KSLA grant number: TFV 2018-0001.

Specification	**Cost**
Salary postdoc	1 220 000
Travels	25 000
Material	105 000
Consumables	95 000
Premises	195 000
Overhead	450 000
Total	**2 090 000**