Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Southern Swedish Forest Research Centre

Diversity and distribution of *Armillaria* species in Dalby Söderskog and Norreskog

Wenzi Ren

Master´s thesis • 30 credits
EUROFORESTER
Master Thesis no. 326
Alnarp, 2020

Diversity and distribution of *Armillaria* species in Dalby Söderskog and Norreskog

Wenzi Ren

Supervisor: Michelle Cleary, SLU, Southern Swedish Forest Research Centre
Examiner: Jörg Brunet, SLU, Southern Swedish Forest Research Centre

Credits: 30 credits
Level: Advanced level A2E
Course title: Master thesis in Forest Science
Course code: EX0928
Programme/education: Euroforester Master Program SM001
Course coordinating department: Southern Swedish Forest Research Centre

Place of publication: Alnarp
Year of publication: 2020
Online publication: https://stud.epsilon.slu.se

Keywords: *Armillaria*, species identification, species distribution, DNA techniques, pairing tests

Swedish University of Agricultural Sciences
Faculty of Forest Sciences
Southern Swedish Forest Research Centre

Abstract

Armillaria is a genus from the phylum Basidiomycota, which can cause *Armillaria* root rot disease. Both broadleaves and conifers are hosts for *Armillaria*. Some *Armillaria* species are important root and butt rot pathogens, causing mortality and yield reduction in forests. Others have more of a role as a saprophyte, helping to degrade woody substrate and therefore have some ecological importance. Knowing which species of Armillaria is present is important to determine any potential impact in forest regeneration.

The aim of this thesis is to study the diversity and distribution of *Armillaria* species in the forest reserves at Dalby Söderskog and Norreskog in southern Sweden Two different methods were used in this study, one is based on the molecular techniques where the ITS region and EF-1α region of samples' DNA were amplified for sequencing. The amplified DNA sequences were queried in the Gene bank. The other method is based on the biological species concept which is realized by pairing tests.

According to the results, *Armillaria gallica*, *A. cepistipes* and additional *Armillaria sp.* were identified in the study area and *A. gallica* is dominating. *Armillaria gallica* is distributed both in Söderskog and Norreskog, while *A. cepistipes* was only found in Söderskog. The morphology of rhizomorphs of *Armillaria* in cultures varied among isolate. Two genets were identified using traditional pairing tests and the genet sizes could be large enough to cover the whole study site. The Armillaria species identified in this thesis have an important role as saprophytes, decomposing dead organic matter, but can also behave as facultative parasites if trees are weakened by other stress factors. Several tree species are currently suffering from other biotic stressors (e.g. *Hymenoscyphus fraxineus* on ash, Dutch elm disease on elm, *Phytophthora* spp. on beech), which may make tees more susceptible to attack by Armillaria species in the future. However, given the typical lower virulence associated with the identified species at Dalby, any increase in inoculum potential that may affect regeneration in the long-term is probably short-lived.

Keywords: *Armillaria*, species identification, species distribution, DNA techniques, pairing tests

Table of Contents

List of Figures

List of Tables

1. Introduction

1.1 The genus *Armillaria*

Armillaria, also called the honey fungus, is a genus in the phylum Basidiomycota. The genus is considered to be one of the world's oldest and largest living organisms (Sipos et al., 2018; Smith et al., 1992). As saprophytes, *Armillaria* species play an essential role in the carbon cycle in ecosystems. Some *Armillaria* species are also significant root and butt rot pathogens of woody plants worldwide causing mortality and yield reduction in forests (Cleary et al., 2013).

After the first record about *Armillaria* during 1729 to 1755 (Yen & Francisco, 1990), the nomenclature and taxonomy of *Armillaria* has been confusing over centuries. The original name was created for the genus in the 1700s by the scientist Martin Vahl, in his work 'Flora Danica'. In 1857, Staude raised the *Armillaria* tribe into a generic rank (Yen & Francisco, 1990). The genus was mixed with several other species and genera in the history (Burdsall & Volk, 1993; Yen & Francisco, 1990). Finally three species concepts were used to describe the genus, based on morphological, biological and phylogenetic recognition, but drawbacks exist in all of them (Heinzelmann et al., 2018).

The genus *Armillaria* has high identifiability according to its specific characteristics. Three distinguishing features for *Armillaria* are: mycelial fans, rhizomorphs, and fruiting bodies (Figure 1). Mycelial fans are white mats of fungal mycelium produced between the bark and wood, commonly having a fan-shaped appearance. Rhizomorphs are unique characteristics for this genus (Koch et al., 2017), appearing root-like with fungal strands of mycelium surrounded by a melanized rind. The morphology of rhizomorphs varies depending on the species and their substrates. Different species in the northern hemisphere have dichotomously or monopodial branched patterns of their rhizomorphs inside the soil (Figure 2). Monopodial branched rhizomorphs are more often associated with saprotrophs. In the northern hemisphere, *A. mellea*, *A. borealis*, and *A. ostoyae* have branched rhizomorphs dichotomously, while *A. gallica*, and *A. cepistipes* have monopodial branched rhizomorphs (Morrison, 2004). Rhizomorphs grow on the root, under the bark or in the soil. They can be small, fragile, or robust and abundant, depending on the species. Fruiting bodies usually develop in clusters near the base of infected trees during autumn.

The stalks have honey-yellow caps with light-colored gills and the spores produced are light yellow to white in color.

Figure 1. Mycelium fans, rhizomorphs and fruiting bodies of *Armillaria*. A) Mycelial fans of *A. ostoyae* under bark (Sipos et al., 2018). B) Rhizomorphs of *A. gallica* under the bark (Photo by Wenzi). C) Fruiting bodies of *A. mellea* in field (http://wikipedia.moesalih.com/File:Armillaria_mellea_041 031w.jpg). D) Rhizomorphs of *A. gallica* growing in soil (Photo by Wenzi).

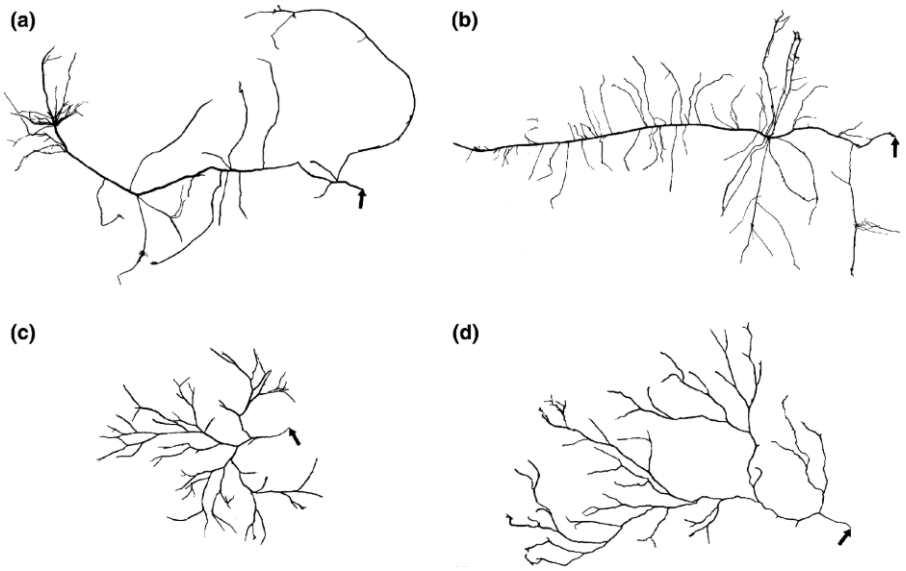

Figure 2. Rhizomorph branching patterns in the northern hemisphere (edit from Morrison, 2004). a) Monopodial branching pattern of *A. gallica*, b) monopodial branching pattern of *A. nabsnona*, c) dichotomous branching pattern of *A. ostoyae*, d) dichotomous branching pattern of *A. borealis.* Arrows indicate the start point of rhizomorph growth.

1.2 *Armillaria* species in Sweden

The genus *Armillaria* has a broad distribution in both hemispheres (Table 1). Over 70 *Armillaria* species are recorded all over the world. Approximately 40 of them are well-described in a way, that the morphology can identify biological species and phylogenetic species (Heinzelmann et al., 2018). These 40 *Armillaria* species are mainly from surveys in America, Europe, Africa, Australia, New Zealand, China, and Japan (Baumgartner et al., 2011). According to Coetzee (Coetzee et al., 2001), the few *Armillaria* species that exist globally were probably introduced by human beings accidently when transporting infected plant resources. Five *Armillaria* species exist in Europe currently, they are *A. borealis*, *A. cepistipes*, *A. gallica*, *A. mellea*, *A. ostoyae* (Heinzelmann et al., 2018). Based on recent research, *Armillaria* has been divided into two genera now: *Desarmillaria*, without annulated fruiting bodies and *Armillaria s. str*. with annulated fruiting bodies (Koch et al., 2017). The genus *Desarmillaria* contains two species, *D. tabescens* and *D. ectypa*. According to Wahlström (1992), all European *Armillaria* species exist in Sweden.

Table 1. Geographic distribution of currently known *Armillaria* species in the world (Heinzelmann et al., 2018).

Geographic distribution	Species
North America	*A. cepistipes*, *A. mellea*, *A nabsnona*, *A. ostoyae*, *A. sinapina*, *A. gallica*, *A. altimontana* *, *A. calvescens* *, *A. gemina* *, *A. mexicana* *
Central and South America	*A. limonea*, *A. novoe-zelandiae*, *A. affinis* *, *A. griseomellea* *, *A. melleo-rubens* *, *A. paulensis* *, *A. procera* *, *A. sparrei* *, *A. tigrensis* *, *A. montagnei* *, *A. umbrinobrunnea* *, *A. viridiflava* *, *A. yungensis* *, *A. puiggarii* *
Europe	*A. borealis*, *A. cepistipes*, *A. gallica*, *A. mellea*, *A. ostoyae*
Africa	*A. gallica* §, *A. mellea* §, *A. camerunensis* *, *A. fuscipes* *, *A. heimii* *, *A. pelliculata* *
Asia	*A. borealis*, *A. cepistipes*, *A fuscipes* §, *A. gallica*, *A. mellea*, *A. nabsnona*, *A. ostoyae*, *A. sinapina*, *A. duplicata* *, *A. jezoensis* *, *A. mellea ssp. nipponica* *, *A. omnituens* *, *A.singula**
Australia & Oceania	*A. limonea*, *A. novae-zelandiae*, *A. aotearoa* *, *A. fellea* *, *A. fumosa* *, *A. hinnulea* *, *A. luteobubalina* *, *A. pallidula* *, Nag.E[#]

* the species is reported only in some geographic area

§ the species speculated to be introduced to some geographic area

A biological species, need further exploration and description.

Tree disease results from the interactions of the host (susceptibility), the pathogen (virulence) and the environment. *Armillaria* species have a wide range of hosts and can infect most woody species causing losses in crops, vineyards, urban settings, forests (Prospero et al., 2004; Morrison et al., 2000), and the pathogenicity and rhizomorph productivity also varies among species (Table 2). The five European *Armillaria* species vary in pathogenicity, rhizomorph production and host preference.

A. ostoyae and *A. mellea* are highly pathogenic, they both act as a primary parasite. *A. ostoyae* is efficient in stump colonization, it can damage young stands and kill them, especially in the northern hemisphere. Rhizomorphs produced by *A. ostoyae* are abundant, while they are thinner and more brittle than those of *A. gallica* and *A. cepistipes.* Conifers are the main host for *A. ostoyae*. Hardwoods could also be a host and records of this appear to be more frequent in North America than in Europe (Williams et al., 1986; Shaw and Kile, 1991; Wahlström, 1992; Guillaumin et al., 1993; Burdsall & Volk, 1993)

A. mellea is an important hardwood pathogen. It could also weaken young conifers in southerly latitudes, but the reports are less frequent in Europe, Asia and North America. Rhizomorphs produced by *A. mellea* are rare. (Guillaumin et al., 1993)

Table 2. Characteristics of the five *Armillaria* species present in Europe (Williams et al., 1986; Shaw and Kile, 1991; Wahlström, 1992; Guillaumin et al., 1993; Burdsall & Volk, 1993; Guillaumin et al., 1993; Burdsall & Volk, 1993; Kwasna, 2008; Lushaj et al., 2010; Baumgartner et al., 2011; Heinzelmann et al., 2017).

Species	Pathogenicity	Rhizomorph	Main Host range
A. ostoyae	Primary parasite; Aggressive pathogen	Abundant but brittle	Conifers
A. mellea	Primary parasite; Aggressive pathogen	Rare in soil	Broadleaves
A. borealis	Depends	Better than *A. ostoyae*	Conifers & broadleaves
A. gallica	Secondary parasite; Weak pathogen	Abundant	Broadleaves
A. cepistipes	Secondary parasite; Weak pathogen	Abundant	Conifers

A. borealis is a parasite on both conifers and broadleaves, but its pathogenicity varies. (Guillaumin et al., 1993). *A. borealis* produces rhizomorphs faster and more consistent than *A. ostoyae* (Guillaumin et al., 1993; Heinzelmann et al., 2017; Lushaj et al., 2010)

A. gallica and *A. cepistipes* are both secondary parasites and have weaker pathogenicity than *A. ostoyae* and *A. mellea*. *A. gallica* can weaken trees and usually co-occurring with more virulent *Armillaria* species. *A. gallica* produces abundant rhizomorphs that can be found under the bark of standing or down trees, and throughout the soil. Broadleaves are the main host for *A. gallica* (Guillaumin et al., 1993; Burdsall & Volk, 1993; Lushaj et al., 2010; Baumgartner et al., 2011)

Pathogenicity of *A. cepistipes* is weaker than that of *A. gallica* (Guillaumin et al., 1993). The species is efficient commonly found co-occurring with *A. ostoyae*. (Guillaumin et al., 1993; Kwasna, 2008)

1.3 *Armillaria* root rot disease

In general, it is difficult to diagnose damage by *Armillaria* root rot disease because symptoms and signs are inconspicuous. Early infection by *Armillaria* induces almost no symptoms on the trees until the root systems are extensively colonized. When infection becomes more severe, the tree crowns show chlorosis and can exhibit dieback. Smaller trees are generally more susceptible to being killed by *Armillaria* compared to older trees. On conifers, resin may be produced around the base of infected trees. Infected roots can be encrusted with resin, soil, and/or rhizomorphs. Conversely, infected broadleaved trees may

appear as sunken cankers at the tree base, covered with loose, necrotic bark. Mycelial fans and rhizomorphs under the bark of diseased trees is somewhat diagnostic to *Armillaria* infections.

Infection by *Armillaria* species includes first a parasitic phase and secondly a saprophytic phase (Soularue et al., 2017). In the parasitic phase, *Armillaria* will penetrate the bark and colonize the cambium when the roots are still alive which disrupts the flow of nutrients in the tree. In the saprophytic phase, *Armillaria* survives on dead roots. All *Armillaria* species have a saprotrophic ability, but not all of them are strong parasites (Heinzelmann et al. 2018). *Armillaria* root rot disease spreads mainly in two ways between hosts; spreading by root contact between healthy and infected trees or via rhizomorphs that grow through the soil, attach onto a healthy root, penetrate and cause infection (Figure 3) (Heinzelmann et al., 2018).

Biotic or abiotic factors can increase possibility of trees infection by *Armillaria*. For example, *A. gallica* infects mostly the root system of dying trees. The potential for trees to be infected by *Armillaria* increases when they are weakened by some other stress factor. (Kile et al. 1991).

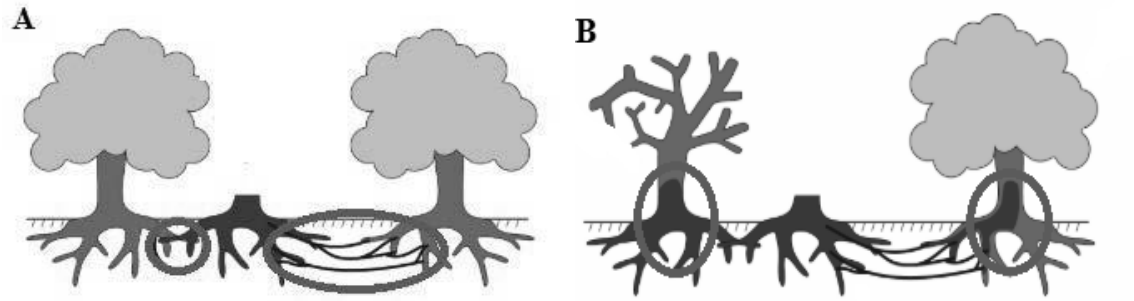

Figure 3. Spread of *Armillaria* root rot disease (edit from Heinzelmann et al., 2018). A) Two methods to infect healthy trees by contacting *Armillaria* infected roots and contacted by rhizomorphs; B) Mycelium of *Armillaria* spreads into healthy trees.

After a tree dies, the whole stump and root system can be colonized by *Armillaria* (Shaw and Kile, 1991) and the fungus survives for several years (decades) depending on the size of the woody substrate. There are many factors, which are influencing the infection process (Guillaumin and Lung 1985), including availability of susceptible trees, and forest management activities like thinning and harvesting which create woody substrate upon which the fungus survives (Morrison, 1972; Rishbeth, 1972b), but also climate (Labb éet al., 2015).

1.4 Identification of *Armillaria* species

In order to determine the potential impact that *Armillaria* may have in a forest, reliable species identification is important. Over the past centuries, morphological, biological, and molecular techniques have been used to describe species. Identification based on the morphology of fruiting bodies was used in early studies. However, morphological techniques are ambiguous because fruiting bodies and rhizomorphs of different *Armillaria* species can be morphologically similar (Shaw and Kile, 1991).

Another possibility for species identification involves 'pairing tests' to characterize the species' compatibility when paired together on nutrient agar. When two samples are somatically incompatible, a dense line develops in the media when the two opposing colonies meet. These two colonies are considered to be different *Armillaria* species or genetically distinct isolates from the same species. In contrast, if the pairing of isolates resulting in fusing of mycelia to become a homogenous colony, they could be considered the genet (Figure 4).

Despite the simplicity of conducting paired-compatibility tests, there are several factors that can influence the interpretations of the interactions; for example, different mycelia shapes, the age of the isolates, and other environmental factors (e.g. temperature and moisture) (Singleton 1992).

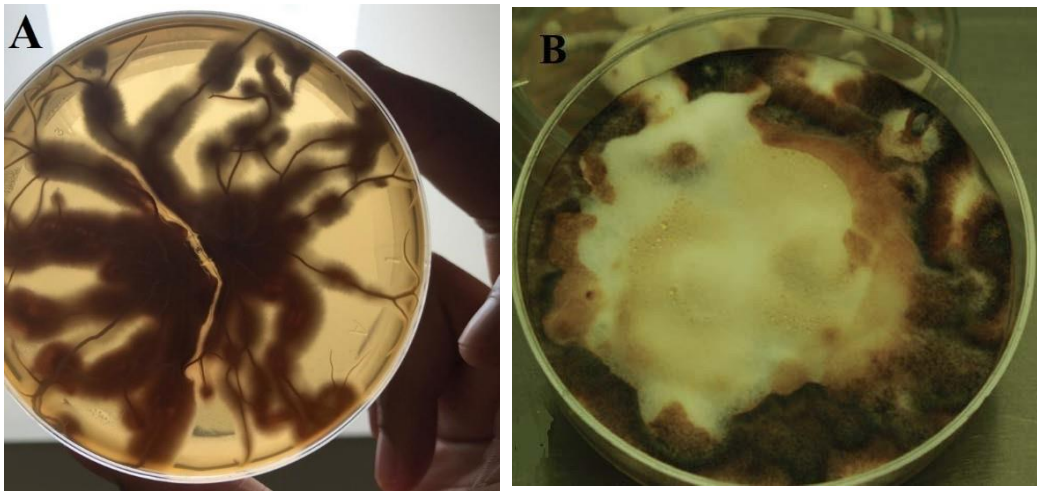

Figure 4. Result of pairing test in culture. A) Incompatible reaction of two isolates showing an obvious inhibition zone between two opposing colonies (photo by Wenzi). B) Compatible reaction of two paired isolates whose hyphae have fused to produce a uniform mycelium colony (Baumgartner et al., 2011).

The third technique for species identification is based on sequencing the fungal DNA (Anderson & Stasovski, 2015). Advanced molecular techniques have greatly improved the ability to accurately detect and identify different species and genotypes of *Armillaria*. Genetic markers are used to improve the specificity (Tsykun, Rigling, & Prospero, 2013). To distinguish six common *Armillaria* species in Europe (including one species from *Desarmillaria*)，a procedure involving three PCR steps including IGS, ITS and EF-1α, and five enzymes (*Nde* I, *Alu* I, *Bsm* I, *Mbo* I, *Hinc* II) are required (Tsykun et al., 2013).

1.5 Aims

The overall aim of this study is to describe the distribution and diversity of *Armillaria* species in mixed broadleaved forests located at Dalby Söderskog and Norreskog in order to better understand the ecological role and potential impact in the future considering that many broadleaved tree species are under stress because of other factors (ash decline, Dutch elm disease, *Phytophthora* spp.).

2. Materials and Methods

2.1 Study site

Dalby Söderskog and Norreskog are located in Skåne, Southern Sweden, 10 km east of Lund (55°41′N, 13°20′E，44-74m a.s.l.). Dalby Söderskog has a size of 37 ha and is protected as a national park since 1918. Prior to protection, it had been a wooded pasture for many centuries. The intensity of grazing and the density of trees have differed considerably over time. Periods of selective cutting were followed by overgrowth, and periods of heavy grazing were followed by abandonment. In the last century, there has been no grazing and continuous overgrowth (personal communication from Jörg Brunet). Even after the forest was protected, there has been some minor cuttings until the late 1900s. During the 1990s there were cuttings of diseased elm (*Ulmus glabra*) and after that only dangerous trees along the walking paths were cut. There has also been clearing of the shrub layer at the entrance and along an ancient earth wall located in the forest. Oak (*Quercus robur*), ash (*Fraxinus excelsior*) and elm (*Ulmus glabra*) were dominant at the beginning of the 20th century, while elm and ash became the dominant species at the end of the century (von Oheimb & Brunet, 2007). The main diameter of trees in the area increased remarkably in this period (von Oheimb & Brunet 2007; personal communication from Jörg Brunet). After 20th century, invasive pathogens replaced the grazing as the main disturbance factors and are playing a role in shaping the forest dynamics. In particular, Dutch elm disease and ash dieback are severely affecting the survival of these of elm and ash, respectively, in the area (Brunet et al., 2014).

Dalby Norreskog is located ca 500 m NE of Dalby Söderskog, with a semi-natural pasture in between the two forests. These three parts together form the historical pasture area Dalby hage. Dalby Norreskog has a size of ca 20 ha and is located on less fertile soil than Dalby Söderskog. Livestock grazing persisted until 1932. In 1979, Norreskog was protected as a nature reserve. Since about 2005, livestock grazing was reintroduced in the western part of Dalby Norreskog. From the history, we can find many differences between Söderskog and Norreskog (Table 3).

Table 3. Differences between Dalby Söderskog and Norreskog (Source: Jörg Brunet).

	Söderskog	Norreskog
Resources	All trees originate from natural regeneration	Some stands are planted, e.g., sycamore, some of the oaks and the beech stand
Thinning	Small cutting at the main entrance and along the prehistoric wall	Heavily thinned around 2008 for removing sycamore
Clear Felling	Better, longer-term continuity forest	Lost most tree cover during the 1600s and 1700s
Big event	No oak dead suddenly	Oak dead suddenly in the late 1980s, early 1990s
	Grazing in Söderskog was stopped in the late 1800s	Continued grazing in Norreskog until 1935; resumed around 2005 in the western part

2.2 Sampling

Rhizomorphs and mycelium samples were collected from different substrate materials in Dalby Söderskog and Norreskog (Table 4). Deadwood was debarked to collect rhizomorphs underneath. At places where no woody material was found, rhizomorphs were collected (Figure 5) from the soil. The location of each sampling point was recorded by GPS (Garmin eTrex Legend® Cx).

Table 4. Sample collection from different substrate materials.

	Stump	Log	Dead standing tree	Soil
Rhizomorph	*	*	*	*
Mycelium	*	*	*	

***** Sample should be collected from this material

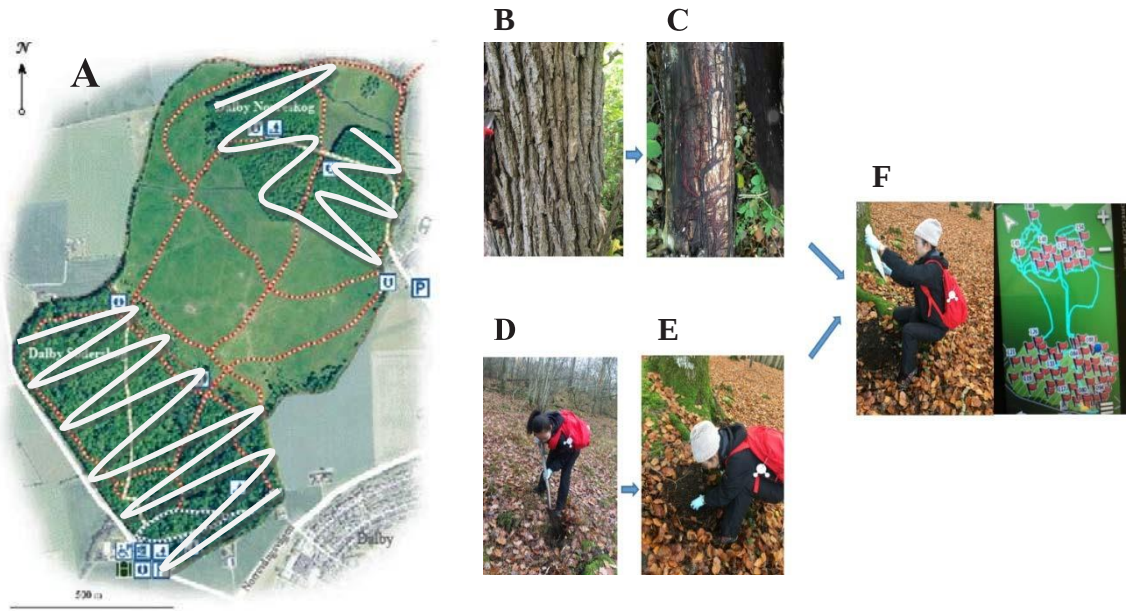

Figure 5. Sample collection procedures: A) Map of Dalby Söderskog and Norreskog, white line indicates the approximate trial followed for collecting samples; B) An *Armillaria* rhizomorph colonized stump with loose bark; C) Debarked tree shows rhizomorphs; D) Digging soil to find the rhizomorphs; E) Collection of rhizomorphs from soil; F) Storing rhizomorphs in bags and marking collection positions by GPS.

2.3 Species identifying procedures through DNA techniques

Isolation

In the lab, rhizomorphs were washed under running tap water and dried with paper towels. The washed samples were surface sterilized in 70% EtOH for one minute, 4% NaCl for five minutes, and again one minute in 70% EtOH. Afterwards, the samples were placed on the paper towel for drying. Four 1-2 mm sections of a rhizomorph were cut and plated on 2% malt extract agar (MEA) amended with 0.01% streptomycin, replicated five times for each strain.

Mycelium fans were sterilized with 70% EtOH for one minute, 4% NaCl for two minutes, and again one minute in 70% EtOH. The surface sterilized mycelium fans were then cut to 4 mm^2 and plated on the media; five replicates were made for each point.

According to their infection strategy, different *Armillaria* species can colonize on the same substrate material (Guillaumin et al., 1993). This means that a sample collected at a certain GPS point could in theory include several species. Based on this fact, randomly 2 to

3 rhizomorphs are collected at the same GPS point and cultured separately on MEA plates. Petri-dishes were sealed with Parafilm® and incubated in the dark at room temperature for over two weeks. Outgrowth of *Armillaria* mycelia were then transferred onto 2% MEA media to obtain isolates growing in pure culture (Figure 6).

To prepare the media for DNA extraction, liquid media was prepared with the 2% malt extract in 50 mL falcon tubes. 1 cm cut pieces of rhizomorphs growing from the pure culture described above were transferred to liquid media, placed on an orbital shaker for 2-3 weeks until the mycelial ball formed.

DNA extraction

DNA was extracted from either mycelium prepared on liquid media, rhizomorphs growing in pure culture, or directly from fresh rhizomorphs obtained from the field. Samples were transferred into Eppendorf tubes and put into the freeze drier for 2 days, then freeze-dried samples were grounded to powder and a sub-sample of the ground tissue was transferred to a new marked Eppendorf tube. DNA was extracted using thermos scientific Genomic DNA Purification Kit according to the manufacturer's protocol. DNA concentration was then measured using the Nanodrop ND-1000 Spectrophotometer (U.S.A). DNA samples were then stored at -20℃.

PCR and DNA sequencing

Polymerase chain reaction (PCR) is a technique used to make large amounts of copies of a specific segment of DNA with the help of primers (short single stranded DNA fragment that flank the target region. In this experiment, ITS region and EF-1α region of the DNA were amplified according to protocols (White et al. 1990, Maphosa et al., 2006). Primers ITS1 and ITS4, EF595F and EF1160R were used. EF595F/EF1160R primers were used after ITS1/ITS4 primers to separate *A. gallica* and *A. cepistipes*, whose results were ambiguous with ITS region. For each reaction, the PCR master mix included 1.6 µL of forward primer (ITS1/EF1160), 1.6 µL reverse primer (ITS4/EF595F), 10.5 µL of Milli Q water, 5.28 µL of dream Taq master mix, and 1 µL template DNA, vortexed them well before using. Positive and negative controls were included in each PCR run, *Phytophthora* was used as positive control to test if the PCR program worked, nuclease-free water was used for negative control to test if there is any PCR product contamination in the master mix. A PCR thermocycler

was used for ITS region with the program including an initial denaturation at 95 ℃ for 2 min, followed by 35 cycles of denaturation at 95 ℃ for 30 s, annealing at 58 ℃ for 30 s, and extension at 72 ℃ for 2 min, and final extension of 72 ℃ for 30 min (Tsykun et al., 2012). For EF-1α region, the PCR program was 1 cycle of 94 ℃ for 2 min, followed by 33 cycles of 94 ℃ for 30 s, 55 ℃ for 30 s, and 72 ℃ for 2 min, and 1 cycle of 72 ℃ for 30 min (Tsykun et al., 2012). Separate amplified PCR products were visualized in 1.2% agarose gel under UV light PCR products that showed bands were then purified and the concentration of each PCR product measured using the Qubit 3.0 Fluorometer. DNA samples which were showed bands in electrophoresis or can be detected under Qubit 3.0 Fluorometer were then sent to KIGene-genetic analyses for sequencing. DNA sequences visualized on ABI chromatograms were manually aligned using the software BioEdit and then the aligned sequence was queried in NCBI (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) gene bank against a reference sequence database. Species were identified based on similarity match > 98.5% compared to the reference database.

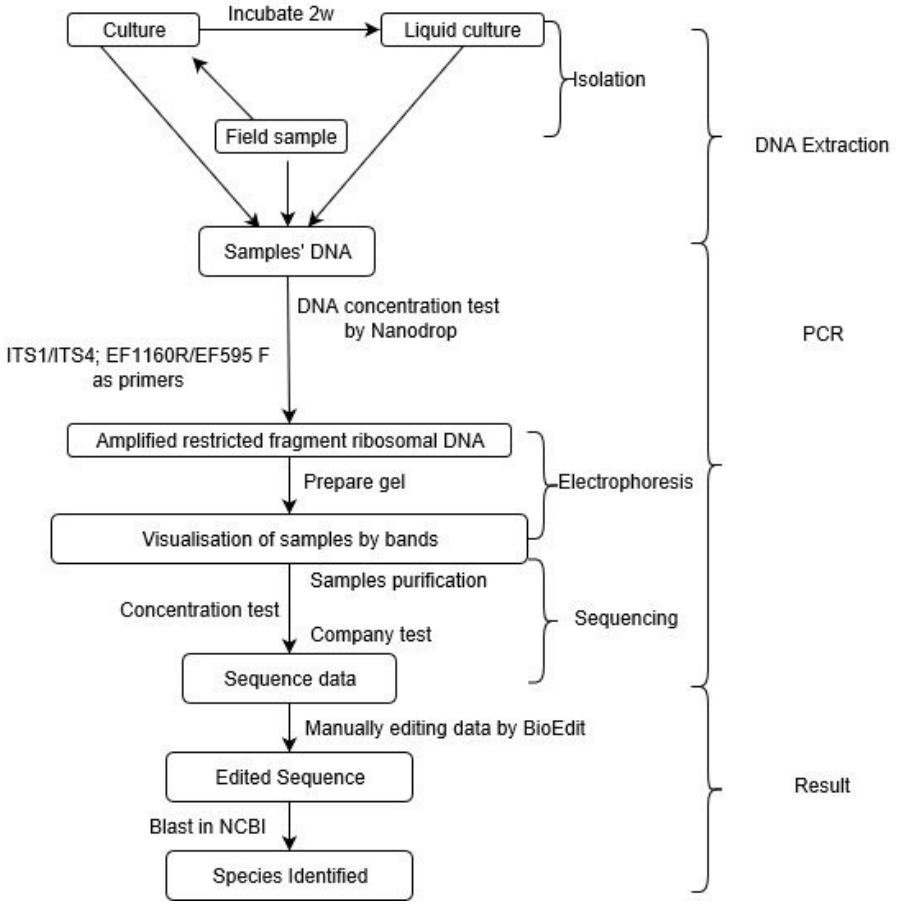

Figure 6. Work trial for *Armillaria* identification through DNA techniques.

2.4 Pairing test

Pairing tests, which could also be called as somatic incompatibility test is used to identify genets. Nine isolates were selected for this test representing both Söderskog and Norreskog; three of them were identified as *A. gallica* and six of them were identified only as *A. sp.* (species which could not be identified to the genus level) in the DNA analysis.

Rhizomorph tips of these picked samples were cut from pure cultures, transferred into MEA, each of them were paired with the eight isolates. The distance between each isolate in petri-dish was 5 mm, and then plates were incubated at room temperature for 40 days. If a demarcation line showed between the opposing mycelium of the paired isolates, then these two isolates could be identified as different genets, if the mycelium of the paired isolates fused with each other, then they can be identified as the same genet (Prospero, et al., 2003).

3. Results

3.1 Species identification

A total amount of 141 field samples were collected, both from mycelial fans and from rhizomorphs (Table 5). Of those, 57 samples originated from Norreskog and 84 samples from Söderskog.

Table 5. Origin of the collected *Armillaria* samples.

Substrate	Mycelium fan	Rhizomorph	Total
Stump	1	16	17
Lying wood	1	28	29
Standing deadwood	1	40	41
Soil	0	54	54
Total	3	138	141

Among the 141 field samples, 83 were successfully isolated. By making replicates and considering the species overlap which was mentioned in Section 2.3, a total of 289 isolates'

were selected for DNA extraction. Of those, 125 samples had sufficient DNA concentration (the concentration can be detected under the high sensitive range using the Qubit 3.0 flurometer) or showed bands in gel electrophoresis (Figure 7), and could be sent for Sanger sequencing. Of the 125 samples, 33 were obtained directly from fresh rhizomorphs obtained from field, 45 from rhizomorphs growing in pure cultures, 65 from mycelium ball growing in liquid cultures; of which 80 from Söderskog and 25 from Norreskog were used for further analysis. Ninety-eight of them were amplified by primer ITS1, 41 of them were amplified by primer EF-1α, 14 samples were amplified by both EF-1α and ITS1/4 primers (Table 6).

According to the sequence results, 40 (32%) out of 125 isolates from 30 sample points (both in Söderskog and Norreskog) were identified as *Armillaria gallica*, e.g. isolate 111 from sample point 85. Three (2.4%) isolates, which belong to three sample points (in Söderskog), were identified as *A. cepistipes*, e.g. isolate 3 from sample point 83. Thirty-five (28%) isolates, belonging to 23 sample points (both in Söderskog and Norreskog), were identified as *A. sp.*, e.g. isolate 161 from sample point 88. Thirty-eight (30.4%) isolates failed; and nine (7.2%) isolates, which belong to six sample points were identified as other fungal species (both in Söderskog and Norreskog), e.g. isolate 163 from sample point 85 was identified as *Ascomycetes* (Table 7).

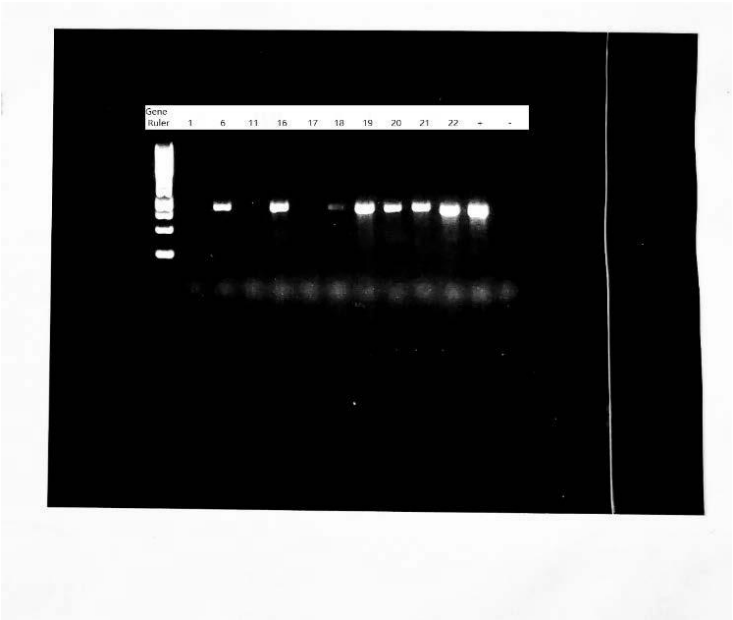

Figure 7. An example of electrophoresis result of DNA samples. Samples 1, 6, 11, 16, 17, 18, 19, 20, 21, 22 showed bands in electrophoresis, while samples 1, 11, 17 had no band in electrophoresis.

Among all sample points, except points with one isolate, only isolates from points 87, 102, 119, 143, 144 can be confirmed as containing one species. All the rest of the points had two or more species at each single sample point, like sample point 89 (at least *A. gallica*) and point 101 (at least *A. gallica* and *A. cepistipes*).

Table 6. The sequence data amount and its resources.

Location	**Söderskog (south)**		**Norreskog (north)**	**Total**
	81		44	125
Sequenced region	**ITS1**		**EF-1**	**Total**
	98		41(14)[1]	125
Isolates' Substrate	**Field samples**	**Rhizomorph cultures**	**Liquid cultures**	**Total**
	33	45	65(17+1)[2]	125

[1]: 14 samples were amplified by both primers

[2]: Liquid cultures were repeated for 17 rhizomorph cultures and 1 field sample for higher accuracy.

Table 7. The putative identification of *Armillaria* species based on DNA sequence data.

Sample ID	**Location**	**Isolate ID**	**Results**		**Putative Identification**
			ITS primer	**EF-1 primer**	
83	south	3	*A. sp.*	*A. cepistipes*	*A. cepistipes*
85	south	4	no significant		can't confirm
85	south	111	*A. sp.*	*A. gallica*	*A. gallica*
		163	*Ascomycetes*	Accession: MH857936.1	
87	south	49	*A. gallica*	*A. gallica*	*A. gallica*
		101	*A. gallica.*		*A. gallica*
		144	*A. gallica*		*A. gallica*
88	south	5	no significant		can't confirm
		161	*A. sp.*	no significant	*A. sp.*
89	south	1	no significant		can't confirm
		2	*A. sp.*	*A. gallica*	*A. gallica*
		74	*A. sp.*		*A. sp.*
		148	*A. sp.*		*A. sp.*
90	south	48	*A. sp.*	*A. gallica*	*A. gallica*
91	south	6	*A. gallica*	empty	*A. gallica*
		85	*A. sp.*		*A. sp.*
92	south	38	no significant		can't confirm
		39	*Ascomycetes*	Accession: MH857936.1	
		147	*Ascomycetes*	Accession: MH857936.1	
97	south	174		*A. gallica*	*A. gallica*
98	south	10	*A. gallica*		*A. gallica*
99	south	11	no significant		can't confirm

		12	*A. sp.*	*A. gallica*	*A. gallica*
		86	no significant		can't confirm
100	south	8	*A. sp.*		*A. sp.*
100	south	104	*A. sp.*	*A. cepistipes*	*A. cepistipes*
101	south	9	*A. cepistipes*		*A. cepistipes*
		109	*A. gallica*		*A. gallica*
		130	no significant	*A. gallica*	*A. gallica*
102	south	15	*A. gallica*		*A. gallica*
		112	*A. sp.*	*A. gallica*	*A. gallica*
103	south	14	no significant		can't confirm
		80	no significant		can't confirm
		102	can't confirm	.	can't confirm
		129	can't confirm		can't confirm
		164	*A. sp.*	*A. gallica*	*A. gallica*
106	south	13	*A. sp.*	empty	*A. sp.*
		84	*A. sp.*	130 bp	*A. sp.*
107	south	134	*A. gallica*	*A. gallica*	*A. gallica*
108	south	23	*A. gallica*		*A. gallica*
		105	no significant		can't confirm
		146	*A. sp.*	*A. gallica*	*A. gallica*
110	south	24		empty	can't confirm
112	south	30	*A. gallica*		*A. gallica*
113	south	22	*A. sp.*		*A. sp.*
		165	*A. sp.*	empty	*A. sp.*
		166	*A. sp.*	*A. gallica*	*A. gallica*
114	south	95	no significant		can't confirm
		121	can't open		can't confirm
		170		*A. gallica*	*A. gallica*
116	south	25	*A. sp.*	*A. gallica*	*A. gallica*
		87	no significant		can't confirm
		131	*A. sp.*		*A. sp.*
		162	*A. sp.*	empty	*A. sp.*
119	south	18	*A. gallica*		*A. gallica*
		167	*A. gallica*		*A. gallica*
122	south	40	*A. sp.*	empty	*A. sp.*
		145	*A. gallica*	empty	*A. gallica*
123	south	21	*A. sp.*		*A. sp.*
		127	*A. sp.*	empty	*A. sp.*
128	south	76	*A. gallica*	no significant	*A. gallica*
129	south	43	*A. sp.*	*A. gallica*	*A. gallica*
130	south	20	no significant		can't confirm
		13	no significant		can't confirm
		135		empty	can't confirm
134	south	50	*A. sp.*	*A. gallica*	*A. gallica*
135	south	19	*A. sp.*		*A. sp.*
135	south	126	*A. gallica*		*A. gallica*

		136	*A. gallica*		*A. gallica*
139	north	27	*A. sp.*	empty	*A. sp.*
		79	*A. sp.*		*A. sp.*
		106	*A. sp.*		*A. sp.*
140	north	103	*A. sp.*	*A. gallica*	*A. gallica*
142	north	28	no significant		can't confirm
		29	no significant		can't confirm
143	south	125	*Ascomycetes*	Accession: KC876225.1	
		128	*Ascomycetes*	Accession: KC876225.1	
144	south	113	*Ascomycetes*	Accession: KC876225.1	
		124	*Ascomycetes*	Accession: KC876225.1	
145	north	116	no significant		can't confirm
		138	*A. sp.*		*A. sp.*
149	north	77	no significant		can't confirm
		132	*A. sp.*		*A. sp.*
		143	*A. sp.*		*A. sp.*
150	north	181		*A. gallica*	*A. gallica*
151	north	41	*A. sp.*		*A. sp.*
152	north	46	no significant		can't confirm
		75	no significant		can't confirm
153	north	107	*A. sp.*	empty	*A. sp.*
		108	*Ascomycetes*	Accession: KC876225.1	
156	north	16	no significant		can't confirm
		133	no significant		can't confirm
157	north	47	*A. gallica*	*A. gallica*	*A. gallica*
		81	*A. gallica*		*A. gallica*
		88	*A. sp.*		*A. sp.*
158	south	31	no significant		can't confirm
		32	*Ascomycetes*	Accession: KY853448.1	
		89	*A. sp.*	no significant	*A. sp.*
160	south	114	no significant		can't confirm
		157	*A. gallica*		*A. gallica*
161	south	142	*A. sp.*		*A. sp.*
		158	*A. sp.*		*A. sp.*
163	north	117	can't open		can't confirm
163	north	169		*A. gallica*	*A. gallica*
164	north	152	*A. sp.*		*A. sp.*
		171	no significant		can't confirm
165	north	99	no significant		can't confirm
166	north	90	can't open		can't confirm
		94	*A. sp.*		*A. sp.*
167	north	91	no significant		can't confirm
		118	can't open		can't confirm
		140	*A. sp.*		*A. sp.*
168	north	93	no significant		can't confirm
		172	*A. sp.*		*A. sp.*
169	north	120	*A. sp.*		*A. sp.*

		123	*A. sp.*		*A. sp.*
171	north	97	no significant		can't confirm
		98	can't open		can't confirm
172	north	115	no significant		can't confirm
172	north	173		*A. gallica*	*A. gallica*
173	north	96	*A. sp.*		*A. sp.*
174	north	154	*A. sp.*		*A. sp.*
		159	*A. sp.*		*A. sp.*
190	north	217		*A. gallica*	*A. gallica*
195	north	179		*A. gallica*	*A. gallica*

Among all the unidentified samples, 14 of them were amplified by both ITS and EF-1α primers, 12 of them had no sequence or too short queries which was unable to be identified, and one of them had no significant similarity in Gene bank. Most (74%) of isolates amplified by ITS1 primer could not be identified because of poor sequence result. 7% of the unidentified isolates had no DNA sequence match in Gene Bank (Table 8, Table 9).

Table 8. Numbers of unidentified isolates and their percentage.

Reasons	**Sequence**			**Reference**	**Method**	**Total**
	No sequence	**Too short query**	**Low ident**	**NSNSI**	**Multiple results**	
Number	6	15	33	5	14	73
%	8.2	20.5	45.2	6.8	19.2	100
	73.9			6.8	19.2	

NSNSI: Nice sequence but no similarity ident in Gene Bank;

Table 9. Detailed reasons for unidentified isolates from PCR products.

Isolate ID	**Results**	**Reasons**	**Isolate ID**	**Results**	**Reasons**	
93	NSSF	NSNSI	161	*A. sp.*	Lower - ident than (by ITS1)	78%; BSEP
16			74			83%
20			148			82%
28			85			83%
29			13			98%; NSEP
4		255 bps	131			89%
1		210 bps	152			98%
11		442 bps	88			89%
86		147 bps	89			75%; NSSF by EF-1α
105		388 bps	162			90%; NSEP
95		240 bps	21			95%

87		246 bps	127			98%; NSEP
13		130 bps; NSEP	19			97%
116		459 bps	96			78%
114		421 bps	154			77%
99		124 bps	159			76%
91		404 bps	27			94%;NSEP
97		219 bps	79			94%
115		460 bps	41			97%
135		170 bps; NSEP	138			78%
38	can't confirm	lower than 92% ident (by ITS)	106		Over 99% ident to- (by ITS1)	*A. gallica* and *A. cepistipes*
5			132			*A. gallica*, *A. cepistipes* and *A. sp*.
14			84			*A. ostoyae*, *A. cepistipes*, and *A. gallica*; BSEP
80			165			*A. sp*., *A. cepistipes* and *A. gallica*; NSEP
77			40			*A. ostoyae*, *A. gallica*; NSEP
46			143			*A. cepistipes* and *A. sp*.
75			107			*A. sp*. and *A. gallica*; NSEP
133			142			*A. gallica* and *A. cepistipes*
31			158			*A. gallica* and *A. cepistipes*
171			140			*A. gallica* and *A. cepistipes*
129			172			*A. gallica* and *A. cepistipes*
102			8			*A. gallica* and *A. cepistipes*
94			22			*A. gallica* and *A. cepistipes*
24		no sequence; NSEP in 24	123			*A. gallica* and *A. cepistipes*
121			NSSF: No significant similarity found; NSNSI: Nice sequence but no similarity ident in gene bank; bps: Base pairs NSEP: No sequence by EF-1α primer BSEP: Bad sequence by EF-1α primer			
90						
117						
118						
98						

3.2 Species distribution

A. cepistipes was found only in Söderskog, at the entrance of the park, along the trails or at the borders. *A. gallica* was broadly distributed in both Söderskog and Norreskog. Other unidentified *Armillaria* species were rare and only present at the border or entrance of the park. Overlap occurred among the different species identified (Figure 8). In general, Dalby Norreskog has less *Armillaria* species diversity than Söderskog.

3.3 Substrate materials

In all 36 identified *Armillaria* samples, half of them were collected from standing dead wood, 25% of them from logs, and 8% from on stumps. Totally, 83% were derived from woody materials and 17% were derived from soil (Table 10).

Table 10. Substrates of identified *Armillaria* species.

Substrate	*A. gallica*		*A. cepistipes*		*A. sp.*		Total	%
	Söder	Norre	Söder	Norre	Söder	Norre		
Stump	3	0	0	0	0	0	3	8.3
Log	3	0	1	0	1	4	9	25.0
Standing dead tree	6	2	1	0	4	5	18	50.0
Soil	0	3	1	0	2	0	6	16.7
Total							36	100

Söder: Söderskog; Norre: Norreskog

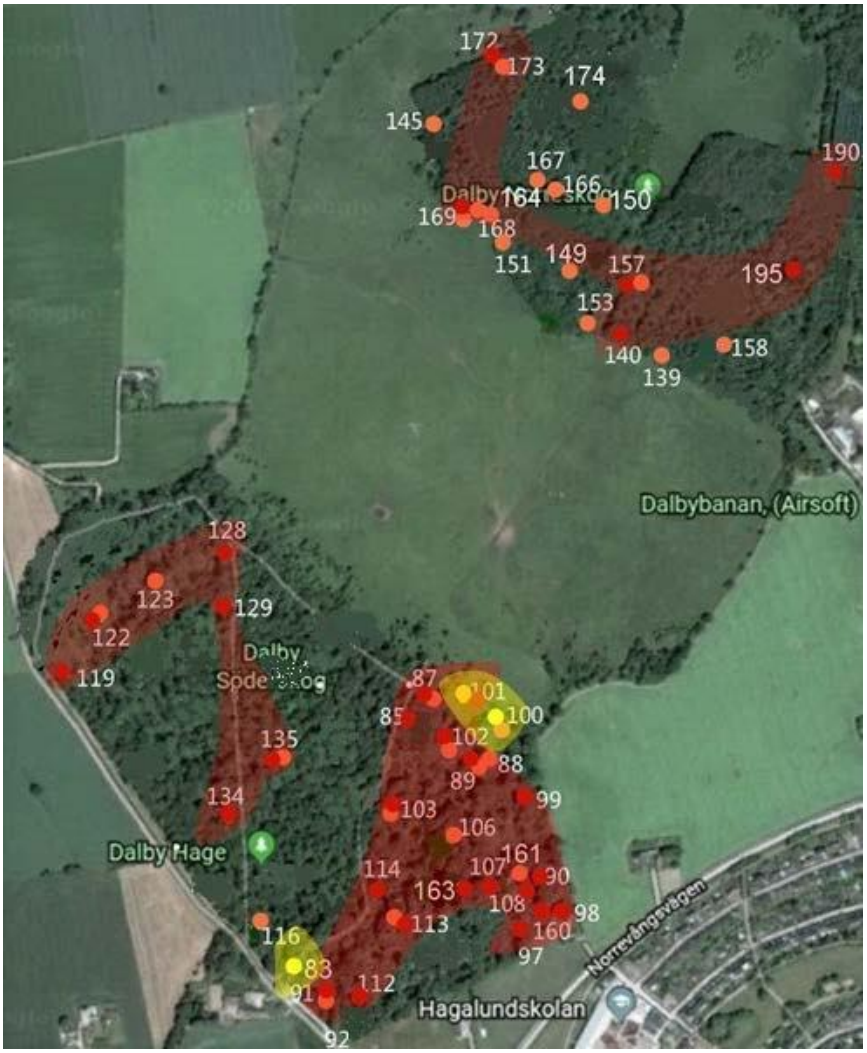

Figure 8. *Armillaria* species distribution in Dalby Söderskog and Norreskog. Only the identified isolates are shown on the map; All *Armillaria* species are marked by circle. The yellow areas are occupied by *Armillaria cepistipes*, the red areas are occupied by *Armillaria gallica*. The orange dot represents *Armillaria sp*.

3.4 Morphology of isolates

The morphology of *A. gallica* in culture varied (Figure 9). For example, samples 99a (isolate ID 12) and 140b (isolate ID 103) were both confirmed as *A. gallica*, but they showed different branching patterns.

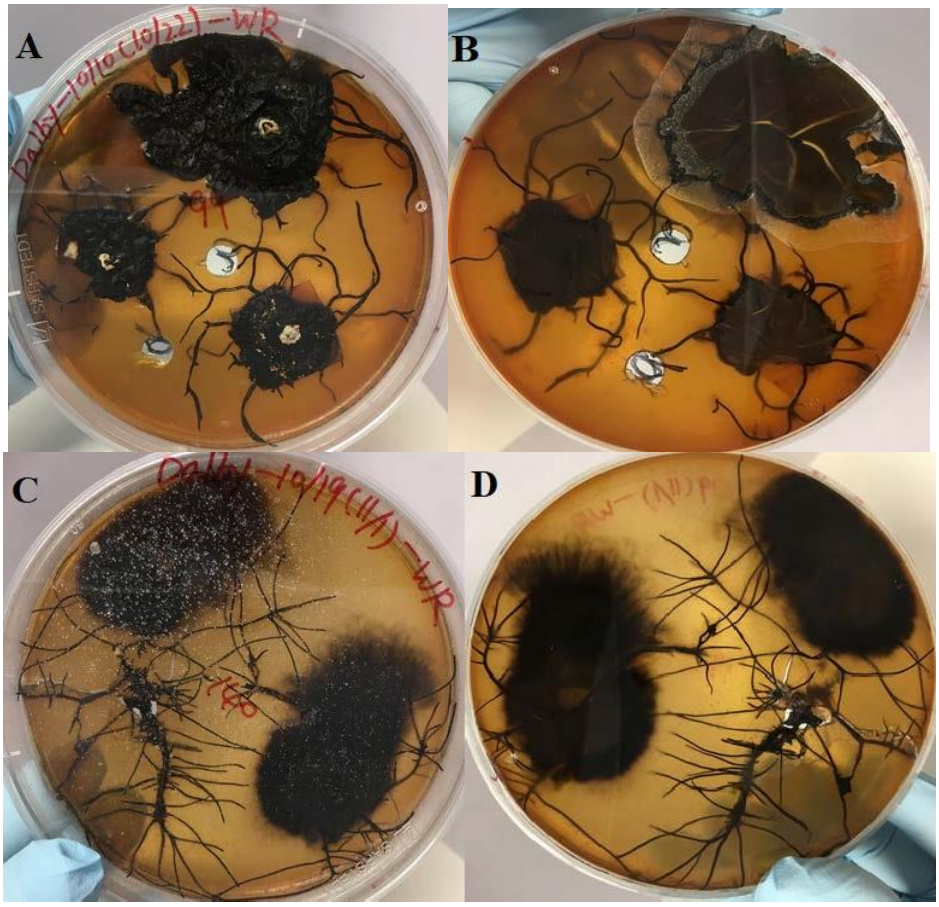

Figure 9. Morphologies of *Armillaria gallica* from different isolates. A), B) are the front and back sites of the isolate from point 99. C), D) are the front and the back sites of isolate from point 140.

Only three isolates were identified as *A. cepistipes*; but only one pure culture was obtained from these (Figure 10). This isolate was extracted from a field sample at point 83 in Söderskog. According to the rhizomorphs in pure cultures, it was hard to see significant differences between *A. gallica* and *A. cepistipes*.

Figure 10. Morphology of *Armillaria cepistipes.*

There were also cases that the same isolate from different cultures have different morphology. For example, both cultures in Figure 11 were extracted from the same piece of mycelium fan at point 145, but they appeared different, where culture of A/B had

larger mycelium spreading area. Even the same isolate in the same petri-dish had different morphology during the growth process (Figure 12).

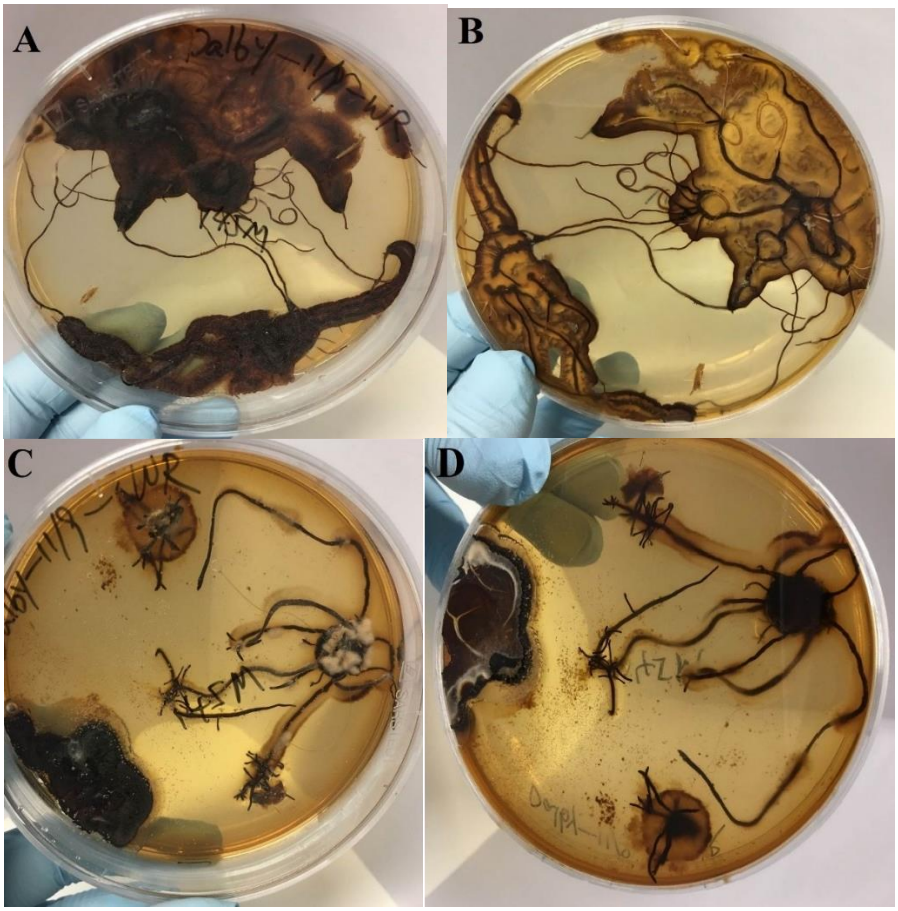

Figure 11. Isolates extracted from the same mycelium fan of point 145.

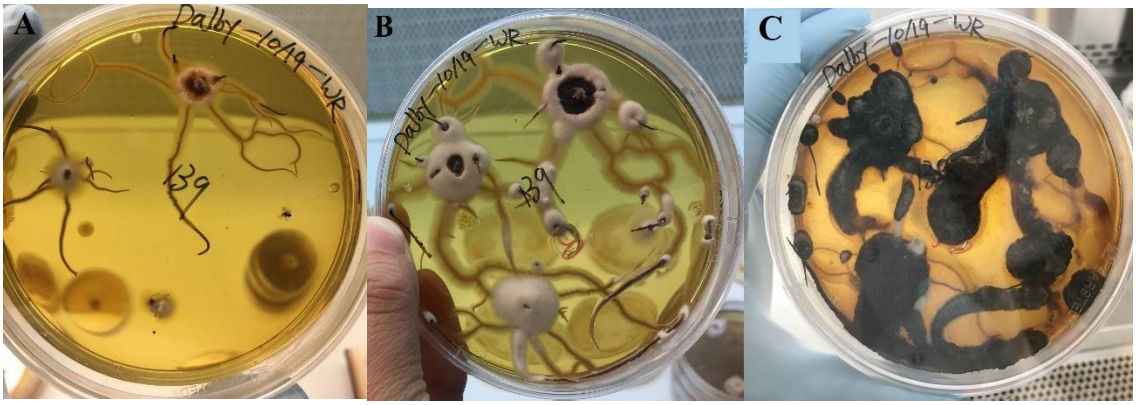

Figure 12. Rhizomorphs of sample 139 in different time. This isolate was plated on October 19th, 2018; Pictures A), B), C) were taken on October 30th, 2018; November 5th, 2018 and March 1th, 2019, respectively.

3.5 Pairing test

In this study, 36 pairs were made from isolates from nine different points in order to identify the genotypes (genets), basing on the sexual behavior of *Armillaria*, three of them were chosen from *A. gallica*, six of them were chosen as unidentified *Armillaria sp.* After 40 days, seven pairs showed compatible or incompatible outcomes, the other paired cultures marked as "." in Figure 13 had no obvious result. Two pairs (102-169 and 166-173) were deemed to be compatible, because mycelium of the two opposing colonies fused; sample 102 was identified as *A. gallica* through molecular techniques, and isolate 169 could be identified as *A. gallica*. Five pairs are incompatible, gaps formed between them. They include: 102 (*A. gallica*), 166 (*A. sp.*), 166 (*A. sp.*), 169 (*A. sp.*), 102 (*A. gallica*), 173 (*A. sp.*), 139 (*A. sp.*), 164 (*A. sp.*), 139 (*A. sp.*), 173 (*A. sp.*) (Fig. 14). Since 173 (*A. sp.*) and 166 (*A. sp.*) fused, they are assumed to be the same isolates. However, they did not fuse with 102 (known as *A. gallica*).

91 A. g								
.	102 A. g							
.	.	123 A.s						
.	.	.	135 A. g					
.	.	.	.	139 A.s				
.	.	.	.	×	164 A.s			
.	×	.	.	.	.	166 A.s		
.	√	.	.	.	.	×	169 A.s	
.	×	.	.	×	.	√	.	173 A.s

Figure 13a. Pairing test results. The green marked sample 91, 102, 135 are *Armillaria gallica*. The yellow marked samples are *Armillaria sp.*."√" means the mycelium of two opposing isolates fused with each other (compatible reaction), "×" means two isolates have an obvious gap between each other (incompatible reaction). A.g represents *A. gallica*. A.s represents *A. sp.*

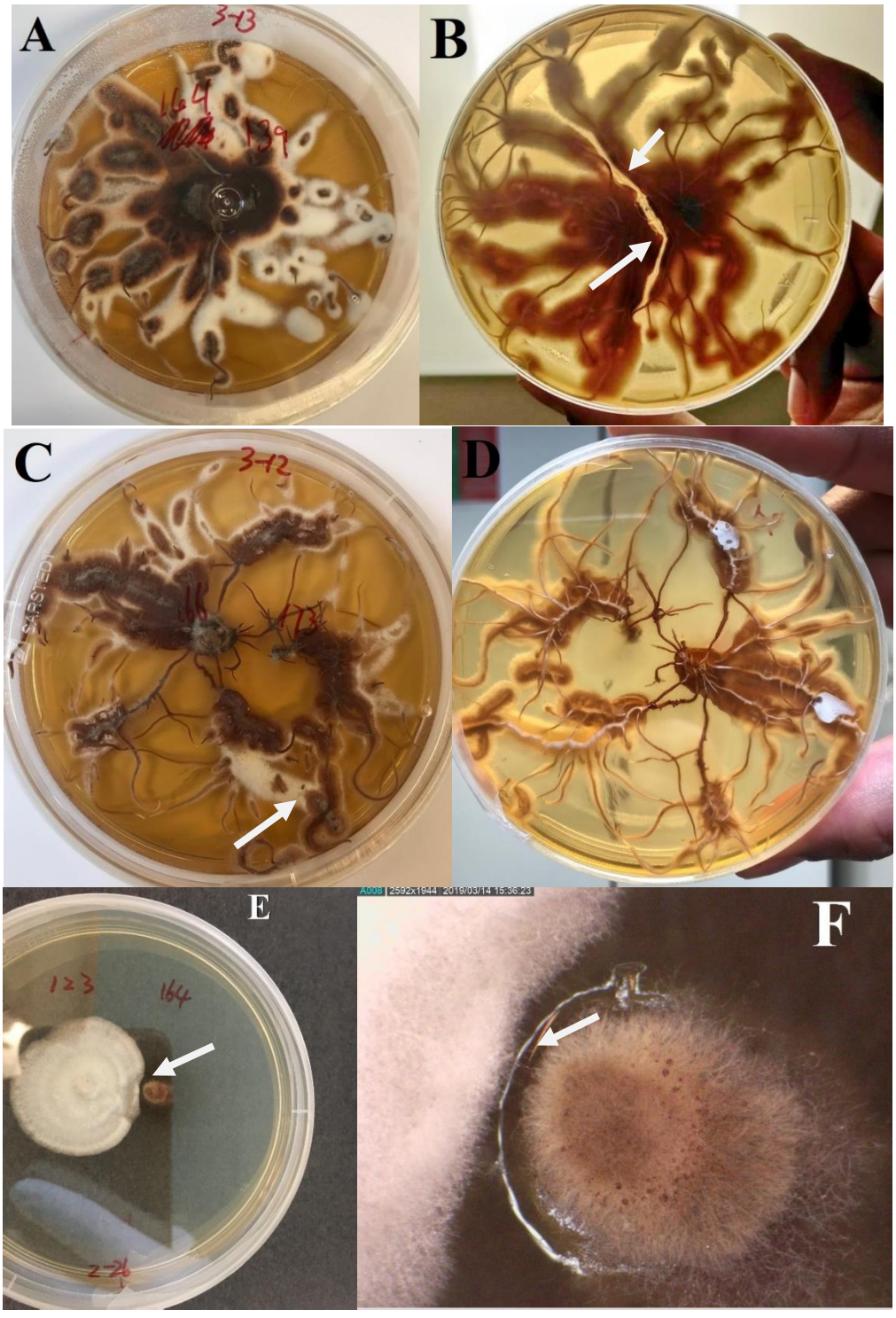

Figure 13b. Compatible and incompatible somatic pairing results. A) and B) show the front side and backside of paired isolates 164 and 139 after 40 days of plating, arrows point out the gap between isolates; C) and D) show the front and backside of paired isolates 166 and 173 after 41days of plating, no gap exists between; E), F) shows a zone of inhibition between two opposing isolates 123 and 164 after 16 days.

4. Discussion

4.1 Identified *Armillaria* species and composition in study areas

In this study, *A. gallica, A. cepistipes*, and additional, but unidentified *Armillaria sp.* were identified. According to current species diversity, *A. gallica* is dominating both Söderskog and Norreskog. *A. cepistipes* is found in low abundance, and is very sporadic in Söderskog. Thus, Söderskog has a more diverse species composition comparing to Dalby Norreskog, however not all samples were analyzed, and several could only be identified to a genus level, and several none at all.

4.2 Ecology of *A. gallica* and *A. cepistipes* in Dalby

According to previous studies (Baumgartner et al., 2011; Guillaumin et al., 1993; Prospero et al., 2004; Kwasna, 2008), *A. gallica* and *A. cepistipes* behave similarly as saprophytes colonizing dead tissue or as a weak pathogen taking advantage of dying trees. Both species can coexist with other *Armillaria* species. For example, *A. cepistipes* was recorded to coexist with *A. ostoyae* as a secondary parasite, which prefers conifers (Prospero et al., 2004), while *A. gallica* is more frequently associated to broadleaves, and has been recorded as a secondary parasite after *A. mellea* (Guillaumin et al., 1993)..

According to Baumgartner et al. (2011), *A. gallica* and *A. cepistipes* usually perform as wood decomposer in forests. In this study, a large proportion (83%) of samples were collected from woody materials which is important for carbon cycling. However, these species could potentially take advantage of hosts under stress. A study from Lithuania showed a high incidence of *A. cepistipes* infection (up to 80%) on trees that are stressed (Lygis et al., 2005). Weakened trees can commonly become more susceptible to attack by other pests and pathogens (Shearer et al., 1993).

Stress factors like ash decline, Dutch elm disease and *Phytophthora spp*. may also influence the development of *Armillaria* root rot in the study area over time, contributing to premature death of those species affected and potentially altering the forest dynamics. In Lithuania, Lygis et al. (2005) suggested that mortality of ash trees (later known to be affected by ash dieback) were highly related to *Armillaria*, especially *Armillaria cepistipes*. Hauptman (2016) also showed suggest similar results. Moreover, the Lithuanian study showed that presence of *A. cepistipes* had a negative influence on natural regeneration of

ash. In Denmark, a high incidence of *A. gallica* infection and mortality was found in ash dieback-affected stands (Skovsgaard et al., 2010). Thus, it can be assumed that as more ash trees become infected by Hymenoscyphus fraxineus, they will succumb to Armillaria in the end, perhaps sooner than they would without the presence of Armillaria already on or surrounding the root base.

4.3 The genet size and age

In this study, two genets were identified: *A. gallica* which was distributed in Norreskog and Söderskog, the other genet located only in Norreskog. Based on rate of the fungus' estimated mycelial growth, at between 0.3 and 1.6 m per year (Lygis et al., 2005), we may be dealing with a single genet that is several hundred years old, but possibly even larger since those tested isolates were only taken from the edge of Norreskog and Söderskog. Both vegetative spread and basidiospores play a role in the distribution of new disease loci (Worrall, 2018).

4.4 Comparison on identification methods

In this study, two different methods, pairing test and molecular techniques were used to identify the species. Pairing tests can be done to help understand the population structure of *Armillaria sp.*. However, it requires quite long and tedious work for maintaining cultures and continuous examination and scoring of plates during a long period of time. Contamination often happen during the plating and incubating of fungi and for this method a testing strain (one with a known identificated) is required.

DNA techniques are the most reliable means for fungal species identification, but still the procedure is not always clear-cut or robust. In particular, it was discovered that more than one genomic region needs to be targeted in order to make the accurate identification, but even then, many errors can occur at several stages in the DNA analysis pipeline.

5. Conclusion

According to this study, *A. gallica* and *A. cepistipes* were identified at Dalby Söderskog. *A. gallica* and *A. cepistipes* play an important role as saprophytes, but may become opportunistic pathogens given the large number of other biotic threats that are stressing elm, ash and possibly other broadeleaved tree species. Over time, tree species composition will

be influenced by the complexity of these interactions and Further investigation as to the remaining unidentified *Armillaria* species and their long-term influence on forest dynamics is warranted.

6. Acknowledgments

First of all, I would like to express sincerest thanks to my supervisor Michelle Cleary, who guided me a new perspective to observe forest, and provided constant support, and care during the process. I would also like to thank Mohammed Elsafy, who guided me on the practical lab work and Simone Prospero from WSL in Switzerland who inspired me when the experiment was stuck. Special thanks to Dalby National Park who granted the permission to collect samples and NordGen institution who funded me on part of the sequencing. Warm thanks to all the persons in the forest pathology lab and the Department.

7. References

Anderson, J. B., & Stasovski, E. (1992) Molecular Phylogeny of Northern Hemisphere Species of *Armillaria*, Mycologia, 84:4, 505-516, DOI: 10.1080/00275514.1992.12026170

Baumgartner, K., Coetzee, M. P. A., & Hoffmeister, D. (2011). Secrets of the subterranean pathosystem of Armillaria. Molecular Plant Pathology, 12(6), 515–534. https://doi.org/10.1111/j.1364-3703.2010.00693.x

Brunet, J., Bukina, Y., Hedwall, P., & Holmström, E. (2014). Pathogen induced disturbance and succession in temperate forests : Evidence from a 100-year data set in southern Sweden. Basic and Applied Ecology, 15(2), 114–121. https://doi.org/10.1016/j.baae.2014.02.002

Burdsall, H. H., & Volk, T. J. (1993). The state of taxonomy of the genus Armillaria. McIlvainea, 11, 4–12.

Cleary, M. R., Arhipova, N., Morrison, D. J., Thomsen, I. M., Sturrock, R. N., Vasaitis, R., Stenlid, J. (2013). Stump removal to control root disease in Canada and Scandinavia: A synthesis of results from long-term trials. Forest Ecology and Management, 290, 5–14. https://doi.org/10.1016/j.foreco.2012.05.040

Coetzee, M. P. A., Wingfield, B. D., Harrington, T. C., Steimel, J., Coutinho, T. A., & Wingfield, M. J. (2001). The root rot fungus Armillaria mellea introduced into South Africa by early Dutch settlers. Molecular Ecology, 10(2), 387–396. https://doi.org/10.1046/j.1365-294X.2001.01187.x

Guillaumin, J. -J, Mohammed, C., Anselmi, N., Courtecuisse, R., Gregory, S. C., Holdenrieder, O., van Dam, B. (1993). Geographical distribution and ecology of the Armillaria species in western Europe. European Journal of Forest Pathology, 23(6–7), 321–341. https://doi.org/10.1111/j.1439-0329.1993.tb00814.x

Hauptman, T., Ogris, N., de Groot, M., Piškur, B., & Jurc, D. (2016). Individual resistance of Fraxinus angustifolia clones to ash dieback. Forest Pathology, *46*(4), 269–280. https://doi.org/10.1111/efp.12253

Heinzelmann, R., Dutech, C., Tsykun, T., Labbé, F., Soularue, P., Prospero. (2019). Latest advances and future perspectives in Armillaria research. Canadian Journal of Plant Pathology, 41(1), 1-23. https://doi.org/10.1080/07060661.2018.1558284

Heinzelmann, R., Prospero, S., & Rigling, D. (2017). Virulence and stump colonization ability of Armillaria borealis on Norway spruce seedlings in comparison to sympatric Armillaria species. Plant Disease, 101(3), 470–479. https://doi.org/10.1094/PDIS-06-16-0933-RE

Koch, R. A., Wilson, A. W., Séné, O., Henkel, T. W., & Aime, M. C. (2017). Resolved phylogeny and biogeography of the root pathogen *Amillaria* and its gasteroid relative , *Guyanagaster*. BMC Evolutionary Biology, 17(33), 1-16. https://doi.org/10.1186/s12862-017-0877-3

Lushaj, B. M., Woodward, S., Kećа, N., & Intini, M. (2010). Distribution, ecology and host range of Armillaria species in Albania. Forest Pathology, 40(6), 485–499. https://doi.org/10.1111/j.1439-0329.2009.00624.x

Lygis, V., Vasiliauskas, R., Larsson, K. H., & Stenlid, J. (2005). Wood-inhabiting fungi in stems of Fraxinus excelsior in declining ash stands of northern Lithuania, with particular reference to Armillaria cepistipes. Scandinavian Journal of Forest Research, 20(4), 337–346. https://doi.org/10.1080/02827580510036238

Maphosa, L. A., Wingfield, B. D. A., Coetzee, M. P. A. A., Mwenje, E. B., & Wingfield, M. J. A. (2006). Phylogenetic relationships among Armillaria species inferred from

partial elongation factor 1-alpha DNA sequence data. Australasian Plant Pathology 35, 513–520. https://doi.org/10.1071/AP06056
Morrison, D. J. (2004). Rhizomorph growth habit, saprophytic ability and virulence of 15 Armillaria species. Forest Pathology, 34(1), 15–26. https://doi.org/10.1046/j.1439-0329.2003.00345.x
Morrison, D. J., Pellow, K. W., Norris, D. J., & Nemec, A. F. L. (2000). Visible versus actual incidence of Armillaria root disease in juvenile*rous* coniferous stands in the southern interior of British Columbia. Canadian Journal of Forest Research, 30(3), 405–414. https://doi.org/10.1139/x99-222
S. Prospero, O. Holdenrieder, D. Rigling. (2004). Comparison of the virulence of *Armillaria cepistipes* and *Armillaria ostoyae* on four Norway spruce provenances. For. Pathol., 34, 1-14. https://doi.org/10.1046/j.1437-4781.2003.00339.x
Prospero, S., Rigling, D., & Holdenrieder, O. (2003). Population structure of Armillaria species in managed Norway spruce stands in the Alps. New Phytologist, 158(2), 365– 373. https://doi.org/10.1046/j.1469-8137.2003.00731.x
Shearer, C. A., Carroll, G. C., & Wicklow, D. T. (1993). The Fungal Community: Its Organization and Role in the Ecosystem. Mycologia, 5(85), 881–882. https://doi.org/10.2307/3760630
Sipos, G., Anderson, J. B., & Nagy, L. G. (2018). Armillaria. Current Biology, 28(7), R297–R298. https://doi.org/10.1016/j.cub.2018.01.026
Skovsgaard, J. P., Thomsen, I. M., Skovgaard, I. M., & Martinussen, T. (2010). Associations among symptoms of dieback in even-aged stands of ash (Fraxinus excelsior L.). Forest Pathology, 40(1), 7–18. https://doi.org/10.1111/j.1439-0329.2009.00599.x
Smith, M. L., Bruhn, J. N., & Anderson, J. B. (1992). The fungus Armillaria bulbosa is among the largest and oldest living organisms. Nature, 356(6368), 428–431. https://doi.org/10.1038/356428a0
Soularue, J. P., Robin, C., Desprez-Loustau, M. L., & Dutech, C. (2017). Short rotations in forest plantations accelerate virulence evolution in root-rot pathogenic fungi. Forests, 8(6). https://doi.org/10.3390/f8060205
Tsykun, T., Rigling, D., Nikolaychuk, V., & Prospero, S. (2012). Diversity and ecology of Armillaria species in virgin forests in the Ukrainian Carpathians. Mycological Progress, 11(2), 403–414. https://doi.org/007/s11557-011-0755-0
Tsykun, T., Rigling, D., & Prospero, S. (2013). A new multilocus approach for a reliable DNA-based identification of Armillaria species. Mycologia, *105*(4), 1059–1076. https://doi.org/10.3852/12-209
von Oheimb, G., & Brunet, J. (2007). Dalby Söderskog revisited: long-term vegetation changes in a south Swedish deciduous forest. Acta Oecologica, *31*(2), 229–242. https://doi.org/10.1016/j.actao.2006.12.001
Worrall, J. J. (1994). Population Structure of Armillaria Species in Several Forest Types. Mycologia, 3(86), 401-407, DOI: 10.1080/00275514.1994.12026427
Yen, S. B., & Francisco, S. (1990). Nomenclature, Taxonomy and Identification.
Microbiology, *28*(9), 1877–1880.