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DO SEED ZONES CONSERVE ADAPTIVE VARIATION?

TESTING THE ADAPTIVE SIGNIFICANCE OF SEED ZONES IN SCOTS PINE

Final report for
Scottish Forestry Trust
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General View of the seedling provenance/progeny trial of native Scots pine used for estimating variation in phenology, response to cold winter temperatures and drought.

Outdoor seedlings fitted with clips for dark acclimation prior to measurement of chlorophyll fluorescence.

INTRODUCTION

The basic principles underlying the choice of seed sources for establishment of forest tree populations for production or conservation are that the material should be genetically adapted to the current environmental conditions at the establishment site, and should contain sufficient genetic variation to allow adaptation to future environmental change. When populations are being established in sites not currently occupied by the tree species concerned, guidelines are required to ensure that suitable seed source populations are chosen to meet these objectives. The conventional solution to this problem is to delimit geographic areas on a map, seed zones, within which seed transfer is permitted from source to establishment site, and between which seed transfer is prohibited.

A number of assumptions underlie the use of seed zones. The first is that geographically proximate sites within a seed zone share environmental attributes, and these environmental attributes differ from those in geographically distant sites located in other seed zones. Put another way, the designated seed zones map directly onto patterns of environmental variation across the species range. The second assumption is that the tree species concerned is differently adapted to the contrasting environmental conditions in the different seed zones that have been designated. The corollary is that transfer of genetic material between seed zones will result in maladaptation of the established tree populations, and a reduction in probability of establishment and effective growth of the transferred material.

The objective of the research programme reported here has been to test these two assumptions for the seed zones which have been drawn up to guide the establishment of New Native Pinewoods in Scotland. In this programme the objective has been to increase the area of native Scots pine (*Pinus sylvestris* L.) woodland through planting using seed sourced from the existing 84 pinewood remnants within Scotland. At the start of this programme seven seed zones were designated within Scotland (Fig. 1). Grant support for planting was only paid if seed had been sourced from a population located in the same seed zone as that of the planting site.
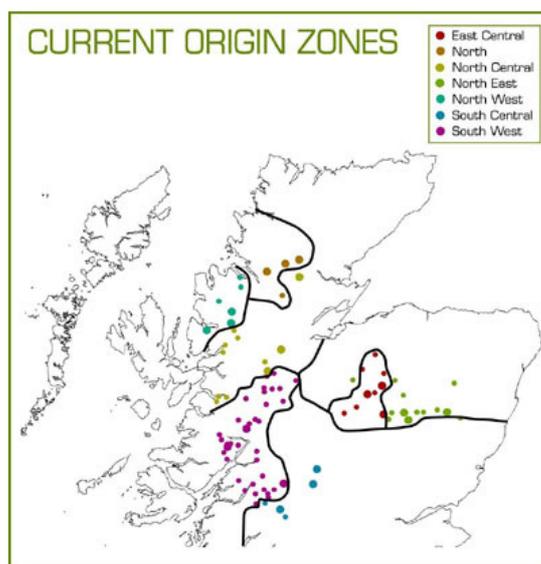

Figure 1 Boundaries of the seven currently designated seed zones for New Native Pinewood establishment, and location of native pinewood stands located within them

The original designation of the seven Scots pine seed zones was based not on knowledge of patterns of environmental variation across Scotland, and associated patterns of adaptive variation in Scots pine, but on the similarity of Scots pine populations for genetically variable resin monoterpene composition. Such similarity is likely to reflect common postglacial ancestry and patterns of gene flow, rather than patterns of adaptation to environmental variation. Thus it is very pertinent to ask whether the seed zones designated under the Native Pinewood grant scheme are likely to have achieved the objective of ensuring establishment of well adapted material in the planting programme.

To address the effectiveness of the Native Pinewood seed zone guidelines we have adopted two approaches. In the first we have gathered data on the patterns of climatic variation across Scotland and asked whether the current seed zones map well onto this pattern of climatic variation. In the second approach we have used common garden experiments to establish the extent of genetic differentiation among native pine populations for characters related to adaptation to environmental variation experience across Scotland, specifically length of the growing season, low winter temperatures and moisture deficit during the growing season.

A. Do Seed Zones Reflect Patterns of Environmental Variation across Native Pinewood sites?

In order to address this question UK Met Office long-term average data have been used to characterise the climate experienced by each of the 84 native pinewood remnants in Scotland. Data for seven variables reflecting differences in temperature, growing season and rainfall have been extracted (Table 1). The raw data indicate that although the native pinewoods occupy a small geographic area, they span a wide range of climatic conditions. Thus the length of the growing season in some eastern, high altitude populations can be less than 150 days, whereas it can approach 300 days at sites on the west coast. Likewise annual rainfall can vary from less than 800mm in the east to almost 3000 mm in the west.

Variable	PC1	PC2
Length of the growing season	-0.45	-0.12
February mean temperature	-0.45	-0.12
July mean temperature	-0.35	-0.47
Annual extreme temperature range	0.10	-0.72
Air frost days per year	0.44	-0.03
Ground frost days per year	0.43	-0.13
Annual precipitation	-0.30	0.46
Percentage of variation	69.20	23.99

Table 1 List of climatic variables used in the principal component (PC) analysis. Values in the table are correlation coefficients that vary between -1 (strong negative correlation) and 1 (strong positive correlation); the further the coefficient is from zero, the stronger the association between the variable and the PC.

The raw data from all 84 native populations has been used in PCA ordination analysis to visualise the pattern of climatic variation. The first two PCA components account for 69% and 24% of the climatic variation and largely reflect differences in temperature and oceanicity of native pinewood sites respectively (Table 1). By plotting PCA1 against PCA2 (Fig. 2) it is possible to arrange the pinewoods in climatic space. By indicating the seed zone to which each population belongs, we can determine the extent to which populations from the same seed zone cluster in climate space, and assess whether seed zones capture the pattern of climatic variation across native pinewood sites.
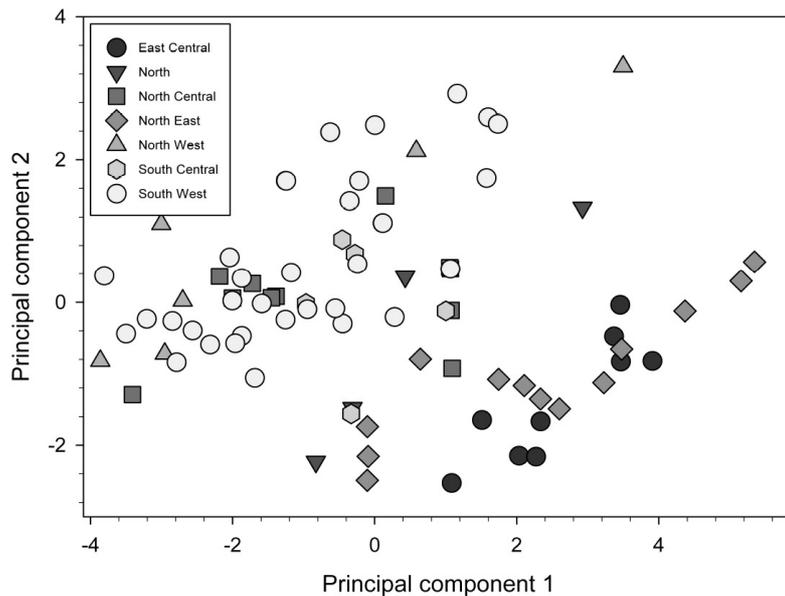

Figure 2 Plot of the first two principal components, which account for 69 and 24% of total variation, respectively, of climatic variation among 84 native pinewood sites. The seven variables used are shown in Table 1. Current seed zones are represented by different symbols The closer the populations are located on the graph, the more similar they are climatically.

Conclusions
Results of the PCA analysis show that although the positions of pinewoods within a seed zone tend to cluster on the plot, there are huge overlaps between seed zones, and two populations within a seed zone may be further apart than two populations located in different seed zones. Thus the designated seed zones do not map well onto climatically distinct regions within Scotland.

B. Are Native Pinewood Populations Genetically Differentiated for Adaptively Important Phenotypic Characters?

i). Genetic Differentiation for Phenology
The previous analysis of climatic variation highlighted huge variation in the length of the growing season among pinewood sites. An adaptively important character is therefore likely to be phenology of growth, with natural selection acting to ensure that bud burst and bud set occur so as to maximise use of the growing season, but minimise growth outside the growing season where trees will be vulnerable to frost damage.

In order to determine whether there is significant genetic differentiation among populations for phenology, we sampled open-pollinated seed collected by family from 4 maternal parents from each of 21 native pine populations across Scotland (Appendix I). Forty offspring were raised per family and seedlings were arranged in a randomised block design in an unheated glasshouse. In spring 2008 the time of emergence of new needles from apical buds was measured using twice weekly recordings between late March and early May. Analysis of variance indicated significant variation for date of bud burst both among populations and among families within population (Table 2) with a 7 day difference between the earliest and latest populations.

Source of Variation	d.f.	Mean Square	Significance
Blocks	39	171	P<0.001
Population	20	1103	P<0.05
Family(Population)	63	608	P<0.01
Within Family	3120	50	

Table 2. Analysis of variance for date of bud flushing in a seedling provenance/progeny trial of *P. sylvestris*. The trial contained 21 populations from the native range within Scotland and four open pollinated families per population. Plants were grown in a cool glasshouse near Edinburgh.

For each of the 21 sites from which populations had been sampled, average annual growing degree days (GDD) were estimated from Met Office data. There was a highly significant regression of mean date of bud burst on GDD (P = 0.0026) explaining 36% of the variation in population means (Fig.3). Timing of bud flush was earlier in populations originating from cooler climates.
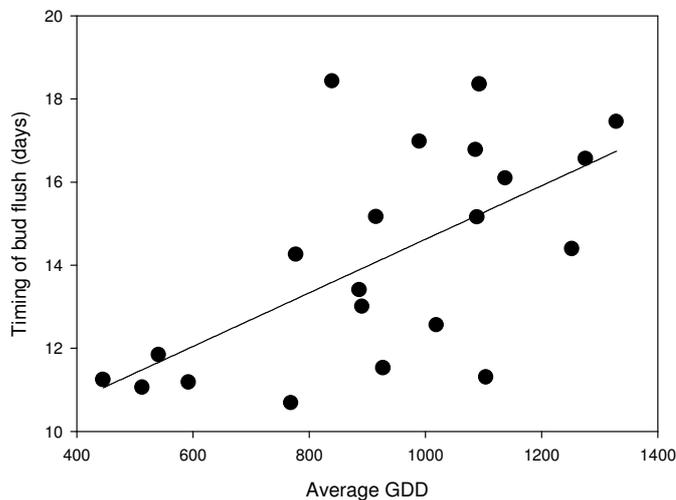
Figure 3 Regression of time of bud flush in 21 native pinewoods on the growing degree days (GDD) at their site of origin. (P=0.0026, R^2=36%).

Conclusions

The results clearly demonstrate that under the same environmental cues, native pine populations differ genetically in their timing of growth. Though there is a relationship between conditions at site of origin and time of bud flush, this is not easily interpretable. All populations are growing under novel climatic conditions and are not expected to show the phenological patterns expressed at their home site because they

are receiving different environmental cues. One possible explanation for the early bud burst of populations from cool origins is that they require a smaller heat sum to flush than populations from warmer areas. Further work on timing of bud flush at the sites of origin would be helpful in uncovering the causes of the genetically determined phenological differences that have been observed.

ii). Genetic Differentiation for Response to Environmental Stress

One way of measuring short term tolerance of plants to fluctuation in environmental factors (possible stress conditions) is to estimate their photosynthetic efficiency, the degree to which they are able to utilise light energy for photosynthesis. If plants are tolerant of the environmental fluctuation or stress, we expect them to be able to utilise a higher proportion of light energy in photosynthesis than if they are intolerant and their photosystems are impaired. This can be measured quantitatively using chlorophyll fluorescence. Leaves or needles are dark adapted, light is then shone onto them, and the proportion of light energy utilised in photosynthesis F_v/F_m is calculated. For plants that are not stressed this ratio is commonly 0.8, but falls below this as efficiency of photosynthesis declines.

Estimation of photosynthetic efficiency from chlorophyll fluorescence measurements offers tremendous scope for revealing genetic differentiation among plant populations in their response to environmental stress. This is because the method can be applied to many individuals within a short time period, allowing comparisons among populations or families to be achieved under uniform environmental conditions, so revealing genetically determined rather than environmentally determined differences. In this project we have employed the power of the chlorophyll fluorescence technique to compare the response of populations and families of native Scots pine to two potential environmental stress factors, low winter temperature and drought.

a). Response to Low Winter Temperature

A climatic characteristic that varies markedly across native pinewood sites is the magnitude of annual temperature fluctuations, associated with the oceanicity of the site. The amplitude of fluctuations is much lower in west coast sites at sea level than in the east and at higher elevations. One way in which the populations may be adaptively differentiated is therefore with respect to their ability to tolerate fluctuations giving rise to low winter temperatures. Populations from higher elevations in a more continental climate may be more tolerant of low winter temperatures than those from more oceanic climates at lower elevations.

In order to test this prediction a subset of 8 native pine populations from the 21 originally collected in 2007 were chosen to cover the range of environments across the climatic range in Scotland (Fig. 4, Appendix I). For each population four maternal, open-pollinated families, each comprising six seedlings, were raised in a randomised block design in the greenhouse. Plants were potted into 11x11x12cm pots in spring 2008 and transferred to an outdoor bench in August 2009 where they were grown in six blocks of 32 seedlings. Temperature data were continuously recorded from the site.
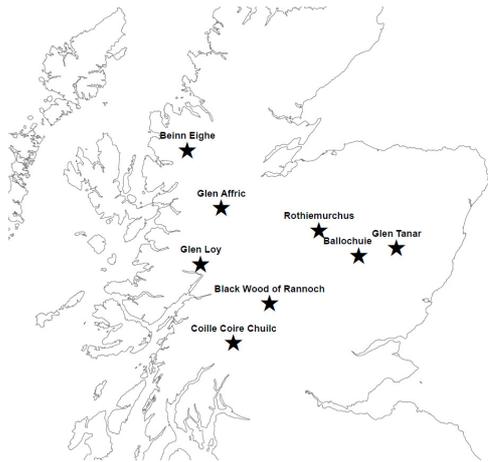
Figure 4 Location of populations included in experiment to assess response to low winter temperatures

Chlorophyll fluorescence was measured on all seedlings in the trial on 14 occasions between September 2009 and May 2010. Measurements were taken between 8:00am and noon on a single day. Two current or previous year needles from opposite sides of the main stem and separate fascicles were used and their mean was taken as the fluorescence value for the plant. Needles were dark adapted for approximately 20 minutes. Fluorescence measurements were made outside using a Handy PEA portable measurement system. The maximum quantum yield of primary photochemistry F_v/F_m was estimated for each plant.

Figure 5 documents the overall decline in average daily temperature to a low of less than -5°C in January, a subsequent rise in February, and then a dip below freezing in March before a steady rise in subsequent months. Figure 6 shows variation for F_v/F_m over the same time period. On average F_v/F_m declines from November until March, then rises in the late spring. However inspection of Figure 6 also suggests that at particular time periods in the winter there is variation in F_v/F_m among populations.

ANOVA for F_v/F_m shows that differences among populations are statistically significant in January and March, coinciding with periods during which average temperature dropped below freezing (Table 3). At these time points the west coast population from Beinn Eighe consistently shows the lowest F_v/F_m ratio. A preliminary analysis of association between photosynthetic efficiency after freezing episodes and environmental factors shows that altitude of population origin accounts for 74% and 44% of variation in F_v/F_m in January and March respectively (Fig. 7).
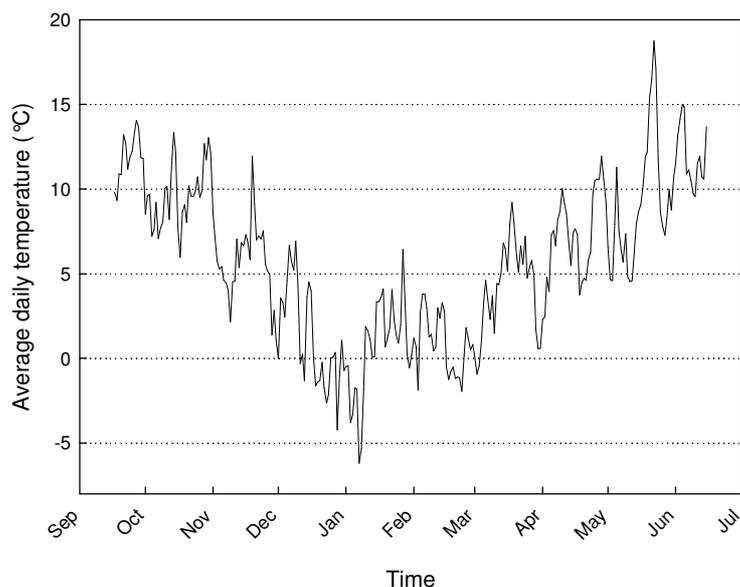
Figure 5 Variation in average daily temperature experienced by eight populations of Scots pine at the Bush estate between September 2009 and May 2010.
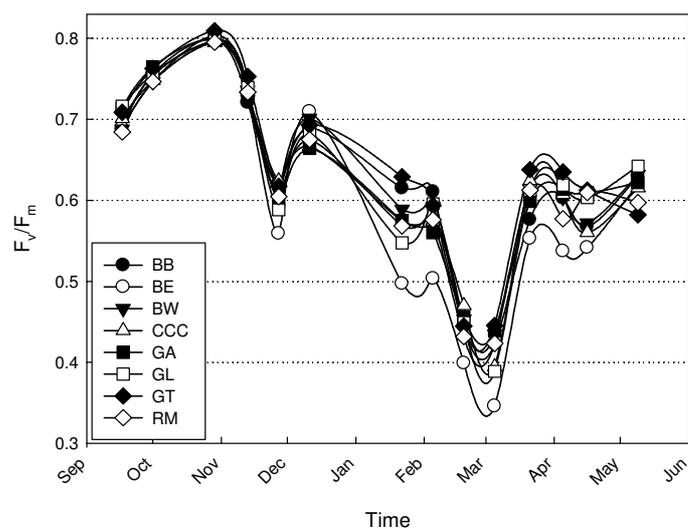
Figure 6 Variation in mean value of F_v/F_m for eight populations of Scots pine grown outside in a common garden experiment between September 17 2009 and May 9 2010. Population abbreviations are given in Appendix 1.

Table 3 ANOVA for variation in photosynthetic efficiency F_v/F_m among eight populations of Scots pine grown outside in a progeny/provenance experiment and measured on January 22 and March 21 2010. ns=not significant, $*=p<0.05$, $**=p<0.01$.

		Mean Square for:	
Factor	d.f	F_v/F_m Jan 22nd	F_v/F_m March 21st
Population	7	0.0398**	0.0180*
Families within Population	24	0.0095n.s.	0.0073n.s.
Blocks	5	0.0080n.s.	0.0298**
Residual	155	0.0162	0.0077
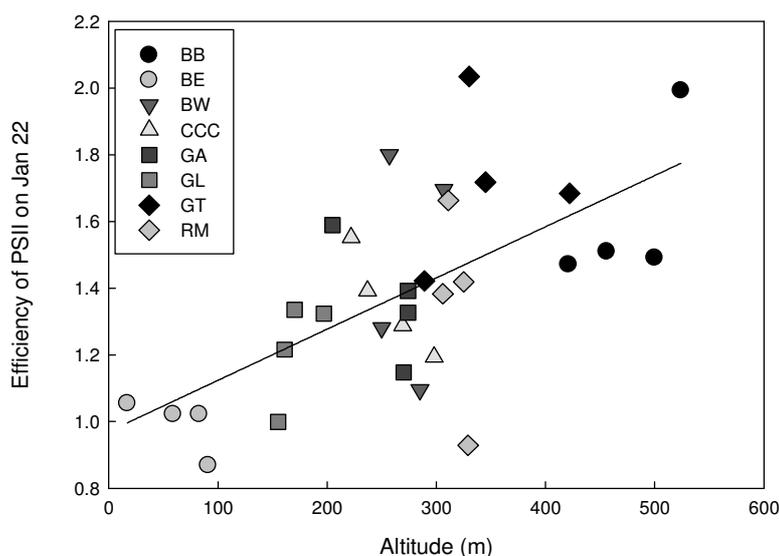
Figure 7 Relationship between altitude at origin and efficiency of photosynthesis ($F_v/F_m/(1-F_v/F_m)$) measured on January 22nd 2010 for eight populations of Scots pine grown outside at the Bush estate in a progeny/provenance experiment. Population abbreviations are given in Appendix 1.

Conclusions

Although further analysis needs to be done, these results suggest that there are genetic differences among native Scots pine populations in response to reductions in average daily winter temperature below freezing. Populations from lower elevations (or perhaps more oceanic climates) are more adversely affected in terms of their photosynthetic efficiency than those from higher elevations. One plausible explanation is that growth rhythms differ between the populations such that those from lower elevation (oceanic) sites do not shut down growth to such a degree in the winter as those from higher elevation (more continental) sites. Populations from low elevation sites are therefore more vulnerable to photosynthetic damage by freezing temperatures.

b). Response to Drought

One of the most striking forms of environmental variation that is found across the range of Scots pine populations in Scotland is in levels of precipitation. Annual rainfall levels vary from 800mm in the east to nearly 3000mm in the west. Equally importantly from the point of view of trees, there is also significant variation in estimated moisture deficit among sites during the growing season. This is calculated as the difference between monthly evaporation and monthly rainfall in the growing season, and is not well correlated with rainfall levels (Appendix I). Variation of this nature suggests that seedlings of Scots pine from different Scottish origins may have been selected for different degrees of drought tolerance. An experiment was therefore set up in which populations of Scots pine from the provenance/progeny trial were subjected to drought treatment, and their response was measured in terms of water deficit in the needles, photosynthetic efficiency and needle browning.

Five populations covering the full range of annual rainfall variation were selected from the 21 populations originally collected (Fig. 8, Appendix I)
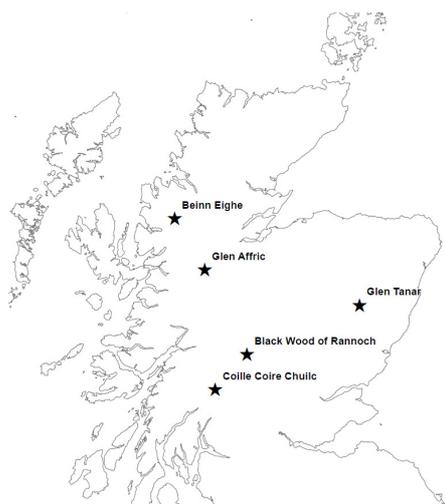
Figure 8 Locations of the five Scots pine populations used in the drought tolerance experiment

Seedlings were potted up in spring 2008 to pots of size 11 × 11 × 12 cm (length × width × depth). The trial was divided into 20 blocks of 20 seedlings each (i.e. one family member per block), and the order of families within blocks was randomized. For the drought study, ten treatment and ten control blocks were chosen. The drought treatment was applied between June 4 and July 9 2009. Droughted blocks were not given any water during this period). Control samples were always watered 2-3 times per week and one day before the measurements. Following the drought treatment all seedlings were watered normally.

To estimate the effects of droughting on the seedlings' water status, water deficit (WD) was measured in 100 control and 100 droughted seedlings (five populations, four families/population, five seedlings/family/treatment) on two occasions (June 8 and 29). Three randomly chosen current-year needles were sampled per plant, and their fresh weight (FW) was measured. After this the needles were kept in deionized water overnight, and their weight at a full turgor was measured (TW). Finally the needles were placed in an oven at ~75 °C for 3 days, after which their dry weight was measured (DW). Water deficit was calculated as WD = (TW-FW)/(TW-DW).

Chlorophyll fluorescence was measured in 10 seedlings per family per treatment on three occasions (June 9-10, June 23-24 and June 30-July 1 2009). The measurements were taken in the glasshouse over two days between 8.00 am and noon, and population and family means were calculated over this time period. Control and treatment blocks were scored alternately so that measurements for each population and family would be spread over the two days (five control and five treatment blocks per day). Technical details of chlorophyll fluorescence measurements were as given above.

The droughted blocks were rewatered normally following the end of the drought stress. Nine weeks after the start of rewatering the proportion of fully brown (dead) seedlings was scored in each population and family.

i). Water deficit
Before the droughting treatment, mean water deficits of populations varied between 0.28 and 0.30. At the end of the treatment (June 29th) mean water deficit per

population was much greater and varied over a wider range (0.47 to 0.56). ANOVA demonstrated a significant drought treatment x population interaction (P<0.01), indicating that the populations responded differently in terms of water loss to the imposition of drought treatment. Neither rainfall levels nor moisture deficit at site of origin accounted for a significant amount of water deficit variation.

ii). Photosynthetic efficiency
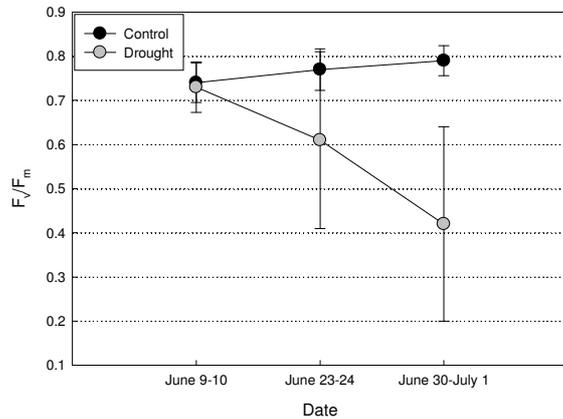
Figure 9. Effect of droughting treatment on photosynthetic efficiency of Scots pine seedlings as measured by the chlorophyll fluorescence ratio Fv/Fm.

Figure 9 shows that the overall effect of the droughting treatment was to significantly reduce the efficiency of photosynthesis as measured by the ratio F_v/F_m. ANOVA indicated that there were significant differences among families but not among populations in their photosynthetic efficiency (F_v/F_m) in response to droughting both on June 23rd and June 30th (P<0.05 in both cases).
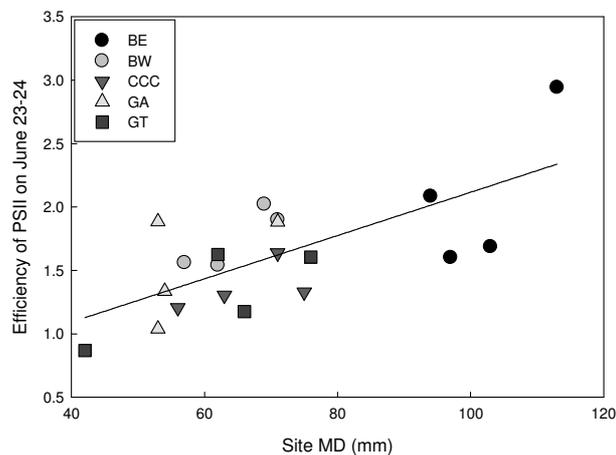
Figure 10. Regression of photosynthetic efficiency Fv/Fm under droughting against moisture deficit during the growing season at the site of origin for families sampled from five populations of Scots pine in Scotland.

Photosynthetic efficiency of families under droughting showed a significant regression on moisture deficit in the growing season at the site of origin (P< 0.001, R^2 = 0.46, Figure 10), but no significant regression on annual rainfall at site of origin. A significant correlation was found between water deficit measured on June 29 and F_v/F_m in individual seedlings on June 23-24 (r=-0.56, p<0.0001) suggesting that these two assessment methods are measuring the same response to drought.

iii). Proportion of Dead Seedlings

The proportion of fully brown seedlings in populations within the drought treatment varied between 0.47 in population GA and 0.65 in population CCC. There were no statistically significant differences in the frequency of surviving seedlings among the five populations (χ^2= 2.73, df=4, p=0.60). However, the proportion of brown seedlings was higher in families with higher WD on June 29 (r=0.54, p=0.014) and with lower F_v/F_m on June 23-24 (r=-0.56, p=0.011). A similar correlation was observed with F_v/F_m on June 30-July 1 (r=-0.53, p=0.017).

Conclusions
Although relatively small in size, this experiment has demonstrated significant genetic differences among native Scots pine populations in response to drought, measured in three different ways. Drought response of families is not related to annual rainfall at their site of origin, but to the moisture deficit during the growing season at their site of origin. Families from sites with greater moisture deficits are less badly affected than those from sites with lower moisture deficits. The results suggest a degree of adaptation to differences in moisture regimes in native pine populations across Scotland.

Publications arising from the work to date:

Matti J. Salmela, Stephen Cavers, Witold Wachowiak, Joan E. Cottrell, Glenn Iason & Richard A. Ennos (2010). Understanding the evolution of native pinewoods in Scotland will benefit their future management and conservation. Forestry, *in revision*

Matti J. Salmela, Stephen Cavers, Joan E. Cottrell & Richard A. Ennos (2010) Fast phenotyping using chlorophyll fluorescence detects drought response in a common-garden trial of five native Scots pine (*Pinus sylvestris*) populations in Scotland. *submitted to Plant, Cell and Environment*

Matti J. Salmela, Stephen Cavers, Joan E. Cottrell & Richard A. Ennos (2010) Seasonal patterns of photosynthesis under extreme winter conditions of 2009/2010 reveal adaptive differentiation among eight native Scots pine populations (*Pinus sylvestris*) in Scotland. *in preparation*

OVERALL CONCLUSIONS AND PRACTICAL RECOMMENDATIONS ARISING FROM THE RESEARCH

<u>Conclusions</u>

1. Existing seed zones for native Scots pine do not reflect climatic patterns of variation across Scotland. There is a need to revise and improve the existing guidelines for sourcing of seed for New Native Pinewoods.

2. There is strong evidence for genetic variation among native Scots pine populations in Scotland in terms of phenology and response to low winter temperature and drought. Geographic patterns of genetic differentiation are not random, as would be expected under genetic drift, but are related to patterns of underlying environmental variability. There appears to be local adaptation of Scots pine to the diversity of environmental conditions within Scotland.

<u>Recommendations</u>

1. Revised guidelines for selecting seed sources for planting New Native Pinewoods within Scotland are required. Given the presence of extensive differences in environmental conditions across Scotland, and the evidence of local adaptation of Scots pine to these environments, seed source guidelines should in future be based on the principle of matching the environments of seed source and seed planting sites.

2. Designating seed zones by drawing lines on maps does not achieve the objective of ensuring that the environments of seed source and seed planting sites are well matched. Geographic proximity does not necessarily equate to environmental similarity. A new method for choosing seed sources of native Scots pine is required.

3. Data on geographic variation for many climatic variables across Scotland is already available from the Met Office, based on direct measurement or extrapolation. Given this database it should be possible to develop an expert system in which the geographic locations of the planting site and possible seed source populations are input. Information on environmental conditions at these sites are retrieved from a database, and compared between planting site and all possible seed source sites. Using rules based on the similarity of environments it should be possible to select the seed source most closely matched in terms of environmental conditions to the proposed planting site.

4. Work to date has indicated that matching planting sites with respect to length of growing season, exposure to low winter temperatures and moisture deficit during the growing season is important. Further work is required to investigate additional aspects of environmental variation to which Scots pine populations in Scotland may be differentially adapted e.g. soil type, degree of soil waterlogging etc. in order to refine the expert system for seed source selection.

Appendix I. Location of maternal families of *P. sylvestris* sampled for the provenance/progeny trial, together with information on ecological variables at the site.

Population	Core pinewood area (ha)	Pinewood zone	Family	Altitude (m)	Latitude	Longitude (W)	Accumulated temperature (degree days)	Moisture deficit (mm)
Abernethy	2452	East Central	AB1832	365	57.2	3.6	873	47
			AB1834	363	57.2	3.6	876	48
			AB1837	311	57.2	3.6	939	61
			AB1839	327	57.2	3.6	919	57
Allt Cull	13	North East	AC1802	512	57.0	3.4	699	13
			AC1803	493	57.0	3.4	723	18
			AC1806	464	57.0	3.4	759	26
			AC1808	435	57.0	3.4	795	33
Amat	181	North Central	AM41	39	57.9	4.6	1202	116
			AM44	201	57.9	4.6	1019	74
			AM45	153	57.9	4.6	1073	86
			AM50	156	57.9	4.6	1070	85
Ballochuie	775	North East	BB74	500	57.0	3.3	717	17
			BB75	524	57.0	3.3	687	11
			BB80	456	57.0	3.3	772	29
			BB97	421	57.0	3.3	816	38
Beinn Eighe	182	North West	BE21	59	57.6	5.4	1210	103
			BE23	91	57.6	5.4	1173	94
			BE26	83	57.6	5.3	1183	97
			BE30	17	57.6	5.4	1259	113
Black Wood of Rannoch	1011	South Central	BW1822	307	56.7	4.3	990	57
			BW1825	257	56.7	4.3	1054	69
			BW1828	250	56.7	4.3	1063	71
			BW1830	285	56.7	4.3	1082	62
Coille Coire Chuilc	67	South Central	CCC1801	298	56.4	4.7	1024	56
			CCC1806	222	56.4	4.7	1125	75
			CCC1807	237	56.4	4.7	1105	71
			CCC1809	269	56.4	4.7	1062	63
Cona Glen	189	South West	CG1862	89	56.8	5.3	1265	100
			CG1864	154	56.8	5.3	1182	83
			CG1867	180	56.8	5.3	1149	76
			CG1868	169	56.8	5.3	1163	79
Crannach	70	South West	CR1881	258	56.6	4.7	1063	65
			CR1885	307	56.6	4.7	999	53
			CR1888	338	56.6	4.7	959	45
			CR1889	280	56.6	4.7	1034	60
Glen Affric	1532	North Central	GA1892	205	57.3	5.0	1073	71
			GA1893	274	57.3	5.0	990	53
			GA1897	274	57.3	5.0	989	53
			GA1900	270	57.3	5.0	994	54
Glen Cannich	301	North Central	GC85	348	57.3	4.9	892	36
			GC88	361	57.3	4.9	876	32
			GC89	182	57.4	4.9	1091	78
			GC90	189	57.4	4.9	1082	77

Appendix I ctd..

Glen Derry	235	East Central	GD94	493	57.0	3.6	726	16
			GD95	492	57.0	3.6	727	16
			GD98	437	57.0	3.6	795	30
			GD99	426	57.0	3.6	809	33
Glen Einig	27	North	GE33	59	58.0	4.7	1171	108
			GE36	69	58.0	4.7	1160	106
			GE37	45	58.0	4.7	1187	112
			GE38	46	58.0	4.8	1186	111
Glen Loy	74	South West	GL1868	155	56.9	5.1	1168	85
			GL1872	161	56.9	5.1	1160	83
			GL1876	197	56.9	5.1	1114	74
			GL1877	170	56.9	5.1	1149	81
Glen Tanar	1564	North East	GT1851	289	57.0	2.9	975	76
			GT1856	422	57.0	2.9	810	42
			GT1858	345	57.0	2.9	906	62
			GT1860	330	57.0	2.9	924	66
Loch Clair	126	North West	LC3	166	57.6	5.4	1070	62
			LC5	153	57.6	5.4	1108	79
			LC7	107	57.6	5.4	1162	91
			LC8	102	57.6	5.4	1168	92
Meggernie	277	South Central	MG1813	286	56.6	4.4	1025	62
			MG1815	264	56.6	4.4	1053	68
			MG1816	287	56.6	4.4	1023	62
			MG1820	385	56.6	4.3	895	37
Rhidorroch	103	North	RD61	175	57.9	5.0	1049	76
			RD63	220	57.9	5.0	998	64
			RD64	193	57.9	5.0	1028	71
			RD70	138	57.9	5.0	1090	85
Rothiemurchus	1744	East Central	RM1841	306	57.1	3.8	951	61
			RM1845	325	57.1	3.8	928	56
			RM1846	329	57.1	3.8	923	55
			RM1848	311	57.1	3.8	944	60
Shieldaig	103	North West	SD11	91	57.5	5.6	1182	92
			SD13	44	57.5	5.6	1243	104
			SD14	56	57.5	5.6	1229	101
			SD16	132	57.5	5.6	1197	68
Strath Oykel	14	North	SO51	38	58.0	4.6	1192	115
			SO55	152	58.0	4.6	1064	86
			SO56	158	58.0	4.6	1058	84
			SO60	63	58.0	4.6	1164	109