Report

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DO SEED ZONES CONSERVE ADAPTIVE VARIATION?

TESTING THE ADAPTIVE SIGNIFICANCE OF SEED ZONES IN SCOTS PINE

Twenty four month progress report for Scottish Forestry Trust
September 2009

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Objectives of the project:

The project will use a combination of molecular markers and growth experiments to assess the effectiveness of designated seed zones in Scots pine for the conservation of adaptive variation, by answering the following questions:

1. Do designated seed zones accurately reflect patterns of neutral and adaptive variation in Scots pine?
2. Is local seed better adapted to the local environment, both now and in the future, than seed from further afield?

Progress from January 2009 to September 2009

1. Assessment of the Climatic Characteristics of all Native Pinewood Populations

The climate data from the total inventory of Native Pinewoods (not just those sampled in this study) have been subject to PCA ordination analysis to find the axes which account best for this climatic variation. The results for the total sample of populations are very similar to those for the sampled populations. The first principal component, accounting for 69% of the variation describes a change from a relatively warm and moist climate with long growing season to a colder and drier climate with a shorter growing season. The second axis, accounting for 24% of the variation, largely reflects differences in the range of temperatures experienced. The positions of the populations on the PCA plot are shown in Fig.1, and the weightings of the first two principal components in Table 1. As before, for the more restricted data set, populations from the same seed zone tend to group together but there is significant overlap in climate space among the seed zones.

Figure 1. PCA plot of all Native Pinewood populations based on climatic variables and classified by seed zone. EC = East Central, N = Northern, NC = North Central, NE = North East, NE = North West, SC = South Central, SW = South West.

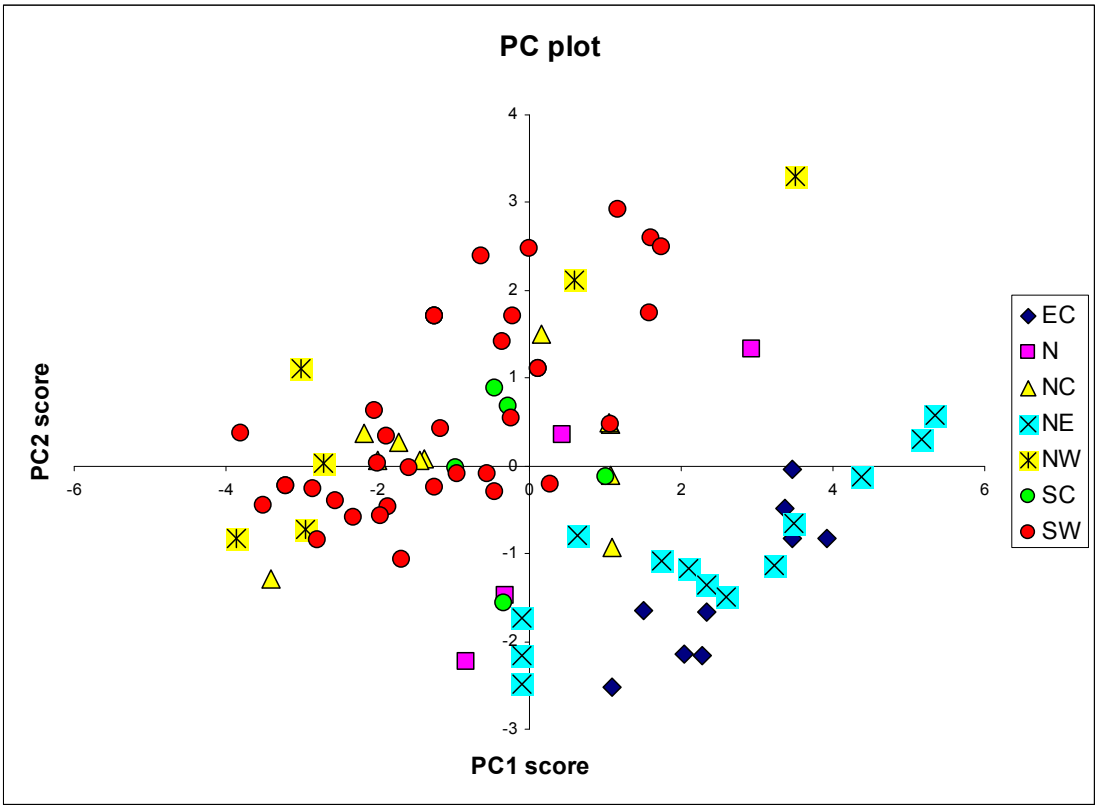


Table 1. Weightings of the Variables used to compute the first two principal components.

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

2. Measurement of adaptive character variation in provenance/progeny trial

Drought response

During the last eight months, analysis of the provenance/progeny trial has concentrated on measuring response of seedlings to drought stress. The primary objective has been to develop a technique that can be used on a large scale to quantify the physiological effect of drought on a population sample of seedlings. The technique chosen was the measurement of chlorophyll fluorescence, which assesses the efficiency of photosystem II responsible for the light-driven part of photosynthesis. Under stress the efficiency of this photosystem is reduced.

An experiment was established to measure chlorophyll fluorescence among a selection of populations and families of Scots pine sampled from across a rainfall gradient within Scotland over the course of a six week droughting experiment. The first aim was to determine whether chlorophyll fluorescence was significantly reduced as a consequence of droughting. The second was to determine whether, under droughting, significant differences in chlorophyll fluorescence could be detected among the populations that spanned the rainfall gradient.

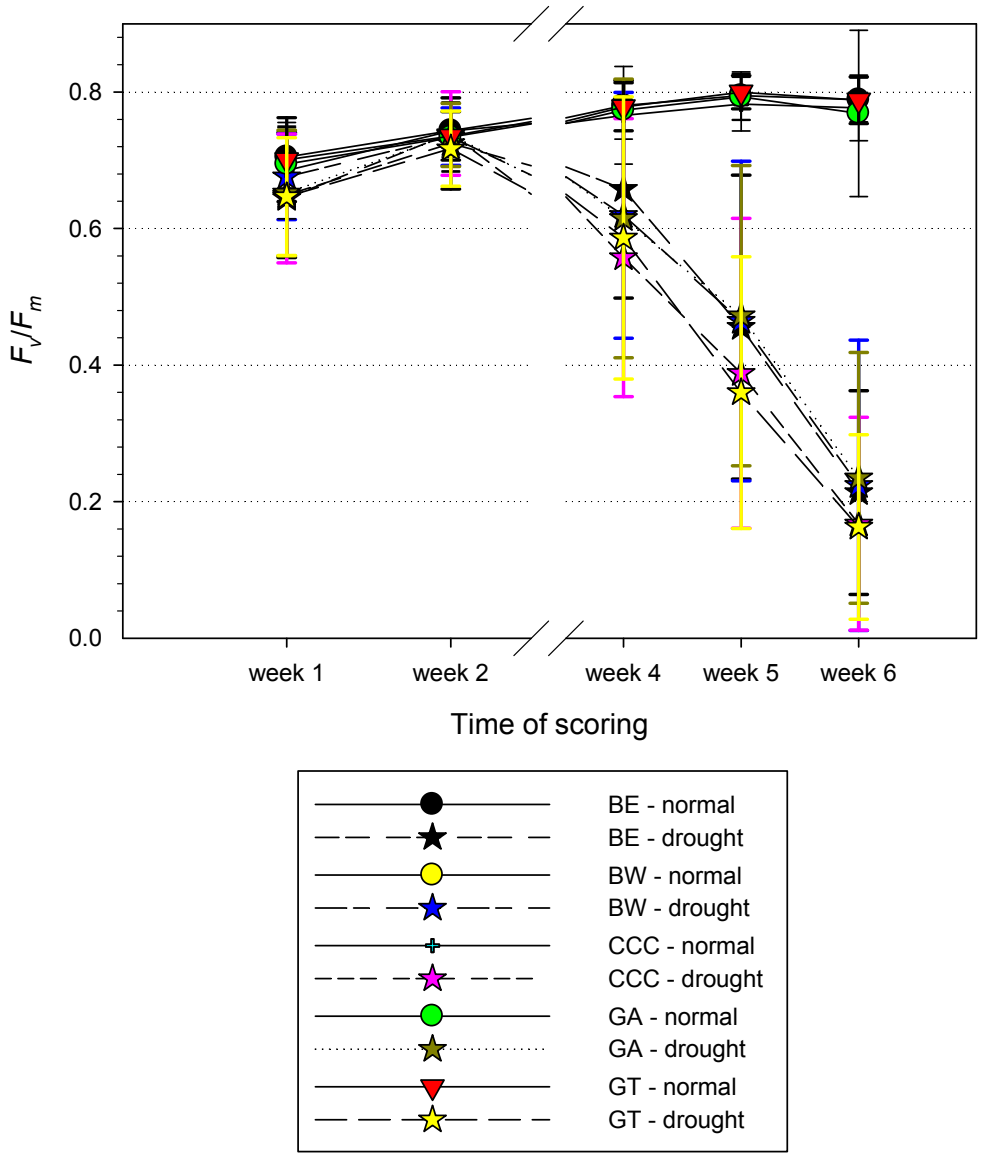
Five populations spanning the rainfall gradient in Scotland were selected (Table 2). Four families per population, each containing 20 offspring were included in the experiment (a total of 400 seedlings). Half of the seedlings from each family were subjected to a droughting treatment (watering withheld over the period June 4th to July 9th 2009), while watering was applied to the remaining seedlings in the family (control). Chlorophyll fluorescence measurements were made on all seedlings once a week over the time course of the experiment. Measurements took place within a two day period each week between the hours of 8:00 and 11:30.

Table 2. Populations used in drought experiment

Population	Seed Zone	Annual Precipitation (mm.)
Coille Coire Chuilc	South Central	2904
Beinn Eighe	North West	2411
Glen Affric	North Central	1685
Black Wood of Rannoch	South Central	1159
Glen Tanar	North East	785

The effects of the droughting treatment on chlorophyll fluorescence over the time course of the experiment are illustrated in Fig. 2. There is a very rapid reduction in chlorophyll fluorescence 3 weeks after the imposition of drought, and chlorophyll fluorescence continues to decline in the droughted treatment for the duration of the experiment.

Fig.2. Chlorophyll fluorescence in populations of Scots pine seedlings in control (normal) and droughted (drought) treatments over the time course of a 6 week droughting experiment. BE = Beinn Eighe, BW = Black Wood of Rannoch, CCC = Coille Coire Chuilc, GA = Glen Affric, GT = Glen Tanar.



Analysis of variance indicates a significant effect of the droughting treatment on chlorophyll fluorescence at week three ($P<0.001$). However no significant difference could be detected in chlorophyll fluorescence among the Scots pine populations ($P= 0.126$) or among families within populations ($P=0.174$) subjected to drought at week three.

At the end of the experiment watering was resumed for the seedlings subjected to drought. After nine weeks the numbers of individuals that were alive and dead in the five populations were scored. There were no significant differences in the frequency of surviving seedlings among the five populations ($\chi^2_{(4)} = 2.73$ n.s.). Seedlings from the two droughting treatments have been stored for later analysis of variation in root/shoot ratio.

The conclusions from this experiment to date are that chlorophyll fluorescence measurements can be applied at the population level to provide a reliable and repeatable physiological measure of the photosynthetic capacity of seedlings. A highly significant effect of droughting can be detected on photosynthetic capacity of Scots pine seedlings. However the experiment was unable to detect significant differences in adaptation to drought stress among the five populations of pine sampled from across the rainfall gradient in Scotland.

Further work on chlorophyll fluorescence

Following the successful application of chlorophyll fluorescence for measuring photosynthetic capacity on a large scale, the technique is currently being used to look at seasonal variation in photosynthetic capacity of Scots pine populations across the year. The objective is to determine whether there are differences in the seasonal patterns of photosynthetic capacity among populations derived from environments with different lengths of growing season. For this experiment eight populations have been chosen spanning the variation in length of growing season (Table 3).

Table 3. Populations used in the Growing Season Experiment

Population	Seed Zone	Length Growing Season (days)
Ballochbuie	North East	108
Glen Loy	South West	187
Glen Affric	North Central	204
Rothiemurchus	East Central	216
Coille Coire Chuilc	South Central	223
Glen Tanar	North East	231
Black Wood of Rannoch	South Central	252
Beinn Eighe	North West	279

Four families comprising six individuals per family (total 192 individuals) were arranged outside in a randomised block design in August 2009. Chlorophyll fluorescence will be measured in these populations/families once a month across the growing season to determine seasonal rhythm, and to analyse whether there are any differences in seasonal rhythm among provenances and families.

3. Analysis of Gene Flow and Mating System using Microsatellite markers

Five pairs of microsatellite primers have now been optimised for Scots pine. For two of these primer pairs all individuals in the trial have been amplified by PCR and scoring of these individuals is underway. Genotyping of individuals at the three remaining microsatellite loci will take place over the coming winter period.

4. Presentation of Research

Over the last eight months the preliminary findings of this research project have been presented at two conferences:

i). To the Conference on the Evidence Base for Environmental Management & Conservation at the University of Stirling, 6th April 2009-09-27

ii). To the Botanical Society of Scotland student symposium held on 22nd September 2009.