Pathogenicity of Two Novel *Corinectria* spp. from Central Siberia on *Abies alba*.

Inoculation tests and risk assessment as invasive species on *Abies alba* in Europe.

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Pathogenicity of Two Novel Corinectria spp. from Central Siberia on Abies alba. Inoculation tests and risk assessment as invasive species on Abies alba in Europe.

Patogenicitet hos två nya arter i släktet Corinectria från centrala Sibirien på Abies alba. Inokulations-test och riskbedömning som invasiva arter på Abies alba i Europa.

Johan Wredh

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Abstract

Two newly discovered species of fungi in the genus *Corinectria*, referred to as *Corinectria* sp. X and *Corinectria* sp. Y was found in Central Siberia in 2006 and sequenced 2020. This thesis aims to broaden the knowledge of the host range of these species in inoculation tests done on cuttings and saplings of *Abies alba*. The two new species are thought to be vectored by the bark beetle *Polygraphus proximus*. Their close relative *Corinectria fuckeliana* is a common wound inhabiting fungi on *Picea abies* in northern Europe but is known to infect a wide range of hosts. The inoculation test on saplings gave a positive result for pathogenicity on *A. alba* and re-isolation of fungi was somewhat successful, barely completing Koch's postulate. An eventual outbreak of the two *Corinectria* spp. on *A. alba* in Europe is most likely going to happen in association with *P. proximus* or another insect vector.

Två nyligen upptäckta svamparter i släktet *Corinectria*, nämnda som *Corinectria* sp. X och *Corinectria* sp. Y upptäcktes i centrala Sibirien år 2006, och sekvenserades 2020. Det här arbetet ämnar bredda kunskapen om möjliga värdarter för dessa arter genom inokulationstest på sticklingar och ungträd av *Abies alba.* De två nya arterna tros spridas med hjälp av barkborren *Polygraphus proximus*. Den närbesläktade svampen *Corinectria fuckeliana* är vanligt förekommande i sår på *Picea abies* i norra Europa men är känd för att infektera ett brett sortiment av värdträd. Inokulationstestet på ungträden gav ett positivt resultat för patogenicitet på *A. alba* och återisolering av svampen var någorlunda lyckat, vilket till viss del färdigställde Kochs postulat. Ett eventuellt utbrott av de två *Corinectria*-arterna på *A. alba* i Europa sker med största sannolikhet i association med *P. proximus* eller någon annan insektsvektor.

Keywords: Corinectria, *Abies alba*, *Polygraphus proximus*, Canker, Siberia, invasive species, insect vector

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Abbreviations

ANCOVA	Analysis of Covariance
EU	European Union
DNA	Deoxyribonucleic acid
GDP	Gross Domestic Product
GLM	Generalized Linear Model
IAFP	Invasive Alien Forest Pathogen
IUCN	International Union for Conservation of Nature

1. Introduction

1.1 Invasive alien forest pathogens

The International Union for Conservation of Nature (IUCN) defines invasive alien species as:

> ”[…] animals, plants or other organisms that are introduced by humans […] into places outside of their natural range, negatively impacting native biodiversity, ecosystem services or human economy and well-being.“ (IUCN 2024).

Invasive alien species poses one of the largest threats to biodiversity, being second only to habitat loss (Wilcove et al. 1998). Invasive alien forest pathogens (IAFPs) are mostly ascomycete fungi that are introduced to an area outside its native region where they cause disease and threaten the biological diversity of forest trees and shrubs. Pathogens can be defined as microbial agents which cause disease. The rate of invasions to Europe, and the spread of invasive species within Europe has been steadily increasing since the year 1800. Over the last 30 years, IAFPs which cause severe damage to trees like root rot and cankers have been increasing at a faster rate than IAFPs which cause gentler infections like defoliation. The extent and rate of invasion of IAFPs are correlated to both ecological, geographical and socioeconomic factors like biodiversity, genetic heterogeneity, temperature, and GDP. Once an IAFP has established itself in a new ecosystem it seems almost impossible to get rid of, meaning the best strategies are prevention and damage control. (Santini et al. 2012).

The global economic costs of invasive species have been estimated to 1.288 trillion dollars over the years 1970 to 2017 (Diagne et al. 2021). Another estimation of the economic losses from IAFPs specifically puts the annual cost in the United States at 2.1 billion dollars, with Dutch elm disease caused by *Ophiostoma novo-ulmi* and chestnut blight caused by *Cryphonectria parasitica* being the main culprits (Pimentel et al. 2000). *O. novo-ulmi* is, like many other tree pathogens, spread by insect vectors, specifically by bark beetles in the genus *Scolytus* (Jürisoo et al. 2021). Recently, DNA from *O. novo-ulmi* have been associated with other beetles like *Xyleborinus saxesenii,* and *Xyleborus dispar*

(Jürisoo et al. 2021). Insects' ability to vector fungal tree pathogens, and the ability of fungi to use different insects as vectors makes wood boring beetles a key player in understanding the pathways of new IAFPs. The main pathways for invasive insects are through wooden packing material, and global trading with live plant material (Panzavolta et al. 2021).

IAFPs outside their native range tend to be more destructive on species closely related to their native hosts than to the native hosts themselves (Parker & Gibert 2004; Loo 2008). An example of this is the aforementioned Dutch elm disease which is thought to originate from Asia where elms have developed resistance and only experience mild symptoms, whereas American and European elms suffer severe effects with high mortality rates (Hubbes 1999).

One way to determine if a specific microorganism is responsible for a disease is testing it for the four criteria of Koch's postulate, which are as follows:

1. The microorganism is present in abundance in all diseased organisms.
2. The microorganism is isolated from the diseased organism and grown in pure culture.
3. The microorganism causes disease when introduced from pure culture to a healthy host.
4. The microorganism must be re-isolated from the diseased host.

Koch's postulate was originally developed to identify animal pathogens but will in this thesis be used to settle the pathogenicity of a potential IAFP.

1.2 *Nectriaceae*

Nectriaceae is a large and ecologically diverse family of ascomycete fungi. Many species in the family are parasitic on woody plants (Mihál et al. 2014). The parasites tend to produce perennial cankers, leading to reduced water and nutrient flow, stunted growth, and potentially death (Sakamoto et al. 2004). Cankers are characterized by swelling of wood, necrotic lesions, deformed and brittle branches, and excessive sap production as a response from the tree (Sakamoto et al. 2004; Ghasemkhani 2012). The family includes parasites on a wide range of tree hosts, including broad leaf trees, conifers, and fruit producing trees like *Malus* spp. (apples) and *Theobroma* spp. (cacao) (Stauder et al. 2020; Mihál et al. 2014; Mohali & Stewart. 2021). Many of the affected trees have important roles in silviculture, horticulture, and food production. One of the genera in the family is *Corinectria*.

1.3 Corinectria

1.3.1 Outbreak in Siberia

In 2006 a new disease of unknown origin and causal agent of *Abies sibirica* dieback was observed in central Siberia. By the year 2017, the geographical extent of the disease had expanded approximately 450 km northwards (Pavlov et al. 2020, figure 1). Fungal isolation, ITS sequencing, and phylogenetic analysis resulted in the discovery of two previously undescribed species within the genus of *Corinectria*. The phylogenetic separation between the two species, referred to as *Corinectria* sp. X and *Corinectria* sp. Y, was well-supported, as was the separation with the closest group. This group included the species *Corinectria fuckeliana* (C. Booth) C. Gonzáles & P. Chaverri, 2017. A following inoculation test of *Corinectria sp. X* on *A. sibirica* gave a positive pathogenicity result. (Pavlov et al. 2020).

1.3.2 *Corinectria fuckeliana*

Since little is known about the two new species, a description of one of its closest relatives, *C. fuckeliana*, will help in understanding their biology. *C. fuckeliana* is a species native to the northern hemisphere with varying phenotypes, lifestyles, and hosts. It seems to be the one of the most common fungi in *P. abies*, occurring in both sound looking wood (Roll-Hansen & Roll-Hansen, 1979) and as a wound invader entering through both wounds and/or branch stubs (Vasiliauskas 1998; Roll-Hansen & Roll-Hansen, 1980). It can act as a saprophyte, obtaining nutrient from dead organic material; endophyte, living inside plants without causing disease; or as a disease-causing pathogen. It has at least three spore phases: one sexual phase producing ascospores called the *Neonectria* phase, one unicellular asexual phase called the *Acremonium* phase, and one multicellular asexual phase called the *Cylindrocarpon* phase (Crane et al. 2009). The ascospores produced in the fruiting bodies seems to play a big role in the dispersal and spread of *C. fuckeliana* (Vasiliauskas & Stenlid 1997), but it is also speculated that wood boring beetles could act as vectors of infection since a positive correlation has been found between the occurrence of *C. fuckeliana* and attacks from the spruce bark beetle *Dendroctonus micans* in *P. abies* (Vasiliauskas & Stenlid 1997). The nature of the association between these organisms is still unknown, but it is not uncommon for species in closely related genera like *Neonectria* to have complex interactions with insects. Examples include the interaction between *Neonectria faginata* and *Neonectria ditisssima* with the beech scale insect *Crypococcus fagisuga* (Crane et al. 2009). *C. fuckeliana* has a wide range of hosts, occurring in various species of the genera *Abies, Larix, Picea* and *Pinus* (González & Chaverri

2017). *C. fuckeliana* does occurs in *Abies alba*, but not as commonly as in *P. abies* (Metzler et al. 2012; Mihál et al. 2014).

1.3.3 *Corinectria* spp. X and Y

The inoculation test with *Corinectria* sp. X on *A. sibirica* performed by Pavlov et al (2020) suggests that the outbreaks recorded in Central Siberia could be caused by these two novel *Corinectria* spp. The relationship between them and interactions with other fungal species or insect vectors is still largely unknown. A following pathogenicity test on *Abies lasiocarpa, Picea abies, Pinus sylvestris*, *Pseudotsuga menziesii*, and *Larix sibirica* gave a positive result for both *Corinectria* sp. X and *Corinectria* sp. Y on only *A. lasiocarpa* (Menkis et al, 2024), suggesting the two species prefer trees in the genus *Abies*. The same study also showed a clear difference in the aggressivity of the species, showing *Corinectria* sp. X to be a more aggressive pathogen on *A. lasiocarpa* than *Corinectria* sp. Y. Since the first isolation of the two species, at least 22 new isolates have been reported.

A possible insect vector is the four-eyed fir bark beetle *Polygraphus proximus* which originates from northeastern China, Korea and Japan. *P. proximus* is currently acting as an invasive species with established populations in Central Siberia (the region where the two *Corinectria* spp. were discovered). In 1999 an insular finding was done on *P. abies* near Saint Petersburg, and in 2006 it was found in plantations of *A. sibirica* in Moscow oblast (Baranchicov et al. 2010; Kerchev 2014; Musolin et al. 2022). It is thought that the main pathway for the spread of *P. proximus* is through un-barked timber transport and wood packaging (Musolin et al. 2022). In its invasion, *P. proximus* has expanded its range of tree hosts, preferring *Abies* species (EPPO 2014; Musolin et al. 2022). *Corinectria* sp. X has been detected in 15 out of 23 samples of *P. proximus* using high throughput sequencing of fungal ITS2 rDNA as means of identification (Menkis et al, 2024).

1.4 *Abies alba*

The genus *Abies* consists of around 40 different species spread across the northern hemisphere (Semerikova et al. 2018). It can be phylogenetically divided into six groups based on chloroplast DNA sequences. *A. alba* (Central Europe) is placed in group IV, *A. lasiocarpa* (Western North America) in group V, and *A. sibirica* (Siberia) in group VI. Out of these three groups, IV is the outgroup, meaning *A. lasiocarpa* and *A. sibirica* share a more recent common ancestor with each other than with *A. alba*. (Semerikova & Semerikov 2013). This fact is important since *A. lasiocarpa* and *A. sibirica* have already been shown to be susceptible to the new *Corinectria* spp. while *A. alba* has not. A positive pathogenicity test on *A.*

alba would expand the phylogenetic spectrum of known hosts for the new *Corinectria* spp.

A. alba is a tree of significant economic importance, being used in construction, paper pulp, turpentine and medical products, and as decorative Christmas trees (Dobrowolska et al. 2017). Christmas tree stands consist of many different exotic species growing in relatively close approximation. For silviculture of *A. alba* it is suggested to use a forestry model with a wide age range (such as continuous cover model) rather than even aged stands (like a clear cut model). *A. alba* needs shade as a sapling and is dependent on shelter trees (Dobrowolska et al. 2017).

The fact that *A. alba* is a tree of high economic and cultural significance, and that species in the family *Nectriaceae* are known to cause damage and reduce growth, makes basic research into the biology of these two novel *Corinectria* spp. valuable

1.5 Purpose

This thesis aims to contribute to the knowledge about the host range of the two newly detected species of *Corinectria* found in Central Siberia by testing their pathogenicity on saplings of *A. alba*.. The hypothesis is that *A. alba* could be susceptible to infection by the two *Corinectria* spp. since at least two other species in the genus of *Abies* are. Furthermore, *Corinectria* sp. X is expected to be more aggressive than *Corinectria* sp. Y, as has been shown through tests on *A. lasiocarpa* (Menkis et al. 2024).

2. Methods

To test the pathogenicity of *Corinectria* sp. X and *Corinectria* sp. Y on *A. alba*, two inoculation experiments were set up: one using cuttings (experiment I) from a single tree, and one using dug up saplings (experiment II). In experiment I, the branches were inoculated with three fungal species: *Corinectria* sp. X, *Corinectria* sp. Y, and *C. fuckeliana*, using two isolates of each fungal species and one control. Each treatment was replicated on nine cuttings resulting in a total of 63 replications. In experiment II, the branches were inoculated with two fungal species, *Corinectria* sp. X *and Corinectria* sp. Y, using the same two isolates as experiment I, and one control. Each treatment was replicated on six saplings resulting in a total of 30 replications. The fungal isolates used for the three different species can be found in table 1.

Table 1. The isolates used in the inoculation experiments.

Corinectria sp. X	*Corinectria* sp. Y	*C. fuckeliana*
N8 (Central Siberia)	N7 (Central Siberia)	ST1 (Uppsala, Sweden)
N21 (Central Siberia)	N15 (Central Siberia)	ST2 (Uppsala, Sweden)

2.1 Inoculation tests

2.1.1 Experiment I

Several lower branches were collected from a medium sized (around ten cm diameter at breast height) *A. alba* on 2024-02-06. The cut ends were put in a plastic bag for transport. They were then stored with the cut end in a bucket of water until the next day. The next day, shoots that measured around 25 cm in length and about 1.5 mm in stem width were made into cuttings and placed in a mesh on top of a box of water (Bräuner Nielsen et al. 2017; Skulason et al. 2017). The shoots were marked with a coloured bead attached to the mesh that corresponded to a specific treatment. The cuttings were spread in a randomized way over four boxes to create a random block design. To inoculate the shoots, the needles were cut, and the bark scraped with a scalpel over a length of about 1.5

cm to expose fresh wood. The inoculum, a piece of aspen wood inoculated with mycelium about one mm thick and one cm long, were placed on the wound with tweezers. The wound and piece of wood were then wrapped in parafilm. The tweezers and scalpel were sterilized in between every inoculation. The control was done using identical pieces of aspen that had been placed on the same agar medium but without mycelial inoculation. The four boxes were then placed in a climate chamber with a 16:8-hour day-night cycle at 20°C to emulate summer.

The data was collected eight weeks after inoculation by taking notes of the state of the cutting (dead top or alive top) The outer bark around the inoculation site was scraped off to look for any obvious necrotic lesions, like presence of necrotic phloem and production of sap.

2.1.2 Experiment II

About 20 days into experiment I, the needles of the cuttings were observed to dry up: first above the inoculation site, and later also below the inoculation site. Since the point of the experiment was to see the fungal infection and colonization on living tissue, experiment I was compacted to two boxes to make space in the climate chamber for a second inoculation experiment. Experiment II was set up on two-to-three year-old saplings. The saplings were picked out based on size and apparent health from an area with several healthy saplings of similar size. Collecting all saplings from a small area would ensure soil properties to be as similar as possible between replicates. A square of soil with the volume of around one litre was dug up around the self-regenerated saplings and placed into a pot, making sure the soil was in direct contact with the bottom of the pot to allow the plants to absorb water from the tray in which they were placed. The reasoning was that a thicker stem diameter would make the data collection easier and more reliable, and having a sapling with roots in soil would prevent the infected tissue from drying. The saplings were watered twice a week by pouring water in their trays. The saplings were collected on 2024-02-28 from the same forest as the cuttings. The inoculation was done one week after collection using the same method and identical inoculation plugs as the cuttings. Since the saplings had been regenerated and growing in the wild where they experienced grazing from deer along with other disturbances, they looked quite different from one another. This made having a consistent method for picking the inoculation point difficult. The internode used as inoculation site was decided by the following criteria: The thickest and longest internode accessible for inoculation and parafilm wrapping without bending and damaging the branch. This resulted in the inoculated internodes being of varying age.

Data was collected eight weeks after inoculation. Measurements were done on:

1. The height of the inoculation site (mm). This was measured from the centre of the inoculation site to the point where the tree emerged from the soil.
2. The length of the internode where inoculation was done. Necrotic lesions tend to stop or spread slower when reaching a node, meaning the size of the internode could have a large effect on the extent of the lesion.
3. The trunk diameter of inoculation site. This was measured since it could influence the rate of expansion of the necrotic lesion.
4. The expanse of the necrotic lesion. Lesions were assessed by carefully cutting of the outer bark with a scalpel and exposing the phloem. The presence of resin and necrotic tissue outside the inoculation site with a sharp transition to the healthy tissue were used as indicators for cankers. The vertical expanse of the lesion was measured from the very top of the necrotic tissue to the verry bottom. Brown, dry surface tissue from the wounding that did not extend outside the inoculation site were not considered as cankers and thus not measured.
5. Presence of brown needles. If the sapling had one or several branches with brown needles that were not present when extracting the plants from the forest, it was noted binomially as a 1 = presence of brown needles, or a 0 = no brown needles.
6. Dead tops. If a canker had surrounded the full circumference of the sapling and killed the top of the tree, it was noted as a dead top. The vertical lesion length of a canker like this was measured from the centre of the inoculation site to its lowest point. This value was then doubled assuming equal spread upwards.

To complete Koch's postulate of linking a microbe to a disease, the *Corinectria* spp. had to be re-isolated from the infected plant. To do this, a small piece of tissue from the inoculation site was cut using a flamed scalpel. The piece was briefly swept through a flame to kill of any surface microbes and put into a petri dish with solid Hagem media (Stenlid 1985). The petri dish was sealed with parafilm and observed over the following days. Any *Corinectria*-like mycelia growing was subsequently sampled and transferred to a new petri dish with Hagem media to prevent eventual contamination from other fungi in the wood tissue. The fungi were then identified morphologically. Using PCR and ITS sequencing would have been ideal to get a more secure result, but these methods were unavailable due to time restraints.

2.1.3 Sources of error

The saplings came with more varying factors than the cuttings. This includes variation in tree genotypes, internode age, soil macro- and microbiome, soil texture and structure, and grazing damage from deer. The plants were dug up during late winter and put in a climate control chamber that emulated summer. Trees in temperate climates tend to enter dormancy during the cold season (Havranek & Tranquilini 1995). Taking dormant trees out of the ground and putting them in the warmth could stress them or leave them vulnerable to other pathogens that could interfere with the experiment.

2.2 Statistical analysis

All statistical analyses were done in R-studio (2023.12.1 +402). The raw data from experiment II can be found in appendix 1. The code and packages used can be found in appendix 2.

2.2.1 Experiment I

No statistical analysis was performed on the data collected from experiment I.

2.2.2 Experiment II

To see the effect of internode size, trunk diameter, and inoculation site height, three individual linear regression models were created with internode length, diameter, and inoculation site height respectively as explaining variables, and vertical length of necrotic lesion as response variable. Since this test was set up to see how the size of the cankers was affected by these factors, a subset of the data was created to only include the data points that had formed cankers, i.e. only the data points inoculated with a fungal treatment.

To test if there was a significant variance in the mean vertical length of necrotic lesion between the two species, an analysis of covariance (ANCOVA) was done by pooling the fungal treatments into their corresponding species (as seen in table 1) and comparing the means of vertical length of necrotic lesion against each other. Internode size and diameter were used as conditioning factors. These variables where not correlated (appendix 3). Since the cankers fully surrounding the circumference of the sapling were measured using a different method, the vertical extent of their necrotic lesion is not comparable to the rest of the dataset. Thus, they were removed from the dataset. This dataset also did not include control replicates and had normally distributed data with equal variance within groups (appendix 3).

The presence of brown needles between treatments was tested by running a logistic, multi factor regression with four dummy variables for the different treatment categories, leaving control as reference variable. The test used a general linear model (GLM).

3. Results

3.1 Experiment I

In experiment I, three of the control replicates had had live tops, but all three appeared to have a failed inoculation where not enough bark was scraped off to enable proper contact with wood and are thus inappropriate to be used in the dataset for any statistical analysis. This was also the case for two of the cuttings inoculated with N7. None of the successful inoculations had a live top (table 2).

One canker-like structure was found in a cutting inoculated with *C. fuckeliana* (ST1 isolate) (figure 1). It had a necrosis spread of 3.2 mm.

Table 2. shoot status. "" indicates failed inoculation where not enough bark was scraped off to enable proper contact with the wood.*

Treatment	Dead	Alive
N7	7+2*	0
N15	9	0
N8	9	0
N21	9	0
ST1	9	0
ST2	9	0
Control	6	3*

Figure 1. A canker-like structure found on a cutting inoculated with C. fuckeliana (ST1 isolate). The orange patch marked "A" is mycelium left over from the inoculum. The brown region marked "B" is necrotic phloem and potentially canker formation. The green region marked "C" is healthy phloem.

3.2 Experiment II

All saplings inoculated with mycelia had well developed cankers, while none of the control saplings showed any signs of canker development (figure 2, figure 3).

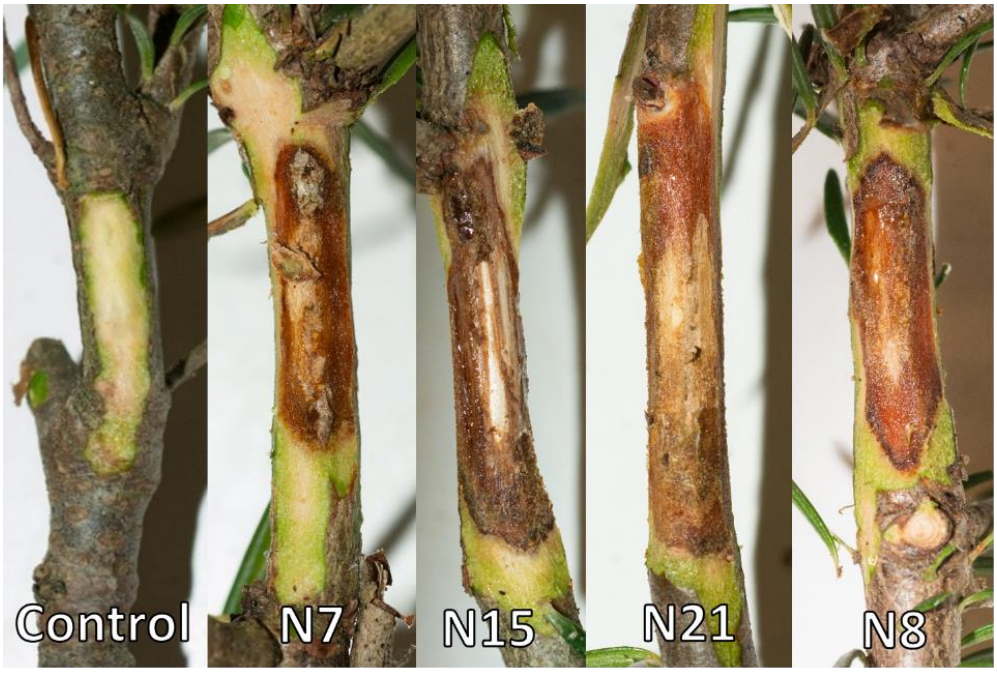

Figure 2. Examples of cankers formed on the saplings of A. alba in experiment II. The treatments used in inoculation are displayed under each example.

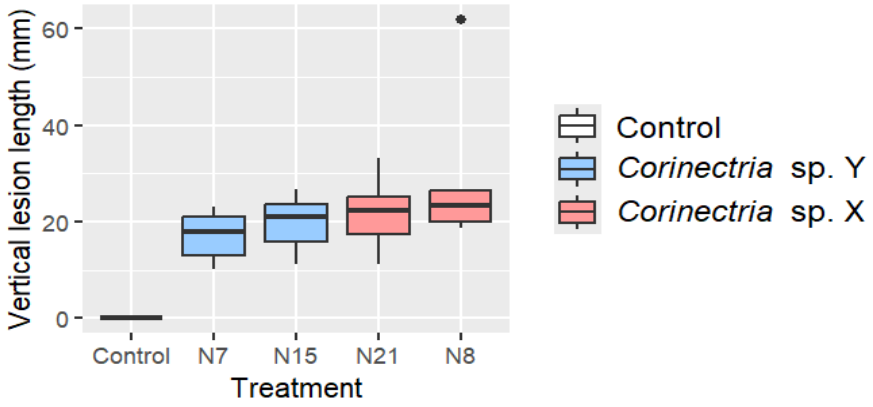

Figure 3. Boxplot showing the vertical extent of necrotic lesion for every treatment. The top and bottom of the boxes shows upper and lower quartile, middle line shows the median, and the lines/dot extending beyond the boxes show the minimum and maximum values. The isolates of Corinectria sp. Y are shown as blue, and the isolates of Corinectria sp. X are shown as red.

The linear regression models gave a significant p-value with 95% confidence for the diameter and internode size effect on vertical lesion length, while the height of the inoculation site had no significant effect on the size of the lesion (figure 4, table 3).

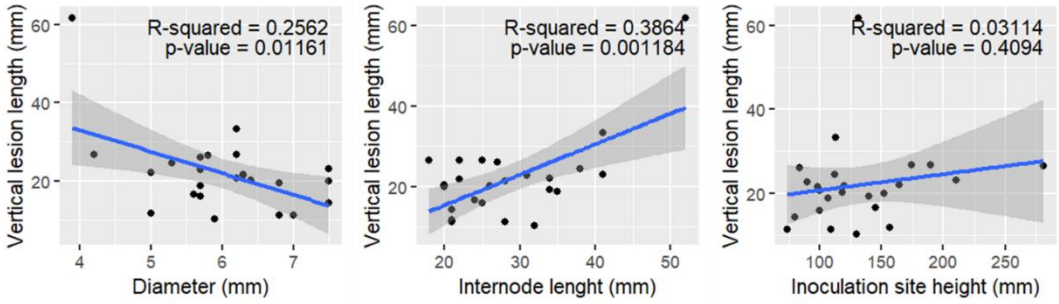

Figure 4. Linear regression models for the effect of diameter, internode size, and height of inoculation site on the vertical length of the necrotic lesion. The blue line is a fitted trend line and the darker grey area around the trend line displays the 95% confidence interval estimates. The models respective multiple R-squared value and p-value are displayed on each graph.

The ANCOVA test with fungal treatments pooled into species gave a p-value of 0.132 (table 4).

No significant correlation was found in the GLM for any of the treatments and the presence of dead needles (table 5).

One sapling inoculated with isolate N8 had a full radial spread of necrosis. This had resulted in the lesion spreading to the closest node downwards, and the needles in the crown drying up (figure 5).

At least one re-isolation of fungi per treatment showed growth of *Corinectria*-like mycelia (figure 6). All other replicates had fast growing mycelia which produced green conidia. This was suspected to be fungi from the genus *Trichoderma*. No *Corinectria*-like mycelia was observed growing from the tissue of the controls.

Figure 5. Sapling inoculated with isolate N8. Necrotic lesion has spread fully around the trunk killing the top. The necrosis has spread all the way to the next lowest node. The area marked "A" is the inoculation site. "B" marks the edge of the necrotic lesion.

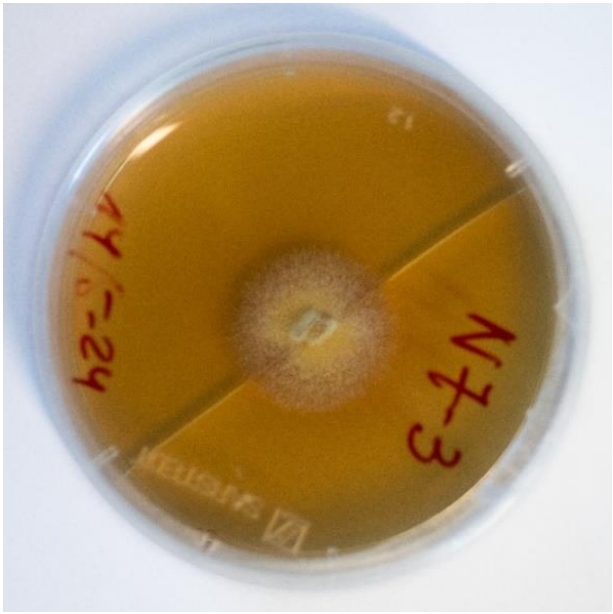

Figure 6. Corinectria-like mycelia growing on Hagem media. The sample has been reisolated from a sapling inoculated with isolate N7.

Table 3. Coefficient, standard error, P-value, multiple R^2 from individual linear regression models. Response variable of all models is length of necrotic lesions in saplings inoculatied with fungal treatment.

Explanatory variable	Coefficient	Std Error	P-value	Multiple R^2
Diameter	-5.552	2.0167	0.0116	0.256
Internode size	0.757	0.203	0.00118	0.386
Inoculation site height	0.0384	0.0457	0.409	0.0311

Table 4. Result of ANCOVA test comparing the difference in means of vertical necrotic lesion length for the treatments pooled into species. Diameter and internode size are used as conditioning variables. The table shows the degrees of freedom, sum of squares, mean of squares (sum sq/df), f value, and p-value.

	Df	Sum sq	Mean sq	F value	Pr(>F)
Species	1	78.2	78.22	2.483	0.132
Residuals	19	598.5	31.50		

Table 5. Result from GLM testing for treatment effect on the pressence of brown needles using dummy variables for the treatments. Control was used as reference variable and is read in the table as intercept. The table shows values for coefficients, standard error, z-value, and p-value.

	Estimate	Std. Error	z value	Pr(> |z|)
(Intercept)	-6.931e-0	8.660e-01	-0.800	0.423
N8	-9.163e-01	1.396e+00	-0.656	0.512
N21	2.258e-16	1.225e+00	0.000	1.000
N7	-1.887e+01	4.390e+03	-0.004	0.997
N15	-1.887e+01	4.390e+03	-0.004	0.997

4. Discussion

4.1 Experiment I

The cuttings used in the experiment were only about 1.5 mm in diameter, about the same width as the inoculation plug. The mechanical damage from the inoculation probably caused the top part of the cuttings to die off to the next lowest node. Some of the cuttings also had a complete dieback where only the needles under the water surface remained green when the data from experiment I was assessed. They were most likely unable to transport water up the stem. Thin branches tend to be more sensitive to embolism than thicker branches (Cochard 1992).

The canker found on the branch inoculated with *C. fuckeliana* could be the result of a fungal infection. *C. fuckeliana* is known to infect *A. alba* (Metzler et al. 2012). The thinness of the branch made sterilizing the outside and re-isolating the fungus unfeasible and unreliable. No conclusion can be drawn from this singular observation.

All in all, this method is not an appropriate way to perform an inoculation experiment on small diameter branches. An alternative way to conduct the inoculation test could include one or more of the following suggestions: using thicker branches to make measuring necrosis easier and more reliable; having a gentler way of inoculating, like removing a needle and using agar plugs as inoculum on the needle stump; and a shorter inoculation time to reduce the risk of natural dieback (although enough time for necrosis to develop is needed). This is what Bräuner Nielsen et al. (2017) did in their inoculation test with *Neonectria neomacrospora* on trees in the genus *Abies*. They had better success than experiment I in this thesis.

4.2 Experiment II

The result of the experiment suggests that both *Corinectria* sp. X and *Corinectria* sp. Y are pathogenic on *A. alba*. The slight differences in means of vertical lesion extent between the species (figure 3) could be a real effect, but it is not considered

significant with 95% confidence in this pathogenicity test (table 4). Considering there is evidence for *Corinectria* sp. X being slightly more aggressive than *Corinectria* sp. Y on *Abies lasiocarpa* (Menkis et al, 2024), one would expect to see a larger difference in canker size between the species in this experiment. Some explanations as to why it is not considered significant in this experiment could be unaccounted for variabilities such as tree genotype, internode age, and soil and shoot microbiome. Microbes interacting with the plants both through the roots and on the foliage can trigger immune responses in plants like systemic acquired resistance and induced systemic resistance, priming the plant for an upcoming infection or insect attack (Bakker et al. 2013; Van Wees et al. 2008). Potential differences in the composition of the saplings associated microbiome could lead to differences in the extent of the fungal infections in experiment II. Having more replicates could also help reveal any true difference between the species. Another explanation is that the difference in aggressivity between the species is smaller or non-existent in *A. alba*.

The sapling with a full radial canker (figure 5) shows that *Corinectria* spp. X can kill the top of a young *A. alba*. This is something that has been observed from these fungi in the other *Abies* sp. on which they have been tested: *A. sibirica*, and *A. lasiocarpa* (Menkis et al. 2024; Pavlov et al. 2020). It can be assumed that several others of the inoculated saplings would develop a full radial canker as well had the inoculation time of the experiment been longer.

Re-isolations were not successful in all replicates. *Corinectria* mycelia grows slower than, for example, Trichoderma, meaning if the piece of wood used in re-isolation was contaminated by fast growing fungi, these could outcompete the *Corinectria* on the plate. The pieces of wood were quickly swept through a flame to sterilize the outside, but any fungi growing in the tissue together with the wood could have been transferred to the agar plate. The wood pieces might not have been in the flame for long enough to kill of all surface microbes leading to contaminants on the outside being transferred to the agar plate. Re-isolations were successful in a few replicates of every isolate, barely completing Koch's postulate.

4.3 Risk of invasion

A. alba might be susceptible to the two *Corinectria* spp., but for an invasion to happen, there must also be pathways for them to reach locations where *A. alba* is growing. The spread could happen trough aerial ascospores, as might be the case of *C. fuckeliana* (Vasiliauskas & Stenlid 1997), but it is unlikely this is what will infect trees over long distances. Most likely it will be vectored by an insect, like *P. proximus*. *P. proximus* has been spreading further and further westwards, with the insular finding as far west as St. Petersburg in 1999 and the invasion of

Moscow oblast (Baranchicov et al. 2010; Kerchev 2014). Currently, *A. alba* is not included in the expanding host range of *P. proximus* (Bernardinelli et al. 2014), but so far, the invasion of *P. proximus* has not extended into the native territory of *A. alba*, so the barrier might be geographical rather than biological.

Since the Russian invasion of Ukraine, legal timber transport from Russia to the European Union (EU) has been prohibited. Russian timber is, however, allowed to be transported to Belarus, which has *A. alba* growing right across its western border in Poland (Wolf 2003). *P. proximus* is able to fly several kilometres, meaning careless management of timber products from regions where it has already established could lead to the beetle, and maybe also the fungi, spreading to the native regions of *A. alba* (Koivula 2024). Since many fungi can be vectored by several insect species, it can not be excluded that another bark boring beetle ultimately could be responsible for the spread of the *Corinectria* spp. to *A. alba*.

To understand the consequences of the *Corinectria* spp. reaching *A. alba* stands in Europe, it can be helpful to compare it to the effects of closely related *C. fuckeliana* when it has reached forestry stands far outside its natural range. In the mid-1990s, *C. fuckeliana* (native to the northern hemisphere) was found to produce cankers on *Pinus radiata* stands in New Zeeland. The cankers are causing deformation of the main trunk and most likely reduces growth, although complete tree death seems to be rare (Dick & Crane. 2009). In 2008 *C. fuckeliana* was also discovered in *P. radiata* stands in Chile where similar symptoms were observed (González & Chaverri. 2017). Growth loss was not estimated to be very large, but since it is affecting and malforming the trunk of the tree, and the main economic value of this part of the tree comes from being straight and uniform when turned into planks, economic losses were still expected (Morales 2009; Dick & Crane. 2009). It is likely something similar could occur on *A. alba* in Europe. Depending on the rate of infection in young trees and how big of a diameter that can develop a full radial canker (like seen in figure 5), forestry stands could experience severe losses in newly planted areas. It is still unknown how the infection of these fungi would be affected by different forestry models, but the continuous cover forestry recommended for *A. alba* by Dobrowolska et al. (2017) could be beneficial in reducing the severity of an eventual invasion, especially if the outbreak is vectored by a bark beetle like *P. proximus*. Assuming bark beetles are niched to prefer a specific age of trees, a large stand consisting of same-aged trees that are the perfect age for the beetle could trigger large swarms leading to dire outbreaks.

Since *A. alba* is being grown and sold as a Christmas tree, it is grown in specific Christmas tree plantations. Christmas tree stands are often comprised of several exotic *Abies* spp. native to different parts of the world growing in close proximity to each other (Bräuner Nielsen et al. 2017). Thus, these plantations are

high risk areas where different *Abies* spp. get in contact with each other and where fungal infections like the *Corinectria* spp. could get transmitted and spread.

The two *Corinectria* spp. are currently of unknown origin. One possibility is that they have been introduced together with *P. proximus*. Since IAFPs tend to be less aggressive on their native hosts (Parker & Gibert. 2004; Loo 2008), it could be that they have gone unnoticed in Northeastern China and Japan. Either because the trees in China and Japan are more resistant to the fungus itself, or because the trees are more resistant to *P. proximus* and the damage of the fungi could be correlated with the damage of *P. proximus*. Another pathogenicity test could be done with the two *Corinectria* spp on the native host trees of *P. proximus*, for example *Abies mariesii*, or *Picea glehnii* (Kerchev 2014). Another possibility is that the two *Corinectria* spp. are native to Central Siberia where they were found. The question then becomes what has caused them to suddenly make themselves visible and problematic. It could have been triggered by the arrival of *P. proximus*, or it could be linked to climate change which in Siberia has led to increased temperatures, aridity and extreme weather (Callaghan et al. 2021).

4.4 Conclusion

In conclusion, the European species *A. alba* is susceptible to infection by the two new *Corinectria* spp.. An eventual invasion into Europe is most likely going to happen in association with *P. proximus* or another bark boring beetle. The outbreak would cause some economic losses by means of reduced growth, timber deformation, and sapling dieback. Christmas tree plantations are high risk zones where several exotic *Abies* spp. grow in close approximation. More inoculation tests need to be done on potential tree hosts, specifically trees that are hosts to *P. proximus* in its native region of Northeastern Asia. Further research is also needed on the loss of growth from the two *Corinectria* spp. and *C. fuckeliana.*

References

Bakker, P. A. H. M., Doornbos, R. F., Zomioudis C., Berendsen, R. L., Pieterse, C. M. J. (2013) Induced Systemic Resistance and the Rhizosphere Microbiome. Plant Pathology Journal 29(2), 136-143. https://doi.org/10.5423/PPJ.SI.07.2012.0111

Baranchikov, Y., Akulov, E., Astapenko, S. (2010). Bark beetle *Polygraphus proximus*: a new aggressive far eastern invader on Abies species in Siberia and European Russia. In: McManus, Katherine A; Gottschalk, Kurt W., eds. 2010. Proceedings. 21st U.S. Department of Agriculture interagency research forum on invasive species 2010; 2010 January 12-15; Annapolis, MD. Gen. Tech. Rep. NRS-P-75. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 64-65. https://www.fs.usda.gov/research/treesearch/37559

Callaghan, T. V., Shaduyko, O., Kirpotin, S. N., Gordov, E. (2021). Siberian environmental change: Synthesis of recent studies and opportunities for networking. Ambio 50(11), 2104-2127. https://doi.org/10.1007/s13280-021-01626-7

Cochard, H. (1992). Vulnerability of several conifers to air embolism, Tree Physiology. 11(1), 73–83. https://doi.org/10.1093/treephys/11.1.73

Crane, P. E., Hopkins, A. J. M., Dick, M. A., Bulman, L. S., (2009). Behaviour of *Neonectria fuckeliana* causing a pine canker disease in New Zealand. Canadian Journal of Forest Research. 39(11), 2119-2128. https://doi.org/10.1139/X09-133

Diagne, C., Leroy, B., Vaissière, AC., Gozlan, R. E., Roiz, D., Jarić, I., Salles, JM., Bradshaw, C. J. A., Courchamp, F. (2021). High and rising economic costs of biological invasions worldwide. Nature 592, 571–576. https://doi.org/10.1038/s41586-021-03405-6

Dick, M. A., Crane, P. E. (2009). *Neonectria fuckeliana* is pathogenic to *Pinus radiata* in New Zealand. Australasian Plant Disease Notes 4, 12–14. https://doi.org/10.1071/DN09005

Dobrowolska, D., Bončina, A., Klumpp, R. (2017). Ecology and silviculture of silver fir (*Abies alba* Mill.): a review. Journal of Forest Research 22(6) 326-335. https://doi.org/10.1080/13416979.2017.1386021

EPPO (2014) Pest risk analysis for *Polygraphus proximus*. EPPO, Paris. http://www.eppo.int/QUARANTINE/Pest_Risk_Analysis/PRA_intro.htm

Ghasemkhani, M. (2012) Genetic basis for resistance against fruit tree canker in apple. Swedish University of Agricultural sciences. Landscape Planning, Faculty Horticulture and Agricultural Science. https://pub.epsilon.slu.se/9096/1/Ghasemkhani_M_120925.pdf

González, C.D., Chaverri, P. (2017). *Corinectria*, a new genus to accommodate *Neonectria fuckeliana* and *C. constricta* sp. nov. from *Pinus radiata* in Chile. Mycol Progress. 16, 1015–1027. https://doi.org/10.1007/s11557-017-1343-8

Havranek, W. M., Tranquillini, W. (1995). 5 - Physiological Processes during Winter Dormancy and Their Ecological Significance. Physiological Ecology. Ecophysiology of Coniferous Forests. 95-124, https://doi.org/10.1016/B978-0-08-092593-6.50010-4

Hubbes, M. (1999). The American elm and Dutch elm disease. The Forestry Chronicle 75(2) 265-273. https://doi.org/10.5558/tfc75265-2

IUCN (2024). Invasive Alien Species. https://iucn.org/our-work/topic/invasive-alien-species [2024-05-21]

Jürisoo, L., Süda, I., Agan, A., Drenkhan, R. (2021). Vectors of Dutch Elm Disease in Northern Europe. Insects 12(5):393. https://doi.org/10.3390/insects12050393

Kerchev, I.A. (2014). Ecology of four-eyed fir bark beetle *Polygraphus proximus* Blandford (*Coleoptera; Curculionidae, Scolytinae*) in the west Siberian region of invasion. Russian Journal of Biological Invasions. 5, 176–185. https://doi.org/10.1134/S2075111714030072

Koivula, M. Vasaitis (Ed.), R. (2024). Alien invasive pathogens and pests in the changing environment: Focus on North Europe: Abstracts of presentations of the panel of Nordic Forest Research Network SNS N2023-01. The potential for arrival and establishment by the Four-Eyed Fir Bark Beetle (*Polygraphus proximus*) in the European Union. Baltic forestry 30(1). https://doi.org/10.46490/BF750

Loo, J.A. (2008). Ecological impacts of non-indigenous invasive fungi as forest pathogens. In: Langor, D.W., Sweeney, J. (eds) Ecological Impacts of Non-Native Invertebrates and Fungi on Terrestrial Ecosystems. Springer, Dordrecht. 81-96. https://doi.org/10.1007/978-1-4020-9680-8_6

Menkis, A., Pavlov, I. N., Litovka, Y. A., Timofeev, A. A., Vasaitis. Vasaitis (Ed.), R. (2024). Alien invasive pathogens and pests in the changing environment: Focus on North Europe: Abstracts of presentations of the panel of Nordic Forest Research Network SNS N2023-01. Occurrence and pathogenicity of *Corinectria* spp. – a canker disease of *Abies* and its association with *Polygraphus proximus*. Baltic forestry 30(1). https://doi.org/10.46490/BF750

Metzler, B., Hecht, U., Nill, M., Brüchert, F., Fink S., Kohnle, U,. (2012). Comparing Norway spruce and silver fir regarding impact of bark wounds. Forest Ecology and Management. 274, 99-107. https://doi.org/10.1016/j.foreco.2012.02.016.

Mihál, I., Cicák, A., & Tsakov, H. (2014). Fungi of the genus *Nectria* sl (*Bionectriaceae, Nectriaceae, Hypocreales, Ascomycota*) in Bulgaria and their phytopathological significance. Silva Balcania 15(2), 26-34. https://ife.sk/wp-content/uploads/2016/10/Mihal_Nectria-BGRE-1.pdf

Mohali, S. R., Steward, J. E. (2021). *Calonectria vigiensis* sp. nov. *(Hypocreales, Nectriaceae*) associated with dieback and sudden-death symptoms of *Theobroma cacao* from Mérida state, Venezuela. Botany 99(11) 683-693. https://doi.org/10.1139/cjb-2021-0050

Morales, R. (2009). Detección de *Neonectria fuckeliana* en Chile, asociado a cancros y malformaciones fustales en plantaciones de *Pinus radiata*. Bosque (Valvidia) 30(2). https://doi.org/10.4067/S0717-92002009000200007

Musolin, D. L., Kirichenko, N. I., Karpun, N. N., Aksenenko, E. V., Golub, V. B., Kerchev, I. A., Mandelshtam, M. Y. , Vasaitis, R., Volkovitsh, M. G., Zhuravleva, E.N., Selikhovkin, A. V. (2022) Invasive Insect Pests of Forests and Urban Trees in Russia: Origin, Pathways, Damage, and Management. Forests 13(4):521. https://doi.org/10.3390/f13040521

Nielsen, U. B., Xu, J., Nielsen, K. N., Talgø, V., Hansen, O. K., & Thomsen, I. M. (2017). Species variation in susceptibility to the fungus *Neonectria neomacrospora* in the genus *Abies*. Scandinavian Journal of Forest Research 32(5), 421–431. https://doi.org/10.1080/02827581.2017.1287300

Panzavolta, T,. Bracalini, M,. Benigno, A,. Moricca, S. (2021) Alien Invasive Pathogens and Pests Harming Trees, Forests, and Plantations: Pathways, Global Consequences and Management. Forests 12(10):1364. https://doi.org/10.3390/f12101364

Parker, I. M., Gilbert, G. S. (2004). The Evolutionary Ecology of Novel Plant-Pathogen Interactions. Annual Review of Ecology, Evolution, and Systematics 35, 675-700. https://doi.org/10.1146/annurev.ecolsys.34.011802.132339

Pavlov, I.N., Vasaitis, R., Litovka, Y.A., Stenlid, J., Jankovsky, L., Timofeev, A. A., Menkis, A. (2020). Occurrence and pathogenicity of *Corinectria* spp. – an emerging canker disease of *Abies* sibirica in Central Siberia. Sci Rep. 10, 5597. https://doi.org/10.1038/s41598-020-62566-y

Pimentel, D., Lach, L., Zuniga, R., Morrison, D. (2000) Environmental and Economic Costs of Nonindigenous Species in the United States. BioScience, 50(1), 53-65. https://doi.org/10.1641/0006-3568(2000)050[0053:EAECON]2.3.CO;2

Roll-Hansen, F., Roll-Hansen, H. (1979). Microflora of sound-looking wood in *Picea abies* stems. European Journal of Forest Pathology. 9, 308-316. https://doi.org/10.1111/j.1439-0329.1979.tb00693.x

Roll-Hansen, F., Roll-Hansen, H. (1980). Microorganisms which invade *Picea abies* in seasonal stem wounds. European Journal of Forest Pathology. 10, 396-410. https://doi.org/10.1111/j.1439-0329.1980.tb00057.x

Sakamoto, Y., Yamada, Y., Sano, Y., Tamai, Y., & Funada, R. (2004). PATHOLOGICAL ANATOMY OF *NECTRIA* CANKER ON *FRAXINUS MANDSHURICA* VAR. *JAPONICA*. IAWA Journal 25(2), 165-174. https://doi.org/10.1163/22941932-90000358

Santini, A., Ghelardini, L., De Pace, C., Desprez-Loustau, M. L., Capretti, P., Chandelier, A., Cech, T., Chira, D., Diamandis, S., Gaitniekis, T., Hantula, J., Holdenrieder, O., Jankovsky, L., Jung, T., Jurc, D., Kirisits, T., Kunca, A., Lygis, V., Malecka, M., Marcais, B., Schmitz, S., Schumacher, J., Solheim, H., Solla, A., Szabò, I., Tsopelas, P., Vannini, A., Vettraino, A. M., Webber, J., Woodward, S., Stenlid, J. (2013), Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytologist 197, 238-250. https://doi.org/10.1111/j.1469-8137.2012.04364.x

Semerikova, S. A., Khrunyk Y. Y, Lascoux, M., Semerikov, V. L. (2018). From America to Eurasia: a multigenomes history of the genus *Abies*. Molecular Phylogenetics and Evolution 125, 14-28 https://doi.org/10.1016/j.ympev.2018.03.009

Semerikova, S.A., Semerikov, V.L. (2014). Molecular phylogenetic analysis of the genus *Abies* (*Pinaceae*) based on the nucleotide sequence of chloroplast DNA. Russian Journal of Genetics 50, 7–19. https://doi.org/10.1134/S1022795414010104

Skulason, B., Hansen, O. K., Thomsen, I. M., Talgø, V. and Nielsen, U. B. (2017). Damage by *Neonectria neomacrospora* and *Adelges piceae* in provenance trials of subalpine fir (*Abies lasiocarpa*) in Denmark. Forest Pathology 47(3). https://doi.org/10.1111/efp.12326

Stauder, C. M., Utano, N. M., Kasson, M. T. (2020) Resolving host and species boundaries for perithecia-producing nectriaceous fungi across the central Appalachian Mountains. Fungal Ecology 47. https://doi.org/10.1016/j.funeco.2020.100980

Stenlid, J. (1985). Population structure of *Heterobasidion annosum* as determined by somatic incompatibility, sexual incompatibility, and isoenzyme patterns. Canadian Journal of Botany. 63(12), 2268-2273. https://doi.org/10.1139/b85-322

Van Wees, S. C. M., Van der Ent, S., Pieterse, C. M. J. (2008) Plant immune responses triggered by beneficial microbes. Biotic Interactions 11(4), 443-448. https://doi.org/10.1016/j.pbi.2008.05.005

Vasiliauskas, R. (1998). Ecology of Fungi Colonizing Wounds of Norway Spruce (*Picea abies* (L.) Karst.), with special Emphasis on *Stereum sanguinolentum* (Alb. & Schw.: Fr.) Fr.. Diss. Swedish University of Agricultural Sciences.

Vasiliauskas, R., Stenlid, J., (1997). Population structure and genetic variation in *Nectria fuckeliana*. Canadian Journal of Botany. 75(10), 1707-1713. https://doi.org/10.1139/b97-885

Vasiliauskas, R., Stenlid, J., Johansson, M. (1996). Fungi in bark peeling wounds of *Picea abies* in central Sweden. European Journal of Forest Pathology. 26, 285-296. https://doi.org/10.1111/j.1439-0329.1996.tb01074.x

Vitousek, P. M. (1990). Biological Invasions and Ecosystem Processes: Towards an Integration of Population Biology and Ecosystem Studies. Oikos, 57(1), 7–13. https://doi.org/10.2307/3565731

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., & Losos, E. (1998). Quantifying Threats to Imperiled Species in the United States. BioScience, 48(8), 607–615. https://doi.org/10.2307/1313420

Wolf, H. (2003). Technical guidelines for genetic conservation and use. Silver fir. Abies alba. EUFORGEN. https://www.euforgen.org/species/abies-alba/ [2024-05-21]

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Appendix 1

Raw data from experiment II.

label	treatment	Lesion_vertical	lesion_radial	diameter	inoc_site_height	internode_size	needle_dieback	dead_top
1	N8	26,6	0,66	4,2	190	18	0	0
2	N8	20	0,25	7,5	152	20	0	0
3	N8	20,2	0,5	6,4	118	26	0	0
4	N8	18,7	0,3	5,7	107	35	0	0
5	N8	26,5	0,5	5,8	281	22	1	0
6	N8	61,8	1	3,9	131	52	0	1
1	N21	26	0,75	5,7	84	27	0	0
2	N21	33,3	0,75	6,2	113	41	0	0
3	N21	11,2	0,25	6,8	74	21	0	0
4	N21	22	0,33	5	164	34	1	0
5	N21	22,7	0,75	5,7	90	31	0	0
6	N21	15,9	0,25	5,7	100	25	1	0
1	N7	10,2	0,33	5,9	130	32	0	0
2	N7	11,7	0,166	5	157	21	0	0
3	N7	21,7	0,5	6,3	120	22	0	0
4	N7	19,3	0,33	6,8	140	34	0	0
5	N7	23	0,33	7,5	210	41	0	0
6	N7	16,6	0,5	5,6	145	24	0	0
1	N15	14,3	0,33	7,5	80	21	0	0
2	N15	24,4	0,66	5,3	112	38	0	0
3	N15	26,6	0,5	6,2	174	25	0	0
4	N15	21,4	0,33	6,3	98	28	0	0
5	N15	20,5	0,5	6,2	100	20	0	0
6	N15	11,2	0,25	7	109	28	0	0
1	Control	0	0	5,4	150	23	0	0
2	Control	0	0	5,8	105	25	0	0
3	Control	0	0	6	58	21	1	0
4	Control	0	0	5	104	12	1	0
5	Control	0	0	5,3	95	21	0	0
6	Control	0	0	6,4	80	36	0	0

Appendix 2

Packages and codes used in statistical analysis:

Packages:

readxl
ggplot2
car

Code:

ANCOVA model:
aov(lesion_vertical ~ treatment + diameter + internode_size)

Linear regression models for conditioning variables:
lm()

Generalized linear models for needle dieback:
glm(needle_dieback ~ dummy_8 + dummy_21 + dummy_7 + dummy_15, family = 'binomial')

Plotting
ggplot()

Testing normality:
qqnorm()
qqline()

Testing equal distribution:
leveneTest()

Appendix 3

Testing for correlation between conditioning factors with linear regression model:

Call:
lm(formula = diameter ~ internode_size, data =
data_saplings_no_control_pooled_cutoff)

Residuals:

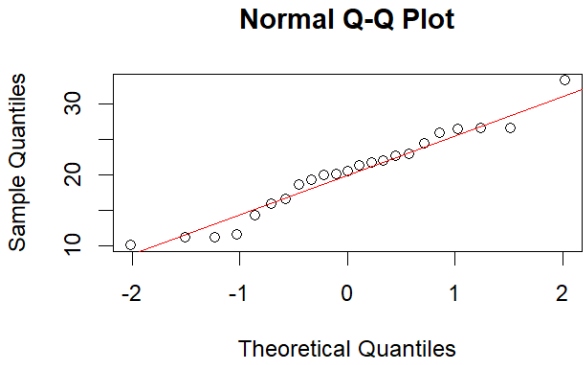
Min	1Q	Median	3Q	Max
-1.83178	-0.43876	0.00419	0.48264	1.45395

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.903411	0.747384	7.899	1.01e-07 ***
internode_size	0.007132	0.026323	0.271	0.789

P-value of internode_size far above 0.05 meaning no correlation between factor.

Testing normal distribution of data used in ANCOVA model with QQ test:



Theoretical quantiles match sample quantiles. Data points fall approximately on the red line representing normal distribution.

Equal variance of data used in ANCOVA model tested with a levene test:

Levene's Test for Homogeneity of Variance (center = median)

	Df	F value	Pr(>F)
group	1	0.0018	0.9666
	21		

P-value far above 0.05 meaning equal variance between groups

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