

**REVIEW**

The viability of a breeding programme for ash in the British Isles in the face of ash dieback

William J. Plumb^{1,2,3} | Timothy L. R. Coker^{1,2} | Jonathan J. Stocks^{1,2} |
 Paul Woodcock⁴ | Christopher P. Quine⁵ | Miguel Nemesio-Gorriz³ |
 Gerry C. Douglas³ | Laura J. Kelly^{1,2} | Richard J. A. Buggs^{1,2}

¹Royal Botanic Gardens Kew, Richmond, Surrey, UK

²School of Biological and Chemical Sciences, Queen Mary University of London, London, UK

³Forestry Development Department, Teagasc, Dublin, Republic of Ireland

⁴Joint Nature Conservation Committee, Monkstone House, Peterborough, UK

⁵Forest Research, Northern Research Station, Midlothian, UK

Correspondence

Richard J. A. Buggs, Royal Botanic Gardens Kew, Richmond, Surrey, UK.
Email: r.buggs@qmul.ac.uk

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Societal Impact Statement

The current ash dieback epidemic in Europe caused by *Hymenoscyphus fraxineus* poses a key question to policy makers: whether or not to commit time and resources to the initiation of a breeding programme for the development of more resistant ash, as a long-term policy of adaptation to the epidemic. Here we review current evidence on the potential viability of such a programme, from a biological perspective. We conclude that a breeding programme for ash aimed at resistance to current strains of *H. fraxineus* in the British Isles is biologically feasible.

Summary

To evaluate the viability and feasibility of a future breeding programme to produce trees resistant to an emerging pest or pathogen, it is helpful to ask the following questions: How much variation in resistance exists in tree populations? To what extent is this resistance heritable? How many genetic loci are involved? What level of resistance is found in other species of the same genus? Here, we survey current knowledge of these issues in relation to the degree of resistance of European ash (*Fraxinus excelsior*) to *H. fraxineus*, the fungus causing ash dieback (ADB). Several studies have found a low frequency of heritable resistance in *F. excelsior* populations, which seems to be determined by many genetic loci. This suggests that a breeding programme is viable and that natural selection may also increase the mean resistance of populations over time. More research is needed on the genetic basis of resistance to ADB to understand how quickly natural selection can operate in woodlands and what acceleration may be possible in breeding programmes, including via use of genetic markers. Hybrid breeding programmes may also be a possibility, as some ash species appear to be more resistant to ADB than is *F. excelsior*, but more research is needed on this issue. We do not yet know if it will be possible to breed *F. excelsior* to have resistance to both ADB and the emerging threat of emerald ash borer. We recommend short-term mitigation measures for the ADB epidemic and future research directions.

KEYWORDS

Agilus planipennis, ash dieback, breeding, evolution, *Fraxinus*, *Hymenoscyphus fraxineus*, natural selection

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1 | INTRODUCTION

The *Fraxinus excelsior* (European ash) populations of Europe are being severely damaged by ash dieback (ADB). This disease is caused by the alien invasive ascomycete fungus *Hymenoscyphus fraxineus*, which has spread through Europe since the late 1990s (Pautasso, Aas, Queloz, & Holdenrieder, 2013). The asexual stage of the fungus grows within living trees, causing local necrosis and the sexual stage develops on dead leaf material producing wind-dispersed ascospores. As a common, long-lived deciduous tree, ash is a major natural capital asset and plays an important role in nature's contribution to people in Europe. Surveys in the UK, where the fungus was first discovered in woodlands in 2012, suggest that stakeholders would like action to be taken to produce ash trees that have high resistance to ADB (Jepson & Arakelyan, 2017a, 2017b). The purpose of this review is to determine whether a breeding programme to produce ash trees with high ADB resistance is biologically feasible.

Throughout this paper, we use the term 'resistance' to refer to any means by which trees may have low susceptibility to a pest or pathogen, including tolerance and avoidance mechanisms. We treat resistance as a trait that may be continuous or categorical, depending on its genetic basis. We survey the relevant evidence currently available for ash and outline what further evidence is needed. Other factors must be taken into account when considering a breeding programme in addition to the biological feasibility of increasing mean resistance in populations. These include the public acceptability of the methods used by the breeding programme, the type of tree desired by end users (e.g. fast-growing trees with little variability and good timber qualities vs. trees with close to the same appearance, diversity and ecological function as pre-epidemic populations) and, related to this, the methods available for deployment of more resistant trees. Such factors have been recently described by Woodcock, Cottrell, Buggs, and Quine (2017) and Sniezko and Koch (2017) and we do not discuss them further here.

A first key question directing our response to a newly established pest or pathogen, such as ADB, is: how much variation in resistance exists in the tree populations and to what extent does this have a genetic basis (Carson & Carson, 1989; Falconer, 1960; Sniezko & Koch, 2017)? A breeding programme is only viable if there is existing variation in resistance, so that more resistant trees can be selected. It is advantageous if there are at least some trees that are sufficiently resistant to survive to reproductive age: if this is not the case, the more resistant trees may have to be kept alive using quarantine or pesticides until they reach reproductive age and it may take several generations before offspring with sufficient resistance to survive unaided to reproductive age are produced. If there is variability in resistance, it is essential for a breeding programme that a proportion of this is due to genetic differences among individuals (i.e. it is heritable). If the variation in resistance is entirely due to environmental variables, then mitigation must revolve around management practices, not breeding.

If variable and heritable resistance exists in host populations, a second key question that will determine the effectiveness of a breeding programme is: how many genetic loci are involved in

resistance? If there is only one locus involved, it may be straightforward to increase the frequency of the trait in the population, but little enhancement of the trait may be possible. If a few loci with large additive effects are involved, then the variants of these loci that confer resistance may be rapidly brought together via breeding to produce more resistant trees (Burdon, 2001; Carson & Carson, 1989). However, resistance based on one or a few loci may not be durable, as it may be easy for the pest or pathogen to evolve counter measures against the host's resistance mechanism (e.g. Sniezko, Smith, Liu, & Hamelin, 2014). On the other hand, if there are many loci involved in host resistance, each with a small effect, a breeding programme over many generations, may result in increased and more durable resistance (Burdon, 2001; Carson & Carson, 1989).

A conceptual framework—albeit with many simplifying assumptions (Morrissey, Kruuk, & Wilson, 2010)—in which to understand the effectiveness of a breeding programme for a quantitative trait may be provided by the breeder's equation:

$$R = h^2 S,$$

where R is the per generation response of the trait to selection, h^2 is the narrow-sense heritability of the trait (the proportion of phenotypic variance in the trait due to additive genetic differences among individuals) and S is the selection differential (Falconer, 1960).

The frequency and narrow-sense heritability of a trait such as disease resistance can be estimated through family studies of phenotypes. These can also provide an indication of whether one or many loci are responsible for variation in the trait. Such studies can give an indication of the viability of a conventional breeding programme, where trees with apparent resistance are crossed and their progeny grown up and examined for resistance, with the most resistant trees selected for further rounds of breeding. Such a breeding programme may be assessed and undertaken without the use of any DNA sequence data or knowledge of the molecular genetic basis of the trait. However, in long-lived trees with little previous history of genetic research it can take decades to assess the parameters governing the success of a breeding programme and even longer to undertake the breeding programme itself. Genomic methods may provide a more rapid approach (Neale, 2007; Sniezko & Koch, 2017).

Genome-wide genotyping of large numbers of phenotyped individuals can be used to statistically determine the genetic loci responsible for a trait and the size of effect they have on the phenotype. Resistance in tree populations can be well suited to a genome-wide association study (GWAS) approach (Neale & Kremer, 2011; Neale & Savolainen, 2004) which analyses differences in allele frequencies between large numbers of healthy and unhealthy individuals. This method has proved successful in, for example, discovering genes involved in response to a fungal pathogen in poplar (Zhang et al., 2018) and associated with *Puccinia psidii* rust disease resistance in *Eucalyptus* (Resende et al., 2017). If we have information about loci associated with resistance, we can use this information to estimate

the breeding values of individuals and choose the optimal parents for breeding programmes (Sniezko & Koch, 2017). Where heritability is low, selection using genotypes may provide more accurate estimation of breeding values than selection based on the phenotypic health status of a tree. Using genotyping, we may also be able to select parent combinations that contain different, and potentially complementary, variants conferring resistance and maximum diversity at other loci. Once these have produced seeds, we can, at a very early stage, select seedlings for pathogen screening trials that carry the variants we want. This can save time and space, by allowing selections to be made more quickly than would be possible by waiting for response to pest or pathogen pressure. Where traits are governed by very many loci, a genomic prediction approach may be used, based on thousands of genome-wide markers: this approach is widely used in crop and livestock breeding and has been attempted for complex traits in *Eucalyptus* and pine (Müller et al., 2017; Resende et al., 2012).

If heritable resistance cannot be found in indigenous tree populations, we need to ask a third key question: what level of resistance occurs in other species of the same genus? If high levels of resistance can be found in other species, this trait may be introgressed into an indigenous species using a hybrid breeding programme. For example, attempts are being made to introgress genes for resistance to chestnut blight into American chestnut (*Castanea dentata*) from the resistant Asiatic species *C. mollissima* (Clark, Schlarbaum, & Hebard, 2012). If no related species exist with genetically based resistance, genetic engineering may be the only way to generate resistant trees.

Answers to the three questions above may also allow us to predict how successful natural selection will be in shifting the mean resistance of a population over time. In most cases if a breeding programme is viable, then natural selection is also likely to be effective (an exception being situations where no trees are naturally able to survive to reproductive age and a breeding programme requires quarantine or pesticides in early generations). The decision to embark on a breeding programme may therefore often be dependent on an assessment of the relative speed at which allele frequencies can be changed by a breeding programme versus natural selection. Detailed and well-parameterised models would be needed to predict this accurately, including parameters such as: the number of loci affecting resistance, the geographical distribution of alleles that increase resistance, the size of environmental effects and the age at which hosts lose juvenile resistance.

In the absence of such information, if a degree of resistance is heritable, a breeding programme is likely to be favoured if observational evidence suggests that few trees can survive to reproduce in nature. By contrast, relying on natural selection will likely be deemed the best policy if many trees are observed to survive to reproduce (Table 1). In this case, management decisions may be taken to seek to enhance natural selection by promoting natural regeneration and avoidance of felling healthy trees that may be more resistant than others. If reliable genetic markers are identified which indicate the level of resistance that a tree might have, these could inform decisions about which trees to cut down and which to preserve in cases where managers would otherwise clear-fell.

Long-term policy decisions about a newly established pathogen must also take into account possible future threats. In the case of ADB, ash populations are also threatened by the emerald ash borer (EAB), *Agilus planipennis*, which is currently found near Moscow in Russia, with fears of it spreading further westwards (Musolin, Selikhovkin, Shabunin, Zviagintsev, & Baranchikov, 2017; Semizer-Cuming, Krutovsky, Baranchikov, Kjær, & Williams, 2019; Straw, Williams, Kulinich, & Gninenko, 2013). This beetle has killed millions of ash trees in North America since its accidental introduction in the late 1990s, leaving American ash species, including *F. pennsylvanica* and *F. americana*, critically endangered (Jerome, Westwood, Oldfield, & Romero-Severson, 2017; Westwood, Oldfield, Jerome, & Romero-Severson, 2017).

Here, we survey what is known regarding the frequency and heritability of resistance to ADB in European ash, *F. excelsior* and the number of genetic loci involved. We also survey what is known about the levels of resistance to ADB found in other *Fraxinus* species. The evidence base that we consider relies heavily on data from parts of Europe that have been exposed to the disease for the longest times. We also consider what is known about resistance of *F. excelsior* to EAB and the possibility of breeding for resistance to both EAB and ADB. We apply this knowledge to the current situation in the British Isles, which was one of the last areas of northern Europe to be affected by ADB and where public policy decisions regarding long-term adaptation to the disease's establishment are still under discussion.

2 | VARIATION IN RESISTANCE TO ADB IN *F. EXCELSIOR*

A large number of studies have taken place across Europe examining variation in resistance to ADB, based on disease symptoms in populations of ash trees (for a comprehensive summary see Table S1 and Note S1). These analyses represent a variety of study sites, including natural woodlands, plantations, seed orchards and progeny trials. A range of different health score measures were used. Considerable variation in health of trees was found, with most studies including both trees that were completely healthy and trees that had died; this suggests high variability in degree of resistance to ADB.

In order to synthesize some of these data, Coker et al. (2019) conducted a meta-analysis of studies of death due to ADB in Europe. They analysed 36 surveys in woodlands including observations of a total of 17,825 mature trees with exposure to ADB of between four

TABLE 1 The frequency and heritability of resistance to a pest/pathogen in existing host populations allows a simple preliminary assessment of possible routes to enhanced resistance within a limited timeframe

	Frequent occurrence of trees surviving to reproduce	Rare occurrence of trees surviving to reproduce
High heritability	Natural selection	Breeding programme
Low heritability	Silviculture and site management	Genetic engineering or hybridisation

and 20 years, finding a maximum mortality of 69%. In plantations established prior to the arrival of the epidemic they analysed 12 surveys of 15,773 trees in total, finding a maximum mortality thus far of 85%. They analysed 10 surveys of naturally regenerated saplings totalling 18,461 trees, finding maximum mortality of 82%. For mature trees, mortality tended to increase with time of exposure to ADB, with a sharp increase in mortality rate at around 10 years after first detection of ADB in a region (Figure 1): this is probably due to the time it takes for ADB to grow and spread within trees after infection. In contrast, for natural regeneration the lowest levels of mortality were found in the two sites with the longest exposure to ADB; Enderle et al. (2017) have suggested that such patterns may reflect the work of natural selection with large numbers of seedlings being killed before they reach sufficient size to be counted in surveys. It has been widely observed that older trees take longer to die when infected by ADB than younger trees do, as shown by Timmermann, Nagy, Hietala, Børja, and Solheim (2017).

The Coker et al. (2019) meta-analysis also gives us information about how quickly natural inoculum pressure can select for trees with resistance. In woodland trees and trials established before the ADB epidemic (Figure 1), around 10 years of ADB presence was needed before mortality rates increased sharply. We might therefore expect to see an increase in the rate at which frequencies of alleles conferring resistance rise in British ash populations after about 15 years (around 2027) when the majority of susceptible

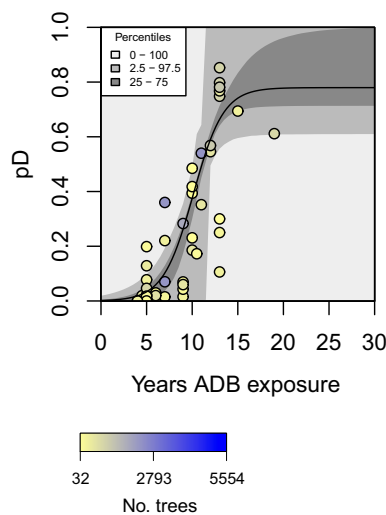


FIGURE 1 Ash mortality in European woodlands and trials planted prior to regional ADB detection. Each point corresponds to one trial or woodland plot. Point colour corresponds to the number of trees sampled (see colour scale). A three-parameter logistic model is fitted to these data (pD—proportion of dead trees) over time. Data points are weighted according to sample size n . Weighted goodness-of-fit = 0.810. Mortality predictions shown in grey are based on bootstrap data: for each iteration, predictions for pD were calculated for t at intervals of 0.5. Of these predictions, the range (lightest grey), the 2.5th and 97.5th percentiles (intermediate grey) and the interquartile range (darkest grey) are plotted, alongside the original model (black). Reproduced from figure 3d in Coker et al. (2019). ADB, ash dieback

mature trees are expected to have died, leaving the more resistant trees to provide seed for natural regeneration. A recent study from Denmark suggests that this increase will start earlier as trees more susceptible to ADB produce fewer seeds than more resistant trees in the years when they are infected but still alive (Semizer-Cuming, Finkeldey, Nielsen, & Kjær, 2019). It is also worth noting from a UK public safety and management perspective, the Coker et al. (2019) analysis suggests that we might expect to see a sharp rise in death rates of mature ash tree in the UK in the years around 2022, given that ADB was first detected in UK woodlands in 2012.

One trial that is of particular interest for the British Isles is a Forest Research mass screening trial that was set up in late 2012 and early 2013. The trial comprises 14 sites located in the southeast of England (where the damage to ADB is currently at its highest) with saplings from up to 15 provenances from the British Isles and Europe planted at each site. After three years, in 2016 a detailed assessment of tree health using a scale of zero to seven (where higher scores indicate healthier trees) was carried out for 28,160 trees at two of the worst affected sites (Stocks, Buggs, & Lee, 2017). In this study seed provenance was found to have a significant ($p \leq 0.001$) effect on tree health. Average tree mortality was 17%. Three percent of trees were healthy. The majority of trees had intermediate levels of damage. The presence of some healthy trees across all provenances (albeit at low frequencies) bodes well for a successful breeding programme for resistance to ADB (Stocks et al., 2017). Comparisons with assessments in future years will be needed to inform us about how accurately the scores made in 2016 reflect each tree's long-term resistance.

Ash tree genotypes from the British Isles are also being screened for ADB resistance in continental Europe. In 2004 a series of ash provenance trials was set up with seed from 44 provenances from across Europe, including six from the British Isles, as part of the 'Realising Ash's Potential' project (https://cordis.europa.eu/project/rcn/58712_en.html). Replicated plantings were made in Belgium, Denmark, England, France, Germany, Ireland and Italy. All of these are now reported to be affected by ADB and should soon yield data on levels of resistance. In addition, 529 ash genotypes from Ireland were planted in Lithuania in 2018 to screen for ADB resistance under heavy inoculum pressure.

Overall, the remaining number of living and healthy trees found so far across Europe, including in areas with long exposure to ADB, is encouraging, especially given the wide range of abiotic environmental variables found across this range. However, we do not yet have data on how many trees can survive from seed to sexually reproducing adult under ADB pressure throughout their life-time. If currently healthy trees do possess heritable resistance, a breeding programme or the long-term effects of natural selection, may allow more resistant individuals to be produced.

3 | HERITABILITY OF RESISTANCE TO ADB

Heritability of resistance can be estimated from field trials in which multiple groups of individuals of known relatedness are exposed to

ADB inoculum. Such trials were set up in the UK in 2016 by Forest Research, using seeds from the Earth Trust Breeding Seedling Orchards (Jo Clark, pers. comm.); 22,080 progeny from 46-half sibling families were planted across three sites. Similar trials were already growing in some European countries before ADB arrived. Some clonal trials have been analysed to estimate broad-sense heritability of ADB resistance, which includes all genetic effects. Some family trials have been analysed to calculate narrow-sense heritability, which includes only additive genetic effects that are the most important to breeding programmes. These studies are summarised in Table S2 and Note S2. Most studies have shown a heritability of 0.4–0.5. This is higher than levels of heritability found for most resistance traits in sheep and cattle (Morris, 1998, 2007), but lower than for some crops (Simmonds, 1991). This level of heritability suggests that there will be a good response to selection, given a sufficient selection differential and selection based on an individual's phenotype may be fairly effective. Phenotypic assessments of trials may also provide sufficient information for the training of models for genotypic selection, given large sample sizes.

It should be noted that these estimates of 0.4–0.5 heritability were from planted trials with fairly uniform environments; heritability is likely to be lower where there is more variability in the environments of individual trees. No one has as yet, to our knowledge, calculated heritability of resistance in mature ash stands in forests (Kjær et al., 2017). Various studies suggest that variable environments affect the amount of damage caused by ADB (see Table S3). Influential factors include: density of stocking (Havrdová, Zahradník, Romportl, Pešková, & Černý, 2017), temperature (Havrdová et al., 2017), altitude (Havrdová et al., 2017), distance to the nearest ash stand (Havrdová et al., 2017), soil characteristics (Havrdová et al., 2017; Pušpure, Matisons, Laiviņš, Gaitnieks, & Jansons, 2017), co-occurrence with other tree species (Havrdová et al., 2017; Pušpure et al., 2017), proximity of water-courses (Havrdová et al., 2017), humidity (Marcais, Husson, Godart, & Cael, 2016), drainage (Pušpure et al., 2017), collar infections by *Armillaria* (Chandelier, Delahaye, Claessens, & Lassios, 2017; Chandelier, Gerarts, San Martin, Herman, & Delahaye, 2016; Enderle et al., 2017; Enderle, Peters, Nakou, & Metzler, 2013; Hauptman, Ogris, Groot, Piškur, & Jurc, 2016; Heinze, Tiefenbacher, Litschauer, & Kirisits, 2017; Lenz, Bartha, Straßer, & Lemme, 2016; Pliura et al., 2017) and other fungi (Langer, 2017). Studies so far suggest that genotype by environment interactions in ADB damage among trial sites are weak (Kjær, McKinney, Nielsen, Hansen, & Hansen, 2012; Pliura, Lygis, Suchockas, & Bartkevicius, 2011; Stener, 2013) whereas a study which simulated spring frost and summer drought treatments showed a significant genotype by environment interaction (Pliura, Lygis, Marčiulyniene, Suchockas, & Bakys, 2015).

Due to the more variable environments of woodlands it is likely that making selections from healthy individuals in woodlands will be less effective for breeding programmes than selections from planted trials and data from woodlands will be less useful in training genotypic models. On the other hand, breeding programmes that are

based only on data from trials run the risk of breeding trees for high resistance to ADB in only environments similar to the trial sites.

4 | ASH BREEDING PROGRAMMES IN EUROPE

Based on the finding that there is some variability of resistance to ADB in European ash and that this has modest levels of heritability, several breeding programmes have already commenced in Europe. Clonal seed orchards of putatively more-resistant trees have been set up in Denmark (Kjær et al., 2017) with the aim of producing seed by 2025. In Sweden, two seed orchards of *F. excelsior* plus-trees selected in 1991 and 1992 are now being monitored for ADB resistance (Cleary, Nguyen, Stener, Stenlid, & Skovsgaard, 2017; Stener, 2013, 2018) and scions have been collected and grafted from healthy ash trees across the entire natural distribution of ash in Sweden (Cleary et al., 2017). These trees may provide the basis of future breeding programmes for ADB resistance in Sweden. Selections of healthy ash trees are also underway on Gotland (Rimvys Vasaitis pers. comm.).

A progeny trial set up in 2005 is being used as the basis of a breeding programme in Lithuania. This started with 27,000 three-year-old trees planted across three sites (Pliura & Baliuckas, 2007; Pliura et al., 2011). Fifty-two of the apparently most resistant genotypes from these trials were cloned in 2010 in 10–40 grafted ramets per genotype and planted in spring 2012 at seven locations in Lithuania. By autumn 2013, 98% of these planted trees had survived but 71% were symptomatic with ADB (Pliura et al., 2017). These are also being augmented by scions and seeds from 167 trees selected from 10 natural Lithuanian populations of *F. excelsior* (Pliura et al., 2017).

In Austria, since 2015 a large breeding programme on *F. excelsior* is underway at the Austrian Research Centre for Forests (BFW) in collaboration with the University of Natural Resources and Life Sciences (BOKU); seeds from more than 700 healthy mother trees have been sampled all over Austria and the more than 30,000 resulting offspring are being evaluated for their resistance to *H. fraxineus* in a nursery trial (Unger, Kirisits, Konrad, Schüler, & Geburek, 2017; www.eschein-not.at). The eventual goal is to identify the mother trees with the highest breeding value and to establish ash seed orchards with the improved material. In 2018, a similar initiative was started to select resistant *F. angustifolia* as well (Heino Konrad pers. comm.).

In the UK, seed orchards of plus-trees set up by the Future Trees Trust came into production just as the ADB epidemic began. The Living Ash Project (livingashproject.org.uk) funded by the Department for Environment, Food and Rural Affairs (Defra) is examining these trees and their progeny for resistance to ADB and is also seeking resistant trees from other UK populations as potential material for a breeding programme (Jo Clark, pers. comm.).

If the various breeding programmes that have been initiated across Europe were to work together and exchange material, it may be possible to more rapidly and cost-effectively produce ash populations with higher mean resistance to ADB. This will be especially true

if some alleles contributing to resistance are found in some areas of Europe and not others.

5 | NUMBER AND IDENTITY OF GENETIC LOCI INVOLVED IN RESISTANCE TO ADB

An indirect estimate of the number of loci involved in resistance to ADB was made by McKinney et al. (2014), who argued from the results of Kjær et al. (2012), 'The approximate normal distribution of the breeding values for the mother trees suggests that the observed resistance is based on the additive effects of a number of genes (quantitative resistance) rather than on a single or a few resistance genes (qualitative resistance), which would have resulted in a more categorical distribution of the breeding values' (McKinney et al., 2014, p. 492). Further studies of family trials and controlled crosses will provide more information on this issue in future, but meanwhile a more rapid approach can be provided by genomic analyses.

To provide a reference for genomic characterisation of trees with differing levels of resistance to ADB, the genome sequence of a UK ash tree has been assembled (Sollars et al., 2017). The nuclear genome is comprised of c. 880 Mb, with 38,852 annotated genes. Thirty-seven other *F. excelsior* trees from across Europe were also sequenced at low coverage; in regions of the genome with between 5× and 30× coverage in all samples (2.3% of the genome) 529,812 variants were found, of which 394,885 were bi-allelic single nucleotide polymorphisms (SNPs) with minor allele frequency above 0.05. This panel of trees allowed estimation of linkage disequilibrium in the genome, finding that SNPs showed little correlation at over 25 kb distance from one another; this means that dense markers would be needed in studies aiming to find associations between genotypes and phenotypes.

So far only one approach has identified loci associated with resistance to ADB: associative transcriptomics on a population of Danish trees (Harper et al., 2016; Sollars et al., 2017). Gene expression markers (GEMs) associated with ADB damage scores were identified, with a subset of markers showing the strongest association used to predict phenotypes in a test panel of 58 trees (Harper et al., 2016; Sollars et al., 2017). The loci identified included those showing similarity to MADS-box proteins (eight GEMs) and a cinnamoyl-CoA reductase gene (two GEMs) that may be involved in hypersensitive response (Harper et al., 2016; Sollars et al., 2017). There are limitations to the transcriptomic approach for predicting resistance. Many genetic variants affecting ADB susceptibility may be inaccessible to transcriptomic studies because they are in non-protein-coding regions of the genome or are not being expressed at sufficient levels to be detected at the time plant material is sampled. The relatively small sample size used to test thousands of loci for association means that statistical power is weak. The transcriptome markers were developed from a set of 213 Danish ash trees and these may differ in their genetic background from the UK and other European populations (Heuertz et al., 2006; Sollars et al., 2017), perhaps due to different population histories following the last ice age. Thus,

further refinement and testing on UK populations of known resistance is needed before these markers could inform a UK breeding programme.

A report of a GWAS based on the Forest Research mass screening trial (see above) is available as a pre-print (Stocks et al., 2019). This used whole genome sequencing of pools of DNA from healthy versus unhealthy trees from different provenances. Health scores were evaluated after five years of ADB inoculum pressure. By using whole genome sequencing, this study had very dense marker coverage of the genome, which means that loci directly responsible for the trait could have been identified. By using pooling within provenances and health categories, a total of 1,250 trees were included but costs were kept as low as possible. By comparison of allele frequencies in healthy versus unhealthy pools, allowing for variation due to provenance, this study has discovered 3,149 SNP loci that may be involved in resistance to ADB in the British Isles. The authors found that 200 of these loci could be used to predict the health of 150 trees with an accuracy sufficient for use in genetic screening for a breeding programme.

6 | SUSCEPTIBILITY OF WORLDWIDE ASH SPECIES TO ADB

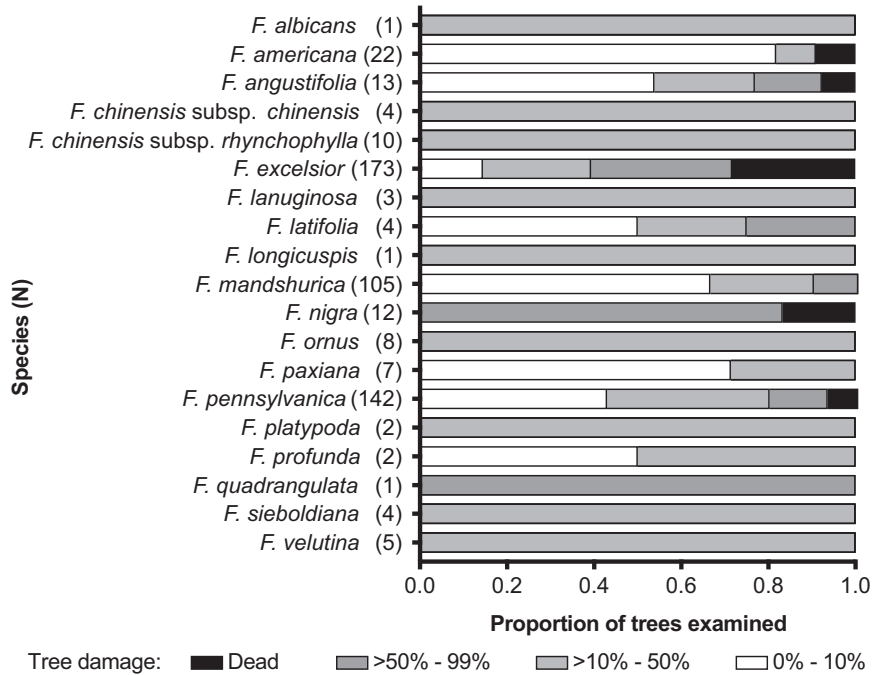
There are approximately 50 *Fraxinus* species worldwide, divided among six sections: *Dipetalae*, *Fraxinus*, *Melioides*, *Ornus*, *Pauciflorae* and *Sciadanthus* (Wallander, 2008, 2013). Nielsen, McKinney, Hietala, and Kjær (2016) examined a large number of species from an arboretum in Denmark, scoring them for percentage crown die-back from natural infections and stem lesion length from controlled inoculations of grafts. Drenkhan et al., (2017 and literature cited therein) described the health of 12 exotic ash species planted in parks in Estonia. These and other smaller studies are summarised in Figure 2, listed in Table S4 and described below. For many species, sample sizes are low and more research is needed.

6.1 | *Fraxinus* species indigenous to Europe

Fraxinus angustifolia is found in central and southern Europe, parts of northwest Africa and southwest Asia (Wallander, 2012). Nielsen et al. (2016) found damage scores from zero to 75% (mean 16%) following natural infection of *F. angustifolia* (including subsp. *angustifolia* and subsp. *syriaca*). Drenkhan et al. (2017) found that for two specimens of *F. angustifolia* subsp. *syriaca* (reported under the synonyms *F. potamophilia* and *F. sogdiana*; see Wallander, 2012), one had minor damage and the other was dead or dying. Hauptman et al. (2016) found variability in ADB susceptibility between *F. angustifolia* genotypes, with 16.7%–83.8% damage following a four-year infection period. Adamčíková, Pažitný, and Pastirčáková (2018) also found variability among *F. angustifolia* genotypes and on average longer lesions after bark inoculation than in *F. excelsior* (Adamčíková et al., 2018).

Fraxinus ornus is found in regions of Southern Europe and southwest Asia (Wallander, 2012). Nielsen et al. (2016) reported 0%–8%

FIGURE 2 ADB damage scores for different ash species taken from two studies. This summary table was produced using susceptibility/damage data taken from Nielsen et al. (2016) and Drenkhan et al. (2017), two European studies covering natural ADB infections for worldwide *Fraxinus* species. Sample sizes for each species are shown in parentheses after the species name. White represents the proportion of trees with 0%–10% damage, light grey is the proportion of trees with >10%–50% damage, dark grey is the proportion of trees with >50%–99% damage and black is the proportion of trees with 100% damage or dead. Further details of the studies can be found in Table S4. Note that the data for *Fraxinus angustifolia* combine all subspecies of *Fraxinus angustifolia*, including those originally reported under synonyms. ADB, ash dieback



crown damage in *F. ornus* and only small lesions in controlled stem inoculations. A single tree examined by Drenkhan et al. (2017) remained healthy over the course of their study. Experiments on 15 seedlings each of *F. excelsior* and *F. ornus* and various anecdotal observations, suggest that *F. ornus* is less susceptible than *F. excelsior* to ADB and only affected by *H. fraxineus* in its leaves, with the fungus failing to enter woody tissue (Kirisits, 2017).

6.2 | *Fraxinus* species indigenous to North America

Nielsen et al. (2016) found *F. nigra* and *F. quadrangulata* (represented by a single tree) developed severe crown dieback. The other North American species (*F. albicans*, *F. americana*, *F. latifolia*, *F. pennsylvanica*, *F. profunda* and *F. velutina*) suffered much less damage on average (see Figure 2 in Nielsen et al. (2016)), although one *F. americana* was apparently killed by ADB. However, some of the species with relatively low crown damage did develop severe lesions from controlled inoculations. For example, *F. velutina* was the most resistant North American species in the arboretum, with only one of five trees showing minor crown damage, but developed the largest lesions (7.5–11 cm). Drenkhan et al. (2017) found *F. nigra* to be badly affected with 86% of trees showing at least 50% crown dieback and 14% scored as dead or dying. *Fraxinus americana* was observed as the most resistant North American species with 73% of trees remaining healthy and only 9% dead or dying. *Fraxinus pennsylvanica* (with 37% of trees healthy) was more susceptible than *F. americana* but less so than *F. excelsior* (with 13% of trees healthy).

Kowalski, Bilański, and Holdenrieder (2015) also detected higher resistance in *F. pennsylvanica* than *F. excelsior*. The mean length of necrotic lesions on rachises was 8.4 cm in *F. excelsior* and only 1.9 cm in *F. pennsylvanica*. Moreover, lesions developed on all inoculated *F.*

excelsior stems (mean length 18.0 cm), but only 6% of *F. pennsylvanica* stems (with a mean length 1.9 cm).

6.3 | *Fraxinus* species indigenous to Asia

As Asiatic ash species may share an indigenous range with *H. fraxineus* they might be expected to have co-evolved with it and be more resistant than naive ash populations in Europe or North America (Cleary et al., 2016; McMullan et al., 2018; Zhao, Hosoya, Baral, Hosaka, & Kakishima, 2013). Nielsen et al. (2016) found that three Asian species (*F. chinensis* subsp. *rhynchophylla*, *F. lanuginosa* and *F. platypoda*) demonstrated high resistance against natural infection and did not develop any symptomatic lesions following controlled inoculations. *Fraxinus paxiana* fared the worst of the Asian species in terms of natural infection, although most trees had relatively low damage (Figure 2 in (Nielsen et al., 2016)), but this species was not included in the controlled inoculations. Despite little damage from natural infection, *F. sieboldiana* developed the largest lesions (>5 cm) of any Asian species in the controlled inoculations. *Fraxinus mandshurica* demonstrated some resistance, with only 25% of the grafts developing minor lesions (2–3 cm).

Preliminary observations of a stand in Estonia containing various species of *Fraxinus* (Drenkhan & Hanso, 2010), found minor symptoms typical of ADB in *F. mandshurica*. These included small bark necroses and dieback of some shoots and twigs, with analysis of symptomatic tissue confirming the presence of *H. fraxineus* (Drenkhan & Hanso, 2010). Drenkhan et al. (2017) examined 92 *F. mandshurica* individuals and the majority (62%) remained healthy, with no dead or dying trees identified. Two *F. chinensis* trees were also examined and appeared resistant to ADB (Drenkhan et al., 2017). Gross and Holdenrieder

(2015) found *H. fraxineus* to be pathogenic to *F. mandshurica* following artificial stem wound inoculations, with 12 of 16 seedlings (75%) developing lesions of ≥ 2 cm external length. However, the seedlings originated from the same provenance (from seed of a single mother tree) and variability between genotypes could help to explain the possibly higher susceptibility of *F. mandshurica* indicated by this study. In the indigenous range of *H. fraxineus*, *F. mandshurica* is not reported to be damaged by the fungus (Zheng & Zhuang, 2014).

7 | HYBRIDISATION AMONG *FRAXINUS* SPECIES

If a breeding programme based on selection within pure *F. excelsior* fails to yield germplasm with durable resistance combined with environmental adaptation another approach may be hybridisation with other ash species that have greater resistance to ADB. This might generate resistant ash trees, but backcrossing may be needed to ensure that these hybrids are: adapted to the British climate, not susceptible to indigenous pests and pathogens and support a similar range of indigenous species in British ecosystems. However, such an approach will only be possible if fertile hybrids can be made successfully. Currently, we do not know of any published hybrids between *F. excelsior* and more resistant species. Hybridization occurs naturally between *F. excelsior* and *F. angustifolia* where the two species meet in their indigenous range in Europe (Fernández-Manjarrés, Gerard, Dufour, Raquin, & Frascaria-Lacoste, 2006; Heuertz et al., 2006; Thomasset et al., 2011). Hybrids between *F. nigra* and *F. mandshurica* were produced in the 1970s at the Agriculture and Agri-Food Canada Morden Research Centre and selections from these were named 'northern treasure' and 'northern gem' (Davidson & Ronald, 2001).

It should be noted that *F. nigra*, *F. mandshurica*, *F. excelsior* and *F. angustifolia* are phylogenetically close (Hinsinger et al., 2013; Wallander, 2008), all belonging to section *Fraxinus* (Wallander, 2012). Thus, it may be that *F. mandshurica* and *F. excelsior* are able to hybridise, but it seems unlikely that hybridisation between *F. excelsior* and species of ash more highly resistant to ADB in other sections of the genus will be simple. It may be that the regeneration of such hybrids will be possible using in vitro culture of immature embryos. More research is needed in this area, which may be especially important once other threats are taken into account.

8 | OTHER THREATS

A breeding programme for ash with resistance to the current ADB epidemic may be a long-term and expensive process, so it is highly desirable that any trees generated are also resistant to other threats. Indigenous pathogens such as *Armillaria* are interacting with ADB to cause tree mortality (Chandelier et al., 2017, 2016; Enderle et al., 2017, 2013; Hauptman et al., 2016; Heinze et al., 2017; Lenz et al., 2016; Pliura et al., 2017) but it may be that enhanced ADB resistance is sufficient to reduce this combined threat. The two most obvious

further threats to ash trees in Europe are the introduction of additional variants and strains of ADB or the mutation of current strains, as well as the introduction of EAB, as we describe below.

The genetic variability of *H. fraxineus* in Europe is very low and only represents only a small subset of the variability of the species in east Asia (Nornex report; McMullan et al., 2018). More virulent strains of the fungus may exist and may invade Europe from Asia in future. Furthermore, new introductions would open the potential for more virulent strains to evolve in Europe. On the other hand, if high pathogenicity has recently evolved in European *H. fraxineus*, other strains may pose a lower risk or might even be beneficial if they were introduced and their genes spread into the current population in Europe. Models of pathogen-host co-evolution suggest that established pathogen populations will evolve towards intermediate virulence (Lenski & May, 1994). When hosts are forest trees, this co-evolutionary dynamic is likely to take centuries: Lygis et al. (2017) tested this hypothesis for 200 European isolates of ADB in Europe but found no evidence for reduction in virulence.

We do not yet know with certainty the level of resistance in *F. excelsior* populations to EAB. So far it seems that *F. excelsior* has an intermediate level of resistance to EAB (Showalter et al. MS, this issue) compared to other *Fraxinus* species. What this means for the viability of current *F. excelsior* populations in the field under EAB pressure is not yet known. The invasive ranges of *H. fraxineus* and EAB now overlap in the region of Russia between Moscow and the border with Belarus, providing a natural experiment on the combined effect of the two threats (Musolin et al., 2017; Semizer-Cuming, Krutovsky, et al., 2019). Any breeding programme for co-occurrence of resistance to two very different threats is likely to be challenging. Simultaneous selection for different traits is difficult if they are negatively correlated (Falconer, 1960). Metabolomic analysis of Danish trees hints that trees with higher resistance to ADB may have lower levels of iridoid glycosides, which may provide defence against herbivores (Sollars et al., 2017); this is suggestive of a possible trade-off between resistance to EAB and resistance to ADB, but is as yet untested. On the other hand, some Asiatic ash species seem to have natural ranges that co-occur with both *H. fraxineus* and EAB and *F. mandshurica* is reported to be a natural host of both ADB and EAB in Asia with neither apparently having a serious negative impact on this species under normal conditions (Musolin et al., 2017; Orlova-Bienkowskaja & Volkovitch, 2018; Wei, Reardon, Wu, & Sun, 2004; Zheng & Zhuang, 2014), suggesting that resistance to both threats is possible.

9 | VIEWS OF THE PUBLIC AND STAKEHOLDERS

It is crucial to the long-term success of a breeding programme that it is politically feasible as well as scientifically possible (Woodcock et al., 2017). In Britain there was considerable public concern about ADB when it was first found in woodlands in Norfolk and subsequent studies have analysed this initial public reaction and its effect on public policy (Fellenor et al., 2017; Potter & Urquhart, 2016;

Tsouvalis, 2018). Studies have also been carried out of the acceptability of genetic solutions to ADB, including face-to-face interviews at countryside events (Jepson & Arakelyan, 2017a) and a YouGov survey (Jepson & Arakelyan, 2017b); these indicate that breeding of native 'tolerant' ash is the most popular course of action in response to ADB, with the planting of non-native, hybrid or genetically modified ash trees being less popular choices.

10 | CONCLUSIONS

Genetically based host resistance is likely to provide a long-term adaptation to the ADB crisis, but we do not yet know the optimal pathway to this goal. A broad base of biological research needs to continue in order to predict the most biologically feasible approach to adapting to ADB and the future threat from EAB. On the evidence currently available, a breeding programme for *F. excelsior* aiming for resistance to current strains of ADB in the British Isles is biologically feasible, as variation in resistance to ADB is found in natural populations and this is moderately heritable. The same conclusion has been drawn by many European studies (e.g. Pliura et al., 2011; McKinney, Nielsen, Hansen, & Kjær, 2011; McKinney et al., 2014; Kjær et al., 2012; Stener, 2013; Enderle, Nakou, Thomas, & Metzler, 2015; Muñoz, Marçais, Dufour, & Dowkiw, 2016; Stener, 2018). Based on this evidence, natural selection is also likely to be effective, so long as more resistant individuals are able to survive in natural environments from seedling to sexual maturity. It is unlikely that hybridisation among ash species will be needed to generate ADB-resistant ash trees for Britain, but Asiatic species could be a source of resistance if required.

Whilst a breeding programme based on phenotypic selection is likely to be effective, more research is needed to understand how efficiently breeding could be accelerated using screening for genetic markers. This will involve better understanding of the genetic basis of ADB resistance, including further testing of the loci identified by Stocks et al. (2019) and investigating the geographic distribution of their alleles. We also need to understand more about the susceptibility of juvenile versus mature trees and how these are correlated. Acceleration via marker-assisted methods is likely to be greatest when juvenile resistance is not a good predictor of future resistance and when large numbers of alleles scattered among many populations need to be combined.

Such research will also help us to answer the critical question of how quickly natural selection can be expected to lead to higher ADB resistance in Britain, relative to a breeding programme. We first need to know if natural regeneration can survive to adulthood under ADB pressure. To then assess how rapidly natural selection will work we would need a detailed model parameterised by quantitative information, including data on: the number and effect sizes of loci affecting resistance, the spatial distribution of alleles that increase resistance, the size of environmental effects and the susceptibility of different ash genotypes at different ages. Such data will also help us to design a breeding programme, if one is required, tailored to policy objectives such as retention of local genetic variation as far as possible or selection for form and timber quality.

Enhancement of natural selection is the aim of current Forestry Commission England guidelines for woodland managers, which recommend that ash trees without ADB symptoms should as far as possible be retained and measures be taken to promote natural regeneration from these trees (Anonymous, 2018). The evidence gathered here suggests that these guidelines are likely to be successful in gaining some increase in the proportion of trees with low susceptibility to ADB in woodlands. It may in future be possible to enhance this process using genetic screening to select which trees should be retained, but more work is needed to test effective markers for this. Cloning of more resistant trees and planting these out, as implemented in some other tree breeding programmes (reviewed by Sniezko & Koch, 2017), may also be a viable short-term measure to enhance local populations of ash (Douglas, McNamara, O'Connell, Dunne, & Grant, 2017).

We do not yet know if the breeding of *F. excelsior* for resistance to both ADB and EAB is biologically possible from standing genetic variation. Dealing with this combined threat will need a great deal of international collaboration (Semizer-Cuming, Krutovsky, et al., 2019). Further research is needed to determine variability and heritability of EAB resistance in *F. excelsior* and whether or not there is a trade-off between ADB and EAB resistance. The fact that species from east Asia are reported to have high resistance to both threats shows that this combination of traits is possible within the genus. It may be necessary to cross *F. excelsior* with Asian ash species in order to gain resistance to both threats and further research is needed to understand if this will be possible.







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AUTHOR CONTRIBUTIONS

R.J.A.B., C.P.Q and P.W. planned and designed the research. W.J.P., T.L.R.C., J.J.S. and R.J.A.B analysed the data. W.J.P., L.J.K., P.W., M.N.-G., G.C.D, T.L.R.C., J.J.S. and R.J.A.B wrote the manuscript.

ORCID

William J. Plumb  <https://orcid.org/0000-0002-1498-0113>
 Timothy L. R. Coker  <https://orcid.org/0000-0002-2516-0639>
 Jonathan J. Stocks  <https://orcid.org/0000-0003-4958-4580>
 Christopher P. Quine  <https://orcid.org/0000-0002-3820-2375>
 Miguel Nemesio-Gorriz  <https://orcid.org/0000-0001-8052-8101>
 Laura J. Kelly  <https://orcid.org/0000-0003-1159-939X>
 Richard J. A. Buggs  <https://orcid.org/0000-0003-4495-3738>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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