



Identifying the scale of suspected hybrid ash (*F. excelsior* x *F. angustifolia*) in Ireland

PROJECT TEAM

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BACKGROUND

Ash imported from 1993 to 2000 have produced poorly growing plantations in a substantial number of cases. This project investigates whether the planting stock was true common ash (*Fraxinus excelsior*) or natural hybrid material involving another continental species (*F. angustifolia*). These plantations are now seeding. The project is investigating the origin of the imported trees and their interbreeding capacity, as the dispersal of hybrid seed into the landscape may give rise to a wild population that would interbreed with native stock, resulting in genetic pollution of native ash germplasm.

OBJECTIVES

The overall objective is to provide a set of tests that can confirm the hybrid nature of ash that is present in suspect stands in Ireland and their potential to interbreed with indigenous ash.

The specific objectives are:

- to examine suspect ash stands in Ireland using known morphological criteria;
- to examine suspect ash stands in Ireland using known molecular criteria;
- to assess the potential threat of confirmed hybrid material to introgress with native stocks of ash.

PROGRESS

Two sites, one in Dublin and one in Meath, have been chosen for intensive study as well as material from France where natural hybridisation of ash occurs. Several tasks have been completed and data are being analysed. Completed studies include a comparison of flowering time (phenology) and the sex ratio of trees in suspect

'hybrid' plantations and native ash trees. Our studies have shown that flowering period of suspect hybrid ash in plantations can overlap with native trees and that seeds are produced in these plantations. By a paternity analysis of the seeds, we aim to determine whether the seeds were produced by pollen from within the stand of imported trees or by pollen from native trees around the stand. Similarly, we are examining seeds on native trees in hedgerows near these plantations to see if they were sired by pollen from the imported trees. DNA has been isolated from parent trees and statistical analyses of the morphological data, combined with the genetic data is underway to determine the paternity of the seeds collected in the suspect hybrid stands. This will also indicate the level of gene flow from plantation to native trees and from natives to plantation trees.

Seeds collected from suspect hybrid trees have been stratified and are now in germination tests to compare the embryo size and plant development from these seeds compared to native ash. To determine those characters which may be diagnostic for hybrid ash we have studied F1 hybrid ash trees which were produced from controlled crosses. The parameters measured were the density of stomatal cells and leaf morphology traits. In addition, DNA has been extracted from parent and F1 hybrid trees and a molecular analysis is in progress.

Work is underway to develop a 'bar code' approach to genotyping ash using molecular markers for potentially adaptive characters which could distinguish *F. excelsior* from *F. angustifolia* and hybrids; the genes involved affect foliar morphology, drought resistance, bud flushing, and dormancy.

A set of guidelines have been drafted as an aid to identify hybrid ash. The features of hybrid ash are:

- Brown buds are associated with hybrid trees but black buds may also be found.
- Narrow leaflets, about 2 cm wide with a spear form, usually bearing between 7 to 15 conspicuous marginal teeth are typical of many hybrids. Common ash bears at least 20 very small teeth and frequently up to 30.
- Leaflet numbers on sun-exposed branches can be as low as 5, but more typically 7, although some branches can exhibit more leaflets; common ash exhibits frequently 9 to 13 leaflets.
- Tendency to produce leaf and bud whorls of three on the same plane especially on slow growing branchlets and twigs. This is also associated with a round, plump

terminal bud in hybrids. Common ash typically has two opposite leaves with a 'snake head pointed bud'.

- Flowering time in hybrid individuals usually starts earlier, (December-January); common ash tends to flower in mid February to March, or even April depending on climatic conditions during the year.

ACTIVITIES PLANNED

- Sequencing of a comprehensive sample of Irish provenances, continental *F. excelsior* and *F. angustifolia* and hybrid populations using expressed sequence tags (ETS) and Internal Transcribed Spacers (ITS) to provide a potential 'bar code approach' to identification.
- Analyses of paternity determination for progeny collected in hybrid plantations.
- Submit a scientific paper for publication on the characterization of F1 hybrid ash.
- Prepare a set of guidelines to aid in the identification of hybrid ash in a leaflet form, and guidelines on how best to mitigate and manage the problem in plantations.
- Determine the viability of seeds from Irish hybrid plantations.
- Complete the morphological analysis of Irish plantation material to compare with putative hybrid zone material from France.
- Complete the genetic fingerprinting (genotyping) of chloroplast markers for two plantations.

OUTPUTS

Posters

Muriel Thomasset, Gerry Douglas, Trevor Hodkinson. 2008. *Hybrid alien ash in Ireland and its potential for interbreeding with native ash*. Postgraduate Ecology Forum conference 10-12 March 2008 at Trinity College Dublin. Conference book, page 40.

Juan F. Fernández-M., Muriel Thomasset, Trevor R. Hodkinson, Nathalie Frascaria-Lacoste, Gerry C. Douglas. 2008 *Identifying the scale of suspected hybrid ash (F. excelsior × F. angustifolia) in Ireland and its potential for genetic pollution of indigenous ash*. Climate Change and Systematics, 1-3 September 2008 at Trinity College Dublin. Conference book, page 24.

Muriel Thomasset, Gerry Douglas, Trevor Hodkinson. 2008. *Hybrid alien ash: F. excelsior × F. angustifolia and its potential for interbreeding with native ash under current and future climatic conditions*. Climate Change and Systematics, 1-3 September, 2008 at Trinity College Dublin. Conference book, page 29.

Juan F. Fernández-M., Muriel Thomasset, Trevor R. Hodkinson, Nathalie Frascaria-Lacoste, Gerry C. Douglas. 2008. *Identifying the scale of suspected hybrid*

ash (F. excelsior × F. angustifolia) in Ireland and its potential for genetic pollution of indigenous ash. Agricultural Biotechnology International Conference August 24-28 2008, University College Cork. Poster 1.18 Book of abstracts, page 8.

Communications

'Keeping Irish ash pure' 2008. ScienceSpin No. 31 p10.

M. Thomasset. Seminar day for first year postgraduate at Trinity College Dublin, 25 March 2008: Presentation: '*Hybrid alien ash in Ireland and its potential for interbreeding with native ash.*'

M. Thomasset, T. R. Hodkinson, G. C. Douglas. 2009. *Assessing the potential for introgression of imported ash with native ash (Fraxinus excelsior L.)* Irish Ag. Forum (in press).

G.C. Douglas, M. Thomasset, T.R. Hodkinson. 2009. *Phenology of alien hybrid ash Fraxinus excelsior × F. angustifolia in Ireland*. Presentation at Treebreedex meeting Noble Hardwood Trees Breeding Italy (in press).

Report

Muriel Thomasset. 2009. Transfer Report from MSc to PhD Programme at TCD. *Hybrid alien ash: Fraxinus excelsior × F. angustifolia in Ireland and its potential for interbreeding with native ash*.