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Pollination success of *Fraxinus excelsior* L. in the context of ash dieback

Anna-Katharina Eisen^{1*} , Devrim Semizer-Cuming^{2,3} , Susanne Jochner-Oette¹ and Barbara Fussi³

Abstract

Key message Paternity analyses show that effective pollination of ash (*Fraxinus excelsior* L.) in a seed orchard and a floodplain forest affected by ash dieback is more likely to be facilitated by healthier males. Thereby, natural selection can have a positive effect on the health of future generations.

Context Ongoing ash dieback and increasing fragmentation of ash populations may result in reduced pollen flow, which can reduce pollination success of future generations of ash trees. Therefore, it is essential to further improve our understanding of gene flow patterns, especially with respect to ash dieback.

Aims In this study, paternity analyses were conducted in a seed orchard and a floodplain forest in Germany in 2018 to explain the relationship between pollination success and the health status of ash trees and distances of effective pollen transport.

Methods Cambium samples (i.e., from twigs and stumps) were collected from 251 ash trees (putative father and mother trees) for genotyping, and the health status of each tree was documented using a scoring system to evaluate vitality. Additionally, seeds were harvested from 12 mother trees per site. Genetic analyses using nuclear microsatellites were performed to determine paternal trees. Paternities were assigned based on the likelihood model implemented in the Cervus 3.0.7 software.

Results Our results showed that the average pollination distance was 76 m in the seed orchard and 166 m in the floodplain forest. In general, pollination success decreased substantially with increasing distance to the mother tree. Despite the dense tree cover in the floodplain forest, pollen were transported over long distances (greater than 550 m), suggesting that non-local sources also play a role in pollination. This is supported by the foreign pollen input identified in the seed orchard (66.5%). Self-pollination was detected only to a very small extent, and thus had no major influence on reproduction. In addition, both healthy and slightly diseased father trees showed similar mating success. However, this was not the case for the severely diseased ash trees (more than 50% of crown damage) because only a few offspring could be assigned to them. Nevertheless, in contrast to the floodplain forest, there was no significant correlation between damage classes and pollination success in the seed orchard.

Conclusion Long-distance pollen transport contributes to the connectivity of ash trees in the landscape. Additionally, both healthy and slightly diseased fathers have a greater contribution to pollination, thus potentially improving the health of the next generation of ash trees. Moreover, gene flow between stepping stone populations is necessary to ensure the positive impact on the genetic diversity of ash populations in the future.

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Keywords Ash dieback, Gene flow, Paternity analysis, Effective pollen transport, Pollination success

1 Introduction

In recent decades, ash dieback, caused by the fungus *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, and Hosoya (Baral et al. 2014) (Syno.: *Hymenoscyphus pseudoalbidus* (Queloz et al. 2011) and its asexual stage *Chalara fraxinea* (Kowalski 2006), has acutely threatened ash populations in Europe (Metzler et al. 2012; McKinney et al. 2014; Kosawang et al. 2018) and its silvicultural future (Enderle 2019). Common ash (*Fraxinus excelsior* L.) is considered a promising tree species under climate change conditions (Kölling 2007; Enderle et al. 2017; LWF 2019; Müller-Kroehling and Schmidt 2019) and presents itself as a versatile and valuable species with high ecological and economical importance (Enderle et al. 2017; Hultberga et al. 2020). However, high mortality rates have already led to a sharp decline in many local ash populations (Lygis et al. 2014; Marçais et al. 2017; Pliūra et al. 2017; Semizer-Cuming et al. 2021). Simulations indicated that up to 75% of ash trees in mixed stands in Europe are expected to die over the next 30 years (Coker et al. 2019). Other studies even suggest that only approx. 1–5% of ash trees are not susceptible and show little or no symptoms related to ash dieback (McKinney et al. 2014; Rigling et al. 2016; Enderle 2019). In this regard, individuals with very low levels of disease symptoms and less than 30% leaf loss are considered partially resistant (i.e., developing less symptoms) and might be suitable for genetic conservation or breeding programs (Lenz et al. 2012; McKinney et al. 2014). Disease development at stand level will therefore depend on the ability of less susceptible genotypes to spread their genes via pollen and seeds (Lobo et al. 2015; Semizer-Cuming et al. 2019, 2021). Partial resistance to ash dieback was not found to be population or provenance based but rather individual based (McKinney et al. 2011, 2014; Enderle et al. 2017; Semizer-Cuming et al. 2019). Therefore, it is essential that genetic connectivity is maintained, even in fragmented landscapes, and that genetic diversity is sufficiently high for the reproduction of trees for future healthy ash populations. To assess tree population responses and the consequences of ecological and biological threats, it is essential to understand pollen dispersal patterns.

Effective gene flow between partially resistant adult trees is necessary to establish healthier next generations of ash trees (Semizer-Cuming et al. 2017, 2019; Fussi 2020). However, ongoing ash mortality and the increasing fragmentation of ash populations lead to a

decrease in pollen flow (McKinney et al. 2014; Semizer-Cuming et al. 2017), which can further limit the genetic diversity of future generations (Fussi et al. 2014). If partially resistant ash trees are highly underrepresented, less healthy genotypes will also succeed and produce offspring. This can lead to decreased natural selection under the prevailing environmental conditions (Eisen et al. 2022b). Reduced genetic diversity can negatively affect the adaptive potential of future generations of ash trees, whereby genetic variation is particularly important for adaptation to new pathogens due to the long generation time of forest trees (Fussi et al. 2014; McKinney et al. 2014). In addition, ash trees are trioecious, i.e., monoecious and dioecious individuals can occur simultaneously (Roloff 1997), which can increase the risk of self-pollination (McKinney et al. 2014; Semizer-Cuming et al. 2021).

Genetic analyses investigating the mating success of ash individuals in populations have been performed only in a few studies to date (Heuertz et al. 2003; Bacles and Ennos 2008; Thomasset et al. 2014; Semizer-Cuming et al. 2017). The relationship between reproductive success and the health status of ash trees in a seed orchard has only been assessed once (Semizer-Cuming et al. 2019): It was demonstrated that health status and reproductive success of ash trees in a seed plantation in Denmark were negatively correlated. Female ash trees severely affected by ash dieback produced a much lower seed quantity compared to healthy ones, whereas damaged males could still sire some offspring. In another study, Semizer-Cuming et al. (2017) examined the genetic connectivity of ash trees in an isolated forest area. Thereby, it was found that about 55–64% of the seeds and 75–98% of the seedlings descended from local ash trees, but 26–45% of the pollen were transported from outside the forest area. Moreover, a positive correlation was found between seed dispersal distance and wind speed, while there was none between pollen dispersal distance and wind speed. Research on distances of effective pollen transport varied widely among studies: Heuertz et al. (2003) conducted a study in a mixed deciduous forest in Romania and determined a distance of 70–140 m, whereas Bacles and Ennos (2008) estimated the distance with up to 2.9 km in a deforested Scottish landscape. Semizer-Cuming et al. (2021) found that 50% of pollen dispersal occurred within 140 m in a Danish forest, 5% of pollen dispersal occurred over a distance of 1.3 km, and 1% was transported farther than 3 km. Similar

results were also obtained from aerobiological investigations on pollen transport and deposition using gravimetric pollen traps, which were conducted in two ash seed orchards in Germany (Eisen et al. 2022a). It was shown that 50% of the ash pollen in the air occurred within a distance of 200 m from the source, whereas only 10% of the total pollen catch reached a distance of 500 m.

In general, pollen dispersal is influenced not only by abiotic and biotic factors such as meteorological conditions, topography, and vegetation but also by tree height and crown size (Scheifinger et al. 2013; Puc 2012; Adams-Groom et al. 2017; Eisen et al. 2022a). Sork and Smouse (2006) also noted that less fragmented landscapes have more pollen input from outside and therefore adjacent pollen sources. The magnitude of influential factors suggests that it is important to improve our understanding of gene flow patterns in fragmented and non-fragmented ash populations further to predict accurate estimates of pollen dispersal (Semizer-Cuming et al. 2017).

Given this background, the objectives of this study are thus to explain the relationship between pollination success and the health status of ash trees and to test whether healthy fathers contribute more to the next generation, as well as to estimate pollen dispersal at sites affected by ash dieback. Based on the results, we discuss the implications of the observed relationship between the extent of damage caused by ash dieback and pollination success in relation to conservation and management of the species. This could be of particular interest for the establishment of future seed orchards where pollen emission from outside should not influence the production of healthy offspring in the orchards.

2 Material and methods

2.1 Study area

The seed orchard Schorndorf (48°46' N, 9°25' E, 420 m a.s.l.) is located in the valley Remstal near Schorndorf, Baden-Württemberg, Germany, and has an area of approx. 2.3 ha (Fig. 1a). The average annual temperature is 10.3 °C (DWD station Stuttgart Schnarrenberg, 1981–2010) and the average annual precipitation 855 mm (DWD station "Winterbach, Rems-Murr-Kr.", 1981–2010). The orchard is located on a NW exposed slope with an inclination of about 6 to 10° (LGRB 2021). It consists of grafts of selected so-called "plus trees," which were selected for growth and stem quality prior to the outbreak of the disease and is characterized by a well-spaced plot design (7 m × 7 m). The plus trees originated from the South German hill and mountain area and the Alps and Alpine foothills in

Baden-Wuerttemberg (Enderle et al. 2014). Initially, 68 clones (416 ash trees) were planted in November 1992 in 25 rows: 36 clones with 8 female or hermaphrodite ramets and 32 clones with 4 male ramets. Due to the negative effects of ash dieback, the majority of the trees (ca. 70%) died (Eisen et al. 2022a). In 2018, the seed orchard consisted of 123 mature ash trees with a maximum height of 17 m (Fig. 1b/Appendix Fig. 7). In the seed orchard, common ash is growing almost exclusively with few other tree species such as cherry and apple trees, wild service trees, or lime trees (especially along the sides). It is surrounded by meadows, which are mostly bordered by a mixed forest consisting mainly of beech, spruce, and pine. There are no other ash trees in the immediate surroundings (within a radius of about 1 km). Due to ash dieback, there is currently no commercial demand for ash seeds.

The floodplain forest is located at the Danube between the cities Neuburg and Ingolstadt, Bavaria, Germany (Fig. 1a). The selected area for our investigations is situated near the Bergheim barrage (48°44' N, 11°16' E, 375 m a.s.l.) and has an area of about 10 ha. The average annual temperature is 7.8 °C, and the average annual precipitation is 715 mm (1961–1990) (Schwab et al. 2018). The calcareous and nutrient-rich substrate of the site enables favorable growing conditions especially for common ash (Doben et al. 1996; Margraf 2004). Common ash is represented in the dense floodplain forest with a share of about 15% (Jochner-Oette et al. 2021). In the selected area, 50 ash trees and 78 tree stumps were sampled for our analyses (Fig. 1c/Appendix Fig. 8).

2.2 Sampling and vitality assessment

In October 2018, a lift truck was used to collect seeds for genotyping in the seed orchard and in the floodplain forest (12 mother trees selected per stand, Table 1) to quantify the proportion of pollination success of diseased and healthy father trees. All investigated ash trees (seed orchard: 123 ash trees and floodplain forest: 50 ash trees) were classified according to the scoring system of Lenz et al. (2012) for assessing the vitality of adult ash trees. Trees in categories 0 and 1 (up to max. 30% leaf loss) were classified as healthy, all other trees as diseased (categories 2 to 4) or dead (category 5). The dead trees were not included in the surveys. In Schorndorf, seeds were collected from nine different clones, representing six healthy and six diseased ash trees. From three clones, two ramets were selected for seed harvest (Table 1). A total of five healthy and seven diseased ash trees were selected in the floodplain forest. The number of analyzed and genotyped seeds per mother tree varied between 48

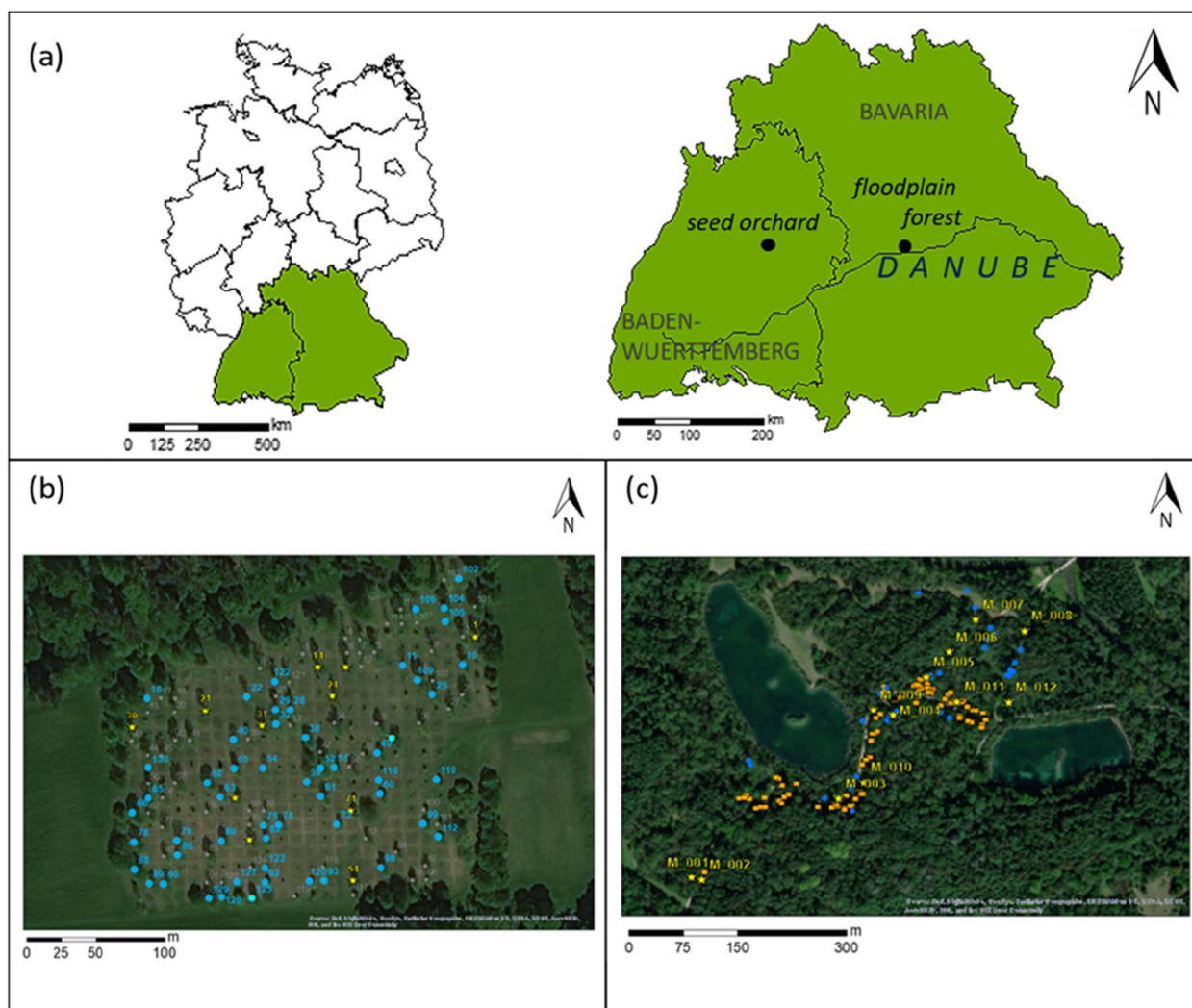


Fig. 1 Study areas. **a** Location of the seed orchard near Schorndorf (48°46' N, 9°25' E, 420 m a.s.l.) and of floodplain forest (48°44' N, 11°16' E, 375 m a.s.l.) in Germany. **b** Seed orchard including investigated ash trees, labeled by tree ID: yellow stars — mother trees, blue dots — ash trees identified as potential father trees by their clone number, gray dots — other investigated ash trees excluded as pollen donors. **c** Study area of floodplain forest including investigated ash trees, labeled by tree ID: yellow stars — mother trees, blue dots — ash trees identified as father trees, orange squares — logged ash trees identified as father trees. Source of the maps: ESRI Data & Maps

and 49 seeds in Schorndorf and between 70 and 76 seeds in the floodplain forest.

In spring 2019, twigs from all 123 mature ash trees (12 mother trees and 111 adult ash trees) were sampled in the seed orchard. In the floodplain forest, twigs or wood from 128 trees were sampled: 50 samples were derived from standing ash trees and 78 from ash stumps. The 78 ash trees had to be removed in January 2019 in order to ensure road safety. Since damage caused by ash dieback had already been observed before, they were classified as diseased. The logged trees were ash trees, which may have served as pollen donors since they were cut after

the pollen season and may have contributed to pollination and seed development. The standing ash trees were sampled at a 25-m radius around the mother trees, while ash stumps were interspersed (Fig. 1c/Appendix Fig. 8). After sampling, the tissue was preserved at -20 °C until further analyses.

Due to the identical genotypes of the clones in the seed orchard, in most cases, a single tree could not be identified as the father (except for the cases where there was only one potential father per clone). Therefore, all ramets of a clone that are possible pollen donors for a mother tree were defined as potential fathers. To exclude the trees that were not candidate fathers (i.e., females and/

Table 1 Characteristics of mother trees and number of analyzed offspring, as well as the potential longest and shortest pollen distance from mother tree to the detected father trees

Study site	Mother tree (clone number_tree ID)	Vitality classification of mother tree in 2018	Gender of mother tree	Number of analyzed offspring	Number of offspring with known father (clone)	Number of (potential) father trees	Shortest distance of pollen transport [m]	Longest distance of pollen transport [m]
Seed orchard	1619_31	1	Female	48	21	34	6.3	117.8
	1619_81	1	Female	48	31	17	7.7	111.9
	1627_01	2	Female	48	10	29	14.7	191.1
	1627_21	2	Female	48	12	26	27.7	126.9
	1663_24	2	Female	48	27	24	27.9	124.3
	1663_94	0	Hermaphrodite	48	9	19	19.9	137.6
	1664_71	0	Hermaphrodite	49	6	13	14.8	110.8
	1866_30	2	Female	49	16	26	14.4	171.7
	0000_62	1	Female	49	29	38	8.2	149.9
	1878_13	2	Female	48	12	17	39.7	139.1
	1879_37	1	Female	49	13	25	28.3	135.1
	1898_14	2	Female	48	5	16	48.1	130.4
	Floodplain forest	M_01	1	Female	70	13	13	14.8
M_02		2	Hermaphrodite	76	10	10	152.0	512.5
M_03		1	Hermaphrodite	70	18	13	19.2	308.8
M_04		2	Female	73	25	14	3.9	347.2
M_05		2	Female	71	24	18	5.8	296.0
M_06		1	Female	72	24	16	32.1	462.7
M_07		0	Female	70	24	11	2.3	319.9
M_08		3	Female	71	8	7	40.6	551.6
M_09		2	Hermaphrodite	74	24	8	24.7	221.1
M_10		2	Hermaphrodite	76	16	13	16.1	253.5
M_11		1	Female	70	19	16	5.5	328.2
M_12		3	Hermaphrodite	74	19	17	0.0	479.8

or trees with no flowering), the gender of the ash trees was determined on site (in the period 2018–2021), and the presence of flowers or flower buds and their phenological stage was recorded thoroughly with binoculars on 19 April 2018 (109th day of the year). Hermaphrodite mother trees were also considered as potential father trees. For the floodplain forest, no phenological data were available in 2018. The gender was determined for the ash trees alive; however, this was not possible for the ash stumps. The evaluated attributes for all ash trees can be found in the [Appendix](#) (Tables 2 and 3). The geographic position (UTM coordinates; 32 N) of each sampled ash tree was recorded using DGPS (Stonex S9 III, Stonex, Paderno Dugnano (MI), Italy) for both sites ([Appendix Fig. 7/](#)[Fig. 8](#)).

2.3 DNA extraction and genotyping of microsatellite markers

For the adult trees, cambium of the twigs and stumps was used for DNA extraction. For the seeds, DNA was

extracted from their embryos. DNA extraction was performed using the cetyltrimethylammonium bromide [CTAB] method (Doyle and Doyle 1990). The DNA content of the samples was determined using a spectrophotometer (GeneQuant pro, Amersham Biosciences, CA, USA). After DNA extraction, polymerase chain reaction (PCR) was performed to examine 15 microsatellite loci in three different multiplexes ([Appendix Table 4](#)) (Brachet et al. 1999; Lefort et al. 1999; Gerard et al. 2006; Aggarwal et al. 2011; Bai et al. 2011; Noakes et al. 2014). However, reproducible results could only be obtained for eleven markers (M2-30; Fp18437; Femsatl 11; Femsatl 12bis; Femsatl 4; Fp14665; Fp21064; FRESTSSR308; FRESTSSR528; Femsatl 19; ASH2429). Femsatl12bis had too many null alleles in floodplain samples, while the same was the case for marker Fp14665 in the seed orchard. These markers were therefore only used for the seed orchard and floodplain, respectively. Subsequently, PCR products were separated by high-resolution capillary electrophoresis using

the GeXP automated sequencer (Beckman Coulter, Inc., Fullerton, CA, USA) and analyzed by software-assisted allele scoring.

2.4 Data analysis

For clone identification, the population genetic software GenALEX 6.0 (Peakall and Smouse 2012) was used. The multilocus genotype of all adult trees was compared and identical genotypes assigned to one clone in the seed orchard. Trees with nonidentical multilocus genotypes with any of the known clones were treated as an individual tree and labeled “0000.” This was the case when the rootstock had grown up to a new tree (two variants: variant 1: The rootstock has grown up after planting, so the ash trees are sexually mature and could be potential fathers; variant 2: Due to ash dieback, the trees have died, and the rootstocks have sprouted. These ash trees were still too young for flowering). Paternity analysis implemented in the Cervus 3.0.7 software package (Kalinowski et al. 2007) was applied to determine the father of each seed. In this context, the allocation is based on Delta (Δ) (Labuschagne et al. 2015). Thereby, the difference of the likelihood-odds ratio (LOD) score between the two most likely parents is examined to find the actual parents. The critical values of Δ were calculated at strict (95%) and relaxed (80%) confidence levels during the simulations (Labuschagne et al. 2015; Semizer-Cuming et al. 2019). For Schorndorf, 100,000 offspring and 70% of sampled potential fathers were simulated. For the alluvial forest, 100,000 offspring and 30% of sampled potential fathers were simulated. The minimum number of loci was set to eight. The error rate was kept at 0.01 (Semizer-Cuming et al. 2019). In addition, self-pollination was explicitly taken into account in the model.

Pollen distances were calculated from the UTM coordinates of the mother and father trees. In the case of the seed orchard, the distances to the mother trees were calculated individually for all potential fathers (ramets) of the same clone, assuming that each of them could be the potential father, since it was not possible to determine the distinct one. Then, we averaged the sum of the distances for each father tree (i.e., each clone). Thus, it was only possible to estimate the average pollination distances to the mother trees. In the floodplain forest,

we were able to calculate the actual pollination distances between fathers and mothers, as all had unique genotypes. The distances were classified at intervals of 10 m. In addition, the relationship between the number of offspring per father tree and the degree of damage to the father trees were calculated. Since ramets of the same clone were showing varying vitality scores, the average vitality score over all ramets per clone was calculated for the seed orchard Schorndorf. In order to estimate the correlation between the damage class and the pollination success, a chi-squared test and exact Fisher test were applied. All analyses and visualizations were performed in RStudio (version 1.2.1335.0) or Microsoft Excel 2016.

3 Results

3.1 Seed orchard

3.1.1 Paternity analysis: relationship between reproductive success and health status

In total, 123 ash trees could be classified into 37 different clones with distinct genotypes. The number of ramets per clone varied from one to eight, with an average of four ramets per clone in the orchard. Twelve of the trees were selected as mother trees. Of the remaining 111 ash trees, 56 were male, 36 were female, and 15 were hermaphrodites. The gender of four ash trees could not be determined accurately. Seventy-six trees had flowers or flowering buds. Thus, 49 trees (36 females and 13 males/hermaphrodites without flowers) were excluded as pollen donors (Appendix Table 2).

Out of the 580 examined offspring, 194 (33.5%) of them could be assigned to their potential fathers (Fig. 2a). Five single trees and 18 clones (54 ash trees) were detected as potential fathers, although for four clones only one tree could be the father in each case with certainty, due to the exclusion procedure (ash trees that were female and/or not flowering were excluded as fathers). Due to its hermaphroditism and the fact that paternity could be found for this clone, one mother tree was also included as a potential father tree. Nine ash trees had no pollination success; three of them have not reached their reproductive age. Regarding the other six ash trees, four were hermaphrodite, and two were male. It was striking that the two male ash trees were very strongly affected by ash dieback (vitality scores 3 and 4).

(See figure on next page.)

Fig. 2 Results of the seed orchard near Schorndorf. **a** Identified father clones with number of ramets (n) resp. identified fathers as well as average vitality score per clone with the average number of offspring per father tree, **b** number of offspring summed allocated to the average vitality score of the father clones, with n representing the number of genotypes, and **c** average number of offspring summed per potential father tree allocated to the vitality score of the potential father trees, with n representing the number of ramets (vitality score: 0 — dark green; 1 — light green; 2 — yellow; 3 — orange; 4 — red). **d** Number of father trees per distance class based on paternity analysis. All potential father trees per mother tree have been counted

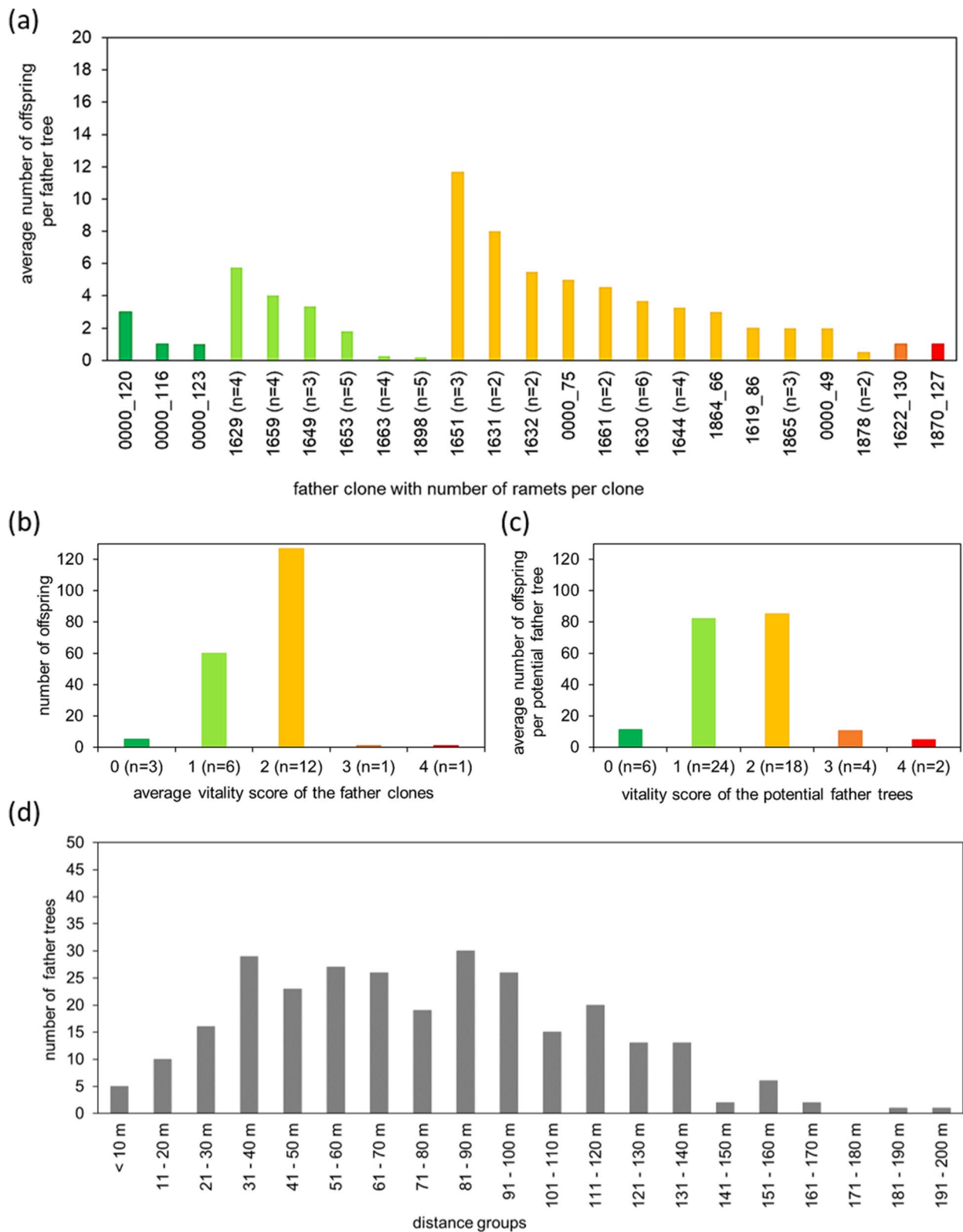


Fig. 2 (See legend on previous page.)

The highest pollination success was observed for the clone *1651* (three potential fathers; average vitality score of 2) with 35 offspring, whereas the lowest pollination success was one offspring associated with 13 trees. Dividing the number of offspring per clone by the number of ramets, the maximum number of offspring is 11.7, and the minimum is 0.3. In addition, the average vitality score over all ramets of a father clone was analyzed; out of the 18 father clones and five single trees, three single trees could be assigned to an average vitality score of 0, six clones and two single trees to an average vitality score of 1, and ten clones to an average vitality score of 2. Only one clone each that sired offspring could be assigned to the vitality classes of 3 and 4 (one offspring each). Overall, father trees in vitality class 2 produced the most offspring (127 offspring) (Fig. 2b). In vitality score classes 0 and 1, in which the ash trees are still considered healthy, the number of identified fathers who sired offspring was substantially below the value of class 2 (vitality score 0: 5 offspring and vitality score 1: 60 offspring). However, no significant correlation could be detected between the damage classes and pollination success (chi-square test: $p=0.999$, Fisher test: $p=0.997$).

Considering the 54 potential father trees as individual trees (regardless of the clone affiliation), 6, 24, 18, 4, and 2 ash trees could be assigned to vitality scores 0, 1, 2, 3, and 4, respectively. With an average of 85.6 offspring, most of the father trees were detected in vitality score class 2 in this case as well. However, the number of fathers that sired offspring in scoring classes 0 and 1 was in total higher (vitality score 0: 11.1 offspring and vitality score 1: 82.0 offspring) than the number of offspring in scoring class 2, with an average of 93.1 offspring. Vitality scores 3 and 4 could be assigned an average of 10.6 and 4.7 offspring from identified fathers, respectively (Fig. 2c). Since vitality classes 1 and 2 had more ramets per clone, it can be assumed that weakly damaged clones have a higher overall potential to produce offspring. However, no significant correlation could be found (chi-square test: $p=0.982$, Fisher test: $p=0.904$).

For the mothers, the average number of offspring that could be assigned to a father tree was 7.5 offspring for vitality score 0 (two mother trees), 23.5 offspring for vitality score 1 (four mother trees), and 13.7 offspring for vitality score 2 (six mother trees).

The analyses identified possible self-pollination for three offspring of the mother trees *1619_31* ($n=2$) and *1898_14* ($n=1$). In the clone *1619* ($n=8$), only the ramet *1619_86* was observed to produce male flowers in addition to female flowers. Thus, due to the number of ramets, it can be assumed that there is a

low probability (11.1%) of self-pollination of the parent tree *1619_31*. Clone 1898 consists of two female and six male ash trees; thus, the probability of self-pollination is again very low, with a value of 12.5%. No self-pollination could be detected for the two hermaphrodite mothers.

3.1.2 Distances of effective pollen transport

The average distance of effective pollen transport of all identified potential father trees to the mother trees was 76.2 m in the seed orchard Schorndorf (Fig. 2d). The largest distance was estimated at 191.1 m and could be detected between the potential father tree *1630_90* and the mother tree *1627_1*. The shortest distance was calculated between the potential father tree *1644_32* and the mother tree *1619_31* at 6.3 m.

In general, it was found that only 5.3% of the identified paternal trees with potential pollination success were located less than 20 m from the mother tree. A total of 23.9% of the potential father trees were located between 21 and 50 m from the mother tree, and 45.1% of the potential father trees were located within a distance of 51 to 100 m. For the distance between 101 and 150 m, potential pollination success was attributed to 22.2% of the father trees and for the distance between 151 and 191.1 m only to 3.5%.

For a better spatial understanding of effective pollen transport, pollination success was investigated with respect to the geographical location of the mother trees. The two mother trees, *1619_81* and *1898_14*, located in different areas of the seed orchard were selected as a representative to describe the general observed pollen dispersal patterns.

For mother tree *1619_81* (vitality score 1), most of the offspring (31 out of 48 seeds) could be assigned to their potential fathers (17 different trees) (Table 1). The mother tree is located in the southwestern area of the orchard, and thus, many potential father trees are within reach (Fig. 3a). The results showed that clone *1629* (four ash trees, average vitality score 1) had the highest pollination success (14 offspring) with this mother tree, but with a distance between 54.6 and 111.9 m, it is not located in the immediate vicinity of the mother tree. At the same time, it was found that the father clones with only one offspring are partly very close to the mother tree (clone *1651* — shortest distance of 34.9 m and clone *1879_75* — distance of 10.5 m). Both clones were linked to an average vitality score of 2.

The mother tree *1898_14* (vitality score 2) had the fewest offspring assigned to it (5 out of 48 seeds). However, for the five offspring, 16 possible father trees must be considered with only a maximum of one to two

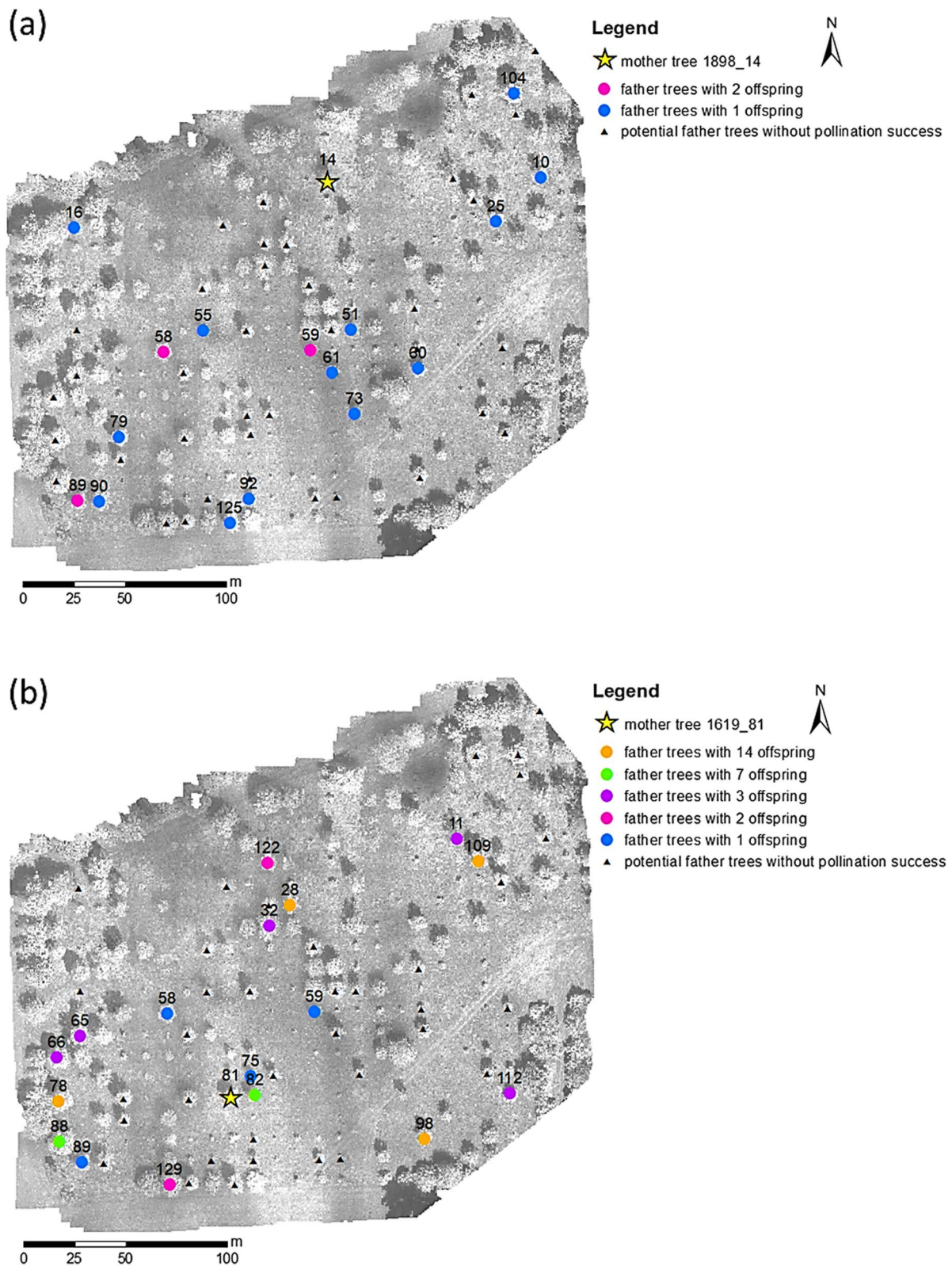


Fig. 3 Mother tree. **a** 1619_81 and **b** 1898_14 with their potential identified father trees and their number of offspring in the seed orchard Schorndorf. Orthophoto taken with drone XR6 (Airborne Robotics, London, UK) equipped with a four-channel multispectral camera (Tetracam Micro-MCA 4, Chatsworth, CA, USA) on 12.07.2018; coordinate system UTM 32N

offspring descended from one father clone. The reason for this could be the location of the mother tree at the northern border of the orchard (Fig. 3b), since 43 offspring could not be assigned to a father clone. The potential father trees identified are not located directly around the mother tree but are mostly distributed in the southern part of the orchard. The closest potential father tree (*I630_51*; vitality score 3) was located at a distance of 48.1 m from the mother tree, 51.6% of the potential father trees were located between 51 and 100 m from the mother tree, and 44.8% of the potential father trees were located between 101 and 130 m from the mother tree.

3.2 Floodplain forest

3.2.1 Paternity analysis: relationship between reproductive success and health status

In the floodplain forest, all sampled trees had unique genotypes. In total, 224 (26.14%) offspring out of 857 examined could be assigned to 73 fathers (Fig. 4 a, b). Forty-three tree stumps and 30 ash trees were detected as father trees. From the 30 living ash trees, 2, 10, 9, 8, and 1 individuals had a vitality score of 0, 1, 2, 3, and 4, respectively (Appendix Table 3). Five of the living ash trees were hermaphroditic; the other 25 were male. Accordingly, no pollination success was detected for 20 living ash trees (out of 50 initially investigated trees), with only six of them being male and one being hermaphroditic; the other 13 were female. Please note that the cambium samples were collected prior to gender determination. Of these seven ash trees (male and hermaphroditic) without pollination success, two were healthy, and five were diseased.

The highest pollination success was observed related to the father tree *tree stump* 19 with 24 offspring, followed by the father tree *A_22* with 20 offspring and the father tree *A_01* with 13 offspring. Twenty-five trees only sired one offspring each. The highest total number of offspring was found in diseased ash trees (vitality scores 2–4). Thus, a significant correlation between the damage classes and pollination success could be found in this case (chi-square test: $p = 0.001$). The tree stumps, which were regarded as diseased individuals ($n = 43$), had 113 offspring (50.5%), and the diseased ash trees ($n = 18$) had 47 offspring (21.0%). In vitality scoring classes 0 and 1, in which the ash trees are

considered as healthy ($n = 12$), the number of offspring was 64 (Fig. 4c). In addition, the average number of offspring per number of ash trees in a vitality scoring class was analyzed; it can be recognized that with an average of 12 offspring, father trees of vitality score 0 could be assigned to the most offspring. The father trees of vitality scoring classes 1, 2, 3, and 4 ($n = 1$) had an average of four, three, and two offspring, respectively, and the current tree stumps had an average of three offspring (Fig. 4d).

For the mothers, the average number of offspring that could be assigned to a father tree was 24, 18.5, 19.8, and 13.5 offspring for vitality score 0 ($n = 1$), 1 ($n = 4$), 2 ($n = 5$), and 3 ($n = 2$), respectively. Additionally, the analyses detected self-pollination for one offspring of the mother tree *M_12* (hermaphrodite; vitality score class 3).

3.2.2 Distances of effective pollen transport

In the floodplain forest, the average distance of effective pollen transport was 165.6 m (Fig. 4e). The longest distance was 551.6 m and could be observed between the *tree stump* 79 and the mother tree *M_08*. The shortest distance (excluding inbreeding) with 2.3 m was calculated between the father tree *A_02* and the mother tree *M_07*.

Overall, 9.6% of the father trees with pollination success were located less than 20 m from the parent tree, and 27.6% were located between 21 and 100 m away. Thus, it can be assumed that the nearest father trees did not pollinate most of the seeds. However, 49.3% of the father trees with pollination success were located within 130 m of the mother tree. A total of 33.3% of pollination success was detected for the trees at more than 200-m distance. For the distance between 301 and 400 m and 401 to 500 m, pollination success could only be detected for 6.4% of the father trees each. For the distance of 501 to 600 m, the value decreased to only 1.3%.

Detailed spatial information was acquired for two selected mother trees, i.e., mother tree *M_04* and *M_08*. For mother tree *M_04* (vitality score 2), 25 seeds out of 73 were attributed to their fathers. This was the mother tree with the highest number of seeds where the respective fathers could be identified. Fourteen father trees

(See figure on next page.)

Fig. 4 Results of the floodplain forest. **a** and **b** Identified **a** living adult trees and **b** father tree stumps (ts) with their vitality score with the number of offspring per father tree. **c** Number of offspring summed allocated to the vitality score of the father trees (n) and (d) average number of offspring summed allocated to the vitality score of the number of father trees (n) (vitality score: 0 — dark green; 1 — light green; 2 — yellow; 3 — orange; 4 — red; tree stumps — brown). **e** Distribution of distance classes derived from paternity analyses for pollen dispersal. The distance classes are in relation to the position of the pollen donor (number of father trees grouped)

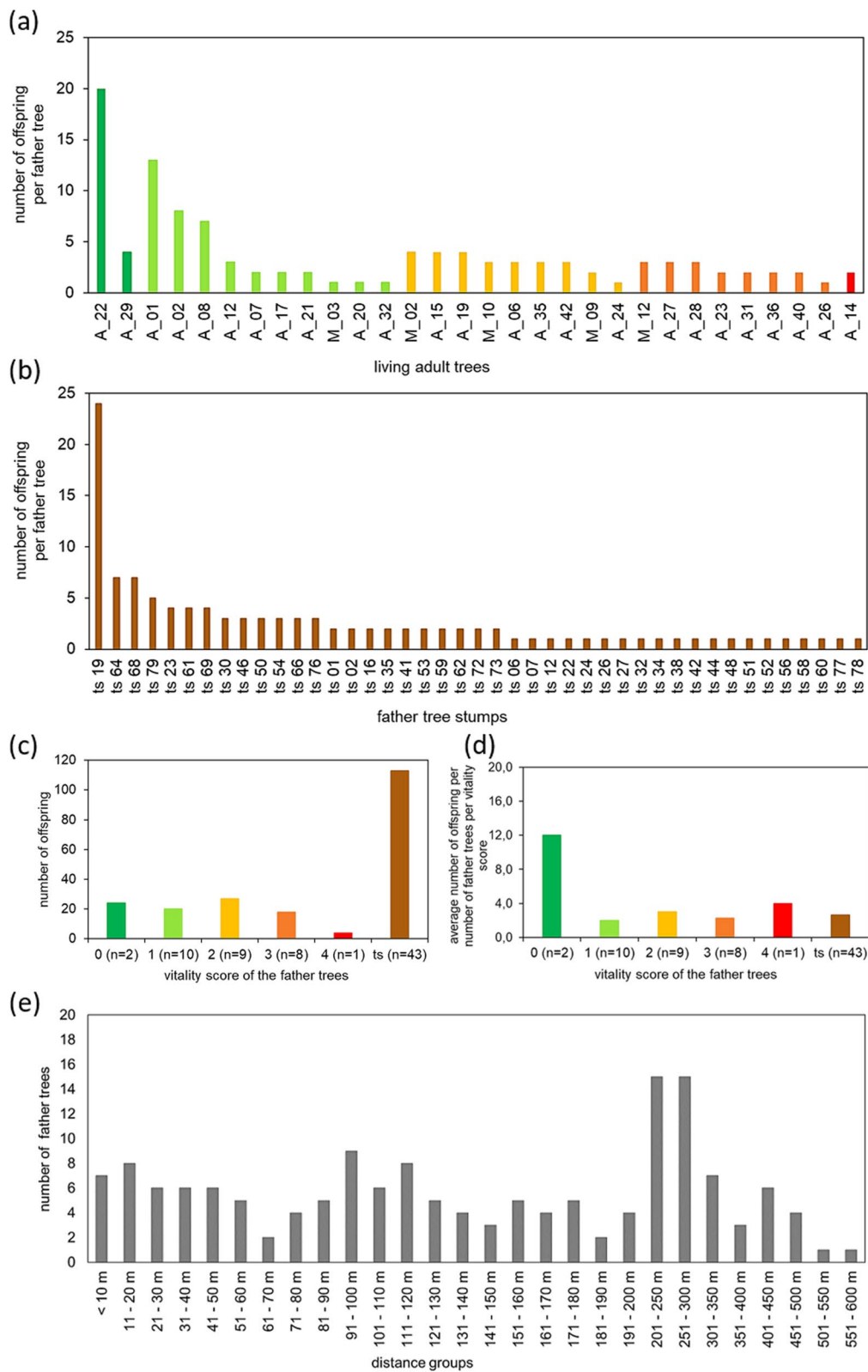


Fig. 4 (See legend on previous page.)

sired 25 seeds (Table 1). The mother tree was centrally located at the study site in a clearing northeast of the lake and was only obscured by other trees to the east and south due to its location (Fig. 5a). Therefore, many of the father trees investigated were within reach to the mother tree. The findings indicated that this mother tree had the highest pollination success (six offspring) with the tree stumps 64 and 68. Both father trees were located on the southwest side of the lake and were not in close proximity, with the distances of 179.9 and 196.8 m, respectively. In contrast, the father ash trees, which were not more than 100 m away from the mother tree, could only be associated with one offspring. In other words, the nearest fathers were not the most successful fathers.

Compared to *M_04*, the mother tree *M_08* (vitality score 3) had less offspring assigned to their fathers. Seven father trees could be detected for the eight offspring. The mother tree is located at the northern edge of the study area; therefore, all identified fathers were positioned in the southwest of the mother tree (Fig. 5b). The closest father (*A_06*) with the pollination success of two offspring was located 40.6 m away from the mother tree. All other more distant father trees had only one offspring. The parent trees, *tree stump 61* and *tree stump 79*, were located the farthest from the mother tree, at a distance of 374.3 and 551.6 m, respectively.

3.3 Comparative summary of the two study sites

The comparison of the results obtained from the seed orchard and the floodplain forest showed that the proportion of all investigated healthy ash trees (vitality scores 0 and 1) differed, with the orchard having 49% healthy trees (Fig. 6a) and the floodplain forest having only 15% (Fig. 6c) or 38% if excluding the tree stumps (Fig. 6e). The results were similar in terms of the proportion of ash trees that could be identified as (potential) fathers (Fig. 6b, d). In this case, the proportion of healthy ash trees was 50% in the seed orchard and 17% in the floodplain forest. However, considering the number of offspring, the analyses showed that at both sites, healthy fathers (vitality scores 0 and 1) as well as those not severely affected by ash dieback (vitality score 2) had almost the same mating success. In contrast, highly diseased fathers (vitality scores 3 and 4), whose proportion in the seed orchard was 14% and in the floodplain forest 30% (excluding the tree stumps; Fig. 6f), could only be assigned to a few offspring. Nevertheless, effective gene flow also takes place between highly susceptible trees.

Although the seed orchard offers the potential to capture most potential father trees, the proportion

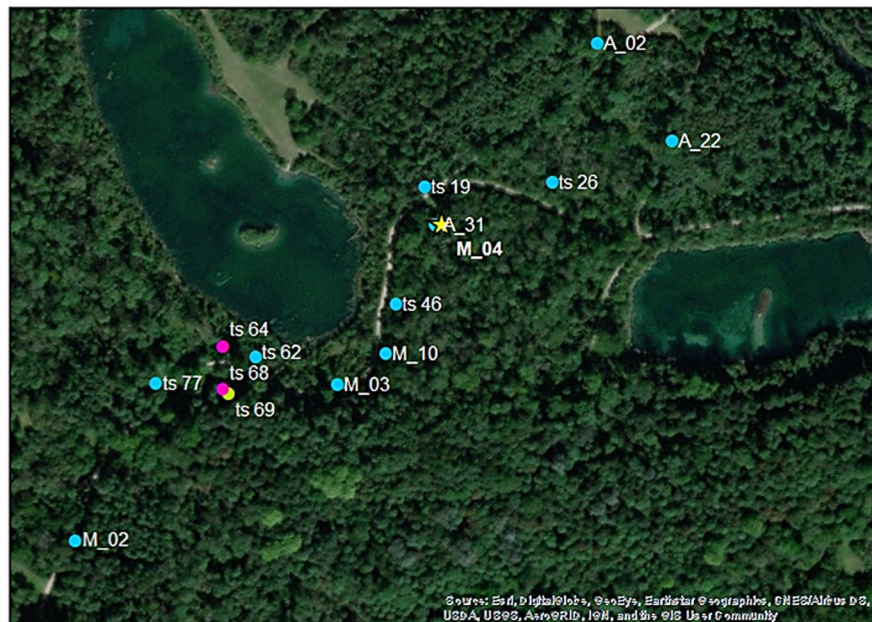
of offspring whose father could be identified was not noticeably lower in the floodplain forest either (orchard: 33.5%; floodplain forest: 26.1%). Our results on genetic connectivity showed that the average distance between identified father and mother trees was 76 m in the seed plantation and 166 m in the floodplain forest. Pollination success generally decreased substantially with increasing distance to the mother tree. Despite the dense tree coverage in the floodplain forest, pollen were transported over long distances (greater than 550 m). Thus, it indicates that local sources were not the only ones playing a role in the pollination. This is supported by the 66.5% of foreign pollen input found in the seed orchard. Nevertheless, we were able to identify the differences in pollen dispersal patterns, especially in the seed orchard. For instance, for mother trees at the border of the plantation (*1627_01*, *1878_13*, *1898_14*, *1627_21*, *1866_30*, *1663_94*), only an average of 22% of the offspring could be assigned to their potential fathers, whereas the proportion for mother trees close to the center (*1663_24*, *1619_31*, *1879_37*, *0000_62*, *1664_71*, *1619_81*) was 44%. A similar pattern was also found in the floodplain forest, though it was not as pronounced (near the center: 30%; at the edge: 21%).

4 Discussion

4.1 Relationship between reproductive success and health status

Overall, our results showed that the ash trees of vitality score 2 produced the most offspring on average, both in the seed orchard and in the floodplain forest (excluding tree stumps). But, taking into account the number of individuals, healthy trees with no or little damage (vitality scores 0 and 1) were not associated with substantially lower pollination success. However, it should be noted that in the case of the Schorndorf seed orchard, only the average vitality score class of the clones could be considered for our assessments in the paternity analyses. Due to the identical genotype of the ramets per clone, the software cannot detect a distinct father tree. As the ramets per clone can have varying vitality scores, the average vitality score has been used. Therefore, it is possible that healthy ash trees will produce more offspring than diseased trees in a clone or vice versa. In the floodplain forest, some of the father trees were only present as tree stumps due to the tree removal before sampling. Therefore, we cannot accurately estimate their vitality score but assume that they were damaged by the fungus to some extent (vitality score > 2). Thus, based on the results, both healthy and relatively

(a)



0 50 100 200 m

(b)



0 50 100 200 m

Fig. 5 Mother tree **a** M₀₄ and mother tree **b** M₀₈ with their identified father trees and their number of offspring in the floodplain forest. Source of the maps: ESRI Data & Maps

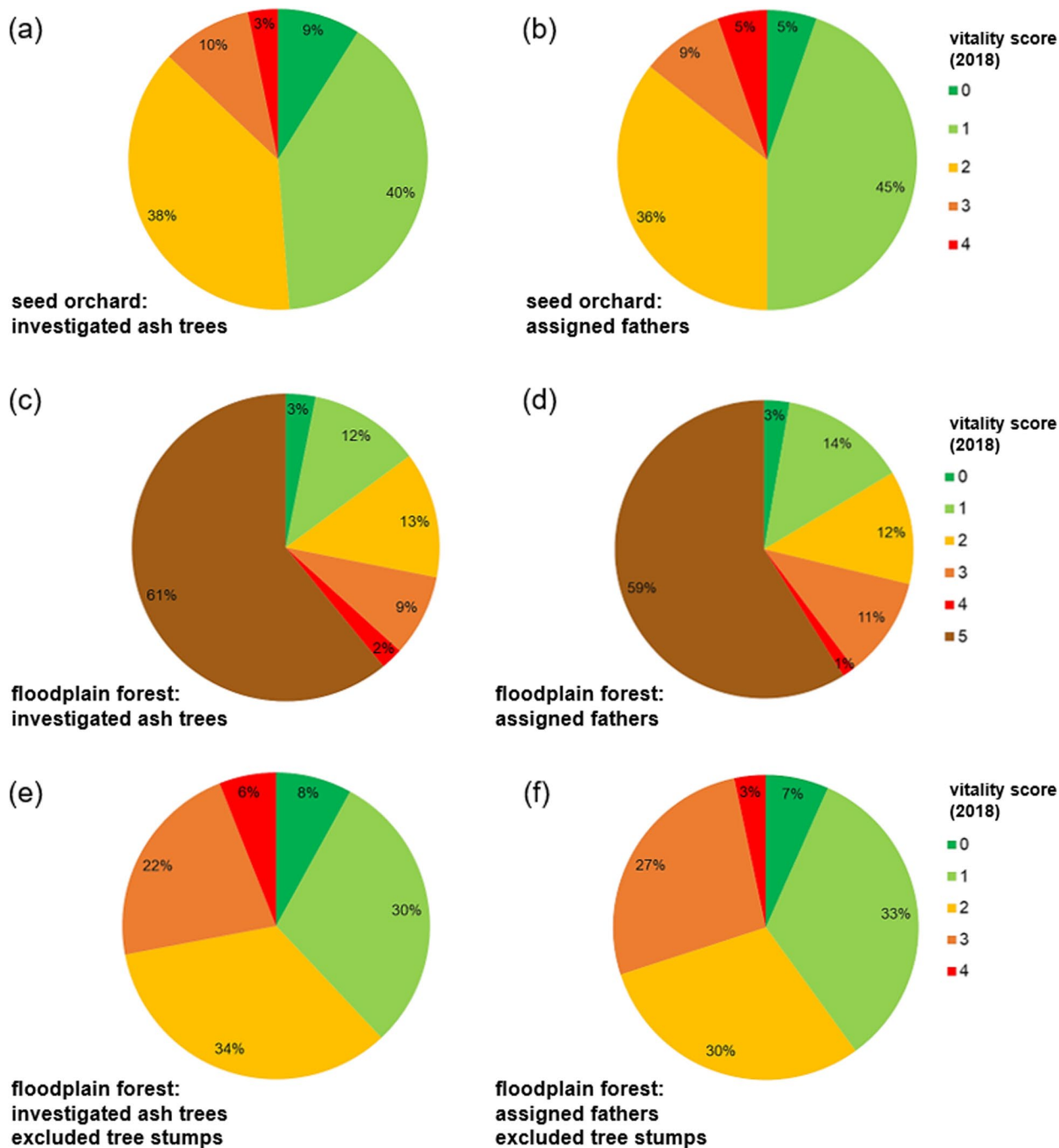


Fig. 6 Vitality of the ash trees in the study sites in 2018. Vitality of **(a)** all investigated ash trees, which could be a pollen donor versus **(b)** all assigned potential fathers in the seed orchard and **(c)** all investigated ash trees, which could be a pollen donor versus **(d)** all assigned fathers in the floodplain forest (vitality score: 0 — dark green; 1 — light green; 2 — yellow; 3 — orange; 4 — red; tree stumps with unknown vitality — brown)

healthy fathers have high pollination success. However, the effect observed by Gassner et al. (2019) that the highest ash pollen emissions occurred 1 to 2 years after the first symptoms of ash dieback noticed in a

region was very weakly perceived in our study. Similar effects of excessive flowering with increasing tree damage were also noted in the context of the forest dieback in the 1980s (Gassner et al. 2019). Thus, it

appears that *Hymenoscyphus fraxineus* infection could at least temporarily increase pollen emission. This can be explained by the fact that stress symptoms in affected trees could lead to increased flowering (Wada and Takeno 2010), which can be regarded as a compromise between reproduction and defensive reaction of the tree (Denancé et al. 2013). Since disease resistance can be partially inherited from parents to offspring (Kjær et al. 2012; Lobo et al. 2015), increased flowering of damaged trees may have a negative effect on the natural regeneration. Thus, Kjær et al. (2012) suggested that only about 1% of the ash trees have the potential to produce offspring with an expected crown damage of less than 10% under the current disease pressure. Since our study revealed low pollination success for the severely damaged father trees and we observed a poor flower development, specifically on severely damaged ash trees in previous studies (Eisen et al. 2022a), it can be assumed that severe damage is associated with decreased pollen production. Nevertheless, even severely damaged male ash trees were capable of producing offspring, which is in line with the findings of Semizer-Cuming et al. (2019; 2021). On the other hand, crown damage caused by ash dieback reduces individual reproductive success (Semizer-Cuming et al. 2021) and thus has a positive effect on natural selection. Therefore, next generations will probably develop a higher resistance to ash dieback. This emphasizes the importance of not removing healthy ash trees during thinning as well as of enriching the gene pool of existing forests through targeted planting of less susceptible ash trees.

Additionally, self-pollination does not seem to have a major effect on reproduction in ash. According to our results, only in a very small proportion of the offspring, self-pollination could be detected with high probability (one offspring in the floodplain forest). Moreover, the study by Saumitou-Laprade et al. (2018) showed that *Fraxinus excelsior* L. might be self-incompatible, preventing loss of genetic diversity, which is critical for the future of ash, especially in the light of ash dieback (Fussi 2020).

Concerning the mother trees, in the seed orchard, the majority of the offspring (52.6%) whose fathers could be identified were assigned to mothers of vitality class 1, and in the floodplain forest, the proportion was 56.3%. Since the total number of seeds produced per tree was not investigated at this point, it is not possible to make a statement about the relationship between health status and reproductive success of the mother trees. However, a couple of studies previously reported that female ash trees severely affected by ash dieback produced much lower amounts of seeds compared to healthy ones (Semizer-Cuming et al. 2019, 2021). Overall, our research indicates

a relationship between reproductive success and health, with healthy and only slightly damaged male ash trees (vitality score ≤ 2) contributing more to pollination.

4.2 Estimation of pollen transport distances

Our results showed that ash trees in Schorndorf had the highest pollination success rate (70%) within a radius of 100 m. In the floodplain forest, over 50% of pollination success occurred within 140 m, whereas only about 8% of father trees had pollination success over distances of more than 400 m. The longest detected distance of pollination in the floodplain forest was more than 550 m, corresponding nearly to the maximum extent of the study area. Thus, our findings are very consistent with previous research; the study by Heuertz et al. (2003), which revealed that effective pollen transport occurred at distances ranging from 70 to 140 m, fits well with our results from Schorndorf. The highest amount of successful pollination occurred at a distance of 76 m between father and mother trees in the seed orchard. In contrast, Semizer-Cuming et al. (2021) reported that 50% of effective pollen transport occurs within 140 m. This observation was reflected in the results of efficient pollen transport in the floodplain forest. In both cases, a sub-area of a large contiguous mixed forest was investigated, where ash occurs with other forest tree species.

Despite the fact that 2018 was a masting year at the Schorndorf seed orchard (Eisen et al. 2022a), a 66.5% foreign pollen input was detected. Seed orchards are collections of breeding populations that have been carefully selected to produce high-quality reproductive material (Fussi et al. 2014). Therefore, pollen input from ash trees from outside the orchard can affect seed quality. A seed orchard should be distinguished above all by the fact that the selected plus trees within the orchard mutually fertilize each other, with preferably no foreign input. Although no ash trees were found in the immediate vicinity of the orchard, it is possible that ash pollen from the further surrounding area contributed to fertilization. This has already been demonstrated in previous studies on effective pollen transport, which detected distances between 1.3 and 2.9 km (Bacles and Ennos 2008; Semizer-Cuming et al. 2021). In the 300-ha landscape heavily deforested described by Bacles and Ennos (2008), pollen immigration represented also between 43 and 68% of effective pollination (depending on the assignment method). Furthermore, Semizer-Cuming et al. (2017) found that 26–45% of pollen were transported from outside when they studied the genetic connectivity of ash trees in an isolated forest site in a fragmented landscape (2 ha). The cause of not local or even long-distance pollen transport could be wind related, which, as already demonstrated in other studies, is the primary factor

responsible for the dispersal of pollen (Laaidi 2001; Eisen et al. 2022a). According to Puc (2012), larger effective pollen transport distances are linked to stronger winds. Semizer-Cuming et al. (2017) found a positive correlation between prevailing wind direction and the mean direction of pollen dispersal in ash. In addition, factors such as tree height and canopy width also influence pollen dispersal (Adams-Groom et al. 2017). Thus, pollination within the orchard may have suffered from many dead trees due to ash dieback. Thus, spacing between trees increased, possibly leading to an increasingly mixed pollen cloud and higher pollen income from outside to pollinate the flowers. This also corresponds well with our pollen dispersal patterns: In the case of mother trees in marginal positions, a greater pollen input from outside was observed, especially in the orchard. Therefore, seeds should be harvested preferably from central trees having a higher probability that both parents originate from plus trees within the orchards. In Germany, the Forest Reproduction Act (FOVG 2002; BGBl. I.S. 1658) recommends that seed orchards maintain a distance of 400 m from phenotypically weak stands of the same species or a species that can be crossed with it (gGA 2019). Thus, one measure to increase pollination within the orchard is to ensure that the distance between foreign ash trees and the seed orchard is substantially greater than 400 m. In addition, protecting seed orchards primarily with conifers such as spruce planted at the borders may aid in reducing pollen input from the outside and avoiding cross-pollination. Deciduous trees or shrubs might also reduce pollen input but are more permeable because leaf formation often begins after flowering of ash or are low in height (in case of shrubs). Another fact is the synchronization of flowering time between clones in the seed orchard. Flowering of male and female flowers has to occur approximately at the same time in order to fertilize successfully. When selecting plus trees from different regions, the flowering time of the plus trees combined in the seed orchard may differ from each other. If there is only little overlap between the flowering time of male and female flowers, pollen from outside may be more fertile at certain times (Mondal et al. 2019). Therefore, we recommend that a large orchard is established with as many trees as possible. This increases the likelihood that the trees flower synchronously and thus ensures that most pollination will occur within the seed orchard.

In the floodplain forest, only 26.1% of the seeds could be assigned to their fathers, implying that pollen are transported over distances greater than 550 m. However, in the approaches based on paternity analyses, it is often difficult or infeasible to sample all trees in a forest that represent a potential pollen source for

reproduction (Bacles and Ennos 2008). Hence, it must be noted that a higher number of parent trees would possibly also shift the mean values, maxima and minima. However, as already discussed, since healthy and only slightly damaged male ash trees (vitality score ≤ 2) contribute more to pollination, there is a chance for gene flow between somewhat isolated individuals or groups of healthy ash trees. Increasingly fragmented ash populations that are expected in the future, as well as already isolated ash trees that are less susceptible to *H. fraxineus*, may still be in genetic exchange with one another, increasing the chance of a healthier next generation. Individuals of the natural regeneration are the result of natural selection and might be able to withstand inter- or intraspecific competition and could therefore be disease resistant (Metzler et al. 2012). Thus, in the long term, those genotypes that can better withstand the negative effects of the fungus will prevail and have the highest fitness under the prevalent environmental conditions (Fussi et al. 2014). However, Buchner et al. (2022) found that the environmental conditions prevailing during long-distance pollen transport play an important role in the successful pollination of female ash flowers. It was demonstrated that pollen viability decreased faster with increased or prolonged UV radiation and under warmer conditions. Instead, viable pollen could still be observed after 28 days at moderate temperatures. Thus, prolonged heat extremes caused by climate change can have serious consequences for the pollen's viability during long-distance pollen transport. Nevertheless, it has been shown that gene flow can occur over longer distances, and successful pollination is possible.

5 Conclusion

In summary, it can be concluded that effective pollen transport occurs over long distances, although with decreasing pollination success. There is still hope for the future of ash since healthy and slightly diseased fathers make a greater contribution to pollination, meaning that gene flow will facilitate the healthier next generation of ash in the future. However, in order to reduce pollen input from outside and avoid cross-pollination on the seed plantations, it is important to establish large seed orchards protected by planting around the border. In addition, female trees should be planted mainly in the center of the orchard to enhance short-distance pollination within the orchard. We recommend harvesting seeds only during masting years due to intensified pollination between plus trees in the orchard.

Appendix

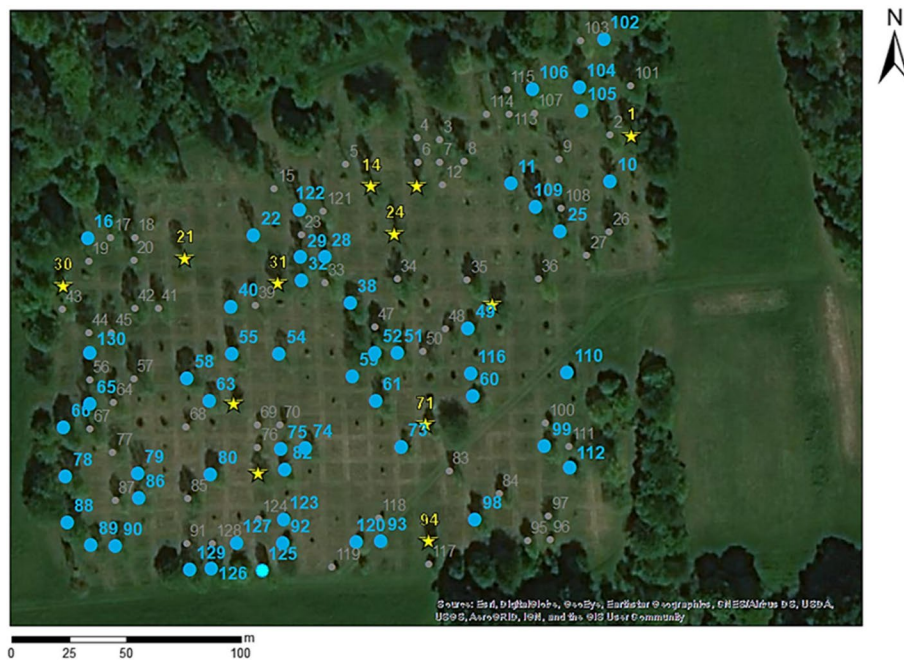


Fig. 7 Seed orchard including investigated ash trees, labeled by tree ID: yellow stars – mother trees, blue dots—ash trees identified as potential father trees by their clone number; gray dots –investigated ash trees excluded as pollen donors. Source of the map: ESRI Data & Maps

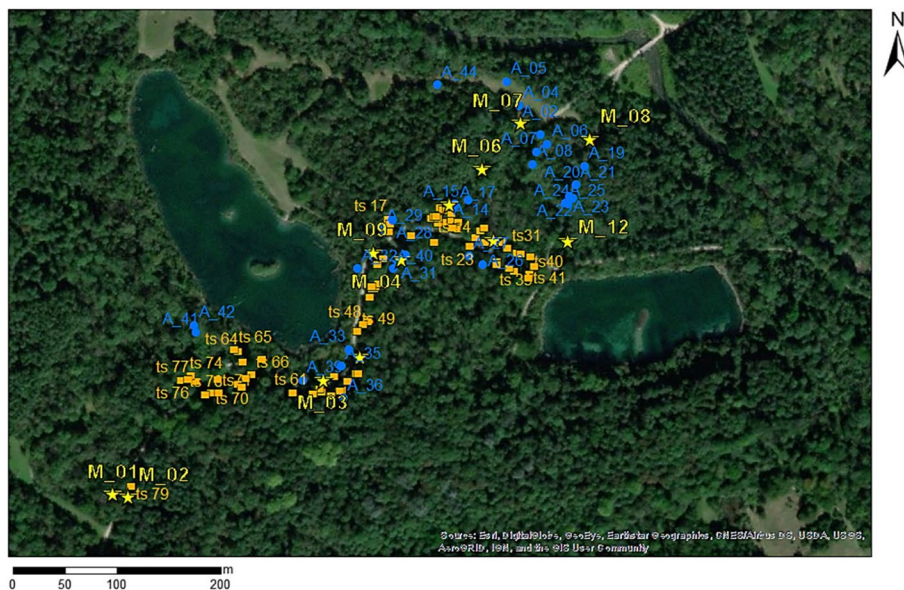


Fig. 8 Study area of floodplain forest including investigated ash trees, labeled by tree ID: yellow stars — mother trees, blue dots — ash trees identified as father trees, and orange squares — logged ash trees identified as father trees. Source of the map: ESRI Data & Maps

Table 2 Ash trees of the seed orchard near Schorndorf

Clone number	Trec-ID	New clone assignment (if necessary)	Gender	Flowered	Vitality score (2018)
1627	1		f	+	2
1664	2		f	+	2
1877	3		f	-	2
1859	4		h	-	3
1637	5		m	+	3
1866	6		f	+	3
1630	8		m	+	2
1878	9		f	+	3
1630	10		m	+	2
1644	11		m	+	3
1878	13		f	+	2
1898	14		f	+	2
1630	16		m	+	4
1879	18		f	+	2
1898	19		m	-	3
1663	20		f	+	2
1627	21		f	+	2
1878	22		h	+	2
1879	23		m	+	4
1663	24		f	+	2
1898	25		m	+	1
1877	26	0000	f	+	1
1866	27		h	+	1
1629	28		m	+	1
1632	29		m	+	2
1866	30		f	+	2
1619	31		f	+	1
1644	32		m	+	2
1866	33		f	+	2
1627	34	1866	h	+	2
1631	35		m	+	1
1664	36		f	+	1
1879	37		f	+	1
1635	38	1653	m	-	1
1898	39		m	-	2
1653	40		m	+	2
1664	41		f	+	2
1631	42		m	-	2
1632	43		m	-	2
1653	44		m	-	2
1661	45	0000	m	+	3
1877	47	0000	m	-	1
1619	48		f	+	1
1878	49	0000	h	+	2
1661	50		m	-	1
1630	51		m	+	3
1865	52		m	+	2
1649	54		m	+	1
1659	55		h	+	0
1627	56		h	+	2
1878	57		f	+	2
1651	58		m	+	2
1651	59	1630	m	+	1
1653	60		m	+	1
1898	61		m	+	1
1877	62	0000	f	+	1
1865	63		m	+	3
1619	64		f	+	1
1644	65		m	+	2
1864	66		h	+	2
1663	67		f	+	1
1866	68		f	+	2
1664	69		f	+	2
1663	70		f	+	1
1664	71		h	+	0
1659	73		m	+	1
1878	74		h	+	1
1879	75	0000	h	+	2
1627	76		f	+	2
1879	77		f	+	1
1629	78		m	+	1
1898	79		m	+	1
1653	80		m	+	1
1619	81		f	+	1
1631	82		m	+	2
1878	83		f	+	1
1619	84		f	+	1
1878	85		f	+	2
1619	86		h	+	2
1664	87		+	+	1
1631	88		m	+	2
1651	89		m	+	2
1630	90		h	+	2
1866	91	0000	f	+	2
1630	92		m	+	1
1865	93		h	+	1
1663	94		h	+	0
1664	95		f	+	1
1866	96		f	+	2
1879	97		f	+	2
1629	98		m	+	1
1649	99		m	+	1
1898	100		f	+	1
1663	101		f	+	2
1653	102		m	+	3
1659	103		f	+	3
1865	104	1659	m	+	1
1653	105		m	+	1
1649	106		m	+	1
1627	107		f	+	1
1619	108		f	+	2
1629	109		m	+	1
1663	110		h	+	1
1877	111		m	+	1
1644	112		m	+	1
1879	113		f	+	1
1651	114	0000	m	+	4
1663	115		f	+	1
1880	116	0000	m	+	0
1879	117		f	+	1
1877	118		h	+	0
1626	(119)		h	+	0
1880	120	0000	m	+	0
1664	121		f	+	3
1661	122		m	+	2
1889	(123)	0000	m	+	0
1646	(124)				0
1898	125		m	+	0
1632	126		m	+	2
1870	(127)		f	+	4
1880	128	0000	m	+	0
1661	129		m	+	1
1622	130		m	+	3

Gender assignments (m, male; f, female; h, hermaphrodite), the presence of flowers on the 109th day of the year (+ or -), and vitality classifications according to Lenz et al. (2012) (categories 0 and 1, healthy; categories 3–4, diseased; category 5, dead). Color codes are as follows: pink indicates the mother trees, blue shows the potential fathers assigned to offspring, green denotes the potential fathers unassigned to any offspring, and white shows all other ash trees excluded as potential fathers

Table 3 Ash trees of the floodplain forest

tree-ID	gender	vitality score (2018)
M_001	f	1
M_002	h	2
M_003	h	1
M_004	f	2
M_005	f	2
M_006	f	1
M_007	f	0
M_008	f	3
M_009	h	2
M_010	h	2
M_011	f	1
M_012	h	3
A_E_001	m	1
A_E_002	h	1
A_E_004	h	2
A_E_005	m	1
A_E_006	m	2
A_E_007	m	1
A_E_008	m	1
A_E_009	m	4
A_E_010	f	2
A_E_011	f	3
A_E_012	h	1
A_E_014	h	4
A_E_015	m	2
A_E_017	m	1
A_E_019	m	2
A_E_020	m	1
A_E_021	m	2
A_E_022	m	0
A_E_024	h	3
A_E_024	m	2
A_E_025	f	3
A_E_026	h	3
A_E_027	h	3
A_E_028	m	3
A_E_029	m	0
A_E_030	f	0
A_E_031	h	3
A_E_032	m	1
A_E_033	m	2
A_E_034	f	4
A_E_035	m	2
A_E_036	m	3
A_E_038	m	1
A_E_039	f	2
A_E_040	m	3
A_E_041	m	2
A_E_042	h	2
A_E_044	m	2
tree stamp 01	(m/h)	diseased
tree stamp 02	(m/h)	diseased
tree stamp 03		diseased
tree stamp 04		diseased
tree stamp 05		diseased
tree stamp 06	(m/h)	diseased
tree stamp 07	(m/h)	diseased
tree stamp 08		diseased
tree stamp 09		diseased
tree stamp 10		diseased
tree stamp 11		diseased
tree stamp 12	(m/h)	diseased
tree stamp 13		diseased
tree stamp 14		diseased
tree stamp 15		diseased
tree stamp 16	(m/h)	diseased
tree stamp 17		diseased
tree stamp 18		diseased
tree stamp 19	(m/h)	diseased
tree stamp 20		diseased
tree stamp 21		diseased
tree stamp 22	(m/h)	diseased
tree stamp 23	(m/h)	diseased
tree stamp 24	(m/h)	diseased
tree stamp 25		diseased
tree stamp 26	(m/h)	diseased
tree stamp 27	(m/h)	diseased
tree stamp 28		diseased
tree stamp 29		diseased
tree stamp 30	(m/h)	diseased
tree stamp 31		diseased
tree stamp 32	(m/h)	diseased
tree stamp 33	(m/h)	diseased
tree stamp 34	(m/h)	diseased
tree stamp 35	(m/h)	diseased
tree stamp 36		diseased
tree stamp 37		diseased
tree stamp 38	(m/h)	diseased
tree stamp 39		diseased
tree stamp 40		diseased
tree stamp 41	(m/h)	diseased
tree stamp 42	(m/h)	diseased
tree stamp 43		diseased
tree stamp 44	(m/h)	diseased
tree stamp 45		diseased
tree stamp 46	(m/h)	diseased
tree stamp 47		diseased
tree stamp 48	(m/h)	diseased
tree stamp 49		diseased
tree stamp 50	(m/h)	diseased
tree stamp 51	(m/h)	diseased
tree stamp 52	(m/h)	diseased
tree stamp 53	(m/h)	diseased
tree stamp 54	(m/h)	diseased
tree stamp 55		diseased
tree stamp 56	(m/h)	diseased
tree stamp 57		diseased
tree stamp 58	(m/h)	diseased
tree stamp 59	(m/h)	diseased
tree stamp 60	(m/h)	diseased
tree stamp 61	(m/h)	diseased
tree stamp 62	(m/h)	diseased
tree stamp 63		diseased
tree stamp 64	(m/h)	diseased
tree stamp 65		diseased
tree stamp 66	(m/h)	diseased
tree stamp 67		diseased
tree stamp 68	(m/h)	diseased
tree stamp 69	(m/h)	diseased
tree stamp 70		diseased
tree stamp 71		diseased
tree stamp 72	(m/h)	diseased
tree stamp 73	(m/h)	diseased
tree stamp 74		diseased
tree stamp 75	(m/h)	diseased
tree stamp 76	(m/h)	diseased
tree stamp 77	(m/h)	diseased
tree stamp 78	(m/h)	diseased
tree stamp 79	(m/h)	diseased

Gender assignments (m, male; f, female; h, hermaphrodite), the presence of flowers on the 109th day of the year (+ or -), and vitality classifications according to Lenz et al. (2012) (categories 0 and 1, healthy; categories 3–4, diseased; category 5, dead). Color codes are as follows: pink indicates the mother trees, blue shows the potential fathers assigned to offspring, green denotes the potential fathers unassigned to any offspring, and white shows all other ash trees excluded as potential fathers

Table 4 Specifications of the fifteen nuclear microsatellite markers used in the study. Markers with no reproducible results are shown in Italics

Primer name	Temp. [°C]	Length [bp]	Label	Multiplex	Repeat motif	Literature
M2-30	55	182–294	Dy751	A	(TG)15(AG)23	Brachet et al. (1999)
<i>Fp12353</i>	55	218–243	Cy5	A	(TAAT)5	Noakes et al. (2014)
<i>Fp21068</i>	55	232–282	Cy5	A	(TTGGT)4	Noakes et al. (2014)
<i>Fp19681</i>	55	209–222	Dy751	A	(CT)8	Noakes et al. (2014)
<i>Fp18437</i>	55	302–321	Dy751	B	(CT)10	Noakes et al. (2014)
Femsatl 11	55	161–234	Cy5	B	(GA)20(TA)4	Lefort et al. (1999)
Femsatl 12bis	55	147–261	Dy751	B	(GA)6CA(GA)8	Gerard et al. (2006)
Femsatl 4	55	155–205	Cy5	B	(CA)2(AG)24	Lefort et al. (1999)
<i>Fp14665</i>	55	260–297	IRD700	B	(CT)8	Noakes et al. (2014)
<i>Fp21064</i>	59	234–241	Cy5	C	(GCC)7	Noakes et al. (2014)
FRESTSSR308	59	194–202	Dy751	C	(AG)8	Aggarwal et al. (2011)
<i>FRESTSSR427</i>	59	224–272	IRD700	C	(TAAT)4	Aggarwal et al. (2011)
FRESTSSR528	59	271–304	Cy5	C	(TTC)4/(CTG)8	Aggarwal et al. (2011)
Femsatl 19	59	142–238	IRD700	C	(CA)6CGGC(CA)13	Lefort et al. (1999)
ASH2429	59	235–255	Dy751	C	(TTC)7	Bai et al. (2011)

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Authors' contributions

Conceptualization, BF and SJO; methodology, BF, DSC, SJO, and AKE; formal analysis and investigation, AKE; data curation, AKE; visualization, AKE; writing — original draft preparation, AKE; writing — review and editing, AKE, SJO, BF, and DSC; funding acquisition, SJO and BF; and supervision, SJO. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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References

- Adams-Groom B, Skjøth CA, Baker M, Welch TE (2017) Modelled and observed surface soil pollen deposition distance curves for isolated trees of *Carpinus betulus*, *Cedrus atlantica*, *Juglans nigra* and *Platanus acerifolia*. *Aerobiologia* 33:407–416. <https://doi.org/10.1007/s10453-017-9479-1>
- Aggarwal RK, Allainguillaume J, Bajay MM, Barthwal S, Bertolino P, Chauhan P, Consuegra S, Croxford A, Dalton DL, den Belder E, Díaz-Ferguson E, Douglas MR, Drees M, Elderson J, Esselink GD, Fernández-Manjarrés JF, Frascaria-Lacoste N, Gäbler-Schwarz S, Garcia de Leaniz C, Ginwal HS, Goodisman MAD, Guo B, Hamilton MB, Hayes PK, Hong Y, Kajita T, Kalinowski ST, Keller L, Koop BF, Kotzé A, Lalremruata A, Leese F, Li C, Liew WY, Martinelli S, Matthews EA, Medlin LK, Messmer AM, Meyer EI, Monteiro M, Moyer GR, Nelson RJ, Nguyen TTT, Omoto C, Ono J, Pavinato VAC, Percy M, Pinheiro JB, Power LD, Rawat A, Reusch TBH, Sanderson D, Sannier J, Sathe S, Sheridan CK, Smulders MJM, Sukganah A, Takayama K, Tamura M, Tateishi Y, Vanhaecke D, Vu NV, Wickneswari R, Williams AS, Wimp GM, Witte V, Zucchi MI (2011) Permanent genetic resources added to molecular ecology resources database 1 August 2010–30 September 2010. *Mol Ecol Resour* 11:219–222. <https://doi.org/10.1111/j.1755-0998.2010.02944.x>

- Bacles CFE, Ennos RA (2008) Paternity analysis of pollen-mediated gene flow for *Fraxinus excelsior* L. in a chronically fragmented landscape. *Heredity* 101:368–380. <https://doi.org/10.1038/hdy.2008.66>
- Bai X, Rivera-Vega L, Mamidala P, Bonello P, Herms DA, Mittapalli O (2011) Transcriptional signatures of ash (*Fraxinus* spp.) phloem. *PLoS One* 6:e16368. <https://doi.org/10.1371/journal.pone.0016368>
- Baral HO, Queloz V, Hosoy T (2014) *Hymenoscyphus fraxineus*, the correct scientific name for the fungus causing ash dieback in Europe. *IMA Fungus* 5(1):79–80. <https://doi.org/10.5598/imafungus.2014.05.01.09>
- Brachet S, Jubier F, Richard M, Jung-Muller B, Frascaria-Lacoste N (1999) Rapid identification of microsatellite loci using 5' anchored PCR in the common ash *Fraxinus excelsior*. *Mol Ecol Notes* 8:160–163
- Buchner L, Eisen A-K, Šikoparija B, Jochner-Oette S (2022) Pollen viability of *Fraxinus excelsior* in storage experiments and investigations on the potential effect of long-range transport. *Forests* 13:600. <https://doi.org/10.3390/f13040600>
- Coker TLR, Rozsypálek J, Edwards A, Harwood TP, Butfoy L, Buggs RJA (2019) Estimating mortality rates of European ash (*Fraxinus excelsior*) under the ash dieback (*Hymenoscyphus fraxineus*) epidemic. *Plants, People, Planet* 1:48–58. <https://doi.org/10.1002/ppp3.11>
- Denancé N, Sánchez-Vallet A, Goffner D, Molina A (2013) Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. *Front Plant Sci* 4:155. <https://doi.org/10.3389/fpls.2013.00155>
- Doben K, Doppler G, Freudenberger W, Jerz H, Meyer RKF, Mielke H, Ott W-D, Rohrmüller J, Schmidt-Kaler H, Schwed K, Unger HJ (1996) Geologische Karte von Bayern, 4th edn. Bayerisches Geologisches Landesamt, Munich.
- Doyle JJ, Doyle LJ (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15
- Eisen A-K, Fussi B, Šikoparija B, Jochner-Oette S (2022) Aerobiological pollen deposition and transport of *Fraxinus excelsior* L. at a small spatial scale. *Forests* 13:424. <https://doi.org/10.3390/f13030424>
- Eisen A-K, Fussi B, Jochner-Oette S (2022b) Die Zukunft der Esche im Aulwald. *Auenmagazin*: 4–9.
- Enderle R, Nakou A, Thomas K, Metzler B (2014) Susceptibility of autochthonous German *Fraxinus excelsior* clones to *Hymenoscyphus pseudoalbidus* is genetically determined. *Ann for Sci* 72:183–193. <https://doi.org/10.1007/s13595-014-0413-1>
- Enderle R, Fussi B, Lenz HD, Langer G, Nagel R, Metzler B (2017) Ash dieback in Germany: research on disease development, resistance and management options. In: Vasaitis R, Enderle R (eds) Dieback of European Ash (*Fraxinus* spp.): Consequences and guidelines for sustainable management, Uppsala, Sewden, pp 89–105.
- Enderle R (2019) An overview of ash (*Fraxinus* spp.) and the ash dieback disease in Europe. *CAB Reviews* 14. <https://doi.org/10.1079/PAVSNNR201914025>
- FOVG (2002) Deutsches Forstvermehrungsgutgesetz vom 22. Mai 2002. BGBl. I p. 1658. <https://www.gesetze-im-internet.de/fovg/BJNR165800002.html>. Accessed 23 May 2023.
- Fussi B, Konnerth M, Cremer E (2014) Genfluss in Waldbeständen LWF Wissen 74:22–26
- Fussi B (2020) So hat die Esche eine Chance! In: LWF aktuell 126: 60–61
- Gassner M, Schmid-Grendelmeier P, Clot B (2019) Ash pollen allergy and aerobiology. *Allergo J Int* 28:289–298. <https://doi.org/10.1007/s40629-019-00105-6>
- Gerard PR, Fernandez-Manjarres JF, Frascaria-Lacoste N (2006) Temporal cline in a hybrid zone population between *Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl. *Mol Ecol* 15:3655–3667. <https://doi.org/10.1111/j.1365-294X.2006.03032.x>
- gGA (2019) Forstvermehrungsgutrecht: Empfehlungen des gemeinsamen Gutachterausschusses (gGA) der Länder für dessen Umsetzung. https://www.ble.de/SharedDocs/Downloads/DE/Landwirtschaft/Saat-und-Planzgut/Empfehlungen.pdf?__blob=publicationFile&v=2. Accessed 25 Mar 2022
- Heuertz M, Vekemans X, Hausman J-F, Palada M, Hardy OJ (2003) Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. *Mol Ecol* 12:2483–2495. <https://doi.org/10.1046/j.1365-294x.2003.01923.x>
- Hultberga T, Sandström J, Felton A, Öhman K, Rönnerberg J, Witzell J, Cleary M (2020) Ash dieback risks an extinction cascade. *Biol Conserv* 244:108516. <https://doi.org/10.1016/j.biocon.2020.108516>
- Jochner-Oette S, Rohrer T, Eisen A-K, Tönnes S, Stammel B (2021) Influence of forest stand structure and competing understory vegetation on ash regeneration—potential effects of ash dieback. *Forests* 12:128. <https://doi.org/10.3390/f12020128>
- Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16:1099–1106. <https://doi.org/10.1111/j.1365-294X.2007.03089.x>
- Kjær ED, McKinney LV, Nielsen LR, Hansen LN, Hansen JK (2012) Adaptive potential of ash (*Fraxinus excelsior*) populations against the novel emerging pathogen *Hymenoscyphus pseudoalbidus*. *Evol Appl* 5:219–228. <https://doi.org/10.1111/j.1752-4571.2011.00222.x>
- Kölling C (2007) Klimahüllen für 27 Waldbaumarten. *AFZ—Der Wald* 23:1242–1245
- Kosawang C, Arny DB, Bussaban B, McKinney LV, Xu J, Kjær ED, Collinge DB, Nielsen LR (2018) Fungal communities associated with species of *Fraxinus* tolerant to ash dieback, and their potential for biological control. *Fungal Biol* 122:110–120. <https://doi.org/10.1016/j.funbio.2017.11.002>
- Kowalski T (2006) *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathol* 36:264–270. <https://doi.org/10.1111/j.1439-0329.2006.00453.x>
- Laaidi M (2001) Forecasting the start of the pollen season of *Poaceae*: evaluation of some methods based on meteorological factors. *Int J Biometeorol* 45:1–7. <https://doi.org/10.1007/s004840000079>
- Labuschagne C, Nupen L, Kotzé A, Grobler PJ, Dalton DL (2015) Assessment of microsatellite and SNP markers for parentage assignment in ex situ African penguin (*Spheniscus demersus*) populations. *Ecol Evol* 5:4389–4399. <https://doi.org/10.1002/ece3.1600>
- Lefort F, Brachet S, Frascaria-Lacoste N, Edwards KJ, Douglas GC (1999) Identification and characterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation in the olive family (*Oleaceae*). *Mol Ecol* 8:1088–1089. https://doi.org/10.1046/j.1365-294X.1999.00655_8.x
- Lenz H, Straßner L, Baumann M, Baier U (2012) Boniturschlüssel zur Einstufung der Vitalität von Alteschen. *AFZ—Der Wald* 3:18–129
- LGRB (2021) LGRB-Kartenviewer. <https://maps.lgrb-bw.de/>. Accessed 16 Dec 2021
- Lobo A, McKinney LV, Hansen JK, Kjær ED, Nielsen LR (2015) Genetic variation in dieback resistance in *Fraxinus excelsior* confirmed by progeny inoculation assay. *Forest Pathol* 45:379–387. <https://doi.org/10.1111/efp.12179>
- LWF (2019) Praxishilfe Band I Klima-Boden-Baumartenwahl, pp 89–90. https://www.lwf.bayern.de/mam/cms04/service/dateien/praxishilfe_baumarten_bf.pdf. Accessed 23 May 2023.
- Lygis V, Bakys R, Gustiene A, Burokiene D, Matelis A, Vasaitis R (2014) Forest self-regeneration following clear-felling of dieback-affected *Fraxinus excelsior*: focus on ash. *Eur J Forest Res* 133:501–510. <https://doi.org/10.1007/s10342-014-0780-z>
- Marçais B, Husson C, Caël O, Dowkiw A, Sainonge F-X, Delahaye L, Collet C, Chanderlier A (2017) Estimation of ash mortality induced by *Hymenoscyphus fraxineus* in France and Belgium. *Balt for* 23:159–167
- Margraf C (2004) Die Vegetationsentwicklung der Donauauen zwischen Ingolstadt und Neuburg: vegetationskundlich-ökologische Studie über den Wandel einer Auenlandschaft 30 Jahre nach Staustufenbau, vol 65. Hoppea, Denkschriften der Regensburgischen Botanischen Gesellschaft, Regensburg, Germany
- McKinney LV, Nielsen LR, Hansen JK, Kjær ED (2011) Presence of natural genetic resistance in *Fraxinus excelsior* (*Oleaceae*) to *Chalara fraxinea* (*Ascomycota*): an emerging infectious disease. *Heredity* 106:788–797. <https://doi.org/10.1038/hdy.2010.119>
- McKinney LV, Nielsen LR, Collinge DB, Thomsen IM, Hansen JK, Kjær ED (2014) The ash dieback crisis: genetic variation in resistance can prove a long-term solution. *Plant Pathol* 63:485–499. <https://doi.org/10.1111/ppa.12196>
- Metzler B, Enderle R, Karopka M, Töpfner K, Aldinger E (2012) Development of ash dieback in a provenance trial on different sites in southern Germany. *Allgemeine Forst- Und Jagdzeitung* 183:168–180
- Mondal S, Srivastava A, Joshi G, Yashavantha Rao HC (2019) Asynchronous flowering in clonal seed orchards—an effective strategy for alternative management. *J Plant Sci Phytopathol* 3:36–41. <https://doi.org/10.29328/journal.jpssp.1001029>
- Müller-Kroehling S, Schmidt O (2019) Eschentriebsterben und Naturschutz: 7 Fragen, 7 Antworten. *Anliegen Natur* 41:145–156

- Noakes AG, Best T, Staton ME, Koch J, Romero-Severson J (2014) Cross amplification of 15 EST-SSR markers in the genus *Fraxinus*. *Conserv Genet Resour* 6:969–970. <https://doi.org/10.1007/s12686-014-0260-2>
- Peakall R, Smouse PE (2012) GenAEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28:2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Pliūra A, Bakys R, Suchockas V, Marčiulynienė D, Gustienė A, Verbyla V, Lygis V (2017) Ash dieback in Lithuania: disease history, research on impact and genetic variation in disease resistance, tree breeding and options for forest management. In: Vasaitis R, Enderle R (eds) *Dieback of European Ash (Fraxinus spp.): Consequences and Guidelines for Sustainable Management*, Uppsala, Sewden, pp 150–165
- Puc M (2012) Artificial neural network model of the relationship between *Betula* pollen and meteorological factors in Szczecin (Poland). *Int J Biometeorol* 56:395–401. <https://doi.org/10.1007/s00484-011-0446-1>
- Queloz V, Grünig CR, Berndt R, Kowalski T, Sieber TN, Holdenrieder O (2011) Cryptic speciation in *Hymenoscyphus albidus*. *Forest Pathol* 41:133–142. <https://doi.org/10.1111/j.1439-0329.2010.00645.x>
- Rigling D, Hilfiker S, Schöbel C, Meier F, Engesser R, Scheidegger C, Stofer S, Senn-Irlet B, Queloz V (2016) Das Eschentriebsterben. Biologie, Krankheitssymptome und Handlungsempfehlungen. Merkblatt für die Praxis, Vol. 57; Eidg. Forschungsanstalt WSL: Birmensdorf, 2016; 8 p.
- Roloff, Pietzarka (1997) *Fraxinus excelsior*. *Enzyklopädie der Holzgewächse* 7. Erg. Lfg. 3/97
- Saumitou-Laprade P, Vernet P, Dowkiw A, Bertrand S, Billiard S, Albert B, Gouyon PH, Dufay M (2018) Polygamy or subdioecy? The impact of diallelic self-incompatibility on the sexual system in *Fraxinus excelsior* (Oleaceae). *Proc R Soc B* 285:20180004. <https://doi.org/10.1098/rspb.2018.0004>
- Scheifinger H, Belmonte J, Buters J, Celenk S, Damialis A, Dechamp C, García-Mozo H, Gehrig R, Grewling L, Halley JM, Hogda K-A, Jäger S, Karatzas K, Karlsen S-R, Koch E, Pauling A, Peel R, Sikoparija B, Smith M, Galán-Soldevilla C, Thibaudon M, Vokou D, de Weger LA (2013) Monitoring, modelling and forecasting of the pollen season. In: Sofiev M, Bergmann K-C (eds) *Allergenic pollen: a review of the production, release, distribution and health impacts*. Springer, Dordrecht, pp 71–126
- Schwab A, Stammel B, Kiehl K (2018) Seed dispersal via a new watercourse in a reconnected floodplain: differences in species groups and seasonality. *Restor Ecol* 26:103–113. <https://doi.org/10.1111/rec.12677>
- Semizer-Cuming D, Kjær ED, Finkeldey R (2017) Gene flow of common ash (*Fraxinus excelsior* L.) in a fragmented landscape. *PLoS One* 12:e0186757. <https://doi.org/10.1371/journal.pone.0186757>
- Semizer-Cuming D, Finkeldey R, Nielsen LR, Kjær ED (2019) Negative correlation between ash dieback susceptibility and reproductive success: good news for European ash forests. *Ann Forest Sci* 76. <https://doi.org/10.1007/s13595-019-0799-x>
- Semizer-Cuming D, Chybicki IJ, Finkeldey R, Kjær ED (2021) Gene flow and reproductive success in ash (*Fraxinus excelsior* L.) in the face of ash dieback: restoration and conservation. *Ann Forest Sci* 78. <https://doi.org/10.1007/s13595-020-01025-0>
- Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. *Landscape Ecol* 21:821–836. <https://doi.org/10.1007/s10980-005-5415-9>
- Thomasset M, Hodkinson TR, Restoux G, Frascaria-Lacoste N, Douglas GC, Fernández-Manjarrés JF (2014) Thank you for not flowering: conservation genetics and gene flow analysis of native and non-native populations of *Fraxinus* (Oleaceae) in Ireland. *Heredity* 112:596–606. <https://doi.org/10.1038/hdy.2013.141>
- Wada KC, Takeno K (2010) Stress-Induced Flowering Plant Signaling & Behavior 5:944–947. <https://doi.org/10.4161/psb.5.8.11826>

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