Faculty Biological and Environmental Sciences
Organismal and Evolutionary Biology Research Programme
Doctoral School in Environmental, Food and Biological Sciences
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University of Helsinki
Finland

Taphrina as model phytopathogenic yeasts infecting the model plant *Arabidopsis* and woody plant *Betula pendula*

Margaretta Christita

ACADEMIC DISSERTATION

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Supervisors:	Docent Kirk Overmyer Organismal and Evolutionary Biology Research Programme University of Helsinki, Finland
	Docent Kaisa Nieminen Natural Resources Institute Finland
Thesis Committee:	Professor Fred Asiegbu Department of Forest Sciences University of Helsinki, Finland
	Professor Jarkko Hantula Natural Resources Institute Finland
Pre examiners:	Professor Stephen Woodward School of Biological Sciences University of Aberdeen, United Kingdom
	Professor Marc-André Lachance Department of Biology Western University, Canada
Opponent	Professor Minna Pirhonen Department of Agricultural Sciences University of Helsinki, Finland
Custos	Professor Kurt Fagerstedt Organismal and Evolutionary Biology Research Programme University of Helsinki, Finland

Cover: Witches' broom disease on *Betula pendula*. Illustration by Sm. Solihah, 2021.

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Abstract

Plants constantly interact with microorganisms, including bacteria, and fungi, including yeasts. Plant-associated yeasts have not been studied as much as plant-associated fungi and bacteria. Yeast-like fungi that interact with plants can be pathogenic, neutral residents, or plant growth promoting. Yeast-like fungi can be found in or on all parts of the plant, and their role in the phyllosphere is currently receiving more attention. The plant-associated yeast species studied in this thesis are *Taphrina*, a genus of dimorphic yeast-like fungi, which are able to switch lifestyle from yeast to filamentous fungi when their environment is favourable. In this thesis *Taphrina* species were investigated as model phytopathogenic yeast-like fungi by observing the interaction of *T. betulina* with *Betula pendula* (European silver birch) and developing a *Taphrina* infection system with the genetic model plant, *Arabidopsis thaliana* (hereafter referred to as *Arabidopsis*). This thesis shows that *T. betulina*, which causes witches' broom disease in birch, was the predominant yeast in the phylloplane of both symptomatic and asymptomatic *B. pendula*. Fifty-seven strains of *T. betulina* with seven different genotypes were found. One of the seven genotypes was found to be dominant and was common in infected trees, but was also found at lower levels in samples from healthier trees. Moreover, since *T. betulina* causes witches' broom disease in *B. pendula*, the tumour morphology in birch infected by *T. betulina was* characterized in this thesis. Further, the distribution of susceptible and resistant birch trees provided evidence that suggests there may be genetic resistance against *T. betulina* in the birch population.

To develop the infection system of *Taphrina* in the model plant *Arabidopsis*, *Taphrina* strain M11, previously isolated from wild *Arabidopsis*, was used. The M11 strain caused symptoms of disease in *Arabidopsis*, including leaf deformations in the form of leaf bending and leaf curling. In addition, this result was supported by several tests that showed activation of auxin- and cytokinin-signalling during infection, which may be part of the pathogenic yeast invasion strategy against its host. A high-quality draft genome assembly for *Taphrina* strain M11 was obtained. In addition, as candidate effector proteins, 767 short secreted proteins (SSPs) were found in the M11 genome. No LysM domain containing SSPs were found, suggesting that chitin has a minor role in *Taphrina*-host interactions. Furthermore, infections with plant hormone signalling mutants showed that jasmonic acid and ethylene are major hormones in plant defence against *Taphrina* M11. In growth tests, M11 became more abundant on *Arabidopsis* leaves compared to related *Taphrina* strains and was able to form biofilms. Furthermore, M11 has been identified as a strain of *T. tormentillae*. Thus, this thesis shows that *Taphrina* has potential as a model of phytopathogenic yeasts infecting the model plant *Arabidopsis* and the woody plant *B. pendula*.

Tiivistelmä

Muihin kasvien kanssa tavattaviin mikrobeihin verrattuna hiivat ovat hyvin vähän tutkittuja. Hiivan tapaiset sienet vuorovaikuttavat kasvien kanssa joko patogeeneinä, pöytävierassuhteessa tai hyödyllisinä symbiontteina. Väitöskirjani kasvien kanssa vuorovaikuttavat hiivalajit kuuluvat sukuun *Taphrina*, jonka sienet kykenevät muuttamaan elintapansa hiivamaisesta rihmamaiseksi.

Taphrina betulina aiheuttaa koivulla tuulenpesiä. Tähän tautiin keskittynyttä tutkimusta on rajallisesti, mutta saatavilla olevat harvat tutkimukset esittävät, että taudin aiheuttamaa negatiivista vaikutusta on mahdollisesti aliarvioitu. Väitöskirjallani oli kolme tavoitetta: 1) Saavuttaa käsitys tuulenpesä-sienitaudista *Betula pendula* -koivuissa löytämällä kasvaimen kasvun lähtöpisteenä toimiva puukudos. 2) Ymmärtää paikallista *T. betulina* infektioekologiaa tarkkailemalla terveessä koivussa olevia *Taphrina*-sieniä ja muita hiivasieniä ja vertailemalla niitä *T. betulina*-infektion oireita ilmentävään koivuun. 3) Määritellä molekulaariset puolustusmekanismit hiivapatogeeniä vastaan käyttämällä *Arabidopsis*-mallijärjestelmää, jolla selvitettiin T. betulina-patogeenin vastaisen puolustusreaktion signalointireittejä.

Tässä väitöskirjassa tutkittiin *T. betulina*-kasvaimia ja määritettiin oireiden kehittymisen kudosspesifisyys infektioiden aikana. *T. betulina*-kannat on eristetty yksinomaan sairaista koivuista ja väitöskirjassa näytetään, että *T. betulina*-sientä löytyy myös terveistä koivuista. *T. betulina* oli hallitseva hiiva kasvin lehdistön pinnalla sekä sairaissa että silmämääräisesti terveissä *B. pendula*-koivuissa. Taphrina-infektiojärjestelmän kehittämiseksi *Arabidopsis*-mallikasvilla käytettiin aiemmin villiltä *Arabidopsis*-kasvilta eristettyä M11 *Taphrina*-kantaa. M11 aiheutti *Arabidopsis*-kasvissa taudinoireita, kuten lehtien epämuodostumia, osoittaen jossain määrin samankaltaisuutta muiden *Taphrina*-lajien lehtioireisiin niiden isäntäkasveissa. Väitöskirja sisältää myös M11-infektoituneen *Arabidopsis*-kasvin infektiofenotyyppien ja hormonivasteiden karakterisoinnin.

Näin ollen tämä väitöskirjatyö osoittaa, että *Taphrina*-suvun lajeilla on potentiaalia malliksi fytopatogeenisista hiivoista, jotka voivat infektoida *Arabidopsis*-mallikasvin ja puuvartisen kasvin *B. pendula*.

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Original publications

This dissertation is based on the following publication or manuscripts:

I. **Christita, M.**, and Overmyer, K. (2021) Genetic resistance and tumour morphology in birch infected with *Taphrina betulina*. Forest Pathology, 51(4), e12709. doi: https://doi.org/10.1111/efp.12709

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Author contributions:

I. **MC** performed all experiments. **MC** and KO designed experiments performed field sampling, data analysis, and wrote the manuscript.

II. **MC** performed indolic compound and GUS assays, morphological assays, and data analysing. TS conducted the field sampling, molecular and genetic identification. KO designed experiments. **MC** and KO wrote the manuscript.

III. **MC** performed *Arabidopsis* infection experiments, hyphae and cell observations, hypersensitive cell death assays, phenotyping of mutants, plant hormone GUS assays, phenotypic assays, and ROS stain assays. AA performed *in planta* growth and biofilm formation assays. KW, JS, and AA performed bioinformatic analyses. TS, PA, LP performed genome sequencing. **MC**, AA, KO analysed the data. **MC**, KO, and AA wrote the manuscript.

Abbreviations

ABA	Abscisic acid
BR	Brassinosteroids
CK	Cytokinin
Col-0	Columbia-0 accession
GA	Gibberellic acid
IAA	Indole-3-acetic-acid
ITS	Nuclear rRNA internal transcribed spacer
JA	Jasmonic acid
MAMP	Microbe-associated molecular pattern
ORFs	Open reading frames
OTU	Operational taxonomic unit
PAMP	Pathogen-associated molecular pattern
ROS	Reactive oxygen species
SA	Salicylic acid
YPD	Yeast peptone dextrose medium

1. Introduction

1.1 Plant pathogen – host interaction

Phytopathogenic organisms that invade plant tissues are diverse and have unique mechanisms of pathogenicity (Agrios, 2005). Microorganisms that are phytopathogenic include viruses, mycoplasma, bacteria, nematodes, protozoa, and fungi including yeasts (Staskawicz et al., 1995).

A concept that is key in understanding plant diseases is referred to as the disease triangle, according to which the occurrence of disease in a plant is caused by the interaction of three main factors, namely the host, the pathogens, and the environment. This model can be used to predict epidemiological outcomes in plant health (Scholthof, 2007). According to this concept, plant disease occurs when the host is susceptible, the pathogen is virulent, and the environment is favourable for infection establishment (Agrios, 2005; Nelson, 1994). However, time is a factor that influences the pathogen infection process in the host, thus determining the development of plant diseases (Islam, 2018).

Phytopathogenic microbes have several mechanisms to invade their hosts. Some filamentous fungi use hyphae to penetrate plant tissue. Furthermore, fungi also produce secondary metabolites to weaken plant immune systems (Howlett, 2006). In contrast, plants naturally have an immune system, which functions to limit pathogen infection. There are several mechanisms often found and widely studied in plant immunity, including hypersensitive response, which is localized induced programmed cell death (PCD) in the host plant at the site of infection. Cell death in plants occurs as a response to pathogen infection, which can be in the form of programmed cell death or uncontrolled death from damage or cellular dysfunction. In the hypersensitive response, PCD occurs rapidly at the infected site, which functions to localize the pathogen infection at the expense of the infected cells (Balint-Kurti, 2019). In contrast, cell death can also occur later in the infection as a symptom of pathogen infection, resulting from cell dysfunction caused by infectious pathogens.

Plants have several defensive tools and mechanisms and use both physical and chemical barriers as protection. Cell wall, cuticles, trichomes, thorns, and wax are physical barriers, which are the first line of defence. Plant chemical barriers are known to be secondary metabolites, which have antimicrobial activity. Adapted pathogens have unique mechanisms of defeating pre-formed defences and invading the host plant. Successful pathogens usually overcome the preformed plant chemical defences. For example, adapted pathogens are often more resistant to host-derived antimicrobials *in vitro* than non-adapted pathogens (Funnell et al., 2010).

Plants also have inducible immunity, which is able to recognize the presence of pathogens and other harmful microbes. *Arabidopsis* is a widely used genetic model plant that has allowed detailed study of the plant immune system. However, there is a lack of studies related to the interaction of yeasts with *Arabidopsis*. The interaction

between plant defence systems and pathogens has been described in the "zigzag model", which proposes that the plant immune response consists of elicitor- and effector- triggered responses (Jones and Dangl, 2006). Elicitors in the plant immune system process are known as pathogen associated molecular patterns (PAMPs) or microbe associated molecular patterns (MAMPs). The term MAMPs is also used because these essential structures are also found in non-pathogenic microorganisms (Henry et al., 2012). PAMPs are essential, conserved structures found in pathogenic microorganisms that induce cellular signalling and immune responses in plants. PAMPs have an important role in the defence processes of plants. PAMP induced defence responses include the production of reactive oxygen species (ROS), production of reactive nitrogen species, induction of antimicrobial compounds, and the synthesis of pathogenesis-related (PR) proteins (Newman et al., 2013). The presence of PAMPs in plants is recognized by the plant's innate immune system by pattern recognition receptors (PRRs) that are localized on the surface of plant cells (Newman et al., 2013; Zhang and Zhou, 2010). This first layer of defence induction is called PAMP-triggered immunity (PTI) (Chisholm et al., 2006). Adapted pathogens use effectors to block PTI and cause susceptibility in the host. In the second layer of defence, the plant responds by developing defences through specific disease resistance (*R*) genes, the so-called effector-triggered-immunity (ETI), which is mediated by nucleotide-binding leucine-rich repeat NB-LRR proteins. Thus, PTI and ETI are considered the primary and secondary layers of innate immunity, respectively (Henry et al., 2012).

1.2 Microbe communities of plants

For over a century, plants have been recognized as an important habitat for microorganisms, including bacteria, oomycetes, filamentous fungi, and yeasts. The interaction of microorganisms with plants occurs both on the plant surface and in the soil. The phyllosphere consists of all aerial parts of plants and is dominated by leaves; moreover, it is a habitat for various types of microbes. Fungi, bacteria and yeasts are residents of the phyllosphere and form unique communities (Vorholt, 2012). In addition, plants constantly interact with specific microbes such as rhizosphere and endophytic microbiota, which have received the most research attention (Andrews and Harris, 2000; Fonseca and Inácio, 2006; Wang et al., 2016). The endophytic microbiota consists of microbes that live inside the host plant tissue (Liu et al., 2016; Rosenblueth and Martínez-Romero, 2006) and the rhizosphere microbiota comprises microbes that colonize plant roots, including plant growth-promoting rhizobacteria (Nihorimbere et al., 2011).

Yeasts are a group of microfungi that grow rapidly and have simple nutritional requirements. Yeasts have been used as model systems in genetics and molecular biology because of the reason above and the characteristics of their cell wall structure, which uses unicellular reproduction or combination both of hyphal and unicellular

reproduction (Lachance and Walker, 2018). Yeasts that have been studied as model systems in certain biological systems include *Schizosaccharomyces pombe, Kluyveromyces lactis, Pichia pastoris,* and *Saccharomyces cerevisiae*. The two species *S. pombe* and *S. cerevisiae* constitute the most important model systems used to define modern molecular and cell biology. For example*, S. pombe* is a yeast model for metal homeostasis in plants (Clemens and Simm, 2003) and also a general model for unicellular eukaryotes (Yanagida, 2002). The genome of *S. pombe* has been completely sequenced. *Saccharomyces cerevisiae* is a model for yeast biofilm formation (Bojsen et al., 2012), a model system for the regulation of a eukaryotic biosynthetic pathways (Braus, 1991), and for studying the interaction of peptide hormones and G protein-coupled receptors (Naider and Becker, 2004). *S. cerevisiae* has been shown to produce an extracellular matrix and form multi-cellular aggregates, which are the basis for biofilm formation (Bojsen et al., 2012). When considering the interaction of yeasts with plants, few true yeasts are relevant. However, dimorphic yeasts, are often associated with plants, and many act as phytopathogens. Dimorphism refers to the ability of microorganisms to switch from unicellular yeast to multicellular filamentous hyphae as a response to environmental change (Nadal et al., 2008). Several dimorphic yeast-like fungi are known to cause disease on plants such as *Ophiostoma novo-ulmi* , the causal agent of Dutch elm disease (Brasier, 1991), and *Mycosphaerella graminicola* causing septoria leaf blotch disease in wheat (Palmer and Skinner, 2002). Symptoms of russet in apple and pear are caused by the yeasts *Rhodotorula glutinis* and *Aureobasidium pullulans* (Heidenreich et al., 1997; Spotts and Cervantes, 2002). In addition, *Taphrina* is a well known, large genus of dimorphic species (Nadal et al., 2008), including *T. deformans,* which causes disease in peach and *T. betulina,* which is well known as the causal agent of witches' broom disease in silver birch.

The presence of yeasts that colonize plants has various influences on plant growth. All plant compartments that have been widely explored have been shown to host a variety of yeasts, including roots (rhizosphere) and leaf surfaces (phylloplane). Yeasts and filamentous fungi may form resident populations on leaves, and have symbiotic interactions with their hosts, for instance by supressing the growth of pathogens (Blakeman and Fokkema, 1982).

1.2.1 Yeast interactions in the phyllosphere

Phyllosphere microbial ecology, or the study of leaf surfaces as habitats for microbes, has been undertaken since the 1950s (Ruinen 1956). The growth of yeast in the phylloplane is dependent on nutrient sources from plant metabolites that are secreted onto the plant surface, including types of sugars such as glucose, fructose, and sucrose. In addition, external compounds that fall on the leaf can help microbes fulfil their nutrient requirements (Limtong et al., 2014).

Microbes residing in the phylloplane have various modes of lifestyles and interactions with the host. A microbe can be a neutral resident, mutualistic, or a pathogen for the host (Wang et al., 2016). Yeasts have been reported as a resident on different plant substrates, including the phylloplane (Inacio et al., 2003; Yurkov et al., 2014), and can modulate plant health both by directly interacting with the host and by reshaping the prokaryotic microbiome (Agler et al., 2016). Yeast communities of the phylloplane are dependent on several factors such as the environment, leaf age, plant growth, and the type and density of competing microorganisms (Into et al., 2020; Lindow and Brandl, 2003).

The leaf surface is used by microorganisms as a habitat; however, it is exposed to fluctuating temperature and humidity, unstable conditions due to rain, and direct exposure to sunlight. The phyllosphere provides limited nutrient resources for residing microbes, resulting in high competition for survival. This is further exacerbated by the defence response from the host and as a result, the phyllosphere is considered a harsh environment for microbes (Fonseca and Inácio, 2006; Lindow and Brandl, 2003). Thus, microbes present in the phyllosphere are specifically adapted to survive under these extreme conditions. Several yeast species have often been found in the phylloplane communities of multiple plant species, for example, *Rhodotorula, Cryptococcus, Sporobolomyces,* and *Diozegia* (Fonseca and Inácio, 2006; Inacio et al., 2005).

1.2.2 Yeasts modulating the health of plant

Yeasts are widespread in natural biotopes. Epiphytic yeast communities form on live and moribund plant parts, which exhibit the highest yeast diversity. The yeast-plant interaction can be beneficial for both the yeasts and the host plant through mutualism. Several types of yeasts are reported to provide positive stimulation of plant growth. In contrast, there are also yeasts that are parasitic to plants. Pathogenic yeasts cause disruption to plant metabolism and abnormal growth. In addition, yeast species are also often found inhabiting the plant phyllosphere as commensal microbes. Although the phyllosphere is a habitat for many microbes, it is a harsh environment for survival. As mentioned in the previous section, microbes, including yeast and fungal pathogens, require the ability to survive in the phyllosphere before invading the host tissue.

Yeast mutualism with the host plant is well studied and can occur through various mechanisms. Yeasts support host plant growth by producing substrates that are antagonistic to fungal pathogens. These antagonist yeasts function to reduce diseases of aerial plant tissues and soil borne fungi by acting as a biocontrol against fungal pathogens (Schisler et al., 2011). For example, *Cryptococcus laurentii* and *Sporobolomyces roseus* are effective against gray mold in apples caused by *Botrytis. cinerea*; they utilize the apple volatile butyl acetate, which stimulates germination and adhesion to membranes of *B. cinerea* conidia (Filonow, 2001). Yeast-like fungi

residing on plants have also been shown to promote plant growth. *Candida valida, Rhodotorula glutinis* and *Trichosporon asahii* colonize sugar beet roots and protect the seedlings from *Rhizoctonia solani* diseases (El-Tarabily, 2004).The plant growth promoting yeast *Candida tropicalis* has proven effective for promoting rice growth (Amprayn et al., 2012).

In addition, yeasts that colonize the plant may enter a symbiosis with other microbes such as mycorrhizae in providing substrates needed for plant growth. Plant-growth-promoting yeasts associated with arbuscular mycorrhizae improve sunflower defence against *Macrophomina phaseolina* diseases (Nafady et al., 2019). However, the use of yeasts as plant growth promoters is still very limited when compared to the use of bacteria and mycorrhizal fungi (Fu et al., 2016).

The dimorphic lifestyle provides fungi with a remarkable plasticity and enables them to colonize their hosts and survive in extreme environments (Selbmann et al., 2014). *Rhodotorula* species are commonly found in the phylloplane (Slavikova et al., 2009). Some studies suggest that *R. glutinis* can be used as a biocontrol for *Botrytis cinerea* through antagonistic interaction (Buck, 2002; Buck and Jeffers, 2004). Other yeast species commonly found as residents in the phylloplane belong to the genera *Cryptococcus* (Inacio et al., 2002; Marchant and Towers, 1986) and *Sporobolomyces* (Bashi and Fokkema, 1976; Pennycook and Newhook, 1978).

1.2.3 Yeast-induced activation of plant hormone responses

Plant hormones are necessary in developmental processes and signalling pathways of plant responses to challenges including to biotic stresses. Yeast activity on plants can induce a plant hormone response. Yeasts have the ability to activate host plant hormonal signalling pathways, including the stress hormones salicylic acid (SA), jasmonates (JA) and ethylene (ET), which are important mechanisms of the plant's response to biotic stress. In addition, hormones classically understood as growth hormones, such as abscisic acid (ABA), auxin, gibberellic acid (GA), cytokinin (CK), and brassinosteroids (BR), are also important parts of plant defence signalling pathways (Bari and Jones, 2009).

The production of the auxin, indole-3-acetic acid (IAA) by yeasts is very complex and IAA production could be a crucial strategy used by yeasts to increase competitiveness. IAA produced by yeasts could inhibit the growth of specific bacterial strains competing for resources; furthermore, the produced IAA could also promote the growth of other yeasts (Fu et al., 2017). Plants and fungi produce many compounds with auxin activity; IAA is the most biologically important and most active auxin in plants (Duca et al., 2014) and is involved in most aspects of plant growth and development (Spaepen et al., 2007). IAA can be synthesized by microbes, including both plant growth-promoters and phytopathogens (Duca et al., 2014). Additionally, there are many

compounds with auxin-like activity involved in promoting the growth of plants. IAA methyl and ethyl esters, indole-3-butyric acid, indole-3-lactic acid and its methyl ester, indole-3-propionic acid, indole-3-pyruvic acid, and p-hydroxyphenyl acetic acid can be activated by the bacterium *Pseudomonas fluorescens* KGPP 207, promoting the growth of ginseng (Ten et al., 2000). IAA stimulates rapid and long-term responses in plants and has been identified in plant-associated bacteria and fungi, including yeasts. A yeast that has been widely studied and shown to produce IAA is *Saccharomyces cerevisiae* (Liu et al., 2016; Rao et al., 2010). Although the model yeast *S. cerevisiae* is commensal with some plants, the role of IAA in host interactions remains unexplored. However, this system shows how IAA is involved in fungal development and induces the formation of pseudohypae in *S. cerevisiae* (Prusty et al., 2004).

The dimorphic phytopathogenic fungus *Ustilago maydis* has been shown to produce IAA. Sosa-Morales (1997) observed four strains of *U. maydis* that are pathogens of maize and produce IAA; furthermore, this production of IAA both promoted pathogenicity of *U. maydis* and was involved in tumour-formation in the host (Guevara-Lara et al., 2000). In addition, the ability of *U. maydis* to produce auxin and cytokinin in culture has been demonstrated (Bruce et al., 2010; Mills and Van Staden, 1978; Wolf, 1952). Examination of auxin production by *U. maydis* in galls of maize showed a higher level of pathogenicity was associated with higher auxin concentrations (Moulton, 1942). Additionally, the existence of a non-pathogenic yeast from the genus *Ustilago*, *U. esculenta* JYC070, with a high IAA-producing ability, shows that yeast IAA can promote plant growth and development by significant increases in shoot and root growth on *Nicotiana benthamiana* (Fu et al., 2017).

1.2.4 Biofilm formation by yeast

Biofilm formation can be a sign of fungal growth on the plant surface. Biofilms are defined as collections of microbial cells that form an aggregate on the surface of the host (Reynolds and Fink, 2001). Biofilms are also described as microbial communities that are attached to the surface and embedded in a matrix that they produce from polymeric materials (Villa et al., 2017). According to Reynold and Fink (2001), the baker's yeast *S. cerevisiae* is a fungal biofilm formation model. In plant-fungal interactions that are pathogenic, biofilms support virulence in fungi that are infecting the host plant. Biofilms provide protection against harsh conditions such as on the phylloplane (Freimoser et al., 2019; Motaung et al., 2020). In general biofilms are more resistant to antibiotics and antimicrobials, including secondary metabolites released by the host as a defence mechanism (Parsek and Fuqua, 2004). In contrast, biofilms formed by beneficial microorganisms will support plant growth (Motaung et al., 2020). For example, biofilm formation by *Pseudomonas* species isolated from the rice phylloplane provide biocontrol against *Rhizoctonia solani* (Akter et al., 2016). A biofilm is formed by *Bacillus subtilis* on the melon phylloplane, providing biocontrol against pathogens (Zeriouh et al., 2014). Further studies have shown that bacteria can

form biofilms on fungal surfaces, as seen in interactions between *Pseudomonas aeruginosa* and the dimorphic opportunistic fungal pathogen *Candida albicans* (Hogan et al., 2009). This shows that the formation of biofilms is essential in the interaction of microorganisms with their environment.

1.3 *Taphrina* species

Taphrina species are dimorphic, with a saprophytic yeast stage and a parasitic mycelial stage on plant hosts, causing characteristic morphological changes on infected plants such as "leaf curl", "witches' brooms", out-growths from female catkins, spots on leaves, or deformed fruits (Bacigalova et al., 2003). In addition, they frequently reside in the phyllosphere for long periods in the haploid budding yeast form and infrequently invade their hosts in their pathogenic dikaryotic filamentous form under favourable, cold and wet, environmental conditions (Figure 1). Cultures of all strains are composed mainly of budding cells; occasionally some hyphal fragments are present.

1.3.1 Morphology and taxonomy of *Taphrina*

The genus *Taphrina* belongs to the order Taphrinales, class of Taphrinomycetes, subphylum Taphrinomycotina, and phylum Ascomycota (Fonseca and Rodrigues, 2011). There is a long history of taxonomic study of *Taphrina*. The classical systematic studies of the genus *Taphrina* were carried out from the late 1800s to the 1940s (Mix, 1936) and are carefully reviewed and summarized in the monograph published by Mix (1949). Later, the genus *Lalaria* was created to accommodate *Taphrina* yeast states (Moore, 1998), but later yet considered as redundant and removed, and re-created for *Taphrina*-like species isolated in their asexual states from symptomless plants not previously associated with a *Taphrina* species (Inacio et al., 2003). *Lalaria* is no longer accepted as a genus, all species previously accepted in the genus are now included in *Taphrina* (Moore, 1998; Selbmann et al., 2014). Still, these species raise the question of possible *Taphrina* species that are non-pathogenic and specialized for residing in the phylloplane.

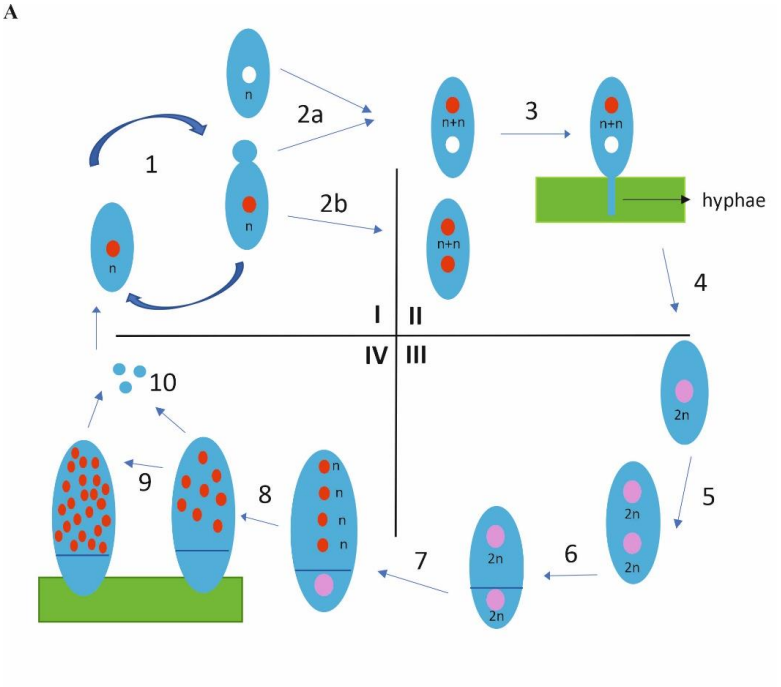
Based on *in vitro* studies, *Taphrina* species can be easily grown on several different artificial media (Fonseca and Rodrigues, 2011; Heneghan et al., 2019). The haploid yeast form is the most common form of cell growth in culture. As for other yeasts, propagation is by budding, but the sexual structures are usually formed only in the biotrophic filamentous state in the host tissue (Kramer, 1960). Yeast budding can be classified as holoblastic or enteroblastic (Sjamsuridzal et al., 1997; von Arx et al., 1982), and all *Taphrina* species engage in enteroblastic budding, which is normally associated with basidiomycetous and not ascomycetous yeasts. On artificial media, *Taphrina* colonies show a cream, pinkish to yellowish colour (Fonseca and Rodrigues, 2011). The existence of *Taphrina* as a yeast-like fungus that interacts with plants has been recorded for more than a century. Before the era of molecular phylogenetics,

Taphrina species were defined phylogenetically based on physiological characteristics and morphological features including colony colour and cell size and morphology, where primarily asci were examined, but yeast cells were also considered. These characters were matched to the key for species identification in the existing genus (Fonseca and Rodrigues, 2011). Moreover, species identification also frequently used the host species and geographical location where *Taphrina* species were isolated. *Taphrina* species can also be identified through their physiological characters, in the form of carbon and nitrogen source utilization. *Taphrina* species have been tested in culture using 32 different carbon compounds including dextrose, sucrose, maltose, melezitose, trehalose, inulin, and pectin (Mix, 1954). Additionally, *Taphrina* species show a characteristic pattern of nitrogen utilization. Most *Taphrina* species use ammonium compounds, nitrates, urea, peptone, asparagine, and specific amino acids (Mix, 1953). Molecular methods are now widely used for identification, also recently including phylogenomics. Several molecular methods used in *Taphrina* phylogenetics include fingerprint PCR with a single primer for microsatellite regions (Rodrigues and Fonseca, 2003) and fluorescent in situ hybridization (FISH) (Tavares et al., 2004). Sequencing of the ITS and the D1/D2 domains of the large subunit rRNA gene are the preferred methods (Komatsu et al., 2010; Rodrigues and Fonseca, 2003).

1.3.2 Characteristics of *Taphrina*

Most members of the subphylum Taphrinomycotina are understudied, with the exception of the genetic model fission yeast (*Schz. pombe*). As members of this subphylum, yeasts of the genus *Taphrina* are of considerable evolutionary interest. The filamentous states of *Taphrina* are parasitic on plants since they can cause malformation in the infected tissue. Typical symptoms caused by *Taphrina* species include leaf blister, leaf curl, leaf spot, and galls or tumours, including witches' broom (Mix, 1949). The plant pathogenic species in the genus *Taphrina* species cause various symptoms in plants many in the Rosaceae, where they cause curly leaves or deformed and aborted fruits, as for example in *Taphrina deformans* on *Prunus persica* or *Prunus dulcis* (Cissé et al., 2013) or *Taphrina tormentillae* on *Potentilla* species (Petrydesova et al., 2016).

A


n
2a
n+n
3
n+n
hyphae
1
2b
n
n
n+n
4
I
II
IV
III
10
2n
9
8
n
n
n
n
5
2n
2n
7
2n
2n
6

B.

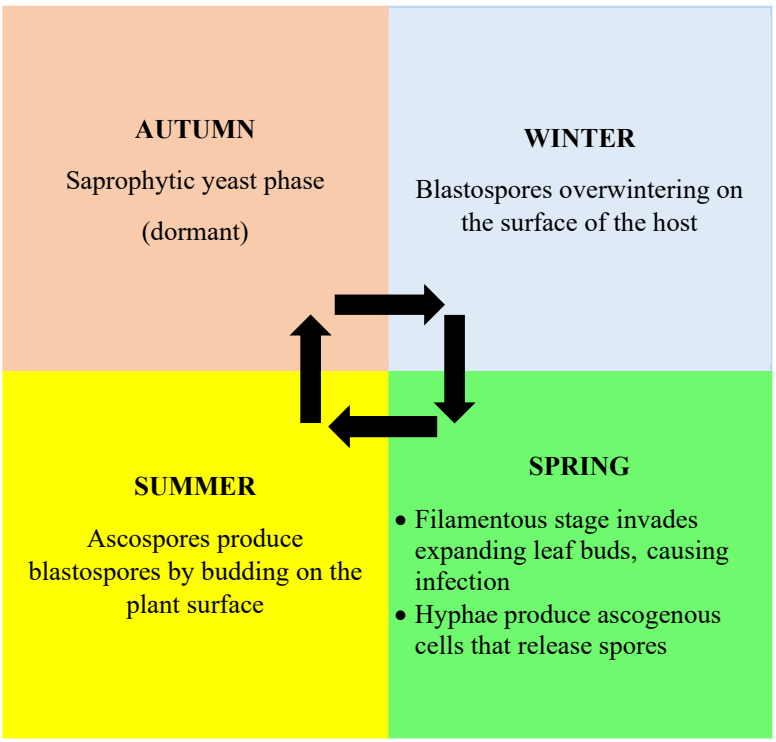

AUTUMN
Saprophytic yeast phase
(dormant)
WINTER
Blastospores overwintering on the surface of the host
SUMMER
Ascospores produce blastospores by budding on the plant surface
SPRING
• Filamentous stage invades expanding leaf buds, causing infection
• Hyphae produce ascogenous cells that release spores

Figure 1. Generalized lifecycle of *Taphrina* species. **A.** Schematic figure of the *Taphrina* lifecycle, based on data summarized by Mix (1949) and Fonseca and Rodrigues (2011). The details of this lifecycle have been observed in some *Taphrina* species and are assumed to be relevant for all *Taphrina* species. However this model is not supported with data for all *Taphrina* species; so the assumption that all *Taphrina* have the same lifecycle must be seen as a working model only. (I) Yeast stage, (II) dikaryon stage, (III) diploid stage, (IV) sporogenesis. (1) Asexual reproduction through blastospore budding, (2a) two different yeast cells conjugate to form the dikaryotic stage, conjugation has only been seen in only few *Taphrina* species, (2b) *Taphrina* species frequently enter the dikaryotic stage by nuclear duplication within a single cell, (3) dikaryotic cells change from yeast to hyphal phase and infectious hyphae enter the host plant extracellular spaces, (4) inside some of the hyphal cells, fusion of two haploid nuclei (karyogamy) occurs to enter the diploid stage; this cell will then form into an ascogenous cell, which will extend out of the surface of the leaf when fully formed, (5) mitosis of the diploid cell into two cells that remain attached, (6) proascus and basal (stalk) cell forming, (7) meiosis produces four haploid nuclei in the ascogenous cell and the stalk cell remains diploid, (8) post-meiotic mitosis, asci produce eight haploid ascospores, sporogenous layer forming on plant cells, (9) in some *Taphrina* species, the eight ascospores begin budding new blastospores inside the ascus, which can become completely filled with spores; (10) spores released from asci. **B.** The development of *Taphrina* life cycle stages by season.

The activity of *Taphrina* species, which are pathogenic to the host, will cause activation of the immune system of the host plant. To invade its host, *Taphrina* will change its growth form from yeast to a filamentous fungus. According to some older studies, penetration of *Taphrina* into the host begins when the bud-conidia produce short hyphae that often directly penetrate the cuticle, but can also enter through stomata, and continue to invade the tissue between the epidermal cells to the parenchyma (Mix, 1936). Thus, the penetration of *Taphrina* in host tissue is not fully understood and can vary between different *Taphrina* species.

T. betulina and other *Taphrina* species are known for the ability to produce the plant hormones auxin and cytokinin. There are little actual data demonstrating the role of these hormones in *Taphrina* pathogenicity and tumour formation. The production of auxin and cytokinin hormones by *T. betulina* was first reported in 1975 (Kern and Naef-Roth,1975), while auxin produced by *T. wiesneri* was first reported in 1961 (Matuyama and Misawa, 1961). IAA biosynthesis in *T. weisneri, T. deformans,* and *T. pruni* is thought to cause hyperplastic disease in plants (Yamada et al., 1990). The production of IAA by pathogenic yeast is a strategy to invade the host. Such species produce IAA from L-tryptophan via indole-3-pyruvic acid (IPyA) and indole-3-acetaldehyde (IAAld) as intermediates. The presence of IAA in these *Taphrina* species is a factor in hyperplastic plant disease (Yamada et al., 1990). However, not all IAA biosynthetic pathways in *Taphrina* species have been studied in depth (Tsai et al., 2014).

1.4 *Betula pendula* and symptoms of witches' broom disease

Betula pendula, or silver birch, is an important timber commodity of Finland, as well as being the national tree. *Betula pendula* grows up to 30 metres high with one main stem and white to silvery bark. Its branches are pendulous, spreading or ascending, but constant throughout the tree. Twigs are glabrous, brown, with pale warts (1 mm in diameter) especially on younger twigs (Atkinson, 1992). Ecologically, birches are typical light-demanding, shade-intolerant pioneer species, with a rapid early growth. In managed birch forests, trees are vigorous until the age of 40-50 years. Birch also have potential as a model woody plant for biotechnology since they have a rapid growth cycle and a small genome (Salojärvi et al., 2017). Birch plantations can also provide protection for seedlings of other species because birch can tolerate a broad range of site conditions.

Witches' broom disease is often found on species of birch, including *B. pendula*, and the name refers to a characteristic symptom of an abnormal broom-like growth, including the rapid development of large numbers of additional shoots of axillary origin (Jump and Woodward, 1994). Additionally, there are tumour-like tissue growths in the shoot. *T. betulina* is known as a pathogen of *B. pendula* causing witches' broom disease (Bacigálová et al., 2005; Dingley, 1970). *T. betulina* invades the woody tissue of birch by hyphae, causing perennial infections (Fonseca and Rodrigues, 2011). However, the woody tissue that is specifically affected and invaded by the pathogen remains unknown. Moreover, the ability of *T. betulina* to produce the plant hormones auxin and cytokinin is suspected to be involved in tumour formation, but this has not been demonstrated.

Unfortunately, many people do not consider witches' broom disease as damaging to birch. To date, the negative impact of witches' broom disease on birch trees has been very poorly studied, although few papers that addressed this have found that it negatively affects birch growth. Infection of *T. betulina* was associated with a significant reduction in wood development, vigour, and quality; height and diameter were reduced by an average of 25% (Spanos and Woodward, 1994). Uncontrolled stem elongation with tumour-like growths on the stem affects the crown architecture of the birch tree (Kostina et al., 2015). In general, most *Taphrina* species infect other plant tissues and only a few, such as *T. betulina*, invade woody tissue and cause witches' broom symptoms, including woody tumour formation.

2. Aims of the study

The main objective of this research was to understand the *Taphrina*-plant interaction. This research observed *Taphrina* interactions with both a natural host plant, in the *T. betulina* - B. *pendula* system, and interactions with a genetic model plant, in the *Taphrina* strain M11 - *Arabidopsis* system.

The specific aims of the study were:

- To study the interaction of the *T. betulina* yeast-phase on the phylloplane of birch.
- To investigate the growth and characteristics of witches' broom in *B. pendula*.
- To explore the possibility of *T. betulina* resistance in *B. pendula* populations.
- To develop *Taphrina* as model plant pathogenic yeasts infecting model plant species.

3. Material and methods

The methods used in the study were described in the indicated manuscripts in Table 1, and a list of species and *Arabidopsis* mutants used is given in Tables 2 and 3.

Table 1. Material and methods used in this study

Material and methods	**Publication or manuscript**
Auxin and cytokinin response activation assay	III
Biofilm formation assay	III
DNA extraction	II, III
Field sampling	I, II, III
Genetic segregation analysis	I
Growth assay	III
Hypersensitive assay	III
Indolic compound assay	II, III
Leaf deformation symptom assay (leaf curling and leaf bending)	III
Leaf press culture	II
Tissue histological staining	I
Reverse genetics: mutant infection assay	III
ROS assay	III
Statistical analysis	I, II, III
Taphrina infection on *Arabidopsis*	III
Witches' broom sampling from birch	I,
Wood sectioning	I
Yeast culturing	I, II, III
Yeast identification	II, III
Yeast re-isolation from birch leaf	II

Table 2. Species used in this study

Species name	**Publication**
Arabidopsis thaliana	III
Taphrina betulina	II, III
Taphrina strain M11	III
T. tormentillae	III
T. carnea (PYCC 5890, a strain of *T. betulina*)	II
T. carnea (PYCC 5705, a strain of *T. tormentillae*)	III
Pseudomonas syringae pv. *tomato*	III
Betula pendula	I, II

Table 3. Mutant *Arabidopsis* used in this study.

Abbreviation	**Mutant name**	**Lost function in**	**AGI code**
coi1-16	*Coronotine insensitive 1-16*	JA perception	AT2G39940
cyp79 b2/b3	*Cytochrome P450, Family 79, subfamily b polypeptide 2 and 3 double mutant*	Camalexin biosynthesis	AT4G39950
ein2	*Ethylene insensitive*	ET signal transduction	AT5G03280
jar1	*Jasmonate resistant*	JA perception	AT2G46370
npr1	*Nonexpressor of PR genes*	SA perception	AT1G64280
pad4	*Phytoalexin deficient*	Camalexin synthesis	AT3G52430
sid2	*Salicylic acid induction deficient 2*	SA biosynthesis	AT1G74710

4. Results and discussion

4.1 Taphrina betulina causing witches' broom on *Betula pendula*

4.1.1 Genetic segregation in birch population

The presence of birches that have witches' broom as a symptom of *T. betulina* infection coexisting with healthy trees in a population raises questions about the potential for genetic segregation of resistance in that population. To investigate this question, individuals of mature birch with a height of over 10 metres at 180 sites in the Helsinki area, including the islands of Isosaari and Suomenlinna, were scored as either showing symptoms or asymptomatic.

The results of tree data collection in the field were mapped and a tree distribution plot was made based on symptoms (Publication I, Figure 2). Only three sites featured only symptomatic trees, while the remaining 156 sites were a mix of susceptible and resistant populations.

Based on these data, two competing models can be used as predictions. The first model is that if susceptibility is determined by the genotype of the pathogen, inoculum load, and environmental conditions, infected individuals will concentrate in places where conditions are favourable to infections. The second model suggests that susceptibility is determined by the genotype of the host, resulting in a mixture of symptomatic and asymptomatic individuals at most sites. These distribution data were used to test the null hypothesis that exposure to pathogens and environmental conditions determines the distribution of susceptibility. Analysis using Pearson's chi-square fit test with Yates' continuity correction rejected the null hypothesis. Thus, mixed populations with resistant and susceptible trees were found at 159 sites in the region around Helsinki, supporting the model that host genotype determines susceptibility to *T. betulina*. This result suggests that there is genetic resistance in birch plants against witches' broom disease. This research does not include a genetic experiment to confirm segregation of genes that confer resistance to *T. betulina*, which will require crossing and analysis of birch trees. Due to time considerations, this will be left for future studies.

Taphrina is a genus of yeast-like fungi that cause disease in many plants other than birch. Several other *Taphrina* species are well known as significant pathogens of economically beneficial plants, as for example *T. deformans* on peach and *T. maculans* on turmeric. *Taphrina* infection has caused economic losses to several crops, particularly peach and turmeric. It has been recorded that peach leaf curl seriously damaged peach trees in Greece, causing an estimated loss of around 15–20% of the possible total production (Thomidis et al., 2010) and 90% yield loss in Russia (Mikhailova and Karpun, 2021). *Taphrina* attacks were also experienced by the

turmeric agricultural sector in India, with losses in leaf area (26%) and in fresh rhizome weight (25%) (Kumar et al., 2020).

Although several studies have shown that witches' broom is an important disease in birch, on the contrary, *Taphrina betulina* is considered more of a curiosity than a serious pathogen of birch (Price and Macdonald, 2012). However, considering that birch is among the most important hardwood trees in both northern Europe (Hynynen et al., 2009; Zohren et al., 2016), and North America (Quigley, 1969; Brisbin and Sonderman 1973), a better understanding of resistance against *Taphrina*, and the role *T. betulina* plays in the ecology of birch in natural forests, is very important.

For commercial purposes, resistance to *T. deformans* has been developed in peach mainly by breeding, but not yet through genetic engineering. For more targeted breeding or genetic engineering, knowledge and understanding of the genes responsible for *Taphrina* resistance are needed. In plant defence systems, PRRs on plant cell surfaces can detect microbes via their PAMPs, and the mostly cytoplasmic plant *R* genes have been adapted to detect effectors (Jones and Dangl, 2006). Resistance to certain pathogens can be achieved by modifying PRRs or *R* genes. Additionally, the genes can potentially be transferred between species (Lacombe et al., 2010), although the plant PRR and *R*-gene systems for *Taphrina* and other yeasts remain unknown (Wang et al., 2016).

It remains unclear if it would be desirable or practical to modify birch for *T. betulina* resistance. However, the birch-*T. betulina* system has advantages as a research model for understanding general mechanisms of plant resistance against *Taphrina*. Resistance to *Taphrina* has been developed mainly in peach. The development of new cultivars that are resistant to peach leaf curl disease caused by *T. deformans* began in the late 1980s in France (Pascal and Monteux-Caillet, 1997) and continues to be developed in several countries, including Bulgaria (Zhivondov et al., 2016). However, in several commercial crops, using genetics to obtain plant resistance to *Taphrina* is difficult because of the different characteristics of plant species. Genetics in turmeric is complicated because turmeric is a triploid organism (Basak et al., 2017; Islam et al., 2007). Genetics in peach has several advantages due to the fact that peach is a diploid organism and has a small genome that has been sequenced. However, peach takes time to flower (about 2-4 years), making genetic studies impractical. For comparison, *B. pendula* is a diploid organism, has a sequenced genome, and can be induced to flower in less than 1 year, making it a great model tree for doing forward genetics, which is the process of using plants with known phenotypes identified in a mutant screens and then mapping the causative genes.

4.1.2 Tumour morphology

Studies on the histology of witches' broom symptoms have been available for more than two decades, however, Jump and Woodward, (1994) only investigated the species *Betula pubescens*. The anatomy and histology of witches' brooms formed in other birch species such as *B. pendula*, had never been investigated. Therefore, this research investigated the morphology of tumour formation that occurred in *B. pendula* caused by *T. betulina* infection. Sectioning and staining samples are very important in this investigation. Sample sectioning at an ultra-thin size and double staining with alcian and safranin was used in this experiment (Publication I). The experimental design used samples of three important parts of the stem; the normal healthy stem, the stem close to a tumour where the tumour is beginning to spread and will show early stages of tumour formation, and the tumour section. This experimental design was used to compare the full infection and tumour formation at multiple stages, all from one branch. The results show that, in general, there is a massive increase in secondary xylem growth in the section near the tumour compared to the normal section. In samples with tumours, the xylem was increasingly wrinkled and tangled (Publication I, Figures 4c, f, i). In tissue with tumours, there was increased growth of the secondary phloem, periderm, with deformation and thickening of the cork cambium (Publication I, Figure 4b, e,). Xylem swelling can be associated with increased vascular connections in the tumour that feeds many new branches and shoots because samples near the tumour did not show xylem thickening and new shoot formation. In addition, the swollen cork tissues also explain that tumour growth starts from the outer branch tissue. However, this study was unable to detect the presence of *T. betulina* hyphae in infected tissues. Different staining methods should be used in future studies to search for infectious hyphae in infected birch. Jump and Woodward, (1994) stained sections of downy birch woody tissues infected with *T. betulina* using the fluorescein-labelled wheat germ agglutinin, which binds chitin. This method did not stain hyphae, although hyphal-like structures were observed (Jump and Woodward, 1994). This is consistent with the suggestion that *Taphrina* species may have less chitin in their hyphae or their chitin is masked by other structures such as beta-glucan or mannoprotein complexes (Publication III), as is further discussed below. Histochemical staining showed that the chemical changes of wood in the tissue were suspected to be an increase in lignin. An increase in lignin accumulation in the tumour tissue of downy birch that was infected with witches' broom disease has been previously reported (Jump and Woodward, 1994). However, further investigation is needed using specific staining for lignification in order to better understand the nature of altered wood chemistry in broom tumours.

Finding the plant tissues where *T. betulina* invades and grows in birch branches is an open question. Suspected localization of *T. betulina* in woody tissues can be predicted by the presence of tumour development and tissue swelling (Publication II). To show the localization of *Taphrina* in birch stem, observation can be done by cutting the tissue and performing specific histology staining, this method was also used to study the histology of witches' broom caused by *T. betulina* infection in *B. pubescens* (Jump and Woodward, 1994).

T. betulina is also known to cause leaf disease. According to Koike and Tanaka (1986), *T. betulina* infects the leaves of *B. maximowicziana* and causes early senescence. Photosynthetic and dark respiration rates go transiently up then decrease sharply. Although it has been shown that *T. betulina* infects *Betula* spp., both on woody tissue and on leaves, there is still little information on non-woody tissues being infected. However, visual symptoms in infected leaves may appear chlorotic or with brown spots. For other *Taphrina* species, symptoms in non-woody tissue can be the presence of tissue hyperplasia, such as leaf curl in peach caused by *T. deformans* (Matuyama and Misawa, 1961), and *T. flavorubra,* which causes deformed fruits in plum (Tsai et al., 2014).

The presence of tumours as a symptom of witches' broom disease is believed to be caused by the ability of fungal pathogens to produce plant hormones as part of their invasion strategy. Auxin and cytokinin are plant hormones that have been widely studied in relation to disease development. Although the involvement of plant hormones from *T. betulina* as a cause of tumour development in woody tissues of birch is still a matter of speculation, several studies in other fungal species have shown that phytohormone production to high concentrations in tumorigenic fungi plays a very important role in plant hyperplasia (Dodueva et al., 2020). However, as is discussed elsewhere in this thesis, plant hormone production is multifunctional and a common feature of many microbes. Hyperplasia is an abnormal tissue growth caused by uncontrolled cell proliferation, usually due to infection with pathogens. Hyperplasia may include crown gall, knots in olive, witches' broom, and leaf curl (Yamada et al., 1990). Auxins and cytokinins are the main phytohormones that control cell proliferation and the development of meristems (Schaller, et al., 2015). This includes the most common and biologically active auxin species in plants, IAA (Schaller, et al., 2015). The production of IAA by pathogenic microbes can take place via several known biosynthesis pathways. In bacteria, IAA is produced from L-tryptophan with indoleacetamide (IAM) as an intermediate, whereas, in some species of *Taphrina,* the IAA biosynthetic pathway is from tryptophan via IPyA and IAAld as intermediates (Yamada et al., 1990).

Witches' broom and tumour formation are symptoms caused by fungal pathogen infection in plants. Several species of *Taphrina* other than *T. betulina* also cause witches' broom disease and tumour formation. *Taphrina wiesneri* is known to cause witches' broom on cherry (Fujii et al., 1968; Seo et al., 2009; Shoji and Sato, 1974), *Taphrina pruni-subcordatae* (Zeller) Mix causes witches' broom on Italian and French prunes (Heinis, 1960), and *Taphrina epiphylla* cause the formation of witches' broom in *Alnus hirsuta*. Moreover, witches' broom and tumour formation can also be caused by several other types of fungi, including *Aciculosporium take*, the causal agent of witches' broom in bamboo (Tanaka et al., 2003), *Moniliophthora perniciosa*, the causal agent of witches' broom disease in cacao (Evans, 2016; Meinhardt et al., 2008),

and *Ustilago maydis* the causal agent smut in maize (Brefort et al., 2009; Skibbe et al., 2010).

Perhaps the most widely studied model of fungal induced tumour formation on plants is corn smut disease caused by *U. maydis*. A pathosystem on the interaction between *U. maydis* and maize is a model of biotrophic interaction (Brefort et al., 2009; Matei and Doehlemann, 2016). The life cycle of *U. maydis* is similar to that of *Taphrina* species, in spite of the fact that they are only distantly related, belonging to different phyla, Basidiomycota and Ascomycota, respectively. Both are dimorphic yeast-like fungi, *U. maydis* has a haploid yeast-like form that can be propagated on artificial media, similar to *Taphrina*. In the yeast phase, both *T. betulina* and *U. maydis* cannot cause disease and infection of the host occurs in the filamentous phase of these fungi. Moreover, *U. maydis* is known to produce auxin, and it has been suggested that auxin biosynthesis stimulates tumour formation in maize (Brefort et al., 2009). Importantly, the reduction of IAA biosynthesis in *Ustilago* did not change the ability to form tumours (Reineke et al., 2008). However, a knockout of two genes in *Ustilago*, an effector and a membrane protein, resulted in a loss of tumour formation during maize infection (Doehlemann et al., 2011). Therefore, assumptions about the function of plant hormones in tumour formation caused by *Taphrina* infection are still questionable.

4.2. Taphrina interaction in *B. pendula* phylloplane

*4.2.1.*Yeast collection from *B. pendula* phylloplane

Taphrina species are among yeasts that can be found in phylloplane (Inacio et al., 2005; Kemler et al., 2017), however, *Taphrina* was most often found from infected plants (Mix, 1949) and was very rarely isolated from asymptomatic plants (Fonseca and Inácio, 2006). In this study, we collected and identified yeasts in three types of samples based on the symptoms found in the leaves and trees from which the leaves were sampled. This research is limited to the interaction of microbes on the plant surface, specifically the interaction of yeasts in the phyllosphere. The types of samples taken were planned in order to compare the distribution of yeast-like fungi, especially *T. betulina* and its interaction with *B. pendula*. Type I samples were symptomatic leaves from symptomatic trees, type II was asymptomatic leaves from symptomatic trees, and type III was asymptomatic leaves from asymptomatic trees. There were a total of nine samples collected (A to I) (Publication II, Table 1).

The presence of yeasts in the birch phylloplane was first visually tested using a leaf press culture and the result showed varied yeast-like colonies with colours of white, beige, orange to pink (Publication II, Figure 1 D-E). Field observations indicate that tumour structure varies in witches' broom disease (Figure 2). There were several characteristics of brooms: for example, some have multiple elongated shoots that have grown from many ectopic axillary buds, which form around the primary infected bud,

to form a central tumour (Publication II, Figure 1B). Some of these brooms differ in the length of the ectopic branches and were called short and long elongated brooms. Others lacked ectopic buds elongating into shoots and only had buds surrounding a central woody tumour and were called tumour-like. Thus, the phenotype of collected type I samples was classified as elongated brooms (EB), short elongated brooms (SE), or tumour-like (TL), while samples from host types II and III which lacked broom symptoms are classified as no broom (NO) (Publication II, Tables S1 and S2). No trees were found that had more than one type of broom. This classification was done to investigate the possibility that different yeasts may be associated with these various structures. However, there were no specific strains associated with different types of brooms. This suggests that host genotype or another factor may determine broom morphology. Additionally, a striking characteristic of the tumour-like broom is that it does not have leaves, so this study did not include leaf samples from tumour-like brooms. The only way to address the issue is to collect and isolate *Taphrina* from the buds of a tumour-like broom, which was not done in this study.

A total of 224 yeasts were isolated. A total of 167 yeasts were identified in 11 species, namely *Taphrina betulina,* a novel species in order Myriangales, *Rhodotorula phylloplana, Rhodotorula bacarum, Rhodotorula laryngis, Cryptococcus wieringae, Cryptococcus tephrensis*, *Kuraishia* sp., *Nakazawaea hosltii, Udinomyces* sp., *Tremellomycetes* sp, and the remaining 51 were unidentified. *B. pendula* is the host for *T. betulina* and thus, as expected, the presence of *T. betulina* yeast dominates as many as 57 strains (26% of all isolates).

The isolation of *T. betulina* from hosts both with and without symptoms suggests that, apart from being a major pathogen for birch trees, some *T. betulina* strains may potentially live as a resident yeasts, without causing disease. This is possible because *Taphrina* has a dimorphic lifestyle and will only switch to the hyphal phase when the environment is favourable. However, in general, *T. betulina* is the main yeast causing witches' broom disease in birch. This is supported by the result that the more severe the symptoms of witches' broom, the more *Taphrina* were isolated. Thus, it is in accordance with the identification of yeast in diseased hosts where the number of *T. betulina* strains was dominant. Other yeast strains that were also commonly found were a novel species belonging to order Myriangiales*, R. phylloplana* and *R. bacarum*. Myriangales (class Dothideomycetes) is an order in subphylum Pezizomycotina, containing filamentous Ascomycetes. During isolation only yeast-like colonies were selected; however, further study is needed to examine the morphology of the novel Myriangiales isolate, in order to determine if it is yeast-like. *Rhodotorula* is a genus of phylloplane inhabiting yeast species (Slavikova et al., 2009; Srisuk et al., 2019; Thapa and Prasanna, 2018) and was shown to act as a biocontrol for *Botrytis cinerea* (Buck, 2002; Buck and Jeffers, 2004).

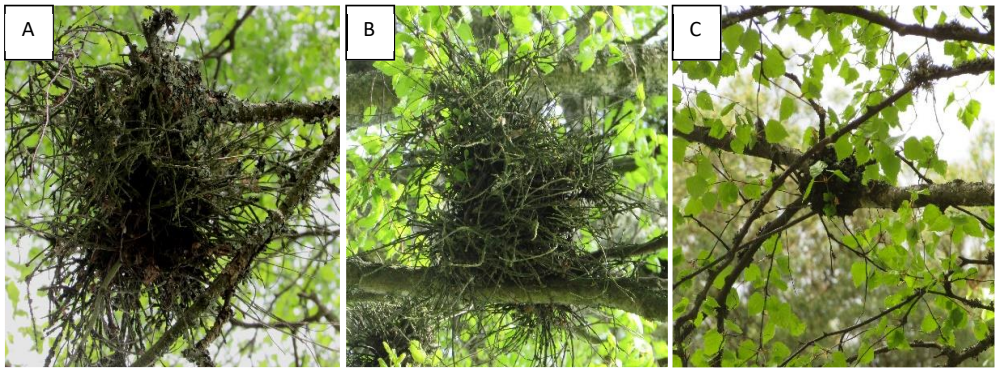


Figure 2. Samples classified as A. elongated brooms (EB), B. short elongated brooms (SE), and C. tumour-like (TL).

4.2.2 *Taphrina betulina* identification and characterization

The yeast strains isolated from the *B. pendula* phylloplane were identified based on the on sequences of the internal transcribed spacer (ITS) region of the nuclear rRNA locus. ITS PCR products were amplified from yeast genomic DNA with the ITS3 and ITS4 primers. This PCR product was digested with TaqI and used as a cleaved amplified polymorphic sequence (CAPS) marker, called as ITC (for ITS Taq1 CAPS marker), which was able to distinguish *T. betulina* with several related *Taphrina* species.

Additionally, the assembly of the *T. deformans* genome (Cissé et al., 2013) was used to identify two pairs of adjacent conserved housekeeping genes (*Rco1-Gyp7* and *Sad1-Rax1*) that are conserved in *T. betulina* and *T. deformans*. Sequences between the gene pair *Rco1-Gyp7* amplified well in several *Taphrina* species and was used for PCR and the product digested with Rsa1 for use as another CAPS marker. For the characterization of *T. betulina* isolates, this new marker, named RGR1 (*Rco1 Gyp7* RsaI), was used. There were two variants identified with the ITC marker, ITC-C and ITC-D, and using the ITC and RGR1 markers together, seven genotypes were identified within the isolated *T. betulina* strains (Publication II).

The genotyping of 57 *T. betulina* strains revealed that strains with the genotype ITS-D RGR-2 were dominant, making up as many as 29 of 57 strains. In addition, *T. betulina* ITS-D RGR-2 strains were found in all host types, with 23 of them isolated from the infected broom samples. This suggests that the presence of this strain may be related to the formation of brooms. However, the ITS-D-RGR-2 strain was also found in asymptomatic hosts, in which both trees and leaves were asymptomatic, suggesting that the yeast can also be a resident on the leaf surface without causing disease. For

further characterization, 22 out of 57 have been selected, which are considered representative of the diversity of sample origin and genotypes.

Morphological characters obtained from observations of colonies grown on 0.2 x PDA media showed pale pink to peach colours (Publication II, Supplementary Figure 4). Additionally, the cell sizes of these newly isolated strains are in accordance with the data presented for *T. betulina* in previous publications and the cell shape is relatively similar between strains. More careful identification of the *Taphrina* strains isolated from birch in this work is needed considering that there are several *Taphrina* species associated with birch, for example *T. betulina, T. nana* (Beck et al., 2016), *T. carnea*, and *T. americana* in North America (Rodrigues and Fonseca, 2003). Strains of these were used for comparison to the 22 selected *T. betulina* isolates. In this study, the most frequently isolated strain of *Taphrina* found from birch in Helsinki was most similar to the strain *T. carnea* (PYCC 5890 =NRRL T-705, this was originally isolated and named *T. nana*, but later shown to be a strain of *T. betulina*), based on the two markers used. However, to more specifically identify the strain that is suspected to be the main cause of witches' broom in *B. pendula*, it is necessary to use additional markers. For this identification, new markers for birch-associated *Taphrina* strains should be developed, since there are genes not covered in the existing markers that would be useful in identifying strains more specifically.

Regarding the former genus *Lalaria*, the *Taphrina* species that previously belonged to the genus *Lalaria* have characteristics that make them different from other *Taphrina* species. *Lalaria* species were represented by strains originating from sources other than infected plant material of a known *Taphrina* host and isolated in the yeast phase (Fonseca and Rodrigues, 2011). There is a possibility that some of the *T. betulina* strains isolated from the birch phylloplane may meet the definition of "*Lalaria*-type" *Taphrina* because some strains were found in asymptomatic trees. However, there are several other characteristics of the "*Lalaria*-type" *Taphrina*. Those isolated were non-pathogenic and seem to be specialized as phylloplane residents only. For example, they could utilize more carbon sources than most *Taphrina* species. Future testing of the carbon-use ability of *T. betulina* strains from birch could help determine whether some strains are specialized in the phylloplane lifestyle and perhaps some of these strains are non-pathogenic (Inacio et al., 2003).

4.2.3 Indolic compounds and IAA signal activation

IAA (indole 3-acetic acid) is a compound produced by plants and which stimulates growth (Sun et al., 2014). In bacteria, IAA is produced through several pathways, with tryptophan as the main precursor in IAA production (Patten and Glick, 1996; Spaepen et al., 2007). In fungi, IAA is known to be produced via tryptophan with the precursor indole-3-pyruvic acid, for example in *U. maydis* (Reineke et al., 2008), while in *Colletotrichum gloeosporioides*, a fungal pathogen causing disease on fruit, produces IAA through the intermediate indole-3-acetamide (IAM) pathway (Maor et al., 2004).

The production of IAA by phytopathogenic yeasts and bacteria is related to their pathogenicity (Cerboneschi et al., 2016; Patten and Glick, 1996; Spaepen et al., 2007). Bacteria use IAA as a colonization strategy including the development of galls (Reineke et al., 2008). *Taphrina* species are known to produce indolic compounds and this study shows the ability of *Taphrina* strain M11 to activate IAA signalling during its interaction with *Arabidopsis* (Perley and Stowe, 1966; Yamada et al., 1990; Publication III).

Since the *Taphrina* infections in *B. pendula* were identified by the presence of gall formation, the production of IAA by *Taphrina* needs to be further documented. A higher production of IAA by *Taphrina* is widely expected to further accelerate the formation of galls, but this has not been well tested and remains a hypothesis for future confirmation.

In plant growth-promoting bacteria, IAA production is a factor that support the growth of host plant (Nassar et al., 2005). Some examples of IAA that have been shown to play a role in host plant growth are the yeast *Williopsis saturnus*, endophytic in maize (*Zea mays* L.) roots (Nassar et al., 2005), and endotypic *Rhodotorula* spp., isolated from the roots of Poplar tree (Xin et al., 2009).

To investigate indolic compound production by yeasts and the activation of a model plant IAA response, 22 strains were selected that were considered representative of the 57 *T. betulina* strains obtained from phylloplane isolation. This study shows that the 22 strains of *T. betulina* tested were all capable of producing indolic compounds with auxin activity. This study also compared the production of indolic compounds between *T. betulina* grown on YPD + 0.1% tryptophan medium and *T. betulina* grown on YPD medium alone. Tryptophan was added to the medium because it is a precursor to IAA in many microbes (Carreno-Lopez et al., 2000; Normanly et al., 1993; Prasanna et al., 2010). Indolic compound production was strongly detected in culture filtrates from strains grown in YPD + 0.1% tryptophan. Further results discussed below suggest that this indolic compound production by yeast strains can trigger activation of IAA signalling. The ability to produce indolic compounds was uncorrelated with the genotype or sample origin of a strain.

To further support the possible auxin production by the 22 *T. betulina* strains, the auxin responsive DR5::GUS reporter system and GUS histochemical staining were used. A strain of *T. betulina* with genotype ITC-D RGR-2 may produce more auxin, as shown by higher auxin response activity in both media (Publication II, Supplemental Figure 5). Considering the result that *T. betulina* genotype ITC-D RGR-2 is suspected to be the main genotype causing infection in *B. pendula*, it is assumed that more pathogenic yeast strains would produce auxin at higher concentrations. In this study, auxin produced by *Taphrina* is suspected to be involved in the pathogenicity toward the host. However, the production of IAA in plant-associated microbes can also be part of a survival strategy in the phylloplane, as well as various other related functions, so

further investigation is needed. This also raises the possibility that there are virulence genes associated with the ITC-D RGR-2 genotype. Further investigation will be needed to identify these gene and their virulence mechanisms.

4.3 *Taphrina* M11 infecting *Arabidopsis*

4.3.1 M11 strain description

The M11 strain of *Taphrina* used here was isolated from the leaf surface of wild *Arabidopsis thaliana* in Helsinki (Wang et al., 2016). The molecular identification results showed that M11 belonged to operational taxonomic unit (OTU) 3. Wang et al (2016) temporarily assigned M11 to OTU 3, based on colony colour and ITS PCR product size. Members of OTU3 were then further identified based on BLAST searches with ITS sequences, which showed that M11 was 99.31% similar to several strains of *T. tormentillae,* including the CBS 332.55 strain of *T. carnea*, which has been shown to also be a strain of *T. tormentillae* (Rodriguez and Fonseca, 2003). This result is already strong evidence that M11 is a strain of *T. tormentillae*. The species delimitation border for yeasts is 98.4%; i.e., any pair of strains with ITS similarity greater than or equal to 98.4% are likely to belong to the same species (Vu et al., 2016).

The *Taphrina* M11 sequencing yielded a high-quality draft genome assembly of 13.6 Mbp in 234 contigs. A total of 5808 proteins were annotated and showed that *Taphrina* M11 shared a larger portion of orthologous proteins with *Taphrina* species pathogenic on *Prunus*, while *Taphrina* M11 itself was found in a wild *Arabidopsis*, which has not previously been known as a host for *Taphrina*.

An investigation of the candidate effector-like genes in the M11 genome identified 18660 short ORFs (open reading frames) (Publication III), of which 767 were SSPs (small secreted peptides), which are candidates for effector proteins-short peptides that have the role to suppress plant immunity, promote susceptibility, and support the pathogen in gaining nutrition and reproducing (Rodriguez-Moreno, Ebert, Bolton, and Thomma, 2018). In addition, they serve as messengers and regulate a variety of processes in plants (Hellmann, 2020). LysM domain is a conserved protein domain known to function in binding the fungal cell wall molecule chitin. LysM domains are found in a wide variety of fungal extracellular effector proteins that bind chitin and block its detection by plants. The LysM domain is also found in the plant PRRs that detect chitin. No LysM domain containing SSPs and CSSPs (cysteine-rich SSPs) were found in the M11 genome.

Fungal cell wall chitin is a target of plant chitinases in fungal immunity. In this process, exochitinases release chitin oligosaccharide PAMPs from fungal cell walls to induce host immune responses. The immune response activation, which includes the secretion of endochitinases, results in hyphal lysis. Thus, related to this process, LysM

effectors protect the fungus against chitinase and other hydrolytic enzymes (Kombrink and Thomma, 2013). The absence of LysM effectors in the M11 genome could cause the presence of M11 in *Arabidopsis* tissue to be easily detected and not protected from the hydrolytic activity of host chitinases. In contrast, this study produced results that suggest *Taphrina* M11 may have only a small amount of chitin in one specific tissue, namely the ascospores, similar to the small amount chitin found in *S. pombe*. This result is also confirmed by the previous sugar composition determined in a biochemical analysis of the cell wall of *Taphrina* (Petit and Schneider, 1983). Therefore, *Taphrina* may not need the LysM effectors as it is not necessary for the *Taphrina*-host interaction. It is suspected that there are other molecules on the surface of the *Taphrina* hyphae that play important roles in the *Taphrina*-host interaction.

Investigation of M11 genome shows that it is enriched with several conserved protein domains previously shown to be characteristic of plant-associated microbes (Publication III, Supplemental Table 5,). Genes encoding proteins containing the protein tyrosine kinase domain were the most common. The protein RNA recognition domain was also frequent; this domain is a common feature in biotrophic pathogens (Pandaranayaka et al., 2019). This result is consistent with the fact that *Taphrina* species are known as biotrophic pathogens (Mix, 1949; Tsai et al., 2014).
To detect the biosynthesis of potential immunoactive fungal cell wall polysaccharides, known fungal and yeast PAMPs from other systems were targeted. Putative cell wall biosynthesis genes in *Taphrina* M11 identified two conserved chitin synthases (Publication III, Figures S3 and S5). In addition, there are two glucans present in the M11 outer cell wall, namely β-glucans (β-1,3-linkages and β-1,6-linkages) and α-glucan (α-1,3-glucan / α-1,4-glucan).

The production of IAA and cytokinins by fungal pathogens is suggested to be related to suppression of plant defence and to the development of gall or tumour symptoms in hosts (Bruce et al., 2010; Gravel et al., 2007; Kazan and Manners, 2009; Reineke et al., 2008). The genes involved in the production of IAA and cytokinins in M11 were investigated. The results show the M11 genome codes for three different enzymatic pathways to produce IAA, they are the indole-3-acetamide (IAM), indole-3-pyruvate (IPyA), and tryptamine (TAM) pathways. This result is unique as, in previous studies, a single pathway was characteristic of *Taphrina*. Based on a literature search, this study is the first to find tryptophan monooxygenase and indole acetamide hydrolase (IAM pathway) in a *Taphrina* genome. Previous studies on *T. deformans* only found TAM and LAD genes of the IPyA pathway (Cissé et al., 2013). It remains unknown why a *Taphrina* strain has several IAA biosynthetic pathways. It is possible that each pathway may have a separate function related to a different specific aspect of the fungal biology or the interaction with the host plant; for example in yeast-to-hyphal transition, defence suppression, tumour formation, or phylloplane survival. To determine the characteristics of *Taphrina* in the IAA biosynthetic pathway, further studies are needed involving more species of *Taphrina*.

4.3.2 M11 growth and biofilm formation

Cell growth and biofilm formation are indicators of a microorganism surviving in host tissues. To determine the capability of growth on *Arabidopsis* and the ability to form biofilms, growth assays and biofilm investigations were done *in vitro*. In this research, growth assays in the *Arabidopsis* phyllosphere were done with four strains of *Taphrina*, namely *Taphrina* M11, *T. betulina* strain PYCC 5889/CBS 119536, *T. carnea* strain PYCC 5705/CBS 332.55 (which is a strain of *T. tormentillae*), and *T. tormentillae* PYCC 5727/ CBS 332.55. *Taphrina* M11 showed the highest growth compared to other *Taphrina* strains. Strain M11 is thought to have adapted well to *Arabidopsis* as a host, possibly because M11 is a *Taphrina* yeast found naturally in wild *Arabidopsis,* in Finland (Wang et al., 2016) and in Germany (Overmyer, Kemen, et al., unpublished results). Similarly, *T. carnea* strain CBS 332.55 and *T. tormentillae* also showed growth on *Arabidopsis*, while in contrast, *T. betulina* did not (Publication III).

Microbes develop biofilms to provide a structure of resistance for cells to unfavourable environmental conditions. Moreover, the biofilm can promote microbial pathogenicity against the host (Padmavathi, Bakkiyaraj, and Pandian, 2017). Further investigation on the formation of biofilms on polystyrene surfaces with diluted PDB medium showed that *T. carnea* strain CBS 332.55 formed biofilms better than other species. *T. tormentillae* showed moderate biofilm formation and M11 formed a low level of biofilm, while *T betulina* showed no biofilm formation after 16 days of growth. The ability of microbes to develop biofilms is influenced by several factors, including leaf surface characteristics. The cuticle is the initial physical barrier for plants, composed of polyester scaffold cutin embedded and sealed with solvent-extractable cuticular waxes (Lewandowska et al., 2020).

Leaf surfaces are coated with cuticular waxes that prevent transpiration water loss and gas exchange as well protect plants from stress due to ultraviolet irradiation and pathogen invasion (Lee and Suh, 2013). As a model plant, *Arabidopsis* has a characteristic cuticle that forms a continuous lipid membrane over the apical epidermal cell walls, with alkanes as the dominant waxes. Additionally, primary alcohols constitute a significant wax fraction on these surfaces (Jenks et al., 2002). Related to the presence of pathogens on the leaf surface, several fungal pathogens release cutinase, which generates PAMPs that lead to pathogen recognition, ROS formation, and defence activation, as in *Arabidopsis* infection caused by *Botrytis* (Serrano et al., 2014). Additionally, other related mechanisms are that cuticle-degrading pathogens can recognize plant surfaces by the presence of cutin monomers that activate fungal cutinolytic activity (Kolattukudy, 1985; Lewandowska et al., 2020). However, the pathogenesis process in a plant occurs specifically and is influenced by very complex factors and mechanisms, but there are no studies that

specifically discuss the relationship between the cuticle wax character of the birch leaf and fungal pathogen invasion. Also, it is not known how well the chemical properties of the polystyrene plates used mimic the conditions on *Arabidopsis* or other plant leaves. Thus, the inability of *T. betulina* to form biofilms under the conditions used in this *in vitro* assay does not mean that *T. betulina* is not able not form biofilms on birch or under other assay conditions.

4.3.3 Plant leaf phenotypic changes as response of infection

Several experimental methods were used in this study to measure both quantity and quality of the response to M11 infection. Experiments conducted by M11 infiltration into *Arabidopsis* leaf tissue showed an infection response including cell death response, leaf curling, and leaf bending. For infiltration infections, cells were suspended in 10mM $MgCl_2$, which acts to osmotically protect the cells and does not induce a significant response in the host plant. As controls for comparison, *T. betulina* was infiltrated into *Arabidopsis* leaves, which is a non-host for this yeast, and $MgCl_2$ infiltration was conducted as a mock experiment or negative control. In these experiments, *Arabidopsis* responded with cell death to both M11 and *T. betulina*. *Arabidopsis* showed a hypersensitive response at early stages of infection to *T. betulina* infiltration, which was clearly visualized with strong trypan blue staining. In contrast, *A. thaliana* showed a weak early cell death response and a longer lasting response to infection with M11, with late symptoms appearing. This infection response resulted in significant development of disease symptoms when compared to *T. betulina* and $MgCl_2$ infiltration as a mock treatment. According to Booker et al. (2004), the smaller the curling index, the higher the curling formation on the leaves. Infiltration of M11 in *Arabidopsis* caused leaf curling with an index of 50% at 14 days post inoculation (dpi), while *T. betulina* and mock treatments show the same curling index of 75% at the same time of measurement (Publication III). This response indicated that M11 caused more curling in the leaves. In addition to leaf curling, leaf bending was also found in this study. The infiltration of M11 to Col-0 reached an average of 40.58^{o} leaf bending, while in *T. betulina* inoculation response, 12.08^{o}, and in mock treatment, 9.80^{o}.

Leaf deformations, including leaf curling and bending, are commonly found in plant-pathogen interaction studies. The presence of leaf deformations is a common result of infections with *Taphrina* species and is the main symptom characteristic of infections with *T. deformans,* the causative agent of leaf curl on peach (Cissé et al., 2013; Giosuè et al., 2000; Matuyama and Misawa, 1961), and on almonds (Syrop, 1975). Although *T. betulina* is a pathogen that causes infection in woody plants, studying leaf deformation as a symptom using *Arabidopsis* as a model plant is relevant because it can be observed in detail and in a fairly short time. Poplar, a woody plant widely used as a model, has been used to study the mechanism of infection that occurs in cases of fungal pathogenicity against woody plants (Cronk, 2005; Jansson and Douglas, 2007).

However, the use of woody plants as model organisms has several drawbacks, including slower growth times and a longer time to reach the stage of treatment and results observation compared to *Arabidopsis*. In addition, for experiments in the laboratory, using *Populus* as a test plant requires a larger area and is more difficult to maintain. On the other hand, *Arabidopsis* has advantages in the form of genetic resources including T-DNA insertion mutants, which are available for most genes. This together with its favourable characteristics, the large amount of available information, and many other available resources, make *Arabidopsis* a valuable model plant for various genetic experiments in plant sciences.

The observed leaf deformation process showed that leaf bending and curling symptoms are a result of long-lasting infection with *Taphrina* strain M11, where symptoms occurred starting at 14 dpi. Thus, this suggests that leaf deformation responses are late symptoms activated slowly when disease is allowed to progress. In contrast, a hypersensitive response was seen in *Arabidopsis* treated with *T. betulina* infection. This seems to stop further *T. betulina* infection as no late symptoms develop. Thus, these findings suggest an infection system of *Taphrina* strain M11 against *Arabidopsis* model plants can be used to explore the genetics of leaf deformation symptom formation.

Hormone imbalance is thought to be the main cause of various leaf deformations in plant infections by fungal pathogens. Cytokinin is a factor of leaf deformation in peaches infected by *T. deformans,* evidenced by diseased leaf tissues containing higher cytokinin concentrations, which were not present in extracts from healthy leaves (Sziraki et al., 1975). The presence of elevated cytokinin concentrations was also found in leaf curl syndrome of Pigeonpea (*Cajanus cajan* MilIsp.; Upadhyaya et al., 1991). Furthermore, the two key steps in the cytokinin biosynthesis pathway were identified in the *Taphrina* M11 genome as tRNA-isopentenyl transferase and cytokinin phosphoribo hydrolase (Publication III).

In the work for this thesis, an experimental system was established using the model plant *Arabidopsis* that can be applied in further research using a genetic approach to test the role of hormones in leaf deformation symptoms caused by M11. For example, mutants with deficient perception or signalling for auxin or cytokinin can be tested to determine if the hormone responses caused by M11 infection are involved in leaf deformation symptoms.

Considering the strong evidence that M11 is a strain of *T. tormentillae*, M11 virulence was tested on *Potentilla norvegica* collected in Finland. For comparison, we tested *T. carnea* strain CBS 332.55 and *T. tormentillae,* which are known pathogens of *Potentilla*. In this experiment, *Potentilla* did not show symptoms in response to any of the *Taphrina* infections. This suggests that *T. tormentillae* has a limited host range, and that the *P. norvegica* used in this experiment was resistant against the strains of

Taphrina that we tested. These results were inconclusive but indicate the need for further M11 virulence tests with other species of *Potentilla*.

4.3.4 ROS activation

ROS are signalling molecules used in response to stress, but also in normal plant development. Staining with 3, 3'-diaminobenzidine (DAB) was used as a ROS assay and performed on the *Arabidopsis* Col-0 wild type accession after experiencing M11 and *T. betulina* infiltration (Publication III), which produced a significant difference between treatment with M11 and *T. betulina*. *T. betulina* accumulated more ROS as shown by a stronger DAB stain after treatment. While the ROS accumulation in *Arabidopsis* infected with M11 was significantly different to the mock, the staining seen was not as strong as that caused by *T. betulina* infections (Publication III). The accumulation of ROS caused by *T. betulina* was consistent with a hypersensitive response, which was seen in a short period of time. These results show that ROS is induced shortly after infection occurs (Lehmann et al., 2015).

Plants have developed an innate immune response adapted to detect the presence of pathogens through pathogen associated molecular pattern (PAMP) triggered immunity (PTI) and effector triggered immunity (ETI) (Chisholm et al., 2006). Innate immune responses include callose deposition, increased pathogenesis related (PR) gene expression, and oxidative bursts producing ROS (Segal and Wilson, 2018). Strong ROS accumulation suggests that *Arabidopsis* has recognized the invading *Taphrina* and has non-host resistance against *T. betulina*. As *Taphrina* is a fungus that grows extracellularly; its introduction to the host occurs in the plant cell wall (Gehrmann, 2013). For *T. betulina,* which is not adapted to *Arabidopsis*, the early response (PTI) stopped the pathogen infection. In contrast, in the process of M11 invasion, it is likely that effector proteins, or other virulence factors, were delivered and suppressed PTI, thus allowing infection to continue. The lack of HR-like cell death and strong ROS accumulation indicates that the defence is being suppressed by M11.

4.3.5 Plant hormone activation and signalling

Pathogens have evolved various strategies to disturb hormonal homeostasis and facilitate infection by counteracting the plant response against pathogens (Ma and Ma, 2016). Production of the plant hormones auxin and cytokinin by pathogenic yeast are part of this strategy. In general, *Taphrina* species are yeasts that are assumed to activate plant hormone signalling pathways in their interaction with plants. The ability of fungi to produce plant hormones *in vitro* has been studied extensively, as has the ability of fungal genomes to encode biosynthesis pathways of plant hormones. This study has further shown that *T. betulina* and *Taphrina M11* are able to activate auxin and cytokinin signalling pathways during an infection (Publication III). Previous studies have shown that auxin signalling was supressed by the plant shortly after an infection occurs, treatment with auxins enhanced pathogen susceptibility, and auxins

are involved in responses to many different pathogens (Fu and Wang, 2011). A plant hormone signalling activation assay was performed with GUS staining and showed more intense colour in *Arabidopsis* with the cytokinin responsive TCS::GUS reporter upon infection with M11. This indicates that M11 was able to activate the host cytokinin signalling. However, staining with the auxin sensing DR5::GUS reporter showed that auxin is slightly, and in some cases not consistently, activated in leaves infected with both M11 and *T. betulina*.

Cytokinins are known to promote cell division in cultured plant cells (Argueso et al., 2009) and are hormones associated with phenotypic changes in plants in response to pathogenic infections. Thus, the result showing activation of TCS::GUS reporter during infections also suggests that the M11 infection activation of cytokinin responses may be involved in phenotypic changes such as leaf curling and bending.

To investigate the role of hormones and other defence signalling pathways in the *Arabidopsis* response to M11 infection, seven mutants were used in these reverse genetic experiments: *coi1-16*, *cyp79 b2/b3*, *ein2, jar1*, *npr1*, *pad4*, and *sid2* (Table 3). Wild-type Col-0 and all mutants were infected with *Taphrina* M11via two methods: by spraying and by hand inoculation using a needleless syringe. There were no visual symptoms in the treatment with the spray method but, in contrast, treatment using the hand inoculation method showed different levels of severity on each mutant.

For symptom analysis, each mutant was treated both with M11 and a mock treatment, following which evaluation of infection results was performed visually and with trypan blue staining to observe cell death and symptom development (Publication III, Figure 4). The M11 infiltration of seven immune signalling mutants produced different results for each mutant. Col-0 and *cyp79 b2/b3* had similar symptoms in terms of the area of infection, while *coi1-16* and *ein2* had similar symptoms characterized by abnormal damage to the leaves inoculated with M11. The response of *coi1-16*, which functions in jasmonic acid perception, and *ein2*, which is required for an ethylene response, showed that ethylene and jasmonic acid insensitivity enhanced the susceptibility phenotype. Ethylene insensitive *ein2* showed the strongest enhanced susceptibility phenotype. This supports that jasmonic acid and ethylene were major hormones in *Arabidopsis* defence against infection with M11 *Taphrina*.

The results of M11 infiltration on *jar1*, *pad4*, *npr1*, and *sid2* were similar and did not show phenotypically abnormal conditions. The *npr1* mutant associated with the SA receptor response to the pathogen shows that salicylic acid was not a major hormone in the *Arabidopsis* defence against the M11 *Taphrina* strain.

5. Conclusions and future perspectives

This study provides in-depth data on the interaction of *Taphrina* with birch and *Arabidopsis* model plants. *Taphrina betulina* is a dimorphic yeast known as a

pathogen that causes witches' broom disease in birch, but apparently, also interacts with birch as a resident yeast. Studies on birch stands in Helsinki show that the mixed populations of different susceptibilities to witches' broom disease support the model that host genotype determines susceptibility to *T. betulina*.

Interaction of *T. betulina* with *Arabidopsis* showed non-host resistance involving hypersensitive response-like cell death and higher accumulation of ROS, resulting in limited growth of *T. betulina* on *Arabidopsis*. *Taphrina* species have a more diverse host range as phyllosphere yeasts compared to as pathogens. Furthermore, the study of yeast phylloplane identification on *B. pendula* leaves showed that *T. betulina* was the dominant yeast. Moreover, *T. betulina* isolated in symptomatic and asymptomatic trees showed that it was a resident yeast in addition to a cause of disease. However, the isolate *Taphrina* M11 was identified as a strain of *T. tormentillae* and was shown to interact as a pathogen against *Arabidopsis* by causing symptoms of leaf curling and leaf bending. Plant hormone signalling experiments showed that jasmonic acid and ethylene were required for the immune response against *Taphrina* M11. *Taphrina* M11 infection of *Arabidopsis* was able to activate cytokinin and auxin responses of the host. There are three different enzymatic pathways in the M11 genome to produce IAA; they are the indole-3-acetamide (IAM), indole-3-pyruvate (IPyA), and tryptamine (TAM) pathways.

The investigation of tumour morphology in birch showed that tumour growth caused by *T. betulina* starts from the outer branch tissue, with increased growth of the secondary phloem, periderm, and with deformation and thickening of the cork cambium. With the results of this study, *Taphrina* can be proposed as a model of phytopathogenic yeasts infecting the model plant *Arabidopsis* and woody plant *B. pendula*.

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