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- Imports of ash plants over the period 1990 to 2000 have resulted in some plantations with poor stem form. One of the identifying features of these plantations may be trees with brown rather than black buds, hence the name 'brown bud ash'.
- 'Brown bud ash' trees are suspected to be derived from the hybridisation of our common native ash (*Fraxinus excelsior* L.) with narrow-leaved ash (*F. angustifolia* Vahl.) in Europe, in geographic regions where the natural distributions of the two species overlap. While the presence of brown buds may be indicative of hybrids, bud colour is variable. We refer to these trees as hybrid/brown bud ash throughout.
- The best ways to identify hybrids are the number of teeth per leaflet and the number of leaflets per leaf. Overall, common ash has 20-30 small teeth per leaflet, while hybrids have 7-15 (Figure 4). Also, our common ash has mostly nine leaflets per leaf, while hybrids tend to have 5-7.
- The presence of these hybrid trees in plantations is a potential threat to the genetic purity of native common ash in Ireland, because of their capacity to further hybridise with native trees and then disperse their seeds. This has been shown by the research outlined in this note.
- The flowering periods of native and hybrid trees overlapped for a period of over four weeks on two different plantations, thereby facilitating hybridization.
- Vegetative bud burst was earlier in the hybrid plantation trees compared to native trees, which makes them vulnerable to bud death from spring frosts. This may explain the poor stem form observed in hybrid / brown bud plantations.

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Identifying and characterising hybrid 'brown bud' ash in Ireland

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Introduction

Over the period 1990-2000 ash plants were imported from continental Europe. Although the imported plants appeared to be of good quality, some plantations from this period have poor growth and form (Figure 1).



Figure 1: Trees in a 12-year-old ash plantation consisting of hybrid ash imported from continental Europe and showing very poor stem form.

Common ash, *Fraxinus excelsior* L. is the only ash species native to Ireland. In continental Europe, another species, *F. angustifolia* (narrow-leaved-ash) occurs with common ash, mostly along rivers and waterways. Poorly-performing ash plantations in Ireland may be derived from seeds collected in areas where natural hybridisation between these two species has occurred and the resulting trees may have brown-coloured buds, rather than the typical black buds of common ash. These plantations are sometimes referred to as 'brown bud ash' or 'hybrid ash'. The work carried out in the CoFoRD-funded ASHGEN project had the objective to enable brown-bud ash plantations to be identified based on morphological features, flowering patterns and genetic markers. Also the flowering pattern in hybrid plantation trees and native ash trees was examined to see if hybrid trees could naturally hybridise with native ash.

Morphological features of hybrid/brown bud ash

Colour and symmetry of buds

Shoots which are growing in full light should be examined for their colour rather than those on suppressed trees or the shoots under the shade of other trees. The buds of common ash are completely black and generally large (Figure 2). The buds of narrow-leaved ash and hybrids are usually brown and somewhat smaller than common ash. However, bud colour can be variable within each species and also in hybrids. In addition, narrow-leaved ash is sometimes found with black buds in areas such as the Loire valley.



Figure 2a: Hybrid/brown bud ash (left) note the brown colour.

Figure 2b: Hybrid/brown bud ash (middle) with brown colour, more rounded buds and trilateral division of the terminal bud.

Figure 2c: Terminal and lateral buds of common ash (right) black in colour

An examination of the terminal buds of common ash shows that they are rather pointed with a 'snake-head' appearance (Figure 2c). Common ash also has bilateral division of the bud scales. The terminal buds of narrow-leaved ash and hybrids may also have bilateral division (Figure 2a), or they can have a more rounded tulip-like appearance, especially on short shoots as in Figure 2b with a trilateral division of the bud scales (Figure 2b and Figure 3b).

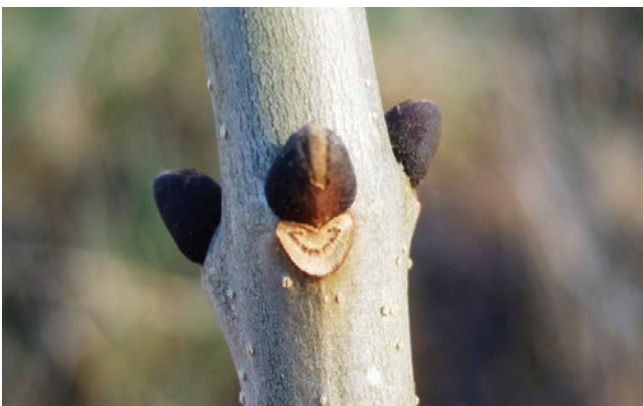


Figure 3a: Lateral buds on short shoots of hybrid/brown bud ash. Note three buds in the same plane.



Figure 3b: Terminal black bud of hybrid/brown bud ash with trilateral division.

Appearance and layout of buds on shoots

In young common ash, the buds occur in pairs, with one bud opposite the other on the stem. Rarely, on some older trees, whorls of three buds may be seen. In narrow-leaved ash and hybrids, the buds may be arranged as in common ash or, alternatively in a group of three buds on the same plane of the stem. Figure 3a shows this arrangement on a shoot from a hybrid ash tree; in this case the buds are black. The paired bud arrangement is generally observed on long vigorous shoots in hybrids, whereas the three bud arrangement is generally observed on shoots less than 10 cm long. In hybrids both types of bud layout can sometimes be seen on the same tree.

Leaf characteristics of common ash and hybrid / brown bud ash

Only sun exposed leaves should be examined to determine the number of leaflets and other leaf traits. Common ash has mostly nine leaflets though there may be as many as 15. Hybrid ash has fewer leaflets (generally 5-7) which are generally narrower than in common ash. Hybrid ash as planted is probably the result of multiple hybridisation events, so the trees do not always show traits which are intermediate between each of the pure parent species. However, in a study of the first generation hybrids between common ash and narrow-leaved ash only three variables were significantly different between the progeny and the parent species. The leaflets of hybrids had an intermediate number of teeth per leaflet (16-17), whereas one parent, (common ash) had an average of 24 and the other parent, (narrow-leaved ash) had 12 (Figure 4). In addition, the hybrids had intermediate values for both the total leaf area and the leaf weight. Figure 4 shows the presence of many small teeth on the leaflets of common ash, with fewer on hybrid ash and pure narrow-leaved ash.



Figure 4a: Leaflet of pure common ash: arrows indicate small teeth, with 20-30 per leaflet.



Figure 4b: Leaflet of hybrid/brown bud ash, with 7-15 teeth per leaflet.



Figure 4c: Leaflet of pure narrow-leaved ash, with 5-12 teeth per leaflet.

Flowering patterns in plantations of imported hybrid ash and native ash

In the course of research carried out in the ASHGEN project we examined the hybridization potential of suspected hybrid material in two plantations by comparing their flowering phenology (flower bud burst and pollen release) with wild common ash in surrounding hedgerows. Data were collected during the flowering period in 2009 and 2010 to compare the results from differing years and varying climatic conditions. The plantations studied were at Kildalkey, Co Meath and Clonee, Co Dublin (which contained imported trees with poor stem form). The trees reached flowering age at approximately 12 years of age and had started to produce seeds. Based on morphological and molecular markers, the plantations were found to be a mixture of tree types: pure common ash, pure narrow-leaved ash and hybrid trees. Flower bud flushing was scored according to a 5-point scale: initial flushing date (stage 1) to the wilting of the flower (stage 5). Stage 2.5 is a key stage, when pollen starts to be released and stigmas are receptive to being pollinated by any available pollen. Mature common ash in the hedgerows of the study sites of Clonee and Kildalkey flowered with greater frequency than the plantation trees in each year, Figure 5. Over the first year of observation (2009), only 47% and 57% of the plantation trees flowered at the two sites and flowering frequency increased in 2010 (Figure 5). The comparisons indicated that the proportion of plantation trees that flowered was significantly lower than the proportion of flowering common ash (native) trees. It should be borne in mind however that not all of the plantation trees had reached flowering age.

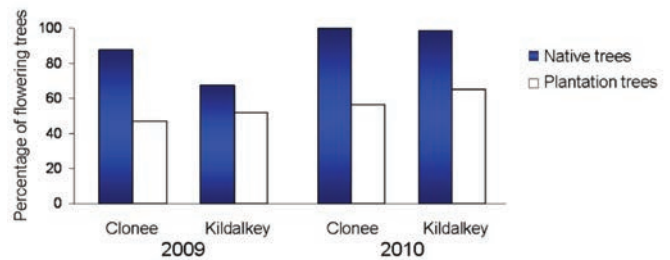


Figure 5: Flowering levels in 2009 and 2010 in common ash (native hedgerow trees) and hybrid ash in plantations at two sites.

January and February of 2010 were much colder and drier than the same months in 2009 (4.3°C average for January 2009 compared to 1.7°C for January 2010). The cold weather in January and February of 2010 did not, however, affect the onset of the flowering period or the time when the flowers reached maturity (Stage 2.5) for the native trees, at either site (Figure 6). Moreover, at the Clonee site only, there were no significant differences between the two years in the flowering pattern (Figure 6). The overall duration of the flowering period (DFP) for native trees was over 60 days in 2009 for the two sites. In 2010, the DFP was approximately 50 days for Clonee and 40 days for Kildalkey (Figure 6).

The flowering of the native trees followed a single peak and trough distribution at both sites and in both years (Figure 6). The majority of individuals showed intermediate flowering dates. At Kildalkey, 50 and 60% of the native flowering trees had an intermediate flowering phenology in 2009 and 2010, respectively. At Clonee, 69.1 and 44.3% of the native trees

which flowered had an intermediate flowering time. However, over the two years at each site approximately 20% of the common ash (native) trees showed an early start and around 15% a late start to flowering (Table 1).

Plantation trees at Kildalkey flowered significantly earlier in 2009 than at Clonee (Figure 6). The duration of the flowering period (DFP) was significantly longer in 2009 for the Kildalkey plantation trees compared to the Clonee plantation. In general, the plantation trees showed less variation in flowering time in 2010 than 2009: an overall decrease in the flowering time period from over 75 days to around 50 days (Figure 6).

There was a large difference between the plantation trees at Clonee and Kildalkey sites in the date of first flushing. The proportion of trees in the flower development category of early, intermediate or late is shown in Table 1. Considering an average response over the two years, 65% of the flowering trees in the plantation at Kildalkey had an early start,

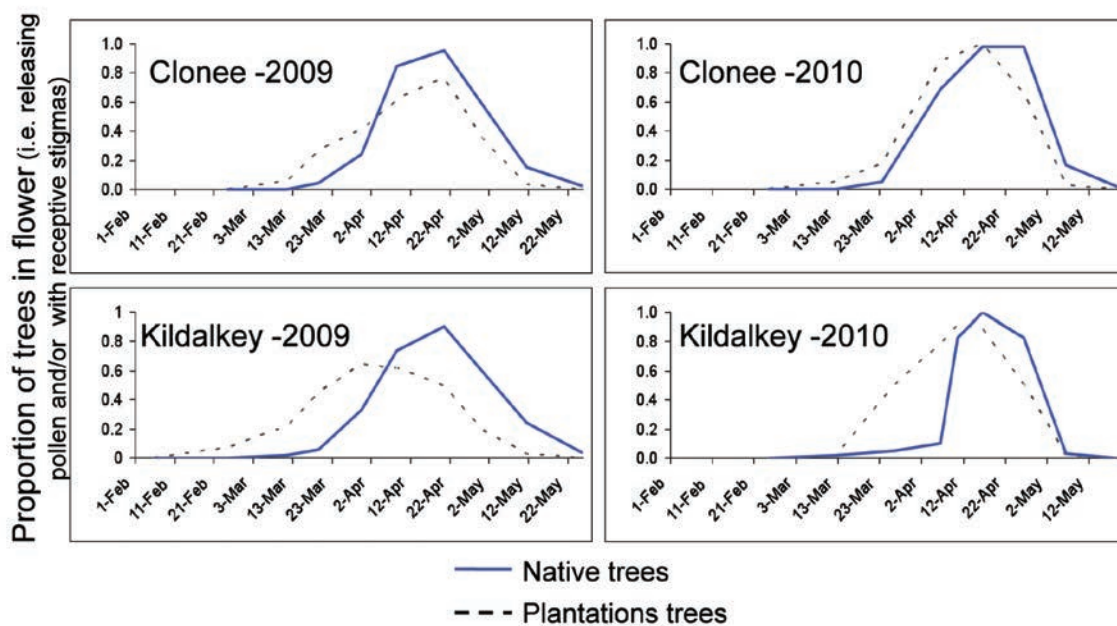


Figure 6: Flowering patterns of hybrid ash (plantation trees) and native ash (hedgerow trees), over two years at two sites.

Table 1: Percentage of individual trees classified as early, intermediate, and late flowering, according their flowering bud burst date, at two plantation sites with imported hybrid ash (Clonee & Kildalkey) and for native hedgerow ash trees around these same plantations, over two years.

Flowering group	CLONEE				KILDALKEY			
	Native		Plantation		Native		Plantation	
	2009	2010	2009	2010	2009	2010	2009	2010
Early	18.3	26.2	32.9	36.6	25.9	23.2	65.9	64.3
Immediate	69.1	44.3	47.1	51.4	50	60	25.5	26.1
Late	12.6	29.5	20	12	24.1	16.8	8.6	9.6

compared to 35% at Clonee. At both sites, for both years, some plantation trees with very late flowering phenology were also observed. The majority of the plantation trees at Clonee (52%) flowered during the intermediate flowering period compared to approximately 25% at Kildalkey. This observation and molecular data suggests that the trees in each plantation may have come from different provenance sources.

Utility of molecular markers in distinguishing hybrids

Molecular markers were used to characterise the plantation trees in order to identify them as either 'hybrid type', '*angustifolia* type' or '*excelsior* type'.

The early flowering trees at Clonee were all identified as 'hybrid type' or as '*angustifolia* type', except for one individual. Trees with intermediate flowering dates were identified as common ash type. Out of the 33 trees that flowered in 2009 and 2010, 25 were classified as early flowering (75.57%) and among these 23 (92%) have been identified as 'hybrid type' or '*angustifolia* type'. None of the trees, which were classified as late flowering, showed *F. angustifolia* morphology.

Do hybrid individuals represent a risk for native ash populations?

Two years of observations have shown that the flowering periods of imported trees in the hybrid / brown bud ash plantations, had significant overlaps with the flowering periods of native hedgerow trees. Therefore, there is a potential for cross pollination of native trees with plantation trees and vice versa. The actual level of gene flow between native and plantation trees was tested using DNA molecular markers. The analysis of progeny (seed) collected from plantation trees and from native trees around these plantations showed that cross pollination occurred. Both the imported hybrid ash trees in plantations and the native hedgerow ash trees surrounding these plantations can sire offspring. This generates future generations of hybrid seeds capable of dissemination and germination in and around plantations

In summary, the phenological and genetic results suggest that if hybrid/brown bud ash is suspected in an area, it should be avoided as an area for seed collection by nurseries and by designated seed collectors. To further minimise the risk of genetic pollution in seeds collected from native ash trees, it is advised to identify late flowering individual trees in advance and to restrict seed collection to these late flowering individuals. An alternative more desirable strategy is to restrict seed collection to registered seed stands and to dedicated seed orchards which are located far away from any plantations containing imported trees.

Critical importance of ash provenance sources

The planting of ash in Ireland is currently suspended due to the threat of the fungus *Chalara fraxinea* which is very severe. It causes the dieback of crown shoots and the death of young and old trees, throughout Europe. It has recently

been confirmed on imported trees in 85 locations in the Republic of Ireland and all trees so affected are being eradicated and destroyed, see:

<http://www.agriculture.gov.ie/forests-service/ash-dieback-chalara/>. This threat of *Chalara* and the threat posed by the further hybridisation of hybrid ash with native trees indicates the importance of using sources of ash trees which are well characterised and documented in all future plantations. Hybrid ash trees are produced in nature in regions where the two parental species of ash coexist i.e. common ash and narrow-leaved ash. Only common ash is found in nature in Ireland. Before the Chalara outbreak, the preferred origin of ash plants (provenance) was from seeds collected in Ireland or from registered seed stands in the UK, so as to ensure that the trees would be well adapted to Irish growing conditions. The provenance certificate which accompanies each consignment of trees from the nursery indicates the origin of the material.

Management options for hybrid /brown bud plantations

Management options for dealing with hybrid ash plantations depending on their age:

- a) *young plantations containing hybrid trees, less than 5 years*
When hybrid individual trees are detected as described above, it is advised to cull all trees with poor stem form, and interplant with ash of a known provenance from Ireland. Pruning of trees is also advised. Many plantations can be managed to achieve the goal of having 120-150 trees per ha of good quality in the final crop, since the initial tree density is usually 3500 trees per hectare.
- b) *plantations containing hybrids aged 5-15 years*
Bad stem defects can be corrected by pruning trees in plantations up to the age of 8-15 years. The stem form of older trees is more difficult or even impossible to correct. The early identification and permanent marking of 350 – 400 trees / ha, as potential crop trees is necessary to obtain an inventory of the standing crop and to manage the plantation properly. Thinning of ash plantations is advised in accordance with standard silvicultural practices (Silvicultural Guidelines for the Tending and Thinning of Broadleaves). Subsequent thinnings should reduce this number to 120-150 trees per ha as the final crop. Common ash is a demanding species requiring regular tending and thinning to enable the production of high quality lumber.
- c) *plantations containing hybrids aged more than 15 years: without tending or thinning having been carried out*
It is unlikely that 15-year old unmanaged plantations of hybrid / brown bud trees will produce trees that are suitable for high quality timber. However, judicious thinning would be advised to enable the production of some hurley butts and quantities of firewood.

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