

Performance of Twenty Four European *Fraxinus excelsior* Populations in Three Lithuanian Progeny Trials with a Special Emphasis on Resistance to *Chalara Fraxinea*

ALFAS PLIŪRA^{1,2*}, VAIDOTAS LYGIS³, VYTAUTAS SUCHOCKAS^{1,2}, AND EDMUNDAS BARTKEVIČIUS²

¹Department of Forest Genetics and Breeding, Department of Silviculture, Institute of Forestry, Lithuanian Research Centre for Agriculture and Forestry, Liepu str. 1, LT-53101 Girionys, Kaunas reg., Lithuania

²Department of Forestry, Lithuanian University of Agriculture, Studentu str. 13, LT-53361 Akademija – Kaunas, Lithuania

³Laboratory of Phytopathogenic Microorganisms, Institute of Botany of Nature Research Centre, Žaliųjų Ežerų str. 49, LT-08406 Vilnius, Lithuania

*Corresponding author: telephone +37037547289, e-mail:genetsk@mi.lt.

Pliūra, A., Lygis, V., Suchockas, V. and Bartkevičius, E. 2011. Performance of Twenty Four European *Fraxinus excelsior* Populations in Three Lithuanian Progeny Trials with a Special Emphasis on Resistance to *Chalara fraxinea*. *Baltic Forestry* 17 (1): 17–34.

Abstract

The main aim of the present study was to estimate genetic variability and the level of genetic control of disease resistance in common ash populations based on progeny testing, and to select resistant populations, families and genotypes for production of reproductive material resistant to dieback caused by *Chalara fraxinea*. A total of 320 half-sib families from ten Lithuanian and fourteen foreign European *Fraxinus excelsior* populations have been tested in three progeny trials established in Telšiai, Pakruojis, and Kėdainiai State Forest Enterprises. Because of severe attacks by *C. fraxinea*, only 10% of trees survived in all progeny trials at the age of eight years. Variance analysis of health condition scores revealed significant effect of genetic factors i.e., populations and families. Differences in resistance among populations were significant and ranged up to 52.8-55.1% in a degree of damage and survival rate of populations varying from 0.13 to 0.59. At the age of eight years, genetic family variance components ranged 5.3-7.5%, while the additive coefficients of genetic variation ranged 29.9-38.7% indicating the existence of strong genetic differences in progenies' resistance to *C. fraxinea* both at populational and family levels. There was a significant site effect on the level of damage which could be due to site conditions and/or different infection pressure from the surrounding stands. However, genotype by environment interaction (G x E) for resistance traits was weak and nonsignificant which indicated that populations and families do not differ much in their performance across environments and thus specific adaptations and genetic variation in ecological response are not pronounced. The obtained high heritability estimates ($h^2=0.40-0.49\pm 0.03$) for health condition indicated that resistance is genetically predetermined and inheritable, and allows forward selection and breeding of the resistant genotypes.

Health condition of foreign populations was worse than that of the Lithuanian ones by 17.5-23.5% in degree of damage and by 48.5-73.5% in survival rate which could be a consequence of more advanced process of natural selection in resistance against *C. fraxinea* in damaged Lithuanian *F. excelsior* populations. The obtained results demonstrated that strategy of the extended introduction of foreign populations to restore Lithuanian ash stands is of doubtful potential. Only few most resistant foreign genotypes could be selected as potential candidates for breeding of 'synthetic' *F. excelsior* populations in order to increase genetic diversity. Among the Lithuanian populations, the best in terms of growth characteristics, resistance, survival, and proportion of most healthy individuals were those originating from Ignalina, Pakruojis, Šakiai and Kėdainiai.

Based on estimated BLUP breeding values, complex breeding indexes and on so-called 'Pyramid selection principle', fifty superior individuals were selected within best families from the best populations for establishing resistant breeding populations of common ash in different provenance regions of Lithuania, as well as for establishing clonal trials and resistant seed orchards of second generation.

Key words: *Chalara fraxinea*, common ash (*Fraxinus excelsior*), dieback, genetic variation, half-sib families, heritability, populations, resistance, selection

Introduction

European forests are repeatedly subjected to negative impacts by previously non-encountered diseases

caused by emerging and invasive fungal pathogens. The most prominent cases include large-scale dieback and decline of noble hardwoods as ash (*Fraxinus*) (e.g., Juodvalkis and Vasiliauskas 2002, Przybyl 2002a,

Barklund 2005, Lygis et al. 2005, Cech and Hoyer-Tomiczek 2007), oak (*Quercus*) (Oszako 2004) and elm (*Ulmus*) (e.g., Brasier 1979, Stipes and Campana 1981), followed more recently by rapid expansion of introduced foliage pathogens of conifers (e.g., Pehl and Wulf 2001, Kirisits and Cech 2006, Barnes et al. 2008, Jankovsky et al. 2009) and *Phytophthora*-caused decline of alder (*Alnus*) (e.g., Santini et al. 2001, Brasier et al. 2004, Jung and Blaschke 2004). To date, all those threats are of high concern also in Lithuania. The emerging diseases are caused by pathogens that have either changed geographical range of their distribution, undergone host shift or have mutated and became more virulent (Anderson et al. 2004, Woolhouse et al. 2005). Massive disease outbreaks deteriorate stability of forest ecosystems and challenge conservation of forest genetic resources and tree breeding.

Since the early 1990s, a large-scale dieback of common ash (*Fraxinus excelsior* L.) has been expanding in Eastern, Central and Northern Europe (Juodvalkis and Vasiliauskas 2002, Przybyl 2002a, Barklund 2005, Heydeck et al. 2005, Thomsen and Skovsgaard 2006, Vasiliauskas et al. 2006, Cech and Hoyer-Tomiczek 2007, EPPO 2007, Schumacher et al. 2007, Halmschlager and Kirisits 2008, Szabo 2009) leading to a great concern for the future of this noble hardwood species. Ash trees are subject to dieback at various ages, in forest stands, as landscape trees, urban plantings and in nurseries (Juodvalkis and Vasiliauskas 2002, Kowalski and Lukomska 2005, Schumacher et al. 2007, Kirisits and Halmschlager 2008). Initial macroscopic symptoms of the disease include necrotic, eye-shaped spots on petioles and the bark of young shoots, discolouration and subsequent wilting of leaves and petioles. Symptoms typically occur in the crown and include elongated cankers on stem and branches as well as dieback of upper parts of the crown (Bakys et al. 2009a, Kowalski and Holdenrieder 2009a,b, Skovsgaard et al. 2010). A fungus *Chalara fraxinea* T. Kowalski was reported as the cause of *F. excelsior* decline (Kowalski and Lukomska 2005, Kowalski 2006) and its pathogenicity to ash has been proven by controlled inoculations (Bakys et al. 2009a, Kowalski and Holdenrieder 2009a). Recently, *C. fraxinea* was linked to the helotialean ascomycete *Hymenoscyphus pseudoalbidus* V. Queloz, C.R. Grünig, R. Berndt, T. Kowalski, T.N. Sieber & O. Holdenrieder (Queloz et al. 2010). In addition, several other fungi with some pathogenic potential were found on diseased trees and the possible influence of coinciding abiotic factors (e.g. drought, frost and changing winter conditions) have been discussed (e.g., Ozolinčius and Stakėnas 2000, Ozolinčius 2002, Przybyl 2002b, Skuodienė et al. 2003, Kowalski and Lukomska 2005, Lygis et al. 2005, Thom-

sen and Skovsgaard 2006, Cech and Hoyer-Tomiczek 2007, Schumacher et al. 2007, Bakys et al. 2009b). Apart from *F. excelsior*, the disease has been observed also on *F. angustifolia* Vahl. (Cech and Hoyer-Tomiczek 2007, Schumacher et al. 2007, Kirisits et al. 2010), *F. ornus* L. (Kirisits et al. 2009), *F. nigra* Marshall, *F. pennsylvanica* Marshall, *F. americana* L. and *F. mandshurica* Rupr. (Drenkhan and Hanso 2010). Molecular tools have been elaborated for rapid detection and identification of *C. fraxinea* (Chandelier et al. 2009, Ios 2009, Johansson 2009).

In Lithuania, the dieback of common ash has started around 1995-1996 in forests of Ukmergė, Jonava and Panevėžys State Forest Enterprises (Juodvalkis and Vasiliauskas 2002, Gustienė 2010). Currently the phenomenon of ash dieback is in its chronic phase and virtually all Lithuanian ash stands are damaged to a greater or lesser extent (Gustienė 2010). Less than 32.6 thous. ha of ash stands out of initial 50.8 thous. ha have survived after sanitary fellings in severely damaged stands (Anon. 2009) and the health condition of the remaining stands continues to deteriorate (Riepšas 2009). No effective means to control spread of the disease have been identified so far, and a question is often being asked by forest owners and managers: what to do with the diseased trees and stands; or even – does it make sense to grow *F. excelsior* at all? According to current recommendations for Lithuanian forest owners and managers (Juodvalkis and Vasiliauskas 2002, Riepšas 2009) it is advised to cut the diseased stands by sanitary fellings. Artificial re-establishment of ash stands is not recommended due to high risk of infections by *C. fraxinea* on newly established plantations. Obviously, the future of common ash, an important ecosystem-forming tree species, is unclear in Lithuania and adequate management measures need to be undertaken.

It is widely accepted that genetic diversity is crucial for disease resistance in forest tree populations (Burdon 2001). The density of remaining viable and flowering trees is extremely low (3-5 trees per ha) in most of the remaining (yet, severely damaged) Lithuanian ash populations. Therefore, the effective population size (N_e) has most likely decreased substantially thus possibly compromising the remaining level of genetic diversity and possibilities for genetically sufficient regeneration of populations. European long-term gene conservation strategies for ash prepared under European Forest Resources Gene Conservation Program (EUFORGEN) emphasized a dynamic gene conservation which is based on capturing sufficient among- and within-population genetic variation, multi-population structure, management and regeneration of gene conservation populations (Pliūra 1999). Howev-

er, under such a dramatic loss of genetic diversity, the dynamic gene conservation is challenged to find new approaches to cope with the complicated situation. The most promising approach would be to create *ex situ* 'synthetic' gene conservation populations in form of progeny trials, clonal trials or seed orchards consisting of selected resistant genotypes.

A substantial variation in the degree of damage of individual trees can be observed in natural populations of *F. excelsior* (McKinney et al. 2010, personal observations). However, recent studies of common ash dieback in the Danish field trials revealed, that only a small fraction (approx. 2-5%) of the wild population could probably have inherent resistance against this devastating disease (McKinney et al. 2010). Burdon (2001) stressed that possibilities for adaptation to new pathogens depend on amount of genetic variation within a species which is the main factor that guarantees potential for genetic and physiologic adaptation and sustainability of populations, in particular in course of recent global changes of climate and environment. The severe ash dieback in Poland and Lithuania could probably be attributed to initially low genetic diversity as studies of maternally inherited chloroplast DNA indicated that only a single DNA haplotype has reached Lithuania and eastern Poland after a last glacial period (Heuertz et al. 2004a). Microsatellite DNA studies of nuclear genome also indicated a rather low genetic diversity as only one to three gene pools dominate in Lithuanian populations (Heuertz et al. 2004b). Because of the dieback, genetic variation has decreased even more, thus the chances for sustainability of populations and genetic adaptation became very small.

Genetic studies of common ash in Denmark have revealed significant genotypic variation in resistance to *C. fraxinea* in clonal (grafted) progenies (McKinney et al. 2010). The comparatively high coefficients of heritability ($h^2 = 0.40$ and 0.49), obtained in 2009 in two progeny trials, indicated that about a half of phenotypic variation in resistance (level of damage) is due to genetic inheritance. Degree of damage of different clones varied from 1 to 68%, and coefficients of genotypic (clonal) variation (CVG) were rather high (61-78%) (McKinney et al. 2010). Even though the average damage score of the 39 tested clones increased from 36.1 to 55.9% during one year of observation (2008-2009), the damage score of most resistant clones remained at 10-13% level, which indicates the existence of genetically-based resistance at a clonal level. However, neither of the tested populations showed clearly higher mean resistance values, and generally, all best-performing genotypes originated from the different populations (McKinney et al. 2010).

The first Lithuanian studies on performance of *F. excelsior* progenies from ten Lithuanian and fourteen foreign (Belgian, Czech, Danish, French, German, Irish and Polish) populations planted in 2005 in three progeny trials showed notable differences in height increment, health condition and other adaptive traits both among populations and among half sib families at juvenile age, with additive genetic variation ranging from 30.3 to 36.8% (Pliūra and Baliuckas 2007). During the course of ash dieback in natural stands and in the progeny trials, genetic differences in susceptibility to *C. fraxinea* became evident as well (personal observations).

The strategy for increasing resistance and restoration of genetic variation could combine both selection of resistant populations, families and genotypes (clones) from native ash stands as well as introduction of possibly more resistant gene pools from foreign *F. excelsior* populations. Engaging different mechanisms of resistance could help to diminish the probability of breakdown of a resistance gained in tree improvement. Thus the strategy in creating a permanent long-term resistance should be based on application of so-called 'Pyramid principle' that integrates two types of resistance inheritance, quantitative and qualitative, i.e. resistance determined by both individual genes and by interaction of many genes, additive effects, epistasis, pleiotropy, etc. (McDonald and Linde 2002a,b, Baniulis et al. 2008). The extensive genetic diversity from native (Lithuanian) populations and wide species distribution range in Europe that is harboured in the above-mentioned Lithuanian 2005 series of progeny trials, constitute the ultimate base for resistance breeding and creating synthetic disease-resistant *F. excelsior* tree breeding populations, as well as for establishing resistance-aimed seed orchards, and for *in vitro* propagation of material for restoration of the threatened Lithuanian ash forests.

The main aim of the present study was to estimate genetic variability and level of genetic control of disease resistance in common ash populations based on progeny testing, and to select resistant populations, families and genotypes for production of reproductive material resistant to dieback caused by *C. fraxinea*.

Materials and methods

Study sites

Studies on genetic variation in resistance and other adaptive traits of common ash were performed in three progeny trials established in 2005 in Telšiai, Pakruojis, and Kėdainiai State Forest Enterprises which represented three different adaptive environments –

the 1st, the 2nd, and the 4th provenance regions of *F. excelsior* (Fig. 2). Site characteristics are presented in Table 1. A fourth progeny trial has been established in Dubrava Experimental-Educational State Forest Enterprise in 2008; however, it was not examined in the present study.

A total of 140 open-pollinated families from ten Lithuanian populations and 180 open-pollinated families from fourteen foreign European (Belgian, Czech, Danish, French, German, Irish and Polish) populations were tested (Figs. 1 & 2, Table 2). Each trial was established in a randomized complete block design, with six blocks each consisting of five to seven single-tree plots of each family. In total, 27,000 three-year-old ash seedlings have been planted (9,000 seedlings in each trial). Seedlings were planted in rows with spacing of 2.5 × 1.5 m.

Each population was represented by a half-sib progeny from 10 to 30 seed trees selected in 1998-2000 in disease-free foreign and diseased Lithuanian natural stands of *F. excelsior*. Minimum distance between the sampled seed trees was 50 m to avoid relatedness. Seed samples from the foreign populations of *F. excelsior* were kindly provided by the partners from the EU 6th framework programme project “RAP: Realizing Ash’s Potential”. Before seeding, the seeds were subjected to warm stratification followed by cold stratification for one year.

Measurements

At the age of four years (in 2006, one year following the establishment of field trials), bud flushing phenology and tree health condition including both damage types – caused by spring frosts and by

Table 1. Characteristics of *Fraxinus excelsior* progeny trials of 2005 year series

No.	Progeny trial name	Area, ha	Latitude, N	Longitude, E	Altitude a.s.l., m	Provenance region	Climate type / continentality index*	Forest site type**
1	Telšiai	3.0	56°03'	22°25'	147	1	Maritime/25	<i>Oxalido-nemorosa</i>
2	Pakruojis	3.0	56°14'	23°53'	53	2	Transitional/27	"
3	Kėdainiai	2.5	55°25'	24°19'	54	4	Transitional/28	"

* according to Chromov (1968)

** according to Karazija (1988)

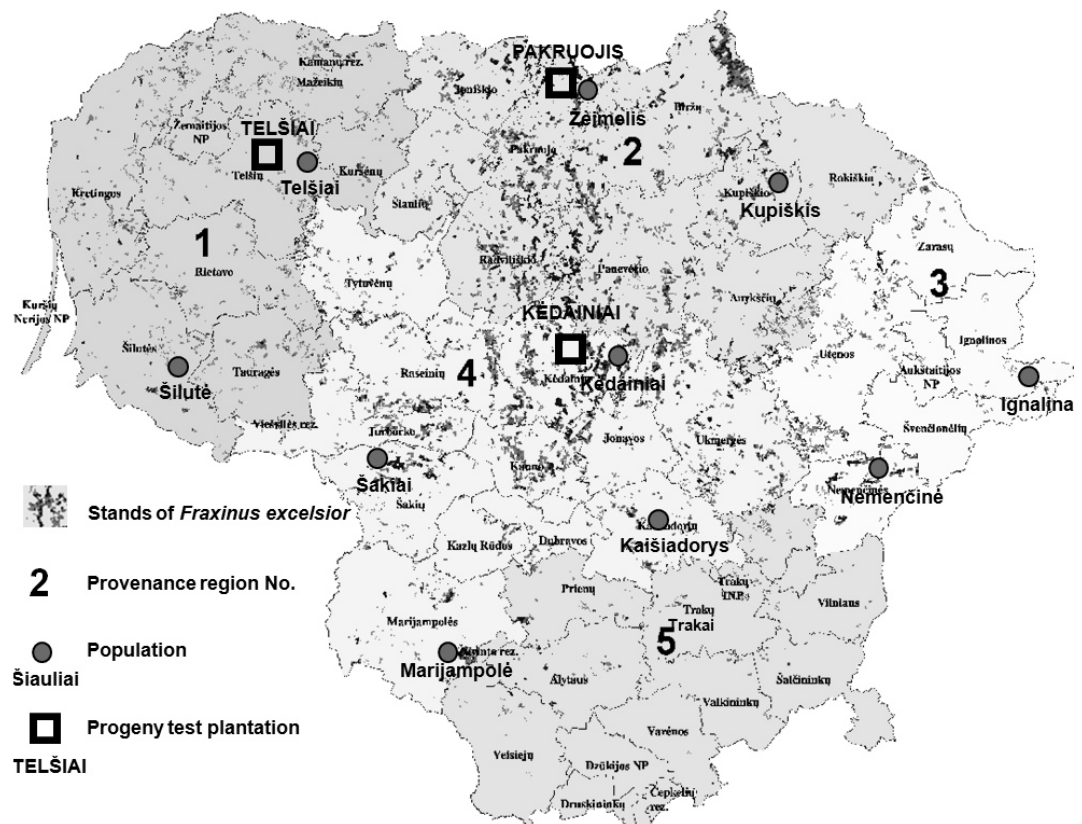


Figure 1. Sites of progeny trials and tested Lithuanian populations of *Fraxinus excelsior*



Figure 2. Sites of *Fraxinus excelsior* progeny trials (1 – Telšiai, 2 – Pakruojis, and 3 – Kėdainiai) and of populations evaluated in the trials

trials (a total of 2,860 trees were evaluated in both trials, 2,318 of them were alive): height, survival (0 – dead tree, 1 – live tree) and health condition focussing on symptoms of *C. fraxinea* (the same classes as above). The scoring scale for estimating the level of tree damage caused by *C. fraxinea* (or, otherwise, – the tree resistance) is presented in Table 3.

Statistical analysis

In order to estimate the significance of effects of a population, a family and a progeny trial (site) to assess their interaction, and to compute variance components of random effects, the variance analysis was done with the MIXED procedure in SAS Software (SAS Institute, Inc., SAS/STAT software, Release 8) which

Table 2. List of Lithuanian and foreign *Fraxinus excelsior* populations tested in progeny trials of 2005-year series

Population-country*	Parent tree No's.	Family No's	Forest district or locality	Latitude N, °	Longitude E, °
Šakiai – LT	34UŠM161-170	Sa1-Sa10	Gelgaudiški s	55,02	23,08
Šilutė – LT	22UŠM171-180	Si1-Si10	Žemaitkiemis	55,25	21,70
Telšiai – LT	39UŠM142-149	Te1-Te10	Eigirdži ai	56,07	22,45
Marijampolė – LT	33UŠM151-160	Ma1-Ma10	Bukta	54,45	23,46
Kaišiadorys – LT	08UŠM111-140	Ka1-Ka30	Būda	54,88	24,36
Žemėli s – LT	40UŠM081-110	Ze1-Ze30	Žemėli s	56,27	24,05
Ignalin a – LT	06UŠM061-070	Ig1-Ig10	Tverečius	55,28	26,57
Nemenčinė – LT	31UŠM071-080	Ne1-Ne10	Sužionys	54,98	25,50
Kupiškis – LT	48UŠM041-050	Ku41-Ku50	Skapiškis	55,86	25,18
Kėdainiai – LT	11UŠM051-060	Ke51-Ke60	Labūnava	55,19	24,00
Farchau – DE	Fa126-4-128-16	Fa126-4-128-16	Herzogtum	53,73	10,72
Ravnholt – DK	F732	F732	Ravnholt	55,26	10,58
Bregentved – DK	F623	F623	Bregentved		
Osterskov – DK	F691	F691	Syddanmark	55,25	9,53
Szczecinek – PL	-	Scz	Szczecinecki	53,7	16,68
Mircze – PL	-	Mir	Hrubieszowski	50,65	23,50
Hagueau – FR	-	Ha610-Ha628	Bas-Rhin	48,82	7,78
Rabstejn – CZ	-	Ra1-Ra9388	Moravskoslez	49,93	17,25
Hoge Bos – BE	-	B1-B15	West-Vlaanderen	50,83	2,95
Donadea – IE	-	D1-D20	Kildare	53,21	-6,45
Currachase – IE	-	Cu1-Cu20	Limerick	54,14	-8,53
Enniskillen – IE	-	En1-En20	Fermanagh	52,36	-7,28
Monterolier – FR	-	M570-M589	Seine-Maritime	49,63	1,35
Morschwiller – FR	-	Mo321-340	Haut-Rhin	47,70	7,27
Val-Saint-Pierre – FR	-	Vs141-160	Aisne	49,78	3,90

* LT – Lithuania, DE – Germany, DK – Denmark, PL – Poland, FR – France, CZ – Czech Republic, BE – Belgium, IE – Ireland

C. fraxinea (classes from 1 – dead tree to 5 – externally healthy tree, Table 3) were evaluated for each seedling; also, their survival (0 – dead seedling, 1 – live seedling) was assessed.



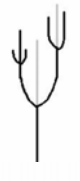



Evaluation of progenies' performance at the age of eight years (in 2010), was done in Telšiai and Kėdainiai progeny trials only, as in the Pakruojis trial over 98% of the trees had died due to combined damages by *C. fraxinea* and spring frosts, and the surviving trees showed very poor growth and health condition. At the age of eight years the following characteristics of each tree were evaluated in Telšiai and Kėdainiai progeny

uses Mixed model equations (MME) and the restricted maximum likelihood (REML) method. *F* tests were carried out to determine when the fixed effects (population and a progeny trial) were significantly different from zero. The significance of the random effects was tested with the *Z* test (SAS Institute 1999). The combined linear model was used for joint analysis of data from both (Telšiai and Kėdainiai) progeny trials together (1):

$$y_{ijlmn} = \mu + z_i + b_j + s(p)_{lm} + p_m + pz_{mi} + s(p)z_{lmi} + \epsilon_{ijlmn}$$

where y_{ijlmn} is an observation of the n th tree from the l th family in the m th population in the i th progeny

Table 3. Scoring of *Chalara fraxinea*-caused damage on seedlings of *Fraxinus excelsior*

Health condition score	Schematic drawing	Description
5		Externally healthy tree: no visible damages by <i>C. fraxinea</i> (according to Bakys et al. 2009a, Kowalski and Holdenrieder 2009a)
4		Slightly damaged tree: sporadic disease symptoms on separate shoots or leaves (according to Bakys et al. 2009a, Kowalski and Holdenrieder 2009a): brown wilted or dry leaves or/and single necrotic lesions on shoots or stem.
3		Moderately damaged tree: dieback of two-to-three shoots (dry or with brown-wilted or dry leaves), some necrotic lesions on shoots or stem; resprouting from below of the damaged shoots
2		Severely damaged tree: the main stem or most of the shoots are dead, the tree is resprouting from the roots (root collar); numerous necrotic lesions occur on stem and resprouted shoots
1		Dead tree: the main stem and all emerged sprouts are dead
0		The tree that has died out during the first year following outplanting in the field trials; no signs of former resprouting could be observed

field trial, μ is the overall mean, z_i is the fixed effect due to the i th progeny field trial, b_j is the effect of the j th block, $s(p)_{lm}$ is the random effect of the l th family in the m th population, p_m is the fixed effect of the m th population, pz_{mi} is the fixed effect of interaction between the j th population and the i th field trial, $s(p)z_{lmi}$ is the random effect of interaction between the l th family in m th population and i th field trial, and e_{ijlmn} is the random residual. The model assumes that random effects are normally distributed with expectation zero and corresponding variances σ_s^2 , $\sigma_{s^*z}^2$ and σ_e^2 .

The following linear model was used for data analysis in a given (individual) progeny trial (2):

$$y_{ijlmn} = \mu + b_j + pm + s(p)_{lm} + pb_{mj} + s(p)b_{lmi} + \varepsilon_{ijlmn},$$

where y_{ijlmn} is an observation on the n th tree from the l th family in the m th population, μ is the field trial mean, b_j is the fixed effect of the j th block, p_m is the fixed effect of the m th population, $s(p)_{lm}$ is the random effect of the l th family in the m th population, pb_{mj} is the fixed effect of interaction between the m th population and the j th block, $s(p)b_{lmi}$ is the random effect of interaction between the l th family in the m th population and the j th block, and ε_{ijlmn} is the random residual. The model assumes that random effects are normally distributed with expectation zero and corresponding variances σ_s^2 and σ_{sb}^2 .

The normality of residuals' distribution and homogeneity of variances were tested with SAS GLM and UNIVARIATE procedures (SAS Institute 1999).

Genetic parameters estimate.

Genetic parameters, family variance components, additive coefficient of genetic variation, heritability and their standard errors were estimated using variances and covariances obtained from analysis of variances, SAS Mixed procedure. The variance components of random effects of families in each progeny trial were derived from corresponding variances and expressed in percent of the total random variation:

$$VC_f = \sigma_f^2 / (\sigma_f^2 + \sigma_e^2) * 100,$$

where VC_f is the relative family variance component, σ_f^2 is the among-family variance, and σ_e^2 is the variance of random residuals.

The additive coefficient of genetic variation was calculated for each individual field trial using a formula:

$$CV_A = \sqrt{4 \cdot \sigma_f^2} \cdot 100 / \bar{X},$$

where σ_f^2 is the among-family variance including population effect, \bar{X} is the phenotypic mean in the trial (Falconer 1989).

Under the assumption that tested families consisted of half sibs, the additive variance was estimated following Falconer (1989) and Falconer and Mackay (1996):

$$\sigma_a^2 = 4 \cdot \sigma_f^2,$$

where σ_a^2 is the additive variance.

The heritability coefficients were calculated using the formula:

$$h_a^2 = \sigma_a^2 / \sigma_{ph}^2,$$

where h_a^2 is the heritability coefficient, and σ_{ph}^2 is the phenotypic variance:

$$\sigma_{ph}^2 = \sigma_f^2 + \sigma_e^2$$

where σ_{ph}^2 is the phenotypic variance and σ_e^2 is the variance of random residuals. Standard errors of individual heritabilities were calculated as described for unbalanced designs in Becker (1984) using harmonic means of observations per family.

Estimation of breeding values

Breeding values of the ash families were estimated using BLUP method with SAS MIXED procedure ("solutions" options) (SAS Institute 1999). The breeding value of each family for a single trait was computed based on Fins et al. (1992):

$$BVI_i = 2h_{fa}(x_i - X_t),$$

where BVI_i is family breeding value index, h_{fa} is family heritability coefficient, x_i is trait mean of i th family, and X_t refers to the phenotypic mean in the trial.

Breeding value of each individual tree for a single trait was computed using a formula:

$$BVI_i = h_a(x_i - X_t),$$

where BVI_i is a breeding value index of the i th tree, h_a is an additive heritability coefficient, and x_i is a trait mean of the i th tree.

Complex breeding value index for each population or family was computed by summarising standardized to mean = 0 and standard deviation = 1 (SAS STANDARD procedure) breeding value indexes of certain traits: health condition (C), survival (S) and tree height (H). The weight coefficients were used to balance weight of breeding value indexes of the different traits. Complex breeding value index ($CBVI_i$) was computed using a formula:

$$CBVI_i = b_C * BVI_C + b_S * BVI_S + b_H * BVI_H$$

For populations, the following weight coefficients were used: health condition (b_C) = 4, survival (b_S) = 2, and tree height (b_H) = 0.5 thus emphasizing the health condition instead of height as population effect for health condition is higher than for height. While in constructing family $CBVI_i$'s the weight coefficients were the following: b_C = 2, b_S = 3, and b_H = 1 thus reducing the weight for health condition and increasing the relative importance of height which is additively inherited.

For an individual tree it was not possible to estimate survival, thus the $CBVI_i$ was computed summarising breeding value indexes of the individual traits, health condition (C) and tree height (H):

$$CBVI_i = b_C * BVI_C + b_H * SVI_H$$

The weight coefficients in this case were the following: b_C = 3 and b_H = 1.

Selection of populations, families and individual genotypes

The selection was carried out step-by-step on three levels: 1) population, 2) family, and 3) individu-

al. In order to guarantee the most stable resistance, a selection procedure was based on a 'Pyramid principle' which integrates two types (quantitative and qualitative) of the inheritance of resistance, i.e. the resistance is determined both by individual genes and interaction of many genes, was applied (McDonald and Linde 2002a,b, Baniulis et al. 2008).

Following the main step-by-step selection scheme, the four best-performing Lithuanian and two foreign populations were selected based on a complex breeding index value and on a proportion of individuals showing the best resistance/adaptability.

In the second step, five to six half-sib families with the highest complex breeding index and the highest proportion of individuals showing the best resistance were picked from each population selected in the first step. This enabled to capture additively inherited resistance.

In the third step, two to three externally healthy individuals (health condition class 5 at the age of eight years) whose height had exceeded the trial mean were picked from the selected families, thus capturing monogenetically as well as poligenetically inherited individual resistance determined by gene interaction (additive, epistasis, dominance, pleiotropy, etc.). If less than two such individuals have occurred per selected family, then one individual with a health condition class 4 from this family, or, if such an individual was absent, an individual with a high health condition score (preferably 5) from another family of similar breeding value was selected.

In order to magnify the genetic gain in height and to increase genetic variation, 11 individuals were picked from the tallest individuals growing in non-selected populations in addition to the main step-by-step selection scheme.

In addition, a total of five control individuals of average resistance (health condition class 3 and 4) were selected from the Kėdainiai, Šakiai, Žeimelis, Rabstejn and Farchau populations.

Results

Contribution of genetic factors to resistance and other adaptive traits of *F. excelsior*

According to the evaluation of our three progeny trials in 2009-2010, only 10% of the outplanted ash trees have survived in all trials altogether because of the severe damages by *C. fraxinea*. Pakruojis progeny trial was excluded from the further analysis as more than 98% of the trees were already dead at the age of eight years. Out of the 2,860 trees that remained in Telšiai and Kėdainiai progeny trials, 2,318 were alive while the rest were dead standing.

As mentioned above, the survival rate of all populations was low in all trials (0.13-0.59); however, differences among populations were clearly pronounced and the survival rate of populations varied from 0.13 to 0.59 points and from 0.25 to 0.56 points in Kėdainiai and Telšiai trials, respectively. Foreign populations showed worse survival than the Lithuanian ones on average by 73.5% and 48.5% (average survival rate 0.24 vs 0.49 and 0.26 vs 0.45) in Kėdainiai and Telšiai trials, respectively (data not shown).

In Kėdainiai progeny trial, the best survival rate was observed in Šilutė (0.59), Kėdainiai (0.57), Telšiai (0.57) and Žeimelis (0.52) populations (Fig. 3), while in Telšiai progeny trial the best survival showed populations of Šakiai (0.56), Telšiai (0.51), Šilutė (0.50), Pakruojis (Žeimelis) (0.48) and Ignalina (0.48) (Fig. 3). Thus, progenies from Šilutė and Telšiai showed the best adaptability/resistance in both progeny trials. Among the foreign populations, the highest survival rate in both progeny trials was observed for the Szczecinek (Poland), Rabstajn (Czech Republic) and Enniskillen (Ireland) populations (Fig. 3). In Telšiai progeny trial, rather good survival was shown also by Morshwiller and Val-Saint-Pierre (France) populations.

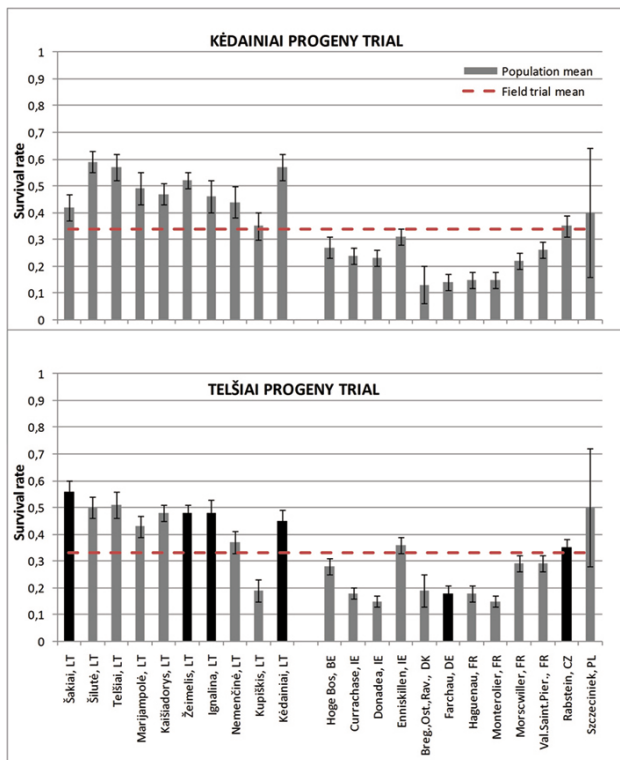


Figure 3. Mean survival rate (0 - dead tree, 1 - live tree) of progenies of Lithuanian and foreign *Fraxinus excelsior* populations in Kėdainiai and Telšiai progeny trials at the age of eight years. Black bars indicate the best-performing populations selected based on complex breeding value indexes (Table 6) for the further breeding. Country codes as in Table 2

In parallel with the rating of survival, the health condition of the surviving trees was also assessed. At the age of eight years, differences among the populations in health condition were significant: the health condition of populations varied from 1.6 to 3.0 and from 1.9 to 3.4 in Kėdainiai and Telšiai trials, respectively (data not shown). The ranking of populations according to their survival rate differed from the ranking according to their health condition (Figs. 3 vs. 4). In Kėdainiai progeny trial, the best ash health condition was observed in Ignalina (mean health condition score = 3.00), Kupiškis (3.00), Kaišiadorys (2.98) and Pakruojis (Žeimelis) (2.94) populations (Fig. 5). In Telšiai progeny trial, the best health condition showed populations of Ignalina (3.41), Pakruojis (Žeimelis) (3.30), Kėdainiai (3.18) and Šakiai (3.18) populations (Fig. 4). These results differed from those obtained during our previous study: for example, at the age of four years (in 2006), progenies from Marijampolė, Ignalina and Kėdainiai populations were of the best health condition in all three trials (Pliura and

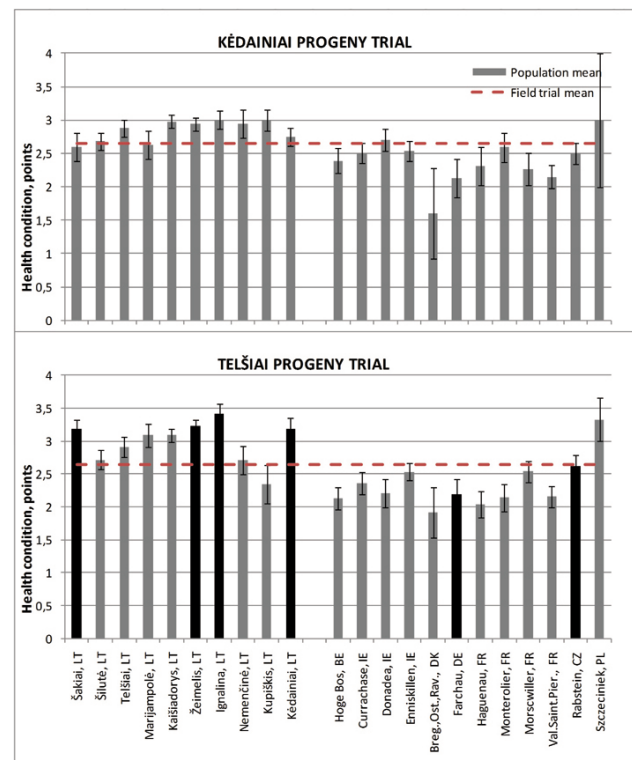


Figure 4. Rating of the mean health condition of progenies of Lithuanian and foreign *Fraxinus excelsior* populations in Kėdainiai and Telšiai progeny trials at the age of eight years. Health condition scores: 1 – dead trees; 2 – severely damaged trees; 3 – moderately damaged trees; 4 – slightly damaged trees and 5 – externally healthy trees. Black bars indicate the best-performing populations selected based on complex breeding value indexes (Table 6) for the further breeding. Country codes as in Table 2

Baliuckas 2007). The ranking of the populations has changed at the age of eight years due to a high, yet uneven, tree mortality across all progeny trials during 2007-2010 year period (Figs. 3, 4). In Pakruojis progeny trial, the overall health condition of ash was worse than that in Kėdainiai and Telšiai progeny trials because of severe spring frost damages combined with the damages by *C. fraxinea*. In general, at the age of eight years, the health condition of the Lithuanian ash populations was better than that of the foreign populations on average by 17.5% and 23.5% in Kėdainiai and Telšiai trials, respectively (data not shown). In Kėdainiai progeny trial, among the foreign populations the best health condition showed Szczecinek (Poland), Donadea and Enniskillen (Ireland), Monterolier (France) and Rabstejn (Czech Republic) populations (Fig. 4). In Telšiai progeny trial, the best were populations from Szczecinek (Poland), Rabstejn (Czech Republic), Enniskillen (Ireland) and

Morshwiller (France) (Fig. 4). However, the performance of the Szczecinek (Poland) population should be evaluated with reservation as very few individuals of this origin were planted in trials and thus the population mean was not objective due to large standard errors (Figs. 3 & 4). The progenies of pooled Bregentved, Osterskov and Ravholt populations (pooling was done as too few seedlings were available from each separate population) from Denmark, and Haguenu and Val-Saint-Pierre populations from France were of the worst health condition in both trials (Fig. 4).

The variance analysis indicated no significant progeny trial (site) effect for tree survival, while this effect was highly pronounced and highly significant ($P < 0.001$) for health condition. This indicated that the health condition is likely influenced by site conditions including also different infection pressure from the surrounding stands (Table 4).

Factor	Df*	Variance ±std. error	Z** or F***- criteria	P****
Phenology at the age of four years				
Families	317	0.039±0.013	2.99	0.0014
Populations	22		24.06	<0.0001
Progeny trials	1		2.86	0.0918
Blocks	3		158.42	<0.0001
Family × progeny trial interaction	316	0.042±0.015	2.86	0.0021
Population× progeny trial interaction	21		2.32	0.0011
Residual		1.049±0.022	47.76	<0.0001
Health condition at the age of four years				
Families	317	0.042±0.013	3.17	0.0008
Populations	22		23.30	<0.0001
Progeny trials	1		3.08	0.0802
Blocks	3		42.77	<0.0001
Family × progeny trial interaction	316	0.028±0.015	1.90	0.0284
Population× progeny trial interaction	21		1.99	0.0069
Residual		1.194±0.025	47.77	<0.0001
Tree height at the age of eight years				
Families	317	36.39±52.44	0.69	0.2438
Populations	21		2.41	0.0008
Progeny trials	1		220.41	<0.0001
Blocks	3		24.70	<0.0001
Family × progeny trial interaction	316	199.45±65.12	3.06	0.0011
Population× progeny trial interaction	21		0.74	0.7866
Residual		2118.0 ±69.31	30.56	<0.0001
Health condition at the age of eight years				
Families	317	0.061±0.033	1.86	0.0314
Populations	22		5.49	<0.0001
Progeny trials	1		25.82	<0.0000
Blocks	3		42.77	<0.0001
Family × progeny trial interaction	316	0.047±0.039	1.20	0.1155
Population× progeny trial interaction	21		1.24	0.2160
Residual		1.761±0.052	34.15	<0.0001
Survival at the age of eight years				
Families	317	0.011±0.002	5.53	<0.0001
Populations	22		11.69	<0.0001
Progeny trials	1		0.05	0.8250
Blocks	3		1.44	0.2284
Family × progeny trial interaction	316	0.003±0.002	1.51	0.0653
Population× progeny trial interaction	21		1.16	0.2790
Residual		0.192±0.003	56.55	<0.0001

*Df – degree of freedom, ** Z – criterion for random effects, *** F – criterion for fixed effects, **** P – probability, calculated for effects of different factors

Table 4. Results from the joint mixed analysis of variances of Lithuanian and foreign *Fraxinus excelsior* populations' progenies traits in Telšiai and Kėdainiai progeny trials

The variance analysis based on the joint statistical model (1) revealed significant effects of genetic factors, populations and families on variation of phenology and health condition in 2006 (at the age of four years), and health condition and survival of progenies in 2010 (at the age of eight years) (Table 4). For tree height, the populational effect was also highly significant ($P < 0.001$), while the family effect was insignificant due to a pronounced family by progeny trial interaction ($G \times E$) (Table 4).

The variance analysis of data from the separate trials, Kėdainiai and Telšiai, showed even stronger populational and family effects on the trait variation (Table 5).

trials their respective ranks were 1, 3, 2 and 3, 2, 1 (data not shown). The ranking of other populations was changing to an even greater extent. Tree health condition of progenies from Kupiškis and Šilutė populations were always of low rank in all progeny trials (data not shown). At the age of eight years, the $G \times E$ interaction for health condition was weak and non-significant (Table 4).

The family variance component (proportion of the total phenotypic variance) ranged from 4.6% (tree survival at the age of eight years in Kėdainiai progeny trial) to 13.1% (tree height at the age of eight years in Kėdainiai progeny trial) in variation of adaptive traits (Table 5). The highest variance component was

Table 5. Family variance components (VC_p), additive coefficient of genetic variation (CV_A), heritability coefficients (h^2) and its standard errors (se), and significance of family and population effects (Z, F and P) in variation of traits of Lithuanian and foreign *Fraxinus excelsior* populations in Kėdainiai and Telšiai progeny trials

Trait	Progeny trial	Family effect			Population effect		CV_A^*	h^2 (se)	
		VC, % \pm std.error	Z	P	F	P			
Phenology at the age of four years	Kėdainiai	7.2	± 1.73	4.2	<0.001	17.2	<0.001	44.6	0.99 (0.01)
	Telšiai	7.3	± 1.44	5.1	<0.001	13.5	<0.001	36.3	0.78 (0.03)
Health condition at the age of four years	Kėdainiai	5.8	± 1.66	3.5	0.003	16.9	<0.001	38.6	0.92 (0.01)
	Telšiai	5.5	± 1.27	4.3	<0.001	11.6	<0.001	29.5	0.60 (0.02)
Health condition at the age of eight years	Kėdainiai	7.5	± 2.73	2.8	0.003	2.6	0.001	29.9	0.40 (0.03)
	Telšiai	5.3	± 2.04	2.6	0.005	5.3	<0.001	38.7	0.49 (0.03)
Tree height at the age of eight years	Kėdainiai	13.1	± 3.65	3.6	<0.001	1.3	0.188	35.8	0.51 (0.03)
	Telšiai	9.8	± 2.71	3.6	<0.001	2.0	0.006	30.3	0.46 (0.03)
Survival at the age of eight years	Kėdainiai	4.6	± 1.28	3.6	<0.001	9.2	<0.001	99.3	0.51 (0.02)
	Telšiai	7.9	± 1.27	6.2	<0.001	8.4	<0.001	109.2	0.59 (0.02)

* CV_A and h^2 were computed including the populational effect (not separated in a statistical model 2 of variance analysis)

It was not possible to calculate the population variance component (in percent) as population effect was considered as fixed. On the other hand, among-population differences were highly expressed and significant (as indicated by high F -criteria), in particular for bud flushing phenology, tree survival and health condition (which reflects combined damages by spring frosts and *C. fraxinea*) in 2006, at the age of four years.

The genotype (at population level) by environment (at progeny trial level) interaction ($G \times E$) was significant only for phenology and health condition at the age of four years (Table 4). This was illustrated by the fact that in respect to their health condition, the Marijampolė, Ignalina and Kėdainiai populations were among the best populations in all progeny trials at this age, but their ranks were different in different trials at the age of four years: in Kėdainiai and Telšiai

obtained for tree height (9.8 and 13.1% in Kėdainiai and Telšiai progeny trials, respectively). In our study, the additive coefficients of genetic variation (CVA) within ash populations were rather high at the age of eight years: 29.9% and 38.7% for health condition, which is of similar magnitude as for tree height: 35.8% and 30.3% in Kėdainiai and Telšiai progeny trials, respectively. The heritability coefficients calculated for health condition at the age of eight years in Kėdainiai and Telšiai progeny trials were rather high ($h^2 = 0.40$ and 0.49 ± 0.03 , respectively; Table 5). As the population effect was included into a statistical model of MIXED procedure and thus excluded from genetic variances in computing genetic parameters, a CVA for health condition at the age of eight years decreased to 25.5-24.5% and heritability coefficients decreased to 0.30-0.21 (± 0.03) (data not shown).

Selection of the most resistant populations

Data from Telšiai progeny trial was used to assess progenies' complex breeding value indexes (*CBVI*s) only, because in Kėdainiai and Pakruojis progeny trials the survival, health condition and growth of the outplanted ash was too poor to obtain the precise values; also, it was not possible to make enough cuttings for grafting. In Telšiai progeny trial, the populations of Ignalina, Pakruojis (Žeimelis), Šakiai and Kėdainiai showed the highest *CBVI*s (Fig. 5) that integrated the health condition, survival, and tree height, and had the highest proportion of healthy individuals.

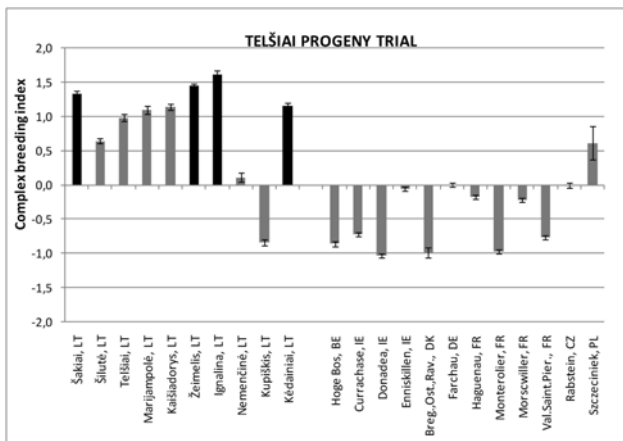


Figure 5. Mean complex breeding value indexes of progenies of Lithuanian and foreign *Fraxinus excelsior* populations in Telšiai progeny trial at the age of eight years. Black bars indicate the best-performing populations selected for the further breeding. Country codes as in Table 2

The *CBVI*s of the foreign populations were much lower than these of the Lithuanian populations (except of the Szczecinek population which was represented by very few individuals and therefore its *CBVI* could not be regarded as reliable). Among the foreign populations, the highest *CBVI*s were obtained for the Rabstejn (Czech Republic) and Farchau (Germany) populations.

In some populations (e.g., Šilutė, Telšiai and Enniskillen, Fig. 6), in addition to a relatively high proportion of externally healthy trees (health condition score 5, Table 3), there was also a high proportion of slightly damaged trees (health condition score 4, Table 3) and moderately damaged trees (health condition score 3, Table 3). Therefore the mean health condition score (Fig. 4) of those populations was higher than that of populations which included a large proportion of externally healthy trees but lower proportion of slightly or moderately damaged trees (Fig. 6). Thus, resistance ranking of populations based on their health condition mean only might not be precise enough.

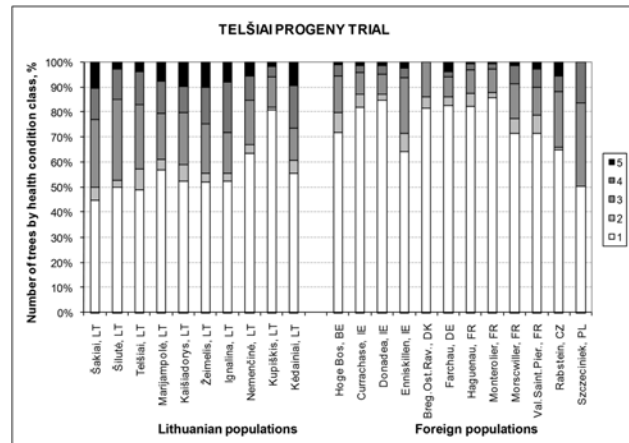


Figure 6. Distribution of number of trees by health condition classes among progenies of Lithuanian and foreign *Fraxinus excelsior* populations in Telšiai progeny trial at the age of eight years. In the chart legend numbers correspond to tree health condition classes as follows: 1 – dead trees; 2 – severely damaged trees; 3 – moderately damaged trees; 4 – slightly damaged trees and 5 – externally healthy trees. Country codes as in Table 2

Therefore, in addition to the *CBVI*, another criterion, a relative number (percentage) of most healthy trees (health condition scores 5 and partly 4) in a given population, was chosen. Subsequently, Šakiai, Žeimelis, Ignalina and Kėdainiai populations were selected as having the highest *CBVI*s and the largest proportion of most healthy trees (Fig. 6).

Similar proportions of trees with the health condition score 5 were found in the Marijampolė and Kaišiadorys populations, however their *CBVI*s were lower and they included fewer slightly damaged trees (health condition score 4) and fewer families with a high proportion of externally healthy trees (less expressed family resistance).

Among the foreign populations, those from Farchau (Germany) and Rabstejn (Czech Republic) were selected as having the highest *CBVI*s and the highest proportion of most healthy (class 5) trees (Fig. 6). The Enniskillen (Ireland) and Morschwiller (France) populations (in Kėdainiai trial) also had a rather large proportion of most healthy individuals, however those populations were not further considered because their native climate differed from the Lithuanian climate: at Enniskillen it is far more maritime, and at Morschwiller it is considerably warmer.

Selection of most resistant half-sib families and individuals

At the second stage of the main selection scheme, five to six families with the highest *CBVI*s and the highest proportion of most healthy individuals were

selected from the four best Lithuanian populations (Ignalina, Kėdainiai, Pakruojis (Žeimelis) and Šakiai) and from the two best foreign populations (Farchau and Rabstejn) (Table 6).

(17.3%) - severely damaged. Subsequently, out of the 27,000 outplanted seedlings in all three trials, only 768 (2.8%) trees could be regarded as externally healthy and slightly damaged (health condition class-

Table 6. Characteristics of best families representing the best-performing Lithuanian and foreign *Fraxinus excelsior* populations in Telšiai trial at the age of eight years. Families chosen to represent the best populations for selection of the best individuals are indicated in bold

Family No	Number of trees	Tree height			Health condition**			Survival rate***		Complex-breeding value index
		Mean, cm	Selection differential***, %	BLUP breeding index	Mean, points	Selection differential, %	BLUP breeding index	Mean	Selection differential, %	
Ra9381	8	145.9	10.0	0.59	3.50	28.0	1.16	2.56	169.4	2.47
Sa5	9	133.9	4.1	0.24	3.80	48.7	2.02	2.23	148.0	2.35
Ze17	8	155.1	21.8	1.28	3.78	44.6	1.85	1.82	120.4	2.25
Ze12	13	126.5	-5.3	-0.31	3.85	60.0	2.49	1.99	131.8	2.13
Sa10	10	159.6	26.5	1.55	3.36	30.3	1.26	1.76	116.5	2.12
Ze6	14	143.6	13.8	0.81	3.64	50.7	2.11	1.69	112.2	2.09
Ze3	8	144.9	11.4	0.67	4.25	59.3	2.46	1.54	102.1	2.03
Ke53	7	147.6	13.6	0.80	3.86	42.2	1.75	1.40	92.9	1.77
Ze9	14	85.1	-45.0	-2.64	3.50	43.8	1.82	2.50	165.2	1.74
Ig4	9	112.9	-11.5	-0.67	4.00	55.8	2.32	1.66	109.8	1.73
Sa6	12	111.8	-16.3	-0.95	3.42	36.4	1.51	1.92	127.3	1.65
Ze27	7	143.6	10.6	0.62	4.14	52.5	2.18	1.16	76.8	1.64
Ke54	9	139.4	7.4	0.44	3.50	35.8	1.49	1.43	94.8	1.63
Ke58	10	99.9	-26.5	-1.56	3.60	38.2	1.59	2.01	133.1	1.59
Ze21	7	134.1	6.0	0.35	4.00	49.8	2.07	1.16	76.8	1.54
Ze18	9	133.8	6.1	0.36	2.82	8.0	0.33	1.66	109.8	1.48
Fa128-14	12	151.3	22.3	1.31	3.21	28.5	1.19	0.98	65.3	1.42
Ze8	8	116.4	-11.3	-0.66	3.11	15.3	0.64	1.82	120.4	1.42
Ig1	8	123.4	-3.5	-0.20	3.25	22.8	0.95	1.54	102.1	1.4
Ig7	7	128.0	-3.4	-0.20	3.57	29.4	1.22	1.40	92.9	1.37
Ze19	8	145.4	16.3	0.95	3.00	16.5	0.69	1.10	73.2	1.29
Ra9388	6	176.7	36.0	2.12	2.86	10.8	0.45	0.77	51.5	1.28
Ra9364	3	232.7	41.1	2.42	4.00	24.0	1.00	0.44	29.9	1.24
Sa4	9	108.1	-16.8	-0.99	3.33	30.5	1.27	1.43	94.8	1.20
Ma5	8	105.8	-17.5	-1.03	3.20	23.1	0.96	1.54	102.1	1.19
Ke56	7	101.6	-20.7	-1.21	3.71	37.0	1.54	1.40	92.9	1.18
Ze16	7	161.7	20.6	1.21	3.50	32.3	1.34	0.62	41.4	1.15
Ig8	6	133.8	1.6	0.09	4.00	42.2	1.75	0.77	51.5	1.09
Ig5	6	152.8	19.7	1.16	4.00	47.4	1.97	0.31	21.2	1.06
Ze30	8	133.9	2.7	0.16	3.00	12.6	0.52	1.10	73.2	1.04
Families, selected in addition to the main step-by-step selection scheme:										
Te7	9	140.0	7.2	0.42	3.10	14.9	0.62	1.92	127.3	1.78
Mo337	5	166.2	17.3	1.01	3.00	6.1	0.25	1.35	89.4	1.39
Ka1	7	133.6	-0.8	-0.05	3.00	12.7	0.53	0.77	51.5	0.73
Ke55	6	116.0	-10.1	-0.60	3.00	13.5	0.56	0.60	39.9	0.46
Vs157	3	236.7	42.9	2.52	3.25	9.4	0.39	-0.38	-24.2	0.47
Cu11	5	132.8	-1.8	-0.11	3.00	14.1	0.58	-0.25	-15.8	-0.07
En2	1	309.0	30.7	1.80	5.00	18.9	0.79	-1.11	-72.5	-0.19
M578	2	222.5	30.6	1.80	2.25	-7.7	-0.32	-0.82	-53.4	-0.26
Do2	1	280.0	25.4	1.49	2.00	-10.5	-0.44	-1.20	-78.4	-0.66
Vs149	1	307.0	34.3	2.02	1.00	-51.4	-2.13	-1.17	-76.7	-0.95

* – first two letters indicate an abbreviated name of a population
 ** – health condition classes from 1 – dead tree to 5 – externally healthy tree (Table 3)
 *** – survival rate was standardized to mean = 0 and standard deviation = 1 to balance its variability in relation to other traits
 **** – selection differential is a difference between a family mean and a trial mean

Out of the 2,860 trees that remained in Telšiai and Kėdainiai progeny trials at the age of eight years, 2,318 (81%) were alive; of which 238 (10.3%) were regarded as externally healthy, 530 (22.8%) - slightly damaged, 1150 (49.6%) - moderately damaged, and 400

es 5 and 4, Table 3). Thus, at the third stage of the main selection scheme, one to three externally healthy individuals which height exceeded the mean of the given trial were selected in the best-performing families (Table 6).

In order to increase genetic gain in height and to increase genetic diversity in ash breeding populations, ten externally healthy individuals showing superior height growth rate (height varied from 294 to 365 cm) were picked out in every population that has not been selected by the main selection scheme. In this way, the best individuals were additionally selected from three Lithuanian (Kaišiadorys, Marijampolė and Telšiai) and seven foreign populations (Val-Saint-Pierre, Morchwiller and Monterolier from France, Rabstejn from Czech Republic, and Currachase, Donadea and Enniskillen from Ireland). In addition, five control individuals of average resistance (health condition score 3 and 4) were selected in the Kėdainiai, Šakiai, Pakruojis (Žeimelis), Rabstejn and Farchau populations.

Discussion

Significant differences among populations revealed in the present study were of similar magnitude as reported to be among *F. excelsior* provenances (or populations) by many other authors for various growth, morphological, phenological and other traits (e.g., Jonsson and Eriksson 1989, Smintina 1993, Giertych 1995a,b, Weiser 1995, Kleinschmit et al. 1996, Abraitis 2000, Ferrazzini et al. 2007). However, no studies on genetic variation in resistance of *F. excelsior* progenies to *C. fraxinea* on populational and/or family levels have been done yet. Recent genetic studies of *F. excelsior* clones in Denmark and Sweden had not revealed any differences among populations in resistance to *C. fraxinea* (Stener 2007, Olrik et al. 2007, McKinney et al. 2010). This absence of differences could probably be due to usage of relatively closely located populations (a narrow geographical scale) which was not the case in the Lithuanian trials investigated herein (a much wider geographical scale).

Studies using DNA microsatellites (Heuertz et al. 2004b) have shown that Lithuanian ash populations belong to a large group of populations that spreads from British islands to East-Central Europe and are comparatively homogenous with a lower among-population differentiation than in Southeastern Europe. Comparative studies of the Random Amplified Polymorphic DNA (RAPD) markers of three Lithuanian and five Polish *F. excelsior* populations have indicated a rather large population differentiation (Nowakowska et al. 2004). The RAPD marker studies of ten Lithuanian ash populations revealed that among-population variation makes up 8.3% of the total marker variation (Žvingila et al. 2005). Taking analysis of a rather small area of the species natural distribution range covered by the above-mentioned studies, such among-population variation may be regarded as considerable al-

lowing expecting also for a rather high among-population variation in disease resistance and other adaptive traits.

The fact that progenies of the Lithuanian populations were in general of better health condition than those from the foreign populations could be a consequence of an earlier-started natural selection process in the Lithuanian ash stands (the longest disease history) in favor of trees with a better resistance against *C. fraxinea*. It should be noted that sound-looking seed trees in Lithuania have been selected in the diseased stands, while in the foreign populations the selection was done before an outbreak of the disease in respective countries.

The significant site (progeny trial) effect on ash health condition indicated that the latter is influenced by site conditions and/or different infection pressure from the surrounding stands. Further studies are needed to evaluate a relative importance of these factors in resistance to infection or disease tolerance.

Weak and non-significant $G \times E$ interaction for health condition at the age of eight years indicated that populations and families do not differ much in their performance across environments and thus specific adaptations and genetic variation in ecological response and resistance are not pronounced. Significant provenance by site interactions have been found for height growth of 22 British and continental European common ash provenances tested at six sites in Wales and England (Cundall et al. 2003) and 52 provenances tested at 26 test sites in the continental Europe (Kleinschmit et al. 1996). In those trials, some provenances were stable in their ranking at different sites whereas others were rather unstable in their performance. However, no data on $G \times E$ interaction in resistance traits have been published yet.

Tree health condition scored at the age of four years (in spring 2006) mainly reflected damages by spring frosts in a combination with sporadic damages by *C. fraxinea*. Whereas scoring of tree damages at the age of eight years mainly reflected extent/severity of those caused by *C. fraxinea* and thus better indicated the level of tree resistance to this pathogen. The high significance of genetic factors (population and family) indicated in variance analysis of data on resistance (health condition and survival) (Table 4) and rather high additive genetic variation coefficients (Table 5) clearly show the existence of genetic variation in resistance of ash to *C. fraxinea* both at populational and family levels. The exclusion of the population effect in computing genetic parameters has caused a reduction of the heritability coefficient (from 0.40-0.49 to 0.30-0.21) and CVA (from 29.9-38.7 to 25.5-24.5%) in health condition indicating that among-population

variation significantly contributes to the overall genetic variation. Very high heritability coefficients for phenology and health condition at the age of four years (Table 5) give an indication that heritability coefficients of all traits most likely were slightly overestimated also due to coefficient 4 was used in computing the additive variance which was based on assumption that progenies consisted of half sibs. Probably, full sibs contributed a significant fraction of the progenies, and thus the coefficient should be reduced to 3 which would result in reduction of additive variances and heritability coefficients by 25%.

Recent genetic studies of grafted *F. excelsior* clones in Denmark and Sweden have revealed significant genotypic variation in resistance to *C. fraxinea* at a clonal level (Stener 2007, Olrik et al. 2007, McKinney et al. 2010), however no studies on genetic variation at a family level have been performed so far. As emphasized by Houle (1992), genetic variation is the most appropriate parameter to compare ability to respond to selection and to make inferences about the forces that maintain genetic variability. In previous studies on common ash, the additive genetic variation was found to be moderate to high, and it was considered to be mostly distributed locally as a result of local adaptation (Broadmeadow et al. 2005). Although comparative studies of adaptive traits performed in Sweden have shown that the additive genetic variation in common ash is lower than that in other broadleaved tree species (Baliuckas et al. 2000), high coefficients of the genetic variation in health condition obtained during our study (Table 5) showed that genetic variation among families was high. In Kėdainiai progeny trial, where the trees have been affected by spring frosts to a greater extent than in Tešiai trial, the additive genetic variation has decreased (Table 5). A similar decrease of genetic variation under the impact of stressors has been reported in our earlier experiments, where CVA's of the Lithuanian *F. excelsior* progenies for height have decreased from 47.6-49.4% (untreated control) to 10.7-30.8% (elevated ozone concentration) (Pliūra et al. 2006).

Comparatively high heritability coefficients calculated for health condition at the age of eight years allow a rather precise detection of a resistant genotype by its phenotype in tree breeding. This narrow sense heritability which accounts the additive component was almost as high as the broad-sense heritability obtained at clonal studies in Sweden and Denmark (Stener 2007, McKinney et al. 2010). Mwase et al. (2008) reported that in ash a tree height is the most heritable trait followed by DBH, while stem straightness and forking appear to be under a weak genetic control. Relatively low heritability values for stem form

(straightness) were also reported by Savill et al. (1999). High heritability of disease resistance revealed during our study was of similar magnitude as reported by Mwase et al. (2008) for growth traits thus indicating that resistance of *F. excelsior* to diseases is genetically based and inheritable at the similar scale as a tree growth. Similar conclusion has been drawn by McKinney et al. (2010). Subsequently, this constitutes a good basis for efficient tree breeding at family as well as at individual levels and for obtaining high genetic gains in increasing the resistance to diseases.

The low survival rate and remaining small fraction of externally healthy trees (2.8%) in Telšiai and Kėdainiai progeny trials indicates that natural selection of the outplanted ash was very intense. Similar results were also obtained in Danish studies (McKinney et al. 2010) where only 2-5% of trees were regarded as healthy. In our case, picking out fifty healthiest trees from the total number of the planted 27,000 seedlings means the extreme selection intensity (one out of the outplanted 540 trees, $i=0.185\%$) that allows a high genetic gain in resistance.

Selection of populations might enable capturing a resistance that is determined by gene/allele complex if it exists in populations. Further studies are needed to evaluate the presence and relative importance of such gene complexes to the resistance of same populations. When selecting the best families within the best populations, in addition to the population resistance, the additively inherited family resistance has been captured. In addition to the additively inherited resistance, the selection of the best individuals in the selected families probably allows capturing an individual resistance determined by individual genes that are present in superior individuals and by interaction of genes such as epistasis or pleiotropy. Studies based on series of controlled crosses between genotypes and analysis of segregation are needed to reveal the effects of such individual candidate genes and/or gene interactions.

Conclusions

Variance analysis of health condition and survival of *F. excelsior* progenies has revealed significant effects of genetic factors i.e., populations and families. At the age of eight years, family variance components ranged from 5.3 to 7.5%, while the additive coefficients of genetic variation ranged from 29.9 to 38.7% indicating the existence of strong genetic differences in progenies' resistance to *C. fraxinea*-caused dieback both at populational and family levels.

Differences in resistance among populations were significant and ranged up to 52.8-55.1% in degree of

damage and survival rate of populations varying from 0.13 to 0.59 points. This provides good possibilities for selection of resistant populations.

The significant site (progeny trial) effect on ash health condition indicated that the latter is influenced by site conditions and/or different infection pressure from the surrounding stands.

Genotype by environment interaction ($G \times E$) for resistance traits was weak and nonsignificant which indicated that populations and families do not differ much in their performance across environments and thus specific adaptations and genetic variation in ecological response are not pronounced.

The high heritability estimates ($h^2=0.40-0.51\pm 0.03$) obtained for health condition and tree height at the age of eight years indicated that resistance is genetically predetermined and inheritable, and allows for straightforward selection and breeding of resistant genotypes.

Health condition of foreign European populations was worse than that of the Lithuanian ones by 17.5-23.5% in the degree of damage and by 48.5-73.5% in the survival rate (average survival rate 0.24 vs 0.49) which could be a consequence of more advanced progress in natural selection in the Lithuanian ash populations (the longest disease history). The obtained results demonstrated that strategy of the extended introduction of foreign populations to restore Lithuanian ash stands is of doubtful potential. Only few most resistant foreign genotypes could be selected as potential candidates for breeding of so-called 'synthetic' *F. excelsior* populations in order to increase genetic diversity.

Using the selected material, resistant breeding and *ex situ* gene conservation populations could be formed over different Lithuanian provenance regions in a form of clonal trials and resistant second-generation seed orchards.

Acknowledgements

The study was supported by the Ministry of Environment of the Republic of Lithuania and the Nordic Forest Cooperation Committee (SNS) project grant SNS-109 and by the the Research Council of Lithuania (project No. LEK-17/2010). Authors are grateful to partners from EC FP6 project "RAP: Realizing Ash's Potential" for sharing seed samples from West-European ash populations. Also, we wish to thank T. Balkus, dr. R. Petrokas, dr. V. Baliuckas, F. Kniečiūnienė, E. Paplauskienė, dr. A. Baliuckienė, V. Kundrotas, dr. V. Verbyla, V. Statkus and other colleagues from Institute of Forestry of Lithuanian Research Centre for Agriculture and Forestry, State For-

est Service, Telšiai, Kėdainiai and Pakruojis State Forest Enterprises for their assistance in collecting seeds, rising plants, establishing progeny trials and field measurements. We are grateful to anonymous reviewers for their constructive comments.

References

- Abraitis, R.** 2000. Common ash (*Fraxinus excelsior* L.) variation in Western Lithuania. *Miškininkystė* 3 (47): 49-60.
- Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak P.** 2004. Emerging infectious diseases of plants: pathogen, pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution* 19: 535-544.
- Anonymous 2009. Lietuvos miškų ūkio statistika [Lithuanian statistical yearbook of forestry] L.R. Aplinkos ministerija, Valstybinė miškų tarnyba. Lututė, Kaunas, 152 pp. (in Lithuanian and English)
- Bakys, R., Vasaitis, R., Barklund, P., Ihrmark, K. and Stenlid, J.** 2009 a. Investigations concerning the role of *Chalara fraxinea* in declining *Fraxinus excelsior*. *Plant Pathology* 58: 284-292.
- Bakys, R., Vasaitis, R., Barklund, P., Thomsen, I. M. and Stenlid, J.** 2009 b. Occurrence and pathogenicity of fungi in necrotic and non-symptomatic shoots of declining common ash (*Fraxinus excelsior*) in Sweden. *European Journal of Forest Research* 128: 51-60.
- Baliuckas, V., Lagerström, T. and Eriksson, G.** 2000. Within- and among population variation in juvenile growth rhythm and growth in *Fraxinus excelsior* and *Prunus avium*. *Forest Genetics* 7(3): 193-202.
- Baniulis, D., Gelvonauskienė, D., Gelvonauskis, B., Rugienius, R., Bendokas, V. and Stanys, V.** 2008. Atsparumo rauplėms genų tyrimas ir molekulinį metodų taikymas obuļu selekcijoje [Studies of scab resistance genes and application of molecular methods in breeding of apple trees]. *Sodininkystė ir daržininkystė* 27(3): 61-75, (in Lithuanian)
- Barnes, I., Kirisits, T., Akulov, A., Chhetri, D. B., Wingfield, B. D., Bulgakov, T. S. and Wingfield, M. J.** 2008. New host and country records of the *Dothistroma* needle blight pathogens from Europe and Asia. *Forest Pathology* 38(3): 178-195.
- Barklund, P.** 2005. Askdöd grasserer över Syd- och Mellansverige [Ash dieback takes over south and mid-Sweden]. *SkogsEko* 3: 11-3, (in Swedish)
- Becker, W.A.** 1984. Manual of quantitative genetics. Academic Enterprises, Pullman, Washington DC, 190 pp.
- Brasier, C.M., Kirk, S.A., Delcan, J., Cooke, D.E.L., Jung, T. and Man In't Veld, W.A.** 2004. *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycological Research* 108(10): 1172-1184.
- Brasier, C.M.** 1979. Dual origin of recent Dutch elm disease outbreaks in Europe. *Nature* 281: 78-79.
- Broadmeadow, M. S. J., D. Ray, and Samuel, C. J. A.** 2005. Climate change and the future for broadleaved tree species in Britain. *Forestry* 78: 145-161.
- Burdon, R.D.** 2001. Genetic diversity and disease resistance: some considerations for research, breeding, and deployment. *Canadian Journal of Forest Research* 31: 596-606.
- Cech, T.L.** 2006. Neuartige Eschenschäden [New damage on ash]. *Forstzeitung* 11: 41 (in German).

- Cech, T. L. and Hoyer-Tomiczek, U. 2007. Aktuelle Situation des Zurücksterbens der Esche in Österreich [Actual situation of dieback of ash in Austria]. *Forstschutz Aktuell* 40: 8-10, (in German).
- Chandelier, A., Andre, F. and Laurent, F. 2009. Detection of *Chalara fraxinea* in common ash (*Fraxinus excelsior*) using real time PCR. *Forest Pathology* 40(2): 87-95.
- Chromov, S.P. 1968. Meteorology and climatology. Hidrometeoizdat, Leningrad, 149 pp. (in Russian)
- Cundall, E.P., Cahalan, C.M. and Connolly, T. 2003. Early results of ash (*Fraxinus excelsior* L.) provenance trials at sites in England and Wales. *Forestry* 76 (4): 385-399.
- Drenkhan, R. and Hanso, M. 2010. New host species for *Chalara fraxinea*. *New Disease Reports* 22: 16.
- Eppo, 2007. *Chalara fraxinea*, Ash Dieback. http://www.eppo.org/QUARANTINE/Alert_List/fungi/Chalara_fraxinea.htm [updated on February, 2010].
- Falconer, D.S. 1989. Introduction to quantitative genetics (3rd edition). Longman Group Ltd., London, 438 pp.
- Falconer, D.S. and Mackay, T.F.C. 1996. Introduction to quantitative genetics (4th edition). Longman Group Ltd., London, 464 pp.
- Ferrazzini, D., Monteleone, I. and Belletti, P. 2007. Genetic variability and divergence among Italian populations of common ash (*Fraxinus excelsior* L.). *Annals of Forest Sciences* 64: 159-168.
- Fins, L., Friedman, S.T. and Brotschol, J.V. 1992. Handbook of quantitative forest genetics. Kluwer Academic Publishers, Dordrecht, The Netherlands, 403 pp.
- Giertych, M. 1995a. Genetyka [Genetics]. In: W. Bugala (Editor), Nasze Drzewa Lesne: Monografia Popularnonaukowe. Tom 17: Jesion wyniosly *Fraxinus excelsior* L. Institute of Dendrology, Poland, p. 355-370 (in Polish)
- Giertych, M. 1995b. Zmienność genetyczna jesionu wyniosłego *Fraxinus excelsior* L. [Genetic diversity of European ash *Fraxinus excelsior* L.]. *Sylvan* 139: 87-91, (in Polish)
- Gustienė, A. 2010. Grybo *Chalara fraxinea* reikšmė uosių džiūties procese [Importance of fungus *Chalara fraxinea* in the process of ash dieback]. *Mūsų girios* 11: 18-19, (in Lithuanian)
- Halmeschlager, E. and Kirisits, T. 2008. First report of the ash dieback pathogen *Chalara fraxinea* on *Fraxinus excelsior* in Austria. *Plant Pathology* 57: p. 1177.
- Heuertz, M., Fineschi, S., Anzidei, M., Pastorelli, R., Salvini, D., Paule, L., Frascaria-Lacoste, N., Hardy, O.J., Vekemans, X. and Vendramin, G.G. 2004a. Chloroplast DNA variation and postglacial recolonisation of common ash (*Fraxinus excelsior* L.) in Europe. *Molecular Ecology* 13: 3437-3452.
- Heuertz, M., Hausman, J.-F., Hardy, O. J., Vendramin, G.G., Frascaria-Lacoste, N. and Vekemans, X. 2004b. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L.). *Evolution* 58: 976-988.
- Heydeck, P., Bemann, M. and Kontzog, H.-G. 2005. Triebsterben an Gemeiner Esche (*Fraxinus excelsior*) im nordostdeutschen Tiefland [Widespread dying of common ash (*Fraxinus excelsior*) in the lowlands of Northeastern Germany]. *Forst und Holz* 60: 505-506, (in German)
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130: 195-204.
- Ioos, R., Kowalski, T., Husson, C. and Holdenrieder, O. 2009. Rapid in planta detection of *Chalara fraxinea* by a real-time PCR assay using a dual-labelled probe. *European Journal of Plant Pathology* 125: 329-335.
- Jankovsky, L., Palovčikova, D. and Tomšovský, M. 2009. Brown spot needle blight associated with *Mycosphaerella dearnessii* occurs on *Pinus rotundata* in the Czech Republic. *Plant Pathology* 58(2): 398.
- Johansson, S.B.K., Vasaitis, R., Ihrmark, K., Barklund, P. and Stenlid, J. 2009. Detection of *Chalara fraxinea* from tissue of *Fraxinus excelsior* using species-specific ITS primers. *Forest Pathology* 40(2): 111-115.
- Jonsson, A. and Eriksson, G. 1989. A review of genetic studies of some important traits in the genera *Acer*, *Fagus*, *Fraxinus*, *Prunus*, *Quercus* and *Ulmus*. Rapportert och Uppsatser Institutionen for Skogsgenetik, Sveriges Lantbruksuniversitet, No. 44, 50 pp.
- Jung, T. and Blaschke, M. 2004. *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread and possible management strategies. *Plant Pathology* 53: 197-208.
- Juodvilkis, A. and Vasiliauskas, A. 2002. Lietuvos uosynų džiūvimo apimtys ir jas lemiantys veiksniai [The extent and possible causes of dieback of ash stands in Lithuania]. *LŽŪU Mokslo Darbai. Biomedicinos Mokslai* 56: 17-22 (in Lithuanian with English summary)
- Karazija, S. 1988. Lietuvos miškų tipai [Lithuanian forest typology]. Mokslas Publishers, Vilnius, 212 pp., (in Lithuanian)
- Kirisits, T. and Cech, L.T. 2006. Entwickelt sich die Dothistroma-Nadelbraune zu einem Forstschutzproblem in Österreich? [Is Dothistroma needle blight going to become an important forest disease in Austria?]. *Forstschutz Aktuell* 36: 20-26.
- Kirisits, T. and Halmeschlager, E. 2008. Eschenpilz nachgewiesen [Ash fungus detected]. *Forstzeitung* 2: 32-33 (in German)
- Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Cech, T.L. and Halmeschlager, E. 2009. The current situation of ash dieback caused by *Chalara fraxinea* in Austria. SDU Faculty of Forestry Journal, Serial A, Special Issue: 97-119
- Kirisits, T., Matlakova, M., Mottinger-Kroupa, S., Halmeschlager, E. and Lakatos, F. 2010. *Chalara fraxinea* associated with dieback of narrow-leaved ash (*Fraxinus angustifolia*). *Plant Pathology* 59: 411.
- Kleinschmit, J., Svolba, J., Enescu, V., Franke, A., Rau H.-M. and Ruetz, W. 1996. Erste Ergebnisse des Eschen-Herkunftsversuches von 1982. [First results of provenance trials of *Fraxinus excelsior* established in 1982]. *Forstarchiv* 67: 114-122 (in German)
- Kowalski, T. and Lukomska, A. 2005. Badania nad zamieraniem jesionu (*Fraxinus excelsior* L.) w drzewostanach Nadleśnictwa Włoszczowa [The studies on ash dying *Fraxinus excelsior* L. in the Włoszczowa Forest Unit stands]. *Acta Agrobotanica* 59: 429-440 (in Polish with English summary)
- Kowalski, T. and Holdenrieder, O. 2009a. Pathogenicity of *Chalara fraxinea*. *Forest Pathology* 39: 1-7.
- Kowalski, T. and Holdenrieder, O. 2009b. The teleomorph of *Chalara fraxinea*, the causal agent of ash dieback. *Forest Pathology* 39: 304-308.
- Kowalski, T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology* 36(4): 264-270.
- Lygis, V., Vasiliauskas, R., Larsson, K.-H. and Stenlid, J. 2005. Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes*. *Scandinavian Journal of Forest Research* 20: 337-346.

- McDonald, B. A. and Linde, C. 2002a. Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* 40: 349–379.
- McDonald, B. A. and Linde, C. 2002b. The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica* 124(2): 163–180.
- McKinney, L.V., Nielsen, L.R., Hansen, J.K. and Kjeær, D. 2010. Presence of natural genetic resistance in *Fraxinus excelsior* (*Oleraceae*) to *Chalara fraxinea* (*Ascomycota*): an emerging infectious disease. *Heredity* (8 September 2010) doi:10.1038/hdy.2010.119.
- Mwase, W.F., Savill, P.S. and Hemery, G. 2008. Genetic parameter estimates for growth and form traits in common ash (*Fraxinus excelsior* L.) in a breeding seedling orchard at Little Wittenham in England. *New Forests* 36(3): 225–238.
- Nowakowska, J., Jablonowski, S., Mockeliunaite, R. and Bieniek, J. 2004. Genetic variability within and among Polish and Lithuanian populations of common ash (*Fraxinus excelsior* L.) based on RAPD analysis. *Baltic Forestry* 10(1): 57–64.
- Olrik, D.C., Kjaer, E.D. and Ditlevsen, B. 2007. Klonforskelle i angreb af asketoptørre [Clonal variation in ash dieback]. *Skoven* 39: 522–525 (in Danish).
- Oszako, T. 2004. Protection of forest against pest insects and diseases. European oak decline study case. Forest Research Institute, Warsaw, Poland. 150 pp.
- Ozolinčius, R. and Stakėnas, V. 2000. Susirūpinimą kelianti uosynų būklė [Condition of common ash forest rising concern]. *Mūsų girios* 6: 8 (in Lithuanian)
- Ozolinčius, R. 2002. Uosynų džiūvimo hipotezės [Hypotheses of ash dieback]. *Mūsų girios* 7: 2–4, (In Lithuanian)
- Pehl, L. and Wulf, A. 2001. *Mycosphaerella*-Nadelpilze der Kiefer: Schadsymptome, Biologie und Differentialdiagnose [*Mycosphaerella*-needle fungi on pines – symptoms, biology and differential diagnosis]. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 53(9): 217–222 (in German)
- Pliūra, A. 1999. European long-term gene conservation strategies: Ash (*Fraxinus* spp). Proceed. of the third EUFORGEN Noble Hardwoods meeting, July 13–16 (1998), Sagadi, Estonia, p. 8–20.
- Pliūra, A. and Baliuckas, V. 2007. Genetic variation in adaptive traits of progenies of Lithuanian and western European populations of *Fraxinus excelsior* L. *Baltic Forestry* 13(1): 28–38.
- Pliūra, A., Balkus, T., Baliuckienė, A. and Baliuckas, V. 2006. Paprastojo uosio genetinis atsakas į skirtingo intensyvumo ozono poveikį juveniliniame amžiuje klimatinėse kamerose. [Genetic response of common ash exposed to different ozone dosage at juvenile age in climatic chambers]. *Miškininkystė* 2(60): 43–53 (In Lithuanian with English summary)
- Przybyl, K. 2002a. Causes of decline of young ash trees and stands. Central Board of State Forests, Warsaw, Poland (in Polish).
- Przybyl, K. 2002b. Fungi associated with necrotic apical parts of *Fraxinus excelsior* shoots. *Forest Pathology* 32: 387–394.
- Queloz, V., Grünig, C.R., Berndt, R., Kowalski, T., Sieber, T.N. and Holdenrieder, O. 2010. Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathology* doi: 10.1111/j.1439-0329.2010.00645.x (published on-line 30 March, 2010).
- Riepišas, E. 2009. Pažeistų uosynų atsikūrimo/atkūrimo būklė ir taikytinos priemonės [Regeneration of damaged common ash stands and recommended measures]. *Mūsų girios* 10: 18–19, (In Lithuanian)
- Santini, A., Barzanti, G.P. and Capretti, P. 2001. A new *Phytophthora* root disease of alder in Italy. *Plant Disease* 85: 560.
- SAS Institute Inc. 1999. SAS/STAT® User's Guide, Version 8, Cary, NC, USA
- Savill, P. S., Spencer, R., Roberts, J. E. and Hubert, J. D. 1999. Sixth year results from four ash (*Fraxinus excelsior*) breeding seedling orchards. *Silvae Genetica* 48(2): 92–100.
- Schumacher, J., Kehr, R. and Leonhard, S. 2009. Mycological and histological investigations of *Fraxinus excelsior* nursery saplings naturally infected by *Chalara fraxinea*. *Forest Pathology* 40: 419–429.
- Schumacher, J., Wulf, A. and Leonhard, S. 2007. Erster Nachweis von *Chalara fraxinea* T. Kowalski sp. nov. in Deutschland - ein Verursacher neuartiger Schäden an Eschen [First record of *Chalara fraxinea* T. Kowalski sp. nov. in Germany - a new agent of ash decline]. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 59: 121–123 (in German)
- Skovsgaard, J.P., Thomsen, I.M., Skovgaard, I.M. and Martinussen, T. 2010. Associations among symptoms of dieback in even-aged stands of ash (*Fraxinus excelsior* L.). *Forest Pathology* 40: 7–18.
- Skuodienė, L., Grybauskas, K., Palionis, V. and Maslinskas, R. 2003. Uosynų būklė ir galimos jų žuvimo priežastys [Condition of common ash forests and probable causes of dieback]. *Miškininkystė* 2 (54): 86–96 (in Lithuanian with English summary)
- Smintina, I. 1993. Provenance trials with ash (*Fraxinus excelsior* L.). Results obtained 10 years after planting. *Revista Padurilor* 108: 10–17.
- Stener, L.G. 2007. Studie av klonskillnader i känslighet för askskottsjuka [Study of clonal differences in susceptibility to ash shoot disease]. Arbetsrapport från Skogforsk nr 648, Uppsala, Sweden, 14pp., (In Swedish)
- Stipes, R. J. and Campana, R. J. (eds.) 1981. Compendium of Elm Diseases. American Phytopathological Society, St. Paul, MN, USA, 96 pp.
- Szabo, I. 2009. First report of *Chalara fraxinea* affecting common ash in Hungary. *Plant Pathology* 58: 797.
- Thomsen, I.M. and Skovsgaard, J.P. 2006. Tøptørre i ask: klimaskade eller svampeangreb? [Crown dieback of ash: climatic damage or fungal attack?]. *Skoven* 38: 408–411, (in Danish)
- Vasiliauskas, R., Bakys, R., Lygis, V., Barklund, P., Ihrmark, K. and Stenlid, J. 2006. Fungi associated with crown dieback of *Fraxinus excelsior*. In: T. Oszako and S. Woodward (Editors), Possible limitation of dieback phenomena in broadleaved stands. Forest Research Institute, Warsaw, Poland, p. 45–53.
- Weiser, F. 1995. Beitrag zur Existenz von Okotypen bei Gemeiner Esche (*Fraxinus excelsior* L.) [Studies into the existence of ecotypes of ash (*Fraxinus excelsior* L.)]. *Forstarchiv* 66: 251–257, (in German)
- Woolhouse, M.E.J., Haydon, D.T. and Aantia, R. 2005. Emerging pathogens: the epidemiology and evolution of species jumps. *Trends in Ecology & Evolution* 20: 238–244.
- Žvingila, D., Verbylaitė, R., Baliuckas, V., Pliūra, A. and Kuusienė, S. 2005. Genetic diversity (RAPD) in natural Lithuanian populations of common ash (*Fraxinus excelsior* L.). *Biologija* 3: 46–53.

Received 23 February 2011

Accepted 05 April 2011

ПОВЕДЕНИЕ ДВАДЦАТИ ЧЕТЫРЕХ ПОПУЛЯЦИЙ *FRAXINUS EXCELSIOR* В ТРЕХ ОПЫТАХ ПО ИСПЫТАНИЮ ПОТОМСТВА В ЛИТВЕ С АКЦЕНТОМ НА УСТОЙЧИВОСТЬ К *CHALARA FRAXINEA*

А. Плюра, В. Лигис, В. Сухоцкас и Е. Баргкявичюс

Резюме

Целью исследований было изучение генетического контроля и изменчивости устойчивости ясеня обыкновенного к болезням и по результатам испытания роста потомства, а также отбор устойчивых популяций, семей и генотипов с целью выведения устойчивого к *Chalara fraxinea* репродуктивного материала для восстановления ясенников в Литве. 320 семей полусибов из 10 литовских и 14 зарубежных популяций ясеня обыкновенного изучались в трёх опытах по испытанию потомства. Из-за эпидемии *C. fraxinea* и других патогенных микроорганизмов, в 8-летнем возрасте в трёх опытах по испытанию потомства сохранилось только 10% деревьев. Вариационный анализ состояния деревьев потомства выявил значительное влияние генетических факторов – популяций и семей. Различия между популяциями по устойчивости к болезням были значительными и составляли до 52,8-55,1% по степени повреждения, а выживаемость популяций была от 0,13 до 0,59. Доля генетического компонента семьи в состоянии здоровья потомства составило 5,3-7,5%, а коэффициент аддитивной генетической вариации был 29,9-38,7%. Эти данные свидетельствуют о существовании генетических различий по стойкости потомства к заболеваниям, вызванным патогенами как на уровне популяций, так и на уровне семей. Статистически значимое влияние местопрорастания на состояние здоровья деревьев означает, что оно зависит от условий окружающей среды и, возможно, от степени инфекционной нагрузки с окружающих насаждений. Взаимодействие генотипа со средой (G x E) по признакам стойкости деревьев, было слабым и незначительным, что указывает на то, что популяции и семья незначительно отличаются по их поведению в окружающей среде и, таким образом, генетическая изменчивость по экологической реакции не проявляется. Высокие значения коэффициента наследуемости ($h^2 = 0,40-0,49 \pm 0,03$) показывают, что резистентность является генетически предопределённой и наследуемой, и позволяет оценить устойчивые генотипы на основе их фенотипов. Все это создаёт хорошие возможности для эффективной селекции по резистентности.

Состояние западноевропейских популяций, по сравнению с литовскими, было хуже на 17,5-23,5% по степени повреждения и на 48,5-73,5% по выживаемости. Это показывает, что вряд ли будет возможно применить перемещение популяций из Западной Европы для восстановления литовских лесов ясеня. Только несколько наиболее резистентных генотипов можно отобрать как кандидатов для включения в синтетическую селекционную популяцию ясеня для повышения генетического разнообразия. Среди исследованных литовских популяций, лучшие, с точки зрения комплекса характеристик - резистентности, выживания и доли самых здоровых индивидов, являются популяции Игналина, Пакруойс, Шакай и Кедайняй.

На основе оценки BLUP комплексных селекционных индексов и по “пирамидному селекционному принципу”, всего 50 превосходящих индивидов были отобраны в лучших по устойчивости к болезням семьях в лучших популяциях для создания резистентных селекционных популяций ясеня обыкновенного в различных регионах прорастания Литвы, а также для создания клоновых лесосеменных плантаций второго поколения.

Ключевые слова: *Chalara fraxinea*, ясень обыкновенный (*Fraxinus excelsior*), генетическая изменчивость, семьи полусибов, наследуемость, популяции, селекция