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The Effects of Intraspecific, interSpecific, and Intergeneric Grafting on the Growth and Development of Fraxinus Excelsior Scions

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Abstract

Fraxinus excelsior (common ash) is a hardwood tree, native to Ireland which has demonstrated adaptability to growing in a wide range of sites. In 2013, there was a total of over 20,000 hectares recorded under ash in Ireland. From an economic, ecological and carbon aspect ash is also a very important tree species. Since the arrival of *Chalara* disease, which has the potential to prove fatal to c.97% of the ash population there is an urgent need to consider how to preserve the remaining resistant trees and propagate new resistant lines. Grafting is the suggested method which has the capacity to produce a tree in a breeding programme which can be field planted within a year, once suitable rootstocks can be determined and produced. This thesis examined the potential for grafting two *Fraxinus excelsior* clones M72 and 98, chosen at random onto *F. excelsior, F. paxiana, F. chinensis, F. japonica, F. platypoda, Syringa vulgaris,* and *Ligustrum ovalifolium* rootstocks to confirm their suitability for large scale vegetative propagation.

It was found that when Clone M72 and Clone 98 were grafted onto *Fraxinus excelsior* rootstocks the survival was 100% and 97% respectively, while the non-grafted Control returned a plant survival rate of 93%. When Clone M72 was grafted onto *Fraxinus chinensis* and *Fraxinus paxiana* rootstocks the resultant graft survival was 68% and 40% respectively. When *Fraxinus platypoda* and *Fraxinus japonica* interstocks were used for grafting Clone 98 survival was 87% and 60% respectively. When *Ligustrum* and *Syringa* rootstocks were used to propagate Clone M72 survival was 37% and 33% respectively. When *Ligustrum* and *Syringa* were used as rootstocks for Clone 98 the result was 30% and 40% respectively. Propagation by budding was not successful.

Vegetative growth of Clone 98 grafted onto *Fraxinus excelsior* rootstocks exceeded the seedling produced plants by 6%, whereas grafted plants of Clone M72 were 28% lower than the control. When Clone M72 was grafted onto *Fraxinus chinensis* rootstocks the reduction was 38%. When it was grafted onto *Fraxinus paxiana* rootstocks the reduction was approximately 79%. When *Fraxinus platypoda* and *Fraxinus japonica* interstocks were used with Clone 98, growth was reduced by 45% and 65% respectively. When rootstocks of *Ligustrum* and *Syringa* were used with Clone M72 and Clone 98 the reduction in growth was between 41% and 52% respectively.

There was a wide variance in bud flushing. It ranged from three to fourty one days over all the treatments.

The mean number of shoots produced per graft was 3.1 and 2.9 respectively for Clone M72 grafted onto *Syringa* and *Ligustrum* and 1.9 and 2.7 respectively for Clone 98 grafted onto *Syringa* and *Ligustrum.* This compared with 3.7 for the Control.

The least number of shoots 1.3 to 1.5 was recorded with Clone M72 on rootstocks of *Fraxinus paxiana* and *chinensis* while interstocks *Fraxinus platypoda* and *Fraxinus japonica* used with Clone 98 resulted in 1.8 and 1.3 shoots respectively.

Flowering percentages were low at 0.01% and 0.02% when recorded over two consecutive years.

It was possible to establish viable grafts on both Asiatic species and related genera. In the case of related genera, their potential to flower and produce seeds would significantly accelerate the establishment of disease free orchards.

Declaration

I certify that this thesis which I now submit for examination for the award of MPhil is entirely my own work with the exception of statistics which have been done by Dr Jim Grant (Teagasc statistician) and has not been taken from the work of others, save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for graduate study by research of Technological University Dublin and has not been submitted in whole or in part for another award in any other third level institution.

The work reported on in this thesis conforms to the principles and requirements of TUD's guidelines for ethics in research.

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Signature _____ Date _____

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Abbreviations

COST	European Co-operation in Science and Technology
DAFM	Department of Agriculture Food and Marine
IGAHM	Irish Guild of Ash Hurley Makers
JFK	John Fitzgerald Kennedy Arboretum
SAS	Statistical Analysis System
NFI	National Forestry Inventory

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Chapter 1: Literature Review

1.1 Study background

The genus Fraxinus belongs to the Oleaceae family and comprises about 65 species (Brickell, 1996), mostly deciduous and rarely evergreen trees (*F. uhdei,* evergreen) found throughout Europe, Asia, and North America. *Fraxinus excelsior* (common ash), is indigenous to Ireland and is also cultivated as an ornamental tree in gardens and large parks (Kew Science, 2017). Ash grows best in rich fertile well drained soil, neutral to alkaline (Horgan *et al.*,2004) and tolerates a wide range of growing conditions, such as urban pollution and exposed sites. Ash is extremely important as it is a productive species in farm forestry with over 20,000 hectares of plantations in Ireland (Mc Cracken *et al.,* 2017). Ash is also a good choice for a specimen tree or in a woodland setting with several cultivars of *Fraxinus excelsior* such as '*jaspidea* and *pendula'* that are worth growing. To date, common ash has been generally pest and disease free in Ireland and Europe however, with increasing world trade in plant materials problems are starting to emerge, such as *Chalara* (ash dieback) and Emerald Ash Borer.

1.2 Importance of ash in Ireland

Common ash (*Fraxinus excelsior*) is the second most common tree species found in hedgerows after hawthorn (*Crataegus monogyna*) and it is also very important; in forests, woodlands, and urban situations (Hendry, 2012). Ash has a broad ranging adaptability to its habitats, in that it will thrive as individual trees in an urban environment, as forest cover, along riverbanks or on arid mountainous slopes (Pautasso *et al*., 2013). It represents 10% of broadleaf afforestation in Ireland (Mc Cracken *et al.,* 2017) with over 20,000 hectares (NFI, 2012).

One of the greatest influences ash has made in Ireland is in the sport of hurling. The hurley stick is called a hurl, hurley or *caman*; and it has timber qualities of strength, shock resistance, with flexibility and 360,000 are needed annually (Mc Cracken *et al.,* 2017). In 2012, data showed that 76% of hurley ash timber was imported (McCracken *et al.,* 2017). It had been hoped that due to plantings of ash over the past 25 years, the demand for hurley timber could have been satisfied from domestic supplies, however, with the arrival of *Chalara* this is unlikely to happen. The Irish guild of ash hurley makers (IGAHM, 2011) stress the need to maintain this cottage industry which supports 400 jobs in the hurley-making industry in Ireland (Teagasc, 2016).

In Ireland, there is no data quantifying the impending loss of ash trees. The only data pertains to the cost to Department of Agriculture Food and Marine (DAFM) for the removal of trees with confirmed outbreaks of *Chalara.* To date 733 hectares of infected plantations (two million ash trees) have been removed and replanted with other species at a cost of €2.6 million (McCracken *et al.,* 2017). In the UK there are142,000 hectares of ash yielding £20 million commercial timber value per annum. This together with the environmental value estimated at £150 million per annum equates to actual value of €1340 per hectare (Cotterill, 2014). On this basis this would value the ash plantations in Ireland at €26.8 million.

1.3 History of C*halara*

The organism causing *Chalara* disease was named *Chalara fraxinea* (asexual stage) and the sexual stage was named *Hymenoscyphus fraxineus.* In common reporting, *Chalara* is referred to as ash dieback. *Chalara* is thought to have originated in Asia, including Japan (Zhao *et al.,* 2013), causing widespread decimation of ash in Europe and is now widespread in Ireland (McCracken *et al.,* 2017). It was first identified in Poland in

1992 (Forestry Commission, 2013) and is present in many European countries including Ireland. *Chalara fraxinea* was first discovered in Ireland in October 2012 on imported plants and the disease was later confirmed by Department of Agriculture, Food and Marine (DAFM, 2018). Once confirmed, it instigated legislation (SI No. 431 of 2012) in conjunction with Northern Ireland restricting the importation and movement of plant material, seed and wood of ash was invoked. A national survey of all recent plantations with imported plants was undertaken (DAFM (2018). The disease was identified on trees in forests, garden centres, farms, roadside plantings, and plant nurseries and such plants were eradicated. This involved the removal of all infected plants and debris on site and its burial. Where infected trees were found in wild hedgerows, they were cut down, along with all other ash trees within 250 metre radius. Confirmed *Chalara* findings have been made in all counties (McCracken *et al.*, 2017).

1.4 The present situation regarding *Chalara* infection.

Since the disease was discovered in Europe, it has progressed across the UK and now to Ireland. No ash trees have been found to be totally resistant to *Chalara.* However different levels of tolerance have been exhibited. Trees showing 10% crown damage comprise 1-5% of the population, while trees with 25% crown damage comprise 10%. In the former category, tree growth is generally unaffected since they are without stem infections and are regarded as being resistant to *Chalara*. Trees that have more than 25% of their crown affected are unlikely to survive (Enderle *et al.*, 2014).

- Genotype, site conditions, and the local environment have an influence on a tree's level of growth capacity, which in turn can influence *Chalara* tolerance / susceptibility. Alsop (2014) reported that trees in prime condition which had more natural resilience to *Chalara.*

- The death of the tree will not occur immediately, as it depends on the level of disease pressure (Alsop, 2014).
- Disease severity can vary from year to year due to seasonal differences and variations between different countries, regions and sites (Alsop, 2014).
- Trees in unfavourable conditions (such as wet sites) tend to be more susceptible (Alsop, 2014).
- Disease tolerance can only be reliably assessed over a few years in areas of disease infection (Alsop, 2014).
- Ash trees of all age classes, from saplings, to semi mature trees are affected.
- Once an infection has been discovered, it has progressed to adjoining areas (Alsop, 2014) The distribution of the disease on a county by county basis is given in (Image1.1).

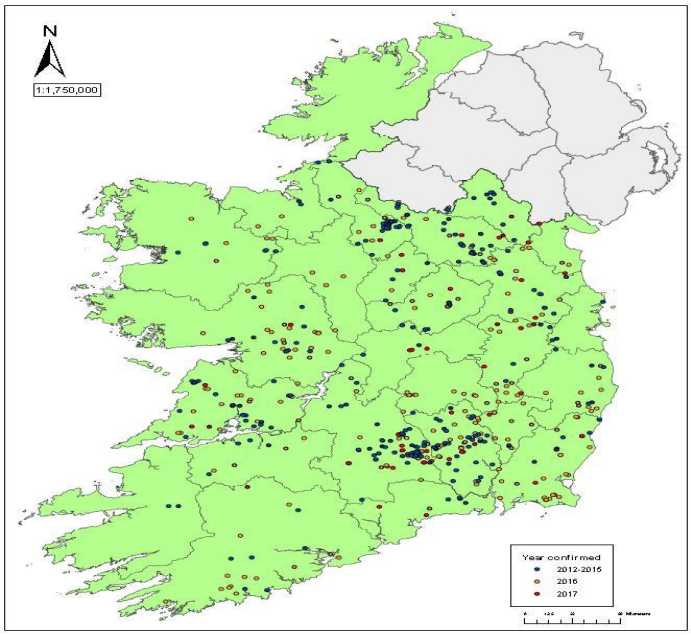

Image 1.1. Distribution map of confirmed findings of Ash Dieback throughout Ireland (as of 31 July 2017).

Source DAFM - Ash Dieback (*Chalara*). Available on the internet at http://www.agriculture.gov.ie/forestservice/treediseases/ashdiebackchalara/.

(Accessed 02/09/2018).

The locations of commercial nurseries and garden centres are not depicted.

Chalara incidences are now reported on the basis of a 10 km x 10 km grid as shown in Image 1.2.

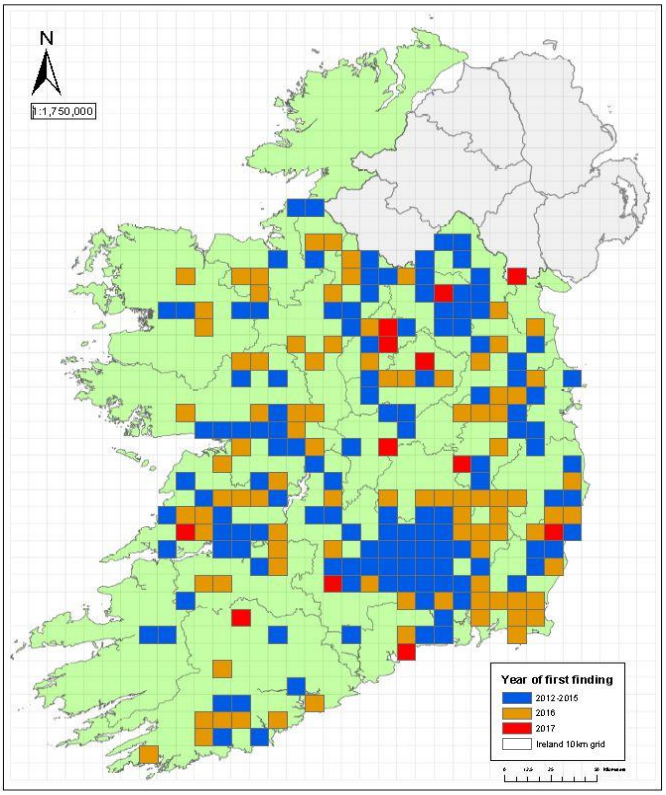

Image 1.2. Distribution map of confirmed findings of *Chalara* in Ireland as of 31st July 2017.

Source DAFM - Ash Dieback (*Chalara*). Available on the internet at http://www.agriculture.gov.ie/forestservice/treediseases/ashdiebackchalara/.

(Accessed 02/09/2018).

The locations of horticultural nurseries and garden Centre's are not depicted**.**

1.5 *Chalara fraxinae* disease:-life cycle and symptoms

The pathogen isolated was initially named *Chalara fraxinea,* in the vegetative stage. Later, the sexual stage (telemorph) was found and named *Hymenoschyphus pseudoalbidus* (Kirisits, 2014). *H. pseudoalbidus*, is closely related to the non-pathogen, *H. albidus*, which is common in Ireland. It has subsequently been renamed as

Hymenoscyphus fraxineus (Baral *et al.,* 2014). *Chalara fraxinea (telemorph Hymenoschyphus fraxineus)* is a virulent fungal pathogen of ash, particularly common in ash (*Fraxinus excelsior). Hymenoschyphus fraxineus* reproduces sexually on ash petioles and on the leaf rachises forming apothecia, which in turn release ascospores infecting healthy leaves from June to October. Disease incubation requires up to one year, but the rachises can remain infectious and release spores from their apothecia for up to five years. It has been reported that spores may also infect the shoot/root collar via the roots and or stem lenticels (Kirisits, 2014). Once in the stem the fungus can develop there over the autumn and winter period. In spring, leaves emerging from above the point of infection wilt and die. The symptoms of the disease have been described by Andersson *et al.,* (2010) and Bakys *et al.,* (2009).

- Dieback of shoots, side branches on main stem and crown loss.
- Wilting and premature foliage loss resulting in black or brown leaves.
- Wounds on the branches, shoots and stems of trees.
- Necrotic lesions starting on the rachises and spreading into the shoots.
- A discolouration of the wood.
- Elongated angular stem lesions often diamond shaped on the shoots.

Dieback of many shoots and loss of foliage over several years generally proves fatal. The disease completes its cycle on the rachis and foliage of *Fraxinus sp.* (Gross *et al.,* 2013). Wind can also cause new infections, through the spread of spores (Cotterill, 2014). The spread of *Chalara* has been modelled by Cambridge University (Downing, 2012). They concluded that between the years 2008-2011 there was a at least 100 days within which weather conditions such as wind direction, rainfall and humidity could have carried the spores across from Europe to southeast England (Downing, 2012). Disease classification can be undertaken by assessing the health status of the tree

crowns. Enderle *et al.* (2014) have developed a classification system based on the percentage of the crown defoliation due to dieback, class 0 has no visible symptoms, class 1 has 1-10% crown loss, class 2 has 11-25% crown loss, class 3 has 26-60% crown loss, and class 4 has 61-99% crown loss; any tree with 100% is dead. In addition Enderle *et al.* (2014), also recorded the amount of epicormic growth within the tree crown the presence of which is indicitave of *Chalara* susceptibility. Examples of disease symptoms are illustrated in Image 1.3.

A **B** **C** **D**

Image 1.3. Disease symptoms of ash dieback Cleary *et al.,* (2017). Symptoms of ash dieback: (a) shoot, branch and stem dieback; (b) bark canker; (c) epicormic shoot necrosis (d) leaf and shoot wilting.

Source: European Ash- Consequences and Guidelines for Sustainable management, Cleary *et al.,* (2017) Available online at https://www.cost.eu/publications/ (Accessed 22/05/2019).

1.6 Disease situation in Europe, UK and Ireland

Chalara is a devastating disease progressing through Europe, with terminal effects for *Fraxinus excelsior, Fraxinus angustifolia* and *Fraxinus ornus* (Alsop, 2014). In Austria, it is most serious because of the importance of the species. The management strategy is to remove stands which are seriously infected but to maintain individual trees which are showing tolerance (Heinze *et al.,* 2017). In Lithuania the disease was discovered in 1995 and 10% of their trees die every year. They undertake sanitary felling and re-assess the sites for re-sprouting and regeneration with a view to propagation of *Chalara* tolerant trees (Pliúra *et al.,* 2017). In Germany *Fraxinus excelsior* accounts for 2% of their forests and their aim is to develop tolerant germplasm (seeds) from tolerant parent trees to establish seed producing orchards. Chemical control of the fungus in the nursery stage shows some promise and may have some value for ornamental ash cultivars used, however it is impractical on a forestry scale (Enderle *et al.,* 2017). Denmark has lost most of its ash stands, with some exception of trees which remain healthy and show disease tolerance. According to Kjaer *et al* (2017) this will secure breeding material to produce new stock adapted to the pathogen. Sweden is examining the balance between host trees and pathogens (Cleary *et al.,* 2017) while also examining the impact of ash dieback on veteran and pollarded trees. They are also assessing veteran trees in grazed, un-grazed and open ground to see if there is a pattern with the disease spread. To date they reported that no tree greater than 140 centimetres in diameter has been affected by the disease (Cleary *et al.,* 2017). In Norway advancement of the disease is being monitored and reports suggest that it is spreading at a rate of 30-50 kilometres per annum using spore sampling to ascertain the speed of the spread (Børja *et al*., 2017). In Belgium the same monitoring techniques are being used as in Norway (Chandelier *et al.,* 2017). In the UK, while initial attempts were made to eradicate infected trees, the current policy is to monitor outbreaks, predict

the spread and genome sequence, to find resistant or tolerant trees. A government funded project has planted 250,000 seedlings over 50 hectares which is comprised of 15 provenances, 10 UK, 2 Irish and 3 European (Clark and Webber, 2017). Genetic markers are also being investigated to determine the level of susceptibility (Sollars *et al.*, 2016). Recently improved genetic markers which will hopefully assist in specifying reduced susceptibility to *Chalara* have been developed (Sollars *et al.*, 2016).

1.7 What are the options to save ash?

The long-term solution is to identify individual trees which are tolerant or resistant to the disease and to propagate from them. No trees have shown complete resistance (Boshier and Buggs, 2015). However it has been shown that resistance is stable within selected clonal lines over time and over several sites (Cleary *et al*., 2017). Studies have shown where disease incidence has been high over the last 20 years such as in Denmark, Lithuania and Poland that resistance is genetically determined (Kjaer *et al.*, 2017). The scientific community in the UK suggested that one option is to plant millions of trees so that 1-2% of the new population will show resistance or tolerance (Kjaer *et al*., 2017). This is based on the assumption that 1% of the estimated 126 million of the ash trees in woodlands plus an additional 27-60 million in non-woodland environments will show *Chalara* tolerance (Clark and Webber, 2017). In addition careful monitoring of the wild population of trees such as those with low level infections to identify tolerant trees is underway in the UK. Other approaches include genetic modification of *Fraxinus excelsior* and intensive selection breeding programmes (Vidal 2015; McEwan, 2016). However genetic would most likely prove to be controversial from the public perspective. (Leake and Spickernell, 2013). Molecular studies could form part of the solution, which will identify genes that are present in

resistant trees, which in turn will assist in initiating a breeding programme and to select resistant trees without exposure to the fungus (Sollars *et al.,* 2016). The most straight forward way to save the ash is to identify resistant trees, propagate them vegetatively and ultimately obtain disease resistant seeds.

Another option is to cross pollinate native trees with resistant Asian species of *Fraxinus*. (Nielsan *et al.,* 2017; Vidal, 2015). However, there are varying susceptibility among *Fraxinus* species, although this information is still somewhat disjointed. *Fraxinus angustifolia*, from Europe and *F. nigra* from North America are heavily affected, to the same degree as *F. excelsior*, whereas *F. pennsylvanica* and *F. americana* from North America, and *F. mandshurica* from Asia, are less susceptible (Kirisits *et al*., 2009; Kräutler & Kirisits, 2012). It is also reported that *F. chinensis* and *F.mandschurica* in their natural habitats show resistance to *Chalara* (Gross and Queloz, 2015).

There are several Asiatic ash species which have shown resistance to *Chalara*. Asiatic species are occasionally found in Ireland as specimen trees. Their origins are specified in Table 1.1.

Table 1.1 Asiatic Ash species origins

Species	Origin in Asia
F. paxiana	China
F. chinensis	China and Korea
F. japonica	Japan
F. platypoda	China

1.8 Grafting

Grafting is defined as the natural or deliberate fusion of plant parts so that vascular continuity is established between them, with the resulting genetically composite

organism functioning as a single plant (Hartmann *et al.*, 2002). It is achieved by connecting two plant parts, the 'scion' which is the chosen material, with the 'rootpiece', which is termed 'rootstock' or sometimes 'stock'. Grafting is commonly used to unite parts of two plants with specific desired traits to produce a new plant. Fruit and nut trees have been grafted, as it can be quite difficult to propagate them efficiently by using cuttings (Propagation Methods and Rootstocks for Fruit and Nut Trees, 2010). Grafted and budded plants can be tailored to deliver desired forms of trees, higher yielding fruit, superior form and adaptation to soils, and ecological conditions (Mcdonald, 1986). With greater emphasis on reducing pesticide use, EU regulations, and environmental laws, disease tolerant rootstocks will play a greater role in plant propagation.

Grafting is an expensive method of propagation. Grafted plants have major advantages in the production of superior and better adapted plants as described below. Many reasons for grafting are enunciated below (Cornell Horticulture, 2014).

1.9 Reasons for grafting

- To propagate plants which are difficult to propagate from cuttings.
- To propagate plants that are sexually sterile and produce few or no seeds.
- To avoid juvenility, which can last several years in fruit trees, and to speed up the onset of flowering.
- Plants produced by seed are not always true to type; however a grafted scion is a true copy.
- Multiple cultivars can be grafted onto one tree such as in 'top-working'. If self-incompatibility is a problem in fruit species, then a pollinator can be grafted onto a single tree to ensure cross pollination for fruit setting.

- To repair an established tree which has been damaged.
- To control the size of the tree and achieve the chosen traits whether it is dwarfing, or invigoration of the scion being grafted, and to promote earlier flowering and fruiting.
- To achieve resistance to pests and diseases. Rootstocks have been identified which show resistance to bacterial diseases such as fireblight on apples; fungal diseases like collar rot on apple which is caused by *Phytophthora;* nematodes on peach and walnut (*Meloidogyne*); and insect pests like *Phylloxera* on grapes (MacDonald, 2014). Selection of rootstocks for resistance to the woolly apple aphid (*Eriosoma lanigerum*) facilitated apple production in Australia and New Zealand where there was a serious outbreak. Re-plant disease in old apple orchards can be reduced by selecting tolerant or resistant rootstocks (Leinfelder and Merwin, 2006).
- Grafting techniques are often used as a tool whereby ornamental trees are joined to make archways or living furniture.
- To accelerate apple tree breeding by reducing the juvenility period (Fischer, 1994).
- It is also claimed that using genetically different rootstocks for perennial crops have the capability to influence traits above and below ground of the grafted plant (Mudge *et al.*, 2009).

1.10 Grafting principles

In dicotyledons, there is a continuous ring of cambium tissue under the bark of the stem. This arrangement is beneficial in bringing the tissues of the rootstock and scion into contact in the grafts, whereas in monocotyledons, this does not occur. The cambium is

a layer of meristematic tissue located between the epidermis and the bark which undergoes cell division uniting the scion and the rootstock. The meristematic cambium produces undifferentiated callus initially and subsequently some cells in the callus differentiate into xylem and phloem vessels, which conduct nutrients and water between the stem and roots. It is critical to ensure the correct orientation of the scion and rootstock occurs, that the scion is dormant and that the rootstock is just about to commence growing (Hartmann *et al.*, 2002). Graft timing must be considered as it varies from species to species (MacDonald, 2014). Generally for plants in the temperate zones grafting is generally carried out in early spring or late summer (MacDonald,2014). Ash is grafted during the dormant season, generally at the end of winter. Often the timing is decided by the successes of the various species (MacDonald, 2014).

There are also genetic limits of grafting, for example:

- It is always possible to graft within clones of the same species (*Fraxinus excelsior* onto *Fraxinus excelsior*)
- It is generally possible to graft various species within a genus (*Fraxinus excelsior* onto *Fraxinus chinensis*).
- Grafting between different plant genera within a plant family is possible. For instance it is possible to graft *Chamaecyparis nootkatensis* and *Thuja orientalis*, both Cupressaceae; *Pyrus communis* and *Cydonia oblonga* both Rosaceae; *Citrus sinensis* and *Poncirus trifoliate* both Rutaceae and *Solanum lycopersicum* and *Solanum tuberosum* in the family Solanaceae (Hartmann an*et al*., 2002).

Grafting between different families of plants is considered impossible (Hartmann *et al*., 2002).

As much cambium contact between the two is required to maxamise success. Ideally both cambial layers should be perfectly aligned and in perfect contact to achieve a strong and successful union (Hartmann *et al*., 2002). Where rootstock and the scion have the same diameters, the cambial layers are easily placed in contact with one another (Image 1.4). Normally, the situation is that the rootstock will have a greater diameter than the scion, so in this instance the cambium from one side of the scion is placed in contact with the cambium on one side of the rootstock (Image 1.5). It is vital that the rootstock and scion are bound together tightly and do not become dislodged.

Image 1.4 Whip graft: scion and rootstock of equal size.

Source available on the internet at http://www.sonneruplund.dk/eng/default.html. (Accessed 23/03/2018).

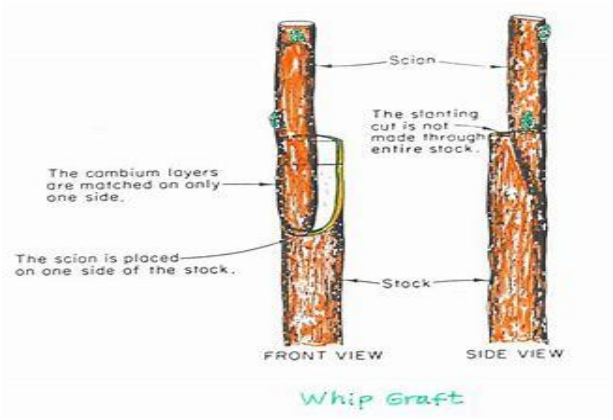

Image 1.5 Whip graft showing rootstock with greater diameter than scion.

Source ndsu.edu PLSC 210: Chapter 12 Plant Propagation: Available on the internet at https://www.ndsu.edu/pubweb/chiwonlee/plsc210/topics/chap12-propagation/chpt12propagation.html. (Accessed 02/09/2018).

The type of graft chosen will depend on the species, material, and the preferred cut chosen by the grafter (Garner, 2013). Consideration should be given to the selection of scions and rootstocks regarding their stem diameters. There is more variation in sapling rootstock diameters in comparison to scion material diameters due to seed source cross pollination (Hartmann *et al* ., 2002). As a general guideline, rootstocks should measure 6-9 mm in diameter, so that they are sufficiently pliable while simultaneously adaquately lignified. Ash rootstocks are normally two years old. The scion material should be freshly cut, graded into different diameter classes and stored in a fridge, between $0\text{-}5^0$ C unless they can be grafted immediately (MacDonald, 2014). Furthermore they should be kept moist to prevent drying out whilst also ensuring no mould is present or develops. It is also important that the scion wood and rootstock stems should be free from soil or grit to prevent impurities from contaminating the scion and rootstock union thus hindering the graft from making a smooth and clean union. Furthermore only pest and disease-free material should be used in the grafting

process. If necessary soluble fungicides may be applied to the scions or rootstocks. In such instances plant materials should be allowed to dry before commencing grafting (Garner, 2013).

Grafting tools are knives, secateurs, tying materials and sealants, depending on the type of grafting union undertaken. The knife is the most important one. It should be comfortable to grip and have a high quality steel blade which can retain a smooth sharp edge. Similarly high quality secateurs should be used as they are essential in the preparation of rootstocks and scion materials. Usually degradable rubber bands, raffia, plastic patches and non-degradable plastic strips are used to secure the graft unions. Once the grafts have been made, the unions are either painted with molten wax or the scion is dipped in it to ensure the graft union is sealed. Paraffin wax which is frequently used should not be greater than 75^{o}C (Hewson, 2012).

1.11 Grafting physiology

For successful grafting, the principal requirement is that a vascular connection is established between the grafted tissues (Hartmann *et al*., 2002). To achieve this it is important that the rootstock and scion are aligned, then success of the graft depends on the vascular tissues of the scion and rootstock growing together therefore both must be kept alive. This vascular connection takes place in five stages as described below.

1.11.1 Stage 1 Alignment of vascular cambiums of the rootstock and scion

As described above, the cambial layers of both the scion and the stock need to be in direct contact to ensure they connect. A clean perfectly executed cut through the wood is critical. Ideally both cambial layers of rootstock and scion should match, however since both are only one to several cells thick this alignment may not occur. What must

occur to move to the next stage is that the cambial layers of scion and rootstock be close enough to ensure that cambial cells from both can form a union.

1.11.2 Stage 2 Wound healing response

In preparing the scion and rootstock for grafting, cells are killed in the rootstock and in the scion to a depth of several cells. This leaves necrotic cell debris in the graft space. The natural response is that the wounded area of the plant parts is isolated from the rest of the plant by production of callus to ensure pathogens do not invade the plant. Callus tissue is formed by cell division mainly in the cambial cells of both the scion and rootstock and this tissue is called wound periderm. Once the callus starts to proliferate from rapidly dividing cambial cells and parenchyma cells, the callus bridge area will form between the rootstock and scion (Hartmann *et al.,* 2002).

1.11.3 Stage 3 Callus bridge formation

The new cambial and parenchyma callus tissue forms in one to seven days on both the scion and rootstock. Without this callus formation, the graft union would fail. This callus continues to proliferate by further cell divisions of the parenchyma cells. These new parenchyma cells soon fill the space between the rootstock and scion. Initially, there is also an adhesion of cells taking place between the cells within the rootstock and scion and across the union area. This adhesive substance (cement) is composed of pectin's, carbohydrates, and proteins. This adhesive substance can bond the rootstock and scion even through many layers of necrotic cell debris. It is still not certain if it is necessary for cell to cell recognition for the process of adhesion to occur. For phloem and xylem of the scion to join up with phloem and xylem of the rootstock, cell to cell recognition may be necessary and cell to cell adhesion may act as a signal in the process (Hartmann *et al.,* 2002). Cell to cell adhesion allows water to pass through the cell walls between the cells of the rootstock and scion, in advance of any vascular

connections across the graft union space. This water movement from rootstock to scion may be sufficient to cause buds in the scion to develop and flush. However, this flushing may not always indicate a successful graft union. In some cases, the flushed buds may desiccate and die because the vascular connections have not been formed across the graft union to provide sufficient water and nutrients for the developing buds.

1.11.4 Stage 4 Wound repair, xylem and phloem differentiation and development of vascular cambium across the callus bridge

After the process of cell adhesion, the xylem and phloem are generally differentiated within the callus bridge area. In this bridge of callus tissue, parenchyma cells from the rootstock and scion become connected initially by plasmodesmata. Plasmodesmata are strands of cytoplasm which extend through the openings in the cell walls and form important connections of communication between cells in the callus bridge. The xylem is normally the first tissue to differentiate to bridge the graft union followed by the phloem. On the perimeter of the recently formed callus, parenchyma cells which are in contact with the cambial cells of the rootstock and scion differentiate into new cambium cells within two to three weeks, post grafting (Hartmann *et al.*, 2002). This allows for secondary development of stem tissues. This stage 4 of graft formation has been discussed in detail by Andrews and Marquez, (1993) and Pina and Errea, (2005). They summarised the events that affect the compatibility of the tissues being grafted as follows:

- Cellular recognition, resulting in the expansion of callus from the rootstock and scion, which in turn will form a bond between both.
- Differentiation of the new cells to form a wedge of callus between the rootstock and scion.
- Formation of new phloem and xylem between the rootstock and scion.

1.11.5 Stage 5 Production of secondary xylem and phloem from the new vascular cambium in the callus bridge

The most recently formed cambial layer in the callus bridge starts the process of laying down newly formed secondary xylem to the inside and phloem on the outside. In this formation of vascular tissues, there is an influence exerted by the rootstock and scion. The development of the newly formed xylem and phloem thereby permits the complete vascular connection between the rootstock and scion. It is vital that this process is completed prior to the new leaves emerging, as failure for this to happen will result in the demise of the plant due to the transpiration losses and the lack of adequate vascular system in place to replace that water (Hartmann *et al.*, 2002). This process can be aided by not overwatering the plant which will have the result of flooding the graft union. It will be obvious that the graft has achieved the first part of viability once the union has begun to callus and the graft buds have begun to swell and later leaves emerge.

1.12 The necessity for different graft types

Different graft types are required due to the material being grafted, and from size and wood quality characteristics:

- Unequal diameter size of scion and stock may dictate the type of graft employed to ensure maximum cambial contact between scion and rootstock (Figure 1.6).
- Some materials do not need as much cambium contact as others to unite. Fraxinus will graft successfully with a splice and with off centre cleft grafts, both of which are relatively simple grafts. Juglans (walnut) requires a lot of cambial contact, thus a saddle graft is more successful in this instance.
- The grafting of incompatible species, whereby an interstock may be used.

1.13 Rootstock selection for traits and qualities

The rootstock may originate from a seedling, a rooted cutting or from a micro-propagated plant. Rootstocks which confer specific characters on the composite grafted plant are generally vegetatively propagated (cuttings, layering).

When selecting rootstocks, it is important to use the tried and trusted combinations of species which are known to give viable grafts. Rootstocks are best produced and selected to match the scion material in stem diameter. For deciduous material, it is essential that the scion material is fully dormant for winter grafts. Normally, rootstocks are 1–2 years old, nursery grown, from source identified seed and either grown for one year in a seedbed then transplanted or grown for another year in the seedbed. The two most important parameters for rootstocks are the stem girth, to suit the girth of the scion material size, and that the root system should be well developed and have a fibrous structure. The propagator's preference and experience can determine as to whether to use one year old or two-year old rootstock.

Rootstocks are normally graded to suit the type of graft to be used. Thin diameter rootstocks, 4-6 millimetre generally are one year old while 6-9 millimetre are two years old. Clonal rootstocks are considered preferential, in comparison to seedlings, as they are more uniform genetically, ensuring a higher grafting success rate; however, they are generally more expensive to develop and produce. It can be prudent to use clonal rootstocks when they have known traits such as resistance to insects, nematodes, and fungi. It is important that winter grafted rootstocks are dried off preferably two to three weeks prior to grafting to reduce the risk of flooding the graft union and the aim should be 50-60% moisture content of the substrate mix (Macdonald, 1986). In this case it was done by shaking the substrate where applicable off the roots and in the instance of

barerooted plants they were removed from the cold store and left in the cold greenhouse for a short while prior to grafting.

1.14 Scion wood

The scion becomes the new shoot system of the graft. The scion is a shoot which has several buds present. Scion-wood for dormant period grafting is collected during the winter, generally January and February and stored in a fridge or suitable cold room while shoots with buds that are used for budding are collected during the growing period and grafted as soon as possible afterwards. The optimal scion-wood is taken from the previous year's growth, ensuring it is lignified sufficiently. It is desirable to collect healthy scion material which does not have any flower buds. The ideal scion diameter should be close in diameter to that of the rootstock.

1.15 Types of grafting

The most common type of graft method is detached scion grafting, which is grafting with excised scions. Examples of these graft types are: whip grafting, side grafting, saddle grafting, cleft grafting, micrografting, interstock grafting and budding.

1.16 Detached scion grafting

In detached scion grafting method, the scion is excised from the parent plant and then grafted onto the chosen rootstock. Several types of cuts are used to maximise the alignment of cambial areas in the stocks and scions.

1.16.1 Whip grafting

Whip grafting, sometimes referred to as splice grafting (Images 1.6 and 1.7) is quite a common method of joining plants such as apples, peaches, plums, cherries and a variation of it is the whip and tongue technique method (Image 1.7). Whip grafting is a relatively easy method of grafting scions and stocks of similar diameter together (Image 1.6). To achieve this graft, the rootstock top is removed approximately 7-10 centimetres above the root collar and a slanted cut of some 45^0 is made in the scion to match. If the thicknesses of the scion and rootstock are different, it is best not to place the scion in the central location; in this instance, ensure one side matches up smoothly. The two cut surfaces are then held together, tied and sealed. The whip and tongue graft work best once the stock and scion are of similar diameter (Image 1.7). The whip and tongue grafting process involves making a sloping cut about three centimetres long at the top of the rootstock and then repeating this process at the base of the scion. Two pieces of material are then aligned to check that the cut length and angles match. To make the tongues start about a third of the way down the tip of the wood of the rootstock, cut into the face; the cut must be straight and about half the length of the first cut and parallel to the first cut (Image 1.7). Make similar cuts in the scion. When the cuts are completed, check that the two pieces fit together snugly and interlock smoothly. Like other grafts, there should be no air spaces between the pieces of wood; with this method of grafting, the wood's natural tension should hold the graft tight in combination with some binding such as rubber bands. The rubber bands used in this thesis were 12.5 millimetre x 4.5 millimetre supplied by Telermaat in the Netherlands.

Image 1.6 Whip grafting

Source sonneruplund.dk. Available on the internet at http://www.sonneruplund.dk/eng/default.html. (Accessed 23/03/2018).

Image 1.7 Whip and tongue graft

Source sonneruplund.dk. Available on the internet at https://www.sonneruplund.dk/podningfoto/whip2.jpg. (Accessed 02/09/2018).

Side grafting is also an easy graft to perform (Lamb *et al.*, 1985). Starting about 7 centimetres above the root collar a downward slice is made leaving this slice attached at the base while the scion having a similar cut, on one side and shorter on the other side is fitted into it (Image 1.8). *Picea* and *Acer palmatum* are generally side grafted.

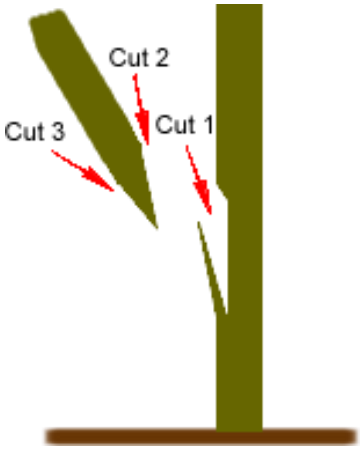

Image 1.8 Side graft

Source : lakeshorebonsai.com. Available on the internet at https://www.bing.com/images/search?q=Side+Grafting&FORM=IRIBIP .

(Accessed 02/09/2018).

1.16.2 Saddle graft

Saddle graft is where the middle is cut from the scion in a wedge shape and the opposite cut made in the rootstock so that the scion sits on top of the rootstock like a saddle (Image 1.9). Like other grafts, the union is tightly bound and sealed. This graft maximises the surface areas of cambial tissue in the stock and scion so that contact is maximised and enhances the chances of placing the cambium of the stock and scion in direct contact. The stock and scion should be about the same size. Some Rhododendrons and Juglans are saddle grafted.

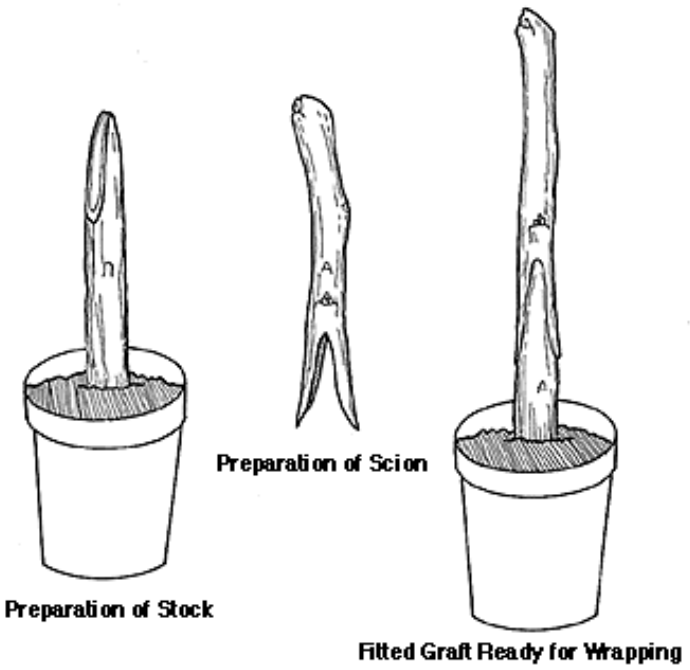

Image 1.9 Saddle graft

Source Growables Grafting Techniques, 2016. Available on the internet at http://www.growables.org/information/GraftingTechniques.htm.

(Accessed 02/09/2018).

1.16.3 Wedge or cleft graft

The wedge or cleft graft is performed by cutting the basal end of the scion in a wedge shape on both sides and then splitting the rootstock to receive the scion (Image 1.10). However when the scion diameter is smaller than that of the rootstock an off-centre cleft graft is made in which just one side of the scion's cambium makes contact with cambium of the rootstock (Image 1.11). This type of graft is sometimes referred to as a cleft graft. The graft is then tied and sealed. If the scions and rootstocks are the same diameters, then the task is much easier. Species suitable for cleft grafting are *Quercus, Acer, Prunus* and *Fraxinus.*

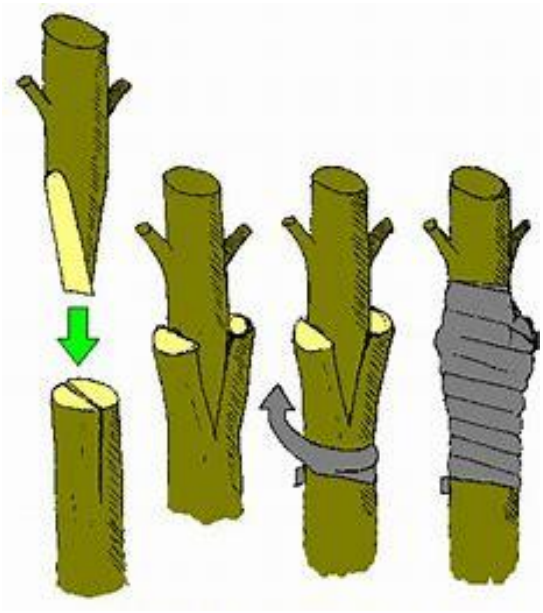

Image 1.10 Cleft or top wedge graft

Source: Pinterest.com. Available on the internet at http://anpsa.org.au/APOL7/qu-graft.gif (Accessed 02/09/2018).

Image 1.11 Off centre cleft graft

Available on the internet at GB-online.co.uk

http://www.gb-online.co.uk/gb-wordpress/?p=545. (Accessed 02/09/2018).

Topworking is grafting a newer variety onto a mature tree (Image 1.12). The type of graft used is a cleft graft. It is mostly used on fruit trees such as apple and pear, which have a productive life of 50 years or more and when the variety on these trees becomes outdated after 20 years. In such cases there is no necessity to grub out the trees, as they can be gainfully utilised by re-grafting new varieties onto the existing trees as shown in Image 1.12.

Image 1.12 Topworking a fruit tree

Source https://tomtheappleman.wordpress.com/category/orchards/

Available on the internet at
https://tomtheappleman.files.wordpress.com/2011/03/010.jpg.

(Accessed 02/09/2018).

1.16.4 Micrografting

One of the major advantages of micrografting is rapid multiplication of material, the elimination of viral problems, multiplication of plants which are difficult to root, and

disease indexing. Around 300 million plants are produced annually by different forms of micropropagation (Clark and Toogood, 1992). Micrografting is a technique where a meristem or shoot tip is placed on a decapitated rootstock which has been grown from tissue cultured seed, or plants in a sterile environment (Image 1.13). The technique is a difficult slow process and is expensive in comparison to normal grafting, due to a low rate of successful grafts. This is because it requires a lot of technical expertise in handling minute and delicate pieces of material while still ensuring that the graft union remains intact. Successful methodologies have now been established for several fruit crops such as apple, almond, grapes, olive, cherry, chestnut, mulberry, peach, pistachio, walnut, and others (Hussain *et al.*, 2014).

Micrografting has been shown to be quite successful in fruit crops. Abousalim and Mantell (1992) achieved 94-100% success using *in vitro* raised rootstocks to micrograft pistachio, while Yildrim *et al.*, (2010) managed to achieve 90-100% by using wild almond seedlings to micrograft selected cultivars. Where micrograft's of fruit trees have been less successful it has been attributed to insufficient contact between the scion and rootstock, incompatibility factors and browning of the cut surfaces caused by phenols (Ramanayake and Kovoor, 1999).

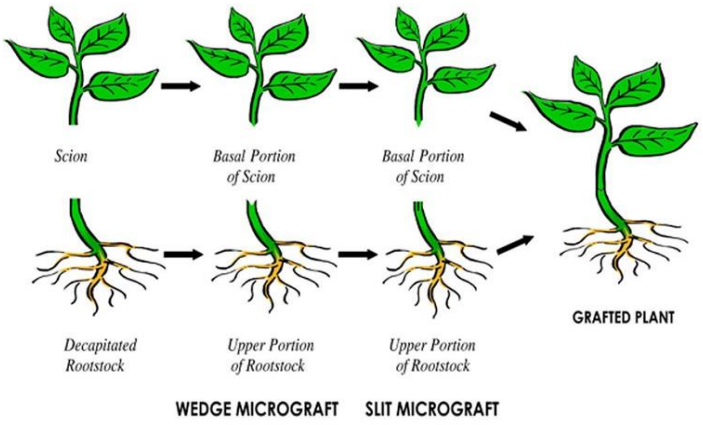

Image 1.13 Micrografting.

Available on the internet at http://slideplayer.com/slide/10626288/36/images/13/WHAT+IS+MICROGRAFTING.jpg. (Accessed 22/05/2019).

1.16.5 Interstock grafting

In some cases, it may be necessary to use an interstock to circumvent incompatibility between the rootstock and scion or possibly to reduce vegetative growth and accelerate flowering. This methodology is sometimes referred to as double working (Image 1.14).

Interstock grafting is a method where a piece of a stem is inserted between the scion and rootstock by means of two graft unions and generally the interstock is a different species or cultivar in comparison to the rootstock or scion. The piece between the scion and rootstock is called an 'interstock' or may also be called 'interstem', 'intermediate stem section' or 'intermediate stock' (Image 1.14). Using an interstock is extra work when grafting, so the benefits must outweigh the time and effort involved.

The principal beneficial result of using the interstock may well be the effect of overcoming incompatibility between the scion and rootstock. The purpose of using some interstocks and rootstocks may be reducing vegetative growth in the scions and

accelerating the onset of flowering in a similar way to that described for using standard rootstocks. Using a dwarfing interstock "Malling 9" with a vigorous rootstock has had the effect of producing a well rooted apple tree (Hartmann *et al.,* 2002).

Much work has been undertaken to determine if the interstock reduces the vigour of the rootstock and in certain instances results in early flowering, an increase in fruit and quality for species such as apples, cherries, plums and pears (Vercammen *et al.,* 2004; Samad *et al.,* 1999; Webster, 1995). Other researchers have stated that employing M27 and M9 as weak interstocks grafted onto vigorous seedling rootstocks resulted in, a reduction of the actual plant development, accelerated plant cropping, and reduced the need for pruning in cases such as apples (Lord *et al*., 1985; Di Vaio *et al.,* 2008).

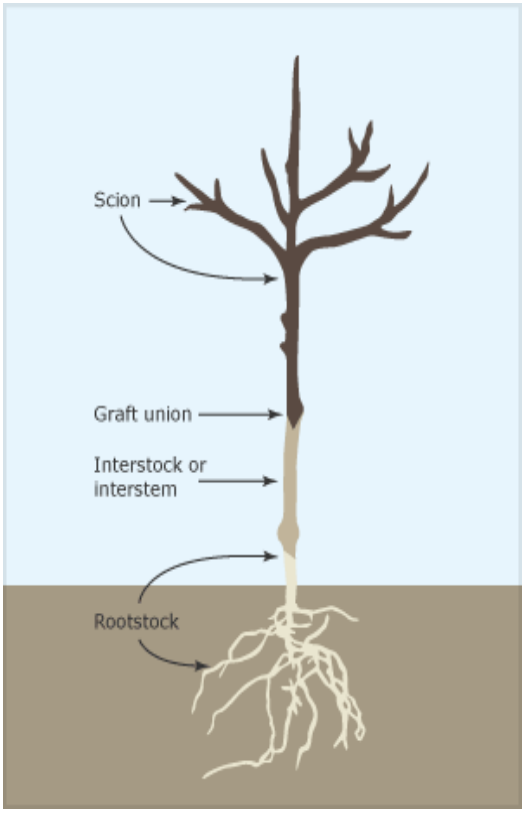

Image 1.14 Interstock grafting

Source: The Ara Encyclopedia of New Zealand is the complete guide to our people, environment, history, culture and society, 2005. Available on the internet at http://www.teara.govt.nz/en. (Accessed 02/09/2018).

1.16.6 Grafting by budding method

Budding, which is another form of grafting, is three times more expensive than cuttings, and fourteen times more expensive than employing seedling propagation (Hartmann *et al*., 2002). This is attributed to the fact that it is a very skilled operation. There are two main types such as T budding and chip budding. It is described as taking a single bud from a shoot of the current year's growth and inserting it just under the bark of the rootstock. Rootstocks used for budding are generally grown in the field or may be potted plants of seed derived material. This operation of budding is generally carried out at the end of the growing season and in the case of ash from late August to mid September. Roses and citrus are generally bud grafted. To undertake this operation, shoots which have several buds present are generally collected from donor plants. Shoot collections should be made as close as possible to the time of budding and should be refrigerated if a delay is anticipated. The buds are normally taken from the axils of the leaves using a shallow slicing cut 5-25 mm below the intended bud and coming out above it in a uniform cut (Image 1.15). According to Macdonald, (1986) selected clones of *Fraxinus excelsior* are sometimes budded onto *Fraxinus excelsior* rootstocks as another form of propagation in comparison to normal grafting.

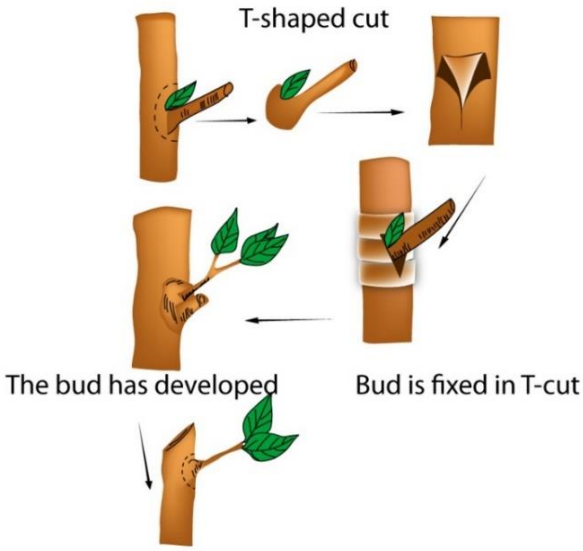

Image 1.15 T- budding

Source Agriculture Form 2 – Kcse online, 2016. Available on the internet at

http://www.kcse-online.info/aH53WC/data/text/Ag2-332000text.html. (Accessed 02/09/2018).

It is beneficial to retain a piece of the petiole with the attached bud, as it is helpful as a handle when inserting the bud. The rootstock is prepared by selecting a clean piece of stem, at the position for inserting the bud, and removing all unwanted growth and buds below the bud insertion point. The stock is prepared by making a T cut through the bark (Image 1.15). Using the grafting knife, the flaps of bark are pulled apart and the bud inserted into this area of cambial tissue. The bud is then tied in position using budding rubber strips, patches or adhesive tape. The plant is then left to await its success or failure. If successful, the bud will grow out (Image 1.15) and then the top of the rootstock is excised, and the growing bud now becomes the new leader of the grafted plant. The nursery stock industry uses this form of grafting for fruit and roses as it is possible to achieve a high daily output. *Acer, Fraxinus, Gleditsia and Tilia* are frequently budded. A grafter can bud 2,000-4,000 plants per day if assisted by another person tying the buds. For this method of propagation, the rootstock should be actively

growing, and the bud used should be mature. These two principles are paramount to achieving a successful budded plant graft.

The other type of budding is ‘Chip’ budding (Image 1.16), which was developed at East Malling Research (Rootstock research at East Malling, 2016). This method is performed by selecting a clean piece of stem on the rootstock where the bud is to be inserted and removing a sliver of wood while leaving a small shelf to receive the intended bud. The scion bud should be excised as a shape to be a mirror image in shape of the sliver of wood that had been excised from the rootstock. In this case, a small piece of wood is retained behind the bud (Image 1.15). To match up the cambial areas due care should be taken to ensure when tying that the bud is in the correct position and has not moved. Chip budding is also suggested for ash propagation by Clark and Toogood, (1992).

Image 1.16 Chip budding

Source forums.gardenweb.com

Available on the internet at https://www.houzz.com/discussions/1492993/summer-budding.

(Accessed 02/09/2018).

1.16.7 Interspecific and intergeneric grafting

Most grafting involves intraspecific grafts i.e. grafting of stock and scions which belong to the same species. On the other hand, intergeneric grafting is the grafting of two plants of different species within the same botanical genus. In the case of this thesis the examples are such, interspecies using *Fraxinus chinensis, paxiana* as rootstocks and intergeneric using *Ligustrum* and *Syringa* as rootstocks. According to Macdonald (1986) intergeneric grafting is often practised when nurse grafting while using root sections as rootstocks. Cultivars of *Syringa vulgaris* are sometimes grafted onto *Fraxinus excelsior* roots or *Ligustrum ovalifolium* seedlings. Thereafter grafted plants are planted deeply to encourage self-rooting and later excised from the nurse plant.

Interspecific grafting has been used to graft vegetables successfully in the cucurbit and solanaceous families (King *et al.*, 2010). A case in point of interspecific grafting is in Latin America (Guatemala) where grafting of coffee is a common propagation practice that has been used on an extensive scale for more than 30 years. To avoid nematode damage to the roots of the coffee plant (*Coffea arabica* L.), a widely practiced system is to graft it onto a rootstock of *C. canephora* (Bertrand *et al.*, 2001).

Intergeneric grafting is a cross between plants in two different genera in the same family. Intergeneric grafting has been used in the horticultural industry to circumvent problems which have arisen due to pest and disease problems in the soil and to increase crop yield (Kubota *et al.*, 2008). *Solanum* lycopersicum (tomato) is highly susceptible to flooding stress and to overcome this problem the tomato plant has been grafted onto an eggplant rootstock (*Solanum melongena*). Eggplant rootstock grafted plants had better plant survival, greater physiological adaptation and also yield was better over the self-grafted and ungrafted plants under controlled flooding stress test (Bhatt *et al.*, 2015). The other positive response was that the rootstock grafting improved the sugar

and starch contents, as well as the fruit yield of the tomato plants under flooding conditions. In the cucurbit family *Citrullus lanatus* (watermelon) has been successfully grafted on rootstocks of *Lagenaria siceraria* (bottle gourd).

Araliaceae plants, which are of interest to the foliage industry, were created by using interspecific and intergeneric combinations to produce new forms. One hundred and one combinations and seven control combinations were tested. Novelty forms of Araliaceae pot plants were created by testing 24 species of 10 genera combinations, intergenerically and interspecifically which resulted in 85 graft combinations which grew. Results indicate that a wide range of intergeneric grafting's are achievable in Araliaceae (Leonhardt, 1996).

Grafting of vegetables originated in Japan and Korea in the 1920's. Vegetables which are commonly grafted are watermelon, cucumber, tomato and eggplant (Lee, 1994). Grafting of vegetables had not been widely practised in Europe until 2005 when the soil fumigant methyl bromide was banned, and nowadays grafted tomato plants are used in commercial production as a routine practice for growing. Grafting focus on vegetables has been on yield and overcoming pest and disease problems. The focus is now diverging to vegetable quality such as appearance, size, shape, colour, flavour and health related compounds like, minerals, vitamins and cartenoids (Rouphael *et al.*, 2010). Grafting practice may become more commonly used as the EU imposes further bans on pesticides. One of the big advantages with this approach is that it allows a pest or disease susceptible scion to be grafted onto a resistant rootstock without a protracted breeding programme for the scion variety. Furthermore, with the growing number of new pests and diseases arriving in Europe, it provides a more flexible solution for dealing with soil borne diseases than breeding new varieties for resistance (Cohen *et al.*, 2007). With the advancement of science in the area of genetics a realistic goal would be

to have rootstocks commercially available with chosen traits and also to have a better understanding of rootstock and scion compatibilities.

1.17 The aftercare of grafted plants

While all stages of the grafting process are important to ensure a high grafting viability success, it is advisable to pay attention to their individual environmental requirements. The aftercare of the grafted plants has a big effect on viability. Post grafting, plants may be placed into boxes within an enclosed glass or plastic enclosure within a glasshouse or polytunnel. This is called "closed case" grafting procedure. The enclosure will contain moist sand or peat and an ambient temperature range of 12.8^{o}C-15^{o}C (Lamb and Nutty, 1981). This regime encourages callusing at the graft union.

The alternative aftercare to closed case is "open case" in which the grafted plant is kept unenclosed in a temperature-controlled greenhouse or an unheated polytunnel.

Other factors in the aftercare of grafted plants are crucial for graft success. If the grafted plants are kept too dry then the roots will fail, if they are too wet, then the graft union will become flooded by exudates from the rootstock into the union area. If shoots, also called suckers or water sprouts form below the graft union are allowed to develop, then these shoots will become dominant and result in graft failure. These epicormic shoots have lain dormant beneath the bark of the rootstock and have been triggered into growth due to the rootstock being cut, water availability and light. If the grafted plants are not kept in suitable environmental growing conditions, then the failure of either of the partners will result in the failure of the graft.

Bench grafting is a description given to any grafting methodology which is performed while the scion and rootstock are unplanted. It is so called, as it is mostly carried out on a bench.

Hot pipe callusing is also another post grafting culture method which directs heat at the graft union, thus aiding the success rate by pre-callusing the union prior to root and shoot activity (Dunn, 1995). In the situation where hot pipe callusing is used, it is important to maintain the graft union at $21^{o} – 27^{o}C$ while the roots plus scion should be maintained at about $5^{o}C$ in order that bud growth does not occur before callus growth and the union has taken place (Hewson, 2012). Pest and diseases should be monitored and dealt with. In the case of field budded plants, budding materials such as tape or rubber bands, budding strips, patches and adhesive tape should be removed once the graft union has been formed.

1.18 How to measure grafting success?

Grafting success is the formation of a permanent union between two plants and the proliferation of growth in the scion. The objectives of grafting are to achieve a high percentage of viable grafted plants while performing the graft quickly and efficiently.

Grafting success depends 45% on preparation, which includes the quality of materials used and their preparation, 10% on the craftsmanship in making the graft and 45% on the aftercare provided to the grafted plant (Hartmann *et al.*, 2002). Preparation includes the rootstock quality, scion quality and the paraphernalia needed to conduct the grafting. It also refers to the type of graft used. Craftsmanship is the skills required in bringing the cambial layers into contact and binding the scion with the rootstock. After care is the monitoring of the grafted plant development and ensuring the temperature and water

requirements are sufficient. Grafting success can easily be measured by the percentage of viable plants surviving.

1.19 Effect of grafting on vegetative growth and fruiting

The desirable results from using grafted plants have been evaluated by many authors in relation to measuring fruit yield, quality aspects and vegetative growth rates (Bertrand *et al.,* 2001., Di Vaio *et al*., 2008., Tetsummara *et al.,* 2015). Using the fruiting potential as a parameter, several studies reported fruit yield increases to varying degrees. Plums are normally grafted on *Prunus cerasifera* rootstocks in Estonia. Using this rootstock resulted in a more vigorous tree that bears fruit late in the season or in earlier years after grafting in comparison to unselected rootstocks (Jänes *et al.,* 2005)**.** In recent times, numerous rootstocks have become available which now produce smaller trees, which are very manageable with regard to harvesting the fruit. Using nine different rootstocks and two cultivars of *Prunus domestica,* a higher average yield was recorded on rootstock's 'Kubanskaja Kometa' and 'Mariana GF 8-1' which was significantly higher than unselected P.*cerasifera* rootstock (Jänes *et al.,* 2005). The rootstock used was claimed to be the governing factor on the vigour of the grafted plants (Botu *et al.,* 2002). In Hungary, (Balzás *et al.*,2011) stated that using interspecific rootstocks, 'RS 841' and the Lagenaria rootstock 'FR Strong' for grafting watermelon independent of the type of rootstock used gave a higher yield by comparison to non-grafted plants. They concluded that the root proliferation of the grafted plants gave more resistance to bad weather conditions and resulted in stability of yield. In one case, a particular rootstock produced 4% lower yield than the non-grafted plants, however, using a different rootstock a 20% higher yield was achieved compared

to non-grafted plants, which illustrates that not all rootstocks are an improvement i.e. they need to be tested (Balzás *et al.*, 2011).

According to Univer *et al.* (2010), extensive trials were carried out in Estonia, Latvia and Lithuania, using 12 different dwarfing rootstocks on an early apple variety 'Auksis' over a five-year growing season. They concluded that the effect of rootstock on fruit weight was modest. Rootstock influence was not the only factor affecting the tree vigour**;** there was also soil, climate, precipitation and the crop husbandry. They stated that some rootstocks had a dwarfing effect.

In Poland, Porebski *et al.* (2006) investigated the effect of various apple rootstocks on growth and fruiting of apple trees. The objective was to test the apple variety 'Rubin' on five different rootstocks. The findings indicated that rootstocks played a significant role in reducing tree growth, bud formation, fruit set, and yield**.**

In the UK East Malling have undertaken research programmes on rootstock research for apples, plums, cherries and quince. The more notable commercially available apple rootstocks are in the following size sequence, M27, M9, M26, MM106, MM111 and M25 (Rootstock research at East Malling, 2016).

Ornamental plants frequently grafted include *Camellia, Hammelis, Wisteria, Thuja, Picea* and *Rosea* (Brickell, 1996).

In Korea, it is accepted common practice that 90% of Cucurbitaceous and 30% of Solanaceae vegetables are grafted onto different species rootstocks (Lee *et al.,* 2010). In relation to fruit quality, and the effects of grafting, research findings have been sometimes contradictory as to whether there are any advantages to using grafting as a method of propagation, however this is species dependent (Davis *et al.,* 2008; Lee *et al.,* 2010). Other research findings concluded that in the case of grafted peppers, other

factors apart from the rootstocks such as harvest period and harvest time also influenced the textural quality of the fruit. In addition, it was recommended to choose the rootstock and scion combination carefully to obtain the desired fruit characteristics (Jang *et al.,* 2013).

In Tanzania, cashew nuts are an important crop, however, there is an issue with the low yield in the island of Flores. The average yield is 0.6 tonne per hectare per year, in comparison to countries like India, Vietnam, Australia, and Nigeria, which record a yield of over one tonne per hectare (Martin *et al.,* 1997). The objective of a study was to utilise old cashew trees by 'topworking'. The conclusions were that the combination of two scions grafted while retaining two productive branches on a tree gave the best growth of new material, the earliest flowering and the highest percentage of flowers yielding fruit in comparison to the other treatments. This side grafting combination gave an almost 82% survival rate, the new shoots started to flower 113 days after grafting with 100% flowering 135 days after grafting, which was significant when compared with non-side grafting of trees; the non-grafted branches which had taken two years to flower. The side grafting also gave a very satisfactory result, in that almost 70% of the flowers yielded fruit. The result was that using side grafting as a method to rejuvenate cashew trees (i.e. topworking) had potential, provided the rootstocks and scions were healthy and that the environmental conditions such as temperature and humidity were within acceptable parameters (Suharto *et al.,* 2012).

In Japan, persimmon (*Diospyros kaki* L.) is produced by grafting scions of designated cultivars onto seedling rootstocks. The resulting grafts tends to have a 4-5 year post grafting period in which the plant goes into a vegetative period without fruiting. When two cultivars 'Fuyu' and 'Hiratanenashi' were tested on a dwarfing rootstock MKR1, they produced flowers soon after field establishment and copiously thereafter (Yakushiji

et al., 2008). The fruit yield per tree over a two-year period appeared to be consistently good and the result concluded that MKR1 rootstocks, which are easy to propagate clonally, are suitable dwarfing rootstock for persimmon cultivation **(**Tetsumura *et al.*, 2015).

In the case of ash tree (*F.excelsior)* grafting's, observational experiences have shown that *F.excelsior* grafted onto *F. excelsior* produced good vegetative growth and flowering which could occur on some plants in the year they were grafted. However, the percentage of flowering trees varied from year to year and was not consistent. The production of flowers is a prerequisite to fruit formation. Years of physiological studies indicate that flowering initiates as a response from signals from the environment and from endogenous factors from organs such as roots and shoot buds. The environmental signals are changes in day length and temperature. The endogenous routes are independent of the environment and are linked to the plant development stage and are often referred to as "autonomous", which indicates the non-impact of environmental factors (Wilkie *et al.*, 2008). Influences of environmental and autonomous factors on flowering vary greatly between and within a species. Some plants pass through a juvenile phase where they are not subject to the environmental factors which trigger flowering (Poethig, 1990). In a study done on the floral initiation of apple (*Malus domestica*) flowering is autonomous with flower initiation governed by the previous season's growth and by interactions of environmental conditions and vegetative growth (Wilkie *et al.*, 2008). This theory of floral initiation in the previous season is also supported by research undertaken by Abbot, *et al*, (1970). Apples can have a juvenile phase where they can take up to six years to flower however grafting them onto dwarfing rootstocks can reduce this juvenile phase (Kotoda *et al.*, 2006).

Previous personal grafting experience in Teagasc has shown that some individual plants of grafted ash flowered and produced seed in its first year of being grafted, and occasionally in subsequent years, albeit sporadically. One explanation of this phenomenon may be that the scion material had been taken from mature trees which had retained adult traits of a capacity to produce flowers and fruits. The objective of this study was to determine the effects of grafting common ash *(Fraxinus excelsior*) onto rootstocks of Asiatic species of Fraxinus and other genera in terms of graft compatibility and the vegetative growth. By using Asian species and other genera as rootstocks, the hypothesis was that vegetative growth of *Fraxinus excelsior* scions would be reduced due to these rootstocks. In the longer term this might shorten the overall period for the onset of flowering and seed production in ash trees. Assuming the identified rootstocks would speed up the onset of flowering and seed production, it would offer the option of grafting *Fraxinus excelsior* scions, which would be selected from trees with resistance to *Chalara* and grafting them onto the optimal species so that the seeds from the grafted resistant trees could be made available more quickly. Asiatic species have co-existed with ash dieback for some time and some of the species such as *Fraxinus mandchurica* and *Fraxinus chinensis* have shown tolerance to dieback disease (Kirisits *et al*. 2009; Kräutler & Kirisits, 2012; Gross and Queloz, 2015). Although dieback of ash shoots leads to a greatly reduced crown of leaves, the fungus *H. fraxineus* can also infect tree trunks at soil level (root collar) (Enderle *et al.,* 2017; Chandelier *et al.,* 2017). Propagating resistant trees of *Fraxinus excelsior* by grafting them onto rootstocks of *Fraxinus excelsior* would leave the rootstocks vulnerable to infection. Therefore, it would be advisable to use rootstocks of Asiatic species which are known to be tolerant of the pathogen. Observational forest assessments in Japan indicate that there are less dieback lesions on the stems of *Fraxinus mandschurica*

than *Fraxinus excelsior* (Zhao *et al.*, 2013). There is no previous knowledge as to the success or failure of the proposed specific grafting of Asian species of Fraxinus or onto species which are in the same botanical family, (Oleaceae) i.e. the genera *Ligustrum* and *Syringa*. *Fraxinus excelsior* rootstocks are proven to be successful (Thompson *et al.*, 2001) and also from personal experience.

Chalara infection has now been confirmed in every county in Ireland (DAFM, 2018). Research has confirmed that a small proportion of ash trees have a natural resistance to *Chalara* (1%) as reported by Kjaer *et al* (2012). Progeny trials studied by Pliūra *et al* (2011) estimate that the level of natural ash dieback resistance to be <5%. Mc Kinney *et al* (2014) estimate that 1-5% of native trees to have some ash dieback resistance and that it is stable in trees that have been propagated vegetatively (Stener, 2013). It has also been shown that resistance is determined genetically i.e. it can be passed onto seed derived progeny (Kjaer *et al.*, 2017).

For this thesis interspecific grafting's, intergeneric grafts and self-grafts were made. Interspecific and intergeneric are referred to as heterografts and self- grafts are referred to as homograft's. The evaluation tasks were to measure the new growth in the heterografts and compare that growth with non-grafted ash as controls and observe and record the compatibility of various graft unions.

The research reported here set out to test the following hypotheses:

- Grafting of *Fraxinus excelsior* onto Asiatic rootstocks produces viable plants.
- Grafting *Fraxinus excelsior* onto *Fraxinus excelsior* with interstocks of Asiatic species produces viable plants.
- Intergeneric grafting will produce viable plants.

- Interspecific and intergeneric grafting results in reduction of vegetative growth and potentially accelerated flowering.

The reason for undertaking this research is to establish a realitively fast methodology of propagation, in comparison to micropropagation so as to ensure that the *Chalara* resistant trees which will be identified in the future can be successfully propagated in sufficient numbers to be established in seed orchards. As Ligustrum and Syringa flower annually in theory if they can be grafted successfully they should flower more often than conventional ash grafted onto ash.

This study concerns the vegetative propagation of *Fraxinus excelsior* and other ash species by grafting method. It concentrates on the effects of the different species of rootstocks on graft viability and their effects on vegetative growth of the scions over two seasons of growth. The aim is to produce viable grafts with multi shoots which will flower early in life thereby accelerating the establishment of seed orchards.

Chapter 2: Materials and Methods

2.1 Introduction

Interspecific and intergeneric grafting was carried out as described previously. The scions of ash trees used in this study were from a collection of common ash (*Fraxinus excelsior*) which had been selected by The National Irish Forestry Board (Coillte) from across the country. The two clones selected as scion material for the grafting experiment were, Clone M72 and Clone 98 and were chosen at random as trees which had not exhibited signs of *Chalara*. The original trees Clone M72 and Clone 98 had been selected for their growth rate, stem form, apical dominance, absence of forks, disease; and such trees are termed plus trees. They were among a national collection of 100 plus trees which had been planted in a clone bank at Teagasc, Kinsealy, Dublin in 2007 and were representative of clonal material available, the theory being that if this is successful then it will work for other clones. The trees in this collection were cut back hard on three occasions to stimulate the production of suitable scion material which ideally should be 6-9 millimeters in diameter, sufficiently long enough to handle comfortably, (100 millimeters), five buds and lignified.

The experimental treatments were interspecific and intergeneric grafts (heterografts) of *Fraxinus excelsior* (Clone M72 and Clone 98) as scions and various species/genera as rootstocks and interstocks. Asiatic species of interstock material, *Fraxinus japonica* and *Fraxinus platypoda* were collected from trees in John Fitzgerald Kennedy Park (JFK) in New Ross, County Wexford. This material was collected on 19th March 2014 and stored in a fridge at 3^{o}C until required. Special permission had been granted by DAFM to collect this material. There was some difficulty with obtaining a sufficient quantity of good quality

grafting material for some species e.g *Fraxinus japonica* and *Fraxinus platypoda* interstocks were not available to test in conjunction with Clone M72. The reason for this was that the trees at JFK had not been culturally managed therefore extension growth was minimal. Control treatment was plants of *Fraxinus excelsior* rootstocks, which were cut back as they would be normally for grafting, and allowed to reshoot to determine the effect of cutting the plants on shoot regrowth. The work sequence is outlined in Image 2.1. The numbers grafted for each treatment are outlined in Table 2.1 and the various grafting and budding combinations are summarised in Table 2.2. In relation to the grafting techniques used, the chosen methods were cleft grafting, which yielded good results (Thompson *et al.,* 2001) and T budding. T budding as a form of vegetative propagation was performed as discussed in the literature review. Bud scions were collected from trees in Kinsealy commencing on 8th September 2014 and finishing on 9th and budded on the same day of collection. T budding was used as described by Rudolf *et al.,* (2008) and Lamb *et al.,* (1985).

Throughout this thesis, the following terms were sometimes used: 'Common ash' refers to *Fraxinus excelsior,* 'Privet' refers to *Ligustrum ovalifolium,* 'Lilac' refers to *Syringa vulgaris.* Clone M72 and Clone 98 refer to two selected genotypes which were used as clonal scions of *Fraxinus excelsior.*

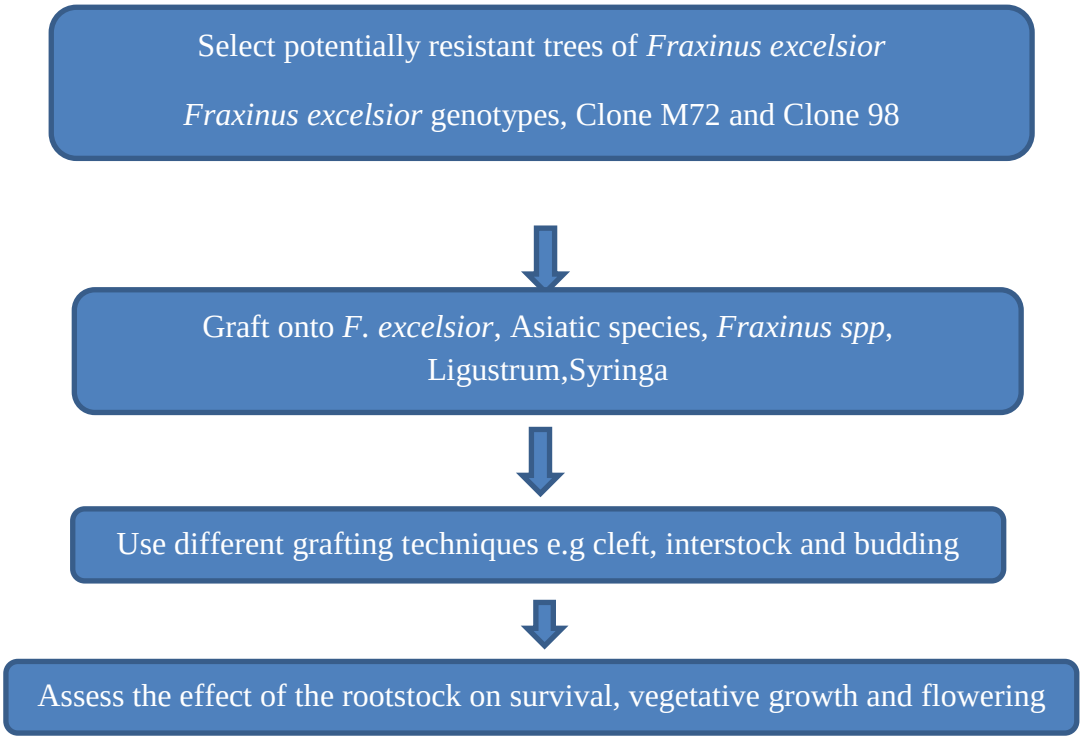

Image 2.1 Experimental work flow chart

The objective was to graft 30 plants for each treatment, however, there was insufficient interstock and rootstock material available for treatments 10, 16, and 17 (Table 2.1). The selected Clones M72 and Clone 98 were grafted onto various rootstocks, Table 2.2.

Table 2.1 The number of grafting and budding combinations originally performed

Treatment	Original Grafting	Number of plants
1	*F. ex* Clone M72 grafted onto *Fraxinus paxiana*	30
2	*F. ex* Clone M72 grafted onto *Fraxinus chinensis*	31
3	*Fraxinus* non – grafted (Control)	30
4	*F. ex* Clone 98 grafted onto *Fraxinus* excelsior	30
5	*F. ex* Clone M72 grafted onto *Fraxinus* excelsior	30
6	*F. ex* Clone M72 grafted onto Ligustrum rootstocks	30
7	*F. ex* Clone 98 grafted onto Ligustrum rootstocks	30
8	*F. ex* Clone M72 grafted onto Syringa rootstocks	30
9	*F. ex* Clone 98 grafted onto Syringa rootstocks	30
10	*F. ex* Clone 98 interstock *Fraxinus japonica* *	25
11	*F. ex* Clone 98 interstock *Fraxinus platypoda* *	30
12	*F.* ex Clone 98 budded onto *Fraxinus paxiana*	32
13	*F. ex* Clone M72 budded onto *Fraxinus paxiana*	30
14	*F. ex* Clone 98 budded onto *Fraxinus excelsior*	30
15	*F. ex* Clone M72 budded onto *Fraxinus excelsior*	30
16	*F. ex* Clone 98 budded onto *Fraxinus platypoda*	10
17	*F. ex* Clone M72 budded onto *Fraxinus platypoda*	7
Total		465

* Rootstock *Fraxinus excelsior*

The various grafting and budding combinations are summarised in Table 2.2.

Table 2.2 Grafting and budding combinations

Scions	Rootstocks used				
Grafted	***F. excelsior***	***F. paxiana***	***F. chinensis***	***Syringa***	***Ligustrum***
Clone M72	√	√	√	√	√
Clone 98	√	NT	NT	√	√
Budded	***F. excelsior***	***F. paxiana***	***F. platypoda***		
Clone M72	√	√	√	NT	NT
Clone 98	√	√	√	NT	NT
Clone 98	****F. japonica***	****F. platypoda***	NT	NT	NT
	√	√	NT	NT	NT

√ = grafting and budding combinations performed

NT = not tested combination

* = In these cases *F. excelsior* was the rootstock and the interstocks were *F. japonica* and *F. platypoda*

2.2 Experiment location

The site location chosen to undertake the grafting experiments was an unheated glasshouse in Teagasc, Kinsealy, Dublin.

2.3 Plant material preparation

Plant material preparation is crucially important in the process of ensuring successful grafted plants, therefore the selection of the best available plant material was the first important factor.

2.3.1 Scion and budding material

The scion material was either in the form of shoots or buds. The shoot scions were 10-15 cm long, with generally 5 buds present, which included the terminal bud. Scions for grafting were collected on 1st April 2014 and placed in a cold store at 3°C and removed, as required, between 1st and 4th April for grafting. For budding, single axillary buds were cut from the donor bud scions which were collected from the trees on the 8th and 9th September and inserted in the rootstocks using a T- bud cut, then wrapped with budding tape as illustrated (Image 1.14). After budding, the potted plants were then put in the unheated glasshouse along with the other randomized plants. For the budding experiment, the Asiatic rootstock species were *Fraxinus paxiana, Fraxinus platypoda* and *Fraxinus excelsior*. The rootstocks for budding had been grown in 3 litre pots for one year in standard Bord na Mona nursery stock growing medium (Appendix 4). The scion material used was clones of *Fraxinus excelsior* (Clones M72 and 98). As controls, bud wood of *Fraxinus excelsior* Clones M72 and 98 were budded onto common ash rootstocks. Rootstocks and scion diameters were recorded at the time of grafting. Smaller diameter scions were used on some combinations to better match diameters of the rootstocks available.

Table 2.3 Rootstock and scion diameters (mm) used for grafting and budding

Clone	Scion (mm)	Rootstock / interstock	Rootstock (mm)
F. ex M72 (6)	8.63	Ligustrum	10.65
F. ex 98 (7)	8.37	Ligustrum	10.20
F. ex M72 (8)	7.33	Syringa	9.28
F. ex 98 (9)	8.04	Syringa	8.17
F. *ex* M72 (5)	7.34	Fraxinus	10.35
F. ex 98 (4)	7.43	Fraxinus	9.32
F. ex M72 (2)	4.70	*F. chinensis*	4.88
F. ex M72 (1)	4.63	*F. paxiana*	4.28
F. ex 98 (10)	7.45	*F. japonica* interstock	10.35
F. ex 98 (11)	8.35	*F. platypoda* interstock	10.35
F. ex 98 (12)	*F. excelsior*	Budded *F.paxiana*	4.89
F. ex M72 (13)	*F. excelsior*	Budded *F.paxiana*	4.89
F.ex 98 (14)	*F.excelsior*	Budded *F. excelsior*	10.59
F.ex M72 (15)	*F. excelsior*	Budded *F. excelsior*	10.59
F.ex M72 (16)	*F. excelsior*	Budded *F. platypoda*	4.80
F.ex. M72 (17)	*F.excelsior*	Budded *F. platypoda*	4.80

( ) = Treatment number

2.3.2 Rootstocks

The ash rootstocks used were bareroot plants of common ash (*Fraxinus excelsior*) and Asiatic species (*Fraxinus paxiana, Fraxinus chinensis* and *Fraxinus platypoda*) and were cleft grafted. Bareroot plants of *Syringa vulgaris* and *Ligustrum ovalifolium* were also used as rootstocks. Common ash rootstocks with a diameter range of 8 – 13 millimeters were obtained in February 2013 from Coillte Nursery located at Ballintemple, Co Carlow. They had been held in cold storage at 3^{o}C until June 2013, then lined out singly in trenches at Kinsealy for 10 months. They were watered and weeds removed by hand as required, then they were lifted and place in cold storage in March 2014, allowed to dry off then used as rootstocks in April 2014. The *Ligustrum ovalifolium* and *Syringa vulgaris* rootstocks were barerooted and obtained from Sylva Company in Belgium in 2014.

The species *Fraxinus chinensis* and *Fraxinus paxiana* were grown at Kinsealy in 2012 from seed collected at John Fitzgerald Kennedy (JFK) arboretum located at New Ross Co Wexford. They were germinated in seed trays in March 2013 with Bord na Móna seed and modular compost. Once the seedings were big enough to handle easily they were pricked off in 25th April 2013 into 350 millilitre rootrainers, which contained Bord na Móna nursery stock growing medium (Appendix 4) and the plants were grown for one year prior to grafting. These plantlets were grown in an unheated greenhouse and were left to partially dry out prior to grafting. Grafting was performed by removing them from the rootrainers as required for cleft grafting in 2014.

2.3.3 Interstocks

In the experiment, with interstocks of *Fraxinus japonica* and *Fraxinus platypoda* and with the scion of *Fraxinus excelsior* Clone 98, common ash rootstocks were

used. The interstock scions of this material were collected from JFK Arboretum, 19th March 2014, stored in a fridge at 3°C until required. The interstock shoots consisted of a piece of stem internode i.e. without axillary buds, so the shoots selected were those with internodes which were as long as possible to facilitate graft matching. It was difficult to find sufficient shoots of *Fraxinus japonica* and *Fraxinus platypoda* with long internodes as interstocks.

2.4 Grafting experiment

A off-centre cleft graft was used in all cases of grafting's reported in this thesis and T budding. This is a relatively simple graft type and one of the most common types (Lamb *et al.*, 1985). It is illustrated in Images 1.11 and 2.2. This off-centre cleft graft involved cutting the barerooted rootstock about 3 centimetres above the root collar and then making a downward incision, about 2.5 centimetres deep close to the bark at an off-centre position to open a cleft. The scion was prepared by making two wedge-shaped sloping incisions about 3 centimetres long, with one side of the scion thicker than the other. The scions were then inserted into the cleft so that the cambial areas of scion and rootstock were in contact Image 1.11 and Image 2.2. Extreme care was taken when performing this graft to avoid splitting the rootstock. Where interstocks were used they were grafted sequentially starting with the rootstock cuts, then the interstock and finally the chosen scion. The graft union was then tied with a rubber band and the entire scion and graft union point was dipped in paraffin wax at 75°C (Hewson, 2012). The rubber bands were of sufficient tightness to ensure that the graft remained in place while also allowing expansion once the union began callusing. In the case where interstocks were used, common ash

rootstocks were first selected, then the interstocks of *F. japonica* and *F. platypoda* were grafted onto the ash rootstocks and thereafter Clone 98 was in turn grafted onto the top of the interstock.

All the grafted and budded plants were then potted into 3 litre pots, which contained Bord na Móna nursery stock growing medium, and grown on for two years (Appendix 4). The grafted plants were then placed in an unheated greenhouse. In relation to their cultural requirements they were watered sufficiently to ensure they were growing, glasshouse was ventilated and in the second growing season the leaf colour was judged to be non-chlorotic therefore no additional plant feeding was deemed necessary.

Budding was performed 8th and 9th September 2014. Bud sticks of *Fraxinus excelsior* Clone M72 and Clone 98 whch had approximately 40 buds of each clone were collected, at the start of each day and placed in a fridge at 3^{o}C until required so that they were maintained fresh. The method used was T budding which is described in the literature review (Image 1.14). Height of budding depends on the material being propagated and, in the case of the experiment reported here in this thesis, it was done at 10 centimetres above ground level.

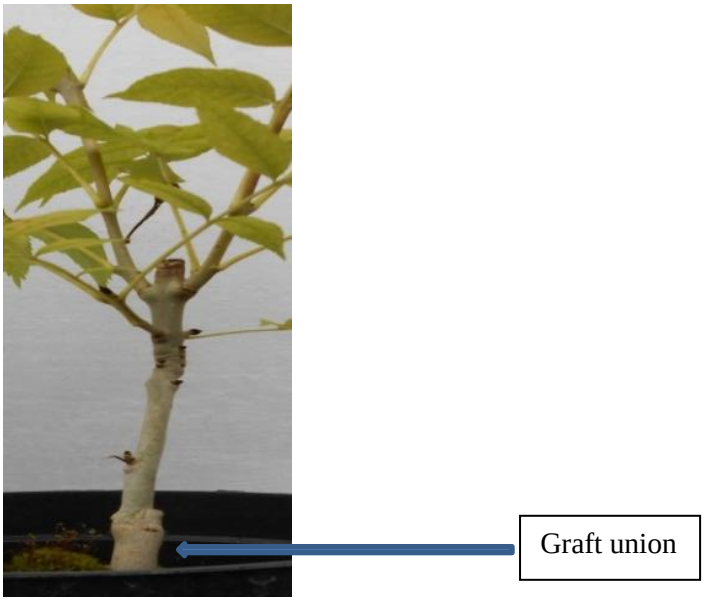

Image 2.2 Off centre cleft grafting image

Image J Mc Namara, 2016

2.5 Experimental design and tasks

When the buds on the scions of the grafted plants had flushed and considered to be viable, the plants in each treatment were randomized into experimental blocks on a bench on 30th May 2014 within the glasshouse at Kinsealy. The budded plants were added to the randomized blocks in September. The experimental design was in six randomized blocks and the block sequence was as follows: Block 3, Block 2, Block 5, Block 6, Block 1, and Block 4. The design consisted of 17 Treatments, with each treatment consisting of seven to 32 replicates, as not all plants survived as viable grafts (Table 2.4). Within each block, the treatment plants were randomly placed according to the design. The viable grafts were distributed across as many blocks as possible (Grant , 2018) i.e. Treatment 2 had 24 surviving plants therefore they could be evenly distributed.

This was a matter of allocating as sensibly as possible to blocks given the small numbers of some treatments. There's no problem allocating more that 1 of a treatment to block but it is desireable to have at least 1 per treatment in each block. After that it was a matter of dividing the available grafts across 6 blocks.

Each graft in a block was assigned a random number and then these were sorted in ascending order to give a position in the block, i.e. the lowest number went to position 1, the next lowest to position 2, etc.

Table 2.4 The number of plants assigned / block

Number of plants per block

Treatment	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6
1	2	2	2	2	2	2
2	4	4	4	4	4	4
3	5	5	5	5	4	4
4	5	5	5	5	5	4
5	5	5	5	5	5	5
6	3	3	3	3	2	2
7	2	2	2	1	1	1
8	4	4	3	3	3	3
9	2	2	2	2	2	2
10	3	3	3	2	2	2
11	5	5	5	4	4	4
12	6	6	5	5	5	5
13	5	5	5	5	5	5
14	5	5	5	5	5	5
15	5	5	5	5	5	5
16	2	2	2	2	1	1
17	2	2	2	1	0	0

The grafted plants were observed on a daily basis and watered as required to ensure they were maintained in good growing conditions. Shoots which developed from below the point of the graft union were routinely removed. Grafted plants were judged to be viable if they produced growing shoots from the

scions. Lilac and privet exhibited an issue with rootstock water shoots which needed to be removed as soon as they appeared. This issue was quite manageable, however they continued to re-appear two years after grafting.

Survival of grafted plants was recorded at the end of the growing seasons in 2014 and 2015. All Treatments were assessed for graft survival on 5th December 2014 and again on 4th December 2015.

The new shoot growths produced by each grafted plant were recorded at the end of the growing season on 5th December 2014, 245 days after completion of grafting. Subsequently in 2015 at the end of the second growing season, recording of growth was carried out on 4th December which was 609 days after completion of grafting. The shoot growth extensions on all the main shoots were measured using a measuring tape from the point of grafting union to the terminal bud (Image 2.3). In addition, the extension growths of all side shoots were also recorded. For grafted plants, the total vegetative growth increment per year was recorded by the addition of the growth measurements recorded for the main shoots plus all side shoots. Generally, in the first year after grafting the scion grew as a single shoot and in year two the grafted plants produced side shoots as well as growth in the main shoot. Shoots were not staked as would sometimes be the case in nurseries to ensure apically dominant plants as the objective in this experiment was to have multiple shoots which would flower earlier. These side shoots were measured and added to the increment of the main shoot to give a total value for the shoot production growth. This data was then statistically analysed using continuous responses were analysed using an ANOVA-type linear model fitted with the Mixed procedure in SAS 9.4 (SAS, 2014).

Budding of *Fraxinus excelsior* onto various rootstocks was performed on 8th and 9th September 2014. They were considered viable on 5th December 2014 if they showed a green colour. Failed buddings appeared brown. Shoot growths from the buds was recorded on 4th December 2015, as described for the grafted plants. Shoots from budded plants were allowed to grow naturally as the objective was to obtain multi shoots.

In 2014, measurements were taken and recorded for the shoot growth for that year and 2015 measurements were taken for the new growth in that year, as illustrated in Image 2.3.

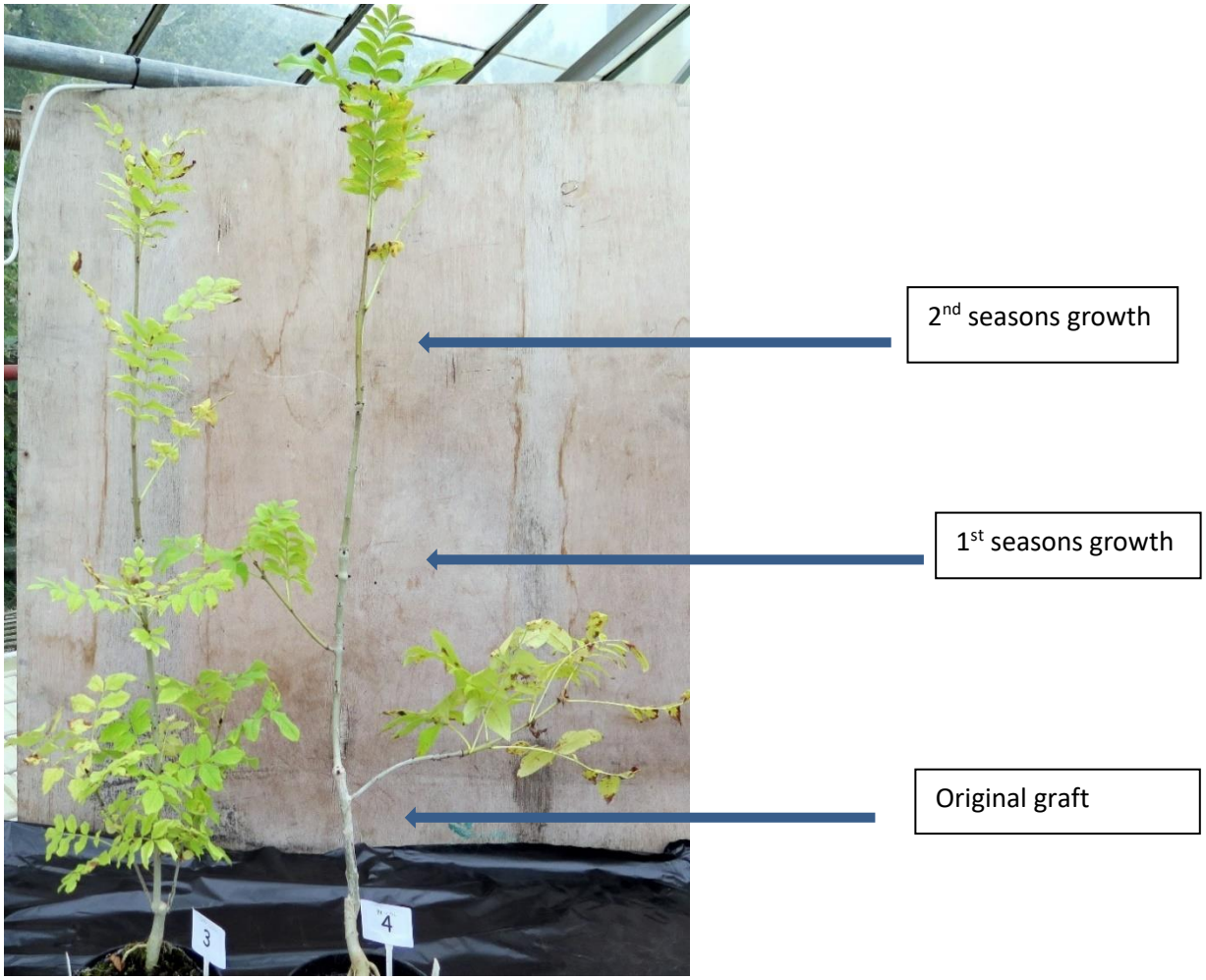

Image 2.3 Growth example of *Fraxinus excelsior* Clone 98 grafted onto *Fraxinus excelsior* rootstock, (Treatment 4) and an ungrafted (Treatment 3).

Image J Mc Namara, 2016

To illustrate the relative effects of rootstock treatment, the vegetative growth increment for each year was computed as follows for the purpose of ranking rootstock effect. The non-grafted (Control) value was used as 100%. Deviations from this were assigned a positive (+) or a negative (-) value. The extent of possible graft incompatibilities between the rootstock and scion was estimated by calculating the extent of the restrictions at the graft unions. This involved measuring stem diameters two centimetres above and two centimetres below the graft unions using

a callipers. This measurement was taken on grafted plants on 9th August 2016 to determine the location and extent of the growth restrictions above and below the graft union position and comparing the responses in self grafts with heterografts. This data was then computed as the percentage change in diameter of scions relative to rootstock diameter. This exercise did not take into account the original differences at time of grafting. Positive values indicated that the scion diameters were greater than the rootstock and negative values where the scion diameter was less than the rootstock diameter.

Bud burst was recorded, starting on 7th April and continuing on the following dates 10th, 12th, 14th 17th, 24th, 27th, 1st May,7th 11th, and 18th 2016 until all buds that were viable had emerged. To assess bud flushing, a UK scale (Hemery, 2011) was used to determine bud emergence which is a 0-4 scale classifying the progression of the bud opening from dormancy to full leaf emergence (Image 2.4).

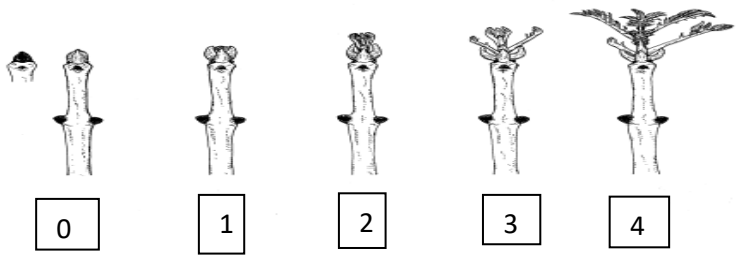

Image 2.4 Ash bud flushing

Available on the internet at

https://gabrielhemery.com/phenology-and-frost/ (accessed 22/05/2019).

(0)-represents bud fully closed, (1)-bud starting to swell and losing its black colour, (2)-leaves beginning to emerge, (3)-first leaves emerged and (4)- leaves fully emerged (Hemery, 2011).

Flowering and fruiting as depicted in images 2.5, 2.6 and 2.7 respectively.

Image 2.5

Image 2.6

Image 2.7

Image 2.5 A branch with pure male inflorescences, only staminate flowers of Fraxinusexcelsior (Wallander, 2013)

Image 2.6 Branch of Fraxinus excelsior with female inflorescences, pistillate flowers with rudimentary stamens (Wallander, 2013)

Image 2.7 Mast fruiting of Fraxinus excelsior (Wallander, 2013)

Available on the internet at http://www.oleaceae.info/index.html. (Accessed: 28/01/2019).

To analyse the data which was collected in 2014 and 2015 the following methodology was applied, using analysis of variance (ANOVA) with Tukey adjustment in SAS Enterprise Guide SAS 9.4 (SAS, 2014). Data shown is mean +SE (n=6). The Tukey-Kramer is an algorithm within the SAS statistical analysis package that reduces the number of false positive results. The LINES procedure the algorithm generated the letters for showing treatment pairs which were of statistical significance.

Continuous responses were analysed using an ANOVA-type linear model fitted with the mixed procedure in SAS 9.4 (SAS, 2014). The residuals from the analysis were checked to ensure that the assumptions of the model were met and, where appropriate, log transformation was used to correct distributional issues. Means from the analysis were separated using pairwise t-tests and multiplicity corrections were made with a

Tukey correction to the p-values. Means on the log scale were back-transformed to medians on the data-scale, along with confidence limits.

The recommended format for the SAS reference was SAS Institute Inc. (2014). *SAS/STAT® 13.2 User's Guide*. Cary, NC: SAS Institute Inc.

Unbalanced data was handled straightforwardly when using software designed for mixed models. It was more of a concern for simple ANOVA type models in the past when balanced data was expected but now it is not a practical concern. Appropriate standard errors are produced easily.

Means were separated by t-tests, with appropriate adjustment (Tukey, simulation) for multiple comparisons. Viability percentages were arrived at by computing the number of plants which were originally grafted and the number surviving for 2014 and 2015.

Survival data was analysed using logistic regression where viability (yes/no) is a binary response. Odds ratios are the natural way to express the outcomes.

Comparisons were made with the Control (Treatment 3) and significance as indicated was shown by the range of values between the confidence limits not including the value 1. All odds ratios are expressed as values greater than 1 and this changes the direction of some comparisons relative to the control.

Comparisons were also made when assessing survival with Treatment 4 and Treatment 5 the logic being that are *Fraxinus excelsior* rootstocks which would be the standard rootstock used.

Plants were measured at the end of the first growing season and results tabulated. Statistical analysis was performed on 2014 growth measurements; however, it should be noted that the vegetative growth measurements represent just the first

year of growth. Treatment, in a one-way classification ANOVA, was highly significant (F statistic, $p<0.0001$) and the tests of differences between means are represented as letter superscripts where means having letters in common are not significantly different.

Comparisons are made with the non-grafted Control (Treatment 3) and significance as indicated is shown by the range of values between the confidence limits not including the value 1. All odds ratios are expressed as values greater than 1 and this changes the direction of some comparisons relative to the control. (2015)

Chapter 3: Results

3.1 Introduction

The main tasks were to record the graft survival rates of the various combinations of scions and rootstocks and then record shoot extension growth, number of shoots produced, stem diameter responses around the graft union, bud flushing, flowering and graft scion, rootstock compatibility. All these tasks were measured numerically, apart from the graft scion compatibility, which was an observational exercise.

3.2 Number of plants grafted and randomised

Table 3.1 shows the number of surviving plants obtained from various combinations of grafting's and buddings.

Table 3.1 The treatments and the various rootstocks they were grafted onto

Treatment Number	Graft combination	Number of plants grafted	Number of plants obtained and randomized
1	F. *ex* Clone M72 grafted onto *Fraxinus paxiana*	30	12
2	F. *ex* Clone M72 grafted onto *Fraxinus chinensis*	31	24
3	*Fraxinus excelsior* non – grafted (control)	30	28
4	F. *ex* Clone 98 grafted onto *Fraxinus excelsior*	30	29
5	F. ex Clone M72 grafted onto *Fraxinus excelsior*	30	30
6	F. *ex* Clone M72 grafted onto *Ligustrum ovalifolium*	30	16
7	F. *ex* Clone 98 grafted onto *Ligustrum ovalifolium*	30	9
8	F. *ex* Clone M72 grafted onto *Syringa vulgaris*	30	20
9	F. *ex* Clone 98 grafted onto *Syringa vulgaris*	30	12
10	F. *ex* Clone 98 interstock *Fraxinus japonica*	25	15
11	F. *ex* Clone 98 interstock *Fraxinus platypoda*	30	27
12	F. *ex* Clone 98 budded onto *Fraxinus paxiana*	32	32
13	F. *ex* Clone M72 budded onto *Fraxinus paxiana*	30	30
14	F. ex Clone 98 budded onto *Fraxinus excelsior*	30	30
15	F. *ex* Clone M72 budded onto *Fraxinus excelsior*	30	30
16	F. *ex* Clone 98 budded onto *Fraxinus platypoda*	10	10
17	F. *ex* Clone M72 budded onto *Fraxinus platypoda*	7	7
Total		465	361

Table 3.1a shows the significant difference when compared to the Control, * indicating statistical significance.

Table 3.1a Table of results

Effect	Point Estimate	95% Wald Confidence Limits			p-value
treat 1 vs 3	5.353	0.234	122.478		0.29
treat 2 vs 3	0.287	0.061	1.357		0.12
treat 4 vs 3	1.725	0.206	14.418		0.61
treat 5 vs 3	5.353	0.234	122.478		0.29
treat 6 vs 3	0.100	0.022	0.446		0.0026
treat 7 vs 3	0.039	0.008	0.178	*	<0.0001
treat 8 vs 3	0.171	0.038	0.780	*	0.023
treat 9 vs 3	0.059	0.013	0.266	*	0.0002
treat 10 vs 3	0.129	0.028	0.603	*	0.0092
treat 11 vs 3	0.689	0.122	3.902		0.67
treat 12 vs 3	5.704	0.250	130.019		0.27
treat 13 vs 3	0.059	0.013	0.266	*	0.0002
treat 14 vs 3	5.353	0.234	122.478		0.29
treat 15 vs 3	5.353	0.234	122.478		0.29
treat 16 vs 3	1.842	0.071	47.647		0.71
treat 17 vs 3	1.316	0.047	36.787		0.87

Logistic regression with Tukey adjustment using SAS 9.4 (SAS, 2014).

Table 3.1a shows that Treatment 7, Treatment 8, Treatment 9, Treatent 10 and Treatment 13 are significantly different to Treatment 3.

To compare the various treatments with other standards, treatments 4 and 5 were used as they were *Fraxinus excelsior* rootstocks which would be the normal rootstock used when grafting ash.

Table 3.1b shows that when Treatment 4 was compared to the various Treatments; Treatment 1, Treatment 6, Treatment 7, Treatment 8, Treatment 9, Treatment 10 and are significantly different to Treatment 4.

Table 3.1c demonstrates that Treatment 1, Treatment 2, Treatment 6, Treatment 7, Treatment 8, Treatment 9, Treatment 10 and are significantly different to Treatment 5.

Table 3.1b Table of results

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 4 vs 1	29.4	4.7	166.7	*	0.0003
Treatment 4 vs 2	6.0	0.9	38.5		0.059
Treatment 4 vs 3	1.7	0.2	14.5		0.61
Treatment 5 vs 4	3.1	0.2	83.7		0.5
Treatment 4 vs 6	17.2	2.8	111.1	*	0.002
Treatment 4 vs 7	45.5	7.1	250.0	*	<0.0001
Treatment 4 vs 8	10.1	1.6	62.5	*	0.013
Treatment 4 vs 9	29.4	4.7	166.7	*	0.0003
Treatment 4 vs 10	13.3	2.1	83.3	*	0.0061
Treatment 4 vs 11	2.5	0.3	18.9		0.37

Logistic regression with Tukey adjustment using SAS 9.4 (SAS, 2014).

Table 3.1c Table of results

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 5 vs 1	90.3	4.8	>999	*	0.002
Treatment 5vs 2	18.7	1.0	360.7	*	0.05
Treatment 5 vs 3	5.4	0.2	122.5		0.29
Treatment 5 vs 4	3.1	0.1	83.7		0.5
Treatment 5 vs 6	53.6	2.9	>999	*	0.008
Treatment 5 vs 7	138.1	7.3	>999	*	0.001
Treatment 5 vs 8	31.3	1.7	591.5	*	0.02
Treatment 5 vs 9	90.3	4.8	>999	*	0.0026
Treatment 5 vs 10	41.3	2.1	791.2	*	0.014
Treatment 5 vs 11	7.8	0.4	165.3		0.19

Logistic regression with Tukey adjustment using SAS 9.4 (SAS, 2014).

3.3 Survival of grafted combinations after the first year of growth, 2014

Table 3.2 shows the survival of various combinations of F. *excelsior* scions when grafted onto different Fraxinus species as rootstocks and interstocks and onto Syringa and Ligustrum. All graft combinations produced plants. There was no block effect in the analysis of survival in both years. It is notable that the plant survival was highest in the range (100-97%) when the rootstock was *Fraxinus excelsior*. Two Control plants of

Fraxinus excelsior (Treatment 3) which had been cut back without making a subsequent graft had died, which indicated that the cutting back procedure could reduce plant survival. On the other hand, the rootstocks of *Fraxinus chinensis* and *Fraxinus paxiana* resulted in a substantial decrease in graft survival, 77%, and 40% respectively. The effect of using interstocks of *Fraxinus platypoda* and *Fraxinus japonica* resulted in 90% and 60% of plant survival, respectively.

The survival of *Fraxinus excelsior* scions on rootstocks of Syringa and Ligustrum were generally lower than when the rootstocks were Fraxinus spp. At the end of the first growing season, Clone M72 produced more plants when grafted onto Syringa and Ligustrum rootstocks than *Fraxinus excelsior* Clone 98. The graft survival for Clone M72 on *Syringa vulgaris* was 67% while Clone 98 had a survival rate of 40%. The graft survival for Clone M72 on Ligustrum was 53% while Clone 98 was 30% (Table 3.2).

Budding survival was assessed on 5th December 2014 and all buddings appeared to be viable, as they exhibited a green colour.

Table 3.2 Survival of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of first year of growth, 2014

Scions	Rootstock	Treatment	No grafts	No grafts Surviving	% Survival
F. *excelsior* (non – grafted (Control)	F. *excelsior*	3	30	28	93 (survival)
F. *excelsior* Clone 98	F. *excelsior*	4	30	29	97
F. *excelsior* Clone M72	F. *excelsior*	5	30	30	100
F. *excelsior* Clone M72	F. *paxiana*	1	30	12	40
F. *excelsior* Clone M72	F. *chinensis*	2	31	24	77
F. *excelsior* Clone 98 with interstock *F. japonica*	F. *excelsior*	10	25	15	60
F. *excelsior* Clone 98 with interstock *F. platypoda*	F. *excelsior*	11	30	27	90
Ligustrum and Syringa rootstocks					
F. *excelsior* Clone 98	*Ligustrum ovalifolium*	7	30	9	30
F. *excelsior* Clone M72	*Ligustrum ovalifolium*	6	30	16	53
F. *excelsior* Clone 98	*Syringa vulgaris*	9	30	12	40
F. *excelsior* Clone M72	*Syringa vulgaris*	8	30	20	67

It also shows that the ungrafted Control had significantly more survival rate when compared to Treatment 1, Treatment 6, Treatment 7, Treatment 8, Treatment 9 and Treatment 10. Table 3.3 a shows the odds limits, confidence values and p-values when comparing Treatment 3 with various Treatments. Treatment 3 is significantly different then Treatment 1, Treatment 6, Treatment 7, Treatment 8, Treatment 9 and Treatment 10.

Table 3.3b and Table 3.3c show the values when compared to Treatment 4 and Treatment 5. The logic of using these comparisons was to ascertain if there were any

signifcant differences when comparing Clone 98 and Clone M72 which were grafted onto *Fraxinus excelsior* rootstocks which would be the normal rootstock used.

Table 3.3 Analysis of survival of *Fraxinus excelsior* 2014

Table 3.3a Odds ratios and confidence limits for the analysis of survival of *Fraxinus excelsior* scions grafted onto various species of rootstocks in comparison to Treatment 3 at the end of first year of growth, 2014. * indicates statistical significance

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 3 vs 1	16.9	3.8	76.9	*	0.0002
Treatment 3 vs 2	3.5	0.7	16.4		0.12
Treatment 4 vs 3	1.73	0.21	14.4		0.61
Treatment 5 vs 3	5.36	0.23	122.5		0.29
Treatment 3 vs 6	10.0	2.2	45.5	*	0.003
Treatment 3 vs 7	25.6	5.6	125.0	*	<0.0001
Treatment 3 vs 8	5.8	1.3	26.3	*	0.023
Treatment 3 vs 9	16.9	3.8	76.9	*	0.0002
Treatment 3 vs 10	7.8	1.7	35.7	*	0.009
Treatment 3 vs 11	1.5	0.3	8.2		0.67

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

Table 3.3b shows comparisons to Treatment 4, where Treatment 1, Treatment 6, Treatment 7, Treatment 8, Treatment 9 and Treatment 10 were significantly different.

Table 3.3b Odds ratios and confidence limits for the analysis of survival of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of first year of growth, 2014 in comparison to Treatments 4. * indicates statistical significance

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 4 vs 1	29.4	4.7	166.7	*	0.0003
Treatment 4 vs 2	6.0	0.9	38.5		0.059
Treatment 4 vs 3	1.7	0.2	14.5		0.61
Treatment 5 vs 4	3.1	0.2	83.7		0.5
Treatment 4 vs 6	17.2	2.8	111.1	*	0.002
Treatment 4 vs 7	45.5	7.1	250.0	*	<0.0001
Treatment 4 vs 8	10.1	1.6	62.5	*	0.013
Treatment 4 vs 9	29.4	4.7	166.7	*	0.0003
Treatment 4 vs 10	13.3	2.1	83.3	*	0.0061
Treatment 4 vs 11	2.5	0.3	18.9		0.37

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

Table 3.3c shows comparisons to Treatment 5, where Treatment 1, Treatment 2, Treatment 6, Treatment 7, Treatment 8, Treatment 9 and Treatment 10 were significantly different.

Table 3.3c Odds ratios and confidence limits for the analysis of survival of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of first year of growth, 2014 in comparison to Treatments 5. * indicates statistical significance

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 5 vs 1	90.3	4.8	>999	*	0.002
Treatment 5vs 2	18.7	1.0	360.7	*	0.05
Treatment 5 vs 3	5.4	0.2	122.5		0.29
Treatment 5 vs 4	3.1	0.1	83.7		0.5
Treatment 5 vs 6	53.6	2.9	>999	*	0.008
Treatment 5 vs 7	138.1	7.3	>999	*	0.001
Treatment 5 vs 8	31.3	1.7	591.5	*	0.02
Treatment 5 vs 9	90.3	4.8	>999	*	0.0026
Treatment 5 vs 10	41.3	2.1	791.2	*	0.014
Treatment 5 vs 11	7.8	0.4	165.3		0.19

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

Table 3.3d displays the Treatment details.

Table 3.3d Treatment details

Treatment	Scion / interstock	Rootstock
3	* Ungrafted	F.*excelsior*
1	F. *excelsior* Clone M72	F. *paxiana*
2	F. *excelsior* Clone M72	F. *chinensis*
4	F. *excelsior* Clone 98	F.*excelsior*
5	F. *excelsior* Clone M72	F. *excelsior*
6	F. *excelsior* Clone M72	*Ligustrum ovalifolium*
7	F. *excelsior* Clone 98	*Ligustrum ovalifolium*
8	F. *excelsior* Clone M72	*Syringa vulgaris*
9	F. *excelsior* Clone 98	*Syringa vulgaris*
10	F. *excelsior* Clone 98 interstock *japonica*	F.*excelsior*
11	F.*excelsior* Clone 98 interstock platypoda	F.*excelsior*

3.4 Vegetative growth measurements of grafted plants at the end of the first growing season 2014, recorded 245 days after grafting

No block effects were found in the compilation of data. The greatest vegetative growth was obtained from *Fraxinus excelsior* control plants which were not grafted but which had been cut back in a similar manner to grafted plants. This was to ascertain what effect the act of cutting back had on the plants and simulate what survival and vegetative growth resulted. This treatment was significantly different when compared to all other treatments (Table 3.4). The vegetative growth obtained with two different scions of *Fraxinus excelsior* clones M72 and 98 were statistically similar but lower than the non- grafted Controls (Table 3.4).

All rootstocks of Asiatic species of ash, as well as interstocks, resulted in significant reduction of vegetative growth in the scions of both Clones M72 and Clone 98 (Table 3.4).

Scion material of M72 grafted onto rootstocks of *Fraxinus paxiana* and *Fraxinus chinensis* resulted in a growth reduction of 95% and 91% respectively compared to non-grafted Control. The rootstocks of *Fraxinus paxiana* resulted in less growth than *Fraxinus chinensis*, however the effect was not significant. However, *Fraxinus paxiana* rootstocks gave significantly lower growth than rootstocks of both Syringa and Ligustrum. Using interstocks of *Fraxinus japonica* resulted in a greater growth reduction than when *Fraxinus platypoda* was used as interstock, 95% and 86% respectively, however they were not significantly different. Table 3.4 also shows that rootstocks of Lilac and Privet resulted in significant reductions in vegetative growth of scion shoots with each Clone of *Fraxinus excelsior* (Clone M72 and Clone 98) when compared to non-grafted Controls (Table 3.4). Furthermore, the rootstocks of Syringa gave a significantly greater reduction in growth compared to Ligustrum when the scion material was Clone 98 as opposed to Clone M72.

Vegetative growth of budded plants was not measured in 2014 as they had been grafted in September and had not grown out.

Table 3.4 Vegetative growth measurements of grafted plants at the end of the first growing season 2014, recorded 245 days after grafting

Scions	Rootstock	Treatment No	Grafts surviving (%)	Mean growth increment in (mm)
F. *excelsior* non – grafted	F. *excelsior*	3	93*(surviving)	338[a]
F. *excelsior* Clone 98	F. *excelsior*	4	97	200[b*]
F. *excelsior* Clone M72	F. *excelsior*	5	100	208[b*]
F. *excelsior* Clone M72	F. *paxiana*	1	40	16[f]
F. *excelsior* Clone M72	F. *chinensis*	2	77	32[def]
F. *excelsior* Clone 98 with interstock F. *japonica*	F. *excelsior*	10	60	18[ef]
F. *excelsior* Clone 98 with interstock F. *platypoda*	F. *excelsior*	11	90	48[cde]
F. *excelsior* Clone 98	*Ligustrum ovalifolium*	7	30	87[c]
F. *excelsior* Clone M72	*Ligustrum ovalifolium*	6	53	51[cd]
F. *excelsior* Clone 98	*Syringa vulgaris*	9	40	38[def]
F. *excelsior* Clone M72	*Syringa vulgaris*	8	67	57[cde]

Logistic regression with Tukey adjustment using SAS 9.4 (SAS, 2014).

*Superscript means with the same letter are not statistically different.

Budded plants were not measured in year one as they had not produced any extension growth at this stage.

3.5 Survival of graft combinations after second year of growth, 2015

At the end of the second year of growth, the survival of the various grafts was recorded (Table 3.5). Overall, the number of grafted plants which survived in 2014 remained much the same in 2015, with a few exceptions. The exceptions were *Fraxinus excelsior* Clone M72 grafted onto *Fraxinus chinensis* where the survival of grafts reduced from 77% in 2014 to 68% in 2015 (Tables 3.2 and 3.5). In the case of *Fraxinus excelsior*

Clone 98 with interstock *Fraxinus platypoda* graft viability reduced from 90% in 2014 to 87% in 2015 (Tables 3.2 and 3.5).

The survival of grafts of common ash grafted onto privet and lilac altered over the two years 2014 and 2015. In the case of *Fraxinus excelsior* Clone 98, grafted onto either privet or lilac, there was no change in the percentage of viable plants from 2014 to 2015. However, for *Fraxinus excelsior* Clone M72, there were some fatalities during the recording period. Survival decreased from 53% in 2014 to 37% in 2015 when the rootstock was privet and from 67% in 2014 to 33% in 2015 when the rootstock was lilac (Tables 3.2 and 3.5).

Buddings, when assessed at the end of the growing season in 2014, appeared to be 100% viable, however, there was a reduction in surviving buddings in 2015. The best performing treatment was *Fraxinus excelsior* Clone M72 budded onto *Fraxinus excelsior* rootstock at 70% survival, and in descending order of survival, *Fraxinus excelsior* Clone 98 budded onto *Fraxinus excelsior* rootstock 30% surviving; *Fraxinus excelsior* Clone 98 budded onto *Fraxinus paxiana* 28% surviving; *Fraxinus excelsior* Clone 98 budded onto *Fraxinus platypoda* 10% surviving; *Fraxinus excelsior* Clone M72 budded onto *Fraxinus paxiana* 7% surviving; and *Fraxinus excelsior* Clone M72 budded onto *Fraxinus platypoda* failed completely (Table 3.5).

Table 3.5 Survival of *Fraxinus excelsior* scions grafted and budded onto various species of rootstocks at the end of second year of growth, 2015

Scions	Rootstock	Treatment	No grafts made	No grafts Surviving	% Survival
F. *excelsior* (Control) non – grafted	F. *excelsior*	3	30	28	93
F. *excelsior* Clone 98	F. *excelsior*	4	30	29	97
F. *excelsior* Clone M72	F. *excelsior*	5	30	30	100
F. *excelsior* Clone M72	F. *paxiana*	1	30	12	40
F. *excelsior* Clone M72	F. *chinensis*	2	31	21	68
F. *excelsior* Clone 98 with interstock F. *japonica*	F. *excelsior*	10	25	15	60
F. *excelsior* Clone 98 with interstock F. *platypoda*	F. *excelsior*	11	30	26	87
Ligustrum and Syringa rootstocks					
F. *excelsior* Clone 98	*Ligustrum ovalifolium*	7	30	9	30
F. *excelsior* Clone M72	*Ligustrum ovalifolium*	6	30	11	37
F. *excelsior* Clone 98	*Syringa vulgaris*	9	30	12	40
F. *excelsior* Clone M72	*Syringa vulgaris*	8	30	10	33
Buddings					
F. *excelsior* Clone 98	F. *excelsior*	14	30	9	30
F. *excelsior* Clone M72	F. *excelsior*	15	30	21	70
F. *excelsior* Clone 98	F. *platypoda*	16	10	1	10
F. *excelsior* Clone M72	F. *platypoda*	17	7	0	0
F. *excelsior* Clone 98	F. *paxiana*	12	32	9	28
F. *excelsior* Clone M72	F. *paxiana*	13	30	2	7

Table 3.6 a has the related statistics for the survival at the end of two years of growth when Treatment 3 was compared with all other Treatments the following were significant, Treatment 1, Treatment 2, Treatment 6, Treatment 7, Treatment 8, Treatment 9, Treatment 10, Treatment 12, Treatment 13, Treatment 14, Treatment 15, Treatment 16 and Treatment 17.

Table 3.6 Analysis of survival of *Fraxinus excelsior* 2015

Table 3.6a Odds ratios and confidence limits for the analysis of survival of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of second year of growth, 2015. * indicates statistical significance

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 3 vs 1	16.9	3.8	76.9	*	0.0002
Treatment 3 vs 2	5.6	1.2	25.0	*	0.26
Treatment 4 vs 3	1.7	0.2	14.4		0.61
Treatment 5 vs 3	5.4	0.2	122.5		0.29
Treatment 3 vs 6	19.2	4.3	90.9	*	0.0001
Treatment 3 vs 7	25.6	5.6	125.0	*	<0.0001
Treatment 3 vs 8	22.2	4.9	100.0	*	<0.0001
Treatment 3 vs 9	16.9	3.8	76.9	*	0.0002
Treatment 3 vs 10	7.8	1.7	35.7	*	0.009
Treatment 3 vs 11	1.9	0.4	10.2		0.43
Treatment 3 vs 12	28.6	6.2	125.0	*	<0.0001
Treatment 3 vs 13	125.0	20.4	1000.0	*	<0.0001
Treatment 3 vs 14	25.6	5.6	125.0	*	<0.0001
Treatment 3 vs 15	5.0	1.1	23.3	*	0.38
Treatment 3 vs 16	71.4	7.8	1000.0	*	0.0002
Treatment 3 vs 17	166.7	6.1	>1000.0	*	0.0025

Logistic regression with Tukey adjustment using SAS 9.4 (SAS, 2014).

Table 3.6 b has the related statistics for the viability at the end of two years of growth when Treatment 4 was compared with all other Treatments the following were significant, Treatment 1, Treatment 2, Treatment 6, Treatment 7, Treatment 8, Treatment 9, Treatment 10, Treatment 12, Treatment 13, Treatment 14, Treatment 15, Treatment 16 and Treatment 17.

Table 3.6c has the related statistics when all Treatments were compared to Treatment 5 and had the same overall result in terms of statistical comparisons as Table 3.6 b.

Table 3.6b Odds ratios and confidence limits for the analysis of survival of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of second year of growth, 2015 in comparison to Treatment 4. * indicates statistical significance

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 4 vs 1	29.1	4.5	179.6	*	0.0003
Treatment 4 vs 2	9.6	1.5	59.8	*	0.015
Treatment 4 vs 3	1.7	0.2	14.4		0.61
Treatment 5 vs 4	3.1	0.1	83.6		0.5
Treatment 4 vs 6	33.3	5.4	206.8	*	0.0002
Treatment 4 vs 7	44.5	7.1	280.1	*	<0.0001
Treatment 4 vs 8	39.4	6.2	239.6	*	<0.0001
Treatment 4 vs 9	29.1	4.7	179.7	*	0.0003
Treatment 4 vs 10	13.3	2.1	84.6	*	0.0061
Treatment 4 vs 11	3.3	0.5	23.5		0.23
Treatment 4 vs 12	48.6	7.8	304.7	*	<0.0001
Treatment 4 vs 13	224.2	26.8	>999	*	<0.0001
Treatment 4 vs 14	44.5	7.1	280.1	*	<0.0001
Treatment 4 vs 15	8.7	1.4	54.7	*	0.021
Treatment 4 vs 16	124.6	10.7	>999	*	0.0001
Treatment 4 vs 17	295.0	9.0	>999	*	0.0014

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

Table 3.6c Odds ratios and confidence limits for the analysis of survival of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of second year of growth, 2015 in comparison to Treatment 5. * indicates statistical significance

Effect	Point Estimate	95% Wald Confidence Limits			p-value
Treatment 5 vs 1	90.3	4.8	>999	*	0.0026
Treatment 5 vs 2	29.8	1.6	562.8	*	0.024
Treatment 5 vs 3	5.3	0.2	122.5		0.29
Treatment 5 vs 4	3.1	0.1	83.6		0.5
Treatment 5 vs 6	103.5	5.5	>999	*	0.002
Treatment 5 vs 7	138.1	7.3	>999	*	0.001
Treatment 5 vs 8	119.1	6.3	>999	*	0.0014
Treatment 5 vs 9	90.3	4.8	>999	*	0.0026
Treatment 5 vs 10	41.3	2.2	790.9	*	0.014
Treatment 5 vs 11	10.4	0.5	211.8		0.13
Treatment 5 vs 12	160.0	8.0	>999	*	0.0008
Treatment 5 vs 13	695.7	30.4	>999	*	<0.0001
Treatment 5 vs 14	138.1	7.3	>999	*	0.001
Treatment 5 vs 15	27.0	1.4	512.8	*	0.028
Treatment 5 vs 16	386.5	13.3	>999	*	0.0005
Treatment 5 vs 17	915.5	14.1	>999	*	0.0014

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

Table 3.6d Treatment details

Treatment	Scion / interstock	Rootstock
3	Ungrafted	F.*excelsior*
1	F. *excelsior* Clone M72	F. *paxiana*
2	F. *excelsior* Clone M72	F. *chinensis*
4	F. *excelsior* Clone 98	F.*excelsior*
5	F. *excelsior* Clone M72	F. *excelsior*
6	F. *excelsior* Clone M72	*Ligustrum ovalifolium*
7	F. *excelsior* Clone 98	*Ligustrum ovalifolium*
8	F. *excelsior* Clone M72	*Syringa vulgaris*
9	F. *excelsior* Clone 98	*Syringa vulgaris*
10	F. *excelsior* Clone 98 interstock *japonica*	F.*excelsior*
11	F. *excelsior* Clone 98 interstock platypoda	F.*excelsior*
12	F. *excelsior* Clone 98 -budded	F. *paxiana*
13	F. *excelsior* Clone M72 - budded	F. *paxiana*
14	F. *excelsior* Clone 98 -budded	F.*excelsior*
15	F. *excelsior* Clone M72 - budded	F.*excelsior*
16	F. *excelsior* Clone 98 -budded	F.*excelsior*
17	F. *excelsior* Clone M72 - budded	F. *platypoda*

To check how the different treatments affected the scion survival after two years of growth and the order ranking, Table 3.7 was compiled. There was no significant differences difference between the graft viabilities of *Fraxinus excelsior* Clones M72 and 98 when grafted onto *Fraxinus excelsior* rootstocks. However, Ligustrum and Syringa had a major reduction effect for each of the *Fraxinus excelsior* Clones M72 and 98.

A summary of the ranking of graft and budding survival for each combination is given in Table 3.7. It shows the highest survival (97-100%) for scions of *Fraxinus excelsior* grafted onto *Fraxinus excelsior*. Using other species of rootstocks resulted in reduced percentages of surviving plants from grafting's and buddings especially rootstocks of Ligustrum and Syringa (range 30-40%) as outlined in Table 3.7 which is an observational exercise of viewing the measurements.

Table 3.7 Graft survival ranking for each of the treatments at the end of the growing season, 2015. Figures in brackets represent treatment numbers.

Scion	Rootstock	Survival %	Ranking
F. *ex* Clone M72 (5)	*Fraxinus excelsior*	100	1
F. *ex* Clone 98 (4)	*Fraxinus excelsior*	97	2
Ash non-grafted (Control) (3)	*Fraxinus excelsior*	93	3
F. *ex* Clone M72 (11)	F. *excelsior*/interstock F. *platypoda*	87	4
F. *ex* Clone M72 – budded (15)	*Fraxinus excelsior*	70	5
F. *ex* Clone M72 (2)	*Fraxinus chinensis*	68	6
F. *ex* Clone 98 (10)	F. *excelsior*/interstock F. *japonica*	60	7
F. *ex* Clone M72 (1)	*Fraxinus paxiana*	40	8
F. *ex* Clone 98 (9)	*Syringa vulgaris*	40	8
F. *ex* Clone M72 (6)	*Ligustrum ovalifolium*	37	9
F. *ex* Clone M72 (8)	*Syringa vulgaris*	33	10
F. *ex* Clone 98 (7)	*Ligustrum ovalifolium*	30	11
Clone 98 – budded (12)	*Fraxinus excelsior*	30	11
Clone 98 – budded (12)	*Fraxinus paxiana*	28	12
F. *ex* Clone 98 - budded (16)	*Fraxinus platypoda*	10	13
F. *ex* Clone M72 budded (13)	*Fraxinus paxiana*	7	14
F. *ex* Clone M72 budded (17)	*Fraxinus platypoda*	0	15

Differences in survival were illustrated when Clone M72 and Clone 98 were grafted onto rootstocks of *Fraxinus excelsior* compared to either Syringa or Ligustrum (Table 3.8). This table contains the figures from which the following deductions were made.

Table 3.8 Clonal comparison grafting survival results, 2015 (count and %, total of 30 per group)

Rootstock	F. *ex* Clone M72 scion	F. *ex* Clone 98 scion	Average % survival of Clones M72 & 98 scions
F. *excelsior*	100	97	99
Syringa vulgaris	33	40	37
Ligustrum ovalifolium	37	30	34

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

Logistic regression for the binomial proportions underlying the data in Table 3.8 with scion and rootstock show no interaction for the two factors. There was no evidence of a difference in the scions ($p=0.85$) but there was a significant outcome for rootstock effects ($p<0.0001$). F. *excelsior* rootstock was more likely (OR = odds ratio) to be viable than Syringa (OR = 66 with confidence interval (12, 333) and was also more likely to be viable than Ligustrum (OR = 76 (13, 500) while there was no significant difference between Syringa and Ligustrum (OR = 1.15 (0.54, 2.44).

3.6 Vegetative growth measurements of grafted plants at the end of the second growing season 2015, recorded 609 days after grafting

The increment of vegetative growth for the year 2015, for all grafted plants is summarized in Table 3.9.

Table 3.9 Vegetative growth measurements of grafted plants at the end of the second growing season 2015, recorded 609 days after grafting

Scions	Rootstock	Treatment	Number of grafts	Mean growth increment (mm)
F. *excelsior* (Control) non –grafted	F. *excelsior*	3	28	585[bc]
F. *excelsior* Clone 98	F. *excelsior*	4	29	620[a]
F. *excelsior* Clone M72	F. *excelsior*	5	30	419[ab]
F. *excelsior* Clone M72	F. *paxiana*	1	12	126[cd]
F. *excelsior* Clone M72	F. *chinensis*	2	21	364[bc]
F. *excelsior* Clone 98 with interstock *F. japonica*	F. *excelsior*	10	15	205[cd]
F. *excelsior* Clone 98 with interstock F. *platypoda*	F. *excelsior*	11	26	322[bc]
F. *excelsior* Clone 98	*Ligustrum ovalifolium*	7	9	347[abc]
F. *excelsior* Clone M72	*Ligustrum ovalifolium*	6	11	295[cd]
F. *excelsior* Clone 98	*Syringa vulgaris*	9	12	279[bcd]
F. *excelsior* Clone M72	*Syringa vulgaris*	8	10	311[cd]
Growth extension on buddings 2015				
F. *excelsior* Clone 98	F. *excelsior*	14	9	273[a]
F. *excelsior* Clone M72	F. *excelsior*	15	21	163[a]
F. *excelsior* Clone 98	F. *platypoda*	16	1	530[a]
F. *excelsior* Clone M72	F. *platypoda*	17	0	0[a]
F. *excelsior* Clone 98	F. *paxiana*	12	9	189[a]
F. *excelsior* Clone M72	F. *paxiana*	13	2	30[a]

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

Means with the same letter are not significantly different according to Tukey (Table 3.9 In this case, the largest increment in vegetative growth of 620 millimetres was recorded for *Fraxinus excelsior* Clone 98 grafted onto *Fraxinus excelsior* rootstocks. It is notable that this graft combination significantly exceeded the growth of non-grafted Control, however the incremental growth of Clone M72 was not significantly different from the non-grafted Control. *Fraxinus excelsior* Clone 98 produced a greater increment of vegetative growth on *Fraxinus excelsior* rootstocks compared to *Fraxinus excelsior* Clone M72. However, this difference was not statistically significant.

Rootstocks of both *Fraxinus chinensis* and *Fraxinus paxiana* had a reduction effect in vegetative growth when compared with common ash rootstocks in the graft combination with *Fraxinus excelsior* M72 as scions, with F. *paxiana* showing a significantly greater vegetative reduction (Table 3.9). Growth on F. *paxiana* and F. *chinensis* were not significantly different from one another with *Fraxinus excelsior* Clone M72 as scions Table (3.9). Syringa and Ligustrum rootstocks reduced the vegetative growth of scions of both *Fraxinus excelsior* Clone M72 and *Fraxinus excelsior* Clone 98, in comparison to when rootstocks of *Fraxinus excelsior* were used (Table 3.10). There were no significant differences between either of the Fraxinus clones on Syringa or Ligustrum rootstocks.

The effects of the different species of interstocks, in combination with scions of *Fraxinus excelsior* Clone 98, showed that the interstocks of *Fraxinus japonica* reduced vegetative growth more than *Fraxinus platypoda*; however, these differences were not significant. Furthermore, the use of Fraxinus interstocks resulted in a similar growth reduction when compared to the effects of either Ligustrum or Syringa rootstocks.

The vegetative increment obtained from buddings was generally lower than for grafted material (Table 3.9). Buds of *Fraxinus excelsior* Clone M72 generally gave poorer

growth on all rootstocks in comparison to *Fraxinus excelsior* Clone 98. In the case of Clone M72 budded onto *Fraxinus platypoda,* all buddings failed. There were no statistical significant differences between any of the budding treatments (Table 3.9).

Table 3.10 displays the average growth increment for 2015 and ranks them in descending order and % decrease from non-grafted Control. This table is an alternative way of viewing the results for discussion purposes.

Table 3.10 Growth extension increase or decrease in comparison to non-grafted Control plants, 2015

Scion	Rootstock	Average increment mm	% Increase/ Decrease
F. *ex* Clone 98	F. *excelsior*	620	+ 6
F. *ex* (Control)	Non-grafted	585	-
F. *ex* Clone M72	F. *excelsior*	419	-28
F. *ex* Clone M72	F. *chinensis*	364	-38
F. *ex* Clone 98	*Ligustrum ovalifolium*	347	-41
F. *ex* Clone 98	F. *excelsior* /interstock F. *platypoda*	322	-45
F. *ex* Clone M72	*Syringa vulgaris*	311	-47
F. *ex* Clone M72	*Ligustrum ovalifolium*	295	-50
F. *ex* Clone 98	*Syringa vulgaris*	279	-52
Clone 98 – budded	F. *excelsior*	273	-53
F. *ex* Clone 98	F. *excelsior* / interstock *japonica*	205	-65
Clone 98 - budded	F. *paxiana*	189	-68
F. *ex* Clone M72 - budded	F. *excelsior*	163	-72
F. *ex* Clone M72	F. *paxiana*	126	-79
F. *ex* Clone M72 budded	F. *paxiana*	30	-95
F. *ex* Clone M72 budded	F. *platypoda*	0*	
F. *ex* Clone 98 budded	F. *platypoda*	530*	

* Denotes these treatments were excluded as there was only one plant surviving. F. *excelsior* Clone 98 budded onto F. *platypoda* although shows 530 millimetres of growth, there was only one plant surviving, therefore it was excluded from ranking. *Fraxinus excelsior* Clone M72 budded onto F. *platypoda* had no plants surviving.

% increase or decrease was compared against the non-grafted (Control).

3.7 Observations on the development of graft unions at the end of the growing season, 2015

To check the responses and suitability of the different graft combinations, a closer examination of all the graft unions was undertaken and comparisons were made showing the development of the union at the end of the second growing season in 2015. It is notable that the self and heterografts performed in this thesis remained viable for at least two years after this study had finished. Arrows in the images that follow indicate the graft union points with the scion above and rootstock below. The scale in each image is marked in 1.0 cm units.

Image 3.1 shows a typical morphology of non-grafted *Fraxinus excelsior* which had re - grown after the shoot had been removed. It shows a swelling at the point where the rootstock was cut. It also shows that the cut rootstock had produced three shoots by the end of the second year of growth, 2015. In 2014, the average growth increment of non-grafted plants exceeded all other treatments.

Image 3.1 Non- grafted *Fraxinus excelsior*

Image provided by J Mc Namara, 2016

Image 3.2 depicts a typical graft union of *Fraxinus excelsior* Clone 98 grafted onto *Fraxinus excelsior* rootstock and in this instance this treatment exceeded all other treatments for vegetative growth. The mean vegetative growth in 2015 was 620 millimetres whereas the non-grafted Control gave 585 millimetres (Table 3.9). It is notable for image 3.2 that there was a smooth transition between the rootstock and scion at the graft union. Measurements of the difference in diameter at two centimetres below and two centimetres above the graft union revealed that the scions were 25% thinner than the rootstocks for this combination two years later.

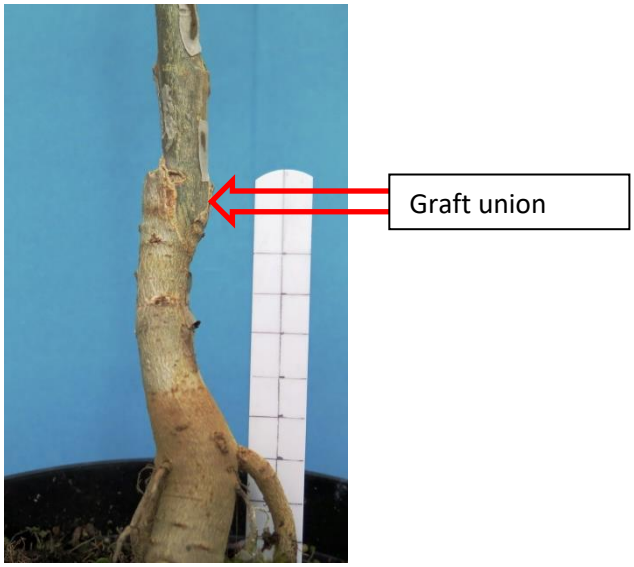

Image 3.2 Clone 98 on *Fraxinus excelsior* rootstock

Image provided by J Mc Namara, 2016

Image 3.3 depicts the grafting union of *Fraxinus excelsior* Clone M72 grafted onto *Fraxinus excelsior* rootstock. The mean vegetative growth was 419 millimetres. In addition, the diameter of the scions at two centimetres above the graft union was 29% thinner than the rootstock. Clone 98 was 25% thinner i.e. generally similar to Clone M72 which shows that these two similar species had the same reaction in that they showed the least incompatibility.

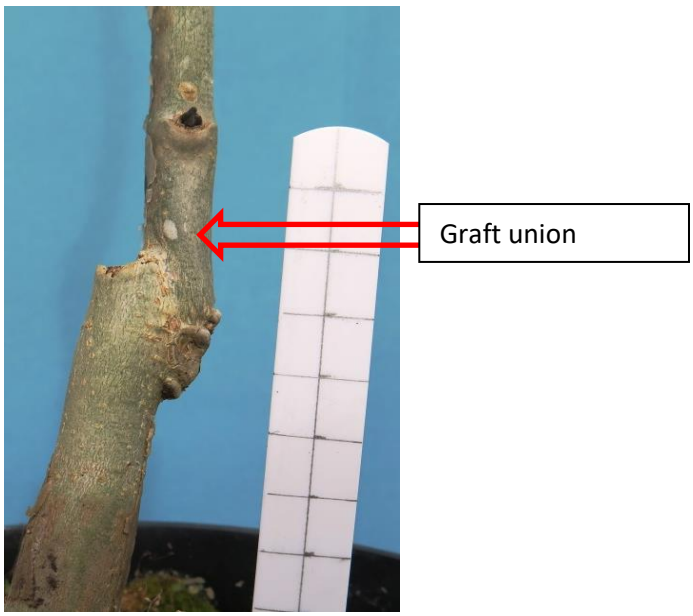

Image 3.3 Clone M72 on *Fraxinus excelsior* rootstock

Image provided by J Mc Namara, 2016

Image 3.4 shows *Fraxinus excelsior* Clone M72 grafted onto *Fraxinus chinensis* rootstock which resulted in vegetative growth increment of 364 millimetres. This combination had a reducing effect on growth compared to *Fraxinus excelsior* rootstocks. The diameter of the scions was 32% lower when compared with the diameter of the rootstock at two centimetres below the graft union Image 3.4. This indicates a greater level of incompatibility than previous combinations described of *Fraxinus excelsior* clones M72 and 98 on *Fraxinus excelsior* rootstocks.

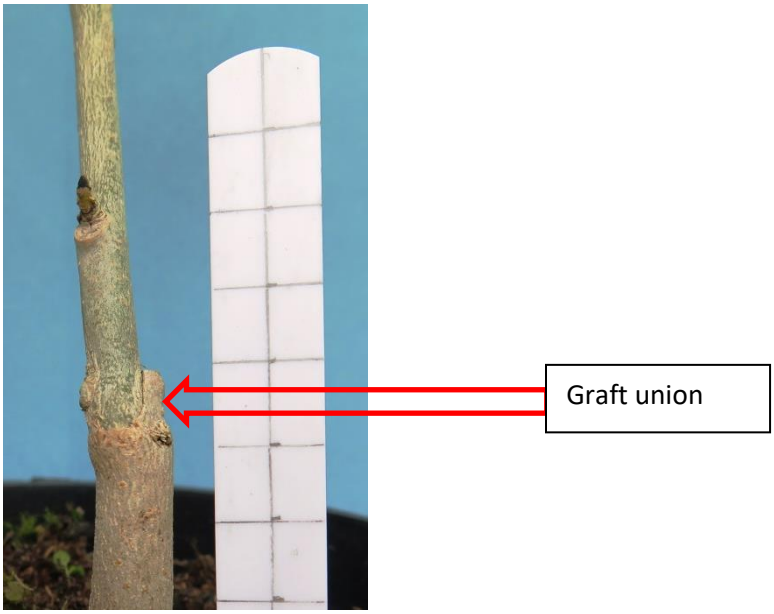

Image 3.4 Clone M72 on *Fraxinus chinensis* rootstock

Image provided by J Mc Namara, 2016

Image 3.5 shows *Fraxinus excelsior* Clone M72 grafted onto *Fraxinus paxiana* and it had resulted in a mean growth increment of 126 millimetres, which would indicate a stronger suppressive effect than *Fraxinus chinensis* as a rootstock as indicated above. The greater suppression of vegetative growth of the rootstock *Fraxinus paxiana* may be due to the vegetative incompatibility between the species. This type of incompatibility, as shown in the bulging graft union, may also be inferred from the difference in the stem diameters above and below the graft union. The scion diameters were 19% less than the rootstock diameters in this combination.

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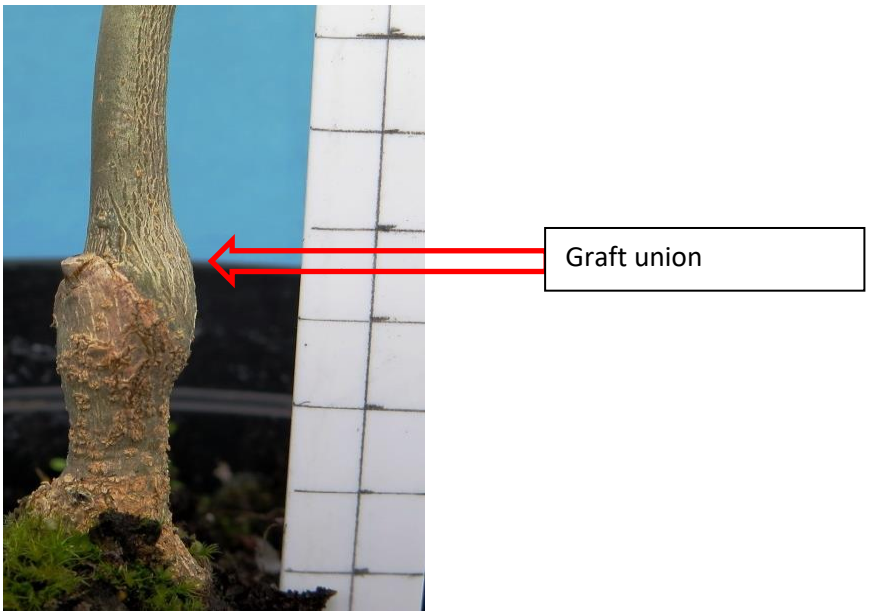

Image 3.5 Clone M72 on *Fraxinus paxiana* rootstock

Image provided by J Mc Namara, 2016

Image 3.6 shows *Fraxinus excelsior* Clone 98 grafted onto interstock *Fraxinus platypoda* and using *Fraxinus excelsior* as the rootstock. Diameter measurements showed that scions were 46% thinner than the rootstock. The interstock was excluded when taking measurement. This indicates a considerable restriction (Image 3.6). The average shoot extension growth was 322 millimetres, which demonstrated that the interstock had a shoot reduction effect when compared to *Fraxinus excelsior* rootstocks.

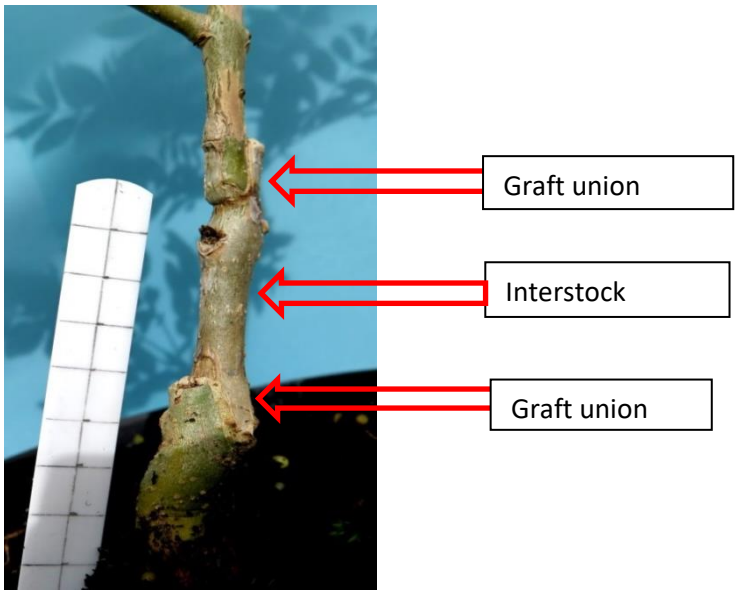

Image 3.6 Clone 98 / *Fraxinus platypoda* interstock on *Fraxinus excelsior* rootstock

Image provided by J Mc Namara, 2016

Image 3.7 shows *Fraxinus excelsior* Clone 98 grafted onto interstock *Fraxinus japonica* which was then grafted onto *Fraxinus excelsior* as the rootstock. Average shoot growth extension was 205 millimetres; note the relatively smooth transition at the upper end of the interstock with the scion of *Fraxinus excelsior* Clone 98. There is a bulge at the basal end of the interstock / rootstock junction (see arrows). There was a 38% reduction in scion diameter when compared to rootstock diameter.

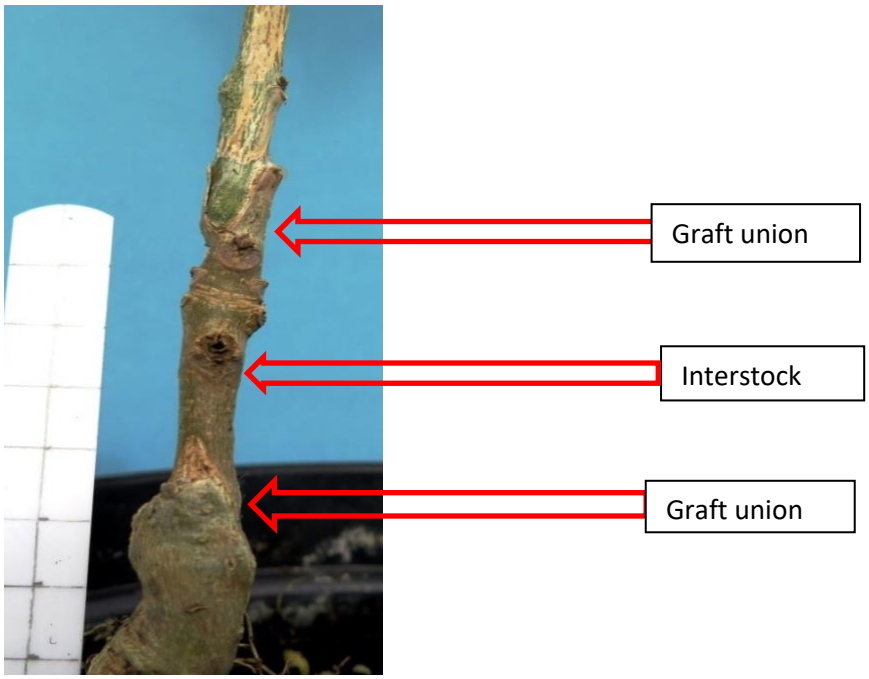

Image 3.7 Clone 98 / *Fraxinus japonica* interstock on *Fraxinus excelsior* rootstock

(Arrows indicate graft unions)

Image provided by J Mc Namara, 2016

Image 3.8 shows the graft union *Fraxinus excelsior* Clone M72 grafted onto *Ligustrum ovalifolium,* which produced a mean increment of 295 millimetres growth extension proving that the rootstock had a reducing influence on growth in comparison to ungrafted plants and to grafts on *Fraxinus excelsior* rootstocks. In this case, the scion growth was 10% greater in diameter than the rootstock diameter. The bulge at the graft union is shown (Image 3.8). The rootstocks of *Ligustrum ovalifolium* produced numerous epicormic shoots from the rootstock and roots over the two-year observation period; they were removed routinely.

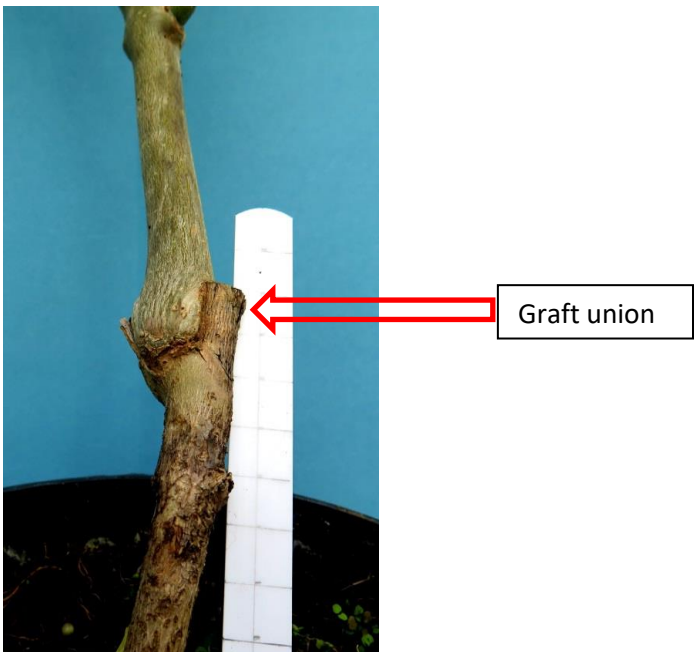

Image 3.8 Clone M72 on Ligustrum rootstock

Image provided by J Mc Namara, 2016

Image 3.9 shows *Fraxinus excelsior* Clone M72 grafted onto *Syringa vulgaris.* There were incompatibility issues, in that the scion was significantly larger than the rootstock (Image 3.9). In this case, the scion grew more vigorously than the rootstock and was 26% greater in diameter. It was noted that the rootstocks of *Syringa vulgaris* produced epicormic shoots from the roots over the two years of observation; these were removed routinely.

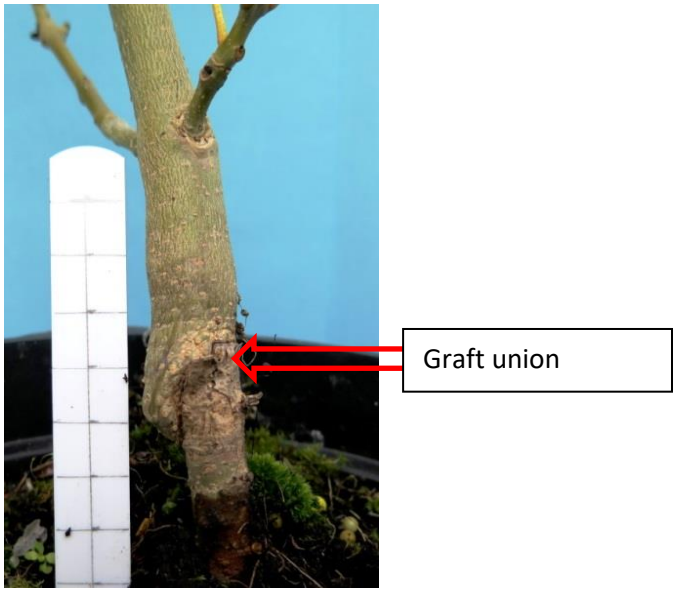

Image 3.9 Clone M72 on Syringa rootstock

Image provided by J Mc Namara, 2016

3.8 Rootstock and scion reduction or increase

Plants were measured above and below the graft unions and the differences were computed as a positive or negative value. Without fully paired sets of measurements at grafting and two years later Table 3.11 is an observational exercise as it would be illogical to use generic numbers to adjust for initial figures. Results are presented as a percentage change between the two positions. For example, the highly compatible grafts of Clone M72 and Clone 98 grafted onto *Fraxinus excelsior* rootstocks gave 29% and 25% reduction respectively in scion stem diameter when compared to the stem diameters of the rootstocks. The overall reduction in scion diameters (negative value) and increase (plus value) in scion diameters is summarized in Table 3.11 for all graft combinations; this data is contained in Appendix 1. It shows that negative values were obtained with all combinations of Fraxinus rootstocks whereas Ligustrum and Syringa rootstocks gave positive values and these were slightly greater with Clone M72 in comparison to Clone 98.

Table 3.11 Rootstock and scion restriction or increase

Scion	Rootstock / interstock	Treatment Number	% change in diameter of scions
Clone M72	*excelsior*	5	-29
"	*paxiana*	1	-19
"	*chinensis*	2	-32
"	*Ligustrum*	6	+10
"	*Syringa*	8	+26
Clone 98	*excelsior*	4	-25
"	*paxiana*- not tested		
"	*chinensis*- not tested		
"	*Ligustrum*	7	+1
"	*Syringa*	9	+8
"	interstock *platypoda*	11	-46
"	interstock *japonica*	10	-38

+ indicates the scion was greater in diameter than the rootstock and – shows the scion was less in diameter than the rootstock.

3.9 Number of shoots produced per plant

Generally, in the first year after grafting, only the terminal bud produced extension growth (data not shown). In the second year, secondary shoots were produced by all the graft combinations. The effects of the various types of rootstocks on their capacity to produce multiple shoots as well as for the ungrafted controls are summarised in (Table 3.12). The greatest number of shoots was produced by the ungrafted plants of *Fraxinus excelsior* (3.7). There was no great difference between the number of shoots produced by scions of *Fraxinus excelsior* Clone 98 (2.7) and *Fraxinus excelsior* Clone M72 (2.5) when grafted onto *Fraxinus excelsior* rootstocks. Clone M72 grafted onto lilac and privet produced 3.1 and 2.9 respectively. The lowest number of shoots was produced on grafts, with rootstocks of *Fraxinus paxiana, Fraxinus chinensis,* and interstocks of *Fraxinus japonica* and *Fraxinus platypoda* in the range 1.3-1.8. Table 3.12 shows that Treatment 3 was significantly different from Treatment 1, Treatment 2, Treatment 10 and Treatment 11 and was not significantly different from the remaining Treatments.

Table 3.12 Mean number of shoots produced per grafted plant at the end of the second year of growth 2015

Scion	Rootstock / interstock	Treatment number	Number of shoots / plant
Control		3	3.7[a]
Clone M72	*excelsior*	5	2.5[ab]
"	*paxiana*	1	1.5[b]
"	*chinensis*	2	1.3[b]
"	*Ligustrum*	6	2.9[ab]
"	*Syringa*	8	3.1[ab]
Clone 98	*excelsior*	4	2.7[ab]
	paxiana- not tested		
	chinensis- not tested		
	Ligustrum	7	2.7[ab]
	Syringa	9	1.9[ab]
	interstock *platypoda*	11	1.8[b]
	interstock *japonica*	10	1.3[b]

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

3.10 Bud flushing results

Bud flushing was recorded in 2015 and 2016 to determine if there were any effects of rootstocks on the rapidity of bud flushing, and if there were any differences between the different graft treatments (Table 3.13). Recording started from dormancy (stage 0) and continued until all viable buds had fully emerged (stage 4; Image 2.4). The scions of

Fraxinus excelsior Clone M72 and Clone 98 required ten and six days respectively to fully flush when grafted onto *Fraxinus excelsior* rootstocks. Interstocks of *Fraxinus platypoda* and *Fraxinus japonica* required seven and twelve days respectively with *Fraxinus excelsior* Clone 98 whch indicates that interstocks did not have a suppresent effect on bud bud flushing. The scions of *Fraxinus excelsior* Clone 98 grafted onto *Ligustrum ovalifolium* were the first to produce fully emerged leaves within three days of breaking dormancy (Stage 1). In contrast, *Fraxinus excelsior* Clone M72 required 18 days to complete bud flushing with Ligustrum rootstocks. Clone M72 grafted onto rootstocks of *Fraxinus paxiana* also required a long period to flush (17 days). The slowest combinations were the budding treatments, with some failing completely (Table 3.13).

Ligustrum and *Syringa* rootstocks when used in conjunction with Clone M72 were not significantly different when compared to one another. The same rootstocks when used with Clone 98 also showed no significant difference when compared to one another. Eventhough Clone 98 grafted onto *Ligustrum* rootstocks (Treatment 7) had the shortest flushing time it was not significant to Treatment 2, Treatment 4, Treatment 9, Treatment 11 and Treatment 3.

Table 3.13 Number of days for grafted plants in various combinations to fully flush, starting at the dormant stage in Spring 2015

Scion	Rootstock / interstock	Treatment Number	Mean number of days to fully flush
Clone M72 (G)	*excelsior*	5	10[c]
	paxiana	1	17[ab]
	chinensis	2	7[dce]
	Ligustrum	6	18[a]
	Syringa	8	19[a]
Clone 98 (G)	*excelsior*	4	6[dce]
	paxiana- not tested		
	chinensis- not tested		
	Ligustrum	7	3[de]
	Syringa	9	8[dc]
	interstock *platypoda*	11	7[dce]
	interstock *japonica*	10	12[bc]
Fraxinus (Control)	*Fraxinus*	3	6[dce]
Clone M72 (B)	*excelsior*	15	41[a]
	paxiana	13	*
	platypoda	17	*
Clone 98 (B)	*excelsior*	14	41[a]
	paxiana	12	20[b]
	platypoda	16	*

Logistic regression with Tukey adjustment using SAS 9.4(SAS, 2014).

(G) Grafted (B) Budded * No data available-did not flush

3.11 Flowering results

The production of flower buds was recorded in spring 2015 and spring 2016. None of the non-grafted produced flowers out of the 28 plants. In the first year after grafting, one plant of *Fraxinus excelsior* Clone 98 and one plant of *Fraxinus excelsior* Clone M72 produced some flowers. In both cases, the selected clones had been grafted onto *Fraxinus excelsior* rootstocks. In addition, one plant of *Fraxinus excelsior* Clone 98 out of 27 flowered when grafted with an interstock of *Fraxinus platypoda* and a rootstock of *Fraxinus excelsior.* None of the plants from the other graft treatments produced flowers, even though vegetative extension growth was reduced in several graft combinations; data extracted from Appendix 2.

Chapter 4: Discussion

European indications are that 1-5% of the *Fraxinus excelsior* population will remain *Chalara* resistant (Mc Kinney *et al.,* 2014). Although *Chalara* has been confirmed in all Irish counties there are no report of significant tree deaths so far. This is due to currently low disease pressure however this situation is expected to alter as inoculation levels build up year after year.

The current challenges are to identify resistant trees and to efficiently and quickly multiply the resistant trees to initiate seed orchards. Seed producing orchards will consist of selected *Chalara* resistant trees which will have been propagated by grafting. The orchards will be designed to optimise cross pollination of trees within the site so that the resistant trees will pollinate each other (Douglas *et al.,* 2013). In this context it is highly desirable that the trees will flower as soon as possible. This cross pollination will result in the progeny of ash having a high level of disease resistance (Kjaer *et al.,* 2017). Propagating resistant trees through seed is the cheapest method and it can be scaled up by establishing many seed orchards throughout Ireland when the mother trees are selected as *Chalara* resistant. However, the first step to the production of seed is by grafted plants and that is estimated to take approximately five to seven years or perhaps longer. Flowering in grafted trees may begin sooner if scions are collected from adult trees that are already flowering. Clearly, it would be highly desirable to shorten the period of vegetative growth in ash in order to accelerate the onset of flowering and seed production. Using various species as rootstocks and interstocks with ash offers the potential to achieve this, based on many examples of similar approaches for other trees and fruit species. Resistant trees have not been identified in Ireland to date, as the disease has not been in the country long enough and because the disease incidence and pressure is not high enough. In 2016, the DNA of the ash genome was sequenced, and

gene markers have been developed which would indicate *Chalara* susceptibility and resistance (Sollars *et al.,* 2016). This test would save time and energy and has huge potential in the identification of disease resistant trees. In Ireland this selection process has begun in that Teagasc have selected what is deemed now to be *Chalara* resistant and grafted these trees to be planted into high disease infestation areas. It has been shown that resistance to *Chalara* is stable in vegetatively propagated ash trees (Stener, 2013). A method for large scale production has been described by Douglas *et al.,* 2017. Personal experience of managing to root hardwood cuttings of ash trees has been unsuccessful however there is another method. It involves firstly micropropagation of the selected trees, followed by a further propagation step of using standard cuttings. Micropropagation would seem to rejuvenate the plants and the results were high rooting percentages from the cuttings. Generally the costs of trees that are propagated vegetatively are higher than for seed produced plants. Because of these costs and the need to produce c7.8 million trees in Ireland to compensate for the impending loss, a production system based on seed is the most feasible way of producing resistant plant material for future forestry needs. An alternative means to seed orchards for producing ash trees with resistance to *Chalara* is to vegetatively propagate individually selected resistant trees. Vegetative propagation has given low rates of rooting in cuttings selected from mature trees in the order of 26% (Cahalan and Jinks, 1992).

Micropropagation of ash is possible however it can be quite challenging, as it tends to be difficult to establish aseptic cultures due to contamination (Thompson *et al.,* 2001). In trials undertaken (Thompson *et al.,* 2001) of micropropagation of ash, 12.5% of the ash cultures from 40 plus ash trees which comprised 13 clones survived up to the seventh culture transfer. However, micropropagation should not be discounted as it could be used to supplement a grafting programme. Therefore, grafting has a major advantage over micropropagation, in that it speeds up the process of producing plants to

establish seed orchards and it is feasible to produce a plant that is big enough for field planting one year after grafting. The aim of this project was to produce viable grafts from Asian rootstocks and interstocks with reduced vegetative growth with multi shoots which ideally would lead to an early onset of flowering.

The grafting method chosen was a modified off-centre cleft graft. Regarding previous work done on off centre cleft grafting of *Fraxinus excelsior* clones onto two-year old *Fraxinus excelsior,* rootstocks a graft viability of 97% was obtained overall from 70 clones, with all clones being successfully grafted (Thompson *et al.,* 2001).

In the case of budding treatments, the viability range was 70% to 0%, however budding should not be discounted as it offers another opportunity to propagate the identified trees in the same calender year. Budding as reported in this thesis was not successful due to the quality of the available rootstocks. Also on a time element it is possible to achieve more grafted plants on a daily basis in comparison to conventional grafting.

In the case of this experiment graft viability ranged from 100% to 0%. The results of the experiment from this thesis after two seasons growth were that the two selected clones of Fraxinus excelsior, Clone M72 and Clone 98 gave similar results in terms of graft viability, 100% and 97% respectively which compared to the findings reported by Thompson *et al.* (2001).

A method of grafting ash which can also be used for grafting small seedlings with stem diameters ranging from 1.5-4.5 millimetres is ‘tube grafting’. This system has shown that very small and young seedlings can be used as rootstocks and scions of single buds (Douglas *et al.,* 1996). The research demonstrated that ‘tube grafting’ which was done in summer period yielded 85% success rate on 20 clones tested (Douglas *et al.,* 1996).

Both winter and summer grafting of *Chalara* resistant ash trees may be needed to build up sufficient stocks of trees for seed orchards.

The influence of rootstocks on flowering and fruiting behaviours of trees has been recently reviewed by (Warschefsky *et al.,* 2016). It gives many examples where specific rootstocks could induce early flowering in crops such as blueberry, pawpaw, pear, sapodilla and apple. The advantages of the various rootstocks to produce vegetables has also been discussed recently (Kumar *et al.,* 2018).

To avoid soil borne diseases which are normally accentuated by successive cropping, vegetable grafting, which originated in Japan and Korea, has now become popular around the world. It was a practice introduced in the late 20th century, and later to North America. In 1990, 59% of the vegetables produced in Japan were from grafted plants and 81% in Korea (Davis *et al.,* 2008). There are over 40 million grafted tomato plants used on an annual basis in North American glasshouses (Kubota *et al.,* 2008). In peppers, grafting can transfer specific traits from the rootstock to influence the phenotype of scion. Grafting eggplant onto *Solanum torvum* has shown to control *verticillium* wilt (Kumar *et al*., 2018). The significance of this in relation to ash is that it has been discovered that *Chalara* can infect a tree through the roots (Kirisits, 2014). It is becoming apparent that using different rootstocks to achieve desired traits has the ability to transfer these traits both above and below ground (Mudge *et al.,* 2009). Therefore, there may be many more species of Fraxinus rootstocks (among the 65 known), and besides the ones tested which could offer possibilities for growth reduction and onset of early flowering when examining the diverse range of rootstocks used in fruit crops (Goldschmidt, 2014). In view of this fact, then the value of Asian species rootstocks could offer possibilities of producing grafted trees in which the rootstock and scion would be resistant to *Chalara*.

To circumvent nematode problems of *Coffea arabica*, interspecific grafting is used in Latin America. Two cultivars of *Coffea arabica* were evaluated on four rootstocks, C. canephora var. Robusta ('T3561' and 'T3757') and C. liberica var. liberica (Hiern) and var. dewevrei (Lebrun). Non-grafted plants of two arabica were used as controls and the trial was evaluated over five years. Viability was 80% for two of the rootstock types and 90% for the remaining two. The control rootstocks had 96% viability. When coffee bean yield was assessed over a four-year period, it was discovered that the four rootstocks reduced yield between 10-48%. Also, it was discovered that the scion stem girth did not increase, similar to observations in this thesis when Asian species of Fraxinus were grafted. As this experiment was conducted over a two year period Fraxinus species resulted in reductions in viability, however there is no data to support seed yield reduction as flowering was so low. However poor productivity was attributed to poor adaptation of the rootstocks chosen to suit the specific site and that more stringent selection of rootstocks was required to adapt to the environment (Bertrand *et al.*, 2001).

In the case of Oleaceae family, much work has been undertaken on olives regarding rootstock production via seed and clonal propagation to achieve size reduction, rooting ability, disease resistance, drought tolerance, salt tolerance, and graft compatibility (Fabbri *et al.*, 2009). Size reduction was possible in olive using selected rootstocks (Fabbri *et al.*, 2009). The genus Fraxinus is also in the Oleaceae family and this study has shown a reduction in vegetative growth with some rootstocks. For example, when vegetative growth was measured in the second growing season for Clone M72 grafted onto *Fraxinus paxiana* and *Fraxinus chinensis* the reduction was 79% and 38% respectively in comparison to the Control (ungrafted). When interstocks of *Fraxinus japonica* and *Fraxinus platypoda* were used with Clone 98 the reductions were 65% and 45% respectively in comparison to the Control (ungrafted). Also when Clone 98 was

grafted onto *Ligustrum ovalifolium* and *Syringa vulgaris* the growth reductions were 41% and 47% respectively. This indicates that Fraxinus rootstock species had an effect in reducing vegetative growth.

Grafting is the recommended method to quickly multiply the future selected *Chalara* resistant trees, however it needs further consideration with regard to the rootstocks employed. Relatively less is known about the diverse rootstocks that could be used. Rootstocks are generally selected for their grafting capacity to alter scion phenotypes (Warschefsky *et al.,* 2016). This involves the practical testing of species and even of genotypes within a species for the desired effects. For example, in the *Malus domestica* (Apple) industry, clonal rootstocks species of *M. baccata, M domestica, M. doumeri, M. halliana, M. hupehensis, M. sargenti, M. sieboldi, M. sieversii, M. sikkimensis, M. sylvestris, M. transisoria, M. toringoides, M. yunnanensis* have been used with the principal targets of tree size control/dwarfing, fruit quality, pest and disease resistance, as well as for drought and cold tolerance (Ignatov and Bodishevskaya, 2011). Dwarfing rootstocks are effective in vegetative growth reduction, thereby ensuring earlier fruiting in apples, therefore, it has been worthwhile selecting rootstocks of apple which displayed less vigorous vegetative growth (Porebski *et al.,* 2006). Disease tolerant rootstocks have been used in apple orchards to overcome the problem of replant disease (Leinfelder and Merwin, 2006). This theory may offer opportunities for Asian ash rootstocks which haven't been investigated yet. The phenotypes of trees have been altered by using growth regulator such as alar which (acid succinamic 2,2-dimethylhydrazine) on apple rootstocks which reduced the heights and the diameters of the stems (Khanizadeh *et al.,* 2007). As Alar is no longer available using paclobutrazol as a retardant may be worth considering. This subject of growth retardants would be worth investigating in relation to their effects on Fraxinus rootstocks and on grafted plants. Rootstock M9 produces higher levels of abscisic acid, which may regulate stomatal opening, and help to alleviate short-term

drought resistance. The more vigorous rootstock MM 111 may have more drought hardy tolerance characteristics because of its more extensive root system (Atkinson *et al.*, 1999).

For commercial production of *Pyrus communis* (Pear) with similar targets as in the apple industry, clonally produced rootstock species have been used (Warschefsky *et al.*, 2016). This goes to prove that a lot more work is necessary in clonal production of ash rootstocks. Experiments undertaken by Fischer, 2009 concluded that in the selection of dwarfing rootstocks for *Pyrus communis* which are vegetatively propagated are required to give consistency in shortening the period for fruit production. Furthermore, genetically engineered rootstocks would prove invaluable against fireblight and in producing rootstocks with herbicide resistance. In this thesis seed produced rootstocks were used therefore it would be worthwhile studying the effects of clonally produced rootstocks as seeding rootstocks generally have varying characteristics.

Similarly for *Prunus persica* (Peach) clonally rootstocks have been used for the same reasons as for apple and pear (Warschefsky *et al.*, 2016). When it came to controlling tree size Hancock *et al.* (2008) identified genes that would give reduced vigour. Byrne (2012) states that tree peach breeding takes 10 years to produce a new variety and that a lot more research needs to be done on the genetics of desirable traits in grafted scions and the influence of rootstocks on the heritability of desirable traits.

Graft compatibility is paramount in achieving high percentage graft viability and permanent graft union, therefore, it is important to select the correct species of rootstock to match the scion. The healing of the graft union may take a year, or, in some cases, the incompatibility may not become obvious for several years (Andrews and Marquez, 1993). Despite the importance of the rootstock in relation to grafting, the actual molecular and physiological aspects of the graft compatibility or incompatibility are not

fully understood (Goldschmidt, 2014). There are limits genetically to what can be grafted to what, so generally it is possible to graft together clones of the same species, various species within a genus sometimes and grafting between different genera within a family is possible but remote (Hartmann *et al.*, 2002). Generally rootstock and scion need to be closely taxonomically related for greater grafting success (Hartmann *et al.*, 2002). However this thesis has shown that intergeneric grafts between Fraxinus and Syringa / Ligustrum were viable even though distantly related taxonomically.

Interspecific grafting research has been undertaken successfully using eggplant (*Solanum melongena*) as rootstocks to graft tomato (*Solanum lycopersicum*) in order to alleviate flood stress, which tomato is very susceptible to. Eggplant rootstocks while conferring flood tolerance to tomato also changed the sugar, starch content and yield of the fruit (Bhatt *et al.*, 2015). Generally, when one species is grafted onto a rootstock of the same species there is no incompatibility (Mudge *et al.*, 2009).

Grafting is a more successful method of asexually propagating Chinese chestnut (*Catania mollissima*) than any other method. Graft incompatibility was investigated for fifteen Chinese chestnut cultivars, nine American, selections, six Japanese cultivars and two Japanese hybrids on two known rootstocks of Chinese chestnut. In total 32 genetically diverse selections of *C. dentata*, *C.mollisima* and *C.crenata* were grafted onto two known rootstocks of *Castanea mollisima*. Interspecific grafting of Chinese chestnut yielded an 80% success rate after two growing seasons. Interspecific grafts of seven American and five Japanese chestnut selections resulted in ≥ 70% success. The percentages are slightly greater than those obtained in this thesis where the viability ranged from 40-87% at the end of the second growing season. Graft morphological abnormalities were also observed at the graft union in interspecific grafting. The results

indicate that genetic incompatibility is not a major cause of graft failure (Huang *et al.,* 1994).

Interstocks can also be used for several reasons such as to circumvent incompatibility between the scion and rootstock and to control the growth vigour (Hartmann *et al.,* 2002). They may also be used to get around issues with incompatibility between the rootstock and scion; either of whose partners has a desired trait. The interstock while reducing vegetative growth may also stimulate flowering. A specific example of this is an interstock of M9 apple rootstock which was grafted between a vigorous rootstock and a vigorous scion, the result being the reduction of vegetative growth, with the consequence that flowering, and seed production increased, in comparison to a grafted plant where an interstock had not been used (Roberts and Blaney, 1967). In addition, other work has shown in apple grafting that using M27 and M9 as weak interstocks grafted onto vigorous rootstocks resulted in a reduction of vegetative growth and an increase in fruiting (Lord *et al.,* 1985). As reported in this thesis interstocks did reduce vegetative growth however no work has been undertaken on this aspect previously therefore the recommendation is to employ the use of other interstocks.

Other researchers working on apples used M27 and M9 as interstocks, have shown that M27 reduced the vegetative growth by 80%, in comparison to the controls; a 50% reduction was obtained when M9 was used. The researchers also discovered that by increasing the interstock length resulted in a reduction in plant vigour and fruit yield (Di Vaio *et al.,* 2008).

Other researchers have stated that using weak interstocks grafted onto vigorous seedling rootstocks in apples resulted in, reducing the need for pruning (Lord *et al*., 1985; Di Vaio *et al.,* 2008). This effect was also noted for interstock grafting using *Fraxinus japonica* and *Fraxinus platypoda* in conjunction with Clone 98 which reduced

vegetative growth by 65% and 45% respectively in comparison to the Control. At the same time, interstocks of *Fraxinus japonica* and *Fraxinus platypoda* grafted onto Clone 98 reduced viability to 60% and 87% respectively.

The effects of interstocks on persimmon was field trialled by Koshita *et al.*, 2006. In this case two years after planting, the total shoot length of the trees was shortened with the use of rootstock candidates (Ac-1, Ac-2, and Y) and also where seedlings of *Diospyros rhombifolia* were used as interstocks to 31, 33, 36, and 16% of the control trees, and the tree height were shortened by 59, 75, 68, and 58% in comparison to those of the control trees, respectively (Koshita *et al.* 2006).

The literature shows the effects of a large range of rootstock species on growth and development of scions of various crops. In relation to Fraxinus, there is no knowledge as to how *Fraxinus excelsior* grafted onto the other Fraxinus species or onto other genera, will perform in the long term. Therefore there may be other species of Fraxinus besides the ones tested, which could offer possibilities for growth reduction and the early onset of flowering when examining the diverse range of rootstocks used in fruit crops (Goldschmidt, 2014) .

Fraxinus excelsior grafted onto *Fraxinus excelsior* will guarantee a high grafting viability success rate, however, ideally a plant which would flower earlier in its life cycle would further speed up production, so it may be the case that the Asian rootstocks examined in this study have a role to play, as they may be capable of inducing early flowering when tested over a few more years than the current study allowed. Using Ligustrum and Syringa as rootstocks may also prove useful in longer term testing because these species produce more flowers and they flower earlier in their lifecycle.

Differences in vegetative growth were observed between the two clones of self grafted *Fraxinus excelsior.* Clone 98 when grafted onto *Fraxinus excelsior* exceeded the Control by almost 6% whereas Clone M72 vegetative growth was reduced by 28%. This demonstrates clonal response differences of scions. Heterografts involving *Fraxinus excelsior* scions grafted onto other species resulted in even greater reductions in vegetative growth. Clone M72 was grafted onto *Fraxinus paxiana* vegetative growth was reduced by 79% and when grafted onto *Fraxinus chinensis* the reduction was 38%.

The principal objectives in testing intergeneric grafting in this thesis was to examine the graft compatibility and hopefully the acceleration of flowering over the long run.

To the knowledge of this author this is the first study in which privet and lilac rootstocks were used for ash scions. Regarding privet it has been used for some time as a 'nurse' rootstock for the propagation of lilac. In this case the privet is the rootstock on which commercial cultivars of lilac are grafted (Rudolf *et al*., 2008). Once the graft union has taken hold, the grafted plants are then planted deeply with the graft union well below soil level so that the adventitious roots begin to be formed by the lilac scions. In this way privet acts as a 'nurse' until the lilac forms its own roots.

Budding when compared with bench grafting by Lipecki *et al.* (2013) on apples found no great differences in tree growth performance, such as tree height, trunk diameter, vegetative growth and the number of shoots which shows that employing budding is a viable option. Although the budding results obtained in this thesis were less successful than grafting the process of budding allows a second chance to undertake grafting in the same year and it is possible to propagate much more daily than conventional off-centre grafting. There is a minor disadvantage with budding, in that it will take one year longer to have a plant ready for field planting.

Another method of grafting which can also be performed is tube grafting. This similar to budding employs single buds grafted onto seedling rootstocks and can also be done in summer (Douglas *et al.*, 1996).

The discussion above has referred to reductions in vegetative growth that are associated with early flowering in fruit trees. According to Prista *et al.* (2003), it is possible to select olive seedlings for earliness in flowering by assessing the vegetative traits early in their development. Based on that research, it is possible to speculate that ash may behave in a similar pattern, with some individuals having a natural capacity for early flowering compared to others. While further growth of the grafted ash trees from the experiment reported here should reveal more clearly the effects of the rootstocks with regards to flowering onset in a longer period of observation. Ash flowers appear before the leaves emerge. In Ireland flowering happens in late April to early May. The flowers emerge in large panicles of 100-400 flowers on shoots from the floral buds on the previous year's growth.

While some research in apples has indicated earlier flowering using interstocks (Vercammen *et al.*, 2004; Samad *et al.*, 1999; Webster, 1995), this did not occur within the period of observations of this thesis.

Research has shown in two tobacco species that cells from either side of a graft can exchange chloroplasts which are responsible for photosynthesis and the entire nucleus of a cell could move across a graft and fuse the genomes of the rootstock and scion (Le Page, 2016). This may have implications in the choice of rootstocks for grafting disease resistant ash in the future if specific genotypes of ash rootstocks can be shown to have specific effects for desired traits such as *Chalara* resistance and early flowering. As there was very little flowering recorded, perhaps in the longer term there may be a relationship between bud flushing and flowering.

In relation to this thesis there was a wide variance overall in the number of days for the plants to flush from three days to fourty one. However, there was very little differences between *Fraxinus excelsior* Clone M72 grafted onto *Fraxinus excelsior* rootstocks (ten days) and *Fraxinus excelsior* Clone 98 grafted onto *Fraxinus excelsior* rootstocks (six days). The non-grafted Controls took ten days to completely flush.

Topworking, which is common practice in apple and pear orchards, also offers major potential for ash once resistant trees have been identified. It would be feasible to graft *Chalara* resistant scion material onto mature already flowering *Fraxinus excelsior* trees in situ which should accelerate flowering in the grafted scions. In addition, it would be prudent to identify resistant ash trees, which flower frequently, and topwork these trees with scion material from trees that are exhibiting *Chalara* resistance.

The results have shown that both clones of *Fraxinus excelsior* could produce flowers when grafted onto common ash but at a low frequency in a two-year period. It is important to note that the scions of the two ash clones were originally derived from fully mature trees and so they had already reached the mature physiological state where flowering was possible. Flowering had occurred in the clonal collection on an infrequent basis and it had been hoped that the frequency of flowering increased in the glasshouse environment. As the experiment was over a short space of time (two years) the hope is that all graft combinations would produce flowers every year in a high frequency. Generally in forestry stands ash trees can take 20-30 years to produce seed from saplings. Once the tree has reached the flowering phase it will generally produce seed annually, however heavy crops tend to be produced every two to five years (Savill, 1991).

The pathogen *H. fraxineus* is endemic in Asia and is thought to have originated there In Asia, the ash tree species have co-evolved over millennia with *Chalara* so that the

populations of several species are genetically tolerant (Lichtarowicz, 2012). By contrast, in Europe, it is a new pathogen and trees have not co-evolved with *Chalara*.

It is also reported that *F. chinensis* and *F. mandschurica* in their natural habitats shows resistance to *Chalara* (Gross and Queloz, 2015). Dormant buds of *F. mandscurica, F. chinensis* and *F. pennsylvanica* have been successfully cryopreserved with recovery rates ranging from 34 – 100%. This methodology could be used to assist with maintaining as much diversity as possible for disease resistant genotypes of *Fraxinus excelsior* (Volk *et al.*, 2009). Similarly, with ash, the aim is to propagate and to produce flowering ash trees as quickly as possible using scions from *Chalara* resistant trees. This will facilitate the interbreeding of many resistant ash trees when they are planted together in seed-producing orchards and from controlled crossings of specific trees with each other.

Therefore the proposal to make interspecific crossings would not incur the public resistance such as with that genetic modification (Jepson and Arakelyan, 2017). Thus a logical option for Europe is to assist nature in the process of evolving ash populations with resistance.

Chapter 5: Conclusions

In summary the clonal scion material of *Fraxinus excelsior* used was chosen at random to test the effectiveness of various rootstocks on graft viability and subsequent vegetative growth. Scions derived from ash seedlings are genetically diverse and would be less likely to show the direct effect of rootstocks.

The experiments were conducted to test the effects of various rootstocks for their ability to produce viable grafts and to reduce vegetative growth in *Fraxinus excelsior.* The objective was to produce a plant with multiple shoots which would flower early in its life cycle. Over the two year growing period assessments were carried out and the results were as follows:-

- When either *Fraxinus excelsior* Clone M72 and Clone 98 were grafted onto *Fraxinus excelsior*, the graft viability was 100% and 97% respectively, and remained at this level over two years
- Furthermore when Clone 98 was grafted onto Syringa, Ligustrum and with the interstock *japonica* the viability remained the same at 40%, 30% and 60% respectively. When Clone M72 was grafted onto *Fraxinus paxiana,* viability was 40%. The un-grafted Control remained at 93% over a two-year growing period assessment. All other treatments dropped viability in the range 33% to 87%.
- Budding viability was extremely low with the best treatment at 30% and the worst failing.
- When the plants were measured for vegetative growth extensions in 2014 the un-grafted Control exceeded all other treatments, however that situation changed in 2015 and Clone 98 grafted onto *Fraxinus excelsior* exceeded the un-grafted

Control by 6%. All other treatments had less vegetative growth than the un-grafted Control ranging from 28% to 79%.

- In 2014 and 2015 there was no significant difference in vegetative growth produced by Clone M72 and Clone 98.
- When the rootstocks and scions were measured for restrictions or increases in stem diameters above and below the graft union, interestingly it showed that negative values e.g. scion was smaller in diameter than the rootstock for all combinations of Fraxinus rootstocks and for interstocks of both Fraxinus species, whereas Ligustrum and Syringa rootstocks gave positive values e.g. the scion was greater in diameter than the rootstock. This would indicate incompatibilities between the rootstock and scion, however these incompatibilities are not of major concern if the graft manages to produce multiple shoots which flower early in life and also conceivably these incompatibilities which could leave the graft union vulnerable to breakage could be managed by keeping the plants in a greenhouse.
- Also, when the number of shoots produced per plant was tabulated *Fraxinus excelsior* Clone M72 grafted onto Syringa rootstocks produced 3.1 shoots per plant which was greater than when it was grafted into *Fraxinus excelsior* at 2.5.
- Regarding bud flushing, the aim was to determine if different rootstocks would influence this trait. Flushing may have an influence in relation to *Chalara* susceptibility. The variances in flushing times over the grafting treatments varied from three days to fourty one days with some plants failing to emerge. Clone 98 grafted onto Ligustrum rootstocks was the fastest to fully flush within three days. There was no difference between ash non-grafted and Clone 98 grafted onto *Fraxinus excelsior* which flushed at six days. The budding

treatments that were assessed exhibited poor results, in that they took 41 days to fully flush.

- Flowering is a highly desirable trait in the context of accelerating the onset of the production of seeds in *Chalara* resistant trees. In 2015 and 2016, all plants were assessed for flowering, as it might be anticipated that grafted plants might flower early in life because the scion material was from mature trees originally and they had flowered sporadically in the clonal conservation collection from which the scion material was collected. Flowering was extremely low in 2015, on an overall basis and it was at 0.01%, and for 2016 it was 0.02% (Appendix 2). However a longer period of observation is required to deliver a fair assessment of the situation.
- The reduction in vegetative growth relative to ungrafted Controls and to Clone 98 was due to the reduced vigour of the scions of Clone M72.

Future work recommenadations include topworking onto trees which exhibit prolific flowering with *Chalara* tolerant scion material, micrografting, cross pollination with Asiatic species and grafting onto other Asiatic species. As there is very little knowledge on rootstocks for desired traits it would be beneficial to start this selection process.

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Appendix 1 Rootstock / scion reduction or increase

Rootstock / scion reduction or increase expressed in mm, used in Chapter 3

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 1	Treat 1	8.54	7.19	-1.35	-15.8
Block 1	Treat 1	8.33	7.87	-0.46	-5.52
Block 1	Treat 2	10.43	8.21	-2.22	-21.28
Block 1	Treat 2	10.16	7.38	-2.78	-27.36
Block 1	Treat 2	13.25	7.33	-5.92	-44.67
Block 1	Treat 3	13.49	9.2	-4.29	-31.8
Block 1	Treat 3	15.88	10.56	-5.32	-33.5
Block 1	Treat 3	16.84	9.22	-7.62	-45.24
Block 1	Treat 3	12.08	8.15	-3.93	-32.53
Block 1	Treat 3	13.21	8.04	-5.17	-39.13
Block 1	Treat 4	14.12	9.05	-5.07	-35.9
Block 1	Treat 4	13.05	8.06	-4.99	-38.23
Block 1	Treat 4	11.59	8.44	-3.15	-27.17
Block 1	Treat 4	12.21	10.55	-1.66	-13.59
Block 1	Treat 4	12.94	9.84	-3.1	-23.95
Block 1	Treat 5	13.04	10.46	-2.58	-19.78
Block 1	Treat 5	14.02	10.3	-3.72	-26.53
Block 1	Treat 5	10.34	9.58	-0.76	-7.35
Block 1	Treat 5	14.24	10.11	-4.13	-29.00
Block 1	Treat 5	15.67	7.65	-8.02	-51.18
Block 1	Treat 6	8.43	10.01	+1.58	+18.74
Block 1	Treat 6	14.04	14.6	+0.56	+3.98

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 1	Treat 7	11.2	14.28	+3.08	+27.5
Block 1	Treat 7	28.49	12.9	-15.59	-54.72
Block 1	Treat 8	Dead			
Block 1	Treat 9	8.28	9.84	+1.56	+18.84
Block 1	Treat 9	13.28	11.09	-2.19	-16.49
Block 1	Treat 10	12.24	5.69	-6.55	-53.51
Block 1	Treat 10	13.33	5.93	-7.4	-55.51
Block 1	Treat 10	12.09	6.77	-5.32	-44.00
Block 1	Treat 11	14.64	5.06	-9.58	-65.43
Block 1	Treat 11	10.89	7.26	-3.63	-33.33
Block 1	Treat 11	11.51	10.46	-1.05	-9.12
Block 1	Treat 11	10.26	7.5	-2.76	-26.9
Block 1	Treat11	14.64	7.29	-7.35	-50.2
Block 1	Treat 12	8.34	10.61	+2.27	+27.21
Block 1	Treat12	7.93	9.96	+2.03	+25.59
Block 1	Treat 13	Dead			
Block 1	Treat 14	Dead			
Block 1	Treat 15	Dead			
Block 1	Treat 16	Dead			
Block 1	Treat 17	Dead			
Block 2	Treat 1	9.8	9.16	-0.64	-6.53
Block 2	Treat 2	11.33	7.59	-3.74	-33.00
Block 2	Treat 2	12.06	8.25	-3.81	-31.59
Block 2	Treat 2	14.6	9.29	-5.31	-36.36
Block 2	Treat 2	12.89	8.43	-4.46	-34.6

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 2	Treat 3	12.64	6.16	-6.48	-51.26
Block 2	Treat 3	18.41	11.89	-6.52	-35.41
Block 2	Treat 3	11.07	7.38	-3.69	-33.33
Block 2	Treat 3	16.92	12.47	-4.45	-26.3
Block 2	Treat 3	Dead			
Block 2	Treat 4	11.69	10.2	-1.49	-12.74
Block 2	Treat 4	12.36	8.68	-3.68	-29.77
Block 2	Treat 4	14.37	10.51	-3.86	-26.86
Block 2	Treat 4	16.97	12.69	-4.28	-25.22
Block 2	Treat 4	14.29	12.31	-1.98	-13.85
Block 2	Treat 5	15.73	9.79	-5.94	-37.76
Block 2	Treat 5	15.22	11.44	-3.78	-24.83
Block 2	Treat 5	12.37	9.34	-3.03	-24.49
Block 2	Treat 5	15.93	8.47	-7.46	-46.82
Block 2	Treat 5	11.26	9.79	-1.47	-13.05
Block 2	Treat 6	8.01	8.23	+0.22	+2.74
Block 2	Treat 6	8.68	11.32	+2.64	+30.41
Block 2	Treat 7	9.88	8.79	-1.09	-11.03
Block 2	Treat 7	11.08	12.69	+1.61	+14.53
Block 2	Treat 8	6.99	7.69	+0.7	+10.01
Block 2	Treat 8	5.44	6.56	+1.12	+20.58
Block 2	Treat 9	8.09	14.96	+6.87	+84.91
Block 2	Treat 9	9.06	12.15	+3.09	+34.10
Block 2	Treat 10	11.02	6.68	-4.34	-39.38
Block 2	Treat 10	14.04	10.1	-3.94	28.06
Block 2	Treat 11	15.3	6.17	-9.13	-59.67

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 2	Treat 11	17.09	8.69	-8.4	-49.15
Block 2	Treat 11	16.59	10.06	-6.53	-39.36
Block 2	Treat11	16.52	9.74	-6.78	-41.04
Block 2	Treat 11	18.5	8.51	-9.99	-54.00
Block 2	Treat 12	Dead			
Block 2	Treat 13	Dead			
Block 2	Treat 14	10.69	7.72	-2.97	-27.78
Block 2	Treat 14	11.18	8.36	-2.82	-25.22
Block 2	Treat 15	11.71	7.69	-4.02	-34.32
Block 2	Treat 15	8.9	6.07	-2.83	-31.79
Block 2	Treat 15	10.86	6.04	-4.82	-44.38
Block 2	Treat 15	10.83	5.93	-4.9	-45.24
Block 2	Treat 16	Dead			
Block 2	Treat 17	Dead			
Block 3	Treat 1	14.49	8.18	-6.31	-43.54
Block 3	Treat 1	8.13	8.06	-0.07	-0.86
Block 3	Treat 2	13.34	9.48	-3.86	-28.93
Block 3	Treat 2	11.36	7.05	-4.31	-37.94
Block 3	Treat 2	13.26	9.34	-3.92	-29.56
Block 3	Treat 2	11.87	6.75	-5.12	-43.13
Block 3	Treat 3	11.96	8.84	-3.12	-26.08
Block 3	Treat 3	10.19	6.21	-3.98	-39.05
Block 3	Treat 3	16.72	10.49	-6.23	-37.26
Block 3	Treat 3	14.79	10.29	-4.5	-30.42

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 3	Treat 3	10.96	5.53	-5.43	-49.54
Block 3	Treat 4	16.23	11.09	-5.14	-31.66
Block 3	Treat 4	12.89	10.09	-2.8	-21.72
Block 3	Treat 4	11.94	8.41	-3.53	-29.56
Block 3	Treat 4	13.04	9.84	-3.2	-24.53
Block 3	Treat 4	11.54	8.28	-3.26	-28.24
Block 3	Treat 5	11.95	8.93	-2.99	-25.27
Block 3	Treat 5	16.88	8.92	-7.96	-47.15
Block 3	Treat 5	13.02	10.32	-2.7	-20.73
Block 3	Treat 5	14.3	11.33	-2.97	-20.76
Block 3	Treat 5	14.24	9.25	-4.99	-35.04
Block 3	Treat 6	11.46	11.65	+0.19	+1.65
Block 3	Treat 6	11.72	10.85	-0.87	-7.42
Block 3	Treat 6	23.34	13.88	-9.46	-40.53
Block 3	Treat 7	9.92	11.06	+1.14	+11.47
Block 3	Treat 8	Dead			
Block 3	Treat 8	Dead			
Block 3	Treat 9	Dead			
Block 3	Treat 9	Dead			
Block 3	Treat 9	14.82	9.93	-4.89	-32.99
Block 3	Treat 10	11.89	6.77	-5.12	-43.06
Block 3	Treat 10	6.3	5.85	-0.45	-7.14
Block 3	Treat 11	18.17	9.69	-8.48	-46.67
Block 3	Treat 11	18.09	8.02	-10.07	
Block 3	Treat 11	15.58	10.61	-4.97	-31.89

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 3	Treat 11	17.05	9.12	-7.93	-46.51
Block 3	Treat 12	7.96	6.47	-1.49	-18.71
Block 3	Treat 12	8.46	8.90	+0.44	+5.20
Block 3	Treat 13	Dead			
Block 3	Treat 14	10.75	4.71	-6.04	-56.18
Block 3	Treat 14	10.07	7.03	-3.04	-30.18
Block 3	Treat 14	14.96	9.35	-5.61	-37.5
Block 3	Treat 14	11.23	7.84	-3.39	-30.18
Block 3	Treat 15	8.52	4.30	-4.22	-49.53
Block 3	Treat 15	12.14	8.54	-3.6	-29.65
Block 3	Treat 16	10.38	6.93	-3.45	-33.23
Block 3	Treat 17	Dead			
Block 4	Treat 1	12.96	10.03	-2.93	-22.60
Block 4	Treat 1	7.32	8.05	0.73	+9.97
Block 4	Treat 2	15.15	7.71	-7.44	-49.10
Block 4	Treat 2	13.96	7.02	-6.94	-49.71
Block 4	Treat 2	11.88	8.97	-2.91	-24.49
Block 4	Treat 3	14.7	9.18	-5.52	-37.55
Block 4	Treat 3	17.89	9.14	-8.75	-48.91
Block 4	Treat 3	15.47	10.43	-5.04	-32.57
Block 4	Treat 3	13.16	8.40	-4.76	-36.17
Block 4	Treat 3	14.93	10.79	-4.14	-27.72
Block 4	Treat 4	12.87	10.45	-2.42	-18.80
Block 4	Treat 4	13.35	9.91	-3.44	-25.76
Block 4	Treat 4	11.34	10.42	-0.92	-8.11

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 4	Treat 4	13.14	8.7	-4.44	-33.78
Block 4	Treat 4	10.88	7.7	-3.18	-29.22
Block 4	Treat 5	15.42	11.58	-3.84	-24.90
Block 4	Treat 5	15.44	10.93	-4.51	-29.20
Block 4	Treat 5	14.63	9.79	-4.84	-33.08
Block 4	Treat 5	10.87	7.61	-3.26	-29.99
Block 4	Treat 5	13.34	9.53	-3.81	-28.56
Block 4	Treat 6	10.28	10.83	0.55	+5.35
Block 4	Treat 6	9.76	10.8	1.04	+10.65
Block 4	Treat 6	Dead			
Block 4	Treat 7	9.08	9.82	0.74	+8.14
Block 4	Treat 7	10.65	12.26	1.61	+15.11
Block 4	Treat 8	Dead			
Block 4	Treat 9	12.58	7.47	-5.11	-40.62
Block 4	Treat 9	8.25	8.92	0.67	+8.12
Block 4	Treat 10	6.85	7.11	0.26	+3.79
Block 4	Treat 10	10.98	5.64	-5.34	-48.63
Block 4	Treat 10	18.05	8.86	-9.19	-50.91
Block 4	Treat 11	14.39	7.98	-6.41	-44.54
Block 4	Treat 11	15.59	5.88	-9.71	-62.28
Block 4	Treat 11	21.05	8.7	-12.35	-58.66
Block 4	Treat 11	17.38	7.88	-9.5	-54.66
Block 4	Treat 11	17.11	7.39	-9.72	-56.8
Block 4	Treat 12	7.36	7.33	-0.03	-0.04
Block 4	Treat 12	7.74	8.14	0.4	+5.16
Block 4	Treat 12	7.92	6.88	-1.04	-13.13

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 4	Treat 12	Dead			
Block 4	Treat 12	Dead			
Block 4	Treat 13	None			
Block 4	Treat 14	11.58	5.61	-5.97	-51.55
Block 4	Treat 14	11.45	6.42	-5.03	-43.93
Block 4	Treat 15	11.24	6.63	-4.61	-41.01
Block 4	Treat 15	15.63	8.59	-7.04	-45.04
Block 4	Treat 15	10.3	6.31	-3.99	-38.73
Block 4	Treat 15	9.45	5.29	-4.16	-44.02
Block 4	Treat 16	None			
Block 4	Treat 17	None			
Block 5	Treat 1	8.83	7.48	-1.35	-15.28
Block 5	Treat 1	14.3	9.33	-4.97	-34.75
Block 5	Treat 2	12.27	10.25	-2.02	-16.46
Block 5	Treat 2	10.07	8.73	-1.34	-13.3
Block 5	Treat 2	12.82	8.95	-3.87	-30.18
Block 5	Treat 2	11.39	8.04	-3.35	-29.41
Block 5	Treat 3	10.87	7.49	-3.38	-31.09
Block 5	Treat 3	12.55	8.83	-3.72	-29.64
Block 5	Treat 3	11.19	8.99	-2.2	-19.66
Block 5	Treat 3	13.56	9.45	-4.11	-30.3
Block 5	Treat 4	14.74	11.77	-2.97	-20.14
Block 5	Treat 4	14.26	9.23	-5.03	-35.27
Block 5	Treat 4	14.37	9.85	-4.52	-31.45
Block 5	Treat 5	10.96	7.65	-3.31	-30.2

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 5	Treat 5	16.72	10.94	-5.78	-34.56
Block 5	Treat 5	10.7	9.6	-1.1	-10.28
Block 5	Treat 5	14.33	10.91	-3.42	-23.86
Block 5	Treat 5	14.22	11.38	-2.84	-19.97
Block 5	Treat 6	9.89	10.25	0.36	+3.64
Block 5	Treat 7	13.24	10.16	-3.08	-23.26
Block 5	Treat 8	9.92	11.56	1.64	+16.53
Block 5	Treat 9	Dead			
Block 5	Treat 10	14.92	7.81	-7.11	-47.65
Block 5	Treat 11	26.84	9.21	-17.63	-65.68
Block 5	Treat 11	12.89	8.19	-4.7	-36.46
Block 5	Treat 11	14.92	7.62	-7.3	-48.92
Block 5	Treat 11	9.66	7.05	-2.61	-27.01
Block 5	Treat 12	Dead			
Block 5	Treat 13	Dead			
Block 5	Treat 14	Dead			
Block 5	Treat 15	Dead			
Block 5	Treat 15	12.03	7.39	-4.64	-38.57
Block 5	Treat 15	10.38	5.79	-4.59	-44.21
Block 5	Treat 15	7.68	5.11	-2.57	-33.46
Block 5	Treat 16	Dead			
Block 5	Treat 17	Dead			
Block 6	Treat 1	12.89	6.47	-6.42	-49.8
Block 6	Treat 1	13.92	10.63	-3.29	-23.63
Block 6	Treat 2	14.93	11.07	-3.86	-25.85

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 6	Treat 2	10.58	8.19	-2.39	-22.58
Block 6	Treat 2	13.82	9.42	-4.4	-31.83
Block 6	Treat 2	13.73	8.67	-5.06	-36.85
Block 6	Treat 3	16.12	10.83	-5.29	-32.81
Block 6	Treat 3	11.64	7.82	-3.82	-32.81
Block 6	Treat 3	13.26	9.45	-3.81	-28.73
Block 6	Treat 3	13.08	8.02	-5.06	-38.68
Block 6	Treat 4	15.62	12.55	-3.07	-19.65
Block 6	Treat 4	13.35	9.76	-3.59	-26.89
Block 6	Treat 4	20.66	17.06	-3.6	-17.42
Block 6	Treat 4	16.26	11.19	-5.07	-31.18
Block 6	Treat 4	15.47	11.87	-3.6	-23.27
Block 6	Treat 5	13.61	9.93	-3.68	-27.03
Block 6	Treat 5	16.27	11	-5.27	-32.39
Block 6	Treat 5	16.81	10.5	-6.31	-37.53
Block 6	Treat 5	16.01	10.62	-5.39	-33.66
Block 6	Treat 5	11.15	7.95	-3.2	-28.69
Block 6	Treat 6	8.20	12.06	3.86	+47.07
Block 6	Treat 7	9.40	11.12	1.72	+18.29
Block 6	Treat 8	10.13	15.69	5.56	+54.89
Block 6	Treat 9	8.28	8.84	0.56	+6.76
Block 6	Treat 10	Dead			
Block 6	Treat 11	13.62	7.6	-6.02	-44.19
Block 6	Treat 11	17.66	8.49	-9.17	-51.92
Block 6	Treat 11	14.76	Dead		
Block 6	Treat 12	Dead			

		Rootstock	Scion	Difference	Scion % reduction / increase
Block 6	Treat 13	6.67	5.23	-1.44	-21.58
Block 6	Treat 14	8.29	5.34	-2.95	-35.58
Block 6	Treat 15	8.12	4.88	-3.24	-39.9
Block 6	Traet 15	11.8	6.95	4.85	-41.1
Block 6	Treat 15	Dead			
Block 6	Treat 16	Dead			

Appendix 2 Flowering assessments for 2015 and 2016

Appendix 2 represents the flowering assessments for 2015 and 2016. 0 means there were no flowers and 1 that it flowered. This appendix is used in section 3.11

Assessed 07-04-2015

Treat number	Block1	Block 2	Block 3	Block 4	Block 5	Block 6	Total	Flowers
1 Clone M72 grafted onto *paxiana*	0-0	0-0	0-0	0-0	0-0	0-0	12	0
2 Clone M72 grafted onto *chinensis*	0-0-0-0	0-0-0-0	0-0-0-0	0-0-0-0	0-0-0-0	0-0-0-0	24	0
3 Ash non – grafted	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0	0-0-0-0	28	0
4 Clone 98 grafted onto *Fraxinus* excelsior	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	1-0-0-0	0-0-0-0-0	29	1
5 Clone M72 grafted onto Ash rootstocks	1-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	30	1
6 Clone M72 grafted onto Ligustrum rootstocks	0-0-0	0-0-0	0-0-0	0-0-0	0-0	0-0	16	0
7 Clone 98 grafted onto Ligustrum rootstocks	0-0	0-0-0	0	0-0	0	0	10	0
8 Clone M72 grafted onto Syringa rootstocks	0-0-0-0	0-0-0-0	0-0-0	0-0-0	0-0-0	0-0-0	20	0
9 Clone 98 grafted onto Syringa rootstocks	0-0	0-0-0	0-0-0	0-0	0-0	0-0	14	0
10 Intergrafting *japonica* interstock - Ash rootstock	0-0-0	0-0-0-0-0	0-0	0-0-0	0	0-0	16	0
11 *platypoda* interstock - Ash rootstock	0-0-0-0-0	0-0-0-0-0	0-0-0-0	0-0-0-0-0	0-0-0-0	0-0-0-0	27	0
12 Clone 98 budded onto Paxiana	0-0-0-0-0-0	0-0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	32	0
13 Clone M72 budded onto Paxiana	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0-0-	0-0-0-0-0	0-0-0-0-0	30	0
14 Clone 98 budded onto Fraxinus excelsior	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	30	0
15 Clone M72 budded onto Fraxinus excelsior	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	30	0
16 Clone Clone 98 budded onto Platypoda	0-0	0-0	0-0	0-0	0	0	10	0
17 Clone M72 budded onto Platypoda	0	0	0-0	0	0	0	7	0

Flowering assessments

Flowering Assessed 15-04-2016

Treatment number	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Total	Flowers
1 Clone M72 grafted onto *Paxiana*	0-0	0-0	0-0	0-0	0-0	0-0	12	0
2 Clone M72 grafted onto *Chinensis*	0-0-0-0	0-0-0-0	0-0-0-0	0-0-0-0	0-0-0-0	0-0-0-0	24	0
3 Ash non – grafted	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0	0-0-0-0	28	0
4 Clone 98 grafted onto *Fraxinus* excelsior	1-1-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0	0-0-0-0	28	2
5 Clone M72 grafted onto Ash rootstocks	0-1-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	30	1
6 Clone M72 grafted onto Ligustrum rootstocks	0-0-0	0-0-0	0-0-0	0-0-0	0-0	0-0	16	0
7 Clone 98 grafted onto Ligustrum rootstocks	0-0	0-0-0	0	0-0	0	0	10	0
8 Clone M72 grafted onto Syringa rootstocks	0-0-0-0	0-0-0-0	0-0-0	0-0-0	0-0-0	0-0-0	20	0
9 Clone 98 grafted onto Syringa rootstocks	0-0	0-0-0	0-0-0	0-0	0-0	0-0	14	0
10 Intergrafting Japonica interstock - Ash rootstock	0-0-0	0-0-0-0-0	0-0	0-0-0	0	0-0	16	0
11 Clone98 Platypoda interstock - Ash rootstock	0-0-0-0-0	0-0-0-0-0	0-0-0-0	0-0-0-0-0	1-0-0-0	0-0-0-0	27	1
12 Clone 98 budded onto Paxiana	0-0-0-0-0-0	0-0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	32	0
13 Clone M72 budded onto Paxiana	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0-	0-0-0-0-0	0-0-0-0-0	30	0
14 Clone 98 budded onto Fraxinus excelsior	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	30	0
15 Clone M72 budded onto Fraxinus excelsior	0-0-0-0-0	1-1-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	0-0-0-0-0	30	0
16 Clone Clone 98 budded onto Platypoda	0-0	0-0	0-0	0-0	0	0	10	0
17 Clone M72 budded onto Platypoda	0	0	0-0	0	0	0	7	0

Appendix 3 Odds ratios and confidence limits

Odds ratios and confidence limits for the analysis of viability of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of first year of growth, 2014. Data used in Table 3.3

Model Information	
Data Set	WORK.TABLE 3.2
Response Variable (Events)	No_grafts_viable
Response Variable (Trials)	No_grafts
Model	binary logit
Optimization Technique	Fisher's scoring
Likelihood Penalty	Firth's bias correction

Number of Observations Read	11
Number of Observations Used	11
Sum of Frequencies Read	326
Sum of Frequencies Used	326

Response Profile

Ordered Value	*Binary Outcome*	*Total Frequency*
1	Event	222
2	Nonevent	104

Class Level Information

Class	*Value*	*Design Variables*									
Treatment	*1*	1	0	0	0	0	0	0	0	0	0
	2	0	1	0	0	0	0	0	0	0	0
	4	0	0	1	0	0	0	0	0	0	0
	5	0	0	0	1	0	0	0	0	0	0
	6	0	0	0	0	1	0	0	0	0	0
	7	0	0	0	0	0	1	0	0	0	0
	8	0	0	0	0	0	0	1	0	0	0
	9	0	0	0	0	0	0	0	1	0	0
	10	0	0	0	0	0	0	0	0	1	0
	11	0	0	0	0	0	0	0	0	0	1
	3	0	0	0	0	0	0	0	0	0	0

Intercept-Only Model Convergence Status
Convergence criterion (GCONV=1E-8) satisfied.

Model Convergence Status
Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	*Intercept Only*	*Intercept and Covariates*	
		Log Likelihood	*Full Log Likelihood*
AIC	389.711	315.387	42.170
SC	393.498	357.043	83.826
-2 Log L	387.711	293.387	20.170

Testing Global Null Hypothesis: BETA=0

Test	*Chi-Square*	*DF*	*Pr > ChiSq*
Likelihood Ratio	94.3245	10	<.0001
Score	86.8121	10	<.0001
Wald	56.1819	10	<.0001

Type 3 Analysis of Effects

Effect	*DF*	*Wald Chi-Square*	*Pr > ChiSq*
Treatment	10	56.1819	<.0001

Analysis of Penalized Maximum Likelihood Estimates

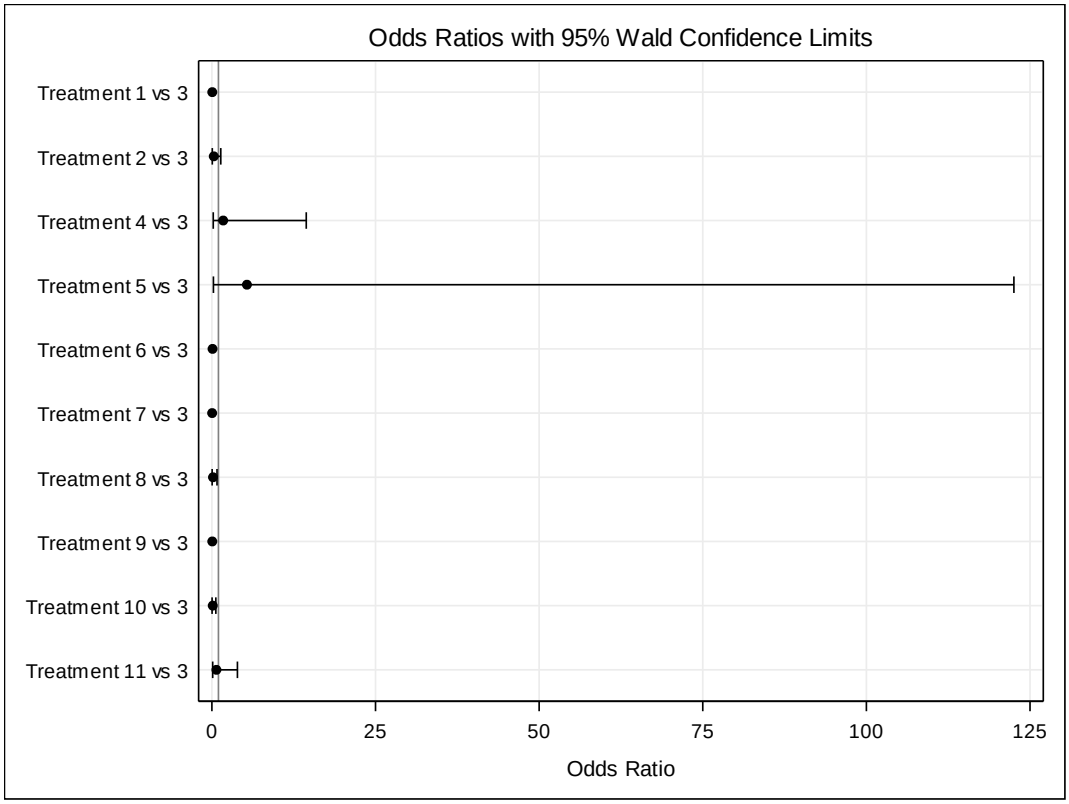
Parameter		*DF*	*Estimate*	*Standard Error*	*Wald Chi-Square*	*Pr > ChiSq*
Intercept		1	2.4336	0.6705	13.1730	0.0003
Treatment	*1*	1	-2.8257	0.7669	13.5762	0.0002
Treatment	*2*	1	-1.2498	0.7933	2.4821	0.1152
Treatment	*4*	1	0.5453	1.0833	0.2534	0.6147
Treatment	*5*	1	1.6778	1.5973	1.1034	0.2935
Treatment	*6*	1	-2.3044	0.7639	9.1010	0.0026
Treatment	*7*	1	-3.2504	0.7787	17.4217	<.0001
Treatment	*8*	1	-1.7646	0.7736	5.2033	0.0225
Treatment	*9*	1	-2.8257	0.7669	13.5762	0.0002
Treatment	*10*	1	-2.0441	0.7847	6.7863	0.0092

Analysis of Penalized Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Treatment	*11*	1	-0.3722	0.8845	0.1771	0.6739
Treatment	*3*	0	0	.	.	.

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
Treatment 1 vs 3	0.059	0.013	0.266
Treatment 2 vs 3	0.287	0.061	1.357
Treatment 4 vs 3	1.725	0.206	14.418
Treatment 5 vs 3	5.354	0.234	122.537
Treatment 6 vs 3	0.100	0.022	0.446
Treatment 7 vs 3	0.039	0.008	0.178
Treatment 8 vs 3	0.171	0.038	0.780
Treatment 9 vs 3	0.059	0.013	0.266
Treatment 10 vs 3	0.129	0.028	0.603
Treatment 11 vs 3	0.689	0.122	3.902



Association of Predicted Probabilities and Observed Responses			
Percent Concordant	77.4	*Somers' D*	0.633
Percent Discordant	14.1	*Gamma*	0.691
Percent Tied	8.5	*Tau-a*	0.276
Pairs	23088	*c*	0.816

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	*Estimate*	*95% Confidence Limits*	
Treatment 1 vs 2	0.207	0.068	0.625
Treatment 1 vs 4	0.034	0.006	0.212
Treatment 1 vs 5	0.011	<0.001	0.208
Treatment 1 vs 6	0.594	0.213	1.652
Treatment 1 vs 7	1.529	0.527	4.437
Treatment 1 vs 8	0.346	0.121	0.990
Treatment 1 vs 9	1.000	0.356	2.806
Treatment 1 vs 10	0.458	0.155	1.350
Treatment 1 vs 11	0.086	0.022	0.330
Treatment 1 vs 3	0.059	0.013	0.266
Treatment 2 vs 4	0.166	0.026	1.070
Treatment 2 vs 5	0.054	0.003	1.033
Treatment 2 vs 6	2.871	0.958	8.604
Treatment 2 vs 7	7.393	2.371	23.050
Treatment 2 vs 8	1.673	0.544	5.146
Treatment 2 vs 9	4.835	1.600	14.608
Treatment 2 vs 10	2.213	0.699	7.008
Treatment 2 vs 11	0.416	0.102	1.691
Treatment 2 vs 3	0.287	0.061	1.357
Treatment 4 vs 5	0.322	0.012	8.689
Treatment 4 vs 6	17.283	2.814	106.162
Treatment 4 vs 7	44.508	7.073	280.074
Treatment 4 vs 8	10.073	1.614	62.856
Treatment 4 vs 9	29.106	4.715	179.668
Treatment 4 vs 10	13.322	2.097	84.650
Treatment 4 vs 11	2.503	0.334	18.770
Treatment 4 vs 3	1.725	0.206	14.418
Treatment 5 vs 6	53.636	2.863	>999.999
Treatment 5 vs 7	138.130	7.262	>999.999
Treatment 5 vs 8	31.261	1.652	591.499
Treatment 5 vs 9	90.331	4.806	>999.999
Treatment 5 vs 10	41.346	2.161	791.192

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	*Estimate*	*95% Confidence Limits*	
Treatment 5 vs 11	7.768	0.365	165.359
Treatment 5 vs 3	5.354	0.234	122.537
Treatment 6 vs 7	2.575	0.895	7.410
Treatment 6 vs 8	0.583	0.206	1.652
Treatment 6 vs 9	1.684	0.605	4.684
Treatment 6 vs 10	0.771	0.263	2.255
Treatment 6 vs 11	0.145	0.038	0.553
Treatment 6 vs 3	0.100	0.022	0.446
Treatment 7 vs 8	0.226	0.077	0.669
Treatment 7 vs 9	0.654	0.225	1.897
Treatment 7 vs 10	0.299	0.098	0.912
Treatment 7 vs 11	0.056	0.014	0.222
Treatment 7 vs 3	0.039	0.008	0.178
Treatment 8 vs 9	2.890	1.011	8.262
Treatment 8 vs 10	1.323	0.440	3.973
Treatment 8 vs 11	0.248	0.064	0.968
Treatment 8 vs 3	0.171	0.038	0.780
Treatment 9 vs 10	0.458	0.155	1.350
Treatment 9 vs 11	0.086	0.022	0.330
Treatment 9 vs 3	0.059	0.013	0.266
Treatment 10 vs 11	0.188	0.047	0.750
Treatment 10 vs 3	0.129	0.028	0.603
Treatment 11 vs 3	0.689	0.122	3.902

Odds ratios and confidence limits for the analysis of viability of *Fraxinus excelsior* scions grafted onto various species of rootstocks at the end of first year of growth, 2015. Data used in Table 3.6

Model Information	
Data Set	WORK.TABLE 3.6
Response Variable (Events)	No_grafts_viable
Response Variable (Trials)	No_grafts
Model	binary logit
Optimization Technique	Fisher's scoring
Likelihood Penalty	Firth's bias correction

Number of Observations Read	21
Number of Observations Used	17
Sum of Frequencies Read	465
Sum of Frequencies Used	465

Response Profile		
Ordered Value	Binary Outcome	Total Frequency
1	Event	245
2	Nonevent	220

Class Level Information																		
Class	Value	Design Variables																
Treatment	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	13	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Class Level Information

Class	Value	Design Variables																
	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Intercept-Only Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates: Log Likelihood	Intercept and Covariates: Full Log Likelihood
AIC	613.715	462.901	65.213
SC	617.857	533.316	135.627
-2 Log L	611.715	428.901	31.213

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	182.8141	16	<.0001
Score	163.2603	16	<.0001
Wald	94.2208	16	<.0001

Type 3 Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Treatment	16	94.2208	<.0001

Analysis of Penalized Maximum Likelihood Estimates

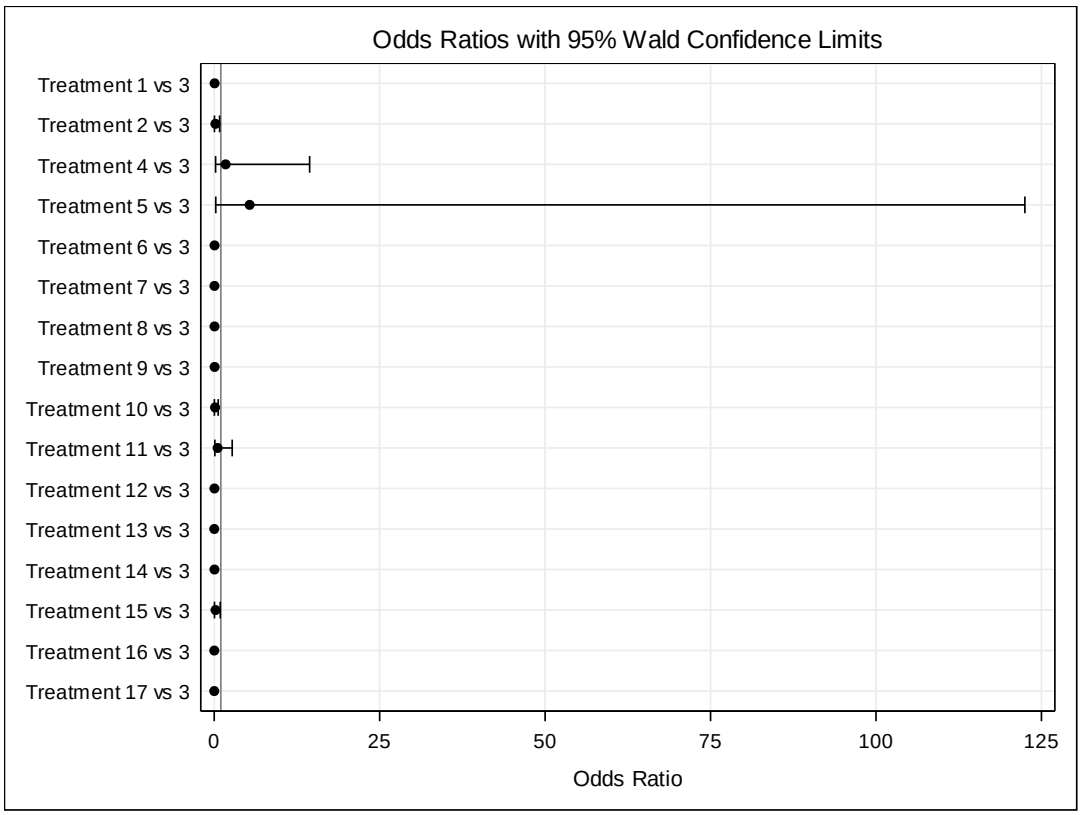
Parameter		DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept		1	2.4336	0.6705	13.1730	0.0003
Treatment	1	1	-2.8257	0.7669	13.5762	0.0002
Treatment	2	1	-1.7169	0.7720	4.9468	0.0261
Treatment	4	1	0.5453	1.0833	0.2534	0.6147
Treatment	5	1	1.6777	1.5972	1.1033	0.2935
Treatment	6	1	-2.9617	0.7697	14.8059	0.0001
Treatment	7	1	-3.2504	0.7787	17.4217	<.0001

Analysis of Penalized Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Treatment	8	1	-3.1027	0.7736	16.0868	<.0001
Treatment	9	1	-2.8257	0.7669	13.5762	0.0002
Treatment	10	1	-2.0441	0.7847	6.7863	0.0092
Treatment	11	1	-0.6605	0.8475	0.6075	0.4357
Treatment	12	1	-3.3393	0.7759	18.5226	<.0001
Treatment	13	1	-4.8672	0.9483	26.3461	<.0001
Treatment	14	1	-3.2504	0.7787	17.4217	<.0001
Treatment	15	1	-1.6168	0.7787	4.3109	0.0379
Treatment	16	1	-4.2794	1.1396	14.1013	0.0002
Treatment	17	1	-5.1418	1.6994	9.1544	0.0025
Treatment	3	0	0	.	.	.

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
Treatment 1 vs 3	0.059	0.013	0.266
Treatment 2 vs 3	0.180	0.040	0.816
Treatment 4 vs 3	1.725	0.206	14.418
Treatment 5 vs 3	5.353	0.234	122.493
Treatment 6 vs 3	0.052	0.011	0.234
Treatment 7 vs 3	0.039	0.008	0.178
Treatment 8 vs 3	0.045	0.010	0.205
Treatment 9 vs 3	0.059	0.013	0.266
Treatment 10 vs 3	0.129	0.028	0.603
Treatment 11 vs 3	0.517	0.098	2.720
Treatment 12 vs 3	0.035	0.008	0.162
Treatment 13 vs 3	0.008	0.001	0.049
Treatment 14 vs 3	0.039	0.008	0.178
Treatment 15 vs 3	0.199	0.043	0.913
Treatment 16 vs 3	0.014	0.001	0.129
Treatment 17 vs 3	0.006	<0.001	0.163



Association of Predicted Probabilities and Observed Responses			
Percent Concordant	80.5	Somers' D	0.667
Percent Discordant	13.9	Gamma	0.706
Percent Tied	5.6	Tau-a	0.333
Pairs	53900	c	0.833

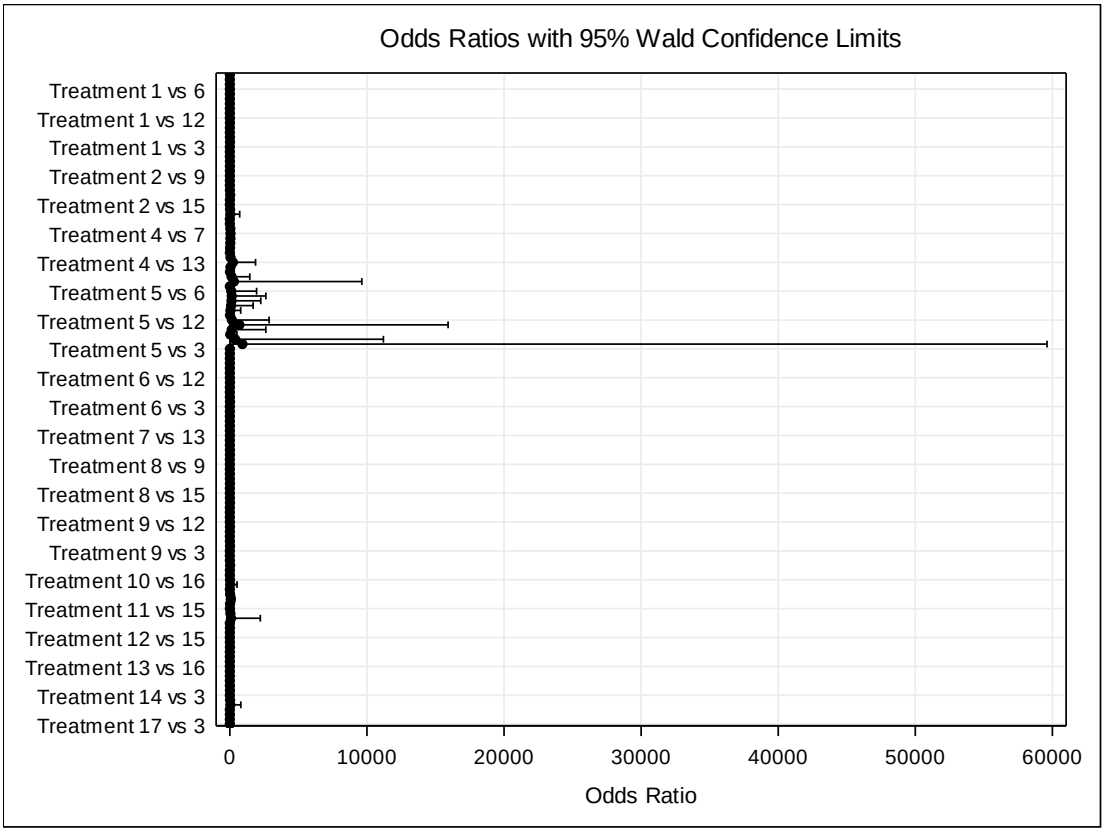
Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Treatment 1 vs 2	0.330	0.116	0.939
Treatment 1 vs 4	0.034	0.006	0.212
Treatment 1 vs 5	0.011	<0.001	0.208
Treatment 1 vs 6	1.146	0.405	3.240
Treatment 1 vs 7	1.529	0.527	4.437
Treatment 1 vs 8	1.319	0.461	3.772
Treatment 1 vs 9	1.000	0.356	2.806
Treatment 1 vs 10	0.458	0.155	1.350
Treatment 1 vs 11	0.115	0.033	0.401
Treatment 1 vs 12	1.671	0.581	4.811
Treatment 1 vs 13	7.703	1.713	34.627
Treatment 1 vs 14	1.529	0.527	4.437
Treatment 1 vs 15	0.299	0.103	0.866
Treatment 1 vs 16	4.279	0.610	30.012

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Treatment 1 vs 17	10.137	0.436	235.692
Treatment 1 vs 3	0.059	0.013	0.266
Treatment 2 vs 4	0.104	0.017	0.648
Treatment 2 vs 5	0.034	0.002	0.634
Treatment 2 vs 6	3.472	1.210	9.961
Treatment 2 vs 7	4.634	1.575	13.634
Treatment 2 vs 8	3.998	1.378	11.594
Treatment 2 vs 9	3.030	1.065	8.626
Treatment 2 vs 10	1.387	0.464	4.149
Treatment 2 vs 11	0.348	0.098	1.229
Treatment 2 vs 12	5.065	1.735	14.786
Treatment 2 vs 13	23.343	5.141	105.984
Treatment 2 vs 14	4.634	1.575	13.634
Treatment 2 vs 15	0.905	0.308	2.662
Treatment 2 vs 16	12.968	1.835	91.651
Treatment 2 vs 17	30.720	1.315	717.666
Treatment 2 vs 3	0.180	0.040	0.816
Treatment 4 vs 5	0.322	0.012	8.689
Treatment 4 vs 6	33.347	5.378	206.787
Treatment 4 vs 7	44.508	7.073	280.072
Treatment 4 vs 8	38.396	6.153	239.594
Treatment 4 vs 9	29.106	4.715	179.667
Treatment 4 vs 10	13.322	2.097	84.649
Treatment 4 vs 11	3.340	0.474	23.534
Treatment 4 vs 12	48.648	7.767	304.720
Treatment 4 vs 13	224.195	26.825	>999.999
Treatment 4 vs 14	44.508	7.073	280.072
Treatment 4 vs 15	8.690	1.381	54.681
Treatment 4 vs 16	124.553	10.660	>999.999
Treatment 4 vs 17	295.047	9.040	>999.999
Treatment 4 vs 3	1.725	0.206	14.418
Treatment 5 vs 6	103.476	5.491	>999.999
Treatment 5 vs 7	138.108	7.263	>999.999
Treatment 5 vs 8	119.143	6.298	>999.999
Treatment 5 vs 9	90.316	4.807	>999.999
Treatment 5 vs 10	41.339	2.161	790.894
Treatment 5 vs 11	10.363	0.507	211.773
Treatment 5 vs 12	150.955	7.961	>999.999
Treatment 5 vs 13	695.675	30.401	>999.999
Treatment 5 vs 14	138.108	7.263	>999.999

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Treatment 5 vs 15	26.964	1.418	512.757
Treatment 5 vs 16	386.486	13.336	>999.999
Treatment 5 vs 17	915.529	14.061	>999.999
Treatment 5 vs 3	5.353	0.234	122.493
Treatment 6 vs 7	1.335	0.456	3.903
Treatment 6 vs 8	1.151	0.400	3.318
Treatment 6 vs 9	0.873	0.309	2.469
Treatment 6 vs 10	0.400	0.134	1.188
Treatment 6 vs 11	0.100	0.028	0.352
Treatment 6 vs 12	1.459	0.503	4.232
Treatment 6 vs 13	6.723	1.487	30.390
Treatment 6 vs 14	1.335	0.456	3.903
Treatment 6 vs 15	0.261	0.089	0.762
Treatment 6 vs 16	3.735	0.530	26.307
Treatment 6 vs 17	8.848	0.380	206.260
Treatment 6 vs 3	0.052	0.011	0.234
Treatment 7 vs 8	0.863	0.292	2.549
Treatment 7 vs 9	0.654	0.225	1.897
Treatment 7 vs 10	0.299	0.098	0.912
Treatment 7 vs 11	0.075	0.021	0.269
Treatment 7 vs 12	1.093	0.368	3.251
Treatment 7 vs 13	5.037	1.095	23.176
Treatment 7 vs 14	1.000	0.334	2.997
Treatment 7 vs 15	0.195	0.065	0.585
Treatment 7 vs 16	2.798	0.392	19.982
Treatment 7 vs 17	6.629	0.282	155.861
Treatment 7 vs 3	0.039	0.008	0.178
Treatment 8 vs 9	0.758	0.265	2.168
Treatment 8 vs 10	0.347	0.116	1.042
Treatment 8 vs 11	0.087	0.025	0.309
Treatment 8 vs 12	1.267	0.432	3.715
Treatment 8 vs 13	5.839	1.282	26.595
Treatment 8 vs 14	1.159	0.392	3.426
Treatment 8 vs 15	0.226	0.077	0.669
Treatment 8 vs 16	3.244	0.458	22.982
Treatment 8 vs 17	7.684	0.328	179.792
Treatment 8 vs 3	0.045	0.010	0.205
Treatment 9 vs 10	0.458	0.155	1.350
Treatment 9 vs 11	0.115	0.033	0.401
Treatment 9 vs 12	1.671	0.581	4.811

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Treatment 9 vs 13	7.703	1.713	34.627
Treatment 9 vs 14	1.529	0.527	4.437
Treatment 9 vs 15	0.299	0.103	0.866
Treatment 9 vs 16	4.279	0.610	30.012
Treatment 9 vs 17	10.137	0.436	235.692
Treatment 9 vs 3	0.059	0.013	0.266
Treatment 10 vs 11	0.251	0.069	0.913
Treatment 10 vs 12	3.652	1.208	11.039
Treatment 10 vs 13	16.829	3.615	78.338
Treatment 10 vs 14	3.341	1.097	10.177
Treatment 10 vs 15	0.652	0.214	1.987
Treatment 10 vs 16	9.349	1.297	67.367
Treatment 10 vs 17	22.147	0.937	523.664
Treatment 10 vs 3	0.129	0.028	0.603
Treatment 11 vs 12	14.567	4.084	51.965
Treatment 11 vs 13	67.133	12.752	353.434
Treatment 11 vs 14	13.327	3.711	47.859
Treatment 11 vs 15	2.602	0.725	9.344
Treatment 11 vs 16	37.296	4.696	296.199
Treatment 11 vs 17	88.349	3.513	>999.999
Treatment 11 vs 3	0.517	0.098	2.720
Treatment 12 vs 13	4.608	1.007	21.087
Treatment 12 vs 14	0.915	0.308	2.721
Treatment 12 vs 15	0.179	0.060	0.531
Treatment 12 vs 16	2.560	0.360	18.203
Treatment 12 vs 17	6.065	0.259	142.215
Treatment 12 vs 3	0.035	0.008	0.162
Treatment 13 vs 14	0.199	0.043	0.913
Treatment 13 vs 15	0.039	0.008	0.178
Treatment 13 vs 16	0.556	0.060	5.185
Treatment 13 vs 17	1.316	0.047	36.798
Treatment 13 vs 3	0.008	0.001	0.049
Treatment 14 vs 15	0.195	0.065	0.585
Treatment 14 vs 16	2.798	0.392	19.982
Treatment 14 vs 17	6.629	0.282	155.861
Treatment 14 vs 3	0.039	0.008	0.178
Treatment 15 vs 16	14.333	2.007	102.348
Treatment 15 vs 17	33.953	1.444	798.303
Treatment 15 vs 3	0.199	0.043	0.913
Treatment 16 vs 17	2.369	0.068	82.778

Odds Ratio Estimates and Wald Confidence Intervals			
Odds Ratio	Estimate	95% Confidence Limits	
Treatment 16 vs 3	0.014	0.001	0.129
Treatment 17 vs 3	0.006	<0.001	0.163



Appendix 4 Bord na Móna Growing Media

Bord na Móna seed and modular compost

Nitrogen -120 milligrammes / litre of compost

Phosphorous – 60 milligrammes / litre of compost

Potash – 200 milligrammes / litre of compost

pH 5.3-5.7

Bord na Móna nursery stock growing medium

Nitrogen **–** 300 milligrammes / litre of compost

Phosphorous – 90 milligrammes / litre of compost

Potash – 300 milligrammes / litre of compost

pH 5.3-5.7