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Characterising 'brown bud' – hybrid ash

Key external stakeholders:

Farm foresters, forest nurseries, forestry advisors, DAFF

Practical implications for stakeholders:

Forest owners should be very careful about the geographic origins of all forest trees, especially ash, which they use to establish their forest plantations. Some imported ash trees from Europe in the period 1990-2002 have shown poor growth and stem form in plantations (see figure above). This is due to the deployment of plants which were not pure common ash (*Fraxinus excelsior)*; rather they were complex hybrids of common ash with the continental species of narrow-leaved ash, *F. angustifolia*. Key morphological characteristics to identify F1 hybrid material are described.

Main results:

- In F1 hybrids, thirteen of the variables were statistically different between the two species but the main differences were a greater leaf area and a lower stomatal density in the leaves of *F. excelsior* compared to *F. angustifolia*.
- Useful characters to distinguish hybrid trees from our native *F. excelsior* were the characters of 'leaf width' and the character of 'number of teeth / leaflet'. In the case of teeth number per leaflet, the reciprocal F1 hybrids had an intermediate number of teeth (16-17) and these numbers were significantly different from either parent: *F. excelsior* (24) and *F. angustifolia* (12), Figure 1; similarly for total leaf area and leaf weight.
- The morphological characters described are useful in providing evidence for the potential presence of hybrid individual trees in the plantations of farmers who suspect they may have them.
- Hybridisation of ash occurs in nature on continental Europe where the two species co-exist and where their geographic ranges overlap. Seeds collected from such areas are undesirable because they may consist of pure F1 hybrids and back crossed hybrids which are not suited for Irish plantations.

Opportunity / Benefit:

This research provided key morphological features which may be used to evaluate the material found in plantations that is suspected to be derived from hybridization. As Ash trees and seeds can be freely marketed across Europe forest nurseries are now aware of the dangers of importing germplasm and plants to Ireland from ash sources which are not pure *F. excelsior* and which may prove to be mal-adapted to Irish growing conditions. Forest owners can now make every effort to ensure that their source of ash seeds / plants is from the recommended list of countries and preferably from registered sources in Ireland and the UK.

Collaborating Institutions:

Trinity College Dublin; Univ Paris VI, France; INRA, France.

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1. Project background:

In the period 1997-2007 we have planted 1.3 million ash trees per year because ash is a hardwood crop with a good economic potential for farm forestry which also produces thinnings for firewood and hurleys. In some years, we have imported ash plants as home production failed to meet full demand. Although the imported plants appeared good, many plantations from this period have produced crooked stems and poor growth (see figure). Contact with European colleagues indicated that common ash (*F. excelsior*) can form hybrids with a related species (*F. angustifolia*), which shares its geographic territory on the continent. The poorly performing material may be progeny from the hybridisation of common ash (*F. excelsior*) with narrow leaved-ash, (*F. angustifolia*). It was observed that imported plant material in these plantations had brown coloured buds rather than the typical black buds of common ash and so, problematic plantations have become known as 'brown bud ash'.
An important aim of this research was to find distinctive morphological characters which would aid in the identification of hybrid plant material, so pure F1 hybrids were analysed.

2. Questions addressed by the project:

- What are the main morphological characteristics of pure common ash, *F. excelsior* compared to pure narrow-leaved ash, *F. angustifolia*?
- What are the main morphological characteristics of F1 hybrids of *F. excelsior* x *F. angustifolia* when each species was used as mother or father species?
- Which morphological characteristics are most useful in the identification of F1 hybrids?

3. The experimental studies:

Measurement were taken from confirmed control plants of each species and compared with F1 hybrids of *F. excelsior* with *F. angustifolia* which were derived from reciprocal crosses with each species as female and male. Ash has compound leaves and measurements were made on whole leaves and component leaflets. We measured dimensional traits (and some computed ratios): leaflet length, width, leaflet area, apex angle of the terminal leaflet, internode length between the second and third leaflet, total leaf area, total leaf length, total leaf width, and total dry leaf weight, and discreet variables: number of leaflets per leaf, number of teeth on the second leaflet from the terminal leaflet and the density of stomata on the underside of leaves. Statistical analysis included both univariate and multivariate methods. Data analysis for basic statistics and data transformation were performed using R Foundation statistical computing software and Statistica v.6.0 .Some variables were log transformed for normalisation. One-way analysis of variance and Tukey HSD post-hoc tests (at 5% probability) were used to make comparisons among the different groups. Observations were also made on trees in plantations which have shown poor growth and crooked stems for the presence of brown buds and the presence of three buds at each node. The latter character is in short shoots on some trees of *F. angustifolia* whereas common ash (*F. excelsior*), has typically two buds per node.

4. Main results:

- Winter buds of common ash are typically black and opposite, there is variation in both traits in hybrids so characterization was concentrated on determining measurable traits. We compared *F. excelsior* with *F. angustifolia* and the F1 hybrids with either parent. Most of the measurements of the whole leaf and leaflets of common Irish ash *F. excelsior* were significantly larger for this species compared to continental narrow-leaved ash *F. angustifolia*; including: leaflet area, leaflet length and width, total leaf dry weight, total leaf area, total leaf length , the angle at the apex of terminal leaflet and the number of teeth per leaflet. For example the mean number of leaflets in *F. excelsior* was 11.95 and was significantly smaller in *F. angustifolia*, with 7.9.
- The density of leaf stomata on the underside of leaves was the only variable found to show significantly higher values in *F. angustifolia*, compared to *F. excelsior*.
- We compared details in offspring of two different types of F1 hybrid; one set had *F. excelsior* as mother, the other had *F. angustifolia* as mother. These offspring were morphologically intermediate

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between the two parental species for most characters, although the ranges of values for hybrids generally overlapped with those of one or other parental species. Only three of the variables were significantly different between the two types of F 1 progeny when compared to each of the parental species. In the case of teeth number per leaflet, the reciprocal F1 hybrids had an intermediate number of teeth (16-17) and these numbers were significantly different from either parent: *F. excelsior* (24) and *F. angustifolia* (12), Figure 1; similarly for total leaf area and leaf weight.

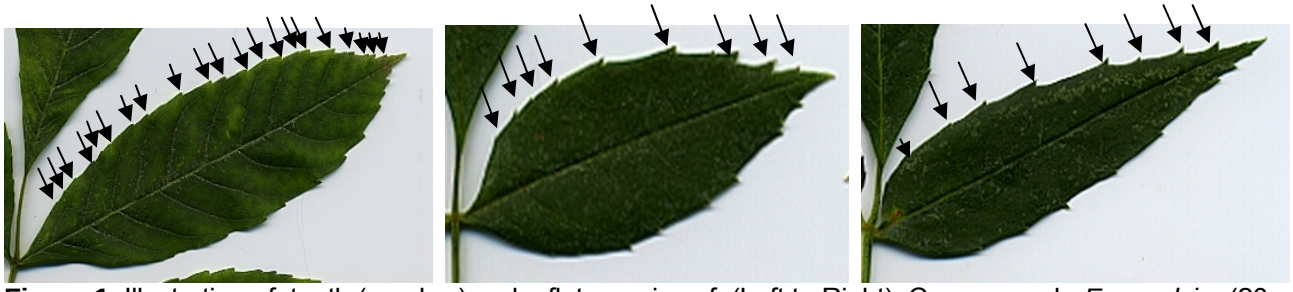

Figure 1. Illustration of teeth (number) on leaflet margins of: (Left to Right); Common ash, *F. excelsior* (20-30); F1 hybrids (7-15), Narrow Leaved ash, *F. angustifolia* (5-12)

- For five of the characters there was no significant difference between *F. excelsior* and the F1 progeny from reciprocal crosses i.e. leaflet length, leaflet width, leaflet area, leaf length, and number of leaflets / leaf. However, for all of these characters in the F1 hybrids, they were significantly different (and greater) than in *F. angustifolia.*
- Hybrids which had *F. excelsior* as mother could be distinguished from those with *F. angustifolia* as mother only by the characters of apex angle of the terminal leaflet and the ratio of leaflet length to width. In each case these characters in the F1 hybrids followed the female parent.
- The ratio of leaflet length to width and the total leaf width were similar in each of the reciprocal hybrid types.
- Although *Fraxinus excelsior* had a significantly lower density of leaf stomata than *F. angustifolia*, the two hybrid types were not significantly different from each other for this character. Stomatal density in the hybrids derived from *F. excelsior* as mother, were more similar in number to pure *F. angustifolia* whereas those hybrids derived from *F. angustifolia* as mother were more similar to pure *F. excelsior* for the density of stomata.

A stepwise discriminant analysis was performed to identify those variables which could best discriminate among the four groups of: *F. excelsior, F. angustifolia*, F1 hybrids with *F. excelsior* as mother and F1 hybrids with *F. angustifolia* as mother. The variable characters of 'leaf width' and the character of 'number of teeth / leaflet' had the most discriminatory power to separate the four groups. The character of 'total leaf area' was also useful as indicated above.

5. Opportunity/Benefit:

End-users of this information are forest owners, COFORD, Department of Agriculture Fisheries and Food, Forestry Inspectors and advisors. Results have shown that pure common ash (*Fraxinus excelsior*) has different morphological features when compared to imported material of narrow leaved ash (*F. angustifolia*) and from hybrids derived from these species. The key morphological features may be used to evaluate the material found in plantations that is suspected to be derived from hybridization. As forest reproductive material can be freely marketed across Europe. Forest nurseries are now aware of the dangers of importing seed germplasm and plants to Ireland from sources which may prove to be mal-adapted to Irish growing conditions and which may be not consist of pure common ash, *F. excelsior*.

6. Dissemination:

A communication in the series 'Coford Connects' has been submitted giving more detailed information covering morphology, flowering, further hybridisation and management suggestions.

Main publications:

Thomasset, M., Fernández-Manjarrés, J.F., Douglas, G.C., Frascaria-Lacoste, N., Raquin, C., Hodkinson, T.R. (2011) 'Molecular and morphological characterization of reciprocal F_1 hybrid ash (*Fraxinus excelsior* □ *F. angustifolia*, Oleaceae) and parental species reveals asymmetric character inheritance'. *International Journal of Plant Sciences* 172, 423-433.

Thomasset, M., Fernández-Manjarrés, J.F., Douglas, G.C., Frascaria-Lacoste, N., Hodkinson, T.R. (2011)

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'Hybridisation, introgression and climate change: a case study for the tree genus *Fraxinus* (Oleaceae)'. In: *Climate Change, Ecology and Systematics*, ed. Trevor R. Hodkinson, Michael B. Jones, Stephen Waldren and John A. N. Parnell. Published by Cambridge University Press. © The Systematics Association 201 pp. 320-342.

Thomasset M. (2011) Introduced hybrid ash: *Fraxinus excelsior* X *F. angustifolia* in Ireland and its potential for interbreeding with native ash. Ph D. Thesis (2011), University of Dublin Trinity College, Ireland.

Popular publications:

Keeping ash trees pure. Science Spin , November 2008.
http://www.sciencespin.com/magazine/archive/2008/11/keeping-irish-ash-trees-pure-/

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