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Genetic improvement of hardwoods for farm forestry

Key external stakeholders:
Tree nurseries, Coillte, farm foresters, forestry consultants, COFORD, The Department of Agriculture Fisheries and Food.

Practical implications for stakeholders:
All hardwood germplasm currently being deployed in the form of plants to farm foresters is from unimproved seed sources

- We have conserved elite breeding germplasm of ash and sycamore from Irish and UK forest estates
- It will form the basis to generate seed which will be genetically improved, resulting in more productive trees with improved stem quality
- Trials have been established which will identify the optimal sources of ash and wild cherry
- Vegetative propagation of ash from selected mature trees has potential for development.

Main results:

- A trial of ash was established consisting of 37 European provenances. First results showed that material from Ireland, UK and northern Europe had a later bud burst date and a greater potential to avoid bud damage / death from late spring frosts.
- Conservation collections of over 100 genotypes of selected ash trees and 180 of sycamore were made by grafting in collaboration with the Future Trees Trust (FTT) – a joint improvement programme with the UK- http://www.futuretrees.org/. They are being used to establish seed–producing orchards to generate improved seeds for deployment in forest plantations.
- Some varieties of wild cherry have been identified with a good early performance.
- Vegetative propagation of ash is achievable by micropropagation of selected mature trees. For some genotypes, large scale vegetative propagation is feasible by cuttings and has potential for further development to a commercial scale.

Opportunity / Benefit:

- The project has secured elite breeding germplasm of ash and sycamore derived from Ireland and the UK in the form of conservation collections of selected plus trees. This material is available for the establishment of seed producing orchards.
- Establishment of seed orchards derived from this material is underway and some seed has been produced already from selected plus trees of sycamore.
- The uptake of technologies to propagate selected ash trees vegetatively has potential for development to supply plants to the nursery markets.
- The best performing provenances of ash and varieties of wild cherry will be identifiable from the established trials after analyzing further periods of growth.

Collaborating Institutions:

Coillte, Future Trees Trust (FTT), Forest Research (UK), COFORD

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1. Project background:

Unlike crops and farm animals, forest trees especially hardwoods, have not been domesticated / improved by selection and breeding. Hardwood trees are long lived perennials which help maintain biodiversity, mitigate climate change by carbon sequestration and provide a wide range of commercial products. Increasingly these products are from plantations rather than natural forests and their high value and demand will require an increase in productivity of high quality timber. A key aspect of productivity for broadleaves is the value placed on the quality of the stems and wood. The sycamore plantation illustrated on page 1 (in Scotland) is 60 years old, stem diameter 60 cms, thinned five times and has an approximate value of € 45,000 / ha. A veneer quality ash tree (*Fraxinus excelsior*) would be valued at 20 times more than a tree that is only suitable for firewood. Gains of up to 20% are also feasible for recoverable wood volume by genetic improvement programmes. About 30% of the planting in Ireland consist of broadleaved species and ash consists of about half of this total. Sycamore (*Acer pseudoplatanus*) and wild cherry (*Prunus avium*) are also high value broadleaved species with fast growth and are suitable for farm forestry. However, all hardwood germplasm currently being deployed is from unimproved seed sources i.e. wild stock.
There is a great diversity of growth and stem qualities among trees in the mature forests throughout Ireland and the UK and genetic improvement is possible by using selected trees to form the base of an improvement programme. Gains made in tree breeding are incremental and permanent as long as the breeding germplasm is secure. Earlier EU supported projects, had identified plus trees of the major broadleaves in Ireland and the UK and work had begun to conserve those tress by grafting propagation. The current project was in collaboration with the British and Irish Hardwood Improvement Programme, consisting of all stakeholders from landowners to timber merchants and universities; it is now a charitable trust in Ireland and the UK in the form of the Future Trees Trust: http://www.futuretrees.org/ . The overall objective is to make available to nurseries (seeds), and ultimately to farm foresters (planting stocks), genetically improved germplasm of the main hardwood species. This collaboration allowed us to have access to a greater diversity of germplasm of plus trees of ash, sycamore and wild cherry collected in the UK estates.

This project had the following objectives: a) selection of trees with superior phenotypes i.e. 'plus trees' b) conservation of selected plus trees by grafting and establishment of *ex situ* conservation collections, c) provision of grafted plus trees to establish breeding populations to produce seed progeny in seed orchards, d) development of technologies to vegetatively propagate plus trees with the view to developing varieties, for large scale deployment after field testing, e) establishment of field trials with different continental sources (provenances) of ash to determine the best provenances for growth in Irish conditions, f) establishment of field tests of wild cherry to determine which selections (made in the UK , and Germany) perform best under Irish conditions.

2. Questions addressed by the project:

- Is it feasible to propagate and conserve a breeding population of ash and sycamore derived from the best phenotypes in Ireland and the UK and establish seed producing orchards?
- Can we identify differences in the performance of different sources of ash provenances (from Europe) when grown in Ireland?
- Can we identify differences in the performance of different genotypes of wild cherry derived from breeding programmes in the UK and Germany when grown in Irish conditions?
- Is it feasible to vegetatively propagate ash trees from selected mature specimens which display superior growth and quality characteristics?

3. The experimental studies:

A provenance test of ash was established at Roosky, Co Roscommon in May 2007. We used 108 plants (2m X 2m) from each of 37 European provenances including controls, in three replications of each provenance;

each replication with 36 trees in an incomplete block and randomized plot design. Survival assessments of trees was made two years after establishment.
The development of bud breaking characteristics of ash (phenology) were categorised at five stages in the progression of bud burst and was recorded on ash material in trees in the provenance trial. Buds were also collected to analyse their sugar and other metabolites during the stages of bud burst using two provenances from North-East of France known for their early flushing traits and two other provenances with known later flushing, from Enniskillen and Loch Tay Scotland.
To make a collection of superior germplasm of ash and sycamore, we received shoots, collected from plus in estates throughout the UK and Ireland. Propagation was by grafting in Spring. For micropropagation of ash we collected developing buds in Spring, surface sterilised them and cultured them *in vitro* on various basal media and with growth regulators. Rooted plantlets were weaned to the glasshouse and established in stoolbeds by cutting them back at least three times in each growing season. Cuttings from stoolbeds had an apical bud plus two nodes and were treated with 0.8% IBA for rooting in two parts peat with one part perlite on a heated bench (20^0C) and covered with light plastic.
Commercial varieties of wild cherry were tested in field trials on three sites (Clonmel, Donard and Loughgall); 24 were from Germany, 7 from the UK and one Irish source. The trials were established (3mX4m) in a mixture with ash, using 48 plants per site/ clone in 6 replications of 4 trees / clone. Tree heights were measured in the third year after establishment.

4. Main results:

The establishment rate for all 37 ash provenances was high ranging from 92 to 100%. We have measured bud burst in Spring over two years and first results indicated a significant provenance effect for the adaptive trait of timing of bud flushing (phenology). Among the top third of provenances with the latest flushing date were material from Ireland, UK, Denmark, Belgium and Lithuania. Late flushing is a desirable trait because such trees may avoid the damage to terminal buds caused by late frosts. Late frosts can result in death of the terminal buds on young trees and will result in subsequent forking of the tree leading to poor quality stems. The earliest provenances to flush were from Italy and some regions of France. First results also indicate differences among provenances in growth rate with Italian material growing poorly.

Collaborative studies showed that bud break and bud development was accompanied by a decrease in the non-structural carbohydrates within buds. The bud-breaking responses of ash provenances with early and late flushing behaviour were recorded in shoots grown in controlled conditions and five metabolites (putrescine, mannitol, trehalose, sucrose and raffinose) showed significant differences between the provenances. Reduced levels of putrescine and increased levels of carbohydrates were recorded in late flushing provenances. The content of sucrose and mannitol was twice as high in the buds of the two known late flushing provenances compared to the content in three early flushing provenances and could be used to differentiate between each type. In addition the levels of putrescine, raffinose and trehalose were also consistently higher in late flushing provenances.

Graft viability of ash was close to 100% for all 90 ash genotypes tested, and the trees propagated are in a conservation collection for further bulking up and the development of seed orchards. Material from approximately 30 UK trees was also propagated. Experiments on vegetative propagation of individual mature plus trees of ash was by micropropagation. We initiated shoot cultures of over 35 genotypes annually but only 10 genotypes survived to the fourth subculture period. Establishment of ash cultures in micropropagation is difficult because of a varied bacterial flora and the physiological mature status of tissues. However, we observed better growth using the basal medium of Ruigini compared to others (MS, DKW and WPM). In addition the cytokinin thidiazuron was needed to initiate cultures but prolonged culturing with high levels resulted in vitrified shoots. This condition, however, could be reversed by culturing shoots in alternative cycles on basal WPM with 3% activated charcoal to restore normal shoots. On this medium we often observed spontaneous rooting, as a possible indicator of physiological rejuvenation. Trees were weaned and first observations of micropropagated ash trees from mature plus trees in a field trial indicate that the material has grown normally, and shown no adverse effects from the propagation procedures.
We have established stool beds using micropropagated plus trees for 10 genotypes in the greenhouse. In this form the stool beds were pruned as low ‘hedges’ to a height of 5 cm so they produce two-three crops of vegetative cuttings per year. Shoots harvested from these hedges rooted at high rates (under plastic on a heated bench, 21^oC); three crops of cuttings could be collected in the same season giving high rooting rates. There was variation in rooting rate among genotypes but the lowest rate remained over 50%. Micropropagation of ash will be useful to bulk up desirable germplasm from seed and more importantly, as a tool to rejuvenate mature trees. Rejuvenated mature trees have shown a capacity for rooting by cuttings.

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This is a cheaper and more practical way to vegetatively propagate plants of ash to test the performance of individual genotypes and also to establish stoolbeds which provide rootable cuttings for more extensive deployment. We have estimated that using stoolbeds, generated from micropropagated plus trees, we could produce 320, 000 rooted ash plants / yr from a glasshouse of 200 m^2.

For sycamore we grafted material from plus trees identified in Ireland and the UK. The average grafting success rate was 57%. This has resulted in the conservation of 79 Irish and 109 genotypes from the UK. The material has been bulked up to four ramets (copies) of each genotype and was established as a seed orchard at AFBI, Loughgall on 2.0ha of land. Grafted material has also been supplied to the Future Trees Trust in the UK. Trees established at Teagasc Kinsealy have flowered after 4 years and produced 6.0 Kg of seed which will be used for progeny testing.

The wild cherry on two trial sites survived well while one was destroyed by deer. Field establishment of wild cherry was generally good giving a field survival of over 90% for 23 / 32 of the varieties. Based on total height growth and increment growth we identified only German varieties in the top 25% of best performers in Irish conditions on two sites. Further periods of growth are required for a full assessment of performance.

5. Opportunity/Benefit:

An important germplasm collection of ash and sycamore has been established in Ireland from the best plus trees available here and the UK. Material from them can be used by forest nurseries or other agencies to establish seed producing orchards. Because the material is from selected trees, the seed progeny, from grafted trees in dedicated seed orchards, can be marketed in the category 'Qualified' according to the EU directive on forest reproductive material. This is the second highest quality level after 'Selected' and 'Source identified' on the genetic quality of forest reproductive material.

Results obtained on the performance of ash provenances and wild cherry varieties are provisional and require more years of growth for a full analysis of important growth traits such as height, stem diameter, stem form and tolerance to disease organisms.

We have demonstrated the capacity to vegetatively propagate selected trees of ash by micropropagation to rejuvenate the trees. Furthermore, we have obtained good rooting rates using conventional cuttings derived from micropropagated plants that were established in stoolbeds. This offers prospects of providing a means to vegetatively propagate selected material of ash on large scale.

6. Dissemination:

Main publications:

Jouve L, Jacques J, Douglas GC, Hoffmann L and Hausman J-F (2007) Biochemical characterisation of early and late bud flushing in common ash (*Fraxinus excelsior* L.) Plant Science: 172 (No5) 962-969.

Cahalane G, Doody P, Douglas G C, Fennessy J, O'Reilly C and Pfeifer A (2007) Sustaining and developing Ireland's forest genetic resources - an outline strategy – COFORD, Dublin, pp. 1-30.

Douglas G C (2006) Genetic improvement and application of biotechnology methods to breeding woody plants. In: Alternative plants for sustainable agriculture. (Eds.) S. Jezowski. M. K. Wojciechowicz, E. Zenkteler. Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland. 9-22.

Douglas GC, Pliura A, Dufour A, Mertens P, Jacques D, J. Fernandez-Manjares JF, Buiteveld J, Parnuta Gh, Tudoroiu M, Curnel Y, Thomasset M, Jensen V, Knudsen M, Foffova E, Chandelier A, Steenackers M. (2013) Common Ash (*Fraxinus excelsior*) IN: Forest Tree Breeding in Europe Current state-of-the-art and Perspectives, Springer, ISBN 978-94-007-61 46-9 pp 403-462 .

Popular publications:

Carey M, Douglas GC and Fennessy J (2010) Hardwood: The British and Irish Hardwood Trust: enhancing the quality of the planting stock of broadleaf species. Crann Spring issue 2010.

Hardwood scheme a 'plus' to industry. Irish Independent: May 2011

Future Trees Trust : http://www.futuretrees.org/

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