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RESEARCH PAPER

Genetic variation and inheritance of susceptibility to *Neonectria neomacrospora* and Christmas tree traits in a progeny test of Nordmann fir

Jing Xu[1] · Ulrik Braüner Nielsen[1] · Fikret Isik[2] · Martin Jensen[3] · Ole Kim Hansen[1]

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Abstract

• ***Key message*** **Pronounced additive genetic variation and high narrow-sense heritability for lesion length caused by *Neonectria neomacrospora* were found in a Nordmann fir progeny test. Significant inbreeding depression was detected in traits important for Christmas tree production. Recurrent selection for multiple traits would be successful for Christmas tree quality traits.**

• ***Context*** The fungal pathogen *N. neomacrospora* causes severe damage in Nordmann fir Christmas trees in Denmark. Family variation in disease susceptibility in the species has not been investigated before. This is the first combined genetic analysis of susceptibility to *N. neomacrospora* and Christmas tree traits in Nordmann fir.

• ***Aims*** Evaluate the genetic variation of susceptibility to *N. neomacrospora* and five Christmas tree traits in Nordmann fir.

• ***Methods*** Five Christmas tree traits were measured on 2413 trees in a progeny test. Artificial inoculation was conducted on detached twigs of full-sib progenies with a *N. neomacrospora* isolate to assess the resistance/susceptibility. Observed variation was partitioned into genetic and environmental causes to understand the heritable control of the traits.

• ***Results*** Pronounced additive genetic variation was observed in susceptibility to *N. neomacrospora* and Christmas tree traits. Narrow-sense heritability for susceptibility to *N. neomacrospora* was 0.63. Significant differences between selfings and outcrossed trees were detected for all traits. Significant improvement for Christmas tree quality could be observed in the breeding process.

• ***Conclusion*** Resistance to *N. neomacrospora* disease and other Christmas tree characteristics can be improved through recurrent selection.

Keywords Polymix mating · Spatial analysis · Narrow-sense heritability · Inbreeding

1 Introduction

Nordmann fir (*Abies nordmanniana* (Steven) Spach) is widely used as Christmas tree in Europe, with a production of approximately 45 million trees per year. Denmark is a major producer with around 11 million trees produced per year, and 9 millions of it for export (Christensen 2019). In 1992, a Danish Christmas tree breeding program started with selection of plus trees and initiation of half-sib progeny trials (Nielsen and Hansen 2013). At the same time, the establishment of a number of clonal seed orchards was initiated and currently there are about 33 ha of clonal seed orchards (Anonymous 2018). The primary traits evaluated in the first-generation half-sib progeny trials included height, flushing, Christmas tree quality, postharvest needle retention, and resistance/tolerance to adelgids (Nielsen

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Contribution of the co-authors Jing Xu: field measurement, inoculation experiment, analysing data, writing manuscript.
Ulrik B. Nielsen: Established the field trial, participated in designing the study and the inoculation experiment, reviewing and writing the manuscript.
Fikret Isik: Assisting analyzing data, reviewing and writing the manuscript.
Martin Jensen: Established the field trial, reviewing the manuscript.
Ole K. Hansen: Established the field trial, designed the study and inoculation experiment, field measurement, pedigree recovery, reviewing and writing the manuscript.

✉ Jing Xu
jx@ign.ku.dk

Extended author information available on the last page of the article

et al. 2002, 2020). In 2007 the final full rotation Christmas tree data from these half-sib trials were available—giving opportunity to estimate breeding values for more than 125 plus trees in the breeding population. Consequently, the best tested plus trees from the first generation were used for polymix crossings in 2009. This study is the first to report the results of this progeny test material.

Since the start-up of the breeding program of Nordmann fir, new challenges have emerged for Christmas tree growers in Denmark; one is the fungal pathogen *Neonectria neomacrospora* (Mantiri et al. 2001). The first description of this fungus was in 1913 on *Abies concolor* in Germany (Wollenweber 1913). During the 1930s to the 1960s, a few reports of this fungus in North America and Norway were made (Nielsen 2020), but no severe damage has been observed until recently. *Neonectria neomacrospora* is a bark parasite feeding on the living cells, resulting in heavy resin flow, cankers, twig blight, and dieback of *Abies* spp. in several European countries since 2008 (Perez-Sierra 2017; Pettersson et al. 2016; Schmitz et al. 2017; Talgø et al. 2013). The fungus quickly caused severe damage in several Nordmann fir seed orchards and numerous Christmas tree stands in Denmark from 2012 and onwards (Proschowsky 2014). Talgø et al. (2013) reported that this fungus is very aggressive in *Abies*—both under field conditions and in inoculation tests. Nielsen et al. (2017) confirmed this by a combined study using field evaluation and artificial inoculation test on 381 *Abies* trees in an arboretum in Denmark, where significant differences were detected among the 39 included taxa. Variation within species has also been reported; Skulason et al. (2017) found that *N. neomacrospora* damage varied among provenances of *Abies lasiocarpa*. Furthermore, our previous studies detected pronounced clonal variation and moderate to high broad-sense heritability estimates of susceptibility to *N. neomacrospora* in three Nordmann fir clonal seed orchards (Xu et al. 2018a). The Danish breeding program of Nordmann fir aims at increasing genetic gain through recurrent selection. The emergence of the *N. neomacrospora* pathogen necessitated the understanding of genetic variation and inheritance of disease susceptibility in an *Abies* species for resistance breeding and selection.

In forest tree breeding, progeny testing is widely used for parental selection (Zobel and Talbert 1984). Polymix breeding followed by paternity analysis of progeny using molecular markers was proposed as an alternative to traditional full-sib breeding (Lambeth et al. 2001). In general, this method offers good estimates of breeding values of parents for backwards selection and a progeny population for forward selection. Furthermore, it is a logistically simpler and less costly breeding strategy (Wheeler et al. 2006).

When testing new genetic plant material, it is good practice to include commercially available material as a baseline for comparison. We had offspring from both the commercial seed stands, where the first generation of plus trees were selected, as well as from the first-generation clonal seed orchards, where these plus trees are grafted. We also included the most widely used seed source from the natural stands of Nordmann fir in Georgia—the Ambrolauri provenance. This allowed us to compare the genetic material from clonal seed orchards with the natural seed sources and understand the current degree of genetic improvement in Nordmann fir.

Non-additive genetic variance is not heritable between generations but could be important for some Christmas tree traits. The most common way to estimate non-additive genetic effects in trees is diallel crossing schemes (Araújo et al. 2012). Since the beginning of the 1990s, efforts have been made to propagate Nordmann fir vegetatively. Somatic embryogenesis (SE) was shown to be a promising technique to mass produce superior clones (Norgaard and Krogstrup 1991). SE plants are being tested in clonal trials, and upscaling of the SE process in bioreactors was successfully demonstrated (Valdiani et al. 2020). Vegetative propagation makes it possible to exploit non-additive genetic variance in Christmas tree traits for deployment.

This study is based on offspring from polymix crossings of Nordmann fir. Full-sib offspring of the population were tested with *N. neomacrospora* via artificial inoculation to study genetic variation in Nordmann fir. Furthermore, several Christmas tree traits were assessed on the full-sib and half-sib offspring and on offspring from commercial seed sources to test several hypotheses.

The primary objectives of this study were to (1) estimate genetic parameters of susceptibility to *N. neomacrospora* and of Christmas tree traits in Nordmann fir, (2) estimate genetic correlations between susceptibility to *N. neomacrospora* and Christmas tree traits, and (3) evaluate the degree of genetic improvement of the traits and draw implications for breeding. Furthermore, we investigated the degree of inbreeding in Nordmann fir and its effect on Christmas tree traits.

2 Material and methods

2.1 Plant materials

Polymix crossings were performed in May 2009 using 14 clones (selected out of 68) from the Nordmann fir clonal seed orchard FP.259 at Silkeborg State forest District (Jutland) in Denmark. The 14 clones were selected based on their breeding values for Christmas tree quality from a half-sib progeny test series (Nielsen et al. 2020). The seed orchard FP.259 was initially grafted using 94 plus trees selected for foliage appearance in the approved Danish seed

stands F.526 and F.527, which were planted around 1902 and are presumed direct imports from the Borshomi area in Georgia (Løfting 1973). Based on progeny test data, the FP.259 seed orchard was thinned to 68 clones before 2009.

The crossing design consisted of two partial diallels using pollen mixtures. The crossings included selfs and some reciprocals (Fig. 1). In other words, each female tree was mated with a pollen mix of five other trees, including itself. Thus, there were (potentially) 14 selfed crosses (the same individual was used as female and male) and 52 outcrossed full-sib families. In addition to the two diallels, two more crosses (half-sibs) were also made using clones C73 and C78 as female parents to mate with pollen mix of the second diallel. In reality, not all crosses produced seeds, and some trees died in the field, so the actual number of selfed crosses was 12, and for outcrossed it was 48 (including 9 reciprocal crosses); thus, in total, there were 51 full-sib families.

In September 2009, individual seed lots were harvested in the FP.259 orchard. Seedling production was initiated in February 2010 at the research facility at Aarhus University. In addition, commercial seed lots from FP.259, two well-known Danish commercial seed stands (F.526 and F.527) and the Ambrolauri Tlugi provenance in Georgia (department 2) were included in the study. The objective was to create a reference plant material using the three commercial seed lots and the wild material from Georgia. An overview of the origin of the parental clones, breeding process, and materials included in this study is provided in Appendix Fig. 6.

During the second growing season, the 14 parent clones (excluding C73 and C78) and a subset of seedlings from the 14 polymix crosses in the two diallels were genotyped with five microsatellites, following a similar procedure as Hansen and Mckinney (2010). The microsatellites were NFH15 and NFF3 (Hansen et al. 2005), Ab12 (Rasmussen et al. 2008), SF b4 (Cremer et al. 2006), and As09 (Lian et al. 2007). A separate paternity analysis was conducted for each of the 14 families using CERVUS 3.0 (Kalinowski et al. 2007; Marshall et al. 1998) to identify paternity for all approximately 1450 seedlings. A list of the five potential male parents in each polymix cross was used to determine the likely male parent for each seedling progeny. The results of the paternity analysis are given in Appendix Fig. 7.

2.2 Establishment of field trial

Two-year-old seedlings were transplanted to a commercial nursery after being individually labelled in March 2012. In addition to genotyped full-sib offspring seedlings, an excess of half-sib progeny from each of the 16 polymix crosses were transplanted to the field trial. Half-sib seedlings were not genotyped. The field trial was established on Fyn island,

Male clones

Female clones	C13	C39	C46	C55	C60	C72	C76	C11	C21	C31	C42	C47	C52	C54	C73	C78
C13	20S	20	20	20	20											
C39		20S	20	20	20	20										
C46			20S	20	20	20	20									
C55	20			20S	40		20									
C60	20	20			20S	20	20									
C72	20	20				20S	40									
C76	20	20	20	20			20S									
C11								20S	20	20	20	20				
C21									20S	20	20	20	20			
C31										20S	20	20	20	20		
C42								20			20S	20	20	20		
C47								40				20S	20	20		
C52	20	20	20			20	20	20		20			20S	40		
C54								20	20	20	20			20S		
C73								20	20	20					20S	20
C78								20	20	20	20					20S

Fig. 1 Crossing design using polymix breeding with paternity analysis of the progeny. Numbers in the cells (e.g. "20") refer to the percentage of the pollen mixture (based on weight) contributed by the male clone. For example, C13 was mated with a pollen mixture of C13, C39, C46, C55, and C60 pollen; all 5 pollen fathers constituted 20% each of the pollen mixture. "S" indicates selfing. Offspring from the two internal 7 × 7 diallels were genotyped with microsatellite markers (green coloured cells). Offspring in red coloured cells were included as half-sibs (not genotyped)

Denmark, about 12 km south-east of Odense city in spring 2014. A total of about 3150 trees were planted. For this study, only 2413 trees were evaluated (Table 1). Seedlings were randomly distributed to two blocks each with 16 rows with 1.1 × 1.1-m spacing.

2.3 Neonectria inoculation experiment

On 13 September 2017, one healthy twig containing three shoots was collected from each of 1176 full-sib offspring in the field trial. Nineteen full-sib trees with little growth were not sampled to avoid damaging the shape of the tree. All twigs were immediately put into plastic bags and shipped to the Hørsholm Arboretum on 14 September 2017. Here the twigs were placed in plastic boxes with water in the bottom (two types were used: SAMLA box from IKEA®; 78 × 56 × 18 cm; item no.698.713.88 and a Bedroller from CURVER®; 61.5 × 40.5 × 19 cm) inside a room at around 20 °C. Plant trays for seedling production (QuickPot® QP D 60T/12) were used inside the plastic boxes to keep twigs upright.

The isolate (NGR1) of *N. neomacrospora* from our previous study (Nielsen et al. 2017) was also used for artificial inoculation in the current study. The isolate was stored in a − 80 °C freezer and multiplied on petri dishes (9 cm in diameter) with potato dextrose agar and incubated for 3 weeks at room temperature until inoculation. Prior to inoculation, left shoot of each twig was removed to give more space for the inoculation set up and also to avoid neighbouring shoots touching each other. Consequently, two shoots (center and right) on each twig were inoculated. One needle in the middle of each shoot was removed to provide a leaf scar where the inoculum plugs (ca. 0.5 cm in diameter) cut from the edges of petri dishes were placed (with the side containing mycelium towards the scar). Inoculation took place between 14 and 16 September 2017. At the end of each inoculation day, the CC containers (https://www.container-centralen.com/our-products/cc-container/), where the plastic boxes continuously had been placed, were covered by a thin plastic to keep humid conditions. Plastic covers were removed on 19 September 2017. The extent of infection by *N. neomacrospora* on the inoculated twigs was evaluated 3 weeks after inoculation, by cutting open the area below the inoculated leaf scars with a knife, then measuring the length of damaged tissue in cm (lesion length) under bark. The total length of the inoculated shoot was also measured.

2.4 Christmas tree traits in the controlled crossing progeny

In the field trial, five Christmas tree traits were assessed on 2413 trees:

- Total tree height in centimetres (9 years from seed);
- Number of terminal buds on the top leader, excluding the center bud which becomes the leader during the next year's growth. Terminal buds form the whorl branches during the next growth year (7 years from seed);
- Christmas tree quality using a visual score from 1 to 9 based on the European grading system for Christmas trees (Nielsen et al. 2010). Scores of 9–7 were assigned to the top quality trees, 6–4 were for low quality but saleable, and scores 3–1 were for cull trees (9 years from seed);
- Density with a score from 1 to 9 was used to assess the crown quality of trees, where 9 was used for the densest tree, with no obvious gaps between whorls plus a conical crown shape like "A" (desirable), while score 1 was assigned to the least dense trees with poor crown quality (large gaps between whorls, non-conical shape) (9 years from seed);
- Flushing score at the start of the 8th growing season was assessed as follows:

0: Bud in winter condition;
1: Bud slowly starting to swell, no green is seen;
2: Bud swollen, some green is seen, but bud scales are still covering the bud;
3: Bud scales dropped, bud not elongating or only very little;
4: Shoot started to elongate, needles brush-like forward pointing;

Table 1 Summary of studied materials. Infection rate is the percentage of trees showing symptoms of *N. neomacrospora* infection in each category of studied materials after inoculation. Christmas tree score is the mean for each category of materials/genetic group. Standard errors of estimates are given in parentheses

Materials/genetic group	Number of trees	Infection rate	Christmas tree score
Full-sib family	1097	93.1%	4.72 (1.51)
Selfs	98	95.3%	3.71 (1.42)
Half-sib family	734		4.50 (1.48)
Tversted F.526 seed stand	115		3.92 (1.28)
Tversted F.527 seed stand	115		3.66 (1.10)
Silkeborg FP.259 orchard	137		4.33 (1.50)
Ambrolauri Tlugi (Georgia)	117		3.87 (1.31)
Total	2413		

5: Shoot elongating, still soft needles;
6: Shoot fully elongated.

2.5 Statistical analysis

The following regular individual tree mixed model was fitted to the *N. neomacrospora* data as well as to the Christmas tree traits measured in the field (Xu et al. 2021):

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \tag{1}$$

where **y** is a vector of observations (e.g. the lesion length in centimetres caused by *N. neomacrospora*), **b** is a vector of fixed effects (e.g. plastic box and shoot length in the artificial inoculation test or block and genetic group effects for Christmas tree traits measured in the field), **u** is a vector of random effects (additive genetic effect, non-additive genetic effect, and row effect for some traits), and **e** is the vector of random residual effects. **X** and **Z** are incidence matrices relating the observations to the fixed and random genetic effects, respectively. The random effects are assumed to follow a multivariate normal distribution with means and variances defined as $u_a \sim N(0, A\sigma_a^2)$, $u_{na} \sim N\left(0, I\sigma_{na}^2\right)$, and $e \sim N(0, I\sigma_e^2)$, where 0 is the expectation, σ_a^2 is the additive genetic variance, σ_{na}^2 is non-additive genetic variance, and σ_e^2 is the residual variance. **A** and **I** are the additive genetic relationship and identity matrices, respectively.

The visual inspection of residual plots in ASReml did not show obvious deviations from normality and homogeneity for the studied traits.

For Christmas tree traits measured in the field, a spatial model based on a two-dimensional separable autoregressive structure was used for residuals to account for within-trial trends. Residual variance was decomposed into spatially dependent residuals and independent residuals. The residual **R** covariance structure was modelled as follows:

$$\mathrm{R} = \boldsymbol{\sigma}_{\boldsymbol{\xi}}^2\left(\Sigma\rho_c \otimes \Sigma\rho_r\right) + \boldsymbol{\sigma}_{\boldsymbol{\eta}}^2\boldsymbol{I} \tag{2}$$

where $\boldsymbol{\sigma}_{\boldsymbol{\xi}}^2$ is the variance of spatially correlated residuals, $\boldsymbol{\rho_c}$ and $\boldsymbol{\rho_c}$ are correlation matrices in the column and row directions, respectively, $\otimes$ is the Kronecker product, $\boldsymbol{\sigma}_{\boldsymbol{\eta}}^2$ is the variance of the independent residuals after accounting for correlated spatial variation, and $\boldsymbol{I}$ is an identity matrix (Costa e Silva et al. 2001). All variance and covariance components were estimated using restricted maximum likelihood in ASReml version 4.0 (Gilmour et al. 2015). The significance of the random effects was tested using a one-tailed likelihood ratio test (LRT). All non-significant effects were dropped from the final models. Wald *F* statistics were used to test the significance of fixed effects. Best linear unbiased predictions (BLUPS) were estimated for the parents for each trait.

Causal additive genetic variances and non-additive genetic variances were estimated from observed variance components. Individual tree narrow-sense heritability (h_i^2) estimates for *Neonectria* susceptibility and Christmas tree traits were calculated using the following equation for the regular model:

$$h_i^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{na}^2 + \sigma_e^2} \tag{3}$$

where σ_a^2 is total additive genetic variance, σ_{na}^2 is non-additive genetic variance (cross effect), and σ_e^2 is residuals. From the spatial model, assuming spatial variance could be separated as part of a correlated environmental variance, only independent residual variances were used to estimate h_i^2 (Bian et al. 2017):

$$h_i^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{na}^2 + \sigma_\eta^2} \tag{4}$$

where σ_η^2 is the independent residual from the spatial model. Standard error of causal variance components and heritability estimates were obtained using the Delta method (Isik et al. 2017).

A multivariate model was fitted to estimate genetic correlations between Christmas tree traits. In a compact form, the mixed model for multiple traits looks like Eq. 1. The only difference between the univariate model in Eq. 1 and the multivariate model is the design matrices (Isik et al. 2017):

$$\mathbf{Y}_{nd} = \left(\boldsymbol{I_d} \otimes \mathbf{X}_{n \times (p+1)}\right)\mathbf{b}_{(p+1)d \times 1} + \left(\boldsymbol{I_d} \otimes \mathbf{Z}_{n \times r}\right)\mathbf{u}_{rd \times 1} + \mathbf{e}_{nd} \tag{5}$$

In this case **Y** is the vector of observations for *d* traits each with *n* rows, $\mathbf{I}_d$ is the identity matrix with dimension *d*, and **X** and **Z** are design matrices with dimensions given. The residuals in the R matrix are correlated for traits measured on the same individual but independent across individuals. Similarly, the additive genetic effects in the G matrix for two traits measured on the same tree are correlated, scaled by additive genetic relationships.

$$R = I \otimes \begin{bmatrix} \sigma_{\varepsilon 1}^2 & \sigma_{\varepsilon 12} \\ \sigma_{\varepsilon 12} & \sigma_{\varepsilon 2}^2 \end{bmatrix} \tag{6}$$

$$G = A \otimes \begin{bmatrix} \sigma_{A1}^2 & \sigma_{A12} \\ \sigma_{A12} & \sigma_{A2}^2 \end{bmatrix} \tag{7}$$

In each of these variance-covariance matrices, variances for the two traits are on the diagonal and the covariances between the traits are in the off-diagonal of the matrix (Isik et al. 2017).

We also correlated *N. neomacrospora* susceptibility predicted breeding values of the 14 parental clones with other Christmas tree traits using R (The R Core Team 2019).

2.6 Inbreeding effect

For all the full-sib individuals, two groups were classified: selfed and outcrossed individuals. Analysis of variance was performed to test if these two groups are significantly different from each other for the Christmass tree and *N. neomacrospora* susceptibility traits using the R *glm* function (The R Core Team 2019). The following equation was used to calculate inbreeding depression (ID) (Lande and Schemske 1985) for each trait:

$$ID = \frac{|\bar{x}_o - \bar{x}_s|}{\bar{x}_o} \times 100\% \quad (8)$$

where $\bar{x}_o$ is the mean of outcrossed individuals and $\bar{x}_s$ is the mean of selfed individuals.

2.7 Genetic gain

In order to calculate genetic gain, a dummy variable named "genetic group" was created. The Pmix group included all the polymix full- and half-sib offspring, excluding the selfs. The Pmix group was compared with the clonal seed orchard FP.259 and the three commercial seed sources (F.526, F.527 and the wild Ambrolauri provenance). A spatial autoregressive residual structure with correlations in row and column directions was used in the model. The model was run in ASReml version 4.0 (Gilmour et al. 2015). Wald *F* statistics was used to test the significance of the genetic groups for each trait. The best linear unbiased estimates (BLUE) for each genetic group were obtained.

3 Results

Summary statistics is provided in Table 1. In total, 2413 trees were included in the study, with a large proportion of trees (1195, including 98 selfs) with known female and male parents, the latter identified using SSR markers. *Neonectria neomacrospora* inoculation of full-sib offspring was effective with an average infection rate of 93.1% twigs in full-sib offspring and 95.3% in selfed offspring. Polymix full- and half-sib offspring (excluding selfs) had the highest Christmas tree quality score (Table 1), followed by offspring from FP.259, while the three seed stands had the lowest Christmas tree score. Spatial models improved the fit statistics of linear mixed models. The improvement in model fit statistics was mainly due to accounting for trends in the column direction, which followed an altitudinal gradient.

3.1 Genetic parameters

Variance components and heritability estimates are presented in Table 2a for the regular model and in Table 2b for the spatial model. There was significant additive genetic variation for lesion length caused by *N. neomacrospora* as indicated by a high narrow-sense heritability (0.63). Non-additive genetic variation was not significant for the trait and was dropped from the final model. For all the Christmas tree traits, adding the spatially correlated residual term resulted in a better model fit. Autocorrelation coefficients in column direction were in general higher than in row direction. Additive genetic

Table 2 Estimates of genetic parameters from the two linear mixed models (a) with independent units and (b) with autoregressive order one spatial residual structure. σ^2_a is additive genetic variance; σ^2_e is residual variance; σ^2_η is random residual variance from spatial model; ρcol and ρrow are autocorrelations for the columns and rows, respectively; h^2_i is individual narrow-sense heritability. Standard errors of estimates are given in parentheses

(a) Genetic parameters model with residual being independent units (regular model)

Traits	σ^2_a(SE)	σ^2_e(SE)	h^2_i(SE)
Lesion length	1.11 (0.08)	0.66 (0.03)	0.63 (0.02)
Height	320 (85.3)	375 (58.5)	0.46 (0.10)
Christmas tree score	0.17 (0.07)	1.96 (0.08)	0.08 (0.03)
Flushing score	0.54 (0.10)	0.36 (0.07)	0.60 (0.09)
Density score	0.41 (0.12)	1.41 (0.10)	0.23 (0.06)
Number of buds	0.49 (0.16)	1.73 (0.12)	0.22 (0.07)

(b) Genetic parameters from model with autoregressive order one spatial residual structure (spatial model)

Traits	σ^2_a(SE)	σ^2_η(SE)	ρrow	ρcol	h^2_i(SE)
Height	339 (59.76)	125 (39.22)	0.85	0.98	0.73 (0.10)
Christmas tree score	0.16 (0.07)	1.62 (0.07)	0.55	0.98	0.09 (0.04)
Flushing score	0.55 (0.10)	0.25 (0.07)	0.63	0.74	0.69 (0.10)
Density score	0.37 (0.11)	1.15 (0.08)	0.77	0.97	0.24 (0.07)
Number of buds	0.50 (0.16)	1.48 (0.12)	0.63	0.98	0.25 (0.07)

Table 3 Additive genetic correlation estimates (r_a) between Christmas tree traits. Standard errors of estimates are given in parentheses. Only significant estimates are presented

Trait1	Trait2	Additive genetic correlation
Height	Number of buds	0.46 (0.15)
Density	Flushing	−0.30 (0.16)
Density	Christmas tree score	0.40 (0.20)
Christmas tree score	Number of buds	0.38 (0.23)

effects were significant for all the traits as suggested by LRTs. Based on the spatial model, the narrow sense heritability for height was highest among the traits (0.73), while Christmas tree quality had the lowest individual narrow-sense heritability (0.09). Flushing had a high heritability (0.69), while density of the tree and number of buds on the leader were under moderate genetic control with heritability estimates of 0.24 and 0.25, respectively.

3.2 Genetic correlations

Multivariate analysis results are presented in Table 3. The number of buds had a moderate positive correlation with height ($r_a = 0.46 \pm 0.15$) and with Christmas tree score ($r_a = 0.38 \pm 0.23$). Tree density had a moderate favourable correlation with Christmas tree score ($r_a = 0.40 \pm 0.20$), and a (favourable) negative correlation with flushing score ($r_a = -0.30 \pm 0.16$).

Pearson correlation between predicted breeding values of *N. neomacrospora* susceptibility and other Christmas tree traits is presented in Fig. 2. Low to moderate positive correlations were detected between *N. neomacrospora* susceptibility and tree density and flushing (0.13 and 0.21, respectively), indicating that earlier flushing trees and the denser trees are more susceptible to the disease. Negative and low correlation between the number of terminal buds with *N. neomacrospora* susceptibility (−0.15) showed that less susceptible trees tend to have more terminal buds. No significant correlation detected between *N. neomacrospora* susceptibility and Christmas tree score and height at a 0.001 significance level.

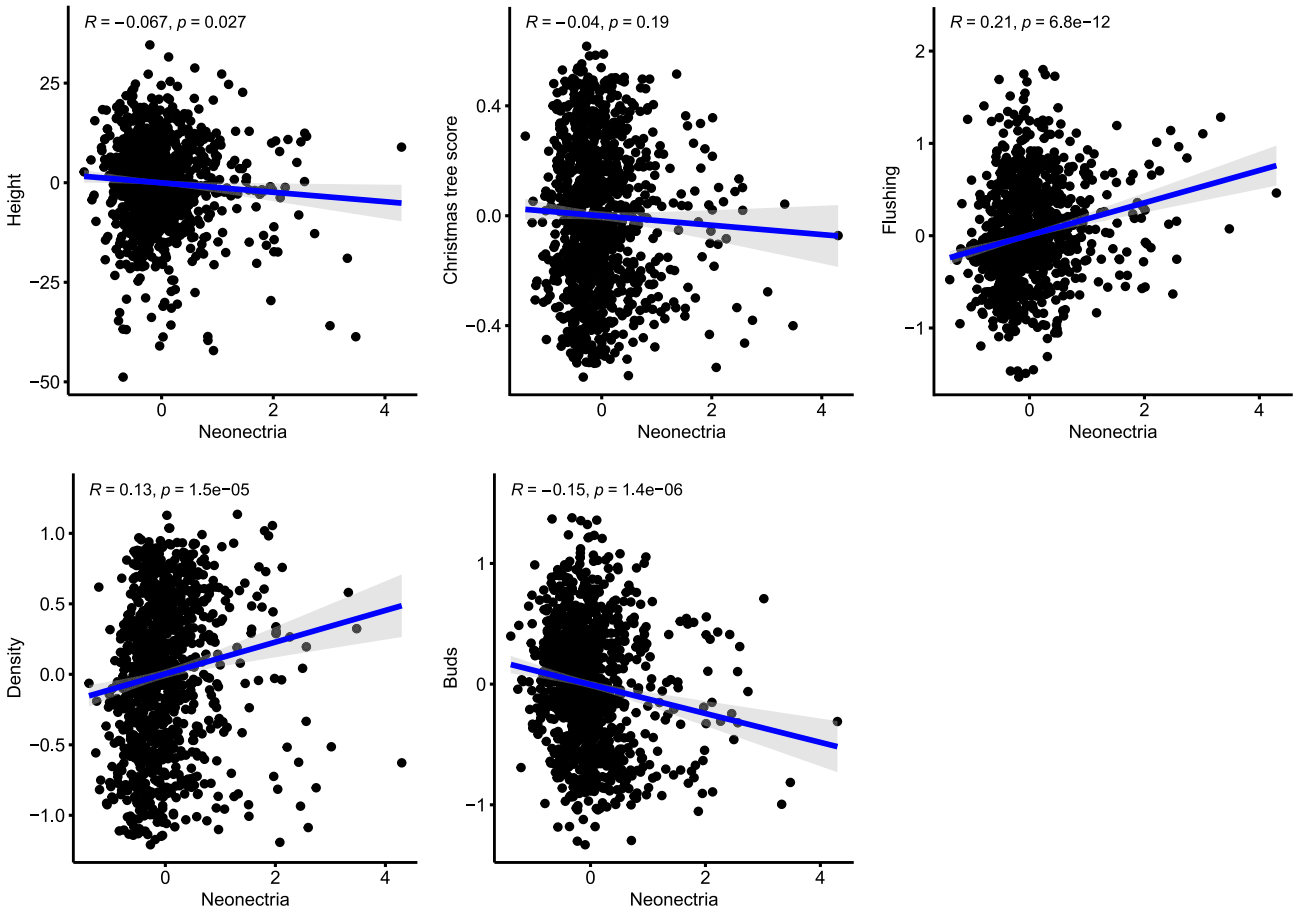


Fig. 2 Scatterplots of each Christmas trait against *N. neomacrospora* lesion length based on predicted values from linear mixed models. Pearson's correlation coefficients presented as R and their significance levels as p (sample size $N = 1079$)

3.3 Inbreeding

One-way ANOVA test for *N. neomacrospora* susceptibility and Christmas tree traits showed that selfed and outcrossed offspring were significantly different from each other ($p < 0.001$). Overall, selfed offspring had 31% longer *N. neomacrospora* lesion length than outcrossed offspring, indicating severe inbreeding depression in susceptibility to *N. neomacrospora* (Fig. 3). Selfed progeny also had lower height (26%), lower Christmas tree quality (21%), a smaller number of buds at the terminal shoots (18%), and less density of branches (14%) compared with outcrossed individuals. Selfed progeny moreover showed 8% earlier flushing than outcrossed progeny, which is not desirable.

3.4 Genetic gain

Breeding values for *N. neomacrospora* susceptibility for the 14 parental genotypes were plotted together with breeding values of the studied Christmas tree traits (Fig. 4). Clones C76, C31, and C52 were the top parental genotypes with highest Christmas tree quality and low breeding values for *N. neomacrospora* susceptibility (lesion length). They also tended to be late flushing and with a larger number of terminal buds and smaller heights (desirable).

The difference between the polymix group (full-sib and half-sib offspring-excluding selfs) and the four commercial seed sources was significant for all the studied Christmas tree traits ($p < 0.001$) (Fig. 5). Results showed that the Pmix group had the second-lowest height, highest Christmas tree quality, and highest density. The Ambrolauri Tlugi provenance (Amb2) had similar performance as F.526 and F.527 with regard to Christmas tree quality and slightly higher for density, while it had the lowest height. BLUE of the Pmix group compared with base populations F.526 and F.527 was about 22% higher for both Christmas tree score and density score, and about 8% lower in height (Fig. 5). The clonal seed orchard FP.259 also performed better in Christmas tree quality and density (17% higher), and height (7% lower) than the parental stands F.526 and F.527. These results indicate a successful breeding program for better Christmas trees.

4 Discussion

4.1 *Neonectria neomacrospora* susceptibility

Tree breeding programs have successfully increased individual resistance to microbial pathogens and can provide long-term management strategies for forest diseases (Sniezko 2006). For this approach to be successful, resistance must be

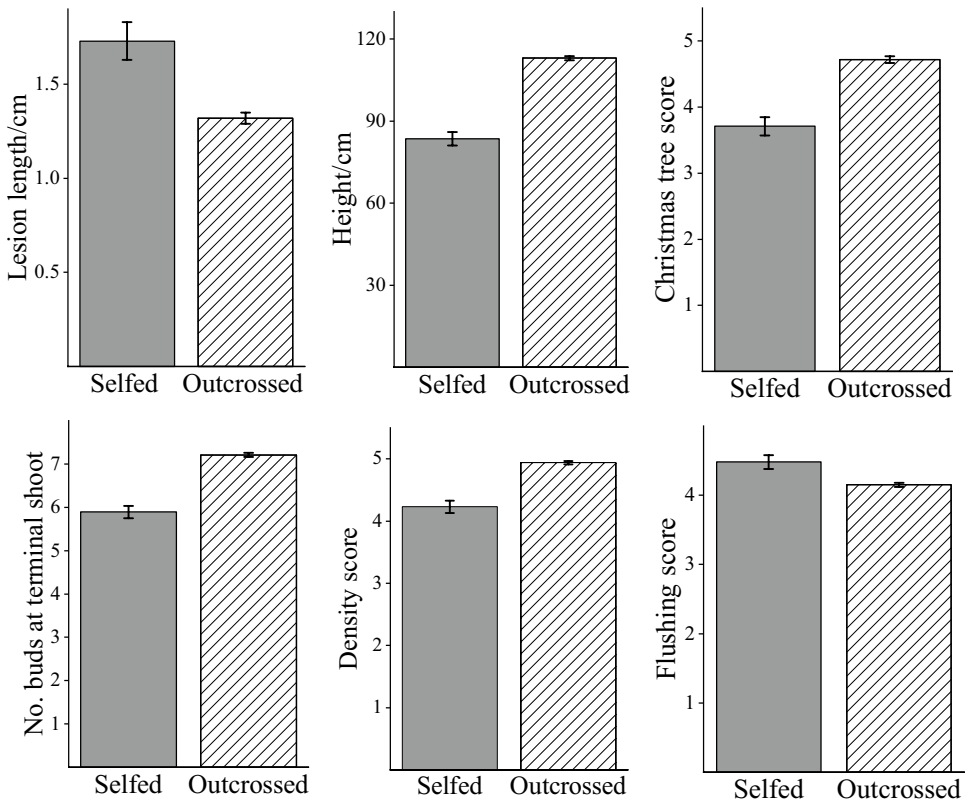


Fig. 3 Mean of *N. neomacrospora* lesion length, height, Christmas tree score, number of buds on the leading shoot, density, and flushing score for selfed and outcrossed polymix offspring. Selfing had adverse effects on all the traits analysed in this study and the differences were significant at a $p < 0.001$ significance level

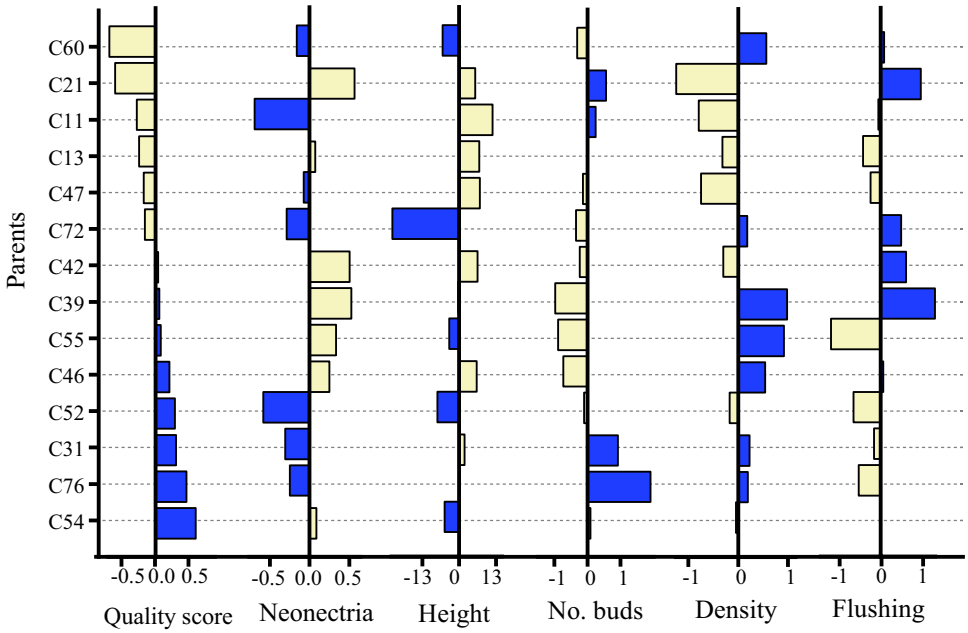


Fig. 4 Breeding values for Christmas tree quality score, *N. neomacrospora* susceptibility, height, number of buds on the leading shoot, density, and flushing score of 14 parents. Parents are sorted by Christmas tree quality score, with the highest breeding values at the bottom. Blue colour indicates positive development of the trait and yellow colour corresponds to negative development

variable, readily detectable, heritable, and durable (Søndreli et al. 2019). In this study, we showed detectable variation in susceptibility to *N. neomacrospora* within Nordmann fir, with high narrow-sense heritability.

High genetic variation among the 1176 full-sib offspring for susceptibility to *N. neomacrospora*, and a high heritability, indicated that selecting resistant genotypes is promising to make improvement for the disease. A similar large variation in *N. neomacrospora* susceptibility was observed among noble fir (*Abies procera* Rehder) families (Hansen et al. 2020), but that study could not deliver heritability estimates. A review by Carson and Carson (1989) suggested that individual narrow-sense heritabilities (h^2) of disease incidence in forest trees seldom are larger than 0.30. However, in recent decades, there is a large volume of studies reporting higher heritability estimates for disease resistance in forest trees. Likewise, in our study, narrow-sense heritability of susceptibility to *N. neomacrospora* in Nordmann fir was high (0.63). In *Pinus taeda*, a relatively

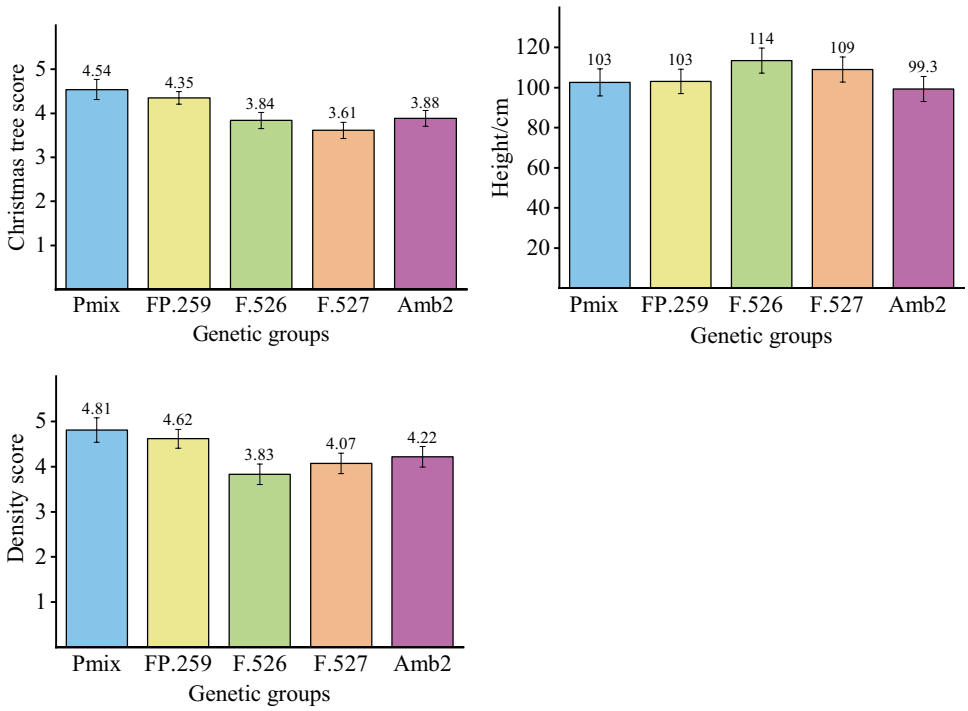


Fig. 5 Best linear unbiased estimates (BLUE) of five different genetic groups for height, Christmas tree quality and density. Pmix: full-sib and half-sibs from the polymix (excluding selfed individuals). Amb2: Ambrolauri Tlugi provenance (department 2). The number on top of each bar is the BLUE value for each group

high heritability (0.56) for fusiform rust resistance (*Cronartium quercuum* f.sp. *fusiforme*) was observed (Isik et al. 2008).

Artificial inoculations in controlled environments, with assessment of lesion length at the point of inoculation, have been instrumental in the study of disease resistance breeding for forest and horticultural pathogens (Garkava-Gustavsson et al. 2013; Gordon et al. 2006; Hurel et al., 2019; McKinney et al. 2012; Shain and Franich 1981; Solheim 1988). McKinney et al. (2012) and Lobo et al. (2015) both found very high correlations (0.85 and 0.93) between necrosis length in the artificial inoculation test and levels of susceptibility in the field trials, which supports and validates the use of lesion length in resistance studies. In a previous work (Xu et al. 2018a), we reported considerable variation in *N. neomacrospora* susceptibility among the 68 clones in the clonal seed orchard FP.259. Ramets of the clones (their identical copies) in the orchard were naturally infected by the pathogen. In the here presented study, twigs of offspring of 14 clones from FP.259 were artificially inoculated and their breeding values were obtained. The correlation between predicted breeding values of the overlapping clones from the two studies was moderately high ($r = 0.61$, $p = 0.02$).

The importance of interactions between host and pathogen has been widely reported (Betts et al. 2016, Best 2018, Kosawang et al. 2020). Genetic variability of virulence of a pathogen may reflect its adaptability to different environments and its ability to infect and reproduce on different hosts (Vanderplank 1963). The virulence level potentially have direct implications for disease outcome and the management of the disease in forest tree breeding populations (McDonald and Linde 2002; Isik et al. 2012). Therefore, more detailed research on the interaction of the host and pathogen is needed.

4.2 Genetic correlations

Pests and pathogens can have significant detrimental impacts on ecology, economy, and aesthetics of forest stands (Carson and Carson 1989). Resistance breeding of forest trees have drawn considerable attention in different tree species (Isik et al. 2008; Koch et al. 2010, 2012; McKinney et al. 2012; Miranda-Fontaina et al. 2007; Stener 2013; van de Weg 1989). However, breeding needs to balance the need for resistance against other economically important traits. We found a moderate favourable positive genetic correlation between flushing score and *N. neomacrospora* susceptibility. In addition, a favourable negative correlation was observed between the number of terminal buds and *N. neomacrospora* susceptibility, suggesting that simultaneous improvement for several traits could be accomplished. No significant correlation was detected between *N. neomacrospora* susceptibility and Christmas tree score, implying that recurrent selection on resistance would not affect the Christmas tree quality.

Phenological traits (flowering, leaf flushing time and autumn leaf senescence) have often been found genetically correlated with disease resistance in forest trees and can give hints about resistance or avoidance mechanisms (Elzinga et al. 2007; Poteri and Rousi 1996; Hurel et al. 2019). Krokene et al. (2012) showed that bud phenology influences whole-plant defensive responses and that stem susceptibility is correlated with fine-scale changes in bud development in different age classes. In our study, inoculations were carried out on detached twigs with current year's developed shoots. However, the chemical components of twigs might differ if they start to develop at different time points. Further work needs to be conducted to better understand the role of phenology in the disease defence in Nordmann fir.

Although the population studied in this work is the most improved in the Danish Christmas tree breeding programme, selected from the first generation (Hansen et al. 2013), we observed a large variation in susceptibility to *N. neomacrospora*. One of the underlying reasons for the observed large variation could be lack of selection on the trait in the first generation and that the trait itself might be controlled by a large number of genes—each with small effects. Many long-term selection experiments in plant and animal breeding suggested that significant genetic variation is maintained even if the sample size of founders was small (Isik and McKeand 2019).

4.3 Genetic variances and inbreeding

In this study, we only found significant additive genetic effect in susceptibility to *N. neomacrospora*. Non-additive genetic effects were non-significant for Christmas tree traits after having excluded selfed offspring from the analysis, which is in line with the results found by Hansen and Nielsen (2010). However, our results should be interpreted cautiously due to the small sample size from the two diallels employed. Large number of parents and their crosses are required to obtain accurate estimates of non-additive genetic variances (Chang et al. 1990; Foster and Shaw 1988; Costa e Silva et al. 2004).

Selfing in forest trees, increasing the expression of deleterious recessive alleles, can result in inbreeding depression of growth, survival, and adult fecundity (Cram 1984; Woods and Heaman 1989; Burgess et al. 1996; Johnsen et al. 2003; Ford et al. 2015). Our results showed substantial inbreeding depression in Christmas tree quality traits and resistance to *N. neomacrospora*. In a previously published work inbreeding depression was observed for filled seeds (40%), growth traits (5–17%), mortality, and axial damage in selfed seedlings of Nordmann fir (Nielsen and Hansen 2010). The next breeding strategy for Nordmann fir should manage relatedness while optimizing long-term genetic gain. Computer generated mating schemes should be employed to achieve these two conflicting objectives as suggested for *Pinus taeda* (Isik and Mckeand 2019).

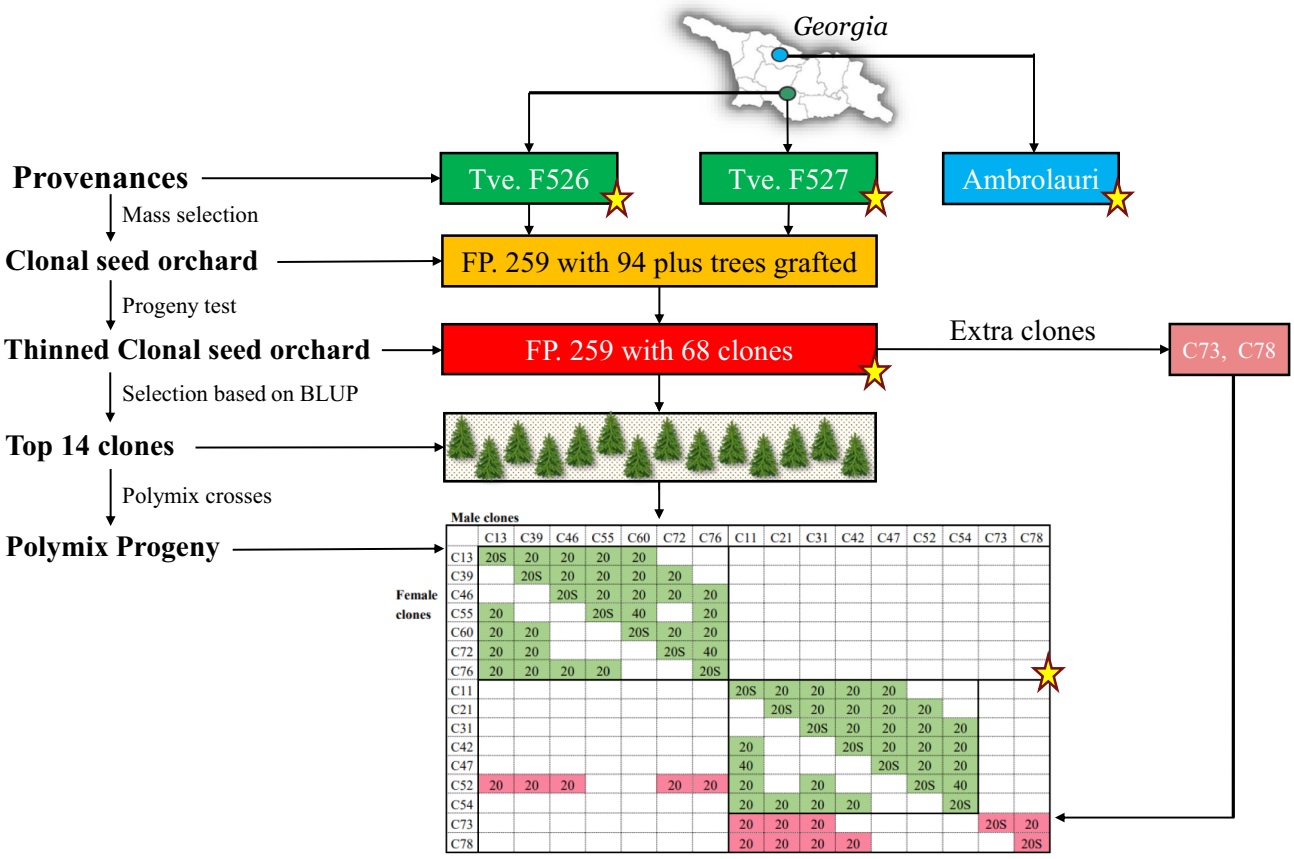
4.4 Genetic gain

Few Christmas tree breeding programs are in the second generation (e.g. Anonymous 2016), and even sparser information is available on realized gain from Christmas tree breeding. The polymix offspring based on top-14 selected clones showed 21% higher in Christmas tree quality and tree density compared with the base population. The improvement of Christmas tree quality in our study was exactly the same as the results from selecting the top 15 (including the 14 clones in current study) out of 128 Nordmann fir clones based on an open pollinated progeny test (Nielsen et al. 2020). Similar estimates were also observed from the closely related species *Abies bornmülleriana* (Xu et al. 2018b). The results suggest that forward selection of the polymix offspring should be performed after paternity determination of the non-genotyped half-sib seedlings to maximize genetic gain. The forward selected seedlings can be grafted into new clonal seed orchards as the material for the subsequent breeding cycle of Christmas trees.

5 Conclusions

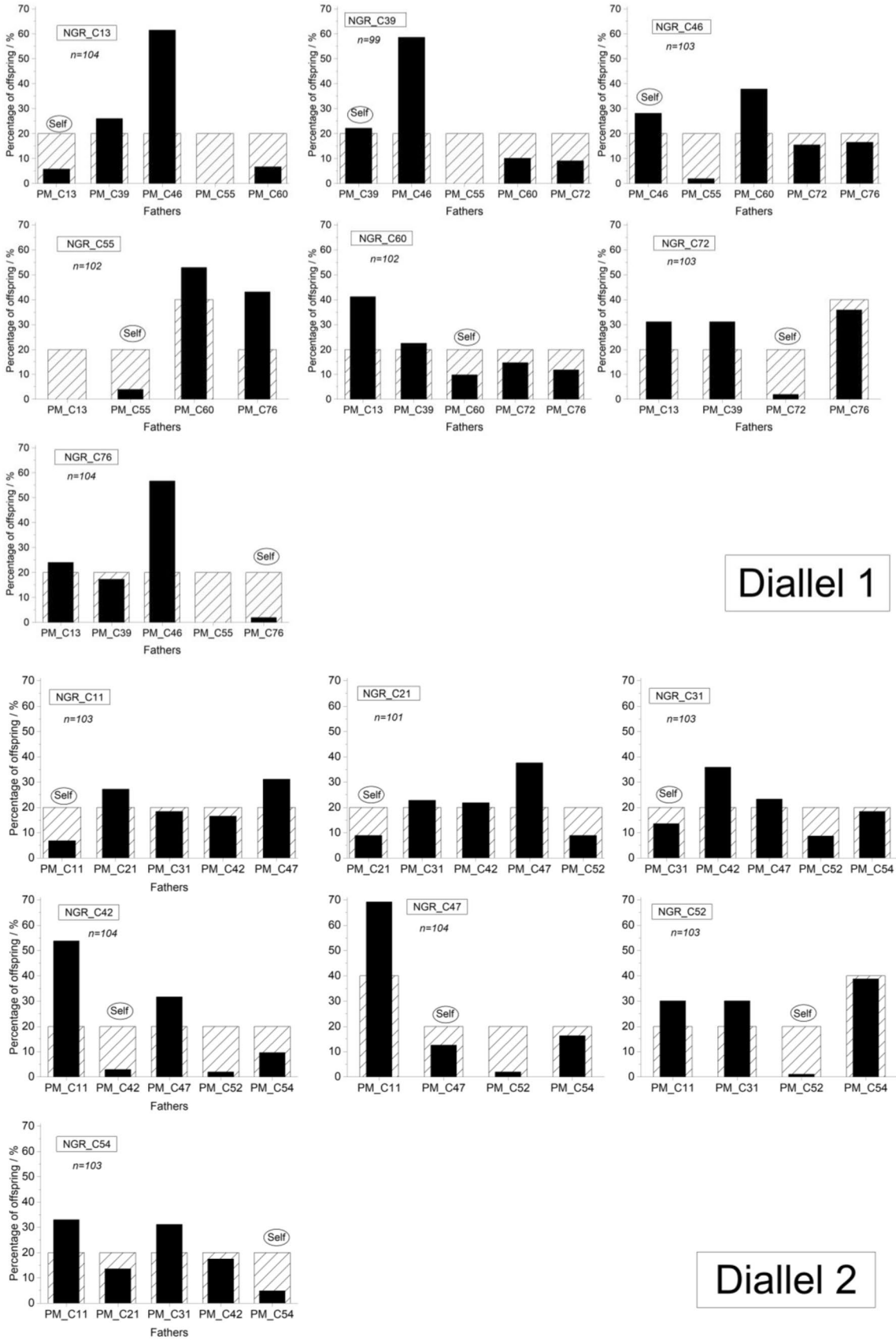
This study provided new and novel information on the genetic basis of susceptibility to *N. neomacrospora* in Nordmann fir. Significant additive genetic variation and high narrow-sense heritability were observed, indicating the trait can be improved through selection. Substantial improvement for tree density and Christmas tree quality has been achieved in the selection process so far. Moderate positive (favourable) genetic correlation between *N. neomacrospora* and flushing showed that simultaneous genetic improvement can be accomplished for both traits. Significant inbreeding depression was detected for all the traits in Nordmann fir. Future breeding of the species needs to balance genetic gain and accumulation of relatedness to avoid negative effects of inbreeding.

Appendix



	C13	C39	C46	C55	C60	C72	C76	C11	C21	C31	C42	C47	C52	C54	C73	C78
C13	20S	20	20	20	20											
C39		20S	20	20	20	20										
C46			20S	20	20	20	20									
C55	20			20S	40		20									
C60	20	20			20S	20	20									
C72	20	20				20S	40									
C76	20	20	20	20			20S									
C11								20S	20	20	20	20				
C21									20S	20	20	20	20			
C31										20S	20	20	20	20		
C42								20			20S	20	20	20		
C47								40				20S	20	20		
C52	20	20	20			20	20	20		20			20S	40		
C54								20	20	20	20			20S		
C73								20	20	20					20S	20
C78								20	20	20	20					20S

Fig. 6 Overview of the origin of parental clones for the polymix study and breeding process. The yellow stars indicate the materials included in this study, which is the polymix offspring, and seedlings from commercial seeds collected from three seed stands (Tve.F.526, Tve. F.527, and Ambrolauri), and one clonal seed orchard FP.259


NGR_C13
n=104
NGR_C39
n=99
NGR_C46
n=103
NGR_C55
n=102
NGR_C60
n=102
NGR_C72
n=103
NGR_C76
n=104
Diallel 1
NGR_C11
n=103
NGR_C21
n=101
NGR_C31
n=103
NGR_C42
n=104
NGR_C47
n=104
NGR_C52
n=103
NGR_C54
n=103
Diallel 2
Percentage of offspring / %
Fathers
Self

◂**Fig. 7** Results from paternity analysis of the fourteen seed lots coming from pollination with pollen mixes in diallel 1 and diallel 2. Black columns show the percentage of offspring sired by the different potential father clones. Hatched columns show the expected percentage, based on the proportion of pollen from the potential fathers in the pollen mixes. "Self" indicates that the seedlings in those columns are the result of selfing

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Data availability The datasets generated and/or analysed during the current study are available in the University of Copenhagen Data Bank, https://doi.org/10.17894/UCPH.E7D968E6-6DA4-4CE9-897D-7022E9536545.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Anonymous (2016) Tree improvement update for Fraser Fir. https://www.ncforestservice.gov/nursery/tip_fraserfir.htm (website accessed 16 March 2020)

Anonymous (2018) Seed stand approval list [Kåringsfortegnelsen]

Araújo JA, Borralho NMG, Dehon G (2012) The importance and type of non-additive genetic effects for growth in *Eucalyptus globulus*. Tree Genet Genomes 8:327–337. https://doi.org/10.1007/s11295-011-0443-x

Best A (2018) Host–pathogen coevolution in the presence of predators: fluctuating selection and ecological feedbacks. Proc R Soc B 285:20180928. https://doi.org/10.1098/rspb.2018.0928

Betts A, Rafaluk C, King K (2016) Host and parasite evolution in a tangled bank. Trends Parasitol 32:863–873. https://doi.org/10.1016/j.pt.2016.08.003

Bian L, Zheng R, Su S, Lin H, Xiao H, Wu HX (2017) Shi J (2017) Spatial analysis increases efficiency of progeny testing of Chinese fir. J For Res 28:445–452. https://doi.org/10.1007/s11676-016-0341-z

Burgess IP, Williams ER, Bell JC, Harwood CE, Owen JV (1996) The effect of outcrossing rate on the growth of selected families of *Eucalyptus grandis*. Silvae Genet 45:97–100

Carson SD, Carson MJ (1989) Breeding for resistance in forest trees—a quantitative genetic approach. Annu Rev Phytopathol 27:373–395

Chang H, Fernando R, Gianola D (1990) An evaluation of maximum likelihood estimates of non-additive genetic variances. Paper presented at the Proceedings of the 4th World Congress on Genetics Applied to Livestock Production, Edinburgh

Christensen CJ (2019) Eksporten af juletræer og klippegrønt i 2018. Nåledrys 110:17–20 (In Danish)

Costa e Silva J, Borralho NMG, Potts BM (2004) Additive and non-additive genetic parameters from clonally replicated and seedling progenies of Eucalyptus globulus. Theor Appl Genet 108:1113–1119

Costa e Silva J, Dutkowski GW, Gilmour AR (2001) Analysis of early tree height in forest genetic trials is enhanced by including a spatially correlated residual. Can J For Res 31:1887–1893. https://doi.org/10.1139/x01-123

Cram WH (1984) Some effects of self-, cross-, and open-pollinations in *Picea pungens*. Can J Bot 62:392–395. https://doi.org/10.1139/b84-059

Cremer E, Liepelt S, Sebastiani F, Buonamici A, Michalczyk IM, Ziegenhagen B, Vendramin GG (2006) Identification and characterization of nuclear microsatellite loci in *Abies alba* Mill. Mol Ecol Notes 6:374–376. https://doi.org/10.1111/j.1471-8286.2005.01238.x

Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G (2007) Time after time: flowering phenology and biotic interactions. Trends Ecol Evol 22(8):432–439. https://doi.org/10.1016/j.tree.2007.05.006

Ford GA, McKeand SE, Jett JB, Isik F (2015) Effects of inbreeding on growth and quality traits in loblolly pine. For Sci 61(3):579–585. https://doi.org/10.5849/forsci.13-185

Foster GS, Shaw DV (1988) Using clonal replicates to explore genetic variation in a perennial plant species. Theor Appl Genet 76:788–794

Garkava-Gustavsson L, Zborowska A, Sehic J, Rur M, Nybom H, Englund JE, Lateur M, Weg E, Van de A Holefors (2013) Screening of apple cultivars for resistance to European Canker Neonectria ditissima. *Acta Hortic* 976. https://doi.org/10.17660/ActaHortic.2013.976.75

Gilmour AR, Gogel BJ, Cullis BR, Welham S, Thompson R (2015) ASReml user guide release 4.1 structural specification. Hemel hempstead: VSN international ltd

Gordon TR, Kirkpatrick SC, Aegerter BJ, Wood DL, Storer AJ (2006) Susceptibility of Douglas fir (*Pseudotsuga menziesii*) to pitch canker, caused by Gibberella circinata (anamorph = *Fusarium circinatum*). Plant Pathol 55:231–237. https://doi.org/10.1111/j.1365-3059.2006.01351.x

Hansen OK, Lamour K, Whetten R, Xu J, Cuenca A, Islam MS, Nielsen UB (2020) Ad hoc breeding of a genetically depauperate landrace of noble fir (*Abies procera* Rehder) using SNP genotyping via high-throughput targeted sequencing. Tree Genet Genomes16 (5). https://doi.org/10.1007/s11295-020-01460-0

Hansen OK, McKinney LV (2010) Establishment of a quasi-field trial in *Abies nordmanniana*—test of a new approach to forest tree breeding. Tree Genet Genomes 6:345–355. https://doi.org/10.1007/s11295-009-0253-6

Hansen OK, Nielsen U, Jensen M (2013) Næste generation i forædlingsprogrammet for nordmannsgran – lavet ved hjælp af pollenmiks-krydsninger. Nåledrys 84:18–23 (In Danish)

Hansen OK, Nielsen UB (2010) Microsatellites used to establish full pedigree in a half-sib trial and correlation between number of male strobili and paternal success. Ann Forest Sci 67:703. https://doi.org/10.1051/forest/2010028

Hansen OK, Vendramin GG, Sebastiani F, Edwards KJ (2005) Development of microsatellite markers in *Abies nordmanniana* (Stev.) Spach and cross-species amplification in the *Abies* genus. Mol Ecol Notes 5:784–787. https://doi.org/10.1111/j.1471-8286.2005.01062.x

Hurel A, de Miguel M, Dutech C, Desprez-Loustau Marie-Laure, Plomion C, Rodríguez-Quilón I, Cyrille A, Guzman T, Alía R, González-Martínez SC, Budde KB (2019) Genetic basis of susceptibility to *Diplodia sapinea* and *Armillaria ostoyae* in maritime pine. bioRxiv 699389 https://doi.org/10.1101/699389

Isik F, Amerson HV, Whetten RW, Garcia SA, Li B, McKeand SE (2008) Resistance of *Pinus taeda* families under artificial inoculations with diverse fusiform rust pathogen populations and comparison with field trials. Can J For Res 38:2687–2696. https://doi.org/10.1139/X08-111

Isik F, Amerson HV, Whetten RW, Garcia SA, McKeand SE (2012) Interactions of Fr genes and mixed-pathogen inocula in the loblolly pine-fusiform rust pathosystem. Tree Genet Genomes 8(1):15–25. https://doi.org/10.1007/s11295-011-0416-0

Isik F, McKeand SE (2019) Fourth cycle breeding and testing strategy for *Pinus taeda* in the NC State University Cooperative Tree Improvement Program. Tree Genet Genomes 15(5):70. https://doi.org/10.1007/s11295-019-1377-y

Isik F, Holland J, Maltecca C (2017) Genetic data analysis for plant and animal breeding. Springer International Publishing, New York. https://doi.org/10.1007/978-3-319-55177-7

Johnsen K, Major JE, Maier CA (2003) Selfing results in inbreeding depression of growth but not of gas exchange of surviving adult black spruce trees. Tree Physiol 23:1005–1008

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Koch JL, Carey DW, Mason ME, Nelson CD (2010) Assessment of beech scale resistance in full- and half-sibling American beech families. Can J For Res 40:265–272. https://doi.org/10.1139/x09-189

Koch JL, Mason ME, Carey DW (2012) Screening for resistance to beech bark disease: improvements and results from seedlings and grafted field selections. Paper presented at the the fourth international workshop on the genetics of host-parasite interactions in forestry: Disease and insect resistance in forest trees. Gen Tech Rep, PSW-GTR-240. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture

Kosawang C, McKinney LV, Nielsen LR, Kjær ED (2020) Variation in aggressiveness of *Hymenoscyphus fraxineus* genotypes amid the ash dieback epidemic. Plant Pathol 69:677–684. https://doi.org/10.1111/ppa.13158

Krokene P, Lahr E, Dalen LS, Skrøppa T, Solheim H (2012) Effect of phenology on susceptibility of Norway spruce (*Picea abies*) to fungal pathogens. Plant Pathol 61:57–62. https://doi.org/10.1111/j.1365-3059.2011.02487.x

Lambeth C, Lee B-C, O'Malley D, Wheeler N (2001) Polymix breeding with parental analysis of progeny: an alternative to full-sib breeding and testing. Theor Appl Genet 103:930–943

Lande R, Schemske D (1985) The evolution of self-fertilization and inbreeding depression in plants. I Genetic Models Evolution 39(1):24–40. https://doi.org/10.2307/2408514

Lian C, Goto S, Hogetsu T (2007) Microsatellite markers for Sachalin fir (*Abies sachalinensis* Masters). Mol Ecol Notes 7:896–898. https://doi.org/10.1111/j.1471-8286.2007.01741.x

Lobo A, McKinney LV, Hansen JK, Kjær ED, Nielsen LR (2015) Genetic variation in dieback resistance in *Fraxinus excelsior* confirmed by progeny inoculation assay. Forest Pathol 45:379–387. https://doi.org/10.1111/efp.12179

Løfting ECL (1973) Statusopgørelse for nordmannsgran [A survey of the present state of Caucasian fir in Denmark]. Forstlige Forsøgsvæsen I Danmark 33:305–326. In Danish.

Mantiri FR, Samuels GJ, Rahe JE, Honda BM (2001) Phylogenetic relationships in *Neonectria* species having *Cylindrocarpon* anamorphs inferred from mitochondrial ribosomal DNA sequences. Can J Bot 79:334–340

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol 40:349–379

McKinney LV, Thomsen IM, Kjaer ED, Nielsen LR (2012) Genetic resistance to *Hymenoscyphus pseudoalbidus* limits fungal growth and symptom occurrence in *Fraxinus excelsior*. Forest Pathol 42:69–74. https://doi.org/10.1111/j.1439-0329.2011.00725.x

Miranda-Fontaina M, Fernández-López J, Vettraino A, Vannini A (2007) Resistance of Castanea clones to *Phytophthora Cinnamomi*: testing and genetic control. Silvae Genet 56:11–21

Nielsen UB, Hansen OK (2010) Response to selfing in seed set, seedling establishment and nursery growth based on controlled crosses of *Abies nordmanniana* Clones. Silvae Genet 59 (2–3). https://doi.org/10.1515/sg-2010-0011

Nielsen UB, Hansen OK (2013) En opdatering på forædlingsprogrammet for nordmannsgran. Nåledrys 84:12–16 (In Danish)

Nielsen UB, Hansen JK, Kromann HK (2010) Impact of site and provenance on economic return in Nordmann fir Christmas tree production. Scan J For Res 26:74–89

Nielsen UB, Kirkeby-Thomsen A, Roulund H (2002) Genetic variation in resistance to *Dreyfusia nordmannianae* Eckst. infestations in *Abies nordmanniana* (Stev.) Spach. For Ecol Manag 165:271–283. https://doi.org/10.1016/S0378-1127(01)00659-4

Nielsen UB, Xu J, Hansen OK (2020) Genetics in and opportunities for improvement of Nordmann fir (*Abies nordmanniana* (Steven) Spac) Christmas tree production. Tree Genet Genomes 16(5). https://doi.org/10.1007/s11295-020-01461-z.

Nielsen KN (2020) Population genomics and biology of the fungal pathogen Neonectria neomacrospora. Ph. D. Thesis, Department of Geosciences and Natural Resource Management, Faculty of Science, University of Copenhagen, 2020

Nielsen UB, Xu J, Nielsen KN, Talgø V, Hansen OK, Thomsen IM (2017) Species variation in susceptibility to the fungus Neonectria neomacrospora in the genus Abies. Scand J Forest Res 32:421–431. https://doi.org/10.1080/02827581.2017.1287300

Norgaard JV, Krogstrup P (1991) Cytokinin induced somatic embryogenesis from immature embryos of Abies nordmanniana Lk. Plant Cell Rep 9:509–513. https://doi.org/10.1007/BF00232107

Perez-Sierra A (2017) Re-emergence of Neonectria neomacrospora on *Abies* spp. in Britain. Paper presented at the Invasive forest pathogens & implications for biology & policy. IUFRO working party 7.02.02. , Niagara Falls, Ontario, Canada. May 7–11 2017.

Pettersson M, Frampton J, Rönnberg J, Talgø V (2016) Neonectria canker found on spruce and fir in Swedish Christmas tree plantations. Plant Health Progress 17(3):202–205. https://doi.org/10.1094/PHP-BR-16-0017

Poteri M, Rousi M (1996) Variation in Melampsoridium resistance among European white-birch clones grown in different

fertilization treatments. Eur J For Path 26:171–181. https://doi.org/10.1111/j.1439-0329.1996.tb00837.x
Proschowsky GF (2014) Naturstyrelsen Nordsjælland - Brancheindsats mod neonectria, Skovplanteringens årsmøde
Rasmussen KK, Andersen US, Frauenfelder N, Kollmann J (2008) Microsatellite markers for the endangered fir *Abies guatemalensis* (Pinaceae). Mol Ecol Res 8:1307–1309. https://doi.org/10.1111/j.1755-0998.2007.02072.x
Schmitz S, Charlier A, Chandelier A (2017) First report of Neonectria neomacrospora on Abies grandis in Belgium New Disease Reports 36:17. https://doi.org/10.5197/j.2044-0588.2017.036.017
Shain L, Franich RA (1981) Induction of Dothistroma blight symptoms with dothistromin Physiological. Plant Pathol 19:49–55
Skulason B, Hansen OK, Thomsen IM, Talgø V, Nielsen UB (2017) Damage by Neonectria neomacrospora and Adelges piceae in provenance trials of subalpine fir (*Abies lasiocarpa*) in Denmark Forest Pathol 47. https://doi.org/10.1111/efp.12326
Sniezko RA (2006) Resistance breeding against nonnative pathogens in forest trees—current successes in North America. Can J Plant Pathol 28:S270–S279. https://doi.org/10.1080/07060660609507384
Solheim H (1988) Pathogenicity of some Ips typographus-associated blue-stain fungi to Norway spruce. Norsk institutt for skogforskning
Stener LG (2013) Clonal differences in susceptibility to the dieback of *Fraxinus excelsior* in southern Sweden. Scand J Forest Res 28:205–216. https://doi.org/10.1080/02827581.2012.735699
Søndreli KL, Kanaskie A, Keriö S, LeBoldus JM (2019) Variation in susceptibility of Tanoak to the NA1 and EU1 lineages of *Phytophthora ramorum*, the cause of sudden oak death. Plant Dis 103(12):3154–3160. https://doi.org/10.1094/PDIS-04-19-0831-RE
Talgø V, Brurberg MB, Stensvand A (2013) Thomsen IM (2013) *Neonectria neomacrospora* is threatening the true fir production in Scandinavia. Proceeding of The 11th International Christmas Tree Research and Extension Conference. Dalhousie University, Nova Scotia, Canada, Faculty of Agriculture, p 28
The R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Valdiani A, Hansen OK, Johannsen VK, Nielsen UB (2020) An efficient bioreactor platform for scaling up the proliferation of Nordmann fir's (*Abies nordmanniana*) somatic embryos. Int J Environ Sci Technol 17:1425–1438. https://doi.org/10.1007/s13762-019-02556-4
van de Weg WE (1989) Screening for resistance to *Nectria galligena* Bres. in cut shoots of apple. Euphytica 42:233–240. https://doi.org/10.1007/BF00034459
Vanderplank JE (1963) Plant Diseases: Epidemics and Control. Academic Press, New York
Wheeler N, Payne P, Hipkins V, Saich R, Kenny S, Tuskan G (2006) Polymix breeding with paternity analysis in Populus: a test for differential reproductive success (DRS) among pollen donors. Tree Genet Genomes 2:53–60. https://doi.org/10.1007/s11295-005-0033-x
Wollenweber HW (1913) '*Ramularia*, *Mycosphaerella*, *Nectria*, *Calorectria*', in Phytopathology. Baltimore, pp. 198–243
Woods JH, Heaman JC (1989) Effect of different inbreeding levels on filled seed production in Douglas-fir. Can J For Res 19:54–59
Xu J, Hansen OK, Thomsen IM, Nielsen UB (2018a) Genetic variation and genotype by environment interaction in the susceptibility of *Abies nordmanniana* (Steven) Spach to the fungus *Neonectria neomacrospora* (Booth & Samuels) Mantiri & Samuels. Ann Forest Sci 75:17. https://doi.org/10.1007/s13595-018-0689-7
Xu J, Nielsen UB, Hansen OK (2018b) Ad hoc breeding of *Abies bornmülleriana* for Christmas tree production using a combination of DNA markers and quantitative genetics—a case study. Tree Genet Genomes 14:5. https://doi.org/10.1007/s11295-018-1276-7
Xu J, Nielsen UB, Hansen OK (2021) Datasets of Christmas tree traits and susceptibility to *Neonectria neomacrospora* results collected in a progeny test of Nordmann fir in Denmark. University of Copenhagen Data Bank. https://doi.org/10.17894/UCPH.E7D968E6-6DA4-4CE9-897D-7022E9536545
Zobel B, Talbert J (1984) Applied forest tree improvement. John Wiley & Sons, New York

Authors and Affiliations

Jing Xu[1] · Ulrik Braüner Nielsen[1] · Fikret Isik[2] · Martin Jensen[3] · Ole Kim Hansen[1]

Ulrik Braüner Nielsen
ubn@ign.ku.dk

Fikret Isik
fisik@ncsu.edu

Martin Jensen
martin.jensen@food.au.dk

Ole Kim Hansen
okh@ign.ku.dk

[1] Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg C, Denmark

[2] Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695-8002, USA

[3] Department of Food Science - Differentiated & Biofunctional Foods, Aarhus University, Agrofoodpark 48, Skejby, 8200 Aarhus, Denmark