The Living Ash Project – Ten Years On

Jo Clark reports on a project that has established the National Archive of Tolerant Material for ash (*Fraxinus excelsior*) that will lead to a sustained breeding programme to increase tolerance and ensure we retain this iconic species for the future.

Ash dieback was first officially recognised in the UK in 2012, although it is now known to have been present several years prior to this (Wylder et al., 2018). Ash dieback is a fungal pathogen, *Hymenoscyphus fraxineus*, that causes dieback of ash and can be particularly severe on young trees and saplings, killing many within one year of infection. Mature trees survive much longer, requiring several years of repeated infection before they die. Nevertheless, research in other countries illustrates that tolerance is found in all populations at a low level, that tolerance is heritable, and that breeding programmes offer an effective way to ensure ash remains a forest species in the short-term.

Since its official recognition, much research has been carried out on ash dieback. The genome of ash has been sequenced (Sollers et al., 2017), silvicultural strategies for dealing with ash dieback have been drawn up (Skovsgaard et al., 2017), different site factors affecting the degree of spread have been analysed (Havrdová et al., 2017; Fuchs et al., 2024) and the ecological role that ash plays in a woodland and its associated biodiversity have been reported (Mitchell et al., 2014). The species composition of woodlands and the growing conditions of ash have a large impact on the severity of dieback symptoms, which are linked to high levels of moisture and humidity (Marçais et al., 2016). Recently, the use of drones to assess the degree of crown dieback has been shown to be effective at monitoring ash dieback at the stand level (Flynn et al., 2024).

Modelling the pathogenicity of ash dieback suggests that up to 75% of ash in mixed stands will die (Coker et al., 2019), but where ash occurs as the dominant species, such as the limestone areas of the Derbyshire Dales, Yorkshire Dales and the Peak District, ash dieback has a much more drastic effect (Figure 1). Situations such as these pose a huge problem for landowners, particularly where there is public access, with the costs of sanitary fellings alone likely to cost the UK £4.8 billion (Hill et al., 2019).

The Living Ash Project – Screening and selection of *Fraxinus excelsior* for resistance to ash dieback

The Living Ash Project (LAP) started work in 2013 with the aim of identifying trees with tolerance to *H. fraxineus*, largely within the existing breeding programmes of the Future Trees Trust and Forest Research, but also from the wider ash population. Concurrently, Forest Research established 14 mass screening trials in areas where ash dieback was reported early on (East Anglia and South East England), with both projects being funded by Defra for five years (Clark and Webber, 2017).

Over 40,000 trees were screened from the pre-existing breeding programmes, which were typically aged between five and 20 years old, and 155,000 newly-planted trees were assessed in the mass screening trials. In addition to this, many of the estates where plus trees had been selected for breeding purposes were also visited, and Ashtag – where the public tagged healthy ash trees and reported them online – was utilised through citizen science to record tolerant trees in the wider environment. From these programmes, approximately 400 trees were selected in 2017 by the project (1% of the breeding programme) and 600 trees selected from six of the mass screening trials, which were then closed.

Figure 1. Ash dieback prevalent in Dovedale National Nature Reserve, summer 2022.

Table 1 illustrates the rate of disease progression in a large (4,200 trees) ash provenance trial planted in 2005 in South East England, which contains 39 provenances from across the entire range of *Fraxinus excelsior*. Here, disease symptoms were first observed in September 2015 on only a few trees, when trees were aged ten, and a full survey was carried out the following summer, and then annually. Each tree was scored using the protocol created by Pliura et al. (2011), where score 5 is a healthy crown showing no symptoms and score 1 is dead (from ash dieback). Disease progression is more rapid in smaller trees as they lack the extensive foliage of larger trees (Timmermann et al., 2017). Eight years later, only 20 trees had no signs of disease, probably recorded as such because trees were too tall to observe the crowns fully. That is to say, every tree almost certainly has some small signs of disease, but a few trees are coping with it very well.

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The culmination of these projects saw the establishment of the National Archive of Tolerant Material on the Public Forest Estate in 2019, which houses almost a thousand trees that were selected as tolerant in 2017 and grafted in 2018 (Figure 2). Three ramets (copies) of each tree were grafted to give an archive of 2,300 trees, as not all grafts were successful. Selections were based on phenotypic observations of crown and stem symptoms, and were mostly selected from within single species research trials, e.g. pure ash stands with high infection pressure.

Table 1. Percentage of trees in each ash dieback category in an ash provenance trial comprising 39 provenances from across the natural range of *Fraxinus excelsior*, planted in 2005. Ash scores are 5: most healthy, to 1: dead.

Score	2016	2017	2018	2019	2020	2021	2022	2023
5	26.9	7.0	3.6	1.6	1.3	0.7	0.6	0.5
4	34.7	25.9	15.5	10.9	7.9	10.4	7.6	6.6
3	23.0	32.7	23.4	17.3	17.0	20.3	17.0	14.9
2	8.2	27.1	41.0	45.2	44.6	35.0	26.2	18.1
1	7.2	7.3	16.5	25.0	29.2	33.6	48.6	59.9

The Living Ash Project Phase 2

Monitoring of research trials has continued in the second phase of the project (LAP2 – Securing Tolerant Material

Figure 2. The National Archive of Tolerant Material, June 2024. Trees were planted at 1 m apart in row, and 2 m apart between rows. Gaps illustrate where trees have died and will be replaced with new selections in 2025.

for Seed Production Purposes), which was again funded by Defra from 2019 to July 2024. Initially, there is a sharp decline in health and many (small) trees died within a year of infection. However, others persist longer, and in some cases, health scores can improve over time. This is not just a response of the tree through epicormic growth, but rather appears to be an active defence as the tree compartmentalises the point of infection and prevents the disease spreading throughout the tree, or possibly the fungus dies in situ, due to high temperatures. This has been observed at a low level in most trials.

All trees in the archive have also been monitored for ash dieback each year (Table 2). These data illustrate the problems with selecting trees early, when several of the trials had only been exposed to ash dieback for three or four years. One year after planting, almost half the trees displayed some signs of ash dieback, and over a third had died. Some of these would have been natural losses from grafting. While healthy trees have slowly declined in number, this appears to have levelled off with about 30% of selections showing very good, sustained tolerance to ash dieback (trees scoring 4 or 5). On such early selections, this number is reassuringly high and tolerance is proving to be durable.

> "About 30% of selections are showing very good, sustained tolerance to ash dieback."

Table 2. Percentage of trees in each dieback category in the National Archive of Tolerant Ash, which were grafted in January 2018 and planted in December 2019. Ash scores are 5: most healthy, to 1: dead.

Score	Jul 20	Oct 20	Jun 21	Oct 21	Aug 23	Jun 24
5	45.8	41.2	21.7	16.8	14.5	14.6
4	5.1	5.3	12.5	16.9	15.3	16.8
3	1.7	3.6	14.3	15.1	14.9	13.2
2	9.5	11.4	10.3	9.8	9.7	6.2
1	37.9	38.5	41.2	41.4	45.6	49.2

Next steps

Less than 30% leaf loss is regarded as partially resistant (McKinney et al., 2014) and useful for breeding programmes. The Living Ash Project has selected trees with less than 10% crown dieback, albeit some selections were made quite early. Although approximately half of these early selections have died, they will be replaced and further supplemented by newer selections that have been recently grafted. Tolerance to ash dieback is widely reported to be moderately heritable (McKinney et al., 2014; Enderle et al., 2015; Muñoz et al., 2016; Semizer-Cuming et al., 2019) and durable (Stener, 2018), and therefore the possibility for a breeding programme for tolerance to ash dieback is feasible. However, such programmes need to be based on hundreds of individuals in order to retain genetic diversity (Kjear et al., 2017). We have selected over 1,000 trees in research trials of ash and in areas of high infection within the wider environment, from across Great Britain. In addition to phenotypic selection, we are hoping to screen all trees with liquid chromatography mass spectroscopy, for which the training panel is currently being built by colleagues at Fera. This

method identifies chemicals in the leaf associated with low and high susceptibility to ash dieback and will be an added screening method for tolerance.

Deployment of tolerant saplings

One of the problems with grafting tolerant ash is that the rootstocks are generally not tolerant, and where disease pressure is high, the pathogen can enter the rootstock through lenticles in the stem. Getting tolerant ash onto their own roots would be a great step forward, however, this is proving very difficult. Micropropagation was first tried, and this was successful from embryos, but getting mature material into tissue culture proved much harder and success rates were very low. A second attempt at vegetative propagation looked at taking cuttings but this proved to be equally difficult and had no success with anything other than very juvenile (up to two years old) material. For now, grafting is the most successful way of propagating tolerant ash and, for the second round of grafting, rootstocks were raised from seed collected from tolerant female ash trees.

Currently, these new tolerant selections are waiting to be planted in clonal archives, which could also function as clonal seed orchards. Graftwood from a mature tree passes this maturity on to the grafted plant, and so clonal orchards typically bear seed earlier than orchards of

Figure 3. A typical diamond-shaped lesion on the stem of a young ash tree, completely healed over and the canopy healthy above the lesion.

Timeline of the Living Ash Project

2013	Forest Research establish 14 mass screening trials, comprising 155,000 trees.
2013	Living Ash Project starts work in August (first phase, LAP1).
↓	Ash resources are assessed and the most tolerant trees selected.
2017	
2018	Approximately 1,000 tolerant trees are grafted (three ramets of each) in January; LAP1 finishes in July.
2019	Second phase of the project (LAP2) commences in August.
2019	The National Archive of Tolerant Material is planted on the Public Forest Estate in Hampshire in December.
↓	Ash resources are assessed and the most tolerant trees selected.
2024	790 new selections are grafted in January, six ramets of each.
2024	July LAP2 finishes.
2025	Replacement grafts will be planted in the National Archive, and at a second archive site in Scotland, in December.

Figure 4. Grafted ash plants, July 2024.

planted seedlings. The grafted plants in the archives are still young (and largely collected from other young trees) and so we must wait for them to reach seed-bearing age (Figure 4). However, some are already flowering and producing small amounts of seed. Research carried out by Eisen et al. (2023) has shown that healthy males have greater fecundity than more diseased males, and heavily infected female trees only produce few seed (Semizer-Cuming et al., 2019), which is good news for promoting natural regeneration. However, while it can take many generations before a healthy ash population emerges, breeding can shorten this time by many years. As reviewed in Plumb et al. (2020) on the feasibility for a breeding programme, such selections made in uniform research trials where disease pressure is high are most likely to succeed, as variation in resistance to ash dieback is found in natural populations and is moderately heritable. Sufficient selections have been made within the Living Ash Project to enable seed production from seed orchards with a degree of tolerance to ash dieback in the short-term, and to commence a sustained breeding programme in order to increase tolerance and ensure we retain ash as a viable timber species for the future. The Living Ash Project has been a large consortium effort and is a source of optimism about the future of this wonderful tree.

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