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Characterisation and conservation of veteran hardwood trees

Key external stakeholders:

Landowners, foresters, Department of Agriculture Fisheries and Food, consultants, land use advisors, nurseries.

Practical implications for stakeholders:

- Veteran trees of oak, ash and sycamore may be viable for many decades and centuries when managed well. They have unique ecological value by providing habitats for plants, birds, mammals, fungi and many specialised invertebrates and microorganisms. They also produce seeds.
- Individual veteran trees of oak and ash have been successfully propagated vegetatively by grafting to extend the life of individual trees; they were returned to the original owners for planting out as shown in the figure above (veteran ash in the background with a young grafted copy in the foreground). Grafted copies of notable veteran trees such as the 'king oak' of Charleville estimated to be 450 years old were established in a living collection at JFK Arboretum Wexford.
- Seeds may be safely collected from veteran trees and used by nurseries because they were shown to be representative of the general gene pool for oak and ash in Ireland and they are capable of producing viable seed and progeny.

Main results:

- Veteran trees are valuable as hosts which provide unique habitats and other ecological functions in Irish landscapes. Individual veterans may be propagated by grafting. Prolonging the lifespan of individual veteran trees is best ensured by safeguarding the root zone around each tree.
- Genetic analyses revealed evidence that veteran trees of oak and ash were no less genetically diverse / unique when compared to the general populations of these species.
- Veteran trees of oak and ash are reproductively active, contributing viable pollen, ovules and seed.
- Progeny from veteran trees is viable and fit in Irish landscapes and seeds from veteran trees may be used by nurseries for establishing plantations.

Opportunity / Benefit:

- The provision of shoot material by scores of owners of veteran trees, for their propagation indicates a great interest among landowners for the preservation and prolongation of the life of individual veteran trees on their lands.
- Best practice for prolonging the life of veteran trees is to provide an exclusion zone around trees to avoid ground compaction.
- Veteran trees provide viable seed and these seeds should be collected from all sectors of the tree to capture the greatest genetic diversity in the progeny for use by forest nurseries.

Collaborating Institutions:

TCD; National Botanic Gardens, Glasnevin (NBG); John F Kennedy Arboretum Wexford (JFK)

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Teagasc project team: Dr. Gerry C. Douglas (Teagasc, project leader) Dr. Evelyn Gallagher (Walsh Fellow)

External collaborators: Dr. Trevor Hodkinson (TCD); Dr. Colin Kelleher (TCD); Mr. G. Michaels (JFK)

1. Project background:

Many farms have veteran trees in pasture or cultivated fields. Typically they are large in girth (>5m oak, >3-4m ash and >3.5m sycamore) with hollowing trunks, cavities and some dead branches. They are living relics of former populations of trees and currently provide a unique ecological habitat. Concerning veteran trees, David Attenborough notes *'there is little else on earth that plays host to such a rich community of life within a single living organism'.* The decaying heartwood is a specialised habitat for a large number of threatened invertebrates and the fruiting bodies of fungi provide an essential breeding ground for insects: fungal gnats, flies and beetles. Decaying wood in branches and hollowing tree trunks is normal and is not a sign of ill health or imminent death. Specialised fungi digest and recycle the deadwood in the centre of trees (heartwood) and rarely colonise the outer rings of living sapwood. The pattern of tree growth is an annual increase in girth with the deposition of new wood on the outer rims of the trunks and branches; therefore, as long as the fungal advance is less than the new growth of wood the tree will remain alive. Notable veteran trees in Ireland have been catalogued: see the Irish Heritage Tree Database at http://www.treecouncil.ie/

Specific genes conferring longevity of life have been identified in other organisms and may be important in veteran trees. The reproductive capacity of veteran trees is not well studied but their capacity to produce viable seed which gives rise to the next generation of trees is an important source of genetic diversity in forests and landscapes. Molecular markers can be used to trace seed dispersal from veteran trees by analyzing putative progeny among surrounding young trees in the same location. Markers in the chloroplast DNA are maternally inherited and are passed exclusively via seeds in ash and oak and are useful in identifying progeny. On the other hand, markers in nuclear DNA are dispersed via pollen and ovules. By using a combination of several nuclear markers and chloroplast markers it is possible to determine the dispersal patterns and gene flow characteristics of veteran trees.

The life span of individual trees such as notable veteran trees of oak, ash, and sycamore may be prolonged by means of their vegetative propagation and was tested in the project. Furthermore the genetic characterization of veteran trees in relation to their capacity to act as male and female parents for future generations of trees is poorly understood and was studied. The project was support by the Department of Agriculture programme 'Conservation of Genetic Resources for Food and Agriculture' and by a Walsh Fellowship for Dr. E. Gallagher.

2. Questions addressed by the project:

- Can veteran trees be propagated vegetatively by grafting to extend the lives of individual trees?
- Are veteran trees unique genetically?
- Do veteran trees pass on their genes and contribute to future generations of trees by producing viable seed?
- Are progeny derived from veteran trees viable in the landscape?

3. The experimental studies:

Vegetative propagation of veteran trees by grafting was undertaken over three Spring seasons, January–March. Scionwood (dormant shoots) was collected, or mailed to us from tree owners using selected tree sources listed in Irish Heritage Tree Database at http://www.treecouncil.ie/ At least 10 grafts were made per genotype. Seedlings of each species (2 year old) were used as rootstocks. A wedge graft was made in all cases by decapitating the rootstock and making an incision in the middle of the rootstock. Shoots with an apical bud were prepared by removing a sliver of stem tissue from two sides at the base followed by immediate insertion into the incision in the rootstock to align the cambial areas. Graft unions were tied with elastic bands and then painted with molten paraffin wax. After grafting, all buds that developed from the rootstock were removed and viability of veteran grafts was recorded after 6 months. For oak grafts we compared the effects of bare rooted rootstocks with container grown rootstocks. We also compared the viability of grafts using rootstocks which were primed into growth with graft viability using dormant rootstocks using four genotypes from 130 yr old trees.

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For diversity assessment in oak, the study trees at Ballytobin estate Kilkenny were separated into four classes: seedlings, saplings, mature trees and veterans and three microsatellite markers were used to assess genetic differentiation by analysis of molecular variance (AMOVA). For analysis of progeny from veteran oaks, DNA was extracted from 70 trees including 25 mature and veteran oak trees and from 40 candidate offspring trees on the same lands. Five trees, including one veteran, were sampled outside the stand as they were considered large and close enough to be possible parent trees. All trees in the site were mapped and parentage was analysed using a likelihood assignment approach with the Cervus software programme.

For ash, DNA was extracted from 74 trees (including veterans) at Stradbally, Laois. Parentage analysis was undertaken using two approaches: a) paternity analyses of seed progeny from known mothers, (up to 20 seeds collected from each tree) and b) analyses of putative progeny trees by a parent-pair analysis with unknown sexes of the parent trees. For the former, the paternity analysis was on progeny from four veteran mothers and 13 candidate veteran father trees. For the latter, the analysis was on molecular data sets of 24 young trees, with 12 candidate veteran mother trees and 13 candidate veteran fathers. As with oaks all ash trees in the site were mapped and parentage was analysed using a likelihood assignment approach with the Cervus software programme.

4. Main results:

Over the course of the project we made 858 grafts of oak (87 genotypes), 180 of ash (18 genotypes) and 223 of sycamore (22 genotypes). Overall, genotype viability for oak was 63%; ash 100% and sycamore 90% giving viable plants of 209 oaks, 163 of ash and 95 of sycamore, some of which were established as a conservation collection at JFK Arboretum Wexford and others were returned to the owners and planted out in their fields. The figure on page 1 shows a veteran ash tree in the background with a viable grafted copy in the foreground, protected against sheep in Co. Offaly. In oak experiments, dormant rootstocks were superior to rootstocks which were growing; bare rooted oak rootstocks gave 39% viable grafts whereas containerized rootstocks gave only 8% viable grafts.

The microsatellite markers used on ash and oak were highly variable and useful to determine estimates of genetic diversity. Lower levels of genetic diversity were found in veteran oak and ash compared to the much larger general populations of each of these species. Veterans may be remnants of earlier populations and may be genetically less rich than younger generations which may have acquired their diversity from long distance wind pollination and from introduced populations of trees from abroad or diverse regions in Ireland.

Genetic diversity and parentage of mature and veteran oak trees (*Quercus robur*) at an estate in Kilkenny were assessed. The markers used were sufficiently variable with 9-14 alleles / locus and all samples were heterozygous. Diversity analysis showed that only 2% of the variation was partitioned among different age classes of the analysed trees, indicating there was a lack of genetic differentiation between age classes and supporting the view that the diversity of the gene pool was maintained in the regenerating younger population of trees, over time.

Overall, veteran oaks were found to be reproductively successful giving rise to viable progeny. Parent-pair analysis revealed that out of 24 trees that were potential parent trees, 15 of them were assigned as a parent to at least one offspring tree, and out of these, five different veteran trees were assigned as parents for a total of four offspring trees. In addition, one veteran tree outside the main study stand was identified as the parent of two offspring found within the strand. Using offspring for which two parents could be assigned, we estimated that the distances among parent trees ranged from 22- 150m with an average pollination distance of 47.8m and seven out of 12 parent pairs were within 50m of each other.

The three nuclear microsatellite molecular markers used on ash were highly variable with 19 - 29 alleles per locus. This allowed estimations of paternity of 37 ash seeds collected from four veteran mother trees. There were 13 candidate father trees in the analysis. Resolving power was not sufficient to assign the father trees for each progeny from each of the veteran mothers. However, paternity was assigned with statistical confidence to four seeds taken from one veteran mother. In this case at least three other veteran trees were male pollen donors while the fourth was a mature ash. Some evidence was found of self pollination, i.e. where the mother tree, on which seeds were collected, was also the most likely pollen donor.

The parentage of 17 young ash trees in woodland surrounding the veteran trees was determined by parent-pair analysis. Of these 17 trees, 12 were assigned parentage, leaving five, which were either the offspring of

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trees outside the stand or for which genotypic data was insufficient to assign parentage. Of the 12 trees which were assigned parentage with statistical confidence, only one was assigned as both first and second parent, suggesting a lower level of selfing among the woodland trees than the result of the seeds (above) would suggest. The average pollination distance was found to be 172m for ash trees.

5. Opportunity/Benefit:

- It is feasible to extend the life span of individual veteran trees of oak, ash and sycamore by grafting shoots from them onto young rootstocks.
- The value of veteran trees in Irish landscapes is that they are living ecological systems which are host to rare and threatened organisms which live within and on them.
- Genetic results have shown that veteran oak and ash trees are representative of the general gene pool of each species in Ireland and do not represent either a unique gene pool by virtue of their character of age-longevity.
- Veteran trees provide the valuable ecological function of contributing seeds and pollen to future generations of trees thereby contributing to the maintenance of genetic diversity of the Irish gene pool for each species
- We demonstrated the presence of viable progeny derived from veteran trees, among saplings and young trees for both oak and ash. This indicates the ecological fitness of progeny from old trees. Therefore they should not be ignored as sources of seeds by seed collectors and nurseries for use in afforestation and amenity planting.
- Landowners can prolong the life of individual veteran trees by safeguarding their root systems from compaction and cultivation. This will help to maintain their viability for many further decades, even centuries. Typically, veteran trees lose large branches as part of the natural process in which the crown volume reduces gradually overtime. Larger pieces of deadwood may be used to protect trees and roots from stock. Most biologically active roots are in the top foot or two of soil and they spread outwards to a distance that is twice the radius of a tree's canopy. An exclusion zone around veteran trees by a living or wire fence offers best protection to exclude and / or manage access by animals / machines. This zone should extend to at least five meters beyond the limit of the tree's canopy.

6. Dissemination:

Main publications:

Gallagher E. (2011) Conservation and molecular characterisation of Irish veteran oak (*Quercus* spp.) and ash (*Fraxinus excelsior* L.) Ph D. Thesis University of Dublin Trinity College, Ireland.

Gallagher E, Douglas GC, Kelly DK, Barth S, Kelleher CT, Hodkinson TR (2012) Old age sex: a parentage study on different age cohorts in a native veteran pedunculate oak (*Quercus robur* L.) woodland using nuclear microsatellite markers. *Biology and Environment*. (accepted and in press).

Douglas GC (2006) Final report to Department of Food and Agriculture, 'Conservation of veteran Irish Hardwood trees'

Popular publications:

E.Gallagher, T.R.Hodkinson, D.L.Kelly, G.C.Douglas (2005) Conservation and molecular characterisation of veteran oak (*Quercus spp*.) and Ash (*Fraxinus* L.) European Vegetation Conference. 20th-23rd June 2005. Conference programme page 48

Gallagher, E., Hodkinson, T., Douglas, G. and Kelly, D. (2006) Conservation and molecular characterisation of veteran oak (*Quercus* spp.) and Ash (*Fraxinus* L.) Irish Plant Scientists' Association Meeting IPSAM. 10th-12th April 2006, Galway. Conference programme page 39

E.Gallagher, T.R.Hodkinson, D.L.Kelly, G.C.Douglas (2005) **Conservation and Molecular characterisation of veteran Oak (Quercus spp.) and Ash (Fraxinus excelsior).** In: "Plants 2010" The Global Partnership for Plant Conservation. National Botanic Gardens in Dublin 23rd -25th October 2005. http://www.botanicgardens.ie/gspc/gppc/posters/oakash.jpg

G.C.Douglas, E.Gallagher, D.L.Kelly, T.R.Hodkinson (2005) **Conservation of veteran trees by grafting and cuttings** In: "Plants 2010" The Global Partnership for Plant Conservation. National Botanic Gardens in Dublin 23rd -25th October 2005. http://www.botanicgardens.ie/gspc/gppc/posters/veteran.jpg

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