Rising Out of the Ashes: Additive Genetic Variation for Crown and Collar Resistance to *Hymenoscyphus fraxineus* in *Fraxinus excelsior*

Facundo Muñoz, Benoît Marçais, Jean Dufour, and Arnaud Dowkiw

First, third, and fourth authors: INRA, UR 0588, Unité Amélioration, Génétique et Physiologie Forestières, CS 40001 Ardon, 45075 Orléans Cedex 2, France; and second author: INRA, Nancy Université, UMR 1136 Interactions Arbres/Microorganismes, IFR 110, F-54280 Champenoux, France.

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ABSTRACT

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Since the early 1990s, ash dieback due to the invasive ascomycete *Hymenoscyphus fraxineus* is threatening *Fraxinus excelsior* in most of its natural range. Previous studies reported significant levels of genetic variability in susceptibility in *F. excelsior* either in field or inoculation experiments. The present study was based on a field experiment planted in 1995, 15 years before onset of the disease. Crown and collar status were monitored on 777 trees from 23 open-pollinated progenies originating from three French provenances. Health status was modeled using a Bayesian approach where spatiotemporal effects were explicitly taken into account. Moderate narrow-sense heritability was found for crown dieback (h^2 = 0.42). This study is first to show that resistance at the collar level is also heritable (h^2 = 0.49 for collar lesions prevalence and h^2 = 0.42 for their severity) and that there is significant genetic correlation (r = 0.40) between the severities of crown and collar symptoms. There was no evidence for differences between provenances. Family effects were detected, but computing individual breeding values showed that most of the genetic variation lies within families. In agreement with previous reports, early flushing correlates with healthier crown. Implications of these results in disease management and breeding are discussed.

An extensive review on the European ash dieback crisis was published recently (McKinney et al. 2014), thus allowing for a brief overview here. Severe dieback of European common ash (*Fraxinus excelsior*) was first reported in Poland and Lithuania in the early 1990s (Lygis et al. 2005; Przybył 2002). The observed symptoms have long been thought to result from a combination of climatic factors and new pathogens and vectors (Pliūra and Baliuckas 2007). It was 14 years after the first report that an ascomycete was identified as the primary causal agent (Kowalski 2006). The disease is now present in at least 26 countries with its current southwestern limit being Central France. First described as a new fungal species (i.e., *Chalara fraxinea*), it was soon suggested that it could be the anamorphic stage of *Hymenoscyphus albidus*, a widespread native decomposer of ash litter (Kowalski and Holdenrieder 2009). However, further investigations were concluded on a distinct and previously undescribed species now referred to as *H. fraxineus* (Baral et al. 2014). Recent findings suggest that the species is invasive and originates from Asia (Bengtsson et al. 2012; Husson et al. 2011; McKinney et al. 2012b; Zhao et al. 2012).

Knowledge on the life cycle of *H. fraxineus* has been improved and summarized by Gross et al. (2012). Sexual reproduction is hypothesized to be mediated through conidia in autumn (A. Gross, *personal communication*) on dead ash petioles in the litter. Apothecia emerge in summer; ascospores are wind-dispersed and germinate on ash leaves forming an appressorium (Cleary et al. 2013). Once in the leaf tissue, the mycelium develops intracellularly, moving through the cells and easily colonizing the phloem, paratracheal parenchyma, and parenchymatic rays (Dal Maso et al. 2012). Symptoms of the disease include wilting of leaves, necrotic lesions on leaves, necrosis on twigs, stems, and branches, and collar lesions (CL). CL have been described quite late (Lygis et al. 2005) and the question of their primary cause—*H. fraxineus* or other fungi like *Armillaria* sp. or *Phytophthora* sp.—is still controversial (Bakys et al. 2011; Enderle et al. 2013; Husson et al. 2011; Orlikowski et al. 2011; Skovsgaard et al. 2010).

With its natural range stretching from Iran to Ireland and from Southern Scandinavia to Northern Spain (Dobrowolska et al. 2011), *F. excelsior* is the most common and the most northern of the three *Fraxinus* species native to Europe, and thus the first one to face this new threat. Detailed quantifications are scarce, but consequences of the disease can be severe. In Lithuania, 10 years after the first report, over 30,000 ha of common ash stands were reported to be affected and mortality was estimated to be 60% state wide while in some parts of the country only 2% of the trees remained visually healthy (Juodvalkis and Vasiliauskas 2002). In Southern Sweden, one fourth of all ash trees were reported as dead or severely damaged 7 years after the first report (Fischer et al. 2010). In some parts of North-Eastern France, only 3% of the trees remained completely healthy 2 years after the first report (Husson et al. 2012). These figures corroborate the findings from Mc Kinney et al. (2011) on 39 clones from 14 Danish populations where only 1 clone out of 39 maintained an average damage level below 10% in two replicated field trials. The computed broad-sense heritability estimates (i.e., 0.40 to 0.49) were however indicative of a strong genetic control, meaning that there is an adaptive potential and that breeding material with low susceptibility would be possible. Evidences of a significant level of genetic variation were also found in a few other comparison experiments involving clones or half-sib families (Kjær et al. 2012; Lobo et al. 2015; Pliūra et al. 2011, 2014; Stener 2013).

The present study explores the genetic variability of common ash for resistance to *H. fraxineus* using open-pollinated (OP) maternal progenies. It is based on a 20-year-old field trial with 23 half-sib families from three French provenances. The trial is located in the

Corresponding author: A. Dowkiw; E-mail address: arnaud.dowkiw@orleans.inra.fr

*The *e-Xtra* logo stands for "electronic extra" and indicates that six supplementary figures and four supplementary tables are published online.

The data and the R analysis package are freely available for download on the following webpage: https://github.com/famuvie/2016_RisingAshes.

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area where ash dieback was first detected in France in 2008. Because the stand has been monitored every year since disease appearance, spatiotemporal components of disease spread could be taken into account to help avoid confusion between disease escape and resistance. Moreover, a Bayesian approach was used instead of the classical frequentist analysis. Bayesian methods accommodate complex spatiotemporal structures in a more straightforward way and their results are directly interpretable in terms of posterior probabilities. In addition, they are also more suitable for modeling data which deviates heavily from normality, as it is frequently the case during early stages of disease spread. In addition, it leverages information from previous knowledge, which can be critical in situations where data are little informative (Mila and Carriquiry 2004). This study is also the first report on the genetic parameters for CL and on the genetic correlation with crown defoliation.

MATERIALS AND METHODS

Experimental design. The studied material consisted of 23 open-pollinated *F. excelsior* maternal progenies from three North-Eastern French provenances (Amancey, eight families; Chalèze, seven families; and Vernois-sur-Mance, eight families). Although located less than 40 km apart (Supplementary Fig. S1), these provenances differ in elevation and soil composition and structure. Mother trees were selected at random within each provenance. Seeds were collected in 1992, cold-stratified, germinated, and raised in nursery until plantation in January 1995.

The trial was established in Devecey, 61 km from the furthest provenance, on a winter tilled agricultural land located 250 m high. Planting was done at a spacing of 4 m × 4 m following a randomized incomplete block design. Depending on plant availability, each family was represented in 2 to 10 blocks plus the border except two families that were represented in the border only. Each block contained 16 families, each represented by four trees that were distributed among four subblocks. Each family was thus represented by 8 to 68 trees in the whole trial including the border (Supplementary Table S1). Since 11 trees died a few years after planting, a total of 777 trees were analyzed here.

Measurements. A first investigation for the presence of *H. fraxineus* was conducted in February 2009 and the trees did not reveal any symptoms. The first signs of infection were observed in February 2010 and the presence of the fungus was confirmed by real-time PCR (French Department of Agriculture, *personal communication*). Crown dieback (CD) was measured in July 2010, July 2011, June 2012, June 2013, and June 2014 using a 0 to 5 ranking scale according to the proportion of dead branches: 0, no dead branches; 1, less than 10% dead branches; 2, 10 to 50% dead branches; 3, 50 to 80% dead branches; 4, more than 80% dead branches; and 5, dead tree. For data analysis, classes were subsequently converted to their median value (i.e., 0, 0.05, 0.30, 0.65, 0.90, and 1). CL numbers were measured in July 2012 and June 2013. Detecting and measuring them required scratching the bark using a triangular scraper; this is why this measurement was conducted for 2 years only. As several lesions can occur on the same tree, basal width of all lesions were cumulated and divided by the basal girth (BG) of the tree to compute CL as a 0 to 1 girdling index (Supplementary Fig. S2). Bud flushing was measured once, 3 years after planting, using a 1 (late) to 5 (early) ranking scale.

Data analysis. We analyzed each symptom independently using a sequence of statistical models of increasing complexity and flexibility. All of the fitted models for CD belong to the family of linear mixed models (LMM). The vector $\boldsymbol{y}$ of n measurements of phenotypic values are described as a linear model with a vector of p fixed effects ($\boldsymbol{\beta}$) and a vector of q random effects ($\boldsymbol{u}$). In matrix form,

$$\boldsymbol{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{u} + \boldsymbol{\varepsilon}$$

where $\mathbf{X}$ and $\mathbf{Z}$ are $n \times p$ and $n \times q$ incidence matrices, respectively, and $\boldsymbol{\varepsilon}$ is the vector of residuals. Furthermore, both $\boldsymbol{u}$ and $\boldsymbol{\varepsilon}$ are assumed to be independent from each other and to follow a zero-mean Gaussian distribution with covariance matrices $\sigma_u^2\mathbf{R}$ and $\sigma^2\mathbf{I}$, respectively, where $\mathbf{R}$ is a $q \times q$ structure matrix, and $\mathbf{I}$ is the $n \times n$ identity matrix.

Several independent random effects with specific variances and structure matrices can be stacked in a single vector $\boldsymbol{u}$ with a block-diagonal covariance matrix where each block is given by the corresponding component and an incidence matrix $\mathbf{Z}$ binding the individual incidence matrices column-wise.

The normality of the residuals is a delicate assumption to make for response variables that are categorical (CD) or restricted to the interval 0 to 1 (CL). However, it is very convenient from a computational point of view, and this approximation is commonly found in other studies (Kjær et al. 2012; Pliūra et al. 2011). We performed a transformation of the CD variable to improve the adjustment to this hypothesis. Specifically, we worked with

$$\mathrm{TCD} = 1 \Big/ \sqrt{\mathrm{CD} + 0.1}$$

No normalizing transformation was found for CL. In consequence, we used a mixture of generalized linear mixed models (GLMM) for this trait. A GLMM is an extension of a LMM for non-Gaussian data. The observations are assumed to be an independent random sample from some distribution of the exponential family conditional to the mean, which is modeled as a nonlinear function of the linear predictor:

$$\boldsymbol{y} \sim f(\boldsymbol{\mu}, \boldsymbol{\theta})$$

$$g(\boldsymbol{\mu}) = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{u}$$

where g is an appropriate link function, $\boldsymbol{\mu}$ is the mean, and $\boldsymbol{\theta}$ is a vector of additional distribution-specific parameters.

Model inference and comparison. All analyses were performed using R (R Core Team 2015). The models were fitted from a Bayesian perspective using the integrated nested Laplace approximation (INLA) methodology (Rue and Martino 2009), implemented in the R-INLA package (Rue et al. 2014). The marginal likelihood, the deviance information criterion (DIC) (Spiegelhalter et al. 2002) and the widely applicable information criterion (WAIC) (Watanabe 2010) were used as model selection criteria. The marginal likelihood was scaled by a factor of –2 (also known as the deviance), so that for all three criteria, lower values are better.

Statistical model for CD. An appropriate statistical model was determined following a model-selection procedure (Supplementary Table S2). The initial reference model included only unstructured random effects (i.e., the matrix $\mathbf{R}$ is an identity matrix for all random effects) and is analogous to what has been commonly used in previous studies on the genetic diversity of resistance to *H. fraxineus* (Kjær et al. 2012; Pliūra et al. 2011). The data were best described by model M5 including fixed effects for the year and bud flush precocity, a random additive genetic effect at individual level and a spatiotemporal (ST) random effect.

The additive-genetic individual effect was modeled as a structured random effect with a known covariance structure given by the family kinship. Specifically, the covariance matrix is

$$\mathbf{R}_a = \sigma_a^2\mathbf{A} \quad (1)$$

where σ_a^2 is the unknown additive genetic variance in the base population and where the additive-genetic structure matrix $\mathbf{A}$ has elements $A_{ij} = 2\Theta_{ij}$, i.e., twice the coefficient of co-ancestry between the individuals i and j (see for example Lynch and Walsh 1998).

The spatiotemporal random effect (ST) was modeled based on a Gaussian spatial process evolving in time, thus allowing for continuous environmental variation. Evaluated in the spatiotemporal locations of the observations, the values of the Gaussian process follow a multivariate normal distribution with a covariance structure

given by the distance, in space and time, between observations. Specifically, the spatiotemporal structure is built as the Kronecker product of separate temporal and spatial processes. The temporal process is simply determined by an autocorrelation parameter ρ_t between observations, while the spatial process is defined by a Matérn covariance function with parameters of shape (i.e., smoothness) ν, spatial scale κ and precision τ^2. The smoothness parameter was fixed to $\nu = 1$ for convenience. The spatial scale parameter is associated with the effective range of the spatial process, so that the correlation between locations at a distance $\rho_s = \sqrt{8}/\kappa$ is approximately 0.13. Finally, the marginal variance of the spatial process is given by $\sigma_s^2 = 1/(4\pi\kappa^2\tau^2)$. This yields a structured random effect with a parametric covariance matrix as follows:

$$\mathbf{R}_{st} = \tau^{-2}\mathbf{Q}(\rho_t, \rho_s) \quad (2)$$

Consequently, while the global temporal trend is captured by the explicit year effect in this model, the ST structure accounts for heterogeneous spatial deviations from the main trend both in space and time.

In order to split the genetic variance into the inter-family and intra-family components and to compare their relative magnitudes, we also fitted a reparameterization of this model introducing an explicit family effect. Computationally, this requires introducing sum-to-zero constraints for each family in the additive-genetic effect for preserving the identifiability of the model.

Statistical models for CL. This variable was considered as the result of two different processes or stages. First is the process determining whether a tree becomes infected or not. Second is the process determining how severely an infected tree is affected by the disease. The two processes were analyzed separately.

The pattern of zeros (i.e., inversely related with the disease prevalence) was modeled with a Bernoulli likelihood while the strictly positive observations (i.e., disease severity) were assumed to follow a continuous distribution with positive support. For the continuous component, beta and gamma were considered as candidate distributions and a preliminary assessment was performed to determine which one fitted the data better. All the potentially explanatory fixed effects were included for this preliminary analysis. The Gamma distribution emerged as the best candidate and was used in all subsequent models.

All other models systematically included an individual additive-genetic and a spatiotemporal (ST) effect as defined in the previous subsection. Two separate variable selection procedures were conducted for the binary and continuous components of CL to assess the relevance of the basal circumference (BC) and the provenance (Supplementary Tables S3 and S4, respectively).

Finally, both components of CL were integrated into a final model that consisted of a mixture of both GLMMs. For a measurement y_{ijk} taken in year i, at the location j for the individual k, we assumed that

$$Pr\left[y_{ijk} = 0\right] = p_{ijk},\ 0 < p_{ijk} < 1$$

$$\left(y_{ijk} \mid y_{ijk} > 0\right) \sim Ga\left(a_{ijk},\ b_{ijk}\right),\ a_{ijk},\ b_{ijk} > 0$$

A hierarchical model for the parameters p_{ijk}, a_{ijk}, and b_{ijk} was specified using appropriate link functions of the expected values of the respective distributions. Specifically, calling $\mu = E[y \mid y > 0] = a/b$, we defined two linear predictors

$$\text{logit}\left(p_{ijk}\right) = \text{Year}_i^{(1)} + s_{ij}^{(1)} + a_k^{(1)}$$

$$\log\left(\mu_{ijk}\right) = \text{Year}_i^{(2)} + s_{ij}^{(2)} + a_k^{(2)}$$

For each linear predictor $p = 1,2$, $\text{Year}_i^{(p)}$ is the fixed effect of the year $i = 2012, 2013$; $s_{ij}^{(p)}$ is a structured spatiotemporal (ST) random effect; and $a_k^{(p)}$ is a structured additive-genetic random effect at individual level (i.e., a vector of individual breeding values, IBV). The ST structure was built as the Kronecker product of separate temporal and spatial zero-mean Gaussian processes, as in equation 2. Finally, the structured additive-genetic effect followed a zero-mean multivariate normal distribution with a known covariance structure given by the family kinship, as in equation 1.

Prior distributions. All the fixed effects had a vague zero-mean Gaussian prior with variance of 1,000.

For the variance σ_a^2 of the additive-genetic effect we used an inverse-gamma with shape and scale parameters of 0.5. This is equivalent to an inverse-chi-square with 1 df, and places the 80% of the density mass between 0.05 and 15, with a preference for lower values.

For the ST structure, the priors were set independently for the spatial and temporal structures. INLA provides a bivariate Gaussian prior for the logarithms of the positive parameters κ and τ^2 of the spatial Matérn field. Its mean and variance were chosen to match reasonable prior judgments about the range and the variance of the spatial field. Specifically, the spatial range was concentrated between 5 and 38 tree spacings, the latter being the length of the shortest side of the field. There might well exist very short-ranged environmental factors affecting one or two trees at a time, but the model would not be able to separate them from random noise. On the other extreme, there certainly are environmental factors with a larger range than the field dimensions, but again, this would be virtually indistinguishable from the global mean of the field. Moreover, the results were found to be very robust to small variations of these prior statements.

Heritability estimates. The narrow-sense heritability is usually computed as the ratio between the additive-genetic variance and the phenotypic variance. However, this can be implemented in practice in different ways, depending on the specific model and the research goals and criteria.

The selected models for CD and for both components of CL yield direct estimates of the additive-genetic variance.

For CD, the phenotypic variance is computed as the sum of the variance components of the model. Namely, the additive-genetic variance, the residual variance, and following accepted guidelines (Visscher et al. 2008), the variance of the ST effect.

Estimating the heritability of the CL symptom is more complex. First, because there are two different traits in the model, and thus two different measures of heritability. But more importantly, because both with binomial and gamma likelihoods, the phenotypic variance is a function of the mean, and thus varies from observation to observation. Furthermore, although the additive-genetic variances are assumed common to the population, the phenotypic variance cannot be decomposed additively into genetic and residual components. Most approaches in the literature dealing with binary variables use the so-called *threshold models*, where the data are assumed to be deterministically 0 or 1 depending on whether some unobserved latent Gaussian variable reaches some threshold (see, for example, Dempster and Lerner 1950). In our case the response variable given the latent structure is random rather than deterministic. Therefore, the residual variability comes from the likelihood distribution, which is in a different scale than the genetic variance parameter. However, it can be shown that this is equivalent to a threshold model (Dempster and Lerner 1950) with an additional residual term following a logistic distribution. This results in the following formula for the heritability of the binomial component in the latent scale:

$$h_{bin} = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_s^2 + \pi^2/3}$$

where $\pi^2/3$ is the variance of a logistic distribution (Nakagawa and Schielzeth 2010), and σ_s^2 is the variance of the ST effect. For the

continuous component we followed the general simulation-based approach for a GLMM described in de Villemereuil et al. (2015).

All heritability estimates have been computed by Monte Carlo simulation given the posterior distributions of the relevant variance parameters. Specifically, we sampled 5,000 independent observations of each variance from their posterior distribution, and derived a posterior density for the heritability.

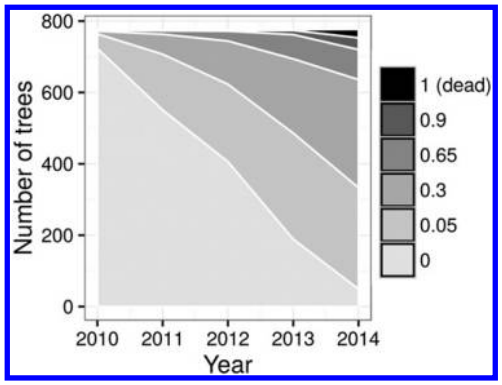

Fig. 1. Crown dieback (expressed as a 0 to 1 intensity) progress over time.

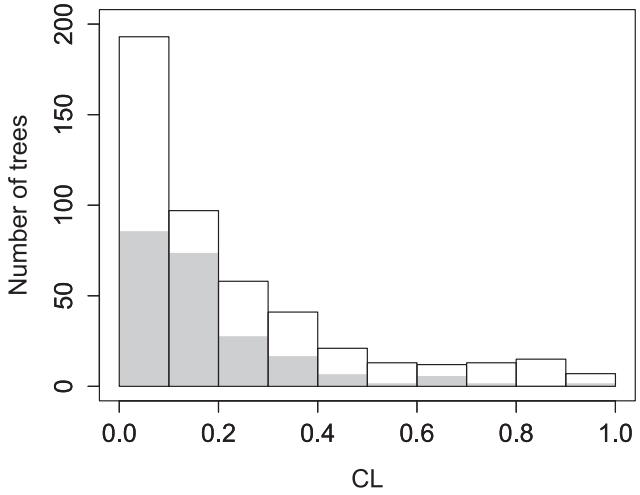

Fig. 2. Distribution of individual values for collar lesions (CL, expressed as a 0 to 1 girdling index) in 2012 (gray bars) and in 2013 (white bars). Individuals without CL (CL = 0) are not shown (i.e., 550 trees in 2012 and 305 trees in 2013).

RESULTS

CD occurrence and severity. From 2010 to 2014, mean CD increased from 0.01 to 0.27. The distribution of individual values remained positively skewed during the whole study time with most trees showing less than 50% dead branches (i.e., CD < 0.5, Fig. 1). In 2010, when disease started to be seen, 53 trees (i.e., 7%) had visible CD including four trees with CD > 0.5. Although one of these four trees died in less than 1 year, the proportion of dead trees reached only 3% in 2014 while the proportion of trees with CD > 0.5 increased to 19%. The annual decrease in the proportion of trees without visible CD was similar in 2011 and 2012 (–24 and –26%, respectively) but it accelerated in 2013 (–53%) and again in 2014 (–74%), leading to a situation where only 49 trees (i.e., 6%) remained free from CD in 2014. When computing the 3,104 values of individual annual increments for CD over the 4 years of the study, only 73 negative values were found (i.e., 2.3%).

CL occurrence and severity. From 2012 to 2013, mean CL increased from 0.05 to 0.14. CL were found on 29% of the trees in 2012 and this proportion doubled in just one year. Mean CL values computed on symptomatic trees only were 0.19 and 0.23 for 2012 and 2013, respectively with only 24 trees with CL > 0.80 in 2013 (Fig. 2). Among them, four had their collar totally rotten in 2013 and they were dead the next year. Fifty-eight negative values were found for the 2012 to 2013 increment in CL with only 12 increments below –0.1 (i.e., 1.5% of all computed increments). Most of these negative increments were probably artifactual due to the impossibility to measure basal girth and lesion length at exactly the same level each year due to the curved shape of the trunk basis.

Although a positive correlation was found between BC and CL occurrence in 2012 (r_Spearman = 0.94, *P* value = 0.017) (Fig. 3), CL occurrence was not clearly related to basal circumference, considering the model comparisons. In 2013, only the very few trees with BC below 30 cm had significantly fewer basal cankers.

Phenotypic correlation between symptoms. A positive correlation between occurrence of CL and CD severity was observed both in 2012 and 2013 (r_Spearman = 1, *P* value = 0.017) (Fig. 3). Despite this trend, CL could be found in many trees without visible CD symptoms. Indeed, 53% of the trees with CD = 0 in 2013 had CL. Conversely, 71% of the trees with CL = 0 in 2013 had CD.

Severities of both traits were significantly correlated also, and correlation increased from 2012 to 2013 (Table 1).

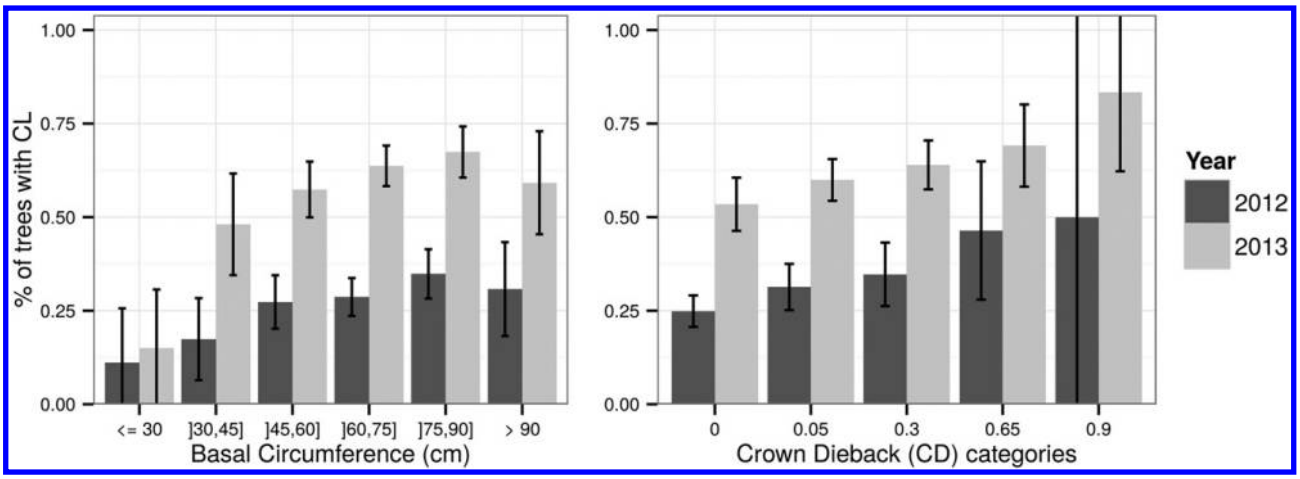

Fig. 3. Occurrence of collar lesions (CL) according to basal circumference categories and crown dieback severity by year. Error bars = 95% confidence intervals for the proportions, considered independently from each other.

Of the 22 trees that were dead in 2014 but still alive in 2013, 21 had CD values higher or equal to 0.65 in 2013 with 14 of them having CL values higher than 0.5 in the same year. Interestingly, one of these 22 trees had no CL and a CD value of only 0.3 in 2013 but this was a small tree (BC = 34 cm).

Inferences for CD. The provenance was not found to be a relevant explanatory variable according to all model-comparison criteria. By contrast, there were clear differences in the mean genetic merit between families (Fig. 4), although most of the genetic variability occurs within families (Fig. 5). The fixed year effect estimates revealed a clear progression of the disease, almost doubling the predicted CD value for a mean individual each year. Specific mean estimated values were 1.0% (2010); 2.6% (2011); 5.2% (2012); 10.7% (2013); and 17.8% (2014) with obvious variation according to bud flush precocity (BF, Fig. 6). Early flushers (high BF values) tended to have lower CD values.

Figure 7 shows the posterior mean ST effect. The scale being inverted by the transformation (TCD), the disease in terms of CD appeared to be consistently more intense in the bottom left corner of the field. The fact that the posterior distributions of the range and variance of the Gaussian process were concentrated well within the support of their corresponding prior indicated that the data were informative enough and the results were not constrained by the choice of the priors (Supplementary Fig. S3).

The posterior modes and 95% highest posterior density (HPD) credible intervals were 0.137 (0.119 to 0.156) for the additive-genetic variance and 0.147 (0.139 to 0.157) for the residual variance. The estimate of the narrow-sense heritability h^2 was 0.42 (0.38 to 0.47). Excluding the ST variance from the denominator increases the estimate up to 0.48 (0.44 to 0.52).

TABLE 1. Phenotypic correlations (*r*) between crown dieback and collar lesions

	2012		2013		2012 and 2013	
r type	*r*	*P* value	*r*	*P* value	*r*	*P* value
Pearson	0.14	3.2e-05	0.31	<2.2e-16	0.29	<2.2e-16
Spearman	0.13	2.4e-04	0.21	4.0e-09	0.23	<2.2e-16
Kendall	0.11	2.6e-04	0.17	3.5e-09	0.20	<2.2e-16

Inferences for CL. For the binary component of CL, including the BC improved DIC and WAIC, particularly in categorical form with an interaction with the year. However, it worsened the deviance. Suspecting some confounding with the ST effect (see Discussion), this variable was not included in the final model. On the other hand, including a provenance effect was clearly not relevant according to all three criteria. Neither BC nor the provenance improved the model for the continuous component.

The plot of predicted against observed values for the binary and continuous components of the mixture model indicated a reasonable goodness of fit of the model (Supplementary Fig. S4). For the continuous component, the model fit yielded a prediction a bit shrinked, but this is expected due to the difficulty to predict values in the extremes of a bounded variable.

The distribution of mean posterior IBV for the binary component by infection status (classified in three categories) was as expected. Trees that did not show any sign of infection got the highest predicted values (Supplementary Fig. S5), while those that showed some level of infection both in 2012 and 2013 got the smallest IBV. The degree of overlapping between these histograms indicates the relative importance of the genetic component with respect to the

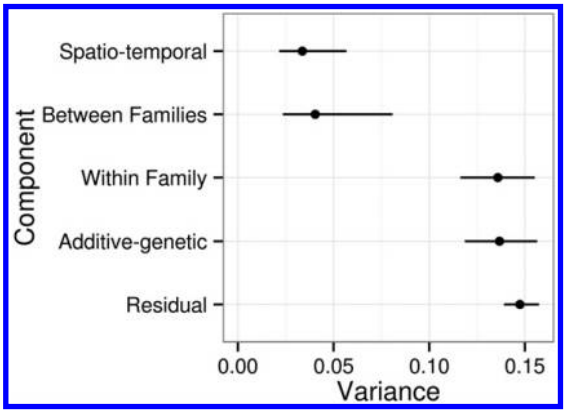

Fig. 5. Posterior modes and 95% highest posterior density credible intervals of the variance components for crown dieback (CD) (transformed: TCD).

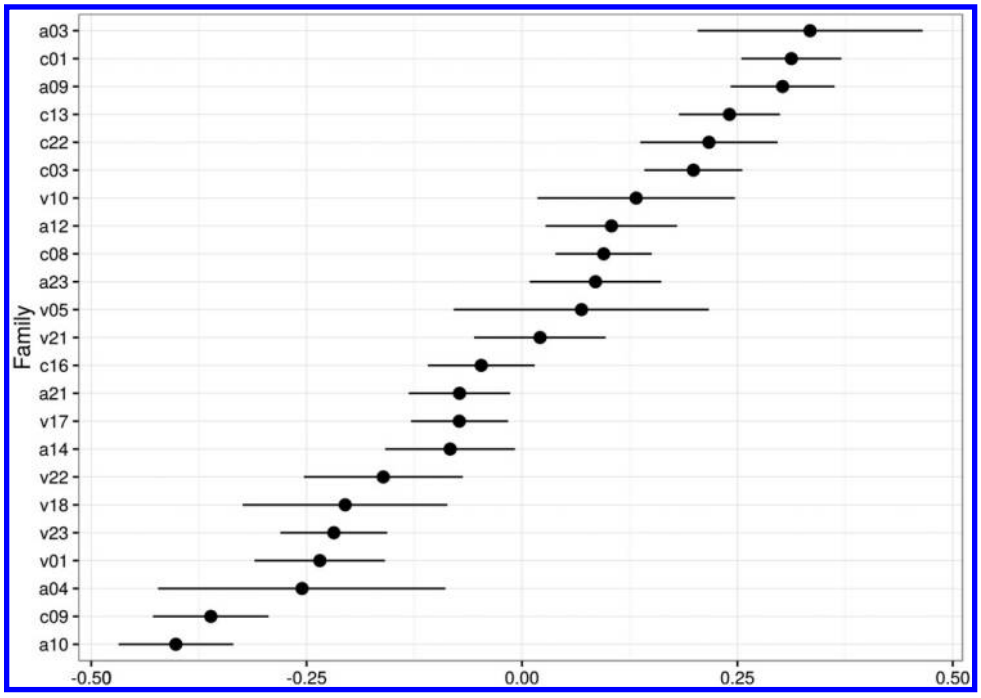

Fig. 4. Posterior means and 95% credible intervals for the family effects for crown dieback (CD) (transformed: TCD; families on the left are the most susceptible).

rest, as a predictor of the phenotype. This is related with the heritability which is presented below.

The ST component displayed very clear trends (Fig. 8), particularly for the binary component, with the bottom rows showing a higher-than-average probability of remaining uninfected in both 2012 and 2013. Similarly, once infected, trees in the top rows were likely to display higher CL values than average. The patterns for both years were very similar, in accordance with the high mean posterior interannual correlation estimates: 0.82 and 0.89 for the binary and continuous components respectively.

In contrast to the model for TCD, the prior distribution played a more significant role in the predicted ST effect, particularly for the binary component (Supplementary Fig. S6). This was expected, as spatially-distributed binary data are very weakly informative.

The mean posterior narrow-sense heritabilities (h^2) of CL were 0.49 and 0.42 for the binary and continuous components, respectively, with a slightly wider 95% credible interval for the binary (0.32 to 0.64) than for the continuous (0.30 to 0.57) components. Excluding the ST variance from the denominator, the posterior mean and credible interval for the heritability of the binary component increases to 0.60 (0.48 to 0.71).

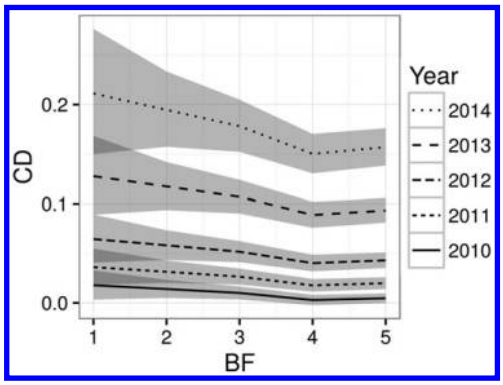

Fig. 6. Posterior mean (lines) and 95% credible intervals (shades) of bud flush precocity (BF) effect on crown dieback (CD).

Genetic correlation between both symptoms. A moderate negative correlation was observed at the genetic level between TCD and CL. The individuals with highest genetic merit for TCD (i.e., those who are predicted genetically better suited to resist the disease symptom in the crown) tend to have higher probability of avoiding CL (r = 0.30, 95% CI: 0.25 to 1), and lower predicted genetic sensitivity to it, in the case of infection (r = –0.40, 95% CI: –1 to –0.34) (Fig. 9).

DISCUSSION

Disease progress and mortality. Only 3% of the trees died 4 years after the disease was detected in the experiment. Mortality rates ranging from 2 to 70% have been reported elsewhere (Enderle et al. 2013; Lobo et al. 2014; McKinney et al. 2011, 2014; Metzler et al. 2012; Pliūra et al. 2011, 2014; Stener 2013), but very few studies allow to relate this rate to the time of exposure to the disease. Only three previous studies allow for comparisons, either because the trees started to be monitored for the disease right after planting or because initial proportions of symptomless trees suggest that the epidemics started recently. Two of these studies reported higher mortality rates despite similar times of exposure to the disease (9% in Enderle et al. 2013, 10% in Pliūra et al. 2011). However, they both analyzed younger trees (10 and 8 years old, respectively) and it is commonly accepted that there is a negative correlation between susceptibility to *H. fraxineus* and tree age (Skovsgaard et al. 2010). By contrast, another study (Pliūra et al. 2014) reported a lower rate of 2% on very young trees (3 years old), but it was measured only 1 year after planting and on grafted material selected for resistance to the disease.

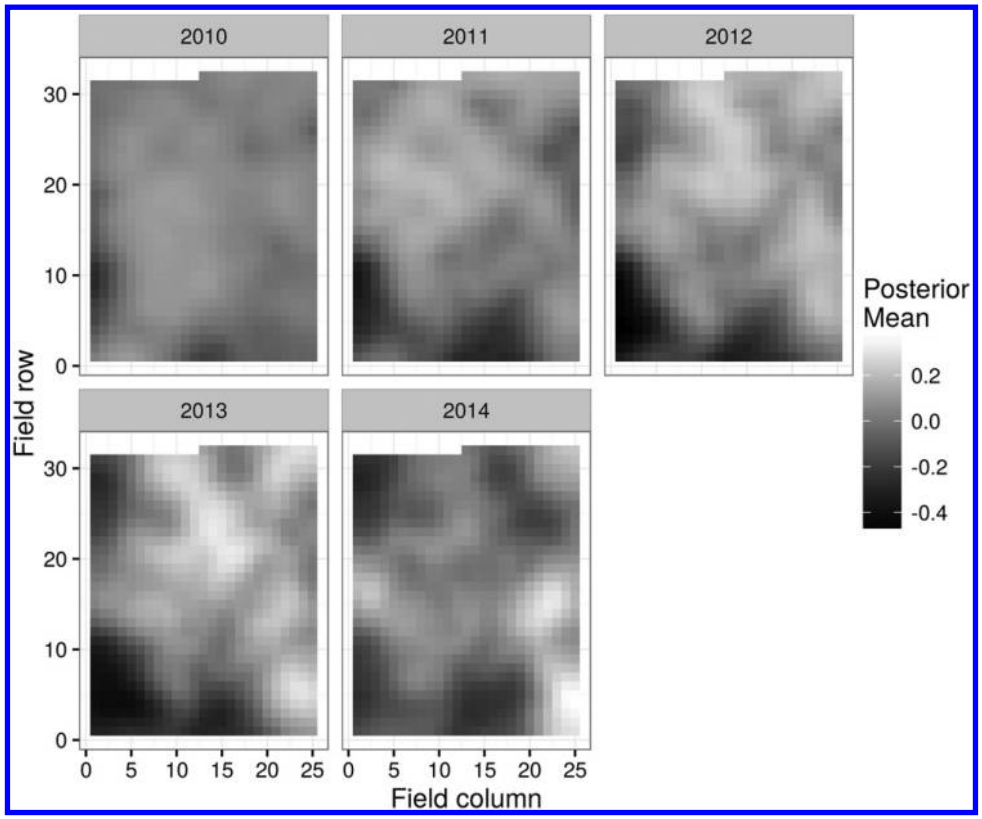

Fig. 7. Posterior mean spatiotemporal Gaussian fields for crown dieback (CD) (transformed: TCD, low values refer to high susceptibility).

Looking at CD only yields a proportion of symptomless trees of 6% in 2014. However, already in 2013, more than 50% of the trees without visible CD had CL. This result is in agreement with observations from Enderle et al. (2013) who found that 15% of otherwise healthy trees were affected by CL. Removing trees without visible CD in 2014 but on which CL were found the year before leads to a rate of symptomless trees of 4% only. Proportions of symptomless trees reported in the literature range from 1% (Lobo et al. 2014) to 58% (Kirisits et al. 2012). Again, comparisons should be made on trees of the same age that underwent similar exposure to the disease and on which monitoring of the disease was not restricted to the crown.

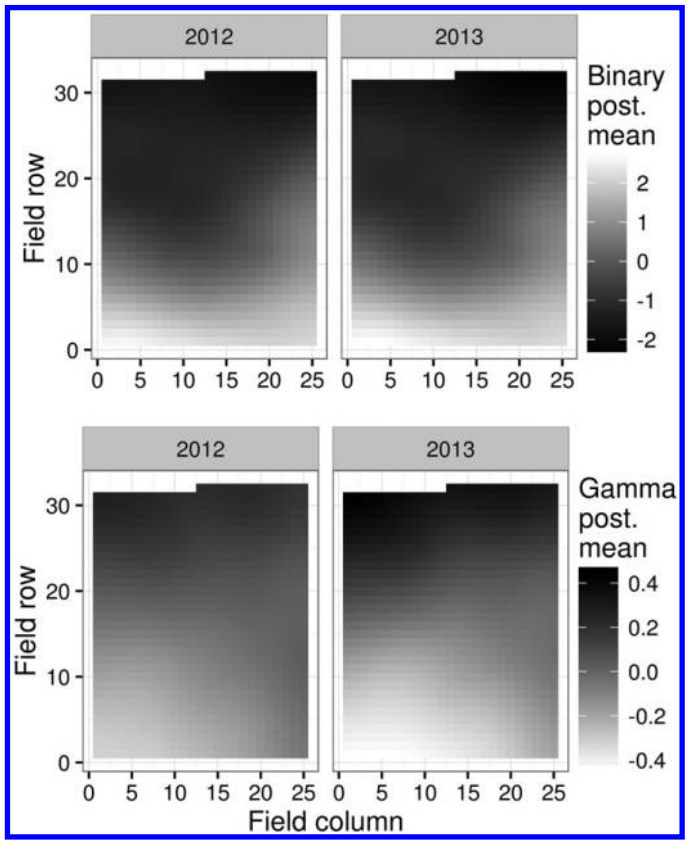

Fig. 8. Posterior mean spatiotemporal Gaussian fields for the binary component (top) and the continuous component (bottom) of the model for collar lesions (latent scale: low values correspond to low susceptibility).

Two studies at least have reported health improvement on some trees (Lobo et al. 2014; Stener 2013). Indeed, a few percent of negative values were found for interannual increments for both CD and CL in the present study. A recovery process clearly acts for CD, when secondary and epicormics shoots are produced in reaction to the disease. By contrast, apparent remission in terms of CL is most certainly artifactual. Indeed, at early stages, CL are often found below ground level and can be missed very easily.

Collar and crown symptoms: Different infection pathways but some common genetic control. In 2013, high proportions of trees showing only one of both symptoms demonstrate that they can occur independently. Nevertheless, a trend for a positive relationship between CD intensity and CL prevalence was observed both in 2012 and in 2013. This result is consistent with previous findings (Bakys et al. 2011; Enderle et al. 2013; Skovsgaard et al. 2010).

Severities of both traits were also significantly positively correlated. Correlation at the phenotypic level has already been demonstrated by Bakys et al. (2011) who reported very high Pearson correlation coefficients (>0.57) between CD and several quantitative measurements of CL, and also by Husson et al. (2012) who reported Spearman correlation coefficients ranging from –0.2 to 0.7 in 60 natural plots. These last authors reported a tendency for higher correlation coefficients to happen in field plots with high overall mean CL. Using PCR assays, they also investigated the possibility that collar and branch lesions may be connected and did not find any evidence to support this hypothesis. Instead, they concluded on separate infection pathways with ascospores potentially infecting the stem base via lenticels in the bark. Both infection pathways certainly require different environmental conditions as the ST Gaussian field revealed higher susceptibility in the upper part of the field experiment for CL whereas it was the reverse for CD. The role of secondary parasites like

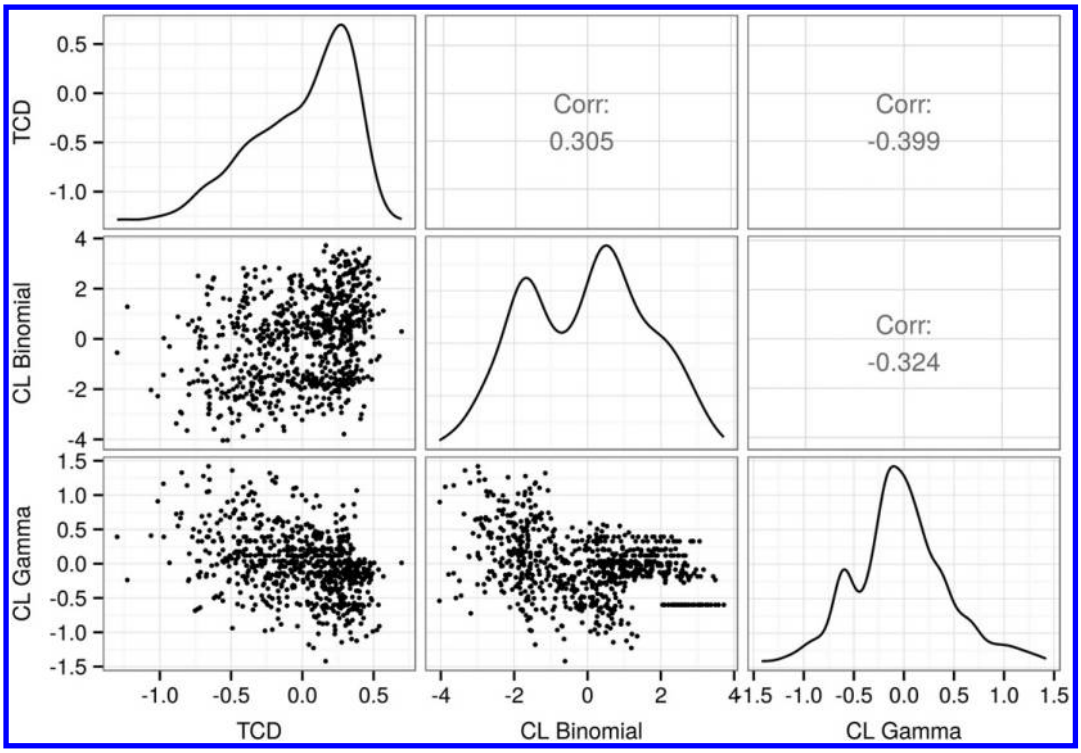

Fig. 9. Correlation between individual breeding values for crown dieback (CD) (transformed: TCD, low values refer to high susceptibility) and the binary (low values refer to high susceptibility) and continuous (low values refer to low susceptibility) components of collar lesions (CL).

Armillaria sp. however requires further investigations as they have been shown to occur at high frequency in ash CL (Bakys et al. 2011; Husson et al. 2012; Lygis et al. 2005; Skovsgaard et al. 2010), thus undoubtedly contributing to the continuous component of CL.

More important, this study is first to go beyond phenotypic correlations and to report on genetic correlations between CD and CL. The computed correlation coefficients are moderate but higher than those computed at the phenotypic level. Partly overlapping (i.e., pleiotropy) or clustered (i.e., linkage) genetic determinisms thus occur which are blurred by different infection processes and environmental requirements.

Genetic components: Their magnitude and how to estimate them properly. Only three studies explored the genetic variability of *F. excelsior* using open-pollinated progenies before this one (Kjær et al. 2012; Lobo et al. 2014; Pliūra et al. 2011). Among them, only one (Pliūra et al. 2011) found significant provenance effects. This outlying result may be due to the fact that these authors studied a large number of provenances (24) covering a large distribution range across Europe. However, as stated by the authors, the observed provenance effect may simply originate from the fact that local (Lithuanian) provenances had undergone natural selection by the pathogen before mother trees were selected whereas other European provenances did not. Other studies, including this one, involved fewer provenances, all of local origins, and did not conclude on significant provenance effects.

All three previous studies concluded on significant family effects and this is confirmed here. However, and because the methodology involves both familial (=mother tree) and individual (=tree) breeding values, the present study is first to be able to demonstrate that most of the genetic variation lies within families.

All estimated narrow-sense heritabilities can be considered as moderate. The one for CD (0.42) falls within the 0.20 to 0.49 range reported by Kjær et al. (2012) and Lobo et al. (2014). Pliūra et al. (2011) computed a much higher (i.e., 0.92) estimate which was based on a global health assessment and was apparently overestimated due to early frosts. For CL, h^2 estimates (0.49, binary component; 0.42, continuous component) cannot be compared with any data from the literature as this is the first time genetic variance components are estimated for this trait. Although moderate, all these heritability estimates are much higher than those reported in *Ulmus* for resistance to *Ophiostoma novo-ulmi* (i.e., 0.14 ± 0.06) (Solla et al. 2015), especially when considering that h^2 was estimated at the intraspecific level here whereas the figures in *Ulmus* come from a mating design involving not only the European *U. minor* species but also the Central-Asian species *U. pumila*.

Interestingly, the methodology implemented here allowed generating h^2 estimates with fairly narrow credible intervals. We believe such procedure (i.e., explicit ST modeling under a Bayesian framework) should be used whenever possible, especially when infection started recently, meaning that (i) measured traits are not normally distributed due to a high proportion of asymptomatic or low infected trees and (ii) disease escape can be confounded with resistance.

Early flushers perform better. There are several indications that health status in the presence of *H. fraxineus* correlates positively with early bud flushing but also with early leaf senescence and leaf coloring in the autumn (Bakys et al. 2013; McKinney et al. 2011; Pliūra and Baliuckas 2007; Stener 2013). A positive relationship was found between early BF and better health status (CD) in the present study also. Although inoculation assays conducted by McKinney et al. (2012a) and Lobo et al. (2015) provided evidence that heritable stricto sensu resistance mechanisms occur in *F. excelsior*, some of the variability observed in the present study may thus come from phenological features leading to what should maybe termed "disease avoidance." A causal correlation between leaf senescence and health status is easy to explain because senescence and subsequent shedding can prevent penetration of the fungus into the branch. The correlation with BF is more difficult to understand, except if early bud flush correlates with early leaf shed. To our knowledge, no one has ever investigated this relationship in common ash.

Implications for disease management and breeding. The present study adds to the common perception that complete resistance to *H. fraxineus* is very rare or absent in *F. excelsior* but that there is significant variability for partial resistance and that it is heritable. Whether partial resistance is preferable to complete resistance will not be discussed here, but partial resistance is not always a guarantee of durability (Dowkiw et al. 2010). Most importantly, confirming that there is heritable genetic variation for susceptibility in *F. excelsior* is a prerequisite to save the species without introgressing resistance genes from exotic species like *F. mandshurica*, the supposed co-evolved host species of *H. fraxineus*.

Regarding natural regeneration, significant genetic variation for susceptibility also raises hopes that natural selection can occur and give rise to resistant populations. This optimistic scenario may however not come true if (i) population sizes are low and/or (ii) juvenile-adult correlations for resistance are weak. These correlations have not been evaluated at the individual level yet, but juvenile trees are known to be more susceptible to the disease (Skovsgaard et al. 2010).

For planting, high levels of intrafamilial variation suggest that seed material from selected parents planted in seed orchards would require further selection. This could be done either in the nursery or directly in the field if planting is done at higher density than usual. Although not common for ash (except for ornamental trees), clonal selection would certainly allow higher and more immediate genetic gain. However, several questions would then arise. First is that of the number of clones to release to ensure sufficient genetic variability, not only to avoid resistance breakdown but also to cope with future threats like the emerald ash borer (*Agrilus planipenis*) and to combine ash dieback resistance with other traits of interest. Second is that of the techniques to use to propagate the selected ash clones and the associated costs. *F. excelsior* is much easier to propagate through grafting than through cuttings. Genetic correlations between CD and CL being moderate, it is necessary considering two separate selection schemes, one for rootstocks (based on CL) and one for grafts (based on CD). Ash propagation through cuttings would certainly deserve more investigation should clonal selection be considered. We believe it is important to note also that selection for early flushing in order to improve resistance would have some drawbacks given the high susceptibility of ash's terminal bud to late frosts. It is important to consider also that all genetic variance estimates were measured at a given point in time and in a given environment. *H. fraxineus* having a sexual stage, new variants of the pathogen appear each year and thus adaptation in the pathogen's populations can occur.

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