Genetic improvement of ash (Fraxinus excelsior)
A European project

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ABSTRACT

The results summarised below are from a European project which had 14 partners (P1-14) in 9 countries: http://www.teagasc.ie/advisory/forestry/rap/index.htm
The work packages were: a) assessing biodiversity from previous provenance trials, from a new European trial and from studies on molecular gene diversity , b) vegetative propagation my cuttings grafting micropropagation, cryopreservation and somatic embryogenesis, c) developing strategies to promote the use of ash more widely.

Measurements and growth data were taken from ash planted in a common provenance / progeny trial, in 1984, in Germany, Belgium and France. Assessments by three partners have indicated that Romanian provenances had very poor stem form and were susceptible to canker.

Morphological and growth data collected by D. Jacques, Gembloux (P14) showed significant variation among provenances for height and circumference data and genotypic heritability varied between 0.32* and 0.76*** on the two sites examined. The coefficient of phenotypic variation decreased with time; it ranged from 15-19% for data collected in 1990 to 5-6% for data collected in 2000. The analysis of data on stem form and crown form showed very highly significant differences among provenances and heritability was characterised as medium to very low (from 0.68 **** to 0.26 NS). Provenance effects were also very highly significant for frost damage at two sites and genotypic heritability was good (from

0.60 *** to 0.72***). The genotypic hertabilities and gains were computed after a multi site analysis of all characters measured. This showed that provenance effect was highly significant for height growth but not for height increment and stem girth. Height heritabilites were 0.78*** from 1990 growth data, decreasing to 0.53** for 2000 data. Provenance effect was at least, significant for crown and stem form giving genotypic heritabilities from 0.44* to 0.77***. Further analyses by J. Dufour, Orleans, (P2) recorded highly significant provenance x site interactions for the traits of height, stem diameter, and stem form across two sites; similarly, a significant provenance x site interaction was noted for height and height increment across two sites (P14).

Frost damage was recorded among provenances in one nursery (P2) which showed a significant rank correlation value of 0.73 between bud flushing date and percentage frost damage. In addition, P 14 analysed flushing data at the level of progeny and found great variability at the nursery level. Further analysis on seven and eight year old trees by P2 on two sites (with 21-67 progenies per site) and 3-4 provenances has shown highly significant provenance and progeny effects for the characters of bud flushing, tree height, and stem-straightness. Using data from half-sib progenies, estimates of heritability were calculated for each provenance for the characters of stem form and flushing date. This showed high heritability values. It also showed significant variability between progenies within provenances compared to variability between the provenances. It indicates that selection for important characters can be made at the level of progeny and provenance for the purposed of genetic improvement. Another important result was that the estimated heritability values varied with the population being studied and for each character under study. Furthermore it was found that the minimum number of progenies for such studies should be greater than 20 so that the estimates of heritability will be more accurate.

A global statistical analysis of data from 16 yr old trees from 46 provenances in France and Germany was undertaken on the characters of height, girth, stem form, forking and frost resistance (P. Mertens Gembloux, P 14). It showed that the provenance, site and interaction effects for girth and height were all very highly significant. The site effect was the most significant, followed by provenance and interaction effects. An analysis of stem form showed a similar effect of site over provenance. In summary these results indicate the strong influence of site on the performance of ash trees, and that selection for important characters such as stem form can be made at the levels of provenance and progeny. However the observed strong site effect, from the global analyses, indicates that selection of the best provenances may be valid for local and regional superiority only.

In a new European provenance trial a “core” collection of 31 provenances was established in France, Italy, Wallonia, Flanders, Germany, Ireland, and UK. Nursery data on flushing of 45 provenances over seven regions indicated that north and western provenances flushed late, south and eastern ones flushed early and there were ‘intermediate’ ones from central Europe. Two provenances appeared unstable (B. de Cuyper, Gerardsbergen P12).

The gene diversity, gene flow pattern and hybridisation in ash was studied using molecular markers, (N. Frascaria, France P6 & B. Heinze, Austria, P 7) for nuclear (1, 8, 5) and more recent chloroplast (3) microsatellite markers. Ash populations were characterised by a high level of intra population diversity and low genetic differentiation between strands. In addition there was also a significant heterozygote deficiency found in French and Austrian studies. Molecular analyses showed a fine scale genetic structure using parentage analysis and autocorrelation tools. It showed that two trees separated by less than 100 m were genetically more similar than two trees chosen at random in the same population. It means that gene flows by pollen and seeds is restricted within the stand. The neighbourhood size estimates showed that any given tree in the studied stand mates at random with 178 individuals. Estimates of gene flow *via* pollen from outside the intensively studied stand were 56% in 2000 and 42% in 2001. However, in small stands pollen gene flow was even higher. It appears that pollen flow combines short distance events and long distances dispersal events in large stands and that in small stands long distance dispersal events were more important. *F. excelsior* hybridises with *F. angustifolia* in sympatric areas. In some hybrid populations intermediate morphologies exist while in others they do not. Molecular grouping methods indicated strong (hybrid) population affinities to either species rather than to an intermediate genetic composition and this results in introgressed hybrid populations rather than hybrid groups.

Plus trees were identified in provenance trials and populations and advances were made in their vegetative propagation through, cuttings, micropropagation, somatic embryogenesis and cryopreservation (Schoenweiss *et al*.,2005a & b). High rooting rates were recorded in cuttings from seedlings and from micropropagated plants of adult trees but not in cuttings from grafted mature trees. This indicated that the micropropagation process induced a rejuvenation effect on ash which restored rooting competence (G. Douglas, Dublin P1). It means there is potential to use micropropagation as a tool to produce stocks of plants from selected trees and to use those plants as 'hedges' for cutting material. This opens the prospect of large-scale vegetative propagation of selected ash trees and the development of polyclonal varieties after appropriate clonal tests on multiple sites. Micropropagation on a pilot scale, at the commercial level was demonstrated (L. Somer, Denmark P 9) and field clonal tests were established. In addition, it was shown that buds from adult trees could be successfully cryopreserved and plants recovered after thawing; this means that selected lines of ash can be preserved for long periods to allow for field test to develop and followed by reactivation of the best lines after testing (Schoenweiss *et al*., 2005b). Throughout the project over 72 clones of selected ash trees were established *in vitro* for several subcultures, however there still remains some difficulties in micropropagating all of the selected genotypes. It was shown that the cytokinin thidiazuron increased the establishment and micropropagation rates in several clones. Rooting of micropropagated shoots could occur spontaneously in shoots cultured with charcoal and was induced by auxins and by reducing the level of cytokinin during plant production in commercial labs.

Somatic embryogenesis in *F. excelsior* was demonstrated for the first time from immature embryos and 40-70% of them could be converted to viable plants (M.

Capuana, Florence P 11). Application of *Glomus* to micropropagated plants resulted in increased shoot growth and plant quality through the mycorrhizal association. Spermidine content was found as an indicator of rooting in cuttings and five biochemical markers in the buds (putrescine, mannitol, trehalose, sucrose and raffinose) showed significant differences between late and early flushing provenance material (L. Jouve, Luxembourg P 8). Flowering and seed formation was observed in grafted ash trees in pots and in six year old seedlings (K. Russell UK, P 13). Restricting water supply to seedlings resulted in flowering in a quarter of the trees. For grafted plants, flowering occurred in about 50% of all clones tested but not every ramet of each clone produced flowers. In the collection of over 60 grafted selected plants which flowered, most were hermaphrodite.

An end-user panel of all users and stakeholders for ash material was consulted in relation to dissemination models for technical information and materials. It consisted of private and public forest owners, sawmillers, nurserymen and researchers. Focus group meetings were also held to determine the reaction to improved genetic resources of ash and the ‘chain of influence’ which would affect the uptake of new material and the developments in genetic improvement. The main requirement specified was that improved material should be demonstrated widely in field trails and followed up with technical data and the official recognition by research authorities (A. Ottitsch, Joensuu P 15).

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