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Rasmus Enderle, Aikaterini Nakou, Kristina Thomas, Berthold Metzler. Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. *Annals of Forest Science*, 2015, 72 (2), pp.183-193. 10.1007/s13595-014-0413-1 . hal-01284165

HAL Id: hal-01284165

<https://hal.science/hal-01284165>

Submitted on 7 Mar 2016

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Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined

Rasmus Enderle · Aikaterini Nakou · Kristina Thomas · Berthold Metzler

Received: 4 March 2014 / Accepted: 10 July 2014 / Published online: 30 July 2014
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Abstract

• **Context** It might be possible to establish a new generation of *Fraxinus excelsior* which is insusceptible towards ash dieback (agent: *Hymenoscyphus pseudoalbidus*) by efficient breeding. However, a considerable number of highly tolerant individuals which have the ability to pass on this trait to their progeny are needed.

• **Aims** The aim of this study was to identify the potential of provenances from southwestern Germany as a source of future selection for resistance or resistance breeding.

• **Methods** In July 2012 and 2013, ash dieback severity was scored by assessing the crown defoliation and the portion of epicormic shoots in the crowns in clonal seed orchards with a total of 1,726 ash trees in southwestern Germany.

• **Results** Ash dieback severity differed strongly between the orchards and the clones. Broad-sense heritability ranged from 0.18 to 0.55 for crown defoliation and from 0.48 to 0.58 for the portion of epicormic shoots between the orchards. Clones

from nearby populations did not show differences in general levels of susceptibility.

• **Conclusion** The study highlights that there is high genetic variation in susceptibility and considerable genetic potential for resistance breeding in provenances from southwestern Germany.

Keywords *Fraxinus excelsior* · *Hymenoscyphus pseudoalbidus* · Ash dieback · Clonal seed orchards · Heritability · Tree breeding

1 Introduction

The ascomycete *Hymenoscyphus pseudoalbidus* (anamorph: *Chalara fraxinea*) is the causal agent of ash dieback (Kowalski 2006; Kowalski and Holdenrieder 2009; Queloz et al. 2011), a disease on *Fraxinus excelsior* L. which seriously endangers the silvicultural utilization of this tree species in Europe. According to present knowledge, the pathogen is an invasive species in Europe which has been introduced from Far East Asia (Husson et al. 2011; McKinney et al. 2012; Zhao et al. 2012; Gross et al. 2014). The disease causes dieback of twigs, leading to defoliation of the crowns. Depending on individual tree vigour and susceptibility, the loss of twigs can be partially compensated for a certain period of time by formation of epicormic shoots.

Strong differences in susceptibility towards the disease have been observed between individual ash trees. A small fraction of trees seems to be partially resistant to the disease, which means that the resistance is of quantitative or partial nature (McKinney et al. 2011; Kjær et al. 2012; McKinney et al. 2012; McKinney et al. 2014). Studies on clones and families from Denmark, Lithuania, Austria and Sweden have shown that susceptibility is genetically determined (McKinney

Handling Editor: Cécile Robin

Contribution of the co-authors Rasmus Enderle: conception of the study, responsible for the data collection, data analyses except modelling and writing the manuscript.

Aikaterini Nakou: constructing and fitting the model.

Kristina Thomas: supervising the seed orchards of Rheinland-Pfalz and contributing to the data collection.

Berthold Metzler: supervising the study and contributing to the data collection.

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et al. 2011; Pliūra et al. 2011; Kirisits and Freinschlag 2012; Kjær et al. 2012; Stener 2013). Moreover, differences in ash dieback severity have been observed between provenances in Lithuania and Germany (Pliūra et al. 2011; Metzler et al. 2012; Enderle et al. 2013). Probably, evolutionary adaptation and selection by foresters will slowly decrease the degree of susceptibility in the ash populations. Resistance breeding programmes may enable the establishment of a future generation of ashes of low susceptibility within a comparatively short period of time. However, a crucial point in resistance breeding is that a considerable number of individuals of low susceptibility need to be involved in order to preserve an adequate genetic diversity in the ash populations (Douglas et al. 2013; Pautasso et al. 2013). Genetic diversity is necessary if populations are to adapt on environmental changes such as new pests. In the case of *F. excelsior*, a particular future threat could be the emerald ash borer (*Agrilus planipennis*), a beetle which killed tens of millions of ash trees in North America (Poland and McCullough 2006) and is spreading westwards from the region of Moscow (Straw et al. 2013). Thus, it is very important to identify ash individuals of low susceptibility and of different provenances to get a better knowledge of the fraction of trees which could be suitable for breeding programmes.

An additional symptom associated with ash dieback is collar rot (Lygis et al. 2005; Skovsgaard et al. 2010; Bakys et al. 2011; Orlikowski et al. 2011; Husson et al. 2012; Enderle et al. 2013). *H. pseudoalbidus* is a colonizer of these basal lesions (Husson et al. 2012), but also, *Armillaria* spp. can often be detected on affected ash collars. According to present knowledge, the resistance to infections in the crown does not prevent from collar rot affection, although prevalence of this symptom is lesser in more resistant trees (Enderle et al. 2013).

Ash trees cover 4.2 and 1.0 % of the total forest area in the German federal states Baden-Wuerttemberg and Rheinland-Pfalz, respectively (Federal Forest Inventory II, Kändler, personal communication). The summed up area of stands in which ash dieback led to noteworthy economic losses in 2012, as stated by regional forestry offices, was 633 ha in Rheinland-Pfalz and 11,451 ha in Baden-Wuerttemberg (Delb et al. 2013a, b).

In this study, ash dieback has been surveyed in four clonal seed orchards in Baden-Wuerttemberg and Rheinland-Pfalz to investigate (i) the general situation of ash dieback and its development, (ii) the interdependency between crown defoliation and the formation of epicormic shoots, (iii) differences in susceptibility between subprovenances of southwestern Germany and (iv) the heritability of susceptibility. The overall aim was to develop a basis of estimation regarding the potential of autochthonous provenances in seed orchards in southwestern Germany as a source of resistant seeds and of future resistance breeding.

2 Material and methods

2.1 Investigated seed orchards

Surveys were carried out in four different clonal seed orchards (Fig. 1). The properties of the seed orchards are presented in Table 1. The orchards comprise grafts of plus-trees which were selected for growth and stem quality prior to the outbreak of the disease. The plus-trees originated from different regions (Fig. 1, Table 1). However, according to the German forest species provenance system, all trees planted in one of the seed orchards, with the exception of Schorndorf, belong to the same provenance. This is why henceforth we refer to the regions of origins as subprovenances. Four subprovenances were present concurrently on the orchard of Kusel and Landstuhl (Fig. 1), but not the same genotypes of these subprovenances were used on the orchards. In average, there were 5.9 clones per subprovenance (min., 1; max., 26). In the orchard of Kusel, the ramets were planted in 13 blocks; in the orchard of Landstuhl, there were ten blocks. Because some of the trees had been removed or excluded from the analyses or had died, the blocks were not complete. In 2012, the mean number of genotypes per block was 57 (standard deviation, 15.7) in the orchard of Kusel and 38.2 (standard deviation, 4.4) in the orchard of Landstuhl.

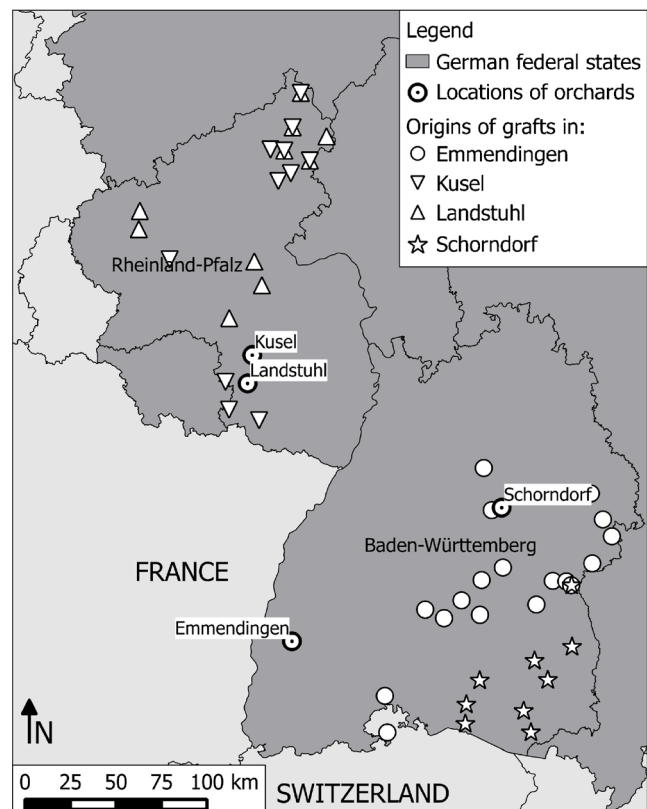


Fig. 1 Locations of the seed orchards and regions of origin of the grafts (subprovenances) planted in the orchards

Table 1 Properties of the investigated seed orchards in 2012

Properties	Seed orchards				Total
Location	Emmendingen	Kusel	Landstuhl	Schorndorf	–
Federal state	Baden-Wuerttemberg	Rheinland-Pfalz	Rheinland-Pfalz	Baden-Wuerttemberg	–
Provenance ^a	81107	81104	81104	81107/81108	–
Year of establishment	1995	1991/93	1992	1992	–
Design	Random	Random blocks	Random blocks	Random	–
Spacing [m×m]	Flexible (7.5×10 to 15×15)	7×7	7×7	7×7	–
Relative stand homogeneity	High	Medium	Low	Low	–
Elevation (m above sea level)	200	400	400	400	–
Stand canopy cover	Open	Open	Open to dense	Open to dense	–
Number of subprovenances	17	11	10	9	43
Number of clones	50	76	51	69	246
Status number (N_s)	42.4	73.4	48.0	60.4	–
Sum of ramets	218	741	382	385	1,726

^a Numbers according to the German forest species provenance system. 81104: Western German mountain area; 81107: Southern German hill and mountain area; 81108: Alps and alpine foothills

Figure 2 shows the distribution of the number of ramets per clone in the orchards. As a measure of the level of imbalance due to the unequal number of ramets per clone, the status number N_s as described by Kjær and Wellendorf (1997) and Lindgren and Mullin (1998) is presented in Table 1. All trees were examined for grafting success, and trees which exhibited indications of unsuccessful grafting, or trees of which the graft union was not clearly visible, were excluded from the survey.

2.2 Data collection

Data collection in the seed orchards took place in July and early August 2012. During this time of year, there is a maximum of foliage, as the formation of Lammas shoots is completed and premature leaf fall has not started. In the orchards of Kusel and Landstuhl, the surveys were repeated in July 2013. Because of the construction of a gas pipe in the orchard of Kusel, 42 ash trees had to be removed during the winter 2012/2013, and these trees were not included in the analyses of the data of 2013. The removals of trees led to a minimal reduction of the status number from 73.4 in 2012 to 73.2 in 2013.

Trees were divided into the following classes of crown defoliation: class 1=1–10 %, class 2=11–25 %, class 3=26–60 % and class 4=61–99 % crown defoliation. Trees without any visible symptoms of ash dieback were assigned to class 0. Crown defoliation has often been used in recent studies to quantify the degree to which a tree is affected by the ash dieback disease (e.g. McKinney et al. 2011; Husson et al. 2012; Kirisits and Freinschlag 2012; Lenz et al. 2012). However, some trees were highly affected by ash dieback but showed a relatively low degree of crown defoliation due to

extensive formation of epicormic shoots (Fig. 3). On the other hand, some trees were highly defoliated but showed only moderate symptoms of ash dieback. Thus, in order to record the degree of disease affection, trees were additionally divided into classes of the estimated portion of epicormic shoots in the crowns, using the same division of classes as for crown defoliation. We defined epicormic shoots in a broad sense, also including small side twigs that substitute for affected terminal shoots of branches. This could be accomplished easily by observing the crowns from opposite sides.

The assessment was carried out by the same person for the orchards in Emmendingen and Schorndorf, whereas three teams consisting of two people each assessed the trees in Kusel and Landstuhl. Prior to the

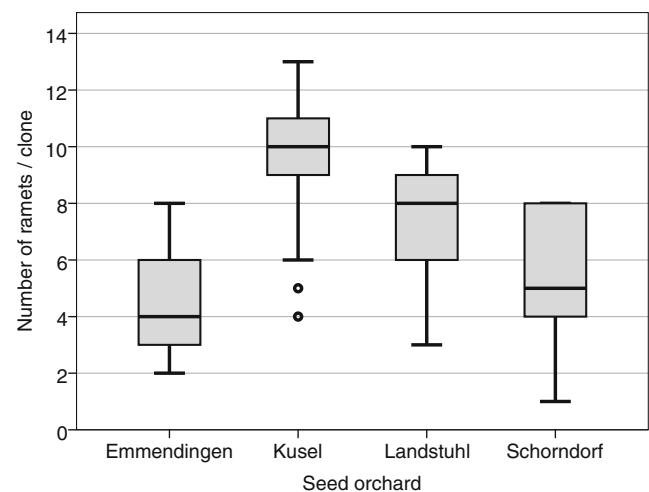


Fig. 2 Distribution of the number of ramets per clone on the four seed orchards



Fig. 3 This tree on the seed orchard of Kusel is highly affected by ash dieback. The proportion of epicormic shoots in the living crown exceeds 60 %. However, due to the excessive formation of epicormic shoots, the inner crown is densely foliated and only the very outer rim is defoliated. The tree was assigned to crown defoliation class 2 (11–25 %)

survey, these teams synchronized assessment by rating about ten trees jointly with the person who assessed the other orchards in order to ensure the comparability of the data.

The individual health condition of the trees might be influenced by collar rots. In order to take this issue into account, every rootstock was examined for the presence or absence of collar rots. The trees showing collar rots were kept in the following analyses, as this symptom is closely connected with the ash dieback disease. No further diagnostics on the collar rots were conducted in order to remain the trees unaffected from artificial influences since further observations of the development of the disease in the seed orchards are foreseen.

2.3 Statistical analyses

In data analyses, the median of the range of the classes of crown defoliation and epicormic shoots was used (class 0, 0 %; class 1, 5 %; class 2, 18 %; class 3, 43 %; class 4, 80 %). The traits for the orchards in Kusel and Landstuhl were analysed by applying the following generalized linear mixed model (GLMM) including Markov chain Monte

Carlo techniques using the MCMCglmm package for R (Hadfield 2010):

$$Y_{ijk} = \mu + c_i + p_j + b_k + p_j : b_k + \varepsilon_{ijk}$$

where Y_{ijk} is the value of the variable (crown defoliation or epicormic shoots), μ is the grand mean, c_i is the random effect of the clone i that was nested in p_j , the random effect of the subprovenance j , b_k is the fixed effect of the block k ; $p_j : b_k$ is the interaction effect of p_j and b_k and ε_{ijk} is the residual of the plot ijk . The model was run with 10,000 iterations. Based on the nature of the database at disposal (large number of data, rather complex structure), we choose an uninformative type of prior (default prior in the MCMCglmm Package). For both orchards and both years, the interaction effect of subprovenances and blocks was not significant. Thus, the model was run again without the interaction effect. The same model, but without the fixed effect of blocks (b_k), was applied for the other orchards, which were planted in a random design. Q–Q plots were examined to ensure that the distribution of the residuals was a close approximation of a normal distribution $N(0, \sigma^2_\varepsilon)$. The convergence of the Markov chains was verified by examining trace plots.

In order to estimate the degree to which the phenotypic variation is due to genetic factors, broad-sense heritability (H^2) was calculated. The broad-sense heritability is the genetic variance divided by the phenotypic variance: $H^2 = \sigma^2_g / \sigma^2_{ph}$ (e.g. Visscher et al. 2008). To obtain σ^2_g , the variance and covariance components due to clones and the variance and covariance components due to subprovenances were added up. In order to assess how the phenotypic mean may change by adaptation (evolvability), the coefficient of genetic variation (CV_G) was calculated as the square root of σ^2_g divided by the total mean of the trait (Houle 1992). The genetic correlation between the two traits crown defoliation and portion of epicormic shoots (the genetic covariance divided by the square root of the product of the clonal variance components of the two traits) was estimated by computing the arithmetic means of the coefficients (Spearman's rho) obtained by correlation per clone. Genetic correlations between the years of data collection were computed in the same way.

3 Results

There were considerable differences in ash dieback severity between the different orchards. Ash dieback was less severe in the two orchards in Rheinland-Pfalz, Kusel and Landstuhl.

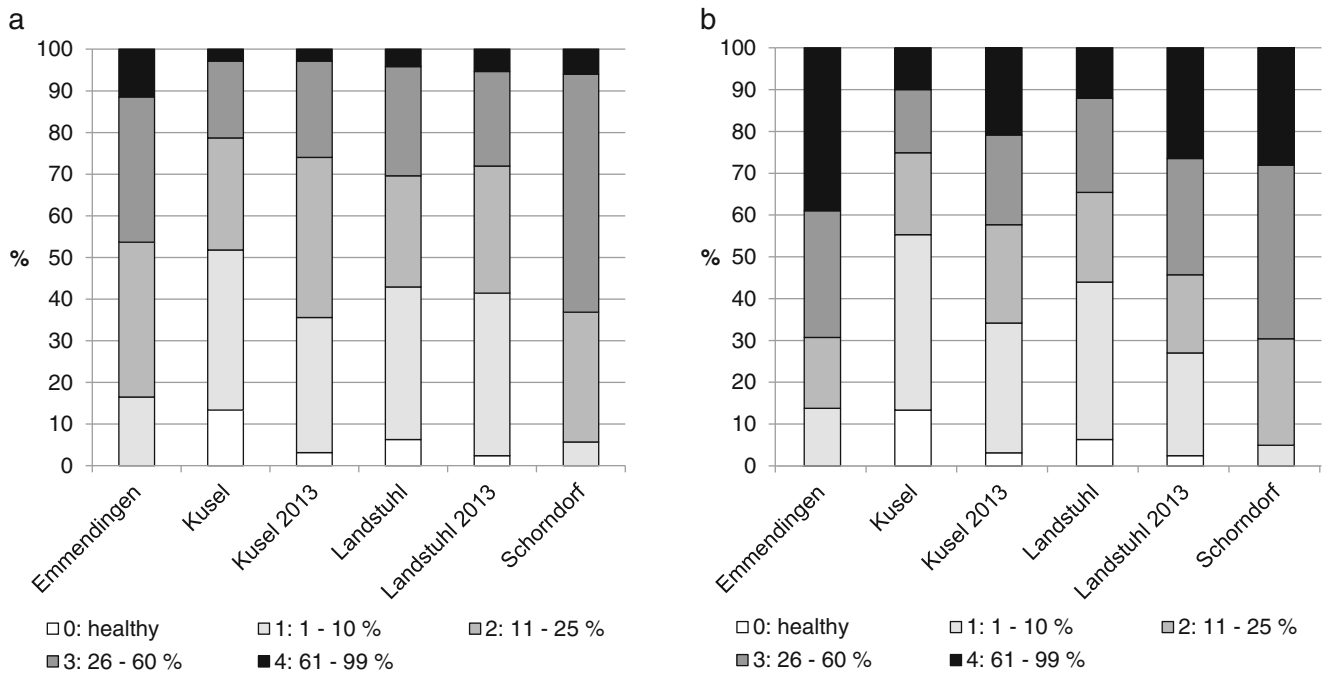


Fig. 4 Proportion of trees in classes of crown defoliation (a) and portion of epicormic shoots (b) for the four orchards in 2012 and 2013

Figure 4a, b shows the proportion of trees in the classes of crown defoliation and epicormic shoots for the four orchards. In 2012, the proportion of trees in crown defoliation class 4 was rather small, ranging from 3.1 % in Kusel to 11.5 % in Emmendingen. In the orchards of Emmendingen and Schorndorf, there was no single completely healthy tree, whereas no symptoms could be detected on 5.9 % of the trees in Landstuhl and 13.1 % of the trees in Kusel. There were no completely symptomless clones. In total, the percentage of epicormic shoots was higher than that of crown defoliation (Fig. 4a, b; Table 2). The proportion of trees belonging to epicormic shoots class 4 ranged from 10.0 % in Kusel to 39.0 % in Emmendingen. The proportion of trees of epicormic shoots class 1 was quite similar to the proportion of trees of crown defoliation class 1. The comparison of the data from 2012 with the data from 2013 shows a substantial decrease of the proportion of symptomless trees. Additionally, the proportion of trees in the class 4 of epicormic shoots roughly doubled, whereas the proportion of trees in the class 4 of crown defoliation remained more or less constant (Fig. 4a, b).

The fixed effects of blocks in the orchards of Kusel and Landstuhl were significant in both years for both variables (see Appendix for detailed listed significance). Moreover, all variance components of the MCMCglmm were significant since the respective credible intervals are disjunct from zero (Table 2). However, compared to the variance due to clones and the residual variance, the response of the subprovenance

effect was very small in all seed orchards. Broad-sense heritability (H^2) and, in all cases but one, the CV_G were generally higher for epicormic shoots than for crown defoliation. Heritability differed more for the latter variable between the orchards. From 2012 to 2013, the H^2 values for crown defoliation had not changed markedly. In contrast, heritability for portion of epicormic shoots slightly increased in the orchard of Kusel and slightly decreased in the orchard of Landstuhl. The CV_G values for both traits decreased for both orchards.

On all seed orchards, crown defoliation was highly correlated with portion of epicormic shoots (Table 3). Coefficients were smaller for the seed orchards which were most affected by the disease (Tables 2 and 3). In accordance to this, the coefficients for 2012 were higher than the coefficients for 2013, when ash dieback became more severe. For all orchards but the orchard of Schorndorf, the coefficients of genetic correlation were smaller than the coefficients of the correlation of clonal means.

Moreover, there were high correlations between the years 2012 and 2013 for the two traits in the two orchards with repeated assessments (Table 4). The coefficients of genetic correlations were smaller than the coefficients obtained from correlation of clonal means and were approximately the same for the two traits. Genetic correlation was higher in the orchard of Landstuhl compared to the orchard of Kusel. The correlation of clonal means was slightly higher for the portion of epicormic shoots than for crown defoliation.

Prevalence of collar rots in 2012 was 3.6 % in Kusel and 10.9 % in Landstuhl. In Emmendingen and

Table 2 Mean values, variance components and genetic estimates for the two traits crown defoliation and portion of epicormic shoots separated by orchards and years

Location year	Emmendingen 2012	Kusel 2012	Kusel 2013	Landstuhl 2012	Landstuhl 2013	Schorndorf 2012
Crown defoliation						
Mean [%]	31.7	17.0	20.8	21.2	21.5	35.2
σ_c^2	203.3	128.6	101.5	94.6	93.2	56.0
95 % CI (σ_c^2)	72.3–365.5	86.0–175.5	65.6–144.1	44.7–153.1	49.0–144.8	23.0–88.9
σ_p^2	124.8	0.5	1.3	9.5	0.0	0.1
95 % CI (σ_p^2)	1.3E–05–370.8	7.7E–17–0.215	8.4E–17–8.072	5.6E–14–55.75	4.4E–17–3.8E–03	6.7E–17–0.037
σ_ε^2	263.5	195.6	187.0	284.4	252.9	246.7
95 % CI (σ_ε^2)	212.5–323.0	174.9–216.2	165.3–207.6	239.9–329.8	213.3–292.2	208.1–282.5
H^2	0.55	0.36	0.35	0.25	0.24	0.18
95 % CI (H^2)	0.38–0.71	0.30–0.49	0.26–0.45	0.14–0.37	0.17–0.38	0.09–0.28
CV _G [%]	57.2	66.9	48.8	48.0	44.9	21.3
Portion of epicormic shoots						
Mean [%]	48.0	20.1	31.7	25.1	37.8	45.1
σ_c^2	479.0	281.8	468.8	318.5	421.5	293.3
95 % CI (σ_c^2)	276.7–713.4	182.2–389.1	320.7–632.5	186.6–476.1	243.0–618.0	179.7–419.2
σ_p^2	1.0	0.7	2.5	0.8	1.8	6.5
95 % CI (σ_p^2)	1.2E–16–2.902	8.7E–17–2.07	7.6E–17–7.706	1.0E–16–0.304	4.5E–17–2.168	4.5E–17–37.89
σ_ε^2	369.2	294.2	341.1	238.3	405.2	316.6
95 % CI (σ_ε^2)	295.2–449.0	263.4–326.7	304.8–380.3	205.2–276.4	351.1–477.4	267.1–365.6
H^2	0.58	0.49	0.57	0.58	0.48	0.49
95 % CI (H^2)	0.43–0.69	0.39–0.58	0.49–0.66	0.44–0.68	0.39–0.63	0.37–0.59
CV _G [%]	45.7	83.6	68.5	71.3	54.5	38.4

σ_c^2 : variance component of clones, σ_p^2 : variance component of subprovenances, σ_ε^2 : residual variance, H^2 : broad-sense heritability, 95 % CI (): respective credible interval, CV_G: coefficient of genetic variation

Schorndorf, no collar rots could be detected. In 2013, collar rot prevalence increased to 8.2 % in Kusel and 16.9 % in Landstuhl. Differences in mean crown defoliation and mean portion of epicormic shoots between trees with and without collar rots are presented in Table 5. The difference in crown damage in 2013 was clearer when the trees which were already affected by collar rots in 2012 were compared to the unaffected individuals.

Table 3 Coefficients of genetic correlation (r_g) and correlation of clonal means (r_c , Spearman's rho, with p values) between crown defoliation and portion of epicormic shoots

Seed orchard	Year	r_g	r_c	p
Emmendingen	2012	0.41	0.54	<0.001
Kusel	2012	0.69	0.89	<0.001
Kusel	2013	0.46	0.77	<0.001
Landstuhl	2012	0.67	0.90	<0.001
Landstuhl	2013	0.62	0.84	<0.001
Schorndorf	2012	0.44	0.40	=0.001

4 Discussion

In the present study, the potential of seed orchards in southwestern Germany as a source of breeding for resistance against *H. pseudoalbidus* was analysed by surveying the two traits crown defoliation and portion of epicormic shoots. The study reports the current fraction of potentially resistant trees in four seed orchards, provides H^2 and CV_G values of susceptibility and investigates the level of susceptibility of clones from different nearby populations. Further, it reveals novel insights in

Table 4 Coefficients of genetic correlation (r_g) and correlation of clonal means (r_c , Spearman's rho, with p values) between the years 2012 and 2013 for the two traits in the two orchards with repeated assessments

Seed orchard	Trait	r_g	r_c	p
Kusel	Crown defoliation	0.32	0.80	<0.001
Landstuhl	Crown defoliation	0.46	0.76	<0.001
Kusel	Portion of epicormic shoots	0.30	0.91	<0.001
Landstuhl	Portion of epicormic shoots	0.46	0.90	<0.001

Table 5 Mean crown defoliation and portion of epicormic shoots in 2013 of collar rot affected and unaffected trees in 2012 and 2013 (*p* values: Mann–Whitney tests)

	Without collar rot 2012	With collar rot	<i>p</i>	Without collar rot 2013	With collar rot	<i>p</i>
Mean crown defoliation [%]	21.1	25.9	0.048	21.2	23.0	0.166
Mean portion of epicormic shoots [%]	33.6	43.5	0.003	33.5	39.9	0.011

the interdependency between crown defoliation and the formation of epicormic shoots.

The H^2 and CV_G values (Table 2) indicate a relative strong genetic determinism of susceptibility to ash dieback in southwestern German populations. Possibly, the H^2 values of the present study are even to a small part underestimated, as it cannot be guaranteed that the upper part of all trees consisted of the scion due to grafting mistakes or resprouting of rootstocks. However, we believe that wrong genotype affiliation, if at all, had a marginal influence on the genetic estimates in this study since the grafting success was thoroughly examined several times during the first growing seasons of the orchards, and all trees which exhibited indications of unsuccessful grafting during the survey, i.e. when the graft union was not clearly visible, were excluded from the analyses. Moreover, the results of the present study are quite in agreement to former studies from northern Europe. McKinney et al. (2011) scored damage due to ash dieback by assessing the percentage of loss of crown foliage, which is comparable to crown defoliation, over a time of 3 years in two stands. They reported H^2 values ranging from 0.25 to 0.54 and CV_G values ranging from 38 to 87 %. From progeny tests in Denmark and Lithuania, quite similar results were reported for narrow-sense heritability (h^2), ranging from 0.37 to 0.52 and from 0.40 to 0.49, and for additive coefficients of variation (CV_A), ranging from 37 to 61 % and from 30 to 39 % (Pliūra et al. 2011; Kjær et al. 2012). Results of a Swedish study on two sites revealed H^2 values ranging from 0.07 to 0.57 and CV_G values ranging from 17 to 55 %, but these values are not directly comparable since they were calculated from ordinal data of ash dieback damage in different parts of the crowns (Stener 2013). As in the former studies (McKinney et al. 2011; Pliūra et al. 2011; Kirisits and Freinschlag 2012; Kjær et al. 2012; Stener 2013), no clone was completely unaffected. This confirms the suggestion that there is no genetically determined total resistance, although some genotypes exhibit a very low degree of susceptibility against ash dieback (Stener 2013). The observed decrease in the CV_G values from 2012 to 2013 can be

explained by the increase of the mean of the traits. Slight annual fluctuations of heritability could also be detected in other studies (McKinney et al. 2011; Kjær et al. 2012; Stener 2013).

Previous studies revealed high correlations between crown defoliation and disease intensity (Metzler et al. 2012; Enderle et al. 2013). However, other reasons for defoliation, such as abiotic factors, can also be important. Moreover, vigorous trees can countervail the loss of twigs as a result of ash dieback by forming epicormic shoots (Fig. 3). Thus, crown defoliation depends to a considerable degree on stand properties and micro-location and is rather an indicator of overall tree vigour than of susceptibility to ash dieback. This is why we believe that the portion of epicormic shoots in the crown, as defined in Section 2.2, is a better indicator of disease infection or susceptibility. Assessing the portion of epicormic shoots was not more time-consuming (after some time of practice) than assessing the crown defoliation. Although epicormic shoots are positively correlated with crown defoliation (Table 3), there are considerable differences in total values as well as in the estimates of H^2 and variance components (Table 2). The H^2 value for crown defoliation was highest in the orchard with the most homogenous stand conditions and considerably smaller in the orchards of relatively low stand homogeneity (Tables 1 and 2). This reflects the influence of the environment on the phenotype. The H^2 values for portion of epicormic shoots differ less between the orchards, indicating that the environment has less influence on this trait.

Assuming that the portion of epicormic shoots is an indicator of susceptibility and crown defoliation is an indicator of overall tree vigour, the positive correlations between the two traits imply that more susceptible trees are less vigorous and/or that trees of lesser vigour are more susceptible. The coefficients of genetic correlations and correlations from clonal means between the traits were higher on the orchards of relative low disease severity (Table 2 and 3 and Fig. 4), indicating that the interdependency between the two traits becomes lesser with increasing disease severity. The coefficients of genetic correlations were, in all orchards but the orchard of Schorndorf, smaller than the coefficients of correlations from clonal means. Apparently,

the interdependency between the traits is only partly due to genetic causes.

The highly significant correlations of clonal means between the years for crown defoliation and portion of epicormic shoots (Table 4) show that the relative rank in the level of susceptibility of the clones was more or less stable over the years, although the total values of these traits increased. This is in agreement with a Danish study, where crown damage was surveyed over a period of 3 years (McKinney et al. 2011). The genetic correlations between the years confirm this result, although they were smaller than the correlations of clonal means (Table 4).

The subprovenances in the orchards of the present study are spatially not far apart from each other (Fig. 1). In the model, the influence of the subprovenances was significant, but very small. Thus, susceptibility to ash dieback seems to not be determined by subprovenances of closely located origins. However, differences in susceptibility were detected in a provenance trial between provenances from southern Germany which differ more in their geographic origin (Enderle et al. 2013). Pliūra et al. (2011) reported differences in susceptibility between Lithuanian and western European provenances, which they traced back to processes of adaptation which had already commenced in the Lithuanian populations.

As expected, our data indicates a rapid increase of disease severity over time (Fig. 4). The number of trees without visible symptoms declined drastically, which could also be observed on other study sites in southwestern Germany. Apparently, new infections on hitherto healthy trees occur even after several years of strong infection pressure (Enderle et al. 2013), which complicate the estimation of the fraction of highly resistant individuals. However, a relative low degree of susceptibility can be assumed for the trees which have remained healthy for several years.

Ash dieback was considerably less severe in the orchards in Rheinland-Pfalz compared to the orchards in Baden-Wuerttemberg (Fig. 4). Since ashes and ash dieback are not as omnipresent here compared to Baden-Württemberg, there was probably less infection pressure on these sites and consequently lower disease incidence. In 2010, the area affected by ash dieback was 5 ha in Rheinland-Pfalz and 2,505 ha in Baden-Württemberg (Schröter et al. 2010a, b), so it seems that ash dieback was present earlier in Baden-Württemberg. Mean crown defoliation on four different sites of a provenance trial in Baden-Wuerttemberg ranged from 22 to 53 % in 2011 (Metzler et al. 2012), which is in agreement to the results of this study.

The data suggest that collar rot-affected trees are more defoliated and have a higher portion of epicormic

shoots than trees without collar rot, although the difference in crown defoliation in 2013 was not significant (Table 5). This might be due to reduced tree vigour in collar rot-affected trees. The difference in crown damage in 2013 was clearer when the trees which were already affected by collar rots in 2012 were compared to the unaffected individuals, indicating that the influence of collar rots increases with time. In another study, no influence of collar rots on radial increment could be detected, possibly because the phenomenon of collar rots was too recent to already have a measurable impact (Enderle et al. 2013). The following aspects complicate the interpretation of the present results: (i) the age of the necroses is unknown (although it appears likely that necroses formation took place within the last 4 or 5 years), (ii) the total lesion sizes and their extent in relationship to the diameter of the stems are unknown and (iii) the tissues of the crowns and of the trunk collars do not belong to the same genotype. Thus, we would like to refrain from drawing further conclusions from these results.

The present study shows that there is high genetic variation in susceptibility to ash dieback in the assessed clones. It highlights that there is a considerable genetic potential for future breeding for resistance in provenances of southwestern Germany. However, it is necessary to monitor the development of ash dieback in the next years to figure out if clones that appear resistant to *H. pseudoalbidus* at the moment will prove to stay unaffected over a longer period of time. The proportion of trees without any visible symptoms (class 0) or with only a small portion of epicormic shoots (class 1) is decreasing (Fig. 4). However, foresters are asked to preserve sound looking ash individuals in the forests (Metzler et al. 2013). Moreover, strong regeneration (up to 100,000 saplings/ha (Roloff and Pietzarka 1997)) is characteristic to *F. excelsior*, raising hope that a sufficient number of highly resistant trees will be available. Under this condition, we assume that it will be possible to establish an autochthonous ash generation with adequate genetic diversity and a high degree of resistance by conventional selection or, much faster, by tree breeding.

Acknowledgments Our thanks go to the Research Institute for Forest Ecology and Forestry of Rheinland-Pfalz for the kind support during the assessments and for providing their seed orchards. For their help during the surveys, we thank Johanna Bußkamp, Jonas Wehrle, Ricardo Rivero, Robert Krämer, Martina Stabel, Dietmar Weber and particularly Hermann Schott, who had good ideas for the development of the disease severity scoring system. We are grateful to Dr. Franziska Peters for revising the English and to anonymous reviewers for valuable suggestions improving the manuscript.

Funding The study was funded by the Forest Research Institute of Baden-Wuerttemberg.

Appendix 1

Table 6 Significance of the fixed effect of blocks for crown defoliation and epicormic shoots and for the orchards of Kusel and Landstuhl in the 2 years of observation

Site	Year	Block	Crown defoliation				Epicormic shoots			
			post.mean	l-95 % CI	u-95 % CI	pMCMC	post.mean	l-95 % CI	u-95 % CI	pMCMC
Kusel	2012	Intercept	30.3	21.8	38.9	<0.001	40.5	28.7	51.6	<0.001
		Block 2	-13.2	-21.9	-4.7	0.006	-15.5	-26.7	-3.2	0.016
		Block 3	-11	-19.8	-2.1	0.014	-15.9	-27.5	-4.4	0.008
		Block 4	-6	-14.7	3.3	0.22	-8.1	-20.6	3.5	0.182
		Block 5	-7.1	-16.8	1.4	0.128	-14.6	-26.9	-3.2	0.02
		Block 6	-11	-20	-2.5	0.022	-20.8	-34.2	-9.9	0.002
		Block 7	-12.6	-21.8	-4.1	0.008	-25.8	-37	-13.7	<0.001
		Block 8	-14.1	-24.4	-6	<0.001	-21.9	-33.4	-9.4	0.002
		Block 9	-14.1	-23.2	-4.7	<0.001	-23.8	-36.8	-12.6	0.002
		Block 10	-21.7	-30.4	-13	<0.001	-28.6	-41.1	-17.7	<0.001
		Block 11	-19.3	-28	-9.3	<0.001	-26.4	-38.7	-14.8	<0.001
		Block 12	-21	-29.9	-11.4	<0.001	-28	-39.3	-15.5	<0.001
		Block 13	-17.7	-26.7	-8.1	<0.001	-28.3	-39.6	-15.4	<0.001
Kusel	2013	Intercept	37.2	28.6	44.6	<0.001	38.8	27.6	51.4	<0.001
		Block 2	-14.9	-23	-6.2	<0.001	-10.4	-23.7	0.8	0.078
		Block 3	-16.7	-25.7	-7.7	<0.001	-4	-16.3	7.7	0.518
		Block 4	-16.6	-24.8	-7.8	<0.001	-9.2	-21.1	2.7	0.128
		Block 5	-6.5	-14.8	2.6	0.138	2.7	-9.9	14.6	0.64
		Block 6	-18.4	-26.9	-9.8	<0.001	-5.1	-17.4	6.6	0.414
		Block 7	-10.6	-19.7	-2	0.02	-2.1	-15.7	9	0.73
		Block 8	-14.1	-22.2	-4.4	<0.001	-4.8	-16.9	7	0.446
		Block 9	-10.9	-19.9	-2.4	0.02	-6.6	-18.2	5.9	0.294
		Block 10	-20.9	-29.8	-12.2	<0.001	-18.3	-30.9	-6.9	0.004
		Block 11	-26.8	-35.2	-18	<0.001	-16.1	-27.5	-3.8	0.01
		Block 12	-23.5	-32.9	-15.1	<0.001	-10.8	-25.9	-0.2	0.078
		Block 13	-22	-30.4	-12.8	<0.001	-7.6	-19.6	4.1	0.198
Landstuhl	2012	Intercept	12.7	6.7	19.6	<0.001	10.1	3.8	17.2	0.002
		Block 2	14.5	7.1	21.4	<0.001	9.2	2.6	15.1	0.01
		Block 3	4.4	-3.5	10.5	0.262	0.7	-5.6	7.4	0.846
		Block 4	4.1	-3.1	11.3	0.294	21.6	14.2	28.1	<0.001
		Block 5	6.1	-1.7	13.8	0.13	16	8.8	23.1	<0.001
		Block 6	15.9	8.3	23	<0.001	29.3	22.7	36.3	<0.001
		Block 7	8.4	1.7	15.8	0.026	14.8	8.2	21.1	<0.001
		Block 8	9.6	2	16.6	0.01	12	6.1	19.3	<0.001
		Block 9	10.7	2.8	18.3	0.004	25	17.7	32.4	<0.001
		Block 10	14.8	7.8	23.3	<0.001	19.3	12.7	26.3	<0.001
Landstuhl	2013	Intercept	8.3	3.1	13.9	0.002	21.3	13.6	29.3	<0.001
		Block 2	9.3	2.3	15.9	0.014	17.8	8.1	26.4	<0.001
		Block 3	5.3	-2.2	12.1	0.146	10.4	0.9	18.7	0.012
		Block 4	18	11.2	25.7	<0.001	20.2	11.2	29.2	<0.001
		Block 5	7.2	-0.6	14.4	0.074	12.9	2.9	21.9	0.014
		Block 6	31.5	24.2	37.6	<0.001	24.9	15.8	34.1	<0.001

Table 6 (continued)

Site	Year	Block	Crown defoliation				Epicormic shoots			
			post.mean	l-95 % CI	u-95 % CI	pMCMC	post.mean	l-95 % CI	u-95 % CI	pMCMC
		Block 7	20.6	13.6	27.4	<0.001	18.1	9	26.7	<0.001
		Block 8	14.3	7.5	21.3	<0.001	14.8	5.6	22.8	<0.001
		Block 9	7.3	0.9	15.2	0.046	26.3	16.6	36.3	<0.001
		Block 10	14.3	6.9	21.3	0.002	15.4	6	24.4	0.002

post.mean estimated mean of the posterior distribution, l-95 % CI lower 95 % credible interval, u-95 % CI upper 95 % credible interval

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