Annex A

Technical Summary

The announcement made by Lord Gardiner coincides with the release of the research report from the Nornex project. Details of the project and its findings are presented below. The full report is available at **http://oadb.tsl.ac.uk/?page_id=964**.

Funding and project background

Title: An open consortium for molecular understanding of ash dieback disease (Nornex)

Project costs: £1,462,266

Funders: Biotechnology and Biological Sciences Research Council and the Department for Environment, Food and Rural Affairs.

Research partners: The research was carried out by a consortium of researchers from John Innes Centre (Lead partner), the University of York, the Genome Analysis Centre, the University of Exeter, FERA science ltd, the University of Copenhagen, Forest Research, the Sainsbury Laboratory, East Malling Research, the Forest and Landscape Institute Norway and the University of Edinburgh.

Technical summary to support announcement

- Associative Transcriptomics has been used to develop gene expression markers related to tolerance to ash dieback. The markers are gene alleles that are present or expressed differently in tolerant trees compared to susceptible trees.
- Tolerance was assessed using a Danish diversity panel of 182 ash trees, scored for visual signs of disease which was then assessed against gene expression and DNA markers.
- Two gene expression markers and a single nucleotide polymorphism marker (SNP) were found to be associated with disease tolerance.
- For the SNP marker, trees with a strong G (guanine) signal are more tolerant. Susceptible trees have a strong A (adenine) signal at this position. This is likely to be due to differential expression of two related genes as opposed to allelic variation of a single gene.
- This is the first time Associative Transcriptomics, which was first developed for use in annual crops, has been used in natural populations or a plant pathology study.
- Initial screening of several small UK populations has indicated that the markers may be more common in UK ash trees compared to trees in other parts of continental Europe. The implications of this finding on the rate of tree decline and death is still to be understood.
- Analysis of metabolites in leaves of selected Danish ash trees identified several secondary metabolites that appear to discriminate between trees that are susceptible versus tolerant to ash dieback.
- The genomes of trees with high and moderate tolerance to ash dieback disease have been sequenced.

- A tree nicknamed "Betty" in a Norfolk Wildlife Trust wood was predicted by the markers to have very high tolerance. Betty is a mature tree that is currently healthy, whilst being adjacent to infected trees.

Detailed Project Results

Pathogen genomics and biology

- The project has produced a genome sequence and transcriptome (Expression map) of several isolates of the ash dieback pathogen *H. fraxineus.*
- Approximately 10,000 protein coding genes have been annotated and made available through the ash dieback open access website.
- Ash dieback is likely to have been introduced into Europe as a consequence of a very limited number of infectious events, but once established has spread widely.
- No breeding between *H. fraxineus* and the UK native *Hymenoscyphus albidus* is evident.
- Strains of *H. fraxineus* present in Europe are closely related to each other but distantly related to isolates from Japan.
- Ash tree leaves can be infected by asexual spores as well as sexual spores.

Ash tree genomics, metabolomics and Associative Transcriptomics

- Metabolite profiling of ash leaves is able to distinguish susceptible and tolerant ash trees.
- Genome sequences were produced for two tolerant Danish trees. The genomes were annotated in collaboration with researchers from Queen Mary University of London, whose work was supported by NERC.
- Associative Transcriptomics (where gene expression is compared to phenotypic variation) was used to identify genetic markers for tolerance. 182 trees from a Danish diversity panel were scored for disease damage in the field and their gene expression analysed. Three markers (1 Single nucleotide polymorphism (SNP) markers and 2 gene expression markers) were identified and were used to successfully predict which trees exhibited tolerance to Ash dieback in a Danish test panel of unrelated trees.
- The markers are associated with MADS box genes which are speculated to be involved in regulating responses to environmental stimuli.
- For the SNP marker, trees with a strong G (guanine) signal tend to be more tolerant. Susceptible trees have a strong A (adenine) signal at this position. This is likely to be due to differential expression of two related genes as opposed to allelic variation of a single gene.
- The SNP marker is predictive within the *F excelsior* species (Danish material) and across the species tested of the *Fraxinus* genus.
- Several small UK ash populations have been screened using the SNP marker:

- 139 uninfected trees from ash clonal orchards in Devon and Oxfordshire were tested. Approximately half of these trees gave a strong G signal, suggesting that they may show some degree of tolerance. These are preliminary results based on a partial analysis, and more research is required to quantify the level of tolerance in the UK Ash population.
- Out of 248 trees from an infected Norfolk Wildlife trust Woodland in Norfolk, 11% possessed the tolerance-associated allele of the SNP marker and 7% displayed a strong G signal. Based on all three markers, the researchers predict 3% of the trees will show some tolerance to the disease (i.e. showing less than 25% canopy damage). More research is needed to understand what impact this may have on disease spread and tree death.
- Tree 148 "Betty" in a Norfolk Wildlife Trust wood was predicted by the markers to have little or no canopy damage. Betty is a mature tree that is currently healthy, whilst being adjacent to infected trees showing extensive damage.
- 156 trees from 39 European woodlands were screened using the markers; three trees possessed the tolerance-associated allele and only 1 showed a strong result signal.

Open Access and Citizen science

- Genomics results from the project were shared via an open access website throughout the project. Those sharing their results saw an increase in collaborative activities compared to those members of the projects who chose not to share incomplete data.
- The project developed a crowd-sourcing citizen science Facebook game Fraxinus that was used to help annotate the ash tree genome.