Vegetative propagation of dieback-tolerant *Fraxinus excelsior* on commercial scale

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Abstract
Ash trees which are tolerant to Hymenoscyphus fraxineus may be selected in all age classes among heavily infected populations. They may be produced also by controlled crossings of disease tolerant trees, because the genetic component of inheritance for disease tolerance is high. For mature and juvenile plant material, the deployment of disease tolerant genotypes could be potentially achieved by vegetatively propagating selected genotypes. We describe a system to vegetatively propagate selected ash genotypes and we discuss the prospects and options for using vegetative propagation on all age classes of trees. Mature trees were rejuvenated through the process of micropropagation to establish mother plants in large trays which were cut back repeatedly (hedged) to produce at least two crops of cuttings per year.

The rooting capacity of ten genotypes was tested by a commercial nursery over a period of three years, to assess the feasibility of using hedged mother plants for efficient propagation. Commercial practise was to treat cuttings with 0.25% IBA, insert them in plug pots and maintain them covered with fine plastic within low plastic tunnels in a non heated greenhouse and without supplementary heating at the cutting base. In the first year, the mean rooting rate was 53 % for the first crop of cuttings and 35 % for the second. In the second and third years the rooting rates improved to over 80% for each crop of cuttings as experience was gained in handling the material. Rooting rate varied among the genotypes.

We assessed the growth and development of micropropagated ash trees in the field from an observation clonal trial, consisting of four mature genotypes which had been established in 2002 in five replicate plots. The micropropagated trees were generally similar in height and dbh to seed derived control trees and developed normally. These observations are discussed in the context of using vegetative propagation as a tool in breeding and for the large scale deployment of ash with tolerance to H. fraxineus.

Keywords: *Fraxinus excelsior, Hymenoscyphus fraxinea, Chalara*, common ash, vegetative propagation, cuttings, rejuvenation, hedges

Introduction

The pathogen H. fraxineus has caused a pandemic of ash dieback disease in Europe. The scale of losses in forest productivity and in ecosystem functions is very great. The production of ash plants with tolerance to this pathogen will be required on a very large scale to restore European forests in the long run. It will also require that tolerant material is generated from parent material that is well adapted to the local and regional environments. Research has shown that tolerance to this pathogen is genetically determined and this opens the opportunity for selection and for breeding ash (Kjaer et al. 2012, Pliūra et al. 2011). The imperative to avoid genetic bottlenecks is discussed by Budde et al.(2016) and the breeding methodologies which are most appropriate for ash have been outlined previously; see 'Breeding methodologies' by Alfas Pliura in Douglas et al. (2013).

Many European countries are now in the process of selecting individual ash genotypes which have displayed a high tolerance to H. fraxineus over several years, within environments with a high disease pressure. The aim thereafter is to establish seed orchards by grafting scion material from selected trees, onto unselected seedling rootstocks. Grafted material has the advantage of accelerating the onset of flowering by comparison with seed derived trees. Progeny tests can reveal the value of individual parent trees from seed orchards. Also, clonal propagation can be very useful for the genetic testing of the components used to establish new orchards, and / or for rogueing existing ones, by ranking of the genotype performance (in clonal tests), and for the subsequent selection of those that are best adapted (Lindgren 2009, 2016).The clonal reproduction of ash plants is necessary to provide planting stocks for genetic testing, as mentioned above and also as a means for bulking up scarce supplies of germplasm which may be produced from controlled crosses and from the limited seed quantities produced in the early years from clonal or seedling seed orchards.

It is important that efficient systems are developed to vegetatively propagate selected genotypes of *F. excelsior* for genetic studies, clonal testing and also for large scale deployment in forests. Research on the tolerance of clonal material to H. fraxineus revealed a great variation among genotypes in disease response; none was entirely unaffected but some remained in good health (Mc Kinney et al. 2011, Lobo et al.2014). Furthermore, the tolerance phenotype was shown to be stable in diverse environments over a period of six years (Stener 2013). Stability in disease tolerance and its strong genetic control opens the potential for the development of clonal lines of ash provided the material can be propagated efficiently. It would be prudent to deploy vegetatively propagated material in the form of polyclonal mixtures and such mixtures should consist of sets of genotypes which have a high level of genetic diversity and a proven adaptation to the target ecological zone(s) (Budde et al. 2016).

The main objective of this study was to show the potential for vegetatively propagating mature ash trees on a commercial scale and the performance of micropropagated trees in the forest. In addition we discuss the options for using vegetative propagation to compliment breeding strategies and the challenges for propagating selected material in various stages of physiological maturity.

Materials and Methods

Propagation of ash clones by cuttings on a commercial nursery

The ash cuttings were produced by micropropagated plants of mature ash trees which had been maintained as hedges by pruning. The micropropagated trees were selected originally as plus trees for a genetic improvement programme and the micropropagation method was described previously (Douglas et al. 2013). After micropropagation, the plants were transferred into a standard nursery grade peat compost with Osmocote fertiliser and trace elements in large plastic crates 60 cm long x 40cm wide x 15.5 cm deep (Figure 1A). The crates had open mesh bases and sides and consisted of numerous slits, 6mm wide and 5cm long. For cutting production, the crates were maintained above ground level on open mesh wire benches to cause air pruning of emerging roots. The micropropagated plants were grown in crates, unpruned for a year after weaning and then pruned back in the second year of growth to the lowest pair of stem buds, within approximately 1.0 - 5.0 cm of soil level, (Figure 1B). Thereafter, for four years, the plants were pruned back to the lowest sets of buds, at least three times each year to form mother plant hedges (Figure 1C). The hedged plants were overwintered outside in crates (December to March) but maintained in an unheated greenhouse during subsequent summer months for cutting production (until December). Hedged mother plants were maintained fully hydrated by an automated overhead watering line in summer. The first of the annual pruning was usually done in March before bud burst when the hedged plants were brought into the unheated glasshouse. The second pruning was in mid to late May, coinciding with the first harvest of cuttings. The third pruning was in mid to late June coinciding with the second harvest of cuttings. Thereafter the hedges were allowed to grow before their removal for overwintering outside in December.

The stock of 10 clones of ash in the form of pruned hedges were transferred a commercial producer of rooted cuttings (Dunnes nursery) in early Spring before bud burst. The mother plants were approximately four to six years old plants when used for the cutting production trials on the commercial nursery. The crates of hedged plants were placed on bricks to facilitate the continued air pruning of the roots and were maintained in an unheated, shaded greenhouse. The plants were top dressed with fresh compost containing slow release fertiliser 'Osmocote Pro' annually and were liquid fertilised with ' Osmocote' as required.

At the beginning of each season (March) the shoots of the previous year were pruned back to stimulate the outgrowth for shoots from dormant buds. Cuttings were collected from all clones on the same dates, when the leaves on new shoots were fully expanded after a growth flush and when the shoots had lignified adequately in all / most of the clones. Two harvests of cuttings were made each year, the first harvest was usually in mid May but was season dependent. Turgid cuttings were collected from fully hydrated hedged plants and were inserted on the same day into compost for rooting. The cuttings consisted of two nodes and most of the cuttings had a terminal bud. The length of the cuttings was in the range 4-8cms; they were freshly trimmed at the base and dipped (to 1 cm) into 'Chrysotop Green' powder containing 0.25% 1BA. The rooting compost consisted of pure peat with dolomite lime (1.2 kg /m^3) and perlite (50L / m^3). Cuttings were inserted into compost in cylindrical jumbo plug pots (50 mm diameter and 60 mm long) in a paper sleeve, open at the base.

Figure 1 A) Micropropagated ash established in the trays for hedging and cutting production; **B)** emerged shoot cuttings from hedged plants; **C)** a 4 year old hedged ash plant in winter; **D)** the low tunnels used in an unheated glasshouse for rooting of cuttings in summer. **E)** Rooted cuttings in summer; **F)** Rooted apical cutting in winter; **G)** Rooted sub apical cutting in winter; **H)** rooted cuttings after one year of growth in 'Rootrainer' pots, note root emergence from the base of tray.

The rooting environment for cuttings was on the unheated floor within low tunnels in a shaded, unheated glasshouse (Figure 1D). The tunnels consisted of translucent plastic suspended on hoops; they were 1.7m wide and 0.6m at the highest point, tapering to ground level. Additional layers of translucent plastic were applied to the tunnels as required on days of intense sunshine. Trays of cuttings (94 / tray) were placed on a woven plastic fabric layer (Mypex) within the tunnels. In 2013 and 2015 an extra layer of translucent plastic was placed over the tunnels and a layer of fine clear plastic was placed directly on top of the cuttings within the low tunnels. In addition, to improve humidity of the rooting environment further, the cuttings were positioned in the centre of the low tunnels and the edge rows of trays contained hydrated plug pots but without ash cuttings. The rooting capacity of the cuttings was evaluated in early September (Figures 1F and 1G) and cuttings were then potted into large 'Rootrainer' pots and were ready for field planting at the end of the following year Figure 1H.

Assessment of micropropagated ash in a pilot scale clonal field trial

We established a field trial consisting of plants from five clonal lines which had been micropropagated. The test material consisted of three clones which were selected as plus trees in mature stands (clones 47, 48, 72) one mature tree which was not selected with a valuable phenotype (F5) and the juvenile clone 8x, micropropagated from a two year old sapling. The clones were micropropagated as previously described and grown in the nursery to the sapling stage (Douglas et al. 2013). The control trees were purchased as a commercial lot of saplings. Trees were planted in March / April 2002 on small mounds in a drained field of soil type brown podzolic, pH 6.8, at a spacing of 2m X 2m in five incomplete randomised blocks. The numbers of sapling trees per block were as follows: control seed derived saplings (16); clone 8x (4); clone F5 (12); clone JK49 (12); clone M72 (4); clone JK47 (4). The trees were maintained by chemical weed control. They were pruned twice to remove side branches to a height of 2.5m. Double leaders and forks were not removed so that the true stem form could be observed.

The traits measured after 14 years of growth were: total tree height (m), diameter at breast height (dbh) in cms, the length (m) of valuable timber produced (i.e. absence of stem defects), height to the first fork (m) and stem straightness using scale 1 to 5 (1= perfectly straight, 5 = crooked). Data was analysed using the Glimmix procedure in SAS 9.4. The analysis was a one-way classification with adjustments for blocking and a weighting was applied to allow for the varying numbers of measurements contributing to each plot means. Mean comparisons were made and multiplicity in the testing was taken into account by using Tukey-Kramer adjustment of the p-value. The significance level was taken as 0.05 and residual checks were made to ensure that the assumptions of the analysis (Normality, constant variance, etc.) were met.

Results

Propagation of ash clones by cuttings on a commercial nursery

Ash cuttings were collected from ten genotypes of micropropagated plants which had been maintained in a hedged condition by pruning. First rooting was observed within two to three weeks of cutting collection on the commercial nursery (Figure 1E). Table 1 summarises the overall rooting percentages obtained from two crops of cuttings within each year, over a period of three years. The dates of cutting collection were based on the observed growth and development of shoots from the hedged plants and on the extent of lignification of the shoots. In the first year (2012), the overall rooting rates were low for the first and second harvest of cuttings (35 to 53%) compared to the second and third years (81 to 94%), Table 1.

Table 1 Overall rooting capacity of cuttings of 10 clones of *F. excelsior* collected from hedges of micropropagated plants and rooted in a commercial nursery

Cutting harvest date	Number of cuttings taken	Number of cuttings rooted	% Rooted
09-June 2012	835	447	53.5
27-July 2012	723	254	35.1
24-May 2013	694	643	92.60
14-June 2013	481	454	94.38
07-May 2015	208	185	88.9
01-June 2015	292	238	81.5

The lower rooting rates in 2012 may have been due to inexperience in handling ash at the nursery i.e., collecting too many cuttings in a sub-optimal stage of development. In addition, the cuttings may have experienced insufficient humidity provided by the single layer of translucent plastic as the cover in the rooting tunnels. For the years 2013 and 2015, fewer cuttings were selected and the humidity was improved by providing a single layer of fine plastic in direct contact with the cuttings as well as an extra layer of translucent plastic as the cover in the rooting tunnels, Figure 1D.

The rooting rates obtained by the commercial nursery varied with the clone and the harvest period. For the first harvest period of each year, the best rooting year was 2013 in which all ten clones rooted at a rate of > 80%; for 2015, seven clones gave > 80% rooting and the remaining three clones rooted in the range 60 to 70% (Figure 2). For the second harvest period of cuttings in each year, the rooting rate was over 85% for all 10 clones in 2013. In 2015 rooting was > 80% for five clones and in the range of 48 to 78% for the remaining five clones (Figure 3).
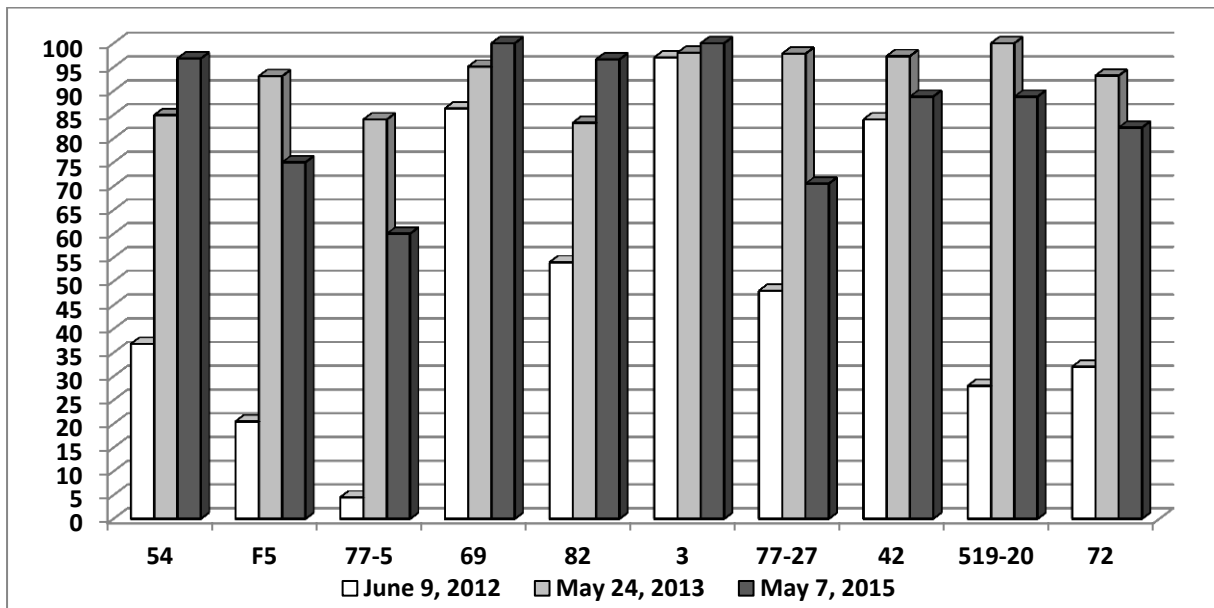

Figure 2 Rooting (%) of ash cuttings for the first annual harvest period, from hedged micropropagated plants of 10 ash clones, over three years in a commercial nursery. Clones with consistent rooting are circled.
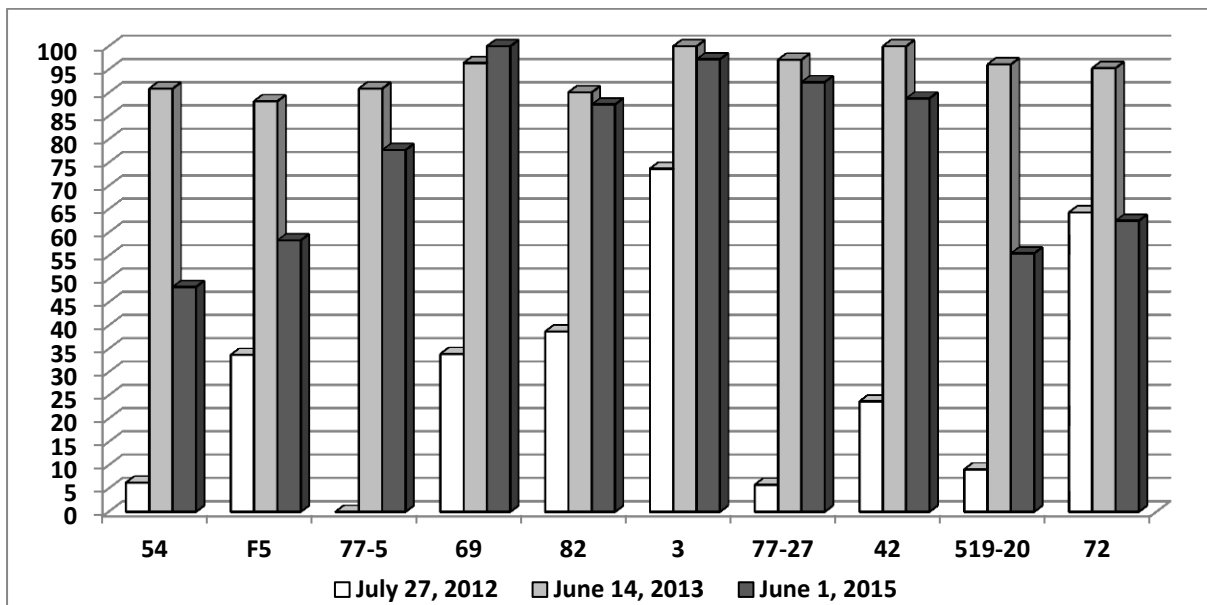

Figure 3 Rooting (%) of ash cuttings for the second annual harvest period, from hedged micropropagated plants of 10 ash clones, over three years in a commercial nursery. Clones with consistent rooting are circled.

The genotypes 69, 3, and 42 had the highest and most consistent capacity for rooting over all three years and harvest periods. In 2012, when we regarded the rooting conditions as sub optimal, these three clones gave the highest rooting rate among all clones with over 80% rooting (Figure 2). Furthermore, these same clones gave > 80 % rooting in each harvest in 2013 and 2015. All rooted plants survived transplantation into Rootrainers in early September (Figure 1F, 1G) and by September of the following year they had grown large enough for field planting (60-70 cm) Figure 1H.

Assessment of micropropagated ash in a pilot scale clonal field trial

Ash clones were planted in the field and their development was observed over a period of 14 years Figure 4. The aim of this pilot test was to record the developmental characteristics of the clones which had been produced by micropropagation compared to seed derived trees. After six years of growth, some flower production was noted and was recorded as 44% for the seedling control trees. The mean flowering frequency among the micropropagated clonal material was 41%: i.e. 82%, 96%, 8%, 0%, 21% for the clones 47, 49, 72, F5, 8x respectively.

Figure 5 The 14 year old clonal trial with micropropagated trees of *F. excelsior*

Table 2 summarises the growth and morphological characteristics after 14 years. Three of the clones were derived from plus trees and two of these performed well in growth and timber quality characteristics such as stem straightness, length of valuable timber and height to the first fork. Two other clones were derived from unselected trees (F5 and 8x) and they showed similar quality characteristics to unselected seedling derived control trees (Table 2). Overall, in terms of height growth and stem diameter the clonally produced trees grew favourably when compared to seed derived trees (Figure 4 and Table 2).

Table 2 Growth traits of micropropagated clones of *F. excelsior*: mature ash plus trees (47, 49, 72) non Plus tree clone F5, unselected juvenile clone 8x and seedling controls after 14 years of growth

| Growth parameter | Micropropagated clones | | | | | Seed |
| | Plus trees | | | Unselected | | Unselected |
	47	49	72	F5	8x	Controls
Tree height (m)	11.1a*	11.0a	10.6a	8.1b	10.3a	10.7a
Diameter at breast height (cm)	11.4ab	11.8a	10.2ab	7.6c	9.9b	10.8ab
Valuable timber length (m)	3.0a	3.1a	0.4c	0.6c	2.1ba	1.4bc
Stem straightness (1-5; 1= excellent)	2.4d	2.1d	4.4a	4.0ab	3.2c	3.6bc
Height. to 1st fork (m)	4.5ab	4.8a	2.1c	3.2bc	3.6bac	3.5bc

*Letters in common are not significantly different, Tukey- Kramer multiple comparisons

Discussion

This study has shown that mature trees of several ash genotypes could be propagated vegetatively in a commercial nursery and that micropropagated ash trees developed normally in the field over 14 years. Chalupa (1990) was first to report the successful field establishment, overwintering and normal development of micropropagated trees of *F. excelsior*. More recent work on micropropagated clones of F. americana reported the normal development of field planted trees over a period of six years and that variation for height growth among 12 clones was twice as great as the variation within the clones (Van Sambeek and Preece 2007).

The present study shows the feasibility of producing common ash by cuttings with the potential for commercialisation. The mother plants, maintained as hedges, produced viable cuttings over several years with two harvests of cuttings per year. The economic viability of producing ash by cuttings depends on the production costs, the selling price and a high rooting percentage for a range of genotypes. The commercial nursery in this study estimated that an overall rooting rate of 80-90% would be required for a profitable operation. They also estimated that approximately 50% of the production cost would be associated with maintenance of the hedged plants. We observed some variation in the rooting capacity among the ten clones and also between the time periods and years of cutting collection. These variables may be attributed to clonal effects and to the varying physiological stage of cutting development. We obtained improved rooting rates in the second and third years of the trials by the application of an additional layer of plastic in contact with the cuttings to increase the humidity. This observation is consistent with previous work on ash cuttings which showed the importance of high humidity during rooting (Jinks 1995). At a commercial scale, a high rooting capacity in each single harvest period is highly desirable. However, different flushing times were observed among the hedged ash clones and this would probably result in each clone reaching the optimal rooting stage at different times. Research is required to optimise the culture conditions of the mother plants and to define the biochemically optimal developmental stage for enabling a high rooting capacity (de Assis et al. 2004; Schwambach et al. 2008). Further efficiencies for commercial production of ash may be possible by adapting the methods used for large scale production of eucalyptus and other tree species. With eucalyptus the intensive production of cuttings is practised using mini and micro cuttings from stock plants grown in hydroponics (de Assis et al. 2004, de Oliveira et al. 2012). For teak, the frequency of pruning the stock plants was shown to be important for both the production of cuttings and their rooting efficiency (Singh et al. 2006). Other work on eucalyptus has shown the potential of genetic markers for selecting genotypes with a high capacity for rooting (Grattapaglia et al. 1996, Marques et al. 2002).

Our starting material for cutting production was mature trees which had been previously rejuvenated through a phase of micropropagation. This system of rejuvenation resulted in cuttings which had a high rooting capacity from the hedged mother plants over several years. Using a micropropagation step to induce physiological rejuvenation has been demonstrated for shrubs such as rhododendron (Marks 1991a,b) and hydrangea (Galopin et al. 1996). Thereafter the mother plants are maintained in a juvenile state by hedging as practised with eucalyptus which are propagated on a scale of hundreds of thousands using 'mini' cuttings (Brondani et al. 2012, Schwambach et al. 2008). A similar process of hedging is used in operational programmes with pine (Majada 2011) and spruce (Armson et al. 1980).

Mature ash trees which display useful traits such as a high tolerance to H. fraxineus in combination with other desirable traits of apical dominance, stem form and growth rates may exist in forests throughput Europe where the disease pressure is high. This valuable material may be conserved in living collections and by cryopreservation of shoots (Schoenweiss et al. 2005) and embryos (Brearley et al. 1995). The stability of disease tolerance has been shown and in clonal material and can be exploited by vegetative propagation (Stener 2013). However, the prudent deployment of clonal selections should be in the form of large polyclonal mixtures which should have a wide genetic diversity to ensure their wide ecological adaptability (Kjaer et al. 2012, Budde et al. 2016). The use of microsatellite markers and the development of new SNP markers will facilitate the identification of sets of genotypes which would constitute a wide genetic diversity in polyclonal mixtures.

To vegetatively propagate mature ash trees by conventional cuttings, it would be necessary to first apply the rejuvenation step of micropropagation. This would generate plantlets in a juvenile state for the production of hedges that would allow the scaling up of production by using the cheaper and more efficient route of conventional cuttings. In this process the micropropagation step may prove the most challenging. Previous research on micropropagation of *F. excelsior* has concentrated on the effects of growth regulators on shoot proliferation resulting in rather low micropropagation rates in the range of 1.5 to 3.2 shoots produced per original shoot cultured / month (Douglas et al. 2013, Schoenweiss, and Meier-Dinkel 2005, Hammatt 1996, Silveira and Cottignies 1994, Pierik and Sprenkels, 1997. Similar results have been reported for other ash

species: *F. ornus* (Arrillaga et al. 1992), *F. angustifolia* (Perez-Parron el al. 1994, Tonon et al. 2001a), *F. americana* (Navarrete et al. 1989) and *F. pennsylvanica* (Kim et al. 1997). The most detailed studies on *F. excelsior* have been by Schoenweiss and Meier Dinkel (2005) and Silveira and Cottignies (1994). The former reported that establishing viable shoot cultures was genotype dependent; just 20% of the genotypes from 16 year old trees were successfully established in the micropropagation phase. Furthermore, they obtained best results for initiating cultures by using emerging buds from grafted plants. Silveira and Cottignies (1994) reported that the optimal time to initiate ash culture from 4 to 7 year old trees was by using apical buds in the months of September, January or March, whereas culturing in May or June failed. Establishing aseptic shoot cultures of ash is hindered by the presence of a rich endogenous and exogenous microflora which can lead to bud necrosis (Donnarumma et al. 2011, Scholtysik et al. 2013). Other factors may be related to the micro morphology of the buds which are selected to initiate cultures (Remphrey 1989, Remphrey and Davidson 1994). It is highly desirable that more efficient methods are developed to consistently establish shoot cultures of ash which are genotype independent, especially from mature and semi mature trees for micropropagation and for rejuvenation. Using shoot meristems may facilitate the establishment of viable shoot cultures by excluding endogenous contaminants and by minimising the physiological influences of the larger tissue masses in whole buds (Ewald and Kretzschmar 1996, Ewald 1998).

Juvenile ash trees which are tolerant of H. fraxineus may be good subjects for selection, breeding and bulking up by vegetative propagation, especially if they have displayed disease tolerance over a few years, in heavily infected forests. Several studies have shown that juvenile saplings of ash are subjected to a high selection pressure and are most susceptible to infection and death by H. fraxineus. Disease pressure is highest close to ground level (Chandelier et al. 2014) and the greatest attrition rate was reported for ash trees in the age class of 5–15 years in Denmark (McKinney et al. 2011) and in Lithuania (Pliura et al. 2011). Selected juvenile trees could be transplanted from the forest to the nursery and converted to hedges as sources of cuttings by repeated pruning as described above. Ash trees generally transplant successfully because of their massive system of fibrous roots and they have a capacity for re-sprouting when cut back. Lygis et al. (2014) reported that 88.6% of stumps in the diameter class 1-10 cm regenerated shoots compared to 35.6% in the 11-20 cm class. Furthermore, previous research indicated the more juvenile nature of coppice shoots; cuttings from coppiced trees gave 47% rooting compared with 26% for shoots taken from the crowns of trees (Cahalan and Jinks 1992).

Several research groups are in the process of establishing seed orchards by selecting parent trees which display field tolerance to H. fraxineus because the heritability of tolerance is high (Pliūra et al. 2011, Kjaer et al. 2012, Lobo et al. 2014). For seed orchards, the selected genotypes are generally propagated by grafting them onto seedling derived rootstocks which are not selected. Graft viability of ash in winter and summer has given success rates of 85-97% for a diverse range of genotypes from mature trees (Douglas et al. 1996, 2001) and even for trees over 100 years old (Obdržálek and Hendrych 2014). Although this approach is conventional, some consideration should be given to the disease tolerance status of the rootstocks because emerging evidence suggests that H. fraxineus may be a causal agent in collar / root rots, either as a sole agent or in concert with other soil pathogens (Husson et al. 2012, Enderle et al. 2013, Marçais et al. 2016, Chandelier et al. 2016). It is not known if genotypes selected with tolerance to crown dieback are also tolerant of collar / root rots. Therefore, there is a case for selecting and vegetatively propagating rootstocks with known resistance to collar rots. The alternative is to select and vegetatively propagate those mature trees which appear tolerant to the combination of crown dieback as well as collar / root rots in forests where both symptoms are common. Using these trees to constitute a seed orchard would require that they would be vegetatively propagated in such a way that each tree has its own (tolerant) root system. However, rooting in the cuttings from the crowns of ash or coppice shoots is low (Cahalan and Jinks 1992) and for large scale propagation the rejuvenation step by micropropagation would be needed to obtain self rooted trees. In general it would be desirable to use genotypes with tolerance to collar rots as the rootstocks for establishing seed orchards, because they would be a potential safeguard against the development of collar rots in future years.

Outdoor seed orchards of ash will be subject to pollen inflows from sources that are situated locally (Morand et al. 2002, Thomasset et al. 2013) and far away (Bacles and Ennos 2008). These studies, using microsatellites indicate pollen flows of up to 70% and more accurate figures from multiple SNP markers will probably reveal higher figures. Consequently, ash seed orchards should be situated in sites which are surrounded by closed forest of another species, as a minimum requirement. It would be more efficient to establish indoor seed orchards to minimise pollen contamination. We have observed viable seed production in grafted trees in the greenhouse where the scion material was from adult trees. Panmixis in indoor orchards should generate ash seeds with the highest levels of tolerance to H. fraxineus. In addition, indoor seed orchards would be the most convenient for generating seed progeny from controlled crosses. In these cases the seeds produced will be valuable and in limited quantities but they could be usefully bulked up vegetatively using a variety of means as

described. High rooting rates would be expected from hedges of this juvenile starting material and we estimate that such hedges should remain highly productive for at least 10 years. Micropropagation would also be an option for seed sources because seed explants are easier to establish in vitro and various seed tissues can be used to regenerate plants. Raquin et al. (2002) have demonstrated that the dormancy requirement of *F. excelsior* could be averted by culturing embryos directly from the seeds. Similarly, Van Sambeek and Preece (2007) have shown that germination in seeds of *F. americana* could be accelerated in vitro by cutting one to two mm from the end of the seeds containing the tips of the cotyledons. Shoot organogenesis has been reported in cultured embryos of *F. excelsior* and was affected by the genotype of the mother tree (Mockeliunaite and Kuusiene 2004) as well as for *F. angustifolia* (Tonon et al. 2001a). Other studies have shown that multiple plants could be regenerated from immature embryos of *F. excelsior* (Capuana et al. 2007) and their epicotyls (Mitras et al. 2009) as well as from hypocotyls of *F. profunda* (Stevens and Pijut 2012). Somatic embryogenesis may be considered as a propagation tool for seeds since the starting tissues to induce them is usually embryos in various stages of differentiation. Somatic embryogenesis has been reported for: *F. excelsior* (Capuana et al. 2007); *F. angustifolia* (Tonon et al. 2001b); *F. americana* (Preece and Bates 1995, Bates et al. 1992) and *F. mandshurica* (Kong et al. 2012; Yang et al. 2013). However, for *F. excelsior* somatic embryogenesis was confined to the seeds derived from one out of four mother trees tested and embryo induction was confined to embryos cultured at an immature stage (Capuana et al. 2007). Further development work is required to make somatic embryogenesis more efficient and applicable to a wide range of genotypes of *F. excelsior*.

Acknowledgements

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