Chapter 9
Common Ash (*Fraxinus excelsior* L.)


9.1 Biology, Ecology and Natural Distribution of Ash

Alfas Pliura

The natural range of common ash covers most of Europe from the shores of the Atlantic Ocean in the West to the Volga River in the East, with the exclusion of the most northern and southern parts (Fig. 9.1). The northern limit is about 64° North in Norway, with the southern margin reaching 37° North in Iran. In mountainous areas, common ash is found in the Pyrenees at 1,750–1,800 m above sea level and in the Swiss Alps at 1,630 m. In Asia (Iran), it can be found at much higher elevations of up to 2,200 m.
Common ash is the largest tree in the genus *Fraxinus* and at maturity (90–120 years) it can reach 20–35 m (maximum 40 m) in height. The mean stem diameter varies from 30 to 70 cm (maximum 150 cm) in adult specimens. The crown is irregular with massive branches, elongated in forest stands.

Common ash is wind pollinated. Flowering starts at 15–20 years on single trees and at around 30 years within stands at irregular intervals. The breeding system is polygamous, ranging from male and female individuals with hermaphroditic intermediates. Morphologically, hermaphroditic individuals are often predominantly male or

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female. Inter-annual variation in sex expression is observed. The fully developed seeds start to disperse by wind in the autumn. According to Heuertz et al. (2003), a mean seed dispersal distance established in Romanian population of *Fraxinus excelsior* was less than 14 m, and moderate pollen flow was in the range 70–140 m. In a French study, the mean seed dispersal distance was about 140 m (Morand et al. 2002b). Seed dormancy usually lasts for two winters but can last for up to six. Stored seed requires combined warm–cold stratification to germinate; see Sect. 9.5.

Common ash requires a rich soil and tolerates a pH as low as 4.5, but prefers soil above 5.5. It is highly tolerant of seasonal water-logging and favours floodplain forests. It is also a typical species of slopes and ravines, growing in association with other characteristic species such as maple, lime and elm. Although dormant trees are very hardy to cold, the young shoots are sensitive to frost.

Common ash exhibits intermediate properties between a pioneer forest tree species and a permanent forest component. Although dispersal and natural regeneration are efficient, the competitive ability of the species is strong only when the ecological requirements are met. Vegetative regeneration is strong after coppicing.

Of the four different ash species growing naturally in Europe, common ash is the most important commercially. Despite the high demand for this quality timber, only a few European countries have gene conservation or tree breeding programmes in place.

Common ash timber is hard, elastic and withstands pressure, shock and splintering. It has a straight grain and there is little distinction between sapwood and hardwood, making it very valuable for furniture, veneer and flooring. It is used widely for tool handles, and for sports equipment such as hockey sticks, oars and hurdles. The formation of “black heart”, a dark stain of the hardwood, can occur in mature trees. This varies within and between individual trees and different sites and reduces the economic value. Ash bark and leaves are astringent and the leaves are used in modern herbal medicine for their laxative properties.

### 9.2 Gene Conservation

Alfas Pliura

On the European scale, common ash has not been considered as an endangered species (Eriksson 2001). However, the natural range and area of ash forests has decreased during the last 4,000 years as the area of agricultural lands has increased. Due to the high economic value of common ash, its silviculture has been actively promoted in the last 30–40 years, supporting natural regeneration, planting and thinning. Common ash genetic resources are threatened by deforestation, loss of suitable habitats, unsustainable exploitation and improper management (i.e. uncontrolled transfer of reproductive material), natural climatic changes, global warming, air pollution, competition with other species, pests, game damage and most recently by ash dieback disease. These pressures could lead to population extinction. Despite the high regeneration potential, the reproduction of some valuable autochthonous
populations is not ensured, and the health status of mature stands in some countries has deteriorated significantly in recent years. Therefore, common ash is considered to be threatened at population level by most countries. In addition, the situation has dramatically worsened recently because of resent ash dieback caused by the pandemic of ash diseases (primarily *Chalara fraxinea*).

EUFORGEN *Fraxinus excelsior* gene conservation strategies and technical guidelines emphasize that genetic conservation should aim at ensuring continuous survival and adaptability of the target species and that these objectives are best met when the Multiple Population Breeding System (MPBS) is applied (Pliura 1999; Pliura and Heuertz 2003). It was recommended that an inventory should be compiled to define the geographical distribution of the species, conservation status, threats and potential use patterns. Then the eco-geographic zones (provenance regions) should be delimited according to climatic variation, topography, soil and vegetation (Weiser 1995). Trees are generally best adapted to the ecological conditions of the region where they evolved. Therefore, local material should be used for plantations wherever possible, unless otherwise recommended as the result of data from provenance trials.

To ensure the adaptive potential of *Fraxinus excelsior* in Europe, EUFORGEN recommended that two complementary gene conservation networks of populations were established, specifically: (1) a network of 20–30 *in situ* populations throughout provenance regions; and (2) a network of *ex situ* populations (progeny trials, provenance trials, collections). Whenever possible, *in situ* conservation activities should be undertaken jointly for other noble hardwoods. Where common ash occurs in large populations in a country, *in situ* conservation is sufficient, with the selection of up to three gene conservation populations/gene reserves of 5–15 ha in size, with at least 100 flowering trees in each provenance.

Gene conservation populations should represent all postglacial migration routes of *Fraxinus excelsior*. A high density of *in situ* gene conservation populations should be established in south-east Europe, especially in Romania and Bulgaria, which have been colonized by populations from different ice-age remnants. In these regions, neutral genetic markers show a differentiation among populations, suggesting that they may have different potentials to cope with future climatic conditions. Special emphasis in selecting populations for conservation should be put on populations which demonstrate higher resistance/tolerance to *Chalara fraxinea*. Specific conservation efforts are also recommended in northern central Europe, due to the high level of differentiation between populations in southern Sweden, although the historical origin of this differentiation still needs to be verified.

Gene conservation strategies and technical guidelines from EUFORGEN have emphasized that *in situ* gene conservation populations need to be managed to increase their adaptive potential by ensuring the natural regeneration of the target species, creating multi-age structure and habitat diversity, and increasing generation turnover (Pliura and Heuertz 2003). To conserve an even-aged mature stand *in situ*, parts of the population should be opened (thinned or cut into narrow
strips of 15–30 m width) to create conditions for natural regeneration. Preferably, this should be undertaken in the year following the mast, when maximum seed is produced by the stand. An area adjacent to the gene reserve could be set aside for natural regeneration, and could later be incorporated as part of the reserve. To promote regeneration in clear-cut strips, randomly selected, abundantly flowering seed trees should be left. If the population consists of some stands or groups of trees of different ages but there is no regeneration, the oldest stands or groups should be cut as soon as the mast years have produced sufficient seed yield or regeneration under the canopy or in areas set aside. Increasing the number of stands or demes (groups of trees) of different ages in the population enhances intra-population genetic variation as the portion of trees involved in regeneration increases. Regeneration can also be stimulated by site scarification and weed control. If these regeneration support measures are not successful, it is recommended that material originating from the population is planted: seeds should be collected from at least 50 trees per population, preferably from central parts of the gene reserve. To prevent gene flow from outside the gene reserve, a buffer zone of 100–150 m should be created by gradually removing mature flowering ash trees within this zone. To secure the sustainability of each population, careful tending is required. Effective treatment including adequate silvicultural measures, protection against disease or insect outbreaks, fire or other factors must be undertaken promptly.

Special measures should be directed to investigating optimal control measures for the pathogen causing ash dieback (Chalara fraxinea) and to generating more resistant populations. Diseased trees (mature and young) should be removed from populations to prevent gene and seed flow from susceptible genotypes. Degraded populations will need to be supplemented by artificial planting with material bred for resistance because the number of remaining healthy trees will be too low (low effective population size $N_e$) to guarantee successful regeneration of genetically diverse and thus sustainable populations. Thinning should be undertaken from below, removing suppressed and damaged trees, thus simulating the natural selection processes in the forest, and stand regeneration. Each gene conservation population must be constantly monitored, including the health status, regeneration success and amount of remaining genetic diversity.

The EUFORGEN Fraxinus excelsior gene conservation strategies and technical guidelines recommended that in situ conservation should be complemented by ex situ measures for populations that are marginal, isolated, endangered, growing under special ecological conditions or carrying rare features. This is of particular importance in the case of dramatic ash dieback in Europe. The most effective form is through progeny trials, which permits joint gene conservation and breeding. On a national scale, 1–3 progeny plantations for conservation (each of 2–4 ha in size) should be established in each provenance region with entries sampled from single trees randomly chosen from 10 to 20 stands within the region and from marginal populations if applicable. These ex situ gene conservation populations can serve as the main breeding populations of Fraxinus excelsior improvement programmes at
the same time. As soon as reproductive age is reached, open pollination of the best individuals selected within each family should ensure the next generation. About 50 optimally adapted individuals should be the founders of each new gene conservation/breeding sub-population.

9.3 Importance of Ash Dieback Disease *Chalara fraxinea* (*Hymenoscyphus pseudoalbidus*)

A. Chandelier, Viggo Jensen, M. Knudsen, Elena Foffova, and Alfas Pliura

9.3.1 Background

Over recent years a severe disease causing the dieback of crown shoots and the death of young and old trees has been reported in many European countries in all growing situations of: forest plantations, roadside trees and in nurseries (EPPO 2008; Halmischlager and Kirisits 2008). Ash dieback disease is known in Poland, Denmark, Sweden, Estonia, Hungary, Norway, Sweden, Lithuania, Germany, Austria, Czech Republic and it may also be present in Estonia, Switzerland, Latvia, Slovenia and France based on symptoms (EPPO 2008). The disease is evident from wilting of shoot tips in summer with a resulting dieback in 1-year-old and 1–2-year-old shoots and the entire crowns of trees. Shoots die either before or just after flushing and in dry summer periods. Necrotic lesions appear on stems around buds or dead twigs and a discolouration is found in the wood of dead shoots near the necrotic areas.

The causal organism is believed to be the ascomycete fungus *Chalara fraxinea* (Kowalski 2006; Kirisits et al. 2009; Kowalski and Holdenrieder 2009a, b), though other fungi have been found in association with dieback disease (Bakys et al. 2009). A detailed study on two 15-year-old sites suggested that a primary disease (*Chalara fraxinea*) was responsible for the dieback symptoms but this disease and tree death were associated with a secondary organism (*Armillaria gallica*) rather than other known pathogens/pests such as *Neonectria galligena*, *Pseudomonas syringae* subsp. *savastanoi* pv. *fraxini*, *Hylesinus fraxini* or *H. varius* when considered collectively (Skovsgaard et al. 2009; Lygis et al. 2005).

The epidemiology of the fungus was investigated when a fungus was isolated from damaged trees and teleomorphs were studied; different molecular methods have been developed to detect the pathogen in infected tissues (Bakys et al. 2009; Chandelier et al. 2010; Loos et al. 2009; Johansson et al. 2009). Morphological characteristics of teleomorph and DNA analysis suggested that the sexual stage of *C. fraxinea*’s telemorph could probably be attributed to the known ascomycete *Hymenoscyphus albidus* (Roberge ex Desm.) W. Phillips (Kowalski and Holdenrieder 2009b). This fungus was known as a harmless saprophyte growing...
on the shed leaf petioles of *F. excelsior* (Kowalski and Holdenrieder 2009a, b). Recent molecular studies have shown that two morphologically very similar fungal taxa exist, i.e. *H. albidus*, the non-pathogenic species, and *H. pseudoalbidus*, the virulent species responsible for the current ash dieback epidemic in Europe; the latter is the sexual teleomorphic stage of *C. fraxinea* (Queloz et al. 2010). *H. pseudoalbidus* is an emergent pathogen. Its origin is still unknown.

A scoring system for recording the disease in young trees has been developed by A. Pliura in Lithuania (Fig. 9.2).

### 9.3.2 Management Suggestions and Breeding

#### Prospects for Resistance

The infection biology of this disease is unknown, consequently, recommendations on limiting its spread and reducing its effects are not developed. The conidia of *C. fraxinea* are sticky and their dispersal may be less effective than the teleomorph, *Hymenoscyphus pseudoalbidus*, which is wind-transmitted (Kowalski and Holdenrieder 2009b). *Chalara fraxinea* may be able to disperse aerially, in soil, water, plants for planting, seeds, or wood (EPPO 2008; Kile 1993). Insect vectors may also have a role in disease transmission.

*Chalara* has been reported from single observations on *Fraxinus angustifolia* subsp. *danubialis* (narrow-leaved ash) and *Fraxinus excelsior* ‘Pendula’ (weeping ash) (Kirisits et al. 2008). Susceptibility of other species e.g. *F. ornus*, to *Chalara fraxinea* is unknown. It is also unknown whether resistance to *Chalara* is present outside the range of the affected areas, and/or if climate conditions would hamper or affect the spread of the disease into new areas. The fungus is difficult to purify and is slow growing on agar. Recently, a protocol was developed to detect *Chalara fraxinea* directly in plant tissue based on a real-time PCR assay. It detected the pathogen and proved to be efficient and rapid. This tool should be useful for early detection, monitoring and for epidemiological studies (Chandelier et al. 2010; Ioos et al. 2009).

The first genetic studies of resistance to this disease were in Danish seed orchards. They revealed significant genotypic variation in resistance to *C. fraxinea* in grafted clonal material (McKinney et al. 2011). The comparatively large coefficients of heritability (from 0.40 to 0.49) indicate that about half of phenotypic variation in the level of damage is due to genetic inheritance. The degree of damage of different clones varied from 1 to 68 %, and the coefficient of genotypic (clonal) variation was rather large (CV_g=61–78 %, McKinney et al. 2011). Even though the average level of damage among 39 clones recorded in 1 year increased from 36.1 to 55.9 %, the damage level among the most resistant clones remained at the level of 10–13 %, This indicates the existence of genetically based resistance at clonal level. However these genotypes originated from different populations, thus no resistant populations have been found yet. Recent detailed studies from progeny trials in Lithuania are summarized in Sect. 9.7.1.
### Resistance Score Scheme Photo Description

<table>
<thead>
<tr>
<th>Resistance score</th>
<th>Scheme</th>
<th>Photo</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td><img src="image1" alt="Tree" /></td>
<td><img src="image2" alt="Photo" /></td>
<td>Completely resistant tree: healthy tree – no signs of damage (brown dry or wilted leaves, brown spots on stem or branches)</td>
</tr>
<tr>
<td>4</td>
<td><img src="image3" alt="Tree" /></td>
<td><img src="image4" alt="Photo" /></td>
<td>Resistant tree: limited damage – single brown dry or wilted leaves or peripheral shoot or/and brown spots on stem or branches</td>
</tr>
<tr>
<td>3</td>
<td><img src="image5" alt="Tree" /></td>
<td><img src="image6" alt="Photo" /></td>
<td>Moderately resistant tree: moderate damage – single or repeatedly dry leading shoot or dry leaves on leading shoot and once or repeatedly resprouted leading sprout/shoot</td>
</tr>
<tr>
<td>2</td>
<td><img src="image7" alt="Tree" /></td>
<td><img src="image8" alt="Photo" /></td>
<td>Tree of low resistance: heavy damage – single or repeatedly dry whole stem to the ground level and once or repeatedly resprouted single or some sprouts/shoots</td>
</tr>
<tr>
<td>1</td>
<td><img src="image9" alt="Tree" /></td>
<td><img src="image10" alt="Photo" /></td>
<td>Tree of very low resistance: completely damaged tree – dry tree with signs of former single or repeatedly dried whole stem and dry resprouted shoot or shoots</td>
</tr>
<tr>
<td>0</td>
<td><img src="image11" alt="Tree" /></td>
<td><img src="image12" alt="Photo" /></td>
<td>Unresistant tree: completely dry tree without visible attempts of former resprouting</td>
</tr>
</tbody>
</table>

**Fig. 9.2** Scoring of resistance/tolerance to *Chalara fraxinea* for young trees of *Fraxinus excelsior*
National and international programmes should be initiated to breed resistant material so that resistant germplasm can be used for restoration of gene conservation populations, breeding populations and general deployment into viable ash forests.

9.4 Other Diseases and Pests of Importance on Ash

Marijke Steenackers, Elena Foffova, Jean Dufour, and Gerry C. Douglas

9.4.1 Bacterial Canker, *Pseudomonas syringae pv. savastanoi*

Ash canker disease can be caused by the bacterium *Pseudomonas syringae pv. savastanoi*, and the fungus *Nectria galligena*. Bacterial cankers are characterized by a general swelling of the stem and presence of brown corky tissue, sometimes with a liquid ooze (Janse 1981). Fungal cankers typically exhibit a massive death of tissue with some swelling at the edges of the canker due to callus production. Ash canker disease caused by *Pseudomonas syringae pv. savastanoi* is known from Ireland to Poland, and from Scotland to Italy and Greece.

The bacterial disease first shows as small cracks in the bark of young shoots. Later, cankers develop and the bacterium becomes systemic in the tree, so that cankers can be initiated on older stems internally without wound infection. Several authors state that the disease is mainly found on trees growing in sites that are unsuitable for ash. Nevertheless, field observations suggest that different genotypes of ash display differences in susceptibility to the disease, which are not induced by environmental factors. An artificial infection technique has been developed, allowing assessment of resistance when the tree is very young. This technique was applied in order to evaluate the susceptibility of 31 European provenances of *Fraxinus excelsior*, belonging to the core collection of a pan-European provenance trial established in Belgium (Research Institute for Nature and Forest) within the EU’s RAP project. Results proved the presence of resistance to bacterial canker within each of the provenances, however great differences were identified between the provenances (M. Steenackers, unpublished data). A species-specific primer has been developed for the detection of *Pseudomonas syringae pv. savastanoi* directly in plant tissue. This technique proved the possible presence of the bacterium in trees showing no symptoms of the disease (M. Steenackers, unpublished results).

9.4.2 Fungal Canker

The fungal canker *Nectria galligena* is more common on ash in moist climates. It enters at leaf scars and may appear at the nodes of shoots as a canker area.
Older cankers produce much corky callus and red perithecia in the cracks. Infective ascospores are produced in rainy periods when they are ejected and disseminated.

### 9.4.3 Pests

In the establishment of plantations, rabbits, hares and deer can destroy young plants and protection is needed. In plantations that are established in pasture fields, slugs and snails have been observed to cause damage by eating the young bark and shoots may die if a strip of bark around the full circumference is eaten. In Europe, hornets (*Vespa crabo*) may cause similar damage to the bark on young trees (J. Dufour 2012, personal communication).

The ash bark beetles feed under the bark and may cause wilting of the trees. Stressed trees are first affected, however, healthy trees may be attacked in stands which are heavily infested (Lozano et al. 1993). The more frequent species *Hylesinus fraxini* is small (adult 2.5–3.5 mm) and feeds on the trunks of younger trees or the thick branches of older trees. The more robust *Hylesinus crenatus* (adult 4–6 mm) lives on bigger trunks (Zúbrik et al. 2008). Both species are distributed throughout continental Europe and also in the British Isles.

The terminal buds of ash may be killed by larvae of the Ash Bud Moth (*Prays fraxinella*). Adults fly in June/July and are common in Europe. However, a study by Kerr and Boswell (2001) concluded that late frost is a more significant factor in killing terminal buds than bud moth.

Adults of the blister beetle (*Lytta vesicatoria*) feed on ash leaves in June in central and southern Europe. It is brilliant green in colour, 15–22 mm in size with an intensive musky scent. In abundant years this pest can cause the total defoliation of ash trees. Other pests which feed on ash leaves are the larvae of ash sawflies, *Tomostethus nigritus* and *Macrophya puctalibum*. During the spring season, adult ash sawflies make small cuts in leaflets of ash trees and lay their eggs; younger larvae chew round holes, while more mature larvae consume entire leaves (Novák et al. 1974).

The sucking insects from the order Hemiptera are another group which may damage ash leaves. Woolly ash aphid (*Prociphilus bumelieae*) are soft yellow-green insects with pear- or globule-shaped bodies; they excrete a wax that causes them to appear wool-covered. Symptoms include curled and deformed leaves, as well as the appearance of honeydew on leaf surfaces (Zúbrik et al. 2008). Other sucking insects affecting ash include the ash psyllids *Psyllopsis discrepans* and *Psyllopsis fraxini*; which cause leaf swelling and a red colour on the borders of rolled leaflets (Zúbrik et al. 2008).

The tiny mite *Eriophyes fraxinivorus* is difficult to detect with the naked eye. They suck on ash flowers and change them to fleshy green galls. In autumn the galls turn brown and woolly and hang on the branches for more than 2 years. Heavily infested trees produce few or no seeds.
9.5 Phenology, Seed Production, Stratification and Seed Orchards

Gerry C. Douglas, Jean Dufour, Muriel Thomasset, and Viggo Jensen

9.5.1 Phenology

Ash begins to flower in early spring (early–mid March in the UK and Ireland) and ends in mid–end May. Anthesis is not always synchronous in adjacent buds (Fig. 9.3).

For the vegetative buds, highly significant provenance effects for the bud flushing dates were observed (Cundall et al. 2003) and see section on Lithuania below. Bud flushing is usually recorded in the field, however after forcing shoots by heat treatments, the relative flushing of early and late provenances was maintained (Jouve et al. 2007). A convenient notation for scoring flushing is given below in Fig. 9.4.

9.5.2 Seeds

Ash seeds contain immature embryos when they are shed in autumn. A small proportion of seeds may germinate if collected and sown in August when the fruits are still green, before dormancy sets in. Alternatively, germination may be obtained in the first year by in vitro culture (Raquin et al. 2002). Fresh seeds have a moisture content of 25–30 %, and can be stored for many years by first reducing the moisture content to 8–10 % and stored refrigerated or at minus 2 °C. A period of stratification/vernalization is required for the embryo to fill the embryo sac by metabolizing the endosperm in advance of germination.

Dry brown seeds must be stratified to achieve uniform germination. Seeds should be mixed with moist peat/sand 1:1 or 2:1 (V/v) and then stored for at least 8 weeks at 16–20 °C followed by 8 weeks of cold stratification at 3 °C (J. Dufour, 2012; Suszka et al. 1996). This operation may be done in outdoor pits for large nursery batches where the natural temperature cycles are sufficient.

9.5.3 Seed Production in Seed Orchards

Significant seed production is possible within 7–10 years in clonal seed orchards. It is important to note the gender of the trees to ensure a good balance between the
different sexual types (male, female and hermaphrodite). A design of the seed orchard with male trees surrounding females may help panmixis.

In western France a clonal seed orchard of grafted trees was planted in 1993 at a spacing of 5 m × 3.5 m, consisting of 32 phenotypically selected clones (531 plants). It began fruiting in 2000. From this surface area of 0.93 ha 42 kg of seeds were obtained in 2004 and 175 kg in 2005 (J. Dufour, 2012)
In Denmark two seed orchards were established from 1947 to 1955 on sites of 1.5 ha. The initial spacing was 4×3 m reduced to 8×12 m. The mean seed yield per year was over 200 kg/ha over 13 harvests (V. Jensen 2012, personal communication). These Danish seed orchards are over 65 years old and still productive.

9.6 Value of Ash and Sources of Planting Stock

Gerry C. Douglas

9.6.1 European Timber Volume, Uses and Values

Statistics and data were provided by partners in the Treebreedex project and they are given in Annexe 9.1.

Ash is harvested as logs for processing and it is used extensively for firewood in many countries because it can be burned fresh. The annual volume of commercially used wood varies with each country. It is <100 m$^3$ for Finland. From other countries the annual volume of commercially used wood has been reported as: 55,000 m$^3$ from Norway; 695,000 m$^3$ from the Czech Republic and 33,000 m$^3$ from Slovakia. For Ireland, 20,000 m$^3$ was an annual harvest for firewood and 3,000 m$^3$ as logs. The statistic of annual production of ash in France was combined with maple to give a mean annual volume of 130,000 m$^3$.

In France, 80% of ash is used for high value veneer and as saw-log for furniture. It is also used for panels and parquet flooring, with smaller quantities used for tool handles. The price obtainable for standing trees which are 50 cm and more in diameter can be 180 Euros/m$^3$ or more for veneer or slicing quality. In Germany, recent prices for veneer quality A-grade logs were approximately 350 Euros/m$^3$ and B-quality veneer logs for 250 Euros/m$^3$. In Ireland ash has a specialized use to make ‘hurleys’ for the national game and, for this purpose, ash prices in 2010 were 430 Euros/m$^3$ for standing trees; and 1,300 Euros/m$^3$ when cut into planks.

9.6.2 Plant Demands and Deployment by Country

The statistics for the areas of ash forests in European countries and the annual plant requirements are summarized in Table 9.1 below. The annual plant requirements by the foresters are also given and broken into afforestation and reforestation needs where possible. However, in the past 3 years ash has not been recommended for planting in many continental countries due to the impact of Chalara fraxinea.
Table 9.1  Summary of ash areas and annual plant needs in Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>Forest areas</th>
<th>Annual plant needs</th>
<th>Years for data</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(m.ha)</td>
<td>Reforestation</td>
<td>Afforestation</td>
</tr>
<tr>
<td></td>
<td>Ash area</td>
<td>million plants (ha)</td>
<td>‘000 (ha) p.a.</td>
</tr>
<tr>
<td></td>
<td>‘000 ha</td>
<td>p.a.</td>
<td></td>
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<tr>
<td>Austria</td>
<td>3.898</td>
<td>0.268</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>0.15</td>
<td>0.09 m plants</td>
<td>38 ha</td>
</tr>
<tr>
<td>(Flanders)</td>
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<td></td>
<td></td>
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<tr>
<td>Belgium</td>
<td>0.55</td>
<td>0.47</td>
<td>0</td>
</tr>
<tr>
<td>(Walloon)</td>
<td></td>
<td></td>
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<tr>
<td>Czech. Rep</td>
<td>2.66</td>
<td>1.2 m (238 ha)</td>
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</tr>
<tr>
<td>Denmark</td>
<td>0.53</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>15.7</td>
<td>0.53 m (500 ha)</td>
<td>515</td>
</tr>
<tr>
<td>Romania</td>
<td>6.3</td>
<td>2.3 m</td>
<td>420</td>
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<tr>
<td>Germany</td>
<td>10.6</td>
<td>See footnote</td>
<td>See footnote</td>
</tr>
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<td>Germany</td>
<td></td>
<td></td>
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<tr>
<td>Italy</td>
<td>8.6</td>
<td>209</td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>0.7</td>
<td>0</td>
<td>1.3 m (333)</td>
</tr>
<tr>
<td>Lithuania</td>
<td>2.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Great Britain</td>
<td>2.6</td>
<td>3.8 m (1,537 ha)</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>0.36</td>
<td>Na</td>
<td>Na</td>
</tr>
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<td>Norway</td>
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<td>Na</td>
<td>0</td>
</tr>
<tr>
<td>Poland</td>
<td>8.8</td>
<td>4.33 m</td>
<td>0</td>
</tr>
<tr>
<td>Slovakia</td>
<td>2.1</td>
<td>1.39 m (250 ha)</td>
<td>1</td>
</tr>
<tr>
<td>Finland</td>
<td>26</td>
<td>Small</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td>25.9</td>
<td>Na</td>
<td>0.18 m</td>
</tr>
<tr>
<td>Sweden</td>
<td>22.5</td>
<td>200 (80)</td>
<td></td>
</tr>
</tbody>
</table>

a Estimate; data were only available for the province of Upper Austria and multiplied with the inverse of the share of this province’s ash standing volume; this number includes reforestation and afforestation

b Austria – this is the sum of all “other” deciduous hardwoods (excluding beech and oak). For comparison, standing volume for ash is 23,705,000 m³ (for the total “other” deciduous hardwoods, it is approx. 57,000,000 m³

c Germany: 2,200 ha of ash were regenerated (naturally and artificially) on average/year between 1983 and 2002

9.6.3 Sources of Ash Available and Used in European Countries

The summary statistics of ash seeds and their sources from ‘Source identified Seed Stands’, ‘Selected Seed Stands’ and from ‘Clonal Seed Orchards’ are given below in Table 9.2. It shows that selected seed stands are available in most countries and these provide a lot of seeds annually.
Table 9.2  Summary statistics on genetic sources and quantities of ash seeds used by European countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Source identified</th>
<th>Selected seed stands</th>
<th>Clonal seed orchards</th>
<th>Estimate of annual seed imports kg</th>
<th>Selected plus trees</th>
<th>Regions of prov’ance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Area/(kg)</td>
<td>No.</td>
<td>Area (ha)</td>
<td>Seed kg/year (period)</td>
<td>No.</td>
</tr>
<tr>
<td>Austria</td>
<td>0</td>
<td>0</td>
<td>137</td>
<td>256a</td>
<td>451 (2000–2010)b</td>
<td>3</td>
</tr>
<tr>
<td>Belgium (Flanders)</td>
<td>1</td>
<td>0.50 ha</td>
<td>1</td>
<td>1.30</td>
<td>15 kg/year</td>
<td>0</td>
</tr>
<tr>
<td>Belgium (Walloon)</td>
<td>27</td>
<td></td>
<td>5</td>
<td>35.44</td>
<td>8 kg (2007–2008)</td>
<td>2</td>
</tr>
<tr>
<td>Czech Rep.</td>
<td>212</td>
<td>540; (2,221 kg (2007–2009))</td>
<td>1</td>
<td>0.5</td>
<td>Too young</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Denmark</td>
<td>8</td>
<td>9.8</td>
<td>4</td>
<td>5</td>
<td>681</td>
<td>261</td>
</tr>
<tr>
<td>France</td>
<td>67</td>
<td>1,000</td>
<td>1</td>
<td>0.93</td>
<td>54 (2007–2008)</td>
<td></td>
</tr>
<tr>
<td>Romania d</td>
<td>79</td>
<td>483</td>
<td>5d</td>
<td>45.9</td>
<td>56 (2005)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Great Britain</td>
<td>6</td>
<td>16.12</td>
<td>29</td>
<td>(2004–2008)</td>
<td>5f</td>
<td>5.43</td>
</tr>
<tr>
<td>Italy total</td>
<td>31</td>
<td>8,349</td>
<td>15</td>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piedmont</td>
<td>15</td>
<td>7,279</td>
<td>15</td>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued)
<table>
<thead>
<tr>
<th>Country</th>
<th>Source identified</th>
<th>Selected seed stands</th>
<th>Clonal seed orchards</th>
<th>Estimate of annual seed imports kg</th>
<th>Selected plus trees</th>
<th>Regions of prov’ance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Area/(kg)</td>
<td>No.</td>
<td>Area (ha)</td>
<td>Seed kg/year (period)</td>
<td>No.</td>
</tr>
<tr>
<td>Lombardia</td>
<td>16</td>
<td>1,070</td>
<td>16</td>
<td>n.a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>9</td>
<td>158</td>
<td>1</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithuania</td>
<td>12</td>
<td></td>
<td>0</td>
<td>0</td>
<td>55 kg (2005)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>58 kg (2008)</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>0</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>216</td>
<td>1,165.2</td>
<td>8</td>
<td>36.77</td>
<td>No data</td>
<td>2</td>
</tr>
<tr>
<td>Finland</td>
<td>2</td>
<td>2.3</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>25</td>
<td>40</td>
<td>2</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Austria – the area is reported as reduced to the ash fraction
*b Austria – highly fluctuating between 0 and 2,200; recently declining because of ash disease
*c Austria – 112 if altitudinal sections are also considered
*d Romania also has four ‘Tested’ seed stands on 57.9 ha
*e Germany imported an average of 0.25 m plants p.a. (period 1999–2009) and exported 1 m plants p.a. (period 2003–2009); seeds are also exported and cultivated plants imported
*f Four breeding seedling orchards + one clonal SO
*g 400 kg in 2000, 10 kg in 2005, 45 kg in 2007 and 990 kg in 2008 (mean 593)
*h One region of provenance and three altitudinal zones for ash
Assessment of Genetic Variation of Adaptive Traits, Growth and Quality Based on Data and Summary Evaluation of Provenance/Progeny Trials in European Countries

The genetic variation of a species, both within and between populations of hardwoods, is influenced by many factors (Jonsson and Eriksson 1989). The mating system, pollen and seed dispersal, role and succession stage in forest ecosystems, site conditions and historical influences such as colonization patterns after the glacial periods of the Quaternary are particularly important. Adaptive variation, expressed in the phenotype in response to natural selection, has been investigated through provenance and progeny trials. These studies revealed that variation between families within provenances/populations was generally high. Phenological traits, including the date of bud burst and set, displayed geographic patterns on a large spatial scale, whereas growth and form, which are influenced by soil and competition conditions, varied at the local level. The existence of different ecotypes of ash (i.e. floodplain, hillside, slope and limestone ecotypes), has been discussed but never been proven by progeny studies/experiments (Kramer et al. 2008). The variation within and among populations has been described for many characters including the rhythm of growth (Baliuckas et al. 2000). This variability is well illustrated by seed morphology and colour in Fig. 9.5.

In 1982, seeds of 52 ash provenances were collected from: Germany (43), Austria (4), Switzerland (3) and Romania (2) to establish the first international provenance tests in several countries. Seeds were sown in 1985 in three nurseries in Germany. Field tests were established between 1986 and 1988 in Germany, in 1988–1989 in France, 1987 in Belgium and the Netherlands. Some of this material was also used in Lithuanian trials. Analyses were carried out on the trial data from the provenance/progeny tests in several countries reported below. In addition, site effects were reported from trials in France, Belgium and the Netherlands and a multi-site analysis was performed from two data sources provided by France and Germany.

An additional international trial on ash provenances/progenies was established in France, Germany, Belgium, Lithuania, Italy, Ireland and the UK in the period 2004–2006 in the frame of an EC project ‘Realising Ash’s Potential’ (RAP series). The series consisted of a minimum core collection of 30 European provenances established in each country, with more than one site in some countries.

9.7.1 Lithuania: Results from Provenance and Progeny Trials

Alfas Pliura

Progeny studies from the RAP series of three provenance/progeny trials in Lithuania were measured and analysed. Material consisted of 340 half-sib families from 24 provenances from Ireland, France, Belgium, Germany, the Czech Republic, Poland and
Lithuania. First results indicated a significant provenance effect for all adaptive traits, bud flushing phenology, height and tree health condition, thus revealing pronounced adaptively significant population variation/structure (Pliura and Baliuckas 2007). Progeny studies have shown that provenances differed also in additive genetic variation, e.g. CV_A for bud flushing phenology; it varied from 10.7 % (in Ignalina population) to 20.5 % (in Sakiai population), while in separate trials CV_A varied from 6.4 to 25.6 % respectively, thus indicating differing adaptive potential of different \textit{Fraxinus excelsior} populations (Pliura and Baliuckas 2007). These trials also revealed that the variation pattern of the main adaptive trait, bud flushing, in the same set of populations was different, depending on site. The data from three sites, each separated by 150–200 km, indicated that progenies of the western-most European provenances had a later bud flushing than East European ones (Pliura and Baliuckas 2007).

The provenances were initially affected by spring frosts and they clearly differed in health status/resistance to \textit{Chalara fraxinea} (Fig. 9.6). The health condition of all Lithuanian progenies in all three trials was better than that of the western European ones. The progenies derived from the provenances of Farchau,
Germany; Rabstejn, Czech Republic and Szczecinek – from Poland, were the healthiest among the western European material (Pliura and Baliuckas 2007). All of these three populations are also among the closest to Lithuania, geographically. The best growth was in provenances from Poland and Denmark. Progenies of the Lithuanian populations, except the Šilutės population, were smaller in height than progenies of the western European populations. This corresponds to the results from earlier studies of Swedish populations of *Fraxinus excelsior*.

![Health status of *Fraxinus excelsior* progenies from Lithuanian and W. European populations in Lithuanian progeny trials (Pliura and Baliuckas 2007). Dashed lines indicate average health status separately for Lithuanian and West European populations.](image-url)
which have shown that progenies from northern latitudes and eastern longitudes had the lowest height (Baliuckas et al. 1999).

9.7.1.1 Susceptibility to Chalara fraxinea

Further detailed studies were made concerning *C. fraxinea* on 8-year-old progenies in two Lithuanian trials from the RAP series of 2005, over the last 3 years. About 10 % of trees have survived, while only 1 % remained completely healthy after 8 years. The differences among populations ranged from 52.8 to 55.1 % in degree of damage and up to 124.2–135.3 % in survival rate (Pliura et al. 2011). Variance analysis of resistance and other adaptive traits has revealed a significant effect of genetic factors, populations and families. Among the populations studied, the best in terms of a complex of characteristics, resistance, survival, and proportion of most healthy individuals were Lithuanian Šakiai, Pakruojis, Ignalina and Kėdainiai populations (health condition 3.2–3.4 scoring points out of 5) and survival of 47–57 %. The progenies from Danish provenances (Bregentved, Osterskov and Ravholt) and those from France (Haguenau and Monterolier) had poorest health scores (about 1.9–2.1 scoring points out of 5) and survival (12–19 %).

The family genetic component at 8 years of age for health condition was 11.6–22.6 %, the additive coefficient of genetic variation was 29.9–38.7 % (Pliura et al. 2011). This indicates the existence of additively inherited genetic differences between families in resistance to diseases (differences in susceptibility and in pathogen tolerance). High heritability estimates obtained ($h^2 = 0.40–0.51$) indicate that resistance is genetically predetermined/inheritable, and this allows for a rather accurate estimation of genotype, based on phenotype in tree breeding. Genotype × environment interaction (both family × site and population × site) for degree of damage and survival was low and insignificant. This indicated an absence of variation in reaction norms in disease resistance and that genetically determined resistance was not affected by environment.

9.7.2 France: Results from Provenance Trials

Jean Dufour

9.7.2.1 Methods

Results were recorded from a (12–13)-year-old test with 50 provenances of ash (*F. excelsior*) on three sites (Trun, Heugas and Soula), and were analysed for growth traits (height, stem girth, flushing date) and quality traits (stem form, crown form, forking, steep branches and game damage).
Table 9.3  Total height and height annual increment (HAI) of ash provenances measured in 2000 and 1998 on three sites, Trun, Heugas and Soula

<table>
<thead>
<tr>
<th></th>
<th>Trun</th>
<th>Heugas</th>
<th>Soula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H00 (cm)</td>
<td>H98 (cm)</td>
<td>H00 (cm)</td>
</tr>
<tr>
<td>Mean</td>
<td>759</td>
<td>662</td>
<td>780</td>
</tr>
<tr>
<td>F value</td>
<td>14.8</td>
<td>11.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Significance level</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Maximum</td>
<td>868</td>
<td>919</td>
<td>987</td>
</tr>
<tr>
<td>Minimum</td>
<td>675</td>
<td>444</td>
<td>437</td>
</tr>
</tbody>
</table>


Form: Stem form (Fo) was assessed only in Trun and Soula, but twice in each site in winter 1995–1996 and 2000–2001, according to a five-level scale graduated from 1 (completely straight) to 5 (very crooked). In the two sites, crown form (Foci) was also assessed in 1995–1996 and 2000–2001 using a five-level scale from 1 (stem axis can be seen up to the top of the tree) to 5 (stem axis disappears at less than 25 % of the total height of the tree). In fact this scoring system is linked with the presence and the height of forks in the tree.

Forking and steep branches: The number of forks and the presence/absence of steep branch has been recorded during winter 2000–2001 in Trun and Soula.

Game damage: Game damage were recorded in Trun and Soula in 1995 with a three-level scale, from 1 (no damage) to 3 (serious damage).

Flushing: Date of flushing has been recorded twice in Trun (1993 and 1998) according to a five-level scale from 1 (terminal bud completely closed with winter dimensions) to 5 (terminal bud completely open, visible growing of the shoot) see Fig. 9.3.

9.7.2.2 Results

Results from a 12 to 13-year-old test with 50 provenances of ash (*F. excelsior*) on three sites in France were analysed for growth traits (height, stem girth, flushing date) and quality traits (stem form, crown form, forking, steep branches and game damage) as described above. It showed highly significant differences between provenances for height and girth (Tables 9.3 and 9.4). The best provenances had a 14 % increase in height and a 24 % increase in stem girth over the mean values on one site. The difference between the best and poorest provenance was 193 cm for height and 109 mm for girth.

For stem form (Fo), there were highly significant differences between provenances in two sites measured in 1995 and 2000 (Table 9.5). Rank correlation (Spearmann) between provenance means for the two notations (1995 and 2000)
are 0.66 *** in Trun and 0.45** in Soula. So, this notation is a good assessment of stem form and it gives a rather precise ranking of the tested provenances for that character. On the contrary, for crown form, F values are slight or not significant in Trun. In Soula, F values are significant but very low, in comparison with the F values for stem form (except in 2000 where it is equal). Rank correlation between provenance means are 0.55*** in Trun and 0.21 (NS) in Soula. Consequently, this notation does not give as good results as the one used for stem form and the differences observed between provenances for that character are difficult to use in practice for improvement.

For the presence of steep branches the differences between provenances were significant in the two sites and the provenance effects for the steep branch character was greater than for the number of forks present. The important trait of flushing date was studied in 1993 and 1998 at one site. Analysis of variance showed a highly significant provenance effect for the 2 years (F values = 11.0 and 10.3) and with rank correlations between provenance means at 0.81.

### 9.7.2.3 France: Site Effects

A multisite analysis of the French trials over three sites showed a very significant site effect and provenance effect for all characters analysed except crown form. The provenance × site interaction had a highly significant effect on all of the growth

#### Table 9.4 Circumference 2,000 and girth annual increment (CAI) in three sites, Trun, Heugas & Soula

<table>
<thead>
<tr>
<th></th>
<th>Trun</th>
<th></th>
<th>Heugas</th>
<th></th>
<th>Soula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C00 (mm)</td>
<td>CAI+ (mm/year)</td>
<td>C00 (mm)</td>
<td>CAI+ (mm/year)</td>
<td>C00 (mm)</td>
</tr>
<tr>
<td>Mean</td>
<td>276</td>
<td>33</td>
<td>290</td>
<td>33</td>
<td>220</td>
</tr>
<tr>
<td>F value</td>
<td>7.0</td>
<td>4.8</td>
<td>7.5</td>
<td>3.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Significance level</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Maximum</td>
<td>343</td>
<td>39</td>
<td>355</td>
<td>42</td>
<td>313</td>
</tr>
<tr>
<td>Minimum</td>
<td>234</td>
<td>29</td>
<td>188</td>
<td>25</td>
<td>159</td>
</tr>
</tbody>
</table>


#### Table 9.5 Results of stem form notation at provenance level in 2 years at two sites, Trun and Soula

<table>
<thead>
<tr>
<th></th>
<th>Trun</th>
<th></th>
<th>Soula</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fo95</td>
<td>Foci95</td>
<td>Fo00</td>
<td>Foci00</td>
<td>Fo95</td>
<td>Foci95</td>
<td>Fo00</td>
<td>Foci00</td>
</tr>
<tr>
<td>Mean</td>
<td>2.32</td>
<td>1.89</td>
<td>2.02</td>
<td>2.57</td>
<td>1.61</td>
<td>1.56</td>
<td>2.40</td>
<td>1.56</td>
</tr>
<tr>
<td>F value</td>
<td>6.0</td>
<td>1.4</td>
<td>13.7</td>
<td>1.5</td>
<td>6.8</td>
<td>1.9</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Significance level</td>
<td>***</td>
<td>N.S.</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.36</td>
<td>2.31</td>
<td>3.70</td>
<td>2.98</td>
<td>3.67</td>
<td>2.08</td>
<td>3.42</td>
<td>2.5</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.85</td>
<td>1.41</td>
<td>1.50</td>
<td>2.13</td>
<td>1.21</td>
<td>1.00</td>
<td>1.91</td>
<td>1.00</td>
</tr>
</tbody>
</table>
characters and for stem form. The provenance effect was more stable from one site to another for the character of presence of steep branches than for the character, number of forks.

9.7.3 The Netherlands: Results from Provenance Trials

Joukje Buiteveld

9.7.3.1 Methods

The Netherlands took part in the long-term international provenance trial, which was established in 26 trial sites in five countries in 1982. Progenies and provenances in this study were tested on two locations: Zuid-Flevoland and Windesheim. A total of 50 progenies and 17 provenances including 14 foreign provenances were used. The 47 progenies (half-sib families) were derived from clones and plus trees from the Netherlands and 3 from Germany. The 14 provenances were derived from Germany, Switzerland and Romania.

Assessments were performed at the ages of 6 and 15 for survival, height, diameter (DBH), stem form, forking, branch angle and susceptibility to ash bark disease caused by a bacterium (\textit{Pseudomonas syringae} subsp. savastanoi pv. fraxini). The traits were assessed according to the following systems:

- Survival as % of dead trees Stem form according to a five classes scale from 1 (completely straight) to 5 (curved stem).
- Forking according to a four classes system (1 = no forking, 4 = forking in the lower (25 % of total height) part of the tree).
- The branch angle according to two classes (1 = flat, 2 = steep)
- Ash bark disease according to a three point scoring system (1 = no infection, reaction, 2 = modest infection, 3 = strong infection).

Results for the 6- and 15-year aged trials were analysed using ANOVA for height and diameter data. The differences between provenances in height and diameter growth were indicated with an LSD test. Form and survival was done using a Generalized Linear Model (GLM). If significant differences could be demonstrated, provenances and progenies were tested with a pairwise t-test (P = 0.05). The calculations were performed with Genstat 6 (Release 6.1 2002; Lawes Agriculture Trust, Rothamsted, UK).

9.7.3.2 Results

The test sites showed large differences for survival rate recorded at 5-year and 15-year-old age after establishment. Analysis shows significant differences in survival
between the provenances, both in Windesheim (P=0.037) and in Zuid-Flevoland (P=0.004). The percentage of losses was more severe in Windesheim (26.4 % at age 15). This high failure rate is mainly caused by damage from mice and rabbits.

In general, highly significant differences between provenances and progenies were shown for both height and diameter (Table 9.6). The average height in Windesheim after 15 years was higher (12.9 m) than in Zuid-Flevoland (11.8 m).

The two best progenies grown in Zuid-Flevoland were derived from plus trees within the reference provenance Echteld-01 with a height of 13.3 and 12.9 m after 15 years. These two progenies had a significantly higher height (12 % above mean) than the trial mean of 11.8 m (P = 0.05).

Foreign material had a predominantly poor height growth. However, two provenances from Germany (Niedersachsen and NordRhein-Westfalen) had a significantly better height than the reference provenance Echteld-01, while the German provenance from Lower Saxony performed significantly worse than the reference (P = 0.05).

In the Windesheim test site the differences between progeny and provenances were even more pronounced, ranging from 14.4 to 10.9 m after 15 years. A progeny from a plus tree within the Echteld-01 provenance had the best growth in height (14.4 m) and was significantly higher, by 11 %, than the trial mean of 12.9 (P=0.05). Again, the foreign provenances showed the poorest height growth. The Romanian provenance appeared to show the lowest height.

In the sites of Zuid-Flevoland and Windesheim respectively. Five and four out of 14 foreign provenances showed a significantly better diameter than the Dutch reference provenance. In general, the Romanian provenance showed a reasonable to good growth in diameter but a poor height growth in both test sites (significantly differing from the reference provenance Echteld-01, P = 0.05).

Correlations between the average height at 6 and 15 years of age appeared to be weak, but significant (P<0.001). (Location=37 % Windesheim R2, R2=Location South Flevoland 28 %) see Fig. 9.7 below.

The statistical analysis for stem form for the Windesheim site showed no significant effect of the progeny or provenance on stem form (P=0.064), but did

Table 9.6 Total height and diameter at age 6 and 15 of ash provenances and progenies in the two test sites Zuid-Flevoland and Windesheim in the Netherlands

<table>
<thead>
<tr>
<th>Zuid-Flevoland</th>
<th>Windesheim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height at age 6 (dm)</td>
<td>33.3</td>
</tr>
<tr>
<td>Height at age 15 (dm)</td>
<td>118.2</td>
</tr>
<tr>
<td>Diameter at age 15 (mm)</td>
<td>92.5</td>
</tr>
<tr>
<td>Height at age 6 (dm)</td>
<td>2.77</td>
</tr>
<tr>
<td>Height at age 15 (dm)</td>
<td>3.67</td>
</tr>
<tr>
<td>Diameter at age 15 (mm)</td>
<td>3.52</td>
</tr>
<tr>
<td>Significance level</td>
<td>***</td>
</tr>
<tr>
<td>Max</td>
<td>38.9</td>
</tr>
<tr>
<td>Min</td>
<td>27.3</td>
</tr>
<tr>
<td>LSD (5 % level)</td>
<td>5.0</td>
</tr>
<tr>
<td>Mean</td>
<td>33.3</td>
</tr>
<tr>
<td>F value</td>
<td>2.77</td>
</tr>
<tr>
<td>Max</td>
<td>38.9</td>
</tr>
<tr>
<td>Min</td>
<td>27.3</td>
</tr>
<tr>
<td>LSD (5 % level)</td>
<td>5.0</td>
</tr>
</tbody>
</table>
for the presence of forks (P = 0.006). Only tree provenances (from Germany, Romania and the Netherlands) differed significantly from the reference provenance and had significantly more trees in class 4 (forks lower part of the tree) or fewer trees in class 1 (no forks).

For the Zuid-Flevoland site, the form results were somewhat similar. Statistical analysis showed here both an effect of the provenance on the stem form (P < .001) as well as on the presence of forks (P = .003). Here the foreign provenances had slightly fewer trees with straight to slightly curved stem form (0–54.2 %) than the Dutch provenances (14.6–63.8 %). However, few significant pairwise differences were found. The Swiss and Romanian provenances had a significantly worse stem form than the reference provenance (P = 0.05). For the position of the branches no significant variation could be observed between the provenances as well as within a provenance. All provenances had a predominantly steep branch angle. In both test sites no strong infection of bark disease (bacterial canker) was observed. Only the Romanian provenance showed a moderate infection at both sites, in 17 and 23 % of the trees.

9.7.3.3 The Netherlands: Site Effects and Conclusions

An analysis of the total data set, based on progenies and provenances present in both test sites, showed a significant interaction between genotype and site for height at age 15 (P < 0.05), but not for diameter growth (P = 0.21). Summarizing, it seems that variation for growth and form within and between ash provenances was high and that the location explained a significant part of the variation found.

Overall, the two test sites showed a similar picture, although not all progenies and provenances were stable across the test sites and showed differences in ranking. Some showed a reasonably good ranking. For instance, three good growing provenances were among the ten best on both sites. The Romanian provenance had the worst stem form, possibly due to late frost. These results seem to be in congruence with the first results from the German sites (Kleinschmit et al. 1986, 2002). In the German site flushing was recorded. Here the Romanian provenances seem to flush early and were
damaged by late frost, resulting in a worse stem form. Also here, the differences between the test sites for mean height was high, ranging from 5.5 to 11 m at the age of 15. Compared with these results the ash provenances showed a good growth on the Dutch sites (average growth in height: 11.8–12.9 m). The age correlation for height at 6 and 15 years of age appeared to be weak. Earlier research on a related species of common ash such as *Fraxinus pennsylvanica* showed that the correlation between 5- and 17-year-old material is low, while it is high for 17- and 20-year-old material (Bresnan et al. 1996).

The interest in the Netherlands in provenance testing is mainly to identify productive and well adapted seed sources for commercial use. The seed supply for ash in ‘selected’ Dutch provenances is limited because a low number of high quality stands are known. Good provenances from abroad can be a valuable addition to the seed sources available in the Netherlands. Based on these first results from this trial three German provenances are considered suitable for plantation in the Netherlands and are therefore included in the Dutch list of recommended tree varieties and provenances (Buiteveld et al. 2004).

### 9.7.4 Belgium: Provenance Trials

Patrick Mertens, Dominique Jacques, and Yannik Curnel

#### 9.7.4.1 Methods

Assessments were made on 52 provenances for height, girth, stem form, forking, frost resistance, steep branch, game damage, and susceptibility to canker on two sites in Belgium. The following measurements have been made since the planting in 1987: Height (H) was measured in spring 1991 and in winter 2000–2001 (respectively H90 and H00) in the two sites and in winter 1995–1996 in Chevetogne (H95). Height increment between spring 1991 and winter 2000–2001 was also computed for the two sites (ACCH). Girth (C): girth was measured in spring 2001 (C00) in the two sites. Form: stem form was estimated in the two sites in winters 1995–1996 and 2000–2001 (respectively Fo95 and Fo00) according to a 5-level scale graduated from 1 (straight) to 5 (crooked). Crown form was also estimated in winter 1995–1996 (Foci95) according also to a 5-level scale, from 1 (stem axis can be seen up to terminal bud) to 5 (stem axis disappears at less than 25% of the crown height).

Forking: Forking was estimated in spring 1991 (F90) and in winter 2000–2001 (F00) in the two sites and in spring 1987 (F87) in Chevetogne according to a 3-level scale in 1987 and 1990 and a 5-level scale in 2000. Except for measures in spring 1987, the higher the quotation, the more forked the tree. The number of forks was also recorded in winter 2000–2001 in the two sites (Nbfo00). Frost resistance:
resistance to frost was recorded in spring 1991 (Gel91) in the two sites and in spring 1989 (Gel89) in Chevetogne according to a 3-level scale, from 1 (terminal bud not frosted) to 3 (terminal bud completely frosted).

Game damage: game damage was estimated in winter 1995 with a 3-level scale, 1 means no damage (Dom95). Steep branch: the presence of a steep branch was recorded in winter 2000–2001 (Stbr00) according to a 2-level scale, 1 for no steep branch and 2 for the presence of steep branch. Sensitivity to canker: this character was observed in winter 2000–2001 (canc00) according to a 3-level scale, from 1 (no attack) to 3 (severe attack).

Individual data per site were submitted to a two-way analysis of variance according to the following linear model (hierarchical classification):

$$X_{ijk} = m + \gamma_i + \beta(\gamma)_{ij} + \varepsilon_{ijk}$$

Where $X_{ijk}$ is the observed phenotypic value of tree $k$ of progeny $j$ (provenance $i$), $m$ is the general mean, $\gamma_i$ is the effect of provenance $i$, $\beta(\gamma)_{ij}$ is the effect of progeny $j$ and $\varepsilon_{ijk}$ is the residual deviation. Once all data were merged, a two-way analysis of variance was realized on individual data according to the following linear model:

$$X_{ijk} = m + \gamma_i + \delta_j + (\gamma \delta)_{ij} + \varepsilon_{ijk}$$

Where $X_{ijk}$ is the phenotypic observed value of progeny $k$ of provenance $i$ in the site $j$, $m$ is the general mean, $\gamma_i$ is the deviation to the general mean attributable to provenance $i$, $\delta_j$ is the deviation to the general mean attributable to the site $j$, $(\gamma \delta)_{ij}$ is the deviation to the general mean attributable to the interaction between provenance $i$ and site $j$ and $\varepsilon_{ijk}$ is the residual deviation.

9.7.4.2 Results from Provenance/Progeny Trials

Detailed results from Belgium were made on assessment of 52 provenances for height, girth, stem form, forking, frost resistance, steep branch, game damage, and susceptibility to canker on two sites. Individual data per site were submitted to a two-way analysis of variance according to the following linear model (hierarchical classification):

$$X_{ijk} = m + \gamma_i + \beta(\gamma)_{ij} + \varepsilon_{ijk}$$

Where $X_{ijk}$ is the observed phenotypic value of tree $k$ of progeny $j$ (provenance $i$), $m$ is the general mean, $\gamma_i$ is the effect of provenance $i$, $\beta(\gamma)_{ij}$ is the effect of progeny $j$ and $\varepsilon_{ijk}$ is the residual deviation.

Once all data were merged, a two-way analysis of variance was realized on individual data according to the following linear model:

$$X_{ijk} = m + \gamma_i + \delta_j + (\gamma \delta)_{ij} + \varepsilon_{ijk}$$
Where $X_{ijk}$ is the phenotypic observed value of progeny $k$ of provenance $i$ in the site $j$, $m$ is the general mean, $\gamma_i$ is the deviation to the general mean attributable to provenance $i$, $\delta_j$ is the deviation to the general mean attributable to the site $j$, $(\gamma\delta)_{ij}$ is the deviation to the general mean attributable to the interaction between provenance $i$ and site $j$ and $\epsilon_{ijk}$ is the residual deviation.

For growth characteristics (height, circumference), differences between provenances were at least significant and genotypic heritability varied between 0.32* and 0.76*** on the two sites, the best results being obtained for the measurements realized in 1990 Table 9.7. On the other hand, the phenotypic variation coefficients decreased with time from 15–19 % in 1990 to 5–6 % in 2000.

Analysis of the data on stem and crown form showed very highly significant differences among provenances; heritability was categorized as medium to very low (from 0.68*** to 0.26 NS). In Halleux, very highly significant stem form differences exist between provenances (Table 9.8). In Chevetogne these differences are only perceptible in 2000. Heritability for form character can be qualified as medium to very low (from 0.68*** to 0.26 NS). Heritability seems to grow with time, especially in Chevetogne. This increase could be explained by the difficulty of judging the stem straightness at a young stage of development, to the subjectivity of the measures and to the change of operator between 1993 and 1998. It can also explain why, in 2000, stem form was very bad in the two sites in contrast to the other year where stem form can be qualified as medium.

Direct genotypic gains were slightly higher for crown form (6.1–7.9 %) than for stem form (1.9–5.8 %). Provenance effect was not significant for forking and for the number of forks except in 1990 in one of the two sites for forking. On the other hand, provenances effects were very highly significant for frost damage on both sites and genotypic heritability was good (from 0.60*** to 0.72***). Finally, genotypic heritability was categorized as low to very low for the steep branch and game damage characters and was medium for canker sensitivity.
9.7.4.3 Belgium: Site Effects

A multi-site analysis of all the studied characters was performed on the two Belgian sites and the corresponding genotypic heritabilities and gains were computed and are summarized in Table 9.9. Site effect was always at least significant except for forking in 1995 and interaction effect was significant only for growth characters, game damage and steep branch character.

The provenance effect, computed at this multi-site level, was at least highly significant for height but was not significant for height increment and girth. Heritability decreases from 0.78*** in 1990 to 0.53** in 2000 for height and can be quantified as low for height increment and girth, respectively equal to 0.35NS and 0.34NS. Provenance effect was at least significant for crown and stem form and genotypic heritability varied between from 0.44* to 0.77***.

The provenance effect was very highly significant in 1990 ($h^2_G = 0.63$), but became non-significant in 2000 for forking ($h^2_G = 0.31$) and was significant for the number of forks ($h^2_G = 0.48$).

Provenance effect was very highly significant for frost and canker sensitivity and genotypic heritability could be quantified as good. On the contrary, no improvement possibilities are allowed for game damage and steep branch characters, the genotypic heritability being close to 0.

9.7.5 Romania: Results from Provenance Trials

Gheorghe Parnuta, and Marin Tudoroiu

9.7.5.1 Methods

In autumn 1975, the nursery test was established using 22 ash provenances: 17 from Romania, 3 from Hungary and 2 from Bulgaria.
With the seedlings obtained, one main trial series was established in two consecutive years – 1977 and 1978 – on 5 different sites. Two trials (Lunca Timișoului and Satu Mare) were established in 1978 and three (Vaslui, București and Comana) in 1978.

The well-known growth parameters (total height, DBH), quality parameters (pruned height, trunk form) and survival, were measured/estimated and calculated. The ‘pruned height’ can be defined as the height measured up to the first green branch. The trunk form was estimated using a three classes scale: 1 – straight and cylindrical trunk, 2 – trunk curved on one side, trunk curved on more than one side.

### 9.7.5.2 Results of Romanian Provenance Trials

The first results from these provenance trials concerned growth data in the nursery for all 22 ash provenances. Many traits were analysed and there were significant differences among provenances for: total height, annual increment, collar diameter, number of lateral branches, terminal bud setting, leaf drop, frost sensitivity, and bud colour in the dormancy period. These results have been summarized by Contescu (1980). Subsequently, data was collected and analysed at 6 years of age (Contescu 1984) followed by results obtained at 10 and 15 years (Smîntînă 1993, 1995; Smîntînă et al. 1994). These results have provided some conclusions regarding the behaviour of the different provenances at each growth stage and have identified the most valuable provenances from the growth point of view and also the best adapted in terms of resistance to frost and physiological drought during winter conditions.

The most recent trial results have analysed data from five trial sites at 30 and 29 years respectively after establishment of the plantation: for total height, pruned

### Table 9.9 Genotypic heritability and gains, and influence of factors ‘provenance’, ‘site’ and their interaction for growth (H90, H00, ACCH, C00), architectural (Fo95, Fo00, Foci95,F90, F00, Nbfo00, Stbr00), game (Dom95) and frost (Gel91) damage and sensitivity to canker (Canc00) characters

<table>
<thead>
<tr>
<th></th>
<th>$h^2_G$</th>
<th>$\Delta Gc$ (%)</th>
<th>Provenance</th>
<th>Site</th>
<th>Provenance X site</th>
</tr>
</thead>
<tbody>
<tr>
<td>H90</td>
<td>0.78</td>
<td>12.7</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>H00</td>
<td>0.53</td>
<td>2.4</td>
<td>&gt;0.95</td>
<td>&gt;0.99</td>
<td>&gt;0.95</td>
</tr>
<tr>
<td>ACCH</td>
<td>0.35</td>
<td>1.6</td>
<td>NS</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>C00</td>
<td>0.34</td>
<td>2.3</td>
<td>NS</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Fo95</td>
<td>0.44</td>
<td>3.8</td>
<td>&gt;0.95</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Foci95</td>
<td>0.58</td>
<td>7.0</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>NS</td>
</tr>
<tr>
<td>Fo00</td>
<td>0.77</td>
<td>4.3</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>NS</td>
</tr>
<tr>
<td>F90</td>
<td>0.63</td>
<td>4.6</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>NS</td>
</tr>
<tr>
<td>F00</td>
<td>0.31</td>
<td>2.2</td>
<td>NS</td>
<td>&gt;0.95</td>
<td>NS</td>
</tr>
<tr>
<td>Nbfo00</td>
<td>0.48</td>
<td>7.0</td>
<td>&gt;0.95</td>
<td>&gt;0.95</td>
<td>NS</td>
</tr>
<tr>
<td>Gel91</td>
<td>0.81</td>
<td>8.0</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>NS</td>
</tr>
<tr>
<td>Dom95</td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Stbr00</td>
<td>0</td>
<td>0</td>
<td>NS</td>
<td>&gt;0.99</td>
<td>&gt;0.95</td>
</tr>
<tr>
<td>Canc00</td>
<td>0.65</td>
<td>5.6</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>NS</td>
</tr>
</tbody>
</table>
height, average volume per tree, trunk form and G×E interaction. They have been published recently and the main results are summarized below and in Pârnuţă et al. 2009. The results allowed us to identify sets of provenances which were best adapted to the test sites with the highest growth performances and which had the best qualitative trunk characteristics. These provenances will be designated as “tested sources” of forest reproductive materials and they may be recommended for use and transfer into the regions of provenance which correspond to the test sites.

Survival rates of trees (age 29–30) were very significantly different between provenances tested in all the trial sites. The best survival percentage ranged from 68 to 84 % in the Vaslui trial site while the poorest ranged from 35 to 64 % in the Bucureşti trial site. The local provenance did not have the best survival rate in any of the testing sites. Two particular provenances (8 – Strehaia and 5 – Pecica) had high survival rates in all five trial sites.

Total height data was also recorded in trials at 29–30 years after establishment, in all 5 trial sites. The total height had very different values according to provenance and also according to the site of the plantation, proving a very high intra- and inter-population variation. The differences among provenances are statistically assured for a transgression probability p<0.001 (highly significant). Specifically for polygene-controlled traits, continuous variation was evident for the genetic component. The analysis of variance revealed very significant differences among provenances in all five test sites, each of them having different site conditions (Vaslui, hilly site conditions; Bucureşti, Lunca Timişului and Satu Mare plain site conditions, and Comana, meadow site conditions). The best performing provenances were different at each site. The highest value for average total height (14.33 m) was recorded from the Bucharest trial site; this was 5 % greater than the mean value from the Comana site and was 22 % greater than the total mean height registered in the Vaslui site. However, there were some provenances which were placed in the top four in more trials: e.g. provenance 11 – Snagov (in four trials), 5 – Pecica and provenance 6 – Caracal (in three trials).

The analysis of variance for the trunk qualitative trait of ‘pruned height’ revealed very significant differences between the provenances tested in all of the trial sites. The mean pruned height calculated as the percentage of the total height (H) was 0.6 at one trial site and as low as 0.46 at another site. In the trial site Bucureşti, local provenance 11 – Snagov was the best and two foreign provenances from Bulgaria and Hungary were poorest.

The variation in the average volume per tree was continuous, typical of quantitative traits. The analysis of variance showed highly significant differences between the provenances tested in the five trials sites. In the trial site Lunca Timisului one provenance produced an average volume per tree 78 % greater than the poorest performing provenance from Bulgaria and was 21.5 % greater than the mean value of the experiment. The values of variation for average volume per tree was between 53.90 and 105.69 dm³ (Vaslui), between 72.84 and 165.30 dm³ (Bucharest) and between 69.73 and 127.91 dm³ (Comana). Concerning average volume per tree, the best performing provenances gave double the volume of the poorest provenances and showed the great potential for volume improvement of ash stands.
One particular provenance (5 – Pecica) gave superior values for several characters in several different site conditions and may be recommended for use in different regions of provenance due to its adaptability. The value for average volume per tree for this provenance was 96 % higher from the Vaslui trial site and 106 % from the Bucharest site than the value recorded for the poorest provenance. On the other hand, for provenance 14 – Griviţa (Galati) which was the second best at the Vaslui site, it was the poorest of all at the trial sites of Bucharest and Comana. These results confirmed the general principle for Romania concerning the movement of basic material, meaning that provenances may be safely moved from the south to the north and from the west to the east of the country.

The character of the tree trunk form took into account straightness, cylindrical form and absence of trunk defects. The analysis of variance of trunk form showed very significant (p<0.001) differences for the provenances tested among four of the trial sites and was only significant among provenances (p<0.05) within one trial site.

9.7.5.3 Romania: Site Effects, G \times E Interaction

For estimating the G×E interaction the bi-factorial analysis of variance was used for all the quantitative and qualitative traits, from the measurements made at age 29. Analysis of variance showed high significant differences between provenances only for two traits (trunk form and survival). Very significant (p<0.001) differences between testing sites were also found for all traits, except the trunk form.

The G×E interaction proved to have no significance for all the analysed traits, meaning that there were no statistically significant differences between provenances with regard to their reaction to the local ecological conditions; so there was a stability of the performances of provenances in different site conditions.

9.7.6 A European Scale Analysis of Results from Ash Provenance Trials Established on Multiple Sites in France and Germany

Patrick Mertens

9.7.6.1 Methods for a Multi-site Analysis on a European Scale

For the global statistical analysis, 66,800 lines of data relating to the oldest European tests of provenance were examined. The goal of this preliminary analysis was to check “the aptitude” of the statistical analysis of the evaluations carried out on 11 experimental sites in France and Germany, comparing 60 sources of ash, at the stage
of 16 years of age. It is necessary to meet the constraints of statistical analysis which are at the threshold of acceptance in homosedasticity (homogeneity of variance), for such large-scale comparisons.

9.7.6.2 Results from a Multi-site Analysis on a European Scale

This analysis was undertaken on the provenance trials established in 1984 in France and Germany. They consisted of 46 ash sources (German, Swiss, Romanian, Austrian) on nine sites at age 16. Height, girth, stem form, forking, frost resistance, game damage, steep branches and canker susceptibility were assessed.

The Provenance, Site and Interaction effects for girth and height are all very highly statistically significant, as shown in Table 9.10. The Site effect is more significant, followed by the Provenance and Interaction effects. This result was obtained due to the slower growth in girth and height of seeds, especially from one provenance source, referenced as number 16, but also due to the large site variability of girth and height average of many seed sources, that are not the same for the two parameters.

The variability of girth reaches a high standard deviation of 8.4 cm at provenance level for a general average of 19.0 cm (coef. var.: 44.0 %); a height standard deviation of 220 cm for a general Provenance average of 772 cm gives 28.5 % coefficient of variation. The next table also shows that sample of height is inferior to the girth one.

In consequence, this first table expresses the small influence of seed sources selection on girth and height growth of the 16-year-old tested ash trees, represented by 46 Provenances in nine experimental sites.

In summary, for the characters of girth and height, there were very highly significant effects for Provenance, Site and Interaction, with the Site effect > Provenance effect > Interaction effect. An analysis of stem form showed a similar influence of Site over Provenance. Based on a stem-form scale of 1–5 it was possible to compare provenance over all sites (Table 9.11).

This result showed only one provenance as straighter compared to all others. The variability (coefficient) value of girth, height and stem form was then analysed.
However, the site and interaction values were similar (24–29 %) and were 4–5 times higher than those due to Provenance effects (4–7 %). The character of stem form had a relatively higher Provenance effect compared to either Site or Interaction effects. It is therefore advisable to use this character in ash provenance comparisons notwithstanding that it is 4–5 times lower than residual variation and one-third lower than site and interaction effects.

Because of the high variability of results from the material used in this multi-site large 16-year-old trial, a separate analysis was done where each ash family was analysed individually using quartiles. It showed that a minimum of 300 trees was necessary to reduce the variability of the observed recordings. Furthermore, it could be concluded that the using traditional selection parameters had a limited value for ash improvement in material tested from seed-derived provenances.

Further analysis showed that a selection of the best provenances may be valid for local superiority only. A progeny analysis in relation to a basis for selection gave a similar result, and could not be seen as superior to selection at the provenance level. Overall, there was a low provenance and progeny effect of selection in relation to wide site variation. Furthermore, the analyses of progenies showed that the best progenies did not come from the best provenances and the best provenances overall were from geographically distant regions.

### Table 9.11  Global analysis of trunk form (stem form) from nine sites at age 16

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Cma</th>
<th>F</th>
<th>P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provenance</td>
<td>45</td>
<td>13.74</td>
<td>29.44</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Site</td>
<td>7</td>
<td>809.7</td>
<td>1,735</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Provenance X Site</td>
<td>315</td>
<td>4.75</td>
<td>10.18</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>Error</td>
<td>35,247</td>
<td>0.467</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*DF* degree of freedom, *Cma* adjusted mean square, *F* fisher test observed value, *P* probability of Fisher test

### Table 9.12  The variability (coefficient) value of girth, height and stem form when analysed as a percentage of provenance and site effect

<table>
<thead>
<tr>
<th>Effect</th>
<th>CV %</th>
<th>Girth</th>
<th>Height</th>
<th>Trunk form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provenance</td>
<td>5.7</td>
<td>3.7</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>27.5</td>
<td>24.4</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>29.0</td>
<td>24.3</td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>44.1</td>
<td>28.5</td>
<td>26.9</td>
<td></td>
</tr>
</tbody>
</table>

as a percentage of Provenance and Site effects from the analysis in the two previous tables. It showed the % coefficient of variation for the height character as being smaller than that for girth (Table 9.12).
9.8 Heritabilities of Important Silvicultural Traits and Age–Age Correlations

Joukje Buiteveld

9.8.1 Summary Data

Results are given and discussed above on heritability values from provenance/progeny tests on multiple sites in relation to several characters. There are few other publications on broad-sense and narrow-sense heritabilities for traits in ash. Earlier reports by two authors are based on a limited number of field trials. Mwase et al. (2008) concluded that height in ash is the most heritable trait followed by DBH, while straightness and forking appear not to be under strong genetic control. The relatively low values for stem form (straightness) are in concordance with results found by Savill et al. (1999). Both studies showed that heritabilities were highest when the trees were only 3–4 years old and decreased with age of the trees. No data is published on estimates of heritabilities on phenological traits like flushing, bud set and length of vegetation period for which it is known that they are under strong genetic control. In addition, broad-sense heritabilities are estimated based on provenance trials by Dufour & Jacques/Mertens (see above). In general, genotypic heritabilities varied enormously for growth characteristics. The character of ‘form’ showed genotypic heritabilities were moderate to low (stem, crown, forking).

9.8.2 Age–Age and Trait–Trait Correlations

Age–age and correlations among traits, mainly growth, form and phenology characteristics are described by a small number of authors. Some strong correlations can be mentioned. Mwase et al. (2008) reported only moderate 3–8 age–age correlation (rg=0.53), but an extremely high (0.89) for 7–8 age–age correlation for height. High age–age correlations were also reported for DBH (0.92 at age 7–8), which suggests that early selection of ash in young trials at age 7 may be effective. Concerning trait–trait correlations, the studies of both Mwase et al. (2008) and Savill et al. (1999) showed that height was positively correlated with stem growth (DBH). Both studies also revealed that straightness and forking are correlated, although less pronounced. Kleinschmit et al. (2002) found that height growth and bud set are significantly correlated ($r=-0.71**$). Simultaneously, the damages due to frost were significantly correlated with bud set ($r=-0.67$).
9.9 Assessment of Genetic Variation, Hybridization, Gene Flow and Inbreeding Using Neutral Molecular Marker Variation

J. Fernandez-Manjares, Muriel Thomasset, Gerry C. Douglas, and Alfas Pliura

9.9.1 Genetic Diversity in Ash Populations

Common ash populations, like many other species with a wide distribution, are mostly outcrossing, with pollen and seed dispersal mediated by wind. They harbour large levels of genetic diversity (Popescu and Postolache 2009; Zvingila et al. 2005). This high amount of genetic diversity is more observable, however, with nuclear markers than with chloroplast markers (Heuertz et al. 2004a; Harbourne et al. 2005) because ash exhibits moderate amounts of diversity compared to other species such as oak. In Romania, haplotype analysis revealed material from three glacial refugia: the Iberian Peninsula, the Italic Peninsula and the Balkan Peninsula, with a total of 12 haplotypes (Heuertz et al. 2004a). Nuclear markers for ash typically exhibit low values of $F_{st}$, indicating a low genetic differentiation; nevertheless, the values are significant at country scales (Heuertz et al. 2001; Morand et al. 2002a; Hebel et al. 2006; Ballian et al. 2008). At larger scales in Europe, there are differences between the Western and South-Eastern European populations (Heuertz et al. 2004b). In general, both nuclear and chloroplast markers possibly exhibit broad patterns of post-glacial colonization (Heuertz et al. 2004a) but these may be confounded with the genetic patterns of the closely related species of *F. angustifolia* Vahl (see hybridization section below). On the other hand, low levels of genetic diversity have been found at the extreme range of the natural distribution in Finland where common ash is found in isolated islands with reduced available habitat (Holtken et al. 2003).

Additive genetic variation appears to be moderate to high in ash, and is mostly distributed locally as a result of local adaptation (Broadmeadow et al. 2005). For example, one common garden in South-Western France has shown evidence of local adaptation along a climatic gradient on the Pyrenees for characters in growth and leaf phenology (flushing and senescence), leaf unfolding and canopy duration (Vitasse et al. 2009a, b, c). Studies in Lithuania (Fig. 9.8), have shown that some populations such as Sakiai and Kupiskis, had high genetic variation of both adaptive traits and RAPD markers, while other populations had differing levels of RAPD and adaptive traits genetic variation, thus indicating differing adaptive potential of individual populations (Zvingila et al. 2005).

At large temporal and spatial scales, common ash may be considered potentially sensitive to climate change as it does not exhibit the same aggressive post-glacial colonization of other more dominant trees (Myking 2002). Also, rapid evolution in common ash may be retarded because of their small populations, low tree density and potential high mortality (Hemery et al. 2010).
Common ash readily hybridizes in sympatric zones with narrow-leaved ash (*F. angustifolia* Vahl) during years in which climate conditions allow for an overlap in the flowering periods of the two species (Gerard et al. 2006a, b, c; Thomasset 2011). Our knowledge on ash hybridization with *F. angustifolia* has greatly improved during the last 10 years and to a large extent, problematic populations can now be detected by the combined use of molecular, morphological and phenological markers (Raquin and Frascaria-Lacoste 2006; Thomasset 2011). However, the risk of populations which consist of backcross generations and cryptic hybrids that do not exhibit many of the characteristics of *F. angustifolia* is not a negligible threat to the purity of either species (Thomasset et al. 2011a; Gerard et al. 2006a). The *F*₁ hybrids showed intermediate morphology in most characters and the range of variation overlapped with the parental species. Furthermore, the traits of leaf mass, leaf area and number of teeth per leaflet showed significant differences from each of the parental species (Thomasset et al. 2011a). The sympatric zones of common ash with *F. angustifolia* occur mostly at the upper ranges of rivers that flow mostly towards the Atlantic (Loire, Seine in France), but also towards the Black Sea (Danube basin and tributaries in Austria, Hungary and Croatia) and the Mediterranean (Saône, Rhone among others). Unfortunately, the precise locations of hybrid zones requires the simultaneous study of morphological, phenological and/or molecular markers, therefore most observations of the precise locations of hybrid zones remain uncertain (Palada-Nicolau et al. 2005; Fernandez-Manjarres et al. 2006; Gerard et al. 2006a). This approach has also been useful in identifying introgressed or cryptic hybrids which also displayed poor stem form in some Irish plantations (Thomasset 2011; Thomasset et al. 2012).
Flowering in common ash is typically in the period February/March and in *F. angustifolia* in the period December/January. Global warming represents a serious concern for hybridization rates. Observations by Penuelas et al. (2002) have shown recent delays in the onset of flowering of *F. angustifolia* by 37.2 days in Spain. A continuation in this trend will increase the period of flowering overlap of *F. excelsior* with *F. angustifolia* and increase the potential for inter-specific hybridization and gene introgression. A case study of the genus *Fraxinus* showed that the future climate in southern England is more likely to be more favourable than the present for *F. angustifolia* and its hybrids with *F. excelsior*. While this scenario did not apply to Ireland, the evolution of the phenology of *F. excelsior* and of introduced *F. angustifolia* or hybrid trees is unknown under future climatic conditions in Ireland (Thomasset 2011b).

### 9.9.3 Gene Flow

Gene flow in common ash is extensive both locally (Heuertz et al. 2003, Morand et al. 2002b) and at large distances (Bacles et al. 2005; Bacles and Ennos 2008). Fertilization in ash is mostly allogamous (P. Gérard et al., unpublished data). Like many wind-pollinated, outcrossing trees, long distance pollen flow poses serious challenges to seed production in orchards, especially in low production years of scarce flowering when local pollen clouds are not saturated with pollen donors from within the seed orchard. In fact, studies in a seed orchard in Germany suggest that a distance of at least 500 m from non-seed orchard trees is needed to minimize the presence of external alleles in progeny (Hebel et al. 2007).

The large levels of genetic diversity and long distance gene flow have implications for the sampling strategy employed in studies on gene flow. It may be most efficient to take samples from 50 trees and as few as eight seeds per tree instead of 20 sample trees and 20 seeds per tree because the levels of genetic diversity and allelic richness are the same. The former scheme will provide a progeny sample with more rare alleles for the same sample size (Miyamoto et al. 2008).

### 9.9.4 Inbreeding

While high levels of inbreeding have been reported for common ash (Morand et al. 2002a; Ferrazzini et al. 2007) it is not clear if these observations are resulting from the current large battery of nuclear microsatellites (Brachet et al. 1999; Lefort et al. 1999). In fact, there is a high level of variability among these microsatellite markers and some of them do not exhibit inbreeding at all, even for some which have shown over 30 alleles (J. Fernandez-M and M. Thomasset, personal observation). These observations coupled with low to non-existent selfing rates and long-distance pollen movement would suggest biases from certain markers. Results from controlled pollinations of common ash showed that 47/48 trees were capable of producing selfed seeds, however natural selfing in mast years was recorded as 0.3–0.7 % (Fraxigen 2005).
9.10 Current Genetic Improvement Programmes

Alfas Pliura

9.10.1 General Objectives

Breeding/genetic improvement programmes for common ash are conducted at some levels in 11 countries: Austria, Belgium, Czech Republic, Germany, Denmark, Great Britain, Ireland, Lithuania, Netherlands and Romania. Initially these programmes were aimed at improving adaptedness, growth and stem quality (stem form, branchiness, etc.). However, due to severe ash dieback caused by outbreaks of *Chalara fraxinea* and associated diseases, the breeding objectives need to be changed, putting emphasis on improvement of resistance/tolerance to disease in many countries. Thus, several objectives for genetic improvement of *Fraxinus excelsior* may be appropriate:

- improvement of resistance/tolerance to diseases (primarily *Chalara fraxinea*)
- adaptability (adjusting of growth rhythm to avoid damage by spring frosts and long-term climate change effects)
- improvement of growth
- improvement of wood quality (improvement of stem form: straightness, reducing forking; reducing branchiness and forking/spike knots).

The strategy for increasing disease resistance and restoration of genetic variation could combine both selection of resistant populations, families and genotypes (clones) from native common ash stands as well as introduction of resistant gene pool from other European populations of common ash. Engaging different mechanisms of resistance could help to diminish the probability of breaking a resistance gained in tree improvement. Thus the strategy in creating permanent long-term resistance should be based on application of the so-called ‘Pyramid principle’ that integrates two types of resistance inheritance, quantitative and qualitative, i.e. resistance determined by both individual genes and by interaction of many genes, additive effects, epistasis, pleiotropy, etc. All aspects conferring resistance to fungal infection and tolerance to the pathogen should be considered.

Generally the absence of forks, the number and thickness of branches, and stem form are the most important characteristics that affect the quality and commercial value of ash wood (Fig. 9.9).

Forking can be related to damage by late frost. Stem straightness is the second major criterion for selection of wood quality traits. Stem crookedness may be due to forking and may be related to lack of apical dominance. Branch angle, the extent of stem self-pruning or other related traits are also important when breeding for high quality ash. As discussed above, there is significant potential for selection for these traits at the level of progeny, provenance and individual.
Improvement of growth and shorter rotations is leading to a higher proportion of juvenile wood and threatens to decrease wood density and the mechanical properties of the wood (stiffness, durability, shrinkage, etc.). Therefore breeders should aim to improve or at least maintain wood properties in ash tree breeding programmes.

Apart from the general concern of finding adaptive variation to cope with climate change, a major line of research where there could be room for improvement is the character to flood resistance. Common ash often grows on areas near to river systems and seedling survival is greatly affected by water dynamics (Kramer et al. 2008). Moreover, experiments have shown that mountain provenances of common ash are less resistant to flooding than lowland provenances of the species, and they are much less resistant than small-leaved ash (Ruedinger et al. 2008).

**Fig. 9.9** Selected tree of ash for progeny testing, generating breeding seedling orchards or clonal seed orchards by grafting shoots (Photo G.C. Douglas)
9.10.2 Existing Trials Network

A total of 117 field trials of *Fraxinus excelsior* of different types exist in TreeBreedex countries (Fig. 9.10, Table 9.13). Some progeny trials have a population/provenance structure and some provenance trials have a family structure that allows both for evaluating provenance performance and for estimation of genetic parameters (heritability, additive coefficient of genetic variation, genetic correlations, genetic gain, etc.) and for carrying out both family and within-family selection.

A substantial part of field trials constitutes national or pan-European series:

- 1982 field trial series: 26 trial sites in five countries, 17 provenances and 50 families are under test (approximately 60 sources).
- 1986–1989 field trial series, provenance trials were established in three countries (Belgium, France, and Germany), 52 provenances are being tested
- 2004–2005 RAP field trial series, provenance/progeny trials were established in seven countries (Belgium, France, Germany, Great Britain, Italy, Ireland and Lithuania), 55 provenances and over 350 families are being tested.
- 2009 year field trial series: five sites in France, 24 provenances are being tested.
A total of 3419 genetic units of *Fraxinus excelsior* are in tests in all field trials; details are available in the Treebreedex database.

### 9.10.3 A Survey of Breeding Strategies for Ash Used in Europe

**Patrick Mertens**

All partners in the Treebreedex project were surveyed in relation to their genetic improvement programmes on ash. The number of programmes in each country are summarized below in Table 9.14. We report this data in terms of the number of programmes adopting a particular approach relative to the total who replied in the survey. In these programmes, 75% of respondents placed an emphasis on wood productivity and 25% on wood quality. The main aims are to produce improved forest planting material or to develop strategic guidelines for deployment by the final users of ash.

### 9.10.4 Programmes with an Emphasis on Wood Productivity

Most programmes initially concentrated on timber production (six programmes). Other objectives were tree conformation (with four programmes) and plasticity (with two programmes). The latter two were considered in one category called ‘Wood productivity’. The timber production objective is to maximize the wood
Volume; this cannot be considered separately from good tree conformation and high tree plasticity.

As a set of secondary traits, stem form, forking (ramification), volume production, pest and diseases tolerances and a tree’s vitality were selected characters in the majority of 12 programmes. The breeding and improvement programmes were reported as justified based on land areas planted each year from: <500 ha (8/12); between >500 ha and <5000 ha (3/12) with the aim of covering the national territory areas (8/10); only 3/12 programmes were for developing regionally adapted material.

The main objectives of most programmes (7/12) are to produce new improved FRM and on publishing strategic guidelines/recommendations to users. The programmes aim to achieve this objective with the accompanying benefit of improvement of the gene pools (10/12) or by conservation efforts (2/12). About half of respondents indicated that ash improvement is being carried out as part of their routine programmes (6/12). Some respondents consider ash improvement as part of their aims to explore emerging or underdeveloped species (3/12) while others do so to fulfil certification requirements (2/12). Germplasm sources for genetic improvement come from diverse origins: natural dispersion areas of ash species (4/12), stands (3/12), single trees (3/12), and from provenance material (1/12). The source material was deployed into seed orchards mainly using open pollination (7/12), into provenance tests with open pollination and half-sib populations (3/12), stands and single trees (2/12).

Material was selected from mature (7/12) and non-mature (5/12) stands with 3/12 programmes indicating the removal of unwanted trees. Half of respondents used seed stands as their conservation stocks (6/12) while others relied on grafted trees (6/12). Respondents indicated that molecular technologies are limited for directly facilitating genetic improvement goals (3/12) but statistical tools were used in all the programmes.

### 9.10.5 Programmes with an Emphasis on Wood Quality

Four programmes have an emphasis on wood quality by improving the population’s traits for the character of the stem form, absence of forking, pest tolerance/resistance and phenology. With regard to wood quality, 50 % (2/4) of respondents aim to improve these traits via the establishment of seed orchards while others (2/4) aimed for identification of suitable provenances by selection in provenance areas or areas of natural dispersion. In addition, the responding countries indicated they had conservation objectives; for this purpose they marked seed stands (2 out of 4
programmes), and they have established grafted orchards (1 out of 4). The molecular technologies are used to aid the identification of material in some cases. However, most of the cases (3/4) have used statistical tools to follow the evolution of the growth, the form, the tolerance to diseases and the phenology.

9.11 Breeding Methodology

Alfas Pliura

As common ash is considered a species of minor importance in most countries, the breeding intensity in general is of low input and follows a simple recurrent scheme. With the recent spread of ash dieback disease, special considerations must be given to modifying breeding strategies to obtain durable resistance (McDonald and Linde 2002a, b). Figure 9.11 describes the available alternatives in combining methods and approaches that can be used in ash breeding programmes. Methods on the right side of the red line in the figure gives higher genetic gain, however they are more expensive and more labour and technology is required.

At present four main breeding systems can be distinguished: Conventional unstructured (C), Open nucleus (ON), Hierarchical OPen Ended (HOPE) and Multiple Population Breeding System (MPBS). Open nucleus breeding means that the breeding population is split into one small and one large population. The selection intensity is higher in the small population than in the large population, which cause a divergence between the two over time. HOPE means that genes are transferred stepwise via crosses from populations with a low level of improvement to higher levels of improvement.

MPBS was first developed by Namkoong (1976, 1984). It means that the breeding population is split into approximately 20 subpopulations, each with some 50 genetic entries (Fig. 9.12). Multiple small populations can give greater gains than single populations by selecting and intercrossing among subpopulations. Separate subpopulations can have different breeding goals and traits of interest. Moreover, artificial selection progress rates can vary among subpopulations. The difference from the sub-lining strategy is that MPBS is aimed at an increase in the among-population additive variance (see Eriksson and Ekberg 2001). It is also advantageous in case of unpredictable global climate warming and changing trait market values (Eriksson et al. 1993; Koski and Tigerstedt 1996). One main difference of MPBS from the open nucleus system is that there is no infusion of newly selected material from forest stands into the breeding population. Such an infusion results in a reduction of genetic gain. A comparison of the two breeding strategies, MPBS and HOPE, reveals that the HOPE has a lower level of genetic diversity (Williams et al. 1995).

If MPBS is planned for long-term breeding it also takes care of gene conservation of the species (Eriksson et al. 1993). From a conservation point of view MPBS combines the capture of the existing adaptedness while keeping satisfactory
Breeding system: Unstructured MPBS HOPE Open nucleus

<table>
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<tr>
<th>Mating</th>
<th>Open pollination</th>
<th>Polycross</th>
<th>Single pair mating</th>
<th>Double pair mating</th>
<th>Factorial</th>
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<td>Asortative, PAM</td>
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<th>Long-term, 40-50 years</th>
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<td>Single-tree plots</td>
<td>5-10 tree row plots</td>
<td>Large plots (&gt;10 trees)</td>
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<td>In single breeding zone</td>
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<th>Unrestricted</th>
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<th>Within-family</th>
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<td>Forward</td>
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<td>Restricted parents</td>
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<th>Reproductive population</th>
<th>Generative propagation</th>
<th>Vegetative propagation</th>
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<tr>
<td>Seedling seed orchards</td>
<td>Grafted seed orchards</td>
<td>Controlled crossing</td>
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<td>Bional (2 clones)</td>
<td>Small number of clones (10-30)</td>
<td>Large number of clones (30-50)</td>
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Fig. 9.11  Available alternatives to combine methods and approaches to be used in ash breeding programmes. The red line connects combinations of methods suitable for low input ash breeding.

Fig. 9.12  Multiple population breeding system for improving of ash with many specifically targeted breeding populations for different sites, plantation types and specific traits (Based on Eriksson 2001)
additive variance in each subpopulation. MPBS prevails in Sweden, Finland, and Lithuania. Some countries use combinations of different breeding systems, e.g. the *Pinus radiata* breeding in New Zealand has elements of MPBS, nucleus breeding and sub-lining (Eriksson and Ekberg 2001).

Most countries started their *Fraxinus excelsior* breeding efforts by establishing individual or a series of provenance trials and testing progenies from native or foreign provenances/populations. Extensive studies have been carried out or are underway to select the best seed sources and to estimate genetic parameters for the development of breeding programmes. This phase has been achieved or is underway in Belgium, Czech Republic, Germany, France, United Kingdom, Ireland, Netherlands and Romania. However, the provenance trials are of limited use for breeding of *Fraxinus excelsior*. With regard to long-term breeding, the concurrent provenance trials are considered as a blind end (Koski and Tigerstedt 1996). It was emphasized that if the breeding programme is based on provenance experimental trials only, it restricts the application of MPBS in breeding programmes and it is not able to generate significant genetic gain (Varela and Eriksson 1995).

Provenance trials have two main objectives:

1. to identify the best seed source
2. to give information on the past evolution of the species (Eriksson and Ekberg 2001).

Information from provenance trials can be used to identify the provenances to be used for selection of material for the founder population in tree breeding. Plus-tree selection in wild forests or in provenance trials has been carried out to create base breeding populations (Fig. 9.9). Clones have been archived in clone banks or in first-generation seed orchards. This phase has been achieved by a few programmes (Germany, Sweden, UK, Ireland, Lithuania, the Netherlands (two seed orchards), France, Romania, Czech Republic, Poland, Slovakia) or is underway, Table 9.13.

### 9.11.1 Mating Options

The concepts and methods of mating in tree improvement vary from country to country. In the first breeding cycle, most countries started with selection of plus trees and open-pollinated (OP) or polycrossed progeny in long-term testing followed by a mixed forward selection model. This is predetermined mainly by the low economic importance of the species in the country, geographic peculiarities of the country and national socio-historical heritage. In most cases, open-pollinated half-sib progenies have been either directly collected from random seed trees of different age or from plus-trees in the forest or in seed orchards/clone banks. The open-pollinated mating model is planned to be used for obtaining the next generation of *Fraxinus excelsior* in most countries as the low input method of tree breeding. However, OP is not a suitable method to improve the resistance to diseases, as it can compromise the achieved genetic gain because of possible significant uncontrolled
gene flow from non-resistant to diseases pollen sources (unimproved stands). For this purpose, controlled crossing should be planned instead. Double-pair mating among 50 members of a breeding population would be a moderate input breeding method facilitating high genetic gain and genetic variation in the next generation. The diallel mating, however, would facilitate a more precise estimate of general combining ability (GCA), specific combining ability (SCA) and other genetic parameters and would increase the possibility to obtain high genetic gain in tree breeding of Fraxinus excelsior.

9.11.2 Testing Options

Long-term testing in many cases is combined with medium- or short-term testing since genetic gain per time depends very much on the length of the testing period. For most broadleaved tree species the period of 7–15 years facilitates obtaining efficient breeding with satisfactory juvenile–mature correlations. The choice of single or multiple tree plot testing design is very much dependent on the testing period and on the number of genetic entries used. Long-term tests and different types of conversions of test trials to other breeding system units would favour a multiple plot design. Usually a large number of genotypes and many test localities is associated with single tree plot design, as it is more cost-effective (Osorio et al. 2003).

Many studies of coniferous and broadleaved tree species show rather low G×E interaction. However, the majority of estimates of G×E refers to individual traits and not to composite traits. Namkoong (1985) and McKeand et al. (1997) reported that a composite trait might show G×E interaction even if the individual components did not show any G×E interaction. These observations call for progeny trials at several localities. Progeny testing of Fraxinus excelsior is done on few locations/sites in each country where the breeding of ash is carried out (France, Germany, UK, Lithuania, Denmark, Ireland, Romania and the Netherlands). Most of Fraxinus excelsior national progeny/provenance trials series have been established under or in co-operation with FP-5, RAP series or other pan-European field trial initiatives (see results above from France and Belgium).

9.11.3 Selection Options

Among-family forward selection followed by within-family selection is mostly used in Scandinavian and Baltic countries. Dominance of forward selection in tree breeding programmes is reasonable, because in most cases forward selection was superior to backward (e.g. Routsalainen and Lindgren 1998). Computer simulations with considerations of costs, time, genetic parameters and annual budget show that the backward selection ("progeny strategy") would yield higher group merit gain
per year (which is expressed as a function of breeding value and gene diversity) than forward selection (“phenotype strategy”) only in cases where the reproductive maturity of parents is shortened to below 12 years (Danusevicius and Lindgren 2001).

Study of selection and mating principles by computer simulation revealed that positive assortative mating with selection restrictions on group co-ancestry enhances gain and also enables the conservation genetic diversity in long-term forest tree breeding (Rosvall and Mullin 2003). Fernandez and Toro (2001) have shown that the method of restricted co-ancestry selection can be effective in cases where there is a strong need to balance expected gain and genetic diversity. The application of this method does not restrict the larger contribution of the best performing families to the selected group (Lindgren et al. 1989).

Backward selection of *Fraxinus excelsior* based on general combining ability (GCA) of maternal plus trees is usually the first step to establish breeding population based on genetic values and to realize the genetic gain in 1.5-generation seed orchards. This phase has been achieved by a few programmes (Germany, Sweden) or is underway (Denmark, France, Belgium, Netherlands, Lithuania, Romania and the UK). Forward selection is preferred for long-term breeding with selection of the best families and then selection of the best individual phenotypes in these families to create second-generation breeding populations. This phase has been achieved by very few programmes (Germany) or is underway (Lithuania and UK). In the case of the UK the approach adopted has been to establish clonal seed orchards and archives together with Breeding Seedling Orchards (BSOs). BSOs combine provenance and progeny tests in one trial set and lies between the approach of a progeny test and a Seedling Seed Orchard (Barnes 1995). Four ash BSOs were established in the UK in 1993. BSOs are a low-cost means of producing seed with some genetic gain by initial phenotypic selection of mature plus trees in the field, testing their families, followed by intensive selection of the best families and individuals to generate seed orchards which will be in the ‘Qualified’ category (details at: http://www.bihip.org/).

### 9.12 Vegetative Propagation and Cryopreservation

Gerry C. Douglas, and Alfas Pliura

Testing of generative progenies is often combined with testing of vegetatively propagated clones as clonal testing significantly improves the precision of estimates and generates higher genetic gain (Burdon 1986; Danusevicius and Lindgren 2002). There are also indications that more pronounced \( G \times E \) interaction is observed in the performance of clones in comparison to half-sib families, so there would be a need to have more testing sites for clonal selection. However, it is suitable only for species that are easy to propagate vegetatively, unless economic interest is very great. Clone testing should be economically well justified, as it requires more resources to
be allocated to get expected results. Vegetative propagation could be a very efficient option in breeding for resistance to diseases such as Chalara as resistance/tolerance of ash is generally observed more at the individual genotype level in comparison to population or family levels (see above Sect. 9.3). Graft viability of ash is close to 100% for all genotypes but cutting propagation from selected trees was low at <26% (Douglas 2001; Cahalan and Jiinks 1992). Grafting of selected trees followed by micropropagation would be feasible to obtain clones of most resistant individuals selected in natural stands or in progeny trials. First observations of micropropagated ash clones from mature trees in a field trial indicate that the material grew normally and that rejuvenation of adult material is possible (Douglas GC 2012, personal communication).

Previous in vitro studies showed that ash embryos can be cultured to overcome embryo dormancy (Raquin et al. 2002) and that it is feasible to cryopreserve common ash shoots (Schoenweiss et al. 2005) and embryos (Brearley et al. 1995). Somatic embryogenesis from embryos has also been demonstrated in F. excelsior (Capuana et al. 2007) and other species (Tonon et al. 2001; Bates et al. 1992). For micropropagation, authors have worked with F. excelsior (Chalupa 1990; Hammatt and Ridout 1992; Hammatt 1997; Silveira and Cottignies 1994; Pierik and Sprenkels 1997; Schoenweiss and Meier-Dinkel 2005), F. americana (Preece et al. 1987, 1989) and F. angustifolia (Perez-Parron et al. 1994). They observed the optimum culture performance on low salt media such as WPM (Lloyd and McCown 1980) and DKW (Driver and Kuniyuki 1984) compared to the standard MS (Murashige and Skoog 1962). OM medium (Ruigini 1984) was successfully used to micropropagate F. ornus (Arrillaga et al. 1992) and we found it gave good propagation rates and healthier longer shoots with two clones of ash (Abbott 2005) and Table 9.15, Fig. 9.13.

Schoenweiss and Meier-Dinkel (2005) reported that establishment of mature trees was difficult and clone dependent. They also reported micropropagation rates of 1.5–2.0, and rooting ex vitro after a period of root induction in vitro. Our own results confirm these observations. Over 2 years we initiated shoot cultures of over 35 genotypes annually but only 10 genotypes survived to the fourth subculture period. Establishment of ash cultures in vitro is difficult because of a varied bacterial flora (Donnarumma et al. 2011) and the physiological mature status of tissues.

Previous research on Fraxinus species has shown that BAP, TDZ and IBA are effective for axillary shoot proliferation (Navarrette et al. 1989; Chalupa 1990). High concentrations of BAP (1–5 mg/l) stimulated shoot proliferation in juvenile and mature explants of F. excelsior (Chalupa 1990; Leforestier et al. 1991; Hammatt and Ridout 1992; Hammatt 1997; Silveira and Cottignies 1994); F. ornus (Arrillaga et al. 1992) and F. angustifolia (Perez-Parron et al. 1994; Tonon et al. 2001). Pierik and Sprenkels (1997) found that shoot length and multiplication rate of mature F. excelsior increased with increasing concentrations of the cytokinin tetra-hydropyranyl benzyladenine (PBA). Navarrete et al. (1989) reported optimal axillary shoot proliferation and elongation in F. americana by combining TDZ
Table 9.15  The effects of four types of media on the propagation rate from single node explants of two genotypes of common ash

<table>
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<tr>
<th>Basal medium (references in text)</th>
<th>Genotype</th>
<th>Total&lt;sup&gt;b&lt;/sup&gt;</th>
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<td>MS</td>
<td>1.45 ± 0.3&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
<td>2.35 ± 0.3&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;**&lt;/sup&gt;</td>
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<td>WPM</td>
<td>1.77 ± 0.2&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>2.31 ± 0.2&lt;sup&gt;a&lt;/sup&gt;A</td>
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<td>DKW</td>
<td>2.05 ± 0.2&lt;sup&gt;a&lt;/sup&gt;A</td>
<td>2.17 ± 0.1&lt;sup&gt;a&lt;/sup&gt;A</td>
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<tr>
<td>OM</td>
<td>3.28 ± 0.2&lt;sup&gt;b&lt;/sup&gt;A</td>
<td>2.78 ± 0.2&lt;sup&gt;b&lt;/sup&gt;A</td>
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Data from both genotypes combined

*±Standard error. **Different letters within columns (lower case) and rows (upper case) indicate a significant difference (p<0.03) according to Tukey’s HSD

All media modified contained B5 vitamins (Gamborg et al. 1968) with sucrose (30 g/L); BA (5.0 mg/L); IBA, (0.2 mg/L) and TDZ (0.55 mg/L)

(3 µM) with BAP (1 µM) and IBA (1 µM) in the culture medium. Kim et al. (1997) found the concentration of BAP and TDZ significantly affected shoot proliferation of *F. pennsylvanica*. We observed that TDZ is needed to initiate cultures but prolonged culturing with high levels of TDZ resulted in vitrified shoots. This condition, however, could be reversed by culturing shoots in alternative cycles on WPM with 3 % activated charcoal to restore normal shoots. On this medium we often observed spontaneous rooting, as a possible indicator of physiological rejuvenation. After spontaneous rooting *in vitro*, the shoots from mature trees were established in the greenhouse and pruned as low ‘hedges’ to a height of 5 cm, Fig. 9.14.

Shoots harvested from these hedges rooted at high rates (under plastic on a heated bench, 21 °C); three crops of cuttings could be collected in the same season giving highrooting rates (Figs. 9.15 and 9.16)

There was variation in rooting rate among genotypes but the lowest rate remained over 50 % (Fig. 9.17). Micropropagation of ash will be useful to bulk up desirable germplasm from seed and as a tool to rejuvenate mature trees so that clonal material can be generated for clonal tests and also to establish stoolbeds which provide rootable cuttings for more extensive deployment.
Before the emergence of ash dieback disease, genetic improvement of ash has concentrated on quality and adaptive characters. The analyses of provenance and progeny trials have demonstrated that there is a large variation among and within the sources of ash for important characters. For genetic improvement, selections of trees with improved characters can be made at the level of progeny, provenance...
and individual genotype. Molecular analyses of ash populations have shown high levels of intra-population diversity. There is low genetic differentiation between stands but on large geographic scales it is significant.

- Provenance effects were clear for important traits such as height growth and stem form and the estimates of genotypic heritability were high. Furthermore, provenance effects were very highly significant for frost damage and canker sensitivity and genotypic heritability was good.
• There were highly significant provenances × site interactions for important traits such as stem height, diameter and stem form. Results from a global analysis of large replicated trials in two countries showed very strong site effects and that selection of best provenances may be valid for local/regional superiority only, whereas vegetative propagation and testing of selected plus trees would have great potential for improvement.

• Priority in breeding should be given now to research the genetic basis for resistance to ash dieback disease, *C. fraxinea* and other associated pathogens. This is needed because dieback is very serious in many parts of mainland Europe, it is spreading and threatens the species. It is not known if genetic resistance is to be found outside the present range of affected areas. The effects of climate in limiting or favouring its spread are also unknown. Gene complexes which are additively inherited as well as individual genes responsible for resistance need to be identified and molecular markers elaborated for mass screening of ash genetic resources for the presence of genetic resistance.

• To combat dieback of ash in Europe, breeding must concentrate on finding the most resistant populations, families and clones, followed by breeding of multiple lines by crossing of the most resistant materials that have been selected or tested.

• The genetic diversity of ash gene pools which show some resistance is probably too low to guarantee and sustain a long-term survival of the species in Europe. Therefore, each country should establish reproductive populations (grafted or seedling seed orchards and/or maternal collections for vegetative mass propagation) out of pooled material from all resistant breeding populations that are being created in different countries.

• Research is needed to determine the possibilities of recovering, restoring and managing the genetic diversity in natural or semi-natural populations by using remaining resistant genotypes in severely affected ash populations.

• Previous recommendations on the transfer of reproductive material, provenance delimitation, seed collection, seed orchard design and management such as those proposed by the project FRAXIGEN (2005) should be reconsidered because of the widespread threat of *C. fraxinea*.

• Following the identification of resistant sources of germplasm, conventional breeding should incorporate resistance and aim at breeding for adaptability, growth, stem form and wood quality.

• The existing network of ash provenance and progeny trials in Europe, as well as seed orchards and selected stands are important resources for joint studies on disease status, plasticity and adaptability in relation to climate change (phenology, drought, frost). These resources should be maintained and further developed for collaborative studies; in particular on genotype × environment interaction at the European scale.

**Acknowledgements and Contributors** The financial support of the Treebreedex project is acknowledged and the information and contact details of statistics from European countries are given below in Annexe 9.1.


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Annexe 9.1 Details of the Principal Providers of Country Statistics in Tables 9.1 and 9.2 on Ash

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<tr>
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