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RESEARCH PAPER

Negative correlation between ash dieback susceptibility and reproductive success: good news for European ash forests

Devrim Semizer-Cuming [1,2] · Reiner Finkeldey [1,3] · Lene Rostgaard Nielsen [2] · Erik Dahl Kjær [2]

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Abstract

• ***Key message*** **European ash (*Fraxinus excelsior* L.) trees with low susceptibility to ash dieback have higher reproductive fitness compared to highly susceptible trees, although most pronounced for female success. Selection at generation turnover therefore supports the future recovery of ash forests.**
• ***Context*** The introduced invasive pathogen *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, and Hosoya cause extensive damage on European ash (*Fraxinus excelsior* L.). Heritable variation in susceptibility to ash dieback has been observed among ash trees in natural and planted populations, but it is not clear how variation in susceptibility influences reproductive fitness.
• ***Aims*** We hypothesize that healthier male and female trees contribute more gametes to the following generation compared to unhealthy ones.
• ***Methods*** We tested the hypothesis by studying gender, seed production, and paternal success in a clonal field trial with 39 replicated clones. In the trial, the susceptibility level of each clone has been recorded in terms of percent crown damage since 2007. We used a linear regression model to explore the relationship between susceptibility and reproductive success (female and male).
• ***Results*** The clones revealed a clear gender dimorphism with an approximate 2:2:1 male/female/hermaphrodite ratio. Females with low levels of crown damage produced substantially more seeds compared to highly damaged females. The male clone with the lowest level of susceptibility was the most effective pollen donor, but highly susceptible males also sired some offspring.
• ***Conclusion*** The results overall represent good news for the potential recovery of ash forests: selection against most susceptible genotypes at generation turnover is expected to facilitate building up disease resistance in ash populations.

Keywords Ash dieback · *Fraxinus excelsior* · Fitness · Gender · Gene pool · Reproductive success

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Contribution of co-authors Devrim Semizer-Cuming: formulation of the hypotheses, data collection, performing the experiments, data analysis, data presentation, writing the original draft, reviewing, and editing
Reiner Finkeldey: reviewing and editing
Lene Rostgaard Nielsen: formulation of the hypotheses, data collection, reviewing, and editing
Erik Dahl Kjær: formulation of the hypotheses, data analysis, reviewing, and editing

✉ Devrim Semizer-Cuming
dsemize@forst.uni-goettingen.de

[1] Department of Forest Genetics and Forest Tree Breeding, Faculty of Forest Sciences and Forest Ecology, Georg-August University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

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

[3] Present address: University of Kassel, Mönchebergstrasse 19, 34109 Kassel, Germany

1 Introduction

Forest tree species have to cope with various threats, such as emerging infectious diseases, climate change, habitat loss, and fragmentation, which in combination can put them under severe pressure. In forest trees, the primary causes of emerging infectious diseases are fungal and fungal-like pathogens, whose numbers increased 13-fold only from 1995 to 2010 due to expanding networks of international trade and travel (Santini et al. 2013). They present a real challenge for conservation by causing rapid and extensive reduction in numerical abundance and by changing the genetic composition of host populations (Altizer et al. 2003). The outcome of an epidemic in terms of host mortality depends not only on pathogen establishment, development, and virulence but also on how a disease outbreak influences the reproductive success and ultimately the fitness of the host species.

Fraxinus excelsior L. or common ash (Oleaceae, hereinafter ash) is an important component of European forest ecosystems and a valuable timber species (Pliûra and Heuertz 2003). Ash has been decimated in continental Europe by an invasive fungal pathogen, *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya (Baral et al. 2014), since the early 1990s (McKinney et al. 2014), when it was first observed in Poland (Przybył 2002). Genome-wide population diversity in *H. fraxineus* indicates an eastern Russian origin of European ash dieback (Sønstebø et al. 2017), where it is asymptomatically associated with Asian *Fraxinus* species (Cleary et al. 2016; Nielsen et al. 2017). High mortality due to the pathogen in the European ash stands of *Fraxinus excelsior* regardless of age (Bakys et al. 2009; Lenz et al. 2016; Skovsgaard et al. 2010) raises concern about the future existence of the species.

Previous research based on clonal ash stands, such as in Denmark (McKinney et al. 2011), Sweden (Cleary et al. 2014; Stener 2013), and Germany (Enderle et al. 2014), has documented a high level of genetic variation in ash dieback susceptibility among ash individuals, giving rise to high broad-sense heritability estimates (e.g. $H^2 = 0.42–0.54$ in Tapsøre, Denmark; McKinney et al. 2011). Analyses based on variance among young half-sib families have further revealed high levels of narrow-sense heritability in dieback susceptibility (e.g. $h^2 = 0.40–0.49$ in Pliūra et al. 2011; $h^2 = 0.37–0.52$ in Kjaer et al. 2012; $h^2 = 0.42–0.53$ in Lobo et al. 2014; $h^2 = 0.42$ in Muñoz et al. 2016), suggesting that disease tolerance is to a large extent inherited from parents to offspring. This is further supported by the results of Lobo et al. (2015), who compared disease symptoms in parents with necrosis development in their offspring. The mechanisms behind the observed ash dieback resistance are not yet well understood. However, since parts of the mechanisms differ from classical induced resistance (Harper et al. 2016) and the variation is of quantitative nature, we use the term tolerance rather than resistance in the present work. While genetic variation in disease tolerance is known to affect individual survival (Lobo et al. 2014), much less is known on how the level of susceptibility of a given tree influences its reproductive success. However, a recent study found a clear overrepresentation of healthy trees among parents of seedlings on the forest floor of a Danish mixed forest (Semizer-Cuming et al. submitted). Sexual selection may therefore provide an important contribution to natural selection against disease susceptibility. This issue is particularly important to clarify, as the frequency of tolerant ash trees in nature is low (McKinney et al. 2011).

Fitness is a measure of the ability to survive and produce successful offspring. Plants that produce more seeds and fertilize more ovules than the population average therefore have higher fitness (Primack and Kang 1989). Female fitness can be estimated by counting the relative number of mature seeds assuming equal germination percentage while the estimation of male fitness requires assignments based on paternity analysis using highly polymorphic codominant markers (Marshall et al. 1998). The aim of the present study is to test the hypothesis that healthier male and female trees have higher gamete contribution to the subsequent generation compared to the unhealthy ones. We study the variation in gender and fertility in a Danish trial with the clonal replication of 39 genotypes that have been characterized in terms of ash dieback susceptibility based on 10 years of measurements. The implications of the observed relationship between disease tolerance and reproductive success are discussed in relation to the conservation and management of the species.

2 Materials and methods

2.1 Study site and sampling

The study site is located in Tuse N s (55° 45′ 57.99″ N 11° 42′ 47.48″ E) in Northern Zealand, Denmark. It is a clonal field trial, established in 1998 based on a randomized complete block design with 39 individual *F. excelsior* genotypes (39 clones). Each clone was grafted onto rootstocks with approximately 26 replications (ramets) and planted in a single-tree, random block design. The initial objective of the trial was to serve as seed orchard, and the spacing was therefore 3 × 6 m. Details of the trial and the origin of the 39 clones can be found in McKinney et al. (2011). In the trial, seeds were collected in October 2012 from the ramets of three open pollinated female clones, representing relatively healthy genotypes (clone nos. 30, 33, 35) and located in the centre of the trial. Seeds were germinated and grown for 2 years in a greenhouse. At the time of seed collection in 2012, the number of ramets per clone had already been reduced to 4–21 due to high mortality caused by ash dieback.

2.2 Phenotypic assessments

All ramets of the 39 clones have been monitored in terms of crown damage since 2007. Crown damage was evaluated according to five-scale damage classes: class 0 represented no crown damage, class 1–3 indicated an increasing damage (class 1 < 10%, class 2 10–50%, class 3 > 50%), and class 4 meant 100% crown damage (dead). For statistical analyses, crown damage scores were converted into percent damage scores (PDS) based on the median values of the class percent ranges (cf. McKinney et al. 2011). Inflorescences of all flowering trees were observed in May 2015, and genders were scored according to a 1–9 scale, corresponding to the proportion of female flowers: (1) 0%, (2) 12.5%, (3) 25%, (4) 37.5%, (5) 50%, (6) 62.5%, (7) 75%, (8) 87.5%, and (9) 100%. Gender of one genotype (clone no. 28) could not be determined due to lack of flowers, and this clone was therefore excluded from further analyses concerning gender. Female reproductive success of all living trees were scored based on the visual observation of relative seed productivity in July 2015 (a year with abundant flowering) using a relative scoring scale from 0 to 9, where 0 corresponded to no seeds and 9 to very heavy seed production.

2.3 DNA genotyping

DNA genotyping was performed in order to assign fathers to offspring by paternity analysis and thereby estimate male reproductive success of the 39 clones. Leaves were collected from the 39 clones and from 285 2-year-old seedlings, and stored at − 20 °C until DNA extraction. DNA extractions were carried out with ~ 40 mg leaf tissues using the DNeasy® 96 Plant Kit (Cat No. 69181) according to the manufacturer's protocol (Qiagen, Hilden, Germany). Microsatellite analyses were performed with nine selected primer pairs: FEMSATL11 and FEMSATL19 (Lefort et al. 1999); FEMSATL12 (Gerard et al. 2006); ASH2429 (Bai et al. 2011); FRESTSSR308, FRESTSSR427, and FRESTSSR528 (Aggarwal et al. 2011); and Fp18437 and Fp21064 (Noakes et al. 2014). The primers were labelled with four different fluorescent dyes (6-FAM: FEMSATL12, Fp18437, and Fp21064; VIC: FEMSATL11, FRESTSSR308, and FRESTSSR528; NED: FEMSATL19 and ASH2429; PET: FRESTSSR427) and combined in three multiplexes (Multiplex-1: FEMSATL11, FEMSATL12 and FEMSATL19; Multiplex-2: Fp18437, Fp21064, and FRESTSSR528; Multiplex-3: FRESTSSR308, FRESTSSR427, and ASH2429). PCR amplifications were carried out in 15 μl reactions using the QIAGEN Multiplex PCR Kit (Cat No. 206143) according to the manufacturer's instructions. PCR amplifications were performed under the following conditions: initial denaturation at 95 °C for 15 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C, 62 °C, and 59 °C (for Multiplex-1, − 2, and − 3, respectively), extension at 72 °C for 60 s, and final extension at 60 °C for 30 min. PCR products were analysed on the ABI 3130xl Genetic Analyser (Applied Biosystems, Foster City, CA, USA). Each individual genotype and each mother-offspring pair were checked for errors, and the analyses were repeated in case of uncertain or non-amplified peaks. Alleles at each locus were binned into their size classes using a combination of manual and automated allele binning (Matschiner and Salzburger 2009) in order to detect potential binning errors.

2.4 Paternity analysis

Paternity analysis was carried out using CERVUS 3.0.7 (Kalinowski et al. 2007). The assignments were made based on Delta (Δ), the difference in LOD scores between the two most likely parents, to increase the certainty to identify the true parent when multiple parents had positive LOD scores (Marshall et al. 1998). The critical values of Δ were calculated at strict (95%) and relaxed (80%) confidence levels during the simulations. The assigned father was considered true when the likelihood of the paternity was 80% or more. The minimum number of loci was set to 8, and error rate was kept at 0.01. Selfing was allowed in the model as no clones were found to be completely female. To increase the reliability of the critical LOD values, 100,000 offspring were simulated. The percentage of sampled potential fathers was set to 70%. Confident paternity assignments were further checked and confirmed with the gender score of each genotype.

2.5 Analysis of gender and the effect of ash dieback susceptibility on reproductive fitness

We applied a general linear analysis of variance model to test for significant differences in gender scores among the 38 clones (excluding clone no. 28 with unassessed gender) using the ramets as replications. For the further analysis, the clones with a gender score (proportion of female flowers) of 0–20% were considered predominantly male (M), whereas the clones with gender score more than 80% were regarded as predominantly female (F). The remaining clones were considered hermaphrodites (H), and these clones were not included in the analysis of either male or female fitness. In this regard, the numbers of predominantly male, predominantly female, and hermaphroditic clones were M = 15, F = 16, and H = 7, respectively. The ratio does not necessarily reflect the situation in native populations, because the clones were specifically selected for the trial in 1998 with the objective of containing equal numbers of males and females.

Female reproductive success was assessed based on seed score (see as explained in "Materials and methods") while male reproductive success was assessed based on realised paternities. The effect of ash dieback susceptibility (observed as

percent crown damage; PDS) on reproductive success (Y) was analysed based on a linear regression model. The relationship between male fertility and disease susceptibility was analysed based on the data from the 15 predominantly male clones, while the relationship between female fertility and disease susceptibility was analysed based on the data from the 16 predominantly female clones. We applied the linear regression model E (Y_i) = β_{0+} β PDS_i, where PDS_i was the susceptibility of the clones to ash dieback. The analyses for males and females were performed independently. In order to isolate the effect of survival in the clonal trial (differences in number of living ramets per clone), we analysed the relationship between reproductive fitness and ash dieback susceptibility at four levels: (i) total female reproductive success per clone (i.e. summed relative seed score across all ramets), (ii) average female reproductive success per living ramet of each clone, (iii) total male reproductive success per clone (i.e. number of realised paternities), and (iv) average male reproductive success per living ramet of each clone. Statistical analyses were conducted using the 'tidyverse' package (version 1.1.1; Wickham 2017) implemented in R (R Core Team 2017).

3 Results

3.1 Gender assessment

The assessment of gender showed significant differences among clones ($F = 48.4$, $p < 0.001$). The average gender scores ranged from purely male (gender score = 0% for clone nos. 11 and 20) to mostly female (gender score = 92% for clone no. 22) (Table 1). The 16 predominantly female clones formed a distinct group with gender scores of > 80% whereas the border between male and hermaphroditic clones was less clear (Fig. 1). The predominantly males did not carry any seed, but several of the predominantly females had sired offspring (Table 1). The level of disease susceptibility was not correlated with the gender score ($t = -0.25$, $p > 0.05$; Fig. 1).

3.2 Paternity assignments

Paternity was assigned with high exclusion probability (0.9999), and no mother-offspring mismatches were detected. Paternity analysis successfully assigned candidate fathers to 144 (51%) of the analysed seedlings; hereof 102 (36%) at strict confident level (95%), and 1 was self-fertilized. The number of successful pollinations varied among males. Eleven clones were not assigned as candidate fathers whereas a single clone (clone no. 18), the healthiest male clone in the trial, was assigned as a pollen donor for 46 offsprings (Table 1), which corresponds to 32% of the total assignments. When correcting for the number of living ramets per clone, the variation in number of sired offspring varied among clones from 0 to 2.9 per ramet (Table 1).

3.3 The effect of ash dieback susceptibility on reproductive fitness

The negative effect of ash dieback susceptibility on female reproductive success was highly significant, and the crown damage score (PDS) alone explained a high proportion of the observed variation in total female reproductive success and average female reproductive success per living ramet ($R^2 = 0.873$ and 0.518) among the females (Table 2; Fig. 2a, b). PDS, in general, explained less variation ($R^2 = 0.504$–0.349) on the male side (Table 2; Fig. 2c, d). The relationships between reproductive fitness and ash dieback susceptibility among the tested clones were significant at all four levels (Table 2).

4 Discussion

In the present study, we used percent damage score based on the level of crown damage to account for susceptibility to ash dieback. Crown damage is caused by the occlusion of branches leading to necrotic tissue and defoliation. The symptoms are clearly recognisable, and thus, it has been successfully used to evaluate, monitor, and quantify the disease level. Significant variation among clones in crown damage has previously been documented in the present trial with an estimated broad-sense heritability of around 0.4 (McKinney et al. 2011), and the same approach to assess disease intensity has been applied in many other studies (Enderle et al. 2014; Kjær et al. 2012; Lenz et al. 2016; Lobo et al. 2014; Marçais et al. 2017; Muñoz et al. 2016). We therefore consider average clonal level of crown damage as a good estimate for the ability of the clones to withstand infections of *H. fraxineus*.

Our study showed that clones with low levels of ash dieback symptoms (percent damage score) had higher reproductive fitness, but the effect appeared to differ between genders. A strong continuous negative relationship between disease susceptibility and female reproductive success was observed, and the rank in the damage score of the clones therefore corresponded relatively close to the opposite rank in seed productivity. However, the relationship between disease susceptibility and male reproductive success was less straightforward, because some male clones with high damage scores had sired offspring (e.g. clone no. 1 had sired eight ovules; Table 1). The relationship was only significant due to a single male (clone no. 18) that was by far the most effective pollen donor and also the male genotype with lowest ash dieback susceptibility (Fig. 2c, d). The difference between the influence of ash dieback on female and male reproductive fitness probably reflects the differences between maternal and

Table 1 Gender score, seed score (SS), sired offspring (Pat), average percent ash dieback damage score (PDS), average PDS score for living ramets per clone (PDS LR), and number of living ramets (LR) of the 39 clones of *Fraxinus excelsior* in the study site

Genotype (clone ID)	Gender[1]	Gender score (%)	Total SS 2015	Total Pat 2012	Average SS per LR 2015	Average Pat per LR 2012	PDS[2] 2012 (%)	PDS[2] 2015 (%)	PDS LR 2012 (%)	PDS LR 2015 (%)	LR 2012	LR 2015
1	M	16.67	0	8	0.00	0.80	87.50	83.50	75.00	45.00	10	6
2	M	15.63	0	0	0.00	0.00	89.06	80.94	75.00	37.50	7	6
3	H	45.45	1	18	0.09	1.29	56.50	58.75	32.86	16.36	14	11
4	M	6.25	0	0	0.00	0.00	83.33	76.39	75.00	46.88	12	8
5	H	57.14	1	0	0.13	0.00	86.76	72.35	75.00	38.13	10	8
6	M	12.50	0	2	0.00	0.13	82.95	75.23	75.00	50.45	15	11
7	H	40.00	0	0	0.00	0.00	79.44	55.56	71.54	33.33	13	12
8	H	25.00	0	3	0.00	0.30	86.84	78.95	75.00	42.86	10	7
9	M	3.13	0	0	0.00	0.00	87.50	77.50	75.00	50.00	10	9
10	M	9.72	0	2	0.00	0.09	72.05	40.45	70.91	27.37	22	19
11	M	0.00	0	1	0.00	0.08	84.72	70.00	75.00	39.00	12	10
12	M	6.62	3	1	0.18	0.06	75.26	49.21	72.35	43.24	17	17
13	H	47.50	32	20	3.20	1.43	41.11	59.44	24.29	27.00	14	10
14	M	1.56	0	2	0.00	0.17	78.33	71.39	67.50	35.63	12	8
15	M	6.25	0	3	0.00	0.19	76.67	64.72	72.19	35.91	16	11
16	H	56.25	1	0	0.17	0.00	86.25	80.94	69.38	37.50	8	6
17	M	3.57	0	0	0.00	0.00	60.00	73.82	24.44	36.43	9	7
18	M	15.18	0	46	0.00	2.88	37.14	51.43	17.50	27.14	16	14
19	M	7.14	0	3	0.00	0.27	56.76	62.65	33.18	29.44	11	9
20	M	0.00	0	9	0.00	0.64	59.52	64.29	39.29	31.82	14	11
21	F	84.38	44	0	4.89	0.00	61.58	67.63	33.64	31.67	11	9
22	F	91.67	26	3	3.71	0.27	60.26	76.58	31.36	36.43	11	7
23	F	81.82	42	2	3.82	0.15	52.86	59.76	23.85	23.18	13	11
24	F	84.38	2	1	0.40	0.14	87.95	84.09	62.14	30.00	7	5
25	F	87.50	4	2	0.80	0.25	89.47	91.05	75.00	66.00	8	5
26	F	86.25	44	0	3.14	0.00	56.82	52.73	47.22	25.71	18	14
27	F	81.25	5	1	0.83	0.17	89.74	87.37	67.50	60.00	6	6
28	NA	NA	0	4	0.00	0.67	92.11	93.68	75.00	60.00	6	3
29	F	82.50	13	1	2.17	0.11	87.38	85.48	52.22	33.33	9	6
30	F	89.58	45	1	6.43	0.08	48.95	76.32	23.46	32.14	13	7
31	F	83.33	20	2	1.67	0.11	86.25	79.17	72.63	37.50	19	12
33	F	88.39	71	1	4.73	0.06	37.50	37.50	21.88	16.67	16	15
34	F	87.50	48	3	3.69	0.20	40.28	51.67	28.33	33.08	15	13
35	F	86.67	122	0	7.63	0.00	8.33	23.00	7.94	15.94	17	16
36	F	88.64	46	2	4.18	0.15	48.44	54.69	36.54	34.09	13	11
37	F	87.50	1	0	0.13	0.00	84.32	84.77	71.25	58.13	12	8
38	F	87.50	15	1	1.36	0.07	77.86	71.90	69.00	46.36	15	11
39	H	31.73	1	1	0.07	0.06	69.74	60.53	59.12	31.43	17	14
40	M	4.17	0	1	0.00	0.20	93.75	93.75	66.00	66.00	5	5

[1] Gender: F, female; M, male; H, hermaphrodite

[2] Differences among clones in PDS were highly significant in both 2012 ($F = 10.8$, $p < 0.001$) and 2015 ($F = 3.8$; $p < 0.001$), unpublished data

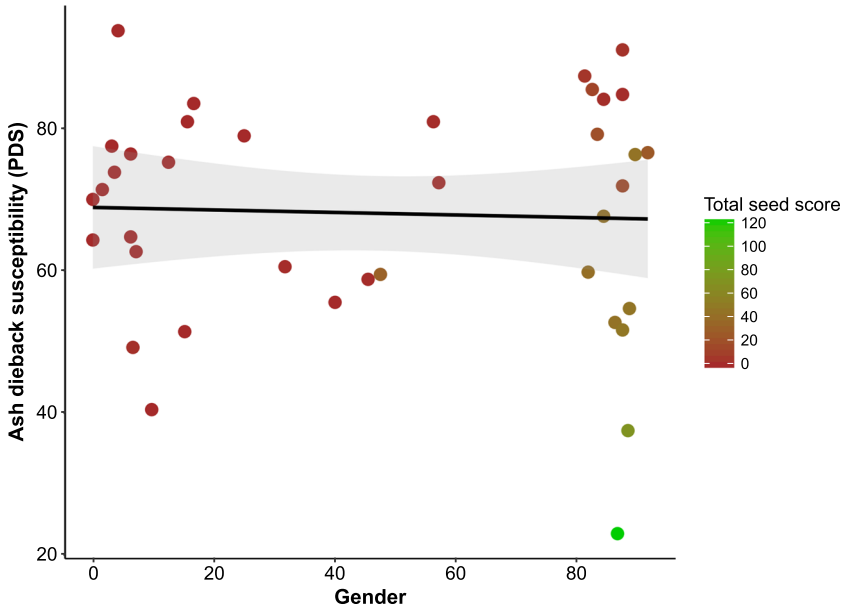

Fig. 1 Gender of the 38 clones of *Fraxinus excelsior*, their level of susceptibility to ash dieback (percent damage score; PDS) in 2015 and relative seed score. Notes: gender of each clone is ranked based on the degree of femaleness (%) flowers (0 = completely male flowers, 100 = completely female flowers). Colour code presents total seed score per clone in 2015, increasing from brown (0 = no seed) towards green (> 100 = abundant seeds).

paternal investments in reproduction. Intersexual difference in reproductive investment is a known phenomenon in dioecious plant species, and the cost of reproduction is generally higher for females than males (Antos and Allen 1999; Cipollini and Whigham 1994; Korpelainen 1992; Obeso 2002). For example, in the desert populations of an evergreen dioecious shrub (*Simmondsia chinensis*), males allocate 10–15% of their resources to reproductive tissues whereas females allocate 30–40% of their resources to reproduction at 100% seed set and female reproductive investment would be equal to that of males at ~ 30% seed set (Wallace and Rundel 1979). In *Salix* species, females also allocate more resources to reproductive tissues than males and therefore have higher reproductive costs in return (Ueno et al. 2006, 2007). Queenborough et al. (2007) studied intersex costs of reproduction in 16 tropical tree species of Myristicaceae in Amazonian lowland forests and concluded that female trees invested 10 times more biomass than male trees in total reproduction. In ash, crown damage due to ash dieback probably limits the available resources for reproduction in both genders. However, highly susceptible males may be able to produce and release pollen before flushing unlike their highly susceptible female counterparts, which may not be able to produce mature seeds while restoring the crown with epicormic shoots during the growing season. The difference among clones in relative female fitness is likely to be even stronger than suggested by the results presented in Fig. 1 and Table 1, because female reproductive success was scored based on a relative seed score from 0 to 9, where a tree with the crown full of seeds (score 9) most likely had more than nine times more seed compared to a tree with very few seeds in the crown (score 1). As it would be expected, differences among clones in terms of mean reproductive success per living ramet were lower than differences in total success for both males and females (Fig. 2) since differences in total success include the effects of differences in survival among genotypes (number of surviving ramets).

Only a small fraction of *Fraxinus excelsior* trees in natural populations can be expected to have a low level of susceptibility (1–5%, McKinney et al. 2014), but the findings of the present study suggest that the frequency of individuals with low susceptibility are likely to increase in the following generations due to the higher reproductive fitness of their parents, especially on the maternal side. Several studies have shown high levels of narrow sense heritability in the level of ash dieback susceptibility (as discussed above in the introduction), and the superior reproductive success of the healthy females and males in our study is

Table 2 Linear regressions for the effect of ash dieback susceptibility (percent damage score; PDS) on reproductive fitness

	Ash dieback susceptibility (PDS)				
	Mean	*df*	R^2	*t*	*p* (> |*t*|)
Sum of seed score across all ramets	70.29	14	0.873	− 9.816	< 0.001***
Sum of paternities	68.70	13	0.504	− 3.632	0.003**
Average seed score per living ramet	54.35	14	0.518	− 3.879	0.002**
Average paternity per living ramet	37.66	13	0.349	− 2.637	0.020*

*0.05; **0.01; ***0.001 significance codes

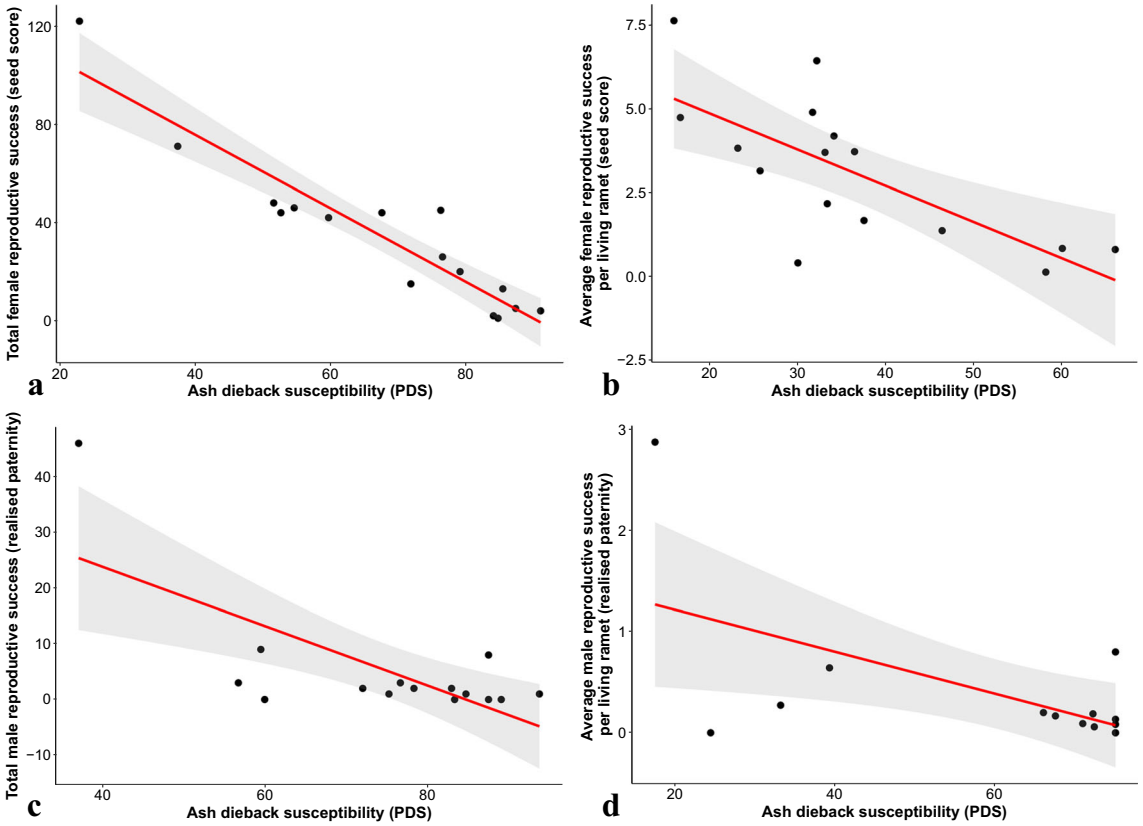

Fig. 2 Relationship between ash dieback susceptibility (percent damage score; PDS) and (**a**) total female reproductive success per clone (seed score), (**b**) average female reproductive success per living ramet per clone (seed score), (**c**) total male reproductive success per clone (realised paternity), and (**d**) average male reproductive success per living ramet per clone (realised paternity). Red lines show fitted regression lines within 95% confidence interval

therefore promising for the future resilience of the European ash forests. Nonetheless, the results indicate that selection against trees with high levels of susceptibility is weaker on the male side because the relationship between susceptibility and male reproductive success is weaker. Flowering in many plant species is regulated by environmental factors and thus stress-induced flowering is not an unknown phenomenon (Wada and Takeno 2010). We did not assess flowering intensity and therefore cannot rule out that diseased trees may have flowered more abundantly than healthy ones. However, we studied successful pollinations and successfully developed seeds, and observed that unhealthy trees were under-represented compared to few healthy male and female clones, which were highly fertile.

Our results present important information from a forest management point of view and suggest landscape managers and foresters to refrain from sanitary clear-cuts of all ash trees in damaged stands, but instead leave healthy trees for natural regeneration. Also, it is important to inform forest owners about the importance of sparing healthy trees in their forests instead of logging them due to concern about timber value. Our results imply that removing unhealthy males would be particularly relevant because unhealthy male trees are more likely to contribute to the next generation than unhealthy female trees. Before the disease outbreak, ash trees could regenerate successfully in European forests with the density of several ten of thousands per hectare (Lygis et al. 2014). Therefore, the potential of ash forests to improve their general level of resistance in the following 1–2 generations should not be underestimated. Still, natural selection and regeneration of ash in forests could be actively supported by the enrichment of damaged stands with offspring from tolerant, healthy ash trees in order to speed up the adaptation processes (Semizer-Cuming et al. submitted). Reliable information on seed and pollen dispersal distances of ash and the factors affecting mating patterns is already at hand (e.g. Semizer-Cuming et al. 2017).

5 Conclusion

Our results show that the healthiest female clones produced substantially more seeds than the unhealthy female clones. This supports the findings from a recent study on ash regeneration in a Danish forest, where healthy trees were overrepresented as the parents of seeds and young seedlings (Semizer-Cuming et al. submitted). This is indeed good news for European ash forests, because higher contribution from healthy trees at generation turnover followed by lower mortality among the next generation of trees with low levels of susceptibility must gradually give rise

to more tolerant ash trees in next generations. We recommend foresters refrain from making complete sanitary clear-cuts of damaged ash stands, but instead leave most resistant individuals, and apply measures for supporting natural regeneration. At the moment, we cannot predict the expected overall progress due to lack of reliable estimates of in situ heritability relevant for the two selection stages. However, the progress may be significant as strong selection seems to be involved in both stages, and effective gene flow is likely to connect surviving trees across large forest landscapes (Bacles and Ennos 2008; Bacles et al. 2005, 2006; Semizer-Cuming et al. 2017). Still, it remains to be revealed whether the process will be fast and efficient enough to ensure the true recovery of the species.

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Data availability The dataset generated and analysed during the current study is available in the University of Copenhagen–Electronic Research Data Archive (UCPH-ERDA), https://sid.erda.dk/public/archives/8a01d83d895d81575baa3c3147339543/published-archive.html.

Compliance with ethical standards

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

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