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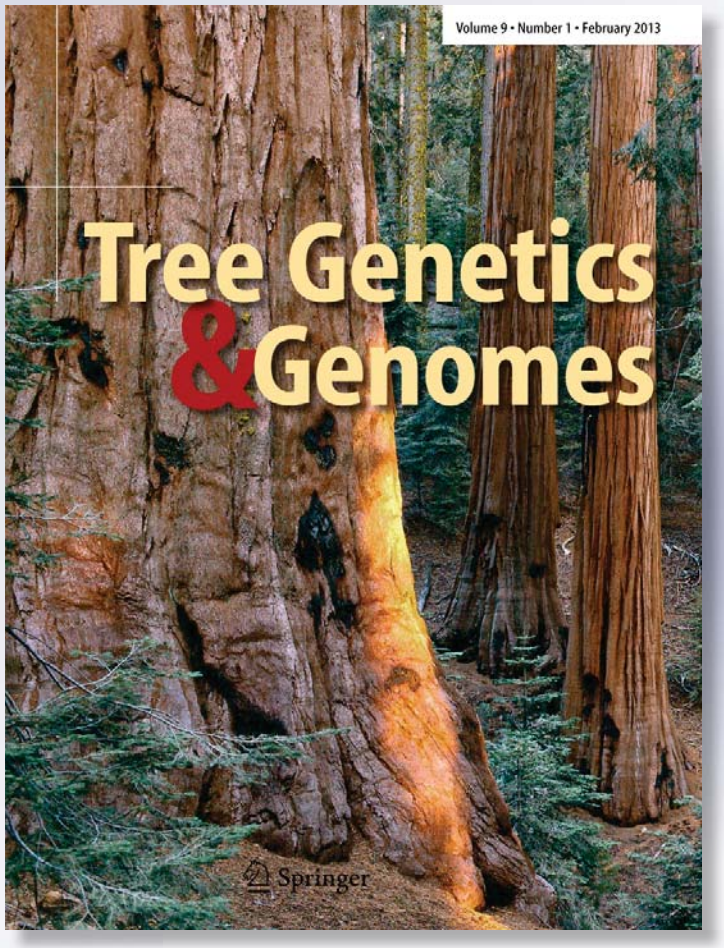

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ORIGINAL PAPER

Genetic variation in resistance to *Phytophthora cinnamomi* in seedlings of two Turkish *Abies* species

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Abstract Genetic variation in resistance to *Phytophthora cinnamomi* was investigated for two fir species endemic to the Republic of Turkey. Open-pollinated families of seedlings of Trojan fir (*Abies equi-trojani*) and Turkish fir (*Abies bornmuelleriana*) were grown from seed in a greenhouse for approximately 15 months, inoculated with rice grains colonized with *P. cinnamomi*, and subsequent mortality assessed biweekly for 16 weeks. Final seedling mortality was higher in Trojan fir (56.4 %) compared to Turkish fir (32.9 %). Mortality in both species varied by geographic origin, decreasing from west (59.8 %, Kazdağı) to east (21.4 %, Karabük). As mortality increased following inoculation, both narrow-sense individual-tree $\left(h_i^2\right)$ and family mean $\left(h_f^2\right)$ heritabilities increased, plateauing at 0.62±0.162 and 0.97±0.011 for Trojan fir and 0.50±0.102 and 0.96±0.01 for Turkish fir, respectively. Terminal and lateral branch bud break assessed under greenhouse conditions were also under strong genetic control. For terminal bud break, individual-tree heritabilities for Trojan and Turkish fir were 0.49±0.146 and 0.45±0.099, respectively, while family mean heritabilities were 0.88±0.035 and 0.88±0.027, respectively. The family mean correlation between bud break and final disease mortality was not significant for lateral buds but positive and significant for terminal buds (r=0.32) suggesting that selection for resistance would either not alter, or slightly reduce, early bud break. These are encouraging results for ongoing tree improvement efforts in North America and Europe to develop planting stock for the Christmas tree industry.

Keywords *Abies bornmuelleriana* · *Abies equi-trojani* · Christmas trees · Root rot · Trojan fir · Turkish fir

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Introduction

Even though the indoor display of evergreen material collected from forests during winter is a centuries-old tradition, the establishment and culture of plantations specifically for Christmas tree production is a relatively new phenomenon (Albers and Davis 1997). During the last 30 to 40 years, the science and technology behind plantation Christmas tree production has developed rapidly, particularly in Europe and North America where annual consumption exceeds 80 million real Christmas trees (Chastagner and Benson 2000). These plantations represent a category of managed forests that are harvested at young ages (generally 3 to 12 years depending on species and region) and are intensively cultured including fertilization, groundcover management, pest control, and crown shaping. While many types of conifers are used for Christmas trees, true firs (*Abies* spp.) have become increasingly popular. Firs are ideally suited for use as Christmas trees due to their natural conical shape, pleasant color and aroma, and strong branches for holding ornaments. Additionally, many fir species have excellent post-harvest needle retention allowing them to be harvested and shipped weeks before their use in consumers' homes (Hinesley and Chastagner 2004).

Some of the most serious diseases afflicting cultivation of firs as Christmas trees are root rots and stem cankers caused

by oomycete species belonging to the genus *Phytophthora* (Chastagner and Benson 2000). The *Phytophthora* species associated with diseases of firs varies with host species and region but includes *Phytophthora cactorum* (Leb. and Cohn) Schröeter, *Phytophthora cinnamomi* Rands, *Phytophthora citricola* Sawada *sensu lato*, *Phytophthora cryptogea* Pethybridge and Lafferty, *Phytophthora drechsleri* Tucker, *Phytophthora gonapodyides* (Petersen) Buisman, *Phytophthora megasperma* Drechsler, and *Phytophthora pseudotsugae* Hamm and Hansen (Benson and Grand 2000, Benson et al. 1976, Chastagner 1997, Shew and Benson 1981).

Cultivation of Fraser fir (*Abies fraseri* [Pursch] Poir) as Christmas trees has developed into a significant industry over the last 40 years in the Southern Appalachian region of the USA. There, significant mortality occurs in many Christmas tree nurseries and plantations due to root rot disease caused primarily by *P. cinnamomi* (Benson and Grand 2000). This disease can be controlled via chemical methods in seedling and transplant beds, but chemical control in plantations is stop-gap at best (Benson et al. 2006) so that severely infested sites are often abandoned for Fraser fir cultivation.

Past efforts to find useful levels of resistance to *Phytophthora* root rot in *A. fraseri* have been unsuccessful; the species is extremely susceptible to *P. cinnamomi*. Lack of genetic variation in Fraser fir to *P. cinnamomi* led to the evaluation of the resistance of other fir species to this disease. Generally, the *Abies* genus is susceptible to *P. cinnamomi* although some variation exists (Frampton and Benson 2012). In an investigation examining variation in resistance of seedlings of 32 *Abies* species, overall mortality of 88 % occurred. Mortality was less frequent in two of the eight taxonomic sections of the genus evaluated, *Momi* (66 %) and *Abies* (79 %) while mortality in all other sections exceeded 93 %. Turkish fir (*Abies bornmuelleriana* Mattf.) and closely related Trojan fir (*Abies equi-trojani* Aschers. et Sint) ranked third and tenth for resistance to *P. cinnamomi*, but mortality in these species was relatively high, 61.3 and 84.2 %, respectively. These results contrasted with a previous study where all Turkish fir seedlings inoculated with *P. cinnamomi* survived (Benson et al. 1997).

While momi fir (*A. firma* Sieb. et Zucc.), a native of Japan, appears to be the most resistant species to *P. cinnamomi* of the genus (Benson et al. 1997; Frampton and Benson 2012), it does not make a desirable Christmas tree due to a coarse branching habit, wide needles, and prickly foliage. Further, it breaks bud 3–4 weeks before *A. fraseri* making it extremely susceptible to spring frost damage. Nevertheless, growers in the Southern Appalachian region have been experimenting with purchasing momi fir to use as rootstock to graft Fraser fir onto and planting the grafts in known *Phytophthora*-infested areas (Hinesley and Frampton 2002, Hibbert-Frey et al. 2010).

While the use of *A. fraseri* grafted onto resistant rootstock is a sound strategy, development of fir that is both resistant and capable of producing a quality Christmas tree is needed in order to more cheaply reclaim areas lost to *A. fraseri* production because of root rot. Turkish fir and Trojan fir are promising species for this role. These species are close relatives to Nordmann fir (*Abies nordmanniana* (Steven) Spach), a widely used Christmas tree species in Europe. Variable results from greenhouse resistance screening trials as well as research field trial results (Frampton 2012, unpublished data) and Christmas tree growers' experiences all indicate that Turkish fir is not uniformly resistant to *Phytophthora* root rot. So, a systematic approach to understand and better use *Phytophthora* resistance within Turkish fir and its relative Trojan fir was undertaken. In this study, using inoculations with *P. cinnamomi*, we demonstrated that resistance in these species is under strong genetic control and that a clinal pattern in the frequency of resistant trees exists in the natural range of these species.

Materials and methods

Plant production and inoculation

Turkish and Trojan fir seeds were obtained from a cone collection trip to northwestern Turkey in fall of 2005 (Frampton and Isik 2006). During the trip, 20 to 40 cones were collected from about 20 trees representing a range of elevations at each of four provenances of Turkish fir (Uludağ, Akyazı, Bolu, Karabük) and two provenances of Trojan fir (Kazdağı, Çan) (Fig. 1). The distance between sampled trees was at least 100 m. Cones were air-dried and seeds extracted by the Turkish Ministry of Environment and Forestry in Ankara, then fumigated and shipped to the USA. After further cleaning, seeds were stored in a freezer at −18°±3 °C until needed. Fraser fir (susceptible control) and momi fir (resistant control) seeds were obtained from the N.C. State University Christmas Tree Genetics Program and purchased from a US seed dealer, respectively.

In March 2007, following a 30-day cold stratification, seeds were sown into flats containing fine vermiculite in a greenhouse in Raleigh, NC, USA. Germinating seedlings were transplanted into a 1:1 (*v*/*v*) mixture of peat and perlite in Ray Leach Pine Cells (66 cm^3, Stuewe and Sons, Inc., Corvallis, OR, USA). As many as 20 germinants per seedlot were transplanted into seedlot plots in each of four randomized blocks in the greenhouse. The seedlings were fertilized weekly with Peters 15–16–17 Peat Lite Special (150–200 ppm N) (J.R. Peters, Allentown, PA USA) and treated with insecticides as needed. The photoperiod was extended to 16 h from August through October by placing 1,000-W halide lamps on a 3-m grid 1.4 m above the trees. The supplemental light was discontinued in October and the thermostat set to 4 °C to induce winter dormancy.

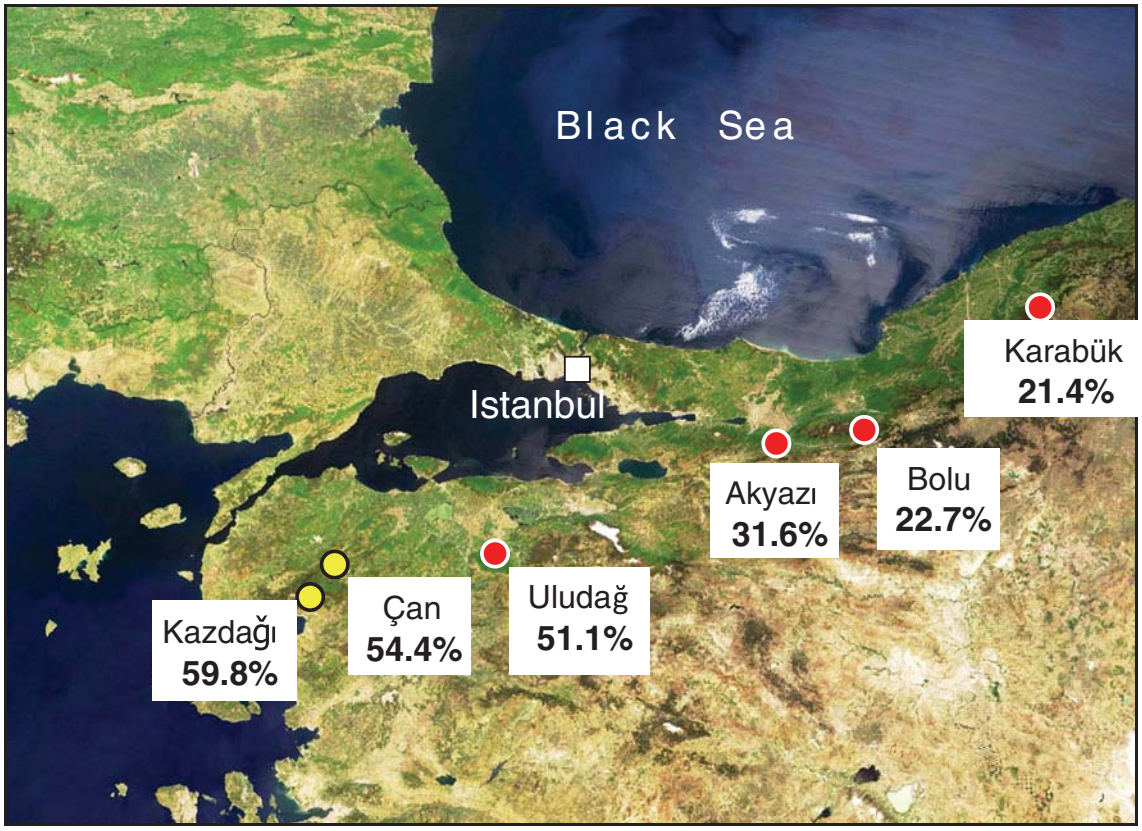

Fig. 1 Location in northwestern Turkey of the two provenances of Trojan fir (Kazdağı, Çan) (yellow circles) and four provenances of Turkish fir (Uludağ, Akyazı, Bolu, Karabük) (red circles) assessed. Seedling mortality 16 weeks after inoculation with *P. cinnamomi* (indicated in *boxes*) varied by geographic origin, decreasing from west (Kazdağı) to east (Karabük)

In November 2007, the dormant seedlings were moved into a walk-in cooler and kept at 2–4 °C for 4 weeks in the dark and watered as needed to prevent desiccation. In December, seedlings were returned to the greenhouse and provided an extended photoperiod with day/night thermostat settings of 24:18 °C. Twenty-eight days after the dormant seedlings received the cold treatment and were returned to the greenhouse, the presence or absence of growth (≥1 mm) of the terminal bud and any lateral branch bud was assessed.

During July of 2008, seedlings were moved from the greenhouse to perform inoculations. Prior to this, the four blocks that had been maintained during greenhouse culture had been slightly reconfigured as necessary to balance the number of seedlings of each family across blocks. One single genotype isolate (23ss04) of *P. cinnamomi* originally derived from Fraser fir was utilized. Previous work has shown that *P. cinnamomi* from a variety of hosts in the USA is clonal, so only one genotype was used for inoculation (Benson, unpublished). Inoculum was prepared and inoculations conducted as previously described (Holmes and Benson 1994; Frampton and Benson 2012). Seedlings were inoculated by making two holes about 2 cm deep and 1 cm from opposite sides of the seedling stem with a glass rod; then, placing a single colonized rice grain into each hole. The medium was pushed back to cover the inoculum.

Seedlings from 105 families of Trojan and Turkish fir were inoculated. Family size averaged 45 seedlings but ranged from 19 to 69 seedlings. A total of 4,858 seedlings were inoculated: 2,819 Turkish fir, 1,911 Trojan fir, 64 Fraser fir, and 64 momi fir. Following inoculation, seedlings were moved into an outdoor lath house and automatically irrigated twice daily (about 23 mm/day). The following non-inoculated control seedlings were cultured in the lath house beside the study: (a) 10 seedlings from three or four families of each provenance (70 and 100 total for Trojan and Turkish fir, respectively); (b) 35 Fraser fir seedlings; and (c) 32 momi fir seedlings. Following inoculation, seedling mortality (completely necrotic shoot) was assessed biweekly for 4 months.

Statistical analyses

Biweekly means for species, provenance, and family were calculated. These means were plotted against time (weeks) to examine the trends (linear, quadratic, etc.) in mortality incidence but also to visually depict the interactions of species and provenances with time. Mortality increased through time but was not linear (Fig. 2), so that its incidence was modeled as a second-order polynomial function of time. The following generalized linear mixed

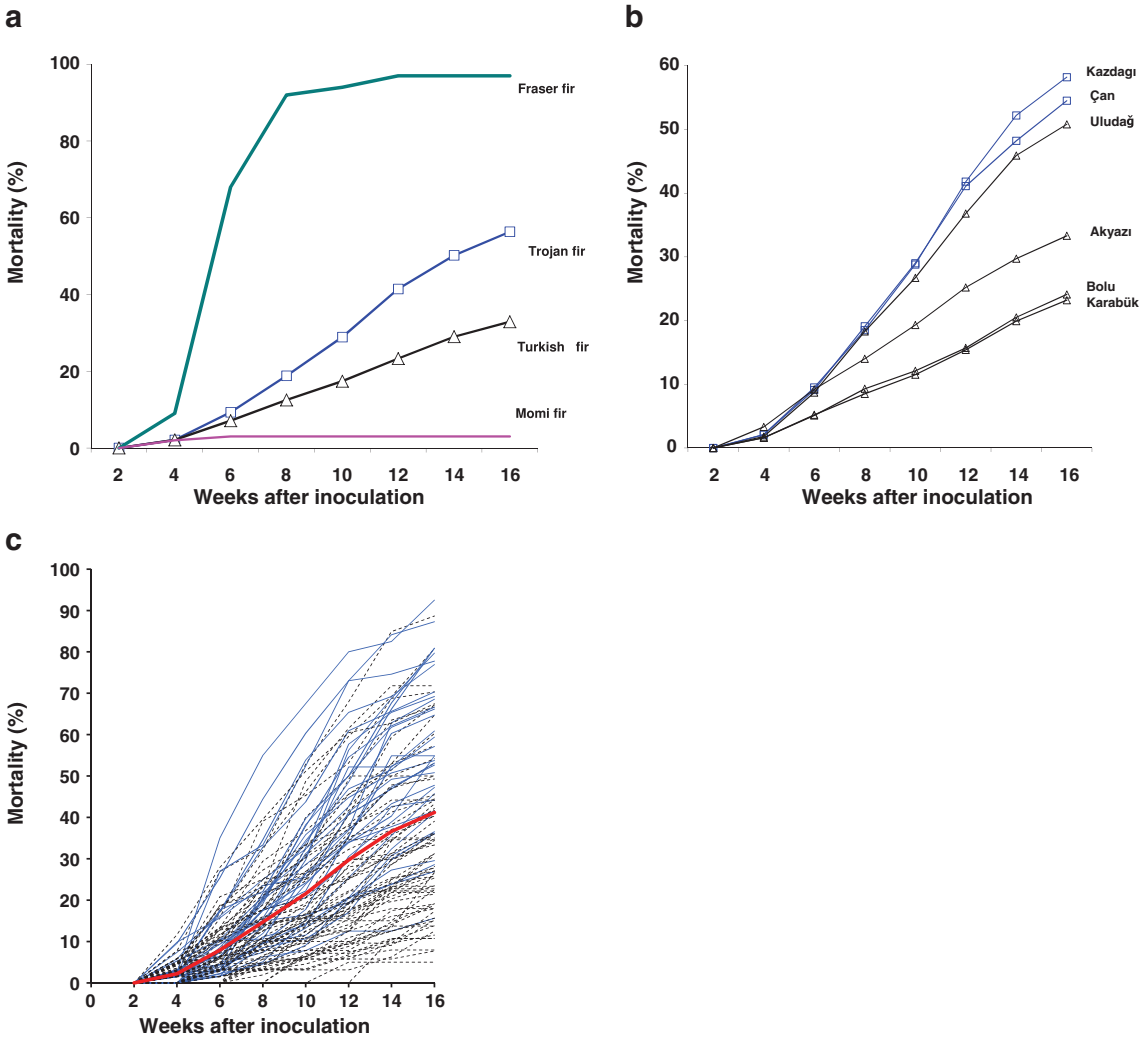

Fig. 2 Mortality curves of fir seedlings over a 16-week period following inoculation with *P. cinnamomi*. **a** Mortality of four fir species (Fraser, Turkish, Trojan, and momi). **b** Mortality of two Trojan fir (Kazdağı, Çan) and four Turkish fir (Uludağ, Akyazı, Bolu, Karabük) provenances. **c** Mortality of 34 families of Trojan fir (*solid blue lines*), 71 families of Turkish fir (*dashed black lines*), and the overall mean (*thick red line*). The average family size was about 45 seedlings

model (generalized estimation equations) was fit to all data of the seedlings inoculated from both species to test the fixed effects (species and provenance) over time. We modeled the probability of mortality (π) while accounting for random replication and family effects and repeated measure:

$$\begin{aligned}\eta_{ijklmn} &= \log[\pi/(1-\pi)] = \mu + R_i + S_j + P(S)_{k(j)} + R \times S_{ij} + R \times P(S)_{ik(j)} + F(SP)_{l(kj)} \\ &+ R \times F(SP)_{il(jk)} + \beta_1 W_n + \beta_2 W \times W_n + \beta_3 (W \times R)_{in} + \beta_4 (W \times W \times R)_{in} + \beta_5 (W \times S)_{jn} \\ &+ \beta_6 (W \times W \times S)_{jn} + \beta_7 (W \times P(S))_{lj(k)n} + \beta_8 (W \times W \times P(S))_{k(j)n} + \beta_9 (W \times F(PS))_{l(jk)n} \\ &+ \beta_{10} (W \times W \times F(PS))_{l(kj)n} + T(SPF)_{ijklm} + e_{ijklmn}\end{aligned} \quad (1)$$

where,

η_{ijklmn}	is the link function [$g(\mu)$] of mortality of the nth time of the mth seedling, jth species, kth provenance, lth family, and in the ith replication
$\log [\pi/(1-\pi)]$	is the logit value or log of odds of mortality
π	is the probability of morality
μ	is the conditional mean
R_i	is the ith replication effect, i=4

S_j	is the jth species effect, j=2
$P(S)_{k(j)}$	is the effect of the kth provenance within the jth species, k=2 or 4
$R\times S_{ij}$	is the ith replication by jth species interaction effect
$R\times P(S)_{ik(j)}$	is the interaction of ith replication and kth provenance within the jth species
$FP(SP)_{l(kj)}$	is the random lth family effect nested within kth provenance within the jth species
$R\times F(SP)_{il(jk)}$	is the interaction of ith replication and lth family
W_n	is the nth linear effect of time (n=1 to 7)
$W\times W_n$	is the quadratic effect of nth time
$W\times R_{in}$	is the interaction of linear nth time and ith replication
$W\times W\times R_{in}$	is the quadratic interaction of the time and ith replication
$W\times S_{jn}$	is the linear interaction of time and jth species
$W\times W\times S_{jn}$	is the quadratic interaction of the time and jth species
$W\times P(S)_{k(j)n}$	is the linear interaction of time and kth provenance within the jth species
$W\times W\times P(S)_{k(j)n}$	is the quadratic interaction of nth time and kth provenance
$W\times F(PS)_{l(kj)n}$	is the linear interaction of nth time and lth family with kth provenance within jth species
$W\times W\times F(PS)_{l(kj)n}$	is the quadratic interaction of nth time and lth family
$T(SPF)_{ijklm}$	is the individual-tree effect (t=1…,5,893)
e_{ijklmn}	is random residual error associated with nth time within mth seedling

β_1 to β_{10} are coefficients related to above terms. These coefficients provide insights about the relative response of species, provenances, and families to inoculation over time. For example, a significant β_7 would suggest that provenances have different rates of disease developments over time.

In the above model, species and provenance effect were considered fixed, while replication and family effects and their interactions were considered random with the expectations $N(0, \mathbf{I}\sigma^2)$. Because biweekly observations were carried out on each seedling, those taken 2 weeks apart are expected to have a higher correlation than observations taken farther apart. In order to account for correlations on repeated measurements, various error variance–covariance structures were evaluated to draw conclusions about the species and provenance effects, assuming default error variance structure (homogeneous and independent errors between measures) may inflate standard errors of model parameters and affect least-squares estimates (Littell et al. 2006). We evaluated autoregressive order 1, compound symmetry, banded Toeplitz with two bands, spatial power Markov, and spatial power exponential covariance structures. Because the generalized chi-square or deviance (model fit statistic) was similar, we used the autoregressive order 1 for the final model (Bolker et al. 2009). We also explored family mean correlations between mortality at week 16, bud break and elevation of mother trees using the CORR procedure of SAS (SAS Institute Inc. 2010).

The probability ($\widehat{\pi}$) of final mortality (16 weeks after inoculation) and the probability of bud break of a single tree were modeled separately for each species with the following generalized linear mixed model using a logit (canonical) link function to partition phenotypic variance into genetic and environmental components:

$$\eta_{ijkl} = \log[\pi/(1-\pi)] = \mu + R_i + P_j + RP_{ij} + F(P)_{k(j)} + RF(P)_{ik(j)} + e_{ijkl} \quad (2)$$

where η_{ijk} is the link function [$g(\mu)$] and μ is the conditional mean, π is the probability of dead seedlings, R_i is the fixed effect of the ith block, P_j is the fixed effect of the jth provenance, RP_{ij} is the fixed interaction effect between ith replication and jth provenance, $F(P)_{k(j)}$ is the random effect of kth family nested within jth provenance with $N(0, I\sigma^2_{f(p)})$, $RF(P)_{ik(j)}$ is the random interaction of the kth family and ith replication with expectations $N(0, \sigma^2_{rf(p)})$, and e_{ijkl} is the random residual with $N(0, I\sigma^2_e)$. The RP_{ij} term was not significant according to the likelihood ratio test and was later dropped from the final model for bud break. The model was run using the GLIMMIX procedure of SAS (SAS Institute Inc. 2010; see the "Appendix"). Using the variance components, we estimated narrow-sense individual-tree $\left(h_i^2\right)$ and half-sib family mean $\left(h_f^2\right)$ heritabilities for mortality and bud break as follows:

$$h_i^2 = \frac{4\sigma^2_{f(p)}}{\left(\sigma^2_{f(p)} + \sigma^2_{rf(p)} + \sigma^2_e\right)} \quad (3)$$

$$h_f^2 = \frac{\sigma^2_{f(p)}}{\left(\sigma^2_{f(p)} + \frac{\sigma^2_{rf(p)}}{b} + \frac{\sigma^2_e}{bn}\right)} \quad (4)$$

where $\sigma^2_{f(p)}$ is the aggregate family variance component across provenances, $\sigma^2_{rf(p)}$ is the replication by family interaction variance, σ^2_e is the fixed error variance, b is the number of replications, and n is the number of seedlings per

Table 1 *F* tests of fixed effects for seedling mortality modeled as a function of linear and quadratic time effects and interactions of time with species and provenance effects (see Eq. 1 for model)

Effect	Numerator DF	Denominator DF	*F* value	Pr>*F*
Species	1	1,006	4.57	0.0328
Prov(Species)	4	1,096	1.74	0.1396
Time	1	39,947	631.88	<0.0001
Time×time	1	39,927	245.82	<0.0001
Time×species	1	39,954	17.62	<0.0001
Time×time×species	1	39,932	7.26	0.0071
Time×Prov(Species)	4	39,931	3.74	0.0048
Time×time×Prov(Species)	4	39,917	1.89	0.1095
Rep	4	364.7	25.40	<0.0001

family per replication. The error variance for binary traits was set to $\pi^2/3=3.29$ in calculation of phenotypic variances as suggested by Gilmour et al. (1985). We assumed that the family variance component is about one quarter of additive genetic variance (Falconer and Mackay 1996). Standard errors of heritabilities were estimated using the delta method (Lynch and Walsh 1998).

Results

Temporal trends in disease mortality of species and provenances

The inoculated seedlings displayed large species differences in mortality (Fig. 2a). The mean mortality of Fraser fir 16 weeks after inoculation was 100 %, but only 3.1 % for momi fir. The two fir species from Turkey were moderately resistant, with 56.4 % mortality for Trojan fir and 32.9 % mortality for Turkish. The mean mortality for Fraser fir increased sharply at week 4 after inoculation, while the trends of mortality for Trojan and Turkish fir were more gradual over time. Over the 16-week assessment period, no mortality was observed in the non-inoculated control seedlings of Fraser and Turkish fir and only negligible mortality in Trojan (one dead seedling) and momi (two dead seedlings) fir.

In the overall analysis of mortality of Trojan and Turkish fir over the entire assessment period (Eq. 1), no significant differences were found among models with different residual variance–covariance structures. They all produced the same generalized chi-square statistic and the same deviance (0.86), a ratio of the generalized chi-square and the degrees of freedom used as a measure of fit (Bolker et al. 2009). *F* test results for mortality of species, provenance within species, and their linear and quadratic interactions with time are given in Table 1. Species varied significantly (Pr<0.0328) for mortality across time; however, the provenance effect pooled across species was not significant (Pr<0.1389). The linear and quadratic effects of time on mortality were highly significant. The increasing trend in mortality is not linear, but the rate of increase (slope) gets steeper with time as shown by the highly significant quadratic effect of time (Pr<0.0001). The time linear and quadratic effects explain a large proportion of variance in mortality as suggested by their larger *F* values compared to those of other terms.

The relative difference between Trojan and Turkish fir species over time was not constant but varied significantly

Table 2 *F* tests of fixed effects for seedling mortality after 16 weeks of inoculation and for terminal and lateral bud break (see Eq. 2 for model)

Effect	DF	Mortality after 16 weeks		Terminal bud break		Lateral branch bud break	
		F	Pr<*F*	*F*	Pr<*F*	*F*	Pr<*F*
Trojan Fir							
Rep	3	2.69	<0.051	63.83	<0.0001	3.91	0.008
Provenance	1	0.53	0.470	3.09	0.089	2.23	0.145
Rep×provenance	3	1.05	0.373	1.41	0.237	1.62	0.183
Turkish Fir							
Rep	3	5.12	0.002	88.76	<0.0001	15.74	<0.0001
Provenance	3	10.69	<0.0001	10.95	<0.0001	5.94	0.001
Rep×provenance	9	0.99	0.450	0.76	0.650	0.55	0.836

Table 3 Mean and range of family means of provenances of Trojan and Turkish fir for mortality 16 weeks after inoculation with *P. cinnamomi*, terminal bud break, and lateral branch bud break

Provenance	# of families	Mortality after 16 weeks		Terminal bud break		Lateral branch bud break	
		Mean	Range	Mean	Range	Mean	Range
Trojan fir							
Çan-Seed stand	16	54.4	15.6–81.9	40.6	21.6–62.9	28.7	14.1–53.0
Kazdağı-Gürgendağ	18	59.8	36.2–92.5	52.4	23.1–79.5	36.2	21.1–52.6
Turkish fir							
Uludağ-NP	20	51.1	10.7–88.7	39.3	16.6–63.9	19.1	7.6–38.4
Akyazı-Dokurcun	20	31.6	7.7–67.4	16.8	7.0–44.8	11.3	3.9–32.8
Bolu-Kökez	12	22.7	7.9–88.7	15.1	8.1–39.1	17.8	7.7–35.2
Karabük-Safranbolu	19	21.4	0.0–45.5	30.4	16.1–47.9	28.2	12.3–65.8

as indicated by the significant time by species linear effect (Pr <0.0001) and the quadratic time by species effect (Pr= 0.0071). Over time, the difference between species mortality increased (Fig. 2a). For example, the difference in mortality between Trojan and Turkish fir was 11.5 % at week 10 and increased to 23.5 % at week 16. The interaction of provenance by time linear effect was significant, but the provenance by time quadratic effect was not.

Disease mortality 16 weeks after inoculation

Provenance effects were highly significant (Pr<0.0001) for mortality at week 16 (Table 2). Provenance means for mortality during the 16-week post-inoculation period are presented in Fig. 2b, and final (week 16) means are presented in Table 3. Trojan fir provenances (Kazdağı and Çan) had clearly higher mortality than three of the Turkish fir provenances (Akyazı, Bolu, and Karabük); however, the Uludağ provenance of Turkish fir showed a similar high mortality response to the pathogen as the Trojan fir provenances (Table 3, Fig. 2b). This source is geographically closer to the Trojan fir natural range (Fig. 1). A west-to-east trend in mortality was evident, being higher (59.8 %) in the most western seed source (Kazdağı) and the lowest (21.4 %) in the most eastern one (Karabük). Large variation in mortality within Trojan and Turkish fir provenances was evident from family mortality plots (Fig. 2c) as well as in the range of family means observed at week 16 (Table 3).

Genetic variances and heritability estimates for disease mortality

In the generalized linear mixed model (Eq. 1) fit to data across the entire assessment period, family effects (nested within species and provenances) and plot effects (interaction of family by replication effect) were treated as random. In this model, the error variance was set to a constant (3.29) for estimation of heritabilities. Family differences explained 12.4 % of the total variance. This family variance is a pooled estimate across species and provenances within species over the entire time period. Individual-tree heritability (0.49± 0.086) and family mean heritability (0.89±0.020) for mortality were high.

Biweekly heritability estimates for mortality of the two species are given in Table 4. Considerable genetic variation

Table 4 Narrow-sense individual-tree $\left(h_i^2\right)$ and half-sib family mean $\left(h_f^2\right)$ heritabilities for mortality observed over time for Trojan and Turkish fir seedlings following inoculation with *P. cinnamomi*

Time	Trojan fir			Turkish fir		
	D/DF[a]	h_i^2	h_f^2	D/DF	h_i^2	h_f^2
Week 4	0.32	0.58 (0.338)	0.96 (0.030)	0.32	0.10 (0.214)	0.77 (0.418)
Week 6	0.82	0.56 (0.187)	0.96 (0.015)	0.72	0.39 (0.131)	0.94 (0.023)
Week 8	0.89	0.33 (0.128)	0.93 (0.031)	0.84	0.37 (0.106)	0.94 (0.019)
Week 10	0.94	0.47 (0.137)	0.96 (0.015)	0.89	0.43 (0.103)	0.95 (0.014)
Week 12	0.96	0.54 (0.144)	0.96 (0.012)	0.91	0.50 (0.106)	0.96 (0.010)
Week 14	0.96	0.53 (0.143)	0.96 (0.013)	0.93	0.50 (0.102)	0.96 (0.010)
Week 16	0.95	0.62 (0.162)	0.97 (0.011)	0.93	0.50 (0.102)	0.96 (0.010)

[a]Variance heterogeneity factor [Deviance/DF]

Table 5 Family variance component, narrow-sense individual-tree heritability estimate $\left(h_i^2\right)$, and family mean heritability estimate $\left(h_f^2\right)$ for terminal and lateral branch bud break of Trojan and Turkish fir seedlings

Trait	Mean	Family variance	h_i^2	h_f^2
Trojan fir				
Terminal bud break	0.48	0.48 (0.164)	0.49 (0.146)	0.88 (0.035)
Lateral branch bud break	0.34	0.31 (0.100)	0.34 (0.102)	0.84 (0.044)
Turkish fir				
Terminal bud break	0.29	0.44 (0.107)	0.45 (0.099)	0.88 (0.027)
Lateral branch bud break	0.21	0.52 (0.121)	0.53 (0.110)	0.90 (0.022)

Standard errors of estimates are given in the parentheses. Variance heterogeneity factor [Deviance/DF] for lateral bud break: Turkish fir=0.90, Trojan fir=1.21. Variance heterogeneity factor [Deviance/DF] for terminal bud break: Turkish fir=0.98, Trojan fir=1.13

observed among families was reflected in high individual-tree and family mean heritabilities for both species. Individual-tree heritability estimates for both species ranged from 0.10 (week 4, Turkish fir) to 0.62 (week 16, Trojan fir). The range of family mean heritability estimates was narrower (0.77 to 0.97) compared to the individual-tree heritability estimates range. Individual-tree heritabilities increased from weeks 4 to 16, especially for Turkish fir. When mean disease mortality was low (early weeks), standard errors of heritability estimates were also high.

In the early weeks after inoculation, the variance heterogeneity factor, D/DF (dispersion parameter), suggested less dispersion than would be expected for a binomial model (Table 4). If the deviance is close to the degrees of freedom (e.g., scale parameter=1), then there is no evidence of over-dispersion or under-dispersion. According to this criterion, the data for the early weeks after inoculation show under-dispersion. In such instances, the standard errors of estimates are biased (overestimated) increasing the *P* values and type II errors. Thus, these genetic parameter estimates and *F* tests for low mortality incidence should be interpreted cautiously.

Terminal and lateral bud break

The incidence of terminal and lateral bud break was assessed at one time only, 28 days after transferring seedlings from a cooler into a warm greenhouse with extended photoperiod. Provenance bud break means are given in Table 3. There were large differences among provenances within the two species, particularly for Turkish fir (F=13.7, Pr<0.0001) (Table 2). The range of terminal bud break among provenance means was 15.1 % (Bolu) to 52.4 % (Kazdağı). There was also a large range for terminal bud break among families within provenances, for example, 16.6 to 63.9 % in the Uludağ provenance. For lateral bud break, provenances differed significantly (F=9.0, Pr<0.0001), but the range among provenances was smaller, 11.3 % (Akyazi) to 36.2 % (Kazdağı). Again, families within provenances varied considerably, especially within the Karabük provenance.

Overall terminal and lateral bud break means, family variance components, and individual-tree and family mean heritability values for two traits are given in Table 5. Turkish fir had noticeably lower bud break than Trojan fir for both terminal buds (48 vs. 29 %, respectively) and lateral buds (34 vs. 21 %, respectively). Heritability estimates for both species were similar. Family mean heritabilities were high (0.84–0.90) with relatively small standard errors.

There was a significant and positive association between family mortality and terminal bud break (r=0.32, Pr< 0.0001) (Table 6). Family mortality also had a positive association with lateral bud break, but the correlation coefficient was marginally significant (r=0.19, Pr<0.051). Family terminal bud break and lateral bud break had a moderately high correlation (r=0.56). Neither family mortality nor bud break was significantly correlated with the elevation of the mother tree.

Discussion

The onset of mortality following inoculation of Trojan and Turkish fir seedlings with *P. cinnamomi* is nonlinear as observed in plots of mortality curves (Fig. 2) and from the

Table 6 Correlations among mortality at week 16 based on family means, bud break based on family means, and elevation of mother trees

	Mortality	Terminal bud	Lateral bud
Terminal bud	0.32 (0.001)		
Lateral bud	0.19 (0.051)	0.54 (<0.0001)	
Elevation	−0.15 (0.112)	−0.11 (0.259)	−0.17 (0.072)

results of the overall model which included time and its quadratic term (Table 1). Mortality accelerates about 4 weeks after inoculation, then gradually slows, and plateaus, or almost does so, after 16 weeks. Previous studies with fir species have also reported this relationship and have used Richards' function to describe it for 32 fir species (Frampton and Benson 2012) and for three provenances of Fraser fir (Frampton and Benson 2004). The present study not only confirmed this relationship at the species and provenance levels but also revealed that mortality at the family level follows a similar trend (Fig. 2c).

The relative ranking of species for resistance to *P. cinnamomi* observed in this study corroborates a previous report (Frampton and Benson 2012). Fraser fir and momi fir are almost uniformly susceptible and resistant, respectively, while Turkish and Trojan fir are intermediate and variable. The frequency of resistance in Turkish fir is higher than in Trojan fir. A novel finding of the present study is that the frequency of resistance increases in a west-to-east trend across the combined geographic ranges of these two species. The cause of this unexpected finding is unknown. Although *P. cinnamomi* is not believed to be native to Turkey, other *Phytophthora* species are present in the country (Balcı and Halmschlager 2003) so a pattern of resistance may have evolved as a result of past exposure to them or to other oomycetes. Alternatively, the observed geographic pattern could be a result of adaptation to environmental factor(s) such as rainfall, temperature, and/or soil characteristics (e.g., texture) with a pleiotropic effect on root rot resistance.

While the range in disease mortality among provenances of these two closely related species is impressive (21.4–59.8 %), the range among families is even more so (0.0–92.5 %) with extremely high family mean heritabilities estimated for both species (0.96–0.97). This is the first report of genetically controlled resistance to *P. cinnamomi* in *Abies* although others have reported genetic resistance to this pathogen in pines (*Pinus radiata* D. Don, Butcher et al. 1984, *Pinus echinata* Mill. and *Pinus taeda* L., Tainter and Baker 1996) as well as in broadleaf tree species such as avocado (*Persea* spp., Douhan et al. 2011), chestnuts (*Castanea* spp. Miranda-Fontaina et al. 2007), and jarrah (*Eucalyptus marginata* Don ex Sm., Stukely and Crane 1994).

Late bud break in the spring is especially important for these species because they generally break bud earlier than Fraser fir, and in many cases, the *Phytophthora*-infested sites where they are deployed in the Southern Appalachians are low-lying and poorly drained areas where frost pockets often occur. The cold treatment and greenhouse conditions that the seedlings received in this study do not represent natural conditions. While it is unknown how the bud break responses measured relate to natural responses, if we assume they mimic natural responses, selection for resistance would either not alter or slightly reduce early bud break. The surviving seedlings of this study have been established in a field trial in the Southern Appalachian region to further assess their adaptability, growth, post-harvest needle retention, and Christmas tree quality. Ultimately, selections will be made and grafted into a clonal seed orchard. Planting stock grown from the seed produced in this orchard will be targeted for sites with known *Phytophthora* problems in the region.

Widespread interest in these *Abies* species from Turkey has risen across the Christmas tree industry. In 2010, the industry-funded Collaborative Fir Germplasm Evaluation Project was organized among universities and grower associations in five regions of the USA plus Denmark (Frampton 2010). During the fall of 2010, another cone collection of Turkish and Trojan fir was carried out, and seedlings are currently being cultured to establish a coordinated provenance–progeny test series across these regions. Ultimately, regional seed orchards grafted from select trees of these trials will be established. Meanwhile, until improved planting stock is available, growers in Europe and the USA have only limited choices in the origin of Trojan and Turkish fir planting stock. If presented a choice when selecting planting stock to regenerate known *Phytophthora*-infested sites, growers should avoid Trojan fir and the Uludağ-NP provenance of Turkish fir.

Use of the results from this investigation has several limitations. A single isolate of *P. cinnamomi* was employed so that interactions among genetic entities of the host and pathogen genotypes could not be detected and, if they exist, may have affected the conclusions. Further, additional *Phytophthora* species are of concern on *Abies* in other regions of the USA and in Europe. Future research utilizing additional pathogen genotypes and species is needed to address host×pathogen genotype interactions.

Additionally, the inoculation techniques used in this study may have overlooked some types of resistance. Young seedlings were inoculated, but they may not possess some resistance mechanisms operative in older trees. The conditions of this trial were different and more favorable for disease development than those under most field conditions because (1) a relatively large amount of inoculum was applied, (2) the medium was kept continuously wet, and (3) the root systems were confined in the container.

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Appendix

SAS GLIMMIX code for model 1

```
title 'First-order Autoregressive Covariance Structure' ;
proc glimmix data=fir.indilong asycov noitprint noclprint ;
 class species prov family rep ;
 model mortality =
    species
    prov(species)
    time
    time*time
    time*species
    time*time*species
    time*prov(species)
    time*time*prov(species)
    rep / dist=binomial link=logit ddfm=kr ;
 random family(species*prov)rep*family(species*prov) ;
 random _residual_ / type=ar(1)(time) subject=treeID(family)residual ;
 nloptions technique = nrridg maxiter =1000 ;
run;
```

SAS GLIMMIX code for model 2

```
 title 'Model 2, Week16 Mortality' ;
 proc glimmix data=fir ;
  by species;
  class rep prov family ;
  model mortality16 = rep|prov / dist=binomial link=logit ddfm=kr ;
  random family(prov)  rep*family(prov) ;
  random _RESIDUAL_  ;
  nloptions technique = nrridg maxiter =1000;
  lsmeans prov /ilink ;
 run;

 title 'Model 2, Budbreak' ;
 Proc GLIMMIX Data=bud.budbreak ;
  by species;
  class rep prov family ;
  model lateral (ref=first)= rep|prov / dist = binary link = logit ;
  random Family(Prov) ;
  random _RESIDUAL_ ;
  nloptions technique = congra maxiter =1000 ;
  output out = two predicted(ilink) = predicted
       stderr(ilink) = stderr lcl(ilink) = lower
       ucl(ilink) = upper ;
  lsmeans prov /ilink;
 run;
```

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