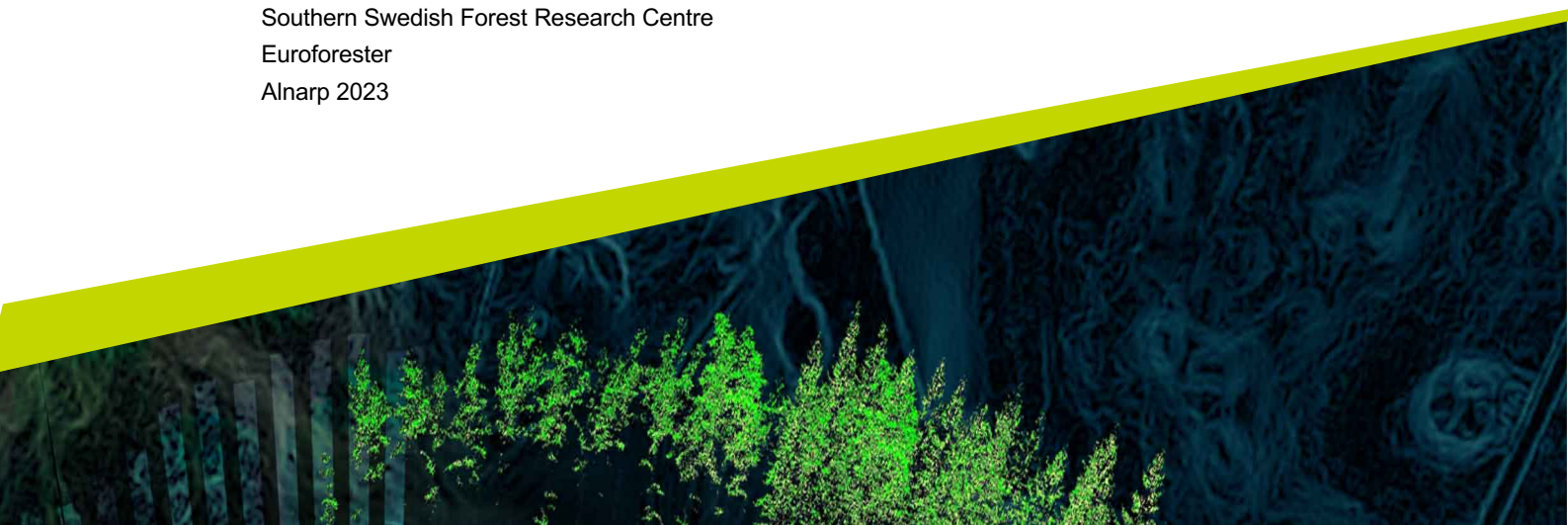
The effect of heat and UVC treatments on seed germination and seed-borne fungi of *Pinus sylvestris*, *Pinus contorta* and *Pseudotsuga menziesii* used in Swedish nurseries

Wiktoria Breza

Master's thesis • 30 credits
Swedish University of Agricultural Sciences, SLU
Southern Swedish Forest Research Centre
Euroforester
Alnarp 2023



The effect of heat and UVC treatments on seed germination and seed-borne fungi of *Pinus sylvestris*, *Pinus contorta* and *Pseudotsuga menziesii* used in Swedish nurseries.

Wiktoria Breza

Supervisor:	**Iva Franić, Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre**
Assistant supervisor:	Michelle Cleary, Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre
Examiner:	Iryna Matsiakh, Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre

Credits:	30 credits
Level:	A2E
Course title:	Master's thesis in Forest Science
Course code:	EX0984
Programme/education:	Euroforester - Master´s Programme
Course coordinating dept:	Southern Swedish Forest Research Centre
Place of publication:	Alnarp
Year of publication:	2023
Keywords:	seed-borne pathogens, artificial regeneration, conifers, thermotherapy

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

Abstract

Pathogen free seeds are critical to produce healthy plants for forest regeneration. However, recent studies have shown that tree seed carry a high diversity of fungi including known and unknown pathogens. There is a need to control seed-borne fungi to minimize the introduction of fungal pathogens, during plant propagation in reforestation nurseries. Thermotherapy treatments, such as heat and UVC light, are recognised as environmentally safe methods for controlling fungal infestation, but effects of such methods on tree seed fungi are poorly understood. Moreover, inadequate dose and duration of these methods can also weaken the viability of the seed by negatively affecting germination.

This study assessed the infection levels and diversity of all and potentially pathogenic fungi (with a focus on known seed-borne pathogen *Sphaeropsis sapinea*) in five seed lots of Lodgepole pine (*Pinus contorta*; PC14 and PC15), Scots pine (*Pinus sylvestris*; PS18 and PS20) and Douglas-fir (*Pseudotsuga menziesii*; PM14) before and after the heat treatment at 55 °C for 8 h and treatments with the UVC light one or three times. Fungi were cultured from 300 seeds per seed lot on nutrient media and identified using Sanger sequencing. Seed germination was also assessed from approximately 300 seeds per seed lot, before and after the treatments were used.

Almost all tested seeds yielded fungi. The high infection level and diversity of fungi, including plant pathogens, was showed for control seed lots of Lodgepole pine and Scots pine. Control Douglas-fir seed lot were highly infected, but fungal diversity was low. High infection level by *S. sapinea* was detected in the control PS18 seed lot. The fungus was also detected in the control PC14 seed lot, but in much lower frequency. The heat treatment reduced fungal infection levels and diversity to varying degrees in Lodgepole pine and Scots pine seeds. This was the case for all fungi, potentially pathogenic fungi and *S. sapinea*. However, the reduction did not occur in Douglas-fir seed lot. Unlike the heat treatment, UVC treatments did not reduce the fungal infection of any tree species. Considering only potentially pathogenic fungi, UVC reduced the infection in PS18 and PM14 seed lots, however the infection levels were increased in PC15 and PS20 seed lots. Occurrence of *S. sapinea* in PS18 seed lot, as well as diversity of all and potentially pathogenic fungi in all seed lots, compare to control treatment, was lower after the UVC treatments. Finally, none of the treatments had a significant negative effect on the germination of the tested seeds.

The study demonstrates that tested thermotherapy treatments can be used to reduce or eliminate fungi in conifer seeds without adversely effecting germination, and that the heat treatment at 55 °C for 8 h gives better results than UVC treatments. However, more research is needed to determine the most appropriate dose and duration, depending on the tree species.

Keywords: seed-borne pathogens, artificial regeneration, conifers, thermotherapy

Acknowledgements

Everyone I mentioned here, in one way or another, made it possible.

I would like to express my sincere gratitude to my main supervisor, Iva Franić. Thank you for your understanding, guidance and important suggestions. Thank you for making me learn so much and acquire new skills. Your support in difficult situations was invaluable. Your knowledge in field of forest pathology and ability to impart knowledge is inspiring.

I would like to express my sincere thanks to my co-supervisor, Michelle Cleary, who, despite her many commitments, gave me valuable suggestions and whose input on this project was very valuable.

I would like to thank all in the Forest Pathology lab in Alnarp for any useful advice and consultation. Muji, my friend, I am happy that we could work together. Thank you for all the kind words and moments.

I would like to thank Pierre Martin for his great support and optimism during this time.

Table of contents

List of tables

List of figures

Abbreviations

DNA	Deoxyribonucleic Acid
MEA	Malt Extract Agar
WA	Water Agar
Cm	Chloramphenicol
EtOH	Ethanol
ITS	Internal Transcribed Spacer
PCR	Polymerase Chain Reaction

1. Introduction

1.1 Artificial regeneration of conifer forest stands using seeds

The progressive historical development of silviculture has been closely related to high demand for timber which has been acquired by large-scale afforestation using artificial regeneration (Chudy & Cubbage, 2020). In 2020, 1,391,200,000 ha of forest area were regenerated in Sweden (Global Forest Resources Assessment; FRA, 2020). For artificial forest regeneration to be successful, several important factors must be considered, among which are: tree species traits and requirements, site characteristics, functions of the stand and estimated costs (Barnett & Baker, 1991; Dey et al., 2008). There are many advantages associated with artificial regeneration in comparison with natural regeneration, such as rapid plant growth which reduces the risk of vegetative competition, better control of the desired species composition, predictable production and high management flexibility. Artificial regeneration can be accomplished by planting seedlings (the most common method) or by sowing seeds directly into the soil (Huss, 2004). In Sweden, seedling planting accounts for 84% of the regeneration area (Svensson, 2019). It is estimated that planting usually results in 70-80% seedling survival in the first three years (Von Sydow, 1997; Sikström et al., 2020). The success of seedling survival depends on site, tree species, pest protection and mechanical site preparation method (Holmström et al., 2019; Sikström et al., 2020). Using seedlings has advantages such as rapid growth and establishment, and the avoidance of seed predators (Allen et al., 2001). However, this method is expensive, which is related to the high production costs as well as the need for manual planting, which is currently difficult to mechanise (Domevscik, 2023). An alternative to seedlings is

direct seed sowing which accounts for 6% of the regeneration area in Sweden (Svensson, 2019). Seeding reduces costs and simplifies mechanisation (Löf et al., 2004), as well as reduces the risk of transferring plant diseases from nurseries to the field (Sánchez et al., 2005). This method has been, however, limited due to the low establishment success, partly driven by high seed predation and the vulnerability of young seedlings (Leverkus et al., 2021), and limited availability of seeds from orchards (Grossnickle & Ivetić, 2017). This has contributed to greater attention to seed processing to increase seed survival and production (Domevscik et al., 2023).

The method of cone collection is determined largely by the species (i.e., size of cones), quantity of cones (Pigott, 2018), terrain and spacing between trees (Fennessy, 2002). Some of the methods of cone collection are mechanical shaking, hand picking or net retrieval system. Also, the period for collecting conifer cones depends, among other things, on the tree species, however, to obtain the desired quality, all cones can only be harvested when they are fully ripe (Miller & Schaefer, 2015). For example, Lodgepole pine (*Pinus contorta* Dougl. ex Loud.) cones are collected in the monthly range: early September - mid November, Scots pine (*Pinus sylvestris* L.) cones: mid-November - early April and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) cones: end August - early October (Fennessy, 2002). Different conifers have different ages of first good seed crop, which for Lodgepole pine and Scots pine is between 15-20 years and Douglas-fir later, as 30-35 years. Seeds between different conifers differ, among other things, in size and colour. Douglas-fir seeds with light brown colour (Farjon, 2010) reach lengths of 4-7mm, whereas brown seeds of Lodgepole pine and Scots pine have very similar, smaller, lengths of 2-5mm and 3-5mm, respectively (Kolotelo, 1997; Farinha et al., 2018). However, the colour of seeds of the same species varies, for example, depending on the region in which they were collected. An example is the seeds of Scots pine, which, depending on where they were collected, can range in colour from light brown to black (Udval & Batkhuu, 2013). After the cone collection, the cones and later seeds are then processed and stored under specific conditions.

Seed processing is based on multiple operations (Fig. 1) such as kiln drying with a temperature ranging between 32–60 °C for most conifers (Aldous, 1972). However, drying temperature depends on initial cone moisture content and sensitivity of the tree species to heat. With some exceptions, such as Eastern white pine (*Pinus strobus* L.), cones must be dried to about 10% moisture content for complete opening (Belcher & Lowman, 1982). The next steps include cone tumbling, dewinging (done mechanically either dry or wet), seed cleaning which includes the removal of small debris and empty seeds, and conditioning, where seed quality can be improved (Bonner, 1991). Once the seeds are processed, they are subjected to germination tests, which are the most reliable tests for assessing the quality and viability of the seeds (Tanaka, 1984). High quality seeds are then stored, which is important to offset years of low conifer production (Bonner, 1991). Seed storage conditions vary depending on their sensitivity to environmental conditions, tree species and how long the seeds need to be stored before planting. Conifer seed, and most commonly pines, are considered "orthodox", so their viability can be maintained for many years when stored at low moisture and temperature (Roberts, 1973). Seed moisture content is the most important factor in seed storage. A moisture content of 5–10% is recommended for successful storage, but for short-term storage (≤ 3 years), higher moisture levels may be acceptable (Bonner, 1987). There is a relationship of moisture content to temperature: at a given moisture content, the higher the storage temperature, the faster the deterioration of seed viability: the lower the storage temperature, the greater the tolerance to high moisture content and the better the retention of viability (Barton, 1961). The standard temperature for short-term storage is 2 °C and for longer periods, -18 °C is best (Barnett & McLemore, 1970). When seeds are stored, it is also necessary to consider seed longevity, which is seed viability after dry storage describing the total seed life span (Rajjou & Debeaujon, 2008). Seeds can be classified into different biological categories according to their life span which varies with the tree species. The seeds of most conifers are classified as microbiotic, which means that their life span is less than three years (Tanaka, 1984). However, under regulated storage conditions, for example subfreezing, the longevity of many tree seeds can be extended. The viability of naturally dispersed seeds of Norway spruce (*Picea abies*

L. H. Karst.), Scots pine and Douglas-fir can be extended only into the first growing season, and sometimes into the second growing season (Tanaka, 1984; Klein, 2003). To enhance germination and seedling growth, several treatments may be carried out, including stratification. During this process, the seeds are most often stored in moist cold conditions, which invokes changes in seed physiology, breaking its dormancy (Udayangani, 2020). In most conifers, the dormancy is shallow which makes them more challenging to germinate (Gosling, 2007).

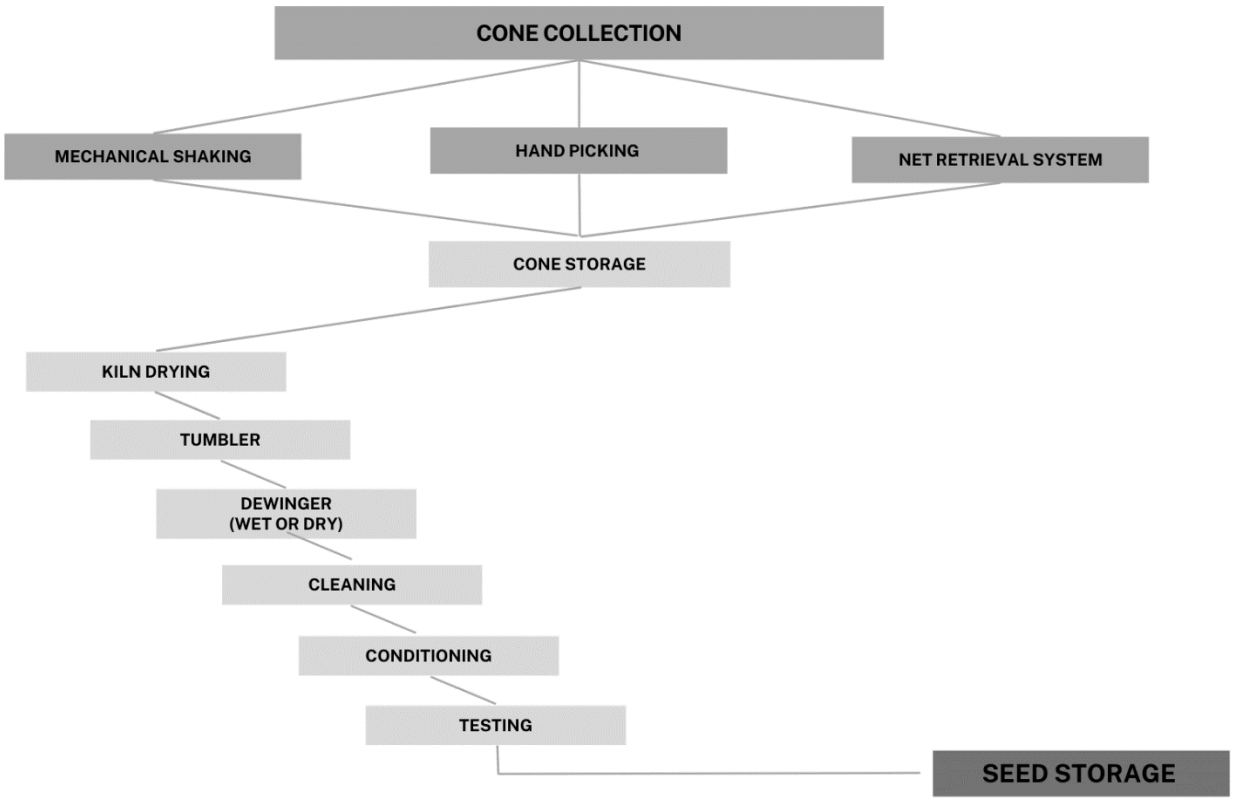


Figure 1 Flow chart of typical extraction and processing of conifer seeds.

Seed orchards are the link between tree breeding and forest regeneration, producing and supplying seeds of the highest quality (Almqvist & Jansson, 2015). Local seed orchards are the main suppliers of conifer seeds to forest nurseries in Sweden (Lindgren, 2007). About 90% of the Scots pine seeds used in plant production, as well as 60% of all planted plants in Sweden, originate from Swedish seed orchards (Prescher, 2007; Swedish Forestry Agency, 2013). The size of the area of seed orchards of two most dominant coniferous tree species in Sweden is 835 ha (Krakau et al., 2013) and 379 ha (Jansson et al., 2013) for Scots pine and Norway spruce,

respectively. The overall seed production in Sweden in 2019 was estimated on 11,000 kg of Norway spruce and 4,000 kg of Scots pine seeds (NordGen, 2021). Biological seed production is often expressed per unit area, i.e., seeds/m^2. As for seed orchards in general, the average of at least 120–150 seeds/m^2 or 10 kg/hectare of Scots pine seeds can be produced (Prescher et al., 2005).

The demand for propagation material results in frequent trade of seeds between countries. Sweden exports around two tonnes of seeds annually (Statistics Sweden, 2021b). In turn, the Swedish Forest Agency (2021a) estimated that between 2017 and 2020, the average annual seed trade into Sweden was 981 kg, where the largest proportion belonged to Norway spruce with a yearly average of around 844 kg, followed by Scots pine with a yearly average of around 96 kg. Most seeds were traded from Belarus (65%), followed by Lithuania (12%) and Poland (10%). Seed trade to Sweden may partly originate from Swedish cones that have been sent abroad for seed extraction (Claes Uggla, The Swedish Forest Agency, 2021). At the same time, the lack of phytosanitary regulation of the international seed trade (Roques, 2010) increases the risk of the spread of potential fungal pathogens that could cause huge economic, as well as environmental losses (Cleary et al., 2019; Franic et al., 2019).

1.2 Fungi associated with conifer seeds and their risk

Fungi belong to one of the most diverse groups of living organisms, with the estimated number of 1.5–5 million species (Choi & Kim, 2017). They have been detected in all climatic zones of the Earth and through the functions they perform, they have a great impact on the ecosystem processes (Osono, 2011; Tedersoo et al., 2014). Some fungi can be saprotrophic, i.e., they decompose organic matter and thus ensure the circulation of nutrients in nature (Hill, 2021). Many fungi form symbiotic relationships with other living organisms, including mutualism and parasitism. In mutualistic relationships both organisms benefit from the relationship. Two common mutualistic relationships involving fungi are mycorrhiza and lichens. In a parasitic relationship, the parasite benefits by taking

the nutrients from the host which in turn gets harmed (Purin & Rillig, 2008). Among parasites are pathogens causing a variety of diseases in forest trees (Hart, 1990). Some pathogens may belong to endophytes; they are latent and may change the character of their relationship with the host during the inhabiting time, for example, during stressful conditions for the host (Bacon et al., 2008). Endophytes are organisms (e.g., bacteria or fungi), which grow inside the plant tissue showing no visible signs of presence (Mengistu, 2020). Some of the pathogens are seed-borne and can be found in all conifer seed components: seed coat, megagametophyte and embryo (Cordell et al., 1989; Kolotelo, 1997). Seed-borne pathogens can either kill the seed, or can be seed-transmitted, i.e., it will not kill the seed, but can grow into the seedling and possibly cause damage later or non-seed-transmitted, i.e., not transmitted to the seedling, but to the environment from where they can infect the seedling (ISPM 38, 2017). Not many fungi are known to be transmitted from seeds to seedlings, which may be because it is a rare occurrence or because knowledge about it is still limited. More studies on seed-transmitted fungi are important because vertically transmitted fungi have the highest chances of establishing in the environment and therefore may pose a phytosanitary risk (Burgess & Wingfield, 2002). The risk is also not negligible for non-seed-transmitted fungi.

Pathogenic fungi can significantly affect the quality and quantity of conifer seeds (Mancini & Romanazzi, 2014) and seedling development (Fraedrich & Miller, 1995). Pathogens account for a high proportion of the detected fungi in seed, with some studies indicating that 20–30% of seed-associated fungi are potential plant pathogens (Cleary et al., 2019; Franic et al., 2019). According to Vujanović et al. (2000), up to six different pathogenic fungi can be detected in Scots pine seeds, which can cause varying degrees of cone and seed infection. One example of a seed-borne pathogen is *Caloscypha fulgens* (Pers.) Boud, which first penetrates the seed of Norway spruce, and kills it even before germination (Sutherland & Van Eerden, 1980; Prochazkova, 2009). This pathogen was first reported in 2002 in Germany on imported conifer seeds from North America (Schröder et al., 2002). According to Sutherland et al. (1987a), the fungus may lead to losses in conifer seedbeds of more than 90%. It causes a seed rot in a few conifers (Woods et al., 1982) but has negligible impact on other developmental stages of trees. Another common

pathogen of conifer seeds is *Sirococcus conigenus* (Pers.) P. F. Cannon & Minter (1983). The pathogen was first reported in Norway in Noble fir (*Abies procera* Rehder*)* seed lot where 31% of seeds were infected (Talgø et al., 2010). This fungus causes shoot blight and can affect several other conifer species in nurseries and in young trees in the field, where the seed-borne inoculum may kill germinating seeds and seedlings (Talgø et al., 2010). Fungi residing inside the seed might be more harmful than those on the surface, such as *Fusarium* spp.*,* often residing inside the seeds of, e.g., pine and Douglas-fir trees (Papavizas, 1985; Graham & Linderman, 1983). A serious problem for seedling production is the seed-borne pathogen *Fusarium circinatum* Nirenberg & O'Donnell, the causal agent of pine pitch canker (Mitchell et al., 2003). This pathogen can cause severe pre- and post-emergence damping off, and lead to dieback and mortality of older pine trees in plantations and forests (Viljoen, 1994; Wingfield, 2008). Its presence has been detected, among others, on the seeds of Douglas-fir; the only conifer besides genus *Pinus* known to be susceptible to this pathogen (EFSA, 2010; Gordon et al., 2006). The spread of this fungus is closely correlated with the local and global transport of plant material, including seeds, as these were the source of its arrival in Europe in the 19th and 20th centuries. The same situation applies to *Sphaeropsis sapinea* (Fries) Dyko & B. Sutton, one of the most threatening seed-borne pathogens (Fabre et al., 2011).

Sphaeropsis sapinea (tip blight pathogen) like many other ascomycete fungi having plastic lifecycles, can exist in an endophytic stage causing symptomless infections for years. However, symptoms of infection with this pathogen may appear when the tree becomes stressed, e.g., by drought, and thereafter tree vitality and therefore host susceptibility is lowered and the fungus can become opportunistic, causing lethal attack (Stanosz et al., 2001; Slippers & Wingfield, 2007). This pathogenic species is currently emerging as a serious problem of pine species in northern Europe (Brodde et al. 2019). The pathogen is associated with cones and seeds (Munck & Stanosz 2010), and frequently forms pycnidia (Peterson, 1977) on more than 80% of cones (Nicholls & Ostry, 1990). In the southern Cape Province of South Africa, it was estimated that between 1923 and 1983 there were 11 outbreaks of *S. sapinea* induced dieback of Maritime pine (*Pinus pinaster* Aiton.) and 25 of

Monterey pine (*Pinus radiata* D. Don; Smith et al., 2002). High levels of pathogen infection were also observed in seed orchards of Corsican pine in France, where 57% of seeds were infected (Decourcelle et al., 2015). Although the fungus is known to be seed-borne, it has not yet been confirmed that it can be transmitted from seeds to seedlings directly, but it seems that trees acquire the fungus from the environment (Slippers & Wingfield, 2007; Stanosz et al., 2007; Bihon et al., 2011). Infected trees typically display symptoms such as yellowing of needles, branch dieback and cankers on the main stem and branches (Kaya et al., 2014*)*. Unfortunately, all seed-borne pathogens, including *S. sapinea*, are still under-researched. There is a growing need to better understand the biology of seed borne fungi (including *S. sapinea*) to introduce the necessary phytosanitary treatments, to ensure healthy seeds for forest regeneration.

1.3 Heat and UVC light as treatments for the elimination of fungi in plants

Several types of treatments to control fungi in plants have been studied. Among these treatments are chemical and non-chemical methods. Chemical methods involve the use of pesticides (e.g., fungicides, nematicides, insecticides) Sinclair, 1993). In the past, fungicides were most used to control fungi on plants and this method is still often considered the most effective. However, chemical methods are not always environmentally safe and with the new Plant Health laws introduced by the EU in 2019 (European Commission, 2019), traditional chemical methods are increasingly being replaced by non-chemical methods, such as treatments with biopesticides and physical treatments (Martin-Garcia et al., 2019). Biopesticides include plant extracts and biocontrol agents, where natural antimicrobial compounds and numerous antagonistic microorganisms are used (Mancini & Romanazzi, 2014). Physical treatments, also known as thermotherapy, use heat energy to kill or inactivate pathogens without affecting plant viability (Baker, 1972). Such methods include treatment by hot water, hot air, moist hot air, solar heat, aerated steam, refrigeration and radiation (Agarwal & Sinclair, 2017). Among

these treatments, heat with hot air and UVC radiation have been used on many plants to control fungal diseases.

Heat treatment can be used to directly kill seed-borne pathogens or retard their growth (Marquenie et al., 2002; Godefroid et al., 2017). Adapted ovens, large chambers or walk-in rooms are typically used for applying heat treatments (Grondeau et al., 1994) which can differ in temperature and duration. Heat is used against fungi residing outside of and inside the seed (Shi et al., 2016) and it affects fungus hyphae (i.e., the growing cells) as well as fungal spores (Van den Brule et al., 2020). It has been shown that heat treatment at 55 °C for 8, 9, 10 and 11 hours can eliminate potentially pathogenic *S. sapinea* and *F. circinatum* from Monterey pine seed coat, embryo and gametophyte, without adversely affecting seed germination (Iturritxa, 2011). In other studies, cereal seeds treated with heat at 50 or 70 °C for up to 14 days eliminated the seed-borne pathogen *Fusarium graminearum* Schwabe, yet the degree of reduction varied with time and temperature (Clear et al., 2002) demonstrating that fungi have different preferences for time and higher/lower temperatures (Agrios, 1997). Another thermotherapy component is ultraviolet (UV) radiation; a natural component of sunlight which can be divided into UVA (315–390 nm), UVB (280–315 nm) and UVC (100–280 nm) comprised of energies between 3 to 124 eV (Diffey, 2002; Paul & Gwynn-Jones, 2003). Among these, UVC light is mainly used to disinfect seed surfaces (El-Gaouth & Wilson, 1995). As opposed to heat treatment which can penetrate the seed to the inside, UVC treatment acts only on the surface of the seed (Birmpa et al., 2013; Luckey, 1980). Because UV radiation can damage DNA, this method can drastically affect the life processes of microbes (including pathogens) (Luckey, 1980). Sterilization by UVC light is widely used in medicine (Siddiqui et al., 2011), horticulture, food processing and agriculture (CleanLight, 2022). UVC light, for example, reduced root infecting fungi (e.g., *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* Kühn and *Fusarium* spp.) of groundnut (*Arachis hypogaea* L.) and mung bean (*Vigna radiata* (L.) Wilczek; Siddiqui et al., 2011; Neelamegam & Sutha, 2015) or cabbage seeds (Brown et al., 2001). However, the application of such treatments on seeds requires adjusting the length and intensity

of this treatments to reduce or eliminate fungi without adversely affecting seed germination.

1.4 Research aims and hypotheses

The main objective of this research was to determine the efficacy of heat and UVC treatments to remove fungi, especially potentially plant pathogenic fungi and *S. sapinea*, present in Scots pine, Lodgepole pine and Douglas-fir seeds and to assess the impact of the treatments on seed germination.

In the present study, three hypotheses were formulated and tested:
H1) Number of seeds per seed lot infected by all fungi, potentially pathogenic fungi and *Sphaeropsis sapinea* is lower for treated than for control seeds.
H2) Occurrence-based diversity (i.e., taxon richness) of all fungi and potentially pathogenic fungi per seed lot is lower for treated than for control seeds.
H3) Number of germinated seeds does not differ between treated and control seeds.

The aim of this study was to collect information on seed-borne fungi in Swedish nurseries and how they can be effectively controlled by applying heat and UVC treatments. The results of this study will allow possible mitigation measures to lower the risk of introduction and spread of potentially harmful fungal pathogens via seed used in reforestation nurseries.

2. Materials and methods

2.1 Study materials

In this study the seeds of three tree species were used: Lodgepole pine, Scots pine and Douglas-fir. Seeds belonged to five different seed lots, which were obtained from the Svenska Skogsplantor. All seeds had previously been stored in optimal conditions. Detailed information on the study materials is provided in Table 1.

Table 1 Source origin and collection date of seed lots of Pinus contorta, Pinus sylvestris and Pseudotsuga menziesii used in the study. In addition, information on the tests and treatments performed on individual seed lots is provided.

Tree species	**Seed lot**	**Origin**	**Collection date**	**Germination capacity test [%] Germination energy test [%]**		
				Treatment		
				Heat at 55 °C, 8 h	**UVC1**	**UVC3**
Pinus contorta	PC14	Larslund, Sweden	2014	X	-	-
Pinus contorta	PC15	Larslund, Sweden	2015	X	X	X
Pinus sylvestris	PS18	Gotthardsberg, Sweden	2018	X	X	X
Pinus sylvestris	PS20	Dal, Sweden	2020	-	X	X
Pseudotsuga menziesii	PM14	La Luzette, France	2014	X	X	X

2.2 Study methods

In this study, seeds were subjected to a heat treatment at 55 °C for 8 h (later referred to as "heat treatment") and two ultraviolet C (UVC) treatments: either once (UVC1) or three times (UVC3). Then, fungal assessment and two germination tests (i.e., germination capacity and germination energy test) were done for all control and treated seeds. Finally, statistical analyses were performed on the data obtained.

2.2.1 Seed treatments

2.2.1.1 Heat treatment at 55 °C for 8 h

A heat treatment at 55 °C for 8 h was carried out for all seed lots, 600 seeds each (e.g., 300 for the fungal assessment and the same number for germination tests), except for one Scots pine seed lot, i.e., PS20 (Table 1). Seeds were placed to sterile glass Petri dishes (Fig. 2) and heat treatment was carried out in a specially designed and dedicated oven. After the treatment, seed samples were sent for germination analysis to the Seed Unit in Svenska Skogsplantor in Lagan, which is the biggest and market-leading supplier of seeds, plants and regeneration services for Swedish forests (https://www.skogsplantor.se/). Fungal assessments were performed on the remaining seeds, approximately 300 per seed lot, in the laboratory.

Figure 2 Seeds after heat treatment at 55 °C for 8 hours in sterile glass Petri dishes and insulated with tape.

2.2.1.2 UVC radiation treatments

Two UVC radiation treatments were carried out one time (i.e., UVC1) or three times (i.e., UVC3) for each seed lot except the Lodgepole pine seed lot PC14 (Table 1). For each treatment, 0.1 kg seeds of each seed lot were used. The seeds were placed on an aluminium tray which was washed with 99.5% alcohol between seed lots. Each seed lot was UVC-treated either once (UVC1) or three times (UVC3) using CleanLight machine (Fig. 3). Germination analyses were performed by the Seed Unit in Svenska Skogsplantor in Lagan. Fungal assessments were performed on the remaining seeds, approximately 300 per seed lot, in the laboratory.

Figure 3 CleanLight machine used to carry out UVC treatments.

2.2.2 Fungal assessment

Fungal assessment was done for all control and treated seeds; in total 300 seeds per seed lot and per treatment. Seed fungi were grown on nutrient media—2% Malt Extract Agar (MEA, 20g/L, Duchefa Biochemie, Haarlem, The Netherlands; WA, Agar bacteriological, 15 g/L; VWR Chemicals, Solon, Ohio, USA and distilled water, 1000ml). This type of medium was chosen because of its effective use in earlier studies to isolate the pathogen *S. sapinea* in *Pinus* sp. seeds (Smith et al., 2002). The media were sterilised in an autoclave at 121 °C for 15 minutes and supplemented with 200 mg/L^{-1} chloramphenicol to prevent the growth of bacteria (Smith et al., 1996). All seeds were placed in 9 cm diameter Petri dishes, five seeds on each plate and stored in the laboratory room in boxes. Then, the isolation of pure fungal cultures and grouping into morphotypes was done. Plates were checked for occurring fungi, depending on the growth rate, daily to every three days, for 21 days in total. Fungi growing out of seeds on 9 cm Petri dishes were first grouped into morphotypes per Petri dish and a representative isolate of a given morphotype was transferred on a small Petri dish with 2% MEA. If necessary, fungal isolates were

subcultured again to obtain pure cultures. Pure fungal cultures were grouped according to macro-morphological characteristics such as colour, texture, form, margin (i.e., the appearance of their edge) and the elevation (Microbiology, 2014).

2.2.2.1 DNA extraction

One representative culture of each morphotype was chosen for DNA extraction. Fungal mycelia were scraped off the nutrient media and placed in 2 ml centrifuge tubes with glass beads. Tubes were placed in liquid nitrogen for a few seconds and homogenized using a MM200 Retsch ball mill (Retsch GmbH, KG, Haan, Germany). After homogenization DNA was extracted from fungal tissues using the E.Z.N.A.® SP Plant DNA Kit (Omega Bio-Tek, Norcross, Georgia, USA), following the manufacturer's protocol for dry samples. This kit accurately isolates high-quality total cellular DNA from a variety of plant species and tissues (Zhu et al., 2006).

2.2.2.2 Amplification of rDNA Internal Transcribed Spacer region and Sanger sequencing

Nuclear Ribosomal Internal Transcribed Spacer (ITS) region which is a conservative region used for fungal identification (Schoch et al., 2012) was amplified in Polymerase Chain Reaction (PCR). Prior to PCR, DNA quantity and quality was measured using a DS-11 UV-Vis Spectrophotometer (DeNovix) and DNA was diluted by mixing 10 µl of extracted DNA and 90 µl of water therefore the dilution was 1:10. This process improves amplification of the marker gene by reducing chimeric read formation and diluting inhibitors of the PCR (Castle et al., 2018). Subsequently, ITS region was amplified in the volume of 25 µl, including 2 µl of DNA, 8.5 µl of ddH20 12.5 µl of DreamTaqPCR Master Mix (2X), ITS1 (forward primer sequence) and ITS4 (reverse primer sequence) primers in the quantity of 1 µl each (Tedersoo et al. 2014). PCRs were carried out using Eppendorf Mastercycler following the conditions: 1 cycle of initial denaturation for 2 min at 95 °C, 35 cycles of denaturation for 1 min at 95 °C, 35 cycles of annealing for 45 s at 55 °C, 35 cycles of extension for 1 min 30 s at 72 °C, and 1 cycle of strand

completion for 5 min at 72 °C. To confirm the presence of amplified DNA in a PCR sample electrophoresis was used. PCR products were sent to Macrogen (Amsterdam, Netherlands) for purification and sequencing using chain termination method (i.e., Sanger sequencing) with the same primers that were used in PCRs.

2.2.2.3 Sequence analysis and identification

In order to trim the low-quality ends and to align forward and reverse DNA sequences into consensus sequences the Bioedit program (Hall, 1999) was used. The Basic Local Alignment Search Tool (BLAST) was used for comparing obtained sequences with sequences in The National Centre for Biotechnology Information (NCBI) database (Geer et al., 2010). Uncultured/environmental sample sequences were excluded to identify the organism more accurately, i.e., without considering possible environmental contamination that may have contaminated the desired cultures (Weir, 2014). In addition, sequences from type material were considered to ensure high level of confidence in the taxonomic identification. This approach is important when working with cultured fungi, where the type material is easily available to the research community from the culture collection (Federhen, 2015). Sequence assignment was based on E-value, which is a measure of likeliness that sequence similarity is not by random chance (Reed, 2022). Fungal sequences were assigned to species if it showed less than 2% divergence from the reference sequence. Subsequently, when the similarity between the fungal and reference sequence was 90–98%, the sequence was assigned to a genus, and when the similarity was below 90% the sequence was assigned to a family. If a blasted sequence was matched to multiple taxa with the same similarity rank, the assignment was ranked down from the species to the next taxonomic level (i.e., genus, family). Assigned taxon names were checked against Mycobank (Crous et al., 2004) to ensure the most recent and accurate names were used. Finally, potential pathogenicity was assigned to the identified fungal taxa using FUNGuild, which is a tool for parsing fungal community datasets into ecological guilds based on their taxonomic assignments (Nguyen et al., 2016). In the case when information was not available on FUNGuild, other scientific studies available at Google Scholar were used to confirm potential pathogenicity of identified fungi.

2.2.3 Germination assessment

A seed germination assessment was done by performing germination capacity test and germination energy test. Germination capacity is defined as the percentage by number of seeds that can complete germination under optimal conditions (after around three weeks of incubation; Domin et al., 2020), while germination energy gives us the percentage by number of fast-germinating seeds in a given sample which germinate within a definite period such as 7-14 days under optimum or stated condition (Willan, 1987). The analysis was carried out on control and treated seeds (Table 1), approximately 300 seeds per seed lot. Seeds of each sample (where samples indicate seed lots, n = five) were placed on moistened filter paper and covered with plastic caps (Fig. 4). The samples were assessed after 7 and 14 days for Scots pine seeds and on days 7, 10, 14 and 21 for Lodgepole pine seeds. Seeds of Douglas-fir were assessed at 7, 14 and 21 days. During the assessment, when radicle, i.e., embryonic root of a plant and the first part of a seedling to emerge from the seed during germination (Chang & Johar, 2022), was longer than 1.5 times the length of the seed, seed was removed.

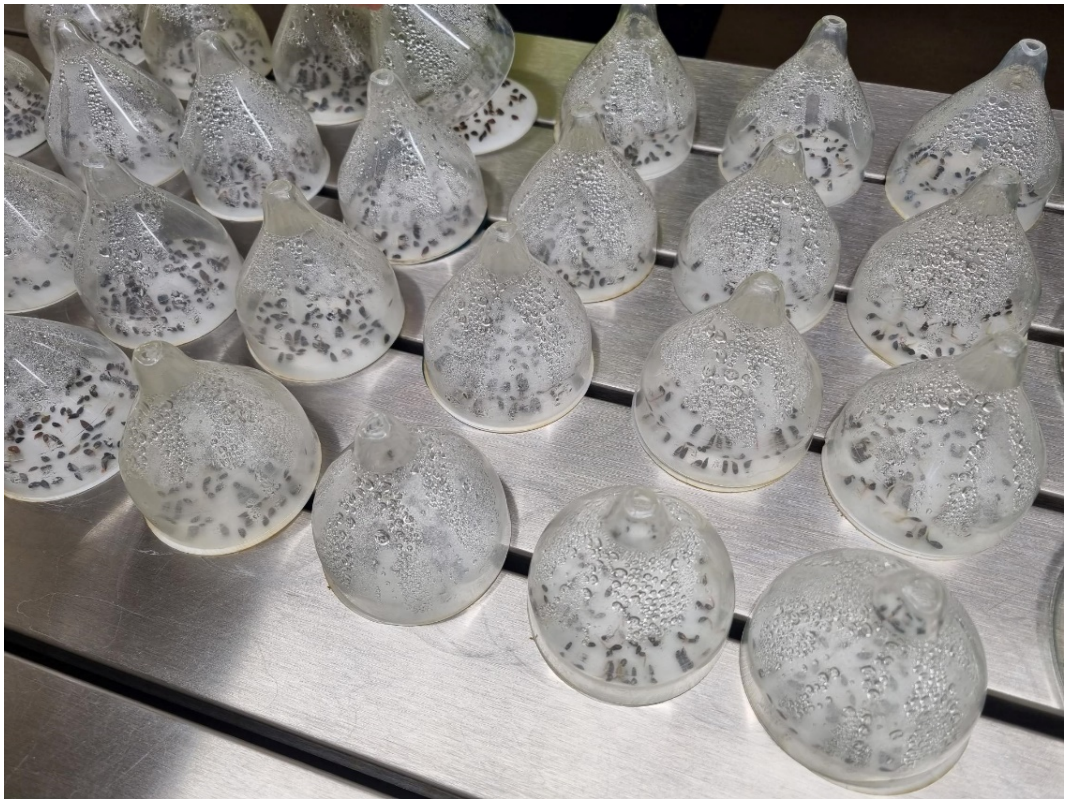


Figure 4 Seed samples placed on the filter paper and covered with plastic caps.

2.2.4 Statistical analysis of data

Statistical tests were conducted in R (R Core Team, 2018). Statistical tests were considered significant at $P < 0.05$.

The differences in response variables between treatment levels (categorical variable with 4 levels: control, heat, UVC1 and UVC3) were tested. The seed infection level (i.e., number of seeds carrying fungi in a sample of 300 seeds), occurrence-based diversity (i.e., number of fungal morphotypes or species in a sample of 300 seeds) and germination (i.e., germination energy and capacity) were assessed per seed lot and across treatments. The seed infection level was measured for *i)* all fungi, *ii)* potentially pathogenic fungi and *iii) Sphaeropsis sapinea*. Occurrence based diversity was measured as the number of morphotypes and species and was assessed for *i)* all fungi and *ii)* potentially pathogenic fungi. A Normality Test was performed to check whether the collected data were distributed normally, which gave confidence that the data met the assumptions for the subsequent statistical tests (Khatun, 2021). For this purpose, density plots and Q-Q plots were performed using the ggpubr package (R Core Team, 2018). Response variables with normally distributed data were analysed using parametric ANOVA test with the (aov) function (Fox, 2016). To test response variables with non-normally distributed data the non-parametric Kruskal-Wallis test was performed using kruskal.(test) function (Hollander & Wolfe, 1973). Moreover, Venn Diagram tool from VennDiagram package (Shade & Handelsman, 2012) was used to show the number of species of all fungi and potentially pathogenic fungi that were, or were not eliminated, across treatments, for each seed lot separately. All the plots were constructed using the ggplot function (package ggplot2; Wickham, 2009).

3. Results

3.1 Overall fungal taxonomy

Fungi were growing from about 84.5% of all control and treated seeds (4,307 seeds out of 5,100). The total number of 6,560 fungal colonies from those seeds was observed and among them 2,262 representatives were isolated. Obtained cultures were assigned to 371 morphotypes. It was observed that more than 80% of morphotypes (311 out of total 371) were represented by more than one isolate, whereas 60 morphotypes were singletons (i.e., represented by only one isolate). The most abundant morphotypes (i.e., ≥ 1% of total number of isolates, n = 22) represented 59.7% of total 6,560 isolates.

DNA was successfully extracted from 227 representative morphotypes among all 371 observed morphotypes. The representative morphotype was chosen in case when the same morphotype occurred in one seed lot for two different treatments. From all DNA samples, the ITS region was successfully amplified in PCR and sequenced. About 93% (210 out of 227) of fungal sequences provided good quality data for the identification of fungi. From obtained sequences, 80.2% were assigned to species, 11.9% to genus and 0.4% to family. Remaining 7.5% of sequences could not be assigned to any taxonomy rank. In total, 63 species belonging to 14 fungal genera and 27 different families were identified. The most abundant fungal taxa (i.e., ≥ 1% of total number of isolates, n = 17) represent 87.3% of total 6,560 isolates (Table 2; Fig. 5). The majority of identified fungal sequences belonged to phylum Ascomycota (92.9%), the remaining sequences were assigned to Mucoromycota (4.8%) and Basidiomycota (1.9%). There was also one plant sequence assigned to

Tracheophyta (0.4%). Identified fungal sequences were assigned to eight fungal classes. Within Ascomycota fungi were assigned to four classes: Eurotiomycetes (41%), Dothideomycetes (36.2%), Sordariomycetes (13.2%) and Leotiomycetes (2.4%). Within Mucoromycota, the identified species belonged to class Mucoromycetes (4.7%). Basidiomycota were assigned to three classes: Cystobasidiomycetes (1%), Agaricomycetes (0.5%) and Tremellomycetes (0.5%).

Potential plant pathogens (as assigned using FUNGuild and available literature) were present in 38.5% of seeds (1,961 seeds out of 5,100). They represented about 31.6% of all isolates (2,075 out of 6,560) and about 22% (50 out of 227) of obtained fungal sequences. The most abundant potentially pathogenic fungi (i.e., ≥ 1% of total number of isolates, n = 4; Table 2) represent 29.6% of total 6,560 isolates. The identified pathogens were assigned to two phyla: Ascomycota (92%) and Mucoromycota (8%).

Table 2 Taxonomic assignments of 17 most abundant fungal taxa to which 22 most abundant fungal morphotypes were assigned, their potential pathogenicity, relative abundances and occurrence across seed lots.

Taxon	Potential pathogenicity	Abundance [%]	Seed lot
Penicillium bialowiezense K.M. Zalessky	X	16.4	PC14, PC15, PS21, PS19
Briansuttonomyces eucalypti Crous, in Crous & Groenewald		15.8	PC14, PC15, PS21, PS19
Mucor hiemalis Wehmer		10.2	PC14, PC15, PS21, PM
Hormonema macrosporum Voronin		9.0	PC14, PC15, PS21, PS19
Rhizopus arrhizus A. Fisch.	X	9.0	PM
***Penicillium* sp.**		4.8	PC14, PC15, PS19
***Thyronectria* sp.**		3.5	PC14, PC15
Neomicrosphaeropsis juglandis D. Pem, Selcuk, Jeewon & K.D. Hyde		3.5	PC14, PC15, PS19
Talaromyces neorugulosus A.J. Chen, Frisvad & Samson		3.4	PC14, PC15
Sphaeropsis sapinea (Fries) Dyko & Sutton	X	3.1	PC14, PS19,
Penicillium nothofagi Houbraken, Frisvad & Samson		1.5	PC14, PC15, PM
Penicillium yezoense Hanzawa ex Houbraken		1.4	PS21
Penicillium spinuloramigenum Thom.		1.3	PC15, PS21,
Epicoccum tritici Hennings		1.2	PS21, PS19
***Fusarium* sp.**	X	1.1	PC14, PS21, PS19,
***Talaromyces* sp.**		1.1	PC14, PC15
Apiospora sp.		1.0	PC14, PC15, PS21, PS19

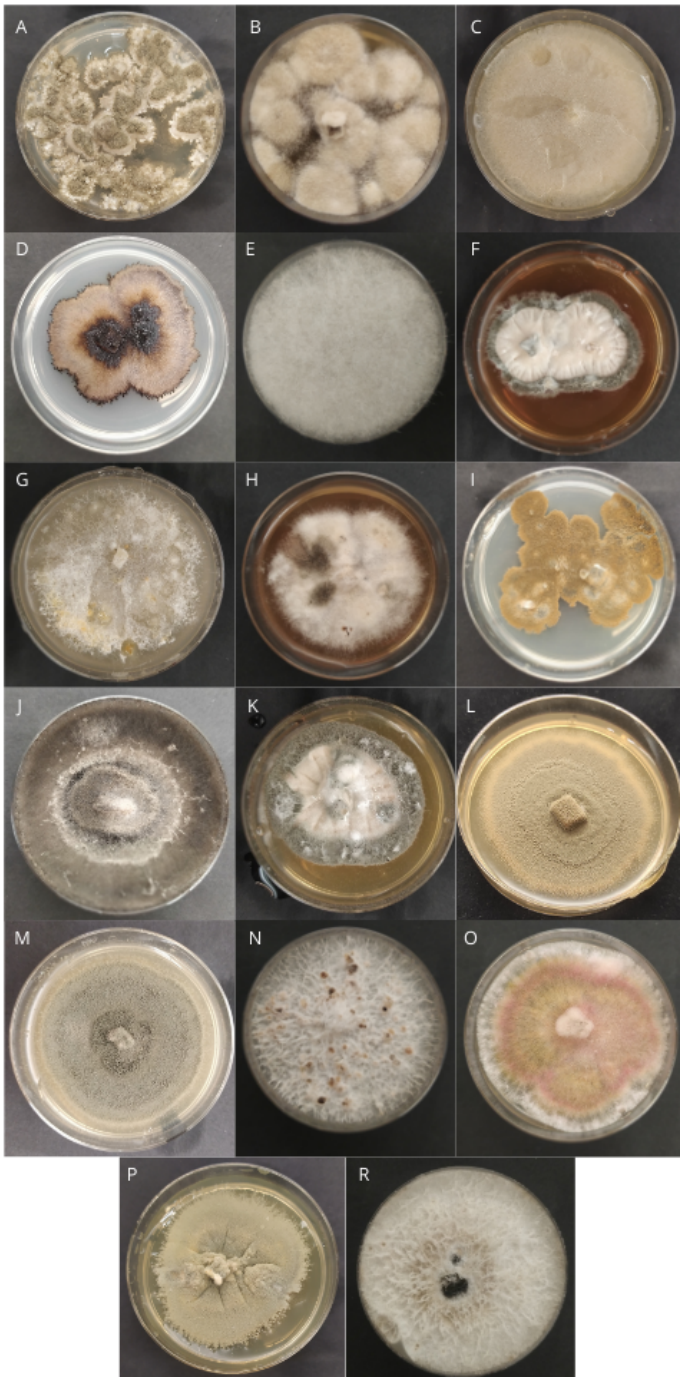


Figure 5 Photos of fungal colonies of the 17 most abundant fungi and their taxonomic assignments: A) Penicillium bialowiezense; B) Briansuttonomyces eucalypti; C) Mucor hiemalis; D) Hormonema macrosporum; E) Rhizopus arrhizus; F) Penicillium sp.; G) Thyronectria sp.; H) Neomicrosphaeropsis juglandis; I) Talaromyces neorugulosus; J) Sphaeropsis sapinea; K) Penicillium nothofagi; L) Penicillium yezoense; M) Penicillium spinuloramigenum; N) Epicoccum tritici; O) Fusarium sp.; P) Talaromyces sp.; R) Apiospora sp..

3.1.1 Control seeds

Fungi were observed on about 98.3% of all control seeds (1,474 seeds out of 1,500). The total number of 2,265 fungal colonies was observed on those seeds and among them 964 representatives were isolated. Obtained cultures were assigned to 165 morphotypes. It was observed that more than 70% of morphotypes (116 out of total 165) were represented by more than one isolate, whereas 49 morphotypes were singletons (i.e., represented by only one isolate). The most abundant morphotypes (i.e., ≥ 1% of total number of isolates, n = 17) represent 70.2% of total 2,265 isolates.

3.1.2 Treated seeds

3.1.2.1 Heat treatment

Fungi were observed on about 43.8% of all heat treated seeds (526 seeds out of 1,200). The total number of 560 fungal colonies was observed on those seeds and among them 191 representatives were isolated. Obtained cultures were assigned to 58 morphotypes. It was observed that more than 58.6% of morphotypes (34 out of total 58) were represented by more than one isolate, whereas 24 morphotypes were singletons (i.e., represented by only one isolate). The most abundant morphotypes (i.e., ≥ 1% of total number of isolates, n = 3) represent 64.1% of total 560 isolates.

3.1.2.2 UVC1 treatment

Fungi were observed on about 96.3% of all seeds treated with UVC light one time (1,155 seeds out of 1,200). The total number of 1,865 fungal colonies was observed on those seeds and among them 603 representatives were isolated. Obtained cultures were assigned to 73 morphotypes. It was observed that more than 82% of morphotypes (60 out of total 73) were represented by more than one isolate, whereas 13 morphotypes were singletons (i.e., represented by only one isolate). The most abundant morphotypes (i.e., ≥ 1% of total number of isolates, n = 18) represent 82.6% of total 1,865 isolates.

3.1.2.3 UVC3 treatment

Fungi were observed on about 96% of all seeds treated with UVC light three times (1,152 seeds out of 1,200). The total number of 1,870 fungal colonies was observed on those seeds and among them 498 representatives were isolated. Obtained cultures were assigned to 75 morphotypes. It was observed that more than 81% of morphotypes (61 out of total 75) were represented by more than one isolate, whereas 14 morphotypes were singletons (i.e., represented by only one isolate). The most abundant morphotypes (i.e., ≥ 1% of total number of isolates, n = 14) represent 80.9% of total 1,870 isolates.

3.2 Differences in fungal seed infection levels across treatments

The differences in fungal seed infection levels per seed lot (i.e., number of infected seeds per SL) were assessed across treatments (i.e., control, heat treatment at 55 ˚C for 8 h, and UVC light treatments repeated one or three times) for all fungi, potentially pathogenic fungi, and *Sphaeropsis sapinea*. Similarly, the differences in fungal seed infection levels were shown across treatments and seed lots, also for all fungi, potentially pathogenic fungi, and *Sphaeropsis sapinea*.

3.2.1 All fungi

No significant differences in the number of infected seeds per SL were observed for all fungi between the seed treatment levels (Kruskal-Wallis: Df = 3, $\chi 2 = 3.499$, P = 0.3208; Fig. 6; Supplementary Table 1). While almost all tested seeds were infected in control and UVC treated seeds (i.e., ~300 seeds per SL), the heat treatment reduced the infection level, in comparison with control treatment, by 55%.

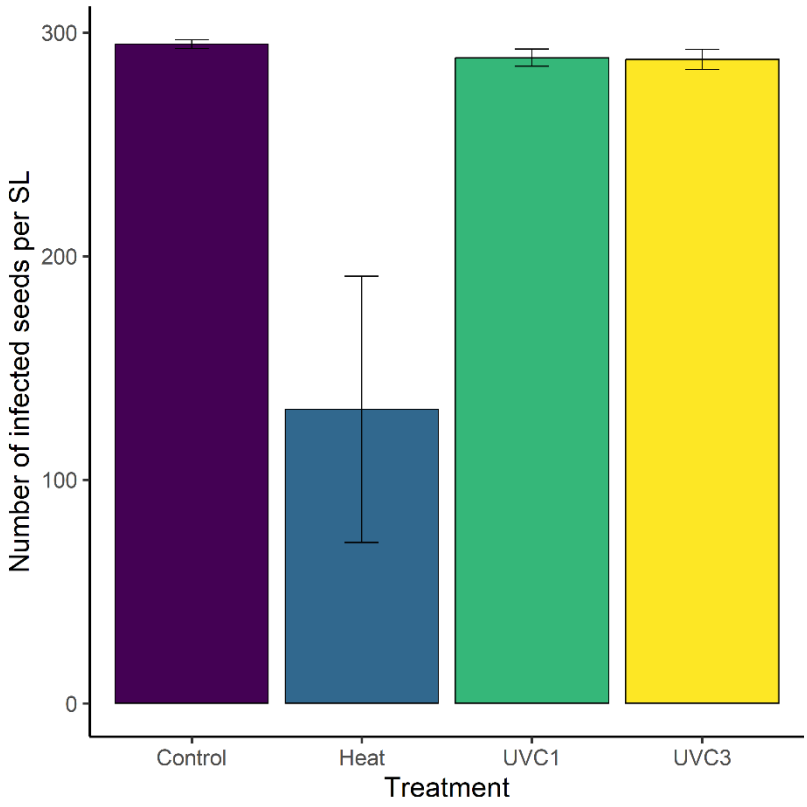


Figure 6 The number of seeds within a seed lot infected with all fungi across four treatments: control (i.e., no treatment; N = 5), heat (i.e., 55 ˚C for 8 h; N = 4), or UVC light one time (i.e., UVC1; N = 4) or three times (i.e., UVC3; N = 4). Values are mean and standard errors.

The infection levels across treatments and seed lots, as shown in Fig. 7, revealed that similarly all seeds were infected for control and UVC treated seeds, while heat treatment reduced the amount of infected seeds for most of the seed lots - by 75% for PC14, by 90% for PC15 and by 58% for PS18. However, the heat treatment did not reduce the infection level for seed lot PM14.

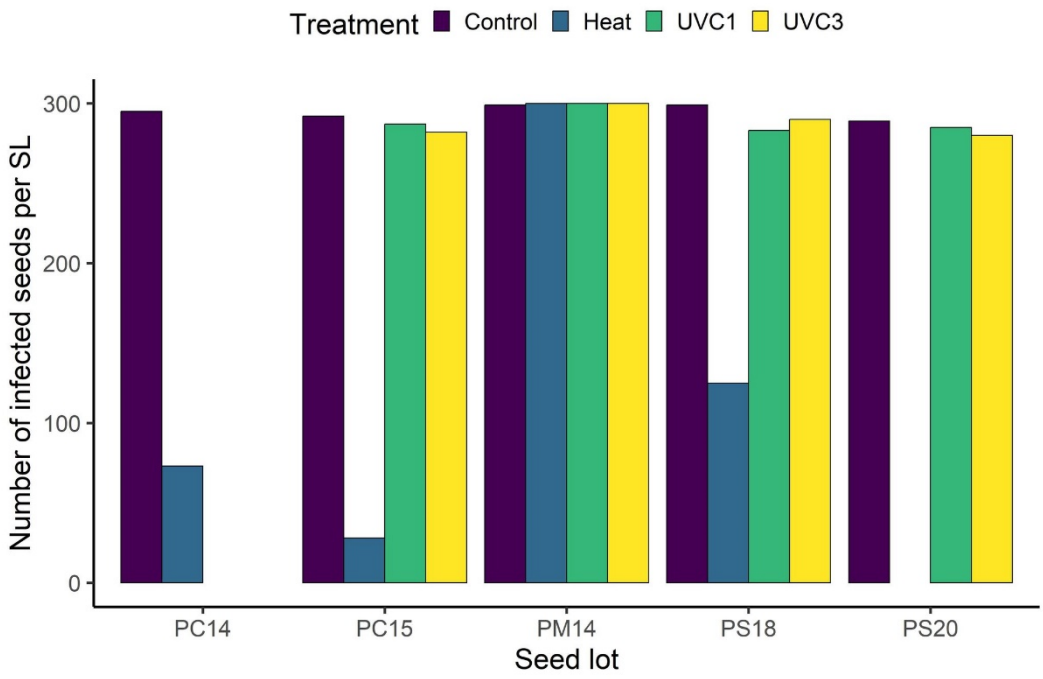


Figure 7 The number of seeds infected with all fungi across four treatments and seed lots. The treatments are control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3). Missing bars are because some seed lots have not been treated, i.e., PC14 with UVC1 and UVC3 and PS20 with heat. Seed lots PC14 and PC15 belong to the tree species Pinus contorta; PM14 to Pseudotsuga menziesii, and PS18 and PS20 to Pinus sylvestris.

3.2.2 Potentially pathogenic fungi

No significant differences in the number of infected seeds per SL were observed for potentially pathogenic fungi between the seed treatment levels (ANOVA: Df = 3, P = 0.964; Fig. 8; Supplementary Table 1). Number of infected seeds for the control treatment was about 145 per SL on average. Compared to control treatment, heat treatment reduced this number of infected seeds by 46%, UVC1 by 11% and UVC3 by 28%.

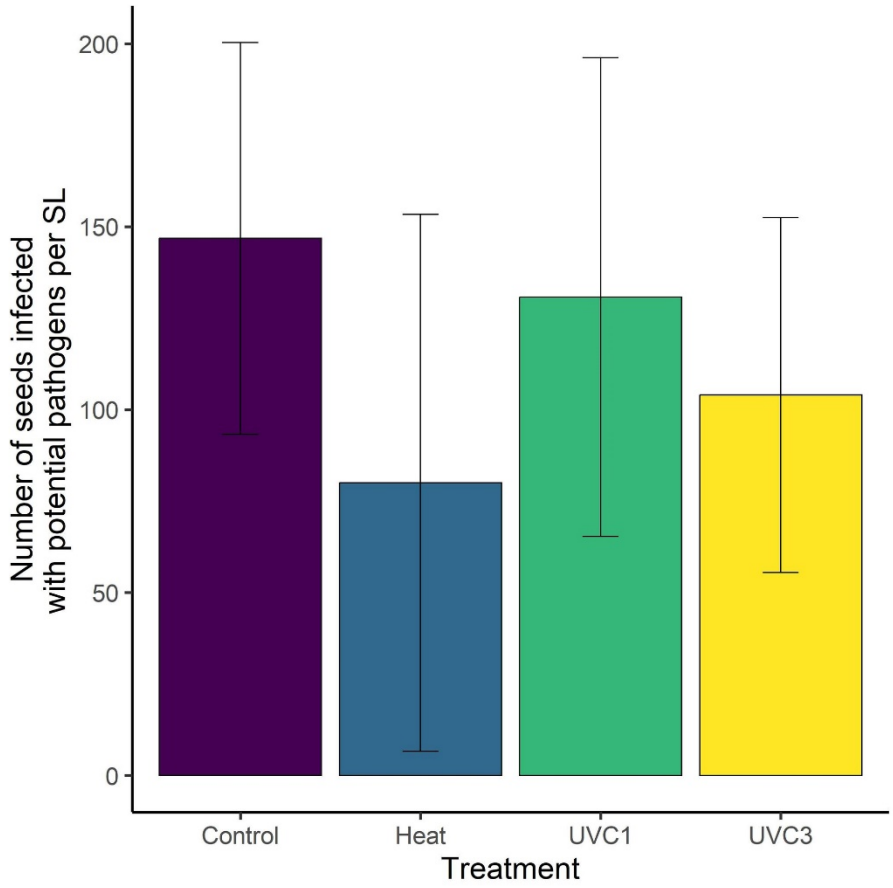


Figure 8 The number of seeds within a seed lot infected with potentially pathogenic fungi across four treatments: control (i.e., no treatment; N = 5), heat (i.e., 55 ˚C for 8 h; N = 4), or UVC light one time (i.e., UVC1; N = 4) or three times (i.e., UVC3; N = 4). Values are mean and standard errors.

The infection level with potentially pathogenic fungi across seed lots and treatments, as shown in Fig. 9, showed for the control treatment less than 70 infected seeds in PC14 and PC15 seed lots, about 120 infected seeds in PS18 seed lot, in comparison with almost all 300 infected seeds in PM14 seed lot, and 255 in PS20 seed lot. Heat treatment, compared to control treatment, reduced the infection level in three seed lots: PC14 by 76%, PC15 by 79% and PS18 by 97%, but not in PM14 in which the infection level was similar as in the control. UVC1 treatment increased the infection level in seed lot PC15 by 475% and in PS20 by 18%, while the infection was reduced in seed lot PS18 by 47%. Similarly, UVC3 treatment increased the infection by 407% in PC15, and reduced it by 54% in PS18 and by

14% in PS20. UVC treatments eliminated all infection by potentially pathogenic fungi in PM14.

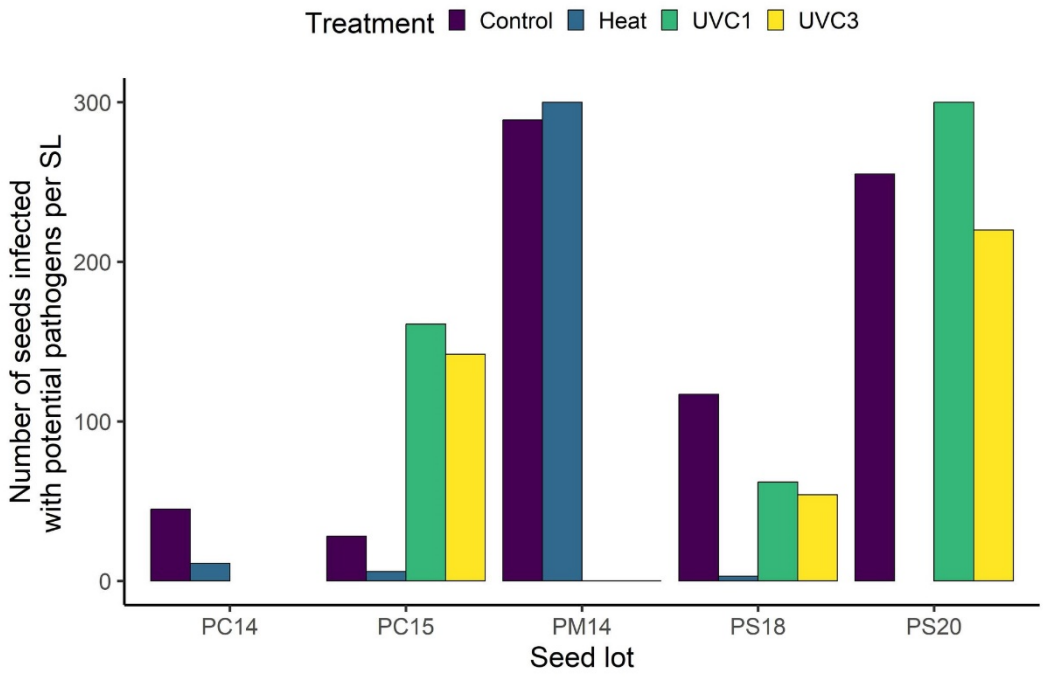


Figure 9 The number of seeds infected with potentially pathogenic fungi across four treatments and seed lots. The treatments are control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3). Missing bars are because some seed lots have not been treated, i.e., PC14 with UVC1 and UVC3 and PS20 with heat. Seed lots PC14 and PC15 belong to the tree species Pinus contorta; PM14 to Pseudotsuga menziesii, and PS18 and PS20 to Pinus sylvestris.

3.2.3 *Sphaeropsis sapinea*

No significant differences in the number of seeds infected with *Sphaeropsis sapinea* per SL were observed between the seed treatment levels (Kruskal-Wallis: Df = 3, $\chi 2 = 0.587$, P = 0.8992; Fig. 10; Supplementary Table 1). Number of infected seeds with *S. sapinea* for the control treatment was less than 20 seeds per SL on average. In comparison with control treatment, all treatments decreased infection level as follows: heat by 95.9%, UVC1 by 72.5% and UVC3 by 60.2%.

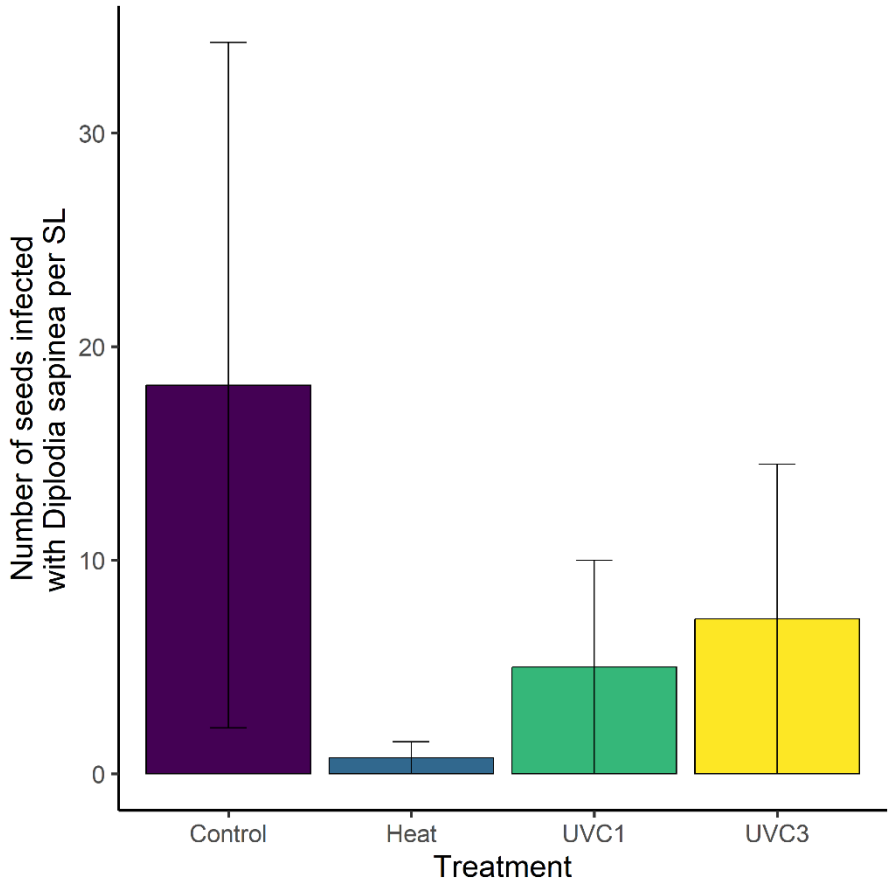


Figure 10 The number of seeds within a seed lot infected with Sphaeropsis sapinea across four treatments: control (i.e., no treatment; N = 5), heat (i.e., 55 ˚C for 8 h; N = 4), or UVC light one time (i.e., UVC1; N = 4) or three times (i.e., UVC3; N = 4). Values are mean and standard errors.

Pathogen infection across seed lots and treatments (Fig. 11) was observed in two out of five seed lots: PC14 and PS18. Considering seed lot PC14, only 9 out of 300 seeds were infected in the control treatment, and after the heat treatment *S. sapinea* could not be isolated from the seeds. A much higher infection occurred in the seed lot PS18 where 82 out of 300 seeds were infected with *S. sapinea*, but this number was reduced after the treatments were applied by 96% after the heat treatment, by 76% after the UVC1 and by 65% after the UVC3 treatment.

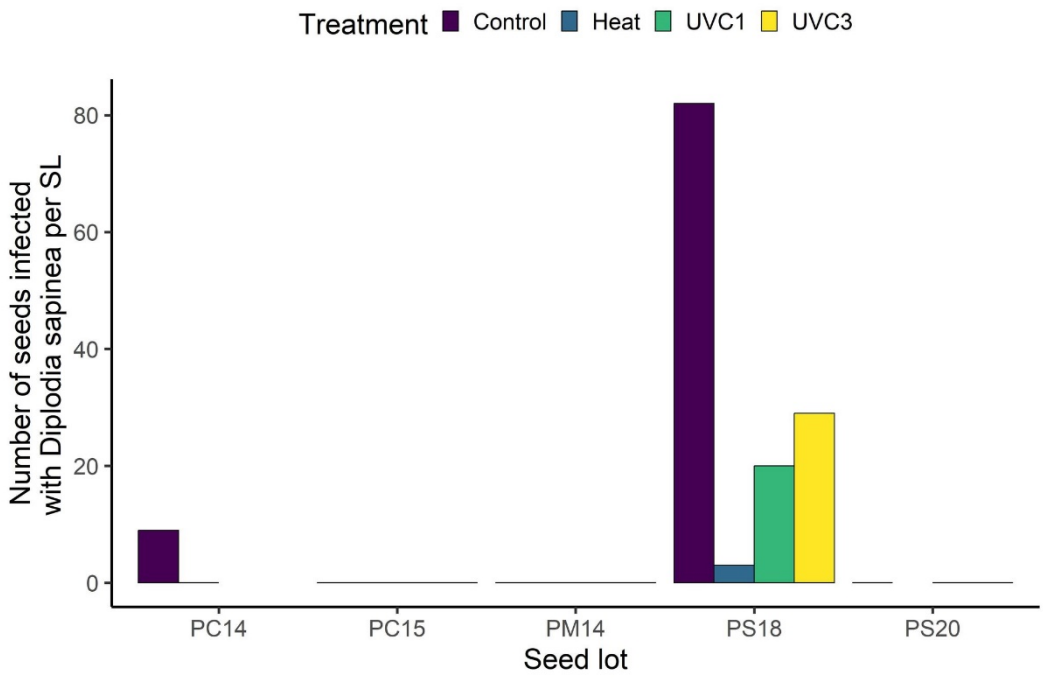


Figure 11 The number of seeds infected with Sphaeropsis sapinea across four treatments and seed lots. The treatments are control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3). Seed lots PC14 and PC15 belong to the tree species Pinus contorta; PM14 to Pseudotsuga menziesii, and PS18 and PS20 to Pinus sylvestris.

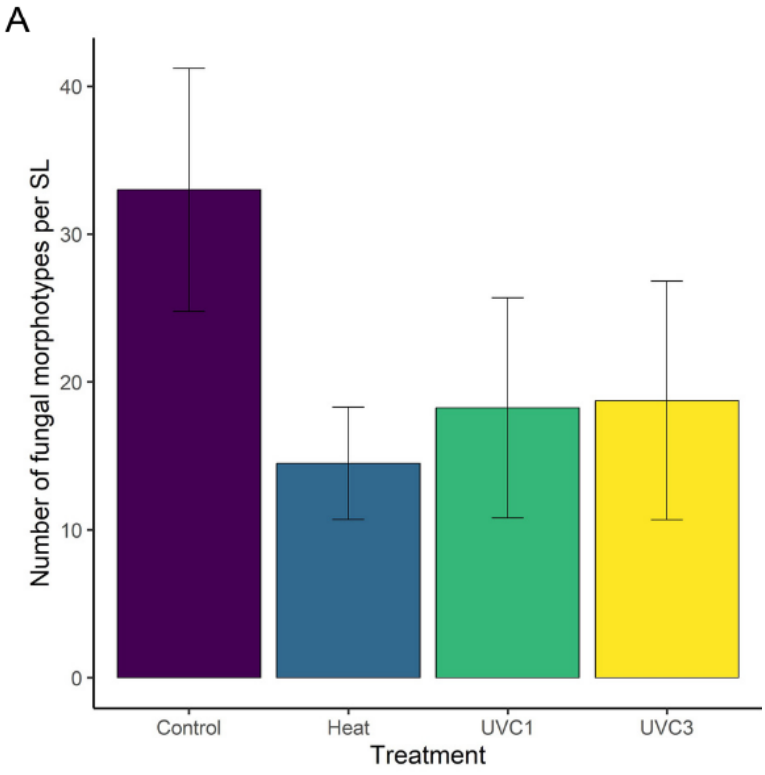
3.3 Differences in fungal occurrence-based diversity across treatments

The differences in fungal occurrence-based diversity per seed lot (i.e., number of fungal morphotypes or species per SL) were assessed across treatments (i.e., control, heat at 55˚C for 8 h, and UVC light one or three times) for all fungi and potentially pathogenic fungi. Similarly, the differences in fungal occurrence-based diversity were shown across treatments and seed lots, also for all fungi and potentially pathogenic fungi. Moreover, species composition of all fungi and potentially pathogenic fungi before and after the treatments was described.

3.3.1 All fungi

3.3.1.1 Number of fungal morphotypes

No significant differences in the number of fungal morphotypes per SL were observed for all fungi between the seed treatment levels (ANOVA: Df = 3, P = 0.3; Fig. 12A; Supplementary Table 2). Number of morphotypes for the control treatment was about 33 per SL on average. This number was reduced by half by all treatments, compared to control treatment: heat reduced it by 56%, UVC1 by 44.7% and UVC3 by 43.2%. Similar results were obtained for when number of fungal species per SL was considered (Fig. 12B).



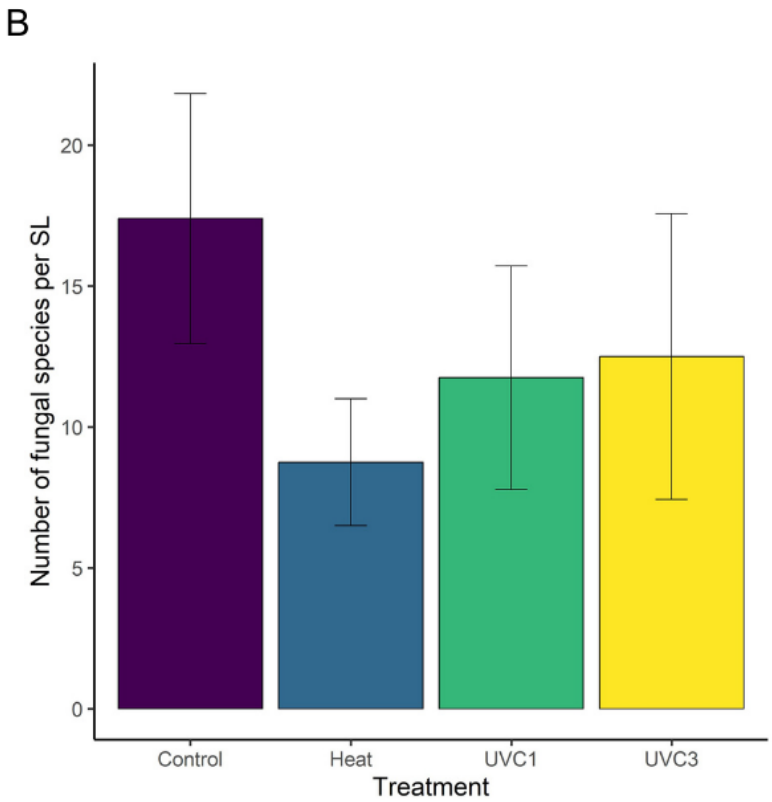


Figure 12 The number of morphotypes (A) and species (B) within a seed lot for all fungi across four treatments: control (i.e., no treatment; N = 5), heat (i.e., 55 ˚C for 8 h; N = 4), or UVC light one time (i.e., UVC1; N = 4) or three times (i.e., UVC3; N = 4). Values are mean and standard errors.

In most cases, the reduction of number of morphotypes after treatments was observed on a seed lot level (Fig. 13A). More than 40 fungal morphotypes were recorded in PC14, PC15 and PS18, as well as 20 morphotypes in PS20 control seeds in comparison with less than 10 in PM14. Heat treatment reduced the number of morphotypes, compared to control treatment, by 70% in seed lots PC14 and PC15, and by half in seed lots PS18 and PM14. UVC1 and UVC3 treatments reduced the number by about 20% in PC15, and by 70% in seed lots PS18 and PM14. UVC treatments increased the number by 20% for seed lot PS20. Similar results were obtained for when number of fungal species per SL was considered (Fig. 13B).

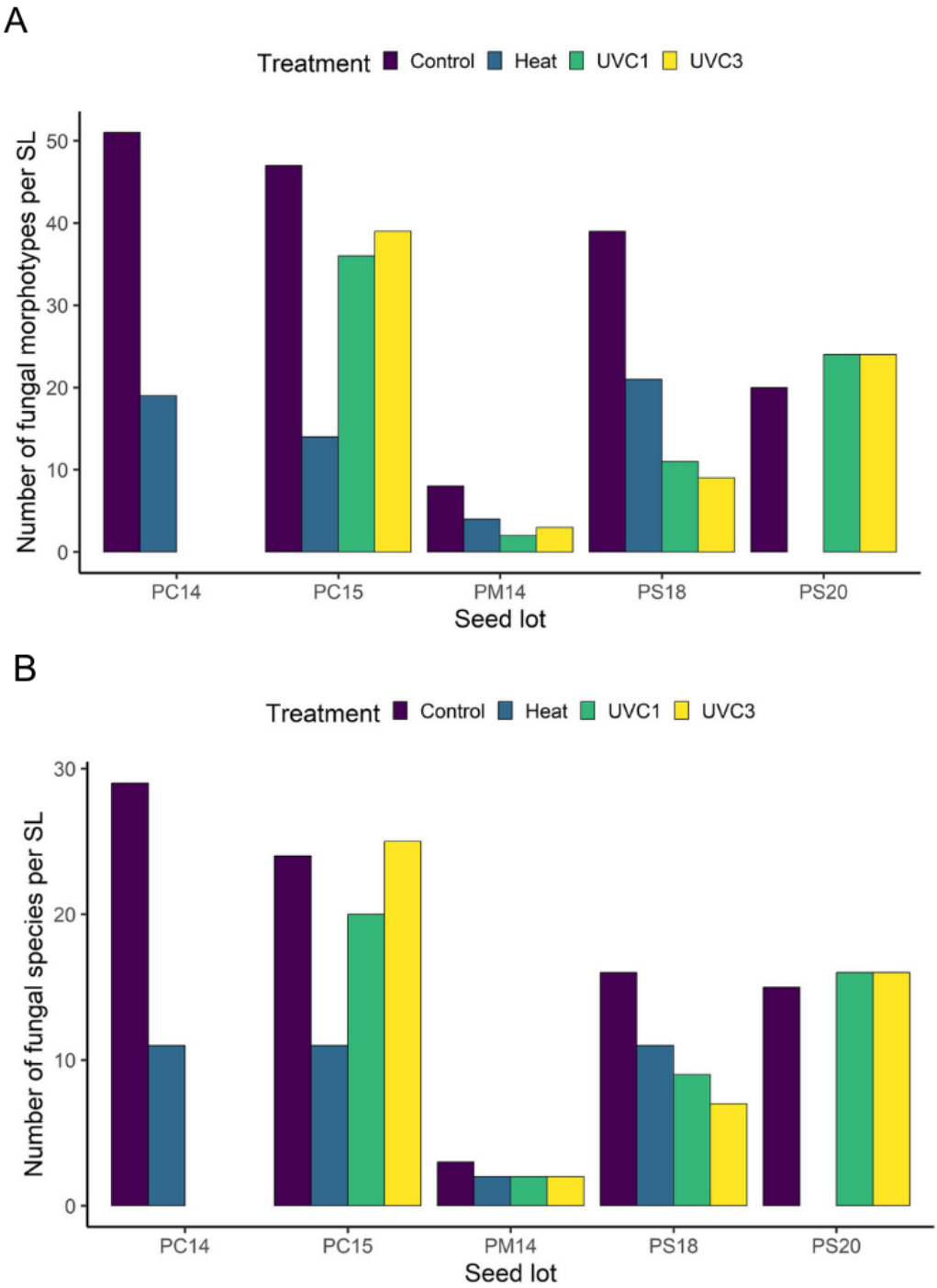
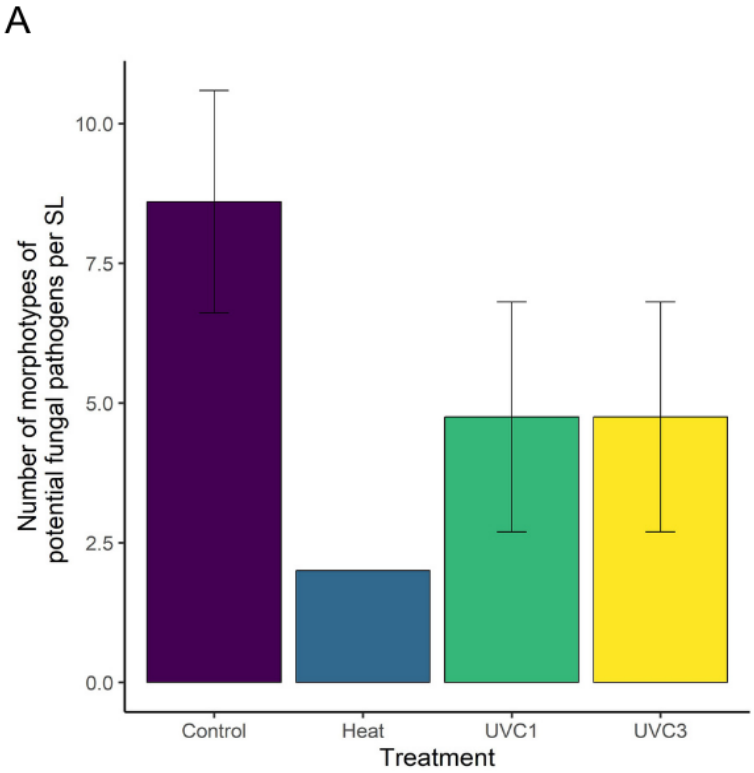


Figure 13 The number of morphotypes (A) and species (B) for all fungi across four treatments and seed lots. The treatments are control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3). Missing bars are because some seed lots have not been treated, i.e., PC14 with UVC1 and UVC3 and PS20 with heat. Seed lots PC14 and PC15 belong to the tree species Pinus contorta; PM14 to Pseudotsuga menziesii; and PS18 and PS20 to Pinus sylvestris.

3.3.2 Potentially pathogenic fungi

3.3.2.1 Number of fungal morphotypes

No significant differences in number of morphotypes of potential fungal pathogens per SL were observed between the seed treatment levels (ANOVA: Df = 3, P = 0.114; Fig. 14A, Supplementary Table 2). Number of morphotypes for the control treatment was less than 10 per SL on average. The overall effect of the heat and both UVC treatments, compared to the control treatment, is as follows: heat reduced the number of morphotypes per SL by 77% and both UVC by about 45%. Similar results were obtained for when number of species of potential fungal pathogens per SL was considered (Fig. 14B).



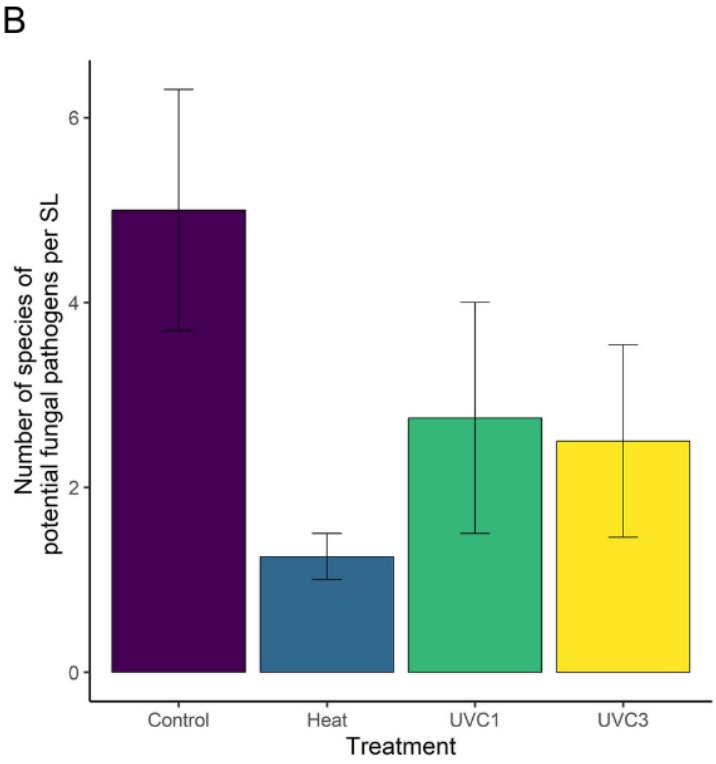


Figure 14 The number of morphotypes (A) and species (B) within a seed lot for potentially pathogenic fungi across four treatments: control (i.e., no treatment; N = 5), heat (i.e., 55 ˚C for 8 h; N = 4), or UVC light one time (i.e., UVC1; N = 4) or three times (i. e., UVC3; N = 4). Values are mean and standard errors.

The number of morphotypes of potential fungal pathogens per SL across four treatments and seed lots, as shown in Fig. 15A, was at a similar level for control treatment in seed lots PC14 (12 morphotypes) and PS18 (14 morphotypes). Six morphotypes were observed in PC15, eight in PS20 and only three in PM14. Heat treatment, compared to control treatment, again resulted in a high reduction of morphotype diversity – numbers were reduced by about 84% in PC14 and PS18 and by 67% in PC15. The heat treatment had no major effect on the number of morphotypes in seed lot PM14. UVC treatments had no major effect on PC15 and PS20 seed lots. However, these treatments reduced the number in PS18 by about 71%. UVC resulted in the absence of any morphotypes in PM14 seed lot. Similar results were obtained for when number of species of potential fungal pathogens per SL was considered (Fig. 15B).

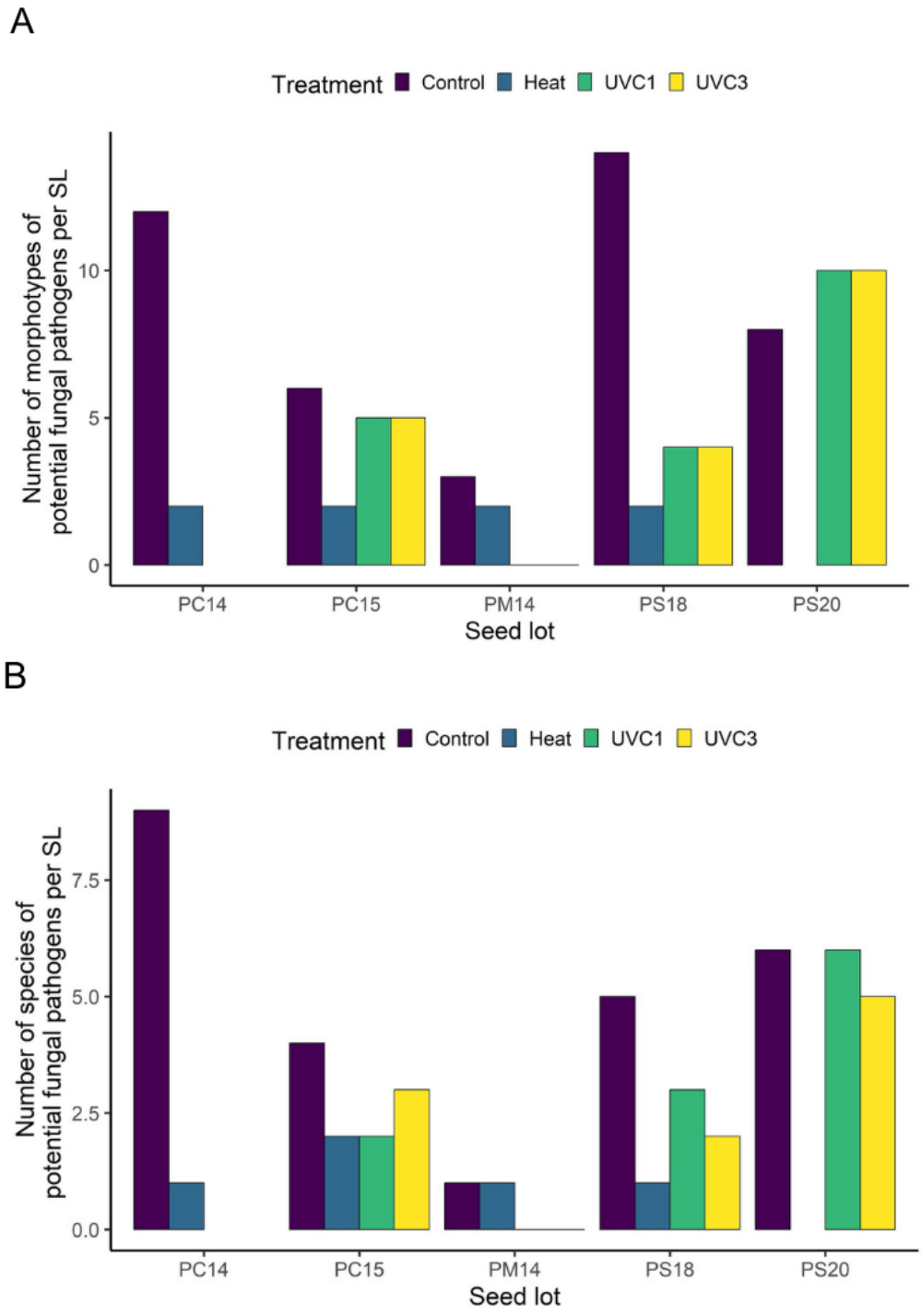


Figure 15 The number of morphotypes (A) and species (B) for potentially pathogenic fungi across four treatments and seed lots. The treatments are control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3; N = 4). Values are mean and standard errors.

3.3.3 Species composition across seed lots and treatments

In total, 36 different fungal species appeared in the seed lot PC14 (Fig. 16), and among these 29 were associated with control seeds and 11 with heat treated seeds. Among all observed species, eight were potentially pathogenic, of which all eight were associated with control seeds and only one with heat. Considering all fungi in general, 25 species were observed only in the control treatment, i.e., were eliminated by heat treatment. These species were: nine *Penicillium* species, i.e., *Penicillium* sp., *Penicillium spathulatum* Frisvad & Samson, *Penicillium bialowiezense, Penicillium terrenum* D.B. Scott*., Penicillium diabolicalicense* Visagie & Seifert*, Penicillium wellingtonense* Houbraken, Frisvad & Samson*, Penicillium lanosum* Westling*., Penicillium robsamsonii* Houbraken & Frisvad and *Penicillium nothofagi*, as well as *Apiospora* sp*., Apiospora kogelbergensis* (Crous) Pintos & P. Alvarado*, Cystobasidium larynges* (Reiersöl) A. M. Yurkov*, Neomicrosphaeropsis juglandis, Pseudocamarosporium lonicerae, Pseudocamarosporium propinquum* (Sacc.) Wijayaw., Camporesi & K.D.Hyde*, Sciadopityaceae, Talaromyces* sp*., Thyronectria* sp. and *Mucor hiemalis*„ including seven potentially pathogenic species: *Allantophomopsiella pseudotsugae* (M.Wilson) Crous, *Alternaria alstroemeriae* E.G. Simmons & C.F. Hill*, Sphaeropsis sapinea*, *Fusarium* sp., *Heterotruncatella spartii* (Senan. et al.) F. Liu, L. Cai & Crous*, Penicillium bialowiezense* and *Phoma* sp. Four fungal species that were detected in the control treatment were not eliminated by heat treatment. These species were: *Briansuttonomyces eucalypti, Hormonema macrosporum, Talaromyces neorugulosus* and one potentially pathogenic, *Pestalotiopsis hollandica* Maharachch., K.D. Hyde & Crous. The following seven species were unique for heat treatment (i.e., possible contamination): *Capronia* sp*., Coniochaeta* sp*., Cystobasidium slooffiae (*E.K. Novák & Vörös-Felkai) Yurkov et al., *Epicoccum* sp*., Neocucurbitaria juglandicola* Jaklitsch & Voglmayr*, Penicillium miczynskii* K.M. Zalessky and *Thyronectria lamyi* (Desmazières) Seeler.

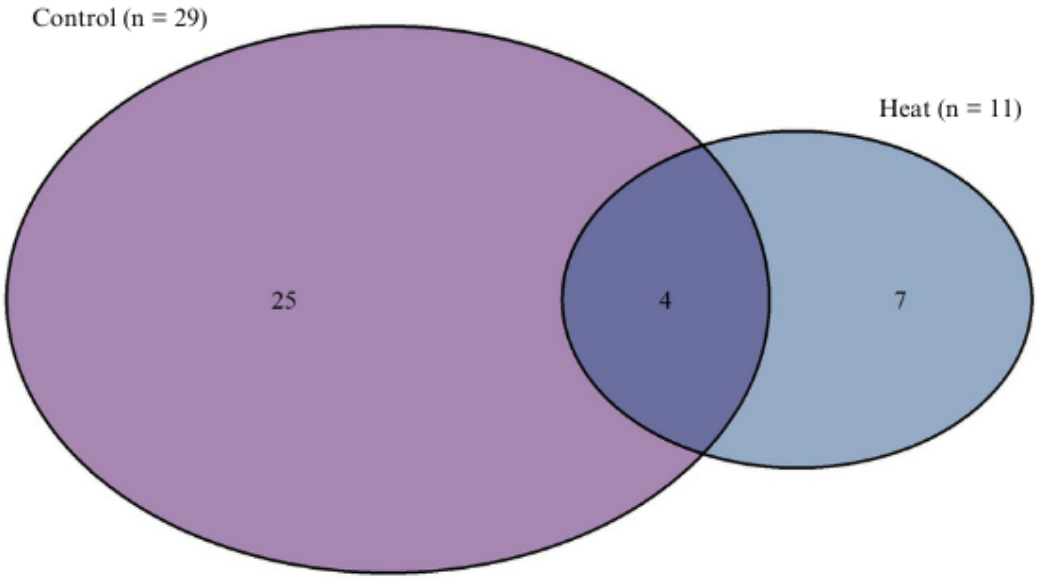


Figure 16 The number of fungal species in Pinus contorta seed lot PC14 that occurred between different treatments: control (i.e., no treatment) and heat (i.e., 55 ˚C for 8 h).

In total, 42 different fungal species appeared in seed lot PC15 (Fig. 17), and among these, 24 were associated with control seeds, 11 with heat treated seeds, 20 with seeds treated by UVC light one time and 25 with seeds treated by UVC light three times. Among all observed species, five were potentially pathogenic, of which four were associated with control seeds, two with heat, two with UVC1 and three with UVC3. Considering all fungi in general, seven species were observed only in the control treatment, i.e., were eliminated by all treatments. These species were: *Apiospora sp*., *Coprinellus sp*., *Neomicrosphaeropsis juglandis*, *Penicillium charlesii* G. Smith, *Samsoniella hepiali* (Q.T. Chen & R.Q. Dai ex R.Q. Dai et al.) H. Yu, *Penicillium speluncae* Visagie & N. Yilmaz, including one potentially pathogenic species: *Talaromyces rugulosus* (Thom) Samson, Yilmaz, Frisvad & Seifert. Four fungal species that were detected in the control treatment were not eliminated by neither of the treatments. These species were *Penicillium miczynskii, Hormonema macrosporum, Thalaromyces neorugulosus* and one potentially pathogenic species, *Penicillium bialowiezense.* The number of species unique for treatments (i.e., possible contamination) were as follows: *Capronia sp., Cystobasidium slooffiae, Neocucurbitaria juglandicola* and *Thyronectria lamyi* for

the heat treatment; *Trichothecium sp.* and *Mucor hiemalis* for the UVC1 treatment; *Penicillium maclennaniae H.Y.* Yip., *Penicillium nothofagi, Pseudocamarosporium propinquum, Thyronectria berberidicola* R.Ma & S.N.Li, *Trichothecium ovalisporum* (Seifert & S.A.Rehner), *Penicillium fellutanum,* including one potentially pathogenic, *Cladosporium subuliforme* for the UVC3 treatment.

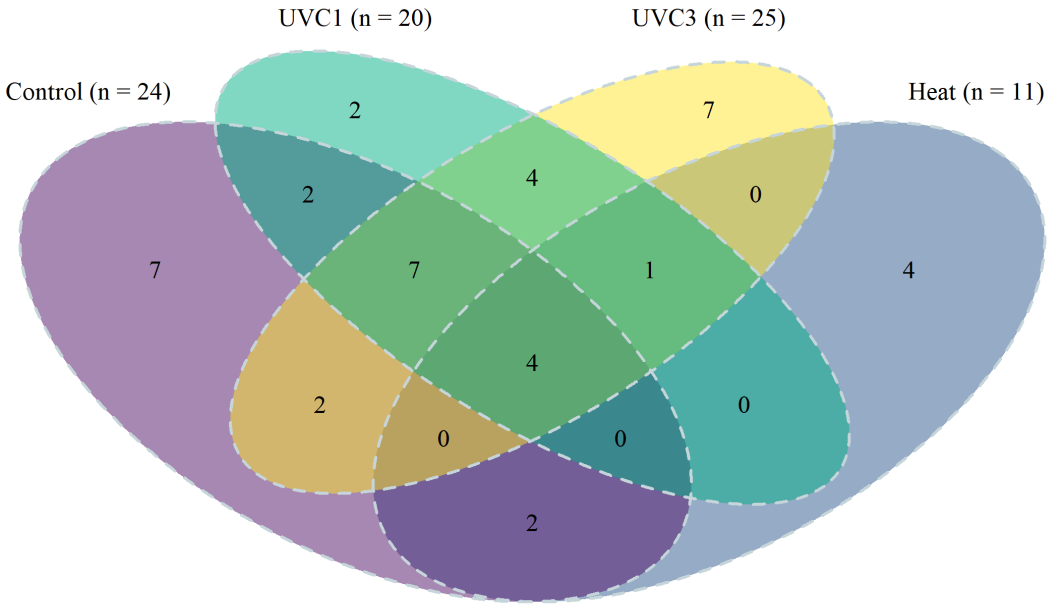


Figure 17 The number of fungal species in Pinus contorta seed lot PC15 that occurred between different treatments: control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3).

In total, 25 different fungal species appeared in the seed lot PS18 (Fig. 18), and among these, 17 were associated with control seeds, 12 with heat treated seeds, nine with seeds treated by UVC light one time and seven with seeds treated by UVC light three times. Among all observed species, six were potentially pathogenic, of which five were associated with control seeds, one with heat, three with UVC1 and two with UVC3. Considering all fungi in general, seven species were observed only in the control treatment, i.e., were eliminated by all treatments. These species were:

Apiospora sp., *Neoascochyta paspali* (P.R. Johnst.) Q. Chen & L. Cai, *Neodidymelliopsis camporesii* D. Pem, Doilom & K.D. Hyde, *Nothophoma sp.* and *Penicillium thomii* Maire, including two potentially pathogenic species: *Alternaria angustiovoidea* E.G. Simmons and *Dothiorella sarmentorum* (Fr.) A.J.L. Phillips, Alves & Luque. Two fungal species that were detected in the control treatment were not eliminated by neither of the treatments. These species were: *Nectria dematiosa* (Schwein.) Berk. and the pathogen *Sphaeropsis sapinea*. The number of species unique for treatments (i.e., possible contamination) were as follows: *Macroventuria sp., Penicillium sp., Penicillium tardochrysogenum* Frisvad, Houbraken & Samson, *Naganishia liquefaciens* (Saito & M. Ota) X.Z. Liu *et al., Aspergillus creber* for the heat treatment, and one potentially pathogenic fungal species, *Penicillium bialowiezense*, for UVC1 treatment.

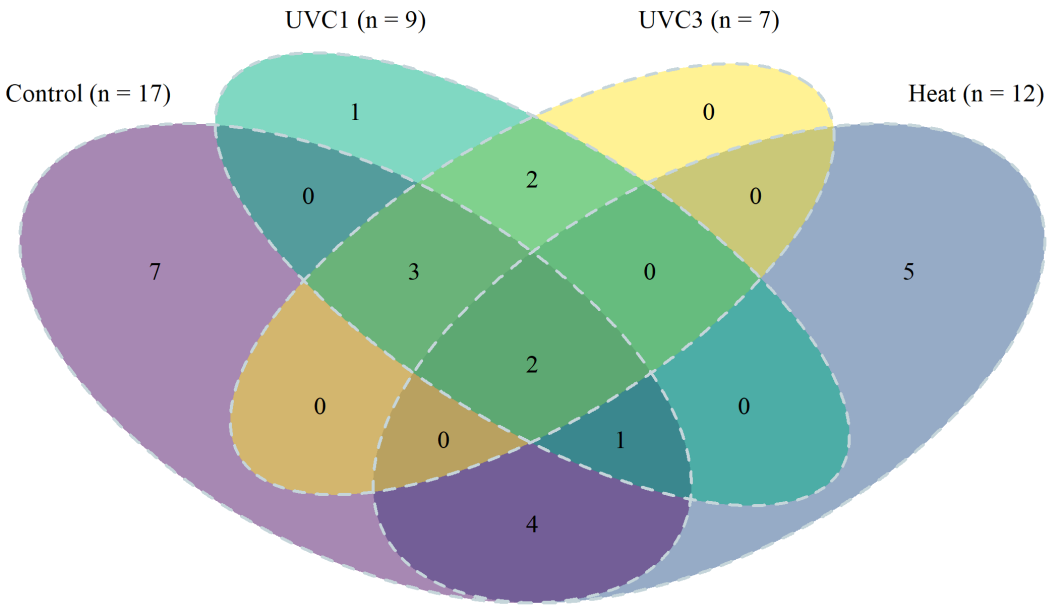


Figure 18 The number of fungal species in Pinus sylvestris seed lot PS18 that occurred between different treatments: control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3).

In total, 16 different fungal species appeared in the seed lot PS20 (Fig. 19), and among these, 15 were associated with control seeds, 16 with seeds treated by UVC light one time and 16 with seeds treated by UVC light three times. Among all observed species, six were potentially pathogenic, of which all six were associated with control and UVC1 treated seeds and five with UVC3 treated seeds. No fungal species was observed that was eliminated by each of the treatments, and in total 14 species survived all three treatments, including: *Apiospora* sp., *Aspergillus oerlinghausenensis, Briansuttonomyces eucalypti, Hormonema macrosporum, Nectria dematiosa, Penicillium fimorum* Houbraken & Frisvad, *Penicillium spinuloramigenum, Penicillium yezoense*, *Mucor hiemalis,* and five potentially pathogenic species, *Cladosporium subuliforme, Fusarium sp., Penicillium bialowiezense, Penicillium thomii* and *Stagonosporopsis lupini* (Boerema & R. Schneid.) Boerema, Gruyter & P. Graaf. One species, *Mucor plumbeus* Bonord., was unique for UVC3 treatment (i.e., possible contamination).

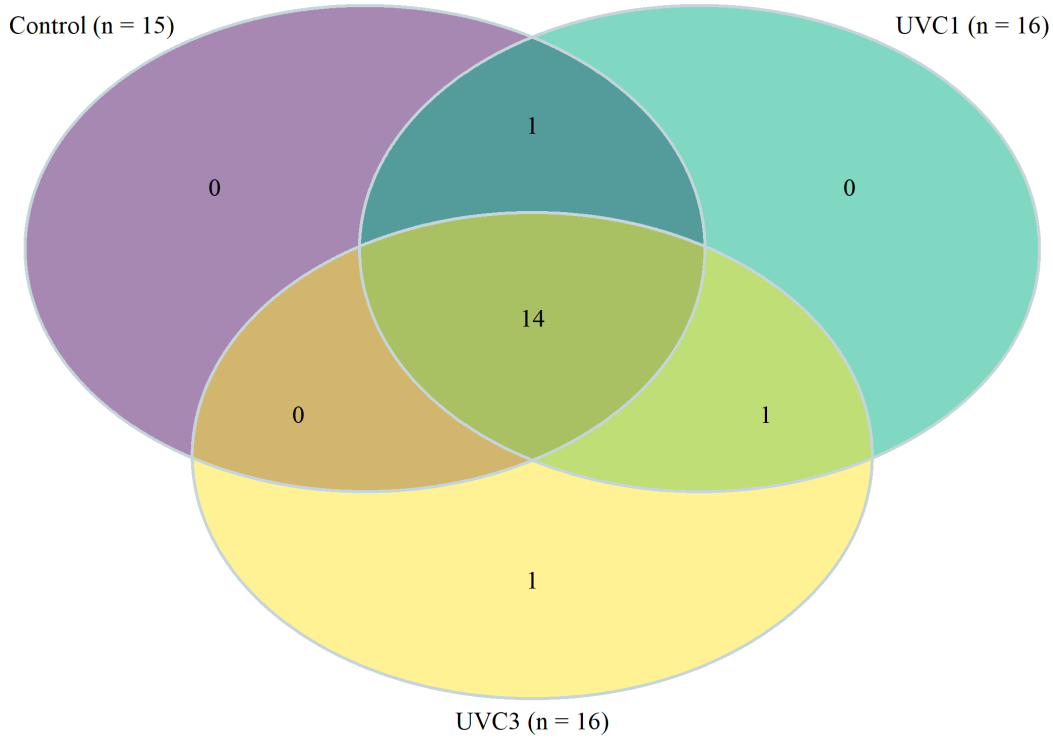


Figure 19 The number of fungal species in Pinus sylvestris seed lot PS20 that occurred between different treatments: control (i.e., no treatment), UVC light one time (i.e., UVC1) or three times (i.e., UVC3).

In total, five different fungal species appeared in the seed lot PM14 (Fig. 20), and among these, three were associated with control seeds, two with heat treated seeds, two with UVC1 treated seeds and two with UVC3 treated seeds. Among all observed species, one was potentially pathogenic, and associated only with control and heat treated seeds. One fungal species, *Trichoderma atroviride*, was observed only in the control treatment, i.e., was eliminated by all treatments. Out of three species that appeared in the control treatment, the heat treatment did not eliminate two of them: *Trichoderma simmonsii* P. Chaverri, F.B. Rocha and the pathogen *Rhizopus arrhizus*. Two species, *Penicillium nothofagi* and *Mucor hiemalis*, were unique for both UVC treatments (i.e., possible contamination).

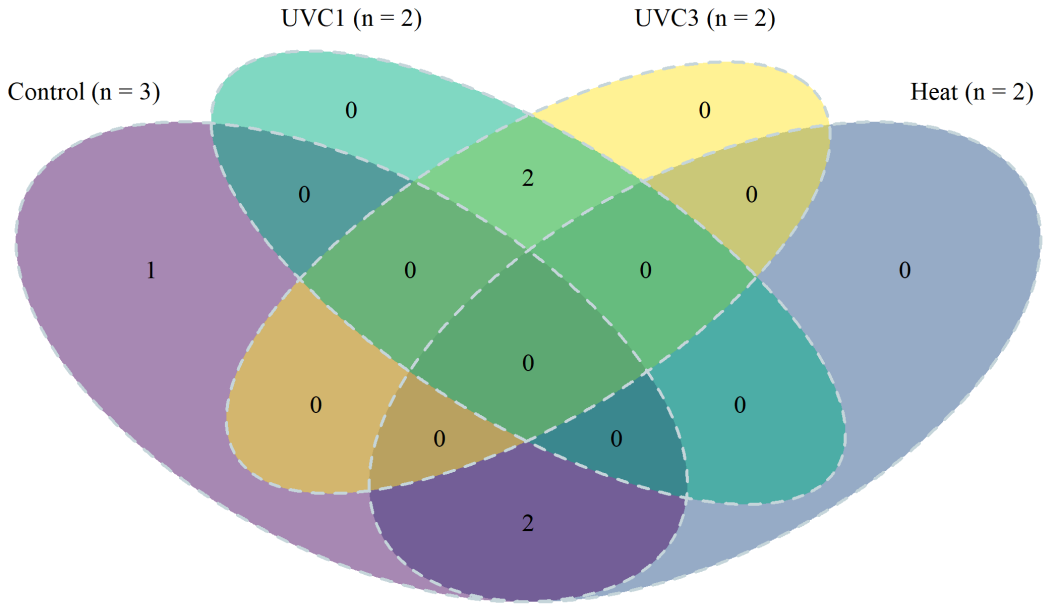


Figure 20 The number of fungal species in Pseudotsuga menziesii seed lot PM14 that occurred between different treatments: control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3).

3.4 Differences in seed germination among treatments

The differences in seed germination per SL (i.e., germination capacity or energy per SL) were assessed across treatments (i.e., control, heat treatment at 55˚C for 8 h, and UVC light treatments repeated one or three times). Similarly, the differences in seed germination were also shown across treatments and seed lots.

3.4.1 Germination capacity

No significant differences in germination capacity per SL were observed between the seed treatment levels (Kruskal-Wallis: Df = 3, $\chi 2 = 2.3802$, P = 0.4973; Fig. 21; Supplementary Table 3). Germination capacity for the control treatment resulted in approximately 90% per SL on average. When considering the overall effect of the treatments, compared to the control, no great differences are observed, as heat treatment reduced germination capacity by only 5%, UVC1 by 3% and UVC3 by 2%.

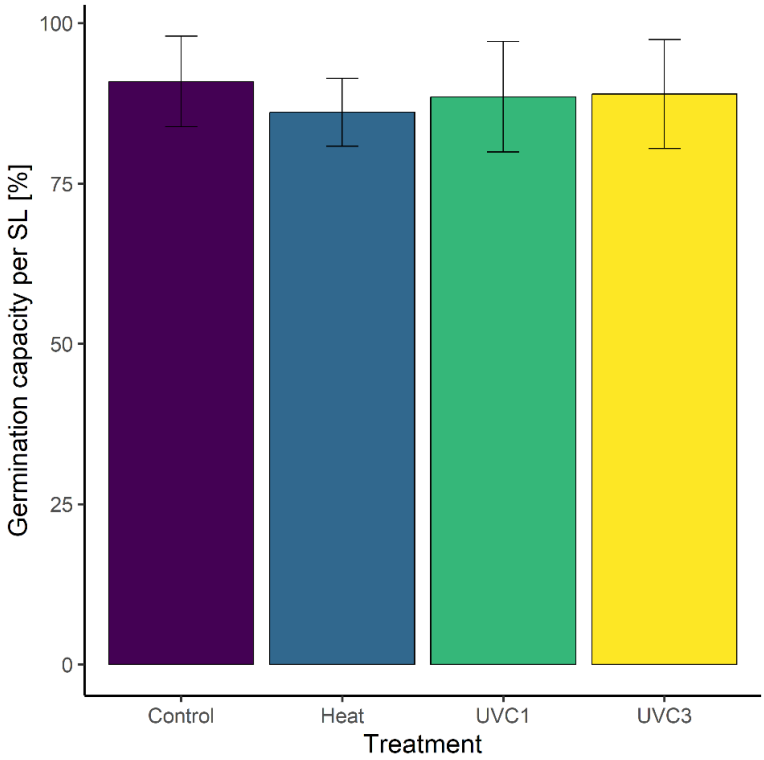


Figure 21 The germination capacity within a seed lot across four treatments: control (i.e., no treatment; N = 5), heat (i.e., 55 ˚C for 8 h; N = 4), or UVC light one time (i.e., UVC1; N = 4) or three times (i.e., UVC3; N = 4). Values are mean and standard errors.

Germination capacity across seed lots and treatments as shown in Fig. 22 was at the similar level between all treatments for seed lots PC14, PC15, PS18 and PS20 and varied between 90-100%. Germination capacity of seeds in the seed lot PM14, was around 60-70%.

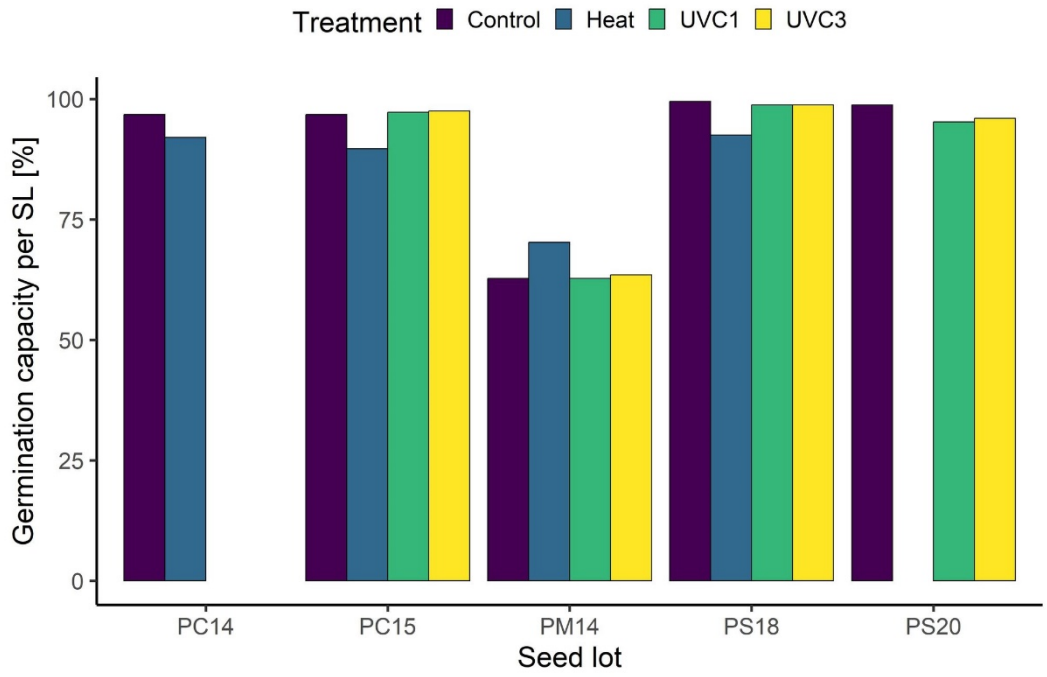


Figure 22 The germination capacity across four treatments and seed lots. The treatments are control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3). Missing bars are because some seed lots have not been treated, i.e., PC14 with UVC1 and UVC3 and PS20 with heat. Seed lots PC14 and PC15 belong to the tree species Pinus contorta; PM14 to Pseudotsuga menziesii; and PS18 and PS20 to Pinus sylvestris.

3.4.2 Germination energy

No significant differences in seed germination energy per SL were observed between the seed treatment levels (Kruskal-Wallis: Df = 3, $\chi 2 = 0.6294$, P = 0.8897; Fig. 23; Supplementary Table 3). Germination energy for the control treatment was around 75% per SL on average. In this case, the heat treatment reduced germination energy value, compared to the control treatment, by 10%, and both UVC treatments slightly increased it by, respectively: UVC1 by 3% and UVC3 by 5%.

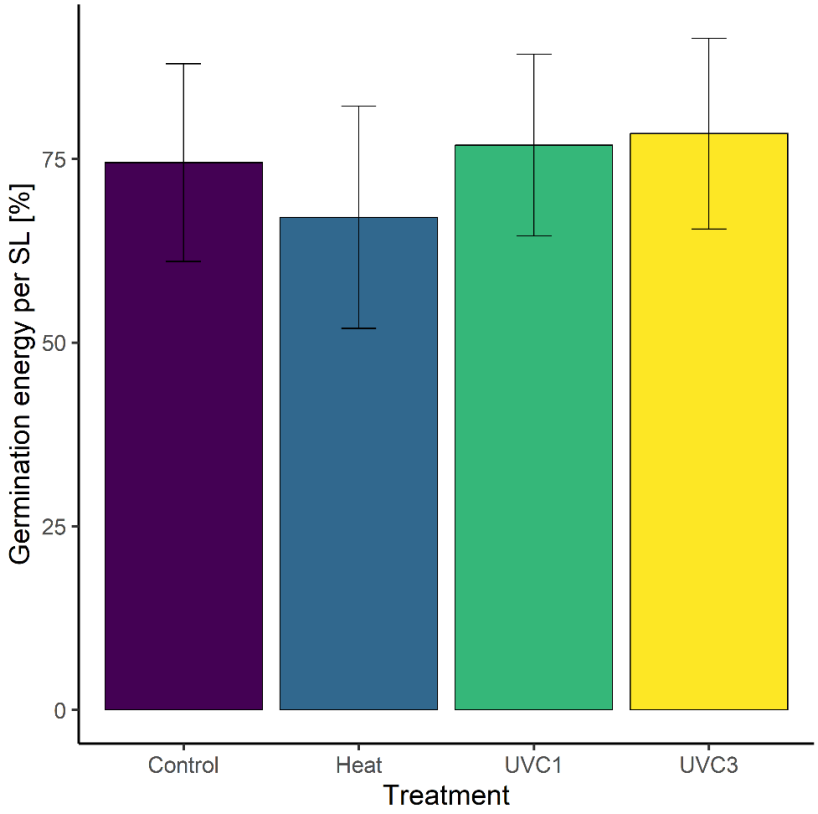


Figure 23 The germination energy within a seed lot across four treatments: control (i.e., no treatment; N = 5), heat (i.e., 55 ˚C for 8 h; N = 4), or UVC light one time (i.e., UVC1; N = 4) or three times (i.e., UVC3; N = 4). Values are mean and standard errors.

Germination energy across seed lots and treatments (Fig. 24) was the highest in seed lots PS18 and PS20, i.e., almost 100% for all treatments. Lower overall germination energy, i.e., around 70% was observed for seed lots PC14 and PC15. In the seed lot PM14 germination energy was about 25% in the control treatment, and the value remained similar after the heat treatment. However, both UVC treatments increased germination energy by half.

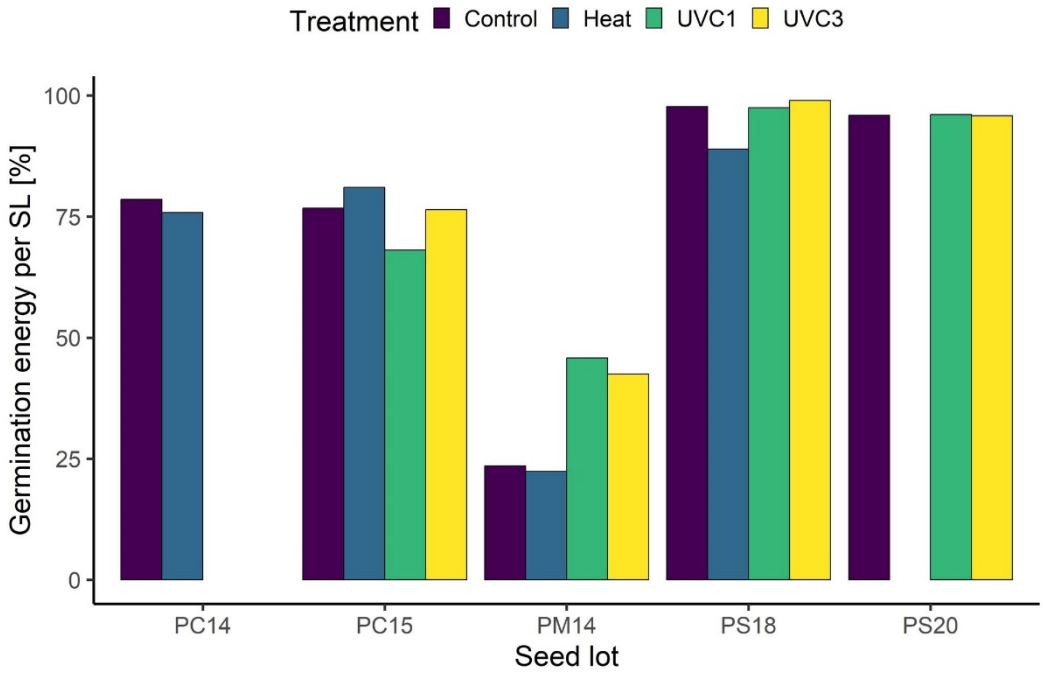


Figure 24 The germination energy across four treatments and seed lots. The treatments are control (i.e., no treatment), heat (i.e., 55 ˚C for 8 h), or UVC light one time (i.e., UVC1) or three times (i.e., UVC3). Missing bars are because some seed lots have not been treated, i.e., PC14 with UVC1 and UVC3 and PS20 with heat. Seed lots PC14 and PC15 belong to the tree species Pinus contorta; PM14 to Pseudotsuga menziesii; and PS18 and PS20 to Pinus sylvestris.

4. Discussion

This study assessed the infection level and diversity of all and potentially pathogenic fungi (with a focus on *Sphaeropsis sapinea*) in seed lots of Lodgepole pine, Scots pine and Douglas-fir trees before and after the heat treatment at 55 °C for 8 h and UVC light treatments. Seed germination was also assessed before and after the treatments to ensure seed viability post treatment. Therefore, this study provides new knowledge for improved risk management of introduction and spread of fungal pathogens with seeds used in reforestation nurseries using environmentally friendly treatments with no effect on seed germination.

This study confirmed a high level of infection by fungi, including potentially pathogenic fungi and *S. sapinea*, as well as high diversity of all and plant pathogenic fungi associated with the seeds of Lodgepole pine and Scots pine, which is similar to previous findings (de la Bastide et al., 2019; Blumenstein et al., 2021). The overall diversity, however, varied across species, with lower fungal diversity in Douglas-fir seeds in comparison with the pine seeds tested. Therefore, the results indicate that seeds of the study tree species, mainly pine seeds, may serve as a pathway for the introduction of pathogenic fungi into forest nurseries, causing diseases of tree seedlings with a negative impact on forest regeneration.

The results in this study show no statistically significant differences in fungal infection levels and diversity among treatments, although the average values show high variation across treatments. Consequently, none of the hypotheses were statistically confirmed. Possible reason for this is the small number of seed lots used in this study, together with the high variation in infection levels and diversity across seed lots. To properly test these differences statistically and obtain more reliable results, the number of seed lots of the studied tree species, should be increased.

The results showed that heat treatment at 55 °C for 8 h reduces fungal infection levels in the Lodgepole pine and Scots pine seed lots by 80% and 50%, respectively. A high reduction of approximately 90% in infection by potentially pathogenic fungi and *S. sapinea* was also observed in the infected seed lots. The overall fungal diversity and the diversity of potentially pathogenic fungi associated with Lodgepole pine and Scots pine seeds were almost always reduced by at least 50%. In summary, heat treatment at 55 °C for 8 h reduced fungal infections and diversity in pine seeds and could be used to minimize the incidence of seed-borne plant pathogens in forest nurseries. However, not all fungi could be eliminated from the seeds of the studied tree species, especially in the case of Douglas-fir, and these should be investigated further to assess the likelihood of their impact on seed and seedling health.

UVC light did not reduce infection levels by all fungi, but it did reduce overall fungal diversity. Moreover, infection levels by plant pathogenic fungi increased by more than 400% in PC15 and with UVC1 by 18% in PS20 post-treatment while pathogenic fungi were reduced by half in seed lot PS18, with UVC3 by 14% in seed lot PS20 and eliminated in the seed lot PM14. The reason for this could be that fungi that survived the UVC treatment (approximately 50% of control fungi) infected the seeds at a higher frequency during storage prior to plating. This can be explained by the higher infection of the surviving fungal species after UVC treatment; thus, it is possible that UVC had a positive effect on sporulation of some infecting fungi, and fungal conidia spread faster, attacking more seeds.

None of the treatments had a significant negative effect on the germination of the tested seeds indicating that the treatments performed in this study can be used to reduce or eliminate fungal infection in conifer seeds of the studied tree species without adversely effecting germination.

4.1 Effect of the seed treatments on fungal infection levels

4.1.1 All fungi

This study showed the high overall fungal infection levels of control seeds of Lodgepole pine, Scots pine, and Douglas-fir. The high level of infection in these tree species is not surprising, as it has already been confirmed in other studies. For instance, in a study by Kowalczyk et al. (2004), 2,710 fungal colonies representing approximately 50 species were isolated from 1,800 seeds of Scots pine. Seeds of Douglas-fir can also host hundreds of various fungi (Bakewell-Stone, 2023), and the infection of a few main fungal species has been described in Lodgepole pine seeds (Drenkhan et al., 2016; Watt et al., 2012).

Our results showed that heat treatment can be highly effective in reducing the infection levels by fungi of Lodgepole pine and Scots pine seeds, but not of Douglas-fir seeds. This variation might be linked to the size of the seed, as, according to Bond et al. (1999), it is the seed size on which the effectiveness of the heat treatment depends. It could be that seeds of a larger size require more time to reach the desired temperature in the centre of the seed, and a longer duration of treatment might be needed to eliminate fungal infection throughout the seed (Shi et al., 2016; Birmpa et al., 2013). Douglas-fir seeds reach lengths of 4-7mm, whereas Lodgepole pine and Scots pine seeds have very similar, smaller, lengths of 2-5mm and 3-5mm, respectively (Kolotelo, 1997; Farinha et al., 2018). Considering this, the lack of the heat treatment effect on Douglas-fir seeds may be due to the larger size of Douglas-fir seeds compared to the seeds of Lodgepole pine and Scots pine.

It was assumed that UVC treatments would also contribute to the reduction of fungal infection in the studied seed lots. However, this assumption has been proven incorrect, as almost 100% of seeds after UVC were infected consistently across all seed lots. The most likely reason for this result was fungi that survived the UVC treatment (around 50% of fungi observed in control seeds) and infected more seeds during storage prior plating. It could be also possible that the fungi that survived the UVC treatment were already present in almost all 300 treated seeds, so that their

infection did not necessarily increase after this treatment, however, more isolates of these fungi were observed after the treatment. The low efficiency of UVC treatments on some fungal species that survived may be due to a series of UV protective mechanisms by which fungi have evolved in response to the selective pressure exerted by radiation (Wong et al., 2019). Fungi produce pigments, such as melanin, carotenoids, and mycosporins, that act as 'sunscreens', minimize damage by radiation exposure, and prevent intercellular damage (Avalos & Limón, 2015; Belozerskaya et al., 2017). Also, phenols and terpenes are considered important defence compounds in conifers (Krokene et al., 2008; Eyles et al., 2010), yet few attempts have been made to correlate UV-induced changes in tissue chemistry with disease resistance (Manning & Vontiedemann, 1995; Witzell & Martin, 2008). This result may also have been influenced by the fact that UVC does not penetrate inside the seed, but acts on the surface (Birmpa et al., 2013; Luckey, 1980), therefore the fungi that appear inside of the seeds were not eliminated. Another reason for this could be that UVC may not have reached each side of the seeds, which can create a problem with small products, such as conifer seeds. It is possible that some fungi on the seed surface that were not exposed to and reached by UVC light survived. The exposure of seeds to the light treatment can be achieved by installing vibrating belts or a vibrating gutter so that UVC light can shine on all sides of the seeds (CleanLight, 2022). The possible insufficient dose of exposure to UVC light should also be considered. Too little exposure to the treatment may lead to poor ultraviolet absorption, thus failing to damage the fungal DNA, which will remain active (Wong et al., 2019). Therefore, fungal growth and survival can be strongly influenced by methodology, various adaptive mechanisms, and exposure to ultraviolet radiation. The effects of UVC on conifers are not well described; therefore, more research is needed to understand the lack of effect of UVC treatments on seed fungal infection of the tree species studied and to ascertain whether this treatment can be used to eliminate fungal infection.

4.1.2 Potentially pathogenic fungi

Control seeds of Douglas-fir were infected by potential pathogens in higher numbers relative to each seed lot of the pines tested. However, other studies have

confirmed that the seeds of all the conifers studied can be a source of high fungal infection (Whittle, 1977; Gordon, 1967). The high number of infected Douglas-fir seeds is related to a potential pathogen that infected many seeds in a relatively short time, that is, *Rhizopus arrhizus*, which is known to be highly sporulating fungal species (Rudramurthy et al., 2023). This was also the result obtained by Mallams (2004), who observed that all the seeds of this conifer tested were mainly infected by only one potential pathogen, *Fusarium sporotrichioides,* which can spread rapidly (Zhou et al., 2006). However, to confirm the high level of Douglas-fir seed infection compared to the study pine seeds, it would be necessary to increase the number of seed lots for each species covering the spatial and genotypic variation of the study tree species.

Heat treatment proved effective in reducing infection of potentially pathogenic fungi in all seed lots except Douglas-fir, where infection levels remained similar as in control seeds. Ramsey (2005) even observed an increase in infection of Douglas-fir seeds after heat treatment at 75 ºC for 30 min. In our study we observed the increased infection of *R. arrhizus* after the heat treatment. Kaerger et al. (2015) showed that this species is thermotolerant, with the temperature at which it reaches optimum growth up to 37 °C or higher. According to Ramsey (2005), some fungal species that are tolerant to heat can grow better and faster at higher temperatures, which correlates with the increased fungal infection of heat-treated seeds in the PM14 seed lot observed in this study.

UVC treatments, reaching only the seed surface (while heat also penetrates inside the seed), eliminated the potential pathogens by 100% on the PM14 seed lot. This study shows that ultraviolet light can eliminate fungal infections of potentially pathogenic fungi in Douglas-fir seeds. In contrast, UVC1 and UVC3 treatments contributed to a higher number of seeds infected with potential pathogens in seed lot PC15 and UVC1 in PS20, which is a surprising result, as UVC has been proven to be harmful to pathogens (McDonald et al., 2000; Falconí & Yánez-Mendizábal, 2017). This result may have been because UVC light had a positive effect on sporulation of the infecting fungi. For example, infection with the potential pathogen *P. bialowiezense* in seed lot PC15 involved 24 control seeds, 175 UVC1-

treated seeds, and 148 UVC3-treated seeds. It has been shown that the production of fungal conidia, which are responsible for the spread of the fungus and infection of the host, can be increased under UV light (De Menezes et al., 2014) which might have been a case with *P. bialowiezense*. Even though ultraviolet radiation is known as one of the main environmental factors that can kill the conidia of several fungal pathogens, more studies should be conducted on the effect of this treatment on fungal infection.

4.1.3 Sphaeropsis sapinea

This pathogen has previously been detected in at least 50 different pine and other coniferous species, such as Douglas-fir (Kaya et al., 2014), fir, spruce, and larch (Sinclair & Lyon, 2005). In our study, we found its infection in two seed lots of pines, Lodgepole pine (PC14) and Scots pine (PS18). Previous studies confirmed that this pathogen is most detected on its main host Scots pine (Brodde et al., 2019) and is commonly isolated from the seeds of this species (Decourcelle et al., 2015; Oskay & Karatas, 2021). In this study, *S. sapinea* did not occur in Douglas-fir seed lot. The pathogen has never been recorded in Douglas-fir seeds but has only been isolated from seedlings of this conifer (Kaya et al., 2014), as well as from shoots and cones (Aday et al., 2014). The pathogen infected only one seed lot from each pine species (seed lots PC14 and PS18 carried *S. sapinea*, whereas seed lots PC15 and PS20 did not). Surprisingly, Lodgepole pine seed lots originated from the same collection site and differed only in the date of collection. The infection rate in seed lot PC14 was low, as only nine control seeds were infected. Thus, it is possible that the lack of observation of this pathogen in the second seed lot of this conifer (i.e., PC15) was due to the overall low infection rate of this pathogen in this tree species. Considering this, further studies with more seeds are required, as it is possible that 300 seeds were insufficient to detect this pathogen in Lodgepole pine. Scots pine seed lot PS20, collected in a forest nursery in Dal, Sweden, was not infected, which was expected because the Svenska Skogsplantor forest nursery had already confirmed that the forest nursery was free of the pathogen. The high infection rate of another Scots pine seed lot, PS18, collected in a forest nursery in Gotthardsberg, Sweden, correlates with the study of Larsson et al. (2021). In this study, *S. sapinea*

was detected in 2019 in the same nursery in Scots pine seedlings. This was the first report of this pathogen in seedlings of this conifer, as well as the first report of the disease caused by *S. sapinea* in Swedish forest nurseries. Our study confirms that Scots pine seeds can be a frequent source of high infection by *S. sapinea* and contribute to spreading of the pathogen in forest nurseries.

Heat treatment successfully reduced the frequency of *S. sapinea* in infected seed lots by 100% in PC14 (9 control seeds were infected) and 96% in PS18 (82 control seeds were infected). High temperatures of more than 35 °C are considered lethal to this pathogen and can reduce its frequency (Decourcelle et al., 2015). For example, Decourcelle et al. (2015) demonstrated that heat treatment at 40 °C for 1 h eliminated the pathogen found on Corsican pine seeds. The heat treatment applied in our study yielded similar results to the study by Iturritxa et al., (2011), where the same temperature and duration of heat were applied to Monterey pine seeds that had been artificially infected with *S. sapinea* prior to treatment which led to eradication of the pathogen from seeds. In summary, the results of our study showed that heat treatment can greatly reduce *S. sapinea* infection in Lodgepole pine and Scots pine. A treatment dose of 55 °C for 8 h proves effective and could be further used for this purpose.

UVC1 and UVC3 treatments reduced pathogen frequency in the infected PS18 seed lot by 76% and 65%, respectively. In a study by Reglinski et al. (2013), for example, the irradiation of Monterey pine seedlings with UVC resulted in a dose-dependent induction of resistance to subsequent inoculation by *S. sapinea*; the longer the UVC exposure time, the lower the percentage of pine seedling dieback. This resulted in lower incidence of disease and reduced size of stem lesions in UVC treated compared with control seedlings. However, in the above-mentioned study UVC light induced resistance of the Monterey pine seedling that then eliminated *S. sapinea*, and in our study the pathogen infection was reduced directly by UVC light. It can therefore be concluded that UVC can be used to reduce *S. sapinea* infection levels in the seeds of Lodgepole and Scots pines.

4.2 Effect of the seed treatments on fungal occurrence-based diversity

4.2.1 All fungi

Overall, the number of fungal morphotypes and species identified in this study was high. A total of 371 morphotypes were recorded in the studied seeds, but approximately 60% of all isolates belonged to 22 dominant morphotypes. This result shows that a small number of fungal morphotypes with many individuals dominated the fungal communities, whereas a larger number of morphotypes was represented by a small number of individuals. Franić et al. (2020) reported that seed-borne fungal communities were dominated by a small number of fungi and that seed-borne fungi are host-specific. Sieber (2007) suggested that co-evolution between fungi and hosts (any part of plants) could have led to communities being dominated by a few fungal species. However, almost all the most dominant fungi were not previously recorded as specific to the tree species on which they were detected in this study. The exception is *S. sapinea,* which, as in our study, was previously isolated mainly from Scots pine.

Morphotype and species diversity varied similarly across treatments and different tree species, with high diversity of Lodgepole pine and Scots pine seeds and a lower diversity of Douglas-fir seeds. The number of fungal species in control seeds of Scots pine in both seed lots was generally lower than that reported in other studies. Kowalczyk et al. (2004), for example, identified 50 fungal species from 2,710 fungal colonies on seven seed samples including 1,800 seeds in total representing different stages of extraction process. The fungal diversity of Lodgepole pine seeds is poorly understood, but studies have shown high fungal diversity in the seedlings of this tree (Karlman, 1981). The lowest number of fungal species in the control Douglas-fir seed lot, that is, three fungal species recorded, did not correlate with the study by Gordon (1969), who detected 26 different fungal species on a similar number of seeds (n = 400). However, in the study by Gordon (1969) also only a few species dominated the community. Obtaining only three fungal species in PM14 control seeds in our study may be explained by the competition between

different fungi. Observed species, i.e., *R. arrhizus* and two species from the genus *Trichoderma*, i.e., *Trichoderma atroviride* and *Trichoderma simmonsii*, are known for their rapid growth and are considered aggressive competitors (Battaglia et al., 2011; Tyśkiewicz et al., 2022), quickly colonizing various substrates and eliminating slower-growing fungi (Oszust et al., 2020). *Trichoderma* spp., for example, are potent mycoparasitic fungi that compete for nutrients, secrete cell wall-degrading enzymes, and excrete secondary metabolites that are active against many pathogenic fungi in plants (Harman & Björkman, 1998; Hjeljord & Tronsmo, 1998). According to Huang (2020), fungal growth is correlated with their abundance which indicates that fast-growing fungi might have a competitive advantage when coexisting with other fungi.

The most frequently occurring species for Lodgepole pine and Scots pine seed lots in the control treatment were *H. macrosporum*, *N. juglandis* and *B. eucalypti*. None of the three fungal species were confirmed to be pathogenic to plants. To date, *H. macrosporum* has been demonstrated on Douglas-fir seeds, where it was, next to *Trichoderma*, the most frequently isolated fungus in a study by Bergmann & Busby (2021). This fungus has been recorded in the needles of infected Japanese black pine (*Pinus thunbergii* Parl.; Liu et al., 2021), but its pathogenicity to this tree was not confirmed, nevertheless, its elimination may prevent the possible risk of spreading this fungus in different pine species. It is known that *N. juglandis* has already been recorded in dead branches of common walnut (*Juglans regia* L.) in Turkey (Pem et al., 2020) and in various diseased plants in Iran (Ahmadpour et al., 2021), and information about *B. eucalypti* is very scarce. In our study, the same *Penicillium* species were observed in the two control seed lots of Lodgepole pine tree, i.e., *Penicillium bialowiezense*, *Penicillium diabolicalicense*, *Penicillium lanosum* and *Penicillium terrenum*. Fungi from this genus have already been reported in Lodgepole pine seeds (de la Bastide et al., 2019), as well as in seeds of different conifers, e.g., Scots pine (Kowalczyk et al., 2004). These ascomycetous fungi are molds that are most often saprotrophic in nature (Chávez et al.; 2006). *Penicillium* spp. can protect plants from dangerous pathogens by inducing defence systems in plants as well as developing systemic acquired resistance (Srinivasan et al., 2020). An example is the study by Yamaji et al. (2001), in which this genus was

shown to protect Sakhalin spruce (*Picea glehnii* (F. Schmidt) Mast.) seedlings from damping-off. Frequently isolated fungi, mainly from PS18 control seeds, were from the genus *Fusarium*, which in turn is known to infect pines. Fungi from this genus are classified as damping-off and root rot-causing species, strongly reducing the production of conifer seedlings, such as Scots pine (Swiecimska et al., 2020) and Douglas-fir (James et al., 1991; Hoefnagels & Linderman, 1999). Its presence has already been reported in control pine seeds (Clubbe, 1980), as well as in at least 60 pine species worldwide (Davydenko et al., 2018). Occurrence of already mentioned *Trichoderma* species, that is, *T. atroviride* and *T. simmonsii* correlate with a study by Bergmann & Busby (2021), where *Trichoderma* spp. were also the most frequently recorded in Douglas-fir seeds without any treatment. Fungi of this genus, including *T. atroviride* (Reithner et al., 2011) and *T. simmonsii* (Ben M' henni et al., 2022), are recognized as promising plant protection alternatives to chemical protection methods, as they are used as fungal Biocontrol Agents (BCA; Copping & Menn, 2000). They have already been used against tree pathogens, such as *F. circinatum* infecting Monterey pine seedlings in forest nurseries in Chile and New Zealand (Reglinski & Dick, 2005, Moraga-Suazo et al., 2011). Moreover, *Trichoderma* spp. also improved the emergence and vitality of the seedlings of this conifer. BCA may also improve the control of Armillaria root rot in pine (Prospero et al., 2021). Consequently, the presence of these fungi on Douglas-fir seeds may have a positive effect on reduction of other pathogenic fungi and their infection, so their elimination is not advisable. All these fungal species show that functional diversity of fungi in seeds is high, as pathogens, saprotrophs and potential biocontrol agents were found in this study.

Our results showed that heat treatment reduced the diversity of seed fungi in Scots pine and Lodgepole pine seeds. One of the largest reductions in individuals recorded was for *Penicillium* sp. and *Thyronectria* sp. in both seed lots of Lodgepole pine, as well as *Fusarium* sp. in the PS18 seed lot. *Thyronectria sp.* has not yet been recorded in this conifer, but among this genus, the pathogenic mold *Thyronectria austroamericana* is known to cause canker in honey locusts (*Gleditsia triacanthos*; Hudelson, 2021). Heat treatment did not eliminate *H. macrosporum*

from any seed lot of pine tested, which was still present in many individuals after treatment.

UVC treatments, compare to control treatment, reduced the overall fungal diversity in the seeds. An example is the already mentioned species *N. juglandis*, which was not reported in the PS18 seed lot after the UVC treatment but counted 200 isolates in the control treatment. Some fungal species, however, remained resistant to the treatment. For instance, *Penicilium* sp. appearing in PC15 and PS20 UVC-treated seed lots. The demonstrated resistance of fungi from the *Penicillium* genus and their frequent presence after UVC correlates with the study of a Laichmanová & Sedláček (2019), where the prevalence of strains of this genus after treatment was also demonstrated. Also, the differences in the number of UVC applications (one time or three times) were very small, as the diversity was at the same level after these treatments.

In some seed lots UVC resulted in the appearance of many individuals of new fungal species in comparison with control seeds. This is best shown in the seed lot PC15, where up to seven new species appeared post-treatment, as well as in the seed lot PM14 in which all fungi recorded after the UVC treatment were new. Examples with the highest number of isolates in these seed lots after treatment are *M. hiemalis* and *P. nothofagi*. In a study by Laichmanová & Sedláček (2019) it was showed that spores of the genus *Mucor* were resistant and stayed viable after the UVC treatment. *M. hiemalis*, one of the most dominant fungi in this study, is not considered as pathogenic to plants. This species was earlier reported on White spruce (*Picea glauca* (Moench) Voss; Mittal, 1986), four different pine species (Mittal et al., 1990), as well as on various beech (*Betula* spp.) trees (Dickinson & Pugh, 1974) and some other deciduous trees, e.g., Pedunculate oak (*Quercus robur* L). *Penicillium nothofagi*, that appeared after the UVC treatment in PM14 seed lot, was recorded in other studies only in the soil (Houbraken et al., 2011c). The presence of these fungi may be also a result of contamination, as both species appeared in other seed lots in the control treatment. Given these exceptions of the appearance of new species or an increase in the abundance of fungi after UVC

treatments, more research should be carried out on the effects of UVC light on different fungal species.

4.2.2 Potentially pathogenic fungi

Several of the species identified in this study have been characterised as potential plant pathogens (Supplementary Table 4) infecting in total almost 40% of seeds in this study. Previous studies have shown that potential pathogens can account for 30% among all fungi identified in the seeds (Cleary et al. 2019; Franic et al. 2019), which suggests comparable diversity. Most of the detected potential pathogens, 10 out of 15, are known to cause damage to trees, especially to conifers from the genus *Pinus*. One of the most dominant potential tree pathogens in this study for control seeds of pines tested was the previously mentioned *S. sapinea*, causing great damage to pine species around the world (Georgieva & Hlebarska, 2017). A significant proportion of the fungal species detected in this study were also non-tree plant pathogens. These species are not considered a direct threat to tree health, but their presence should be controlled. However, it is possible that the identification of species in this study is not totally accurate, as the ITS has its limitations in providing sufficient resolution for some highly specific fungal genera, such as *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma*, because these taxa have narrow or no barcode gaps in their ITS regions (Samson & Pitt, 2000; Lücking et al., 2020).

In this study, heat treatment reduced the overall diversity of potential tree pathogens compared to control seeds. For example, potential pathogen *H. spartii* was eliminated by the heat in PC14 seed lot. This species is known to cause dieback and canker of Stone pine (*Pinus pinea* L.) in Tunisia (Hlaiem et al., 2019) and Black Spot Needle Blight of *Pinus sylvestris var. mongolica* trees in China (Wang, 2022). Heat treatment has similarly eliminated *A. pseudotsugae*, a potential pathogen of mainly pines but also larches. This species has so far only been recorded in Europe, where it causes Phomopsis disease by attacking the young shoots of the plants (Wajihi, 2019). In PS18, previously mentioned *Fusarium* sp. was also eliminated by the heat treatment which correlates with the study of Bennett & Colyer (2010). In their study, *Fusarium oxysporum* f. sp. *vasinfectum* infection on cotton seed was

reduced by the heat treatments at 30, 35 and 40 °C for up to 24 weeks as well as at 60, 70 and 80 °C for 2 to 14 days. Another species eliminated by the heat found in all seed lots except PM14, *P. thomii*, is known as a potential seed-borne pathogen causing diseases of Eastern white pine trees in North America (Mittal, 1986), but the symptoms of its infection are unknown. Heat treatment, however, did not eliminate *P. hollandica*, which appeared in seeds of Lodgepole pine. This fungus is a potential pathogen of Mediterranean cypress (*Cupressus sempervirens* L.) in Spain, as it infests its shoots (Silva et al., 2020). It has also been recorded on various pine tree species (Jiang et al., 2022; Maharachchikumbura et al., 2011), Bull banksia (*Banksia grandis* Willd.) in Australia and on Japanese umbrella-pine (*Scapdopitys verticillate* Sieb. & Zucc.) in the Netherlands (Jiang et al., 2022).

The UVC treatment, similarly, to heat treatment, also resulted in a reduction of the diversity of potential tree pathogens. For example, *D. sarmentorum*, eliminated from the seed lot PS18, is a potential pathogen of common walnut causing branch dieback (Iqbal, 2022). This species was also observed on ash trees (*Fraxinus* spp.), where it caused tree decline, wood cankers, bud necrosis, shoot and branch dieback displayed through bleached, necrotic or discoloured canes in infected trees (Ivanova, 2018). Another example of a potential pathogen of common walnut also found in our study is *C. subuliforme* Bensch, Crous & U. Braun (Yang, 2023), which was not eliminated by the UVC treatment in the seed lot PS20. UVC treatments, compare to heat, did not eliminate potentially pathogenic *P. thomii* from any seed lot, as well as *Fusarium* sp. from both seed lots of Scots pine. The reason for the lack of effect of UVC on *Fusarium* sp. could be that this treatment is known to penetrate only the seed surface, and fungi from this genus mainly reside inside the seed (Papavizas, 1985; Graham & Linderman, 1983).

4.3 Effect of the seed treatments on seed germination

The heat treatment at 55 ˚C for 8 h and the UVC light treatments performed in this study did not adversely affect the germination capacity and germination energy of Lodgepole pine, Scots pine and Douglas-fir seeds. This result implies that these

treatments can be used on the seeds of these tree species to reduce or eliminate the infection by potentially pathogenic fungi without affecting seed germination.

The lack of negative effect of heat treatment on seed germination was also confirmed by Nunez & Calvo (2000). In their study, seeds of Scots pine and Aleppo pine (*Pinus halepensis* Mill.) treated with heat even at 70 °C germinated at the same level as control seeds, but the treatment was shorter, i.e., only 1-5 minutes. Also, Knapp & Anderson (1980) observed no negative effect of heat treatment at 45-65 °C for 15 minutes on Lodgepole pine seed germination. It can therefore be concluded that heat treatments of different durations and temperatures can be used on conifer seeds to control fungal infection without adversely affecting germination. However, maximum and minimum heat treatment doses (temperature x duration) should be studied in more depth.

UVC radiation has so far mainly been used to eliminate fungal infection on crops (Sadeghianfar et al., 2019; Kondrateva et al., 2020), and there is very little research on the use of this treatment on conifer seeds and its effect on germination. Results of our study do not correlate with the study by Foroughbakhch Pournavab et al. (2019), who showed that Martinez pinyon (*Pinus maximartinezii* Rzed.) seeds were highly sensitive to UVC treatments, which negatively affected their germination. More research should be carried out to investigate the effects of using UVC light on conifer seeds so that in the future we can effectively control fungal infection without negatively affecting germination.

4.4 Traditional plating as a method for the fungal identification

One of the most common methods for assessing fungi in seeds is plating. This method allows for obtaining fungal cultures from tree seeds by putting these seeds on a particular nutrient medium. There are many advantages using this method, such as providing complete information on fungal communities from different areas, distribution of fungi and fungus-host relationships (Gautam et al., 2022). With this method, we have a high sensitivity of fungi, however, only if the medium

is correctly selected. Also determination of the fungus at species level is possible. It's also considered as an economical method and demands less specialised equipment than more advanced techniques (Gautam et al., 2022). However, in addition to these advantages, there are also disadvantages that may contribute to the choice of other methods for fungal characterisation. Traditional plating is very time-consuming. The detailed process of sampling, culturing, isolation and identification requires a commitment of time. This method is also resource-consuming (Figdor & Gulabivala, 2011). If the medium is chosen incorrectly, the growth of some fungi may be limited due to low sensitivity, which is then a limitation in identifying fungi (Mendonca et al., 2022). It was also previously studied that a range of uncultivable species is unknown from fruiting bodies (Nilsson et al., 2018). Lastly, there is a risk of contamination in using this method (Figdor, 2011).

Due to limitations in the use of traditional culture-based methods, DNA and RNA based high-throughput sequencing (HTS) approaches are becoming essential (Nilsson et al., 2019). While sequencing DNA provides a genetic profile of a cell/organism, sequencing RNA identifies only the genes that are actively transcribed in the cells (Arroyo Mühr et al., 2021). Within that, the amplicon sequencing (also known as metabarcoding), using PCR, enables to analyze genetic variation in a specific genomic region (Tedersoo et al., 2002). While sequencing information has traditionally been elucidated using a low-throughput technique called Sanger sequencing, HTS is much more efficient given the sequencing volume. The Sanger method only sequences a single DNA fragment at a time, in turn, HTS is massively parallel, enabling hundreds of millions of DNA molecules to be sequenced (Paul et al., 2018). HTS also offers greater discovery power to detect rare variants with deep sequencing, as well as higher sequencing depth enabling higher sensitivity (Nilsson et al., 2019). However, DNA and RNA using HTS methods, are less cost-effective compared to the culture-based method (Nilsson et al., 2019). Ultimately, by betting on more modern methods to identify multiple species more quickly and efficiently at once, the implementation of HTS technology will be invaluable to practitioners' work in the future.

5. Conclusions and recommendations

Results of the present study show that seeds of Lodgepole pine and Scots pine carry abundant and highly diverse fungi, including potential plant pathogens and specifically *Sphaeropsis sapinea*. Fungal diversity, however, varies among tree species, as Douglas-fir seed fungi were less diverse. The seeds of study tree species are also a source of frequent fungal infection. This study shows that heat at 55 °C for 8 h can reduce the level of fungal infection, as well as the overall fungal diversity. UVC treatments also reduce the overall fungal diversity, while infection levels remain high. Both treatments performed result in a reduction of infection by potentially pathogenic fungi, including *S. sapinea*. Heat and UVC treatments performed have no negative effect on seed germination, indicating their safe use in reducing or eliminating fungal infection in conifer seeds of study tree species.

The results obtained point to the risk of introducing pathogenic fungi into forest nurseries causing diseases of tree seedlings with a negative impact on forest regeneration. Also, frequent import and use of latently infected pine seeds has been identified as a factor promoting the global movement of *S. sapinea*. The high demand for Scots pine seeds in Sweden leading to frequent seed trade may contribute to the easy and rapid spread of the pathogen. Thus, there is a high risk that *S. sapinea*, as well as different dangerous plant pathogens, could be moved to new areas when seeds are being exchanged for reproduction purposes. In addition to the trade, it is extremely important to prevent the infection of seedlings with this pathogen already in orchards. Treating seeds prior to sowing will result in disease-free seedlings. At the same time, treating conifer seeds with heat and UVC prior export can highly prevent the spread and further pathogen infection.

This study emphasizes the need for further research of the effects of heat and UVC treatments on conifer seed fungi. In this study the number of 300 seeds per each seed lot (five seed lots in total) was tested, which is considered as the minimum number of seeds required for a 95% probability of detecting at least one infected seed in a seed lot containing 1% contaminated seeds. It is important for practitioners to know what fungi can be find in the seed lots to minimize the risk of introducing harmful fungi to the forest nurseries and elsewhere (in case when seeds are shipped abroad). Therefore, testing more seeds and seed lots would be needed to understand more comprehensively possible risks associated with the movement of reforestation seeds. The reason for further research using more seeds and seed lots also arose from the high variation across seed lots and treatments found in the results of this study. More comprehensive information is needed to reach a general assumption that these treatments can be used on seeds to control fungi, especially pathogenic ones.

More research should be carried out to determine whether shorter time of heat treatments would also be effective in controlling fungi. Reducing the time of this treatment would streamline and facilitate the work for practitioners. It is also needed to get sufficient information on the possibilities of using UVC as a treatment in nurseries. The results of this study showed that UVC seems to be less effective than heat treatment. However, it may still be worthwhile to see if UVC can be optimized, which can be done by fine tuning the conditions: adjusting light intensity and duration, as well as coverage by installing a vibrating device while performing UVC so it can reach each side of the seed.

This study did not consider seed size as a possible factor which can influence the efficacy of the heat treatment for eliminating seed-borne fungi (Bond et al., 1999). As our results show that the heat treatment had no effect on Douglas-fir seeds, it is suggested that the effect of the size of the seeds tested should be equally investigated.

More studies on seed-borne fungal communities, i.e., determination of fungus-host associations, and investigation of heat and UVC tolerance of fungi will allow to

apply the most appropriate treatment to control fungi, as well as to assess the effect of these treatments.

In summary, the results of this work indicating the high risk and frequency of seed infection by fungi, especially pathogenic ones, prove that heat and UVC treatment, especially heat, should be practically implemented by practitioners in Sweden. The approach of heat-, and UVC-treating infected seeds prior to sowing and prior export will reduce or even eliminate the potential risk of disease transmission to Swedish nurseries, as well as the risk of plant pathogens spread during the seed trade.

References

Aday, G., Lehtijärvi, A., Kaya, Ö. & Doğmuş-Lehtijärvi, T. (2014). First Report of *Diplodia pinea* on *Pseudotsuga menziesii* in Turkey. Plant Disease. 98, 689–689. doi: 10.1094/PDIS-07-13-0765-PDN.

Agarwal, V.K. & Sinclair, J.B. (1997). Principles of Seed Pathology (2nd ed.). CRC Press. doi: 10.1201/9781482275650 .

Agrios, G.N. (1997). Plant Pathology (4th ed.). Academic Press.

Ahmadpour, S.A., Mehrabi-Koushki, M., Farokhinejad, R., Asgari, B., Javadi Estahbanati, A., Mirabolfathy, M. & Rahnama, K. (2021). New records of fungal species of the family *Didymellaceae* from Iran. Mycologia Iranica. 8, 119–133. doi: 10.22043/MI.2022.359850.1229.

Aldous, J.R. (1972). Nursery practice. Her Majesty's Stationery Office, London. Forestry Commun. Bull. 43–184.

Allen, J.A, Keeland, B.D, Stanturf, J.A, Clewell, A.F & Kennedy, Jr. H.E. (2001). A guide to bottomland hardwood restoration. USDA Forest Service General Technical Report SRS. 40–132. doi: 10.2737/SRS-GTR-40.

Almqvist, C. & Jansson, G. (2015). Effects of pruning and stand density on cone and pollen production in an experimental *Pinus sylvestris* seed orchard. Silva Fennica. 49, 1243. doi: 10.14214/sf.1243.

Arroyo Mühr, L.S., Dillner, J., Ure, A.E., Sundström, K. & Hultin, E. (2021). Comparison of DNA and RNA sequencing of total nucleic acids from human cervix for metagenomics. Scientific Reports. 11, 18852. doi: 10.1038/s41598-021-98452-4.

Avalos, J. & Limón, M.C. (2015). Biological roles of fungal carotenoids. Current Genetics. 61, 309-324. doi: 10.1007/s00294-014-0454-x.

Bacon, C.W., Glenn, A.E. & Yates, I.E. (2008). *Fusarium verticillioides*: managing the endophytic association with maize for reduced fumonisins accumulation. Toxin Reviews. 27, 411–446. doi: 10.1080/15569540802497889.

Baker, K.F. (1972). Seed Pathology. In: Kozlowski T. (eds) Seed Biology, vol. 2. Academic Press, New York.

Bakewell-Stone, P. (2023). *Pseudotsuga menziesii* (Douglas-fir). CABI Compendium. CABI. doi: 10.1079/cabicompendium.45266.

Barnett, J.P. & McLemore, B.F. (1970). Storing southern pine seeds. Forestry. 68, 4–27. doi: 10.1093/jof/68.1.24.

Barnett, J.P. & Baker, J.B. (1991). Regeneration Methods. In: Duryea, M.L. & Dougherty, P.M. (eds) Forest Regeneration Manual. Forestry Sciences, vol. 36. Springer, Dordrecht. doi: 10.1007/978-94-011-3800-0_3.

Barton, L.V. (1961). Seed preservation and longevity. Leonard Hill Books Ltd.

Battaglia, E., Benoit, I., van den Brink, J., Wiebenga, A., Coutinho, P.M., Henrissat, B. & de Vries, R.P. (2011). Carbohydrate-active enzymes from the zygomycete fungus *Rhizopus oryzae*: a highly specialized approach to carbohydrate degradation depicted at genome level. BMC Genomics. 12, 38. doi: 10.1186/1471-2164-12-38.

Belcher, E.W. & Lowman, B.J. (1982). Energy considerations in cone drying. Tree Planters' Notes. 33, 31–34.

Belozerskaya, T.A., Gessler, N.N. & Aver'yanov, A.A. (2017). Melanin Pigments of Fungi. In: Mérillon, J.M. & Ramawat, K. (eds) Fungal Metabolites. Reference Series in Phytochemistry. Springer, Cham. doi: 10.1007/978-3-319-25001-4_29.

Ben M'henni, Y., Salem, I.B., Souli, M., Tounsi, S., Debieu, D., Fillinger, S. & Boughalleb-M'hamdi, N. (2022). Biocontrol and Growth Promotion Potential of Combined Application of *Trichoderma simmonsii* and *Aspergillus westerdijkiae* Against Apple Tree Dieback Disease. PhytoFrontiersTM. 2, 268–279. doi: 10.1094/PHYTOFR-01-22-0005-R.

Bennett, R.S. & Colyer, P.D. (2010). Dry Heat and Hot Water Treatments for Disinfesting Cottonseed of *Fusarium oxysporum* f. sp. *vasinfectum*. Plant Disease. 94, 1469–1475. doi: 10.1094/PDIS-01-10-0052.

Bergmann, G.E. & Busby, P.E. (2021). The core seed mycobiome of *Pseudotsuga menziesii* var. *menziesii* across provenances of the Pacific Northwest, USA. Mycologia. 113, 1169–1180. doi: 10.1080/00275514.2021.1952830.

Bihon, W., Slippers, B., Burgess, T., Wingfield, M.J. & Wingfield, B.D. (2011). Sources of *Diplodia pinea* endophytic infections in *Pinus patula* and *P. radiata* seedlings in South Africa. The Journal of Pathology. 41, 370–375. doi: 10.1111/j.1439-0329.2010.00691.x.

Birmpa, A., Sfika, V. & Vantarakis, A. (2013). Ultraviolet light and Ultrasound as non-thermal treatments for the inactivation of microorganisms in fresh ready-to-eat foods. International Journal of Food Microbiology. 167, 96–102. doi: 10.1016/j.ijfoodmicro.2013.06.005.

Blumenstein, K., Bußkamp, J., Langer, G.J., Langer, E.J. & Terhonen, E. (2021). The Diplodia Tip Blight Pathogen *Sphaeropsis sapinea* Is the Most Common Fungus in Scots Pines' Mycobiome, Irrespective of Health Status—A Case Study from Germany. Journal of Fungi. 7, 607. doi: 10.3390/jof7080607.

Boerema, G.H, de Gruyter, J. & van de Graaf, P. (1999). Contributions towards a monograph of Phoma (Coelomycetes) IV — Supplement. An addition to section Heterospora: *Phoma schneiderae* spec. nov., synanamorph *Stagonosporopsis lupini* (Boerema & R. Schneid.) comb. nov. Persoonia - Molecular Phylogeny and Evolution of Fungi. 17, 281–285.

Bond, W.J., Honig, M. & Maze, K.E. (1999). Seed size and seedling emergence: an allometric relationship and some ecological implications. Oecologia. 120, 132–136. doi: 10.1007/s004420050841.

Bonner, F.T. (1987). Cone storage and seed quality in longleaf pine. U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 4, 341. doi: 10.2737/SO-RN-341.

Bonner, F.T. (1991). Seed Management. In: Duryea, M.L. & Dougherty, P.M. (eds) Forest Regeneration Manual. Forestry Sciences, vol. 36. Springer, Dordrecht. 4, 51–73.

Brodde, L., Adamson, K., Julio Camarero, J., Castaño, C., Drenkhan, R., Lehtijärvi, A., Luchi, N., Migliorini, D., Sánchez-Miranda, Á., Stenlid, J., Özdağ, Ş. & Oliva, J. (2019). Diplodia Tip Blight on Its Way to the North: Drivers of Disease Emergence in Northern Europe. Frontiers in Plant Science. 9, 1818. doi: 10.3389/fpls.2018.01818.

Brown, J.E., Lu, T.Y., Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Collins, D.J., Wilson, M.A., Igwegbe, E.C.K., Chalutz, E. & Droby, S. (2001). The effect of low dose ultraviolet light-C seed treatment on induced resistance in cabbage to black rot (*Xanthomonas campestris* pv. campestris). Crop Protection. 20, 873–883. doi: 10.1016/S0261-2194(01)00037-0.

Burgess, T.I. & Wingfield, M.J. (2002). Quarantine is important in restricting the spread of exotic seed-borne tree pathogens in the southern hemisphere. International Forestry Review. 4, 56–65.

Cannon, P.F. & Minter, D.W. (1983). The nomenclatural history and typification of *Hypoderma* and *Lophodermium*. Taxon. 32, 572–583. doi: 10.2307/1221727.

Castle, S.C., Song, Z., Gohl, D.M., Gutknecht, J.L., Rosen, C.J., Sadowsky, M.J. & Kinkel, L.L. (2018). DNA template dilution impacts amplicon sequencing-based estimates of soil fungal diversity. Phytobiomes. 2, 100–107. doi: 10.1094/PBIOMES-09-17-0037-R.

Chang, F.J. & Johar, I. (2022). Radicle Civics - Unconstituting Society. In: Chang, F.J. & Johar, I. (eds) Building 21st-Century Civic Infrastructures. Taylor & Francis Online, vol. 17. doi: 10.4324/9781003199816-22.

Chávez, R., Bull, P., Eyzaguirre, J. (2006). The xylanolytic enzyme system from the genus *Penicillium*. Journal of Biotechnology. 123, 413–433. doi: 10.1016/j.jbiotec.2005.12.036.

Choi, J. & Kim, S.H. (2017). A genome Tree of Life for the Fungi kingdom. Proceedings of the National Academy of Sciences of the United States of America. 114, 9391–9396. doi: 10.1073/pnas.1711939114.

Chudy, R.P. & Cubbage, F.W. (2020). Research trends: Forest investments as a financial asset class. Forest Policy and Economics. 119, 102273. doi: 10.1016/j.forpol.2020.102273.

Claes Uggla, The Swedish Forest Agency. (2021). https://www.skogsstyrelsen.se/en/statistics/.

Cleanlight. (2022). Edible seed treatment with UVC light. Cleanlight. https://cleanlight.nl/edible-seed-treatment-with-uvc-light/.

Clear, R.M., Patrick, S.K., Wallis, R. & Turkington, T.K. (2002). Effect of dry heat treatment on seed-borne *Fusarium graminearum* and other cereal pathogens.

Canadian Journal of Plant Pathology. 24, 489–498. doi: 10.1080/07060660209507038.

Cleary, M., Oskay, F., Doğmuş, H.T., Lehtijärvi, A., Woodward, S. & Vettraino, A.M. (2019). Cryptic Risks to Forest Biosecurity Associated with the Global Movement of Commercial Seed. Forests. 10, 459. doi: 10.3390/f10050459.

Clubbe, C.P. (1980). Colonization of wood by micro-organisms. Department of Botany and Plant Technology Imperial College of Science and Technology London SW7 2BB. 1-236.

Copping, L.G. & Menn, J.J. (2000). Biopesticides: a review of their action, applications and efficacy. Pest Management Science. 56, 651–676. doi: 10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U.

Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, R.S. Jr. & Toko, H.V. (1989). Forest Nursery Pests. USDA Forest Service. 680, 184.

Crous, P.W., Gams, W., Stalpers, J.A., Robert, V. & Stegehuis, G. (2004). MycoBank: an online initiative to launch mycology into the 21st century. Studies in Mycology. 50, 19–22.

Davydenko, K., Nowakowska, J.A., Kaluski, T., Gawlak, M., Sadowska, K., García, J.M., Diez, J.J., Okorski, A. & Oszako, T. (2018). A Comparative Study of the Pathogenicity of *Fusarium circinatum* and other *Fusarium* Species in Polish Provenances of P. sylvestris. L. Forests. 9, 560. doi: 10.3390/f9090560.

de la Bastide, P.Y., LeBlanc, J., Kong, L., Finston, T., May, E.M., Reich, R., Hintz, W.E. & von Aderkas, P. (2019). Fungal colonizers and seed loss in lodgepole pine orchards of British Columbia. NRC Research Press. 97, 23–33. doi: 10.1139/cjb-2018-0153.

de Menezes, H.D., Pereira, A.C., Brancini, G.T.P., de Leão, H.C., Massola Júnior, N.S., Bachmann, L., Wainwright, M., Bastos, J.K. & Braga, G.U.L. (2014). Furocoumarins and coumarins photoinactivate *Colletotrichum acutatum* and *Aspergillus nidulans* fungi under solar radiation. Journal of Photochemistry and Photobiology. 131, 74–83. doi: 10.1016/j.jphotobiol.2014.01.008.

Decourcelle, T., Piou, D. & Desprez-Loustau, M.L. (2015). Detection of *Diplodia sapinea* in Corsican pine seeds. Plant Pathology. 64, 442–449. doi: 10.1111/ppa.12263.

Derr, H.J. & Mann, W.F. (1971). Direct seeding pines in the South. Department of Agriculture.

Dey, D.C., Jacobs, D., McNabb, K., Miller, G., Baldwin, V. & Foster, G. (2008). Artificial Regeneration of Major Oak (*Quercus*) Species in the Eastern United States—A Review of the Literature. Forest Science. 54, 77–106. doi: 10.1093/forestscience/54.1.77.

Dickinson, C.H. & Pugh, G.J.F. (1974). Biology of Plant Litter Decomposition. Academic Press. doi: 10.1016/B978-0-12-215001-2.X5001-6.

Diffey, B.L. (2002). Human exposure to solar ultraviolet radiation. Journal of Cosmetic Dermatology. 1, 124–130. doi: 10.1046/j.1473-2165.2002.00060.x.

Domevscik, M., Häggström, B., Lim, H., Öhlund, J. & Nordin, A. (2023). Large-scale assessment of artificially coated seeds for forest regeneration across Sweden. New Forests. 54, 255–267. doi: 10.1007/s11056-022-09920-2.

Domin, M., Kluza, F., Góral, D., Nazarewicz, S., Kozłowicz, K., Szmigielski, M. & Ślaska-Grzywna, B. (2020). Germination Energy and Capacity of Maize Seeds Following Low-Temperature Short Storage. Sustainability. 12, 46. doi: 10.3390/su12010046.

Drenkhan, R., Tomešová-Haataja, V., Fraser, S., Bradshaw, R.E., Vahalík, P., Mullett, M.S., Martín-García, J., Bulman, L.S., Wingfield, M.J. & Kirisits, T. (2016). Global geographic distribution and host range of *Dothistroma* species: A comprehensive review. The Journal of Pathology. 46, 408–442. doi: 10.1111/efp.12290.

EFSA, Panel of Plant Health (2010). Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options by EFSA. Wiley. 8, 1495. doi: 10.2903/j.efsa.2010.1495.

El-Gaouth, A. & Wilson, C.L. (1995). Biologically-based technologies for the control of postharvest diseases. Postharvest News and Information. 6, 5–11.

European Commission. (2019). Regulation (EU) 2016/2031. Plant Health Law.

Eyles, A., Bonello, P., Ganley, R. & Mohammed, C. (2010). Induced resistance to pests and pathogens in trees. New Phytologist. 185, 893–908. doi : 10.1111/j.1469-8137.2009.03127.x.

Fabre, B., Piou, D., Desprez-Loustau, M.L. & Marcais, B. (2011). Can the emergence of pine Diplodia shoot blight in France be explained by changes in pathogen pressure linked to climate change? Global Change Biology. 17, 3218–3227. doi: 10.1111/j.1365-2486.2011.02428.x.

Falconí, C.E. & Yánez-Mendizábal, V. (2018). Efficacy of UV-C radiation to reduce seedborne anthracnose (*Colletotrichum acutatum*) from Andean lupin (*Lupinus mutabilis*). Plant Pathology. 67, 831–838. doi: 10.1111/ppa.12793.

Farinha, A.O., Branco, M., Pereira, M.F.C., Auger-Rozenberg, M.A., Maurício, A., Yart, A., Guerreiro, V., Sousa, E.M.R. & Roques, A. (2018). Micro X-ray computed tomography suggests cooperative feeding among adult invasive bugs *Leptoglossus occidentalis* on mature seeds of stone pine *Pinus pinea*. Agricultural and Forest Entomology. 20, 18–27. doi: 10.1111/afe.12225.

Farjon, A. (2010). A Handbook of the World's Conifers. Brill Academic Publishers. doi: 10.1163/9789004324510_001.

Federhen, S. (2015). Type material in the NCBI Taxonomy Database. Nucleic Acids Research. 43, 1086–1098. doi: 10.1093/nar/gku1127.

Fennessy, J. (2002). The Collection, Storage, Treatment and Handling of Conifer Tree Seed. Coford. 3, 1–6.

Figdor, D. & Gulabivala, K. (2008). Survival against the odds: microbiology of root canals associated with post-treatment disease. Endodontic Topics. 18, 62–77. doi: 10.1111/j.1601-1546.2011.00259.x.

Foroughbakhch Pournavab, R., Bacópulos Mejía, E., Benavides Mendoza, A., Salas Cruz, L.R. & Ngangyo Heya, M. (2019). Ultraviolet Radiation Effect on Seed Germination and Seedling Growth of Common Species from Northeastern Mexico. Agronomy. 9, 269. doi: 10.3390/agronomy9060269.

Fox, J. (2016). Applied Regression Analysis and Generalized Linear Models (3rd ed.). Sage Publications.

Fraedrich, S.W. & Miller, T. (1995). Mycoflora associated with slash-pine seeds from cones collected at seed orchards and cone-processing facilities in the south-eastern USA. European Journal of Forest Pathology. 25, 73–82. doi: 10.1111/j.1439-0329.1995.tb00321.x.

Franić, I., Eschen, R., Allan, E., Hartmann, M., Schneider, S., & Prospero, S. (2020). Drivers of richness and community composition of fungal endophytes of tree seeds. FEMS Microbiology Ecology. 96, 166. doi: 10.1093/femsec/fiaa166.

Franić, I., Prospero, S., Hartmann, M., Allan, E., Auger-Rozenberg, M.A., Grünwald, N.J. & Eschen, R. (2019). Are traded forest tree seeds a potential source of nonnative pests? Ecological Applications. 29, 01971. doi: 10.1002/eap.1971.

Gautam, A.K., Verma, R.K., Avasthi, S., Sushma, Bohra, Y., Devadatha, B., Niranjan, M. & Suwannarach, N. (2022). Current Insight into Traditional and Modern Methods in Fungal Diversity Estimates. Journal of Fungi. 8, 226. doi: 10.3390/jof8030226.

Geer, L., Marchler-Bauer, A., Han, L., He, J., He, S., Shi, L.C.W. & Bryant, S. (2010). The NCBI BioSystems database. Nucleic Acids Research. 38, 492–496. doi: 10.1093/nar/gkp858.

Georgieva, M. & Hlebarska, S. (2017). A review of *Sphaeropsis sapinea* occurrence on *Pinus* spp. in Bulgaria. Bioscience and Biotechnology. 6, 1–4.

ggplot2 Based Publication Ready Plots. https://rpkgs.datanovia.com/ggpubr/.

FAO. 2020. Global Forest Resources Assessment 2020: Main report. Rome. doi: 10.4060/ca9825en.

Godefroid, S., Van De Vyver, A., Stoffelen, P. & Vanderborght, T. (2017). Effectiveness of dry heat as a seed sterilisation technique: Implications for ex situ conservation. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology. 151, 1054–1061. doi: 10.1080/11263504.2016.1231140.

Gordon, L.R. (1967). Fungi associated with Douglas-fir seed during cone development, seed processing, and storage. Oregon State University. 2-83.

Gordon, T.R., Kirkpatrick, S.C., Aegerter, B.J., Wood, D.L. & Storer, A.J. (2006). Susceptibility of Douglas fir (*Pseudotsuga menziesii*) to pitch canker, caused by *Gibberella circinata* (anamorph = *Fusarium circinatum*). Plant Pathology. 55, 231–237. doi: 10.1111/j.1365-3059.2006.01351.x.

Gosling, P. (2007). Raising trees and shrubs from seed. Forestry Commission.

Graham, J.H. & Linderman, R.G. (1983). Pathogenic seedborne *Fusarium oxysporum* from Douglas-fir. Plant Disease. 67, 323–325. doi: 10.1094/PD-67-323.

Grondeau, C., Samson, R. & Sands, D. (1994). A Review of Thermotherapy to Free Plant Materials from Pathogens, Especially Seeds from Bacteria. Critical Reviews in Plant Sciences. 13, 57–75. doi: 10.1080/07352689409701908.

Grossnickle, S.C. & Ivetić, V. (2017). Direct seeding in performance review—a reforestation field. Reforesta. 4, 94–142. doi: 10.21750/REFOR.4.07.46.

Hall, T.A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series. 41, 95–98. doi: 10.14601/phytopathol_mediterr-14998u1.29.

Harman, G.E. & Bjo¨rkman, T. (1998). Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. In: Harman, G.E. & Kubicek, C.P. (eds) *Trichoderma* and *Gliocladium*. Taylor & Francis, vol. 2. London, United Kingdom. 229–266.

Hart, B.L. (1990). Behavioral adaptations to pathogens and parasites: Five strategies. Neuroscience & Biobehavioral Reviews. 14, 273–294. doi: 10.1016/S0149-7634(05)80038-7.

Hill, R. (2021). How we discovered a hidden world of fungi inside the world's biggest seed bank. The Conversation. http://theconversation.com/how-we-discovered-a-hidden-world-of-fungi-inside-the-worlds-biggest-seed-bank-156051.

Hjeljord, L. & Tronsmo, A. (1998). *Trichoderma* and *Gliocladium* in biological control: an overview. In: Harman, G.E. & Kubicek, C.P. (eds) *Trichoderma* and *Gliocladium*. Taylor & Francis, vol. 2. London, United Kingdom. 131-152. doi: 10.1201/9781482267945.

Hlaiem, S., Boutiti, M. & Mohamed Lahbib, B.J. (2019). *Heterotruncatella spartii* causal agent of dieback disease on *Pinus pinea* in Tunisia. Plant Pathology. 9. doi: 10.5943/ppq/9/1/17.

Hoefnagels, M.H. & Linderman, R.G. (1999). Biological Suppression of Seedborne *Fusarium* spp. During Cold Stratification of Douglas Fir Seeds. Plant Disease. 83, 845–852. doi: 10.1094/PDIS.1999.83.9.845.

Hollander, M. & Wolfe, D.A. (1973). Nonparametric Statistical Methods. John Wiley & Sons. doi: 10.1002/bimj.19750170808.

Holmström, E., Gålnander, H. & Petersson, M. (2019). Within-site variation in seedling survival in Norway spruce plantations. Forests. 10, 181. doi: 10.3390/f10020181.

Houbraken, J., Frisvad, J.C. & Samson, R.A. (2011). Taxonomy of *Penicillium* section Citrina. Studies in Mycology. 70, 53–138. doi: 10.3114/sim.2011.70.02.

https://www.ncbi.nlm.nih.gov/refseq/about/prokaryotes/

https://www.skogsplantor.se/

Huang, Y.L. (2020). Effect of Host, Environment and Fungal Growth on Fungal Leaf Endophyte Communities in Taiwan. Journal of Fungi. 6, 244. doi: 10.3390/jof6040244.

Hudelson, B. (2021). Thyronectria Canker. UW-Madison Division of Extension - University of Wisconsin - Madison.

Huss, J. (2004). Stand Establishment, Treatment and Promotion – European Experience (1st ed.). In: Burley, J. (eds.) Encyclopedia of Forest Sciences. Academic Press, vol. 1-4. United Kingdom. 14–27. doi: 10.1016/B0-12-145160-7/00224-6.

Iqbal, S., Hernández, Y., Lolas, M.A., Elfar, K., Eskalen, A., Latorre, B.A. & Díaz, G.A. (2022). *Dothiorella sarmentorum* Causing Branch Dieback of English Walnut in Maule Region, Chile. Plant Disease. doi: 10.1094/pdis-03-22-0636-pdn.

ISPM 38. (2017). International movement of seeds. Secretariat of the International Plant Protection Convention.

Iturritxa, E., Ganley, R., Wright, J., Heppe, E., Steenkamp, E., Gordon, T. & Wingfield, M. (2011). A genetically homogenous population of *Fusarium circinatum* causes pitch canker of *Pinus radiata* in the Basque Country, Spain. Fungal biology. 115, 288–95. doi: 10.1016/j.funbio.2010.12.014.

Ivanová, H. (2018). Identification and characterization of the fungus *Dothiorella sarmentorum* on necrotic shoots of declining ash in Slovakia. Folia Oecologica. 45, 53–57. doi: 10.2478/foecol-2018-0006.

James, R.L., Dumroese, R.K. & Wenny, D.L. (1991). *Fusarium* diseases of conifer seedlings. Pacific Forestry Centre.

Jansson, G., Danusevičius, D., Grotehusman, H., Kowalczyk, J., Krajmerova, D., Skrøppa, T. & Wolf, H. (2013). Norway spruce (*Picea abies* (L.) H. Karst.). In: Pâques, L.E. (eds.) Forest Tree Breeding in Europe. iForest - Biogeosciences and Forestry, vol. 25. 123–176. doi: 10.1007/978-94-007-6146-9.

Jiang, N., Voglmayr, H., Xue, H., Piao, C.G. & Li, Y. (2022). Morphology and Phylogeny of *Pestalotiopsis* (Sporocadaceae, Amphisphaeriales) from Fagaceae Leaves in China. Microbiology Spectrum. 10, e03272-22. doi: 10.1128/spectrum.03272-22.

Kaerger, K., Schwartze, V.U., Dolatabadi, S., Nyilasi, I., Kovács, S.A., Binder, U., Papp, T., de Hoog, S., Jacobsen, I.D. & Voigt, K. (2015). Adaptation to thermotolerance in *Rhizopus* coincides with virulence as revealed by avian and invertebrate infection models, phylogeny, physiological and metabolic flexibility. Virulence. 6, 395–403. doi: 10.1080/21505594.2015.1029219.

Karlman, M. (1981). The introduction of exotic tree species with special reference to *Pinus Contorta* in Northern Sweden: review and background. College of Forestry, Swedish University of Agricultural Sciences.

Kaya, A.G.A., Lehjärvi, A., Kaya, Ö. & Dogmus-Lehjärvi, T. (2014). First Report of *Diplodia pinea* on *Pseudotsuga menziesii* in Turkey. Plant Disease. 98, 689. doi: 10.1094/pdis-07-13-0765-pdn.

Khatun, N. (2021). Applications of Normality Test in Statistical Analysis. Open Journal of Statistics. 11, 113. doi: 10.4236/ojs.2021.111006.

Klein, K. (2003). *Pseudotsuga menziesii* (Douglas –Fir). In: Aday, G., Lehtijärvi, A., Kaya, Ö. & Doğmuş-Lehtijärvi, T. (eds.) First Report of *Diplodia pinea* on *Pseudotsuga menziesii* in Turkey. Plant Disease, vol. 98. 689–689. doi: 10.1094/pdis-07-13-0765-pdn.

Knapp., A.K. & Anderson, J.E. (1980). Effect of Heat on Germination of Seeds from Serotinous Lodgepole Pine Cones. The American Midland Naturalist. 104, 370–372. doi: 10.1093%2Faobpla%2Fplz077.

Kolotelo, D. (1997). Anatomy & Morphology of Conifer Tree Seed. British Columbia.

Kondrateva, N., Kasatkina, N., Kuryleva, A., Baturina, K., Ilyasov, I. & Korepanov, R. (2020). Effect of treatment of seeds of grain crops by ultraviolet radiation before sowing. IOP Conference Series: Earth and Environmental Science. 433, 012039. doi: 10.1088/1755-1315/433/1/012039.

Kowalczyk, A., Kacprzak, M. & Mańka, M. (2004). Fungi inhabiting Scots pine (*Pinus sylvestris*) seeds in various stages of extraction process. Phytopathology. 33, 53-70.

Krakau, U.K., Liesebach, M., Aronen, T., Lelu-Walter, M.A. & Schneck, V. (2013). Scots pine (*Pinus sylvestris* L.). In: Pâques, L.E. (eds.) Forest Tree Breeding in Europe. iForest - Biogeosciences and Forestry, vol. 25. 267–323. doi: 10.1007/978-94-007-6146-9.

Krokene, P., Nagy, N. & Krekling, T. (2008). Traumatic resin ducts and polyphenolic parenchyma cells in conifers. In: Schaller, A. (eds.) Induced Plant Resistance to Herbivory. Springer, vol. 7. Dordrecht, Netherlands, 147–69. doi: 10.1007/978-1-4020-8182-8_7.

Laichmanová, M. & Sedláček, I. (2019). Fungal species associated with fruit and vegetables transported to the J.G. Mendel station and the influence of UV-C treatment on their fungal community. Czech Polar Reports. 9, 78–87. doi: 10.5817/CPR2019-1-7.

Larsson, R., Menkis, A. & Olson, Å. (2021). *Diplodia sapinea* in Swedish forest nurseries. Plant Protection Science. 57, 66–69. doi: 10.17221/68/2020-PPS.

Leverkus, A.B., Lázaro González, A., Andivia, E., Castro, J., Jiménez, M.N. & Navarro, F.B. (2021). Seeding or planting to revegetate the world's degraded land: systematic review and experimentation to address methodological issues. Restoration Ecology. 29, e13372. doi: 10.1111/rec.13372.

Li, D., Zhang, X., Gu, X., Zhang, Q., Zhao, L., Zheng, X. & Zhang, H. (2019). The infection of grapes by *Talaromyces rugulosus* O1 and the role of cell wall-degrading enzymes and ochratoxin A in the infection. Physiological and Molecular Plant Pathology. 106, 263–269. doi: 10.1016/j.pmpp.2019.03.006.

Liaquat, F., Liu, Q., Arif, S., Shah, I.H., Chaudhary, H.J. & Munis, M.F.H. (2019). First report of brown rot caused by *Rhizopus arrhizus* on tomato in Pakistan. Journal of Plant Pathology. 101, 1263–1263. doi: 10.1007/s42161-019-00320-8.

Lindgren, D., Karlsson, B., Andersson Gull, B. & Prescher, F. (2007). Swedish seed orchards for Scots pine and Norway spruce.

Liu, Y., Qu, Z.L., Liu, B., Ma, Y., Xu, J., Shen, W.X. & Sun, H. (2021). The Impact of Pine Wood Nematode Infection on the Host Fungal Community. Microorganisms. 9, 896. doi: 10.3390/microorganisms9050896.

Löf, M., Thomsen, A. & Madsen, P. (2004). Sowing and transplanting of broadleaves (*Fagus sylvatica* L., *Quercus robur* L., *Prunus avium* L. and *Crataegus monogyna* Jacq.) for afforestation of farmland. Forest Ecology and Management. 188, 113–123. doi: 10.1016/j.foreco.2003.07.013.

Luckey, T.D. (1980). Hormesis with Ionizing Radiation. CRC Press. doi: 10.1201/9780429276552.

Lücking, R., Aime, M.C., Robbertse, B., Miller, A.N., Ariyawansa, H.A., Aoki, T., Cardinali, G., Crous, P.W., Druzhinina, I.S., Geiser, D.M., Hawksworth, D.L., Hyde, K.D., Irinyi, L., Jeewon, R., Johnston, P.R., Kirk, P.M., Malosso, E., May, T.W., Meyer, W., Öpik, M., Robert, V., Stadler, M., Thines, M., Vu, D., Yurkov, A.M., Zhang, N. & Schoch, C.L. (2020). Unambiguous identification of fungi:

where do we stand and how accurate and precise is fungal DNA barcoding? IMA Fungus. 11, 14. doi: 10.1186/s43008-020-00033-z.

Maharachchikumbura, S.S.N., Guo, L.D., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011). *Pestalotiopsis*—morphology, phylogeny, biochemistry and diversity. Fungal Diversity. 50, 167–187. doi: 10.1007/s13225-011-0125-x.

Mallams, K.M. (2004). Fungal contaminants on selected conifer seed, J. Herbert Stone Nursery. Pacific Northwest Research Station.

Mancini, V. & Romanazzi, G. (2014). Seed treatments to control seedborne fungal pathogens of vegetable crops: Seed treatments to control seedborne fungi. Pest Management Science. 70, 860–868. doi: 10.1002/ps.3693.

Manning, W.J. & Vontiedemann, A. (1995). Climate change – potential effects of increased atmospheric carbon dioxide (CO2), ozone (O3), and ultraviolet-B (UV-B) radiation on plant diseases. Environmental Pollution. 88, 219–45. doi: 10.1016/0269-7491(95)91446-r.

Marquenie, D., Michiels, C., Geeraerd, A., Schenk, A., Soontjen, C., Van Impe, J. & Nicolaï, B. (2002). Using survival analysis to investigate the effect of UV-C and heat treatment on storage rot of strawberry and sweet cherry. International journal of food microbiology. 73, 187–96. doi: 10.1016/S0168-1605(01)00648-1.

Martín-García, J., Zas, R., Solla, A., Woodward, S., Hantula, J., Vainio, E.J., Mullett, M., Morales-Rodríguez, C., Vannini, A., Martínez-Álvarez, P., Pinto, G., Alves, A., Amaral, J., Wingfield, M.J., Fourie, G., Steenkamp, E.T., Ahumada, R., Šerá, B., Sanz-Ros, A.V., Raposo, R., Elvira-Recuenco, M., Iturritxa, E., Gordon, T.R. & Diez, J.J. (2019). Environmentally friendly methods for controlling pine pitch canker. Plant Pathology. 68, 843–860. doi: 10.1111/ppa.13009.

McDonald, K.F., Curry, R.D., Clevenger, T.E., Unklesbay, K., Eisenstark, A., Golden, J. & Morgan, R.D. (2000). A comparison of pulsed and continuous ultraviolet light sources for the decontamination of surfaces. IEEE Transactions on Plasma Science. 28, 1581–1587. doi: 10.1109/27.901237.

Mendonça, A., Santos, H., Franco-Duarte, R. & Sampaio, P. (2022). Fungal infections diagnosis – Past, present and future. Research in Microbiology. 173, 103915. doi: 10.1016/j.resmic.2021.103915.

Mengistu, A.A. (2020). Endophytes: Colonization, Behaviour, and Their Role in Defense Mechanism. International Journal of Microbiology. 2020, 6927219. doi: 10.1155/2020/6927219.

Microbiology. (2014). Morphology and general properties of fungi. https://nios.ac.in/media/documents /dmlt/Microbiology/Lesson-51.pdf.

Miller, D.L. & Schaefer, R.M. (2015). Cone and Seed Maturity and Collection Guidelines for Northern Idaho. 1-54.

Mitchell, C.E. & Power, A.G. (2003). Release of invasive plants from fungal and viral pathogens. Nature. 421, 625–7. doi: 10.1038/nature01317.

Mittal, R. (1986). *Penicillium thomii*. Canadian Journal of Forest Research. 17, 1026–1034.

Mittal, R.K., Anderson, R.L. & Mathur, S.B. (1990). Microorganisms associated with tree seeds: World Checklist 1990. Forestry Canada.

Moraga-Suazo, P., Opazo, A., Zaldúa, S., González, G. & Sanfuentes, E. (2011). Evaluation of *Trichoderma* spp. and *Clonostachys* spp. strains to control *Fusarium circinatum* in *Pinus radiata* seedlings. Chilean Journal of Agricultural Research. 71, 412– 17. doi: 10.4067/S0718-58392011000300011.

Munck, I.A. & Stanosz, G.R. (2010). Longevity of inoculum production by *Diplodia pinea* on red pine cones. Forest Pathology. 40, 58–63. doi: 10.1111/j.1439-0329.2009.00607.x.

Neelamegam, R. & Sutha, T. (2015). UV-C irradiation effect on seed germination, seedling growth and productivity of groundnut (*Arachis hypogaea* L.). International Journal of Current Microbiology and Applied Sciences. 4, 430–43.

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S. & Kennedy, P.G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology. 20, 241–248. doi: 10.1016/j.funeco.2015.06.006.

Nicholls, T.H. & Ostry, M.E. (1990). *Sphaeropsis sapinea* cankers on stressed red and jack pines in Minnesotaand Wisconsin. Plant Disease. 74, 54–56. doi: 10.1094/PD-74-0054.

Nilsson, R.H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P. & Tedersoo, L. (2019). Mycobiome diversity: high-throughput sequencing and identification of fungi. Nature Reviews Microbiology. 17, 95–109. doi: 10.1038/s41579-018-0116-y.

NordGen. (2021). Statistics: Forest Seeds and Plants in the Nordic Region. https://www.nordgen.org/wp-content/uploads/2021/12/FULLTEXT01-1.pdf.

Núñez, M.R. & Calvo, L. (2000). Effect of high temperatures on seed germination of *Pinus sylvestris* and *Pinus halepensis*. Forest Ecology and Management. 131, 183–190. doi: 10.1016/S0378-1127(99)00211-X.

Oskay, F. & Karataş, A. (2021). *Pinus nigra* subsp. pallasiana *ve Pinus sylvestris* tohumlarında *Diplodia sapinea*'nın yoğunluğu. Turkish Journal of Forestry. 22, 218–228. doi: 10.18182/tjf.799849.

Osono, T. (2011). Diversity and functioning of fungi associated with leaf litter decomposition in Asian forests of different climatic regions. Fungal Ecology. 4, 375–385. doi: 10.1016/j.funeco.2011.02.004.

Oszust, K., Cybulska, J. & Frąc, M. (2020). How do *Trichoderma* genus fungi win a nutritional competition battle against soft fruit pathogens? A report on niche overlap nutritional potentiates. International Journal of Molecular Sciences. 21, 4235. doi: 10.3390/ijms21124235.

Papavizas, G.C. (1985). *Trichoderma* and *Gliocladium*: Biology, Ecology, and Potential for Biocontrol. Annual Review of Phytopathology. 23, 23–54. doi: 10.1146/annurev.py.23.090185.000323.

Paul, F., Otte, J., Schmitt, I. & Dal Grande, F. (2018). Comparing Sanger sequencing and high throughput metabarcoding for inferring photobiont diversity in lichens. Scientific Reports. 8, 8624. doi: 10.1038/s41598-018-26947-8.

Paul, N.D & Gwynn-Jones, D. (2003). Ecological roles of solar UV radiation: towards an integrated approach. Trends in Ecology & Evolution. 18, 48–55. doi: 10.1016/S0169-5347(02)00014-9.

Pem, D., Jeewon, R., Selcuk, F., Ulukapi, M., Bhat, D.J., Doilom, M., Lumyong, S. & Hyde, K. (2020). Ribosomal and Protein Gene Phylogeny Reveals Novel Saprobic Fungal Species from *Juglans regia* and *Urtica dioica*. Frontiers in Microbiology. 11, 1303. doi: 10.3389/fmicb.2020.01303.

Peterson, G.W. (1977). Infection, epidemiology, and control of Diplodia blight of Austrian, Ponderosa, and Scots pines. Phytopathology. 67, 511–514. doi: 10.1094/Phyto-67-511.

Pigott, D. (2018). Cone collecting Methods and Safety. 2–9. https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/forestry/tree-seed/tree-seed-centre/whitebark-pine/collecting_whitebark_pine_cones_2018.pdf.

Prescher, F, Lindgren, D., Wennström, U., Almqvist, C., Ruotsalainen, S. & Kroon, J. (2005). Seed production in Scots pine seed orchards. In: Fedorkov, A. (eds.) Status, monitoring and targets for breeding programs, Proceedings of the Meeting of Nordic Forest Tree Breeders and Forest Genetics. Russian Academy of Sciences. Russia, Moscow. 65–71.

Prescher, F. (2007). Seed orchards – genetic considerations on function, management and seed procurement. Acta Universitatis Agriculturae Sueciae. 1-49.

Prochazkova, Z. (2009). Quality assessment methods for *Picea abies* seeds. Dendrobiology. 61, 111–115.

Prospero, S., Botella, L., Santini, A. & Robin, C. (2021). Biological control of emerging forest diseases: How can we move from dreams to reality? Forest Ecology and Management. 496, 119377. doi: 10.1016/j.foreco.2021.119377.

Purin, S. & Rillig, M.C. (2008). Parasitism of arbuscular mycorrhizal fungi: reviewing the evidence. FEMS Microbiology Letters. 279, 8–14. doi: 10.1111/j.1574-6968.2007.01007.x.

R Core Team. (2018). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org.

Rajjou, L & Debeaujon, I. (2008). Seed longevity: survival and maintenance of high germination ability of dry seeds. Comptes Rendus Biologies. 331, 796–805. doi: 10.1016/j.crvi.2008.07.021.

Ramsey, A.C. (2005). Ecology of fungal endophytes in Douglas-fir and ponderosa pine roots in eastern Washington. University of Washington. 1-80.

Reed, G. (2022). How to Interpret BLAST Results. The Computational Biology Magazine.

Reglinski, T. & Dick, M. (2005). Biocontrol of forest nursery pathogens. New Zealand Journal of Forestry. 50, 14– 21.

Reglinski, T., Taylor, J.T., Ah Chee, A., Northcott, G. & Spiers, M. (2013). Biochemical responses to ultraviolet-C radiation and methyl jasmonate in *Pinus radiata* seedlings that accompany induced resistance to *Diplodia pinea*. Plant Pathology. 62, 851–858. doi: 10.1111/ppa.12008.

Reithner, B., Ibarra-Laclette, E., Mach, R.L. & Herrera-Estrella, A. (2011). Identification of Mycoparasitism-Related Genes in *Trichoderma atroviride*. Applied and Environmental Microbiology. 77, 4361–4370. doi: 10.1128/AEM.00129-11.

Roberts, E.H. (1973). Predicting the storage life of seeds. Seed Science and Technology. 1, 499–514.

Roques, A. (2010). Alien forest insects in a warmer world and a globalised economy: Impacts of changes in trade, tourism and climate on forest biosecurity. New Zealand Journal of Forestry Science. 40, 77–94.

Rudramurthy, S.M., Singh, S., Kanaujia, R., Chaudhary, H., Muthu, V., Panda, N., Pandey, A., Thakur, S., Kaur, H., Ghosh, A., Agarwal, R. & Chakrabarti, A. (2023). Clinical and Mycologic Characteristics of Emerging Mucormycosis Agent *Rhizopus homothallicus*. Emerging Infectious Diseases. 29, 1313–1322. doi: 10.3201/eid2907.221491.

Sadeghianfar, P., Nazari, M. & Backes, G. (2019). Exposure to Ultraviolet (UV-C) Radiation Increases Germination Rate of Maize (*Zea maize* L.) and Sugar Beet (*Beta vulgaris*) Seeds. Plants-Basel. 8, 49. doi: 10.3390/plants8020049.

Samson, R.A. & Pitt, J.I. (2000). Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification. CRC Press. doi: 10.1201/9781482284188.

Sánchez, M.E, Andicoberry, S. & Trapero, A. (2005). Pathogenicity of three *Phytophthora* spp. causing late seedling rot of *Quercus ilex* ssp. ballota. Forest Pathology. 35, 115–125. doi : 10.1111/j.1439-0329.2004.00392.x.

Schmitz, S., Zini, J., Etienne, M., Moreau, J.M., Chandelier, A., & Cavelier, M. (2006). Effectiveness of thiophanate-methyl, trifloxystrobin and vinclozolin on canker caused by *Phoma exigua* Desm. on ash tree seedlings. BASE. 10, 25-31.

Schoch, C.L. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences. 109, 6241–6246. doi: 10.1073/pnas.1117018109.

Schroder, T., Kehr, R. & Huttermann, A. (2002). First report of the seed-pathogen *Geniculodendron pyriforme*, the imperfect state of the ascomycete *Caloscypha fulgens*, on imported conifer seeds in Germany. Forest Pathology. 32, 225–230. doi: 10.1046/j.1439-0329.2002.00288.x.

Shade, A. & Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. Environmental Microbiology. 14, 4–12. doi: 10.1111/j.1462-2920.2011.02585.x.

Shi, Y., Meng, S., Xie, X., Chai, A. & Li, B. (2016). Dry Heat Treatment Reduces the Occurrence of *Cladosporium cucumerinum*, *Ascochyta citrullina*, and *Colletotrichum orbiculare* on the Surface and Interior of Cucumber Seeds. Horticultural Plant Journal. 2, 35–40. doi: 10.1016/j.hpj.2016.02.004.

Siddiqui, A., Dawar, S. & Zaki, M. (2011). Role of ultraviolet (UV-C) radiation in the control of root infecting fungi on groundnut and mung bean. Pakistan Journal of Botany. 43, 2221–2224.

Sieber, T.N. (2007). Endophytic fungi in forest trees: are they mutualists? Fungal Biology Reviews. 21, 75–89. doi: 10.1016/j.fbr.2007.05.004.

Sikström, U., Hjelm, K., Holt Hanssen, K., Saksa, T. & Wallertz, K. (2020). Influence of mechanical site preparation on regeneration success of planted conifers in clearcuts in Fennoscandia – a review. Silva Fennica. 54, 10172. doi: 10.14214/sf.10172.

Silva, A.C., Diogo, E., Henriques, J., Ramos, A.P., Sandoval-Denis, M., Crous, P. W. & Bragança, H. (2020). *Pestalotiopsis pini* sp. nov., an Emerging Pathogen on Stone Pine (*Pinus pinea* L.). Forests. 11, 805. doi: 10.3390/f11080805.

Sinclair, J.B. (1993). Control of Seedborne Pathogens and Diseases of Soybean Seeds and Seedlings. Pesticide Science. 37, 15–19. doi: 10.1002/ps.2780370104.

Sinclair, W.A., Lyon, H.H. & Johnson, W.T. (2005). Diseases of Trees and Shrubs. Cornell University Press.

Slippers, B. & Wingfield, M.J. (2007). The Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact. Fungal Biology Review. 21, 90–106. doi: 10.1016/j.fbr.2007.06.002.

Smith, H., Wingfied, M.J. & Coutinho, T.A. (2002). The role of latent *Sphaeropsis sapinea* infections in post-hail associated die-back of *Pinus patula*. Forest Ecology and Management. 164, 177– 84. doi: 10.1016/S0378-1127(01)00610-7.

Smith, H., Wingfield, M.J., Crous, P.W. & Coutinho, T.A. (1996). *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. South African Journal of Botany. 62, 86– 8. doi: 10.1016/S0254-6299(15)30596-2.

Srinivasan, R., Prabhu, G., Prasad, M., Mishra, M., Chaudhary, M. & Srivastava, R. (2020). *Penicillium*. In: Amaresan, N., Senthil Kumar, M., Annapurna, K., Kumar, K. & Sankaranarayanan, A. (eds.) Beneficial Microbes in Agro-Ecology. Academic Press, vol. 32. 651–667. doi: 10.1016/B978-0-12-823414-3.00032-0.

Stanosz, G.R., Blodge, J.T., Smith, D.R. & Kruger, E.L. (2001). Water stress and *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings. New Phytologist. 149, 531–538. doi: 10.1046/j.1469-8137.2001.00052.x.

Stanosz, G.R., Smith, D.R. & Leisso, R. (2007). Diplodia shoot blight and asymptomatic persistence of *Diplodia pinea* on or in stems of jackpine nursery seedlings. Forest Pathology. 37, 145–154. doi: 10.1111/j.1439-0329.2007.00487.x.

Statistics Sweden. (2021b.) URL: http://www.statistikdatabasen.scb.se/pxweb/sv/ssd/START__HA__HA0201__HA0201B/ExpTotalKNAr /?rxid=f45f90b6-7345-4877-ba25-9b43e6c6e299.

Sutherland, J.R., Miller, T. & Quinard R.S. (1987a). Cone and seed diseases of North American Conifers. North American Forestry Commission publication. 1, 77.

Sutherland, J.R. & Van Eerden, E. (1980). Diseases and insect pests in British Columbia forest nurseries. Joint Report. 12, 55.

Svensson, L. (2019). Quality of Regrowth 2018/2019. Statistical report JO0311 SM 1901. Swedish Forest Agency.

Swedish Forest Agency. (2021a). URL: https://www.skogsstyrelsen.se/globalassets/statistik/statistiska-meddelanden/sm-levereradeskogsplantor-2020.pdf

Swedish Forestry Agency. (2013). Swedish statistical yearbook of forestry 2013. Swedish Forestry Agency.

Świecimska, M., Tulik, M., Šerá, B., Golińska, P., Tomeková, J., Medvecká, V., Bujdáková, H., Oszako, T., Zahoranová, A. & Šerý, M. (2020). Non-Thermal Plasma Can Be Used in Disinfection of Scots Pine (*Pinus sylvestris* L.) Seeds Infected with *Fusarium oxysporum*. Forests. 11, 837. doi: 10.3390/f11080837.

Talgø, V., Brodal, G., Klemsdal, S.S. & Stensvand, A. (2010). Seed borne fungi on *Abies* spp. Seed Science and Technology. 38, 477–493. doi: 10.15258/sst.2010.38.2.20.

Tanaka, Y. (1984). Assuring Seed Quality for Seedling Production: Cone Collection and Seed Processing, Testing, Storage, and Stratification. In: Duryea, M. L. & Thomas, D. L. (eds.) Forest Nursery Manual: Production of Bareroot Seedlings. Dr W. Junk Publishers, vol.4. Oregon, Corvallis. 386, 4.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Põldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Pärtel, K., Otsing, E., Nouhra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T.W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S. & Abarenkov, K. (2014). Global diversity and geography of soil fungi. Science. 346, 1256688. doi: 10.1126/science.1256688.

Tedersoo, L., Bahram, M., Zinger, L., Nilsson, R.H., Kennedy, P.G., Yang, T., Anslan, S. & Mikryukov, V. (2022). Best practices in metabarcoding of fungi: From experimental design to results. Molecular Ecology. 31, 2769–2795. doi: 10.1111/mec.16460.

Tyśkiewicz, R., Nowak, A., Ozimek, E. & Jaroszuk-Ściseł, J. (2022). *Trichoderma:* The Current Status of Its Application in Agriculture for the Biocontrol of Fungal Phytopathogens and Stimulation of Plant Growth. International Journal of Molecular Sciences. 23, 2329. doi: 10.3390/ijms23042329.

Udayangani, S. (2020). Difference Between Stratification and Scarification. https://www.differencebetween.com/difference-between-stratification-and-scarification/.

Udval, B. & Batkhuu, N.O. (2013). Seed and Cone Characteristics of Scots pine (Pinus sylvestris L.) from Diverse Seed Sources in Northern Mongolia. Eurasian Journal of Forest Research. 16, 57–62.

Vaghefi, N., Pethybridge, S.J., Ford, R., Nicolas, M.E., Crous, P.W. & Taylor, P.W.J. (2012). *Stagonosporopsis* spp. associated with ray blight disease of Asteraceae. Australasian Plant Pathology. 41, 675–686. doi: 10.1007/s13313-012-0161-3.

Valdés, I., Rodríguez, J., Portela, A. & Jiménez, P. (2014). First report of *Alternaria alstroemeriae* on *Alstroemeria* sp. in Colombia. New Disease Reports. 29, 21. doi: 10.5197/j.2044-0588.2014.029.021.

Van den Brule, T., Lee, C.L.S., Houbraken, J., Haas, P.J., Wösten, H. & Dijksterhuis, J. (2020). Conidial heat resistance of various strains of the food spoilage fungus *Paecilomyces variotii* correlates with mean spore size, spore shape and size distribution. Food Research International. 137, 109514. doi: 10.1016/j.foodres.2020.109514.

Viljoen, A., Wingfield, M.J. & Maras, W.F.O. (1994). First report of *Fusarium subglutians* f. sp. pini on pine seedlings in South Africa. Plant Disease. 78, 309–312. doi: 10.1094/PD-78-0309.

Von Sydow, F. (1997). Abundance of pine weevils (*Hylobius abietis*) and damage to conifer seedlings in relation to silvicultural practices. Scandinavian Journal of Forest Research. 12, 157–167. doi: 10.1080/02827589709355397.

Vujanovic, V., St-Arnaud, M. & Neumann, P.J. (2000). Susceptibility of cones and seeds to fungal infection in a pine (*Pinus* spp.) collection. Forest Pathology. 30, 305–320. doi: 10.1046/j.1439-0329.2000.00211.x.

Wajihi, A.H., Lee, S.Y., Das, K., Eom, A.H. & Jung, H.Y. (2019). First Report of *Allantophomopsiella pseudotsugae* Isolated from Soil in Korea. The Korean Journal of Mycology. 47, 29–34. doi: 10.4489/KJM.20190004.

Wang, S.R., Zhang, H., Chen, Y.Z., Zhang, Y.D., Li, D.B., Huang, Y., Zhang, G. & Yang, J. (2022). First Report of Black Spot Needle Blight of *Pinus sylvestris* var. mongolica Litv. Caused by *Heterotruncatella spartii* in China. Plant Disease. 106. doi: 10.1094/PDIS-12-21-2667-PDN.

Watt, M.S., & Bloomberg, M. (2012). Key features of the seed germination response to high temperatures. New Phytologist. 196, 332–336. doi: 10.1111/j.1469-8137.2012.04280.x.

Weir, B.S. (2014). How to get good fungal and bacterial identifications from GenBank sequences. NCBI. https://www.rhizobia.co.nz/ids-using-genbank.

Whittle, A.M. (1977). Mycoflora of cones and seeds of *Pinus sylvestris*. Transactions of the British Mycological Society. 69, 47–57. doi: 10.1016/S0007-1536(77)80114-9.

Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis. Springer. doi: 10.1007/978-0-387-98141-3.

Willan, R.L. (1987). A Guide to Forest Seed Handling, with Special Reference to the Tropics. Food and Agriculture Organization of the United Nations.

Wingfield, M.J., Hammerbacher, A., Ganley, R.J., Steenkamp, E.T., Gordon, T.R. Wingfield, B.D. & Coutinho, T.A. (2008). Pitch canker caused by *Fusarium circinatum*—A growing threat to pine plantations and forests worldwide. Australasian Plant Pathology. 37, 319–334. doi: 10.1071%2FAP08036.

Witzell, J. & Martin, J.A. (2008). Phenolic metabolites in the resistance of northern forest trees to pathogens – past experiences and future prospects. Canadian Journal of Forest Research. 38, 2711–27. doi: 10.1139/X08-112.

Wong, H.J., Mohamad-Fauzi, N., Rizman-Idid, M., Convey, P. & Alias, S.A. (2019). Protective mechanisms and responses of micro-fungi towards ultraviolet-induced cellular damage. Polar Science. 20, 19–34. doi: 10.1016/j.polar.2018.10.001.

Woods, T.A.D., Farris, S.H. & Sutherland, J.R. (1982). Penetration of Sitka spruce seeds by the pathogenic fungus *Caloscypha fulgens*. Canadian Journal of Botany. 60, 544–548. doi: 10.1139/b82-073.

Yamaji, K, Fukushi, Y, Hashidoko, Y, Yoshida, T. & Tahara, S. (2001). *Penicillium* fungi from *Picea glehnii* seeds protect the seedlings from damping-off. New Phytologist. 152, 521–531. doi: 10.1046/j.0028-646X.2001.00280.x.

Yang, Y., Luo, W., Zhang, W., Mridha, M.A.U., Wijesinghe, S.N., McKenzie, E.H.C. & Wang, Y. (2023). *Cladosporium* Species Associated with Fruit Trees in Guizhou Province, China. Journal of Fungi. 9, 250. doi: 10.3390/jof9020250.

Yong-Dong, Z.Y., Huang, Y.H., Manawasinghe, I.S., Wanasinghe, D.N., Liu, J.W., Shu, Y.X., Zhao, M.P., Xiang, M.M. & Luo, M. (2021). *Stagonosporopsis pogostemonis*: A Novel Ascomycete Fungus Causing Leaf Spot and Stem Blight on *Pogostemon cablin* (Lamiaceae) in South China. Pathogens. 10, 1093. doi: 10.3390%2Fpathogens10091093.

Zhou, J.H., Du, B., Liu, C.Q., Yang, S.C., Wang, Y.Y. & Zhu, Y.Y. (2006). First report of *Fusarium sporotrichioides* causing root rot of *Erigeron breviscapus* in China. Plant Pathology. 55. doi: 10.1111/j.1365-3059.2006.01470.x.

Zhou, Z.C., Tang, X., Hu, S., Zhu, W., Wu, X., Sang, W.J., Peng, L. & Ding, H. (2023). First Report of Grey Spot on Tobacco caused by *Alternaria alstroemeriae* in China. Plant Disease. doi: 10.1094/PDIS-11-22-2705-PDN.

Zhu, T.Y., Wang, B. & Guo, Q. (2006). Isolate high quality DNA from varieties of plant specimens with E.Z.N.A.TM plant DNA systems from Omega Bio-Tek. VWR International. 16, 10–11.

Popular science summary

Pathogen free seeds are critical to produce healthy plants for forest regeneration. However, recent studies have shown that tree seed carry a high diversity of fungi including known and unknown pathogens. There is a need to control seed-borne fungi to minimize the introduction of fungal pathogens, during plant propagation in reforestation nurseries. Thermotherapy treatments, such as heat and UVC light, are recognised as environmentally safe methods for controlling fungal infestation, but effects of such methods on tree seed fungi are poorly understood. Moreover, inadequate dose and duration of these methods can also weaken the viability of the seed by negatively affecting germination.

This study assesses the infection level and diversity of all and potentially pathogenic fungi (with a focus on *S. sapinea*) in seed lots of Lodgepole pine (*Pinus contorta* Dougl. ex Loud.), Scots pine (*Pinus sylvestris* L.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) before and after the heat at 55 °C for 8 h and UVC light one or three times. Seed germination was also assessed before and after the treatments.

The results showed that almost all tested seeds yielded fungi. The high infection level and diversity of fungi, including plant pathogens was showed for control seed lots of Lodgepole pine and Scots pine, and high infection levels and lower diversity for Douglas-fir seed lot. High incidence of *S. sapinea* was detected in the control PS18 seed lot, and negligible in the control PC14 seed lot. Overall, the heat treatment reduced fungal infection level and diversity to varying degrees in Lodgepole pine and Scots pine seeds, including potentially pathogenic fungi and *S. sapinea*. However, the reduction did not occur for the Douglas-fir seed lot. UVC treatments did not affect the fungal infection of any tree species. Considering only potentially pathogenic fungi, UVC reduced the infection in PS18 and PM14 seed lots, however increased in PC15 and PS20 seed lots. Occurrence of *S. sapinea* in PS18 seed lot, as well as diversity of all and potentially pathogenic fungi in all seed lots, compare to control treatment, was usually lower. The results also showed that none of the treatments had a significant negative effect on the germination of the tested seeds.

The study demonstrates that heat treatment at 55 °C for 8 h and UVC light one or three times can be further used to control fungal infestation, however, more research is needed to determine the most appropriate dose and duration, depending on the tree species. Performed in this study treatments can be used to reduce or eliminate fungal infection and diversity on conifer seeds without adversely effecting germination.

Keywords: seed-borne pathogens, artificial regeneration, conifers, thermotherapy

Appendix

Supplementary Table 1 *– Results of One-Way ANOVA Test (Number of seeds infected by potential pathogens per SL) and Kruskal-Wallis Test (Number of infected seeds per SL, Number of seeds infected with Sphaeropsis sapinea per SL) for the differences in fungal seed infection levels among treatments. Degrees of freedom (Df), chi square (χ2), P values, sum of squared errors (SSE) and mean squared error (MSE) are shown.*

Model	**Response variable**	**Factor**	**Df**	**χ2**	**P**	**SSE**	**MSE**
Number of seeds infected by potential pathogens per SL ~ Treatment	Number of seeds infected by potential pathogens per SL	Treatment	3	-	0.964	3735	1245
Number of infected seeds per SL ~ Treatment	Number of infected seeds per SL	Treatment	3	3.499	0.3208	-	-
Number of seeds infected with *Sphaeropsis sapinea* per SL ~ Treatment	Number of seeds infected with *Sphaeropsis sapinea* per SL	Treatment	3	0.587	0.8992	-	-

Supplementary Table 2 *- Results of One-Way ANOVA Test (Number of fungal morphotypes per SL, Number of fungal species per SL, Number of morphotypes of potential fungal pathogens per SL, Number of species of potential fungal pathogens per SL) for the differences in fungal presence/absence-based diversity among treatments. Degrees of freedom (Df), P values, sum of squared errors (SSE) and mean squared error (MSE) are shown.*

Model	Response variable	Factor	Df	P	SSE	MSE
Number of fungal morphotypes per SL ~ Treatment	Number of fungal morphotypes per SL	Treatment	3	0.3	928	309.3
Number of fungal species per SL ~ Treatment	Number of fungal species per SL	Treatment	3	0.515	176.1	58.69
Number of morphotypes of potential fungal pathogens per SL ~ Treatment	Number of morphotypes of potential fungal pathogens per SL	Treatment	3	0.114	100.4	33.45
Number of species of potential fungal pathogens per SL ~ Treatment	Number of species of potential fungal pathogens per SL	Treatment	3	0.139	33.5	11.167

Supplementary Table 3 *- Results of Kruskal-Wallis Test (Germination capacity per SL [%], Germination energy per SL [%]) for the differences in seed germination among treatments. Degrees of freedom (Df), chi square (χ2) and P values are shown.*

Model	Response variable	Factor	Df	χ2	P
Germination capacity per SL [%] ~ Treatment	Germination capacity per SL [%]	Treatment	3	2.3802	0.4973
Germination energy per SL [%] ~ Treatment	Germination energy per SL [%]	Treatment	3	0.6294	0.8897

Supplementary Table 4 *– Potential plant pathogens detected in this study, their distribution, symptoms and known hosts.*

Potential plant pathogen	Distribution	Known host/s	Symptoms	Reference/s
Penicillium bialowiezense	-North America (Canada, USA), -Central America (Panama), -Europe (Poland, Germany)	-Brussel, -*Lactarius luteolus*, -*Polyporus* sp., -Coconut, -Christmas fern (*Polystichum acrostichoides (*Michx.) Schott), -Redwood (*Sequoia* sp.)	-Decay of brussels sprouts	-Varga et al. (2008), -Scholtz & Korsten (2015), -Peterson et al. (2004)
Rhizopus arrhizus	World	-Tomato	-Brown rot	-Liaquat et al. (2019), - Munjal & Sharma (1975), - Mittal & Sharma (1982), -Garbowski (1936), -Urosevie (1961), -Mason & Van Arsdel (1978), -Gordon (1967), -Mittal et al. (1990)
Sphaeropsis sapinea	World	-50 pine species (*Pinus* spp.), -Douglas-fir, -Fir (*Abies* sp.), -Larch (*Larix* sp.), -Spruce (*Picea* sp.)	-Tip blight, -Stem and branch canker, -Dieback, -Yellowing of needles	-Adamson et al., (2015), -Kaya et al., (2014), -Munck & Stanosz (2010), -Whitehill et al., (2007), -Phillips et al., (2013)
Fusarium sp.	World	-Scots pine, -Douglas-fir	-Damping-off	-Swiecimska et al. (2020), -James et al. (1990)
Penicillium thomii	World	-Eastern white pine (*Pinus strobus* L.), -*Gyrineum roseum (*Tod.), - *Trichothecium roseum (Pers.)*	Unknown	-Mittal (1986), -Nicoletti et al., (2014)
Pestalotiopsis hollandica	-Europe (Spain)	-Mediterranean cypress (*Cupressus sempervirens* L.*),* -Bull banksia *(Banksia grandis),* - Japanese umbrella-pine *(Sciadopitys*	-Blighted shoots	-Silva et al., (2020), -Jiang et al., (2022)

		verticillate Sieb. & Zucc)		
Stagonosporopsis lupini	-North and South America, -Europe (UK)	-White lupin *(Lupinus albus* Forssk.*),* -Andean lupin *(Lupinus mutabilis* Sweet*)*	-Leaf Spot and Blight	-Boerema et al., (1999), -Vaghefi et al., (2012), -Yong-Dong, (2021)
Alternaria angustiovoidea	-North America	-Leafy spurge (*Euphorbia esula* L.), -Artichoke (Cynara cardunculus var. scolymus), -Corn, -Aowpea, -Okra (*Abelmoschus esculentus* L.), -Safflower (*Carthamus tinctorius* L.), -Zinnia (*Zinnia* sp.)	-Dead plants, -Chlorosis and wilting of leaves on cuttings of leafy spurge	-Ming-Yang et al, (1990)
Allantophomopsiella pseudotsugae	-Europe (Germany, UK, Netherlands, Norway, Iceland)	-Conifers: mostly pines, larch	-Phomopsis disease	-Wajihi et al., (2019)
Cladosporium subuliforme	-North America (Cuba), -Asia (China)	-Pepper (*Capsicum* sp.), -Passion fruit (*Passiflora edulis* Sims*),* -Common walnut *(Juglans regia L)*	-Yellow leaf spot, -Leaf blight symptom	-Yang, (2023)
Heterotruncatella spartii	-Africa (Tunisia), -Asia (China)	-Stone pine (*Pinus pinea* L.*),* *-Pinus sylvestris var. mongolica*	-Dieback disease, canker, -Black Spot Needle Blight	-Hlaiem et al., (2019), -Ren-Wang, (2022)
Dothiorella sarmentorum	-Europe (Slovakia), -South America (Chile)	-Ash (*Fraxinus* sp.), -Common Walnut, -Grapes (*Vitis* sp.), -Pistachio (*Pistacia* sp.)	-Declining tree, -Wood cankers, -Bud necrosis, -Shoot and branch dieback displayed through bleached, necrotic or discoloured canes in infected trees, -Branch dieback	-Ivanova, (2018), -Iqbal, (2022), -Gonzalez-Domıngueza et al., (2017)
Phoma sp.	-Europe (Belgium, France, UK)	-Ash	-Cancer	-Schmitz et al. (2006)
*Talaromyces rugulosu*s	-Asia (China)	-Grapes	-Decay	-Li et al., (2019)
Alternaria alstroemeriae	-South America (Colombia), -Asia (Japan, China),	-*Alstroemeria* sp., -Tobacco (*Nicotiana* sp.)	-Black spot disease, -Grey spot	-Yamagishi et al., (2009), -Valdes et al., (2014),

	- Australia			-Cheng Zhou et al., (2023), -Simmons & Hill, (2007)

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.