

Slutrapport

Projektrubrik: Ecology and conservation of red-listed wood-decay fungi of oak

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Populärvetenskaplig sammanfattning

The overall objective of the project was to generate new knowledge and provide conservation and management guidelines for threatened wood-inhabiting oak fungi. To achieve this objective, we implemented four working packages (WP) in order (a) to increase the ecological and genetic knowledge on threatened wood-inhabiting oak fungi; (b) to evaluate whether veteranisation (a purposeful damaging of trees owing to create decayed wood habitats) of ca. 100 year-old oaks may allow fungi of conservation interest to establish in wood; (c) to evaluate whether inoculating rare oak fungi into the wood of mature oaks can be a viable conservation measure. During the project work, we communicated stakeholders, obtained permissions for sampling in protected areas, collected fruiting bodies and wood samples, analysed materials, and disseminated results by publishing research papers. In WP1, we studied genetics of red-listed wood-decay fungi *Hapalopilus croceus* and *Fistulina hepatica*. In *H. croceus*, we studied the mating type system as it influences the probability of in- or outbreeding and may affect the species' fitness. We have found that *H. croceus* possesses a bipolar mating type system, which increases the probability of finding a compatible mate, but also increases the chance of inbreeding due to its current rareness, fragmented distribution, and declining habitat. Our work also resulted in collection of multiple *F. hepatica* cultures, analysis of which is under way. In WP2, digitalization and analysis of unique ecological data on red-listed wood-decay oak fungi (such as *H. croceus*, *F. hepatica* and other) from the 1970s onwards by Stellan Sunhede was completed, organized and two manuscripts describing these observations are in preparation. This information may have direct implications for species conservation and management as may help to understand at what conditions these fungi reproduce and disseminate in environment. In WP3, we analysed the effect of oak veteranisation on the development of wood fungal communities including fungi of conservation interest. Oak wood was found to be exceptionally rich in fungal endophytes, but due to slow development of fungal communities, it was too early to identify if any of the types of veteranisation will be more advantageous regarding fungi of conservation interest or for other fungi forming favourable decay conditions for desired biodiversity. To provide conservation guidelines for the management of younger oak trees, will require follow up studies to clarify how different types of veteranisation will affect species of conservation interest. In WP4, we collected and analysed wood sampled from mature oaks at Halltorps Hage nature reserve in Öland that were inoculated 18 years ago with red-listed wood-decay fungi. These fungal species were found to have established in wood of living oaks, demonstrating that fungal inoculation can be a viable management measure owing to save and sustain protected species.

Resultat

The project generated new fundamental and applied knowledge on red-listed wood-decay fungi associated with oak. The project also led to the establishment of new research contacts and international collaboration. The results of the project including international collaboration were published in three research papers, which are attached to this report. In addition, three manuscripts are in preparation and are expected to be published in the nearest future.

1) The paper by Redr et al. (2020) "The mating type system of the rare polypore *Hapalopilus croceus*". *Fungal Ecology* 45: 100941, describes the identification of the mating type system, which in fungi influences the probability of in- or outbreeding and thus affects the genetic variation in the species' populations and fitness. Fungal basidiomycetes (such as *H. croceus*) can have one of the three mating systems: self-compatible homothallism with no mating types, and bipolar and tetrapolar outcrossing types with 50 and 25% self-compatible spores, respectively. We identified the mating type system of *H. croceus* as being bipolar. Fungi with a bipolar mating system have a higher likelihood for inbreeding and lower for outcrossing due to increased sibling spore compatibility, resulting in a decrease in heterozygosity compared to species with a tetrapolar mating system. Although bipolarity increases the probability of finding a compatible mate, which may be selected for in rare species, inbreeding of *H. croceus* is likely to be reinforced by the bipolar mating system due to its current rareness, fragmented distribution, and declining habitat.

2) The paper by Menkis et al. (2021) "Endophytes dominate fungal communities in six-year-old veteranisation wounds in living oak trunks". *Fungal Ecology* In Press doi: 10.1016/j.funeco.2020.101020, describes veteranisation as a method, which is used to create microhabitats in younger trees, and how veteranisation affects oak fungi including red-listed species. We used DNA-metabarcoding and analysed the early fungal succession in six-year-old veteranisation wounds in ca. 100 year-old living oak trunks. The results showed that veteranisation had only a minor effect on the richness of fungal taxa, but it had a positive effect on their abundance, indicating a higher activity and build-up of fungal biomass. Different types of veteranisation partly selected for different groups of fungi, demonstrating that the choice of veteranisation treatment may directly affect the speed and magnitude of wood decay, and thus the formation of decayed wood habitats which are mainly created by basidiomycetes. The development of decay is a slow process and it was too early to identify if any of the types of damage will be more advantageous regarding fungi of conservation interest, but the presence of at least three red-listed fungi, i.e. *Fistulina hepatica*, *Inocutis dryophila*, and *Xylobolus frustulatus*, suggests that the wood of ca. 100 year-old oaks is a potentially suitable habitat for colonisation.

3) The paper by Nordén et al. (2020) "Ten principles for conservation translocations of threatened wood-inhabiting fungi". *Fungal Ecology* 44: 100919, is an international collaboration effort describing principles of conservation translocation of red-listed fungi owing to save these species. When combined with other conservation or restoration measures, translocation can be an effective measure for preventing further population decline in the short term, and preventing species extinctions in the long term. The study highlights the uncertainties and risks that are connected to fungal translocations and propose ten principles adhering to the precautionary principle.

4) Two manuscripts, that are in preparation, describe unique ecological data on red-listed wood-decay oak fungi *Fistulina hepatica* and *Hapalopilus croceus*. Analyses include field observations from 1970s onwards (more than 100,000 records) on periodicity of fungal fruitbody formation, fruitbody size, geographical orientation and location on the tree, the presence and vicinity of fruiting bodies of other fungal species and how the climatic factors could affect the fruitbody production each year. This

knowledge may have implications for species conservation and management as it may help to understand at what conditions these fungi reproduce and disseminate in environment.

5) The manuscript, that is in preparation, titled "Fungal inoculations to initiate formation of hollows and to reintroduce threatened species in 100 years old oaks – results after two decades", reports results of artificial inoculation of red-listed and other wood decay fungi in healthy oaks, which was done 20 years ago, and demonstrates that this method is generally successful. We detected all fungal species inoculated in a large number of inoculated holes, but more extensive establishment of red-listed species and decay development appears to be very slow in living trees.

Målbeskrivning

We consider that the overall objective of the project was achieved as it generated new fundamental and applied knowledge on red-listed wood-decay fungi associated with oak, thereby expanding our understanding on genetics, ecology, and conservation management of these fungal species. Firstly, the genetic studies provided the evidence on the presence of bipolar mating type system in the red-listed fungal species *H. croceus*, which can have implications for conservation management. For example, this shows that in a long run inbreeding in *H. croceus* is likely to be reinforced by the bipolar mating type system and that diversification of suitable habitats or even artificial inoculations may be needed in the future to ensure fitness and viability in the *H. croceus* population. Secondly, the digitalization and analysis of unique field observations on red-listed wood-decay fungi associated with oak, significantly expanded the available knowledge on reproduction strategies, mycelial longevities in colonized wood and environmental conditions needed for these fungal species to reproduce. This information can benefit practitioners and nature managers aiming to increase the reproduction and dissemination of red-listed oak fungi. Thirdly, the new knowledge accumulated in an oak veteranisation study is a valuable addition to overall fungal ecology and fungal community dynamics. Specifically, it showed that fungal community succession in oak wood is generally a slow process and largely different from the one as compared to coniferous tree species. Although this study was done relatively early i.e. only six years following the treatments, the results suggest that veteranisation has in general a positive effect on the establishment of wood decay fungi including red-listed species that with time are likely to lead to wood decay and the formation of hollows. Furthermore, the exposure of sapwood and heartwood partly selected for different communities of fungi with implications for management of oak trees and their associated biodiversity. Fourthly, the study of inoculation of red-listed fungi in oak wood demonstrated that this conservation strategy is viable as fungi that were inoculated were able to establish and were recovered from the wood after 20 years period. This shows that fungal inoculations (or translocations) can be a useful tool owing to save the species that otherwise may face extinction.

Kommunikation och nyttiggörande av resultat

The results of the project were included in three research articles and three additional manuscripts are in preparation, which directly contributes to the dissemination of gathered knowledge to research community and society. The project work also contributed to the establishment of international collaboration and communication of results on fungal inoculations in Swedish oaks (see publication by Nordén et al. 2020) to this research network. A seminar at the Dept. of Forest Mycology and Plant Pathology, SLU was conducted describing the results of the project to PhD students, postdocs and senior researchers. Results of the project were also presented to participants of the conservation

course October 2019 focused on fungi of conservation interest on Öland. Results of the project were communicated to the nature management unit of the Kalmar county board and to the nature management practitioners at the company ProNatura, which were also involved in the project work. The new knowledge and ideas generated during the project work resulted in a new research project titled "The hidden diversity of endophytic fungi in trees in Norway" funded by the Norwegian biodiversity information centre. The DNA sequence information generated during the project work were deposited in the GenBank database and made publicly available, which will benefit research community and society. New findings of red-listed fungal species were reported to Artportalen at the Swedish species Information Centre at SLU.