Genotypie and environmental variability in the growth and photosynthesis of Sitka spruce (Picea sitchensis [Bong,] Cam)

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Genotypic and environmental variability in the growth and photosynthesis of Sitka spruce (*Picea sitchensis* [Bong.] Carr.)

Ph.D. Thesis

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Abstract

In 1990, clonal seedlings from twelve full-sib families of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) were planted at four contrasting sites throughout the UK; Newcastleton and Wauchope in the Scottish Borders; Scootmore in Morayshire; and Llandrindod in Wales.

There were large differences in nutrient concentrations between sites, with Newcastleton generally having the highest nitrogen- and phosphorus- based nutrient concentrations and Wauchope the lowest. Both sites had a similar soil structure, with the soil at Scootmore having a lower ability to capture moisture. Scootmore experienced the highest temperatures during the growing season and Wauchope the lowest. There was little difference in precipitation levels between sites but there were large differences between years.

After 10 years of growth in the field, height, diameter and wood density (using the Pilodyn® technique) were measured. The resulting data showed that Newcastleton and Wauchope were similar and had greater tree growth, whereas Scootmore and Llandrindod were also similar but had smaller tree growth but higher wood density. However, a high mortality rate at Llandrindod excluded this site from further analysis. The 10-year data were used to classify the clones into clusters, and a clone representative of each cluster was chosen for further analysis.

The selected clones showed the same growth patterns between sites; C20177 had largest growth rate; C20211 had smallest growth rate but higher wood density; C20208 was intermediate in terms of growth and wood density. However, large variation was apparent at each site, a result of environmental impacts on the growth rates. Chlorophyll, total nitrogen (N) and phosphorus (P) were extracted from differently aged needles, at different heights in the canopy for each clone at each site. N and P did not vary between clones or heights in the canopy, although did increase with increasing needle age. The distribution of chlorophyll followed light intensity patterns (increasing in shaded older and lower canopy needles) and was significantly higher in C20177. All foliar constituents were higher in Newcastleton trees and lowest in Scootmore. Foliar constituents reflected the nutrient concentration in the soils, although Scootmore had lower foliar concentrations than expected, which may be a result of the reduced moisture availability at this site. The lower uptake of nutrients at Scootmore explains the lower growth rate at Scootmore.

Photosynthetic light response curves showed highest photosynthetic and respiration rates at Newcastleton and showed lowest rates at Wauchope. There was little difference in rates between clones or position in the canopy. Various parameters of light efficiency showed little difference between clones, position in the canopy or site. Differences in total non-structural carbohydrates were evident between sites (Wauchope had the highest concentrations) and between clones (C20177 had the lowest). Lower carbohydrate levels in C20177 reflected the greater growth rates by this clone.

Destructive biomass sampling of above-ground organs reflected the results of the earlier height, diameter and wood density measurements, indicating that the clones had not changed growth patterns between the years 2000 and 2004. Between sites, only C20177 showed a change in carbon (C) allocation, with a switch from allocating the majority of C to the trunk at favourable sites (Newcastleton) to allocating the majority of C to branches under nutrient or water stress (Wauchope and Scootmore respectively). C allocation below-ground showed no clear pattern between clones, although fine root density was lowest for C20177, suggesting this clone was less effected by nutrient or water stress. Differences in C allocation below-ground between Wauchope and the other two sites also suggested that nutrient concentration had a greater effect on coarse roots.

Parameters were calculated from the observed data and used to simulate photosynthetic rates of Sitka spruce in a process-based model of tree evapotranspiration. A comparison between observed and simulated data showed that the model predicted seasonal, site and clonal differences but the absolute values were overestimated at Newcastleton and underestimated at Wauchope and Scootmore. A sensitivity analysis showed that six parameters largely affected the output of the model and, with the majority of these parameters extrapolated from the literature, they would explain the large differences between simulated and observed data.

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Declaration

I declare that no part of the following thesis has been previously submitted for a degree in this or any other university, and is entirely my own research except where duly acknowledged and with the following exceptions:

- The 10-year tree height, trunk diameter and wood density data in chapters 3 and 4 were provided by Forest Research, UK.
- The process-based evapotranspiration model, ForestETp, in chapter 9 has been primarily developed by Forest Research, UK, and the University of Antwerp, Belgium (primarily by G. Deckmyn).

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Abbreviations & Acronyms

ϕ	Apparent Quantum Efficiency
A_{max}	Maximum Rate of Photosynthesis at Light Saturation
A_{max_N}	Maximum Rate of Photosynthesis at Light Saturation per Unit of Nitrogen
A_{net}	Net Photosynthesis
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
C	Carbon
CO_2	Carbon dioxide
DBH	Diameter at Breast Height
DGDG	Digalactosyl Diglyceride
ETp	Evapotranspiration
FR	Forest Research
G_{min}	Minimum Stomatal Conductance
H	Parameter Describing the Curvature Response to High Temperatures
IUFRO	International Union of Forest Research Organisations
J_m	Electron Transport
k	Convexity
K_n	Nitrogen Allocation Co-Efficient
LAI	Leaf Area Index
LCP	Light Compensation Point
LSE	Light Saturation Estimate
MGDG	Monogalactosyl Diglyceride
N	Nitrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
N_b	Nitrogen Not Used in Photosynthesis
NH_4	Nitrate
P	Phosphorus
PAR	Photosynthetically Active Radiation
PCA	Principal Component Analysis
PGA	Phosphoglyceric Acid

P_i	Inorganic Phosphate
PIDS	Polarisation Intensity Differential Scatter
PO_4	Phosphate
PPFD	Photosynthetic Photon Flux Densities
Q	Photon Flux Density
QCI	Queen Charlotte Islands
QE	Quantum Efficiency
R_{day}	Dark Respiration
R_{day_N}	Dark Respiration per Unit of Nitrogen
Rubisco	Ribulose Bisphosphate Carboxylase-Oxygenase
RuBP	Ribulose Bisphosphate
S	Parameter Describing the Response of Electron Transport to Low Temperatures
TNC	Total Non-Structural Carbohydrates
UK	United Kingdom
USA	United States of America
UV	Ultra Violet
V_m	Photosynthetic Capacity
VPD	Vapour Pressure Deficit
X_n	Ratio of Photosynthetic Capacity to Leaf Nitrogen
d.wt	Dry Weight
f. wt	Fresh Weight

Units

cm	Centimetres
g	Grams
J	Joules
km	Kilometres
l	Litres
m	Metres
M	Molar
mg	Milligrams
ml	Millilitres
mm	Millimetres
mmol	Millimole
mol	Mole
nm	Nanometres
°C	Degrees in Celsius (Temperature)
s	Seconds
V	Volts
W	Watts
µg	Micrograms
µl	Microlitres
µmol	Micromole

1. Summary

1.1 Genotype versus environment

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) is a native species of North America, with its natural distribution spread along the Pacific coast, in a narrow strip from California to Alaska. The growth requirements of Sitka spruce are broad, allowing it to establish on a diverse selection of sites (Roche & Haddock, 1987). The species has flourished since its arrival in Britain in 1831, as it is particularly well-adapted to the oceanic climate, and has become one of the most widely planted and important timber species (Holmes, 1987).

Experiments by the International Union of Forest Research Organisations have shown that Sitka spruce from various provenances have different growth qualities when cultivated on a common site. Sitka spruce from warmer provenances, such as those from California and southern Oregon, performed better at warmer sites (Nanson, 1984; Pederick, 1984; Roman-Amat, 1984), whilst Sitka spruce from colder provenances, such as those from British Columbia and Alaska, were much hardier and outperformed the warmer provenances at the colder sites (Alexandrov, 1984; Kleinschmit & Svolba, 1984). In the UK, Australia and New Zealand, growth is inversely correlated to latitude of provenance (Lines & Samuel, 1984; Miller & Shelbourne, 1984; Pederick, 1984), whereas in Latvia, growth was positively correlated with increasing latitude (Pirags, 1984).

Environmental factors explain the majority of the growth variation in Sitka spruce, with climate and attack by white pine weevil (*Pissodes strobi*) accounting for over two-thirds of the variation in tree height, trunk diameter and seedling survival in British Colombia, Canada (Ying, 1997). The effect of climate changes with increasing tree age, from a temperature related effect in younger spruce trees to a moisture related effect in older trees (Xu *et al.*, 2000). Abundant precipitation is one of the most important factors during the growing season of Sitka spruce (Roche & Haddock, 1987) and drought is also a major factor in determining plant growth and survival in Norway spruce (*Picea abies*) in southern Europe; CO_2 assimilation decreased and there was a decline in radial increment of the trunk (Vygodskaya *et al.*, 1995). Temperature is a major determinant of the geographical location of many species. The distribution of *Tilia cordata,* and many other European species, was limited by low summer temperatures (Pigott & Huntley, 1978, 1980, 1981) or

by the tolerance of low winter temperatures (Grace, 1987), and the growth of Norway spruce increased with increasing temperatures during the growing season (Mäkinen *et al.*, 2001). Nutrient availability also impacts on growth, with soil nitrogen deficiency causing a decrease in chlorophyll content, photosynthesis, Rubisco activity, stomatal conductance, and needle size and number in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Chandler & Dale, 1993, 1995; Murray *et al.*, 2000). Phosphorus deficiency generally inhibited shoot growth and greatly diminished wood volume in trees (Conroy *et al.*, 1990).

1.2 Process-Based Tree Growth Models

The Farquhar model is one of the most widely used and developed process-based models (Farquhar *et al.*, 1980; von Caemmerer & Farquhar, 1981). The model is a comprehensive description of the biochemical processes of photosynthesis that are compatible with studies of gas exchange measurements. The Farquhar model was further developed to include limitation by thylakoid activity (Sage, 1990) and incorporated into models simulating canopy photosynthesis of *Quercus alba* (Harley & Baldocchi, 1995), *Picea mariana* (Rayment *et al.*, 2002), *Picea abies* (Falge *et al.*, 1996), and *Picea sitchensis* and *Pinus radiata* (Wang & Jarvis, 1990). The Farquhar model was also coupled with carbon allocation models (Aber & Federer, 1992; Bartelink, 1998; Dewar, 1997; McMurtrie & Wolf, 1983; Reynolds & Thornley, 1982) and combined with the pipe-model theory (Mäkelä, 1999; Valentine, 1999; West, 1993). However, the above models were developed for a generic tree and validated with data from one species and for different environments, but did not consider genetic variation within species.

1.3 Aims of the research contained within this thesis

Using Sitka spruce as a test species, the impact of both genotype and environment are investigated in relation to photosynthesis and tree growth.

Forest Research had a unique set of clonal experiments, planted in 1990. The experiment consisted of eight Sitka spruce stock plants randomly selected from within six unrelated full-sib families. The stock plants were the hybrids of 'plus' trees, trees with excellent heritable growth characteristics, and each family was a cross of unique parents. Fifteen seedlings were propagated from each stock plant, so in total there were 720 trees from eight stock plants within six families. The trees were planted randomly within a site and

replicated at four sites across the UK. With 48 clones from six families, planted across four contrasting sites, the effect of genotype and environment could be thoroughly investigated.

Forest Research has been developing a process-based tree growth model to forecast plant response to climate change and site management, and to select tree lines best suited to particular environments. The model predicts canopy photosynthesis, evapotranspiration and carbon allocation. The model is split into several modules: the weather generator, which downscales monthly climate data into daily time steps; the canopy light environment, calculating the light intensity through the canopy; the canopy water environment, calculating rainfall interception by the canopy and other tree structures; the soil environment, calculating the movement of water within the soil; the gas exchange model, calculating CO_2 assimilation; and carbon allocation.

By taking gas exchange and physiology measurements of different clones, within different families and at different sites, the model equations can be modified to take in account genetic variability and the differences in growth, yield and environmental interactions. The primary aim of this project was to quantify genetic variability in the growth and physiology of Sitka spruce and to extend the predictive capabilities of existing process-based models of tree growth.

1.4 Thesis plan

The thesis begins with an introduction to the present literature, indicating the environmental and genetic impacts on photosynthesis and growth, with additional information on the development of existing process-based models. A background to Sitka spruce, its growth requirements and use in British forestry is also included. Chapter 3 investigates the differences in various climate and soil properties at each site. A comparison of these properties between sites and between clones within site is included. Chapter 4 explains the background to the Forest Research clonal experiments, a description of the field trials, previous data collection and an analysis of these data showing the effect of the environment and the growth differences between different families of Sitka spruce. Chapter 5 focuses on the selection of Sitka spruce clones from the data, as representative of the various growth patterns that are seen within the experiments. A comparison of the selected clones both within and between sites is also presented. Chapter 6 investigates the differences between the foliar nitrogen, phosphorus and chlorophyll contents of the

selected clones both within and between sites. The effect of soil nutrient concentrations on the foliar concentrations is discussed. In Chapter 7, the differences in gas exchange and related photosynthetic parameters of the clones, normalised for foliar nitrogen concentration, are investigated. In Chapter 8, the carbon allocation of the clones is calculated from destructive biomass sampling and differences between the clones and sites are discussed. Chapter 9 includes a description of the Forest Research process-based tree growth model. Comparisons of model simulations with observed data are discussed. A sensitivity analysis of various parameters is also presented. The final chapter is a discussion of the results from Chapter 3 to Chapter 9, and the conclusions that can be drawn from these data.

2. Literature

2.1 Sitka spruce

2.1.1 Natural distribution of Sitka spruce

Sitka spruce (*Picea sitchensis* [Bong.] Carr.) is a native species of North America, with the natural distribution spread along the Pacific coast, in a narrow strip from California to Alaska (Fig 2.1). The natural distribution covers a distance of 3000 km and 22 degrees of latitude and is dependent on abundant moisture during the growing season, with Sitka spruce particularly limited to areas where there is no summer drought (Roche & Haddock, 1987). The annual precipitation ranges from 1345 mm to 2980 mm, with higher precipitation in the north, and July precipitation ranges from 4 mm to 175 mm. The annual heat sums, based on a threshold of 5 °C, range from 2511 °C in Oregon to 851 °C in Alaska and frost-free days vary from 194 to 111 at the same sites (Roche & Haddock, 1987). Sitka spruce is less competitive in warmer, drier areas and is replaced by Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*), with the white pine weevil also becoming a pest. Sitka spruce is also a low elevation species, rarely found above 500 m and grows best on deep, moist, well-drained soils. However, it occurs on a wide variety of soils such as alluvial soils along river banks, on coarse textured soils and on soils with a thick accumulation of organic matter, with the pH generally ranging from 4.0 to 5.7 (Harris, 1978). Sitka spruce is mainly associated with western hemlock but towards the south it is associated with red wood (*Sequoia sempervirens*), Port-Orford cedar (*Chamaecyparis lawsoniana*), western white pine (*Pinus monticola*), Douglas-fir, shore pine (*Pinus contorta*) and western red cedar (*Thuja plicata*), and towards the north with Alaskan cedar (*Chamaecyparis nootkatensis*), mountain hemlock (*Tsuga mertensiana*) and sub-alpine fir (*Abies lasiocarpa*). Over a small part of its range in British Colombia, Sitka spruce is sympatric with white spruce (Roche & Haddock, 1987).

2.1.2 The use of Sitka spruce in British forestry

David Douglas first brought Sitka spruce to Britain in 1831, after visiting the spruce forests in British Colombia (Holmes, 1987). The species has flourished since its arrival, as it is particularly well adapted to the British oceanic climate and because of its ability to grow on a wide range of soils (Roche & Haddock, 1987). It has now become widely planted and

an important timber species because of its versatility, vigour and good quality timber (Holmes, 1987).

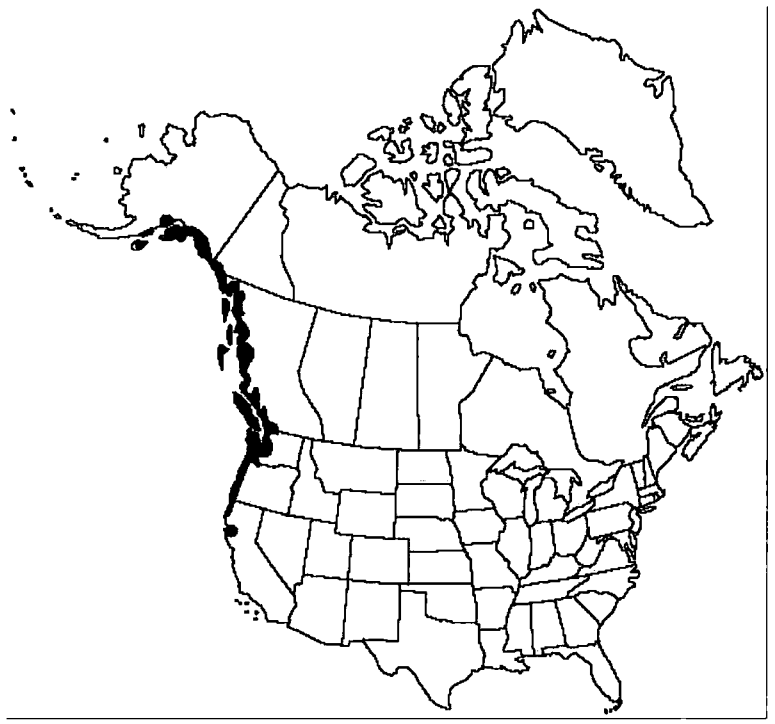


Fig 2.1: Natural distribution of Sitka spruce in North America (from 'Flora Online': http://flora.huh.harvard.edu:8080/flora/index.jsp).

Sitka spruce is the most important species growing in upland Britain and a woodland census in 1980 estimated that 48 % of the coniferous high forest area in Scotland was Sitka spruce plantation. In the early 1980s, over 70 % of the trees planted annually in Scotland were Sitka spruce (Low, 1987). During 1982 to 1986, the annual cut of Sitka spruce was 1.32 million m^2, with 605 000 m^2 as small round wood and 715 000 m^2 as saw logs (Brazier, 1987). Round wood is mainly used for pulping and particleboard, and saw logs are used in house construction, fencing, sheds, agricultural buildings, pallets and packaging (Brazier, 1987).

2.2 Environmental effects on plant growth

2.2.1 Climate effects

Temperature

Temperature is one of the most important climatic variables, with numerous studies illustrating the restriction on plant distribution imposed by temperature. In 1944, research on *Ilex aquifolium* illustrated an eastern distribution limitation in Britain, an area where it can tolerate the low temperatures (Grace, 1987), a similar distribution to ivy (*Hedera*), foxglove (*Digitalis*) and primrose (*Primula*) (Pigott, 1975). *Tilia cordata* in western Europe and *Cirsium acaule* in Britain are limited in their distribution by low summer temperatures (Pigott, 1970; Pigott & Huntley, 1978, 1980, 1981), whereas the distribution of *Fagus* species in both northern America and western Europe is determined by both the minimum temperatures of January and July (Huntley *et al.*, 1989). Birch (*Betula pubescens*) establishment strongly correlates with temperature, occurring only when temperatures rise above 5°C and to a much greater extent at 10°C (Woodward, 1987) (Fig 2.2), and during a 14-year study on *Verbena officinalis*, a population minimum occurred following a very cold January (Woodward, 1997).

During periods of cold temperatures, the ability to resist frost and damage caused by cold temperatures often determines plant distribution. The primary site of injury is the cell membrane (Senser & Beck, 1973) where a decrease in temperature causes a change in the hydrophobic matrix (Lyons, 1973), which in turn leads to a metabolic imbalance (Alberdi & Corcuera, 1991). In cold sensitive plants, photosynthesis is adversely affected, as the electron transport in the thylakoids is uncoupled (Alberdi & Corcuera, 1991). Limitation to photosynthesis can also be caused by end product synthesis during cold temperatures, as a result of increased inhibition of enzymes (Stitt, 1991).

The lowest temperature for plant survival depends on the previous exposure to low temperatures (Grace, 1987). During seasonal periodic stress plants have time to adapt (Alberdi & Corcuera, 1991), a process known as cold acclimation - a non-heritable modification of the structure and function of a plant, induced by cold temperatures, which minimises the damage caused by low temperatures (Fig 2.3).

Cold acclimation depends on the species, the season in which the cold temperatures occur and is induced by decreasing day lengths (Alberdi & Corcuera, 1991). During acclimation, carbohydrates, amino acids, glycinebetaine, proteins and lipids accumulate in the cytoplasm to reduce the risk of internal freezing. In *Picea*, during the cold-hardening period there are a larger number of divisions in the chloroplasts (Senser & Beck, 1984), although it is unknown how it affects freezing tolerance. In Scots pine trees from colder habitats, more biomass was allocated to the roots and they had lower growth rates than their warmer habitat counterparts (Oleksyn *et al.*, 1998).

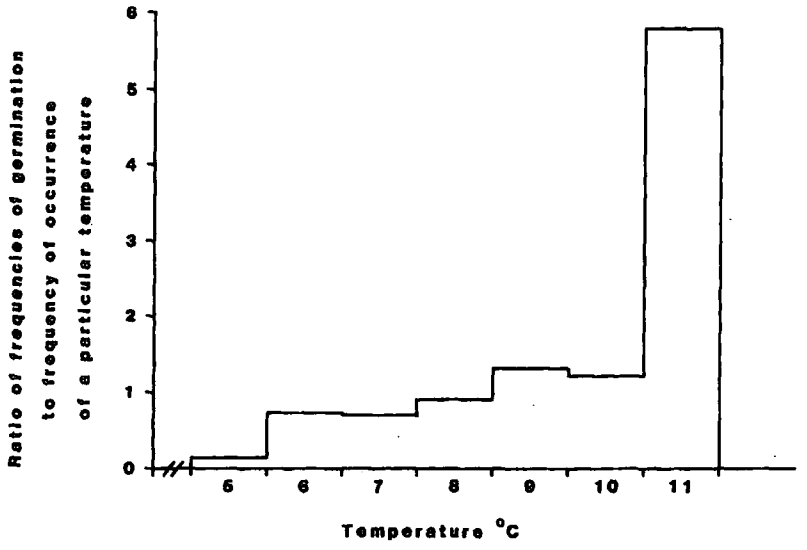


Fig 2.2: Relationship between temperature and seed germination of birch (*Betula* sp.) (after Woodward (1987) and Kullman (1979)).

During the summer months, cold temperatures disrupt the development of the embryos during reproduction. Firstly, bad weather will reduce the number of pollinator visits, a particular problem if the stigma is receptive only for a short period (Pigott, 1970). Secondly, cold temperatures can delay development of the embryo and, if development is not fully complete by the time of detachment, the fruit or seed will not be viable (Pigott, 1975). Thirdly, if the temperatures are too low, germination cannot occur. Flowering and germination will only occur in *V. officinalis* above a certain temperature (Woodward, 1997), and in the case of *T. cordata* growing in oceanic climates, pollen will not germinate and the seed cannot be fertilised if temperatures decline below a threshold (Pigott & Huntley, 1981).

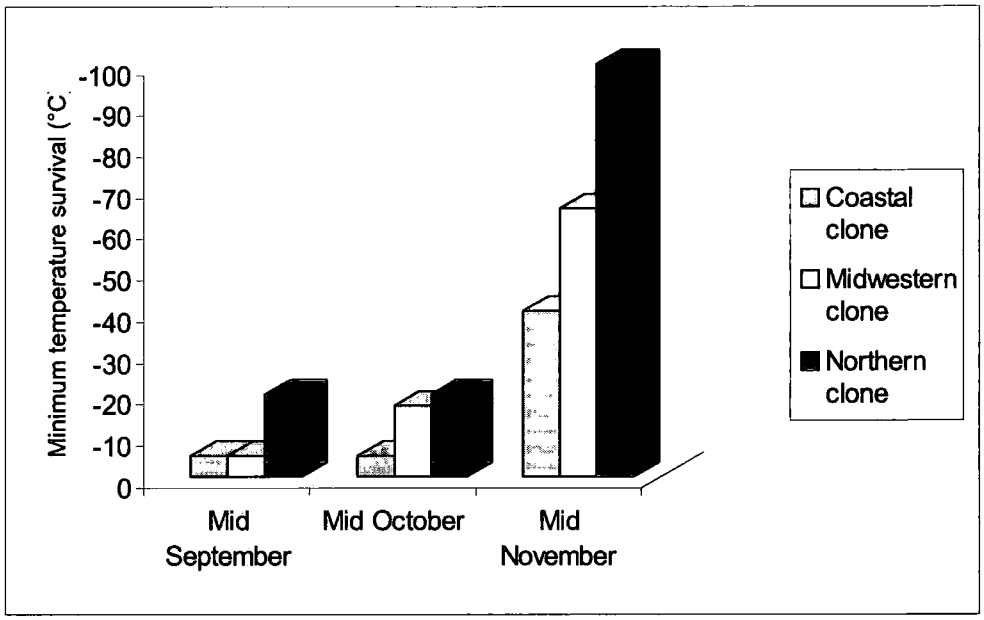


Fig 2.3: Cold acclimation for three clones of red-osier dogwood (*Cornus sericea*; after Begon *et al.* (1996) and Weiser (1970)).

Although dependent on species, there is an eventual decrease in photosynthesis at high temperatures. The stomata will close to reduce water loss if temperatures rise too high but this also reduces the carbon dioxide (CO_2) concentration in the leaf and will lead to a decrease in the rate of photosynthesis (Woodrow *et al.*, 1990). Elevated temperatures can also change the canopy structure of trees by altering the total leaf area, distributions of leaf area and leaf age classes, which affect the transpiration rate and photosynthesis (Kellomäki & Wang, 1998).

Rubisco has an affinity for both CO_2 and oxygen (O_2), and at temperatures of around 20°C, there is a higher affinity for CO_2. With an increase in temperature, the solubility of CO_2 and O_2 are altered (Stitt, 1991) and the affinity of Rubisco to O_2 increases (Jordan & Ogren, 1984). However, two thirds of the decrease in photosynthesis with increasing temperature is attributed to a decrease in the rate of Rubisco activity (Grub & Mächler, 1990), with only a third due to the change in solubility of the gases (Jordan & Ogren, 1984). However, by increasing the demand for carbohydrates within the plant there is an increase in the rate of photosynthesis, which suggests there is limitation by carbohydrate accumulation.

If carbohydrates accumulate, the photosynthetic pathway is inhibited and physical damage to the photosynthetic apparatus may also occur (Tissue *et al.*, 1993). In *Trifolium pratense* carbohydrate accumulation decreased photosynthesis and, in monoecious cucumbers (*Cucumis sativus* cv. Chipper), an increase in starch led to a decrease in carbon exchange

mechanisms during vegetative growth and flowering (Peet *et al.*, 1986). Similarly, when the export of carbohydrates was blocked from the leaf of *Amaranthus edulis* there was a decrease in the rate of photosynthesis, with soluble sugar concentrations increasing six fold (Azcón-Bieto & Osmond, 1983; Blechschmidt-Schneider *et al.*, 1989).

When there is an accumulation of sucrose, there is a decrease in inorganic phosphate (P_i) availability (Arp, 1991). With a low P_i concentration there is a decrease in the rates of photophosphorylation, electron transport and phosphoglyceric acid (PGA) production (Blechschmidt-Schneider *et al.*, 1989). Therefore, the rate of ribulose bisphosphate (RuBP) regeneration will be depressed (Azcón-Bieto, 1983) and will lead to photosynthetic inhibition.

A large concentration of sucrose also induces higher formation rates of starch grains, which are of a larger and irregular shape. This accumulation of starch can damage or alter the structure of the thylakoid membranes in the chloroplast or compresses the cytosol, leading to an increase in the diffusive resistance to CO_2 (Stitt, 1991).

Moisture

Water availability is an important factor of plant distribution. In the USA, tundra and forest formations occur where water deficits are low and coniferous forests occur only where water deficits are at their lowest, whilst grasslands and shrublands are at the opposite extreme and occur where the annual water deficit is high (Stephenson, 1990). Norway spruce showed a decrease in CO_2 assimilation by over 15 % during dry spells over a 100-year period in southern Europe (Vygodskaya *et al.*, 1995). Drought during the summer months limited photosynthesis in both mountain and coastal populations of *Pistacia lentiscus* L. in Spain (Flexas *et al.*, 2001) and during an extreme drought in 1995 in northern England, plant survival on a limestone grassland was limited to areas of deep soils in the valley bottom and greater moisture availability (Buckland *et al.*, 1997).

During drought the water potential may fall below a threshold value and different processes, as opposed to cell expansion and leaf growth, may occur, e.g. leaf abscission (Woodward, 1987). A correlation between abscission and soil water potential has frequently been observed in trees (Woodward, 1987) but is species-dependent.

The main effect of water stress is stomatal closure (Stitt, 1991). The effect of stomatal closure on transpiration and photosynthesis is well documented, although the mechanisms underlying the response to environmental variation are not (Whitehead, 1998). Stomatal conductance varies with needle age, depth in the canopy and shoot order (Leverenz *et al.*, 1982), so the effect of water stress varies depending on the age of the leaf and its position in the canopy. With control imposed by stomatal closure, the water deficit decreases causing an increase in Rubisco activity, although the rate of CO_2 assimilation decreases (Chaves, 1991; Woodrow *et al.*, 1990). Other factors often accompany drought. Excess light and high temperatures damage the photo-reaction centres through photoinhibition (Chaves, 1991) – in *Pinus radiata*, drought caused a reduction in the electron flow to photosystem II (Conroy *et al.*, 1986).

Dehydration in a plant causes a change in the partitioning of carbon from starch to sucrose, creating molecules of a low molecular weight, probably a mechanism of osmotic adjustment (Chaves, 1991). Acclimation to drought stress in *Pinus radiata* occurred with an increase in atmospheric CO_2 availability, caused by an elevation in the amount of carbohydrate, which helped facilitate osmotic adjustment (Conroy *et al.*, 1986).

Wind

Wind is an important factor for ventilating plant surfaces, mixing air near the leaf with new air from the atmosphere, bringing fresh supplies of CO_2 to leaves and transporting heat and water vapour away. However, species living in windy places often have dwarf or prostrate growth forms (Grace, 1981). Dwarf forms also occur in areas of drought, so water stress has been proposed as a mechanism in both cases; evaporation for drought and transpiration for wind. However, an increase in wind does not necessarily increase transpiration because, as the wind cools the leaf, the gradient of water vapour diffusion slows down (Grace, 1981).

Experiments have shown that shaking, an analogy for wind stress, prevents the lamina from expanding properly, affecting plant growth (Grace, 1981). The apical end of the shoot is very sensitive to displacement, which is likely to occur in windy environments. If this happens, lower shoots outgrow upper shoots and leads to small bushy growth forms (Grace, 1981).

Wind can also cause physical micro- or macro- scopic damage. Microscopic damage is caused by collision of the leaves, altering the wax deposition on the cuticle and inducing the rupture of epidermal cells. This can lead to a higher conductance to water vapour and an increase in transpiration, which in turn may lead to water stress. Integrity of the cuticle is important during periods of limited water availability.

In the Cairngorms, Scotland, the meristem temperature of the vegetation was measured along an altitudinal transect, running through native pine forest at low elevations through to dwarf shrubs at high elevations (Grace *et al.*, 1989). In the forest and amongst dwarf vegetation at high elevation, the meristem temperature was similar to the air temperature, but at mid altitudes, there was a large difference between meristem temperature and air temperature. The dwarf structure at high elevations was aerodynamically smoother than taller vegetation at mid elevations.

2.2.2 Light intensity

Generally, an increase in solar irradiance increases the rate of photosynthesis (Campbell *et al.*, 1988). With continuing high irradiance, there is a near linear increase in the rate of CO_2 fixation until a threshold level is reached, a result of light saturation or end product accumulation (Stitt, 1991). As the control by stomatal conductance increases with increasing irradiance (Stitt *et al.*, 1991), the inhibition may also be a result of water deficit or reduction of the internal CO_2 in the leaf. In *Trifolium pratense*, an increase in light caused a decrease in the activity of Rubisco, suggesting the limiting process is the accumulation of carbohydrates (Grub & Mächler, 1990).

Using radiolabelled CO_2 with ^{14}C, Hodge *et al.* (1997) investigated the effects of different light treatments on *Lolium perenne* grown in sand and soil. In sand, total plant biomass was unaffected by an increase in light but there was a change in the partitioning. There was an increase in root growth, causing an increase in root to shoot ratio. *L. perenne* grown in soil also demonstrated a difference in the carbon partitioning, but showing a decrease in the root to shoot ratio, a result of an increase in shoot growth. Where nutrients were limiting, increased photosynthesis of *L. perenne* was used to increase root growth for greater nutrient accumulation.

Plants adapted to low light environments have high photosynthetic efficiencies, low respiration rates and low light saturated photosynthetic rates (Boardman, 1977), a result of changes in nitrogen partitioning. Nitrogen is invested in more light harvesting proteins in the thylakoid membranes to compensate for low irradiance (Stitt, 1991).

2.2.3 Atmospheric CO_2 concentration

An increase in atmospheric CO_2 concentration increases photosynthesis. However, after 39 weeks grown in elevated CO_2, *Pinus radiata* could not sustain the high growth rates that were seen in the first few weeks (Conroy *et al.*, 1990). The acclimation was a result of decreased Rubisco activity, caused by end product inhibition and a decline in the availability of P_i (Ceulemans & Mousseau, 1994). For *P. radiata* grown with phosphorus addition there was no acclimation under elevated atmospheric CO_2 concentration, and there was a further increase in needle density, plant dry weight and photosynthesis (Conroy *et al.*, 1988; Conroy *et al.*, 1986). For *P. sylvestris*, after three growing seasons in elevated atmospheric CO_2 concentration, there was no evidence for an increase in the rate of photosynthesis or for any acclimation (Wang & Kellomäki, 1997).

Generally, with increased CO_2 concentration there is an increase of 38% and 40% for coniferous and an increase of 63% and 61% for broadleaf trees in biomass and in the rate of photosynthesis respectively, and a change in the carbon partitioning with more biomass allocated to the roots (Ceulemans & Mousseau, 1994; Conroy *et al.*, 1990). Birch (Pettersson & McDonald, 1992), deciduous trees (Lee & Jarvis, 1995), sweet chestnut (*Castanea sativa*) and beech (*Fagus sylvatica*) (El Kohen *et al.*, 1993) also show an increase in dry weight in elevated CO_2. The increase in dry weight can have an inhibitory effect on the light harvesting processes. In *Trifolium subterraneum*, increased atmospheric CO_2 led to a decrease in the chlorophyll *a*:*b* ratio. As the carbohydrate level increases, the starch grains become irregularly shaped and larger in volume. These starch grains can disrupt the configuration of the grana, affecting light harvesting properties (Cave *et al.*, 1981). Elevated atmospheric CO_2 concentration increased photosynthesis but there was no change in above-ground growth of *Populus tremuloides* (Kubiske *et al.*, 1998). Below-ground, however, there was an increase in fine root production but only in high nitrogen conditions and only in early leaf fall genotypes.

Any decrease in photosynthesis under elevated atmospheric CO_2 is generally thought to be nutrient stress related (Curtis, 1996). Addition of phosphorus stop plants from acclimating, but if nitrogen concentration cannot be maintained then the dry weight will decline (El Kohen *et al.*, 1993) and will lead to Rubisco reduction. In tomato (*Lycopersicon esculentum* (Mill.) cv. Findon cross) plants, Rubisco activity declined under elevated atmospheric CO_2 (Besford, 1990) and nitrogen addition was required to increase Rubisco activity (Besford *et al.*, 1990).

Water is also important for the increase in biomass and well-watered plants rarely acclimate (Lee & Jarvis, 1995; Townsend, 1995). An increase in atmospheric CO_2 causes a decrease in the stomatal conductance, increasing the ability to withstand drought but may also increase the leaf temperature. Ultimately this leads to photoinhibition, but the increase in leaf area increases the size of the canopy and therefore shading, protecting the leaf (Lee & Jarvis, 1995).

There was a decrease in the rate of respiration with an increase in atmospheric CO_2 in *Malus domestica* and *Quercus prinus* (Bunce, 1992). Elevated atmospheric CO_2 alters intracellular pH, suppressing the respiratory enzymes and leading to a recycling of respired CO_2 before it leaves the leaf (Wullschleger *et al.*, 1992). Elevated CO_2 concentration alters the ratio of CO_2 to O_2 and there is a subsequent decrease in the rate of oxygenase activity of Rubisco, leading to a decrease in the respiration rate.

2.2.4 Topographical effects

Altitude

An increase in altitude causes a decrease in air temperature and evaporation, and an increase in wind speed (Grace, 1987). The metabolic rates of plants from higher altitudes are affected less by temperature change, giving a comparative advantage over their lowland counterparts. *Populus* genotypes from low altitudes were restricted to altitudes where temperature did not decline too low, as they lacked the metabolic mechanism to survive lower temperatures (Criddle *et al.*, 1996). *Sedum rosa*, a high altitude species, and *Sedum telephium*, a low altitude relative, both grow well throughout the altitudinal range of Britain. However, the growth rate of *S. telephium* is reduced at higher altitudes and increased at lower altitudes, a result of the temperature difference between altitudes

(Woodward & Pigott, 1975). *Geum urbanum* and *Geum rivale* extend throughout Britain, although only *G. rivale* is found at higher altitudes, as the growth of *G. urbanum* ceases at higher altitudes due to cold temperatures (Graves & Taylor, 1986).

Plant species in the Austrian Alps at higher elevations have a higher photosynthetic capacity and CO_2 efficiency use than species at a lower elevation. The high elevation species have higher nitrogen contents and, therefore, have a larger quantity of assimilatory tissues for higher rates of CO_2 assimilation (Körner & Diemer, 1987). The populations of *Picea abies* at higher elevations had higher assimilation rates, higher respiration, higher needle nitrogen concentration and higher chlorophyll content, in comparison to lower altitude populations and these differences were correlated with mean annual temperature (Oleksyn *et al.*, 1998). Slow mineralization of litter, an increase in leaching and frequent water-logging at higher elevations leads to reduced nitrogen availability. It has been suggested that the relationship between altitude and foliar nitrogen concentration is a result of genetic adaptation in high altitude populations (Oleksyn *et al.*, 1998).

G. rivale has a larger root system than its low altitude relative and could be a mechanism to allow greater nitrogen uptake or used as a carbohydrate store for regeneration of above-ground parts after winter (Graves & Taylor, 1986). Alternatively, the change in root:shoot ratio may be due to a decrease in the shoot system. At different altitudes, there is a statistically significant relationship between leaf temperature and leaf extension (Woodward *et al.*, 1986). Low elevation species had higher leaf extension rates but growth stopped below a threshold temperature, whilst plants from higher altitudes had lower leaf extension rates but growth did not cease at low temperatures. Better utilisation of nitrogen also occurred with slower above-ground growth (Oleksyn *et al.*, 1998).

Latitude

Latitude, like altitude, is not a direct functional factor affecting plant growth. Colder temperatures are experienced at higher latitudes but other factors also differ with latitude - quality and quantity of solar radiation, climate extremes, site fertility, and length of growing season (Reich *et al.*, 1996). In Western Poland, there was a positive correlation between foliar nitrogen concentration and latitude in *P. sylvestris*, and a decrease in nitrogen content with increasing temperature. However, the foliar nitrogen concentration also depended on site quality, with needles of trees grown at polluted sites containing

lower nitrogen contents (Reich *et al.*, 1996). In herbaceous and woody plants, a greater percentage of foliar nitrogen and greater respiration rates are found with increasing latitude, although evergreen conifers had the greatest foliar nitrogen content at mid latitudes (Reich *et al.*, 1996).

Populus genotypes from different latitudes had different metabolic properties, which determined their geographic range (Criddle *et al.*, 1996), whilst *P. abies* showed growth differences at different latitudes but little difference when grown in a common environment (Oleksyn *et al.*, 1998).

Solar radiation varies in latitudinal bands, with a decrease in radiation as latitude increases (Woodward, 1987) (Fig 2.4). Mean irradiance at different latitudes does not vary greatly although there are large monthly ranges. Plant growth depends on the ability to intercept solar radiation and convert it into carbohydrates and dry matter, and is therefore, directly related to leaf area index, incoming solar radiation and the efficiency of radiation to dry matter conversion (Woodward, 1987).

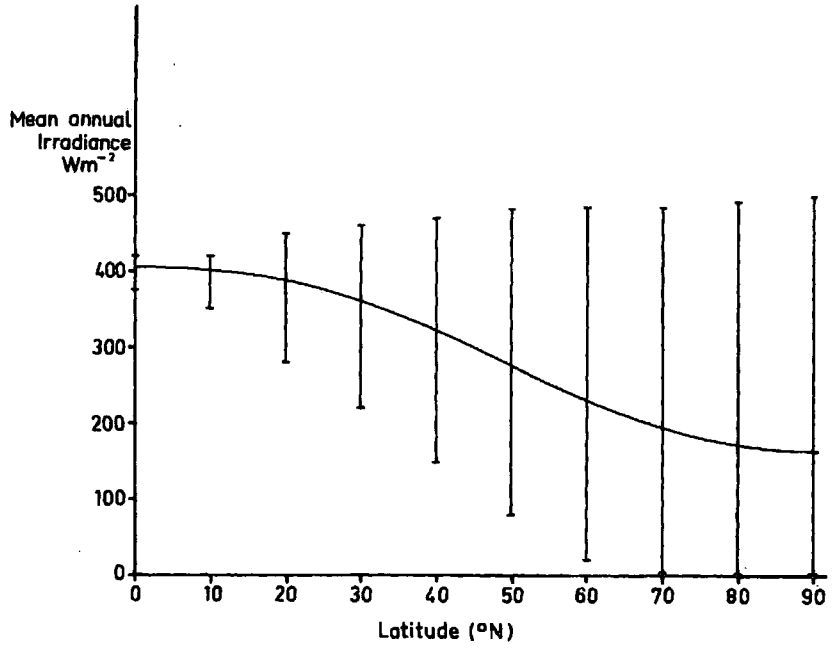


Fig 2.4: Mean annual irradiance incident on earth's atmosphere, for the northern hemisphere, indicating maximum monthly range (after Woodward (1987)).

The plant response to latitude and altitude appears to be similar. Both show an increase in respiration with higher values but with altitude there is also a mirrored effect of an increase

in photosynthesis. Within higher latitudes, there is no change in mean or maximum photosynthesis, although the increase in nitrogen content suggests there is an increase in assimilatory tissues.

Aspect

In New Zealand, north-facing slopes received 80% more radiation, had stronger winds, were dryer, had larger evapotranspiration and were warmer than south-facing slopes (Radcliffe & Lefever, 1981). In Colorado, USA, the south-facing slopes that received more radiation, had stronger winds, greater water loss and greater soil desiccation (Isard, 1986). *Dryas octopetala* was absent from the south-facing slopes although an abundant shrub in the area. Snow cover and soil moisture were the primary controllers. Snow provided a lot of meltwater but it evapotranspired very quickly and snow cover for long periods could lead to desiccation (Isard, 1986). In the UK, in Derbyshire, solar radiance was most affected by slope in the winter months, although the temperature difference between the slopes was greatest in the summer months (Rorison *et al.*, 1986a). *Arrhenatherum elatius* was dominant on the south-facing slope and *Centaurea nigra* on the north-facing slope but, when grown in a controlled climate environment, there was no difference in the growth rate. In the field, soil type and location affected both species. In particular, *A. elatius* was not favoured on the south-facing slope but, when watered, the locational effect was removed. Greater radiation and stronger winds on the south-facing slope caused greater water loss and desiccation, affecting the growth of *A. elatius*. The north-facing slope was much more favourable for growth because the moisture concentration and possibly the atmospheric humidity were much more ideal (Rorison *et al.*, 1986b). In the field, the south-facing slope was most favourable to both species for nitrogen accumulation and there were smaller temperature responses than on north-facing slopes, with the root component being the most responsive (Rorison *et al.*, 1986b).

2.2.5 Nutrient effects on plant growth and photosynthesis

Nitrogen

Nitrogen is important for the rate and functioning of photosynthesis, as nitrogen is partitioned into Rubisco, the main enzyme of photosynthesis, or chlorophyll (Evans, 1989). Plants store nitrogen in leaves and roots, and will remobilise it from storage when needed,

especially during growth. The amount remobilised does not depend on available nitrogen at the present time but by the amount available in the previous year (Millard, 1996).

The concentration of nitrogen available is positively correlated with the activity of Rubisco (Evans, 1989), so low nitrogen availability leads to a decline in photosynthesis and growth (Arp, 1991). Soil nitrogen deficiency caused a decrease in chlorophyll content, photosynthesis, Rubisco activity, stomatal conductance, and needle size and number in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Chandler & Dale, 1993, 1995; Murray *et al.*, 2000). In lodgepole pine (*Pinus contorta* ssp. *latifolia*) low nitrogen availability also led to low needle nitrogen and chlorophyll content, lower photosynthesis and lower Rubisco activity and content (Tissue *et al.*, 1993).

With the addition of nitrogen, Rubisco synthesis and activity, plus electron transport activity, is stimulated (Besford *et al.*, 1990), negating the deleterious effects of low nitrogen concentration (El Kohen *et al.*, 1993). Foliar nitrogen, leaf area index and dry matter increased in above ground parts during fertilisation experiments of *Pinus radiata* (Beets & Whitehead, 1996). Increasing nitrogen availability also had a positive effect on the photosynthesis of Scots pine (*Pinus sylvestris* L.) (Wang & Kellomäki, 1997), loblolly pine (*Pinus taeda* L.) (Murthy *et al.*, 1997) and *Picea mariana* (Paquin *et al.*, 2000), as well as increasing the rate of carboxylation of Rubisco in flush (current year needles) and 1-year-old needles of black spruce (*Picea mariana* Mill. B.S.P.) (Paquin *et al.*, 2000). However, increasing nitrogen availability had no effect on the growth of Sitka spruce at Aber forest in Wales, UK (Emmett *et al.*, 1995), or on the growth of Balsam fir (*Abies balsamea*) (Evans *et al.*, 2001). And in Japanese red pine (*Pinus densiflora* Sieb. et Zucc.), high nitrogen treatment led to a decrease in photosynthesis, due to a decrease in carboxylation efficiency and a decrease in Rubsico content and activity (Nakaji *et al.*, 2001).

The use of nitrogen in photosynthesis is a carefully balanced process, with reallocation of nitrogen in low nitrogen conditions. For example, a greater proportion of nitrogen is partitioned into the thylakoids on sites with lower irradiances (Evans, 1989) to improve the light harvesting abilities, making the most efficient use of the available nitrogen.

The efficient use of nitrogen differs between species and functional groups, with evergreen trees demonstrating lower nitrogen efficiency than their deciduous counterparts. Several

reasons have been suggested for this. Firstly, the thick and impermeable mesophyll cell walls have a large resistance to CO_2 diffusion and may limit the photosynthetic capacity of evergreens. Secondly, evergreen trees may allocate a smaller proportion of nitrogen to photosynthetic enzymes and, finally, these enzymes may have a lower specific activity. It was suggested that more nitrogen was used in plant defence or in cell wall proteins of the thick mesophyll cells in evergreen plants, which may be especially important for long-term leaf survival (Hikosaka *et al.*, 1998).

Phosphorus

Phosphorus availability is positively correlated with Rubisco activity. Loblolly pine trees grown in low phosphorus environments had similar rates of decreasing photosynthesis as trees grown in low soil nitrogen concentration, and the maximum rate of assimilation was closely correlated with needle phosphorus concentration (Loustau *et al.*, 1999). Plants grown in phosphorus-deficient soils had an inhibited shoot growth and trees had a greatly diminished wood volume (Conroy *et al.*, 1990). Phosphorus deficiency also led to a dysfunction of photosystem I (Conroy *et al.*, 1986) and a decrease in the efficiency of photosystem II (Loustau *et al.*, 1999). In *P. radiata*, although the needle density was not affected by phosphorus deficiency (Conroy *et al.*, 1986), there was a decrease in dry weight (Conroy *et al.*, 1988). A decrease in available phosphorus, although initially not limiting and not affecting needle size, caused an eventual decrease in Rubisco activity of Sitka spruce, a result of a decline in the formation of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), and a decline in the regeneration of RuBP (Chandler & Dale, 1993). Topa & Cheeseman (1992) found low soil phosphorus concentrations did not alter shoot or root dry weight of *Pinus serotina* until after six weeks, although there was a decrease in the growth rates and CO_2 exchange rates during the first two weeks. After six weeks, the CO_2 exchange rates had recovered, even though the needle growth and inorganic phosphate (P_i) in the cells were still declining. In maritime pine (*Pinus pinaster*), after 12 weeks of phosphorus deficiency, there was a decrease in maximum carboxylation rate, maximum electron transport rate and quantum yield, but after 22 weeks there was an attenuation of these effects (Loustau *et al.*, 1999). In the C_4 plant *Amaranthus edulis*, low foliar phosphorus concentration resulted in low P_i concentration in the chloroplast, affecting rates of photophosphorylation, electron transport

and PGA reduction, and leading to a reduction in the regeneration of RuBP and photosynthesis (Blechschmidt-Schneider *et al.*, 1989).

2.3 Genetic effects on plant growth

Variation in the growth of trees from different provenances has been found when grown on a common site. The lipid content of Norway spruce (*Picea abies*) foliage from 19 provenances differed when grown on two sites in England. The environmental impact was the largest cause of variation but trees from provenances with the lowest extreme winter temperatures had a higher ratio of monogalactosyl diglyceride (MGDG) to digalactosyl diglyceride (DGDG) in comparison to provenances with warmer extreme winter temperatures. This was attributed to trees from warmer areas having to turn more of the MGDG into DGDG to provide protection from winter injury (Wellburn, 1997).

Research on 14 provenances of Sitka spruce grown at eight sites also illustrated that environmental factors explain the majority of growth variation, with climate and attack by white pine weevil (*Pissodes strobi*) accounting for over two-thirds (Ying, 1997). In the early stages of seedling growth, there was a latitudinal pattern in the response of the provenances, especially at sites with a strong oceanic climate and where free of weevil attack. As the seedlings aged, the pattern changed to a longitudinal effect at sites that were harsher, more northerly and further inland (Ying, 1997). Southerly clones of Sitka spruce outperformed northerly clones, when grown in the same conditions (Centritto *et al.*, 1999) but these differences were only seen in elevated atmospheric CO_2 concentration and not in ambient conditions. The same southerly clones also showed higher initial nitrogen use efficiency and a higher initial growth rate (Centritto & Jarvis, 1999). However, provenance had no effect on photosynthetic capacity.

40 % of variation in the vigour characteristics was accounted for between provenances and 60% within, during common site experiments of different provenances of Sitka spruce (Fletcher, 1992), showing considerable variation between trees from different areas of the natural populations but also a large proportion between trees within the same area. The International Union of Forest Research Organisations (IUFRO) experiments on Sitka spruce showed considerable growth differences between various provenances from Canada and northwest USA when cultivated on a common site. Sitka spruce from warmer provenances performed better at milder sites but was more susceptible to frost (Lines,

1987; Lines *et al.*, 1971; Nanson, 1984; Pederick, 1984; Roman-Amat, 1984), whilst Sitka spruce from colder provenances was much hardier but not able to attain the same growth rate (Alexandrov, 1984; Kleinschmit & Svolba, 1984). Variation was also evident in the provenances' susceptibility to green aphid (*Elatobium abietinum* [Walk.]) attack (Carter & Nichols, 1984; Day, 1984).

Clones from 16 open pollinated maternal families of black spruce were grown in present and future predicted climates (elevated atmospheric CO_2 concentrations and higher temperatures), and in conditions of low and high nitrogen concentrations. An increase in seedling survival and growth occurred in both elevated atmospheric CO_2 concentration and high nitrogen conditions, with no significant differences between families among treatment conditions. However, there were significant differences in the seed mass, seed germination, seedling survival and growth between families, with the survival rate ranging from 48 to 78 %. Although the families could be split into fast growing or slow growing trees, the differences between seed mass, germination or survival could not be distinguished in the same way (Wang *et al.*, 1994).

In 24 clones of the aspen hybrid *Populus tremula* x *Populus tremuloides* and one clone of European aspen (*Populus tremula*), differences were found in the stability of the growth response. The clones could be split into three groups, based on results of height and trunk diameter data from four sites; low stability clones, which grew very well on good sites (i.e. forest sites as opposed to agricultural land); average stability clones, which grew well on poor sites but better on good sites; and high stability clones, which grew equally well on all sites but had a lower growth rate (Yu & Pulkkinen, 2003). Clones of trembling aspen (*Populus tremuloides*) showed that early-leaf fall genotypes had significantly higher photosynthesis, less leaf area, with leaf area development decreasing earlier in the season, in comparison to late-leaf fall genotypes, and had significantly greater fine root length (Kubiske *et al.*, 1998).

2.4 Process-based models

2.4.1 Photosynthesis Models

The Farquhar model was one of the most widely used and developed models (Farquhar *et al.*, 1980; von Caemmerer & Farquhar, 1981). The model was a comprehensive description

of the biochemical processes of photosynthesis that was compatible with studies of gas exchange measurements. The fundamental core of the model splits CO_2 assimilation into two parts: the carboxylation reactions and the oxygenation reactions. These reactions could be limited by the partial pressures of CO_2 and oxygen, which determined the partitioning of Rubisco activity; or by the concentration of Rubisco and by the rate of RuBP regeneration, which was related to the supply of NADPH and ATP. The rate of NADPH and ATP production was dependent on the total phosphate concentration and the consumption rate of NADPH and ATP was determined by the electron transport rate. Electron transport was dependent on the quanta absorbed and the threshold of the upper limit, related to the properties of the thylakoid membrane. All of the equations describing the above processes were integrated at the chloroplast level and extended to the leaf, by summing the contributions of each chloroplast. The respiration rate ran in parallel to the CO_2 assimilation rate.

Temperature and external CO_2 partial pressure were included but had a low impact on assimilation rates. The most important factor affecting photosynthesis was light intensity and its distribution throughout the leaf. The electron transport rate was dependent on the internal and external shading. Nitrogen content was an important factor controlling CO_2 assimilation. The carboxylase reaction rate and the electron transport capacity were the two key parameters, as these show important genotypic and phenotypic variation (Farquhar *et al.*, 1980; von Caemmerer & Farquhar, 1981).

The Farquhar model was further developed to describe the limitation of Rubisco activity by the thylakoid reactions or P_i regeneration (Sage, 1990). RuBP consumption, electron transport and P_i regeneration were regulated to avoid limitation by one of the processes. The model was used to investigate the response to alteration in the light intensity and the partial pressure of CO_2. Increasing light intensity increased electron transport rates and increased photosynthesis to saturation point, when Rubisco activity decreased. Decreasing the CO_2 partial pressure reduced the Rubisco activity. P_i regeneration, controlled by starch and sucrose synthesis, was affected by light and decreasing CO_2 partial pressure.

With slight modification of the temperature dependencies of the parameters, CANOAK was developed from the Farqhar model. The two-module model described the CO_2 and water vapour transfer from leaf to canopy, in a one-dimensional, multi-layer canopy. The first module computed the leaf and soil energy transfer, turbulent diffusion and radiative

transfer. The second module calculated photosynthesis, stomatal conductance and respiration (Baldocchi & Harley, 1995). The model effectively described the complex effects of light, temperature and relative humidity of sun and shade leaves of *Quercus alba* L. and *Acer rubrum* L. at Oak Ridge, USA (Harley & Baldocchi, 1995), although there was considerable error with variation of atmospheric CO_2 concentration.

The Farquhar model has also been used to calculate photosynthesis of *Picea mariana* at the leaf, branch and canopy level (Rayment *et al.*, 2002) and calculate photosynthesis of *Picea abies* at the branch level (Falge *et al.*, 1996). The results for *P. mariana* demonstrated the same seasonal dynamics but the results for *P. abies* showed errors in light acclimation, in the damage caused by pollutant deposition, needle age and cold stress effects.

The model MAESTRO was developed to predict radiation absorption, photosynthesis and transpiration for a tree stand (Wang & Jarvis, 1990). The model predicts the radiation adsorption, photosynthesis and transpiration in the crown of each tree using seven independent sub-models. Radiation, calculated from hourly positions of the sun, was split into direct beam and diffuse radiation. The leaf area density and leaf angle were calculated for three age classes and three ecological types (sun, intermediate and shade) and radiation absorption subsequently calculated. For each leaf age and type, conductance of water vapour and CO_2, transpiration and photosynthesis were calculated. Photosynthesis was calculated using the biochemical equations of the Farquhar model. The model was validated with data from *Picea sitchensis* and *Picea radiata* stands. Hourly calculations showed large deviations from the measured data but daily calculation differed by less than 10 %.

MAESTRO was used to investigate the linear relationship between net primary production and absorbed photosynthetically active radiation (PAR) (Medlyn, 1998). The model showed that daily values of photosynthetic light use efficiency varied regardless of the canopy structure, rejecting the hypothesis that the linear relationship was due to the structure of the canopy, exposing leaves to non-saturating PAR. MAESTRO did show that variability decreases with an increase in time scale, although efficiency varied across sites with different leaf area indices or light climate.

McMurtrie and Wang (1993) investigated the effects of temperature and CO_2 at the canopy level using MAESTRO and comparing with another, already established, model.

BIOMASS also used the Farquhar model of photosynthesis but varied in the detail of canopy structure and irradiation. Calculations were made daily and assumed a homogeneous canopy, with the foliage divided into three horizontal layers, subdivided into sunlit and shaded fractions. Both models showed the same photosynthetic response with increasing temperature and with ambient and elevated atmospheric CO_2 concentrations. A comparison of the predictions of MAESTRO and BIOMASS showed an agreement within 10 % across a range of conditions. However, neither model incorporated photosynthetic inhibition at high sucrose concentrations or the effect of sink strength on the rate of photosynthesis.

The PHOTOS model also calculated photosynthesis within a heterogeneous canopy (Zhang & Xu, 2003). The tree crown was considered a cone shape, with six vertical layers and one to three horizontal sections in eight directions. The model also calculated within crown and between crown shading. The computed photosynthetic rates, net primary production and growth was in good agreement with the measured data of a Chinese fir plantation (*Cunninghamia lanceolata* (Lamb.) Hook.).

STANDFLUX, a three dimensional microclimate and gas exchange model, considered the canopy to be series of concentric cylinders and vertical layers of homogeneous leaf and stem densities, but also considering leaf and stem angles (Falge *et al.*, 2000). The calculated canopy conductance agreed with measured data of *Picea abies* within 20 %.

'Big leaf' models scaled up photosynthesis from chloroplast to leaf to canopy and did not take into account spatial heterogeneity in the canopy (multi-layer models). The theory underlying this approach was that simple biochemical models, treating canopies as hemispherical surfaces with uniform properties, work well provided that the photosynthetic machinery of leaves was distributed in approximate proportion to total photosynthetic potential (Lloyd *et al.*, 1995). Therefore, if the photosynthetic capacity between leaves was in proportion to the profile of absorbed irradiance or nitrogen distribution then the equation describing leaf photosynthesis would represent the canopy photosynthesis (De Pury & Farquhar, 1997). Using the Farquhar model, a curvature factor was added to account for the differences in irradiance across the canopy and an empirical model of stomatal conductance response to light, temperature and humidity was also added (Lloyd *et al.*, 1995). The output of the model was compared with eddy covariance data of part of the Amazon rainforest. As the rainforest canopy had nitrogen allocation and photosynthesis in

proportion to irradiance, the model was able to provide a good description of hourly gas exchange rates.

De Pury and Farquhar (1997) compared the outputs of a 'big leaf' model with a single layer model of sunlit and shaded leaves and a multi-layer model. Using the multi-layer model as standard, the big leaf model consistently over estimated photosynthesis and did not follow the observed trends with increasing irradiance. This was because the curvature factor varied with canopy leaf area index and foliar nitrogen content. However, the predictions of the sun/shade model closely followed that of the multi-layer model. Analyses by Raulier *et al.* (1999) also illustrated that big leaf models incurred a bias of 15 % to 26 % when compared to multi-layer models, a result of the assumption of proportionality between photosynthetic capacity and irradiance. By modelling the degree of light saturation in leaves of a heterogeneous canopy, the predicted light response curves of photosynthesis concluded that the big-leaf model assumption of full acclimation of photosynthesis to irradiance was not justified (Kull & Kruijt, 1998).

Three micrometeorological models were used to simulate daily rates of photosynthesis and transpiration in a maize crop (Sinclair *et al.*, 1976). The most complex model simulated wind speed, CO_2 concentration, water vapour, air and leaf temperature, and radiation distribution in a heterogeneous canopy. A simplified model did not include the same vertical gradients of micrometeorological factors, but predictions of CO_2 assimilation were within 12 % of the rates predicted by the complex model. A big-leaf model, however, agreed within 5 % of the rates predicted by the complex model.

2.4.2 Carbon allocation models

Thornley (1972) developed an assimilation model describing the partitioning of photosynthate and attempted to explain the growth pattern upon the mechanisms by which the substrate is transported around the plant and how it was subsequently used. At low concentrations the rate of utilisation was proportional to the concentration and at high concentrations the utilisation rate became saturated. The supply of the substrate was assumed limited by photosynthesis, which was limited by nitrogen availability. However, growth was dependent upon a single substrate in the leaves, which ultimately led to an imbalance.

A further development on the Thornley growth model was a root to shoot partitioning model (Reynolds & Thornley, 1982). The total plant matter was divided into structure (shoot and root dry matter) and storage (carbon and nitrogen compounds). Partitioning co-efficients were used to describe the division of new dry matter between roots and shoots. Results from the model were similar to the observed root to shoot ratio of tomato plants.

A partitioning model including respiration was developed by McMurtrie and Wolf (1983). Photosynthesis was calculated from radiation and partitioned into foliage dry weight, fine root dry weight and stem dry weight. The rate of tissue production in each compartment was proportional to the amount of substrate left after respiration and also included losses from leaves and roots including root exudates. The model was used to explore the disparities of estimated dry matter production and that observed, to help identify the factors that limit growth.

In 1992, a model called PnET was developed to predict net primary production in temperate and boreal forests (Aber & Federer, 1992). There were four major components: climate calculation, photosynthesis, water balance and carbon allocation, each calculated on a monthly time step. The model predicted annual net primary production well in comparison to experimental data.

In 1997, a model was developed for estimating stand growth for the benefit of foresters, called 3-PG. The gross primary production was calculated from the photosynthetically active radiation and the canopy quantum efficiency coefficient. It also included: estimation of below ground carbon allocation, using a simple relationship of growing conditions on root growth and turnover; a sub-model to calculate changes in stem numbers per unit area with time; allometric ratios to determine allocation of carbon to foliage and stems; an equation explaining the age related decline in net primary production associated with hydraulic conductance; and a ratio of net to gross primary production (Landsberg & Waring, 1997). The results showed that there was excellent correspondence between the simulated and measured cumulative stem biomass for *Pinus radiata* at sites in Australia and New Zealand. A similar model exists that calculates dry matter partitioning based on five sub-models including allometric ratios between stem diameter, tree height and branch biomass, and between tree diameter and root biomass. Results showed that the root to shoot ratio had a strong impact on partitioning, which depended on the nutrient availability

and therefore the condition of the site. The model provided a feedback mechanism between growth conditions and partitioning (Bartelink, 1998).

Dewar (1997) created a tree growth model based on a cereal crop model called RESCAP. The model was simple, with growth being either light limiting or water limiting. Light limitation was calculated from the amount of radiation intercepted by the canopy and the water limitation was calculated by the maximum rate of water extraction by the roots. This model was combined with a soil water balance model, assuming that daily maximum rainfall interception by the canopy is proportional to the canopy leaf area index. Results, using *Pinus radiata*, showed that the model could realistically simulate plant growth.

2.4.3 Pipe model theory

The pipe-model theory stated that each unit measure of the foliage on a tree was attached via an active pipe extending to the base of the trunk and into the root system (Shinozaki *et al.*, 1964), so the sapwood area of a tree was proportional to foliage biomass.

Using this theory, a model was developed, where dry matter growth was equal to pipe dry matter growth, height increment was equal to the increase in pipe length and basal area was equal to the total pipe basal area (Valentine, 1985). This model was further developed by including a constant ratio between foliar dry matter and total cross-sectional area of active pipes (Valentine, 1988). Therefore, the development of a stand was a result of an increase in basal area because of growth of the active pipe area and the subsequent increase in total foliar dry matter. At foliar dry matter maximum, the continuing growth in basal area was a result of aggregated basal area of disused pipes (Valentine, 1988), where live branches have withered and shed but still leaving their pipes in the stem (Chiba, 1998). Using empirical evidence from *Picea taeda*, the pipe-model theory, in the growth model 'Pipestem' (Valentine, 1999), showed accurate predictions in above ground dry matter.

Using the pipe-model theory, West (1993) developed a two part model comprising of a partitioning model and a biomass growth model to predict dry matter partitioning of Eucalyptus trees. Firstly, the light absorption was calculated over the year and used to determine the photosynthetic output. The losses, in terms of leaves, branches and stems, were determined and the volume of the crown was changed accordingly. With all predicted variables, the partitioning model predicted the growth in biomass, stem diameter and

height, and stem sapwood of each tree. A mortality sub-model was applied to determine the number of trees that died during the year. Overall, the model simulated the growth behaviour of a monoculture of Eucalyptus trees well.

Mäkelä (1999) combined carbon balance (by regulating gross growth or increasing senescence) with the pipe-model theory to develop a model in two parts; carbon balance modelled in terms of total mass increment, and structural balance in terms of the distribution of total mass to different plant parts. The conservative structural relationships in plants were re-established after a disturbance, through adaptive allocation of growth. The model also assumed a functional balance between the roots and the shoots, although the woody structure was additionally constrained according to the pipe-model theory (the connection of sapwood for transport between the roots, stems and branches). The model was then used to investigate the effect of disturbance or environmental change. Recovery after defoliation depended on how quickly the carbon balance was returned - the quicker the rebalance the quicker the recovery. However, the model overestimated growth in cases when the disturbance caused a decrease in the availability of water or nutrients.

3. Environmental differences between and amongst three Sitka spruce clonal experiments

Abstract

Environmental differences were investigated between and amongst three Sitka spruce clonal experiments located at different sites in Scotland: Newcastleton and Wauchope, both in the Scottish Borders, and Scootmore, in Moray. Soil nutrient concentrations were highest at Newcastleton and lowest at Wauchope. Despite large differences in nutrient concentrations between sites, nutrient concentrations within sites were fairly uniform. Silt was the largest proportion of soil at all three sites, with Newcastleton and Wauchope having equal proportions of sand and clay in the soil. At Scootmore, a larger proportion of the soil was sand than clay. Again, the proportions of soil constituents were largely uniform across the site. Precipitation during the growing season was very similar between sites and between years 2001 and 2002. In 2003, there was a large decrease in precipitation during the growing season, with the greatest decline at Scootmore. Scootmore had the highest mean temperatures during the growing season. However, Wauchope had the highest absolute temperature, and the lowest mean and absolute temperature.

3.1 Introduction

3.1.1 Nutrient effects on plant growth and photosynthesis

Nitrogen is a major limiting factor in plant growth. In elevated atmospheric carbon dioxide (CO_2) studies of loblolly pine (*Pinus taeda* L.), Tissue *et al.* (1993) demonstrated that photosynthesis only increased if nitrogen input was increased; increased carbon alone could not increase plant growth as a result of limiting nitrogen availability. The addition of nitrogen under ambient atmospheric CO_2 conditions will also increase plant growth. For example, when *Populus tremuloides* trees were grown under high nitrogen conditions, the trees grew to a height twice as tall as those grown under low nitrogen conditions (Kubiske *et al.*, 1998). Acceleration of shoot and root growth of Scots pine (*Pinus sylvestris* L.) was also seen under high nitrogen conditions, compared to accelerated root growth only under low nitrogen conditions (Iivonen *et al.*, 1999). In Sitka spruce (*Picea sitchensis* (Bong.)

Carr.), tree height has previously been shown to increase following nitrogen addition, particularly after 10 years of growth (Miller & Miller, 1987).

Increased soil nitrogen may increase plant growth primarily through enhancing photosynthesis, by increasing chlorophyll and Rubisco concentration in the foliage. Fertilisation enhanced photosynthesis in black spruce (*Picea mariana* Mill. B.S.P.) (Paquin *et al.*, 2000) and *Eucalyptus grandis* (Grassi *et al.*, 2002), and fertilised trees of *P. tremuloides* had significantly higher photosynthesis than the unfertilised trees (Kubiske *et al.*, 1998). Increasing soil nitrogen, increased photosynthesis in Scots pine (Wang & Kellomäki, 1997), by up to 15 % during fertilisation experiments by Tissue *et al.* (1993). Increasing soil nitrogen also had a positive effect on the photosynthesis of loblolly pine (Murthy *et al.*, 1997), and increased the rate of carboxylation of Rubisco in flush and 1-year-old needles of black spruce (Paquin *et al.*, 2000). However, in Japanese red pine (*Pinus densiflora* Sieb. et Zucc.), high nitrogen treatment led to a decrease in photosynthesis, due to a decrease in carboxylation efficiency and a decrease in Rubsico content and activity (Nakaji *et al.*, 2001). Increasing nitrogen availability had no effect on the growth of *P. sitchensis* at Aber forest in Wales, UK (Emmett *et al.*, 1995), or on the growth of Balsam fir (*Abies balsamea*) in Vermont, USA (Evans *et al.*, 2001).

Soil nitrogen deficiency has been shown to cause a decrease in chlorophyll content, photosynthesis, Rubisco activity and stomatal conductance in Sitka spruce (Chandler & Dale, 1993; Murray *et al.*, 2000). In *P. taeda*, low soil nitrogen availability has been shown to lead to a low needle nitrogen and chlorophyll content, lower photosynthesis and lower Rubisco activity and content (Tissue *et al.*, 1993).

3.1.2 Climatic effects on plant growth

Climate is a major determinant of growth, and it explains two thirds of the variation in height, diameter and survival of Sitka spruce in British Colombia, Canada (Ying, 1997). The effect of climate changes with increasing tree age, from a temperature related effect in younger trees to a moisture related effect in older trees (Xu *et al.*, 2000).

Temperature is a major determinant of the geographical location of many species. For example, the absolute minimum winter temperature affects the survival of *Verbena officinalis*, and the temperature must be 16 °C for the plant to flower and greater than 14 °C

for seed germination to occur (Woodward, 1997). The distribution of *Tilia cordata* and many other European species is limited to where the mean maximum temperature in the warmest month, July, is 16 °C or above (Pigott, 1975). Temperature can also affect plant growth, with leaf extension in Alpine plants only occurring above a certain temperature (Woodward *et al.*, 1986). An increase in daytime temperature from 10 °C to 20 °C and an increase of mean temperature from 7.6 °C to 14 °C led to an increase in leaf area and dry weight for *Sedum rosea* (L.) and *Sedum telephium* L. ssp. *fabaria* Syme (Woodward, 1975). In southern Finland, an increase in radial trunk growth of Norway spruce (*Picea abies* (L.) Karst) was correlated with higher than normal temperatures during May (Mäkinen *et al.*, 2001).

Precipitation also affects plant growth. The growth of Sitka spruce is dependent on abundant moisture during the growing season and the greatest growth occurs where there is no drought (Roche & Haddock, 1987). In Buxton, England, during an extreme drought in 1995, the species that survived on the limestone grassland were found on deeper soils, but the same species on shallower soil did not survive (Buckland *et al.*, 1997). And in Mallorca, Spain, the photosynthesis of *Pistacia lentiscus* L. growing in both montane and coastal sites was limited in summer due to drought (Flexas *et al.*, 2001). Norway spruce in southern Europe showed a decrease in CO_2 assimilation of 15 to 25 % during dry spells over a 100-year period, with fluctuations in the trunk radial increment, as a result of water stress (Vygodskaya *et al.*, 1995). Drought can affect plants in a number of ways. In *Pinus radiata* D. Don., drought led to a reduction in the electron flow to photosystem II (Conroy *et al.*, 1986), while in many plants drought causes a decrease in water potential and, if it reaches below a threshold, leaf abscission will occur (Woodward, 1987). The stomatal closure during periods of reduced moisture availability restricts CO_2 assimilation but the decrease in photosynthesis with water deficits could be a result of other factors. Drought may be accompanied by excess light and by high temperatures, both damaging to photo-reaction centres (Chaves, 1991).

3.1.3 Hypotheses

- With nutrient availability having a large impact on growth and photosynthetic rates, it is important to investigate the nutrient availability at each site. The concentration of the

nitrogen-based nutrients (nitrate and ammonia) and phosphate in the soil are expected to differ between sites but will remain uniform throughout each site.

- With moisture abundance being important during the growing season, soil moisture status throughout the growing season will be important. Using particle size as an indication of the soil's capacity to hold moisture, differences are expected between sites that will reflect the site's moisture retention properties, which may be reflected in the tree growth at each site.
- Moisture availability also depends on the precipitation received at each site. Precipitation during the growing season is expected to differ between sites, potentially impacting on tree growth.
- Temperature is an important determinant for the growth and survival of *P. sitchensis*. Differences in mean maximum and mean minimum temperature during the growing season are expected between sites, and may explain the different growth rates experienced at each site.

3.2 Method

3.2.1 Soil nutrient extraction and determination of concentrations

Soil cores

Soil cores were taken during October 2002 at Newcastleton and Wauchope, and during September 2003 at Scootmore, using a corer of 50 cm depth (for map of sites see Chapter 4). One core was taken from each selected clone (Chapter 4) at each site, within 0.5 m of the root crown. The cores were wrapped tightly in polythene bags to maintain integrity and stored at 4 °C within two hours. In the laboratory, the cores were split into A and B horizons where appropriate and were analysed within five days (120 hours).

Nitrate and ammonia extraction

5 g (± 0.01 g) of fresh soil and 50 ml of 1 M potassium chloride (BDH Lab Supplies, Poole, Dorset, UK) were placed into a 100 ml polythene bottle, and shaken mechanically for one hour. The solution was then filtered through Whatman No 2 paper (BDH Lab Supplies, Poole, Dorset, UK) (Skalar, 1995), the extract stored at 4 °C, and subsequently analysed within 48 hours. If analysis was delayed, the extract was stored at – 20 °C to

maintain sample integrity and, when ready for use, defrosted overnight and analysed within 24 hours.

Phosphate extraction

1 g (± 0.01 g) of fresh soil and 200 ml of Truog extracting solution were placed into a 500 ml polythene bottle, and shaken mechanically for 30 minutes. Truog extracting solution was prepared daily by adding 10 ml of 0.5 M sulphuric acid (BDH Lab Supplies, Poole, Dorset, UK) and 15 g ammonium sulphate (BDH Lab Supplies, Poole, Dorset, UK) to five litres of distilled water (Skalar, 1995). After shaking, the solution was filtered through Whatman No 2 paper (BDH Lab Supplies, Poole, Dorset, UK) and the first 25 ml of extract was discarded. The remaining extract was stored at 4 °C and analysed within 48 hours, or stored at –20 °C until required, defrosted overnight and analysed within 24 hours.

Soil nutrient concentration determination

The nitrate and ammonia concentrations were determined using a SAN^{plus} 4000 segmented flow analyser (Skalar Analytical, Breda, The Netherlands), connected to a SA1000 autosampler (Skalar Analytical, Breda, The Netherlands), matrix photometer (6250; Skalar Analytical, Breda, The Netherlands) and an interface unit (SA8600; Skalar Analytical, Breda, The Netherlands). SAN^{plus} v6.2 software was used to run the analyser and to calculate concentrations of nitrate and ammonia. 1 M potassium chloride was used as a blank. Standard curves of ammonia were made by diluting ammonium chloride with 1 M potassium chloride to give a range of 0.02 to 1.00 mg N l^{-1} and the standard curves of nitrate were made by diluting sodium nitrate with 1 M potassium chloride to give a range of 2 to 10 mg N l^{-1}. The phosphate concentrations were also determined using a SAN^{plus} 4000 segmented flow analyser, using Truog extracting solution as a blank and standard curves made by diluting potassium dihydrogen *o*-phosphate with Truog extracting solution to give a range of 0.01 – 0.50 mg P l^{-1}.

Nitrate concentration was determined by passing the sample through a column of granulated copper-cadmium to reduce the nitrate to nitrite. The total nitrite concentration was determined by diazotising with sulphanilamide and coupling with α-naphthylehylenediamine dihydrochloride to produce a pink-coloured complex, which is measured at a wavelength of 540 nm.

Ammonia concentration was determined by a modified Berthelot reaction, by chlorinating the ammonia to monochloroamine, then reacting with salicylate to form 5-aminosalicylate and oxidation to form a green coloured complex. The absorbance of the green complex was measured at a wavelength of 660 nm.

Phosphate concentration was determined by mixing ammonium molybdate, potassium antimony tartrate and phosphate from the sample to form an antimony-phospho-molybdate complex, which produced a blue colour when reduced by ascorbic acid. The absorbance of the blue complex was measured at a wavelength of 880 nm. All reagents were prepared according to manufacturers instructions (Skalar, 1995).

All chemicals used were of analytical grade, obtained from BDH (BDH Lab Supplies, Poole, Dorset, UK) or from Sigma (Sigma Chemical Company Ltd, Fancy Road, Poole, Dorset, UK), unless stated otherwise.

3.2.2 Particle size determination

The soil was air dried and sieved through a 2 mm sieve. 0.5 to 0.7 g was placed in a 50 ml plastic tube and, to oxidise the organic material, 20 ml of 20 % hydrogen peroxide was added. The tubes were then covered and placed in a boiling water bath. After two hours, the tubes were removed, centrifuged at 4000 rpm for four minutes and the supernatant decanted off. Due to the high organic content, the process to oxidise the organic material was completed again. After the second centrifugation and removal of supernatant, 20 ml of distilled water was added, the solution recentrifuged at 4000 rpm for four minutes and the supernatant discarded once more. Finally, 20 ml distilled water and 2 ml sodium hexametaphosphate solution were added to prevent coagulation of the particles.

The samples were analysed in a Coulter® granulometer (LS230; Coulter Electronics Ltd, Luton, UK). The granulometer uses lasers to calculate the proportion of the different sized particles in the sample, from the pattern of the light defraction of each particle. The granulometer also uses polarisation intensity differential scatter (PIDS) for accurate determination of particles smaller than 1 μm, by measuring the scatter of polarised light from each particle.

The percentage of silt, clay and sand were calculated from the granulometer output. Clay particles were ≤ 0.002 mm, silt particles were 0.06 mm > 0.002 mm, and sand particles were 2.00 mm > 0.006 mm diameter.

3.2.3 Precipitation and temperature data

Precipitation and maximum and minimum temperature were provided from the Meteorological Office Land Surface Observation Stations Data via the British Atmospheric Data Centre. Meteorological stations were located as close to the field sites as possible but were limited to those that recorded current observations. As a result, the stations were 13 km, 12 km and 9 km from Newcastleton, Wauchope and Scootmore, respectively. Data were available for all three years at Wauchope and Scootmore but only for 2001 for Newcastleton, although the station was currently active. Other Meteorological stations with current data were located too far from Newcastleton to be considered suitable for the analysis in relation to the present study.

Daily precipitation data were summed from the beginning of June to the end of August and the mean monthly precipitation calculated for the growing season. Mean minimum and maximum temperatures were calculated for each month of the growing season, from daily observations, and the absolute maximum and minimum temperatures were also recorded for the growing season.

3.3 Results

3.3.1 Soil nutrient concentrations

Generally, the nutrient concentrations in the A horizon were higher at Newcastleton and lowest at Wauchope (Fig 3.1). There were significant differences in the soil concentrations of nitrate between sites for all clones and in the ammonia soil concentrations of C20208 (Table 3.2).

In the B horizon soils, Wauchope had the lowest concentrations of the three nutrients (Fig 3.2). Generally, Newcastleton had higher nitrate concentrations and Scootmore higher ammonia and phosphate concentrations. There were only significant differences in nitrate concentration between sites for C20177 and C20211 (Table 3.2).

At Newcastleton and Wauchope, the nutrient concentrations for each clone showed a similar pattern in the A horizon soils (Fig 3.1); C20211 had the highest nitrogen-based nutrient concentrations; C20177 had the highest phosphate concentration but also the lowest nitrate concentration; C20208 had the lowest ammonia and phosphate concentration.

The B horizon soils at Newcastleton showed very different results (Fig 3.2); C20177 had the highest nitrogen-based nutrient concentrations; C20208 had the highest phosphate concentrations and lowest nitrogen-based nutrient concentrations; C20211 showed the lowest phosphate concentration.

At Wauchope, C20211 had the highest concentration of all the three nutrients in the B horizon soils (Fig 3.2), C20177 had the lowest nitrate and ammonia concentrations, and C20208 having the lowest phosphate concentration.

At Scootmore, C20211 had the highest nitrate and phosphate concentration in the A horizon soils, C20177 had the highest ammonia concentration but also the lowest nitrate and phosphate concentrations, and C20208 had the lowest ammonia concentration (Fig 3.1).

In the B horizon soils at Scootmore (Fig 3.2), C20177 had the highest nitrate concentration and lowest phosphate concentration, C20208 had the highest phosphate concentrations and lowest nitrate concentration, C20211 had the lowest ammonia concentration.

There were no significant differences in the nutrient concentrations between clones for either horizon at all sites (Table 3.1).

3.3.2 Particle size of soils

The percentage of different particle sizes in the A horizon varied between sites. Silt was the highest percentage of the soil at all sites, with the greatest proportion at Wauchope (80 %) and smallest at Scootmore (60 %; Fig 3.3). Newcastleton and Wauchope had a similar percentage of clay and sand in the A horizon soils (20 %). Scootmore had a lower percentage of clay (10 %) but higher percentage of sand (40 %). There were significant differences in the clay, silt and sand percentages of C20177 between sites, in the clay

percentage of C20208 between sites, and in the silt and sand percentages of C20211 between sites (Table 3.3).

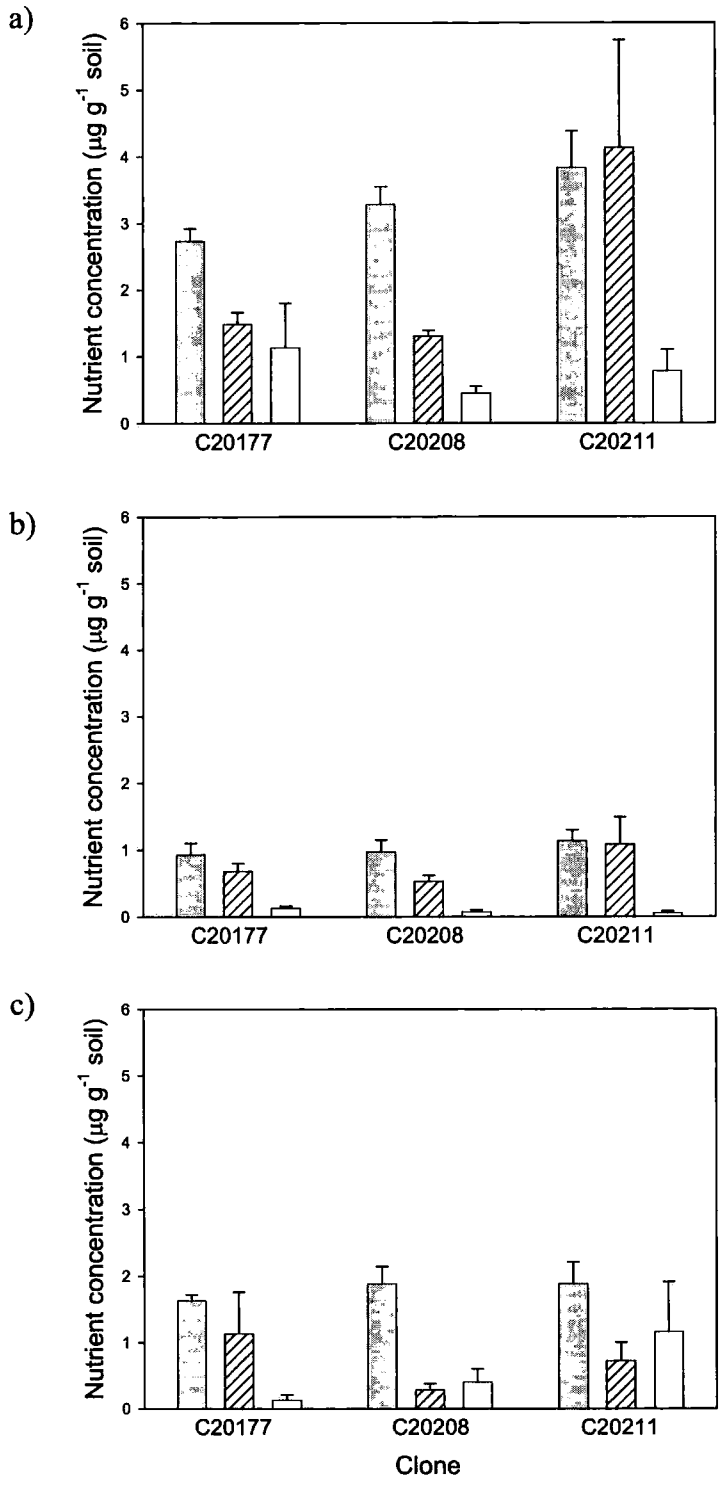


Fig 3.1: Mean nitrate (grey bars), ammonia (hashed bars) and phosphate (white bars) concentrations (+1s.e., n = 4) in the A horizon soil surrounding the root zone of clones C20177, C20208 and C20211, at a) Newcastleton, b) Wauchope and c) Scootmore.

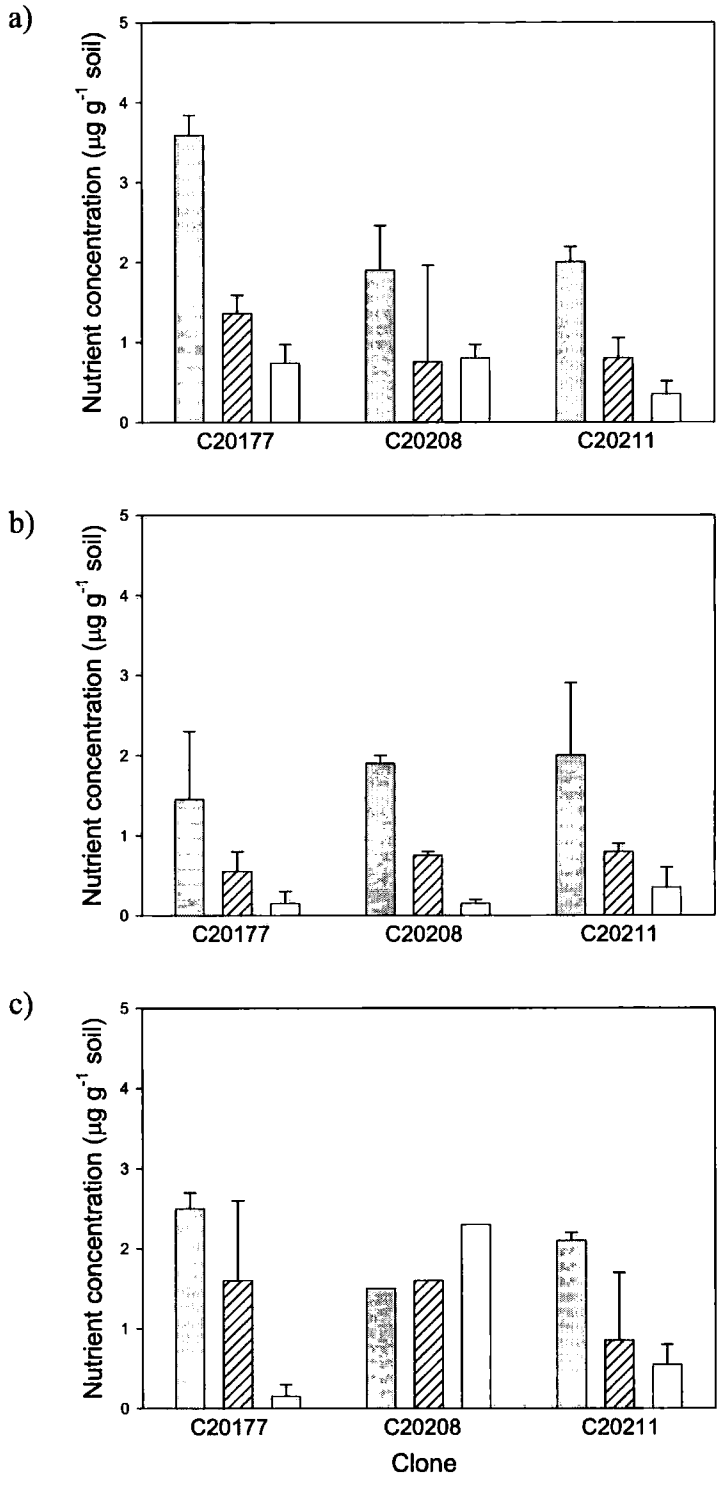


Fig 3.2: Mean nitrate (grey bars), ammonia (hashed bars) and phosphate (white bars) concentrations (+1s.e., n = 4) in the B horizon soil surrounding the root zone of clones C20177, C20208 and C20211, at a) Newcastleton, b) Wauchope and c) Scootmore.

Table 3.1: χ^2 and P values from the Kruskal-Wallis test, investigating differences in the nutrient concentrations between clones at the three sites.

		A Horizon			B Horizon			
		Nitrate	Ammonia	Phosphate	Nitrate	Ammonia	Phosphate	d.f
Newcastleton	χ^2	4.149	1.911	0.481	0.554	3.835	0.611	2
	P	0.126	0.385	0.786	0.758	0.147	0.737	
Wauchope	χ^2	1.378	2.563	3.292	0.286	0.682	0.515	2
	P	0.502	0.278	0.193	0.867	0.711	0.773	
Scootmore	χ^2	0.885	3.666	2.053	3.600	0.400	3.053	2
	P	0.643	0.160	0.358	0.165	0.819	0.217	

Table 3.2: χ^2 and P values from the Kruskal-Wallis test, investigating differences in the nutrient concentrations between sites for each clone. Results highlighted in grey are significant at the 0.05 probability level and results in bold and highlighted in grey are significant at the 0.01 probability level.

		A Horizon			B Horizon			
		Nitrate	Ammonia	Phosphate	Nitrate	Ammonia	Phosphate	d.f
C20177	χ^2	**9.649**	3.877	5.838	6.124	3.918	5.312	2
	P	**0.008**	0.144	0.054	0.047	0.141	0.070	
C20208	χ^2	**9.118**	8.656	4.074	5.250	4.050	4.810	2
	P	**0.010**	0.013	0.130	0.072	0.132	0.090	
C20211	χ^2	8.511	5.967	5.596	6.545	1.104	1.905	2
	P	0.014	0.051	0.061	0.038	0.576	0.386	

As in the A horizon, silt was the greatest proportion in B horizon soils at each site, being about 80 % of the soil at Wauchope but only 50 % to 60 % at Scootmore (Fig 3.4). Newcastleton and Wauchope shared a similar percentage of clay and sand (20 %), with Scootmore showing a smaller proportion of clay (10 %) and a higher proportion of sand (40 %). There were no significant differences in the percentage of different particle sizes between sites (Table 3.3).

There was no significant difference in the percentage of the soil that was clay, silt or sand between clones within each site, in either horizon (Table 3.4).

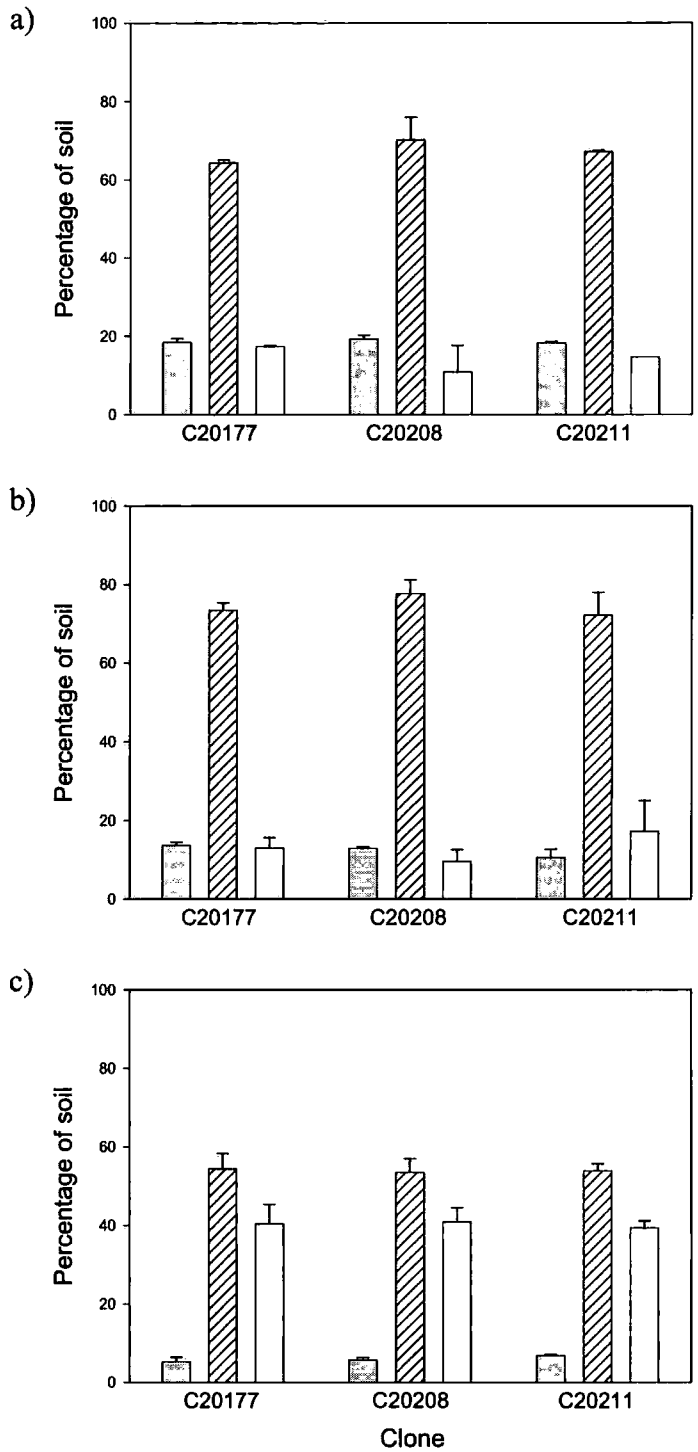


Fig 3.3: Mean clay (grey bars), silt (hashed bars) and sand (white bars) percentages (+1s.e., n = 4) in the A horizon soil surrounding clones C20177, C20208 and C20211, at a) Newcastleton, b) Wauchope and c) Scootmore.

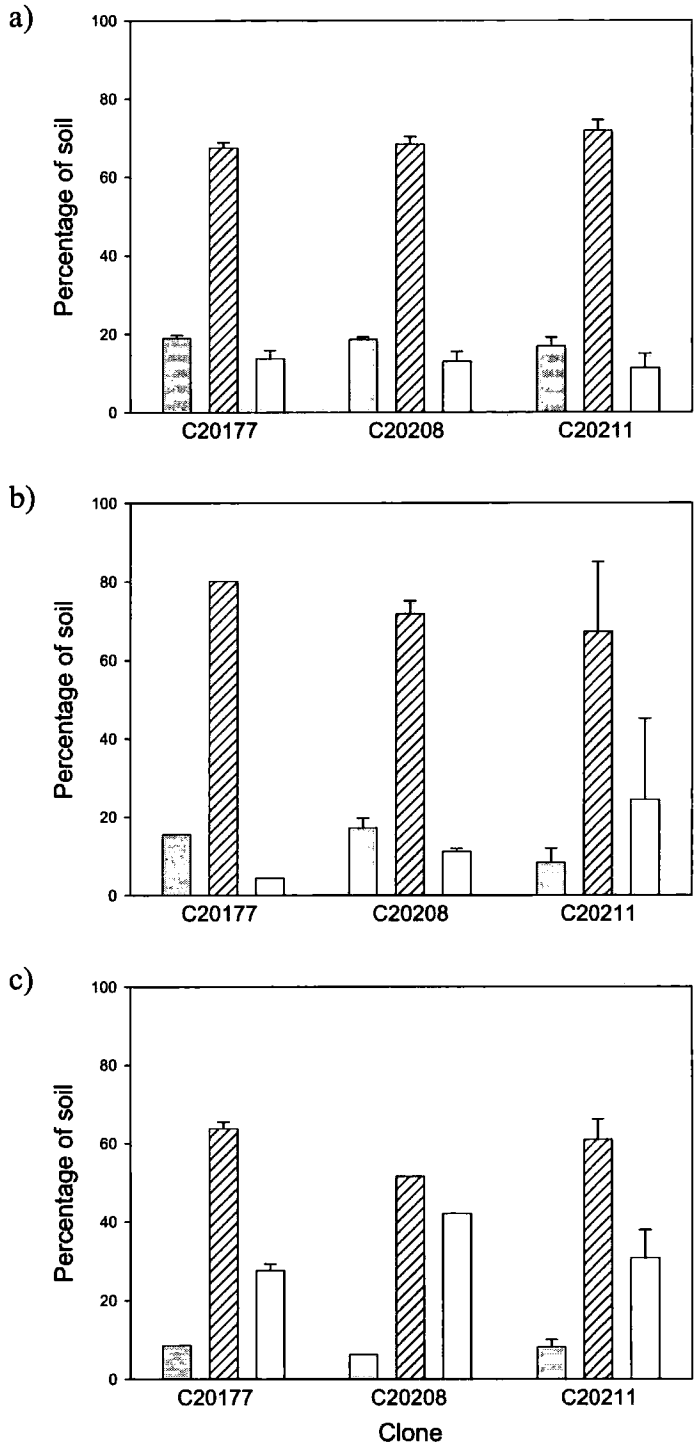


Fig 3.4: Mean clay (grey bars), silt (hashed bars) and sand (white bars) percentages (+1s.e., n = 4) in the B horizon soil surrounding clones C20177, C20208 and C20211, at a) Newcastleton, b) Wauchope and c) Scootmore.

Table 3.3: χ^2 and *P* values from the Kruskal-Wallis test, investigating differences in the particle size soil proportions between clones at each site.

		A Horizon			B Horizon			
		Nitrate	Ammonia	Phosphate	Nitrate	Ammonia	Phosphate	d.f
Newcastleton	χ^2	0.857	2.000	2.059	0.426	1.867	0.157	2
	P	0.651	0.368	0.357	0.808	0.393	0.925	
Wauchope	χ^2	2.214	0.714	0.714	2.381	0.857	1.238	2
	P	0.331	0.700	0.700	0.304	0.651	0.538	
Scootmore	χ^2	1.085	0.269	0.346	2.000	2.000	2.000	2
	P	0.581	0.874	0.841	0.368	0.368	0.368	

Table 3.4: χ^2 and *P* values from the Kruskal-Wallis test, investigating differences in the particle size soil proportions between sites for each clone. Results highlighted in grey are significant at the 0.05 probability level and results in bold and highlighted in grey are significant at the 0.01 probability level.

		A Horizon			B Horizon			
		Nitrate	Ammonia	Phosphate	Nitrate	Ammonia	Phosphate	d.f
C20177	χ^2	6.000	6.000	6.000	4.286	3095	4.286	2
	P	0.050	0.050	0.050	0.117	0.213	0.117	
C20208	χ^2	6.000	5.500	5.333	2.143	2.381	2.381	2
	P	0.050	0.064	0.069	0.343	0.304	0.304	
C20211	χ^2	4.900	6.111	6.162	3.778	1.806	1.806	2
	P	0.343	0.047	0.046	0.151	0.405	0.405	

3.3.3 Precipitation during the growing season

During the growing season in 2001, the precipitation at all three sites was similar, with precipitation slightly higher at Scootmore and slightly lower at Wauchope (Fig 3.5). The data for Wauchope and Scootmore show, again, there was little difference in the amount of precipitation received during the growing season of 2002. When compared with the data from 2001, Scootmore had almost identical precipitation between the two years and Wauchope showed a slight increase from 2001 to 2002. Between the growing seasons of 2002 and 2003 there was a large decrease in precipitation received at both Wauchope and Scootmore. At Wauchope, there was a decrease of 55 % and at Scootmore a decrease by

66 % in 2002 compared to 2001, with Wauchope receiving the higher deposition of the two sites.

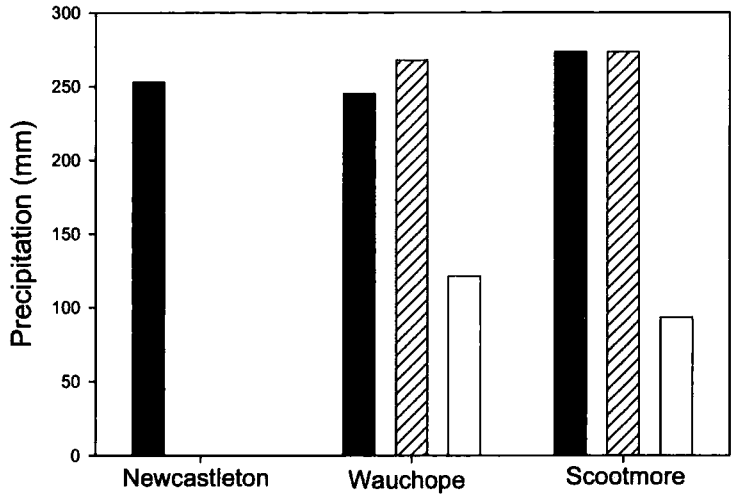


Fig 3.5: Total precipitation during the growing season (June to August) in 2001 (grey bars), 2002 (hashed bars) and 2003 (white bars). Note: Precipitation data during 2002 and 2003 not available for Newcastleton.

3.3.4 Temperature during the growing season

The mean maximum temperature during the three months (June to August) of the growing season of 2001 was approximately 2 °C higher at Newcastleton than at either Wauchope or Scootmore (Fig 3.6). Wauchope and Scootmore show very similar mean maximum temperature during the growing season of 2001.

Between June and July, for all three sites there was a large increase in mean maximum temperature (an increase of 3 °C at Newcastleton, 2.5 °C at Wauchope and 2 °C at Scootmore). Between July and August, there was very little change.

Wauchope and Scootmore both showed a continuous increase in temperature during the growing season of 2002. Scootmore had the highest mean maximum temperatures but converged with Wauchope in August.

During 2003, Scootmore again had the higher temperatures. At both sites, there was an increase between June and July. There was little change between July and August.

Although, Scootmore had the highest mean maximum temperatures, Wauchope had the highest absolute maximum temperature for all three years (Newcastleton has been excluded; Table 3.5). Between the three years, there was a general increase in temperature during the growing season. At Wauchope, there was a large increase (4.7 °C) between 2002 and 2003. At Scootmore, increase was larger between 2001 and 2002, with an increase of 7.9 °C.

Newcastleton had the lowest mean minimum temperatures during the growing season of 2001, with Wauchope and Scootmore showing almost identical values (Fig 3.7). There was an increase in minimum temperatures between June and July, of approximately 2 °C for all three sites, with a slight decrease, between July and August.

During the growing season of 2002, there was a continuous increase in minimum temperature at Wauchope and Scootmore, with Wauchope showing the lowest temperatures of the two sites.

In 2003, Wauchope again had the lowest temperatures. Both sites showed an increase in minimum temperature between June and July, but a decrease between July and August.

Between Wauchope and Scootmore, Wauchope had the lowest absolute minimum temperature in 2001 and 2003 but Scootmore had the lowest in 2002 (Table 3.9). Both Wauchope and Scootmore showed an increase in absolute minimum temperature between the three years.

Table 3.5: Absolute maximum and minimum temperatures for the growing season (June to August) during years 2001 to 2003 for each site.

	2001		2002		2003	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Newcastleton	25.7	-1.6	n/a	n/a	n/a	n/a
Wauchope	25.2	0	24.3	4.3	29	2.5
Scootmore	15.7	2.5	23.6	1.8	24.7	4.7

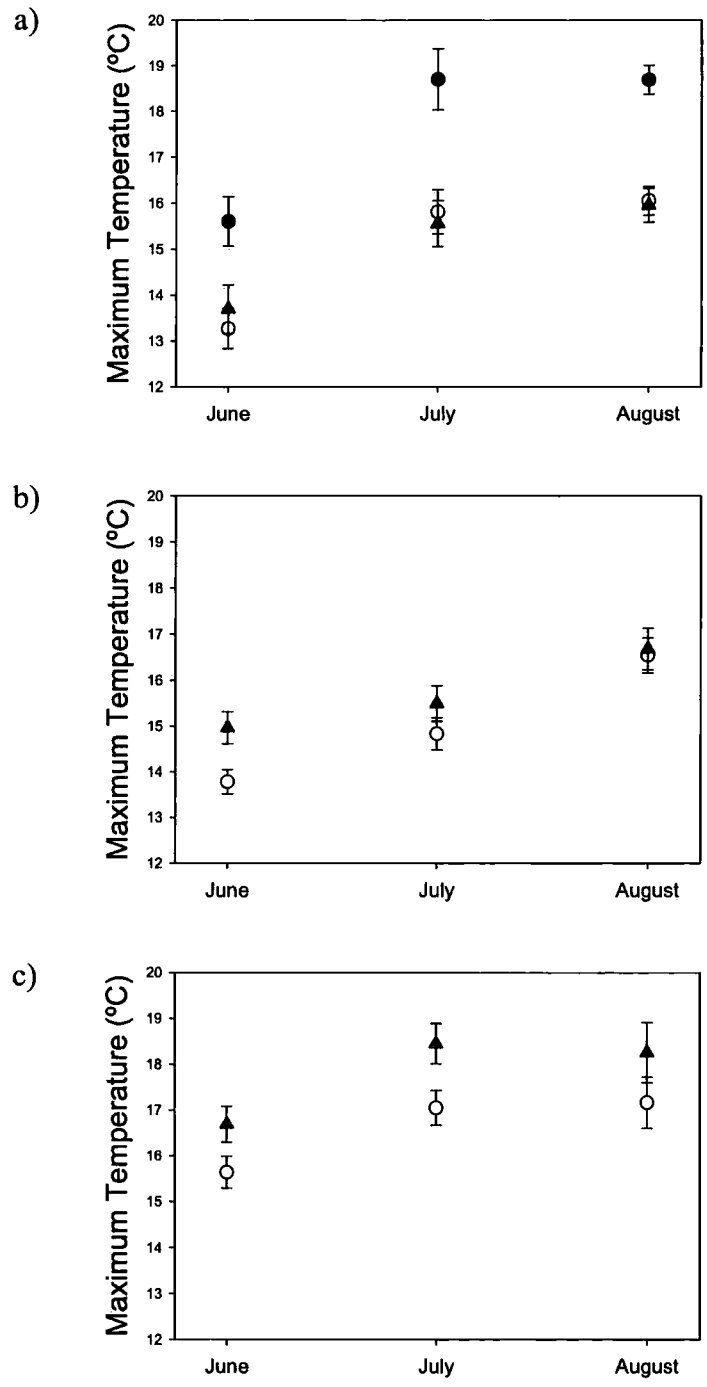


Fig 3.6: Mean maximum temperature (± 1s.e.) during the growing season (June to August) for a) 2001, b) 2002 and c) 2003, for Newcastleton (●), Wauchope (○) and Scootmore (▲).

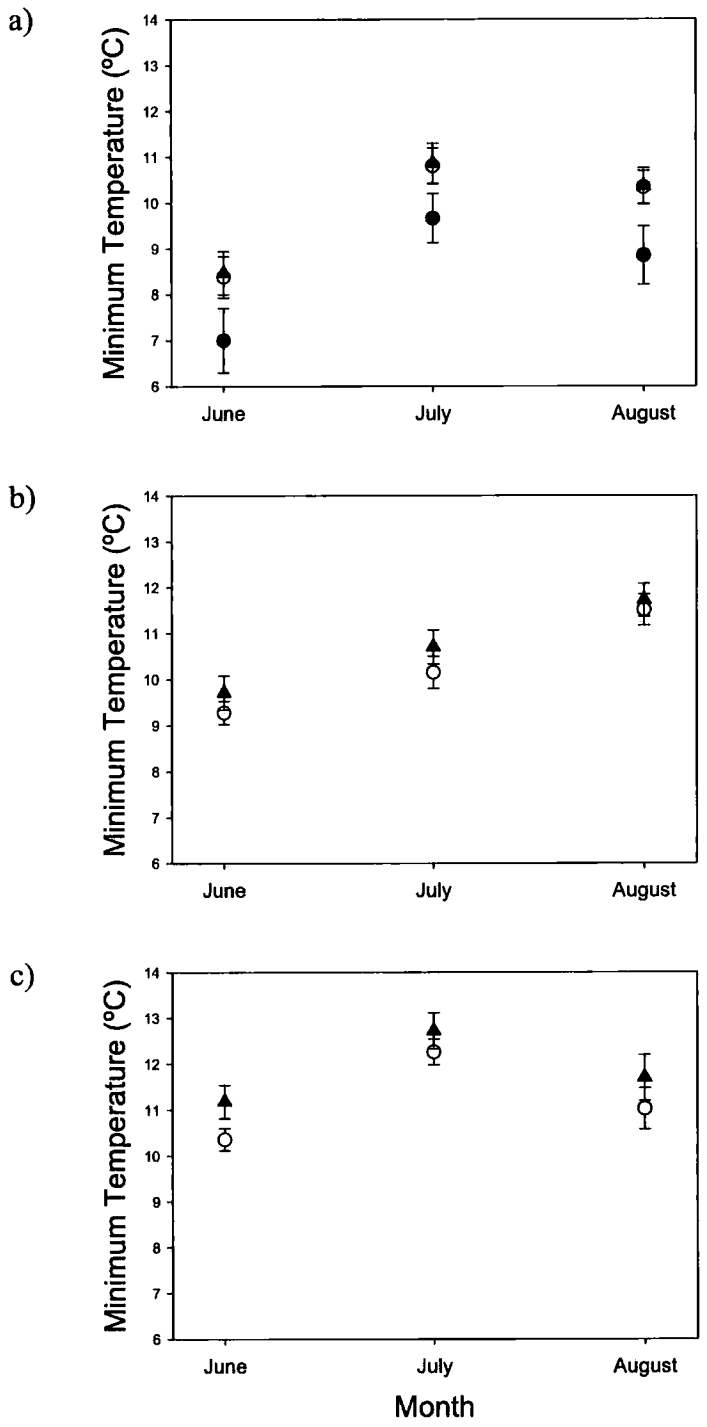


Fig 3.7: Mean minimum temperature (± 1s.e.) during the growing season (June to August) for a) 2001, b) 2002 and c) 2003, for Newcastleton (•, continuous line), Wauchope (○, dashed line) and Scootmore (Δ, dotted line).

3.4 Discussion

Newcastleton generally had the highest concentration of nutrients in the A horizon and B horizon soil. Wauchope had the lowest nutrient concentrations, with the nutrient concentrations at Scootmore being higher than those at Newcastleton for some clones, particularly in the case of phosphate concentration. The higher nutrient concentrations at Newcastleton may explain the increased growth rate of the trees at this site in comparison to the trees at Wauchope and Scootmore (Chapter 4). Greater nitrogen availability has been found to increase tree height in *P. tremuloides* (Kubiske *et al.*, 1998) and accelerated shoot growth in *P. sylvestris* (Iivonen *et al.*, 1999). However, the soil at Scootmore had greater nutrient concentrations in comparison to the soil at Wauchope. If greater nutrient concentration did increase tree growth at Newcastleton, then the trees at Scootmore are expected to be growing faster than the trees at Wauchope. However, the opposite occurred, suggesting that nutrient concentration alone cannot have caused the difference in tree growth between the sites.

Within sites, there is no general pattern in the nutrient concentration of the soil of either horizon amongst the clones. The distribution of nutrients within each site is fairly uniform, suggesting that differences in nutrient availability cannot be causing the differences in clonal growth (Chapter 4).

Silt was the greatest proportion of soil at the three sites, for the soil of all clones and for each horizon. The percentage of clay and sand was very similar at Newcastleton and Wauchope but sand percentages were higher than clay percentages at Scootmore. Overall silt percentages were highest at Wauchope and lowest at Scootmore, and clay percentages lowest at Scootmore. The increased percentage of sand at Scootmore suggests that the soil was more free-draining and unable to capture and retain as much moisture as the other two sites. With moisture during the growing season an important factor in the growth of *P. sitchensis* (Roche & Haddock, 1987), the decreased growth rate at Scootmore may be linked to a reduced ability to hold moisture.

There was little difference in precipitation receipt during the growing season of 2001 at all three sites and 2002 at Scootmore and Wauchope. Monthly precipitation at all sites was within the July precipitation levels found throughout the natural range of *P. sitchensis* (Roche & Haddock, 1987). Precipitation receipt during 2001 and 2002 was therefore

unlikely to be causing a detrimental affect on the growth and photosynthesis of the clones. However, there was a large reduction in precipitation during the growing season of 2003 at Wauchope and Scootmore. *P. sitchensis* and other conifers are dependent on abundant soil moisture, particularly during the growing season (Mäkinen *et al.*, 2001; Roche & Haddock, 1987), and this reduction in precipitation may have impacted on photosynthesis and tree growth. Any impact in photosynthesis remains unmeasured as, due to unavoidable logistical constraints, Scootmore was the only site sampled in 2003 and this site was not sampled in any other year (Chapter 7).

Wauchope and Scootmore experienced similar maximum temperatures during the growing seasons of 2001 to 2003, with Newcastleton exhibiting higher temperatures in 2001. Increased radial growth of *P. abies* in southern Finland was correlated with high temperatures in May and trunk diameter increase of *P. sylvestris* in northern Britain was correlated with summer temperatures (Hughes *et al.*, 1984; Mäkinen *et al.*, 2001). With higher temperatures at Newcastleton, the greater height and trunk diameter of the trees, in comparison to the trees at Wauchope and Scootmore, could be explained by the higher temperatures experienced at Newcastleton. The mean maximum temperatures at Wauchope and Scootmore increased between each growing season, with the highest temperatures in 2003. Temperature also impacts on photosynthetic rates (Fredeen & Sage, 1999; Lamhamedi & Bernier, 1994; Leonardos *et al.*, 1996; Ludlow & Jarvis, 1971; Neilson & Jarvis, 1975). The differences in temperature during the growing seasons of different years may have altered photosynthetic rates. Newcastleton was the only site at which photosynthesis was measured over two consecutive years. However, since the meteorological data at this site were only available for one year, it is not possible to say if temperature had impacted on photosynthetic rates.

Wauchope experienced lower mean minimum temperatures during the growing seasons of 2001 to 2003, in comparison to Scootmore. In 2001, the mean minimum temperature was lowest at Newcastleton. With increasing temperature causing an increase in photosynthesis, then lower temperatures should induce lower photosynthetic rates. Therefore, at Newcastleton the lower minimum temperatures may lead to lower photosynthetic rates in comparison to the other sites, and lower photosynthesis at Wauchope in comparison to Scootmore. Lower temperatures had not impacted on tree

growth if the minimum temperatures of 2001 are typical, as Newcastleton has the lower minimum temperatures but the highest tree growth (Chapter 4).

3.4 Conclusions

Nutrient concentrations in both the A and B horizon soils were higher at Newcastleton and lower at Wauchope. Although there were large differences between sites, the distribution of nutrients within the sites was fairly uniform, with no significant differences in the nutrient concentration available to each clone.

Silt was the largest component of the soil, and was highest at Wauchope and lowest at Scootmore. Newcastleton and Wauchope shared a similar and equal proportion of soil that was clay and sand. Scootmore had a smaller percentage of clay but a higher percentage of sand suggesting that the soil at Scootmore was more free-draining. There was little difference in the particle size distribution within each site.

There was little difference in the precipitation between sites during the growing seasons of 2001 and 2002, and between the years. During the growing season of 2003, there was a large decrease in precipitation, with the largest decline at Scootmore.

Generally, there was an increase in temperature during the three months of the growing season. Scootmore had the highest mean maximum temperatures but Wauchope had the higher absolute maximum temperatures between 2001 and 2003. Wauchope also had the lowest minimum temperatures and the lowest absolute temperature in 2001 and 2003.

4. Growth differences between full-sib families of Sitka spruce at four sites

Abstract

Height, trunk diameter and wood density data after ten years of growth were available for twelve full-sib families of Sitka spruce (*Picea sitchensis* (Bong.) Carr.), grown at four sites throughout the UK. Principal component analysis (PCA) showed that two sites, Newcastleton and Wauchope, were similar in terms of tree growth, with both sites having larger trees but lower wood density than the other two sites. The other two sites, Scootmore and Llandrindod, also showed a similar response, with trees generally smaller but having higher wood density. There was an apparent trade-off between tree size and wood density. There was a large environmental effect. The difference in PCA output was significant between sites for each family. Any differences that were not significant were between Newcastleton and Wauchope, or between Scootmore and Llandrindod, reaffirming the similarity amongst the two pairs. The similarity amongst each pair appeared to be a result of previous site use. Overall, the families had increased tree growth with respect to the control trees at all sites.

4.1 Introduction

4.1.1 Genetic variation in Sitka spruce

As with any species, Sitka spruce shows natural genetic variation. There are large differences in the growth rate between individuals, with 40% of variation in vigour characteristics accounted for between provenances and 60% within provenances (the geographic region of the seed origin) (Fletcher, 1992). In a series of experiments conducted by the International Union of Forest Research Organisations (IUFRO), Sitka spruce from various provenances in Canada and northwest USA had different growth rates when cultivated on a common site. The main factor causing growth variation was the temperature that the trees experienced in the area from which each provenance was derived. Sitka spruce from warmer provenances performed better at milder sites, but was more susceptible to frost (Nanson, 1984; Pederick, 1984; Roman-Amat, 1984), whereas Sitka spruce from colder provenances, although never able to attain such good rates of

growth, was much hardier (Alexandrov, 1984; Kleinschmit & Svolba, 1984). Within the UK, southern provenances were more at risk from frost damage, although those individuals that survived still attained greater growth rates (Lines, 1987; Lines *et al.*, 1971). Variation was also evident in the susceptibility of the provenance to attack by green aphid (*Elatobium abietinum* [Walk.]) (Carter & Nichols, 1984; Day, 1984).

Within provenances there was considerable variation. For example, Sitka spruce of Queen Charlotte Islands (QCI; British Columbia, Canada) origin had superior growth at lower elevations in comparison to individuals of the same origin grown at higher elevations (Fletcher, 1992).

4.1.2 Forest Research (FR) progeny experiments

In 1948, the Forestry Commission set up a genetics section to investigate and exploit natural variation to improve the speed and quality of timber production and to determine the provenance best suited to particular sites (Samuel *et al.*, 2000). Progeny trials have been running for many years, investigating the heritability of what are considered to be good growth characteristics. Recently, trees considered to have heritable, good growth characteristics have been cross-pollinated and cloned in numerous nursery and field trials, to help identify trees within provenances that will perform best on individual sites. Commencing in 1963, trees were selected for excellent vigour and stem traits from British Sitka spruce forests. Due to the high level of variation at the level of the individual tree, low level phenotypic selection plus progeny testing was employed to identify individuals that could be used for the improvement of timber (Fletcher, 1992). Over the next 20 years, over 1800 trees were selected, mostly of QCI origin, although records are incomplete. QCI provenance was preferred due to the similarity in latitude and climate to the UK and the provenance testing concluded that Sitka spruce of QCI origin should be planted on sites where the temperature in September drops below –5 °C (Fletcher, 1992). Since 1967, a regular programme of open-pollinated half-sib progeny testing has been running and superior stock trees have been selected based on the 15 year trunk diameter, wood density and stem form measurements of these experiments (Lee, 2001). In Scotland, the breeding programme has resulted in a 14% increase in wood volume at Kilmichael, Argyll at 27 years, whilst at Aultmore, Moray and Whitrope, Scottish Borders, there was an increase by 13% in trunk diameter after 22 years (Lee, 1992). Although it is possible to see the superiority of the progeny after 20 years or more of growth in the field, the length of time

it takes to gain results increases the length of time before the progeny can be used commercially. With regular measurements of the growth characteristics the trials can be used to predict, after a shorter period of time, the outcome after 20 years. Using a progeny test site in Garcrogo Forest, south-west Scotland, planted in 1972, it was calculated that, based on family means, mid-rotation diameter could be selected for using height at 5 years, although this increased to 7 to 9 years for individual trees (Lee *et al.*, 2002a). For both individuals and family means, mid-rotation wood density could be predicted from the mean weighted density of the outer 4 rings from a 9-year-old tree (Lee *et al.*, 2002b). From a range of sites across north and west England, Scotland and central Wales, trees selected at 6 or 10 years for their vigour characteristics retained their superiority at 15 years (Lee, 1992).

There is only a limited amount of improvement that can be gained from open pollination of superior phenotypes. By using both male and female gametes from selected trees the genetic gain can effectively be doubled (Shelbourne, 1969). FR now employs this approach in its current progeny testing. In the Borders region of Scotland, a progeny test of specific crosses has been replicated at two sites and, after 4 years growth in the field, there was a 46% increase in height when compared with the QCI control (Mboyi & Lee, 1999). This increase in height was mainly due to the greater lammas growth of the progenies (the free growth that occurs after predetermined growth has been completed), which was very small in the control trees. However, there was an associated increase in frost damage with increased lammas growth. The progeny showed only a small increase in frost susceptibility (5%) and by selecting the crosses that showed the greatest overall height there was a decrease of 4% in frost damage compared to the control.

4.1.3 Hypotheses

The above field experiment, although showing a large increase in height, was based on two sites with similar rainfall, slope and daylength. This increase may not be so great or may be even greater if replicated on sites that are very different from these test sites. This may also be true of the open pollinated trials; although these showed an increase in various growth characteristics, the data are only available for one site. Therefore using the clonal trial at the two sites mentioned above plus two further replicated sites at very different locations, the following were investigated:

- differences in tree height, trunk diameter and wood density between sites caused by changes in the environment
- differences in tree height, trunk diameter and wood density between full-sib families within and between sites, to explore the genotypic response to the environment.

4.2 Experiment Background

Data are available for four FR experimental sites within the UK (Fig 4.1): Scootmore in north-east Scotland, Newcastleton and Wauchope in the Scottish Borders, and Llandrindod in Wales. Table 4.1 contains detailed site information. The same experiment was replicated at each site. 'Plus' trees (trees with excellent heritable growth characteristics) were cross-pollinated, with each parent used once in a unique combination (Table 4.1c). The resulting seeds were grown in a nursery and eight seedlings selected from the crosses that exhibited the growth characteristics of the parents. The eight seedlings created a full-sib family and, from each seedling within a family, 15 cuttings were taken and grown into seedlings, effectively creating clones (Fig 4.2). For clarity, each set of 15 individuals will be referred as a 'clone', with a unique number identifying each set. With eight clones in each family and with 12 full-sib families, 94 clones were created, with 1410 individuals in total (two families has only seven sibs). Each site was split into two plots. Each plot contained six full-sib families and the same families were planted on each site. Therefore, the same clones were planted on the same plot at each site. The plots were split into 15 fully randomised blocks and each block contains one individual of each clonal set, planted at a distance of 2 m from each neighbour in all directions. Each block also contained two control trees of unimproved Sitka spruce of QCI origin. The design of the blocks varied between sites (Table 4.2). At Wauchope and Llandrindod there were thirteen and seven sets of clones missing respectively.

For all sites individual tree height, trunk diameter at breast height (1.3 m; DBH) and wood density data after 10 years growth in the field were available. Missing data represented tree mortality or, for wood density data only, individual trees considered too small to measure. Height was measured using a hypsometer and DBH with a girth tape. Wood density was measured indirectly using a Pilodyn® machine (PROCEQ, Zürich, Switzerland), where a blunt pin is fired into the trunk with a fixed force of 6 joules and the penetration distance is inversely related to the wood density.

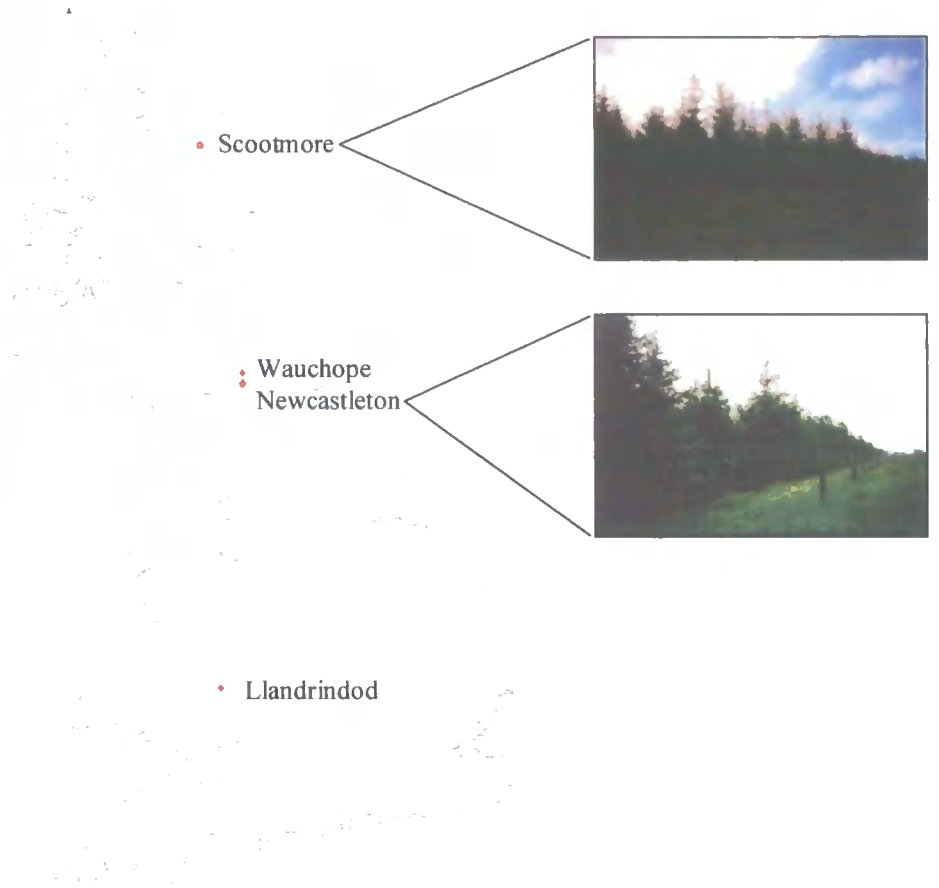


Fig 4.1: Map showing locations of clonal experiment plots.

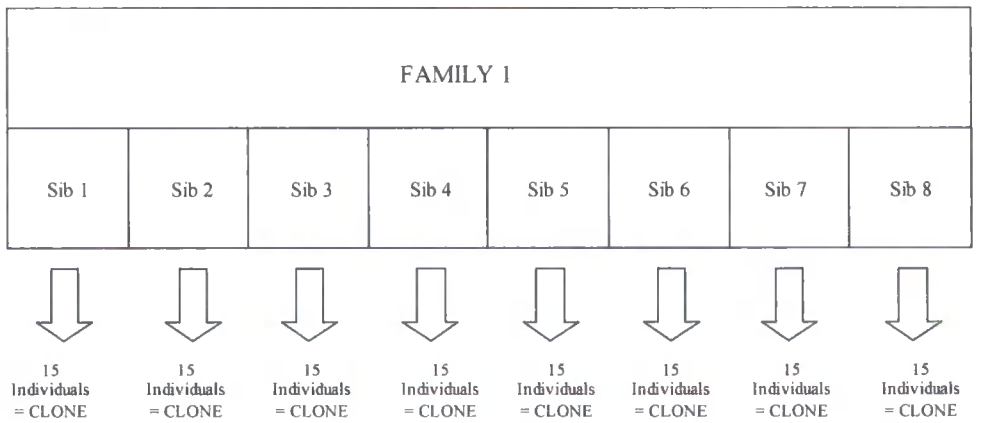


Fig 4.2: Diagram showing the split of trees into individuals and sibs in each family.

Table 4.1: Table of the site information for all four sites, a) general site characteristics, b) the mean height, mean diameter at breast height (DBH) and mean wood density ± 1 s.d. for each plot, and c) the clones that belong to each family and their parental cross.

a)

Site	Grid ref	Aspect	Altitude (m)	Soil Type
Newcastleton	NY507889	SE	180	Upland brown earth, cultivated
Scootmore	NJ172392	NE	240	Iron pan, shallow layer of peat
Wauchope	NT556054	NW	320	Iron pan / peaty gley
Llandrindod	SO212662	SE / N	360 / 450	Shallow upland brown earth

b)

Site	Plot 1			Plot 2		
	Mean Height (m) ± s.d.	Mean DBH (cm) ± s.d.	Wood Density (mm) ± s.d.	Mean Height (m) ± s.d.	Mean DBH (cm) ± s.d.	Wood Density (mm) ± s.d.
Newcastleton	6.71 ± 0.91	11.31 ± 1.97	19.30 ± 3.00	6.69 ± 0.79	12.09 ± 1.95	18.84 ± 3.49
Scootmore	4.75 ± 1.08	6.66 ± 1.91	12.77 ± 3.29	4.90 ± 1.25	6.97 ± 2.47	13.87 ± 3.68
Wauchope	5.96 ± 0.81	10.99 ± 1.94	18.73 ± 2.73	5.76 ± 0.76	10.92 ± 2.08	17.67 ± 5.06
Llandrindod	4.82 ± 0.89	8.03 ± 1.91	10.29 ± 2.22	5.81 ± 1.44	9.15 ± 2.54	14.44 ± 3.05

Table 4.1 cont'd

c)

Site	Plot 1			Plot 2		
	Family	Crosses	Clone Numbers	Family	Crosses	Clone Numbers
Newcastleton	1	689 x 493	C20121-28	7	1150 x 1492	C20169-76
	2	1102 x 1370	C20129-36	8	1615 x 141	C20177-84
	3	94 x 1500	C20137-44	9	2000 x 563	C20185-92
	4	321 x 1463	C20145-52	10	2099 x 980	C20193-200
	5	727 x 769	C20153-60	11	2122 x 1773	C20201-04, 06-08
	6	946 x 543	C20161-68	12	3159 x 492	C20209-14, 16
Scootmore	1	689 x 493	C20121-28	7	1150 x 1492	C20169-76
	2	1102 x 1370	C20129-36	8	1615 x 141	C20177-84
	3	94 x 1500	C20137-44	9	2000 x 563	C20185-92
	4	321 x 1463	C20145-52	10	2099 x 980	C20193-200
	5	727 x 769	C20153-60	11	2122 x 1773	C20201-04, 06-08
	6	946 x 543	C20161-68	12	3159 x 492	C20209-14, 16
Wauchope	1	689 x 493	C20121-27	7	1150 x 1492	C20169-76
	2	1102 x 1370	C20129-36	8	1615 x 141	C20177-84
	3	94 x 1500	C20137-42	9	2000 x 563	C20185-92
	4	321 x 1463	C20145-49, 51-52	10	2099 x 980	C20193-97, 98-200
	5	727 x 769	C20153-60	11	2122 x 1773	C20201-04, 06, 08
	6	946 x 543	C20161,63-65, 67-68	12	3159 x 492	C20209-11, 13, 14, 16
Llandrindod	1	689 x 493	C20121-28	7	1150 x 1492	C20169-76
	2	1102 x 1370	C20129-36	8	1615 x 141	C20177-84
	3	94 x 1500	C20137-44	9	2000 x 563	C20186-88, 90-92
	4	321 x 1463	C20145-52	10	2099 x 980	C20193, 95-96, 99-200
	5	727 x 769	C20153-60	11	2122 x 1773	C20201-03, 06, 08
	6	946 x 543	C20161-68	12	3159 x 492	C20209-11, 13, 14, 16

Table 4.2: Layout of the two plots within the clonal experiment at each site. Each number represents the number of trees in a row (first number) or column (second number). Each tree was planted 2 m apart.

	Layout of trees	
Site	Plot 1	Plot 2
Llandrindod	7 x 7	9 x 5
Newcastleton	5 x 10	5 x 10
Scootmore	5 x 10	12 x 4
Wauchope	9 x 5	8 x 5

4.3 Method

Using SPSS® release 10 (SPSS Inc, Chicago, USA), the data for tree height, DBH and wood density were standardized for both sites. A preliminary investigation using principal components analysis (PCA) was run using all three variables to examine the differences between sites and between families within each site. Using the mean PCA score from axis 1 for each family, the distance from the site mean of axis 1 scores was calculated to compare the positions of the same families at different sites. The range of PCA scores for axis 1 was also calculated to determine the variation for each family at each site. A oneway Analysis of Variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) *post hoc* test was then employed to determine significant differences between families within and between sites.

4.4 Results

4.4.1 Principal Components Analysis (PCA)

The PCA extracted three axes with eigenvalues of 2.453, 0.370 and 0.176, explaining 82%, 12% and 6% of the variation respectively. The first axis, explaining the majority of the variation, had a large positive loading for each variable. The second axis had large and small negative loadings for height and DBH respectively, and a large positive loading for wood density. The third axis had small positive loadings for height and wood density and a

negative loading for DBH (Table 4.3). Due to the small percentage of variation it explained, the third axis was omitted from further analysis.

Table 4.3: The eigenvalues, percentage of variance explained and the loadings of the PCA axes, using standardised height, DBH and wood density data.

	Component		
	1	2	3
Eigenvalues	2.453	0.370	0.176
% of variance explained	81.78	12.35	5.88
Loadings			
Standardised Height	0.886	-0.425	0.184
Standardised DBH	0.942	-0.008	-0.335
Standardised Wood Density	0.883	0.435	0.173

The different sites occupied different spaces when plotted using the mean PCA scores of axes 1 and 2 for all individuals of each full-sib family (Fig 4.3). For the first axis, the families of Newcastleton and Wauchope had mostly positive scores, whereas Scootmore and Llandrindod had mostly negative scores. The positions on the second axis were less distinct with Wauchope and Scootmore having mostly positive scores and Newcastleton and Llandrindod mostly negative. There was less distinction between Newcastleton and Wauchope and between Scootmore and Llandrindod, but there was a definite distinction between the two pairs.

The mean PCA axis 1 score for each family within each site is shown in Fig 4.4. Newcastleton and Wauchope showed similar positions as did Llandrindod and Scootmore, particularly for families 1 to 6, which were the families found on plot 1 at all sites. Llandrindod and Scootmore had the lowest mean differences and Newcastleton had the highest with the exception of family 3. The results of the oneway ANOVA (Table 4.4) showed that the families were occupying different PCA spaces within each site. The *post*

hoc test showed that there were some families that were not statistically significantly different (Fig. 4.4, not all results are shown) and these were usually Llandrindod and Scootmore or Newcastleton and Wauchope. The exceptions to this were with families 7 and 9, where Llandrindod and Wauchope showed similar distances from the mean.

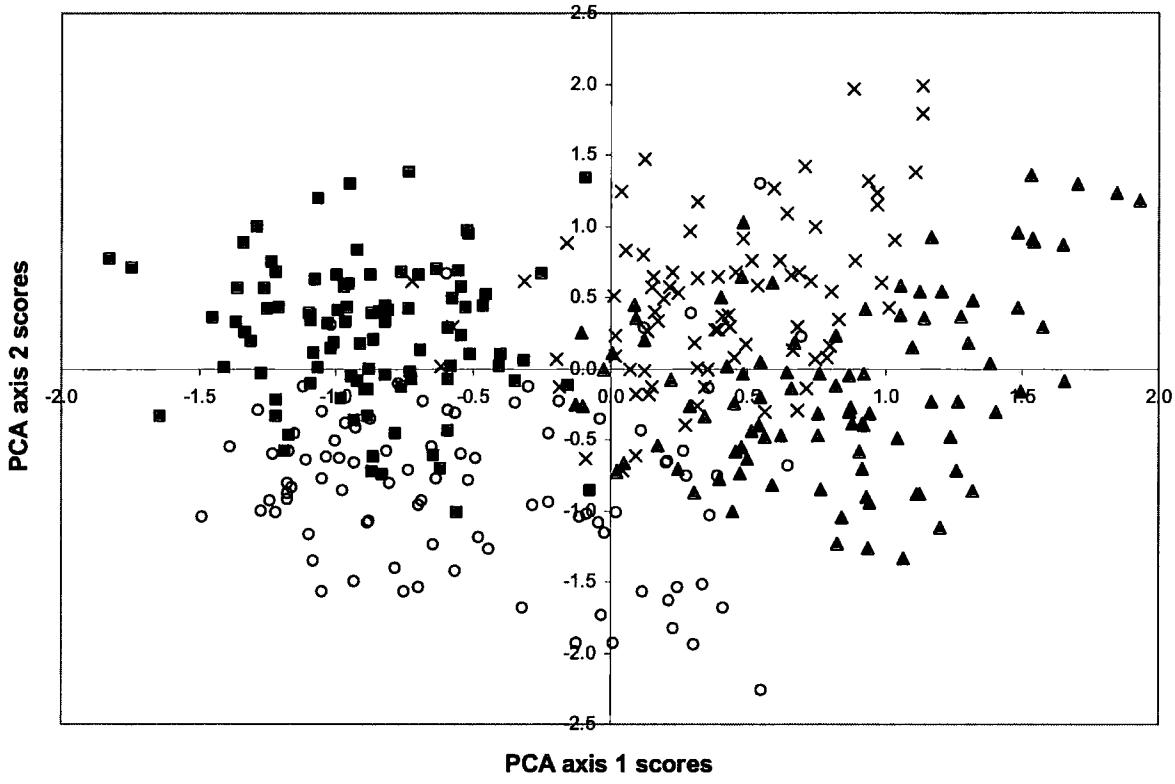


Fig 4.3: Scatterplot of PCA axis 1 scores against PCA axis 2 scores. Each point represents the mean of the 15 individuals for each clone. Error bars have been omitted for clarity. o represents Llandrindod, ▲ Newcastleton, ■ Scootmore and x Wauchope.

The difference between mean PCA axis 1 score of each family and the site mean is shown in Fig 4.5. Families 3 and 12, and the control, had negative differences for each site, whilst families 8 and 11 were all positive. The other families had a mixture of positive and negative differences. However, for families 1 to 6 at Llandrindod the difference was always negative and for families 7 to 12 always positive, which corresponds to plot 1 and plot 2 respectively. All other sites had a mixture of positive and negative differences within each plot. Newcastleton and Wauchope had very similar differences within families 2 and 12, Newcastleton and Scootmore had similar differences within family 4, and within family 8 Newcastleton and Llandrindod were very similar. For families 5 and 11 Scootmore and Wauchope had almost identical differences with Newcastleton also showing a similar value. There were no obvious relationships between families that

showed a similar position from the site mean but there did appear to be a slight grouping of Newcastleton and Wauchope.

Table 4.4: Results of oneway ANOVA investigating differences of PCA axis 1 scores between sites for each family. d.f. is degrees of freedom

Family	Within d.f.	Between d.f.	F	*P*
1	3	419	264.360	<0.001
2	3	433	435.322	<0.001
3	3	373	302.379	<0.001
4	3	428	230.870	<0.001
5	3	428	340.519	<0.001
6	3	358	210.689	<0.001
7	3	396	83.902	<0.001
8	3	395	178.407	<0.001
9	3	384	100.242	<0.001
10	3	342	87.140	<0.001
11	3	306	120.444	<0.001
12	3	309	93.754	<0.001
Control	3	167	57.710	<0.001

The range of PCA axis 1 scores for each family at each site are shown in Fig 4.6. Scootmore had the largest range for all families, except family 7, with Wauchope usually having the smallest range. Newcastleton and Llandrindod swap in their ranking depending on the plot. In plot 1 (families 1 to 6) Newcastleton usually had the larger range and for plot 2 (families 7 to 12) Llandrindod had greater ranges than Newcastleton. The control followed the same pattern as plot 2. Very few of the families had identical ranges at all sites, although Wauchope and Llandrindod had a very similar range for family 1, and family 5 at Newcastleton, Wauchope and Scootmore had almost equal ranges.

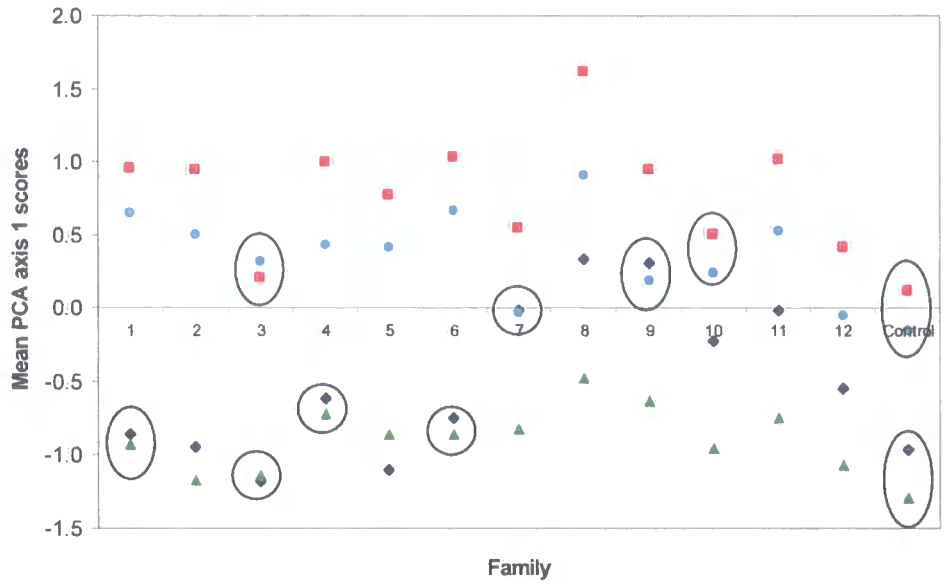


Fig 4.4: The mean PCA axis 1 scores for each family at all sites. ♦ represents Llandrindod, ■ Newcastleton, ▲ Scootmore and ● Wauchope. Error bars have been omitted for clarity. Ringed points show a **non-significant** result from Tukey's HSD *post hoc* test.

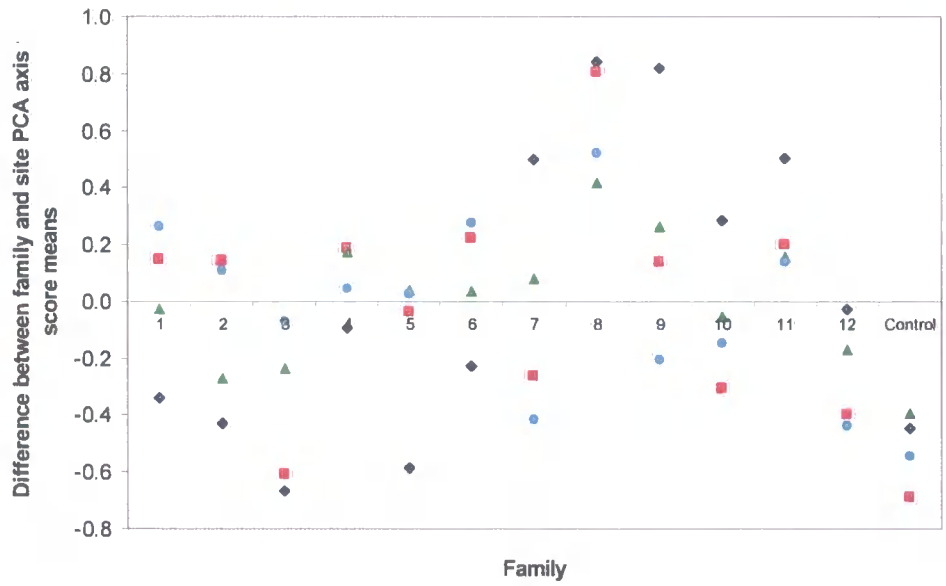


Fig 4.5: The difference between mean PCA axis 1 scores for each family and the mean PCA axis 1 score for the site, for all sites. ♦ represents Llandrindod, ■ Newcastleton, ▲ Scootmore and ● Wauchope. Error bars have been omitted for clarity.

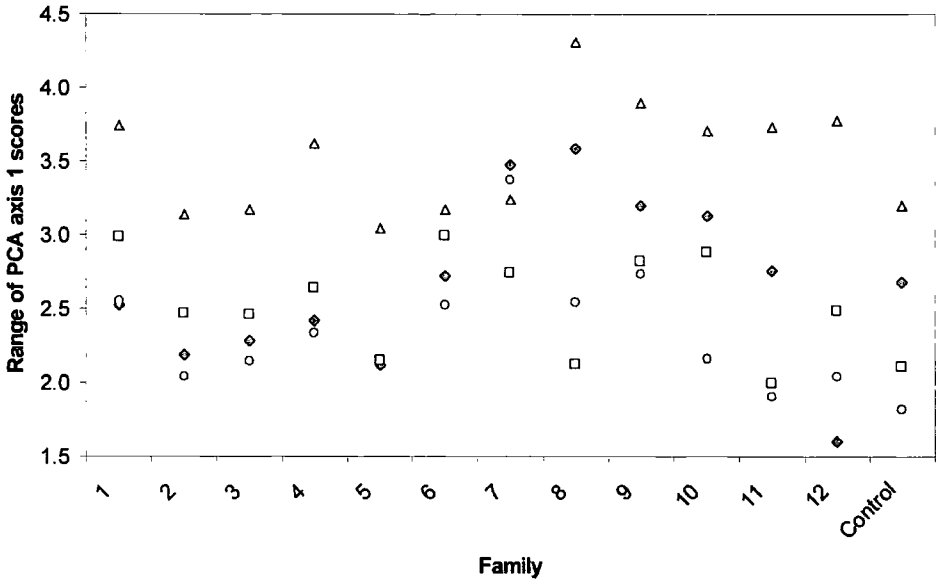


Fig 4.6: Range of PCA axis 1 scores for all individual trees belonging to each family for all sites. ♦ represents Llandrindod, □ Newcastleton, △ Scootmore and ○ Wauchope.

4.5 Discussion

The results of the PCA show that there was one main axis explaining over 80% of the variation that, based on the loadings, described a gradient running from tall trees with a large DBH but low wood density (positive PCA axis 1 scores) to short trees with a small DBH and high wood density (negative PCA axis 1 scores). The second axis, explaining a much smaller percent of the variation, described a gradient running from tall trees with large DBH and high wood density (positive PCA axis 2 scores) to short trees with thin trunks and low wood density (negative PCA axis 2 scores). However, the loadings showed that height and wood density had the largest effect, with DBH being almost negligible. The third axis, explaining very little of the variation, was affected mainly by the DBH with a lesser but equal effect of height and wood density. As axis 1 explained the majority of the variation, it can be concluded that the majority of the trees were investing in their growth in a particular method. Trees either grew quickly, gaining greater height and thicker trunks, although at the cost of the wood density, or the trees grew more slowly, were shorter and thinner, but with higher wood density. A negative correlation between wood density and height or diameter had been found previously (Pfeifer, 1984; Wood, 1986) and it seems that generally Sitka spruce invests in size or wood density but not both.

The scatterplot of PCA axis 1 scores against PCA axis 2 scores showed how the trees respond to the different environmental conditions at each site. At Newcastleton, the trees were mainly tall with large diameters but low wood density, with some that were smaller and thinner but still with low wood density. At Wauchope, the majority of the clones were similar to the Newcastleton trees but with some tall trees having large diameters and high wood density. At Scootmore, the trees were small and thin with high wood density, with some that were taller with larger diameters but still had high wood density. Llandrindod was similar to Scootmore but some of the trees were small and thin but with low wood density. From this analysis, Newcastleton and Wauchope appeared to be similar sites in terms of tree growth. The two sites were situated within a similar region in the UK, with similar climates (Mboyi & Lee, 1999) and both were previously old pastures. Llandrindod and Scootmore also showed similar responses and, although they were situated in very different locations geographically, both were re-stock sites, i.e. the land had previously been used for timber production. However, all sites were distinct in Fig 4.3, so there was a definite environmental effect, allowing the same clones to behave differently at each site.

By focusing attention on the PCA axis 1 scores, it was possible to investigate the differences caused by the environment at each site. The mean scores for each family at each site showed the same pattern, in that Newcastleton and Wauchope were similar sites, as were Scootmore and Llandrindod. The results of the oneway ANOVA showed that there were significant differences between sites for each family, but where the *post hoc* test had shown there were no significant differences it was usually between Newcastleton and Wauchope or Scootmore and Llandrindod. The exceptions to this were families 7 and 9, where Llandrindod and Wauchope were not significantly different. Both families were found on plot 2 of the FR experiments and at Llandrindod this plot had a particularly high mortality rate (29%). The increased light availability to the surviving trees may have increased tree height and DBH, making it very similar to the sites with greater heights and DBHs – Newcastleton and Wauchope. The significant results of the oneway ANOVA showed that the environmental effect at each site was strong. If the environment was not exerting a large effect then each clone would grow at the same rate and in the same manner, and the oneway ANOVA would show no or few significant differences between sites within families. All the family means were greater than the control mean for each site, which suggested that phenotypic selection for superiority was successful, as has been shown at many sites before (Lee, 1992; Mboyi & Lee, 1999). However, at Llandrindod

there were two families with means less than the control, suggesting that clones suitable for enhanced growth at one site may not be suitable for others, although generally the success was high for most families.

Although the clones and their families were significantly different between sites, there still may be a genetic effect exerted on the trees if the families were in the same relative positions with respect to the PCA axes within each site. By calculating the distances of the family mean from the overall site mean, if the distance was the same it was possible to say the families were in the same relative position. The results showed a mixed response. Families 3, 8, 12 and the control were either all positive or negative for each site, showing that the families were all performing in the same way, i.e. they were growing larger or smaller than the mean. However, the distances of the families from the mean at each site were not very similar, suggesting that the genetic effect was not very strong. For the other families there was a mixture of positive and negative values suggesting that the environmental effect was stronger than the effect exerted by the genotype. There were some families that were showing very similar distances from the mean at several sites (families 2, 4, 8 and 12) and there were two families that had three sites showing very similar differences. Newcastleton, Wauchope and Scootmore were all very similar for families 5 and 11, with Wauchope and Scootmore almost identical. However, there were no families where all sites showed the same differences so the environment was having the largest impact on the growth of the trees. There was also no obvious relationship between which sites had the same or very similar distances. However, Newcastleton and Wauchope had the most similar distances leading to the suggestion that the clones within the two sites were responding in the same way.

An interesting pattern emerged for Llandrindod, where the differences between the family and site means were all negative for families 1 to 6 and all positive for sites 7 to 12. Families 1 to 6 corresponded to plot 1 and families 7 to 12 to plot 2. Plot 1 and 2 were found at different locations within the site and this may have affected the growth ability of the trees depending upon their location. The mortality rate was much higher on plot 2, increasing light availability, which may have led to increased growth, so the trees were always larger than the site mean.

The range of the PCA axis 1 scores showed that Scootmore had the largest variation with respect to the growth variables for all families, which suggested that Scootmore had a

larger environmental impact affecting the growth of the trees. The range would be small if the environmental impact was insignificant or the genetic factor was greatly imposed. Wauchope had the smallest ranges, so all trees within each family were growing in a similar manner, suggesting that the effect from the environment is not as great as the other sites. Newcastleton and Llandrindod swapped in their ranking between plots, with an increase in the ranges on plot 2 (families 7 to 12) for Llandrindod. This again would seem to correspond with the difference in location and mortality rates at Llandrindod manifesting itself within quite different growth performances. There was little difference in the family ranges of PCA axis 1 scores between the two plots at Newcastleton, suggesting that there was little variation in the environmental or genetic impact experienced within each plot. Therefore, it appeared that Llandrindod was causing the change in ranking between the plots.

The large ranges for each family suggested that there was large variation at the individual tree level. Surprisingly, the control ranges were generally smaller than the family ranges. As the control was unimproved Sitka spruce of QCI origin, it would consist of a mixture of genotypes and would therefore be expected to show more variation than the individuals of each family. However, as the control was growing more slowly it may be that the trees had not yet reached a stage in their growth where the genetic variation is fully expressed. Newcastleton was the best growing site in terms of height and diameter, indicating that the trees were growing fastest on this site. The control range was larger than some of the families at Newcastleton, indicating that it had more variation. On a faster growing site, the control may have reached a stage in its growth where the genetic variation was starting to show.

4.6 Conclusions

Newcastleton and Wauchope have been shown to be similar sites in terms of the tree growth strategy, with larger trees but lower wood density than the other two sites. Scootmore and Llandrindod also showed a similar pattern in their growth although with smaller trees and higher wood density. It appeared that the trees at the pairs of sites that group together were investing their growth differently, with a trade off between size and wood density. There was a large environmental effect, swamping that of the genotype, as the mean PCA axis 1 scores for all sites were significantly different for all families and the

distance of the family mean from the site mean showed that the positioning within site was different for all families. Where there was some non-significant results it was usually between Newcastleton and Wauchope or Scootmore and Llandrindod, again suggesting that the sites could be split into two pairs. Newcastleton and Wauchope were similar sites in respect to their location, rainfall, slope, daylength and past use, so it was not surprising they were showing a similar clonal response. Scootmore and Llandrindod were contrasting sites in that they were located in very different regions of the UK with differing climatic conditions. However, their previous land use was as conifer plantation and this appeared to be the major factor affecting the growth of the trees. Overall, it seemed that the clonal trial had been successful in improving the rate of growth at all sites. However, the environment still had a large effect on the growth of the clones, although the genotype did appear to have an impact, especially when the sites had the same use history.

5. Similarities and differences between and amongst clones of Sitka spruce grown at four contrasting sites

5.1 Introduction

5.1.1 Environment versus genetics

The environment has a large impact on tree growth. Fourteen provenances of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) were grown at eight different sites across British Columbia, Canada, where the majority of variation in growth could be explained by environmental factors, with climate and attack by white pine weevil (*Pissodes strobi*) accounting for over two-thirds of this environmental variation (Ying, 1997). Environment was also the largest cause of differences in lipid concentration in 19 provenances of Norway spruce (*Picea abies*) when grown on two different sites in England (Wellburn, 1997).

Different provenances of Sitka spruce, however, do show a similar response between different sites, indicating that genetic control of growth is stronger than the environmental effects. In the early stages of seedling growth, there was the same latitudinal pattern in the response of fourteen Sitka spruce provenances at eight sites across British Columbia, Canada. The similarity was particularly striking at those sites with a strong oceanic climate and were free of weevil attack. As the seedlings aged, the pattern amongst the provenances changed to a longitudinal effect although it was evident only at sites that were harsher, more northerly and further inland (Ying, 1997). In 24 clones of the aspen hybrid *Populus tremula* x *Populus tremuloides*, differences were found in the stability of the growth response. The clones could be split into three groups, based on results of height and diameter data from four sites; low stability clones, which grew very well on good sites and poorly on poor sites, indicating a large environmental impact on growth; average stability clones, which grew well on poor sites but better on good sites; and high stability clones, which grew equally well on all sites, indicating a small environmental impact and strong genetic control on growth (Yu & Pulkkinen, 2003).

During common site experiments of different provenances of Sitka spruce, 40 % of the variation in the vigour characteristics was accounted for between and 60% within

provenances (Fletcher, 1992), showing considerable variation between trees from different areas of the natural populations but also a large proportion of variation between trees within the same area. Although provenances may show the same growth patterns between different sites, there is considerable difference in growth within provenance, suggesting that environmental differences within the site are affecting tree growth.

5.1.2 Hypotheses

Using the same height, trunk diameter and wood density data detailed in Chapter 3, it was hypothesised that:

- The clones can be classified into different groups at each site and that the groups represent different positions on the gradient running through the PCA main axis as detailed in Chapter 3.
- The data could be split into the same groups at each site.
- A clone selected as representative of each group will show the same clone ranking for height, trunk diameter and wood density at each site.
- The different sites will generally show differences in the three variables regardless of clone, as a result of the environmental differences.

5.2 Methods

The data used in Chapter 3 were also used for the selection of the clones. For the data analysis, only Newcastleton and Scootmore sites were used. Wauchope was excluded from the analysis because of its similarity in geographic and climatic position to Newcastleton, and Newcastleton was the superior site in terms of height growth and survival. Llandrindod was excluded due to the high mortality rate of the site (19% overall; 8% plot 1 and 29% plot 2) and dissimilarity between the two plots. Newcastleton and Scootmore were contrasting sites, where Scootmore was the colder site (Fig 5.1) and also had lower rainfall, therefore potentially having the shorter growing season (Fig 5.2). The sites and the plots were analysed separately and all trees considered individually, and only identified as an individual of a particular clone during the final selection stage.

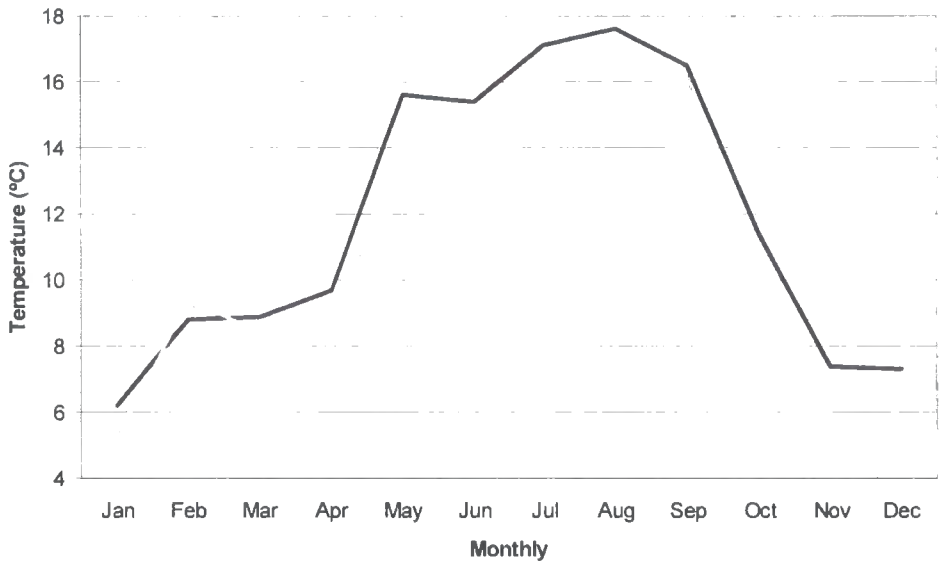


Fig 5.1: Mean monthly maximum temperature for Newcastleton (—) and Scootmore ( ) during 1998. Calculated from daily temperature data, taken from nearest Met Office weather station to site, provided by the British Atmospheric Data Centre.

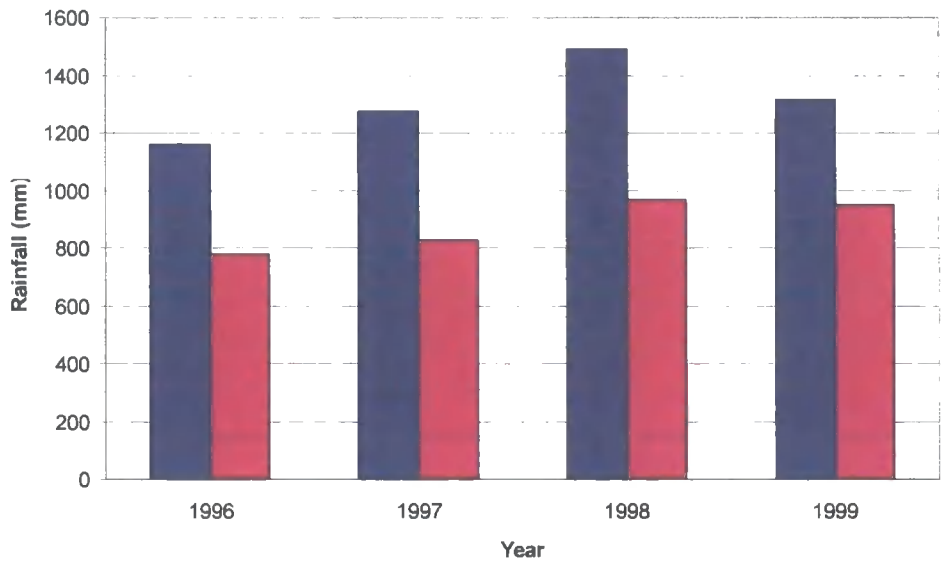


Fig 5.2: Yearly rainfall for Newcastleton (■) and Scootmore (■) during the period 1996 to 1999. Calculated from daily rainfall data, taken from nearest Met Office weather station to site, provided by the British Atmospheric Data Centre.

As the PCA indicated that there were no visible clusters within each site, a hierarchical cluster analysis using Ward's method was performed on all three variables, to group

individual trees into clusters based on similar growth characteristics. The hierarchical analysis partitioned the individuals into a number of clusters so the total within-group sum of squares around the centroid was as small as possible. To begin with each individual was considered a separate cluster and, using an agglomerative algorithm, clusters were amalgamated so that the increase in the sum of squares was the smallest possible. This continued until all individuals were combined into one cluster. The resulting dendrogram illustrated how similar clusters were by the length of the branching arm at each split. A large difference was represented by a long arm and, where this occurred, it was considered to be a split into separate clusters.

Within the hierarchical cluster analysis, once an individual was assigned to a group it could not be moved, even if this would improve the total within-group sum of squares. However, this was achieved by subsequently using a K-means cluster analysis. This approach had the same objective as the hierarchical cluster analysis but used an iterative relocation algorithm. Using randomly picked centroids, the individuals were split into groups, which were then modified by moving individuals from one group to another if it reduced the sum of squares. Overall it produced a 'tighter' cluster, with a smaller sum of squares, than the hierarchical technique.

The advantage of using the hierarchical cluster analysis and the resulting dendrograms was it indicated how many clusters there were in the data. This information was fed into the K-means cluster analysis, which required the final number of clusters as an input. The mean values of the variables for each hierarchical cluster could be used to create hypothetical individuals used as the initial centroids for the K-means clusters. This was required to achieve clusters with the smallest sum of squares overall, which may not have happened if random centroids were used.

After the data had been split into clusters using the cluster analyses, the clone number was identified for each tree. A cluster was then assigned to each clone, based on its 15 individuals. If all individuals did not belong to the same cluster, then the clone was assigned to the cluster the majority of the individuals belonged to. One clone was selected from each cluster using the following procedure.

1. A representative clone within each cluster was chosen for the Newcastleton site, where clones with the largest number of individuals assigned to the cluster (i.e. a maximum of 15) were primarily selected.
2. The clones chosen for Newcastleton were then compared with the same clones at the Scootmore site. Although these clones did not necessarily belong to the same cluster at Newcastleton, they had to be in different clusters within Scootmore.
3. If the clones were not in different clusters at Scootmore, step 1 had to be repeated to find the next suitable clone, and was continued until the representative clones at Newcastleton were in different clusters at Scootmore.
4. Where there were several clones belonging to the same cluster and they all had the same number of individuals belonging to that cluster, the clone with the smallest sum of squares was used.
5. The representative clones for each cluster had to belong to different families and be present at all sites (including Wauchope and Llandrindod) otherwise steps 1 to 5 had to be repeated.

At the end of this analysis there was a set of clones for plot 1 and a set of clones for plot 2, each belonging to a different cluster at Newcastleton and Scootmore and a different family for all four sites.

This research only utilised one plot. For plot selection the ranking of the clones was considered, i.e. how many individuals belonged to the same cluster assigned to each clone. For example, if plot 1 had 15 individuals all belonging to the same cluster for each clone and plot 2 only had 10 individuals, plot 1 would be preferred.

5.3 Results

5.3.1 Hierarchical Cluster Analysis

At Newcastleton, the dendrogram for plot 1 showed four distinct clusters (Fig 5.3a). After an initial very distinct split, each cluster could be further split into two distinct clusters. Within plot 2, there were two very distinct clusters again and one of these could be further split into two less distinct clusters (Fig 5.3b).

The resulting dendrograms of the hierarchical cluster analysis for Scootmore showed that for plot 1 there were two very distinct clusters and both could be further split into two, less distinct, clusters (Fig 5.3c). Within plot 2, the dendrogram showed two distinct clusters and one of these could be split again (Fig 5.3d), although the difference between the two new clusters was small.

At both Scootmore and Newcastleton, within plot 1 there were four clusters and within plot 2 there were three clusters.

5.3.2 K-Means Cluster Analysis

The K-means cluster analysis illustrated that there were no clones where all individuals were classified into the same cluster, except for clones C20180 and C20177 in plot 2 at Newcastleton. At both sites, the two plots showed different responses with respect to the classification of a clone's individuals into clusters. Plot 2 had a large number of clones where 10 or more individuals were classified into the same cluster. However, for plot 1 the majority of clones had fewer than nine individuals classified into the same cluster, resulting in a larger sum of squares.

5.3.3 Clone selection

Plot 1

The clones initially chosen for plot 1 at Newcastleton were C20158 for cluster 1, C20133 and C20148 for cluster 2, C20123 for cluster 3 and C20168 for cluster 4 (Fig 5.4a). When comparing with Scootmore (Fig 5.4c), C20123 and C20133 were rejected as both were split between two clusters, and C20168 was rejected as it was in the same cluster as C20148. The next clone chosen for cluster 3 at Newcastleton was C20138, which was acceptable at Scootmore. For cluster 4, there were three more possibilities. Two were dismissed at Scootmore as they belonged to the same cluster as a previously chosen clone. The remaining clone, C20134, was accepted as, although it belonged to the same cluster as C20138, that particular cluster was made up of a large number of individuals and the cluster with no representative clone was so small the analysis was unable to find a clone with the majority of individuals belonging to that particular cluster.

Plot 2

The clones initially chosen for plot 2 at Newcastleton were C20174, C20193, C20211 and C20213 for cluster 1, C20189, C20200 and C20216 for cluster 2, and C20177 and C20180 for cluster 3 (Fig 5.4b). Both C20177 and C20180 have all 15 individuals classified into cluster 3, so the clone with the smallest sum of squares was chosen (C20177). When the other representative clones at Newcastleton were compared with those at Scootmore (Fig 5.4d), C20200 was split between two clusters and C20189 belonged to the same cluster as C20177. Both were rejected and C20216 chosen as representative of cluster 2. For cluster 1, C20174 belonged to the same cluster as C20216 at Scootmore and was rejected. C20211 was chosen from the three remaining clones as it had the smallest sum of squares. However, C20211 and C20216 belong to the same family. C20211 was selected because of its smaller sum of squares and larger number of individuals classified into the cluster assigned to the clone. Of the next choices for cluster 2, C20208 had the smallest sum of squares.

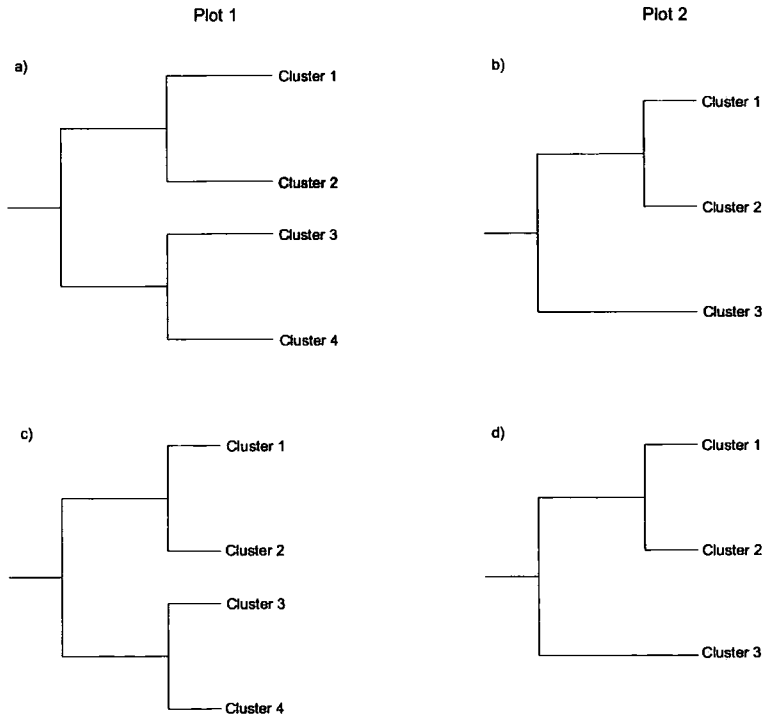


Fig 5.3: Dendrograms from the hierarchical cluster analysis for a) Newcastleton plot 1, b) Newcastleton plot 2, c) Scootmore plot 1 and d) Scootmore plot 2.

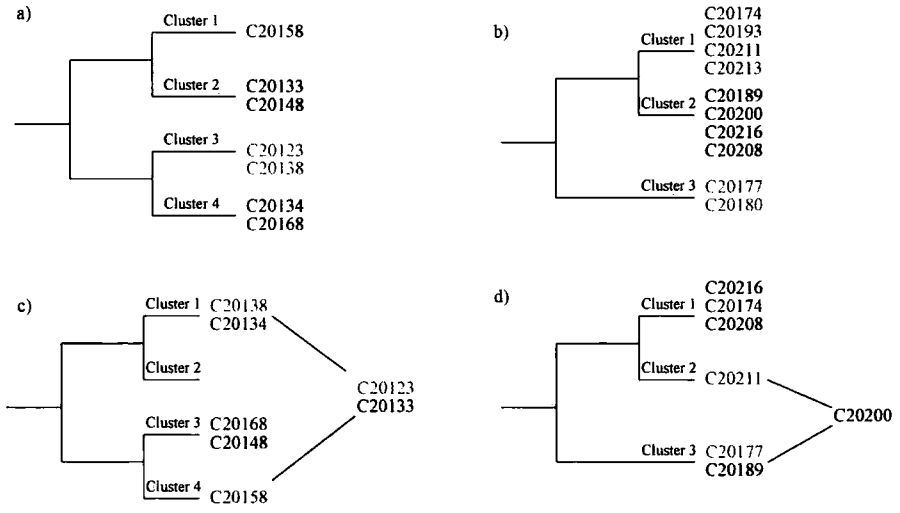


Fig 5.4: Dendrograms showing the clone selection for a) Newcastleton plot 1, b) Newcastleton plot 2, c) Scootmore plot 1 and d) Scootmore plot 2 (Red denotes clones in cluster 1, blue denotes cluster 2, green denotes cluster 3 and purple denotes cluster 4 at Newcastleton).

5.3.4 Plot selection

The K-means cluster analysis showed that the clones at plot 2 had a greater number of individuals classified in the cluster assigned to the clone and, hence, had a smaller sum of squares than plot 1. Due to the better structure of the data, plot 2 was selected.

5.3.5 Clone and site differences

The clones that were used for the physiological research were C20177, C20211 and C20208. The mean values for the three variables and the standard error of each clone is shown in Fig 5.5, for all sites, including Wauchope and Llandrindod. Height at Newcastleton, diameter at breast height (DBH; 1.3m) at Newcastleton, Wauchope and Llandrindod and wood density at all four sites showed the same pattern; C20211 had the lowest values, C20177 had the highest values, and C20208 with values in between. However, height at Scootmore, Llandrindod and Wauchope and DBH at Scootmore showed a different pattern; C20211 still had the lowest values but C20208 had the highest with C20177 in between. The error bars for height and DBH at Llandrindod are large and overlap, illustrating that the clones were not greatly different from each other.

In comparison to the PCA in Chapter 3, the three clones represent different positions along the main gradient. C20177 individuals are large trees, with greater height and trunk diameter, but with lower wood density, representing the positive end of the PCA main axis. C20211 individuals are small trees, with smaller height and trunk diameter, but with high wood density, representing the negative end of the main axis of the PCA. C20208 individuals lay between the positive and negative end of the PCA main axis, with a medium height, trunk diameter and wood density.

There were also differences in the growth responses of the clones at each site. All clones at Newcastleton were growing faster and had greater height and trunk diameters, but smaller wood density than the clones at the other sites. Scootmore clones were smaller in height and trunk diameter but had the greatest wood density. Height, trunk diameter and wood density of the clones at Wauchope and Llandrindod were intermediate between those of the clones at Newcastleton and Scootmore. However, the clones at Wauchope had greater height and trunk diameters, but smaller wood density, in comparison to the clones at Llandrindod.

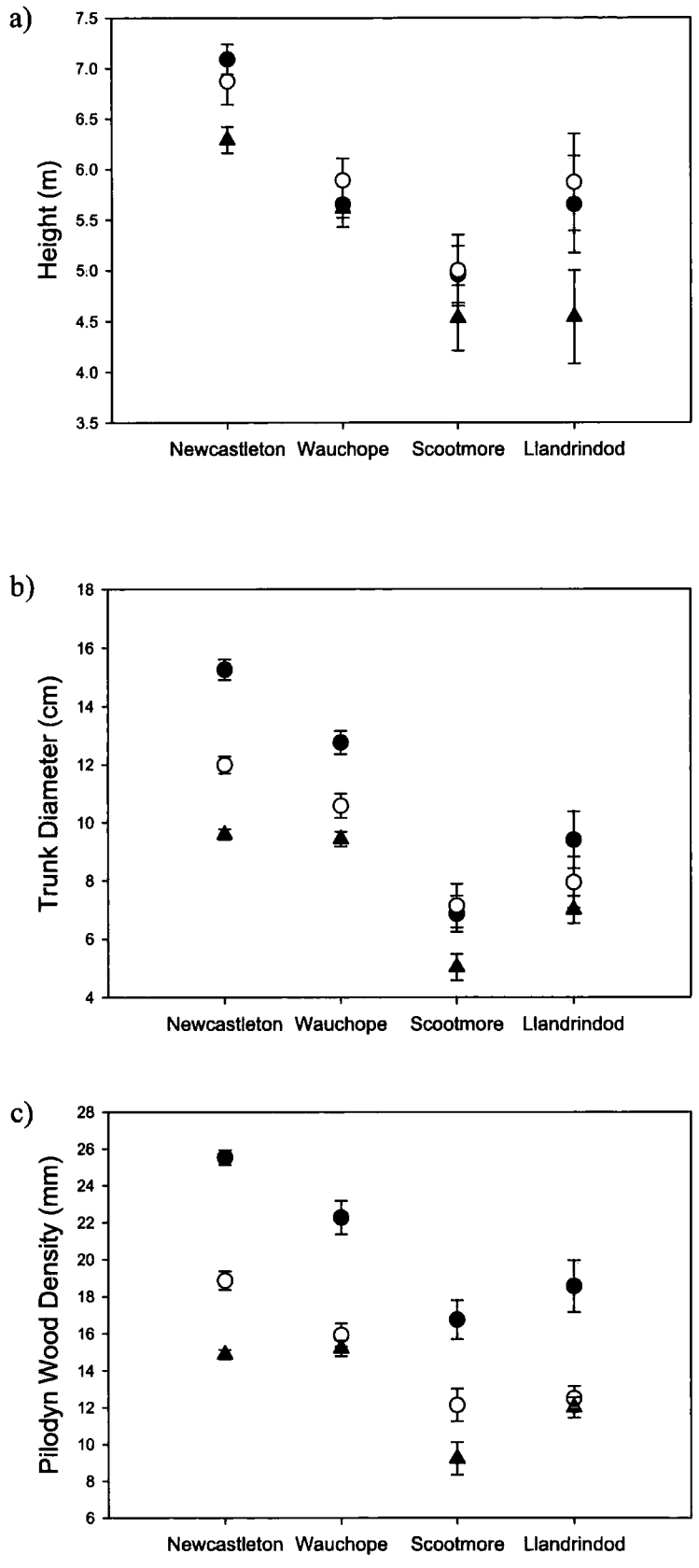


Fig 5.5: Mean ± 1 s.e. (n = 15) of a) height, b) diameter and c) wood density for C20177 (•),C20208 (○)and C20211 (▲) at all four sites.

5.4 Discussion

The cluster analysis proved successful by classifying all individuals into three or four clusters depending on the plot. The same clustering was found at both sites, although Newcastleton appeared to be the superior in terms of distinction between clusters, with the branching arms longer than at Scootmore. The same number of clusters found on each plot at both sites suggested that there was a common growth pattern between the sites. However, the individuals showed large variation suggesting a large environmental effect was confounding the identical growth of each individual and clone. Large variation within populations of Sitka spruce had been found before (Fletcher, 1992) and, with only two clones that have all 15 individuals assigned to the same cluster, the data agrees with these previous findings. Plot 2 appeared to have less of an environmental effect as the clones had a larger number of individuals (10 or above) that were grouped into the same cluster, in comparison to the clones growing on plot 1. However, both plots were very similar in structure and situated in the same location, and, therefore, the plots were subjected to the same environmental pressures. As there were different clones growing on the two plots, the particular crosses on plot 2 may be less susceptible to the effects caused by the environment.

The selected clones showed the same pattern in ranking between sites and between growth variables. Usually C20211 had the lowest value and C20177 had the highest, with C20208 in between. This pattern was particularly shown by DBH and wood density data, suggesting that the two were tightly linked. A strong negative correlation had been found before between DBH and wood density (Pfeifer, 1984; Wood, 1986), and agrees with the results here, as a high value represented a low wood density and a high value of wood density was linked to a large DBH. DBH at Scootmore did show a change in the ranking of the clones, with C20177 and C20208 swapping places and C20208 having the larger DBH, and could a result of the harsher conditions at Scootmore (lower temperatures and lower precipitation).

A change in the order of clones at each site suggested that there was a strong environmental effect, as the clones would have been in the same order if the genetic effect were stronger. By far the largest environmental effect seems to be on height. Scootmore, Llandrindod and Wauchope all showed the same order and share the same ranking as DBH

at Scootmore. Newcastleton showed a different ranking but the same as the DBH and wood density at the other sites. C20211 always had the lowest values but where the environment did exert a large effect then C20177 swapped places with C20208, which then had the largest values. Not only did the change in the ranking of the clones between sites suggest that there was a large environmental effect masking the genetic effect but there was also a lot of variation in individual height and DBH, shown by the larger error bars. If the growth of clones was determined mainly by the genetic control then all individuals would grow in a similar way and the error bars minimised. On this basis, wood density seemed to be under a tight genetic control, as all sites showed the same order of clones and the error bars were small.

With reference to the gradient running through the first PCA axis in Chapter 3, clone C20177 was towards the positive end, tall trees with large DBH but low density, and C20211 was at the negative end, small trees with small DBH but high wood density. Clone C20208 was an intermediate clone.

The three variables showed the same pattern between sites. Height, trunk diameter and the Pilodyn® measurement of wood density were all higher at Newcastleton and lowest at Scootmore. The variables at Wauchope and Llandrindod were between the measurements at Newcastleton and Scootmore, but with Wauchope having the higher measurements. The measurements at Wauchope were closer to those at Newcastleton, whilst the measurements at Llandrindod were closer to those at Scootmore. This suggested Newcastleton and Wauchope were similar sites, and Scootmore and Llandrindod were also similar but with some environmental aspect resulting in a lower height and diameter growth rate.

5.5 Conclusions

At Newcastleton and Scootmore, the individual trees could be split into 4 clusters on plot 1 and split into 3 clusters on plot 2. The clusters were more distinct at Newcastleton and on plot 1.

A greater number of trees were classified into the same cluster on plot 2, resulting in a smaller sum of squares. Plot 2 was therefore chosen for further analyses.

Clones C20177, C20208 and C20211 were chosen from plot 2, as these clones had the greatest number of individual trees classified into the same cluster and the clones belonged to different clusters at Newcastleton and Scootmore.

The three clones generally showed the same pattern at each site. C20177 had the greater height, trunk diameter and Pilodyn wood density measurement, with C20211 having the lowest measurements. The height, trunk diameter and wood density measurements of C20208 were intermediate of those of C20177 and C20211. Wood density and trunk diameter had the strongest relationship, showing the same clonal patterns between sites.

The variables also showed the same pattern between sites. Newcastleton had the greatest height, diameter and wood density measurements, with Scootmore having the lowest. Wauchope and Llandrindod measurements were in between those of Newcastleton and Scootmore but with Wauchope having the greater measurements between the two sites.

6. Chlorophyll concentration, nitrogen content and phosphorus content in the foliage of three clones of Sitka spruce grown at three sites.

Abstract

Chlorophyll, total nitrogen and total phosphorus were extracted from the needles of three different genotypes of Sitka spruce, at three different sites, taken from different positions in the canopy and from different needle age classes. Chlorophyll *a* and *b* both decreased with increasing height in the canopy and increased with increasing needle age. Older needles showed signs of shade adaptation, with an increase in the ratio of chlorophyll *a*:*b* with increasing needle age. Needle total nitrogen concentration increased with increasing canopy height, whilst total phosphorus concentration did not differ between heights. Both nitrogen and phosphorus concentrations decreased with increasing needle age. There was little difference in the nitrogen and phosphorus concentration between the three genotypes, but C20177 did have higher concentrations of chlorophyll. The needles from Newcastleton contained the highest concentration of chlorophyll, nitrogen and phosphorus, with the needles from Scootmore having the lowest.

6.1 Introduction

6.1.1 Nitrogen availability and partitioning throughout the canopy

Soil nitrogen deficiency has been shown previously to cause a decrease in chlorophyll content, photosynthesis, Rubisco activity and stomatal conductance in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Chandler & Dale, 1993; Murray *et al.*, 2000). Needle size and number also decreased with soil nitrogen deficiency (Chandler & Dale, 1995), although needle cell size remained the same as non-deficient needles, suggesting nitrogen deficiency affects cell division and not cell expansion. In loblolly pine (*Pinus taeda* L.), low soil nitrogen availability led to a low needle nitrogen and chlorophyll content (Tissue *et al.*, 1993) but nitrogen addition had no effect on the growth of Sitka spruce at Aber forest in Wales, UK (Emmett *et al.*, 1995), or on the growth of Balsam fir (*Abies balsamea*) in Vermont, USA (Evans *et al.*, 2001).

Nitrogen is partitioned into Rubisco, the main enzyme of photosynthesis, or chlorophyll, both of which are correlated positively with foliage nitrogen content (Evans, 1989). With variation in the photosynthetic photon flux densities (PPFD) through the tree crown (Norman & Jarvis, 1975; Šprtová & Marek, 1999), and with foliar nitrogen partitioned to optimise photosynthesis (Evans, 1989), different concentrations of Rubisco and chlorophyll would be expected throughout the canopy. In the top of the canopy, where photosynthesis is higher (Jarvis *et al.*, 1976; Leverenz & Jarvis, 1979, 1980b; Šprtová & Marek, 1999), greater quantities of nitrogen will be partitioned to Rubisco (Sage & Pearcy, 1987). In the lower canopy, where PPFD is lower, more nitrogen will be used in chlorophyll and in the light-harvesting complex (Boardman, 1977; Leverenz & Jarvis, 1980b; Šprtová & Marek, 1999). The lower chlorophyll *a:b* ratio found in shade foliage indicates an increase in the relative amount of light harvesting complex, which is more efficient at capturing low light intensities (Šprtová & Marek, 1999). However, although there is an increase in the partitioning of nitrogen to chlorophyll at low irradiances, there is a decrease in the electron transport rate per unit of chlorophyll (Evans, 1989; Lewandowska *et al.*, 1977), resulting in the lower photosynthetic rates that are found in the shaded lower canopy (Boardman, 1977).

6.1.2 Phosphorus deficiency and acclimation of photosynthesis

Phosphorus availability can affect plant growth rates by altering photosynthetic rates. In Sitka spruce, phosphorus deficiency led to a reduction in Rubisco activity (Chandler & Dale, 1993). In maritime pine (*Pinus pinaster* Ait.), the rate of electron transport, the rate of Rubisco carboxylation and the carboxylation efficiency increased with increasing soil phosphorus content (Loustau *et al.*, 1999). In *Pinus radiata* D. Don., phosphorus-deficient seedlings showed a change in chlorophyll *a* fluorescence and a decrease in photosynthesis (Conroy *et al.*, 1986). However, after 22 weeks of growth in phosphorus-deficient soil, *P. radiata* had acclimated to low phosphorus concentrations and had the same photosynthetic rates as seedlings that were not phosphorus-deficient. Also in *P. radiata*, phosphorus deficiency caused a decrease in growth and foliar surface area but did not affect maximum rates of photosynthesis, unless grown under elevated atmospheric concentrations of CO_2 (Conroy *et al.*, 1988), and phosphorus-deficient seedlings exhibited a higher light-saturated photosynthesis than non-deficient seedlings after acclimation (Conroy *et al.*, 1986). A similar response was also found in pond pine (*Pinus serotina*

Michx.), where phosphorus deficiency initially caused a decrease in relative growth rates and carbon exchange rates, but while there was a partial recovery of carbon exchange rates after six weeks (up to 85% of the non-deficient rates), the relative growth rate remained much lower (Topa & Cheeseman, 1992).

6.1.3 Hypotheses

- Chlorophyll content was expected to decrease with increasing height in the canopy, as a result of higher light intensities at the top of the canopy. Chlorophyll content was expected to increase with increasing needle age, as a result of increased shading horizontally along the branch towards the interior of the crown.
- Total foliar nitrogen was not expected to vary between needles at different heights in the canopy or in needles of different ages because where chlorophyll was expected to be lower, photosynthesis was expected to be higher and Rubisco content would also be increased.
- Foliar phosphorus content was not expected to vary between different heights in the canopy or between needle age classes.
- Differences of chlorophyll content, nitrogen content and phosphorus content within the needles were expected to reflect the differences in the growth of the three clones.
- The chlorophyll content, nitrogen content and phosphorus content within the needles were expected to reflect the environmental differences between the three sites. With soil nitrogen and phosphorus concentrations highest at Newcastleton (Chapter 3), the foliar concentration of both nutrients was expected to be highest at Newcastleton. With nutrient concentrations lowest at Wauchope, then foliar concentrations of nitrogen and phosphorus were expected to be lowest at Wauchope.

6.2 Method

The needles placed in the conifer leaf chamber for the gas exchange measurements (Chapter 7) were removed after analysis and frozen at –20 °C within one hour. Needles were kept frozen until analysis.

6.2.1 Moisture content determination in needles

For calculating chlorophyll and carbohydrate content on a dry weight basis and for producing the dried needle tissue for total nitrogen and phosphorus determination, 0.1 g fresh needles were placed in an oven at 85 °C for at least 48 hours and until there was no further weight loss. After placing the needles in a desiccator whilst cooling, the needles were re-weighed and the moisture content determined gravimetrically, by subtracting the final weight from the initial weight.

6.2.2 Chlorophyll content determination in needles

The method used was taken from Chandler and Dale (1993), with some modifications. The chlorophyll was extracted twice from 100 mg of needles by grinding in a pestle and mortar, initially with liquid nitrogen to aid tissue disintegration, followed by 5 ml ice-cold 100% acetone and approximately 0.3 g of acid washed sand, again to aid tissue distintegration. The extract was centrifuged at 6000 rpm (Econospin, Sorall Instruments, Du Pont, Wilmington, Germany) for four minutes. The supernatant was decanted into a graduated plastic bottle and another 5 ml 100% acetone added to the centrifugation tube and the pellet resuspended. After centrifuging again at 6000 rpm for three minutes, the supernatants were combined and made up to 15 ml total volume. The absorbance of the supernatant was measured at 662 nm and 645 nm on a spectrophotometer (Shimadzu UV150-02, Shimadzu Seisakusho Ltd., Kyoto, Japan), using 100% acetone as a blank. Chlorophyll content of the extracts (μg ml^{-1}) was calculated from Lichtenthaler & Wellburn (1983):

$$\text{Chlorophyll } a = 11.75A_{662} - 2.35A_{645} \qquad [6.1]$$

$$\text{Chlorophyll } b = 18.61A_{645} - 3.96A_{662} \qquad [6.2]$$

where A_{xxx} was the absorbance at xxx nm. The concentrations were then multiplied by 15 (ml) and divided by fresh weight to give results in mg g^{-1} fresh weight. The fresh weight to dry weight ratio, determined in section 6.2.1, was then used to calculate results as mg g^{-1} dry weight. All procedures were carried out in dim light and on ice, to reduce pigment degradation during extraction.

All chemicals used were of analytical grade, obtained from BDH (BDH Lab Supplies, Poole, Dorset, UK) or from Sigma (Sigma Chemical Company Ltd, Fancy Road, Poole, Dorset, UK).

6.2.3 Total nitrogen and phosphorus determination in needles

Foliage total nitrogen and phosphorus contents were determined using a micro-Kjeldahl digestion and a spectrophotometric assay, based upon the method of Hind (1993) and Skalar (1995).

10 mg (±0.4 mg) of oven-dried, ground needle tissue was added to 1 ml digestion acid and left overnight at room temperature. The digestion acid consisted of concentrated sulphuric acid containing 3.5% w/v Kjel tablet (1.5 g potassium sulphate and 7.5 μg selenium; Thompson and Copper Ltd, Liverpool, UK) and 72% w/v salicylic acid, or sulphuric acid containing 3.5% w/v potassium sulphate, 0.4% w/v copper sulphate and 72% w/v salicylic acid. The digestion acid containing the Kjel tablet was used for samples from Newcastleton and Wauchope but, as a result of contamination of the Kjel tablets, the digestion acid containing copper sulphate was used for samples from Scootmore.

The digestion was heated at 100 °C for two hours in a heating block (Skalar 5620/40 digester and Skalar 5600 controller; Skalar Analaytical BV, 4800 Breda, Netherlands). The digestion was left to cool before 3 ml of 30% v/v hydrogen peroxide was added and then re-heated at 330 °C for two hours. After cooling, the digestion mixture was decanted into a 250 ml volumetric flask and made up to volume with distilled water. The digestion oxidises organic nitrogen and phosphorus to ammonium and phosphate, respectively. Potassium sulphate increases the reaction temperature, selenium or copper sulphate increases the oxidation of the organic matter and salicylic acid converts nitrate into 5-nitrosalicylic acid so that nitrate-nitrogen is also included in the total nitrogen determination (Bremner, 1996). Hydrogen peroxide fully oxidises the organic matter.

Total nitrogen and phosphorus concentrations were determined as concentrations of ammonium (NH_4^+) and phosphate (PO_4^{3-}), by using automated spectrophotometry. A SAN^{plus} 4000 segmented flow analyser (Skalar Analytical, Breda, The Netherlands) was connected to a SA1000 autosampler (Skalar Analytical, Breda, The Netherlands), matrix photometer (6250; Skalar Analytical, Breda, The Netherlands) and an interface unit

(SA8600; Skalar Analytical, Breda, The Netherlands), with SAN^{plus} v6.2 software used to run the analyser and calculate concentrations of NH_4^+ and PO_4^{3-}.

Ammonia and phosphate concentrations were determined using the method described in Chapter 3, section 3.2.1. Blanks were made by diluting 1 ml of the digestion acid mixture, without the addition of any sample, with distilled water to a volume of 250 ml. Standard curves were made by diluting ammonium chloride with digested blank to give a range of 0.02 to 1.00 mg N l^{-1} and diluting potassium dihydrogen *o*-phosphate with digested blank to give a range of 0.01 – 0.50 mg P l^{-1}.

All chemicals used were of analytical grade, obtained from BDH (BDH Lab Supplies, Poole, Dorset, UK) or from Sigma (Sigma Chemical Company Ltd, Fancy Road, Poole, Dorset, UK), unless stated otherwise.

6.2.4 Statistical analysis

Using SPSS® release 10, a two-way analysis of variance (ANOVA) was used to investigate the effect of site and clone on the chlorophyll, total nitrogen and total phosphorus concentrations. The same statistical test was also used to examine the effect of height and needle age on the chlorophyll, total nitrogen and total phosphorus concentrations at different heights in the canopy and for different age classes. Significant differences are reported at the 0.05 probability level.

For ease of visually determining differences in the data, the mean value of chlorophyll, total nitrogen and total phosphorus content for each age class, at each height in the canopy, for each clone at each site were ranked. A black filled circle was used to represent each parameter value, with the size corresponding to the position in the ranking, so the highest value of each parameter had the largest circle.

6.3 Results

6.3.1 Moisture content of needles

Moisture content generally ranged from 40% to 60% for all needle samples throughout the canopy and at all three sites. Moisture content was subsequently used for calculation of

chlorophyll on a dry weight basis and was not subjected to any further analysis. The results are presented in Appendix 1.

6.3.2 Chlorophyll *a* concentration in needles

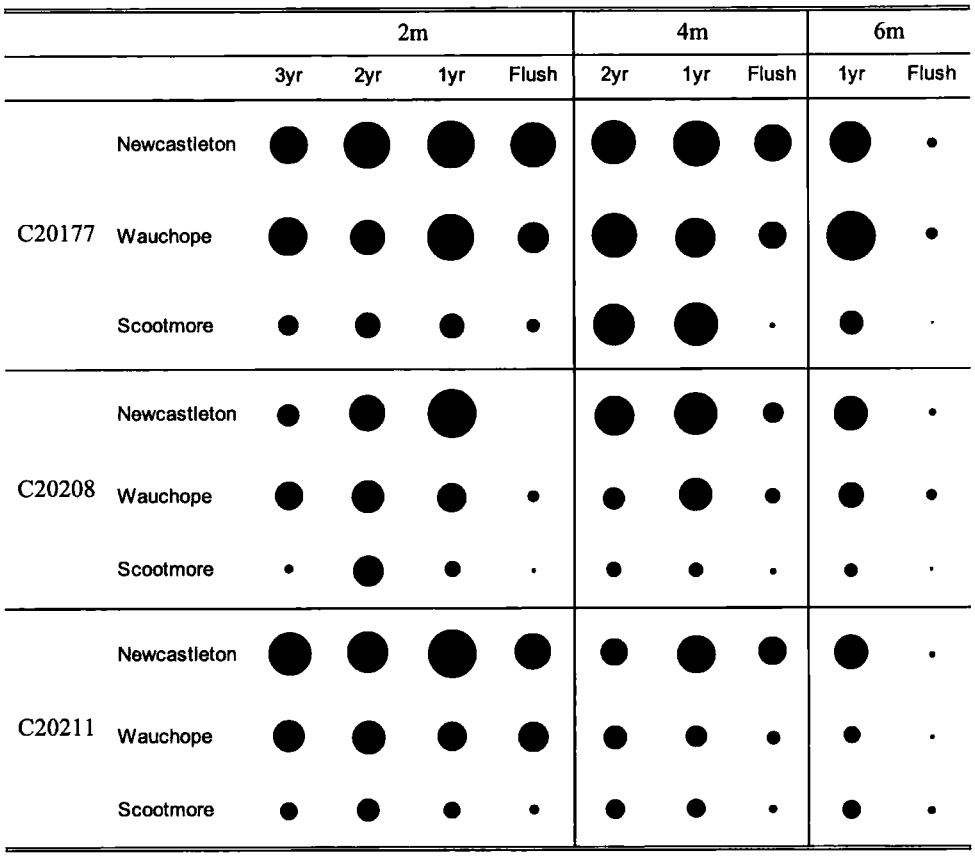
All three clones showed a similar range of concentrations but C20177 had slightly higher chlorophyll *a* concentrations, particularly at Wauchope (Table 6.1, actual mean chlorophyll *a* in Appendix 2). There was very little difference between C20208 and C20211 at all three sites. Differences between clones were significant for 3-year-old needles at 2 m and 2-year-old needles at 4 m (Appendix 3).

For each clone, the chlorophyll *a* concentrations were highest at Newcastleton and lowest at Scootmore, with significant differences between sites for flush, 1-year-old and 3-year-old needles at 2 m and for 2-year-old needles at 4 m (Appendix 3).

Regardless of clone, there was little difference between the chlorophyll *a* concentrations of the three heights, although there was slight decrease with increasing height in the canopy. This was most evident with C20211 at Newcastleton and Wauchope. Conversely, C20177 at Scootmore showed an increase in chlorophyll *a* concentrations between 2 m and 4 m. Differences in the chlorophyll *a* concentrations between heights were significantly different for all clones at Newcastleton and C20211 at Scootmore (Appendix 3).

There was an increase in chlorophyll *a* concentration with increasing needle age, although less apparent at 2 m. At 2 m, C20208 showed a decrease in chlorophyll *a* concentration with increasing needle age and, generally, there was little difference between the needle age classes. There were significant differences between needle ages for C20208 at all sites, C20177 at Wauchope, and C20211 at Newcastleton and Wauchope (Appendix 3).

Table 6.1: Rank of chlorophyll *a* concentration (mg g^{-1} d. wt) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest chlorophyll *a* concentration.

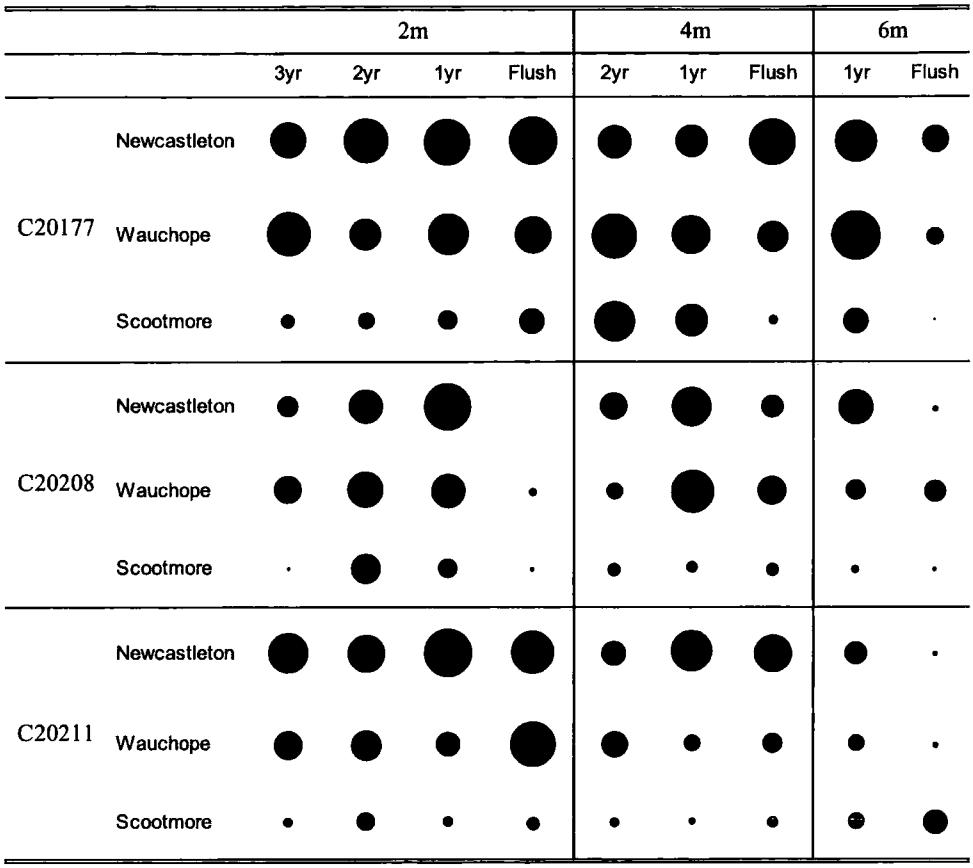


6.3.3 Chlorophyll *b* concentration in needles

There was little difference in the chlorophyll *b* concentrations of each clone, although there were slightly higher concentrations in C20177, in particular at Wauchope (Table 6.2, actual mean chlorophyll *b* in Appendix 2). Differences between clones were significant for the oldest needles at each height only (Appendix 3).

Chlorophyll *b* concentrations were highest, for all clones, at Newcastleton and lowest at Scootmore. The chlorophyll *b* concentrations at Wauchope were most similar to those at Newcastleton. The differences between concentrations at each site were significant for flush, 1-year-old and 3-year-old needles at 2 m, and for 2-year-old needles at 4 m (Appendix 3).

Table 6.2: Rank of chlorophyll *b* concentration (mg g^{-1} d. wt) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest chlorophyll *b* concentration.



There was little difference in the chlorophyll *b* concentrations between the three heights in the canopy, although there was a slight decrease with increasing height. A significant difference exists for C20177 at Wauchope only (Appendix 3).

Chlorophyll *b* concentration varied considerably with increasing needle age. At 2 m, there was generally a decrease with increasing needle age and at 6 m, there was an increase in chlorophyll *b* with increasing needle age. At 4 m, there was a mixture of increasing and decreasing concentrations between needle ages. There were few significant differences between needle ages, with significant differences for C20208 at Newcastleton and C20177 at Wauchope (Appendix 3).

6.3.4 Chlorophyll *a:b* ratio in needles

There was very little difference in the chlorophyll *a:b* ratio between clones (Table 6.3 actual mean chlorophyll *a:b* ratio in Appendix 2), with no significant differences (Appendix 3).

Table 6.3: Rank of chlorophyll *a:b* ratio for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest chlorophyll *a:b* ratio.

		2m				4m			6m	
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
	Newcastleton	●	●	●	·	●	●	·	●	●
C20177	Wauchope	·	●	●	●	·	●	●	·	·
	Scootmore	●	●	●	·	·	●	·	●	●
	Newcastleton	●	●	·		●	●	●	●	·
C20208	Wauchope	●	·	·	●	●	·	·	●	·
	Scootmore	●	●	·	·	·	●	·	●	·
	Newcastleton	·	●	·	·	●	·	·	●	·
C20211	Wauchope	●	●	●	·	·	●	·	·	·
	Scootmore	●	●	●	·	●	●	·	●	·

The differences between sites varied between the three heights in the canopy and between clones. At 2 m, Newcastleton had the lowest chlorophyll *a:b* ratio and Scootmore the highest, regardless of clones. At 4 m, Newcastleton had the highest ratio in C20177 and C20208 but Wauchope had the lowest in C20177 and Scootmore the lowest in C20208. For C20211, the highest ratio was at Scootmore, with Newcastleton and Wauchope showed a very similar, lower, ratio. At 6 m, C20177 and C20208 showed very little difference in chlorophyll *a:b* ratio between sites, but for C20211 Newcastleton had the highest and

Wauchope the lowest. There were significant differences between sites for 1-year-old needles and 3-year-old needles at 2 m (Appendix 3).

There was very little difference in the chlorophyll *a*:*b* ratio between heights, although there was a decrease between 4 m and 6 m for C20177. A significant difference was present only for C20211 at Newcastleton (Appendix 3).

The chlorophyll *a*:*b* ratio increased with increasing needle age. There were significant differences for all clones at Scootmore, C20211 at Newcastleton and Wauchope, and C20177 at Newcastleton (Appendix 3).

6.3.5 Total nitrogen concentration in needles

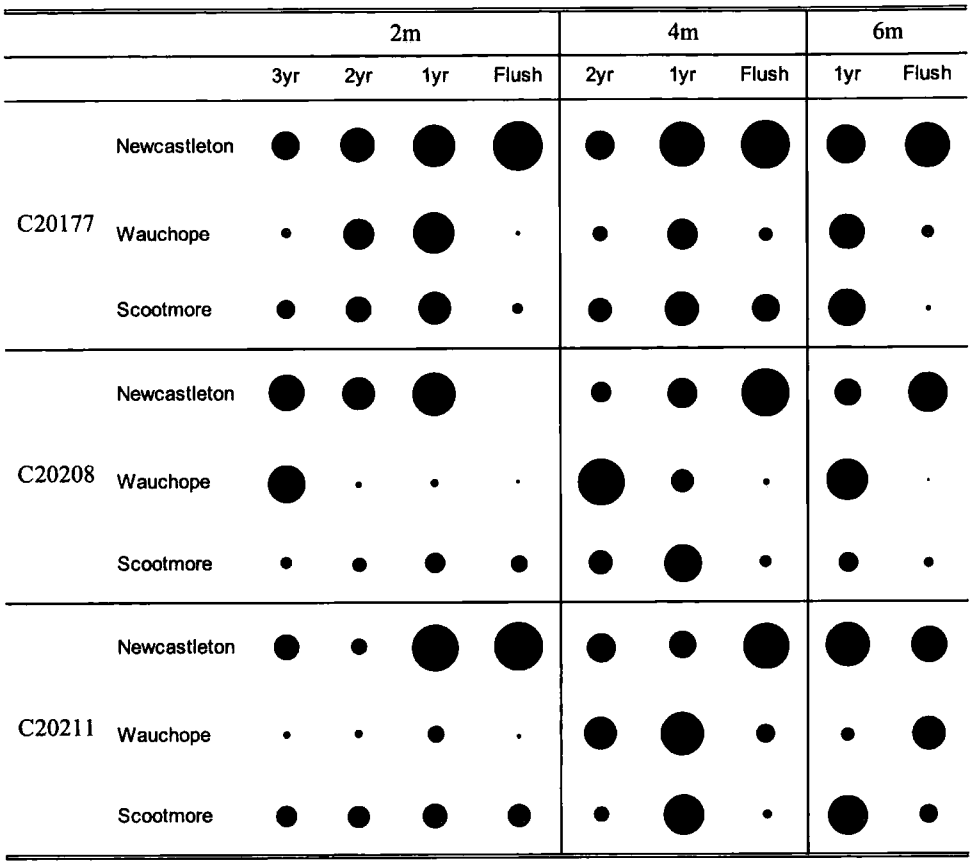
There was very little difference in the total nitrogen concentration of the clones (Table 6.4, actual mean nitrogen content in Appendix 2), with a significant difference between clones for flush needles at 2 m only (Appendix 3).

The clones showed different responses of total nitrogen concentration between sites. The nitrogen concentration of C20177 and C20211 was highest at Newcastleton, with Wauchope and Scootmore showing lower but similar concentrations. With C20208, the nitrogen concentration was lowest at Scootmore, with Newcastleton and Wauchope showing higher and very similar concentrations. There were significant differences between sites for 1-year-old and 2-year-old needles at 2 m, and for flush needles at 4 m and 6 m (Appendix 3).

There was little difference in the nitrogen concentration between heights in the canopy, although there was a slight decrease between 4 m and 6 m. However, there were no significant differences between heights (Appendix 3).

Generally, there was a decrease with increasing needle age. However, there are many exceptions; C20177 at 6 m at Wauchope and Scootmore, C20208 at all heights at Wauchope and at 6 m at Scootmore, C20211 at 6 m at Newcastleton and Scootmore and at 4 m at Wauchope and Scootmore. There were significant differences between needle ages for C20208 and C20211 at Newcastleton, and C20177 at Scootmore (Appendix 3).

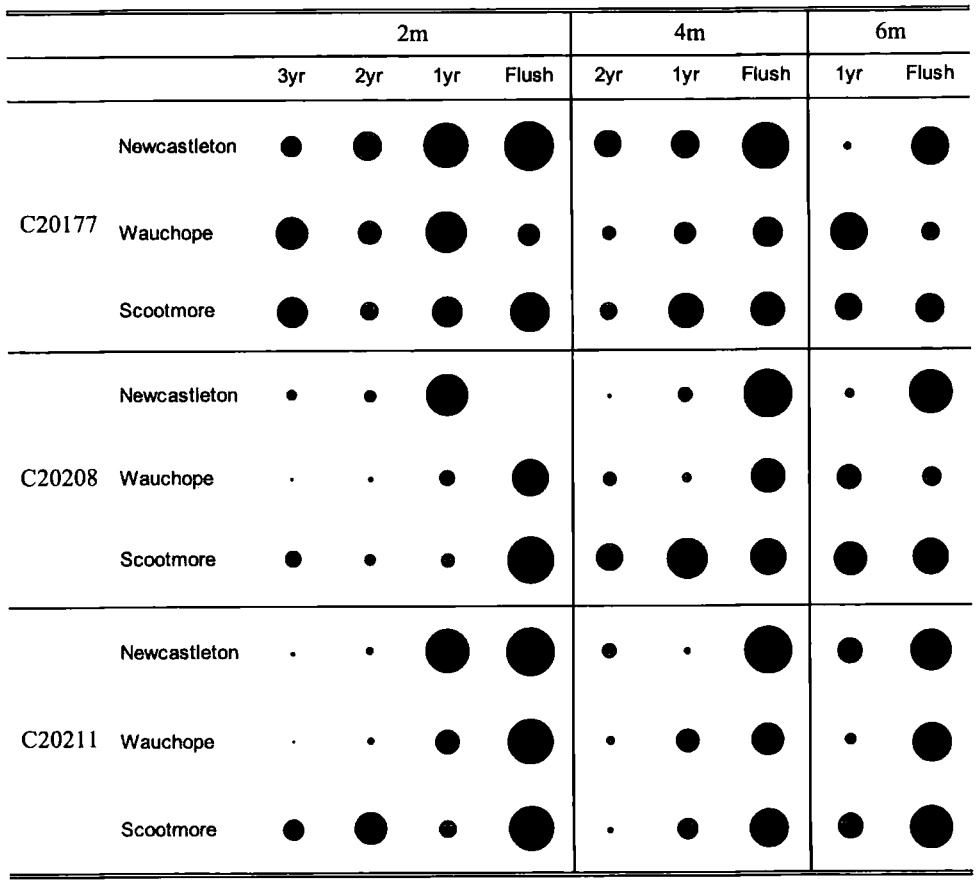
Table 6.4: Rank of foliar nitrogen concentration (mg g^{-1} d. wt) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest nitrogen concentration.



6.3.6 Total phosphorus concentration in needles

There was very little difference in the total phosphorus concentration between the clones (Table 6.5, actual mean phosphorus content in Appendix 2), with only one significant difference for 2-year-old needles at 2 m in the canopy (Appendix 3).

Table 6.5: Rank of foliar phosphorus concentration (mg g^{-1} d. wt) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest phosphorus concentration.



Between sites, Newcastleton had the slightly higher phosphorus concentrations for all three clones. There were significant differences between sites for 1-year-old needles at 2 m and flush needles at 4 m (Appendix 3).

There was very little difference in the total phosphorus concentration between the different heights in the canopy, with a very small decrease between 4 m and 6 m. There were no significant differences between heights (Appendix 3).

There was a decrease in total phosphorus concentration with increasing needle age for all clones at all sites. The only exception was at Wauchope for C20177 at 6 m. There were significant differences between needle ages for C20208 and C20211 at Newcastleton and Scootmore (Appendix 3).

6.4 Discussion

The range of chlorophyll contents reported here were comparable with figures already published for Sitka spruce (Chandler & Dale, 1993; Murray *et al.*, 2000). Chlorophyll *a* and chlorophyll *b* concentrations showed a slight decrease with increasing height in the canopy and could be explained by the light gradient. Light intensity increases with increasing height and, with greater light availability, the needles at the top of the canopy require less chlorophyll to capture the quanta needed for photosynthesis to occur. In the lower canopy, with much greater shading, less light is available and more chlorophyll is required to capture the equivalent quanta. C_3 plants grown in low light intensities partitioned more nitrogen into chlorophyll pigments (Evans, 1989). The decrease in chlorophyll *a* with increasing height was only significant at Newcastleton and for C20211 at Scootmore, suggesting that there was little difference in the chlorophyll content between heights at most sites. A similar response was found with *Picea abies*, where no significant differences were found between the chlorophyll *a* content of sun and shade needles (Šprtová & Marek, 1999). Even fewer significant differences were found in chlorophyll *b* content between heights, suggesting that light availability was having a very small effect. This was in contrast to the research of Šprtová and Marek (1999), who found that the chlorophyll *b* content was significantly higher in shaded needles of *P. abies*. As the trees were still in their juvenile stage, light may not have impacted on the needle physiology, as high light intensities were able to penetrate through to the lower canopy.

The chlorophyll *a* content increased with increasing needle age and may also reflect light availability. Older needles closer to the trunk are subjected to greater mutual shading and will need more chlorophyll for capturing the required quanta for photosynthesis. However, with increasing height there was less shading, so at 6 m in the canopy there was little mutual shading occurring in the older needles, but these needles also showed an increase in chlorophyll *a* in older needles. This change between age classes could be a result of physiological changes that occur as needles age (Ludlow & Jarvis, 1971), requiring higher concentrations of chlorophyll to capture enough light for photosynthesis. The response of chlorophyll *b* content with increasing needle age was not as straightforward. With increasing needle age, there was an increase in chlorophyll *b* content at 6 m and could be explained by the same age-related physiological changes that cause the increase in chlorophyll *a*. However, at 2 m, there was a decrease in chlorophyll *b* with increasing

needle age and a mixture of increases and decreases at 4 m, which were harder to explain. A decrease in chlorophyll *b* content may be caused by gaps in the canopy allowing high light intensity sun flecks to penetrate, requiring less chlorophyll in the needles. However, a similar response would be expected of the chlorophyll *a* content, which was not seen.

Although there was little difference in the chlorophyll *a*:*b* ratio between heights, there was a slight decrease between 4 m and 6 m. As the ratio increased within low light environments with other *Picea* species (Kayama *et al.*, 2002), this again suggested that the needles at the top of the canopy were adapted to high light environments. As the chlorophyll *a*:*b* ratio also increased with increasing needle age, this suggested that the needles are adapting to the horizontal light environment along the branch, with older needles adapted to lower light intensities. A phosphorus deficiency in *Pinus radiata* caused an increase in the ratio (Conroy *et al.*, 1986), so the increase in chlorophyll *a*:*b* ratio may also reflect a change in the nutrient status of the needles at different positions in the canopy.

The total foliar nitrogen content reported here was comparable with nitrogen contents of Balsam fir (Evans *et al.*, 2001) but indicative of nitrogen deficiency as the values were lower than those of the optimum concentration reported for Sitka spruce (Emmett *et al.*, 1995; Jalkanen *et al.*, 1998). There was a slight increase in foliar nitrogen content between 4 m and 6 m in the canopy, although there was little difference between the three heights. Plants optimise the partitioning of nitrogen to maximise photosynthesis, with the major use of nitrogen in Rubisco (Evans, 1989). With higher rates of photosynthesis expected in the upper canopy (Jarvis *et al.*, 1976; Leverenz & Jarvis, 1979, 1980b; Šprtová & Marek, 1999), the majority of nitrogen would be partitioned into Rubisco at 6 m, decreasing with increasing depth in the canopy. In this case, a decrease in foliar nitrogen content might be expected in the lower canopy. However, foliar nitrogen content was increased in lower irradiances in Balsam fir (Evans *et al.*, 2001) and the results here had also shown an increase in chlorophyll in the lower canopy, another major source of foliar nitrogen, therefore leading to little difference in the foliar nitrogen content at different heights in the canopy.

There was a decrease in foliar nitrogen content with increasing needle age. As there was an increase in chlorophyll content with increasing needle age, the higher foliar nitrogen

content in younger needles must be due to an increase in Rubisco. Therefore, higher photosynthetic rates were expected in younger needles.

The total foliar phosphorus content of the clones was comparable with the range of values previously reported for Sitka spruce (Jalkanen *et al.*, 1998). Foliar phosphorus content showed the same pattern seen with total foliar nitrogen content, with the three heights in the canopy showing similar concentrations and with a decrease in foliar phosphorus content with increasing needle age. Low foliar phosphorus content led to a reduced photosynthetic rate in Sitka spruce (Chandler & Dale, 1993), *Pinus serotina* (Topa & Cheeseman, 1992), *Pinus radiata* (Conroy *et al.*, 1988) and *Pinus pinaster* (Loustau *et al.*, 1999). In the present study, therefore, photosynthesis was expected to show little difference between different heights in the canopy but would be higher in current year needles in comparison to older needles. Generally, there were few differences in the concentrations of the various foliar constituents between the three clones. However, where there were differences, C20177 usually had the higher concentrations. C20177 had the higher chlorophyll *a* and chlorophyll *b* concentrations, but this led to very little difference in the chlorophyll *a*:*b* ratio. There was very little difference in the concentrations of foliar nitrogen and foliar phosphorus between the clones.

Chlorophyll content was generally highest in needles at Newcastleton and lowest in needles at Scootmore. The chlorophyll *a*:*b* ratio also generally showed the highest values in needles at Newcastleton but the lowest ratios varied between needles at Wauchope or Scootmore. Total foliar nitrogen content was also highest in needles at Newcastleton and the lowest concentration in needles at Scootmore. The foliar nitrogen concentration in needles at Wauchope varied with clone, showing similar values to Newcastleton in C20208 and similar values to Scootmore in C20177 and C20211. The higher foliar nitrogen and phosphorus contents reflected higher soil nitrogen and phosphorus contents at Newcastleton. The increased availability of nitrogen at Newcastleton also led to increased chlorophyll contents in the foliage. However, Scootmore had higher soil concentrations of nitrogen and phosphorus, in comparison to Wauchope, but Scootmore had the lowest foliar concentration of both nutrients. This suggested that the trees were able to make use of the available nutrients at Wauchope and some factor was limiting uptake at Scootmore. Alternatively, the lower concentrations at Wauchope may have been a result of nutrient depletion, caused by the higher uptake by the trees. The soil cores were taken during the

same month at Newcastleton and Wauchope, but were taken during an earlier month at Scootmore. The difference in timing did not allow an exact comparison between sites and nutrient concentrations at Newcastleton and Wauchope may have been different in the preceding month.

6.5 Conclusions

Chlorophyll concentration of needles generally decreased with increasing height in the canopy and increased with increasing needle age, suggesting chlorophyll content is highest in shaded needles. Chlorophyll *a*:*b* ratio increased with needle age and depth in the canopy, again suggesting that older needles and needles in the lower canopy are shade-adapted.

Foliar nitrogen content increased with increasing height in the canopy and increased in younger needles. Foliar phosphorus content did not differ between different heights in the canopy but did decrease with increasing needle age.

C20177 had the highest concentration of chlorophyll. There was little difference in the concentration of foliar nitrogen and phosphorus between clones.

The needles at Newcastleton had the higher concentrations of chlorophyll, foliar nitrogen and foliar phosphorus, with Scootmore having the lower concentrations. The higher concentrations in the needles at Newcastleton were reflected by the higher soil nutrient concentrations at this site. Soil nutrient concentrations were not reflected by foliar nutrient concentrations at Scootmore or Wauchope.

7. Light response and carbohydrate concentration of needles from three clones of Sitka spruce grown at three sites

Abstract

Photosynthetic light response curves of Sitka spruce were taken *in situ* from three different genotypes, at three different sites, at different positions in the tree canopy and for different needle age classes at each of these positions. The photosynthetic rates were normalised for nitrogen content and five different parameters were subsequently calculated from the curves: light saturated photosynthesis (A_{max_N}), dark respiration (R_{day_N}), light compensation point (LCP), light saturation estimate (LSE) and quantum efficiency. A_{max_N} and quantum efficiency were highest at Newcastleton and lowest at Scootmore, whilst R_{day_N} was also highest at Newcastleton but lowest at Wauchope. LCP and LSE showed little difference between sites. Within sites, there was little difference in any of the parameters between clones or between different heights in the canopy, with only R_{day_N} showing a slight increase with increasing height. At Newcastleton, A_{max_N}, R_{day_N} and LCP showed an increase with increasing needle age but at Wauchope and Scootmore there was a decrease with increasing needle age. LSE and quantum efficiency decreased with increasing needle age at Newcastleton but increased with increasing needle age at Wauchope and Scootmore. Carbohydrate analyses of the needles showed highest concentrations in the needles at Wauchope, with similar concentrations at Newcastleton and Scootmore. C20177 had the lowest concentrations of carbohydrates and C20208 and C20211 had similar but higher concentrations. There was little difference in concentrations between heights but there was an increase in carbohydrates with increasing needle age.

7.1 Introduction

7.1.1 Gas exchange of conifers

Conifers have typical C_3 photosynthesis, with carbon dioxide (CO_2) affinity and chlorophyll concentration comparable to herbaceous plants, although the photosynthetic rates in conifers are reduced by up to one fifth (Cannell, 1987). In Sitka spruce, this reduction has been attributed to several factors. Firstly, within the canopy there is mutual shading, a result of the compact arrangement of the needles (Norman & Jarvis, 1975).

Secondly, within needles, there are gradients of photon flux density that prevents light saturation in all photosynthetic units, but particularly in the abaxial area of the needle (Jarvis *et al.*, 1976). Thirdly, the maximum stomatal conductance for CO_2 is lower in conifers than in herbaceous C_3 plants (Beadle *et al.*, 1983). Finally, it is has been predicted that there are low intrinsic rates of electron transport and carboxylase activity in spruce needles (Lewandowska *et al.*, 1977; Lewandowska & Jarvis, 1978).

Light is the dominating conditioning environmental influence upon photosynthesis, with canopies exposed to large differences in irradiance. Needles at the top of the canopy are physiologically and anatomically acclimated to higher irradiances ('sun' needles), whereas needles in the lower canopy are shade-adapted ('shade' needles) (Jarvis *et al.*, 1976). Shade-adapted plants tend to have high photosynthetic efficiencies, low respiration rates, low compensation points, low saturation estimates and low light-saturated photosynthetic rates (Boardman, 1977); these adaptations would be expected in 'shade' needles. This has been found in the needles of Norway spruce (*Picea abies* [L.] Karst.), where light saturated photosynthesis, apparent quantum efficiency, light compensation point and dark respiration were higher in 'sun' needles compared to 'shade' needles (Šprtová & Marek, 1999). In conifers in general, the maximum rate of photosynthesis is higher in needles in the upper canopy, whilst respiration and light compensation point become progressively lower with increasing depth into the canopy (Jarvis *et al.*, 1976). Rapid saturation of photosynthesis occurs in 'shade' needles, lowering their ability to use excess energy at high light intensities, hence a lower efficiency and a lower compensation point. The higher respiration of 'sun' needles could be an expression of higher energy expenditures, connected to both growth and maintenance, resulting from greater assimilation rates (Šprtová & Marek, 1999). In Sitka spruce, higher photosynthetic capacities and respiration rates have been found in 'sun' foliage (Leverenz & Jarvis, 1979, 1980b), although other research has suggested there is little difference between the photosynthetic rates of the two foliage types (Leverenz & Jarvis, 1980a), with 'shade' shoots as efficient at utilising light as 'sun' needles (Jarvis *et al.*, 1976). Similarly, another study of Sitka spruce, found the light response curves of 'shade' needles more convex than 'sun' needles. A small convexity indicates a large range of photon flux densities within the needles, so not all the photosynthetic units are saturated. Therefore, it appears that 'sun' needles are less efficient at utilising intermediate photon flux densities and may not be as productive as the 'shade' needles (Leverenz & Jarvis, 1979).

7.1.2 Gas exchange with needle age

A decrease in the photosynthetic activity of older needles is evident in Black spruce (*Picea mariana* [Mill] BSP) (Lamhamedi & Bernier, 1994) and Lodgepole pine (*Pinus contorta* [Dougl.] ssp. *latifolia* Engelm.) (Schoettle & Smith, 1999), as well as in Sitka spruce (Ludlow & Jarvis, 1971; Rayment *et al.*, 2002). There have been several suggestions to account for this decline in activity with age. A strong gradient of light occurs horizontally along the branches, from the current year needles at the end of the branches in higher light intensities to the older shaded needles at the interior of the crown (Rayment *et al.*, 2002). With acclimation to the local light environment, needles adapt to the most economical photosynthetic capacity and a decrease in photosynthesis would be expected in older needles in a way analogous to the adaptation of 'sun' and 'shade' foliage. If older needles were shade-adapted, then older needles would be expected to be more productive than current year needles at lower light intensities. However, younger needles of Lodgepole pine were exhibiting higher photosynthetic rates in the shade, suggesting other factors may be involved in the age-related decline of photosynthesis (Schoettle & Smith, 1999).

The decrease of photosynthetic rates in older needles has been attributed to physiological and anatomical changes in aging needles, with decreases in stomatal and mesophyll conductances, accumulation of wax in stomatal cavities and non-reversible winter chloroplast damage all suggested as possible causes (Lamhamedi & Bernier, 1994; Ludlow & Jarvis, 1971).

7.1.3 Differences in gas exchange of differing clones

Differences in photosynthesis have been found between different provenances and clones of conifers. In Sitka spruce there are distinctions between provenances, although these are not statistically significant; more northerly spruces exhibit higher photosynthetic and respiration rates but, unexpectedly, are slow growing (Ludlow & Jarvis, 1971). In 10 different clones of interior spruce (*Picea glauca* (Moench) Voss x *P. engelmannii* Parry ex Engelm.) from five full-sib families, significant differences in net photosynthesis, stomatal conductance and water-use efficiency were evident both between and within families (Fan & Grossnickle, 1999), and when studied over the period of cold acclimation in autumn, striking differences in freezing tolerance between families were also present.

7.1.4 The nitrogen-photosynthesis relationship

Soil nitrogen deficiency has been shown to cause a decrease in photosynthesis and Rubisco activity in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Chandler & Dale, 1993; Murray *et al.*, 2000). Increasing soil nitrogen also had a positive effect on the photosynthesis of Scot pine (*Pinus sylvestris* L.) (Wang & Kellomäki, 1997) and loblolly pine (*Pinus taeda* L.) (Murthy *et al.*, 1997), as well as increasing the rate of carboxylation of Rubisco in flush and 1-year-old needles of black spruce (*Picea mariana* Mill. B.S.P.) (Paquin *et al.*, 2000). In loblolly pine low soil nitrogen availability led to a low needle nitrogen and a lower photosynthetic rate (Tissue *et al.*, 1993).

Foliar nitrogen is partitioned into Rubisco, the main enzyme of photosynthesis, or chlorophyll, both of which are correlated with foliage nitrogen content (Evans, 1989). However, maximum photosynthesis was related significantly to nitrogen content in younger needles but not in older needles of lodgepole pine (*Pinus contorta* ssp. *latifolia*) (Schoettle & Smith, 1999). And in Japanese red pine (*Pinus densiflora* Sieb. et Zucc.), high nitrogen treatment led to a decrease in photosynthesis, due to a decrease in carboxylation efficiency and a decrease in Rubisco content and activity (Nakaji *et al.*, 2001).

With variation in the photosynthetic photon flux densities (PPFD) through the tree crown (Norman & Jarvis, 1975; Šprtová & Marek, 1999), and with foliar nitrogen partitioned to optimise photosynthesis (Evans, 1989), different levels of Rubisco and chlorophyll would be expected throughout the canopy. In the top of the canopy, where photosynthesis is higher (Jarvis *et al.*, 1976; Leverenz & Jarvis, 1979, 1980b; Šprtová & Marek, 1999), greater quantities of nitrogen will be partitioned to Rubisco (Sage & Pearcy, 1987). In the lower canopy, where PPFD is lower, more nitrogen will be used in chlorophyll and in the light-harvesting complex (Boardman, 1977; Leverenz & Jarvis, 1980b; Šprtová & Marek, 1999). However, although there is an increase in the partitioning of nitrogen to chlorophyll at low irradiances, there is a decrease in the electron transport rate per unit of chlorophyll (Evans, 1989; Lewandowska *et al.*, 1977), resulting in lower photosynthetic rates in the shaded lower canopy (Boardman, 1977).

7.1.5 Carbohydrate accumulation and photosynthetic inhibition

With different rates of photosynthesis at different positions in the canopy and for different needle ages, different concentrations of total non-structural carbohydrates would be expected throughout the canopy. Carbohydrate concentrations also correspond to the natural environment (Wiemken & Ineichen, 2000), particularly shown through the seasonal dynamics of carbohydrate concentrations. In Norway spruce (*Picea abies* L. [Karst.]), starch concentration decreased following bud burst in spring, while at the same time there was an increase in sucrose, but with glucose and fructose showing no seasonal pattern (Wiemken & Ineichen, 2000). A similar pattern was seen in red spruce (*Picea rubens* Sarg.), with a decrease in starch concentration through the summer after a peak in spring (Schaberg *et al.*, 2000), and changes in carbohydrate concentration were also strongly correlated with frost hardiness in Scots pine (*Pinus sylvestris* L.) and Norway spruce (Aronsson *et al.*, 1976). Interestingly, site had no effect and soil nitrogen content did not affect carbohydrate concentrations in Norway spruce (Wiemken & Ineichen, 2000). Foliage has a critical role in the storage of carbohydrates, so a loss of foliage leads to a decline in capacity for both the production and storage of carbohydrates, which can, in turn, lead to a decrease in plant growth and vigour. Indeed, extensive foliage loss has been linked to the decline in growth and increased mortality rate seen in field grown red spruce in the USA (Schaberg *et al.*, 2000).

Carbohydrates can also impact on photosynthesis through inhibition by accumulation. Accumulated carbohydrates in red clover (*Trifolium pratense* L. cv. Renova) and wheat (*Triticum aestivum* L.) caused a decrease in photosynthesis, which was restored when carbohydrate export was able to resume (Azcón-Bieto, 1983; Grub & Mächler, 1990). In the C_4 plant *Amaranthus edulis* L., blocking of sucrose export resulted in a carbohydrate increase of five- to six-fold and a large decline in photosynthesis, which did not recover until after 14 hours in the dark (Blechschmidt-Schneider *et al.*, 1989). Accumulation of sucrose leads to a decrease in internal phosphorus concentration (P_i), a result of the inhibition of sucrose-phosphate synthetase. A reduction in P_i affects the rates of photophosphorylation and electron transport, which in turn leads to a decrease in ribulose 1,5 bisphosphate regeneration and consequently photosynthesis (Lawlor, 2001).

7.1.6 Hypotheses

- With photosynthetic rates differing between clones in previous research, it was expected that there would be differences in the photosynthesis of the three different Sitka spruce clones.
- Photosynthetic rates were also expected to differ with increasing height in the canopy, as a result of the light environment, and to decrease with increasing needle age.
- Photosynthetic differences were expected between sites, as the environment impacts on the photosynthesis, although photosynthesis per unit of nitrogen was not expected to differ.
- Lower concentrations of carbohydrates were expected to reflect the sink demand, where there is faster growth. Differences in carbohydrates throughout the canopy and between sites were expected to reflect the needle or tree growth rates.

7.2 Method

7.2.1 Gas exchange measurements

The set up of the clonal experiments, with regards to breeding background and experimental set-up, have been discussed in Chapter 4. The selection of clones for the gas exchange experiments and the description of the three sites have been discussed in Chapter 5.

Gas exchange measurements were taken after bud burst in late May, several weeks into the growing season, during June and July in 2001, between June and August in 2002 and during July in 2003. Data were collected at Newcastleton in 2001 and in the early part of the 2002 growing season, at Wauchope in the latter half of 2002 and at Scootmore in 2003. The same protocol was used for each of the three clones at each site.

To gain access into the canopy, scaffolding towers were erected adjacent to each tree. Each tower consisted of three 2 × 2 × 2 m units allowing a total of 6 m into the canopy to be reached and allowing access to the upper part of the canopy. A platform was constructed at the top of each unit, providing three working areas (plate 7.1). The trees had been brashed in 1999 to a height of about 2 m above the forest floor, making 2 m the lowest part of the canopy. With 2 m representing the lower part of the canopy and 6 m the upper part, a

height of 4 m was chosen as representative of the middle canopy whilst also allowing for ease of access, with platforms at each chosen height. As a result of the brashing, the trees could only be accessed from one side, dictating where the scaffolding towers could be placed. At Newcastleton and Wauchope, the trees could be reached from the north-east or south-west sides and at Scootmore the north-west or south-east sides. In all cases, the most southerly, most suitable branch was used. Each branch was chosen on its quality and how well it represented the canopy height, with branches that were damaged, broken, dying or had prematurely stopped growing discounted from the analysis.

Plate 7.1: Photograph showing 6 m high scaffolding tower, with platforms at 2 m intervals.

Gas exchange was measured using an infra-red gas analyser (LCA4; ADC Bioscientific Ltd, Hoddesdon, Herts, UK) and an attached conifer leaf chamber (PLC4C; ADC Bioscientific Ltd, Hoddesdon, Herts, UK)(Plate 7.2). The leaf chamber was modified to allow the use of larger branches, up to a diameter of 10 mm. During measurements, a 5 cm segment of branch was clamped into the chamber, avoiding the trapping of needles in the jaw gaskets, and for branches with diameters less than 10 mm, the excess width was filled with Blu-Tack® (Bostik, Leicester, UK). The LCA4 was calibrated with 700 ppm CO_2 (Cryoservice, Worcester, UK) at least once a week. Air with ambient atmospheric CO_2 concentration was supplied, via plastic tubing, from 3 m above the top of the canopy.

7.2.2 Photosynthetic light response curves

An artificial light source (20W 12V Cool beam, dichroic 'white light' bulb; Osram, Germany) was attached to the top of chamber and using various arrangements of iconel-coated null density glass filters (ADC Bioscientific Ltd, Hoddesdon, Herts, UK), a sequential range of 10 light intensities was obtained: 1750, 1150, 730, 430, 290, 230, 140, 90, 57 and 0 μmol Q m^{-2} s^{-1}. Using these 10 light intensities, a light response curve of photosynthesis (μmol CO_2 m^{-2} s^{-1}) was obtained for each needle age class that could be safely reached at the three different heights in the canopy. At 2 m, a maximum of four age classes could be reached (flush [current year's needles], 1-year-old, 2-year-old and 3-year-old needles), at 4 m, a maximum of three classes could be reached (flush, 1-year-old and 2-year old needles) and at 6 m, at maximum of two age classes could be reached (flush and 1-year-old needles)(Fig 7.1). Control of temperature, humidity and ambient CO_2 was not possible in the use of the conifer chamber.

Plate 7.2: Photograph showing the LCA4 set up on a scaffolding platform, with an artificial light source attached to the conifer leaf chamber and the five null density glass filters.

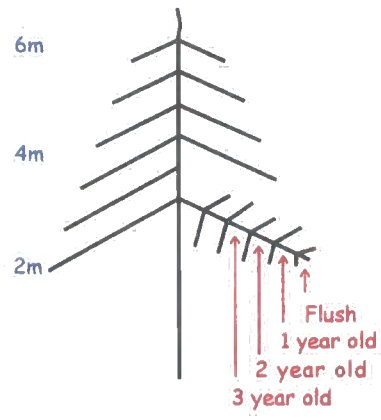


Fig 7.1: Diagram showing the positioning of the three heights in the canopy and the different needle age classes.

The needles within the chamber were removed from the branch after analysis, immediately chilled and then frozen at –20 °C within two hours. Projected area of the needles was calculated by scanning the needles on a flatbed scanner and the area calculated using Delta-T Scan software (Delta T Devices Ltd, Burwell, Cambridge, UK).

Photosynthesis was normalised for foliar nitrogen content. The foliar nitrogen concentration from Chapter 6 was recalculated as mol N m^{-2}. The photosynthesis measurements were then divided by this value to give photosynthesis as μmol CO_2 mol^{-1} N s^{-1}.

7.2.3 Calculation of light response curves

Light response curves and their parameters were calculated using Photosyn Assistant© v1.1.2 (Dundee Scientific, Dundee, UK). Using a non-rectangular hyperbola, the program calculated various parameters. Apparent quantum efficiency (ϕ) was calculated from the initial slope of the light response curve, light compensation point (LCP) and apparent dark respiration (R_{day}) from the axes intercepts, and light saturated photosynthesis (A_{max}) from the upper asymptote (Fig 7.2). An additional parameter (k) described the bending of the curve between the linear gradient and the maximum value. The parameters were determined by fitting a quadratic equation (equation 1) to minimise the sum of squares (Prioul & Chartier, 1977).

$$A = \frac{\phi \cdot Q + A_{\max} - \sqrt{(\phi \cdot Q + A_{\max})^2 - 4 \cdot \phi \cdot Q \cdot k \cdot A_{\max}}}{2k} - R_{day} \qquad [1]$$

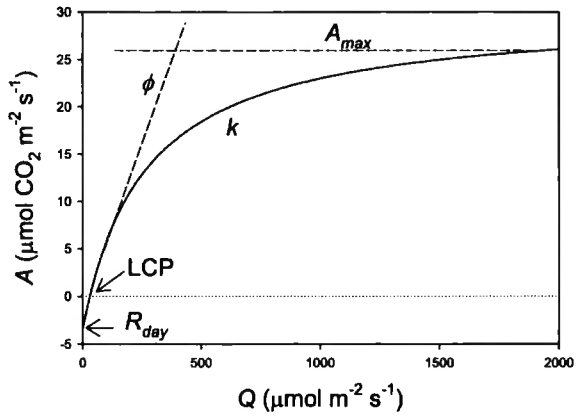


Fig 7.2: An idealised response curve of photosynthesis (A) to photon flux density (Q) and associated parameters; light saturated photosynthesis (A_{max}), dark respiration (R_{day}), convexity (k), light compensation point (LCP) and quantum efficiency (ϕ).

By estimating the initial values of R_{day}, ϕ, k and A_{max} from a linear regression of the first part of data, a Neider-Mead simplex routine was used to calculate the four parameters by the least squares method. LCP was then extrapolated from the x-axis intercept and the light saturation estimate (LSE) from the linear function of ϕ and R_{day} to its intersection with A_{max} (Walker, 1989). k was not included in subsequent analyses.

7.2.4 Statistical analyses of light response curves

Five parameters were extrapolated from the light response curve: A_{max}, R_{day}, LCP, LSE and quantum efficiency (ϕ). Using SPSS® release 10 (SPSS Inc, Chicago, USA). A two-way analysis of variance was used to investigate the effect of site and clone on the photosynthetic parameters for each needle age class at each height in the canopy. A two-way analysis of variance was also used to investigate the effect of height and needle age on the photosynthetic parameters for each clone at each site. Differences were considered significant at the 0.05 probability level.

As temperature and humidity could not be controlled in the conifer leaf chamber, temperature and water vapour may have impacted on the photosynthetic rates. To account for any variability, temperature and vapour pressure deficit (VPD) was used as a covariate in the two-way analysis of variance for A_{max}.

For ease of visually determining differences in the data, the mean value of the parameters for each age class, at each height in the canopy, for each clone at each site were ranked. A

black filled circle was used to represent each parameter value, the size corresponding to the position in the ranking, so the highest value of each parameter had the largest circle.

7.2.5 Total non-structural carbohydrate content determination in needles

The extraction method was based upon Farrar (1993). 100 mg of needles were lightly crushed, added to 10 ml of 90% aqueous ethanol (Fisher Scientific, UK) and heated at 60 °C for 1 hour in a water bath or heating block (Skalar 5620/40 digester and Skalar 5600 controller; Skalar Analytical BV, 4800 De Breda, Netherlands). The extract was decanted and the residue re-extracted a further two times. All extracts were combined. The tissue carbohydrates were further extracted in 10 ml distilled water at 30 °C for two hours. The ethanol and water extracts were stored separately at 4 °C and analysed for carbohydrate content within 24 hours.

Starch was subsequently extracted from the same tissue by adding the needles to 6 ml amyloglucosidase solution, buffered at pH 4.5. The enzyme solution was made by adding 0.4925g amyloglucosidase (10 units ml^{-1}) to a buffer of 490 ml 0.2M sodium acetate and 510 ml 0.2M acetic acid (Dawson *et al.*, 1989). The extraction medium was heated at 55 °C in a water bath for 24 hours.

Carbohydrate concentrations of all three extracts were determined using the phenol-sulphuric acid assay (Dubois *et al.*, 1956). Using a dry, thick walled, glass test tube, 50 µl 80% phenol w/w was added to 1 ml of extract (diluted according to carbohydrate content) and 5 ml 98% sulphuric acid was carefully but forcefully pipetted into the tube, ensuring the mixing of the tube contents. The assay was left for 20 minutes to allow the colour to develop and the reaction to cool. Using 1 cm^3 quartz glass cuvettes, the absorbance of the solution was measured at 485 nm on a spectrophotometer (Shimadzu UV150-02, Shimadzu Seisakusho Ltd., Kyoto, Japan), using assayed water and assayed enzyme solution as blanks for the ethanol/water extractions and enzyme extraction respectively. A standard curve of glucose ranging from 0 to100 µg ml^{-1} was used to calculate the concentration of glucose equivalents in each sample from all three extraction methods. The moisture content, determined in section 6.2.1, was then used to calculate results as mg g^{-1} dry weight.

All chemicals used were of analytical grade, obtained from BDH (BDH Lab Supplies, Poole, Dorset, UK) or from Sigma (Sigma Chemical Company Ltd, Fancy Road, Poole, Dorset, UK), unless stated otherwise.

7.2.6 Statistical analyses of carbohydrates

A two-way analysis of variance was used to investigate the effect of site and clone on the non-structural carbohydrate concentration for each needle age class at each height in the canopy. A two-way analysis of variance was also used to investigate the effect of height and needle age on the carbohydrate concentration for each clone at each site. Differences were considered significant at the 0.05 probability level.

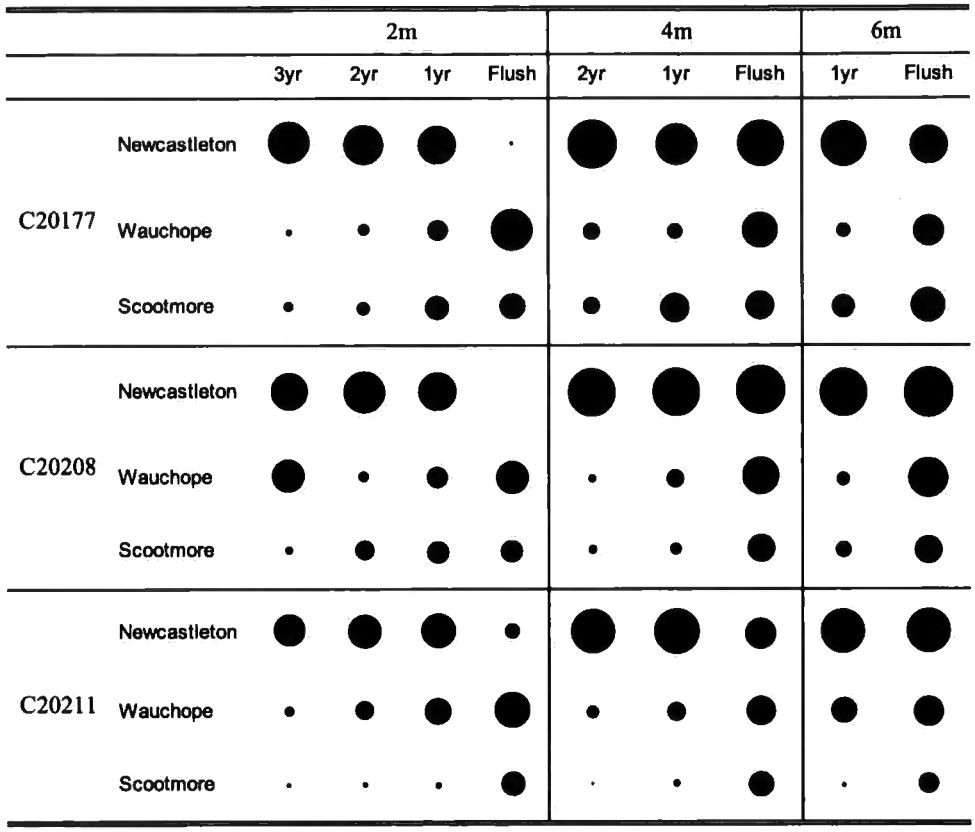
7.3 Results

7.3.1 Light saturated photosynthesis per mol of nitrogen (A_{max_N}; μmol CO_2 mol^{-1} N s^{-1})

A_{max_N} was highest at Newcastleton and lowest at Scootmore for all clones (Table 7.1, actual mean values of A_{max_N} are given in Appendix 4). Within site, there was little difference in the A_{max_N} between clones. The differences were significantly different for all needle ages at each height with the exception of flush needles at 4 m, but there were no significant differences between clones (Appendix 5).

The A_{max_N} did not largely differ between heights in the canopy but did show a decrease with increasing needle age at Wauchope and Scootmore. At Newcastleton, particularly at 2 m, there was an increase in A_{max_N} with increasing needle age. There were no significant differences between the different heights but there were significant differences between needle ages for each clone at Wauchope and Scootmore (Appendix 5).

Table 7.1: Rank of A_{max} (μmol CO_2 mol^{-1} N s^{-1}) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest A_{max}.



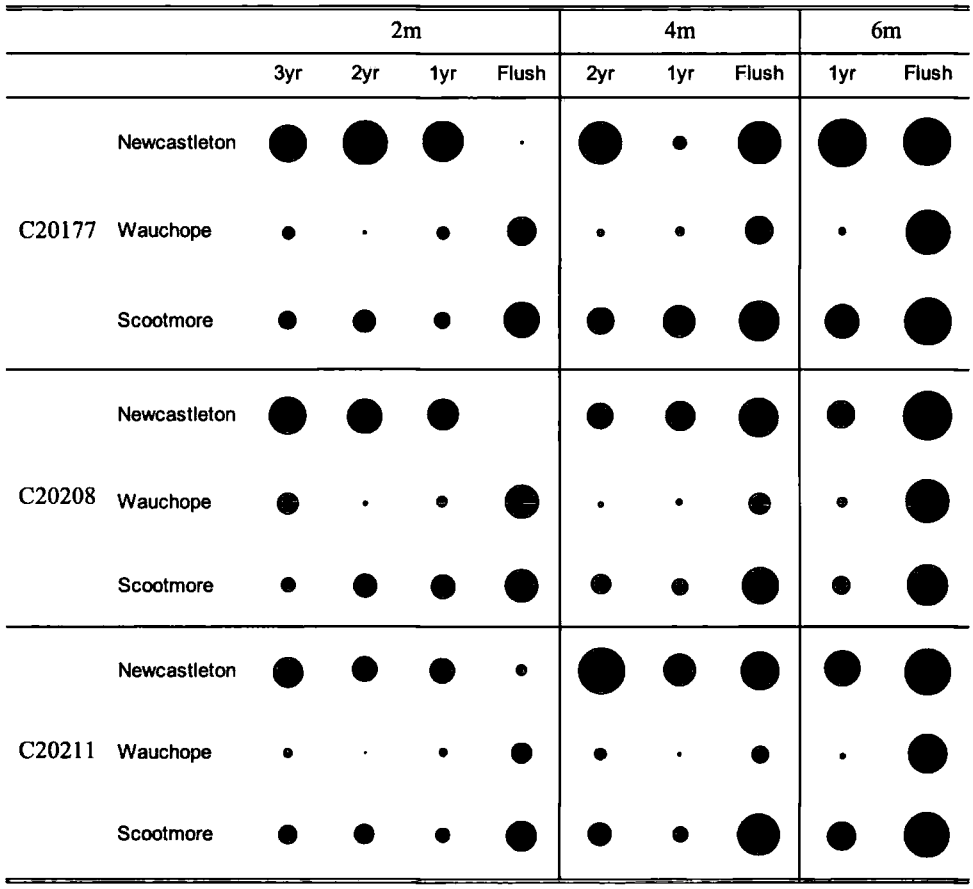
There was little difference in the results using either temperature or VPD as covariates. With VPD as covariate, there were significant differences between sites for all needle ages at each height in the canopy, with the exception of flush and 2-year-old needles at 4 m. With temperature as covariate, there were significant differences between sites for flush needles and 2-year-old needles at 2 m, 1-year-old needles at 4 m, and flush needles at 6 m. There were no significant differences between clones with either temperature or VPD as covariates (Appendix 5).

7.3.2 Dark respiration per mol of nitrogen (R_{day_N}; μmol CO_2 mol^{-1} N s^{-1})

R_{day_N} was highest at Newcastleton and lowest at Wauchope for all clones (Table 7.2, actual mean values of R_{day_N} are given in Appendix 4). Within site, there was little difference in the R_{day_N} between clones. The differences were significantly different for all needle ages at each height with the exception of needles at 6 m, and 1-year-old and 2-year-old needles at 2 m and 4 m (Appendix 5). There were no significant differences between clones.

There was a slight increase in R_{day_N} with increasing height, most noticeable between 2 m and 6 m at Wauchope and Scootmore. There was a decrease with increasing needle age at Wauchope and Scootmore, whilst at Newcastleton, there was an increase in R_{day_N} with increasing needle age. There were significant differences between heights and between needle ages at each site (Appendix 5).

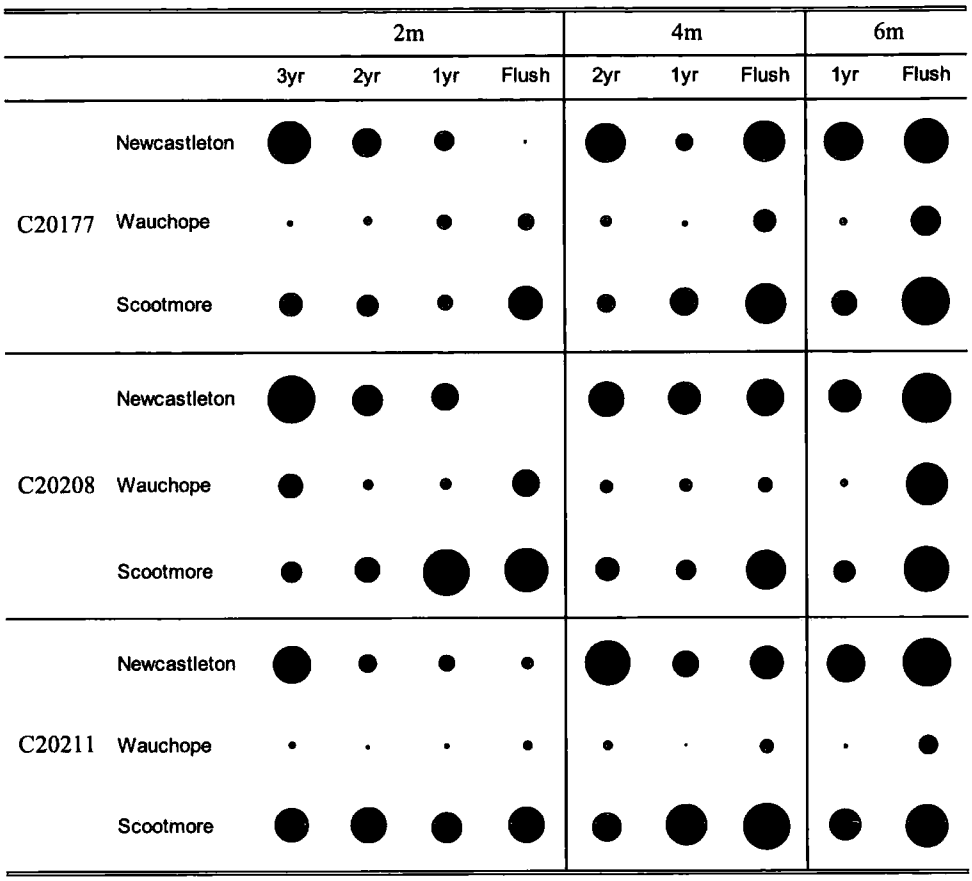
Table 7.2: Rank of R_{day} (μmol CO_2 mol^{-1} N s^{-1}) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest R_{day}.



7.3.3 Light compensation point per mol of nitrogen (LCP; μmol *Q* mol^{-1} N s^{-1})

There was little difference between the LCP of Newcastleton and Scootmore, with Wauchope showing lower values (Table 7.3, actual mean values of LCP are given in Appendix 4). There was little difference between the LCP of the clones. There were significant differences between sites for all needle ages at each site, with the exception of 1-year-old needles at 2 m and 6 m, but there were no significant differences between clones (Appendix 5).

Table 7.3: Rank of LCP (μmol Q mol^{-1} N s^{-1}) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest LCP.



There was little difference in the LCP between the different heights but there was a decrease with increasing needle age at Wauchope and Scootmore. At Newcastleton, particularly at 2 m, there was an increase with increasing needle age. There were significant differences between heights for C20211 at Newcastleton and C20177 at Scootmore, with C20208 at Wauchope and C20177 at Scootmore showing a significant difference between needle ages (Appendix 5).

7.3.4 Light saturation estimate per mol of nitrogen (LSE; μmol Q mol^{-1} N s^{-1})

LSE was highest at Newcastleton. For C20211, LSE was lowest at Wauchope, but with little difference in the LSE between Wauchope and Scootmore for C20177 and C20208 (Table 7.4, actual mean values of LSE are given in Appendix 4). There was little difference between the LSE of the different clones, although the LSE of C20211 was slightly lower than the LSE of the other two clones at Wauchope. There were significant differences between sites for all needle ages at each height, with the exception of 1-year-old needles at

2 m and flush needles at 4 m. There was a significant difference between clones for 1-year-old needles at 4 m (Appendix 5).

Table 7.4: Rank of LSE (μmol Q mol^{-1} N s^{-1}) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest LSE.

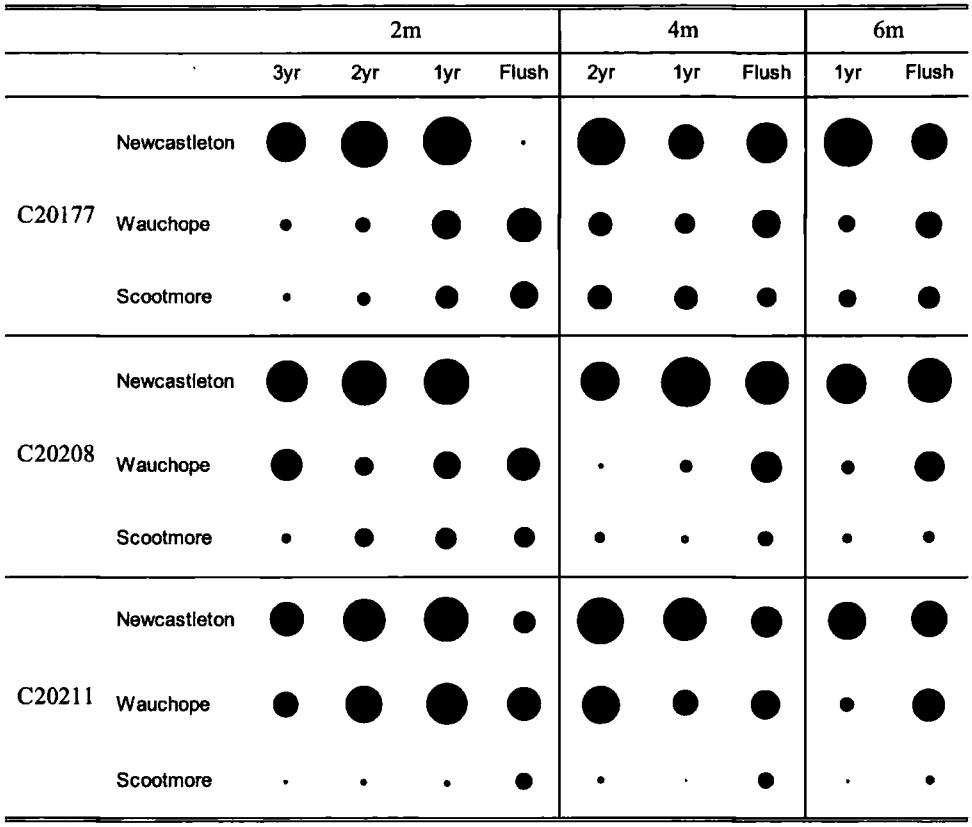
		2m				4m			6m	
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	●	●	●	·	●	●	●	●	●
	Wauchope	·	●	•	●	•	•	●	•	●
	Scootmore	●	●	•	●	•	●	●	•	●
C20208	Newcastleton	●	●	●		●	●	●	●	●
	Wauchope	●	•	•	●	•	•	•	•	●
	Scootmore	•	●	●	●	•	•	●	•	●
C20211	Newcastleton	●	●	●	•	●	●	●	●	●
	Wauchope	·	•	●	●	·	·	●	•	•
	Scootmore	●	●	●	●	·	●	●	•	●

There was little difference in the LSE between different heights but there was a decrease with increasing needle age, again with the exception of LSE at Newcastleton, which showed an increase with increasing needle age. There were few significant differences; C20208 and C20211 at Newcastleton between heights, and C20208 at Wauchope and C20177 and C20208 at Scootmore between needle ages (Appendix 5).

7.3.5 Quantum efficiency per mol of nitrogen (QE; mol^{-1} N)

Newcastleton had the highest QE (Table 7.5, actual mean values of QE are given in Appendix 4). For C20177, Wauchope and Scootmore both had similar, lower values, whilst the QE was lowest at Scootmore for C20208 and C20211. For C20211, the QE at Wauchope was similar to the QE at Newcastleton.

Table 7.5: Rank of QE (mol^{-1} N) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest QE.



There was little difference between clones within Newcastleton and Scootmore, but at Wauchope, C20211 had slightly higher QE in comparison to the other clones. There were significant differences between sites for each clone but no significant differences between clones (Appendix 5).

There was little difference between the heights in the canopy, but a decrease with increasing needle age at Wauchope and Scootmore. At Newcastleton, there was an increase with increasing needle age. There were significant differences between heights and between needle ages for C20177 at Wauchope and significant differences between needle ages for C20177 at Scootmore (Appendix 5).

7.3.6 Total non-structural carbohydrate (TNC) concentration in needles

C20177 had the lowest TNC concentration, with C20208 and C20211 showing similar concentrations (Table 7.6, actual mean TNC concentrations are given in Appendix 4). There were no significant differences between clones (Appendix 5).

Between sites, Wauchope had the highest TNC concentrations. Newcastleton and Scootmore showed very similar concentrations in C20177 and C20211, but Newcastleton had the smallest concentration in C20208. There are significant differences between sites for flush needles at all heights in the canopy and 2-year-old and 3-year-old needles at 2 m (Appendix 5).

There was little difference in the total carbohydrate concentration between heights, with a significant difference for C20208 at Newcastleton only (Appendix 5).

Flush needles had the lowest total carbohydrate concentration at each height, with an increase in concentration with increasing needle age, with the exception of C20177 and C20208 at 4 m and 6 m at Wauchope, and C20208 at 4 m at Scootmore. There were significant differences for C20208 at Newcastleton and Wauchope, and C20211 at Wauchope and Scootmore (Appendix 5).

There was little difference in the ethanol and water soluble carbohydrate concentrations between clones. Starch concentrations were similar between C20177 and C20208 but were higher in the needles of C20211. There were few significant differences (Appendix 5).

Ethanol-soluble carbohydrate concentrations were highest at Wauchope and lower but similar at Newcastleton and Scootmore. Water-soluble carbohydrate and starch concentrations were similar at Wauchope and Scootmore, whilst water-soluble carbohydrate concentrations were highest at Newcastleton but lower for starch. There were significant differences in the concentrations of the three carbohydrates for nearly all needle ages at each height (Appendix 5).

Ethanol-soluble carbohydrate, water-soluble carbohydrate and starch concentrations did not differ largely between heights in the canopy but increased with increasing needle age. There are few significant differences between heights and between needle ages (Appendix 5).

Table 7.6: Rank of TNC (mg g^{-1} d. wt) for all clones, at each site, for each height in the canopy and needle age class. The largest black circle represents the highest TNC.

		2m				4m			6m	
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	●	●	●	·	●	●	·	●	●
	Wauchope	●	●	●	●	·	●	●	·	●
	Scootmore	●	●	●	·	●	●	·	●	●
C20208	Newcastleton	·	●	●		●	●	·	●	·
	Wauchope	●	●	●	·	●	●	●	●	●
	Scootmore	●	●	●	·	●	●	●	●	·
C20211	Newcastleton	●	●	●	·	●	●	·	●	·
	Wauchope	●	●	●	●	●	●	●	●	●
	Scootmore	●	●	●	·	●	●	·	●	●

7.4 Discussion

There were some distinct differences in the photosynthetic parameters between sites. Newcastleton had higher growth rates than the other two sites (Chapter 5), therefore it was not unexpected that A_{max_N} was higher at this site. Higher growth and photosynthetic rates at Newcastleton also led to a higher R_{day_N} rate, as greater respiration occurred where activity was highest. Newcastleton also had the highest LCP, LSE and QE. With higher R_{day_N}, higher photosynthesis was required to reach the point where CO_2 assimilated equalled the CO_2 respired, requiring a greater quantity of light and hence a higher LCP. With higher A_{max_N}, a greater quantity of light was required to reach saturation and hence the higher LSE at Newcastleton. Newcastleton also has a higher QE, showing that the needles at this site are more efficient in utilising light during photosynthesis.

The clones at Scootmore show the lowest growth rate and, therefore, lower A_{max_N} was expected. With lower growth rates and lower A_{max_N} rates at Scootmore and therefore lower activity, lower R_{day_N} would also be expected. However, although not as high as

Newcastleton, Scootmore did not show the lowest R_{day_N}. A low A_{max_N} but higher R_{day_N}, may explain the lower growth rates at Scootmore. LSE was also lower, as less light was required to reach the lower A_{max_N} rates. QE was also lower at Scootmore, showing the needles at this site were less efficient at utilising light for photosynthesis.

R_{day_N} was lowest at Wauchope, which was unexpected as the needles did not show the lowest activity and the trees at this site did not show the lowest growth rates (Chapter 4). During the particular year in which the gas exchange was measured at Wauchope, the needles may have been slower growing, therefore showing lower activity and a lower R_{day_N} rate. With decreased R_{day_N} and A_{max_N} still at a high rate, carbohydrates would be accumulating in the needles. There was also a low LCP, a result of the low respiration rate. The LSE was also low, with very similar results to the clones at Scootmore. However, with higher A_{max_N} in comparison to the clones at Scootmore, the QE was higher at Wauchope, showing that the needles at Wauchope are more efficient at utilising light and reach saturation at a faster rate.

The clones did not show distinct differences in their photosynthetic parameters, particularly with A_{max_N}, R_{day_N} and LCP. However, at Wauchope, C20211 had lower LSE but increased QE, whilst C20208 and C20177 had higher LSE and lower QE. With the clones photosynthesising at the same rate, saturation at a lower light concentration resulted in a greater efficiency in the light use and, hence, a greater QE. Saturation at a higher light concentration resulted in a lower efficiency and lower QE.

There were also few differences in the photosynthetic parameters between the different heights in the canopy. A decrease in photosynthesis with increasing depth in the canopy was expected. Plants from low light environments had reduced electron transport, resulting in a lower photosynthesis per unit of nitrogen (Evans, 1989), and the photosynthetic parameters were characteristically low in shade-adapted plants (Boardman, 1977), in the lower canopy of white spruce (Man & Lieffers, 1997), and in shaded needles of Norway spruce (*Picea abies* [L.] Karst.) (Šprtová & Marek, 1999). It may be that the needles in the lower canopy were not shade adapted, a result of the young age of the trees and a less dense canopy. However, previous research on Sitka spruce has shown that QE was not affected by the position in the canopy (Jarvis *et al.*, 1976; Leverenz & Jarvis, 1979).

There was a decrease in the photosynthetic parameters with increasing needle age at Wauchope and Scootmore. A light gradient runs horizontally along a branch, from needles at the end in high light intensity to shaded needles close to the trunk (Rayment *et al.*, 2002). Older needles, therefore, were shaded and the decrease in photosynthesis was a reflection of the decrease in light intensity. However, the decrease could also have been a result of the physiological changes that occur as needles age (Lamhamedi & Bernier, 1994; Ludlow & Jarvis, 1971). With a decrease in A_{max_N} and activity as needles age, then a reduced R_{day_N} was expected, as well as less light needed to reach compensation point or saturation. Conversely, the clones at Newcastleton did not show a decrease with increasing needle age, except with A_{max_N}. The increase in R_{day_N} in older needles may be a result of damage to older needles, consequently increasing maintenance respiration. However, if the older needles were damaged, then a decrease in QE would also be expected but it increased in older needles.

The concentrations of TNC were comparable with those previously reported for *P. abies* (Wiemken & Ineichen, 2000) but higher than those of *Picea rubens* (Schaberg *et al.*, 2000), although the starch contents were comparable. Wauchope had the highest TNC concentration, with Newcastleton and Scootmore having similar concentrations. The higher concentrations in the needles of the clones at Wauchope were a result of a high A_{max_N} rate but a low R_{day_N} rate. Although A_{max_N} was higher at Newcastleton, and producing greater carbohydrate concentrations, activity was higher, resulting in the use of a large proportion of the TNC. Scootmore had lower A_{max_N} but also lower activity, so fewer carbohydrates used, resulting in the same concentration of TNC as in the needles at Newcastleton. At Newcastleton, the TNC may have been exported to other parts of the tree to avoid inhibition by the accumulation carbohydrates, a result of the higher A_{max_N}.

There was little difference in the TNC concentration between clones, although C20208 and C20211 had higher concentrations than C20177. With the C20177 trees being taller and having a larger trunk diameter, the increase in growth may have resulted in a greater use of the TNC. There were few differences between the different heights in the canopy, showing that the sink strength was equal throughout the canopy. There was also an increase in TNC with increasing needle age. Flush needles had higher sink strength than the older needles, a result of needle growth. Flush needles were also photosynthesising at a higher rate, making the flush needles the largest source and sink in the canopy.

7.5 Conclusions

A_{max_N}, R_{day_N}, LSE and QE were all highest at Newcastleton, and lowest at Wauchope for R_{day_N} and LSE but lowest at Scootmore for A_{max_N} and QE. LCP showed very little difference between sites.

All five parameters showed little difference between clones.

There was little difference in A_{max_N}, LCP, LSE and QE between heights in the canopy, with R_{day_N} showing a slight increase with increasing height.

A_{max_N}, R_{day_N}, LCP, LSE and QE all increased with increasing needle age at Newcastleton but decreased with increasing needle age at Wauchope and Scootmore.

TNC concentrations of C20208 and C20211 were similar and higher than the TNC concentrations of C20177. The clones at Wauchope had the highest TNC concentrations, with lower but similar concentrations at Scootmore and Newcastleton. There was little difference in concentration with increasing height in the canopy but there was an increase in TNC concentration with increasing needle age. The different extractions of carbohydrates all showed similar patterns.

8. Carbon partitioning in three clones of Sitka spruce grown at three sites

Abstract

Twelve trees from three clones of Sitka spruce were felled at three sites. Dry mass was determined for branches, foliage and trunk. Root cores were taken from three distances from the root collar of each tree and the dry mass of coarse and fine root density calculated. Wood density and root density was calculated for each tree. Wood density was greatest for clone C20208 at Wauchope and Scootmore, greatest for C20211 at Newcastleton, and lowest for C20177 at all three sites. Carbon allocation above-ground was greatest for C20177 at Newcastleton and Wauchope, greatest for C20211 at Scootmore and lowest for C20211 at all sites. Bigger trees contained more carbon, although they had the lowest wood density. The majority of carbon was allocated to the trunk, except for C20177 at Wauchope and Scootmore, which allocated more carbon to the branches. This suggested that under water or nutrient stress, C20177 changed allocation patterns. While there was no pattern seen in coarse root density, fine root density was lowest for C20177 at all sites, with carbon allocation below-ground also lowest for this clone. Carbon allocation to coarse roots showed the same pattern amongst clones at Newcastleton and Scootmore, suggesting that differences in allocation to coarse roots were mainly affected by soil nutrient concentration.

8.1 Introduction

8.1.1 Environmental effects on carbon allocation and dry mass partitioning

Generally, under conditions of stress, plants partition more carbon (C) to the root systems (Negi *et al.*, 2003). During fertilization experiments, seedlings of *Populus nigra* grown under low nutrient conditions allocated 24% more C to the roots than the high nutrient availability counterparts (Glynn *et al.*, 2003), and the root biomass of *Pinus radiata* showed an inverse relationship with nutrient availability (Rodríguez *et al.*, 2003). In slow-growing Arctic vegetation, increasing nutrient availability corresponded with an increase in root biomass, giving advantage over the faster-growing species (van Wijk *et al.*, 2003). However, with 40-year-old *Larix leptolepis*, there was no difference in root biomass

between different nutrient concentrations (Son & Hwang, 2003), and in loblolly pine (*Pinus taeda*), growing in north-west Florida, fertilization had no effect on fine root biomass (Lee & Jose, 2003). With *Eucalyptus saligna*, increased nutrient availability increased C assimilation, almost all of which was allocated above-ground (Giardina *et al.*, 2003).

Nutrient stress in *Pinus palustris* increased root:shoot ratio by 69 %, showing an increase in below-ground biomass in relation to above-ground biomass (Jose *et al.*, 2003). Water stress also caused a similar but smaller impact, with an increase in root:shoot ratio by 19 % (Jose *et al.*, 2003). Stem and needle biomass were also affected by nutrient and water stress, with more biomass partitioned above-ground in high nitrogen and high water conditions (Jose *et al.*, 2003).

The C allocation in *Pinus contorta* post-fire was independent of tree density or age (Litton *et al.*, 2004), but three years after thinning, fine root biomass of *Quercus ilex* increased by 100 % (Lopez *et al.*, 2003), and the foliage mass of *Pinus radiata* was not affected by silvicultural regime, tree size or tree status (dominant or subdominant) (Rodríguez *et al.*, 2003). Differences in silvicultural regime can alter competition for light, moisture and nutrients. In *P. radiata*, C allocation to the stem was increased when in competition for light or nutrients (Rodríguez *et al.*, 2003). Increased light availability has also been shown to significantly increase the C partitioning below-ground in *Lolium perenne* (Hodge *et al.*, 1997). When grown in sand culture, the total biomass was unaffected, therefore increasing the root:shoot ratio of *L. perenne* (Hodge *et al.*, 1997). However, when grown in soil, total biomass increased, with an increase in shoot growth and, therefore, there was a decline in the root-shoot ratio (Hodge *et al.*, 1997).

An increase in atmospheric ozone concentration decreased the wood biomass of *Betula pendula* saplings by 22 % in the first growing season but there was no further change in the second growing season (Karlsson *et al.*, 2003). Root biomass also decreased by 30 % in the first growing season but the biomass of the foliage increased during both growing seasons (Karlsson *et al.*, 2003). However, elevated atmospheric CO_2 concentration resulted in an increased uptake of C in a *Pinus taeda* forest in North Carolina, USA. Eighty-three percent of the increased C was allocated to the trunk and the majority of the remainder to the root system (Schäfer *et al.*, 2003). In *Castanea sativa* and *Fagus sylvatica* biomass increased with increased atmospheric CO_2 concentration, but in *C. sativa* the additional C was

partitioned mainly to the roots and in *F. sylvatica* the additional C was allocated equally amongst all organs (El Kohen *et al.*, 1993). In *Pinus sylvestris*, elevated atmospheric CO_2 increased late wood density (Kilpelainen *et al.*, 2003) but in *Pseudotsuga menziesii* elevated CO_2 did not alter biomass production or allocation to any organ (Olszyk *et al.*, 2003). However, elevated temperature decreased biomass allocation to the leaves (Olszyk *et al.*, 2003). During 1993 to 1999, May temperature was discovered as an important predictor of total tree biomass production in a hardwood forest in Quebec, Canada (Côté *et al.*, 2003).

8.1.2 Genotypic effects on carbon and biomass allocation

Twenty-three percent of the variation in biomass in *Acer rubrum* seedlings was accounted for by differences among the geographic origins (Mohan *et al.*, 2004). A common garden experiment of *Picea abies* from 54 populations from different altitudes showed a decrease in seedling dry mass with increasing altitude of seed origin, regardless of the elevated photosynthetic rates (Oleksyn *et al.*, 1998). The allocation of dry mass also differed with a doubling of partitioning to the root with increasing altitude of origin (Oleksyn *et al.*, 1998).

In three populations of *Eucalyptus microtheca* from north-west, central and south-east of Australia, biomass decreased under water deficit with the effect greater in the north-west and central populations (Li & Wang, 2003). In *Eucalyptus cloeziana* from humid and dry provenances grown under 100 %, 70 % and 50 % field capacity water regimes, there was no alteration in biomass production (Ngugi *et al.*, 2003). However, when compared with a species from a dryer environment, *Eucalyptus argophloia*, the species from the dryer environment produced twice as much biomass under 100 % field capacity and three times as much biomass at 70 % and 50 % field capacity than *E. cloeziana* (Ngugi *et al.*, 2003).

The seedling biomass of northern provenances of *Picea abies* all decreased with elevated atmospheric CO_2 concentration, with no difference between provenances (Vanhatalo *et al.*, 2003). Slow-growing clones of *Betula pendula* increased biomass in elevated atmospheric CO_2 concentration but there was a decrease in fast-growing clones (Vanhatalo *et al.*, 2003). The biomass of six clones from two populations of *P. abies* was enhanced by elevated atmospheric CO_2 but did not alter allocation patterns (Spinnler *et al.*, 2003), and elevated atmospheric CO_2 increased whole plant dry matter production in two families of *Pinus radiata* but only when phosphorus was in adequate supply (Conroy *et al.*, 1990). However,

allocation differed with more C allocated to the trunk in one family, while the other allocated more C to roots and branches (Conroy *et al.*, 1990). In ambient atmospheric CO_2 concentration, there was very little difference between two northerly clones and two southerly clones of *Picea sitchensis* (Centritto *et al.*, 1999). In elevated atmospheric CO_2, there was an increase in biomass for all clones with no change in allocation but southerly clones out-performed northerly clones (Centritto *et al.*, 1999).

8.1.3 Hypotheses

- With environmental effects largely influencing carbon allocation, it was expected that the carbon allocation would reflect the differences between the three sites, regardless of clone.
- With Newcastleton having the highest nutrient concentration and Wauchope the lowest (Chapter 3), a greater proportion of carbon allocated to above-ground organs was expected at Newcastleton and below-ground at Wauchope.
- With differences in height, diameter and wood density seen after 10 years of growth (Chapter 4), differences in carbon allocation were expected between clones at each site, and the same pattern was expected between clones at each site.

8.2 Methods

8.2.1 Destructive biomass sampling

Four individual trees of each clone were randomly selected and felled at the three sites. Branches were removed from each whorl of each tree, counted and weighed. The canopy was split into three equal sections and three branches from each section were randomly selected and sealed in pierced polythene bags. The branches were stored at 4 °C within eight hours of cutting and analysed within 96 hours of felling.

The trunks were cut into 2.5 m long logs and transported to the Centre for Timber Technology and Construction (Building Research Establishment, Watford, UK) for analysis of wood density.

8.2.2 Foliage and branch analysis

Within laboratory conditions, all foliage from current year, 1-year, 2-year and 3-year-old needle age classes were removed from the three branches of each section of the canopy. Needles were separated into needle age classes, weighed and dried at 65 °C for at least 48 hours. After cooling in a desiccator, needles were re-weighed and percentage dry weight calculated.

Branches were stripped of any remaining foliage and a 10 cm long section cut from one branch of each section of the canopy. The branch section was weighed and dried at 65 °C for at least 48 hours. After cooling in a desiccator, the branch section was re-weighed and percentage dry weight calculated.

8.2.3 Wood density calculation (kg m^{-3})

A small disc of variable thickness (10 to 100 mm long) was cut from each log using a chain saw. The disc was debarked and submerged in water for 48 hours until fully saturated. The volume (m^{-3}) of the disc was then calculated by measuring the displacement caused by the disc in a container of water. The disc was then dried in an oven at 103 °C for 48 hours or until there was no change in weight to ensure complete removal of water. The disc was weighed and the density calculated (equation 8.1).

$$\text{Density (kg m}^{-3}) = \frac{\text{Mass (kg)}}{\text{Volume (m}^{-3})} \qquad [8.1]$$

8.2.4 Coarse and fine root density (mg roots cm^{-3} soil)

For each felled tree at each site, using a chamber auger, three cores were taken at 0.1 m, 0.5 m and 1.0 m from the root collar in a north-west direction. The core was taken to a depth of 1 m. The cores were stored in PVC piping and placed in a black plastic bag, to avoid the formation of moulds and retain humidity. The cores were stored in a cool (4 °C) and dry environment.

The core was separated into 10 cm long sections and the soil suspended in water. The soil-root mixture was poured into stacked sieves with a mesh size range of 2 cm^2 to 0.2 mm^2 and washed using a jet of water aided by hand manipulation. Roots were then removed

individually and water carefully removed by blotting on tissue paper. The roots were separated into coarse (≥ 5mm diameter) and fine (< 5mm diameter) roots, weighed and dried in an oven at 65 °C for 48 hours. Percentage dry weight of the roots was then calculated. Root density was calculated as mg roots cm^{-3} soil, from the measured root weight within the known volume of the auger.

8.2.5 Carbon allocation

Carbon mass was reported as equivalent to the dry weight of the various components of the tree, although it was recognised that this measurement will also include other elements and that the dry weight was not entirely comprised of carbon.

The carbon allocated to needles was calculated per age class, as the dry weight proportion of the needle sample per branch. The carbon mass for each age class was summed to gain the carbon mass per branch and then multiplied by the number of branches in the section of the canopy the original needle samples were taken from. The foliage carbon mass for each section of the tree was summed to gain the foliar carbon mass for the whole of the canopy.

The fresh weight of the branches in each section was determined by subtracting the fresh weight of the whole of the foliage in the section from the weight of the section of the canopy (branches plus foliage). The carbon mass of the branches was calculated as the dry weight proportion of the branches. The branch carbon mass for each section of the tree was summed to calculate the branch carbon mass for the whole canopy.

The carbon mass of the trunk was calculated from the trunk volume and wood density measurements. The trunk volume was calculated from the diameter measurement of each log and the length of each log, assuming that each log was a cylinder. By knowing the volume and wood density of the trunk, it was possible to calculate the carbon mass.

With the total fresh weight of the root system unknown, it was not possible to calculate the total carbon mass for the roots. Carbon allocation to roots was, therefore, expressed as a dry weight density (mg roots cm^{-3} soil).

8.2.6 Statistical analyses

Using SPSS® release 10, an analysis of variance was used to investigate the effects of site and clone on wood density, root density and carbon allocation. As a result of small sample sizes, a Kruskal-Wallis test was used. Significant differences were reported at the 0.05 probability level.

8.3 Results

8.3.1 Wood Density

Wood density was highest at Scootmore and lowest at Newcastleton for all clones (wood density values in Appendix 6). However, there was only a significant difference between sites for C20208 (Appendix 7).

C20177 had the lowest wood density at all three sites. C20208 had the highest wood density at Wauchope and Scootmore, with C20211 having the highest wood density at Newcastleton. The differences between the clones were significant at all sites (Appendix 7).

8.3.2 Root Density

The fine root density (mg roots cm^{-3} soil) was highest at Scootmore and lowest at Wauchope for all clones at all distances from the root collar, with the exception of C20211, which had the lowest root density at 0.1 m from the root collar at Newcastleton (Table 8.1). There were no significant differences between sites (Appendix 7).

The coarse root density (mg roots cm^{-3} soil) was much more variable, with highest and lowest density differing both within clones at different sites and between clones on the same site (Table 8.1). At 0.5 m from the root collar only, the lowest coarse root density for all clones was at Wauchope. The only significant difference between sites was for C20211 (Appendix 7).

The clone with the highest fine root density varied between sites and between distances from the root collar, although at 0.5 m C20211 had the highest density at all sites (Table 8.2). At 0.5 m and 1.0 m, C20177 had the lowest fine root density at all sites. There were

no significant differences in fine root density between clones at any site or position (Appendix 7).

The highest and lowest coarse root density was even more varied, with no pattern between clones at each site (Table 8.2). The clones with the highest and lowest coarse root density (C20208 and C20211, respectively) were the same at Newcastleton at 0.1 m and 0.5 m. At 1.0 m, there was little difference in the root density between clones, particularly at Newcastleton and Wauchope. There were no significant differences in coarse root density between sites for any clone at any position (Appendix 7).

Table 8.1: The site (Newcastleton (●), Wauchope (○) or Scootmore (▲) with the highest and lowest fine and coarse root density for each clone at three distances from the root collar (0.1 m, 0.5 m and 1.0 m).

		Fine Roots		Coarse Roots	
Distance	Clone	Highest	Lowest	Highest	Lowest
0.1 m	C20177	▲	○	▲	●
	C20208	▲	○	●	▲
	C20211	▲	●	○	●
0.5 m	C20177	▲	○	●	○
	C20208	▲	○	▲	○
	C20211	▲	○	▲	○
1.0 m	C20177	▲	○	●	○▲
	C20208	▲	○	▲	●○
	C20211	▲	○	●▲	○

Table 8.2: The clone (C20177 (●), C20208 (○) or C20211 (▲) with the highest and lowest fine and coarse root density for each clone at three distances from the root collar (0.1 m, 0.5 m and 1.0 m).

		Fine Roots		Coarse Roots	
Distance	Site	Highest	Lowest	Highest	Lowest
0.1 m	Newcastleton	●	▲	○	▲
	Wauchope	▲	●	●	○
	Scootmore	●	▲	●	○
0.5 m	Newcastleton	▲	●	○	▲
	Wauchope	▲○	●	●▲	○
	Scootmore	▲	●	○	●
1.0 m	Newcastleton	○	●	●○▲	
	Wauchope	○	●	●○▲	
	Scootmore	▲	●	○	●▲

8.3.3 Above-ground carbon allocation

C20177 contained the largest quantity of carbon and C20211 the lowest at Newcastleton and Wauchope (Table 8.3). At Scootmore, C20211 contained the lowest carbon concentration but C20208 had the largest.

The greatest allocation of carbon was in the trunk for all clones at Newcastleton (Table 8.3). At Wauchope and Scootmore, the greatest allocation carbon was in the trunk for C20208 and C20211 but in the branches for C20177. For all clones at all sites, the lowest quantity of carbon was allocated to the needles.

Amongst the clones at each site, the greatest proportion of carbon allocated to needles was in C20211 and the lowest in C20177 (Table 8.3). The greatest proportion of carbon allocated to the branches and trunk was in C20177 and C20208, respectively. The lowest proportion of carbon allocated to the branches and trunk was in C20211 and C20177, respectively. There were no significant differences between sites or clones in C allocation to trunk, foliage and branches, or total C in above-ground organs.

Table 8.3: Carbon allocation (kg per total above-ground weight of tree) and percentage of total above-ground carbon allocation to foliage, branches and trunk, and total above-ground carbon allocation for each clone at each site.

		Foliage		Branch		Trunk		Total	
Site	Clone	kg	%	kg	%	kg	%	kg	%
Newcastleton	C20177	0.59 ±0.04	**0.88**	24.65 ±8.09	**36.64**	42.03 ±7.96	**62.48**	67.27	100
	C20208	0.90 ±0.23	**1.71**	15.29 ±1.64	**29.25**	36.08 ±2.31	**69.04**	52.26	100
	C20211	1.01 ±0.07	**3.54**	7.94 ±0.84	**27.98**	19.42 ±2.53	**68.47**	28.36	100
Wauchope	C20177	0.65 ±0.16	**1.67**	20.88 ±9.05	**53.56**	17.46 ±1.71	**44.77**	38.99	100
	C20208	0.60 ±0.25	**1.93**	10.16 ±5.74	**32.99**	20.05 ±3.75	**65.08**	30.80	100
	C20211	0.75 ±0.04	**2.85**	9.31 ±0.68	**35.65**	16.06 ±0.93	**61.50**	26.12	100
Scootmore	C20177	0.51 ±0.10	**2.54**	10.98 ±2.71	**54.71**	8.58 ±3.33	**42.75**	20.06	100
	C20208	0.71 ±0.19	**3.26**	8.31 ±5.34	**38.43**	12.61 ±8.60	**58.31**	21.63	100
	C20211	0.37 ±0.17	**7.62**	1.89 ±0.90	**39.35**	2.54 ±1.09	**53.03**	4.79	100

8.3.4 Below-ground carbon allocation

Fine roots

The greatest carbon allocation to fine roots at Newcastleton and Wauchope was in C20208 and in C20211 at Scootmore (Appendix 6). The lowest allocation was in C20177 at all three sites.

The distance from the root collar where the greatest allocation to fine roots was found differed between sites for each clone (Table 8.4). Only C20177 had the greatest fine root allocation at the same position (0.1 m from the root collar) at each site. For C20208, the greatest root allocation was found at the same position at Newcastleton and Wauchope, and for C20211 the C allocation to fine roots was greatest at the same distance at Newcastleton and Scootmore. The same pattern was present for the lowest fine root allocation, with the allocation lowest at the same position at all sites for C20177, at Newcastleton and Wauchope for C20208, and at Newcastleton and Scootmore for C20211.

Table 8.4: Percentage C allocation to fine and coarse roots, for three distances (0.1m, 0.5 m and 1.0 m) from the root collar, for each clone (C20177, C20208 and C20211) at the three sites (Newcastleton, Wauchope and Scootmore).

		Fine Roots			Coarse Roots		
		0.1 m	0.5 m	1.0 m	0.1 m	0.5 m	1.0 m
C20177	Newcastleton	43.04	30.97	25.99	58.95	27.24	13.81
	Wauchope	32.36	29.12	38.52	50.89	40.00	9.11
	Scootmore	21.08	38.00	40.92	57.62	18.08	24.31
C20208	Newcastleton	38.67	30.82	30.51	80.36	12.71	6.93
	Wauchope	32.96	32.89	34.15	49.46	8.08	42.46
	Scootmore	39.58	31.72	28.70	75.22	17.11	7.67
C20211	Newcastleton	46.54	36.52	16.94	89.41	6.96	3.62
	Wauchope	23.17	44.65	32.18	4.08	21.44	74.48
	Scootmore	15.58	40.63	43.80	24.04	48.71	27.25

There was little similarity between the clones with the greatest C allocation to fine roots at each site, although C20177 and C20211 had the highest allocation at 0.1 m and 1.0 m,

respectively, at Newcastleton and Scootmore (Table 8.5). C20211 and C20177 also at Newcastleton and Scootmore had the lowest root allocation at 0.1 m and 1.0 m, respectively (Table 8.6).

Table 8.5: The clone (C20177 (●), C20208 (○) and C20211(▲)) with the greatest C allocation to fine and coarse roots for the three positions (0.1 m, 0.5 m and 1.0 m from the root collar) at each site (Newcastleton, Wauchope and Scootmore).

	Highest C allocation to fine roots			Highest C allocation to coarse roots		
	0.1 m	0.5 m	1.0 m	0.1 m	0.5 m	1.0 m
Newcastleton	●	▲	▲	●	○	▲
Wauchope	▲	○	○	●	▲	○
Scootmore	●	○	▲	●	▲	○

Coarse Roots

The greatest carbon allocation in coarse roots was in C20208 at Newcastleton and Scootmore, and in C20177 at Wauchope (Appendix 6). The lowest carbon allocation to coarse roots was in C20211 at Newcastleton and Scootmore, and in C20208 at Wauchope.

At Newcastleton and Wauchope, the highest coarse root allocation for all clones was at 0.1 m from the root collar (Table 8.4). At Scootmore, each clone showed a different response. The lowest coarse root allocation varied greatly between clones at all sites, with no pattern emerging between or among the clones.

At 0.1 m from the root collar, C20177 had the greatest root allocation (Table 8.5) and C20208 the lowest at all three sites (Table 8.6). C20211 had the greatest allocation at 0.5 m and C20208 at 1.0 m at Wauchope and Scootmore, whilst at Newcastleton C20208 had the highest allocation at 0.5 m and C20211 at 1.0 m. The clone with the lowest allocation at 0.5 m and 1.0 m varied between sites, although C20177 had the lowest allocation at 1.0 m at Wauchope and Scootmore.

Table 8.6: The clone (C20177 (●), C20208 (○) and C20211(▲)) with the lowest fine and coarse root density (mg roots cm^{-3} soil) for the three positions (0.1 m, 0.5 m and 1.0 m from the root collar) at each site (Newcastleton, Wauchope and Scootmore).

	Lowest C allocation to fine roots			Lowest C allocation to coarse roots		
	0.1 m	0.5 m	1.0 m	0.1 m	0.5 m	1.0 m
Newcastleton	▲	○	●	○	▲	○
Wauchope	○	●	▲	○	○	●
Scootmore	▲	●	●	○	●	●

8.4 Discussion

Wood density was highest at Scootmore and lowest at Newcastleton, which was in agreement with the 10-year Pilodyn® measurement data in Chapter 4. The trees at Newcastleton have larger trunk diameters but lower wood density, showing an inverse relationship between diameter and wood density, a relationship commonly seen in trees (Wood, 1986). There were no significant differences between sites, suggesting that the environmental differences were causing little effect on the wood density. Moisture availability affected C allocation in *Pinus palustris* (Jose *et al.*, 2003), with water stress causing an increase in the root systems and high water availability increasing C allocation to the above-ground organs. With precipitation levels lower at Scootmore (Chapter 3), a lower wood density was expected. However, with precipitation abundant during the year and during the growing season at all three sites then water availability was unlikely to have impacted on wood density. Temperature was also similar between sites (Chapter 3) and was unlikely to be causing differences in wood density. Nutrient levels were different between sites (Chapter 3), with concentration highest at Newcastleton and lowest at Wauchope. *Populus nigra* and *Pinus radiata* allocated more C to the roots under nutrient limitation (Glynn *et al.*, 2003; Rodríguez *et al.*, 2003), so a reduced wood density was expected at Scootmore. However, nutrient availability had no effect on *Larix leptolepis* and loblolly pine (Lee & Jose, 2003; Son & Hwang, 2003) and even increased C allocation above-ground in *Eucalyptus saligna* (Giardina *et al.*, 2003). It appeared that nutrient availability was not affecting wood density or that other factors were causing a larger effect.

Fine root density was highest at Scootmore and lowest at Wauchope. Previous research has shown that low nutrient availability stimulated fine root growth (Glynn *et al.*, 2003; Rodríguez *et al.*, 2003), therefore greater fine root density was expected at Wauchope and the lowest density at Newcastleton. Water stress also increased the fine root system in *Pinus palustris* (Jose *et al.*, 2003). There was little difference in precipitation levels between sites during the growing season but annual precipitation was lower at Scootmore, with little difference between Wauchope and Newcastleton. The difference in annual precipitation explained the higher root density at Scootmore. The soil at Scootmore also contained a higher percentage of sand (Chapter 3), suggesting that the soil is more free draining and therefore unable to hold as much water as the soils at Wauchope and Newcastleton. Coarse root density varied greatly between distance from the root collar and between sites, with no consistent or explainable pattern. It appeared that the larger, regional, environmental impacts were not affecting coarse root growth.

C20208 had the highest wood density at Wauchope and Scootmore but C20211 had the highest wood density at Newcastleton. C20177 has the lowest wood density at all sites. C20177 had the largest trunk diameter at each site and agreed with the inverse relationship between diameter and wood density (Pfeifer, 1984; Wood, 1986). C20211 had the highest wood density at Newcastleton and also had the smallest diameter and Pilodyn® measurement, again fitting the diameter-wood density relationship. C20208 had the highest wood density at Scootmore and Wauchope suggesting that changes have occurred during the three years after height, diameter and Pilodyn® distance were measured to the destructive biomass sampling. To further confound matters, C20208 at Scootmore had the largest diameter after 10 years of growth and was therefore expected to have the lowest wood density.

Within each site, C20177 had the lowest fine root density but the highest fine root density response between clones was varied. With nutrient concentration, water availability and temperature uniform throughout each site, it appeared that C20177 differed from the other clones by allocating more C to above-ground organs and less to the root systems. The clone with the highest fine root density varied between C20208 and C20211, suggesting that these clones were showing a similar pattern in allocation, allocating more C to below-ground organs in comparison to C20177. The differences in fine root density were a result of the clone's response to the environment at each site. The response of the clones in

coarse root density was not consistent and showed no pattern within any site, suggesting the differences in genotype did not affect coarse root density.

At Newcastleton and Wauchope, carbon allocation was highest above-ground in C20177 and lowest in C20211. At Scootmore, above-ground C allocation was also lowest in C20211 but highest in C20208. The trees that were taller and had larger trunk diameters also contained the largest C concentration and those that were smaller had the lowest C concentration. Therefore, at Newcastleton and Wauchope, C20177 had the tallest trees, the largest diameters and the greatest C content. At all three sites, C20211 had the shortest trees, smallest diameters and, therefore, the lowest C content. At Scootmore, C20208 had the greatest C content but at this site C20208 also had the tallest trees and largest diameters. There were no significant differences between clones within each site, suggesting that there was little difference between the clones. A previous comparison of two northerly clones and two southerly clones of *Picea sitchensis* also illustrated very little difference in carbon content between clones (Centritto *et al.*, 1999).

The greatest proportion of C was allocated to the trunk in all clones at Newcastleton but only for C20208 and C20211 at Wauchope and Scootmore. C20177 at Wauchope and Scootmore allocated the greatest proportion of carbon to the branches. All clones at all sites allocated the smallest proportion of C to the needles. Differences in above-ground allocation have been found previously in *Pinus radiata*, with more C allocated to the trunk in trees from one family, while the trees from another family allocated more to branches (Conroy *et al.*, 1990). Although the clones were showing differences in C allocation above-ground, it also depended on the site. At Newcastleton, all clones showed the same allocation but environmental differences at Wauchope and Scootmore produced a differentiation in allocation patterns amongst the clones.

An increase in C allocation to the branches was not accompanied by an increase in C allocation to the foliage. Amongst the clones, C20211 allocated the greatest proportion of C to foliage and C20177 the smallest, even though at Wauchope and Scootmore C20177 allocated the largest proportion of C to the branches. There was an inverse relationship between C allocation to foliage and C allocation to branches, as C20211 has the smallest C allocation to the branches but the largest to the foliage, whilst C20177 has the largest C allocation to branches but the smallest to the foliage.

At Newcastleton and Wauchope, C20208 allocated proportionally more C to fine roots than the other clones. At Scootmore, C20211 allocated proportionally more C to fine roots and at all sites C20177 allocated the least C to fine roots. Again, the clones at Newcastleton and Wauchope were behaving in the same way. As Newcastleton and Wauchope were similar sites in terms of geographic location, climate and land-use history, it was expected that the clones would respond in a similar way.

There were differences in C allocation to fine roots between the sites and clones at different distances from the root collar. C20177 showed the same pattern at each site, with greatest C allocation to fine roots at the same distance from the root collar. C20208 also showed the greatest C allocation to fine roots at the same distance but at Newcastleton and Wauchope only, and C20211 showed the greatest C allocation to fine roots at the same distance at Newcastleton and Scootmore. The clones were showing the same pattern at Newcastleton, again suggesting that the conditions were more favourable at this site, with a small environmental effect on the C allocation to fine roots. Wauchope and Scootmore showed more variation, suggesting that there were greater environmental effects. Precipitation levels were lower at Scootmore and nutrient concentration lower at Wauchope, possible explaining the increased variation in C allocation.

The greatest C allocation to coarse roots at Newcastleton and Scootmore was in C20208 and at Wauchope in C20177. The lowest C allocation to coarse roots at Newcastleton and Scootmore was in C20211 and C20208 at Wauchope. Newcastleton and Scootmore were showing the same pattern suggesting that precipitation levels may not be causing differences in coarse root allocation. Wauchope was different from the other two sites, and with nutrient availability lower at this site, suggests that the differences in C allocation to coarse roots were a result of nutrient concentrations. However, all clones at Newcastleton and Wauchope showed the greatest C allocation to coarse roots at the same distance from the root collar, suggesting that these sites were similar. The distance at which C allocation to coarse roots was greatest showed no pattern at Scootmore, and there was no pattern in the distance from the root collar, which had the lowest C allocation to roots, suggesting there was little genotypic control over C allocation to coarse roots and the distribution of coarse roots for each tree.

8.5 Conclusions

The environment affected wood density, with each site showing different wood densities. Wood density showed an inverse relationship with trunk diameter, so that the clone with largest trunk diameter at each site also had the lowest wood density and the clone with the smallest trunk diameter had the highest wood density.

Fine root density showed a clear pattern amongst sites, with fine root density highest at Scootmore and lowest at Wauchope. This response was probably a result of precipitation levels, as Scootmore had the lowest annual precipitation and Wauchope the highest. Nutrients did not appear to have an effect, as low nutrient availability increases fine root biomass but Wauchope had the lowest nutrient concentrations and lowest fine root density. There was no consistent pattern in coarse root density.

The greatest carbon allocation above-ground was in the same clone at Newcastleton and Wauchope, and the lowest carbon allocation above-ground was in the same clone at all three sites. C allocation was positively related to tree height and trunk diameter.

For each clone at each site, with the exception of C20177 at Wauchope and Scootmore, the greatest allocation among above-ground organs was in the trunk. For C20177 at Wauchope and Scootmore the greatest allocation was in the branches. For all clones, the lowest allocation was in the foliage. There was an inverse relationship between C allocated to the trunk and C allocated to the foliage.

The greatest C allocation to fine roots was in the same clone at Newcastleton and Wauchope, and the lowest C allocation was in the same clone at all three sites. The greatest and lowest C allocation to coarse roots was in the same clone at Newcastleton and Scootmore. This suggested that the fine root C allocation of the clones were affected by climate effects, and therefore showed a similar response at Newcastleton and Wauchope, but were not greatly affected by the nutrient concentrations. The similarity of C allocation to coarse roots at Newcastleton and Scootmore suggested that nutrient concentration affected C allocation to coarse roots.

9. Process-based modelling of sun and shade photosynthesis of three clones of Sitka spruce at three sites

Abstract

A process-based model of tree evapotranspiration was used to simulate the net photosynthetic rates (A_{net}) of Sitka spruce. A_{net} was calculated for sun and shade foliage on a daily basis and validated with data from three Sitka spruce clones at three sites. A_{net} of sun foliage was highest at Newcastleton. The A_{net} of sun foliage was lowest at Scootmore for clones C20177 and C20211, and lowest at Wauchope for C20208. There was little difference in the A_{net} of shade foliage between sites. Within Newcastleton and Scootmore, C20177 had the highest A_{net} and C20211 the lowest A_{net} of sun foliage. C20208 had the highest and C20211 had the lowest A_{net} for the sun foliage at Wauchope and for the shade foliage at all sites. The differences between sites and between clones were greater during the growing season and smaller during winter. The model overestimated A_{net} between 29 % and 70 % at Newcastleton and underestimated A_{net} between 10 % and 71 % at Wauchope and Scootmore. The error between simulated and observed values was a result of errors in the weather input data and errors in certain parameters. A sensitivity analysis of the parameters indicated that six largely influenced the photosynthetic output of the model (LAI, nitrogen concentration at the top of the canopy, ratio of photosynthetic capacity-to-leaf nitrogen, V_m activation energy, S and H). The effects were greater in sun foliage except for LAI, which had greater effects on the shade foliage photosynthesis.

9.1 Introduction

9.1.1 The Farquhar model

The Farquhar model is one of the most widely used and developed models (Farquhar *et al.*, 1980; von Caemmerer & Farquhar, 1981). The model is a comprehensive description of the biochemical processes of photosynthesis that was compatible with studies of gas exchange measurements. The fundamental core of the model split CO_2 assimilation into two parts: the carboxylation reactions and the oxygenation reactions. These reactions may be limited by Rubisco activity, by the concentration of Rubisco and by the rate of RuBP regeneration. The equations describing the carboxylation and oxygenation reactions are

integrated at the chloroplast level and extended to the leaf, by summing the contributions of each chloroplast.

The Farquhar model was further developed to describe the limitation of Rubisco activity by the thylakoid reactions or P_i regeneration (Sage, 1990). A slight modification to the temperature dependencies of the equations allowed the model to effectively describe the complex effects of light, temperature and relative humidity of sun and shade leaves of *Quercus alba* L. and *Acer rubrum* L. at Oak Ridge, USA (Harley & Baldocchi, 1995). The Farquhar model has also been used to simulate the photosynthesis of *Picea mariana* at the leaf, branch and canopy level (Rayment *et al.*, 2002) and estimate photosynthesis of *Picea abies* at the branch level (Falge *et al.*, 1996). The results for *P. mariana* demonstrated the observed seasonal dynamics but the results for *P. abies* showed errors in light acclimation, in the damage caused by pollutant deposition, needle age and cold stress effects. The model also showed considerable error in simulating the photosynthetic response of *Q. alba* and *A. rubrum* at different atmospheric CO_2 concentrations.

Using the Farquhar model to describe photosynthesis, a model was designed to predict radiation absorption, photosynthesis and transpiration for *Picea sitchensis* and *Picea radiata* stands (Wang & Jarvis, 1990), using three needle age classes and three ecological types of needles (sun, intermediate and shade), different leaf area densities and leaf angles. Hourly calculations showed large deviations from the observed data but daily calculation differed by less than 10 %. Other models based on the Farquhar model also found good agreement (within 20 %) between observed and simulated data (Falge *et al.*, 2000; Zhang & Xu, 2003).

9.1.2 Carbon allocation and the pipe-model theory

The Farquhar photosynthetic model has also been coupled with carbon allocation models to describe dry matter partitioning. The photosynthetic products were partitioned into root and shoot dry matter (Reynolds & Thornley, 1982; Thornley, 1972), using allometric ratios between stem diameter, tree height and branch biomass, and between tree diameter and root biomass (Bartelink, 1998), after considering losses from leaves and root and through respiration (McMurtrie & Wolf, 1983). Resulting models were used to calculate net primary production in temperate and boreal forests (Aber & Federer, 1992; Landsberg & Waring, 1997).

Carbon allocation models often included the principles of the pipe-model theory to describe dry matter partitioning. The pipe-model theory states that each unit measure of the foliage on a tree was attached via an active pipe extending to the base of the trunk and into the root system (Shinozaki *et al.*, 1964), so the sapwood area of a tree was proportional to foliage biomass. Models include a constant ratio between foliar dry matter and total cross-sectional area of active pipes, and dry matter growth was equal to pipe dry matter growth, height increment was equal to the increase in pipe length and basal area was equal to the total pipe basal area (Valentine, 1985, 1988). However, trees reach a foliar dry matter maximum but the basal area continues to increase. The increase in basal area was a result of aggregated disused pipes, where live branches have withered and shed but their pipes remain (Chiba, 1998). Allocation models incorporating the pipe-stem theory have been used to accurately simulate the growth of *Picea taeda* (Valentine, 1999) and *Eucalyptus* (West, 1993).

9.1.3 Hypotheses

Using a process-based model of evapotranspiration (ForestETp), with photosynthesis described using the Farquhar model, daily photosynthesis was calculated for Sitka spruce. Using parameters calculated from each clone at each site, it was hypothesised that:

- ForestETp would accurately predict the photosynthesis at each site and for each clone, when compared with the observed data (Chapter 7).
- The simulated photosynthesis would show the same pattern between sites as the observed photosynthetic rates (i.e. the site showing the highest photosynthetic rates will also show the highest simulated rates).
- The simulated photosynthesis would show the same pattern amongst the clones as the observed data (i.e. the clone showing the highest photosynthetic rates would also show the highest simulated rates).

9.2 Methods

9.2.1 Model description

For an in-depth description of the ForestETp model, see Evans *et al.* (2005).

The macro-climate module

The module is a stochastic-deterministic, site-scale module that provides daily time step climate input from readily available monthly time step climate data.

Precipitation is generated using a two-state Markov process to determine the occurrence of a rain day. On a rain day, the module assumes a single rainfall event of uniform intensity and when the temperature falls beneath zero, this is assumed to be in the form of snow. A two-step gamma probability distribution function describes the amount of rainfall occurring on a rain day.

Air temperature is generated from an auto-correlation intensity process coupled with a uniformly random generated distribution around the observed mean and constrained within the observed standard deviation. Wind speed and relative humidity are estimated using a modelling technique analogous to mean air temperature.

Solar radiation is approximated using spherical geometry and is corrected for the impact caused by the atmosphere's constituent gases and dust particles. The solar radiation is further attenuated and scattered by cloud cover and corrected for latitude, aspect and slope. The radiation is separated into direct and diffuse radiation, with a further separation into the photosynthetically active elements.

Canopy radiative transfer module

The canopy is differentiated into different layers and within each layer a distinction is made between sunlit and shaded leaves. A radiative transfer scheme simulates the transmittance, reflectance and adsorption of long wave, near infra-red and direct and diffuse photosynthetically active radiation (PAR), and separates the penetration of direct and diffuse radiation through the canopy of sunlit and shaded leaves. The composition of wavelengths does not change, regardless of cloudiness or aerosol composition, but does consider changes through canopy absorption. The module irradiance equations follow De Pury & Farquhar (1997). For both sunlit and shaded leaves, the area within the canopy is calculated and the mean irradiance, mean layer assimilation, transpiration and conductance rate generated. The irradiance absorbed by the sunlit leaves is calculated with direct beam, scattered beam and diffuse radiation. The shaded leaves are assumed to receive diffuse radiation only. Leaf angle is also taken into consideration, as is sunfleck penetration. The

profile of nitrogen follows the predicted distribution of absorbed irradiance through each layer and, with the inclusion of seasonal variation, non-uniform profiles of photosynthetic capacity can be developed.

Canopy Water Environment Module

The model considers that there is only a single rainfall event per day. The canopy structure (i.e. the different layers), the mean evaporation and rainfall rates are used to calculate the rainfall interception and the wet canopy evaporation rates. Following the equations of Gash *et al.* (1995), during each rainfall event there is a period of wetting up followed by canopy saturation followed by a period of drying out after the rainfall ceases. The water holding capacity is dependent on the size and shape of the canopy. Also defined are the rainfall falling directly to the soil without touching the canopy, the rainfall dripping onto the soil surface from the saturated canopy, the stem storage rate and the proportion of rain diverted to stem flow that will end up on the soil surface. Evaporation from the wet canopy is defined using a modified Penman-Monteith equation (the ground heat-sink and canopy transpiration terms have been removed).

Soil Environment Module

The module of soil-water balance works on a daily timestep, multi-horizon capacity, which requires the input of climate data with soil survey and laboratory measured physical data. The model allows the vertical and lateral movement of water but does not include the effect of slope, nor does it allow excess soil water to move into profiles from spatially adjacent profiles. For each soil type and within each horizon, movement of water is dependent upon the depth, the content of clay, sand and silt, organic content and the initial water content. The model will simulate the formation of transient perched water tables and generate surface run-off. Root water uptake is calculated from the transpiration demand, the distribution of the roots and the soil water content using the 'sink function' of Jarvis (1989). This assumes a root adaptability factor, a ratio between actual to potential root water uptake proportional to a dimensionless water stress index, which adjusts the stress in one part of the root system by increasing the uptake where conditions are more favourable. The root length distribution is assumed to be logarithmic with depth and the root water uptake is distributed along the root depth according to stress, determined by the water availability, in each horizon (Feddes *et al.*, 1974).

Soil surface evaporation is dependent upon the total incident radiation on the soil surface, which is separate for the soil surface underneath the canopy and not underneath the canopy. The resistance to evaporation during the soil drying out period is generated from a matrix model calculating the soil moisture in both liquid and gaseous phases within the soil pores at different depths (Campbell, 1985). Soil temperature is assumed not be equal to the air temperature.

Gas exchange module

Gas exchange is based on the model describing C_3 photosynthesis by the regulation of Rubisco and electron transport in the leaf developed by Farquhar et al. (1980) and von Caemmerer and Farquhar (1981), with additions from Long (1991), McMurtrie and Wang (1993) and Friend (1995).

Photosynthesis is tightly coupled with the Ball-Berry method of stomatal conductance (Aphalo & Jarvis, 1993; Woodrow *et al.*, 1990) to provide a robust phenomenological description of stomatal conductance. The Ball-Berry model is dependent upon the level of photosynthetic activity, so that the stomata are only open as much as is needed, indirectly affected by the environmental variables.

The temperature effects on the kinetic properties of carboxylation and RuBP regeneration are taken into account by changes in the CO_2 solubility and the affinity of Rubisco to oxygen, calculated using the kinetic constants of McMurtrie and Wang (1993). The module explicitly describes the role of nitrogen by influencing the Rubisco concentration and the soluble leaf proteins used in electron transport (Friend 1995).

The Ball-Berry method for calculating stomatal conductance is an iterative process to resolve the values of the internal concentration of CO_2 (C_i) and assimilation, by altering the value of stomatal conductance. Assimilation and stomatal conductance are inter-dependent and C_i is a function of the interaction between CO_2 assimilation and stomatal conductance to CO_2, regulated by the leaf boundary layer and the mesophyll cell surface resistances to CO_2 transfer.

9.2.2 Calculation of parameters

Parameters derived from the collected data are shown in Table 9.1. Rooting depth (cm) was calculated from root cores (Chapter 8) and was the maximum depth where roots were found. Tree height (m) was the mean height of the clone, calculated from the heights of the individual trees that were used during the gas exchange measurements (Chapter 3). Canopy storage (mm), quantity of precipitation captured by the canopy, was calculated as the LAI multiplied by 0.2 mm (Gash *et al.*, 1995). Nitrogen at the top of the canopy (mmol m^{-2}) was simply calculated from foliar nitrogen concentrations in the needles at 6 m in the canopy. Light compensation point (LCP; mmol mol^{-1} N s^{-1}) was calculated from the x-axis intercept of the light response curves of gas exchange, normalised for tissue nitrogen concentration (Walker, 1989).

All other photosynthetic and canopy parameters (Table 9.2) were extrapolated from the ECOCRAFT database (Medlyn *et al.*, 1999) or from the literature (De Pury & Farquhar, 1997). N_b (mmol m^{-2}) was the non-photosynthetic foliar nitrogen concentration; χ_n (mmol mol^{-1} s^{-1}) was the ratio of photosynthetic capacity-to-leaf nitrogen; K_n was the nitrogen allocation co-efficient; V_m activation energy (J mol^{-1}) was the energy required for the activation of Rubisco; J_m activation energy (J mol^{-1}) was the energy required for the activation of electron transport; S (J K^{-1} mol^{-1}) was the electron transport response to low temperatures; H (J mol^{-1}) was the curvature temperature response to higher temperatures; θ explains the curvature of electron transport to irradiance; G_{min} (mol m^{-2} s^{-1}) was the minimum stomatal conductance.

Rain stemflow is defined as the proportion of water that runs along the branches and trunk, and ultimately ends up on the soil surface. Storage trunk is the quantity of rain that is collected by the trunk and does not end up on the soil surface. The %PAR parameter describes the percentage of radiation that is photosynthetically active and the spectral reflectance factor describes the proportion of radiation that is reflected and not absorbed.

Soil parameters were calculated for each horizon (Table 9.3). Clay, silt, sand and organic content were calculated directly from the laboratory analysis (Chapter 3).

9.2.3 Meteorological data

Meteorological data were obtained from the Met Office Land Surface Observation Stations via the British Atmospheric Data Centre. Data were available from Met stations close to the Wauchope and Scootmore sites. However, for Newcastleton, there were no Met stations located near to Newcastleton and the climate data was calculated via the weather generator module by interpolating data from the Met stations nearest to Newcastleton.

9.2.4 Model Simulations

The model was run for a period of 10 years, starting from 1998 and finishing in 2008. To compare between simulated and observed data, only the results from 2001, 2002 and 2003 during the growing season were used. The model was run once for each clone at each site, so a total of nine simulations were made.

9.2.5 Sensitivity Analysis

For each parameter inputted during each simulation, a sensitivity analysis was conducted. Each parameter was altered by ± 10 %, and the photosynthesis subsequently simulated between 2001 and 2003 for each altered parameter, using only one alteration per simulation.

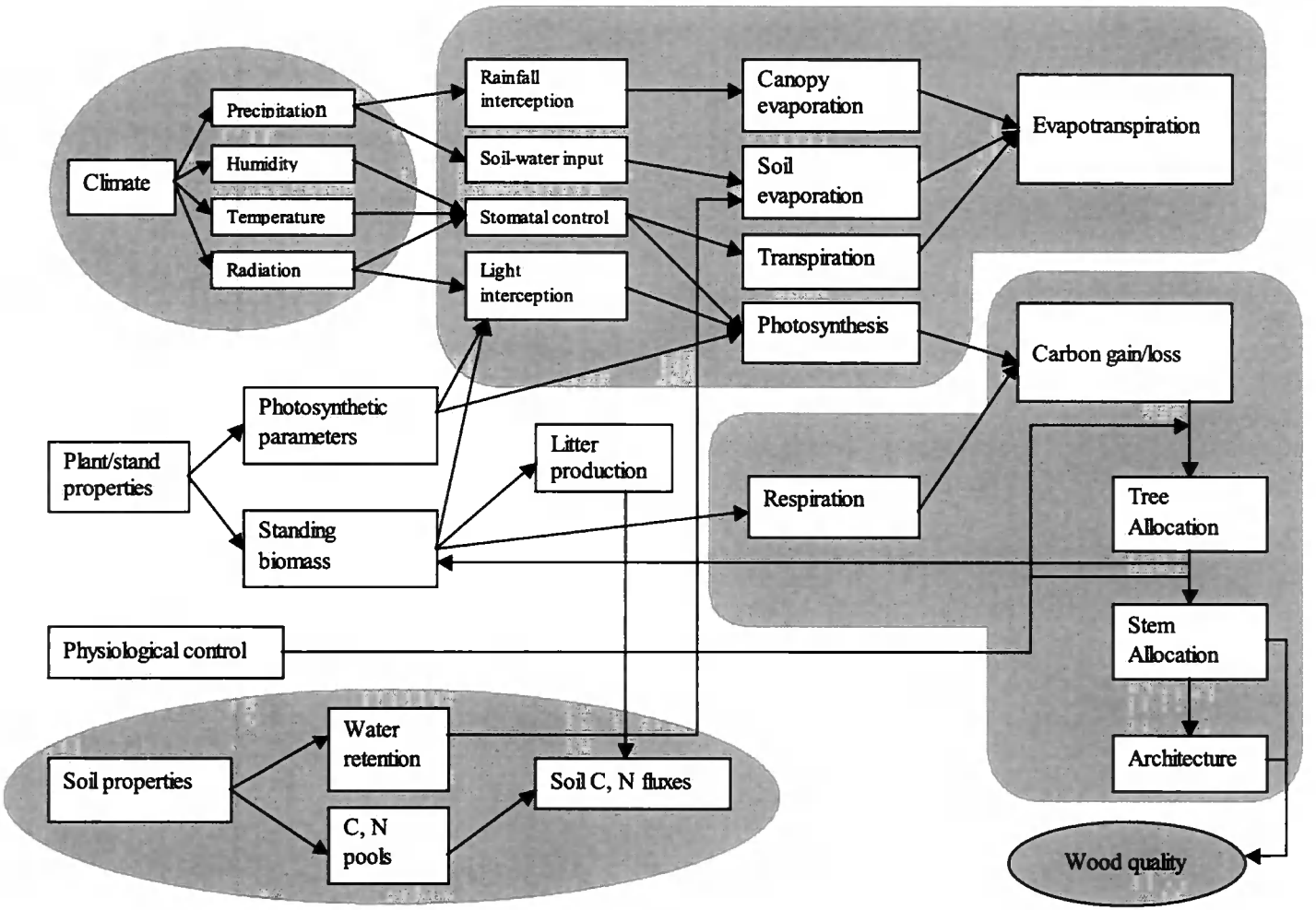


Fig 9.1: Overview of the ForestETp model, showing the modules and the links between them.

Table 9.1: Model parameters calculated from gas exchange, foliar analyses or tree characteristics data.

	Newcastleton			Wauchope			Scootmore		
	C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211
Rooting depth (cm)	50	50	50	50	50	50	50	50	50
LAI (m^2 m^{-2})	2.1	2.5	1.8	2.0	2.9	1.6	2.4	2.7	1.6
Tree height (m)	7.1	6.3	6.9	5.7	5.7	5.9	5.0	4.5	5.0
Canopy storage (mm)	0.42	0.50	0.36	0.40	0.58	0.32	0.48	0.54	0.32
Nitrogen concentration at top of canopy (mmol m^{-2})	168.51	135.83	154.77	128.28	117.30	150.69	117.62	119.27	133.12
Light compensation point (mmol mol^{-1} N s^{-1})	0.923	1.479	1.012	0.355	0.464	1.117	1.012	0.204	1.138

Table 9.2: Parameters extrapolated from the literature

Ec/R	0.15
Storage trunk (mm)	0.25
Rain stemflow	0.1
V_m activation energy (J mol^{-1})*	54000
J_m activation energy (J mol^{-1})*	55400
S (J K^{-1} mol^{-1})*	670
H (J mol^{-1})*	200500
K_n**	0.713
N_b (mmol m^{-2})**	25
χ_n (mmol mol^{-1} s^{-1})	1.16
G_{min} (mol m^{-2} s^{-1}) *	0.043
Stomatal conductance slope*	6.44
Spectral reflectance factor**	0.15
θ**	0.7
Radiation: % PAR	45
PPFD conversion factor	4.5

*Medlyn & Jarvis (1999)
**De Pury *et al.* (1997)

Table 9.3: Soil parameters for the soil environment module

	Newcastleton	Wauchope	Scootmore
Horizon one			
Depth (cm)	20	5	30
Clay content (%)	15.84	1.99	2.45
Silt content (%)	60.66	11.93	22.46
Sand content (%)	12.79	1.99	16.77
Organic content (%)	10.71	84.09	58.32
Horizon two			
Depth (cm)	30	22	20
Clay content (%)	16.60	6.13	6.87
Silt content (%)	63.31	37.53	51.77
Sand content (%)	11.62	6.98	27.32
Organic content (%)	8.47	49.36	14.04
Horizon three			
Depth (cm)	-	23	-
Clay content (%)	-	8.42	-
Silt content (%)	-	48.05	-
Sand content (%)	-	11.28	-
Organic content (%)	-	32.25	-

9.3 Results

9.3.1 Differences in simulated data between sites

Sun foliage

The simulated data showed that all clones had the highest A_{net} at Newcastleton (Figs 9.5-9.7). C20177 and C20211 had the lowest A_{net} at Wauchope, while the A_{net} of C20208 was very similar at Wauchope and Scootmore but with A_{net} slightly higher at Scootmore during the summer and slightly higher at Wauchope during the winter. For C20211, during winter the A_{net} at all three sites was similar and for C20177 the A_{net} was similar during winter at Wauchope and Scootmore.

Shade foliage

The A_{net} of C20177 at Newcastleton and Scootmore was very similar, with the A_{net} at Wauchope being lower. There was little difference between sites during winter (Figs 9.5-9.7). The A_{net} of C20208 showed very little difference between sites. The A_{net} of C20211 was highest at Newcastleton and very similar at Wauchope and Scootmore. There was little difference between sites especially during winter.

9.3.2 Differences in simulated data between clones

Sun foliage

At Newcastleton, there was little difference between the A_{net} of the clones. C20177 had the highest A_{net}, particularly during summer (Figs 9.2-9.4). During summer, C20211 had the lowest A_{net}, whilst C20208 had the lowest A_{net} during winter. At Wauchope, C20208 had the highest A_{net}, with little difference between the A_{net} of C20177 and C20211. During winter, all clones had a very similar A_{net} rate. At Scootmore, there was little difference between the clones, although C20177 had slightly higher A_{net} and C20211 slightly lower A_{net} during summer. During winter, there was little difference, although C20208 had slightly lower A_{net} rates.

Shade foliage

At all three sites, C20208 had the highest A_{net} and C20211 the lowest (Figs 9.2 – 9.4). However, during the winter, there was very little difference in the A_{net} rates between clones.

9.3.3 Sensitivity analysis

The sensitivity analysis showed that six of the ETp parameters largely affected the simulated photosynthetic response (Table 9.4).

A 10 % increase in LAI increased gross photosynthesis by just under 5 %, while a decrease in LAI caused a decrease in photosynthetic rates by about 5 %. An increase in N concentration at the top of the canopy and an increase in the ratio of photosynthetic capacity to leaf N caused a similar increase in photosynthetic rates and a decrease in either parameter caused a similar decrease in photosynthesis. For LAI, N concentration and the ratio of photosynthetic capacity to leaf N, the increase in photosynthesis with a 10 % increase in parameter value was a very similar percentage to the decrease in photosynthesis with a 10 % decrease in parameter value.

An increase in V_m activation energy caused a decrease in photosynthetic rates, while a decrease in parameter value caused an increase in photosynthetic rate. A 10 % decrease in parameter value caused a slightly higher percentage change than a 10 % increase in parameter value.

An increase in S caused an increase in photosynthetic rates and a decrease in S caused a decrease in photosynthesis, while an increase in H caused a decrease in photosynthesis and a decrease in H caused an increase in photosynthesis. In both cases, the decreasing photosynthetic response was much greater than the increasing photosynthetic response; so decreasing S largely decreased the photosynthetic rates, while an increase in H caused a large decrease in photosynthetic rates.

Table 9.4: Percentage difference between simulated A_{net} and simulated A_{net} with an increase or decrease of 10 % of each ETp parameter for sun and shade foliage.

	Sun A_{net}		Shade A_{net}		Gross Photosynthesis	
	+10%	-10%	+10%	-10%	+10%	-10%
LAI	2.85	-3.41	8.50	-9.32	4.37	-4.98
Tree height	-0.18	0.26	-0.01	0.03	-0.14	0.20
Canopy storage	-0.14	0.16	-0.01	0.02	-0.11	0.12
Leaf nitrogen not photosynthetic	-0.29	0.31	0.00	0.01	-0.22	0.24
Photosynthetic capacity to leaf nitrogen	8.59	-8.78	0.65	-0.79	6.44	-6.62
Nitrogen concentration at top of canopy	8.79	-9.01	0.17	-0.32	6.68	-6.87
Light compensation point	-1.15	1.14	-3.06	3.22	-1.60	1.64
Vm activation energy	-11.64	13.25	0.02	-0.01	-8.37	9.51
Jm activation energy	0.00	0.02	-1.13	0.97	-0.31	0.28
Electron transport temperature response	0.07	-8.61	6.64	-5.28	1.78	-7.59
Jm curvature parameter	-8.61	0.07	-5.28	6.58	-7.60	1.77
Nitrogen allocation coefficient	-1.71	1.75	-0.15	0.14	-1.36	1.40
Stomatal conductance min CO_2	0.61	-0.58	0.15	-0.15	0.48	-0.46
Stomatal conductance slope	0.99	-1.12	0.07	-0.06	0.76	-0.85

9.3.4 Differences between observed and simulated photosynthesis during the growing season

The ETp model overestimated photosynthesis between 29 % and 71 %, and underestimated between 10 % and 70 % during the growing season (Table 9.5). Generally, the model overestimated photosynthesis at Newcastleton and underestimated photosynthesis at Wauchope and Scootmore. Within each site, there was little difference in over- or under-estimation of photosynthesis between sun and shade foliage, or between clones.

Table 9.5: Mean observed photosynthetic rates (μmol m^{-2} s^{-1}), mean simulated photosynthetic rates and percentage difference between them for each clone at each site for sun and shade foliage.

		Sun foliage			Shade foliage		
Site	Clone	Mean Observed	Mean Simulated	Difference (%)	Mean Observed	Mean Simulated	Difference (%)
	C20177	5.67	8.72	**54**	3.05	2.31	**-24**
Newcastleton	C20208	4.65	7.96	**71**	2.11	2.75	**30**
	C20211	6.11	7.90	**29**	2.89	1.93	**-33**
	C20177	9.88	4.98	**-50**	4.18	1.98	**-53**
Wauchope	C20208	8.68	6.02	**-31**	3.66	3.02	**-17**
	C20211	10.01	4.83	**-52**	5.08	1.51	**-70**
	C20177	9.31	6.85	**-26**	4.99	2.75	**-45**
Scootmore	C20208	6.89	6.23	**-10**	4.41	3.04	**-31**
	C20211	4.46	6.64	**49**	3.58	1.74	**-51**

9.4 Discussion

The simulated A_{net} was highest at Newcastleton, lowest at Wauchope for C20177 and C20211 and lowest at Scootmore for C20208. However, the observed data showed a different pattern. The A_{max} (not normalised for nitrogen) was highest at Scootmore and lowest at Newcastleton for all clones. With C20177 and C20211 showing a higher A_{net} at Scootmore in comparison with Wauchope, this suggested that the simulated A_{net} at Newcastleton was too high. The model consistently overestimated the A_{net} of sun foliage at Newcastleton, leading to the higher overall mean at Newcastleton. The model underestimated the A_{net} of sun and shade foliage at Wauchope and Scootmore, leading to a lower overall mean at these two sites and, therefore, a lower A_{net} than at Newcastleton.

The differences between the observed and simulated data were similar between sun and shade foliage, with a general underestimation by the model. The differences between simulated and observed ranged from 10 % to 71 %, with only five results below 30 % difference. This disparity between simulated and observed was much greater than the

differences between simulated and observed of a range of process-based models. The simulated data of other photosynthetic models and observed data generally agree within 20 % for *Cunninghamia lanceolata* (Zhang & Xu, 2003), *Picea abies* (Falge *et al.*, 2000), *Quercus alba* and *Acer rubrum* (Harley & Baldocchi, 1995). Another process-based photosynthetic model (MAESTRO; Wang & Jarvis, 1990), based on the equations of Farquhar *et al.* (1980), found that the daily photosynthetic simulations of *Picea sitchensis* and *Picea radiata* differed from the observed data by less than 10 %.

In the present study, the errors in the simulated data were most likely a result of errors in the parameters. Parameters for the ETp module were extracted from the available literature or the ECOCRAFT database (Medlyn & Jarvis, 1999) if they could not be calculated from the collated data. V_m activation energy, J_m activation energy, H, S and both stomatal conductance parameters were all extrapolated from the ECOCRAFT database for Sitka spruce growing in a similar environment. However, it was not always possible to obtain parameters for Sitka spruce and parameters from other *Picea* or *Pinus* species were used. The spectral reflectance factor, the curvature of electron transport to irradiance and the nitrogen parameters were all extrapolated from De Pury and Farquhar (1997). There were differences between species, in particular differences between broadleaf and deciduous trees (Reich *et al.*, 1995), which could have an impact on the overall photosynthetic response.

The sensitivity analysis showed that there were six parameters that largely affected the photosynthetic output of the model. An increase in LAI increased photosynthesis, a direct response of an increase in photosynthetic area leading to an overall increase in photosynthesis. An increase in V_m activation energy caused a decrease in photosynthesis, most likely a result of greater light energy required for Rubisco activity, limiting the photosynthesis of days with lower light availability. An increase in nitrogen concentration increased photosynthesis. Rubisco content and photosynthesis are positively related to nitrogen content (Evans, 1989), so an increase in nitrogen increased Rubisco content and photosynthesis. An increase in the ratio of photosynthesis-to-foliar nitrogen also increased photosynthesis. With an increase in this parameter, for each unit of nitrogen the photosynthetic capacity was increased, and the nitrogen use efficiency increased. The two parameters describing the temperature response of the electron transport effected photosynthesis in different ways. An increase in S increased photosynthesis, while an

increase in *H* decreased photosynthesis. *S* described the low temperature electron transport response; therefore an increase *S* lowered the optimum temperature for electron transport and, hence, increased photosynthetic rates at a given temperature. *H* described the high temperature electron transport response; therefore an increase in *H* increases the optimum temperature for electron transport and, hence, decreased photosynthetic rates at a given temperature. Generally, increasing or decreasing a parameter value had a similar effect on photosynthesis. However, the effect of decreasing *S* was greater than the effect of increasing the parameter value. The opposite was true for *H*, with an increase in the parameter having a greater effect on photosynthesis. The effect of altering the six parameters was greater for sun foliage, with the exception LAI, which had a much greater effect in the shade foliage.

Of the six parameters that have the greatest impact on photosynthesis, only two were calculated from the observed data; LAI and nitrogen concentration at the top of the canopy. The other four parameters were extrapolated from De Pury and Farquhar (1997) or the ECOCRAFT database. With large effects caused by these parameters, it seems that the error between simulated and observed photosynthesis were caused by the errors in these parameters. *S*, *H*, and *Vm* activation energy were all estimated from *Pinus sylvestris* growing in Finland, extrapolated from the ECOCRAFT database. The ratio of photosynthetic capacity-to-leaf nitrogen was taken from De Pury and Farquhar (1997). This relationship has been shown to be very robust in deciduous species but very weak in evergreen species, often with no relationship present (Reich *et al.*, 1995). This parameter was calculated from data from crop species, and was most likely too high for an evergreen such as Sitka spruce.

The photosynthetic rates at Newcastleton were consistently overestimated by the model but underestimated at Wauchope and Scootmore. The major difference between the sites in the model simulations was the climate input data. As meteorological data were not available for Newcastleton, it was interpolated from the nearest weather stations, which will have brought error into the model simulations. The apparent underestimation of photosynthesis by the model could partly be explained the differences in the measurement of photosynthesis by the model and by the field data. The model reported photosynthesis as the maximum net rate during the day, whilst the observed data reported photosynthesis as the light-saturated photosynthetic rate. It was very possible that, on many days,

photosynthesis was not light-saturated and, therefore, the simulated data showed a lower photosynthetic rate than the observed data.

As a result of photosynthesis data only being available during the summer months, it was only possible to compare the simulated and observed data for the period June to August. The observed data showed C20177 had the highest A_{max}, with little difference between C20208 and C20211, although C20208 had slightly higher A_{max} in shade needles. The simulated data showed the sun foliage of C20177 had the highest A_{net} at Newcastleton and Scootmore, and C20211 the lowest, agreeing with the observed data. The simulated data of the sun foliage at Wauchope and the simulated data of shade foliage at all sites showed that C20208 had the highest and C20211 the lowest A_{net}. Although there was large variation in the absolute photosynthetic values, the model was generally showing the same clonal response within sites at Newcastleton and Scootmore for the sun foliage. At Wauchope, although the response of C20211 agreed with the observed data, C20208 had the highest simulated photosynthetic rates when C20177 had the highest observed photosynthetic rates. The difference between simulated and observed was most likely a result of the nitrogen parameters. The photosynthetic capacity-to-leaf nitrogen ratio and non-photosynthetic nitrogen were constant for all simulations. However, nitrogen concentration at the top of the canopy was directly calculated from the foliar nitrogen concentrations and differed between clones. With photosynthetic rates directly related to nitrogen concentration (Evans, 1989), the differences in nitrogen concentration at the top of the canopy were likely to have affected photosynthetic rates.

Other factors that may have affected photosynthesis include needle age, which was not taken into account by the model. Needle age was found to affect the simulated response of photosynthesis of *P. abies* (Falge *et al.*, 1996) and may explain the disparity between observed and simulated photosynthetic rates of the three clones. The model does not include a planting density function, therefore the effect of the neighbour's proximity would not be taken into account when calculating parameters such as light availability, temperature, wind or precipitation. The sensitivity analysis showed that temperature based parameters varied the photosynthetic rate considerably when altered, the variation in immediate climate surrounding the individual tree in the field may have caused the differences been observed and simulated data.

9.5 Conclusions

The A_{net} of sun foliage of all clones was highest at Newcastleton, and lowest at Wauchope for C20177 and C20211. The A_{net} of sun foliage of C20208 was very similar between Wauchope and Scootmore. There was little difference in the A_{net} of shade foliage of all clones between sites, although A_{net} was highest at Newcastleton for C20177 and C20211.

At Newcastleton and Scootmore, C20177 had the highest A_{net} of sun foliage and C20211 the lowest. At Wauchope, C20208 had the highest A_{net} of sun foliage, with little difference between C20177 and C20211. For all sites, C20208 had the highest A_{net} and C20211 the lowest in shade foliage.

The differences between sites and, within site, the differences between clones were much smaller during the winter months and greatest during the growing season.

The sensitivity analysis indicated six parameters were largely affecting photosynthetic rates (LAI, nitrogen concentration at the top of the canopy, ratio of photosynthetic capacity-to-leaf nitrogen, V_m activation energy, S and H). The effects were larger in the sun foliage, except for LAI, which had larger effects on the shade foliage.

The model largely overestimated photosynthesis between 29 % to 71 % at Newcastleton and underestimated between 10 % to 70 % at Wauchope and Scootmore. The errors were caused by parameters extracted from the literature, errors in the meteorological data and differences in the reporting of simulated and reported photosynthetic rates.

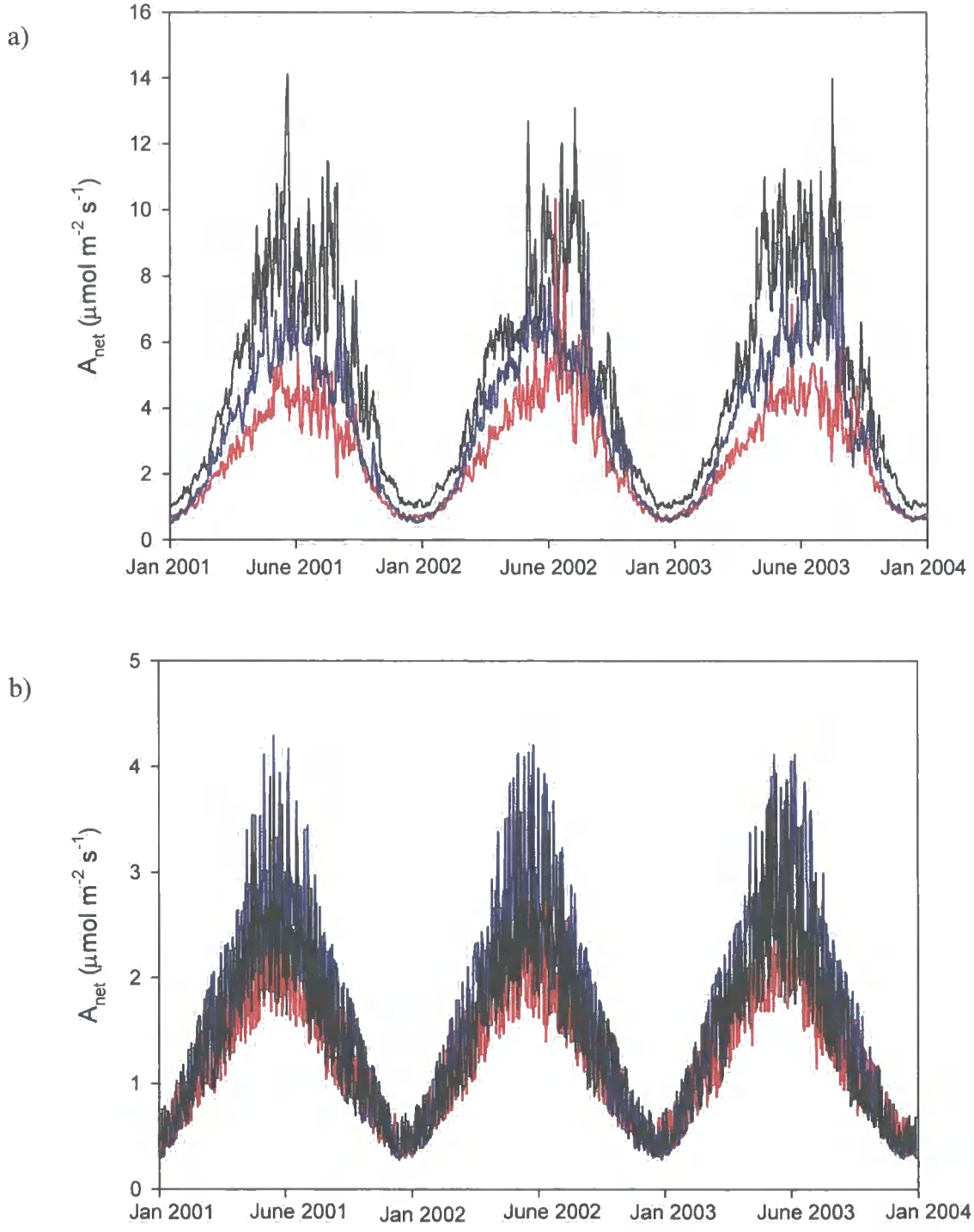


Fig 9.2: Net photosynthesis (A_{net}) from the beginning of 2001 to the end of 2003 for C20177 at Newcastleton (black), Wauchope (red) and Scootmore (blue) in a) sun foliage and b) shade foliage.

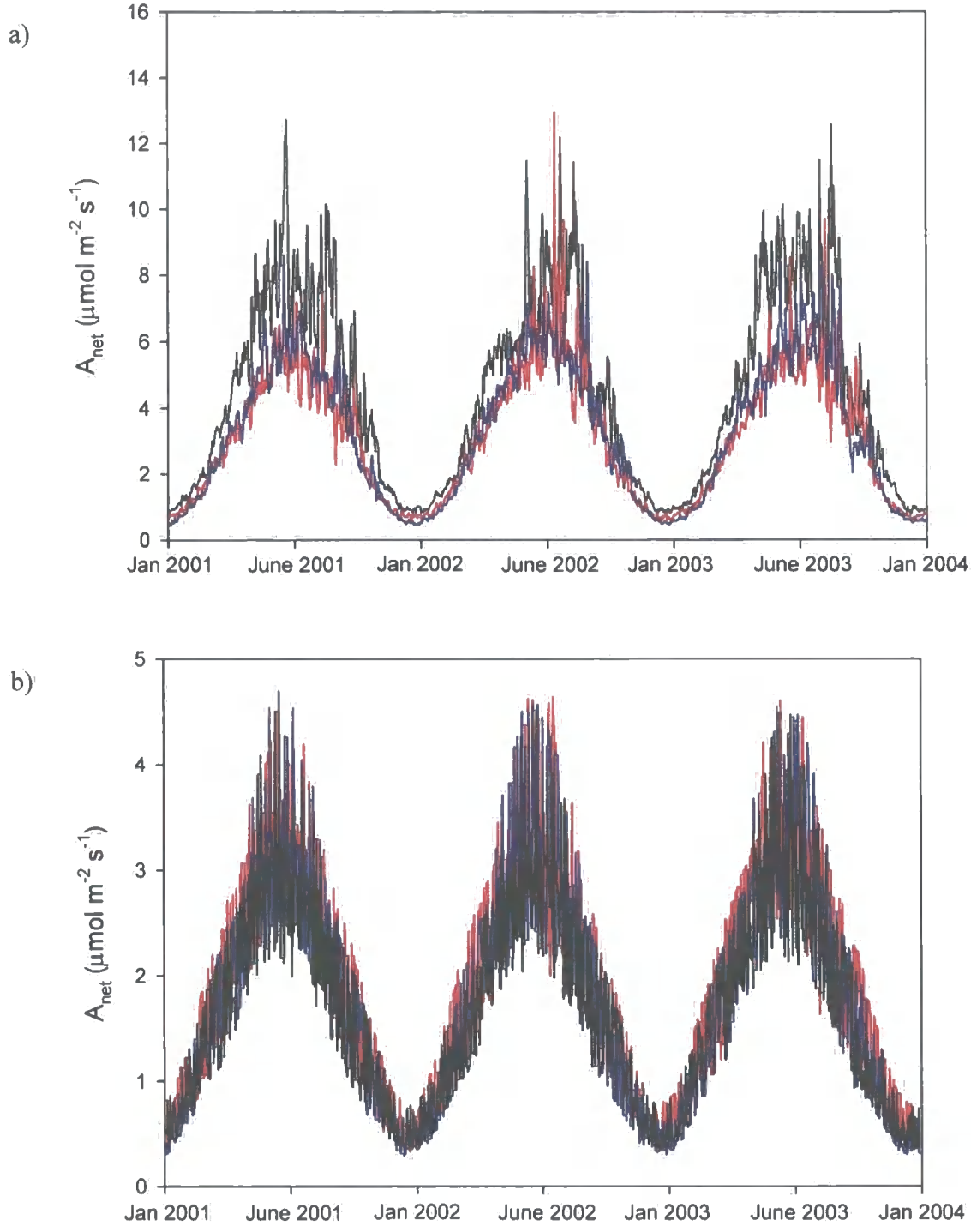


Fig 9.3: Net photosynthesis (A_{net}) from the beginning of 2001 to the end of 2003 for C20208 at Newcastleton (black), Wauchope (red) and Scootmore (blue) in a) sun foliage and b) shade foliage.

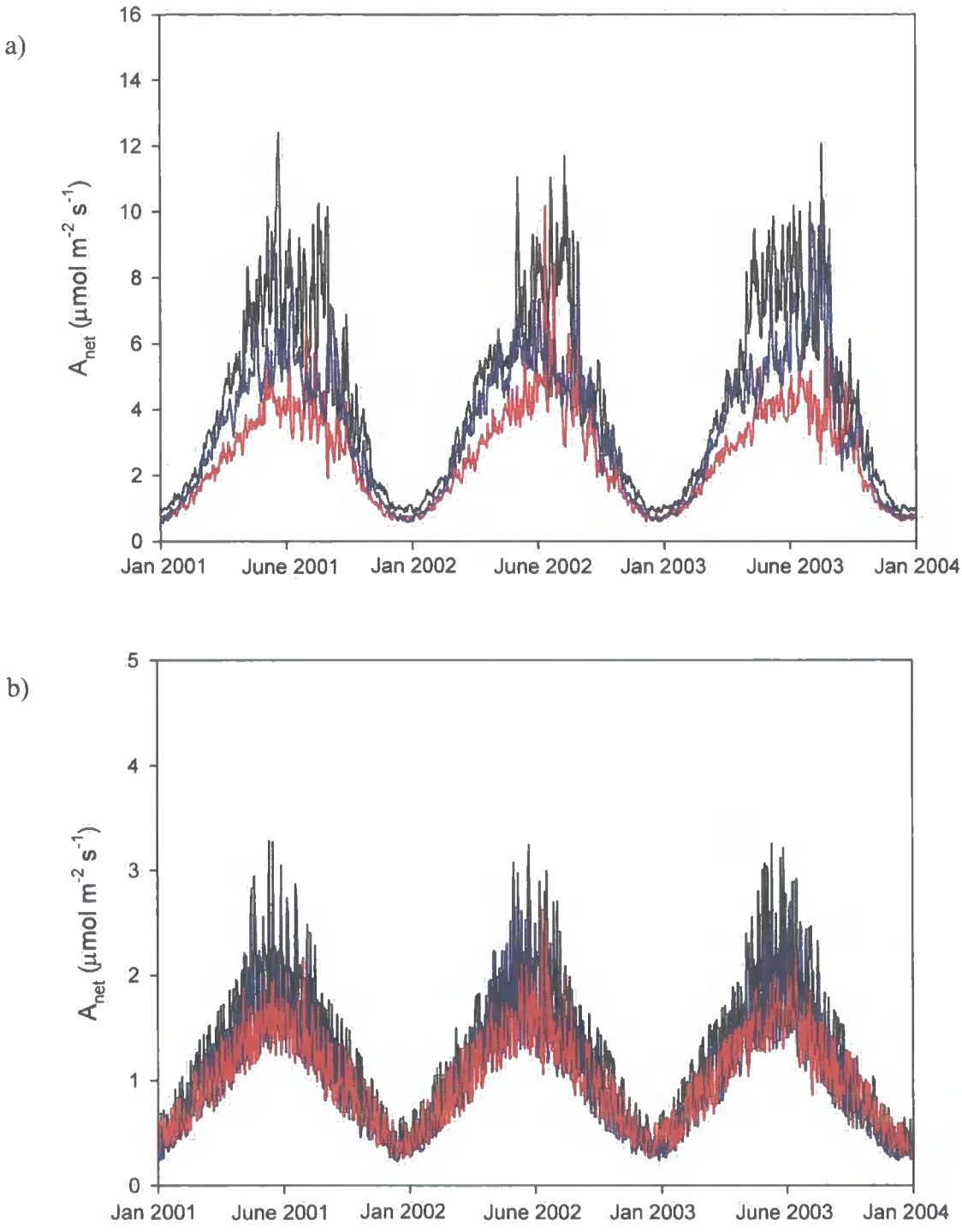


Fig 9.4: Net photosynthesis (A_{net}) from the beginning of 2001 to the end of 2003 for C20211 at Newcastleton (black), Wauchope (red) and Scootmore (blue) in a) sun foliage and b) shade foliage.

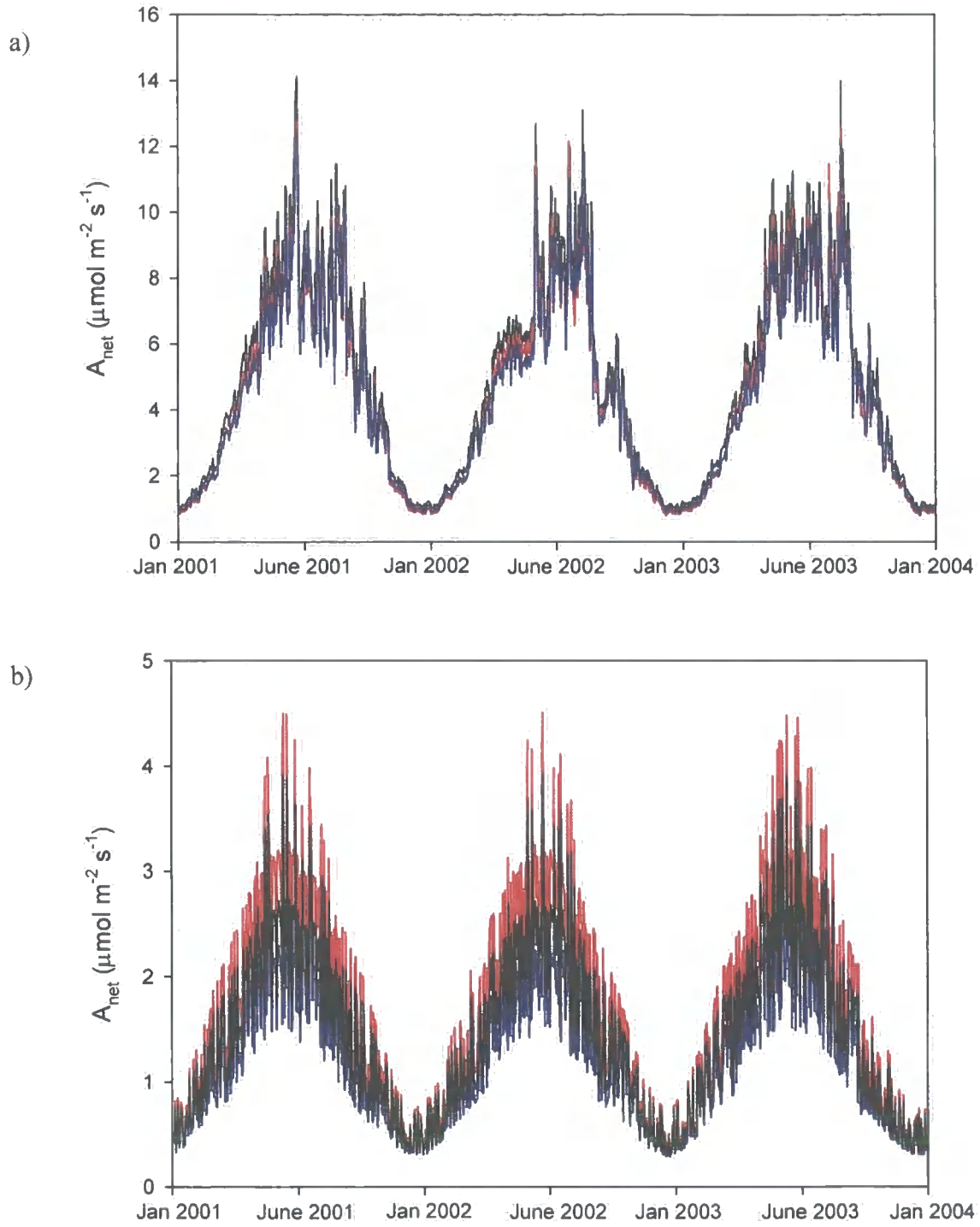


Fig 9.5: Net photosynthesis (A_{net}) from the beginning of 2001 to the end of 2003 at Newcastleton for C20177 (black), C20208 (red) and C20211 (blue) in a) sun foliage and b) shade foliage.

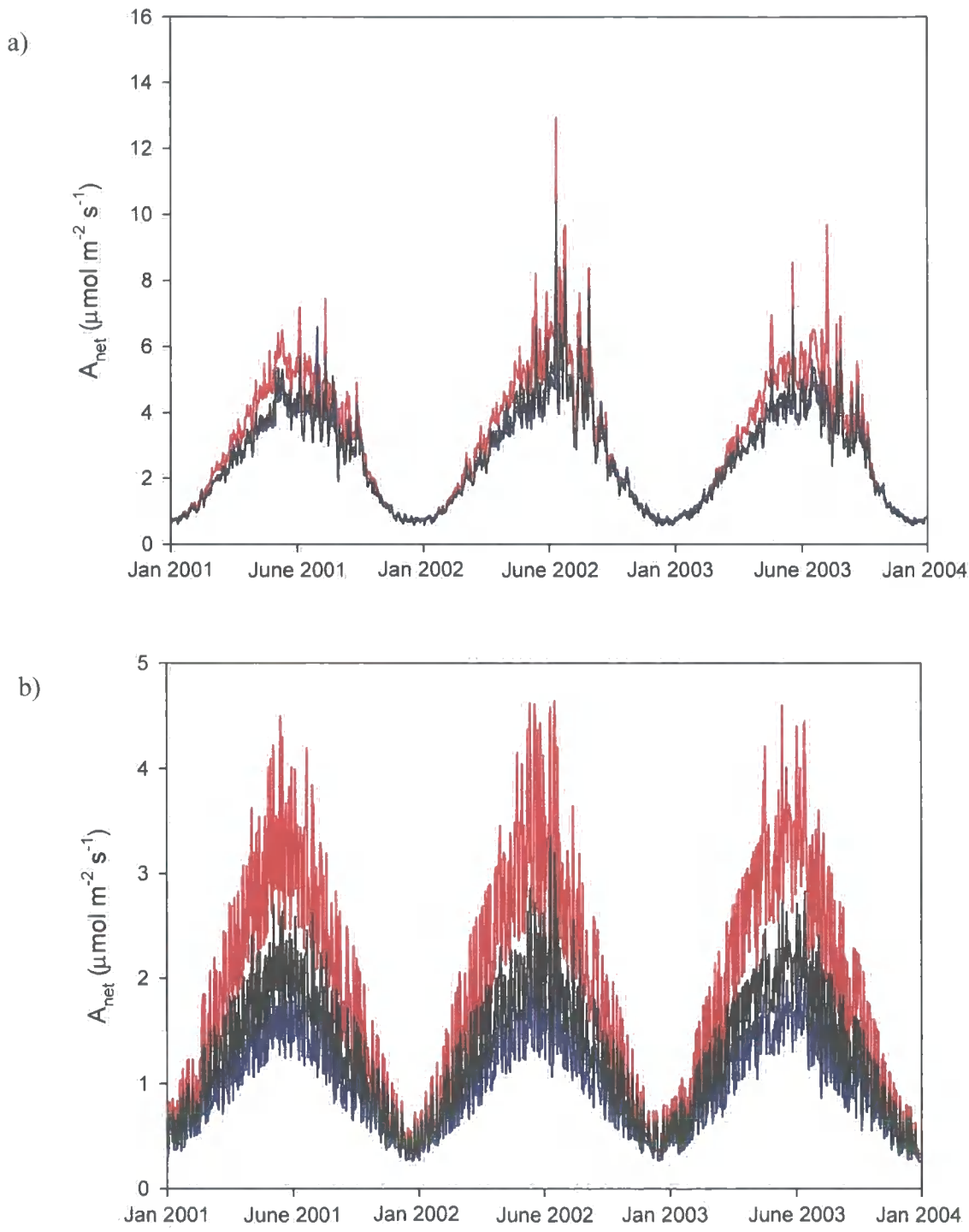


Fig 9.6: Net photosynthesis (A_{net}) from the beginning of 2001 to the end of 2003 at Wauchope for C20177 (black), C20208 (red) and C20211 (blue) in a) sun foliage and b) shade foliage.

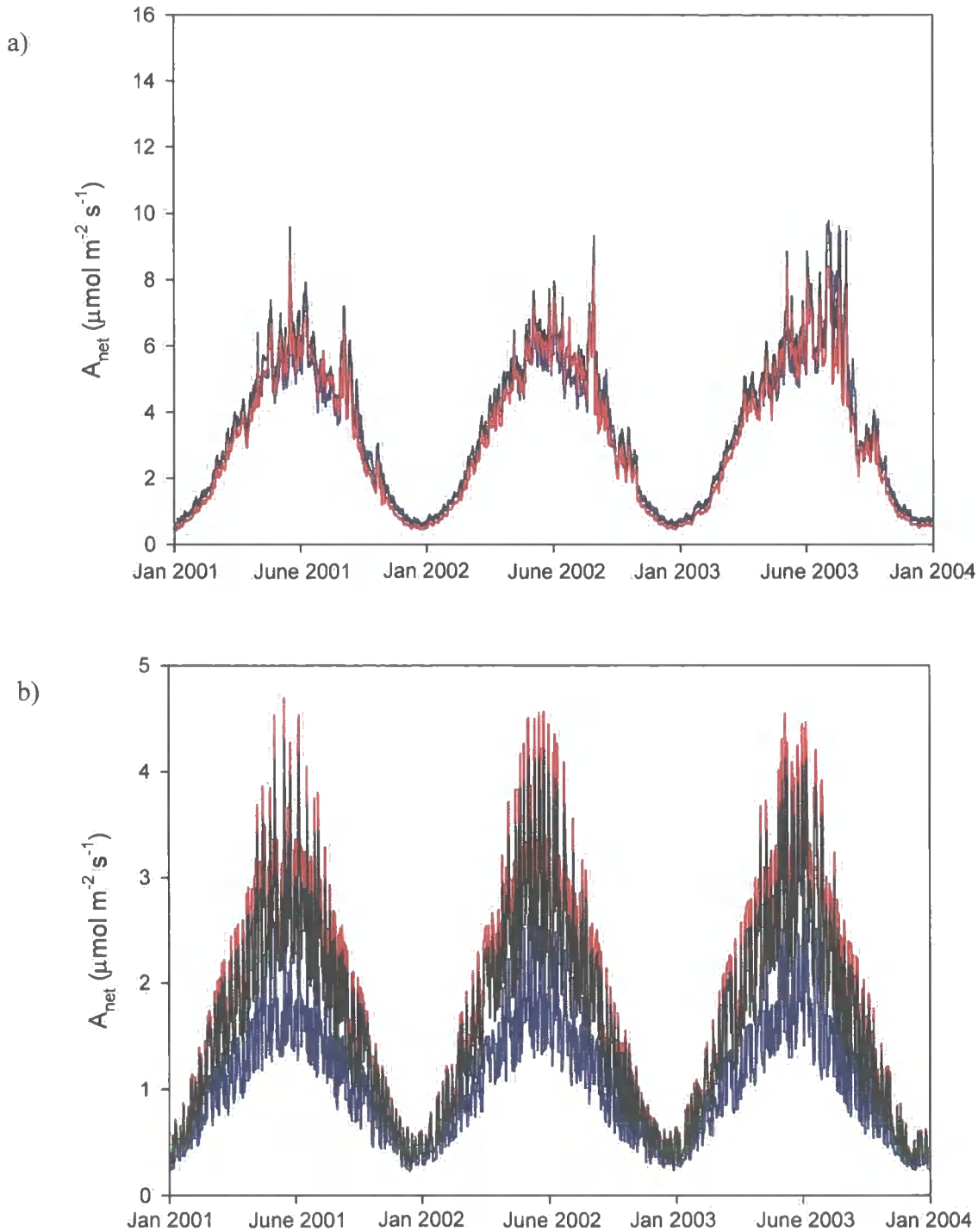


Fig 9.7: Net photosynthesis (A_{net}) from the beginning of 2001 to the end of 2003 at Scootmore for C20177 (black), C20208 (red) and C20211 (blue) in a) sun foliage and b) shade foliage.

10 Final Discussion

The main aim of the research presented herein was to investigate the environmental and genotypic variation of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) by measuring photosynthetic rates, various physiological variables and carbon allocation, for three different clones of Sitka spruce at three different sites. Parameters derived from these data were also used to assess the predictive capabilities of a process-based tree growth model. This chapter presents the main conclusions, puts them into a wider biological context and sets priorities for further work.

10.1 Environmental variation

The detailed analyses in Chapter 4, of tree height, trunk diameter and wood density for all four sites, over which the clonal experiment was replicated, indicated a growth gradient running from tall trees with large diameters and low wood density to short trees with small diameters and high wood density. A second, weaker, gradient also existed; running from tall trees with large diameters and high wood density to short trees with small diameters and low wood density. A scatterplot of the two gradients illustrated that trees growing at Newcastleton were tall, of large diameter and low wood density. The trees at Wauchope also showed a similar growth pattern, but with some tall trees having a large diameter and high wood density. The trees at Scootmore and Llandrindod were shorter, with small diameters and high wood density, although some trees at Llandrindod also exhibited low wood density. The mean height, trunk diameter and wood density data also showed the same pattern between sites, regardless of clone. Newcastleton and Wauchope are situated in a similar geographic location (Mboyi & Lee, 1999), with a similar climate and land use history - the similarity of environment explained the similar tree growth response at both sites. Scootmore and Llandrindod are located in very different parts of the UK, with different precipitation levels and air temperatures, but both sites were previously coniferous forest before the present experiment was planted. The trees at Scootmore and Llandrindod showed similar growth responses and here the similarity in land use history appeared to be the major factor affecting tree growth.

Within each site, the growth variables of the twelve full-sib families showed considerable scatter, indicating a large variation in growth at the individual tree level. With each family

consisting of eight full-sibs, the growth response of the individuals within each family was expected to have been very similar. As there was large variability in tree growth, it suggested there was a large environmental impact at the individual level. A large variation in growth at the individual tree level has previously been found in Sitka spruce (Fletcher, 1992) and so was not unexpected. And in clones of hydrids of *Populus trichocarpa* and *Populus deltoides*, 30 to 65 % of growth variation in the stem was attributed to environmental differences when grown on two contrasting sites (Wu & Stettler, 1997).

The classification of each individual tree into clusters based on the growth characteristics within each site also indicated a large environmental impact (Chapter 5). The 15 individuals of each set of clones should have been classified into the same cluster at each site if the environment exerted little control over tree growth. However, only two clones had all 15 individuals classified into the same cluster and this only occurred at one site.

Nitrogen-based nutrients and phosphate are two important environmental factors that can affect tree growth. Increased nitrogen availability increased root and shoot growth in *Pinus sylvestris* (Iivonen *et al.*, 1999), and increased the height of Sitka spruce (Miller & Miller, 1987) and *Populus tremuloides* (Kubiske *et al.*, 1998). Soil concentrations of nitrogen-based nutrients and phosphate concentrations were calculated in two soil horizons at each site (Chapter 3). Concentrations were highest at Newcastleton and lowest at Wauchope, with intermediate concentrations at Scootmore. The higher concentration of nitrogen-based nutrients at Newcastleton may have been the cause of the increased tree height at this site. However, with tree growth similar between Newcastleton and Wauchope, a similarity in nutrient concentrations was expected but was not evident. However, the nutrient concentrations were only provided for one month in one year and did not indicate nutrient concentrations for any other year. In previous years, nutrient concentrations may have been similar at Newcastleton and Wauchope. Alternatively, the higher nutrient concentration at Scootmore, in comparison to Wauchope, may potentially have caused an increase in tree growth but other environmental factors were preventing the trees at this site from utilising the nutrients available. However, higher nitrogen availability has been shown not to affect the growth of Sitka spruce (Emmett *et al.*, 1995) and *Abies balsamea* (Evans *et al.*, 2001). Therefore, higher nitrogen concentration does not always coincide with increased growth.

Chlorophyll, nitrogen and phosphorus concentrations were highest in needles at Newcastleton and lowest in needles at Scootmore (Chapter 6). Low soil nitrogen

availability has previously been shown to reduce needle nitrogen and chlorophyll content in Sitka spruce (Chandler & Dale, 1993; Murray *et al.*, 2000) and in *Pinus taeda* (Tissue *et al.*, 1993), suggesting that the needles reflect the soil nutrient availability. Therefore, the higher concentrations at Newcastleton were a reflection of the soil nutrient concentrations. This would also suggest that needles at Wauchope would have the lowest chlorophyll and nitrogen concentrations but the analysis did not show this. However, rather than the needles at Wauchope not following this relationship, the trees at Scootmore may not have been able to utilise the available nitrogen and hence exhibit a lower needle nitrogen content.

A_{max_N}, R_{day_N}, LSE and QE were highest at Newcastleton, A_{max_N} and QE were lowest at Scootmore, and R_{day_N} and LSE lowest at Wauchope. LCP did not differ between sites (Chapter 7).

Increased nitrogen availability increased photosynthesis in *Picea mariana* (Paquin *et al.*, 2000), *P. tremuloides* (Kubiske *et al.*, 1998), *P. sylvestris* (Wang & Kellomäki, 1997) and *P. taeda* (Murthy *et al.*, 1997), and the higher nitrogen availability at Newcastleton resulted in higher A_{max_N}.. The higher R_{day_N} was a result of the greater photosynthetic activity. The higher needle chlorophyll content at Newcastleton resulted in a higher quantity of light was required to saturate each chlorophyll unit, resulting in higher LSE.

The relationship between the photosynthetic variables, foliar constituents, environmental variables and growth at Wauchope and Scootmore was less straightforward. Although there were fewer nutrients at Wauchope, in comparison to Scootmore, the photosynthetic rate was higher. However, foliar nitrogen is partitioned into Rubisco, the main enzyme of photosynthesis, or chlorophyll, both of which are correlated with foliage nitrogen content (Evans, 1989). Therefore, the higher nitrogen content in the needles at Wauchope resulted in a higher concentration of chlorophyll, and hence a higher photosynthetic rate, regardless of the nutrient availability.

Photosynthesis is inhibited above a threshold temperature of about 30 °C in Sitka spruce (Leonardos *et al.*, 1996; Ludlow & Jarvis, 1971; Neilson *et al.*, 1972) and mean air temperature was highest at Scootmore, which may have impacted on the photosynthetic rates at this site. However, the temperature at Scootmore during the gas exchange measurements and the ambient temperature during any part of the year did not increase

above the maximum temperature for photosynthesis of Sitka spruce (Ludlow & Jarvis, 1971). However, although nitrogen availability was not the lowest, the foliar nitrogen content was lowest, resulting in the lower concentrations of chlorophyll and, therefore, lower photosynthetic rates. Phosphorus content was also lowest in the needles at Scootmore. Phosphorus deficiency caused a reduction of photosynthesis in Sitka spruce (Chandler & Dale, 1993), *Pinus pinaster* (Loustau *et al.*, 1999) and *Pinus radiata* (Conroy *et al.*, 1986), so the lower photosynthetic rates at Scootmore may be a result of a lower phosphorus concentration.

Precipitation during the growing season is an important factor in the growth of Sitka spruce (Roche & Haddock, 1987). Precipitation levels, although some variation was present, did not vary greatly between sites (Chapter 3). There was very little difference in the structure of the soil at Newcastleton and Wauchope, but Scootmore had a larger proportion of sand and a lower proportion of clay in comparison (Chapter 6). Emmett *et al.* (1995) found that the main limitation on the growth of Sitka spruce at Aber Forest, UK, was soil water availability irrespective of nitrogen application. The larger proportion of sand at Scootmore made the soil more free draining, resulting in lower water availability, and this may explain the reduced uptake of nutrients by the trees at this site, and therefore the lower photosynthetic and growth rates.

The destructive biomass sampling showed an inverse relationship between wood density and trunk diameter, a trend that has been shown before (Pfeifer, 1984; Wood, 1986). Wood density was highest at Scootmore and lowest at Newcastleton (Chapter 8), agreeing with the results of the 10-year height, diameter and Pilodyn® wood density data (Chapter 4). There appear to be two strategies in which trees invest above-ground C. A tree can increase its height and diameter but at the expense of wood density, or increase wood density but have a reduced height and diameter. With slightly higher annual precipitation and nutrient concentration at Newcastleton, these conditions appeared to favour the first option, whereas the lower annual precipitation and nutrient concentration at Scootmore favour the second option. At Wauchope, the height, diameter and wood density were intermediate between Newcastleton and Scootmore. The annual precipitation was similar to Newcastleton but the nutrient concentration much lower. The effect of lower nutrient availability appeared to slightly reduce height and diameter, and slightly increase wood density.

Fine root density was highest at Scootmore and lowest at Wauchope, suggesting that the trees at Scootmore were under high environmental stress, as plants generally increase root density under stress conditions (Negi *et al.*, 2003). With nutrient levels and root density lowest at Wauchope, nutrient availability did not appear to be affecting root density. Annual precipitation was lower at Scootmore, and with fine root density affected by water availability (Jose *et al.*, 2003), it appeared that moisture was the main factor affecting the root density.

Newcastleton had the largest carbon (C) content in above-ground organs and Scootmore the lowest. However, Scootmore had the highest C content in below-ground organs and Wauchope the lowest. Moisture availability and nutrient concentration can affect the partitioning of C, with water stress in *Pinus palustris* (Jose *et al.*, 2003) and nutrient stress in *Populus nigra* and *Pinus radiata* (Glynn *et al.*, 2003; Rodríguez *et al.*, 2003) causing an increase in C allocation to below-ground systems (Negi *et al.*, 2003). At Scootmore, annual precipitation was lower and the trees were responding by increasing C allocation below-ground. Newcastleton had the highest nutrient concentration and higher annual precipitation resulting in an increase in above-ground partitioning. However, the below-ground allocation was lowest at Wauchope. Although Wauchope had the highest annual precipitation, the site also had the lowest nutrient concentration, suggesting that Wauchope would have increased C allocation below-ground. For these genotypes of Sitka spruce it appeared that precipitation levels were more important than nutrient concentration for C allocation below-ground.

Using parameters from data collected in Chapters 3, 6 and 7, a process-based evapotranspiration model (ForestETP) largely overestimated photosynthesis at Newcastleton and largely underestimated photosynthesis at Wauchope and Scootmore. There were several reasons for this. Firstly, observed weather data were available for Wauchope and Scootmore, but at Newcastleton the data were interpolated from nearby Met stations using the weather generator module. Secondly, the sensitivity analysis suggested that there were six parameters that greatly affected the model output, four of which were not calculated from the data but extrapolated from data available in the literature. The underestimation of photosynthesis by the model was most likely a result of errors in these parameters and errors in the daily weather input. The model predicted highest photosynthetic rates during the growing season, declining to a minimum in winter

although photosynthesis was still occurring. Sitka spruce has been shown to photosynthesise during the winter months and can assimilate greater CO_2 during winter (Ludlow & Jarvis, 1971). At each site, sun foliage photosynthesis was greater than the shade foliage, agreeing with the observed data, showing that the model effectively predicts differences in sun and shade foliage.

10.2 Genotypic variation

The PCA (Chapter 4) and cluster analyses (Chapter 5) identified three clones that were distinct in their growth patterns. The individuals of C20177 were tall, with large trunk diameters and low wood density; the individuals of C20211 were small, with small diameters and high wood density; and the growth variables of the individuals of C20208 were mid-way between those of C20177 and C20211. The three clones showed the same pattern at each site, showing that the genotypic effect on growth was determining the growth pattern of the clones regardless of the environmental effect. Experiments in Belgium (Nanson, 1984), Australia (Pederick, 1984), France (Roman-Amat, 1984), Bulgaria (Alexandrov, 1984), Northern Germany (Kleinschmit & Svolba, 1984) and Britain (Lines, 1987) have all shown similar results in the growth rate of different provenances of Sitka spruce. Sitka spruce from more southerly provenances grew faster than more northerly provenances. In a study investigating the growth, leaf traits and canopy architecture of different clones from crosses of *Populus trichocarpa* and *Populus deltoids,* grown in a common garden experiment in the USA, growth was found to be largely genetically determined (Wu & Stettler, 1996). When grown on contrasting sites, 15 to 30 % of the growth variance was attributed to genotypic differences (Wu & Stettler, 1997). In particular, the genetic correlations of differences in stem growth traits and some leaf traits suggested that part of the genetic response to the different environments was shared (Wu & Stettler, 1997; Wu & Stettler, 1998). For *Populus trichocarpa* clones collected from contrasting environments but grown on a common site, leaf and crown traits were shown to be under high genetic control (Dunlap & Stettler, 1998). The clones in this experiment mainly originate from the same provenance (Queen Charlotte Islands) but there was still genotypic distinction between them. Fletcher (1992) reported that 40 % of growth variation was accounted for between provenances but 60 % was within provenances, and this research has capitalized on this variation by selecting three clones with distinct growth patterns.

The nutrient concentrations in the soil available to each clone varied between sites and between horizons but there was no pattern in which clone had the highest or lowest nutrient concentrations. However, the variation was small, with no significant differences. There was also little difference in particle size in the soil surrounding the clones, with no significant differences at the 5 % level. The differences in growth between clones within each site were not attributed to differences in the soil structure or nutrient availability.

C20177 had the higher chlorophyll *a* and chlorophyll *b* concentration, in particular at Wauchope, suggesting that C20177 had a greater capacity for photosynthesis. There was little difference in the total nitrogen content or total phosphorous content between clones (Chapter 6). With no difference in the soil nitrogen or phosphorus concentrations between clones, the foliar concentrations were a reflection of this. With little difference between clones in the nitrogen content, C20177 appears to proportion a larger concentration of nitrogen to chlorophyll and less to Rubisco, potentially lowering the photosynthetic capacity of the clone. However, if light was the limiting factor of photosynthesis, then higher chlorophyll contents would be beneficial and C20177 would have a higher photosynthetic rate.

There was little difference in the A_{max_N}, R_{day_N} and LCP between clones at all sites, and little difference in LSE and QE between clones at Newcastleton and Scootmore (Chapter 7). At Wauchope, C20211 had slightly lower LSE and slightly higher QE in comparison to the other clones. The difference in chlorophyll contents between C20177 and the other clones did not affect the photosynthetic capacity of any clone. The higher chlorophyll content of C20177 suggested that the trees were shaded (Boardman, 1977; Evans, 1989; Leverenz & Jarvis, 1980b; Šprtová & Marek, 1999) and required higher chlorophyll content to photosynthesise at a similar rate to the other clones. However, C20177 was the tallest clone and was unlikely to be shaded. The lack of distinction in the photosynthetic parameters between clones suggested that the differences in growth were attributable to genotypic differences in allocation.

The total non-structural carbohydrates (TNC) content reflected the growth rate of each clone. C20177 had the lowest concentration of soluble TNC, reflecting the greater utilisation of carbohydrates, a result of higher growth rates. C20211 had the highest starch concentration, as a lower growth rate required fewer carbohydrates and the soluble TNC was converted starch for storage.

C20177 had the largest trunk diameter and the lowest wood density (Chapter 8), agreeing with the Pilodyn® measurements and fitting the inverse relationship between diameter and wood density (Pfeifer, 1984; Wood, 1986). C20211 had the highest wood density at Newcastleton and also the smallest diameter, again agreeing with the Pilodyn® measurements and the diameter-wood density relationship. However, C20208 had the highest wood density at Wauchope and Scootmore but had an intermediate trunk diameter and even the largest diameter at Scootmore, showing a deviation from the expected relationship. The clones were under greater stress at Wauchope (low nutrients) and Scootmore (lowest annual precipitation of all the sites), suggesting that the diameter-wood density relationship was not robust and that C20208 outperformed the other clones, in terms of wood density, under stress conditions. In contrast, research on *Populus* clones between two sites, growth traits were genetically stronger on the less favourable site (Wu & Stettler, 1998). C20177 also had the lowest fine root density at all sites, suggesting that this clone was less affected by stress conditions, as an increase in fine root density occurred in drought and low nutrient conditions (Glynn *et al.*, 2003; Jose *et al.*, 2003; Negi *et al.*, 2003; Rodríguez *et al.*, 2003).

At Newcastleton and Wauchope, C20177 had the greatest height and diameter, and also the greatest above-ground C allocation. At Scootmore, C20208 had the greatest height and diameter but also the greatest above-ground C allocation. C20211 had the lowest above-ground C allocation and also the smallest height and diameter. Irrespective of the wood density, the clones with the largest volume contained the largest quantity of C. The majority of the C was allocated to the trunk but at Wauchope and Scootmore C20177 allocated the greatest proportion of C to the branches. As the clones were under more stress at Wauchope and Scootmore, it appeared that C20177 allocated more C to branches during stress conditions.

C20208 allocated the greatest proportion of C into fine roots at Newcastleton and Wauchope, C20211 allocated the greatest proportion of C into fine roots at Scootmore, and C20177 allocated the smallest proportion of C into the fine roots at all sites. With C20177 allocating the least C to fine roots it appeared that C20177 was less affected by the differences in environmental conditions, as below-ground stress increases fine root systems. With Newcastleton and Wauchope showing the same clonal response, this suggested that the two sites had similar environmental pressures and that Scootmore was

very different. The climatic variable that seemed to be responsible was precipitation as annual precipitation levels were similar at Newcastleton and Wauchope, but lower at Scootmore.

The evapotranspiration model predicted that C20177 had the highest photosynthetic rates and C20211 the lowest in sun foliage at Newcastleton and Scootmore (Chapter 9), which agreed with observed data. For sun foliage at Wauchope and for shade foliage at all sites, the model simulated C20208 as having the highest photosynthetic rates and C20211 the lowest. This differed from the observed as C20177 had the highest photosynthetic rates at Wauchope and C20211 the lowest, although there was very little difference in the photosynthetic rates of shade foliage between clones. The difference appeared to be a result of the differences in nitrogen concentration at the top of the canopy.

10.3 Further research

This study has shown that, whilst the environment plays a major part in the growth of Sitka spruce, genotypic selection could influence the overall outcome. For example, C20177 was less affected by environmental stress, whilst C20208 and C20211 appeared more susceptible. Although C20177 generally outperforms the other clones in terms of height and trunk diameter, this clone had a reduced wood density. There is a trade-off between volume and wood density. However, the trees were only in a juvenile growth stage at 15-years-old and, with a harvesting age of about 30-years-old, there is still a long time before the wood is required for timber. During the next 15 years, further research would be required to investigate if the genotypic differences found here remain until felling. With little significant difference found in the photosynthetic rates of the clones, if logistics require limitation, it would be prudent to further investigate the overall tree carbon allocation and carbon allocation between organs. Interestingly, with *Populus* clones, leaf and branch traits were under stronger genetic control at the top of the canopy than the lower canopy (Wu & Stettler, 1996) and also in younger than older leaves (Dunlap & Stettler, 1998). Further investigation into the genetic control of growth and carbon allocation at different positions and needle ages in the canopy may provide a more beneficial way of selecting for Sitka spruce clones that perform equally amongst difference environments.

The tree growth model would also benefit from further development. More accurate parameters, particularly photosynthetic and climate parameters, are required to investigate if the model can precisely predict the photosynthetic rates of Sitka spruce. A carbon allocation submodel is also available and it would be beneficial if the model can accurately predict wood volume and density for each clone at each site. An accurate prediction would enable foresters to select the best genotype, in terms of timber production, for each site based on easily measurable photosynthetic rates.

References

Aber, J.D. & Federer, C.A. (1992) A generalized, lumped-parameter model of photosynthesis, evapotranspiration and net primary production in temperate and boreal forest ecosystems. Oecologia, 92, 463-474.

Alberdi, M. & Corcuera, L.J. (1991) Cold acclimation in plants. Phytochemistry, 30, 3177-3184.

Alexandrov, A.H. (1984) Sitka spruce provenance experiment in Bulgaria. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 57-58. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Aphalo, P.J. & Jarvis, P.G. (1993) An analysis of Ball's empirical model of stomatal conductance. Annals of Botany, 72, 321-327.

Aronsson, A., Ingestad, T., & Lööf, L.-G. (1976) Carbohydrate metabolism and frost hardiness in pine and spruce seedlings grown at different photoperiods and thermoperiods. Physiologia Plantarum, 36, 127-132.

Arp, W.J. (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO_2. Plant, Cell and Environment, 14, 869-875.

Azcón-Bieto, J. (1983) Inhibition of photosynthesis by carbohydrates in wheat leaves. Plant Physiology, 73, 681-686.

Azcón-Bieto, J. & Osmond, C.B. (1983) Relationship between photosynthesis and respiration. Plant Physiology, 71, 574-581.

Baldocchi, D.D. & Harley, P.C. (1995) Scaling carbon dioxide and water vapour gas exchange from leaf to canopy in a deciduous forest. II. Model testing and application. Plant, Cell and Environment, 18, 1157-1173.

Bartelink, H.H. (1998) A model of dry matter partitioning in trees. Tree Physiology, 18, 91-101.

Beadle, C.L., Hart, J.W., & Jarvis, P.G. (1983) The extraction and activity of carboxylases in Sitka spruce and some other conifers. Photosynthetica, 17, 321-333.

Beets, P.N. & Whitehead, D. (1996) Carbon partitioning in *Pinus radiata* stands in relation to foliage nitrogen status. Tree Physiology, 16, 131-138.

Begon, M., Harper, J.L., & Townsend, C.R. (1996) Ecology, 3rd edn. Blackwell Science Ltd, Oxford.

Besford, R.T. (1990) The Greenhouse Effect: Acclimation of tomato plants growing in high CO_2, relative changes in Calvin Cycle enzymes. Journal of Plant Physiology, 136, 458-463.

Besford, R.T., Ludwig, L.J., & Withers, A.C. (1990) The Greenhouse Effect: Acclimation of tomato plants growing in high CO_2, photosynthesis and Ribulose-1, 5-Bisphosphate Carboxylase protein. Journal of Experimental Botany, 41, 925-931.

Blechschmidt-Schneider, S., Ferrar, P., & Osmond, C.B. (1989) Control of photosynthesis by the carbohydrate level of the C_4 plant *Amaranthus edulis* L. Planta, 177, 515-525.

Boardman, N.K. (1977) Comparative photosynthesis of sun and shade plants. Annual Review of Plant Physiology, 28, 5-377.

Brazier, J.D. (1987) Man's use of Sitka spruce. Proceedings of the Royal Society of Edinburgh, 93B, 213-221.

Bremner, J.M. (1996). Nitrogen-Total. In *Methods of Soil Analysis. Part 3 Chemical Methods* (eds D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston & M.E. Sumner), Vol. 5, pp. 1085-1121. Soil Science Society of America, Wisconsin.

Buckland, S.M., Grime, J.P., Hodgson, J.G., & Thompson, K. (1997) A comparison of plant responses to the extreme drought of 1995 in northern England. Journal of Ecology, 85, 875-882.

Bunce, J.A. (1992) Stomatal conductance, photosynthesis and respiration of temperate deciduous seedlings grown outdoors at an elevated concentration of carbon dioxide. Plant, Cell and Environment, 15, 541-549.

Campbell, G.S. (1985) Soil Physics With BASIC: Transport Models for Soil-Plant Systems. Developments in Soil Science 14.

Campbell, W.J., Allen, L.H., & Bowes, G. (1988) Effects of CO_2 concentration on Rubisco activity, amount, and photosynthesis in soybean leaves. Plant Physiology, 88, 1310-1316.

Cannell, M.G.R. (1987) Photosynthesis, foliage development and productivity of Sitka spruce. Proceedings of the Royal Society of Edinburgh, 93B, 61-73.

Carter, C.I. & Nichols, J.F.A. (1984) The Green spruce aphid and Sitka spruce provenances in Britain. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 15-19. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Cave, G., Tolley, L.C., & Strain, B.R. (1981) Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. Physiologia Plantarum, 51, 171-174.

Centritto, M. & Jarvis, P.G. (1999) Long-term effects of elevated carbon dioxide concentration and provenance on four clones of Sitka spruce (*Picea sitchensis*). II. Photosynthetic capacity and nitrogen use efficiency. Tree Physiology, 19, 807-814.

Centritto, M., Lee, H.S.J., & Jarvis, P.G. (1999) Long-term effects of elevated carbon dioxide concentration and provenance on four clones of Sitka spruce (*Picea sitchensis*). I. Plant growth, allocation and ontogeny. Tree Physiology, 19, 799-806.

Ceulemans, R. & Mousseau, M. (1994) Tansley Review No. 71: Effects of elevated CO_2 on woody plants. New Phytologist, 127, 425-446.

Chandler, J.W. & Dale, J.E. (1993) Photosynthesis and nutrient supply in needles of Sitka spruce [*Picea sitchensis* (Bong.) Carr.]. New Phytologist, 125, 101-111.

Chandler, J.W. & Dale, J.E. (1995) Nitrogen deficiency and fertilization effects on needle growth and photosynthesis in Sitka spruce. Tree Physiology, 15, 813-817.

Chaves, M.M. (1991) Effects of water deficits on carbon assimilation. Journal of Experimental Botany, 42, 1-16.

Chiba, Y. (1998) Architectural analysis of relationship between biomass and basal area based on pipe model theory. Ecological Modelling, 108, 219-225.

Conroy, J.P., Küppers, M., Küppers, B., Virgona, J., & Barlow, E.W.R. (1988) The influence of CO_2 enrichment, phosphorus deficiency and water stress on the growth, conductance and water use of *Pinus radiata* D. Don. Plant, Cell and Environment, 11, 91-98.

Conroy, J.P., Milham, P.J., Mazur, M., & Barlow, E.W.R. (1990) Growth, dry weight partitioning and wood properties of *Pinus radiata* D. Don after 2 years of CO_2 enrichment. Plant, Cell and Environment, 13, 329-337.

Conroy, J.P., Smillie, R.M., Küppers, M., Bevege, D.I., & Barlow, E.W. (1986) Chlorophyll *a* fluorescence and photosynthetic and growth responses of *Pinus radiata* to phosphorus deficiency, drought stress and high CO_2. Plant Physiology, 81, 423-429.

Côté, B., Belanger, N., Courchesne, F., Fyles, J.W., & Hendershot, W.H. (2003) A cyclical but asynchronous pattern of fine root and woody biomass production in a hardwood forest of southern Quebec and its relationships with annual variation of temperature and nutrient availability. Plant and Soil, 250, 49-57.

Criddle, R.S., Breidenbach, R.W., Fontana, A.J., Henry, J.-M., Smith, B.N., & Hansen, L.D. (1996) Plant respiration responses to climate determine geographic distribution. Russian Journal of Plant Physiology, 43, 698-704.

Curtis, P.S. (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. Plant, Cell and Environment, 19, 127-137.

Dawson, R.M.C., Elliot, D.C., Elliot, W.H., & Jones, K.M. (1989) Data for Biochemical Research, 3rd edn. Oxford University Press, New York.

Day, K.R. (1984) Systematic differences in the population intensity of green spruce aphid, *Elatobium abietinum* (Walker), in a provenance trial of Sitka spruce, *Picea sitchensis* (Bong.) Carr. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 165-171. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

De Pury, D.G.G. & Farquhar, G.D. (1997) Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. Plant, Cell and Environment, 20, 537-557.

Dewar, R.C. (1997) A simple model of light and water use evaluated for *Pinus radiata.* Tree Physiology, 17, 259-265.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., & Smith, F. (1956) Colorimetric method for the determination of sugars and related substances. Analytical Chemistry, 28, 350-356.

Dunlap, J.M. & Stettler, R.F. (1998) Genetic variation and productivity of *Populus trichocarpa* and its hybrids. X. Trait correlations in young black cottonwood from four river valleys in Washington. Trees, 13: 28-39.

El Kohen, A., Venet, L., & Mousseau, M. (1993) Growth and photosynthesis of two deciduous forest species at elevated carbon dioxide. Functional Ecology, 7, 480-486.

Emmett, B.A., Brittain, S.A., Hughes, S., & Kennedy, V. (1995) Nitrogen additions ($NaNO_3$ and NH_4NO_3) at Aber forest, Wales: II. Response of trees and soil nitrogen transformations. Forest Ecology and Management, 71, 61-73.

Evans, C.A., Miller, E.K., & Friedland, A.J. (2001) Effect of nitrogen and light on nutrient concentrations and associated physiological responses in birch and fir seedlings. Plant and Soil, 236, 197-207.

Evans, J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia, 78, 9-19.

Evans, S., Randle, T., Henshall, P., Archangeli, C., Pellenq, J., Lafont, S., & Vials, C. (2005) Recent advances in the mechanistic modelling of forest stands and catchments. In *Annual Report and Accounts (04-05)*, Forest Research, pp 98-111.

Falge, E., Graber, W., Siegwolf, R., & Tenhunen, J.D. (1996) A model of the gas exchange response of *Picea abies* to habitat conditions. Trees, 10, 277-287.

Falge, E., Tenhunen, J.D., Ryel, R., Alsheimer, M., & Köstner, B. (2000) Modelling age- and density-related gas exchange of *Picea abies* canopies in the Fichtelgebirge, Germany. Annals of Forest Science, 57, 299-243.

Fan, S. & Grossnickle, S.C. (1999) Clonal variation in gas exchange and freezing tolerance development of interior spruce (*Picea glauca* (Moench) Voss x *P.engelmannii* Parry ex Engelm.) during autumn acclimation. Scandinavian Journal of Forest Research, 14, 240-250.

Farrar, J.F. (1993). Carbon Partitioning. In *Photosynthesis and production in a changing environment. A field and laboratory manual* (eds D.O. Hall, J.M.O. Scurlock, H.R. Bolhàr-Nordenkampf, R.C. Leegood & S.P. Long). Chapman & Hall, London.

Farquhar, G.D., von Caemmerer, S., & Berry, J.A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta, 149, 78-90.

Feddes, R.A., Bresler, E. & Neuman, S.P. (1974) Field test of a modified numerical model for water uptake by root systems. Water Resources Research 10, 1199-1206.

Fletcher, A.M. (1992). Breeding improved Sitka spruce. In *Super Sitka for the 90s* (ed D.A. Rook), Forestry Commission Bulletin 103, pp. 11-24. HMSO, London.

Flexas, J., Gulías, J., Jonasson, S., Medrano, H., & Mus, M. (2001) Seasonal patterns and control of gas exchange in local populations of the Mediterranean evergreen shrub *Pistacia lentiscus* L. Acta Oecologica, 22, 33-43.

Fredeen, A.L. & Sage, R.F. (1999) Temperature and humidity effects on branchlet gas-exchange in white spruce: an explanation for the increase in transpiration with branchlet temperature. Trees, 14, 161-168.

Friend, A.D. (1995) PGEN: an integrated model of leaf photosynthesis, transpiration, and conductance. Ecological Modelling 77, 233-255.

Gash, J.H.C., Lloyd, C.R. & Lachaud, G. (1995) Estimating sparse forest rainfall interception with an analytical model. Journal of Hydrology, 170, 79-86.

Giardina, C.P., Ryan, M.G., Binkley, D., & Fownes, J.H. (2003) Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. Global Change Biology, 9, 1438-1450.

Glynn, C., Herms, D.A., Egawa, M., Hansen, R., & Mattson, W.J. (2003) Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos, 101, 385-397.

Grace, J. (1981). Some effects of wind on plants. In *Plants and their Atmospheric Environment* (eds J. Grace, E.D. Ford & P.G. Jarvis), pp. 31-56. Blackwell, Oxford.

Grace, J. (1987) Climatic tolerance and the distribution of plants. New Phytologist, 106, 113-130.

Grace, J., Allen, S.J., & Wilson, C. (1989) Climate and the meristem temperatures of plant communities near the tree-line. Oecologia, 79, 198-204.

Grassi, G., Meir, P., Cromer, R., Tompkins, D., & Jarvis, P.G. (2002) Photosynthetic parameters in seedlings of *Eucalyptus grandis* as affected by rate of nitrogen supply. Plant, Cell and Environment, 25, 1677-1688.

Graves, J.D. & Taylor, K. (1986) A comparative study of *Geum rivale* L. and *G.urbanum* L. to determine those factors controlling their altitudinal distribution. I. Growth in controlled and natural environments. New Phytologist, 104, 681-691.

Grub, A. & Mächler, F. (1990) Photosynthesis and light activation of Ribulose 1,5-bisphosphate Carboxylase in the presence of starch. Journal of Experimental Botany, 41, 1293-1301.

Harley, P.C. & Baldocchi, D.D. (1995) Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. I. Leaf model parametrization. Plant, Cell and Environment, 18, 1146-1156.

Harris, A.S. (1978) Distribution, genetics and silvical characteristics of Sitka spruce. In Proceedings of IUFRO Joint Meeting of Working Parties, Vol. 1, pp. 95-122. British Columbia Ministry of Forests, Vancouver.

Hikosaka, K., Hanba, Y.T., Hirose, T., & Terashima, I. (1998) Photosynthetic nitrogen-use efficiency in leaves of woody and herbaceous species. Functional Ecology, 12, 896-905.

Hind, G. (1993). Thylakoid components and processes. In *Photosynthesis and production in a changing environment. A field and laboratory manual* (eds D.O. Hall, J.M.O. Scurlock, H.R. Bolhàr-Nordenkampf, R.C. Leegood & S.P. Long). Chapman & Hall, London.

Hodge, A., Paterson, E., Thornton, B., Millard, P., & Killham, K. (1997) Effects of photon flux density on carbon partitioning and rhizosphere carbon flow of *Lolium perenne*. Journal of Experimental Biology, 48, 1797-1805.

Holmes, G.D. (1987) Foreword. Proceedings of the Royal Society of Edinburgh, 93B, v-vi.

Hughes, M.K., Schweingruber, F.H., Cartwright, D., & Kelly, P.M. (1984) July-August temperature at Edinburgh between 1721 and 1975 from tree ring density and width data. Nature, 308, 341-344.

Huntley, B., Bartlein, P.J., & Prentice, I.C. (1989) Climatic control of the distribution and abundance of Beech (*Fagus* L.) in Europe and North America. Journal of Biogeography, 16, 551-560.

Iivonen, S., Rikala, R., Ryyppo, A., & Vapaavuori, E. (1999) Responses of Scots pine (*Pinus sylvestris*) seedlings grown in different nutrient regimes to changing rootzone temperature in spring. Tree Physiology, 19, 951-958.

Isard, S.A. (1986) Factors influencing soil moisture and plant community distribution on Niwot Ridge, Front Range, Colorado, USA. Artic and Alpine Research, 18, 83-96.

Jalkanen, R.E., Redfern, D.B., & Sheppard, L.J. (1998) Nutrient deficits increase frost hardiness in Sitka spruce. Forest Ecology and Management, 107, 191-201.

Jarvis, N.J. (1989) A simple empirical model of root water uptake. Journal of Hydrology 107, 57-72.

Jarvis, P.G., James, G.B., & Landsberg, J.J. (1976). Coniferous Forest. In *Vegetation and the Atmosphere, Volume 2: Case Studies* (ed J.L. Monteith), pp. 171-240. Academic Press, London.

Jordan, D.B. & Ogren, W.L. (1984) The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase. Planta, 161, 308-313.

Jose, S., Merritt, S., & Ramsey, C.L. (2003) Growth, nutrition, photosynthesis and transpiration responses of longleaf pine seedlings to light, water and nitrogen. Forest Ecology and Management, 180, 335-344.

Karlsson, P.E., Uddling, J., Skarby, L., Wallin, G., & Sellden, G. (2003) Impact of ozone on the growth of birch (*Betula pendula*) saplings. Environmental Pollution, 124, 485-495.

Kayama, M., Sasa, K., & Koike, T. (2002) Needle life span, photosynthetic rate and nutrient concentration of *Picea glehnii*, *P. jezoensis* and *P. abies* planted on serpentine soil in northern Japan. Tree Physiology, 22, 707-716.

Kellomäki, S. & Wang, K.-Y. (1998) Sap flow in Scots pine growing under conditions of year-round carbon dioxide enrichment and temperature elevation. Plant, Cell and Environment, 21, 969-981.

Kilpelainen, A., Peltola, H., Ryyppo, A., Sauvala, K., Laitinen, K., & Kellomäki, S. (2003) Wood properties of Scots pine (*Pinus sylvestris*) grown at elevated temperature and carbon dioxide concentration. Tree Physiology, 23, 889-897.

Kleinschmit, J. & Svolba, J. (1984) IUFRO Sitka spruce provenance experiment in Northern Germany. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A.

McKnight), pp. 131-144. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Körner, C. & Diemer, M. (1987) *In situ* photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. Functional Ecology, 1, 179-194.

Kubiske, M.E., Pregitzer, K.S., Zak, D.R., & Mikan, C.J. (1998) Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO_2 and soil N availability. New Phytologist, 140, 251-260.

Kull, O. & Kruijt, B. (1998) Leaf photosynthetic light response: a mechanistic model for scaling photosynthesis to leaves and canopies. Functional Ecology, 12, 767-777.

Kullman, L. (1979) Change and stability in the altitude of Birch tree limit in the southern Swedish Scandes. Acta Phytogeographica Suecica, 65, 121.

Lamhamedi, M.S. & Bernier, P.Y. (1994) Ecophysiology and field performance of black spruce (*Picea mariana*): a review. Annales des Sciences Forestieres, 51, 529-551.

Landsberg, J.J. & Waring, R.H. (1997) A generalised model of forest productivity using simplified concepts of radiation-use efficiency, carbon balance and partitioning. Forest Ecology and Management, 95, 209-228.

Lawlor, D.W. (2001). Chapter 7. The chemistry of photosynthesis. In *Photosynthesis*, 3rd edn., pp. 139-183. BIOS Scientific Publishers Ltd, Oxford, UK.

Lee, H.S.J. & Jarvis, P.G. (1995) Trees differ from crops and from each other in their responses to increases in CO_2 concentration. Journal of Biogeography, 22, 323-330.

Lee, K.H. & Jose, S. (2003) Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. Forest Ecology and Management, 185, 263-273.

Lee, S.J. (1992) Likely increases in volume and revenue from planting genetically improved Sitka spruce. In *Super Sitka for the 90s* (ed D.A. Rook), Forestry Commission Bulletin 103, pp. 61-74. HMSO, London.

Lee, S.J. (2001) Selection of parents for the Sitka spruce breeding population in Britain and the strategy for the next breeding cycle. *Forestry*, 74, 129-143.

Lee, S.J., Woolliams, J., Samuel, C.J.A., & Malcolm, D.C. (2002a) A study of population variation and inheritance in Sitka spruce. II. Age trends in genetic parameters for vigour traits and optimum selection ages. *Silvae Genetica*, 51, 55-65.

Lee, S.J., Woolliams, J., Samuel, C.J.A., & Malcolm, D.C. (2002b) A study of population variation and inheritance in Sitka spruce. III. Age trends in genetic parameters and optimum selection ages for wood density, and genetic correlations with vigour traits. *Silvae Genetica*, 51, 143-151.

Leonardos, E.D., Tsujita, M.J., & Grodzinski, B. (1996) The effect of source or sink temperature on photosynthesis and C_{14} partitioning in and export from a source leaf of *Alstroemeria*. Physiologia Plantarum, 97, 563-575.

Leverenz, J.W., Deans, J.D., Ford, E.D., Jarvis, P.G., Milne, R., & Whitehead, D. (1982) Systematic spatial variation of stomatal conductance in a Sitka spruce plantation. Journal of Applied Ecology, 19, 835-851.

Leverenz, J.W. & Jarvis, P.G. (1979) Photosynthesis in Sitka spruce. VIII. The effects of light flux density and direction on the rate of net photosynthesis and the stomatal conductance of needles. Journal of Applied Ecology, 16, 919-932.

Leverenz, J.W. & Jarvis, P.G. (1980a) Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Carr.). IX. The relative contribution made by needles at various positions on the shoot. Journal of Applied Ecology, 17, 59-68.

Leverenz, J.W. & Jarvis, P.G. (1980b) Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Carr.). X. Acclimation to quantum flux density within and between trees. Journal of Applied Ecology, 17, 697-708.

Lewandowska, M., Hart, J.W., & Jarvis, P.G. (1977) Photosynthetic electron transport in shoots of Sitka spruce from different levels in a forest canopy. Physiologia Plantarum, 41, 124-128.

Lewandowska, M. & Jarvis, P.G. (1978) Quantum requirements of photosynthetic electron transport in Sitka spruce from different light environments. Physiologia Plantarum, 42, 277-282.

Li, C.Y. & Wang, K.Y. (2003) Differences in drought responses of three contrasting *Eucalyptus microtheca* F. Muell. populations. Forest Ecology and Management, 179, 377-385.

Lichtenthaler, H.K. & Wellburn, A.R. (1983) Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochemical Society Transactions, 11, 591-592.

Lines, R. (1987) Seed origin variation in Sitka spruce. Proceedings of the Royal Society of Edinburgh, 93B, 25-39.

Lines, R., Mitchell, A.F., & Pearce, M.L. (1971). Provenance: Sitka spruce. In *Forestry Commission Report on Forest Research, 1970*, pp. 42-44. HMSO, London.

Lines, R. & Samuel, C.J.A. (1984) The main IUFRO experiments with Sitka spruce in Britain: ten year results. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 21-36. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Litton, C.M., Ryan, M.G., & Knight, D.H. (2004) Effects of tree density and stand age on carbon allocation patterns in postfire lodgepole pine. Ecological Applications, 14, 460-475.

Lloyd, J., Grace, J., Miranda, A.C., Meir, P., Wong, S.C., Miranda, H.S., Wright, I.R., Gash, J.H.C., & McIntyre, J. (1995) A simple calibrated model of Amazon rainforest productivity based on leaf biochemical properties. Plant, Cell and Environment, 18, 1129-1145.

Long, S.P. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO2 concentrations: Has its importance been underestimated? Plant, Cell and Environment, 14, 729-739.

Lopez, B.C., Sabate, S., & Gracia, C.A. (2003) Thinning effects on carbon allocation to fine roots in a *Quercus ilex* forest. Tree Physiology, 23, 1217-1224.

Loustau, D., Ben Brahim, M., Gaudillère, J.-P., & Dreyer, E. (1999) Photosynthetic responses to phosphorus nutrition in two-year-old maritime pine seedlings. Tree Physiology, 19, 707-715.

Low, A.J. (1987) Sitka spruce silviculture in Scottish forests. Proceedings of the Royal Society of Edinburgh, 93B, 93-106.

Ludlow, M.M. & Jarvis, P.G. (1971) Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Carr.). I. General characteristics. Journal of Applied Ecology, 8, 925-953.

Lyons, J.M. (1973) Chilling injury in plants. Annual Review of Plant Physiology, 24, 445-466.

Mäkelä, A. (1999) Acclimation in dynamic models based on structural relationships. Functional Ecology, 13, 145-156.

Mäkinen, H., Nöjd, P., & Mielikäinen, K. (2001) Climatic signal in annual growth variation in damaged and healthy stands of Norway spruce [*Picea abies* (L.) Karst.] in southern Finland. Trees, 15, 177-185.

Man, R. & Lieffers, V.J. (1997) Seasonal photosynthetic responses to light and temperature in white spruce (*Picea glauca*) seedlings planted under aspen (*Populus tremuloides*) canopy and in the open. Tree Physiology, 17, 437-444.

Mboyi, W.M. & Lee, S.J. (1999) Incidence of autumn frost damage and lammas growth in a 4-year-old clonal trial of Sitka spruce (*Picea sitchensis*) in Britain. *Forestry*, 72, 135-146.

McMurtrie, R. & Wolf, L. (1983) Above- and below-ground growth of forest stands: a carbon budget model. Annals of Botany, 52, 437-448.

McMurtrie, R.E. & Wang, Y.-P. (1993) Mathematical models of the photosynthetic response of tree stands to rising CO_2 concentrations and temperatures. Plant, Cell and Environment, 16, 1-13.

Medlyn, B. (1998) Physiological basis of the light use efficiency model. Tree Physiology, 18, 167-176.

Medlyn, B.E., Badeck, F.-W., De Pury, D.G.G., Barton, C.V.M., Broadmeadow, M., Ceulemans, R., De Angelis, P., Forstreuter, M., Jach, M.E., Kellomäki, S., Laitat, E., Marek, M., Philippot, S., Rey, A., Strassemeyer, J., Laitinen, K., Liozon, R., Portier, B., Roberntz, P., Wang, K., & Jarvis, P.G. (1999) Effects of elevated [CO_2] on photosynthesis in European forest species: a meta-analysis of model parameters. Plant, Cell and Environment, 22, 1475-1495.

Medlyn, B.E. & Jarvis, P.G. (1999) Design and use of a database of model parameters from elevated [CO_2] experiments. Ecological Modelling, 124, 69-83.

Millard, P. (1996) Ecophysiology of the internal cycling of nitrogen for tree growth. Zeitschrift fur Pflanzenernahrung und Bodenkunde, 159, 1-10.

Miller, H.G. & Miller, J.D. (1987) Nutritional requirements of Sitka spruce. Proceedings of the Royal Society of Edinburgh, 93B, 75-83.

Miller, J.T. & Shelbourne, C.J.A. (1984) Sitka spruce provenance trials in New Zealand. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 187-196. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Mohan, J.E., Clark, J.S., & Schlesinger, W.H. (2004) Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO_2. Global Change Biology, 10, 233-247.

Murray, M.B., Smith, R.I., Friend, A., & Jarvis, P.G. (2000) Effect of elevated [CO_2] and varying nutrient application rates on physiology and biomass accumulation of Sitka spruce (*Picea sitchensis*). Tree Physiology, 20, 421-434.

Murthy, R., Zarnoch, S.J., & Dougherty, P.M. (1997) Seasonal trends of light-saturated net photosynthesis and stomatal conductance of loblolly pine trees grown in contrasting environments of nutrition, water and carbon dioxide. Plant, Cell and Environment, 20, 558-568.

Nakaji, T., Motohiro, F., Dokiya, Y., & Takeshi, I. (2001) Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. Trees, 15, 453-461.

Nanson, A. (1984) Sitka spruce main provenance experiment in Belgium. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 5-9. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Negi, J.D.S., Manhas, R.K., & Chauhan, P.S. (2003) Carbon allocation in different components of some tree species of India: A new approach for carbon estimation. Current Science, 85, 1528-1531.

Neilson, R.E. & Jarvis, P.G. (1975) Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Carr.). VI. Response of stomata to temperature. Journal of Applied Ecology, 12, 879-891.

Neilson, R.E., Ludlow, M.M., & Jarvis, P.G. (1972) Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Carr.). II. Response to temperature. Journal of Applied Ecology, 9, 721-745.

Ngugi, M.R., Hunt, M.A., Doley, D., Ryan, P., & Dart, P. (2003) Dry matter production and allocation in *Eucalyptus cloeziana* and *Eucalyptus argophloia* seedlings in response to soil water deficits. New Forests, 26, 187-200.

Norman, J.M. & Jarvis, P.G. (1975) Photosynthesis in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) V. Radiation penetration theory and a test case. Journal of Applied Ecology, 12, 839-878.

Oleksyn, J., Modrzynski, J., Tjoelker, M.G., Zytkowiak, R., Reich, P.B., & Karolewski, P. (1998) Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. Functional Ecology, 12, 573-590.

Olszyk, D.M., Johnson, M.G., Tingey, D.T., Rygiewicz, P.T., Wise, C., Van Ess, E., Benson, A., Storm, M.J., & King, R. (2003) Whole-seedling biomass allocation, leaf

area, and tissue chemistry for Douglas-fir exposed to elevated CO_2 and temperature for 4 years. Canadian Journal of Forest Research, 33, 269-278.

Paquin, R., Margolis, H.A., Doucet, R., & Coyea, M.R. (2000) Physiological responses of black spruce layers and planted seedlings to nutrient addition. Tree Physiology, 20, 229-237.

Pederick, L.A. (1984) Growth of Sitka spruce provenances to 11 years in Victoria, Australia. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 1-4. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Peet, M.M., Huber, S.C., & Patterson, D.T. (1986) Acclimation to high CO_2 in monoecious cucumbers. II. Carbon exchange rates, enzyme activities, and starch and nutrient concentrations. Plant Physiology, 80, 63-67.

Pettersson, R. & McDonald, A.J.S. (1992) Effects of elevated carbon dioxide concentrations on photosynthesis and growth of small birch plants (*Betula pendula* Roth.) at optimal nutrition. Plant, Cell and Environment, 15, 911-919.

Pfeifer, A.R. (1984) Sitka spruce IUFRO provenances in Ireland: results to 9 years. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 151-164. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Pigott, C.D. (1970). The response of plants to climate and climatic change. In *The Flora of a Changing Britain* (ed F.H. Perring), pp. 32-44. Classey, Faringdon.

Pigott, C.D. (1975) Experimental studies on the influence of climate on the geographical distribution of plants. Weather, 30, 82-90.

Pigott, C.D. & Huntley, J.P. (1978) Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. I. Distribution in North-West England. New Phytologist, 81, 429-441.

Pigott, C.D. & Huntley, J.P. (1980) Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. II. History in North-West England. New Phytologist, 84, 145-164.

Pigott, C.D. & Huntley, J.P. (1981) Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. III. Nature and causes of seed sterility. New Phytologist, 87, 817-839.

Pirags, D. (1984) IUFRO Sitka spruce ten provenance experiment in the Latvian SSR. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 173-174. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Prioul, J.L. & Chartier, P. (1977) Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO_2 fixation: A critical analysis of the methods used. Annals of Botany, 41, 789-800.

Radcliffe, J.E. & Lefever, K.R. (1981) Aspect influences on pasture microclimate at Coopers Creek, North Canterbury. New Zealand Journal of Agricultural Research, 24, 55-66.

Raulier, F., Bernier, P.Y., & Ung, C.-H. (1999) Canopy photosynthesis of sugar maple (*Acer saccharum*): comparing big-leaf and multilayer extrapolations of leaf-level measurements. Tree Physiology, 19, 407-420.

Rayment, M.B., Loustau, D., & Jarvis, P.G. (2000) Measuring and modelling conductances of black spruce at three organizational scales: shoot, branch and canopy. Tree Physiology, 20, 713-723.

Rayment, M.B., Loustau, D., & Jarvis, P.G. (2002) Photosynthesis and respiration of black spruce at three organisational scales: shoot, branch and canopy. Tree Physiology, 22, 219-229.

Reich, P.B., Kloeppel, B.D., Ellsworth, D.S., & Walters, M.B. (1995) Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. Oecologia, 104, 24-30.

Reich, P.B., Oleksyn, J., & Tjoelker, M.G. (1996) Needle respiration and nitrogen concentration in Scots Pine populations from a broad latitudinal range: a common garden test with field-grown trees. Functional Ecology, 10, 768-776.

Reynolds, J.F. & Thornley, J.H.M. (1982) A shoot:root partitioning model. Annals of Botany, 49, 585-597.

Roche, L. & Haddock, P.G. (1987) Sitka spruce (*Picea sitchensis*) in North America with special reference to its role in British forestry. Proceedings of the Royal Society of Edinburgh, 93B, 1-12.

Rodríguez, R., Espinosa, M., Hofmann, G., & Marchant, M. (2003) Needle mass, fine root and stem wood production in response to silvicultural treatment, tree size and competitive status in radiata pine stands. Forest Ecology and Management, 186, 287-296.

Roman-Amat, B. (1984) IUFRO Sitka spruce provenance experiment in France: results after 7 years in the field. In *IUFRO International Sitka Spruce Provenance Experiment: Sitka Spruce Working Group S2.02.12, Edinburgh, Scotland* (eds C.C. Ying & L.A. McKnight), pp. 119-129. B.C. Ministry of Forests, Canada & Irish Forestry Board, Ireland.

Rorison, I.H., Gupta, P.L., & Hunt, R. (1986a) Local climate, topography and plant growth in Lathkill Dale NRR. II. Growth and nutrient uptake within a single season. Plant, Cell and Environment, 9, 57-64.

Rorison, I.H., Sutton, F., & Hunt, R. (1986b) Local climate, topography and plant growth in Lathkill Dale NRR. I. A twelve-year summary of solar radiation and temperature. Plant, Cell and Environment, 9, 49-56.

Sage, R.F. (1990) A model describing the regulation of Ribulose-1,5-Bisphosphate Carboxylase, electron transport, and triose phosphate use in response to light intensity and CO_2 in C_3 plants. Plant Physiology, 94, 1728-1734.

Sage, R.F. & Pearcy, R.W. (1987) The nitrogen use efficiency of C_3 and C_4 plants. Plant Physiology, 84, 954-958.

Samuel, C.J.A., John, A., & Lee, S.J. (2000) Fifty years of tree breeding in Great Britain. Forest Research, Northern Research Station, Roslin.

Schaberg, P.G., Snyder, M.C., Shane, J.B., & Donnelly, J.R. (2000) Seasonal patterns of carbohydrate reserves in red spruce seedlings. Tree Physiology, 20, 549-555.

Schäfer, K.V.R., Oren, R., Ellsworth, D.S., Lai, C.T., Herrick, J.D., Finzi, A.C., Richter, D.D., & Katul, G.G. (2003) Exposure to an enriched CO_2 atmospheric alters carbon assimilation and allocation in a pine forest ecosystem. Global Change Biology, 9, 1378-1400.

Schoettle, A.W. & Smith, W.K. (1999) Interrelationships among light, photosynthesis and nitrogen in the crown of mature *Pinus contorta* ssp. *latifolia*. Tree Physiology, 19, 13-22.

Senser, M. & Beck, E. (1973) On the mechanisms of frost injury and frost hardening of spruce chloroplasts. Planta, 137, 195-201.

Senser, M. & Beck, E. (1984) Correlation of chloroplast ultrastructure and membrane lipid composition to the different degrees of frost resistance achieved in leaves of spinach, ivy and spruce. Journal of Plant Physiology, 117, 41-55.

Shelbourne, C.J.A. (1969) Tree Breeding Methods. In *Forest Research Institute Technical Paper 55*. New Zealand Forest Service.

Shinozaki, K., Yoda, K., Hozumi, K., & Kira, T. (1964) A quantitative analysis of plant form - the pipe model theory. I. Basic analyses. Japanese Journal of Ecology, 14, 97-105.

Skalar (1995) The SAN^{plus} segmented flow analyser. Soil and Plant Analysis. Skalar Analytical B.V., De Breda, Netherlands.

Sinclair, T.R., Murphy, C.E., & Knoerr, K.R. (1976) Development and evaluation of simplified models for simulating canopy photosynthesis and transpiration. Journal of Applied Ecology, 13, 813-829.

Son, Y. & Hwang, J.H. (2003) Fine root biomass, production and turnover in a fertilized *Larix leptolepis* plantation in central Korea. Ecological Research, 18, 339-346.

Spinnler, D., Egli, P., & Körner, C. (2003) Provenance effects and allometry in beech and spruce under elevated CO_2 and nitrogen on two different forest soils. Basic and Applied Ecology, 4, 467-478.

Šprtová, M. & Marek, M.V. (1999) Response of photosynthesis to radiation and intercellular CO_2 concentration in sun and shade shoots of Norway spruce. Photosynthetica, 37, 433-445.

Stephenson, N.L. (1990) Climatic control of vegetation distribution: the role of water balance. The American Naturalist, 135, 649-670.

Stitt, M. (1991) Rising CO_2 levels and their potential significance for carbon flow in photosynthetic cells. Plant, Cell and Environment, 14, 741-762.

Stitt, M., Quick, W.P., Schurr, U., Schulze, E.D., Rodermel, S.R., & Bogorad, L. (1991) Decreased Ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with antisense rbcS 2: Flux-control coefficients for photosynthesis in varying light, CO_2 and air humidity. Planta, 183, 555-566.

Thornley, J.H.M. (1972) A model to describe the partitioning of photosynthate during vegetative plant growth. Annals of Botany, 33, 419-430.

Tissue, D.T., Thomas, R.B., & Strain, B.R. (1993) Long-term effects of elevated CO_2 and nutrients on photosynthesis and rubisco in loblolly pine seedlings. Plant, Cell and Environment, 16, 859-865.

Topa, M.A. & Cheeseman, J.M. (1992) Effects of root hypoxia and a low P supply on relative growth, carbon dioxide exchange rates and carbon partitioning in *Pinus serotina* seedlings. Physiologia Plantarum, 86, 136-144.

Townsend, J. (1995) Effects of elevated CO_2, water and nutrients on *Picea sitchensis* (Bong.) Carr. seedlings. New Phytologist, 130, 193-206.

Valentine, H.T. (1985) Tree-growth models: Derivations employing the pipe-model theory. Journal of Theoretical Biology, 117, 579-585.

Valentine, H.T. (1988) A carbon-balance model of stand growth: a derivation employing pipe-model theory and the self-thinning rule. Annals of Botany, 62, 389-396.

Valentine, H.T. (1999) Estimation of the net primary productivity of even-aged stands with a carbon-allocation model. Ecological Modelling, 122, 139-149.

van Wijk, M.T., Williams, M., Gough, L., Hobbie, S.E., & Shaver, G.R. (2003) Luxury consumption of soil nutrients: a possible competitive strategy in above-ground and below-ground biomass allocation and root morphology for slow-growing arctic vegetation? Journal of Ecology, 91, 664-676.

Vanhatalo, M., Back, J., & Huttunen, S. (2003) Differential impacts of long-term CO_2 and O_3 exposure on growth of northern conifer and deciduous tree species. Trees - Structure and Function, 17, 211-220.

von Caemmerer, S. & Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta, 153, 376-387.

Vygodskaya, N., Puzachenko, Y., Kozharinov, A., Zavelskaya, N., Tchernyshev, M., Tatarinov, F., Varlagin, A., & Milukova, V.N. (1995) Long term effects of climate on *Picea abies* communities in the South European taiga. Journal of Biogeography, 22, 433-443.

Walker, D.A. (1989) Automated measurement of leaf photosynthetic O_2 evolution as a function of photon flux density. Philosophical Transactions of the Royal Society London B, 323, 313-326.

Wang, K.-Y. & Kellomäki, S. (1997) Effects of elevated CO_2 and soil-nitrogen supply on chlorophyll fluorescence and gas exchange in Scots pine, based on a branch-in-bag experiment. New Phytologist, 136, 277-286.

Wang, Y.P. & Jarvis, P.G. (1990) Description and validation of an array model - MAESTRO. Agricultural and Forest Meteorology, 51, 257-280.

Wang, Z.M., Lechowicz, M.J., & Potvin, C. (1994) Early selection of black spruce seedlings and global change: which genotypes should we favour? Ecological Applications, 4, 604-616.

Weiser, C.J. (1970) Cold resistance and injury in woody plants. Science, 169, 736-750.

Wellburn, A.R. (1997) Environmental factors and genotypic changes in lipids contribute to the winter hardiness of Norway spruce (*Picea abies*). New Phytologist, 135, 115-121.

West, P.W. (1993) Model of above-ground assimilate partitioning and growth of individual trees in even-aged forest monocultures. Journal of Theoretical Biology, 161, 369-394.

Whitehead, D. (1998) Regulation of stomatal conductance and transpiration in forest canopies. Tree Physiology, 18, 633-644.

Wiemken, V. & Ineichen, K. (2000) Seasonal fluctuations of the levels of soluble carbohydrates in spruce needles exposed to elevated CO_2 and nitrogen fertilization and glucose as a potential mediator of acclimation to elevated CO_2. Journal of Plant Physiology, 156, 746-750.

Wood, P.E. (1986) Variation and inheritance of wood properties of Sitka spruce. *MSc Thesis,* Oxford Forestry Institute, Oxford.

Woodrow, I.E., Ball, J.T., & Berry, J.A. (1990) Control of photosynthetic carbon dioxide fixation by the boundary layer, stomata and ribulose 1,5-bisphosphate carboxylase/oxygenase. Plant, Cell and Environment, 13, 339-347.

Woodward, F.I. (1975) The climatic control of the altitudinal distributions of *Sedum rosea* (L.) Scop. and *S. telephium* L. II. The analysis of plant growth in controlled environments. New Phytologist, 74, 335-348.

Woodward, F.I. (1987) Climate and Plant Distribution. Cambridge University Press, Cambridge.

Woodward, F.I. (1997) Life at the edge: a 14-year study of a *Verbena officinalis* population's interactions with climate. Journal of Ecology, 85, 899-906.

Woodward, F.I., Körner, C., & Crabtree, R.C. (1986) The dynamics of leaf extension in plants with diverse altitudinal ranges. I. Field observations on temperature responses at one altitude. Oecologia, 70, 222-226.

Woodward, F.I. & Pigott, C.D. (1975) The climatic control of the altitudinal distribution of *Sedum rosea* (L.) Scop. and *S. telephium* L. I. Field observations. New Phytologist, 74, 323-334.

Wu, R. & Stettler, R.F. (1996) The genetic resolution of juvenile canopy structure and function in a three generation pedigree of *Populus*. Trees, 11, 99-108.

Wu, R. & Stettler, R.F. (1997) Quantitative genetics of growth and development in *Populus*. II. The partitioning of genotype x environment interaction in stem growth. Heredity, 78, 124-134.

Wu, R, & Stettler, R.F. (1998) Quantitative genetics of growth and development in *Populus*. III. Phenotypic plasticity of crown structure and function. Heredity, 81, 299-310.

Wullschleger, S.D., Norby, R.J., & Gunderson, C.A. (1992) Growth and maintenance respiration in leaves of *Liriodendron tulipifera* L. exposed to long-term carbon dioxide enrichment in the field. New Phytologist, 121, 515-523.

Xu, P., Ying, C.C., & El-Kassaby, Y.A. (2000) Multivariate analyses of causal correlation between growth and climate in Sitka spruce. Silvae Genetica, 49, 257-263.

Ying, C.C. (1997) Effects of site, provenance, and provenance and site interaction in Sitka spruce in coastal British Columbia. Forest Genetics, 4, 99-112.

Yu, Q. & Pulkkinen, P (2003) Genotype-environment interaction and stability in growth of aspen hybrid clones. Forest Ecology and Management, 173, 25-35.

Zhang, X.-Q. & Xu, D. (2003) Eco-physiological modelling of canopy photosynthesis and growth of a Chinese fir plantation. Forest Ecology and Management, 173, 201-211.

Appendix 1

Table 1: Mean **% foliar moisture content** and standard error (s.e.) for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	56.38	56.02	56.45	35.75	52.64	57.44	64.48	47.54	60.51
		s.e.	1.45	3.03	3.91	29.88	2.10	1.78	7.40	2.35	4.37
	Wauchope	Mean	59.75	61.11	63.16	65.01	59.94	57.66	62.27	59.46	58.63
		s.e.	1.74	0.85	1.38	0.68	2.06	0.51	1.27	-	2.89
	Scootmore	Mean	55.43	55.99	59.28	65.66	55.81	60.98	60.16	57.17	59.91
		s.e.	1.41	1.10	0.82	0.80	0.58	5.67	0.79	0.07	0.53
C20208	Newcastleton	Mean	55.31	58.43	62.44	-	54.25	54.33	72.32	53.18	66.51
		s.e.	2.07	1.54	1.41	-	2.06	0.51	1.27	-	2.89
	Wauchope	Mean	56.58	57.04	54.48	63.09	55.33	56.19	61.69	54.78	60.09
		s.e.	0.57	1.16	1.86	0.27	1.56	1.49	1.43	1.95	2.87
	Scootmore	Mean	52.89	55.53	54.90	61.37	52.98	54.69	60.36	55.43	59.37
		s.e.	0.91	5.92	0.77	1.23	0.78	0.61	0.88	1.44	1.14
C20211	Newcastleton	Mean	52.67	50.78	56.35	50.09	44.89	49.63	64.72	47.69	58.43
		s.e.	1.59	3.49	6.26	11.89	4.09	2.49	5.48	1.61	1.61
	Wauchope	Mean	53.79	56.04	56.76	63.47	53.32	56.11	58.45	55.04	56.74
		s.e.	1.03	0.99	1.56	0.42	0.74	1.81	1.20	-	1.97
	Scootmore	Mean	52.67	52.76	56.16	62.23	54.36	55.97	60.72	56.09	60.42
		s.e.	1.60	1.21	1.06	1.11	-	1.03	0.49	0.51	0.99

Appendix 2

Table 1: Mean **chlorophyll *a*** concentration (mg g^{-1} d. wt) and standard error (s.e.) for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	4.30	5.29	5.31	4.76	4.75	4.91	4.28	4.40	2.52
		s.e.	0.32	0.41	0.68	0.98	0.30	0.34	0.47	0.19	0.39
	Wauchope	Mean	4.37	4.13	4.93	3.86	4.81	4.39	3.72	6.75	2.69
		s.e.	0.27	0.18	0.35	1.05	0.03	0.62	0.61	-	0.50
	Scootmore	Mean	3.23	3.69	3.65	2.70	4.47	4.67	1.98	3.61	1.61
		s.e.	0.13	0.28	0.17	0.38	0.51	1.91	0.15	0.04	0.11
C20208	Newcastleton	Mean	3.43	4.14	5.41	-	4.39	4.51	3.25	4.12	2.13
		s.e.	0.54	0.12	0.76	-	0.13	0.33	0.47	0.24	0.29
	Wauchope	Mean	3.74	4.03	3.76	2.62	3.56	4.01	2.97	3.67	2.58
		s.e.	0.09	0.28	0.27	0.07	0.26	0.43	0.49	0.05	0.37
	Scootmore	Mean	2.43	3.88	3.02	1.92	2.92	2.84	2.03	2.75	1.76
		s.e.	0.15	1.21	0.21	0.16	0.10	0.39	0.24	0.34	0.24
C20211	Newcastleton	Mean	4.65	4.40	5.96	4.20	3.72	4.34	3.74	4.09	1.99
		s.e.	0.14	0.26	1.42	0.98	0.48	0.18	0.37	0.67	0.26
	Wauchope	Mean	3.89	4.08	3.83	3.84	3.62	3.27	2.70	3.05	1.96
		s.e.	0.15	0.21	0.33	0.97	0.44	0.17	0.27	-	0.19
	Scootmore	Mean	3.12	3.59	3.11	2.45	3.21	3.15	2.14	3.19	2.26
		s.e.	0.02	0.24	0.18	0.43	-	0.25	0.29	0.55	0.45

Table 2: Mean **chlorophyll *b*** concentration (mg g^{-1} d. wt) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	2.48	3.35	3.51	4.14	2.38	2.37	3.67	2.91	2.14
		s.e.	0.25	0.70	0.87	0.33	0.28	0.32	0.80	0.51	0.61
	Wauchope	Mean	3.09	2.34	2.89	2.52	3.12	2.60	2.29	6.62	1.82
		s.e.	0.32	0.15	0.46	0.76	0.13	0.48	0.42	-	0.23
	Scootmore	Mean	1.79	1.80	1.85	2.06	2.79	2.36	1.50	2.06	1.03
		s.e.	0.12	0.20	0.20	0.42	0.54	0.89	0.23	0.14	0.15
C20208	Newcastleton	Mean	1.94	2.41	3.92	-	2.10	2.71	1.99	2.44	1.43
		s.e.	0.40	0.17	0.99	-	0.18	0.62	0.29	0.37	0.30
	Wauchope	Mean	2.17	2.47	2.41	1.47	1.81	2.95	2.23	1.90	1.96
		s.e.	0.21	0.36	0.43	0.01	0.11	0.50	0.45	0.38	0.56
	Scootmore	Mean	1.14	2.25	1.85	1.35	1.72	1.63	1.72	1.47	1.25
		s.e.	0.12	0.73	0.28	0.30	0.17	0.27	0.31	0.22	0.25
C20211	Newcastleton	Mean	2.79	2.55	4.80	3.03	2.04	2.82	2.57	1.99	1.36
		s.e.	0.24	0.32	2.05	0.52	0.38	0.38	0.43	0.77	0.25
	Wauchope	Mean	2.19	2.29	2.04	3.49	2.07	1.80	1.87	1.80	1.38
		s.e.	0.29	0.20	0.18	1.35	0.37	0.27	0.12	-	0.18
	Scootmore	Mean	1.50	1.83	1.55	1.73	1.52	1.44	1.59	1.80	2.04
		s.e.	0.04	0.16	0.26	0.44	-	0.17	0.32	0.69	0.68

Table 3: Mean **chlorophyll *a:b*** ratio and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	1.76	1.88	1.73	1.18	2.08	2.21	1.31	1.70	1.46
		s.e.	0.13	0.25	0.16	0.33	0.16	0.19	0.18	0.24	0.20
	Wauchope	Mean	1.43	1.79	1.82	1.54	1.55	1.72	1.75	1.02	1.45
		s.e.	0.08	0.11	0.24	0.04	0.07	0.08	0.28	-	0.10
	Scootmore	Mean	1.85	2.10	2.05	1.37	1.66	2.04	1.41	1.76	1.65
		s.e.	0.18	0.09	0.17	0.10	0.18	0.23	0.13	0.10	0.16
C20208	Newcastleton	Mean	1.84	1.75	1.56	-	2.15	1.85	1.63	1.81	1.57
		s.e.	0.12	0.10	0.18	-	0.19	0.22	0.10	0.22	0.11
	Wauchope	Mean	1.75	1.70	1.63	1.78	1.98	1.43	1.39	2.01	1.56
		s.e.	0.13	0.19	0.15	0.06	0.15	0.15	0.16	0.38	0.28
	Scootmore	Mean	2.17	1.76	1.74	1.62	1.75	1.80	1.26	1.89	1.49
		s.e.	0.11	0.05	0.13	0.23	0.19	0.13	0.10	0.10	0.14
C20211	Newcastleton	Mean	1.72	1.81	1.61	1.36	1.97	1.65	1.59	2.58	1.58
		s.e.	0.13	0.12	0.16	0.09	0.20	0.16	0.15	0.36	0.13
	Wauchope	Mean	1.84	1.81	1.91	1.24	1.78	1.92	1.44	1.69	1.44
		s.e.	0.17	0.16	0.16	0.23	0.11	0.26	0.07	-	0.10
	Scootmore	Mean	2.09	1.99	2.15	1.53	2.10	2.19	1.42	1.94	1.17
		s.e.	0.07	0.08	0.20	0.11	-	0.08	0.13	0.44	0.17

Table 4: Mean **foliar nitrogen** concentration (mg g^{-1} d. wt) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	8.57	9.11	10.35	23.92	8.69	11.24	15.98	9.60	11.22
		s.e.	0.28	0.60	0.94	4.54	1.15	2.06	3.71	0.97	1.27
	Wauchope	Mean	7.36	8.83	10.08	6.56	7.68	8.86	7.53	9.16	7.48
		s.e.	1.59	1.57	1.90	1.32	0.61	0.61	0.57	-	0.65
	Scootmore	Mean	7.89	8.27	9.00	7.44	8.25	9.15	8.37	9.39	6.84
		s.e.	0.23	0.29	0.61	0.33	0.70	0.50	0.68	0.69	0.22
C20208	Newcastleton	Mean	9.29	9.01	10.48	-	8.05	8.70	13.26	8.36	9.61
		s.e.	1.02	0.45	0.93	-	0.21	0.84	1.99	1.00	1.39
	Wauchope	Mean	9.41	6.89	7.21	5.39	12.05	8.18	6.85	10.08	5.25
		s.e.	0.97	0.21	0.57	0.37	3.55	0.90	0.73	0.42	0.80
	Scootmore	Mean	7.47	7.61	8.07	7.84	8.24	9.40	7.47	8.00	7.41
		s.e.	0.19	0.34	0.15	0.21	1.07	0.39	0.44	0.03	0.51
C20211	Newcastleton	Mean	8.32	7.78	12.30	13.84	8.60	8.55	11.99	10.86	9.36
		s.e.	0.52	0.48	1.42	1.39	0.83	0.54	1.28	1.42	0.49
	Wauchope	Mean	6.98	7.20	7.79	6.51	9.00	10.74	7.95	7.49	9.05
		s.e.	0.40	0.46	0.17	1.12	0.24	2.75	1.01	-	2.30
	Scootmore	Mean	8.08	8.10	8.26	8.18	7.64	9.68	7.23	9.64	7.89
		s.e.	0.43	0.11	0.35	0.52	-	0.91	0.75	0.74	0.61

Table 5: Mean **foliar phosphorus** concentration (mg g^{-1} d. wt) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	1.19	1.35	2.05	4.19	1.31	1.34	2.49	0.95	1.63
		s.e.	0.21	0.13	0.33	0.79	0.41	0.11	0.45	0.09	0.21
	Wauchope	Mean	1.45	1.26	1.75	1.25	1.08	1.21	1.36	1.61	1.16
		s.e.	0.38	0.08	0.27	0.36	0.21	0.10	0.13	-	0.12
	Scootmore	Mean	1.37	1.16	1.43	1.70	1.14	1.50	1.50	1.31	1.35
		s.e.	0.06	0.07	0.07	0.18	0.18	0.18	0.14	0.37	0.09
C20208	Newcastleton	Mean	1.02	1.07	1.81	-	0.77	1.11	2.71	0.97	1.83
		s.e.	0.15	0.14	0.28	-	0.12	0.12	0.47	0.16	0.39
	Wauchope	Mean	0.67	0.81	1.12	1.59	1.08	1.01	1.49	1.28	1.18
		s.e.	0.07	0.16	0.17	0.44	0.28	0.18	0.15	0.14	0.21
	Scootmore	Mean	1.13	1.03	1.08	2.11	1.31	1.71	1.58	1.46	1.58
		s.e.	0.08	0.11	0.07	0.16	0.17	0.36	0.12	0.18	0.09
C20211	Newcastleton	Mean	0.71	0.94	1.83	2.84	1.09	0.93	2.54	1.30	1.80
		s.e.	0.05	0.10	0.24	0.56	0.35	0.08	0.31	0.17	0.05
	Wauchope	Mean	0.60	0.93	1.28	2.07	0.96	1.26	1.44	1.06	1.70
		s.e.	0.04	0.13	0.15	0.27	0.08	0.32	0.08	-	0.15
	Scootmore	Mean	1.19	1.44	1.14	2.05	0.83	1.19	1.67	1.30	1.82
		s.e.	0.14	0.20	0.10	0.28	-	0.13	0.18	0.35	0.36

Appendix 3

Results highlighted in grey are significant at the 0.05 probability level and results in bold and highlighted in grey are significant at the 0.01 probability level.

Table 1: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **chlorophyll *a*** (mg g^{-1} d. wt) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	**6.805**	0.430	0.409	1.672	**6.216**	1.195	1.879	2.432	1.869	2
	P	**0.004**	0.654	0.667	0.211	**0.006**	0.315	0.167	0.118	0.170	
Site	*F*	**18.956**	2.410	**8.005**	**5.906**	2.053	1.141	**16.605**	2.007	2.749	2
	P	**<0.001**	0.103	**0.001**	**0.009**	0.149	0.332	**<0.001**	0.165	0.078	
Clone × Site	*F*	1.102	0.516	0.388	0.156	0.999	0.261	0.906	1.762	2.833	4
	P	0.373	0.724	0.816	0.924	0.426	0.901	0.470	0.183	0.039	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 2: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **chlorophyll *a*** (mg g^{-1} d. wt) between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	**8.474**	3.333	4.223	0.393	0.225	5.104	0.439	0.411	0.219	2
	P	**0.001**	0.048	0.021	0.680	0.800	0.015	0.648	0.666	0.805	
Needle Age	*F*	2.009	**8.884**	2.884	**5.525**	4.659	1.395	2.752	3.285	3.836	3
	P	0.127	**<0.001**	0.046	**0.007**	0.011	0.271	0.057	0.031	0.022	
Height × Age	*F*	0.882	1.140	0.551	3.115	0.558	0.608	0.518	0.377	0.126	3
	P	0.483	0.333	0.650	0.052	0.649	0.617	0.673	0.770	0.944	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 3: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **chlorophyll *b*** (mg g^{-1} d. wt) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	**5.595**	0.258	0.003	1.183	**4.817**	0.547	1.195	**4.818**	0.307	2
	P	**0.008**	0.774	0.997	0.187	**0.017**	0.584	0.314	**0.022**	0.737	
Site	*F*	**15.143**	2.617	**4.306**	**3.611**	0.520	1.855	**5.349**	2.135	0.518	2
	P	**<0.001**	0.085	**0.020**	**0.044**	0.601	0.172	**0.009**	0.149	0.601	
Clone × Site	*F*	1.542	0.702	0.308	1.048	1.337	0.718	1.425	1.903	2.137	4
	P	0.215	0.595	0.871	0.391	0.284	0.585	0.245	0.156	0.098	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 4: *F* statistic and *P* value from the two-way ANOVA, investigating differences of chlorophyll *b* (mg g^{-1} d. wt) between canopy height, needle age and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	2.674	2.615	2.664	**7.087**	0.582	2.087	0.755	0.374	0.465	2
	P	0.080	0.089	0.080	**0.005**	0.567	0.082	0.477	0.690	0.633	
Needle Age	*F*	1.706	**3.866**	1.315	**8.151**	0.773	0.512	0.958	1.316	0.202	3
	P	0.180	**0.018**	0.281	**0.001**	0.521	0.678	0.423	0.284	0.894	
Height × Age	*F*	0.633	0.438	0.337	**9.160**	1.464	1.513	1.287	0.500	0.017	3
	P	0.642	0.649	0.799	**0.001**	0.252	0.239	0.294	0.685	0.997	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 5: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **chlorophyll *a*:*b*** between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	2.490	1.072	1.659	1.284	0.407	1.323	0.130	2.491	0.550	2
	P	0.099	0.352	0.203	0.297	0.670	0.280	0.878	0.113	0.582	
Site	*F*	**5.276**	1.170	**3.303**	0.336	2.844	1.406	1.037	0.683	0.478	2
	P	**0.011**	0.321	**0.047**	0.718	0.077	0.259	0.364	0.518	0.624	
Clone × Site	*F*	0.779	0.213	0.333	0.726	0.944	1.108	1.442	1.172	0.429	4
	P	0.547	0.930	0.854	0.547	0.455	0.369	0.239	0.358	0.787	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 6: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **chlorophyll *a*:*b*** ratio between canopy height, needle age and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	1.158	2.484	**5.778**	2.541	0.993	0.155	0.541	0.598	1.112	2
	P	0.324	0.099	**0.006**	0.107	0.386	0.857	0.587	0.555	0.345	
Needle Age	*F*	**3.811**	2.590	**3.873**	0.585	1.102	**3.262**	**4.118**	**5.997**	**8.849**	3
	P	**0.017**	0.070	**0.015**	0.632	0.369	**0.041**	**0.013**	**0.002**	**<0.001**	
Height × Age	*F*	1.672	0.060	2.602	0.923	1.545	0.361	1.598	0.901	0.160	3
	P	0.174	0.942	0.063	0.450	0.231	0.782	0.207	0.450	0.922	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 7: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **total foliar nitrogen** (mg g^{-1} d. wt) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	0.472	1.235	2.262	**3.997**	0.289	0.594	0.198	0.198	1.901	2
	P	0.628	0.302	0.117	**0.033**	0.751	0.558	0.821	0.823	0.165	
Site	*F*	2.415	**3.822**	**5.427**	1.464	0.365	0.091	**9.249**	0.094	**10.027**	2
	P	0.106	**0.030**	**0.008**	0.253	0.698	0.914	**0.001**	0.910	**<0.001**	
Clone × Site	*F*	0.919	0.311	0.554	1.119	0.823	1.167	0.120	0.658	0.934	4
	P	0.465	0.869	0.697	0.363	0.523	0.342	0.975	0.630	0.456	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 8: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **total foliar nitrogen** (mg g^{-1} d. wt) between canopy height, needle age and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	0.510	2.872	1.779	0.084	2.005	0.172	0.706	1.960	0.252	2
	P	0.604	0.071	0.180	0.920	0.159	0.843	0.500	0.155	0.779	
Needle Age	*F*	0.218	**4.210**	**2.944**	1.190	2.820	0.454	**3.480**	2.594	2.653	3
	P	0.883	**0.013**	**0.043**	0.342	0.063	0.717	**0.026**	0.067	0.071	
Height × Age	*F*	0.827	1.127	0.228	0.263	1.151	0.988	0.939	1.637	1.777	3
	P	0.515	0.336	0.876	0.851	0.351	0.417	0.432	0.197	0.177	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 9: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **total foliar phosphorus** (mg g^{-1} d. wt) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	1.222	3.330	2.751	1.885	0.273	0.558	0.309	0.104	1.048	2
	P	0.309	0.046	0.076	0.176	0.763	0.577	0.736	0.902	0.362	
Site	*F*	1.017	1.937	**4.708**	0.768	0.312	1.951	**6.509**	0.594	1.392	2
	P	0.374	0.157	**0.015**	0.476	0.735	0.158	**0.004**	0.563	0.262	
Clone × Site	*F*	0.995	2.151	0.349	0.304	0.876	0.857	0.223	0.987	1.198	4
	P	0.425	0.092	0.843	0.823	0.492	0.499	0.924	0.441	0.330	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 10: *F* statistic and *P* value from the two-way ANOVA, investigating differences of **total foliar phosphorus** (mg g^{-1} d. wt) between canopy height, needle age and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	0.422	0.479	0.112	0.621	0.088	1.167	0.364	0.627	0.950	2
	P	0.659	0.624	0.895	0.549	0.916	0.330	0.698	0.540	0.400	
Needle Age	*F*	0.932	2.748	1.522	1.039	**3.083**	**12.419**	1.398	**5.913**	**4.352**	3
	P	0.432	0.059	0.221	0.399	**0.048**	**<0.001**	0.259	**0.002**	**0.013**	
Height × Age	*F*	1.010	0.587	1.178	0.861	1.081	1.437	0.023	5.090	0.535	3
	P	0.413	0.562	0.329	0.479	0.378	0.259	0.995	0.005	0.662	
Residual DF		43	32	46	18	22	22	36	37	25	

Appendix 4

Table 1: Mean A_{max} (μmol CO_2 mol^{-1} N s^{-1}) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	130.96	129.75	123.98	14.93	280.82	116.60	112.12	142.84	121.06
		s.e.	12.05	36.79	32.85	1.62	135.79	26.25	43.40	55.39	33.82
	Wauchope	Mean	27.91	37.84	50.96	132.49	46.00	42.01	92.66	41.13	86.48
		s.e.	10.19	7.21	16.23	15.21	1.87	6.41	10.52	-	12.07
	Scootmore	Mean	36.01	39.46	59.59	63.93	45.30	72.85	71.46	54.08	92.03
		s.e.	4.62	5.88	9.98	9.42	5.42	17.17	7.27	3.63	10.04
C20208	Newcastleton	Mean	102.87	134.13	127.31	-	194.33	233.68	192.86	244.15	328.92
		s.e.	25.58	14.88	24.68	-	10.26	60.96	75.22	37.07	127.57
	Wauchope	Mean	87.74	36.85	50.50	87.84	35.32	46.42	95.82	39.74	128.88
		s.e.	69.45	7.57	6.13	19.38	8.04	7.74	9.94	3.33	28.46
	Scootmore	Mean	30.42	48.34	53.10	52.84	35.90	38.23	79.49	44.07	67.46
		s.e.	9.70	11.42	11.45	8.29	8.19	8.17	11.14	10.55	5.55
C20211	Newcastleton	Mean	87.71	88.22	80.54	41.23	132.03	154.14	87.52	123.31	151.53
		s.e.	30.32	37.49	37.85	11.49	37.81	45.15	28.65	57.48	43.71
	Wauchope	Mean	36.77	46.45	58.46	100.56	39.00	47.14	81.16	64.23	84.13
		s.e.	4.05	3.28	7.57	39.70	6.30	4.94	14.33	-	18.86
	Scootmore	Mean	21.71	23.99	26.73	56.72	13.14	30.03	61.76	20.77	51.97
		s.e.	5.18	4.15	5.44	9.26	-	0.91	2.69	4.99	23.79

Table 2: Mean R_{day} (μmol CO_2 mol^{-1} N s^{-1}) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	-16.33	-25.79	-19.93	-1.44	-22.25	-4.59	-14.02	-38.86	-46.91
		s.e.	4.62	19.56	13.49	0.17	15.72	0.72	4.54	22.83	26.98
	Wauchope	Mean	-4.51	-1.79	-4.12	-9.50	-3.34	-3.44	-9.09	-3.17	-24.21
		s.e.	2.61	0.38	0.84	1.11	1.65	2.48	3.67	-	11.19
	Scootmore	Mean	-6.05	-6.42	-5.54	-13.67	-8.88	-12.26	-19.77	-13.39	-43.05
		s.e.	0.58	1.35	0.74	1.53	1.87	2.34	4.18	6.11	11.30
C20208	Newcastleton	Mean	-16.14	-13.52	-10.50	-	-8.58	-9.58	-16.67	-11.65	-49.60
		s.e.	5.56	3.08	3.92	-	1.73	2.06	4.36	3.58	13.51
	Wauchope	Mean	-6.41	-2.27	-6.87	-13.16	-2.54	-2.83	-6.40	-3.60	-23.06
		s.e.	3.45	0.42	2.92	2.18	1.03	1.06	2.19	0.15	5.39
	Scootmore	Mean	-4.88	-6.65	-6.73	-12.96	-6.23	-5.85	-21.65	-6.07	-21.16
		s.e.	1.48	1.19	1.06	2.45	1.07	1.24	6.93	0.91	5.55
C20211	Newcastleton	Mean	-9.92	-7.20	-7.09	-3.84	-26.15	-9.84	-16.80	-12.15	-44.25
		s.e.	3.11	2.81	2.96	0.16	10.14	4.77	5.51	3.79	12.62
	Wauchope	Mean	-3.44	-0.81	-3.97	-6.28	-4.02	-2.06	-5.91	-2.76	-17.07
		s.e.	0.53	1.63	1.63	1.63	1.15	0.33	1.42	-	6.07
	Scootmore	Mean	-6.20	-6.23	-4.96	-9.87	-6.48	-5.39	-21.36	-9.14	-28.32
		s.e.	0.71	1.31	0.49	2.11	-	1.60	4.53	5.90	21.22

Table 3: Mean **light saturation estimate** (μmol Q mol^{-1} N s^{-1}) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	13255.57	9088.62	5156.77	1152.87	18144.25	8700.12	7278.61	6322.37	8037.06
		s.e.	1319.52	1936.41	897.38	269.68	6651.98	2049.73	2471.40	3022.21	2126.99
	Wauchope	Mean	1263.65	4428.56	3208.91	5283.08	3069.53	2652.14	5053.56	2406.19	4401.70
		s.e.	269.70	2238.87	801.47	1229.56	273.65	225.51	1279.04	-	629.13
	Scootmore	Mean	4212.92	3596.85	3509.56	5295.77	2580.15	4488.19	5942.46	3187.83	8142.41
		s.e.	513.79	437.43	643.27	805.04	241.39	978.51	1115.12	710.69	1119.16
C20208	Newcastleton	Mean	15155.63	10018.01	77.16.26	-	21899.53	22152.76	11655.86	19477.25	30565.68
		s.e.	7422.51	2062.43	969.24	-	2588.84	3880.09	2878.83	2286.82	12445.73
	Wauchope	Mean	7720.33	3438.61	2987.73	5244.47	3541.91	3958.19	4710.37	2131.41	8269.02
		s.e.	4784.20	587.04	610.54	708.18	1247.71	754.07	425.22	816.21	983.50
	Scootmore	Mean	3176.63	4681.50	7009.49	6732.86	3747.42	3372.03	8059.67	3809.77	8991.74
		s.e.	610.61	928.77	3336.56	929.22	363.91	375.52	1284.06	299.17	1340.97
C20211	Newcastleton	Mean	10182.95	4977.02	4027.28	2896.05	85.34.47	9095.26	5475.19	9224.95	12198.28
		s.e.	3034.07	1535.11	1487.86	1158.42	1307.35	2033.65	1653.13	3770.75	4194.24
	Wauchope	Mean	1985.02	2555.18	3558.32	3239.69	2014.74	2095.14	4131.28	2848.02	2793.27
		s.e.	479.58	326.22	938.69	1298.86	622.89	468.04	605.55	-	795.79
	Scootmore	Mean	4412.48	3856.25	4286.00	6645.07	2193.26	9414.54	6492.17	3331.84	10843.42
		s.e.	2052.18	842.20	732.51	1419.69	-	4434.40	2106.08	298.67	3726.62

Table 4: Mean **light compensation point** (μmol Q mol^{-1} N s^{-1}) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	1483.81	746.04	443.82	107.61	1233.1	334.31	901.10	936.20	1683.72
		s.e.	442.74	283.93	156.53	44.72	711.65	65.01	262.62	356.07	559.15
	Wauchope	Mean	155.93	180.73	285.10	343.50	209.68	158.35	557.50	172.94	817.32
		s.e.	70.80	82.04	104.70	7.67	106.43	91.35	332.41	-	327.93
	Scootmore	Mean	615.49	494.40	303.66	959.16	422.11	707.28	1233.75	647.80	2567.09
		s.e.	88.62	68.79	58.82	157.91	77.67	193.73	273.14	335.71	639.84
C20208	Newcastleton	Mean	2244.89	826.89	703.75	-	963.73	914.22	1051.20	985.27	4142.91
		s.e.	1434.46	180.30	137.39	-	250.84	215.10	126.32	320.12	1398.79
	Wauchope	Mean	632.45	200.53	334.31	691.74	247.91	233.64	291.16	170.21	1384.96
		s.e.	249.76	30.90	117.42	139.23	125.74	90.73	95.45	45.73	410.06
	Scootmore	Mean	446.53	636.75	2127.16	1517.23	576.33	442.20	1507.16	474.24	1693.08
		s.e.	79.53	182.75	1749.54	404.03	74.49	44.66	323.62	45.73	455.46
C20211	Newcastleton	Mean	1117.94	382.91	264.79	230.94	1403.71	588.88	938.19	931.16	2854.38
		s.e.	449.59	108.83	108.33	40.38	319.61	299.27	338.40	262.25	996.60
	Wauchope	Mean	163.95	137.47	177.75	188.03	197.72	82.82	278.12	117.40	423.43
		s.e.	37.62	19.15	61.62	55.61	75.48	19.01	55.75	-	140.10
	Scootmore	Mean	941.29	1006.09	856.26	1026.32	724.65	1260.15	1953.61	868.19	1423.97
		s.e.	350.03	474.98	278.73	318.35	-	338.91	399.57	248.64	1394.67

Table 5: Mean **quantum efficiency** (mol^{-1} N) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	0.61	1.37	1.54	0.07	1.39	0.42	0.43	1.36	0.47
		s.e.	0.05	0.91	0.92	0.01	0.89	0.11	0.18	0.73	0.17
	Wauchope	Mean	0.10	0.14	0.28	0.46	0.18	0.15	0.27	0.14	0.21
		s.e.	0.02	0.04	0.05	0.05	0.03	0.02	0.06	-	0.03
	Scootmore	Mean	0.09	0.12	0.18	0.22	0.19	0.19	0.15	0.14	0.16
		s.e.	0.01	0.02	0.01	0.03	0.04	0.05	0.01	0.01	0.01
C20208	Newcastleton	Mean	0.73	0.94	1.13	-	0.57	1.97	0.70	0.93	0.75
		s.e.	0.30	0.15	0.33	-	0.05	1.23	0.29	0.29	0.21
	Wauchope	Mean	0.34	0.15	0.26	0.35	0.08	0.11	0.33	0.12	0.29
		s.e.	0.26	0.02	0.06	0.03	0.01	0.02	0.03	0.01	0.11
	Scootmore	Mean	0.10	0.15	0.16	0.15	0.10	0.09	0.14	0.10	0.10
		s.e.	0.02	0.04	0.04	0.03	0.03	0.01	0.01	0.02	0.02
C20211	Newcastleton	Mean	0.46	0.76	0.94	0.17	1.08	0.75	0.32	0.43	0.48
		s.e.	0.13	0.41	0.56	0.03	0.55	0.24	0.11	0.22	0.15
	Wauchope	Mean	0.20	0.51	0.63	0.43	0.54	0.19	0.27	0.12	0.39
		s.e.	0.03	0.29	0.37	0.11	0.36	0.03	0.03	-	0.12
	Scootmore	Mean	0.07	0.08	0.08	0.14	0.09	0.03	0.14	0.06	0.09
		s.e.	0.01	0.02	0.03	0.03	-	0.01	0.03	0.03	0.07

Table 6: Mean **total non-structural carbohydrate concentrations** (mg g^{-1} d. wt) and s.e. for all needle ages (flush – 3yr), heights in the canopy (2 m – 4 m), sites (Newcastleton, Wauchope and Scootmore) and clones (C20177, C20208 and C20211).

			2m				4m			6m	
			3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush
C20177	Newcastleton	Mean	131.62	126.97	104.63	59.37	163.37	128.09	80.90	119.06	99.00
		s.e.	18.03	13.35	8.70	12.83	13.85	12.81	13.09	14.43	9.31
	Wauchope	Mean	133.05	164.30	135.90	109.96	103.74	151.97	156.73	106.88	164.27
		s.e.	11.98	25.82	24.30	12.46	25.12	5.07	28.13	-	23.39
	Scootmore	Mean	130.86	121.90	123.74	95.57	126.27	179.76	105.22	136.06	109.84
		s.e.	12.46	7.79	10.11	5.29	7.96	43.99	7.80	4.34	10.27
C20208	Newcastleton	Mean	92.43	123.90	99.16	-	140.23	126.80	68.03	151.48	99.44
		s.e.	14.38	4.11	15.09	-	12.15	8.69	4.60	12.90	12.17
	Wauchope	Mean	237.50	152.48	163.56	91.41	180.65	139.71	164.59	138.80	213.27
		s.e.	25.88	14.24	19.14	5.19	22.26	8.47	21.35	18.65	17.39
	Scootmore	Mean	161.26	176.84	147.45	89.69	135.11	139.36	161.18	139.30	98.16
		s.e.	6.98	42.07	6.39	7.97	10.72	11.10	32.64	10.57	8.29
C20211	Newcastleton	Mean	131.92	132.08	141.42	100.09	153.19	140.12	106.47	145.20	83.82
		s.e.	7.67	8.56	26.54	22.13	14.09	9.45	20.52	11.21	12.03
	Wauchope	Mean	203.83	217.08	144.19	142.79	194.90	153.10	139.56	242.41	170.84
		s.e.	21.95	21.32	20.66	17.42	25.33	14.13	12.29	-	23.50
	Scootmore	Mean	135.99	139.02	132.36	93.15	147.18	158.97	96.08	134.91	115.35
		s.e.	10.84	5.50	10.80	5.56	#DIV/0!	4.74	8.04	14.59	13.85

Appendix 5

Results highlighted in grey are significant at the 0.05 probability level and results in bold and highlighted in grey are significant at the 0.01 probability level.

Table 1: *F* statistic and *P* value from the two-way ANOVA, investigating differences of A_{max} (μmol CO_2 mol^{-1} N s^{-1}) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	**9.749**	**11.208**	**8.519**	**12.388**	4.385	12.496	2.852	5.438	4.865	2
	P	**0.001**	**<0.001**	**0.001**	**<0.001**	0.023	<0.001	0.070	0.015	0.014	
Site	*F*	0.849	0.562	1.035	0.828	0.310	0.627	1.436	0.332	1.899	2
	P	0.438	0.574	0.365	0.450	0.736	0.540	0.251	0.722	0.165	
Clone × Site	*F*	0.809	0.377	0.467	0.947	0.238	1.515	0.709	0.545	1.452	4
	P	0.529	0.824	0.760	0.435	0.914	0.220	0.591	0.705	0.238	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 2: *F* statistic and *P* value from the two-way ANOVA, investigating differences of A_{max} (μmol mol^{-1} N s^{-1}) between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	1.514	2.156	1.970	1.488	0.192	0.555	0.710	0.000	0.413	2
	P	0.232	0.132	0.151	0.252	0.826	0.582	0.498	>0.999	0.666	
Needle Age	*F*	1.798	0.112	0.289	**14.553**	3.905	4.246	3.742	4.207	**10.694**	3
	P	0.162	0.952	0.833	**<0.001**	0.022	0.016	0.019	0.012	**<0.001**	
Height × Age	*F*	0.611	0.559	0.539	1.288	0.341	0.138	0.981	2.028	0.251	3
	P	0.657	0.577	0.658	0.309	0.796	0.936	0.413	0.127	0.860	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 3: *F* statistic and *P* value from the two-way ANOVA, investigating differences of R_{day} (μmol CO_2 mol^{-1} N s^{-1}) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Site	*F*	**7.618**	1.849	1.352	**6.648**	1.853	2.717	**5.199**	0.934	1.800	2
	P	**0.002**	0.171	0.270	**0.006**	0.178	0.080	**0.010**	0.412	0.181	
Clone	*F*	0.576	0.420	0.391	1.525	0.295	0.083	0.011	0.359	0.186	2
	P	0.568	0.660	0.679	0.240	0.747	0.920	0.989	0.704	0.831	
Clone × Site	*F*	0.475	0.420	0.448	0.936	0.235	1.653	0.108	0.279	0.165	4
	P	0.754	0.793	0.773	0.440	0.916	0.184	0.979	0.887	0.955	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 4: *F* statistic and *P* value from the two-way ANOVA, investigating differences of R_{day} (μmol CO_2 mol^{-1} N s^{-1}) between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	1.655	**4.543**	**5.877**	0.661	**3.884**	1.490	**6.692**	0.528	2.519	2
	P	0.203	**0.018**	**0.005**	0.529	**0.036**	0.247	**0.003**	0.594	0.101	
Needle Age	*F*	0.179	**5.131**	2.195	1.510	**6.048**	2.592	**5.805**	**7.122**	**4.618**	3
	P	0.910	**0.005**	0.101	0.246	**0.004**	0.078	**0.002**	**0.001**	**0.011**	
Height × Age	*F*	0.141	**3.487**	2.505	0.545	**3.099**	1.425	1.979	0.980	1.292	3
	P	0.966	**0.043**	0.071	0.658	**0.048**	0.262	0.135	0.413	0.299	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 5: *F* statistic and *P* value from the two-way ANOVA, investigating differences of light saturation estimate (μmol *Q* mol^{-1} N s^{-1}) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Site	*F*	**7.453**	**8.935**	1.581	4.821	**11.065**	**19.658**	2.510	**6.691**	**4.424**	2
	P	**0.002**	**0.001**	0.218	0.018	**<0.001**	**<0.001**	0.095	**0.007**	**0.020**	
Clone	*F*	0.663	2.006	1.433	0.308	1.001	**3.422**	1.754	1.139	2.657	2
	P	0.522	0.148	0.250	0.738	0.382	**0.044**	0.187	0.344	0.085	
Clone × Site	*F*	0.429	1.013	0.581	0.678	0.778	**5.603**	0.759	1.527	1.697	4
	P	0.786	0.412	0.678	0.575	0.550	**0.001**	0.558	0.239	0.173	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 6: *F* statistic and *P* value from the two-way ANOVA, investigating differences of light saturation estimate (μmol *Q* mol^{-1} N s^{-1}) between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	2.695	3.972	3.695	0.293	0.213	0.319	0.492	0.362	0.240	2
	P	0.079	0.029	0.033	0.750	0.810	0.730	0.615	0.699	0.788	
Needle Age	*F*	2.452	0.464	1.430	2.147	3.284	1.565	5.935	3.573	1.877	3
	P	0.076	0.709	0.246	0.130	0.040	0.226	0.002	0.023	0.159	
Height × Age	*F*	0.558	1.895	0.580	0.061	0.923	1.024	1.597	1.244	1.978	3
	P	0.694	0.167	0.631	0.980	0.446	0.401	0.207	0.308	0.143	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 7: *F* statistic and *P* value from the two-way ANOVA, investigating differences of light compensation point (μmol *Q* mol^{-1} N s^{-1}) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Site	*F*	3.507	3.787	1.232	5.304	3.642	6.337	11.067	2.598	4.482	2
	P	0.042	0.031	0.303	0.013	0.041	0.005	≤0.001	0.104	0.019	
Clone	*F*	0.317	0.080	0.937	1.186	0.087	0.943	0.221	0.041	0.798	2
	P	0.731	0.923	0.400	0.324	0.917	0.399	0.802	0.960	0.458	
Clone × Site	*F*	0.478	1.212	0.541	0.052	0.116	2.273	0.775	0.118	1.227	4
	P	0.752	0.321	0.706	0.984	0.976	0.082	0.548	0.974	0.318	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 8: *F* statistic and *P* value from the two-way ANOVA, investigating differences of light compensation point (μmol *Q* mol^{-1} N s^{-1}) between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	1.718	2.261	6.227	0.211	2.718	0.618	4.605	0.592	0.311	2
	P	0.191	0.121	0.004	0.812	0.088	0.548	0.017	0.559	0.735	
Needle Age	*F*	2.114	2.520	2.475	1.099	3.893	2.082	7.169	1.218	0.631	3
	P	0.112	0.075	0.073	0.375	0.023	0.132	0.001	0.317	0.602	
Height × Age	*F*	0.443	2.241	1.682	0.381	2.979	1.168	2.142	0.760	0.411	3
	P	0.777	0.123	0.184	0.768	0.054	0.344	0.112	0.524	0.747	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 9: *F* statistic and *P* value from the two-way ANOVA, investigating differences of quantum efficiency (mol^{-1} N) between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Site	*F*	**11.604**	**3.836**	**4.251**	**25.028**	1.873	**4.268**	**6.065**	2.473	**7.101**	2
	P	**<0.001**	**0.030**	**0.021**	**<0.001**	0.175	**0.022**	**0.005**	0.114	**0.003**	
Clone	*F*	0.903	0.064	0.073	1.067	0.282	0.903	0.979	0.195	0.352	2
	P	0.416	0.938	0.930	0.361	0.757	0.415	0.385	0.825	0.706	
Clone × Site	*F*	0.352	0.322	0.251	1.412	0.168	1.284	0.698	0.225	0.665	4
	P	0.840	0.861	0.907	0.266	0.952	0.295	0.598	0.921	0.621	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 10: *F* statistic and *P* value from the two-way ANOVA, investigating differences of quantum efficiency (mol^{-1} N) between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	0.518	0.298	0.102	**4.909**	0.785	0.684	0.695	2.732	0.402	2
	P	0.600	0.745	0.903	**0.020**	0.469	0.515	0.506	0.078	0.673	
Needle Age	*F*	1.481	1.030	1.042	**8.565**	2.880	0.848	3.117	1.130	2.474	3
	P	0.233	0.392	0.383	**0.001**	0.059	0.483	0.038	0.349	0.085	
Height × Age	*F*	0.435	1.092	0.550	2.047	0.150	0.558	2.430	0.404	0.392	3
	P	0.782	0.348	0.651	0.143	0.929	0.648	0.081	0.751	0.759	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 11: *F* statistic and *P* value from the two-way ANOVA, investigating differences of total carbohydrate concentrations between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	3.091	2.100	0.676	3.209	2.206	0.485	0.585	3.028	0.611	2
	P	0.060	0.136	0.515	0.060	0.131	0.620	0.562	0.075	0.548	
Site	*F*	**17.698**	**5.777**	2.402	**4.433**	0.593	1.610	**7.492**	1.626	**19.656**	2
	P	**<0.001**	**0.006**	0.103	**0.024**	0.560	0.215	**0.002**	0.226	**<0.001**	
Clone × Site	*F*	**5.012**	1.559	0.919	1.669	1.181	0.289	2.226	3.355	1.613	4
	P	**0.003**	0.204	0.462	0.203	0.343	0.883	0.084	0.034	0.193	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 12: *F* statistic and *P* value from the two-way ANOVA, investigating differences of total carbohydrate concentrations between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	0.713	**7.763**	0.600	0.007	3.072	2.690	1.073	0.347	1.102	2
	P	0.496	**0.002**	0.553	0.993	0.067	0.090	0.353	0.709	0.348	
Needle Age	*F*	0.546	**12.712**	1.268	0.103	**7.214**	**5.878**	2.246	1.327	**9.549**	3
	P	0.654	**<0.001**	0.297	0.957	**0.002**	**0.004**	0.100	0.280	**<0.001**	
Height × Age	*F*	1.108	0.143	0.848	1.865	**4.093**	1.267	0.870	2.509	1.080	3
	P	0.365	0.868	0.475	0.172	**0.019**	0.310	0.465	0.074	0.375	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 13: *F* statistic and *P* value from the two-way ANOVA, investigating differences of ethanol soluble carbohydrate concentrations between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	3.091	1.761	0.897	**4.026**	0.728	1.352	1.097	0.564	2.563	2
	P	0.060	0.185	0.416	**0.032**	0.493	0.272	0.344	0.579	0.092	
Site	*F*	**14.769**	**5.309**	**6.203**	**5.406**	2.372	**3.626**	**7.034**	**4.122**	**21.280**	2
	P	**<0.001**	**0.009**	**0.005**	**0.012**	0.114	**0.037**	**0.003**	**0.035**	**<0.001**	
Clone × Site	*F*	**5.615**	1.410	0.6006	0.565	0.984	0.212	1.413	**3.055**	2.127	4
	P	**0.002**	0.248	0.661	0.644	0.434	0.930	0.248	**0.046**	0.099	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 14: *F* statistic and *P* value from the two-way ANOVA, investigating differences of ethanol soluble carbohydrate concentrations between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	0.405	2.351	1.269	0.311	2.191	2.509	0.574	0.373	1.284	2
	P	0.670	0.111	0.294	0.737	0.136	0.104	0.568	0.691	0.295	
Needle Age	*F*	1.408	1.436	0.309	0.597	**5.806**	4.790	1.812	0.200	**4.253**	3
	P	0.254	0.250	0.819	0.625	**0.004**	**0.010**	0.162	0.896	**0.015**	
Height × Age	*F*	0.812	0.167	0.484	1.003	**3.265**	0.822	1.118	2.349	0.185	3
	P	0.525	0.847	0.695	0.414	**0.041**	0.496	0.355	0.088	0.906	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 15: *F* statistic and *P* value from the two-way ANOVA, investigating differences of water soluble carbohydrate concentrations between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	1.928	1.975	0.138	1.453	0.410	1.370	1.325	1.858	5.069	2
	P	0.162	0.152	0.872	0.255	0.668	0.268	0.278	0.186	0.012	
Site	*F*	14.341	8.538	16.251	1.255	12.506	7.274	23.300	20.591	48.089	2
	P	<0.001	0.001	<0.001	0.305	<0.001	0.002	<0.001	<0.001	<0.001	
Clone × Site	*F*	0.262	0.942	0.513	1.248	0.140	0.071	0.355	2.536	2.373	4
	P	0.900	0.450	0.726	0.317	0.966	0.991	0.839	0.078	0.072	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 16: *F* statistic and *P* value from the two-way ANOVA, investigating differences of water soluble carbohydrate concentrations between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	0.949	0.247	0.732	0.902	0.253	0.565	1.860	1.501	0.868	2
	P	0.395	0.783	0.486	0.423	0.779	0.577	0.170	0.236	0.432	
Needle Age	*F*	2.407	4.280	2.415	2.272	2.605	0.069	6.460	13.040	9.250	3
	P	0.080	0.012	0.079	0.115	0.077	0.976	0.001	<0.001	<0.001	
Height × Age	*F*	1.078	1.293	0.265	0.590	1.006	1.160	0.824	1.336	0.107	3
	P	0.379	0.289	0.851	0.630	0.409	0.347	0.489	0.277	0.955	
Residual DF		43	32	46	18	22	22	36	37	25	

Table 17: *F* statistic and *P* value from the two-way ANOVA, investigating differences of starch concentrations between clone, site and clone by site interaction.

		2m				4m			6m		
		3yr	2yr	1yr	Flush	2yr	1yr	Flush	1yr	Flush	Between DF
Clone	*F*	3.278	4.020	0.576	1.947	3.633	0.514	0.781	1.775	1.427	2
	P	0.051	0.026	0.567	0.167	0.041	0.603	0.465	0.254	0.199	
Site	*F*	2.617	1.239	2.342	7.971	9.261	3.025	2.357	2.820	1.066	2
	P	0.089	0.300	0.109	0.002	0.001	0.062	0.108	0.088	0.356	
Clone × Site	*F*	0.882	0.942	1.196	0.380	0.693	0.469	5.537	1.137	0.960	4
	P	0.486	0.450	0.327	0.769	0.604	0.758	0.001	0.373	0.442	
Residual DF		31	40	40	22	25	34	38	17	34	

Table 18: *F* statistic and *P* value from the two-way ANOVA, investigating differences of starch concentrations between canopy height, needle and height by age interaction.

		Newcastleton			Wauchope			Scootmore			
		C20177	C20208	C20211	C20177	C20208	C20211	C20177	C20208	C20211	Between DF
Canopy Height	*F*	0.291	**5.666**	0.211	1.319	0.580	1.157	0.856	0.322	0.073	2
	P	0.749	**0.008**	0.810	0.292	0.568	0.333	0.433	0.727	0.929	
Needle Age	*F*	1.303	**12.691**	2.601	0.124	0.548	**5.332**	0.573	**4.103**	**4.597**	3
	P	0.286	**<0.001**	0.063	0.945	0.654	**0.006**	0.637	**0.013**	**0.011**	
Height × Age	*F*	1.195	0.507	**4.668**	1.560	0.804	1.190	1.321	0.680	1.577	3
	P	0.327	0.607	**0.006**	0.234	0.505	0.336	0.283	0.570	0.220	
Residual DF		43	32	46	18	22	22	36	37	25	

Appendix 6

Table 1: Wood density (trunk), fine root density at 0.1 m, 0.5 m and 1.0 m from root collar, coarse root density at 0.1 m, 0.5 m and 1.0 m from root collar, and total fine and coarse root density, for each clone at each site.

Site	Clone	Wood Density (kg m^{-3})	Fine Root Density (mg roots cm^{-3} soil)				Coarse Root Density (mg roots cm^{-3} soil)			
			0.1 m	0.5 m	1.0 m	Total	0.1 m	0.5 m	1.0 m	Total
Newcastleton	C20177	292.3 ±27.8	0.86 ±0.15	0.62 ±0.21	0.52 ±0.08	2.00	3.67 ±3.20	1.70 ±0.54	0.86 ±0.39	6.23
	C20208	372.5 ±12.9	0.82 ±0.29	0.74 ±0.31	0.98 ±0.24	2.54	3.86 ±2.46	3.03 ±0.96	0.69 ±0.43	7.58
	C20211	375.6 ±26.4	0.45 ±0.26	0.81 ±0.30	0.87 ±0.19	2.13	2.28 ±1.63	0.72 ±0.21	0.96 ±0.15	3.96
Wauchope	C20177	315.6 ±12.0	0.51 ±0.30	0.41 ±0.01	0.40 ±0.11	1.32	4.28 ±2.36	0.68 ±0.53	0.37 ±0.13	5.32
	C20208	390.5 ±10.4	0.59 ±0.10	0.58 ±0.12	0.61 ±0.13	1.78	0.79 ±0.38	0.13 ±0.07	0.68 ±0.46	1.59
	C20211	383.8 ±10.7	0.70 ±0.05	0.56 ±0.06	0.50 ±0.04	1.76	2.36 ±2.03	0.54 ±0.22	0.24 ±0.09	3.13
Scootmore	C20177	348.2 ±16.9	1.64 ±0.34	1.29 ±0.40	0.60 ±0.08	3.52	1.01 ±6.42	0.78 ±0.42	0.41 ±0.28	11.20
	C20208	445.3 ±12.7	1.14 ±0.31	2.19 ±0.57	1.58 ±0.42	4.90	0.62 ±0.43	3.25 ±0.16	11.30 ±10.88	15.17
	C20211	392.7 ±37.1	0.94 ±0.19	2.45 ±1.20	2.64 ±1.12	6.02	0.96 ±0.45	1.95 ±1.07	1.09 ±0.57	4.01

Appendix 7

Table 1: Results of Kruskal-Wallis test, investigating the effects of site on wood density (kg m^{-3}) for each clone.

Clone	χ^2	d.f.	*P*
C20177	5.856	2	0.053
C20208	12.362	2	0.002
C20211	2.243	2	0.326

Table 2: Results of Kruskal-Wallis test, investigating the effects of clone on wood density (kg m^{-3}) at each site.

Site	χ^2	d.f.	*P*
Newcastleton	7.691	2	0.021
Wauchope	14.689	2	0.001
Scootmore	12.551	2	0.002

Table 3: Results of Kruskal-Wallis test, investigating the effects of site on fine root density (mg roots cm^{-3} soil) at 0.1 m, 0.5 m and 1.0 m from the root collar for each clone.

	0.1 m			0.5 m			1.0 m		
Clone	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
C20177	2.756	2	0.252	4.622	2	0.099	2.222	2	0.329
C20208	2.222	2	0.329	4.356	2	0.113	4.356	2	0.113
C20211	1.867	2	0.393	3.467	2	0.177	6.489	2	0.039

Table 4: Results of Kruskal-Wallis test, investigating the effects of clone on fine root density (mg roots cm^{-3} soil) at 0.1 m, 0.5 m and 1.0 m from the root collar at each site.

	0.1 m			0.5 m			1.0 m		
Site	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Newcastleton	2.489	2	0.288	0.356	2	0.837	5.067	2	0.079
Wauchope	0.800	2	0.670	3.384	2	0.184	1.156	2	0.561
Scootmore	2.409	2	0.288	1.156	2	0.561	4.356	2	0.113

Table 5: Results of Kruskal-Wallis test, investigating the effects of site on coarse root density (mg roots cm^{-3} soil) at 0.1 m, 0.5 m and 1.0 m from the root collar for each clone.

	0.1 m			0.5 m			1.0 m		
Clone	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
C20177	1.067	2	0.587	1.689	2	0.430	1.156	2	0.561
C20208	3.384	2	0.184	5.600	2	0.061	0.202	2	0.904
C20211	0.022	2	0.989	0.622	2	0.733	4.267	2	0.118

Table 6: Results of Kruskal-Wallis test, investigating the effects of clone on coarse root density (mg roots cm^{-3} soil) at 0.1 m, 0.5 m and 1.0 m from the root collar at each site.

	0.1 m			0.5 m			1.0 m		
Site	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Newcastleton	1.156	2	0.561	5.067	2	0.079	0.267	2	0.875
Wauchope	1.067	2	0.578	1.412	2	0.494	0.605	2	0.739
Scootmore	2.756	2	0.252	3.200	2	0.202	0.800	2	0.670

Table 7: Results of Kruskal-Wallis test, investigating the effects of site on carbon allocation to foliage, branch and trunk, and total above-ground carbon allocation for each clone.

	Foliage			Branch			Trunk			Total		
Clone	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
C20177	0.857	2	0.651	2.571	2	0.276	4.751	2	0.102	4.571	2	0.102
C20208	1.143	2	0.565	1.838	2	0.399	3.714	2	0.156	3.714	2	0.156
C20211	4.571	2	0.102	3.714	2	0.156	3.714	2	0.156	3.429	2	0.180

Table 8: Results of Kruskal-Wallis test, investigating the effects of clone on carbon allocation to foliage, branch and trunk, and total above-ground carbon allocation at each site.

	Foliage			Branch			Trunk			Total		
Site	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Newcastleton	3.429	2	0.180	3.714	2	0.156	3.714	2	0.156	3.714	2	0.156
Wauchope	0.000	2	1.000	2.000	2	0.368	1.143	2	0.565	2.000	2	0.368
Scootmore	1.143	2	0.565	3.714	2	0.156	3.429	2	0.180	3.429	2	0.180