

# Summary report on Forest Research aspen clonal trials

Victoria Stokes, Joan Cottrell and  
Stuart A'Hara

Forest Research is the Research Agency of the Forestry Commission and is the leading UK organisation engaged in forestry and tree related research. The Agency aims to support and enhance forestry and its role in sustainable development by providing innovative, high quality scientific research, technical support and consultancy services.



Clone 48 growing at the Kilmichael site after 21 growing seasons.

## Contents

Summary.....	4
Background.....	5
Experiment sites.....	6
Genetic verification of clones.....	8
Growth and performance assessments.....	8
Analysis.....	9
Results.....	9
Genetic verification of clones.....	9
Growth and performance.....	10
Discussion.....	19
Future management.....	20
Management of the existing experiment sites.....	20
Establishment of an aspen seed orchard.....	21
Conclusions.....	22
Acknowledgements.....	22
References.....	23

## Summary

Two experiments were established in the mid-1990s trialling a range of Scottish aspen clones (*Populus tremula*), commercial poplar clones/cultivars and Swedish aspen hybrids on two contrasting sites in Scotland. After more than two decades the height and diameter growth of the Scottish aspen clones were significantly lower than those of both the commercial poplar clones/cultivars and the Swedish aspen hybrids.

The performance of Scottish aspen did not differ between seed zones of origin; no seed zone was superior to the others overall. There was no evidence that aspen from the local seed zone was better adapted and more productive than aspen from distant seed zones at either experiment site.

Twenty-one of the Scottish aspen clones trialled were common to both sites. There was a 2-3 fold difference in growth performance between the best and worst clones, but there was some consistency in performance of the common clones between sites. Clones 105, 70, 34/69 and 75 had large diameters at both sites; clones 105 and 70 also had the largest heights at both sites. The performance of individual clones was not related to proximity of the clone's source to the experiment site; locally sourced clones did not perform better than those from further afield. The best performing clones grew well on both of the experiment sites, which had strongly contrasting environmental and climatic conditions.

Genetic testing (DNA fingerprinting) has been used to ensure that all clones in the study are unique genotypes and are pure *P. tremula*. This has identified that the exceptionally high growth rates shown by one clone are due to it being a hybrid aspen, while some genetic anomalies in another top-performing clone suggest that it too may be a hybrid. A DNA test was also carried out to identify the sex of each clone.

It is recommended that, with the landowners' consent, the existing experiment sites are retained as Forest Research experiments, and that work to develop methods of inducing flowering of aspen in Britain is undertaken.

We suggest that a seed orchard is established to conserve better performing Scottish aspen clones, to be situated in East Anglia, an area conducive to flowering of aspen. Some of the better-performing clones tested in these experiments would be suitable for inclusion in a seed orchard; propagation of material from the experiment trees could be carried out, ensuring a similar number of male and female clones were selected. Consideration should be given to extending the number of clones included in a seed orchard by sampling phenotypically superior clones from across Scotland and concentrating efforts on those that have a greater inclination to flower.

## Background

European aspen (*Populus tremula* L.) is Scotland's only native poplar species. It has a very wide distribution, covering Europe, northern Africa, and western Asia, and extending to the Bering Sea in the north. Within Britain it is frequent in northern and western Scotland but becomes less common moving south and eastwards, and is rare in the south of England (Jobling 1990). It is naturally associated with oak and birch woodlands, and sometimes with Scots pine woodlands. A full review of the ecology, conservation and management of aspen is given by MacKenzie (2010).

Over recent decades there has been increasing interest in the use of aspen in native woodland schemes in Scotland, including native pinewoods. Work by Hollingsworth and Mason (1991) showed success in bulking up stocks of aspen trees through the use of controlled vegetative propagation from root cuttings and suckers. More recently there has been interest in the commercial use of aspen as a fast growing broadleaved species for use in farm woodlands and short rotation forestry schemes, as high yields have been reported (Worrell 1995b).

However, there has been relatively little research on aspen compared to some other native broadleaved species. Despite its wide distribution in Scotland, aspen rarely sets seed and reproduces by vegetative propagation (suckering); it usually exists as small stands or single individuals (MacKenzie 2010). The genetic diversity within British populations has not been heavily studied, and information is required in order to conserve the genetic variability and identify suitable planting stocks (Worrell 1995a, Easton 1997). In addition, research is required on the interaction between genotype and site conditions; the phenotypic plasticity. Provenances that show superior traits and performance on a range of site types could be selected for future schemes.

During the early 1990s a programme of work was initiated to learn more about the potential variation within the native aspen population, both in relation to traits such as growth rate and form, and in terms of resistance to bacterial canker. In spring 1993, a range of Scottish aspen clones were identified from eight defined seed zones (Figure 1) and work was carried out to propagate planting material from these. The zones were defined by the boundary between Regions of Provenance 10 and 20 (see Herbert et al. 1999) as the first divider, with subsequent divisions along major watercourses (Mason et al. 2002). Two experiments were established with the aim of comparing the performance of these native Scottish clones with that of three commercial poplar clones/cultivars that are widely used throughout Britain ('Robusta', 'Beaupre' and 'Fritzi Pauley'). Seven Swedish aspen hybrids (*P. tremula* x *P. tremuloides*) were also planted at one of the sites for further comparison.



This report summarises results from the two experiments more than 20 years after planting. The aims are to:

- 1 Compare the performance of Scottish aspen clones with Swedish aspen hybrids and with commercial poplar clones/cultivars.
- 2 Identify whether performance of Scottish aspen is influenced by the seed zone it originates from.
- 3 Identify top-performing Scottish aspen clones and investigate their tolerance to differing site conditions.

## Experiment sites

The experiments were established at two new planting sites, Moray and Kilmichael. These sites were selected as they represent the extremes of the strong east-west gradient in climatic conditions; the site locations, conditions and establishment are summarised in Table 1. The Moray site was ploughed before planting in 1994, and the Kilmichael site was mounded, before planting in 1995.

At both sites individual clonal plots consisted of four ramets planted in a line at 3.0 m spacing. The Moray experiment trialled 31 Scottish aspen clones and the Kilmichael site trialled 79 Scottish aspen clones, see Appendix 1 for details of the clones planted at the two sites. Twenty-one clones were common to both sites. Both sites also trialled three commonly planted commercial poplar clones/cultivars:

*P. nigra* x *P. deltoids* cv 'Robusta'

*P. deltoids* x *P. trichocarpa* 'Beaupre'

*P. trichocarpa* cv 'Fritzi Pauley'

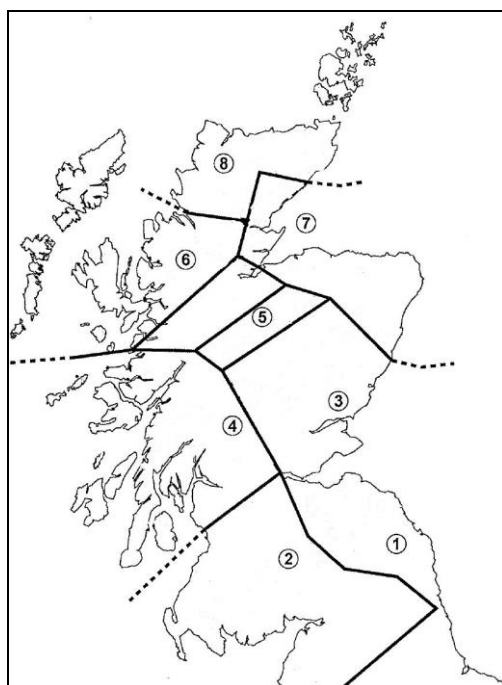
In addition, the Kilmichael site also trialled seven Swedish aspen clones (*P. tremula* x *P. tremuloides*) produced in the Skogforsk programme at Ekebo, Southern Sweden.

At the Moray site there were 5 replicate blocks and at the Kilmichael site there were 4. Both sites were fenced against rabbits and deer.

**Table 1. Site conditions**

	<b>Moray</b>	<b>Kilmichael</b>
Grid reference	NJ 154618	NR 884955
County	Moray	Argyll
Altitude (m)	20	30
Aspect	NW	NW
Slope	Level	Level
Soil	Poorly drained sandy alluvium	Typical brown earth
Vegetation	Upland grass, herbaceous	<i>Juncus</i> , mosses, grasses
ESC climatic factors*:		
Accumulated temperature	1236.6	1404.9
Continentality	4.7	4.3
DAMS	11.0	12.4
Moisture deficit (mm)	134.2	115.2
Rainfall (mm)	684	1692
Site type	New planting	New planting
Previous land use	Poor quality grazing	Ex-agricultural
Preparation	Ploughed	Mounded
Planting date	Spring 1994	Spring 1995
Scottish aspen clones	31	79
Commercial poplar clones/cultivars	3	3
Swedish hybrid aspen clones	0	7
Replicate blocks	5	4

\* Pyatt et al. (2001).



**Figure 1. Map showing the eight seed zones into which the distribution was divided. Reproduced from Mason et al. (2002).**

## Genetic verification of clones

The opportunity was taken to verify the unique genetic status of each of the clones included in the trials, as this was not carried out in the 1990s when the original material was collected and propagated. A leaf sample was collected for each clone in the trial at both experiment sites, DNA extracted, and DNA fingerprinting with 8 microsatellite markers was carried out. Aspen is diploid, therefore each sample generates a 16 allele fingerprint. Results were cross-checked to ensure that each clone had a unique fingerprint, and that this matched between the two experiments (if planted at both sites). Each genetic fingerprint was checked for possession of private alleles (alleles that were not found in any other aspen clone tested in this study) as this is an indicator that hybridisation between *P. tremula* and another poplar species may have occurred. A further DNA test was carried out to identify the sex of each sample (Pakull et al. 2015).

## Growth and performance assessments

An assessment of the trees at each site was carried out in March 2016, 22 growing seasons after planting at Moray, and 21 growing seasons after planting at Kilmichael. At each site the height (m) of all surviving trees in each plot was recorded and the diameter at breast height (1.3 m) was recorded in cm. The form of each tree was also scored using the scale:

- 1 = good straight form, potential “final crop” tree, stands out as among the best formed individuals in the trials;
- 2 = no major defects but some are allowed;
- 3 = significant defects, a candidate to remove in early thinning. Tree stands out as poorly formed compared to the majority in the trials.

At the Moray site 54 plots had been partially or wholly damaged by felling for a new house; these plots were excluded from the presented results and data analysis.



## Analysis

The data were prepared using statistical software GenStat 13 (Payne et al. 2009). Analysis used a generalised linear mixed model (GLMM) fitted using procedure GLIMMIX in statistical software SAS (SAS Institute 2011).

The three growth measures (height, diameter and form score) were analysed for each site independently. The data were assumed to be normally distributed, whereas survival was modelled assuming a binomial distribution with logit link, i.e.,  $\text{logit}(p) = \log(p/(1-p))$  where  $p$  is proportion of plants alive.

Comparing the 'Source' for all data: A fixed effect was fitted for Source (Scottish aspen, Swedish hybrids or commercial poplar clones/cultivars), with random effects for variation between Zones within Sources and between Clones within Zones.

Comparing the 'Zone' for Scottish data: A fixed effect was fitted for Zone, with random effects for variation between Clones within Zones.

Comparing the 'Clone' for Scottish data: A fixed effect was fitted for Clone, with random effects for variation between Zones.

## Results

### Genetic verification of clones

The 'DNA fingerprint' of each clone in the trials was analysed and cross-checked between the two experiment sites (where the clone was included at both trials) and against the aspen clone bank at the Forest Research Northern Research Station, if present.

While the majority of clones did have a unique genetic fingerprint, the exercise did highlight some anomalies:

- In three cases two clone identities were found to have the same DNA fingerprint; the original records confirm that in each case these were collected from the same location but had been allocated different clone identity numbers in the 1990s. The results of these clone pairs have been included in the trials, with the clone identity numbers combined (31/72, 38/68 and 34/69).
- Clones originating from zone 2 were allocated duplicate identity numbers that already existed at one of the experiment sites. We cannot be certain of the identity of material from zone 2 and the results were excluded from this report.

Further work may allow re-identification of the zone 2 clones. None of the affected clones was a particularly high performer in terms of growth.

- The DNA fingerprint of clone 1, originating from Tummel and known for many years to be an exceptional performer in terms of growth at both experiment sites, was found to have a very high number of private alleles. The leaf morphology and phenology were also recorded at the NRS clone bank during 2016 and are anomalous compared to the rest of the clones. The conclusion of this work is that clone 1 is not aspen, *Populus tremula*, but is a hybrid of *P. tremula* and *P. tremuloides*. The productivity of this hybrid is known to exceed that of *P. tremula*; yields of over 20 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup> have been reported for a 20-25 year managed rotation in Sweden (Karacic, 2005). The results for clone 1 were therefore excluded from the statistical analysis to prevent bias.
- Clone 70, another particularly fast-growing clone, was found to have two private alleles not present in any of the rest of the aspen material tested, but present in the commercial poplar 'Beaupre'. Beaupre is a *P. trichocarpa* x *P. deltoides* cross suggesting that clone 70 could possibly be a hybrid between *P. tremula* and one of these poplars; further investigations on leaf morphology, phenology and genetic markers will be carried out the spring. The clone was included in the analysis for this report but results should be treated with caution.

## Growth and performance

How did the Scottish aspen perform compared with commercial poplar cultivars and with Swedish aspen?

At Moray, there was no significant difference in survival between the Scottish aspen clones and the commercial poplar cultivars (Table 2). However, for diameter, height and form score, the commercial poplar cultivars performed significantly better than the Scottish aspen clones (Table 2).

At Kilmichael survival of the Scottish aspen was significantly higher than survival of the Swedish aspen hybrids and the commercial poplar cultivars (Table 2). For those that survived, diameter, height and form score of the Swedish aspen hybrids and commercial poplar cultivars were significantly better than for the Scottish aspen clones (Table 2). The Swedish hybrid material had the best growth performance at Kilmichael.

For both Scottish aspen and commercial poplar cultivars, the survival rate was higher at Kilmichael than Moray, but growth was better at Moray; the low survival rates observed at Moray were largely due to vole damage shortly after planting.

Although 'clone 1' has been shown to be a hybrid (probably *P. tremula* x *P. tremuloides*) and was excluded from the analysis, it is worth noting that the performance far exceeded that of the aspen clones at both sites and was similar to that of the Swedish

hybrids, also *P. tremula* x *P. tremuloides*. The mean heights of 'clone 1' were 18.2 m and 16.0 m at Moray and Kilmichael respectively, and mean diameters were 33.5 cm and 23.6 cm respectively.

**Table 2. Mean survival, diameter, height and form score for each source at Moray and at Kilmichael after 22 and 21 growing seasons respectively. At each site, sources that share a superscript letter are not significantly different from each other.**

Site	Source	Mean Survival (%)	Mean DBH (cm)	Mean Height (m)	Mean Form Score
Moray	Scottish aspen	62.54 <sup>a</sup>	14.97 <sup>b</sup>	10.75 <sup>b</sup>	2.59 <sup>b</sup>
	Commercial poplar clones/cultivars	61.86 <sup>a</sup>	39.98 <sup>a</sup>	19.97 <sup>a</sup>	1.98 <sup>a</sup>
	p value	0.9710	0.0002	0.0083	0.0481
Kilmichael	Scottish aspen	84.3 <sup>a</sup>	8.86 <sup>b</sup>	6.54 <sup>c</sup>	2.64 <sup>b</sup>
	Commercial poplar clones/cultivars	43.5	24.29 <sup>a</sup>	14.15 <sup>b</sup>	1.37 <sup>a</sup>
	Swedish hybrids	51.23 <sup>b</sup>	30.99 <sup>a</sup>	17.38 <sup>a</sup>	1.69 <sup>a</sup>
	p value	0.0039	<0.0001	<0.0001	<0.0001

## Did the performance of Scottish aspen differ between seed zones?

There were no significant differences in performance of Scottish aspen between zones for survival, diameter, height or form score at either site. Figures showing results for each zone are shown in Appendix 2. There is no evidence to suggest that material sourced from the local seed zone performed better at each experiment site than material sourced from distant seed zones (the Moray experiment is situated in zone 7 and Kilmichael is in zone 4).

## Which were the top-performing Scottish aspen clones at each site?

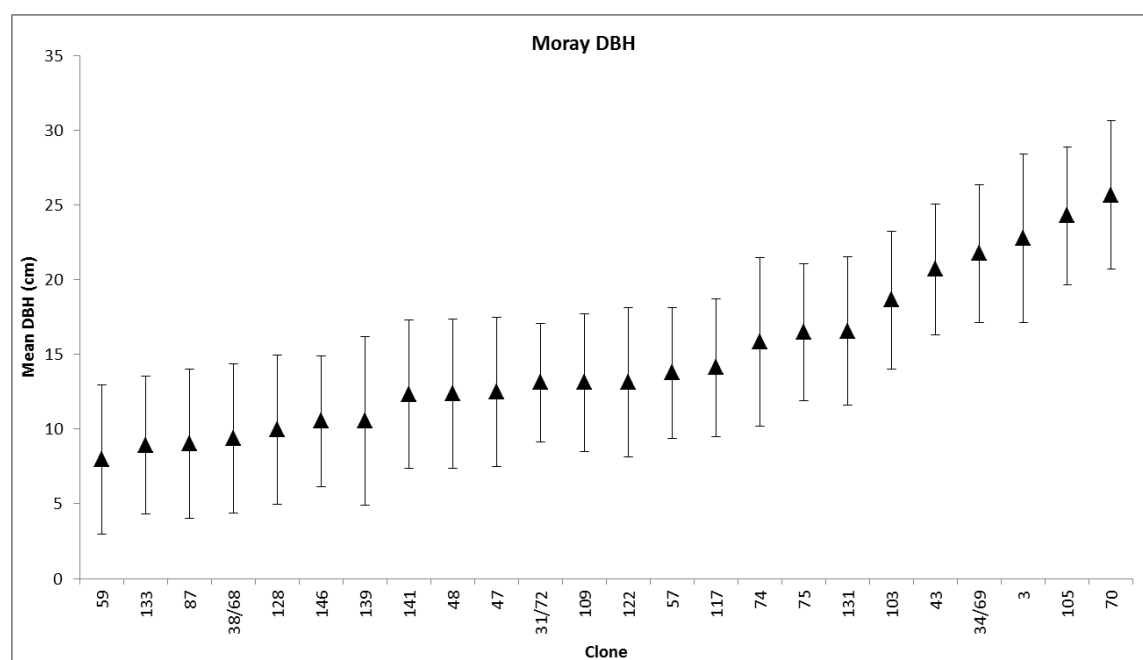
The mean survival, height, diameter and form score for each clone at each site are summarised in Appendix 3.

At Moray there were no significant differences in survival between clones (data shown in Appendix 3), but there were significant differences between clones in diameter, height and form score (Figures 2-4, individual contrasts between clones are not shown for

simplicity). Clones 105, 70<sup>1</sup>, 3, 34/69 and 43 had particularly good growth rates; clones 43, 48 and 105 had particularly good form.

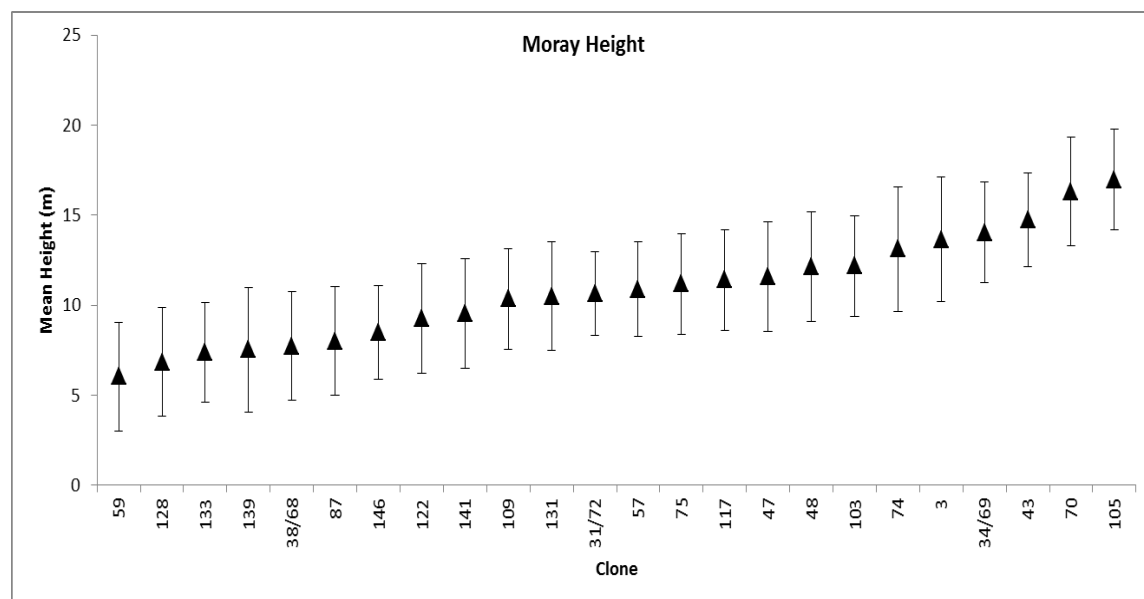
At Kilmichael there were significant differences in survival, diameter, height and form score between clones (Figures 5-8, individual contrasts between clones are not shown for simplicity). The best growth rates were recorded for clones 105, 70<sup>1</sup>, 117, 107, 75 and 100, and the best form scores for clones 48, 98, 110, 105, 75.

There was no evidence to suggest that individual locally-sourced clones were better adapted to the local conditions at each experiments site; none of the better performing clones at each site originated from the local area (zone 7 for Moray or zone 4 for Kilmichael). The consistently best performing clones 105 and 70<sup>1</sup> originated from zones 6 (North-west Scotland) and 1 (South-east Scotland) respectively.

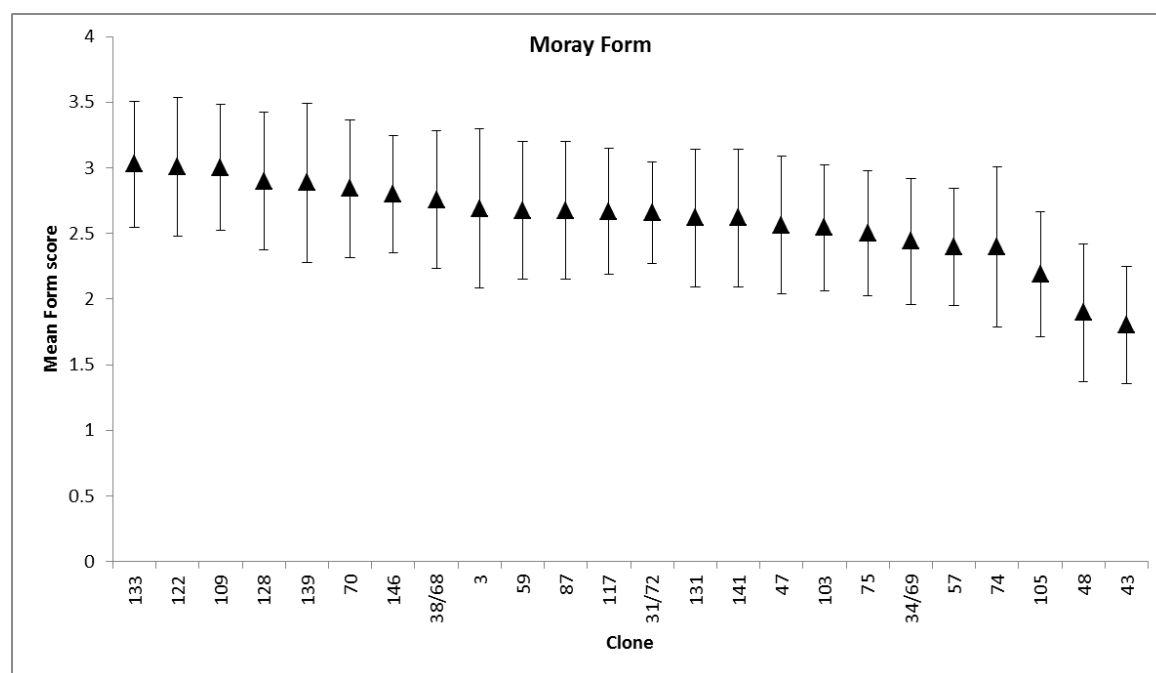


**Figure 2. Mean diameter (cm) of Scottish aspen clones at Moray after 22 growing seasons. Clones are ranked from left to right in order of improving performance.  $p < 0.0001$ ; bars are 95% confidence intervals.**

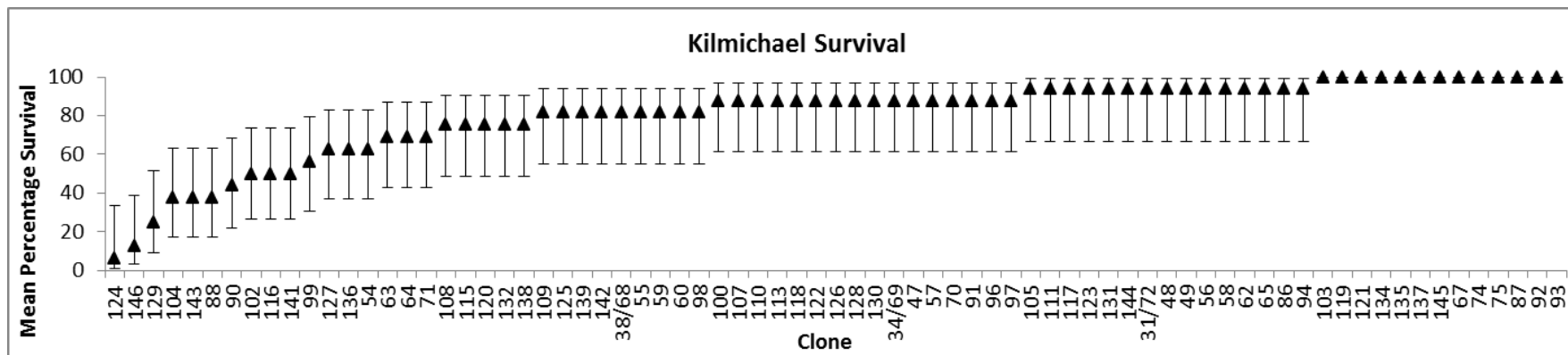
<sup>1</sup> Clone 70 may be a hybrid.



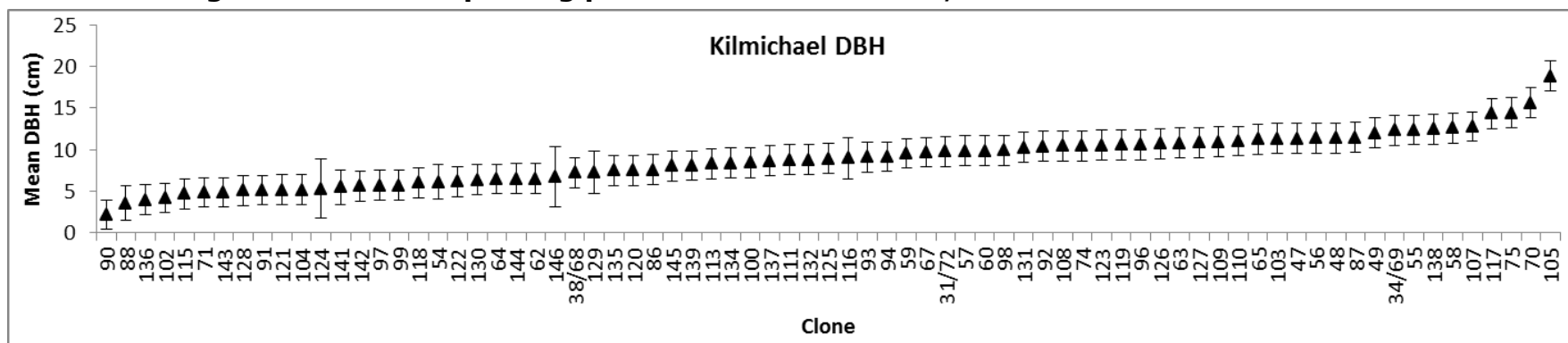
**Figure 3. Mean height (m) of Scottish aspen clones at Moray after 22 growing seasons. Clones are ranked from left to right in order of improving performance.  $p < 0.0001$ ; bars are 95% confidence intervals.**



**Figure 4. Mean form score of Scottish aspen clones at Moray after 22 growing seasons. Clones are ranked from left to right in order of improving performance.  $p = 0.0254$ ; bars are 95% confidence intervals.**

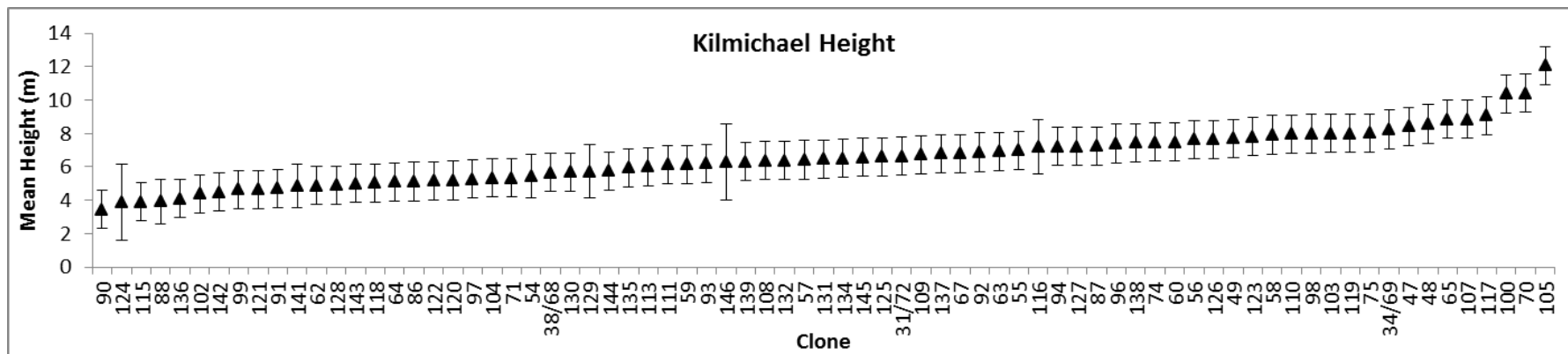


**Figure 5. Mean percentage survival of Scottish aspen clones at Kilmichael after 21 growing seasons. Clones are ranked from left to right in order of improving performance.  $P=0.0254$ ; bars are 95% confidence intervals.**

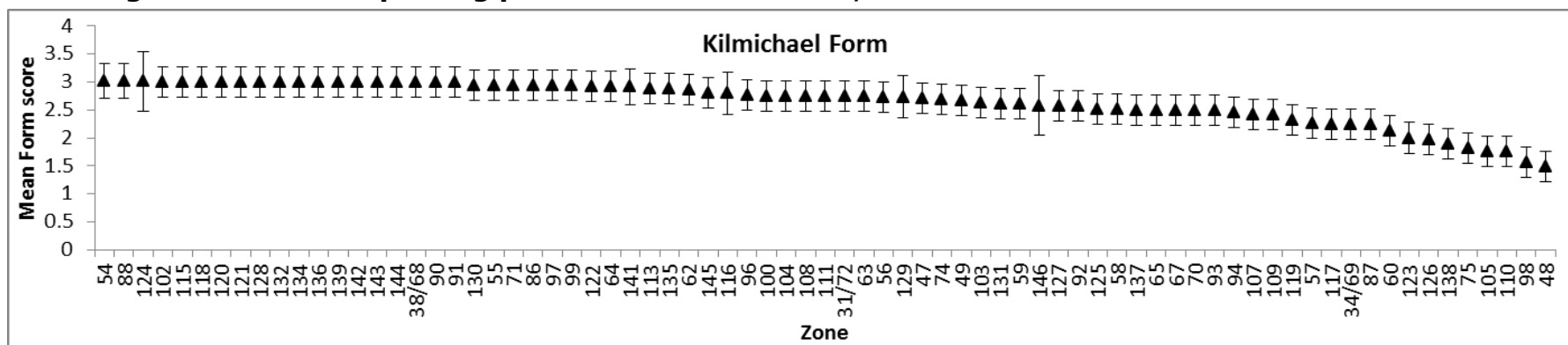


**Figure 6. Mean diameter (cm) of Scottish aspen clones at Kilmichael after 21 growing seasons. Clones are ranked from left to right in order of improving performance.  $P=0.0254$ ; bars are 95% confidence intervals.**





**Figure 7. Mean height (m) of Scottish aspen clones at Kilmichael after 21 growing seasons. Clones are ranked from left to right in order of improving performance.  $P=0.0254$ ; bars are 95% confidence intervals.**



**Figure 8. Mean form score of Scottish aspen clones at Kilmichael after 21 growing seasons. Clones are ranked from left to right in order of improving performance.  $P=0.0254$ ; bars are 95% confidence intervals.**

### Comparison of clones that were grown at both sites.

Twenty-one of the clones trialled were grown at both Moray and Kilmichael, to test comparative performance on two sites with very different climatic conditions (see Table 1). Comparison of the performance of these clones between the two sites allows further investigation of site adaptation and plasticity of the clones.

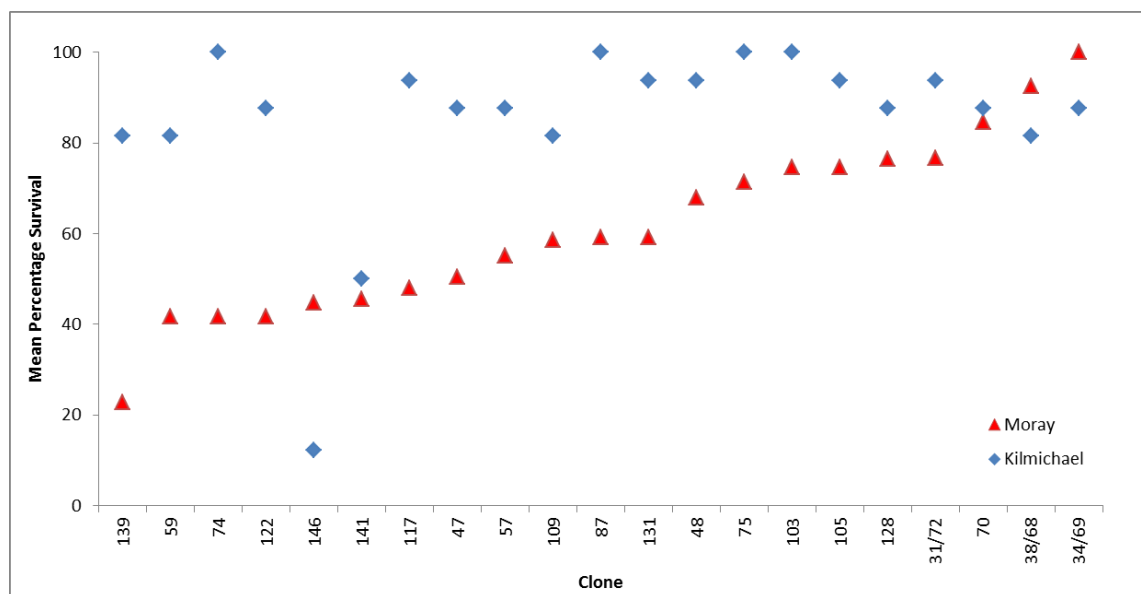
Figures 9-12 show the mean survival, diameter, height and form score of the 21 common clones, ranked from left to right based on improving performance at Moray.

For the 21 common clones, survival at Kilmichael was generally higher than at Moray (Fig. 9) due largely to the vole damage following planting at Moray, noted earlier. The survival at Kilmichael was generally high, over 80%, and there does not appear to be any correlation with the ranking of clones by survival at Moray.

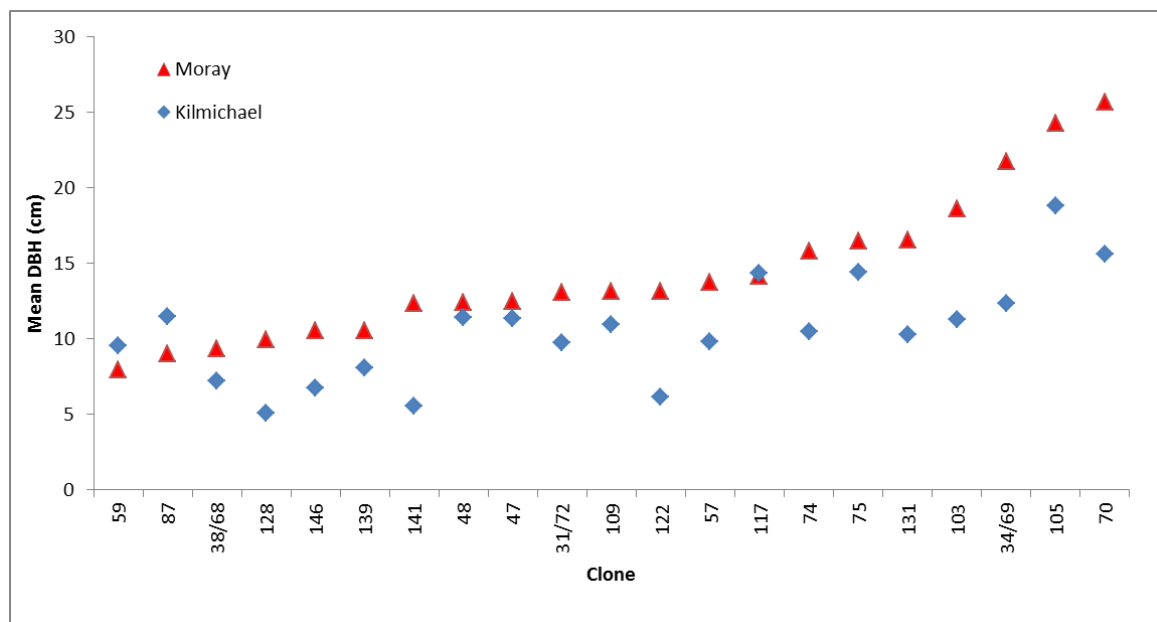
The diameter of the 21 common clones was generally lower at Kilmichael than at Moray, with the difference usually being larger than would be expected for the 1-year age gap (Fig. 10). There was some variation in ranking of the clones between sites; clones 105, 70<sup>1</sup>, 34/69 and 75 had large diameters at both sites, while other clones, such as 117 and 131 were ranked highly at one site, but not at the other. Clones 59 and 87 had the lowest diameter measurements at Moray, but were the only two clones that achieved larger diameters at Kilmichael than at Moray, despite being a year younger; clone 87 had the 6<sup>th</sup> largest diameter of the 21 common clones at Kilmichael, but the 2<sup>nd</sup> lowest diameter at Moray.

The ranking of the 21 clones by height was more consistent between the two sites (Fig. 11). Heights achieved at Moray were larger than at Kilmichael, in some cases by several metres (reflecting the site conditions). Again clones 105 and 70 were the top performers at both sites, with some variation in ranking of the other clones. The consistently high performance of clone 70, alongside the unique genetic signature noted above suggests that it may be a hybrid. As was seen for diameter, the heights of clones 59 and 87 at Kilmichael were comparatively better than at Moray, where they had not performed well.

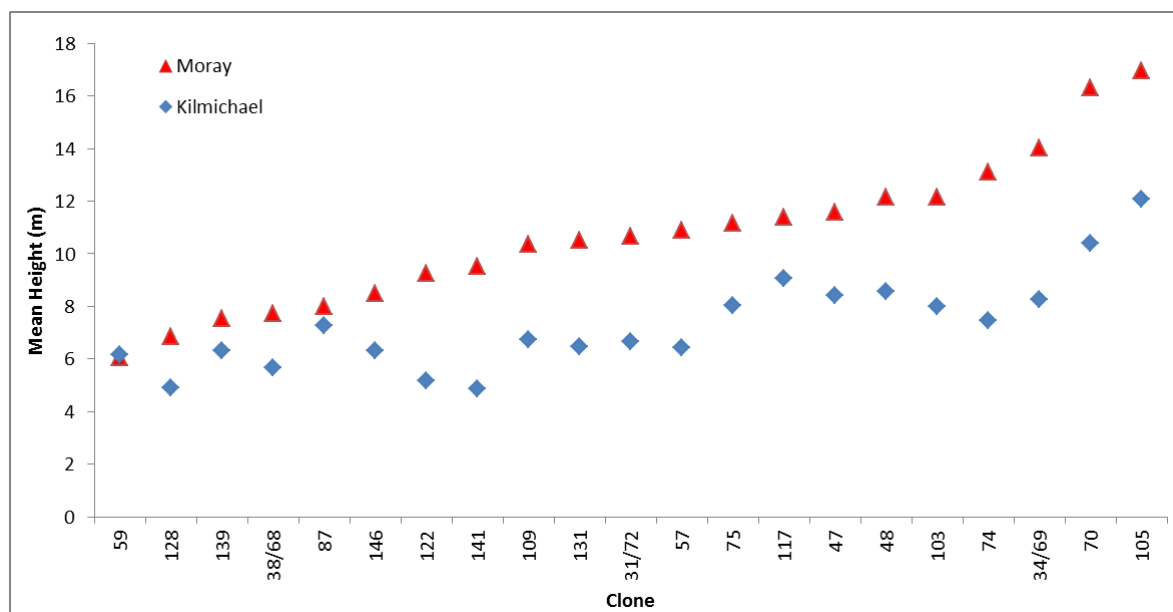
The range in form score between clones was not high and scores were generally poor. Although there was consistency in form score ranking of the best clones (48 and 105) and the worst (122) between sites, the form scores of the other common clones did not appear to be correlated between sites (Fig. 12).



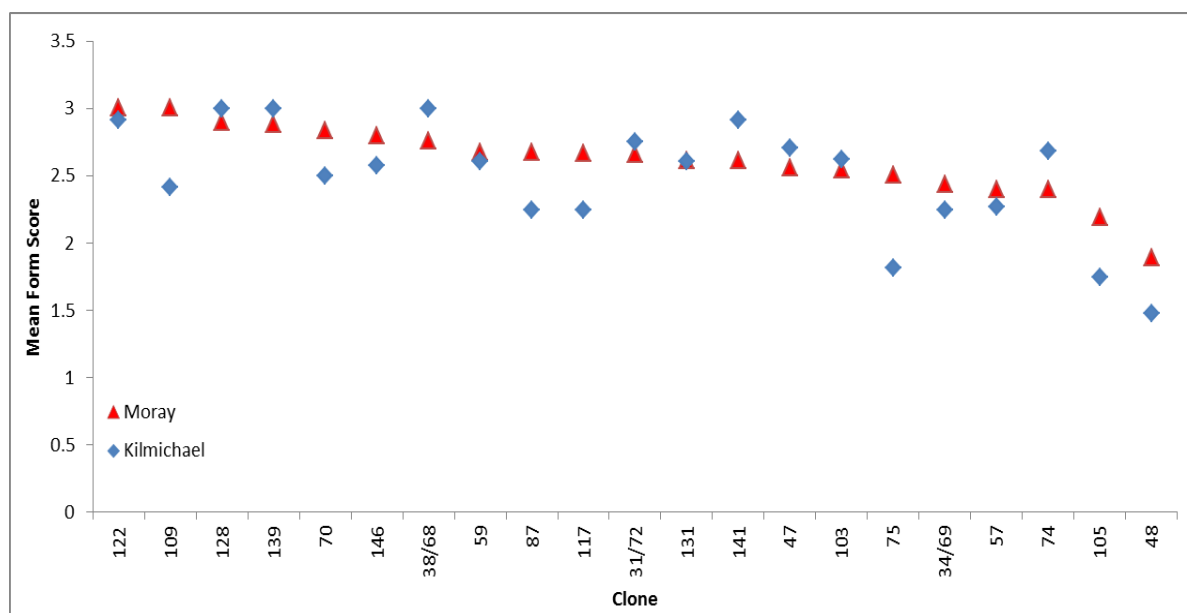
**Figure 9. Mean percentage survival for 21 common clones at Moray and Kilmichael after 22 and 21 growing seasons respectively. Clones are ranked from left to right based on improving performance at Moray. Error bars not shown for clarity.**



**Figure 10. Mean diameter (cm) for 21 common clones at Moray and Kilmichael after 22 and 21 growing seasons respectively. Clones are ranked from left to right based on improving performance at Moray. Error bars not shown for clarity.**



**Figure 11. Mean height (m) for 21 common clones at Moray and Kilmichael after 22 and 21 growing seasons respectively. Clones are ranked from left to right based on improving performance at Moray. Error bars not shown for clarity.**



**Figure 12. Mean form score for 21 common clones at Moray and Kilmichael after 22 and 21 growing seasons respectively. Clones are ranked from left to right based on improving performance at Moray. Error bars not shown for clarity.**

## Discussion

Overall the native Scottish aspen clones did not perform as well as either the commercial poplar clones/cultivars, or the Swedish aspen hybrids, both of which have been improved through selective breeding programmes. The selection criteria for the Scottish clones used in these trials was systematic, aiming to sample a geographic range of material, and not based on performance or growth. Despite this, the best performing Scottish clones did achieve heights that were approaching those of the commercial poplar clones/cultivars at both Moray and Kilmichael, but the diameters of the best Scottish clones were much smaller than those of the commercial poplar clones/cultivars at both sites. The Swedish hybrids, grown at Kilmichael only, had the best growth rates, but the survival was poor at just over 50%, suggesting that they are less well-adapted to the challenging site conditions than the Scottish aspen.

No significant effect of seed zone overall was found on performance of the clones, and locally-sourced material did not perform better at each experiment site. This may reflect the fact that the zones used were broad geographical regions, and did not take into account the immediate environmental conditions at the site where the material was collected, such as altitude, aspect and exposure. Clones from within one seed zone may have been growing in a wide range of different site types. Further in-depth analysis may indicate that individual clones originating from sites with similar microclimate conditions to those of the trial site are better adapted and perform accordingly. Our results contrast with those reported by Mason et al. (2002) in which there was a significant effect of seed zone on 6-year survival, height and diameter performance of aspen at these sites. This may be partially due to the inclusion of 'clone 1' from Tummel, now suspected of being a hybrid, but it may also indicate that significant effects of seed zone exist during the establishment phase but these do not persist in the longer-term.

None of the individual clones that have been identified as being particularly good performers originate from the local area near to the experiment site. The top performing clones 105 and 70 originate from seed zones 6 and 1 respectively (north west and south east Scotland) and both experiment sites are a considerable distance from their sites of origin. However, clone 59 performed particularly badly at Moray but not at Kilmichael; this clone originated in zone 4, where the Kilmichael experiment is situated, and may be poorly adapted to conditions at the Moray site.

There was a wide range in performance of Scottish aspen clones, with a 3-fold difference in diameter and a 2-3 fold difference in height between the best and worst clones at each site. However, there was some consistency in performance between sites, evident when comparing the 21 common clones; clones 105, 70, 34/69 and 75 had the best growth at both sites. This consistency suggests that the particularly good clones that were grown only at Moray (3 and 43) or only at Kilmichael (100) may also grow well on the other site, and perhaps at a range of other sites. However, the performance of some

clones is clearly sensitive to site conditions; clones 59 and 87 demonstrate that performance ranking of some clones can differ markedly between sites.

The superior performances of the Swedish hybrid material (*P. tremula* x *P. tremuloides*), 'clone 1' (thought to be the same hybrid) and 'clone 70' which is possibly a hybrid, demonstrate their potential for highly productive growth on certain sites. Although these may not be appropriate for growth on native woodland sites, their potential should not be overlooked where restriction of planting to native species is not important. However, survival may be poor on challenging sites.

## Future management

### Management of the existing experiment sites

Consideration should be given to the future management of these experiment sites. Both experiments are on privately owned land, and one of the experiments has been badly damaged by recent house construction, the 20-year lease having come to an end. Much of the material held in these trials is not represented in the small NRS clone bank, or (as far as we are aware) in other clone banks.

The experiment sites have provided 20-year growth and survival data for the clones and allowed comparisons to be made between clones, between seed zones and between Scottish aspen and material from other sources. The sites are not appropriate for retention as seed orchards because the material was selected systematically (rather than based on good performance). In addition, although we have no data on flowering of the clones at the experiment sites, flowering of aspen is known to be rare in Scotland (Worrell et al. 1999). However, the experiments continue to have value and our suggestion is that they are retained as long as landowners are in agreement, and are maintained in a good condition, with some work carried out to secure them for future use.

Removal of the Swedish material at the Kilmichael site, and removal of commercial poplar clones/cultivars should be considered at both sites; vigorous root growth may become invasive, making any future collection of root material for propagation from the aspen clones difficult. In addition 'clone 1' should be removed (and clone 70 if it is also proven to be a hybrid). Control of suckering would also need to be carried out. Relabelling of the clones would be required for long-term security, and to ensure any queries relating to the zone 2 clones are addressed. The experiments would remain the responsibility of the Forest Research Long-term Experiments project.

One potential use for these experiments would be to consider experimental work on induction of flowering of aspen at Scottish sites. Some preliminary work has been carried out in the past, investigating methods of stressing trees to induce flowering, such



as droughting and garotting. Future research carried out at these sites could expand on the existing work, and also investigate viability of any seeds produced, to try to induce successful flowering of aspen in Scotland.

### Establishment of an aspen seed orchard

As the experiment sites are not suitable for use as seed orchards, we suggest that conservation of the better performing clones identified in this report is made a priority. This would involve propagation of material from the existing trees and transfer of the propagated material to a secure clone bank on a suitable site. As flowering is a pre-requisite for a seed orchard, we suggest that a suitable site should be selected in East Anglia, where conditions are conducive to flowering.

The number of clones selected for a seed orchard is a trade-off; a larger number of clones provides greater diversity, and spreads risk, but will inevitably mean including some clones that are less productive. Selection of the top performing 25% or 33% of the clones at each site would ensure a reasonable genetic diversity, while concentrating efforts on the good performers. The gender of each clone has been established during the genetic fingerprinting analysis and it would be important to include a balance of male and female clones.

Propagation of the selected clones would be by root cuttings taken from the trees at the experiment sites. Extreme care would need to be taken to ensure that invasive root material from adjacent plots was not sampled; it has been shown that roots of vigorous clones can have a wide lateral spread. As all of the clones have been DNA fingerprinted any subsequent uncertainty following propagation by root cutting could be checked.

Finally, the clones that were included in the clonal trials were selected with the purpose of determining whether material from different zones showed evidence of adaptive differentiation and therefore the aim was to base the trial on an even geographic representation of clones from across Scotland. Therefore no attempt was made to include phenotypically superior clones. It is therefore worth considering another sampling of Scottish aspen based on the following criteria; phenotypic superiority, willingness to flower and an even representation of both sexes. These could be used to extend the diversity of clones used to establish a new seed orchard. Subsequent monitoring of their growth would allow any poorly performing untested clones to be removed.

## Conclusions

- Scottish aspen clones did not perform as well as commercial poplar clones/cultivars or Swedish hybrids.
- There was a wide range in performance of Scottish aspen, but the best clones showed promise and could be grown productively.
- Performance was not related to seed zone, and locally-sourced material did not appear to have any advantage at either site. Further work examining the influence of source microclimate conditions may be beneficial.
- There were differences in the ranking of the clones at the two sites, but the best and worst clones were broadly consistent despite the very different climatic conditions.
- The consistent good performers that have been identified have high plasticity and are likely to grow well on a range of site types.
- Genetic testing has confirmed the unique identity of each clone; DNA fingerprints have been generated allowing identity of any future propagated material to be checked.
- The extreme top-performer noted in previous reports has been confirmed to be a hybrid rather than native aspen. Another top-performer, clone 70 may also be a hybrid.
- Further research on the induction of flowering in Scottish aspen could be carried out at the experiment sites.
- Some of the best performing clones in these trials are suitable for inclusion in a Scottish aspen seed orchard, which should be located in East Anglia where conditions are conducive to flowering.
- Consideration should be given to extending the number of clones included in a seed orchard by sampling phenotypically superior clones across Scotland and concentrating efforts on those that have a greater inclination to flower.

## Acknowledgements

The experiments were designed and managed by Bill Mason and Alan Harrison of Forest Research, and established and assessed by Forest Research Technical Services staff. Statistical analysis was carried out by Tom Connolly of Forest Research. The Moray and

Kilmichael experiment sites are both privately owned and we would like to thank the landowners for allowing use of the sites. Funding for the assessment, genetic testing and analysis was provided by Forestry Commission Scotland.

## References

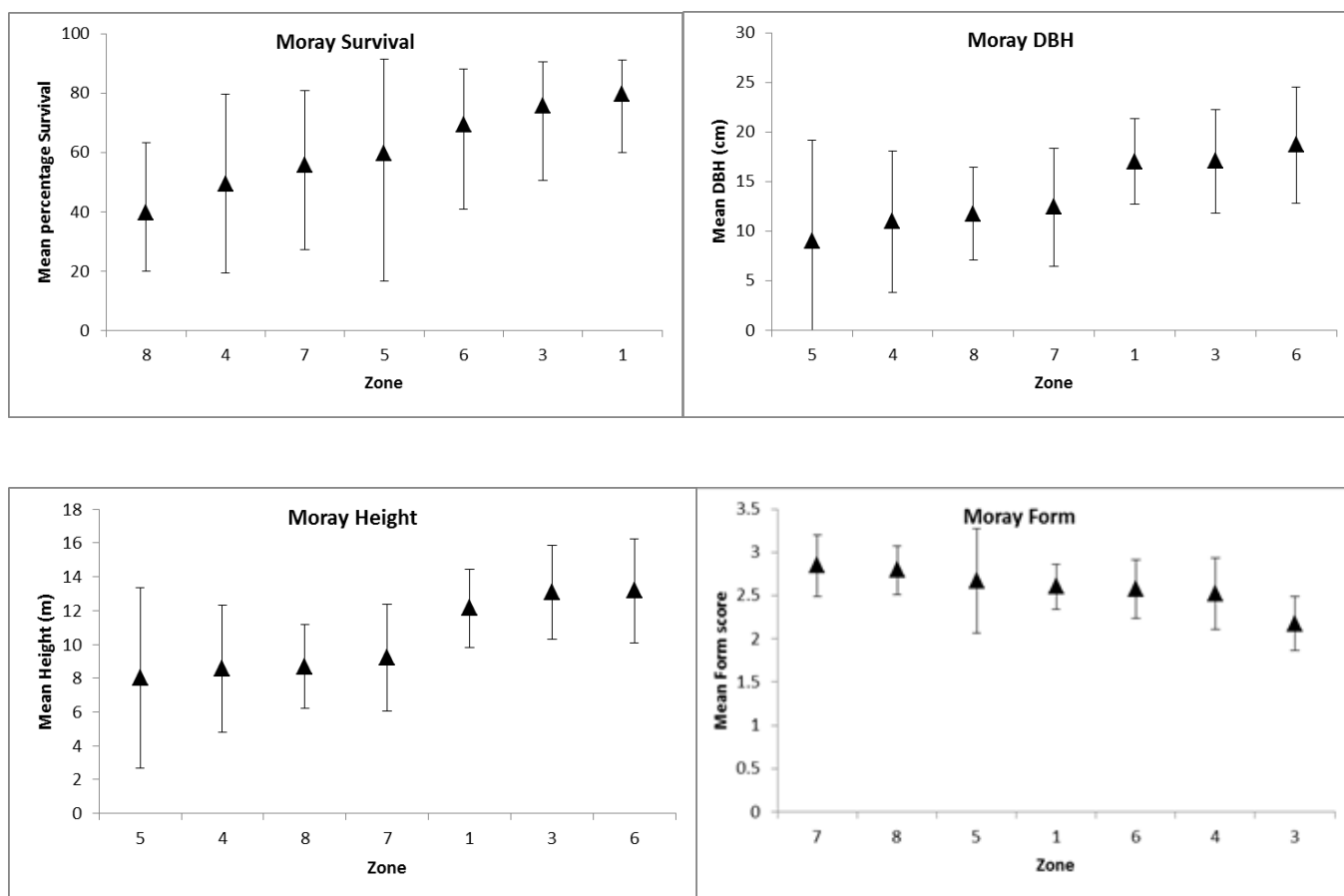
- Easton, E.P. (1997) Genetic variation and conservation of the native aspen (*Populus tremula* L.) resource in Scotland. Unpublished Ph.D. thesis, University of Edinburgh.
- Herbert, R., Samuel, C.J.A., and Patterson, G.S. (1999) Using local stock for planting native trees and shrubs. Forestry Commission Practice Note 8, Forestry Commission, Edinburgh.
- Hollingsworth, M.K. and Mason, W.L. (1991) Vegetative propagation of aspen. FC Research Information Note 200. FC Edinburgh.
- Jobling, J. (1990) Poplars for wood production and amenity. Forestry Commission Bulletin No. 92. HMSO, London.
- Karacic, A. (2005) Production and ecological aspects of short rotation poplars in Sweden. Doctoral thesis, Swedish University of Agricultural Sciences, Uppsala.
- MacKenzie, N.A. (2010) Ecology, conservation and management of Aspen: a literature review. Scottish Native Woods (Aberfeldy).
- Mason, B., Easton, E. and Ennos, R. (2002) Variation in aspen in Scotland: genetics and silviculture. In: The biodiversity and management of aspen woodlands (eds. P. Cosgrove, and A. Amphlett), pp 45-55. Grantown-on-Spey, UK: The Cairngorms Local Biodiversity Action Plan.
- Pakull, B., Kersten, B., Lüneburg, J. and Fladung, M. (2015), A simple PCR-based marker to determine sex in aspen. *Plant Biol J*, 17: 256–261.
- Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B., and Soutar D.M. (2009) GenStat for windows (13th Edition) introduction. VSN International, Hemel Hempstead.
- Pyatt, G., Ray, D. and Fletcher, J. (2001) An Ecological Site Classification for Forestry in Great Britain, Forestry Commission Bulletin 124, Forestry Commission, Edinburgh.
- SAS Institute (2011) The SAS system for Windows. Release 9.2. SAS Institute, Cary, NC.
- Worrell, R. (1995a) European Aspen (*Populus tremula* L.): a review with particular reference to Scotland I. Distribution, ecology and genetic variation. *Forestry* 68: 93-106.
- Worrell, R. (1995b) European Aspen (*Populus tremula* L.): a review with particular reference to Scotland II. Values, silviculture and utilisation. *Forestry* 68: 230-243.
- Worrell, R., Gordon, A.G., Lee, R.S. and McInroy, A. (1999) Flowering and seed production of aspen in Scotland during a heavy seed year. *Forestry* 71(1): 27-34.

## Appendix 1. Summary of Scottish aspen clone identities trialled at each site

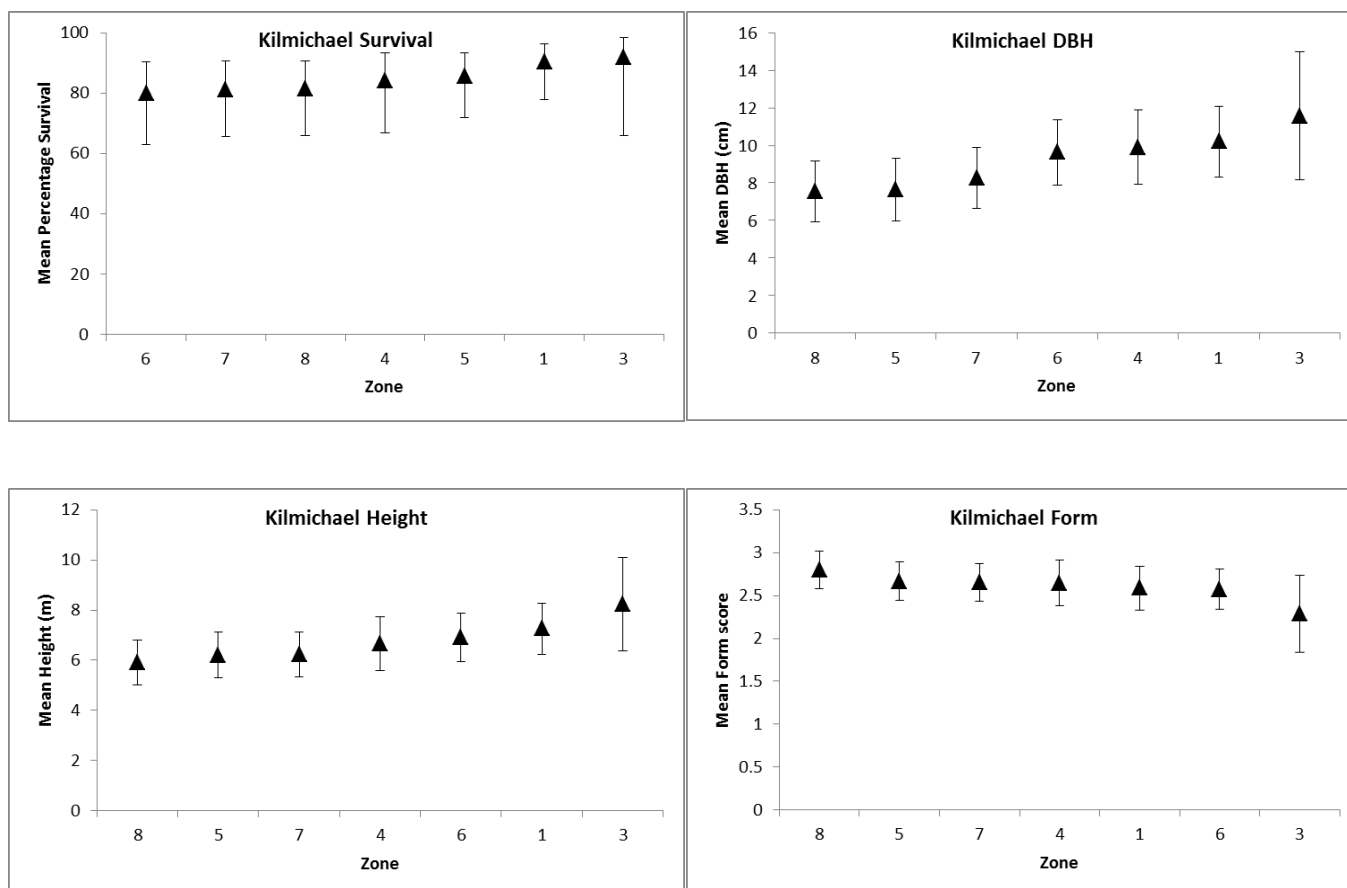
Zone	Moray	Kilmichael	Zone	Moray	Kilmichael
<b>1</b>	31		<b>6</b>		110
	34				111
	38				113
		64			115
		65			116
		67	<b>7</b>	117	117
		68			118
		69			119
	70	70			120
		71			121
	72	72		122	122
	74	74			123
	75	75			124
<b>3</b>	3				125
	43				126
	47	47			127
	48	48		128	128
		49			129
<b>4</b>		54			130
		55	<b>8</b>	131	131
		56			132
	57	57		133	
		58			134
	59	59			135
		60			136
		62			137
		63		138	138
<b>5</b>		86		139	139
	87	87		140	140
		88		141	141
		90			142
		91			143
	92	92			144
	93	93			145
		94		146	146
		95		147	147
		96			
		97	<b>Commercial poplars</b>	Robusta	Robusta
		98		Beaupre	Beaupre
		99		Fritzi Pauley	Fritzi Pauley
		100			
<b>6</b>		101	<b>Swedish hybrids</b>		894065
		102			844002
	103	103			844006
		104			844007
	105	105			844010
	107	107			85412
		108			85432
	109	109			

Appendix 2. Survival, diameter, height and form score results for Scottish aspen originating from each seed zone at (a) Moray and (b) Kilmichael.

**a) Moray: Zones are ranked from left to right in order of improving performance for each measure. Bars are 95% confidence intervals. p values are: 0.0932 for survival; 0.1983 for diameter; 0.0582 for height; 0.0845 for form score.**



**b) Kilmichael: Zones are ranked from left to right in order of improving performance for each measure. Bars are 95% confidence intervals. P values are: 0.7614 for survival; 0.0757 for diameter; 0.1746 for height; 0.4797 for form score.**





Appendix 3. Mean percentage survival, diameter (cm), height (m) and form score for each clone at (a) Moray and (b) Kilmichael.

a) Moray

Clone	Mean percentage survival	Mean diameter (cm)	Mean height (m)	Mean form score
3	68.0	22.8	13.65	2.69
43	100.0	20.7	14.74	1.80
47	50.5	12.5	11.59	2.56
48	68.0	12.4	12.14	1.90
57	55.2	13.8	10.89	2.40
59	41.7	8.0	6.03	2.67
70	84.7	25.7	16.32	2.84
74	41.7	15.8	13.12	2.40
75	71.4	16.5	11.18	2.50
87	59.3	9.0	8.00	2.67
103	74.7	18.6	12.17	2.54
105	74.7	24.3	16.98	2.19
109	58.7	13.1	10.35	3.00
117	48.1	14.1	11.40	2.67
122	41.7	13.2	9.27	3.01
128	76.5	10.0	6.84	2.90
131	59.3	16.6	10.50	2.62
133	29.1	8.9	7.38	3.03
139	22.7	10.5	7.53	2.89
141	45.6	12.3	9.53	2.62
146	44.7	10.5	8.50	2.80
31/72	76.7	13.1	10.65	2.66
34/69	100.0	21.8	14.04	2.44
38/68	92.6	9.4	7.73	2.76

## a) Kilmichael

Clone	Mean percentage survival	Mean diameter (cm)	Mean height (m)	Mean form score
47	87.7	11.3	8.43	2.71
48	93.9	11.4	8.57	1.48
49	93.9	12.0	7.72	2.67
54	62.7	6.1	5.45	3.02
55	81.5	12.4	7.00	2.94
56	93.9	11.4	7.64	2.73
57	87.7	9.8	6.42	2.27
58	93.9	12.6	7.92	2.52
59	81.5	9.6	6.15	2.60
60	81.5	9.9	7.49	2.13
62	93.9	6.5	4.88	2.85
63	69.0	10.8	6.91	2.75
64	69.0	6.5	5.10	2.92
65	93.9	11.2	8.84	2.50
67	100.0	9.7	6.81	2.50
70	87.7	15.6	10.40	2.50
71	69.0	4.9	5.34	2.94
74	100.0	10.5	7.47	2.69
75	100.0	14.4	8.03	1.81
86	93.9	7.6	5.12	2.94
87	100.0	11.5	7.26	2.25
88	37.3	3.6	3.92	3.02
90	43.6	2.2	3.45	3.00
91	87.7	5.1	4.72	3.00
92	100.0	10.4	6.88	2.56
93	100.0	9.1	6.22	2.50
94	93.9	9.2	7.21	2.46
96	87.7	10.6	7.40	2.77
97	87.7	5.7	5.27	2.94
98	81.5	9.9	7.99	1.56
99	56.3	5.7	4.64	2.94
100	87.7	8.4	10.39	2.75
102	50.0	4.2	4.39	3.00
103	100.0	11.3	7.99	2.63
104	37.3	5.2	5.34	2.75
105	93.9	18.8	12.06	1.75
107	87.7	12.8	8.84	2.42
108	75.3	10.5	6.38	2.75
109	81.5	10.9	6.72	2.42
110	87.7	11.0	7.97	1.75

111	93.9	8.8	6.14	2.75
113	87.7	8.3	6.03	2.88
115	75.3	4.7	3.91	3.00
116	50.0	9.0	7.21	2.80
117	93.9	14.3	9.07	2.25
118	87.7	6.0	5.04	3.00
119	100.0	10.6	8.01	2.31
120	75.3	7.5	5.18	3.00
121	100.0	5.2	4.66	3.00
122	87.7	6.2	5.16	2.92
123	93.9	10.5	7.80	2.00
124	6.1	5.3	3.88	3.02
125	81.5	8.9	6.61	2.52
126	87.7	10.7	7.64	1.98
127	62.7	10.9	7.22	2.56
128	87.7	5.1	4.89	3.00
129	24.7	7.2	5.73	2.73
130	87.7	6.4	5.69	2.94
131	93.9	10.3	6.47	2.60
132	75.3	8.8	6.38	3.00
134	100.0	8.4	6.49	3.00
135	100.0	7.5	5.94	2.88
136	62.7	4.0	4.09	3.00
137	100.0	8.7	6.79	2.50
138	75.3	12.4	7.43	1.90
139	81.5	8.1	6.33	3.00
141	50.0	5.5	4.86	2.91
142	81.5	5.6	4.49	3.00
143	37.3	4.9	5.01	3.00
144	93.9	6.5	5.76	3.00
145	100.0	8.0	6.58	2.81
146	12.3	6.7	6.31	2.58
31/72	93.9	9.8	6.65	2.75
34/69	87.7	12.3	8.24	2.25
38/68	81.5	7.2	5.66	3.00

Alice Holt Lodge  
Farnham  
Surrey GU10 4LH, UK

Tel: 0300 067 5600

Fax: 01420 23653

Email: [research.info@forestry.gsi.gov.uk](mailto:research.info@forestry.gsi.gov.uk)

**[www.forestry.gov.uk/forestresearch](http://www.forestry.gov.uk/forestresearch)**

Northern Research Station  
Roslin  
Midlothian EH25 9SY, UK

Tel: 0300 067 5900

Fax: 0 131 445 5124

Forest Research in Wales  
Edward Llwyd Building  
Penglais Campus  
Aberystwyth  
Ceredigion  
SY23 3DA

Tel: 01970 621559

If you need this publication in an alternative format,  
for example in large print or another language, please  
telephone us on 0300 067 5046 or send an email request  
to: [diversity@forestry.gsi.gov.uk](mailto:diversity@forestry.gsi.gov.uk)