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The physiological and genetic basis of productivity and water use in Poplar in current and future climates

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ABSTRACT

FACULTY OF SCIENCE
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THE PHYSIOLOGICAL AND GENETIC BASIS OF PRODUCTIVITY AND WATER USE IN POPLAR IN CURRENT AND FUTURE CLIMATES.

by Stephen Matthew Bunn

Poplar (*Populus spp.*) is a fast growing tree genus, widely distributed across the Northern hemisphere. It is a valuable economic crop used traditionally for papermaking and wood veneer and more recently has aroused new interest as a potential renewable energy resource in the form of biomass for wood fuelled power stations.

Renewable energy sources are likely to become increasingly important as moves are made to reduce CO_2 emissions and conserve dwindling fossil fuel reserves. Consequently it is important to focus research on biomass crops in order to optimise their potential for the future. Information regarding optimum yields, water requirements and how these species will perform under predicted global climate change is required.

This thesis presents data from field and glasshouse experiments to determine the physiological traits associated with high yield. Data is also presented from CO_2 enrichment experiments carried out in open top chambers and FACE facilities to investigate root growth and water use including preliminary data which may be useful in eventually identifying the genetic control of physiological traits in poplar.

A field trial at two contrasting sites in the UK using five poplar genotypes with contrasting physiological traits showed that large leaf size and rapid leaf expansion were good indicators of high yield while rate of leaf production was not. A glass house study using rhizotrons to observe root growth showed high yielding genotypes to have correspondingly high rates of root elongation and density.

Root measurements made on an F2 population of poplar grown under elevated CO_2 in open top chambers provided data that will form the basis of future QTL mapping projects in collaboration with research groups in the USA.

Data presented from a study of sap flow and water use in poplars grown in a Free Air CO_2 Enrichment facility in Italy showed that elevated CO_2 effects on water use in poplar are likely to vary throughout the growing season. Water use was increased at the start of the growing season under elevated CO_2, but was reduced towards the end of the growing season compared to plants in ambient conditions.

The implications of these findings are discussed in relation to the economic viability of poplar as a biomass crop for renewable energy and the possible consequences of global climate change for the future of poplar cultivation are explored.

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Definitions and Abbreviations

A	Net photosynthetic CO_2 assimilation
AC	Alternating current
A/ci curve	the resulting curve from a plot of photosythetic assimilation (A) against intercellular CO2 concentration (ci)
ANOVA	Analysis of variance
biomass	The total quantity of matter in an organism
C	Carbon
CE	Controlled Environment
ci	Intercellular carbon dioxide concentration
cm	centimeter (0.01 meters)
CO_2	Carbon Dioxide
Cp	Specific heat capacity of water (4.186J/g/K)
d	Day
DAP	Days after planting
DC	Direct current
DETR	Department for the Environment, Transport and the Regions
dT	delta T - temperature differential
DTI	Department of Trade and Industry
E	Transpiration
eCO_2	Elevated carbon dioxide
et al.	and others
ETSU	Energy Technology Support Unit
EU	European Union
F1	Progeny resulting directly from the breeding cross of two parent genotypes
F2	Progeny resulting from a second breeding cross using F1 genotypes as parents
FACE	Free Air CO_2 Enrichment
g	gram (0.001 kg)
GH	Glasshouse
Greenhouse effect	Effect in which short wavelength solar radiation entering the earths atmosphere is re-irradiated from the earths surface in the longer infra-red wavelengths and is then re-absorbed by components of the atmosphere to become an important factor in heating the total atmosphere.
g_s	Stomatal conductance
ha	Hectare (10,000 square meters)
hr	hour
IPAR	Intercepted photosynthetically active radiation
IPCC	Intergovernmental Panel on Climate Change
IRGA	Infrared gas analyser
J max	The maximum rate of regeneration of RuBP from Fructose 6-phosphate
K	Kelvin - SI unit of temperature
KAP	Kite Ariel Photography
kg	kilogram (SI unit of mass)
Ksh	Sheath conductance of the heater in a sap flow gauge
L	Litre - measure of volume equal to 0.001 cubic meters
LAI	Leaf area index
LER	Leaf expansion rate
LOD	Measure of the likelihood that a QTL exsists at a point on a chromosome - defined as the log of the ratio of the likelihood of there being one vurses no QTL at a particular point.
m	meter (SI unit of length)
MAFF	Ministry of Agriculture Fisheries and Food

MAS	Marker assisted selection
min	minute (60 seconds)
MJ	megajoule (1 million joules)
ml	millilitre (0.001 Litres)
mm	millimeter (0.001meters)
µm	micrometer (0.000001 meters)
µmol	micromole (0.000001 moles)
mol	mole - the SI unit of the amount of a substance (as distinct from its mass or weight). A measure of the actual number of atoms or molecules in an object.
NOx	Nitrogen Oxides
NPP	Net primary production
°C	Degrees Celcius
OTC	Open top chamber
P	P - value. Statistical value indicating the likelihood of observing the value obtained for the test statistic if the null hypothesis is true.
P.	*Populus*
PAR	Photosynthetically active radiation
PAR	Photosythetially active radiation
POPFACE	Poplar Free Air CO_2 Enrichment
ppm	parts per million
qf	Heat lost from a sap flow gauge due to the cooling effect of sap flow
qr	Radial heat loss from a sap flow gauge by conduction across the gauge
QTL	Quantitative trait loci
qv	Vertical heat loss from a sap flow gauge by conduction through the stem
RAPD	Random amplified polymorphic DNA (pronounced 'rapid') - A technique using single, short synthetic primers for PCR. The primer, whose sequence has been chosen at random, initiates replication at its complementary sites on the DNA, producing fragments up to about 2,000 bases long, which can be separated by electrophoresis and stained with ethidium bromide. A primer can exhibit polymorphism between individuals, and polymorphic fragments can be used as markers.
r_b	boundary layer resistance
RER	Root elongation rate
RFLP	Restriction fragment length polymorphism - genetic variation at the site where a restriction enzyme cuts a piece of DNA. Such variations affect the size of the resulting fragments. These sequences are used as markers on physical maps and linkage maps.
Rhizotron	Clear sided container allowing observation of roots below soil level
RUBISCO	Ribulose 1,5-bisphosphate carboxylase - the enzyme that catalyses the initial reaction of the calvin cycle, involving the fixation of carbon dioxide to ribulose 1,5-bisphosphate.
s	seconds (SI unit of time)
SD	Standard deviation
SOx	Sulphur Oxides
spp.	Species (plural)
SRC	Short rotation Coppice
SRIC	Short rotation intensive culture
St_L	Stomatal limitation
STS	Sequence tagged sites - defined as short DNA segments that occur only once in a genome and whose exact location and order of bases are known. Because each is unique, STS's are helpful for chromosome placement of mapping and sequencing data from many different laboratories. STS's serve as markers on a genetic map.
UK	United Kingdom
USA	United States of America
V	volt - (SI unit of electric potential)

Vc max	The maximum rate of carboylation of RuBP
W	Watt - SI unit of power
WUE	Water use efficiency
x	represents a breeding cross made between two species
<	less than
>	greater than

Chapter 0

General Introduction and Literature Review.

Section 0.1 - General Introduction:

This section is intended to set the scene for the work reported in this thesis showing its relevance to current concerns and issues regarding our environment and exploring how a greater understanding of the *Populus* species may help to address some of these concerns in the future. Section 0.2 reviews research into poplar productivity and introduces the work detailed in chapter 1, while section 0.3 reviews how poplars may be affected by future climate change and introduces the experiments detailed in chapters 2 & 3.

Renewable Energy from Biomass:

Advantages of renewable energy from biomass:

Demands on global energy resources have continued to grow throughout the twentieth century, and look set to increase still further in the future (El Bassam, 1998). However, the preservation and protection of our environment has become one of the most important priorities of the last few decades, during which time energy production has been identified as a major factor contributing to global climate change (IPCC, 2001). Furthermore, it is predicted that global reserves of fossil fuels will be unable to indefinitely sustain current levels of exploitation (Hansen, 1991). Important precedents were set by the Kyoto Protocol (1997), introducing global targets for limiting and reducing emissions of CO_2. However, these plans seem to have suffered a recent setback with America, (under the presidency of George W. Bush), refusing to honour its commitments as laid down

in the Kyoto Agreement. One of the reasons that America is reluctant to cut its CO_2 emissions is its enormous economic dependency on affordable energy. In fact due to the inability and reluctance of the global society to drastically reduce its energy consumption there has been considerable interest and research into potential sources of renewable energy that will enable us to keep up with increasing energy demands, whilst substantially reducing emissions of green house gases.

Currently most of our energy comes from fossil fuels such as oil and gas. This is technically biomass that has been compressed and concentrated over millions of years, but problems arise when the carbon released as a result of fossil fuel combustion exceeds the rate at which carbon is fixed by living plants. The resulting increase in atmospheric carbon (mainly in the form of CO_2) adds to the so-called green house effect - the driving force behind global climate change.

Approximately 200 billion tonnes of carbon are fixed each year by photosynthesis, (approximately ten times the energy equivalent used each year in the world). However, biomass currently represents under 15% of global primary energy consumption, and much of this is consumed in the developing world with much less efficiency than is technically possible or economically feasible (Hall & House, 1995). However, biomass has much greater potential for use in the developed world, where modern farming practices and technology are available to fully exploit energy locked up in biomass as a result of photosynthesis.

The advantages of energy from biomass over energy from fossil fuels are twofold. Firstly, biomass energy (provided it is properly managed) can be considered to be a

renewable resource - new plants can be grown as the previous crop is being converted to energy. Secondly, energy production from biomass can be considered as a closed system (figure 1) (El Bassam, 1998). Although combustion of biomass fuel results in a release of carbon into the atmosphere, the same quantity of carbon will be fixed by the replacement crop. Therefore energy from biomass can be considered to be 'carbon neutral'.

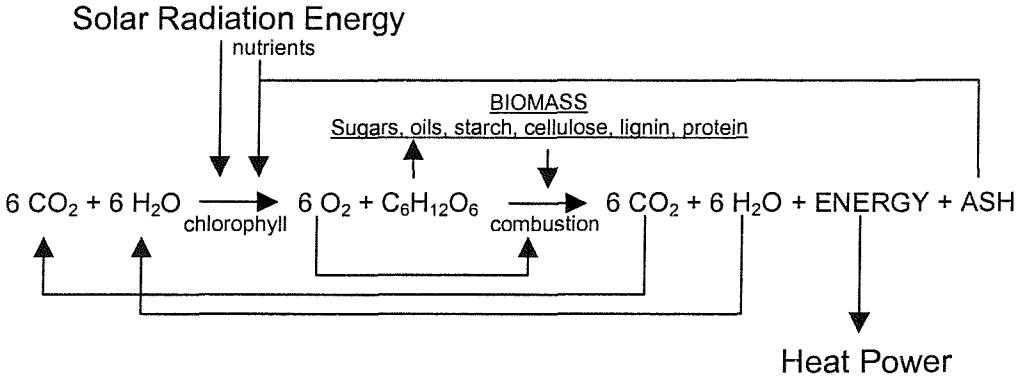


Figure 0.1: The almost closed cycle of energy production from biomass. Reproduced from [El Bassam, 1998]

Further impetus for research into biomass crops has come from UK government and EU commitments to reduce CO_2 emissions as part of the Kyoto agreement. Reductions of 20% in the UK, and 6% across Europe need to be achieved on 1990 emission levels. The UK government have also made a commitment that 10% of the countries energy supply will come from renewable sources by the year 2010, and biomass crops could make up to half of this total.

Biomass crops:

71 species of plant have been identified as potential biomass crops (El Bassam, 1998). They include plant species that can be cultivated to produce solid, liquid or gaseous fuel. Crops with high starch content such as maize and potato can be used to produce ethanol. Oil crops such as rape and sunflower have the potential to produce biodiesel, and all plants containing lignocellulose (i.e. woody crops) can provide energy directly as solid fuels, or indirectly after conversion to gas or liquid fuel. However, many of the plant species with sufficiently high yield potentials to become successful biomass crops utilise the C_4 photosynthetic pathway, which is particularly suited to warm climates. Many of these C_4 crops are not suitable for growth in the temperate climate of the UK and Northern Europe.

Poplar (*Populus spp.*) and willow (*Salix spp.*) are the most advanced biomass crops in the UK (Armstrong, 1997). They are both fast growing tree species, which have the ability to coppice. Plantations of poplar and willow coppice can be harvested every two to five years. The wood is cut in the winter to a few inches above ground level, leaving a stump (or stool) from which new shoots appear in the spring. This management practice is known as short rotation coppice (SRC) and it enables two or three harvests to be obtained from the same rootstock, without the need for replanting. Another feature, which makes poplar and willow particularly suitable for large-scale cultivation, is the ease with which they can be clonally propagated. Cuttings (typically 25 cm long) are taken from the harvested wood and inserted into the ground, leaving only 2.5 to 3 cm protruding. Dormant buds along the stem sprout into new shoots and roots develop from previously undifferentiated cells in the cambium. First year growing shoots are cut back to

ground level, and can be used to establish new plantations. Cutting back after the first year of growth induces the generation of several new shoots from a single stool resulting in a multi-stemmed crop which facilitates agricultural harvesting methods.

Coppiced stools cannot support maximum productivity indefinitely, but need to be grubbed up and re-planted after two or three rotations. The optimum number of years that an established coppice is grown before harvesting, and the optimum number of harvests from a single rootstock will depend on the spacing of the crop and the environment in which it is grown. These factors are currently being investigated in the UK by the Energy Technology Support Unit (ETSU) and the Forestry Commission (Armstrong, 1997).

The Future of Biomass:

In order that poplar and willow can be grown commercially for energy production in the UK, they must be able to compete economically with traditional fossil fuels and other forms of renewable energy such as wind and solar power. Productivity is a fundamental factor that will determine the economic potential of these crops (Hall & House, 1995; El Bassam, 1998), and this in turn is determined by the combined influences of genotype and environment (Ceulemans, 1989; Ceulemans & Deraedt, 1999).

If the government's commitments to renewable energy from biomass are to be achieved and built upon in the future, we require a detailed understanding of

suitable biomass crops (Hall & House, 1995). Physiological traits that improve the suitability of these plants as biomass crops need to be identified; for example traits that aid mechanised harvesting, or improve productivity (Hansen, 1991). Once the factors that influence this suitability are known, they can be combined and selected in future breeding programs (Walsh *et al.*, 1996).

The future of biomass energy in the UK has already taken an important first step. Significant investment has been made into the establishment of an 8MW biomass fuelled power station at Eggborough, North Yorkshire, enabling farmers to invest in biomass crops in the knowledge that there will be a customer for their product (Pitcher *et al.*, 1998).

Why Poplar?

Poplars have been chosen as the subject of this Ph.D. thesis for a number of reasons: Firstly, renewed interest into biomass for renewable energy has raised the profile of poplar research, resulting in a number of research groups undertaking complementary work in this area. Secondly, poplars are a large and diverse group of plants. There are over 30 species native to Europe, Asia and North America, many of which have been inter-bred, resulting in an even larger number of hybrid genotypes (Ceulemans, 1990). Furthermore, poplars are among the fastest growing trees at temperate latitudes and produce wood that is used in a variety of industry, as well as a basis for energy production. These uses include the manufacture of furniture, packaging material and boxes; as a veneer for plywood, matches, baskets, fibre- and particle board, and as a raw material for paper making. Recently,

poplars have also been used in experiments to determine their suitability for 'bio-remediation' of contaminated land (Jordahl *et al.*, 1997; Gordon *et al.*, 1998).

Section 0.2 - Productivity of Poplar:

Poplar breeding for increased productivity:

Previous studies have shown that the progeny of some interspecific crosses exhibit heterosis (or hybrid vigour) (Hinckley *et al.*, 1989). Consequently much interest has been focused on producing new hybrid genotypes with increased productivity. Major breeding programs for Populus are well established both in Belgium at the Game and Wildlife Institute, and in the USA at the University of Washington, Seattle. There are also smaller programs underway in France, and Italy.

Poplars are ideal subjects for tree breeding due to their relatively early sexual maturity (flowering after just 4 to 6 years), the ease with which they hybridise, their fast growth rates and the way in which stocks of new hybrids can be rapidly generated by vegetative propagation. Most active breeding programs have concentrated on three species of poplar, P. deltoides, P. nigra, and P. trichocarpa. Of these, TxD crosses have been seen to be the most productive in Europe and the UK (Ceulemans *et al.*, 1996; Souch & Stephens, 1998; Tabbush & Beaton, 1998), although susceptibility to rust limits the useful life of any individual genotype at present (Lonsdale & Tabbush, 1998).

Examples of increased productivity in hybrid poplar:

Country	Species	Yield dry ton ha^{-1} $year^{-1}$	Crop System
Survey across Europe	*Populus*	10-12	Closely spaced single-stem trees.
Pacific North West	*Populus* hybrids of *interamericana (t x d)*	27.5	closely-spaced single stem trees (nutrient and water added)
UK	*Populus t x d* Beaupre	13	SRC poplar

Physiological factors influencing productivity in Poplar:

Hybrid poplar can exhibit outstanding growth rates on favourable sites. An understanding of the physiological basis for this high rate of production is necessary in order to maximise yields in the future. The physiological factors that influence potential productivity are as follows:

Light interception

Biomass production can be directly related to the interception of photosynthetically active radiation (PAR) by the foliage. This relationship can be quantified by the empirical model:

$$NPP = IPAR\ \varepsilon$$

NPP - net primary production

IPAR - absorbed photosynthetically active radiation

ε - Energy conversion efficiency (grams of dry matter produced per megajoule of energy)

The conversion efficiency will vary for different genotypes, but under adequate moisture and nutrient conditions it will remain fairly constant through the growing season. Reported vales for ε range from 0.32 (Landsberg & Wright, 1989) to 3.14 g/MJ (Cannell, 1989).

Phenology

The time of bud break, leaf senescence and bud set will determine the length of growing season. In some poplar genotypes these variables have been found to be directly related to the length of the frost-free season in the trees natural habitat (Pauley & Perry, 1954). This variation has been attributed to the genetic make up of the individual (Weber *et al.*, 1985; Michael *et al.*, 1988), but can also be governed by environmental factors such as soil and climate (Weber *et al.*, 1985; Dunlap *et al.*, 1992; Dunlap & Stettler, 1996).

Phenological information is an essential factor in most light interception and growth models.

Leaf Area Development

Another factor controlling light interception is the rate of leaf area development. This is governed by two factors; leaf production (i.e. the rate at which new leaves are produced), and leaf expansion. Rate of leaf expansion is a result of the combination of cell division and cell expansion, which can vary considerably between genotypes, depending upon supply of photosynthate, nutrition, photomorphogenic factors and genetic composition (Van Volkenburgh & Taylor, 1996).

Leaf Area Index

Leaf area index (LAI) (The leaf area per square meter of ground) can be used to indicate how well a plant can intercept the light falling on its canopy. Crown architecture, total leaf area, leaf area distribution within the crown, leaf and branch morphology, and orientation are all factors which influence light interception and hence biomass production. Hybrid poplar exhibit significant genotypic differences in these factors (Isebrands & Michael, 1986; Hinckley *et al.*, 1989; Ceulemans, 1990; Ceulemans *et al.*, 1990; Hinckley *et al.*, 1993), resulting in large variations of LAI and light interception between genotypes.

Photosynthesis

The photosynthetic rate of a canopy can be defined as the sum of the photosynthetic rates of all the leaves in the canopy (Ceulemans & Saugier, 1990).

This depends upon a number of variables such as total leaf area, leaf orientation and photosynthetic activity, as well as environmental factors, especially the radiation environment. Photosynthesis not only varies between genotypes, but also between trees grown individually and as part of a stand. The arrangement of leaves within the stand makes the stand more efficient than the individual tree in converting light into dry matter (Isebrands & Michael, 1986; Heilman *et al.*, 1996).

Root Growth Characteristics

Root growth accounts for a significant proportion of carbon allocation within the plant. An adequate root system is essential for support, as well as to provide a vital supply of water and nutrient transport into the plant. However, excessive root growth represents an unnecessary carbon sink, which might otherwise have been allocated to harvestable above ground biomass.

Considerable variation has been observed among different poplar genotypes in the relative quantity of carbon allocated to root systems, in the rate and pattern of development on cuttings, in tolerance to flooding, and in root system configuration (Dickmann *et al.*, 1988; Smit, 1988; Hinckley *et al.*, 1989; Heilman *et al.*, 1994).

The very low wood density of the coarse roots of poplar together with both the succulence and fineness of the fine roots (<0.6mm diameter) means that the root systems are developed with minimal carbon allocation. Also, in contrast to other tree species, the fine roots of poplar may be relatively long-lived (Dickmann & Pregitzer, 1992). All these factors enable poplars to allocate a high proportion of

their assimilated carbon to above ground biomass without sacrificing the effectiveness of the root system.

Identifying Physiological Traits Associated with High Yield in *Populus*.

Rapid growth and high productivity make hybrid poplar (*Populus spp.*) an ideal biomass crop, known to perform well across North America (Heilman & Stettler, 1985; Ranney *et al.*, 1987), Canada, (Balatinecz, 1979) and Europe (Perttu, 1984; Hummel *et al.*, 1988). At least 30 different species of poplar native to North America, Europe and Asia (Ceulemans, 1990) provide a vast gene bank from which selective breeding is capable of producing new hybrids with enhanced productivity. Most notable are the interamericana genotypes (*P. trichocarpa* x *P. deltoides*) which have been reported to yield up to 27.6 dry tonnes.$ha^{-1}.yr^{-1}$ (Heilman & Stettler, 1985; Ranney *et al.*, 1987), and average 20.3 tonnes $ha^{-1}yr^{-1}$ under experimental conditions (Ceulemans, 1990). The ease with which *Populus* may be propagated from vegetative cuttings also enhances its potential for large-scale cultivation. Commercial planting involves the use of hardwood pencils or whips, which are easy to generate, store and transport.

One significant problem that remains however is the *consistent* attainment of high yields that are commercially viable in large scale planting, across a variety of climatic and site conditions. Long-term and widespread planting of energy forestry therefore requires a supply of genetically improved material, suitable for growth at a large number of temperate sites. To achieve this, active breeding programmes must be coupled to an understanding of enhanced yield at a physiological level. The reasons for this are two-fold. Firstly, physiological 'traits' may be identified

and used as selection criteria in breeding programmes (Bradshaw, 1996). Such traits may now be placed as 'molecular markers' on genetic maps and used, in theory, in marker-assisted selection (MAS) (Strauss *et al.*, 1992; Tuskan, 1992) or in determining complex quantitative traits (QTL) (Kearsey & Farquhar, 1998). A second reason for identifying the physiological basis of yield is the development of models that are able to predict genotype performance in conditions that may arise in the future. These may include raised atmospheric carbon dioxide (Ceulemans & Mousseau, 1994) and changes in the patterns of rainfall (Rowntree, 1993).

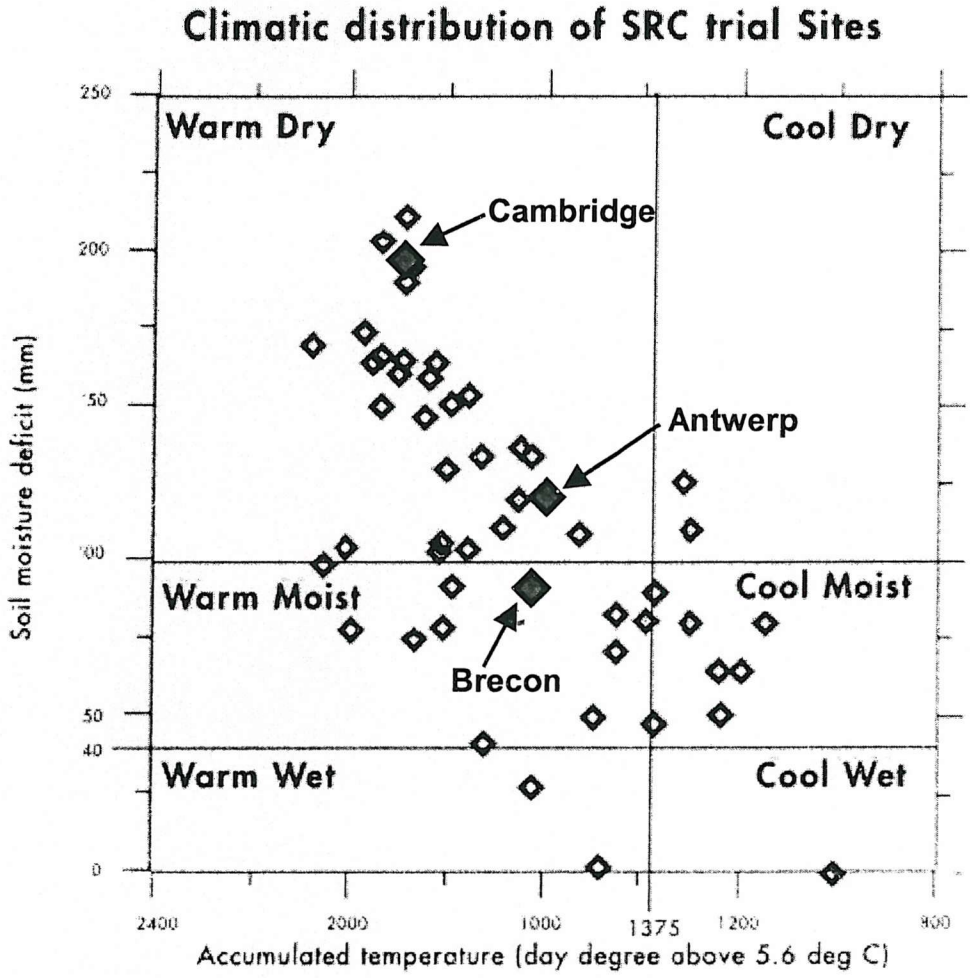
The aim of this study was to determine important physiological characteristics related to yield in five genotypes commonly grown across Europe and currently the subject of a large site-clone trial in the UK. To achieve this, two sites of contrasting climate were compared. In contrast to many other studies of *Populus*, trees were grown in short rotation coppice, rather than as standards, thus simulating a growth form considered highly suitable for energy forestry.

Background information relating to the Forestry Commission field sites:

The two Forestry Commission sites were part of a network of 49 sites established across the UK under contract to ETSU (Energy Technology Support Unit) for the Department of Trade and Industry (DTI) between 1994 and 1996. They were commissioned in order to investigate the suitability of different varieties of poplar and willow for planting as biomass crops with a view to using the biomass as a renewable energy fuel. The research was intended to help increase the percentage

of energy produced from renewable sources in line with EU and Government policy (DTER 1999; EU 1997).

Poplars and willows were chosen since these species have been identified as most reliable for use as energy crops (Potter, 1990). The use of short rotation coppice as a substitute for fossil fuels is environmentally beneficial, as it produces no net CO_2 emissions from its combustion and it also produces low NO_x and SO_x pollutants when compared to fossil fuels (Armstrong, 1997). Sites were chosen to cover the major soil types suitable for poplar and willow cultivation at elevations of less than 250m above sea level, across a range of climatic conditions. Diagram 0.1 shows the climatic distribution of all 49 sites in the UK. Data points corresponding to the Brecon and Cambridge sites used for the research detailed in this chapter are highlighted.



Climatic distribution of site/yield trial sites

Diagram 0.1: Climatic distribution of all Forestry Commission / ETSU Short Rotation Coppice trial sites (from Armstrong 1997). The data points corresponding to the field trial sites at Brecon and Cambridge are indicated. It can be seen that the two sites were quite different in terms of soil moisture deficit with Cambridge being significantly drier than Brecon. Also indicated is a third site at Antwerp, Belgium which was visited as part of this experiment.

Two experimental designs were employed by the Forestry Commission at these sites. 42 sites were planted with three genotypes of each species (poplar and willow) in a randomised block design using 3 replicated blocks of each genotype. These were termed 'extensive sites'. The remaining seven sites were planted with 16 genotypes of each species, again using three replicated blocks of each in a randomised block design. These were known as 'intensive ' sites. The work presented in this chapter relates to research undertaken at two of these intensive sites.

The sites were established and managed according to the following protocol:

1 Sites were sprayed with a contact herbicide to eliminate perennial weeds prior to ploughing

2 Ploughed sites were power-harrowed immediately before planting with 25cm unrooted cuttings.

3 Residual herbicides were applied immediately after planting and the sites kept weed free by further applications of herbicide throughout the year (Willoughby & Clay, 1996).

4 At the end of the first growing season failed cuttings were replaced and all trees stumped back to 10cm above ground level to encourage the stools to become multi stemmed. Harvesting was planned to follow a standard 3-year cutting cycle.

Data collected by the Forestry Commission related to non-destructive yield assessment based on diameter / yield relationship previously established by

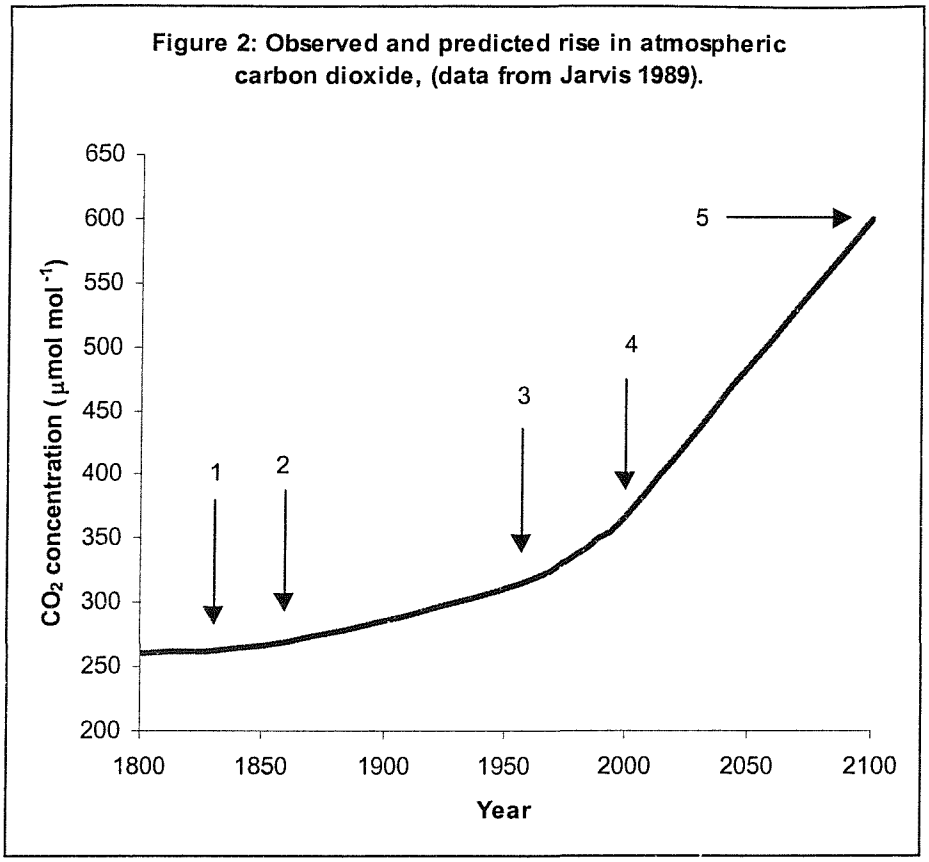
destructive sampling, and insect and disease monitoring. The aim of the trial was to provide data for the construction of yield models that could be used in the future to advise planners, energy producers and growers of the most suitable genotypes to plant in a particular area and to indicate the likely average yield.

Section 0.3 - Productivity in the future:

Predicted increase in atmospheric CO_2.

Jarvis (1989) described the continuing increase in atmospheric carbon over the past 200 years, followed by predictions for the future in his review article "Atmospheric carbon dioxide and forests" (Jarvis, 1989). The following graph (figure 0.2) summarises this information:

Figure 2: Observed and predicted rise in atmospheric carbon dioxide, (data from Jarvis 1989).



Key:

1 - Intensification of agriculture over large areas results in extensive deforestation (especially N. America).

2 - Start of Industrial Revolution and subsequent exploitation of fossil fuels.

3 - Introduction of infrared gas analyser.

4 - Present day.

5 - Predicted increase to 600 μmol mol^{-1} in the next 60 – 100 years.

It should be noted however that these predictions are uncertain. There is uncertainty regarding the future use of fossil fuels (even now steps are being taken to try to limit this use with current research into renewable energy), and uncertainty as to the fate of CO_2 released into the atmosphere. Only 56% of the carbon released as a result of fossil fuel combustion in the past 100 years has contributed

to the increase in atmospheric carbon dioxide. The remainder is unaccounted for, but may be sequestered in the oceans, ultimately to be deposited in the deep ocean.

Consequences of increased atmospheric carbon dioxide on plants.

Present concentrations of carbon dioxide in the atmosphere are a limiting factor in C3 photosynthesis. Increases in CO_2 tend to stimulate photosynthesis, enabling plants to fix carbon at a higher rate, which usually results in increased growth. This increase in photosynthesis can be demonstrated using an infrared gas analyser to measure carbon uptake in a leaf exposed to increasing levels of carbon dioxide. The variable relationship between photosynthetic assimilation (A) and intercellular CO_2 concentration (ci) is explained in chapter one.

The effects of elevated carbon dioxide on poplar trees:

Research into the above ground effects of elevated CO_2 on poplar has consistently revealed that biomass, leaf area and photosynthesis all increase with elevated CO_2 (Ceulemans *et al.*, 1996; Ceulemans *et al.*, 1997; Chen *et al.*, 1997; Will & Ceulemans, 1997). It has also been shown that factors such as stem volume, branch number and branch biomass increase, while other factors such as height and leaf area index are affected differently depending on genotype (Ceulemans *et al.*, 1996). Ceulemans also noted that responses varied depending on duration of exposure, suggesting possible acclimation to elevated CO_2. A subsequent long term study revealed further evidence of acclimation as some genotypes were found

to have reduced RUBISCO activity after 17 months of exposure to high CO_2 (Ceulemans *et al.*, 1997).

Less research has taken place into below ground responses to CO_2, but from the studies that have been done on poplar (Bosac *et al.*, 1995) and other species (Crookshanks *et al.*, 1998) it is clear that there are similar effects on roots as there are on shoots. Increased root length, root number, and root diameter have all been observed in the presence of elevated CO_2 as have root dry weight, and branching density. However, root: shoot dry weight ratios were not found to be affected by CO_2, indicating that the partitioning of carbon between above and below ground resources is unaffected.

Previous studies carried out in controlled environments and open top chambers have also shown that increased CO_2 can reduce stomatal conductance (Bremer *et al.*, 1996; Curtis, 1996; Bunce *et al.*, 1997; Hamerlynck *et al.*, 1997; Anderson & Tomlinson, 1998) and reduce sap flow (Bremer *et al.*, 1996; Bunce, 1996; Kellomaki & Wang, 1998). This would suggest a positive effect of CO_2 on water use and water use efficiency, with large implications for global forest function in the future. However, the relevance of these results has been questioned (Norby *et al.*, 1999). This is because young trees and seedlings have been used in many studies, where growth response to CO_2 is large, but smaller effects may be observed as trees age (Hattenschwiler *et al.*, 1997). It is also well known that the coupling of plant to atmospheric conditions is disturbed in such experiments. Large forced air flows found in open top chambers may alter the relationship between stomatal conductance and transpiration as predicted by Jarvis and

McNaughton (1986) and Bunce *et al.* (2000), leading to over-estimations of the importance of stomata in determining total tree water use (Jarvis & McNaughton, 1986; Bunce, 2000). Consequently, more work is required to investigate water use in mature trees grown in free air enriched CO_2 for long periods of time.

The molecular genetic basis of root growth responses in *Populus* to elevated CO_2

Hybrid poplars grown as short rotation coppice and single stand trees offer a good potential source of renewable energy. However, little research has so far been done to determine the molecular genetic basis of productivity in these trees, how it is affected by stress and climate, and how it could be manipulated in the future (Wu *et al.*, 1997).

The global spread of Populus includes species living in flood plain, temperate, boreal and montane forests throughout the Northern Hemisphere. Genetic variation is high in the genus, which consists of more than 30 species (Ceulemans, 1990). However, very little is know about how the genome influences the growth and physiology of roots which play a critical role in the establishment and persistence of these trees, and consume a significant portion of their carbon budget (Pregitzer & Friend, 1996), and determine effects of water uptake and nutrient gain.

There is plenty of evidence to show that increased availability of CO_2 in the atmosphere stimulates photosynthesis and leads to significant increases in above and below ground biomass (Pritchard *et al.*, 1999). The reason for net photosynthetic enhancement is related to a number of factors which are connected to the characteristics of the primary carboxylating enzyme (ribulose bisphosphate

carboxylase-oxygenase) (Lawlor & Mitchell, 1991). Increased CO_2 activates rubisco which constitutes the rate limiting step in photosynthesis, and reduces the competitive influence of oxygen and photo-respiratory carbon loss (Atkinson, 1996).

The degree to which root growth is enhanced relative to shoot growth varies greatly between genus and even between species of the same genus (Ceulemans & Mousseau, 1994). Much of this variation may depend upon the type of experiments used to quantify this. Many elevated CO_2 experiments use potted seedlings and it has been noted that the size and shape of the pot may influence the whole plant response to elevated CO_2 (Bernstson *et al.*, 1993; McConnaughay *et al.*, 1993). Another complication when comparing effects on root growth in elevated CO_2 experiments is the availability of nutrients. A plant growing in nutrient deficient soil will increase carbon partitioning to its roots in order to obtain nutrition from a wider area, but under conditions of free nutrient supply more carbon will be allocated to the above ground biomass (Eamus & Jarvis, 1989). When root responses to elevated CO_2 have been studied without restricting the roots inside pots it has been found that the number of roots, root length, root growth and fine root mass all increased (reviewed by Ceulemans & Mousseau (1994)).

Root morphology (or phenotype) is determined by a combination of genetic programming and environmental influences. Plant hormones such as auxins, cytokins and ethylene are known to influence root growth (Torrey, 1974; Abeles *et al.*, 1992) and these are produced by the switching on and off of genes within the plant itself. Environmental factors can influence root growth in two ways. They

can act as triggers for the genetic switches that produce plant hormones, or they can act directly on the plant. It is not always clear which of these methods is responsible for a particular phenotypic response, and very often it will be a combination of both. For example, root cap cells are known to exhibit gravitropism (i.e. gravity influences their direction of growth) (Moore & Evans, 1986). Chemical gradients within the soil will affect the density of root growth, causing increased growth in areas of high nutrients (Fitter *et al.*, 1988). Light, through the action of photosynthates has been shown to affect root extension, gravitropism and lateral root production (Hart & MacDonald, 1980). Soil moisture (Sharp & Davies, 1985), temperature gradients (Fortin & Poff, 1991; Bassow *et al.*, 1994), soil composition and neighbouring plant roots (Mahall & Callaway, 1991) are all known to play a part in determining root system growth and morphology.

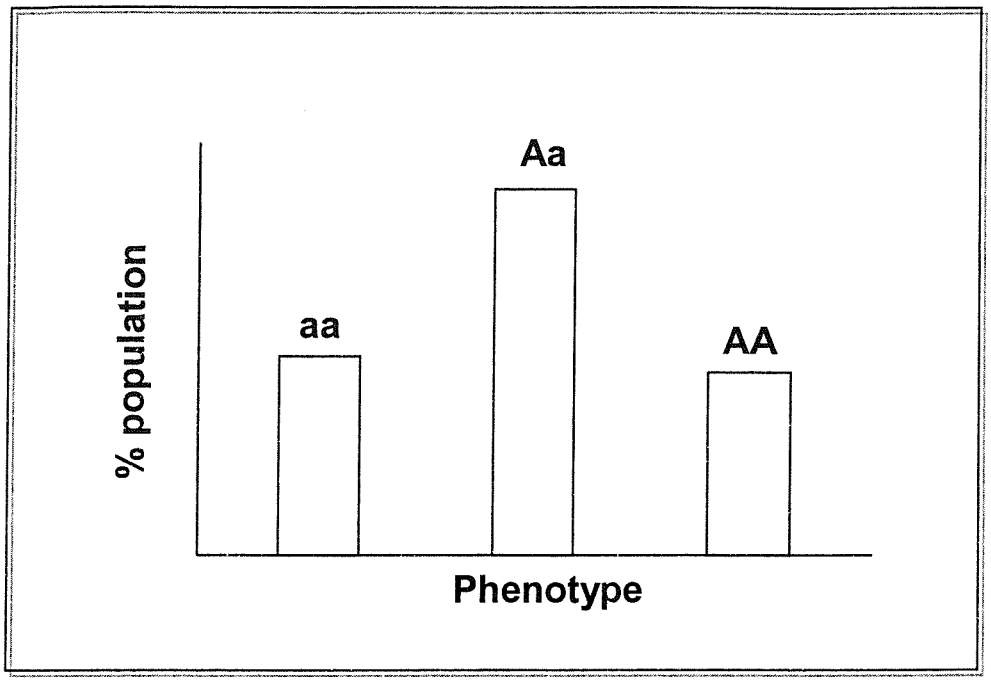
The mechanism of enhanced root growth in elevated CO_2 has been variously attributed to increased cell expansion (Ferris & Taylor, 1994); increased rate of cell expansion with no overall increase in mature cell size (Crookshanks *et al.*, 1998); increased cell division (Kinsman *et al.*, 1997) and increased root branching by initiation of lateral root primordia (Ferris & Taylor, 1994; Gebauer *et al.*, 1996). Irrespective of the mechanism, the importance of below ground responses to elevated CO_2 must not be overlooked since the root system not only provides structural support for increased above ground biomass, but must also be capable of supplying the extra water and nutrients required to support this increased growth. This is an especially important consideration for the future of biomass crops such as poplar, as an understanding of the genetic control of root responses to elevated CO_2 will enable plant breeders to make better informed decisions when choosing

new varieties for the future. Indeed, more knowledge of the genes that govern root growth characteristics may enable plant breeders to use marker-assisted selection to improve new varieties since root traits are hard to identify using conventional screening of field plantations. ***Until now, there have been no studies that attempt to investigate the underlying genetic control of root responses to elevated CO_2.***

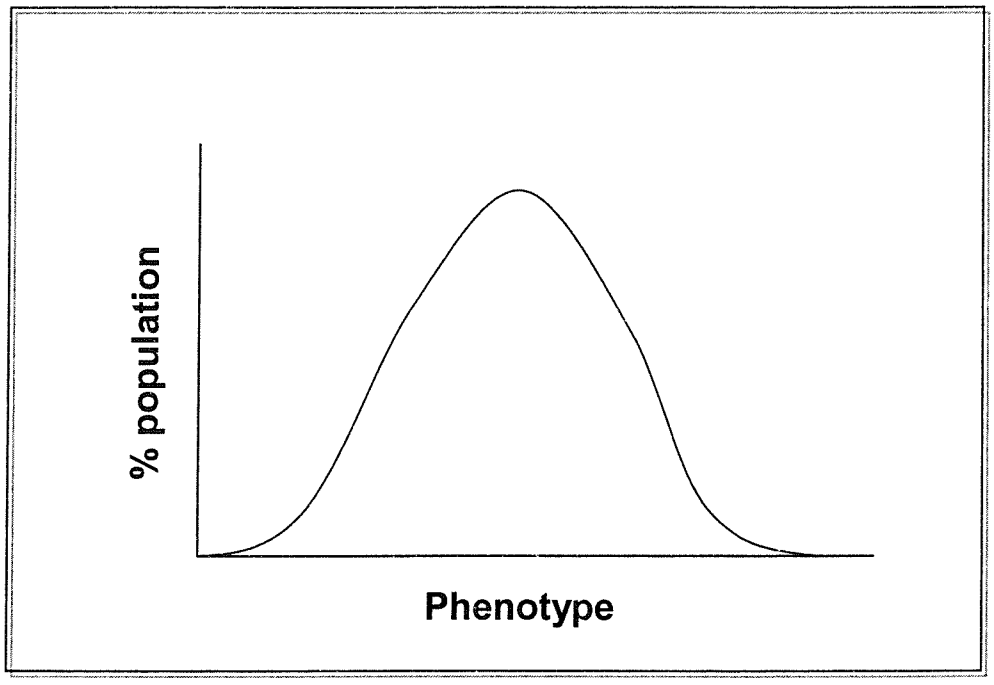
Various complex traits important for plant responses to stress are 'quantitative' rather than 'qualitative', suggesting that they are determined by several rather than single gene products. Many complex traits such as root density and root growth rate are unlikely to be described by a single gene inheritance but can be identified using quantitative genetics (Bradshaw *et al.*, 1994; Bradshaw & Stettler, 1995). Unlike Mendelian genetics where a single trait such as flower colour is determined by the presence or absence of a single gene product, quantitative genetics attempts to explain the variation in continuous traits such as height which can be infinitely variable within a given range (Falconer & MacKay, 1996) (Diagram 0.2). For example, consider a number of genes that by their presence or absence can have either a positive or negative affect on a particular trait such as tree height. The combination of the positive (+) and negative (-) effects of all these genes will determine the final height of the tree:

Trait:	Gene combination:
Very tall tree:	+ + + + +
Medium tree:	+ - - + +
Very small tree:	- - - - -

Diagram 0.2 - Qualitative and quantitative genetics:



Characteristics determined by a single gene have discrete 'qualitative' phenotypes.



Characteristics determined by multiple genes have continuous 'quantitative' phenotypes.

Using quantitative genetics at the molecular level has become widely investigated in both plant and animal systems (Prioul *et al.*, 1997) and is a promising method for analysing continuously variable plant growth traits (Bradshaw & Stettler, 1995). However, an F2 pedigree is required for molecular level investigation of quantitative traits. (F2 pedigree is the name given to a second generation produced after intercrossing or self fertilisation of the first generation (F1) progeny of a given mating pair (Hartle, 1991)). In the case of family 331 the F1 progeny were intercrossed, as self-fertilisation is impossible in *populus* since they are dioecious (see diagram 2.2). Ideally breeding parents in whom the traits of interest are segregating (i.e. at opposite extremes of the range of phenotypes) should be used to produce this pedigree. The pedigree must also have segregating variation for a collection of genetic markers, which should ideally cover the whole genome, allowing the construction of a genetic map (Bradshaw *et al.*, 1994). Finally, It must be possible to score the trait of interest in every individual of the population.

In Poplar a linkage map consisting of 343 RFLP (restriction fragment length polymorphism), STS (sequence tagged site) and RAPD (random amplified polymorphic DNA) markers has been constructed using two contrasting species, *P. trichocarpa* T. & G. and *P. deltoides* Marsh (Bradshaw *et al.*, 1994). The presence or absence of these markers in a particular individual infers the presence or absence of a particular sequence of DNA in that genotype. If the marker is located within or close to a gene sequence then the presence of the marker indicates the presence of that particular gene. Since the position of the marker on the genome is already known it can be used to pinpoint the location of that gene to an area of a particular chromosome. This is the first, most basic step in QTL mapping, but there are 3

problems with this approach. Firstly, false positives will occur if the significance level of the statistics is set too low. Secondly, more than one marker will be associated with a QTL due to the fact that all genes on a chromosome will be linked to some degree. Finally, since the QTL may or may not be on the same allele as any given marker its effect and exact position cannot be known, (although the strongest association will be with the closest marker) (Kearsey & Farquhar, 1998). Several techniques (interval mapping, multiple regression, marker regression and composite interval mapping) have been developed to overcome these problems.

Interval mapping is a process whereby intervals between adjacent markers along a chromosome are scanned and the likelihood profile of a QTL being at any particular point in each interval is determined. The value obtained by this analysis is referred to as the 'LOD' score and is defined as the log of the ratio of the likelihood of there being one vs. no QTL at a particular point (Lander & Botstein, 1989). The significance level of this test has to be adjusted to avoid false positives and this is made depending on the scanned length of the genome and spacing between mapped markers (Lander & Botstein, 1989; Bradshaw & Stettler, 1995). This is the most widely used approach since the software package that performs these calculations (MAPMAKER/QTL) is freely available (Whitehead-Institute, 1993).

Multiple regression (Haley & Knott, 1992) produces similar results to LOD mapping, but is faster and easier to program and has been adapted to take into account fixed effects such as sex or environment. Finally, marker regression

simultaneously fits a model to all the marker means on a given chromosome (Kearsey & Hyne, 1994). This is fast and integrates all the marker information into a single test, but does not provide an overall test of the model.

Many QTL papers use only LOD scores found by interval mapping in order to predict QTL e.g. (Bradshaw & Stettler, 1995; Newcombe & Bradshaw, 1996; Zheng *et al.*, 2000). However, the main problem with this technique is that the significance level needs to be set correctly in order to avoid false positives, and despite stringent efforts to do this correctly the chance of error is still considered to be quite high. A technique called 'composite interval mapping' can be used to help rule out this uncertainty (Basten *et al.*, 1994; Zeng, 1994). This technique initially tests for QTL in the same way as interval mapping, by comparing phenotype and genotype data for each individual in order to determine the likelihood of a QTL occurring between markers on a genetic map. A probability threshold now has to be set above which it can be confidently stated that a QTL actually exists. The next step is to scramble the data by randomly assigning phenotypes to different genotypes within the population, and then repeat the test. This time, any likely QTL that are found will definitely be 'fake' since they occur entirely due to chance (because the data they are based on is fictitious). This process is repeated as many times as possible (up to 1000 is recommended) (Zeng, 1994). The result of these permutations will be a series of 'fake QTL', each with a probability value. Since it is known that all of these QTL are fake, their statistics give an indication of where the probability threshold needs to be set in order to distinguish an actual QTL from the original data analysis. If any of the potential QTL identified from the real data have a probability greater than any of the 'fake QTL' it is likely that these really

are quantitative trait loci. However if they all fall within the same probability range as the 'fake QTL' it is likely that they also exist purely by chance. The computer program used to carry out composite interval mapping is called QTL CARTOGRAPHER (Basten *et al.*, 2000).

Water-use in poplar grown in elevated CO_2 concentrations.

Under current climatic conditions it has been estimated that the worlds forests could sequester about 60 – 87 Gt of carbon from the atmosphere over the period 1995 – 2050 (IPCC, 1995). This is equivalent to 12 -- 15% of the projected (IPCC 1992 scenario) cumulative fossil fuel emissions over the same period. However, current climatic conditions are changing (IPCC, 1995). In particular, increased atmospheric CO_2 may stimulate tree growth further (Jarvis, 1989), a response that may be associated with increased water-use. Annual rainfall is decreasing in some parts of the world (DETR, 1998) and this may limit the net primary productivity of forests, particularly in areas such as southern Europe, where large reductions in rainfall are suggested. There remain therefore, important unresolved issues on whether forests will continue to respond positively to increased atmospheric CO_2 and whether water will be used more efficiently in such trees.

Previous studies carried out in controlled environments and open top chambers have shown that increased CO_2 can reduce stomatal conductance (Bremer *et al.*, 1996; Curtis, 1996; Bunce *et al.*, 1997; Hamerlynck *et al.*, 1997; Anderson & Tomlinson, 1998) and reduce sap flow (Bremer *et al.*, 1996; Bunce, 1996; Kellomaki & Wang, 1998). This would suggest a 'positive' ameliorative effect of

CO_2 on water use, with large implications for global forest function. But the relevance of these results for estimating whole-tree and canopy water use in the carbon dioxide conditions that will prevail in the next few decades has been questioned (Norby *et al.*, 1999). This is because young trees and seedlings have been used in many studies, where growth response to CO_2 is large, but this may only be transitory with rather smaller effects observed as trees age (Hattenschwiler *et al.*, 1997). It is also well known that the coupling of plant to atmospheric conditions is disturbed in such experiments. Large forced air flows may alter the relationship between stomatal conductance and transpiration as predicted by Jarvis and McNoughton (1986) and Bunce *et al.* (2000), leading to over-estimations on the importance of stomata in determining total tree water use. Interestingly, stomatal conductance in trees may be less responsive to elevated CO_2 than in herbaceous species (Saxe *et al.*, 1998). In one of the few studies where a mature forest has been exposed to elevated CO_2 (Ellsworth, 1999), stomatal conductance and shoot water relations of *Pinus taeda* were unaltered by this treatment. A summary of the literature regarding the effects of elevated CO_2 on sap flow, transpiration and stomatal conductance is shown in table 1:

Table 1 - The effects of elevated carbon dioxide on sap flow, transpiration and stomatal conductance:

Abbreviations: CE: Controlled Environment; OTC: Open Top Chamber; GH: Glass House; FACE: Free Air CO_2 Enrichment; NE: No Effect; ↑: Increased; ↓: Decreased;

Species	Environment	CO_2 / ppm	Sap flow	Transpiration	Stomatal conductance	Comments	Reference
Quercus rubra	CE	400, 530, 700			↓	↑ Water use efficiency	(Anderson and Tomlinson 1998)
17 spp.	Field	Natural CO_2 spring			↓	No effect on stomatal density No effect on stomatal index	(Bettarini, Vaccari et al. 1998)
Tradescantia		350, 670			↓	Extra cells identified in stomatal complexes of plants grown in ↑CO_2. ↑ Stomatal index when extra cells counted as part of the stomatal complex, but no change when counted as epidermal cells.	(Boetsch, Chin et al. 1996)
Veronia baldwini	OTC		↓	↓			(Bremer, Ham et al. 1996)
Andropogon gerardii	OTC		↓	↓	↓		(Bremer, Ham et al. 1996)
Sorghestrum nutans	OTC		↓	↓	↓		(Bremer, Ham et al. 1996)
Quercus petraea	OTC	350, 615			↓		(Broadmeadow, Heath et al. 1999)
Quercus rober	OTC	350, 615				↓ stomatal response to LAVPD	(Broadmeadow, Heath et al. 1999)
Fagus sylvatica	OTC	350, 615				↓ stomatal response to LAVPD	(Broadmeadow, Heath et al. 1999)
Malus domestica	FACE	350, 700			NE		(Bunce 1992)
Quercus prinus	FACE	350, 700			NE		(Bunce 1992)
Quercus robur	FACE	350, 700			NE		(Bunce 1992)
Acer saccharinum	FACE	350, 700			↓	(↓ stomatal conductance only above 33°C) All species had reduced rates of dark respiration per unit of mass.	(Bunce 1992)

Species	Environ-ment	CO_2 / ppm	Sap flow	Transpiration	Stomatal conductance	Comments	Reference
Medicago sativa		350, 700		↓			(Bunce 1995)
Dactylis glomerata		350, 700		↓			(Bunce 1995)
Medicago sativa	CE	350, 700	↓	↓		Conductance of excised stem segments ↓ in elevated CO_2	(Bunce 1996)
Glycine max	CE	350, 700	↓	↓		Conductance of excised stem segments ↓ in elevated CO_2	(Bunce 1996)
Medicago sativa	OTC	350, 700		NE	↓	Used soil – vegetation – atmosphere model to determine field transpiration rates without artificial ventilation in chambers	(Bunce, Wilson et al. 1997)
Dactylis glomerata	OTC	350, 700		NE	↓	Used soil – vegetation – atmosphere model to determine field transpiration rates without artificial ventilation in chambers	(Bunce, Wilson et al. 1997)
Zea mays		350, 700		↓		Leaf water potential unchanged	(Bunce and Ziska 1998)
Amaranthus hypocondriacus		350, 700		↓		Leaf water potential unchanged	(Bunce and Ziska 1998)
Review article					↓	No significant effect on stomatal conductance in stressed plants	(Curtis 1996)
Quercus suber	GH	350, 700			NE	Elevated CO_2 had no effect on stomatal conductance under drought and non drought conditions.	(Damesin, Galera et al. 1996)
Sorghum bicolor	OTC	359, 705		↓		Used sap flow to determine transpiration rate per unit leaf area..	(Dugas, Prior et al. 1997)
Glycine max	OTC	359, 705		↓		Greater reduction of transpiration in C3 *Glycine max* than C4 *Sorghum bicolor*	(Dugas, Prior et al. 1997)
Spring Wheat	FACE	370, 550			↓		(Garcia, Long et al. 1998)
Andropogon gerardii		350, 700			↓	↑ leaf water potential regardless of soil water availability (occasionally ↑ stomatal conductance in drought)	(Hamerlynck, McAllister et al. 1997)
Symphiocarpus orbiculatus		350, 700			↓	↑ leaf water potential regardless of soil water availability (occasionally ↑ stomatal conductance in drought)	(Hamerlynck, McAllister et al. 1997)
Salvia pitcheri-were		350, 700			↓	↑ leaf water potential under drought conditions (occasionally ↑ stomatal conductance in drought)	(Hamerlynck, McAllister et al. 1997)

Species	Environ-ment	CO_2 / ppm	Sap flow	Transpiration	Stomatal conductance	Comments	Reference
Spring Wheat	FACE	370, 550		↓ (wet) ↑ (dry)		↑ Water Use Efficiency	(Hunsaker, Kimball et al. 1996)
Pinus sylvestris	GH		↓			(↑ sap flow in low light)	(Kellomaki and Wang 1998)
Andropogon gerardii	OTC	337, 658		↓	↓		(Kirkham, He et al. 1991)
Populus tremuloides	OTC	350, 700			↓		(Kull, Sober et al. 1996)
Prunus serofina	CE	360, 650					(Loats and Rebbeck 1999)
Fraxinus pennsylvanica	CE	360, 650				↑ photosynthesis	(Loats and Rebbeck 1999)
Liriodendron tulipifera	CE	360, 650			↓	↑ photosynthesis	(Loats and Rebbeck 1999)
Pinus taeda	OTC					No effect on water use per unit sap wood area. ↑ Water use due to increased leaf area and sap wood area	(Pataki, Oren et al. 1998)
Rumex obtusifolius	GH	350, 600			↓		(Pearson and Brooks 1996)
Betula pendula	CE	350, 700				↑ Water Use Efficiency (difference diminished with age of leaf)	(Pettersson and McDonald 1992)
Gossypium hirsutum	GH	350, 450, 700		↓	↓		(Reddy, Reddy et al. 1998)
Review Article						Stomatal conductance in trees is remarkably less responsive to ↑ CO_2 than in herbaceous species. Stomata of a number of tree species have been shown to be unresponsive to ↑ CO2. Positive effects of ↑ CO_2 on leaf area can be at least as important in determining canopy transpiration as negative direct effects of ↑ CO_2 on stomatal conductance.	(Saxe, Ellsworth et al. 1998)
Lollium perenne	GH	350, 700		↓		↑ Water Use Efficiency ↓ transpiration + ↑ leaf area = no change in total plant transpiration	(Schapendonk, Dijkstra et al. 1997)

Species	Environ-ment	CO_2 / ppm	Sap flow	Transpiration	Stomatal conductance	Comments	Reference
Triticum aestivun	FACE		↓	↓			(Senock, Ham et al. 1996)
Arachis hypogaea	GH	375, 700				↑ Omega (W) (gKPa kg^{-1}) Omega (W) = (accumulated biomass / transpired water) x saturation deficit	(Stronach, Clifford et al. 1994)
Quercus pubescens	Field	Natural CO_2 spring	↓		↓	↑ osmotic potential ↓ symplastic fraction of water	(Tognetti, Giovannelli et al. 1996)
Quercus ilex	Field	Natural CO_2 spring	↓		NE	↑ osmotic potential ↓ symplastic fraction of water	(Tognetti, Giovannelli et al. 1996)
Quercus pubescens	Field	Natural CO_2 spring	↓			↓ hydraulic resistance Sap flow : foliage area ratio unchanged	(Tognetti, Giovannelli et al. 1996)
Picea sitchensis		350, 600			↓	↑ Water Use Efficiency ↑ Xylem pressure potential	(Townend 1993)
Glycine max	CE				↓	↓ sensitivity of stomata to leaf / air vapour pressure difference	(Wilson and Bunce 1997)

Populus is an important economic species, grown commercially for wood, chipboard products, paper pulp and veneer. More importantly, *Populus* may be grown on a large scale where it can be used as a source of renewable, biomass energy, in line with the Kyoto protocol (article 3.3), utilising afforestation and management to help stabilise CO_2 emissions globally (EU, 1997). Smith et al., (2000) concluded that biomass crops such as poplar and willow offer the best land-use change for carbon mitigation, suggesting that across Europe, a 6 % offset from 1990 emissions could be achieved by this use of agricultural land (Smith *et al.*, 2000). Under current atmospheric conditions, water use from Poplar has been shown to exceed that of many other species (Dolman *et al.*, 1998), and since its rapid growth rates are known to increase when exposed to elevated CO_2 (Ceulemans *et al.*, 1995), future increases in water-use seem likely. Elevated CO_2 leads to enhanced leaf area in poplar (Bosac *et al.*, 1995; Tognetti *et al.*, 1999; Ferris *et al.*, 2001) although reduced stomatal conductance has also been observed (Kull *et al.*, 1996; Will & Ceulemans, 1997; Tognetti *et al.*, 1999). It remains unknown whether positive effects of increased CO_2 on leaf area will compensate for reductions in stomatal conductance in determining tree and forest water use.

Many of these unanswered questions can only be addressed in long-term field experiments where trees are grown to canopy closure and where responses are more than short-term. FACE (free air carbon dioxide enrichment) provides the approach in which this may be achieved. Few data exist from FACE experiments for trees but Senock et. al. (1996) and Hunsanker et al., (2000) report slight reductions of sap flow and evapotranspiration in wheat (*Triticum aestivum*) grown

under FACE conditions, although in neither study was this significant ($P>0.05$) (Senock *et al.*, 1996; Hunsaker *et al.*, 2000).

The aim of this research was to answer these questions, determining the effects of future CO_2 concentrations on both leaf-level stomatal conductance and tree-level water use and using these data to estimate seasonal forest water use for this fast growing, biomass tree. This was achieved at the POPFACE (Poplar Free Air CO_2 Enrichment) facility in central Italy during the summer of 2000 on *Populus* in their second year of growth and exposure to enriched CO_2 conditions, as the canopy closed.

Measurement of sap flow using the stem heat balance method:

The stem heat balance method:

This method utilises a heating element wrapped around the stem in which sap flow is to be measured. Thermocouples positioned around the heater measure heat loss due to conduction through the wood, and heat lost due to conduction through the insulation material surrounding the heater and stem. Provided the power input to the heater is known, rate of sap flow can be calculated from the rates of heat loss from the system.

Diagram 0.3 shows the constant power heat balance sap flow sensor installed on a plant stem. Each sensor is connected to a 4 twisted pair cable:

Pair 1 - Power to the heater

Pair 2 - Radial thermopile signal

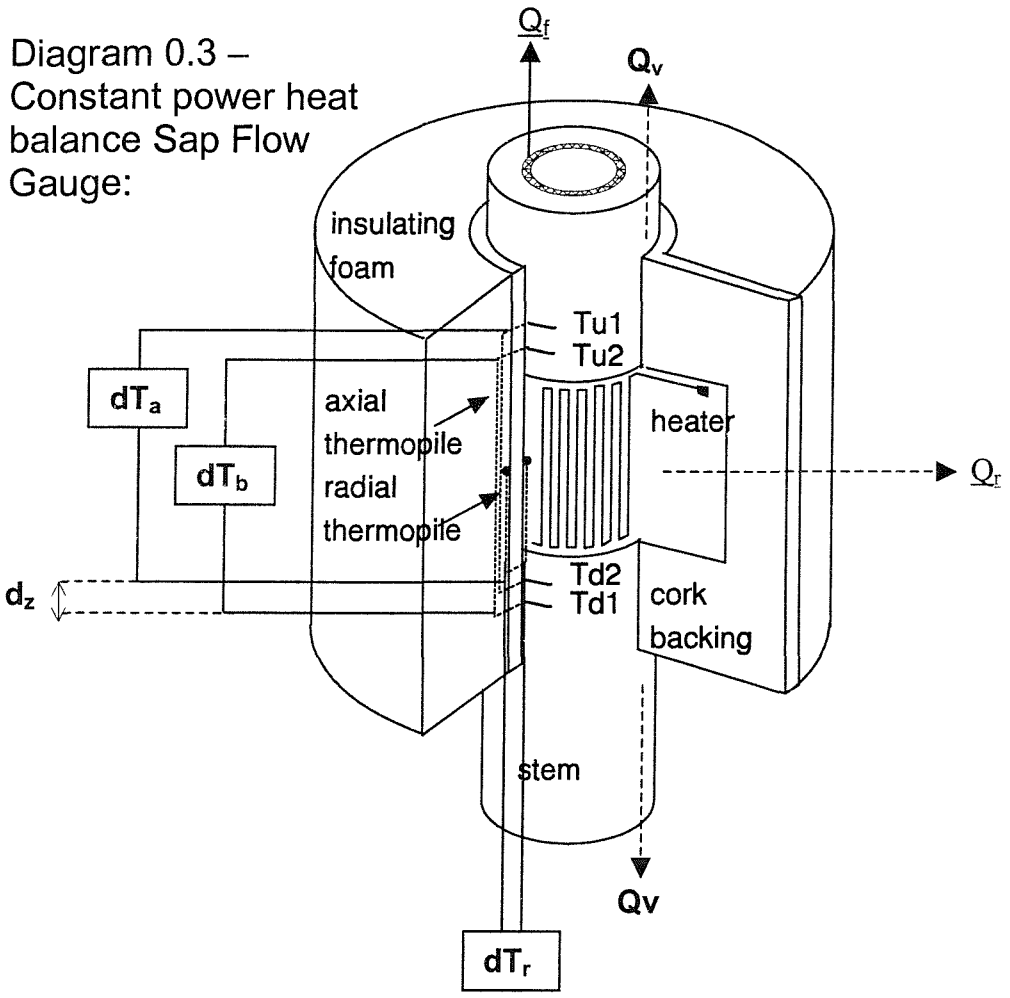
Pair 3 - Upper axial thermopile signal

Pair 4 - Lower axial thermopile signal

Individual gauges can typically be used only on stems with diameters lying within narrow limits, and so a number of different sized gauges are required to match the variability in stem size and growth. The stem heat balance sap flow method has the advantage over other methods (e.g., trunk sector heat balance and heat pulse methods) by allowing non-invasive measurements of the un-calibrated mass flow of water in small diameter stems (Dugas, 1990). With this method, heat is applied to the entire circumference of the stem encircled by an heating tape, and the mass flow of sap is calculated by the balance of the heat flux into and out of the heated section of the stem (Sakuratani, 1981, Baker and Van Bavel, 1987). A foam insulation and weather shield surrounding the stem extend above and below the heater to minimize external gradients across the section of the stem, and reduce solar heating of the stem to a negligible level. Heat input to the stem section is thus limited to the electrical power supplied to the heater (P), and the heat balance of the stem section is: $P=qv+qr+qf$, where qv is the rate of vertical heat loss by conduction in the stem, qr is radial heat loss by conduction across the gauge itself, and qf is the heat uptake by the moving sap stream. The value of qf is determined by subtracting qv and qr from P; all of which are measured quantities. The value of P is calculated from the electrical resistance and voltage across the heater, while qv and qr are determined from measurements of temperature gradients along the stem and across the gauge. Finally, qf is converted to the mass flow

rate of sap. Some researchers use an additional term (Qs) which is the heat storage component of wood. However, the effect of this on the results is so small that it is usually ignored unless trying to measure very low rates of sap flow so this variable was omitted from the calculations used in this experiment. See appendix 4 for details of constant power heat balance equations.

Diagram 0.3 – Constant power heat balance Sap Flow Gauge:



Sap Flow is found by calculating heat loss from the gauge:

$Qf = P - Qv - Qr$

heat loss due to sap flow	=	power input	-	heat loss by conduction in the stem	-	heat loss by conduction across the gauge

Advantages of heat balance method:

Hall et. al. (1998) compared transpiration measurements made using three different techniques (heat balance, heat pulse and deuterium tracing). They reported that the heat balance technique was the best, as it was non-invasive and depended only upon the bulk heat transfer properties of wood, and so was unaffected by variations in the radial distribution of xylem vessels often found in woody plants.

Attaching the sap flow gauges:

The construction of the gauges allows them to be fitted around the branch or stem to be measured. It is important to use a gauge of similar internal diameter to the diameter of the branch to be measured in order to get a snug fit. Some researchers reported the removal of loose bark and dead tissue from the area to which the gauge was to be fitted, and also the application of a 'silicon based electrical insulating compound' in order to eliminate air spaces and improve conduction of heat to the stem (Kellomaki & Wang, 1999). Several papers underlined the importance of protecting the gauges from the sun and rain (Hall *et al.*, 1998; Allen *et al.*, 1999; Smith *et al.*, 1999). Allen and Hall built conical shelters from polythene sheeting, taping one end to the tree above the gauge and sealing the join with grafting wax. They report this method to be highly effective at keeping the gauges dry. Smith et. al. used black netting over their plastic shelters in order shade their gauges from the hot sun in Kenya.

Scaling up

Transpiration measurements from coppiced poplar stems, found using heat balance sap flow gauges were scaled up to estimate stand transpiration (Hall *et al.*, 1998; Allen *et al.*, 1999). The following equation was used:

$$\mathbf{T} = \sum^{i=u}{}_{i=1} \mathbf{S_i}\, \mathbf{L}^{*} / \mathbf{L_i}$$

Where: $\mathbf{T}$ = stand transpiration (mm day^{-1})

$\mathbf{S_i}$ = sap flow in the $_i$th stem (kg day^{-1})

$\mathbf{L}^{*}$ = leaf area index

$\mathbf{L_i}$ = leaf area of the $_i$th stem

Leaf area measurements

Most papers relating to sap flow measurements reported estimates of total leaf area and leaf area index. A number of different methods were used to achieve this:

1. The canopy was divided into 3 layers, and the leaf area in each layer was estimated from calculations of mean area per leaf, and a relationship between number of leaves per branch, and branch basal diameter. (Zhang *et al.*, 1999).

2. Maximum leaf area index was calculated by collecting falling leaves in containers of known surface area during the autumn fall. LAI determined

during experiment by measurement of canopy light interception. (Meiresonne *et al.*, 1999).

3. Leaf area determined by relationship between stem diameter and leaf area, found by destructive sampling of non-experimental trees. Good linear relationships were found, but repetitive sampling at intervals throughout the growing season showed that the actual relationship varied depending on time of year. (Hall *et al.*, 1998; Allen *et al.*, 1999)

Values of transpiration from poplar stands found by previous researchers are detailed in the following table:

Species	Location	Daily transpiration (mm / day)	Total growing season transpiration (mm / season)	Stand age (years)	Reference
P. tristis	Wisconsin USA	4.4 – 4.8	-	2 and 5	Hansen 1988
P. trichocarpa x P.deltoides	Bucks and Avon, UK	1 – 8	-	3	Hall et.al. 1998 Hall & Allen 1997
P. euramericana	N. Italy	-	158	4	Anselmi 1982
P. trichocarpa x P.deltoides	Washinton state USA	3.6 (av), 4.8 (max)	430 – 550	4	Hinckley et. al. 1994
P. deltoides	Michigan state USA	- -	128 365	2 3	Lui et.al. 1988

		-	603	6	Lui & Dickmann 1992
P. trichocarpa x P.deltoides	East Flanders, Belgium	1.9 (av), 5 (max)	320	13	Present study
P. tremuloides	Sascatchewan, Canada	5 – 6 (max)	400	70	Black et.al. 1996

From (Meiresonne *et al.*, 1999)

Maximum daily transpirations rates range from 5mm/day (13 year old trees, Flanders Belgium), to 8mm/day (3 year old trees, Avon UK). There is no daily transpiration given for trees growing in mediteranean climates. The value of seasonal transpiration given for 4 year old poplar growing in northern Italy is very low compared to other results which suggests either P. euramericana uses less water, or that the trees used to take these readings were not irrigated while other experiments were.

The Aims of this Thesis:

1 To investigate the physiological basis of productivity in hybrid poplar grown as short rotation coppice.

2 To investigate the genetic basis of these productivity traits and attempt to identify the quantitative trait loci (QTL) responsible for them.

3 To address the question "Will predicted rises in atmospheric CO_2 lead to increased water use in large scale poplar plantations in the future?"

Chapter 1:

Identifying Physiological Traits Associated with High Yield in *Populus*.

Introduction:

This chapter details a field experiment carried out at 2 contrasting sites in the UK. Physiological measurements were made on 5 contrasting poplar genotypes throughout the growing season and these were compared to the overall productivity of those genotypes.

The aims of this chapter were:

1. To establish physiological traits associated with high yield.
2. To compare physiology of poplars grown in different climates

Materials and Methods

Experimental sites

Two sites, established short rotation coppice trials, to assess the viability of a range of genotypes in SRC by the Forestry Commission in 1995 (Armstrong, 1997), were chosen for their diverse climatic conditions (Table 1.1), Plate 1.1 & 1.2.

Table 1.1: Contrasting climatic conditions at field sites. Soil Moisture and Accumulated temperature (Brecon & Cambridge) (Armstrong, 1997), Rainfall (Ian Tubby, Personal Communication), Antwerp data (William Deraedt, personal communication).

	Location	Soil moisture deficit (mm)	Accumulated temperature (day degree above 5.6°C)	Total Rainfall (mm) (March to November 1998)
Brecon	51°58' N 3°15' E	92.3	1624	26.3
Cambridge	52°10' N 0°05' E	196.2	1883	19.5
Antwerp	51°05' N 4°22' E	121.2	1610	

Soil type at the UK sites were classified into soil series and sub group by the Forestry Commission (Armstrong unpub.). The soil at Brecon was found to belong to the series "Milford", subgroup "Typical brown earth (541)". Cambridge soil was part of the "Windrush" series, subgroup "Pelo-calcareous alluvial gley soil (814)"

The sites were planted in randomised blocks containing 16 *Populus* genotypes with 3 replicated plots per genotype. Each plot contained 100 trees (10 x 10) spaced alternately at 1.5m and 0.75m between rows and 0.9m along the rows, giving a

planting density of almost 10,000 trees ha^{-1}. Four trees close to the centre of each plot were selected for detailed physiological measurements giving a replication of 12 trees per genotype. This study was conducted between June and September 1998 when the trees were in their third year of growth after initial coppicing. A large stepladder was used, enabling measurements to be taken from the most rapidly growing material at the top of the canopy.

Similar measurements were taken from trees at a site near Antwerp (Belgium 51°05'N, 4°22'E). This had been established using a similar protocol to the UK, but was in its second year of growth after coppicing.

Plate 1.1: View of Forestry Commission ETSU field trial at Trumpington, near Cambridge. Different species of coppiced poplar and willow trees planted in 10 x 10 blocks form a discernable patchwork effect. Trees in third year of growth after coppicing.

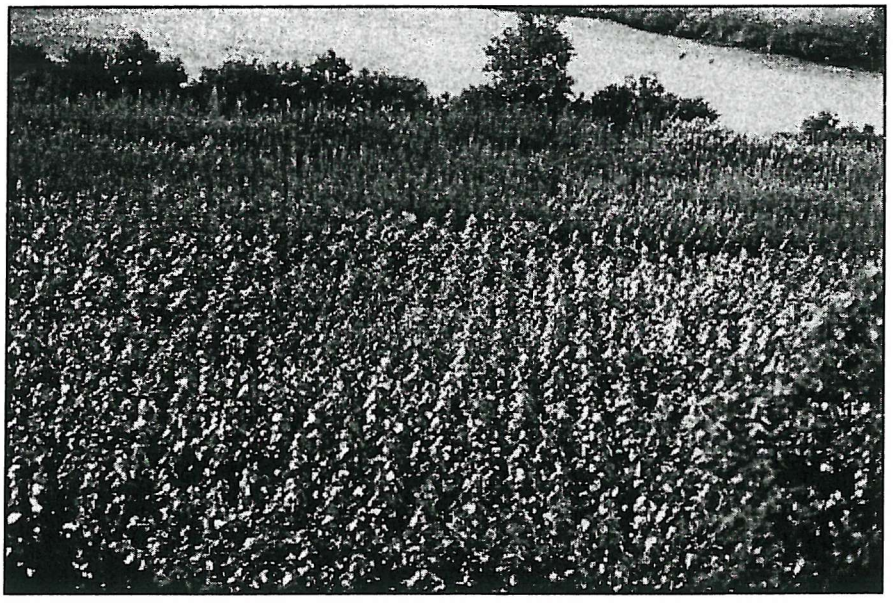


Plate 1.2: Ariel view of Forestry Commission ETSU field trial site at Trefeinon, near Brecon, Wales. Trees in third year of growth after coppicing. (Photo taken from nearby tree top).

Plant Material
Five of the sixteen available *Populus* genotypes planted at each site were selected for assessment due to their varied parentage, as shown in Table 1.2.

Table 1.2: Origins of experimental genotypes of *Populus*.

* Genotypes not yet released to commercial growers.
† (Steenackers *et al.*, 1992) ‡(Armstrong, Unpublished)

Genotype	Maternal parent	Paternal parent	Date of cross	
71.015/1 *	P. deltoides, S.333-44, Michigan, USA	P. trichocarpa, Unknown	1971	†
Beaupre	P. trichocarpa, V.235	P. deltoides, S.1-173		‡
Gaver	P. deltoides, S.71-3	P. nigra, Gibecq	1960	‡
Hoogvorst *	P. trichocarpa, V.235, Washington, USA	P. deltoides, S.620-225, Lower Peninsular, Michigan, USA	1969	†
Trichobel	P. trichocarpa, V.235	P. trichocarpa, V.24		‡

Measurements

Each site was visited three times during the growing season, with the exception of the site at Antwerp, which was visited once. On each visit the following measurements were made.

Leaf length / area relationships. A Licor 3000 (Licor Inc., Nebraska, USA) 'portable leaf area instrument' was used to measure the area and length of 120 leaves of each genotype, at each site (plate 1.3). The relationship between leaf

length and leaf area for each genotype at both sites was determined, allowing all future area measurements to be determined accurately from leaf length alone.

Leaf expansion rates (LER). Leaf lengths of eight of the newest fully unrolled leaves in each plot (2 samples per tree, averaged to give 12 reps per genotype) were measured and recorded using a paper ruler made from 1mm square graph paper (plate 1.4). The leaves were labelled with weather proof tags attached with soft string to the petiole, and re-measured after 3 days. Leaf expansion rate for individual leaves (LER, $cm^2\ day^{-1}$) was calculated from these measurements. The measurements were then combined to give mean rates of leaf expansion throughout the season.

Rate of leaf production. Rate of leaf production was assessed from counts of unrolled leaves made using the weatherproof tags as a marker.

Epidermal cell areas. Six fully mature leaves from trees not used for other measurements, were sampled from each plot at each site (18 per genotype per site) during September 1998. The petioles were cut with a razor blade under water to prevent wilting during the sampling procedure and the length of each leaf was measured in order to calculate leaf area. This was recorded on a glass microscope slide with a permanent marker along with the genotype identity of the leaf. An area of the adaxial leaf surface between the first and second primary vein branches was sprayed with acrylic clear lacquer (Halfords Ltd., Redditch UK). When dry the lacquer was removed from the leaf surface with clear sticky tape and placed onto the labelled microscope slide, for later analysis (plate 1.5). Individual cell size was measured using a 'camera lucida' and microscope (Leitz Wetzlar, Germany). This projected an image of the microscope slide onto a sheet of paper,

at a magnification of 180x. A Delta T image analyser was used to find the area of five randomly selected cells traced from the camera lucida image.

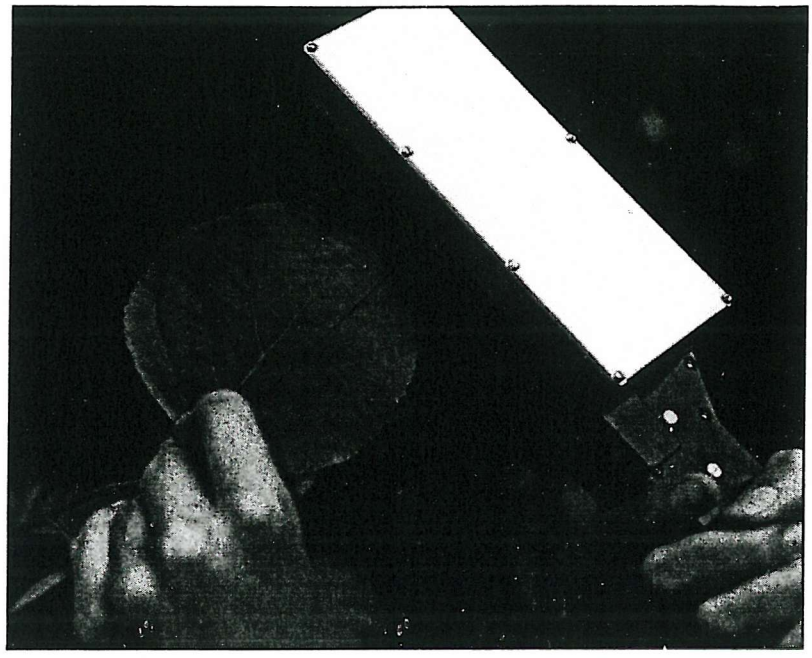


Plate 1.3: Using a Licor 3000 portable leaf are meter to construct leaf length / area relationships.

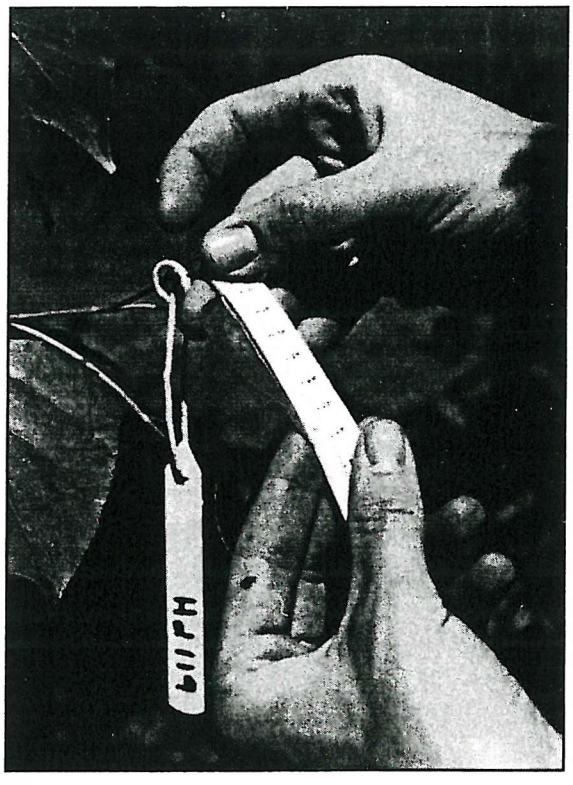


Plate 1.4: Using a paper ruler to prevent damage to delicate new leaves during measurement.

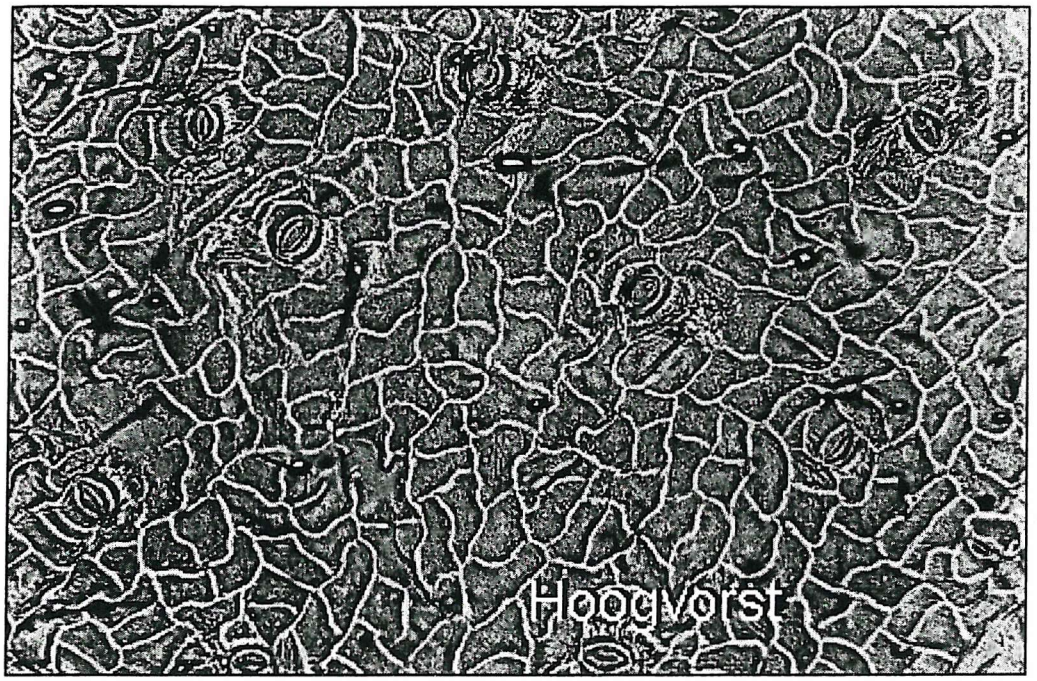


Plate 1.5: An example of an epidermal leaf imprint taken using acrylic spray lacquer. Slides such as this were used to measure stomatal index, stomatal density and epidermal cell density.

Plate 1.6: Photograph taken looking up through the canopy of Gaver. Leaf area index measurements were used to quantify canopy light interception.

Stomatal index & Stomatal density. The ratio of number of stomata to number of epidermal cells per unit area (stomatal index, Willmer & Fricker 1996) was found for each sample by counting the numbers of each cell type in a quarter of the projected image. Stomatal density (number of stomata per unit area) was found similarly using the same samples.

Epidermal cell density. The number of stomata and epidermal cells per unit area was found by counting cells in a known area of the microscope's field of view. Epidermal cell number per leaf was estimated using the area measurements based on the leaf length/area relationships found with the LiCor LA3000, multiplied by the epidermal cell density.

Canopy light interception. Measurements of photosynthetically active radiation (PAR) were taken using two Sunfleck Ceptometers (Delta T devices Ltd., Cambridge, UK).

Eight consecutive readings were taken at different positions around the centre of each plot, while eight readings were taken simultaneously from an area of ground with no tree cover, by a second operator. Measurements were always conducted under diffuse light conditions (full cloud cover, dawn or dusk) in order to eliminate the effects of solar elevation angle and canopy structure (Sunfleck Ceptometer user manual 1989, Appendix A) (plate 1.6).

Calibration of the two ceptometers was undertaken to allow direct comparison of their readings. Readings were taken simultaneously with both Ceptometers, held side by side, under a range of light intensities. The readings were plotted against each other in order to calculate the relationship between the two devices.

The fraction of PAR transmitted through the canopy τ was calculated by:

$$\frac{PAR(inside)}{PAR(outside)}$$

Then used to estimate the leaf area index 'L' of the plot:

$$L = -\frac{\ln \tau}{\mathrm{A}}$$

Where A is derived from a (the absorbtivity of leaves to light in the PAR band):

$$\mathrm{A} = 0.283 + 0.785a - 0.159a^2$$

Photosynthesis and Gas exchange measurements. An infra red gas analyser (PP systems IRGA, Hitchin, Herts, UK) was used during each visit to record rates of photosynthetic assimilation (A), stomatal conductance (g_s) and transpiration (E) of the newest fully expanded leaf on the main stems of four sample trees (plate 1.7). The atmosphere of the IRGA chamber attachment was set at ambient CO_2 concentration (~360ppm). A 12v battery operated quartz-iodide light attachment, (photon flux – 800 $\mu mol.m^{-2}s^{-1}$) was used to provide fully saturated light conditions for each measurement. Boundary layer resistance (r_b) was set to 0.3 m^{-2} s^{-1} mol^{-1}. Temperature within the chamber could not be set, but was monitored and recorded. Instantaneous water use efficiency (WUE) was calculated as A/E and stomatal limitation (St_L) was calculated as Ci / Ca (intercellular / ambient CO_2 concentration).

During the July field visit, further detailed measurements of A vurses intercellular CO_2 concentration (c_i) were taken (see appendix 3 for an explanation of A/ci curves). Photon flux was maintained at 800 μmol m^{-2} s^{-1} as before, and all other variables were set as for the ambient CO_2 measurements. Carbon dioxide concentrations were controlled within the chamber by a lap top computer linked to the IRGA, using a 'sparklet' bulb of compressed CO_2 (ISI, Vienna, Austria) attached to an automatic flow regulation system. A, g_s and E were measured at the following CO_2 concentrations: Ambient (360 μmol. mol^{-1}), 250, 50, 600, 800, 1000, and 1200 μmol. mol^{-1}, following the method of (Bryant *et al.*, 1998). A portable generator was used to maintain a constant power supply to the IRGA, lamp and computer.

Since air and leaf temperature varied between measurements a temperature-dependant biochemical model - Photosyn Assistant v.1.1 (Dundee Scientific, Dundee, UK) was used to interpret the A / c_i curves generated. Values of $V_{c\ max}$ and J_{max} were obtained as *in vivo* measures of Rubisco activity and electron transport capacity of the Thylakoid, respectively.

At the end of their three-year rotation (February 1999), the experimental sites at Brecon and Cambridge were harvested. Detailed yield measurements were made on the individual stools used in this experiment (12 stools per genotype at each site).

Plate 1.7: Making photosynthesis and gas exchange measurements using a PP systems IRGA.

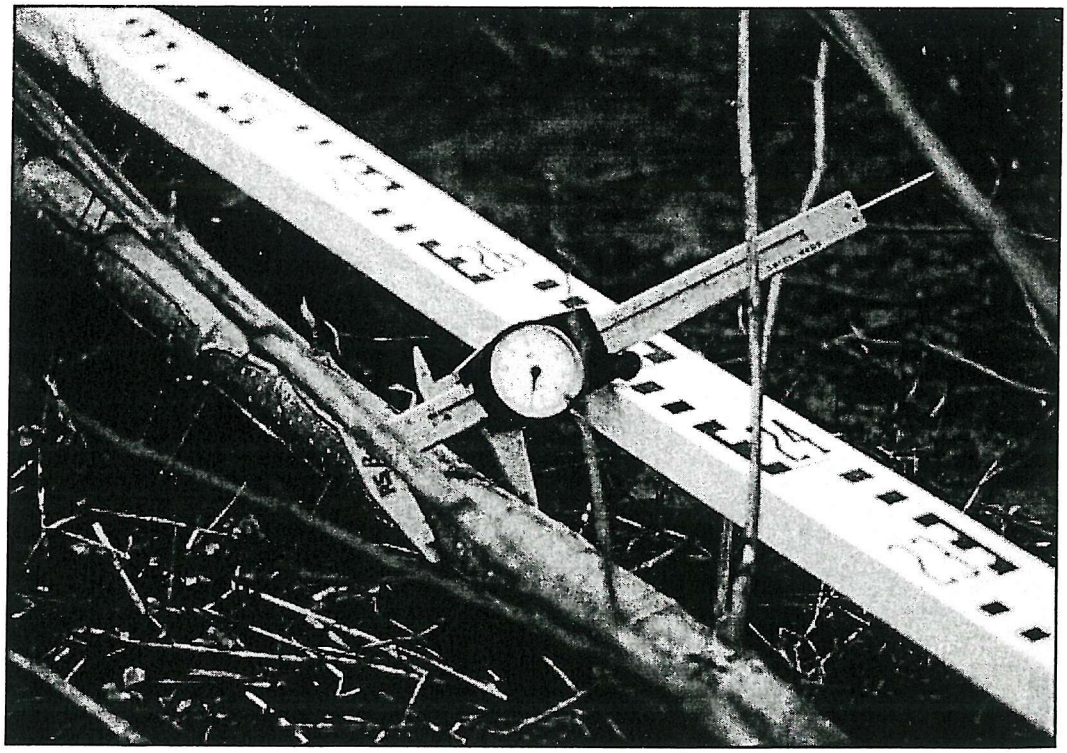


Plate 1.8: During harvest, tree height and mid height diameter were recorded and used to calculate stem volume using Huber's formula.

Stem Volume. Stem volume was measured using Huber's formula (Hamilton, 1975) (plate 1.8):

$$V = \frac{\pi d_m^{\ 2}}{40000} \times L$$

where: V = volume (m3)

L = length (m)

d_m = mid diameter (cm)

π = 3.1415927

Dry mass of harvested wood. Fresh weight of each harvested stool (including main stems and all side shoots) was measured straight after cutting, using a sling and balance in the field (plate 1.9). Each stool was then individually chipped (including all stems and shoots) into a polythene sack. The chippings were mixed in order to evenly distribute wood of different densities, then a sample (approx. 1kg) of the chips were taken and accurately weighed. The samples were individually bagged and removed to the laboratory for drying and weighing. Dry mass of each harvested stool was found using the following formula:

$$dm_s = \frac{fw_s}{fw_c} \times dm_c$$

Where: dm_s = dry mass of harvested stool

dm_c = dry mass of chippings

fw_s = fresh weight of harvested stool

fw_c = fresh weight of chippings

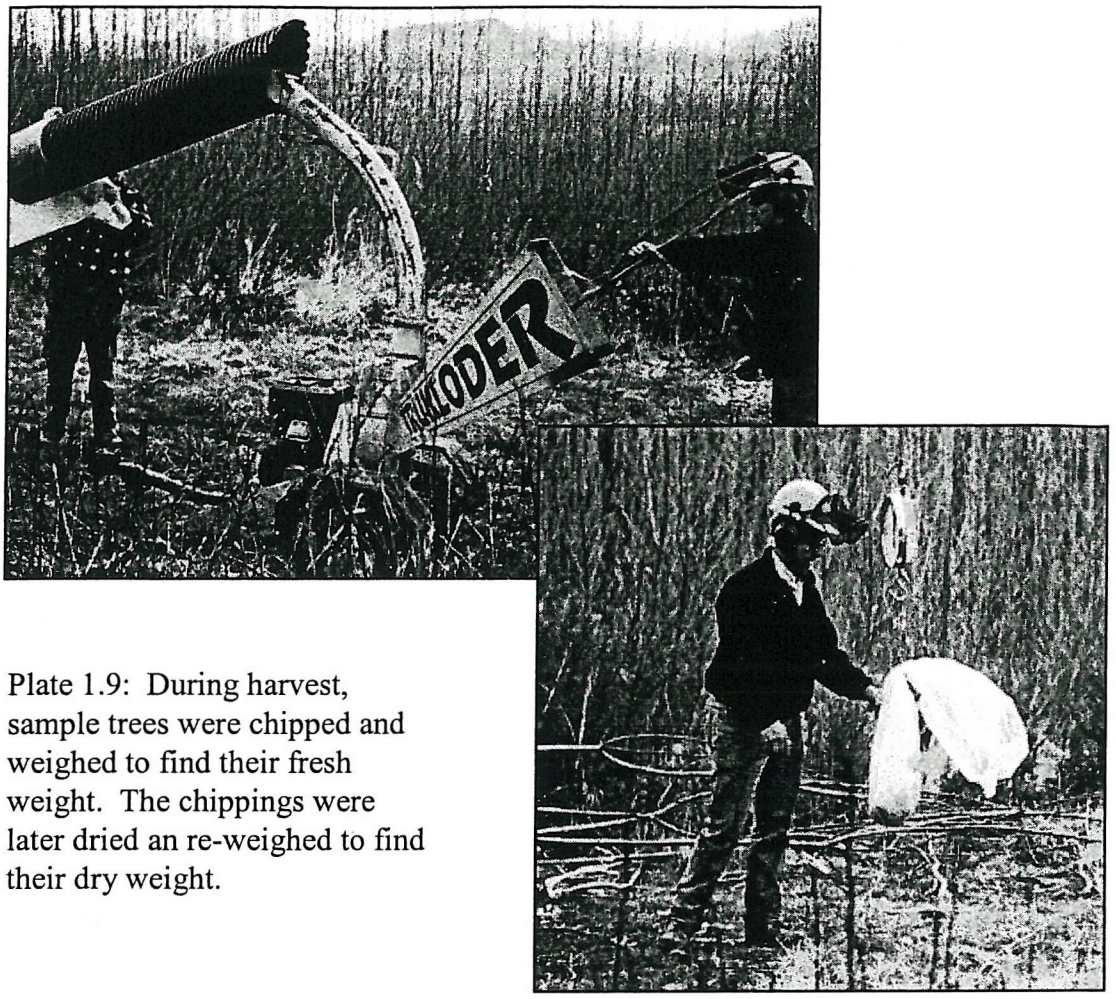


Plate 1.9: During harvest, sample trees were chipped and weighed to find their fresh weight. The chippings were later dried an re-weighed to find their dry weight.

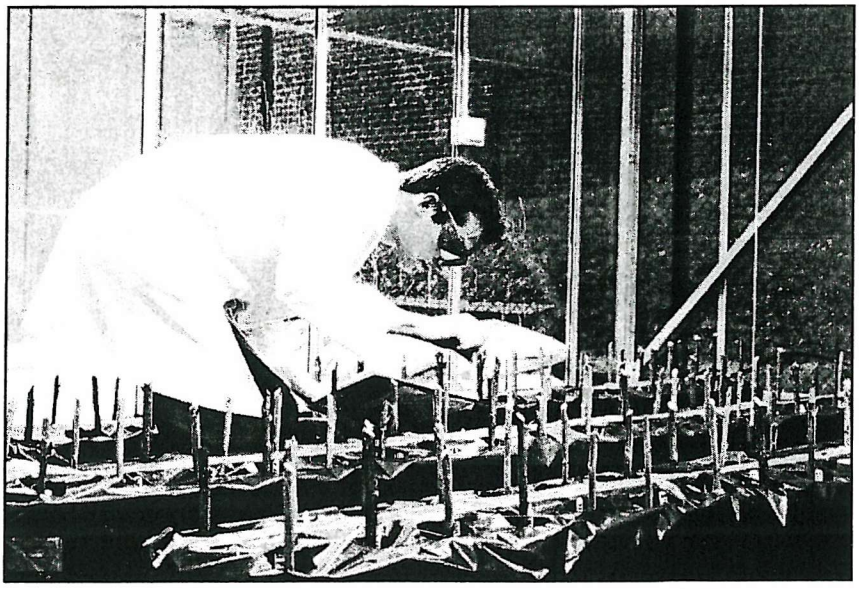


Plate 1.10: Poplar cuttings were planted in mini rhizotrons in a glass house in order to observe and measure root growth.

Glasshouse experiment to assess genotypic differences in root development.

Root production. A mini rhizotron experiment was set up under glasshouse conditions (plate 1.10). Fifteen 25cm cuttings (pencils) of each of the five genotypes examined in the field were planted into clear sided tubes (50cm deep, 15cm diameter). The tubes were sealed at the bottom with muslin and plastic netting, filled with general-purpose compost (William Sinclair Horticultural Ltd. Lincoln, UK) and placed in a randomised block design, on batons to allow drainage. An automatic irrigation system was set up, controlled by a Gardena 1060 water computer (Erin-Gardena Ltd., Letchworth UK), programmed to deliver 300mls of water to each pot twice a day. Sodium lamps were set on a 17 hour light cycle from 06:00 to 23:00, and the clear sides of the rhizotrons were covered with black plastic. Photon flux was always maintained above 150 μmol m^2 s^{-1}, and occasionally reached over 600 μmol m^2 s^{-1} on sunny days. Minimum temperatures ranged between 20°C to 22°C. Soil moisture was recorded daily using a rapitest moisture meter (Rapitest, Corwen, Wales) and converted to volumetric water content by calibration against an ML1 Theta probe (Delta T devices Ltd., Cambridge UK). Soil water content always remained above 0.25 m^3 m^{-3}.

Root elongation rate (RER). This was measured by marking the position of all visible root tips with a permanent marker on to acetate sheets taped against the clear sides of the rhizotron (plate 1.11), at the same time each day. At the end of the experiment (day 29), the acetate sheets were removed and the mean growth rates were measured using a ruler against the traced roots. The fastest growing root in each rhizotron was found, and used to calculate maximum 'root elongation rate' for each genotype.

Plate 1.11: Root elongation rate and root density were measured using acetate sheets overlaid onto the clear plastic window of the mini-rhizotron.

Plate 1.11Root density. The number of roots observed in each rhizotron was recorded and used to give an indication of root density for each genotype. This was investigated further by washing compost from the root systems of each individual plant, and measuring the total root length using an image analyser (Delta T devices Ltd., Cambridge, UK).

Dry mass of shoots and roots. (Excluding original hardwood cutting) were also measured, along with total leaf area; found by photocopying the freshly cut leaves, and analysing the copies with the Delta T image analyser.

Photosynthesis measurements. An infra red gas analyser (PP systems IRGA, Hitchin, Herts, UK) was used to take instantaneous measurements of photosynthetic assimilation for each plant. Readings were taken between 10:00 and 15:00 hours during the period when stomata are fully open. A 12v battery operated quartz-iodide light attachment, (photon flux – 800 μmol m^{-2} s^{-1}) was used to provide fully saturated light conditions. All IRGA variables were set as previously described for field measurements.

Results

The two UK sites, one in Wales and one in England, contrast markedly in temperature, rainfall and soil moisture deficit, as illustrated in Table 1.2. These sites were chosen for this study as they represent 'extremes' in the UK where poplar is likely to be grown in SRC energy forestry (Armstrong, 1997). In particular, rainfall during the experimental period, was approximately 25 % greater at the Brecon site, whilst accumulated degree-days were greater at the Cambridge site. Figure 1.1 shows the biomass yield data for the five genotypes at two contrasting sites in the UK. Large differences in the performance of genotypes were apparent between sites ($P < 0.001$), although at both sites, the *P. trichocarpa* x *P. deltoides* genotypes out-performed the other genotypes, with one exception. That was for the unusually low yield of Beaupre at the Cambridge site, thought to result from a heavy infestation by the poplar rust, *Melampsora* (Lonsdale & Tabbush, 1998). Gaver (*P. nigra* x *deltoides*) gave relatively low yields at both sites, suggesting that there may be only limited conditions where this genotype is suitable for large-scale planting in the UK. One other consistent effect was that higher yields were recorded at the Cambridge site, despite lower rainfall. For each genotype, the relationship between leaf length and leaf area was constructed; enabling rapid measurements of leaf length to be used to estimate leaf area for each genotype and at each site. The data in Figure 1.2 reveal these relationships, where a power curve gave a better fit than a linear relationship for all genotypes except Gaver. Results from Brecon indicate leaves had a larger area for a given length than those at Cambridge, providing strong evidence that for all five genotypes, leaves were of a more cordate shape at the Brecon site. Figure 1.3 compares leaf production and leaf expansion during July 1998. There was no significant

difference between sites or site genotype interaction ($P > 0.05$) so the data from both sites have been combined in this figure to simplify the comparison. ANOVA showed a significant difference between genotypes for the expansion rates of young leaves (dotted bars, $P < 0.001$). Leaves of Hoogvorst exhibited the highest expansion rate, averaging over $8cm^2$ per day, and Gaver the lowest with between 2 and 4 cm^2 per day. Leaf production during June / July (Figure 1.3, solid bars) also showed significant genotype variation ($P < 0.001$). Gaver produced leaves at the fastest rate (> 0.2 per day), with Beaupré and Hoogvorst, the slowest at less than 0.15 leaves per day.

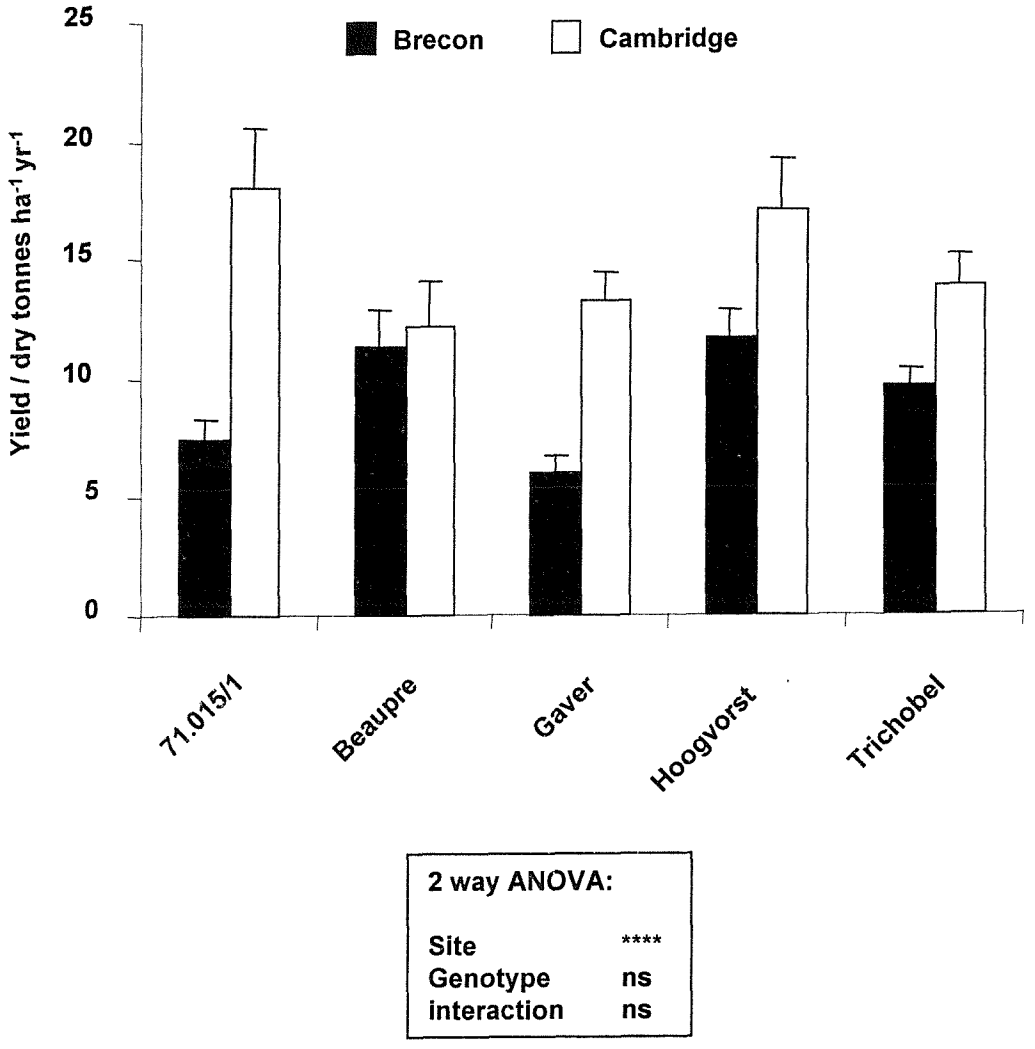


Figure 1.1: Mean annual increment of dry mass from five *Populus* genotypes harvested from Brecon and Cambridge, February 1999. ANOVA **** $P < 0.001$. Bars represent standard errors.

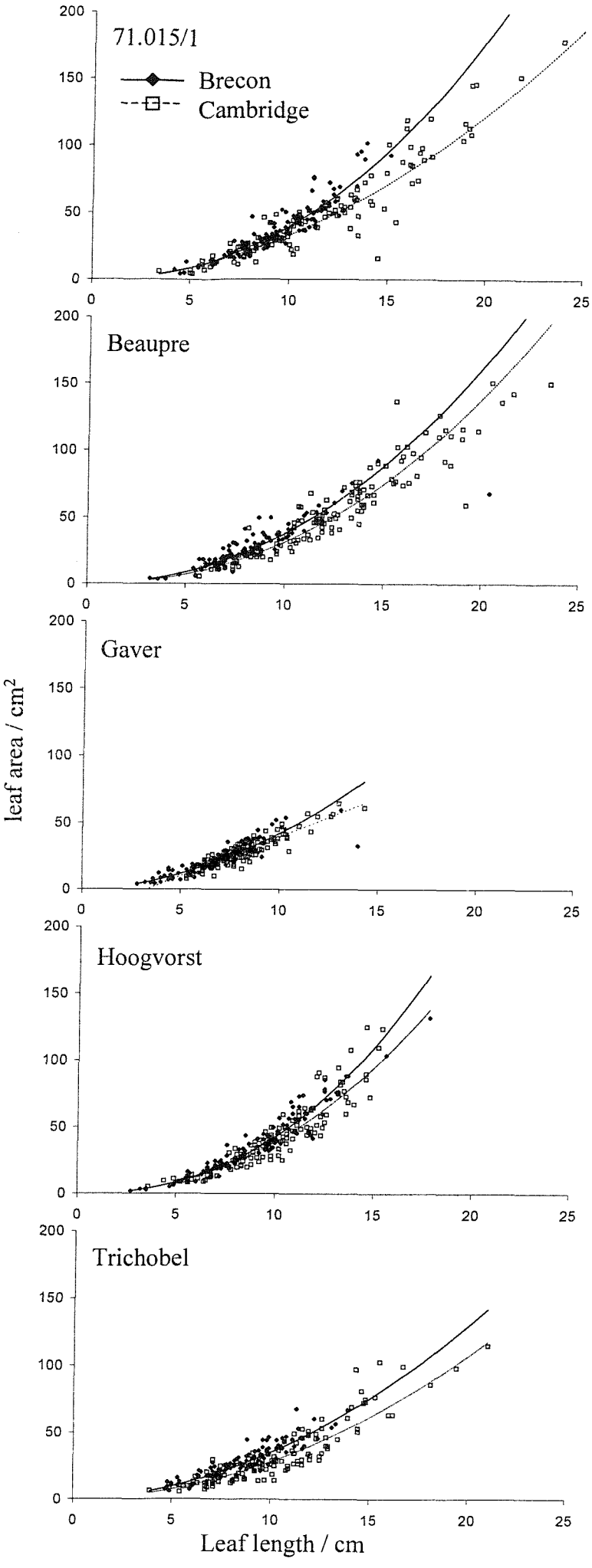


Figure 1.2: Relationships between leaf length and leaf area for five *Populus* genotypes. Measurements taken from trees at Brecon and Cambridge, May 1998.

Brecon:

71.015/1, $y=0.2579x^{2.1848}$ $R^2 = 0.90$;
Beaupré, $y=0.2998x^{2.0988}$ $R^2 = 0.85$;
Gaver, $y=0.6112x^{1.8362}$ $R^2 = 0.85$;
Hoogvorst, $y=0.1983x^{2.3324}$ $R^2 = 0.95$;
Trichobel, $y=0.4549x^{1.8874}$ $R^2 = 0.83$.

Cambridge:

71.015/1, $y = 0.4203x^{1.8961}$ $R^2 = 0.87$;
Beaupré, $y = 0.2223x^{2.1483}$ $R^2 = 0.89$;
Gaver, $y = 5.8878x^{-19.461}$ $R^2 = 0.83$;
Hoogvorst, $y = 0.2424x^{2.2034}$ $R^2 = 0.90$;
Trichobel, $y = 0.3296x^{1.9311}$ $R^2 = 0.83$;

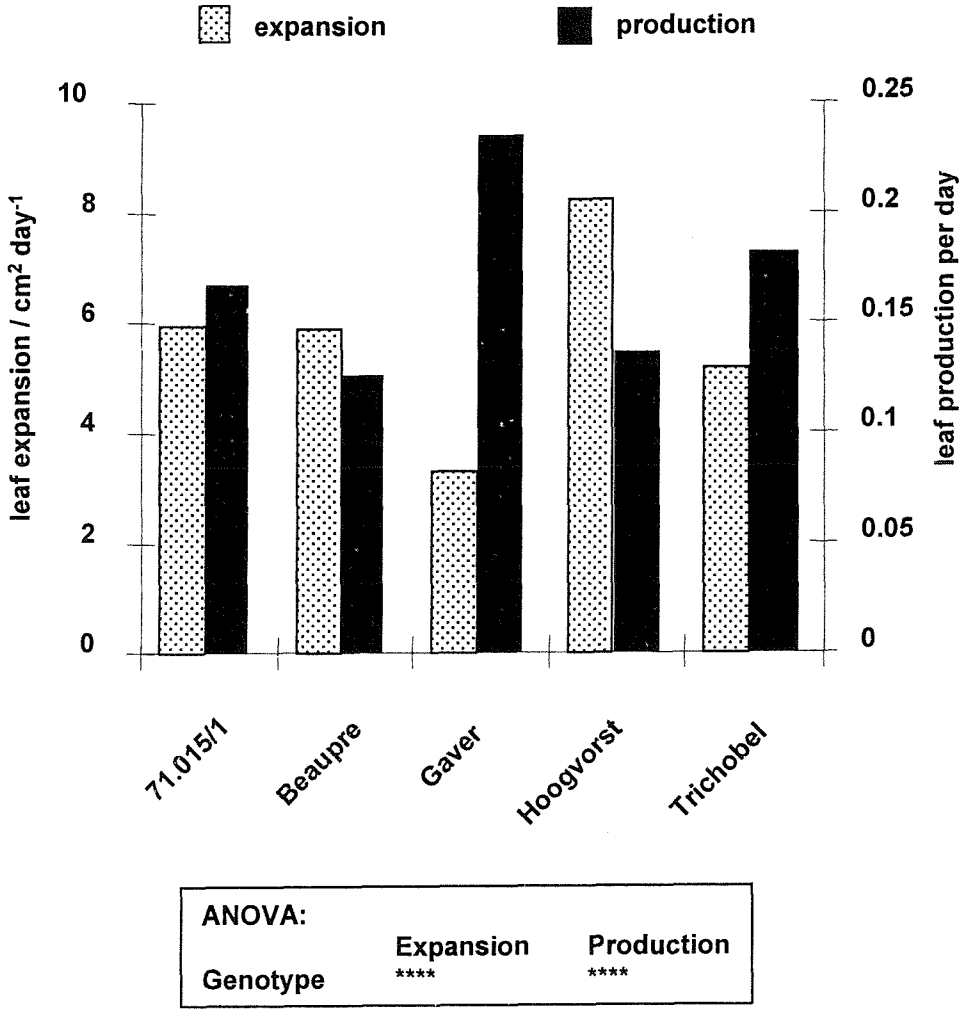


Figure 1.3: Comparison between rates of leaf expansion (dotted bars) and number of leaves produced per day (solid black) for five *Populus* genotypes. ANOVA: expansion **** $P < 0.001$; production **** $P < 0.001$. Brecon and Cambridge data combined since there was no significant site effect or interaction for original data.

There was a significant difference in cell areas for different genotypes ($P < 0.001$, Figure 1.4). Trichobel had the largest leaf cells (around 1800 μm^2) while Gaver had the smallest at around 1000 μm^2. There was a significant effect of site, and a significant site x genotype interaction; Gaver, Hoogvorst and Trichobel appeared larger at Cambridge, while Beaupré and 71.015/1 had significantly larger cell areas at Brecon ($P < 0.001$). Figure 1.5 illustrates the relationship between cell number and leaf area and that between cell size and leaf area. From this it is clear that cell number may be a good predictor of leaf size, whilst cell area appeared a less reliable indicator.

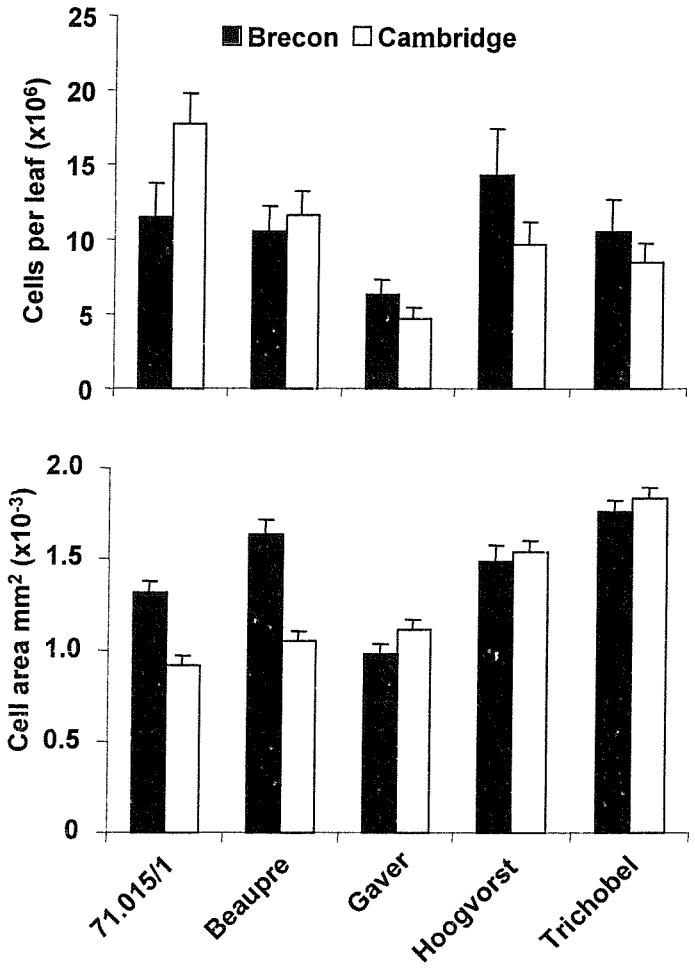
Stomatal index & stomatal density are shown in Figure 1.6 (a & b) where a significant effect of genotype ($P < 0.05$), site ($P < 0.01$), and an interaction ($P < 0.005$) on stomatal index. 71.015/1 and Beaupré were most affected by site, with stomatal index significantly higher ($P < 0.05$) at Cambridge than at Brecon. Stomatal density was not significantly affected by site ($P > 0.05$), but there was a significant effect of genotype ($P < 0.001$) and a significant interaction ($P < 0.001$). Gaver and Hoogvorst were not affected by site, but Beaupre and 71.015/1 had approximately twice the number of stomata per square mm at Cambridge than they had at Brecon (Figure 1.6). There was no significant difference in leaf area index (LAI) values between genotypes at Brecon and Cambridge after 3 years growth ($P > 0.1$, data not shown). However, LAI values for the same genotypes grown at Antwerp for 2 years, did show a significant difference ($P < 0.001$, Figure 1.7). At this site, Hoogvorst had the highest and Gaver the lowest values of LAI.

When analysing the results of the gas exchange measurements it was found that data collected from Cambridge was inconsistent with previous findings from the

glass house, and with data collected at the same time from Brecon. No significant differences ($P > 0.05$) were found between the genotypes for any measure of gas exchange due to large standard errors within the data sets. However, analysis of the data from Brecon (table 4) revealed significant differences between genotypes for A ($P < 0.05$), WUE ($P < 0.05$) and St_L ($P < 0.001$), but not for g_s ($P > 0.05$). Computer analysis of the A/Ci curves from Brecon (figure 1.8) revealed that values of $V_{c\,max}$, shown in table 3, were significantly different ($P < 0.05$) between genotypes, while values of J_{max} were not ($P > 0.05$).

a)

b)



2 way ANOVA:	a) cell no.	b) cell area
Site	ns	****
Genotype	ns	****
Interaction	ns	****

Figure 1.4: a) Mean number of epidermal cells per leaf for five *Populus* genotypes, estimated from epidermal cell imprints taken from trees at Brecon & Cambridge, July 1998. Bars show standard errors, ANOVA (ns) not significant. b) Mean epidermal leaf cell areas for the same genotypes, calculated from the same epidermal imprints. ANOVA *** $P < 0.001$.

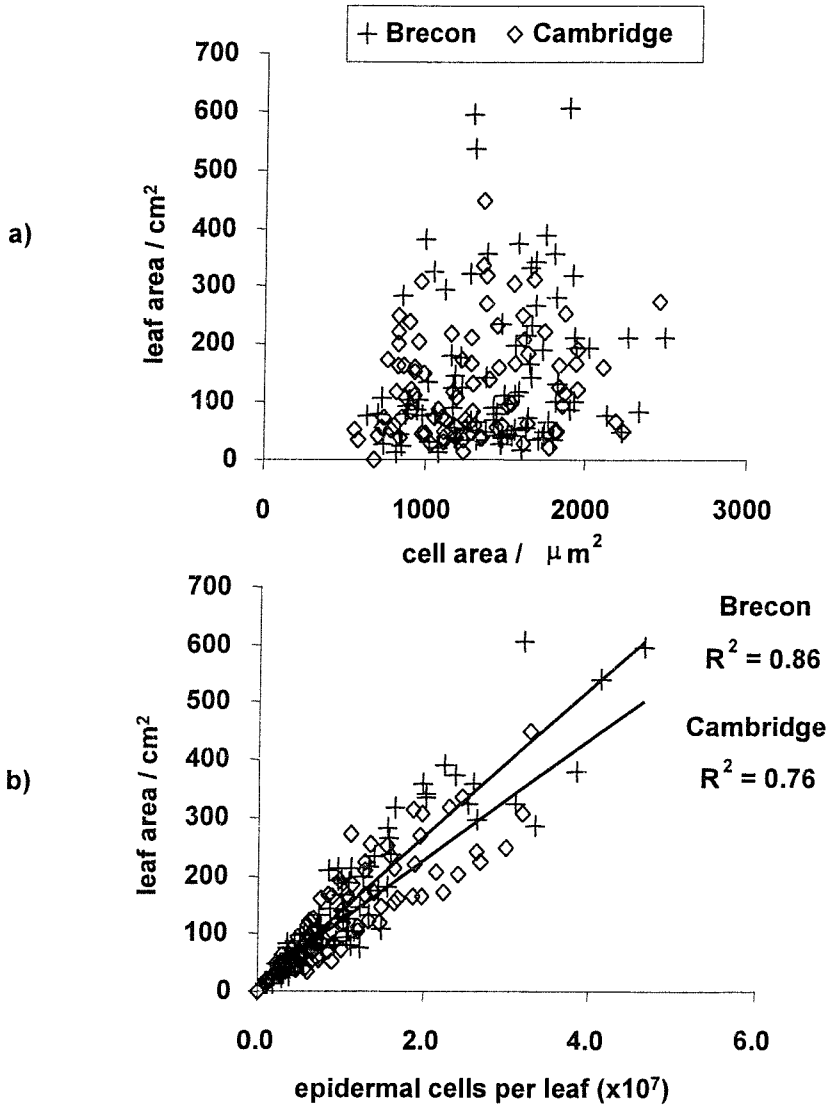


Figure 1.5: a) Leaf area plotted against epidermal cell size shows no correlation. b) Epidermal cell number plotted against surface area of leaf from which epidermal imprint was taken. All genotypes combined to show correlation between the two measurements. $R^2 = 0.86$.

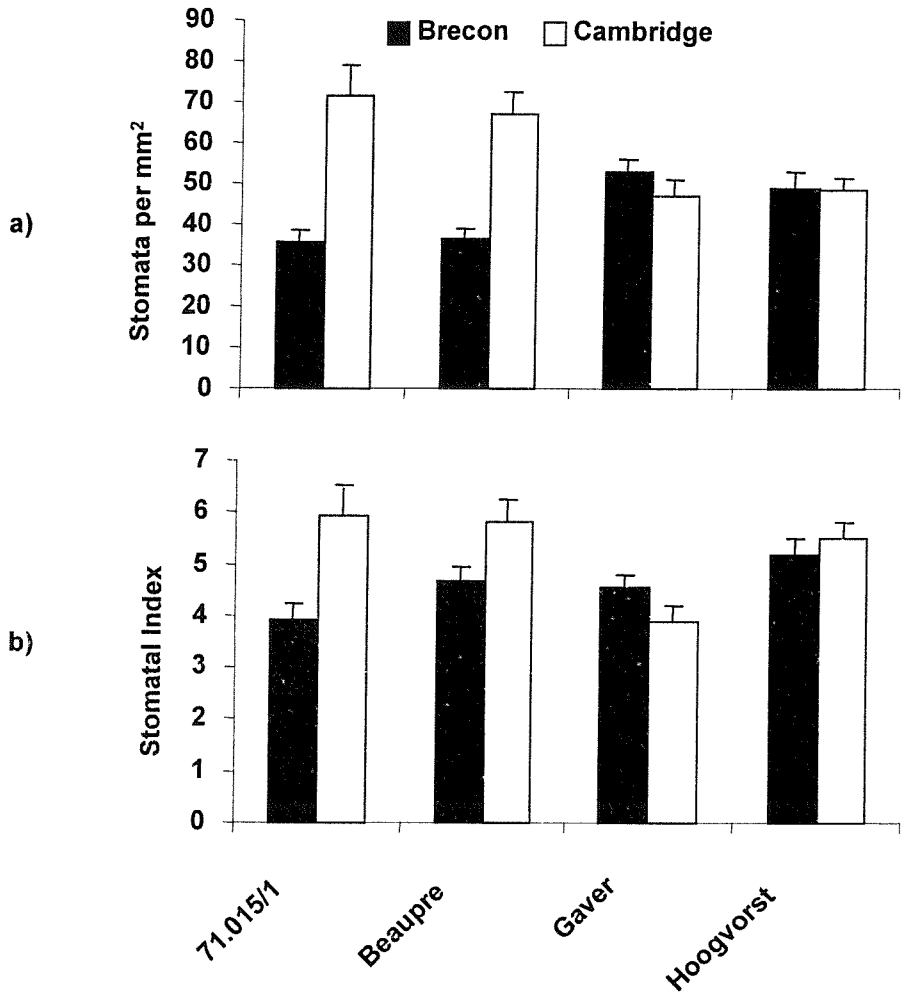


Figure 1.6: Mean values of stomatal density (a) and stomatal index (b), calculated from epidermal cell imprints for four *Populus* genotypes, (no data for Trichobel due to absence of stomata on adaxial leaf surface). Bars show standard errors. ANOVA ns - not significant, * P<0.05, ** P<0.01, *** P<0.005, **** P<0.001.

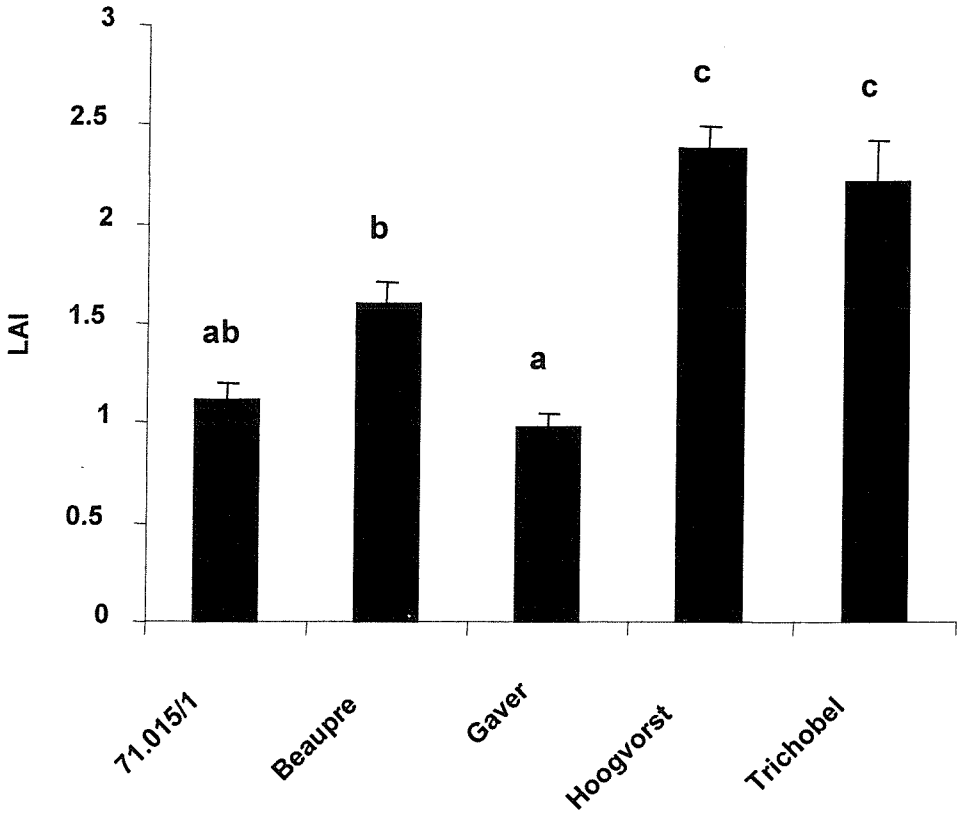


Figure 1.7: Mean values of leaf area index (LAI) five *Populus* genotypes, taken from Antwerp, September 1998. One way ANOVA **** $P < 0.001$, letters in lower case show results of Tukey post hoc analysis, bars show standard errors.

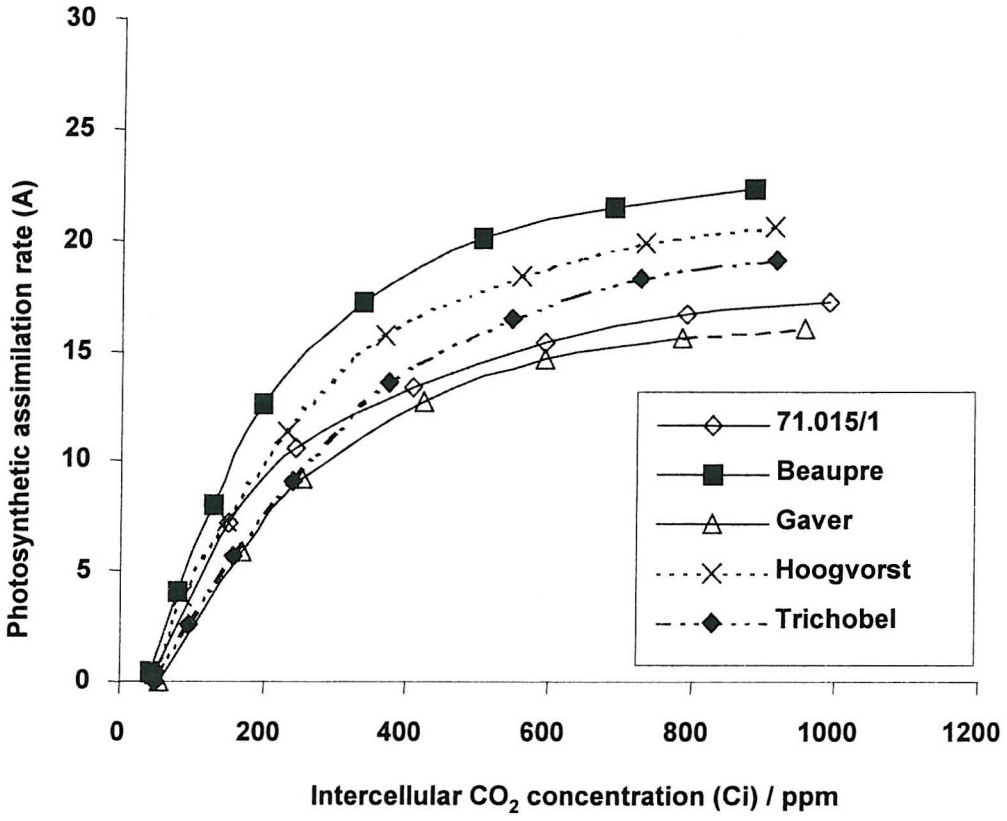


Figure 1.8: Examples of typical A/Ci curves obtained from Brecon, July 1998 for five *Populus* genotypes.

Table 1.3: Mean values of $V_{c\,max}$ and J_{max} from temperature dependant *A* / c_i curve analysis with Photosyn Assistant v.1.1 (Dundee Scientific, Dundee, UK). One way ANOVA indicates that there is a significant difference between genotypes for $V_{c\,max}$ ($P < 0.05$), but not for J_{max} ($P > 0.05$). Letters in superscript show results of a Tukey post hoc analysis. Data from Brecon only.

	$V_{c\,max}$	± standard error	J_{max}	± standard error
71.015/1	46.2 ^{ab}	1.14	163	21.07
Beaupre	52.9 ^{b}	1.34	221	12.42
Gaver	42.5 ^{a}	2.45	156	16.09
Hoogvorst	51.8 ^{b}	2.22	215	27.71
Trichobel	50.0 ^{ab}	1.65	151	16.07
ANOVA	✳		ns	

Table 1.4: Photosynthesis and Gas exchange data from Brecon, July 1998.
A – photosynthetic assimilation, $\mathbf{g_s}$ – stomatal conductance, **WUE** – water use efficiency, $\mathbf{St_L}$ – stomatal limitation. ANOVA significance: ✳ $P < 0.05$, ✳✳✳ $P < 0.001$, ns not significant. Superscripts show results of LSD post hoc tests.

	A ($\mu mol.m^{-2}.s^{-1}$)	$\mathbf{g_s}$ ($\mu mol.m^{-2}.s^{-1}$)	**WUE**	$\mathbf{St_L}$
71.015/1	10.70 ^{ab}	357.16	0.00598 ^{b}	0.680 ^{bc}
Beaupré	12.56 ^{b}	281.16	0.00637 ^{b}	0.642 ^{c}
Gaver	9.16 ^{a}	370.50	0.00421 ^{a}	0.778 ^{a}
Hoogvorst	11.36 ^{b}	365.33	0.00550 ^{b}	0.699 ^{bc}
Trichobel	9.08 ^{a}	275.33	0.00500 ^{ab}	0.723 ^{ab}
ANOVA	✳	ns	✳	✳✳✳

Work in the mini-rhizotron revealed that the five genotypes differed significantly in root length ($P < 0.005$) (Figure 1.9). A post hoc Tukey test showed that the principal difference lay between Hoogvorst and all the others, except Trichobel, ($P < 0.05$). These genotypes had a greater total root length than the others. Root elongation rate (RER) was also significantly different across the genotypes with Hoogvorst exhibiting the fastest growth rate, although only Beaupre roots grew significantly slower than those of Hoogvorst, as shown by the post hoc Tukey test. Both root and shoot dry matter production differed significantly between genotypes ($P < 0.001$, Figure 1.10). A Tukey post hoc analysis showed that Hoogvorst produced significantly more biomass in shoots ($P < 0.05$), and roots ($P < 0.05$) than all of the other genotypes. Trichobel produced more shoot biomass than 71.015/1 and Beaupré ($P < 0.05$), but was similar to these genotypes in root biomass production. Total leaf production (figure 1.11a) was highest for Gaver, being significantly greater than all other genotypes except Hoogvorst ($P < 0.001$). However, this bore no relationship to total leaf area, where Gaver was as low as all other genotypes except Hoogvorst which had almost twice as much leaf area as any of the others (fig 1.11b). There were no significant effects of genotype on the partitioning of biomass between roots and shoots.

Rate of photosynthetic assimilation was not significantly different between genotypes ($P>0.05$), and as such bore no relationship to biomass production (fig. 1.12a). However, total photosynthetic assimilation (rate of photosynthesis per unit area x total leaf area) did show a good correlation with biomass production ($R^2 = 0.7$) (fig. 1.12b).

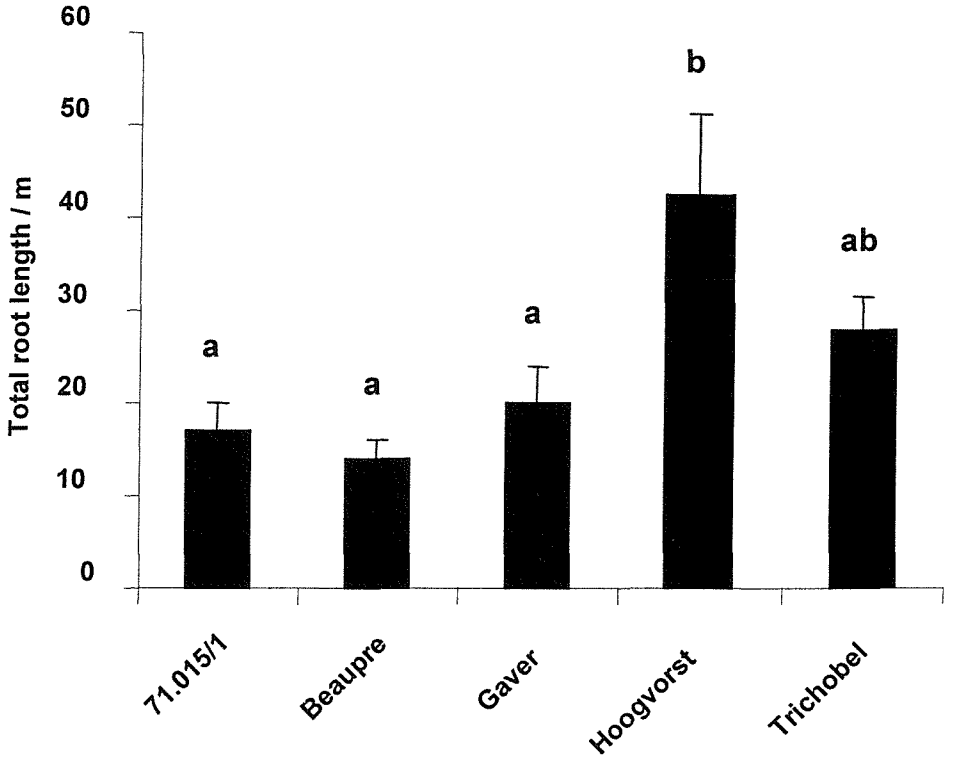


Figure 1.9: Mean total root length after 30 days growth of five *Populus* genotypes grown in mini rhizotrons under glass house conditions. One way ANOVA ** $P < 0.01$. letters in lower case show results of Tukey post hoc analysis, bars show standard errors.

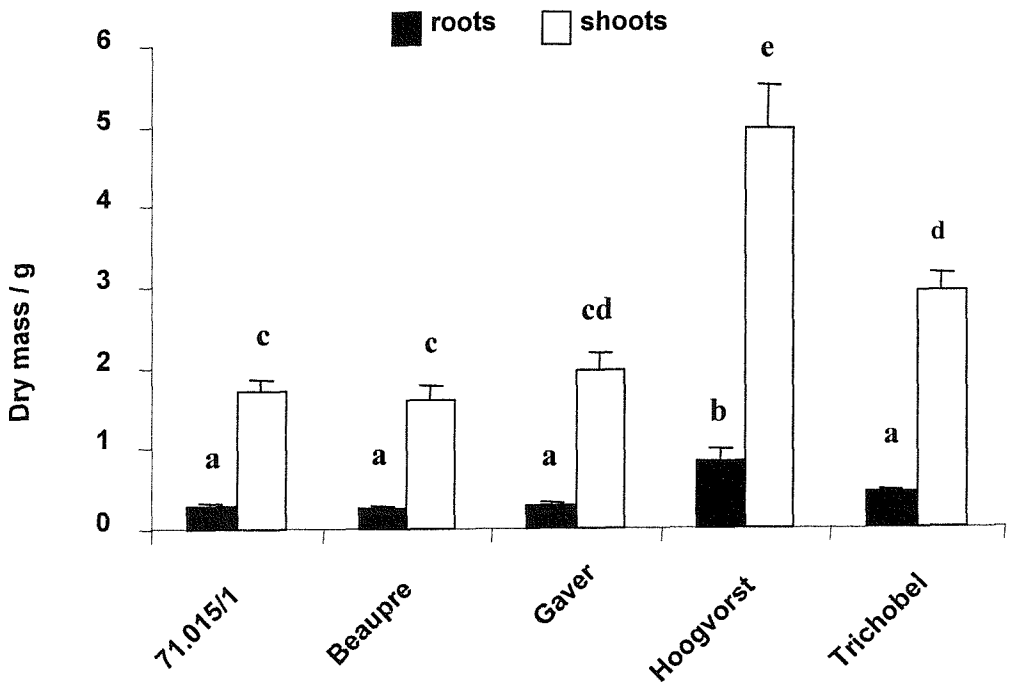


Figure 1.10: Mean dry mass of roots and shoots of five *Populus* genotypes grown in mini rhizotrons under glass house conditions. Letters in lower case show results of Tukey post hoc analysis, bars show standard errors.

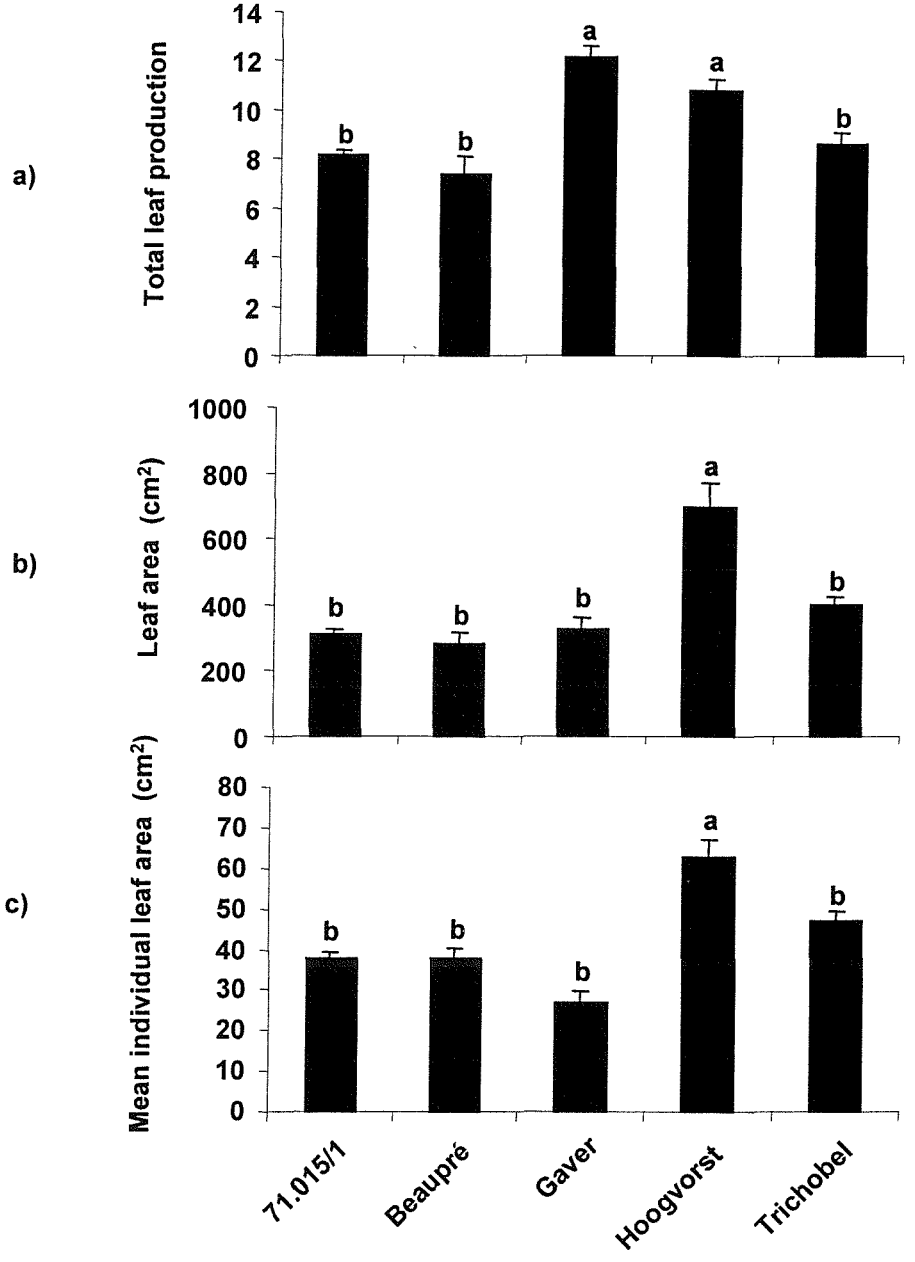


Figure 1.11: a) Mean total leaf production, b) mean total leaf area and c) mean individual leaf area (total leaf area / number of leaves) of five *Populus* genotypes grown in mini rhizotrons under glass house conditions. ANOVA **** $P < 0.001$. letters in lower case show results of Tukey post hoc analysis, bars show standard errors.

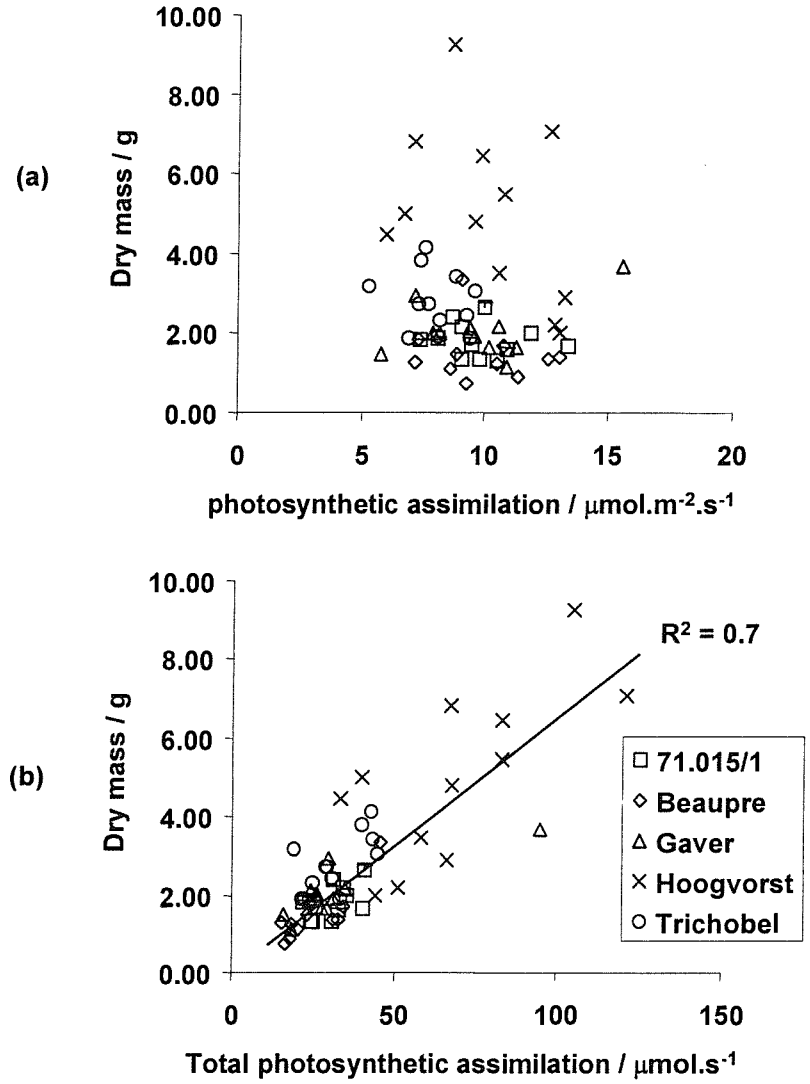


Figure 1.12: Relationships of shoot dry mass with a) rate of photosynthesis per unit leaf area, and b) total photosynthetic assimilation per plant (rate of photosynthesis per unit leaf area x total leaf area) for five *Populus* genotypes grown in mini rhizotrons under glass house conditions.

Discussion

Biomass yields from the field and glasshouse experiments support previous findings that *P. trichocarpa* x *deltoides* hybrids (Beaupre and Hoogvorst) consistently out-perform other genotypes (Heilman & Stettler, 1985; Ceulemans *et al.*, 1992; Souch & Stephens, 1998). One exception was the poor performance of Beaupré in the glasshouse and at Cambridge compared to its performance in the field at Brecon. However, it is likely that this was the result of a rust infection at the Cambridge site and poor quality plant material which came from a plantation that had been severely affected by rust (*Melampsora* spp.) in the previous year. (All other genotypes used in the glasshouse study were obtained from healthy nursery stock). Yield in the field varied between 5.8 and 17.8 t ha^{-1} y^{-1}, values within the range reported by Hansen (Hansen, 1991) for other field studies, with yield at Cambridge appearing consistently higher than at Brecon. Many environmental factors varied considerably between the field sites. However, it is likely that the warmer climate and unlimited water supply from the high water table on the flood plain of the river Cam were largely responsible for the consistently higher yields obtained from the Cambridge site. This was counter to expectations since the site was chosen for its low rainfall compared to Brecon.

Only one other published study has considered the productivity of the highest yielding genotype Hoogvorst, and in their intensive SRC trial Deraedt and Ceulemans (Deraedt & Ceulemans, 1998) also found that this genotype out-performed sixteen other *Populus* genotypes. Thus, there is strong evidence to

support the idea that Hoogvorst represents a highly desirable genotype, with large productivity on at least three sites and in a glasshouse experiment.

The aim of the work reported here was to identify, at a physiological level, the factors influencing productivity of poplar when grown in a multi-stemmed coppice. The relationships between particular leaf-level physiological traits and productivity were most clearly distinguishable between Hoogvorst and Gaver. Hoogvorst (*trichocarpa* x *deltoides*) demonstrated its enhanced productivity in both the field trial and the glasshouse experiment, whilst Gaver (*deltoides* x *nigra*) produced consistently lower yields. The exponential relationships constructed for leaf length and leaf area allowed for quick and convenient measurements of leaf area in the field. The relationship curves fitted the data with values of R^2 ranging from 0.83 to 0.95. Interestingly, similar measurements taken from an identical field trial at Cambridge revealed different relationships for each genotype, indicating that leaf morphology is altered by environment as shown in *Populus* by Dunlap and Stettler (Dunlap & Stettler, 1998) for wet and dry sites.

Several pieces of evidence suggest that leaf area development is an important trait for productivity in *Populus*. Although this confirms earlier work on single-stem *Populus* (Ridge *et al.*, 1986; Ceulemans *et al.*, 1994), here we have analysed the basis of this leaf area effect in greater detail. For example, a comparison between figure 1.3 and figure 1.1 shows clearly that rapid expansion rates of individual leaves were more important than the production rate of leaves. The effect of this can also be seen from the Antwerp study where leaf area index was highest in Hoogvorst and lowest in Gaver again corresponding to trends in yield. Values of LAI were in general, surprisingly low at between 1.5 and 2.5 (Ceulemans reports LAI of 10 and above for single stem short rotation intensive culture (SRIC) poplar

in its third year of growth (Ceulemans, 1990)). The method used to determine LAI from intercepted PAR involves assumptions about the random arrangement of leaves in the canopy that may not be valid in an SRC crop. Gower *et al.*, (Gower *et al.*, 1999) have suggested that non-randomly distributed or 'clumped' canopies may result in the underestimation of LAI and could explain the low values reported here. Interestingly at Brecon and Cambridge, there were no significant genotype differences in LAI (data not shown). This was unexpected, but perhaps reflects the fact that the canopy in year 3 was entirely closed whereas the year 2 canopy at the Antwerp site was still developing. In the glasshouse study the importance of leaf area development was confirmed with Hoogvorst displaying significantly larger root and shoot biomass associated with the production of large individual leaf areas (Figure 1.9). The data obtained from epidermal imprints showed a good correlation between large leaves and high numbers of epidermal cells, as suggested by Van Volkenburgh and Taylor (Van Volkenburgh & Taylor, 1996). Epidermal imprints were taken from a range of different sized leaves and it is only when the epidermal cell number is plotted against the corresponding leaf area that the significance of this relationship can be seen (figure 1.5). Conversely, epidermal cell area was seen to consistently fall within a relatively small range of values (typically between 1.0×10^{-3} and 2.0×10^{-3} mm ^{2}), irrespective of total leaf area (Figure 1.4b). There were significant differences between genotypes for cell expansion (data shown in Figure 1.4b), which reflect differences in productivity to a limited extent but appear to bear no relationship to final leaf size. In conclusion, large cell size may indicate the potential of a genotype for enhanced productivity, but increased cell number would appear to be a better predictor of final leaf size and hence, stemwood production. This perhaps in part reflects the difference between

genetic and environment effects on the processes of cell expansion and production. Cell expansion is a largely biophysical process, determined by water uptake into cells and the relationship of this to vacuolar cell solutes and biophysical changes in wall structure (Cosgrove, 1999). This process is highly sensitive to environmental variation, including changes in water supply (Davies & Zhang, 1991), light (Van Volkenburgh & Cleland, 1980), nitrogen (Taylor *et al.*, 1993) and atmospheric carbon dioxide (Ranasinghe & Taylor, 1996). In contrast, cell production is determined by metabolic events that act to regulate the cell cycle and these are more likely to be tightly controlled by gene expression and likely to be less sensitive to environmental variation. This idea is confirmed by a recent study on the effects of water deficit on cell cycle activity, where cell production was sensitive, but only following long-term 'chronic' exposure to the drought treatment (Schuppler *et al.*, 1998).

It is interesting to consider whether 'leaf cell number' has any value as a trait for selection of productive *Populus* genotypes. Dickmann and Keathley (Dickmann & Keathley, 1996) have described the concept of a poplar genotype for enhanced yield and differentiate between the use of 'physiological' and 'morphological' traits in such a concept. The former are problematic since the appropriate 'rate limiting' steps in any physiological process are often difficult to identify whilst the latter may provide much more robust measures. The genotype for poplar includes several shoot morphological characteristics related to stemwood yield, including leaf area. Riemenschneider *et al.*, (Riemenschneider *et al.*, 1994) also acknowledged the high heritability of leaf morphology traits in their study of 166 genotypes of black cottonwood (*Populus trichocarpa*). However, they were unable

to show that selection was improved over and above the use of plant height alone as a selection trait. In traditional forest breeding, height has been the major trait used for selection in nursery trials for obvious reasons; it is easily assessed and may be used on thousands of seedlings at the initial screening stage. Despite this, the advent of advanced molecular genetic techniques and the availability of molecular genetic maps for several forest tree species is beginning to demonstrate that traits for vegetative growth may be identified as QTL (quantitative trait loci), suggesting that 'molecular markers for yield' may one day replace traditional assessments of plant height in tree improvement programmes. Marques *et al.*, (Marques *et al.*, 1999) have identified several QTL for vegetative traits in an AFLP linkage map of *Eucalyptus* spp., including nine QTL for rooting and four for the production of stable adventitious roots. In *Populus* the molecular genetic map is advanced, with current work focussed on establishing microsatellite markers to cross species boundaries (Bradshaw *et al.*, 1994). This progeny has already been used to identify QTL for leaf characteristics, including leaf size and shape (Wu & Stettler, 1997). Four QTL for individual leaf size were identified and of these, QTL for leaf size on sylleptic branches were found to be clustered with those for stem diameter, on linkage group L. The production of sylleptic branches in *Populus* is known to be linked to high yield (Ceulemans, 1990; Scarascia-Mugnozza *et al.*, 1999), although in *Salix* Ronnberg-Wastljung and Gullberg (Ronnberg-Wastljung & Gullberg, 1999) have suggested the opposite – that syllepsis is negatively correlated with stemwood yield. They suggest that this may be because phenotypic effects driven by environmental variation are of greater importance than genotype for this trait in *Salix.* Despite this, there is still good evidence for *Populus* and *Salix* that individual leaf size is an important determinant of yield and reason to believe

that QTL for such traits may be used in future for marker assisted selection. The data presented here suggest that cell number could be a valuable marker for productivity, although further work to confirm this is needed in the appropriate mapping populations such as those described by Cevera *et al.*, (Cervera *et al.*, 1997).

The data for photosynthetic rate per unit leaf area and the relationships between A and C_i provide clear evidence for genotypic differences in photosynthetic characteristics within *Populus.* These are well-documented (Ceulemans & Isebrands, 1996), but whether they are of value as physiological traits for yield is questionable. Lawlor (Lawlor, 1995) has suggested that although photosynthetic fixation per unit leaf area is the ultimate driving force for all carbon gain and dry matter production, it is often poorly correlated with enhanced yield in any breeding programme. This is because it is difficult to identify the rate-limiting steps within photosynthetic metabolism that determine photosynthesis under field conditions. In this sense, the development of leaf area is a better indicator of yield, although in biomass crops, as opposed to those that require the process of flowering, there is some evidence that leaf level photosynthetic characteristics may be of value (e.g., (Faville *et al.*, 1999)). In *Populus* Isebrands *et al.*, (Isebrands *et al.*, 1988) have shown clearly that rates of leaf photosynthetic fixation, as demonstrated here, may be highest for 'superior' genotypes, and that in contrast to agronomic crops, this is not associated with reduced leaf area. Although even in Miscanthus, it would appear that leaf extension rate in relation to thermal time, rather than rates of CO_2 fixation, provides an excellent predictor of biomass yield (Clifton-Brown & Jones, 1999). The data presented here represent the first full characterisation of gas exchange parameters for the *trichocarpa* x *deltoides* genotype, Hoogvorst. In many

respects, Hoogvorst and Beaupré appear similar, when considering leaf level photosynthetic characters. For example, A and V_{cmax} (Table 1.4) were highest in Beaupré and these did not differ significantly from Hoogvorst. A and V_{cmax} were both significantly lower in the *deltoides* x *nigra* hybrid, Gaver, shown to have a significantly lower stemwood production. Instantaneous leaf level water use efficiency (WUE, Table 1.4) was also highest in Beaupré, although this did not differ significantly from Hoogvorst and the *deltoides* x *trichocarpa* numbered hybrid. It has been suggested that WUE may be a useful trait for selection. One possibility is the use of delta C13 discrimination, as an integrated measure of plant water use efficiency, as proposed by Farquhar and Richards (Farquhar & Richards, 1984), but few, if any studies have linked this trait to a molecular marker.

In summary, this work has shown that fast growing genotypes within *Populus* are characterised by a number of leaf-level traits that appear robust across environments and may in future be used to identify superior genotypes related to yield. Of these traits, the development of leaf area appears most tractable, with highly productive genotypes characterised by high LAIs. Furthermore, detailed analysis showed that leaf expansion and individual leaf size were more important in explaining high LAI than leaf production in both SRC and glasshouse trials. At the level of the cell, leaf cell number proved to be tightly linked to leaf size. Future work should focus on these traits, possibly using available mapping populations to identify QTL.

Chapter 2

The molecular genetic basis of root growth responses in *Populus* to elevated CO_2.

Introduction:

This chapter details an experiment to determine the presence of QTL controlling root growth in poplar family 331, and to investigate the effect of elevated CO_2 on these QTL. This analysis therefore is a novel approach to understanding plant response to environmental stress at the level of the gene. No work has previously been published on the rooting characteristics of these parents or their progeny at either ambient or elevated CO_2 concentrations, although QTL for height, height increment, basal area, height: diameter ratio and spring bud flush (in ambient CO_2 conditions) have all been mapped (Bradshaw & Stettler, 1995).

The aims of this study chapter were threefold:

1 To grow Family 331 in the UK for the first time.

2 To begin to assess responses to elevated CO_2 at a genetic level.

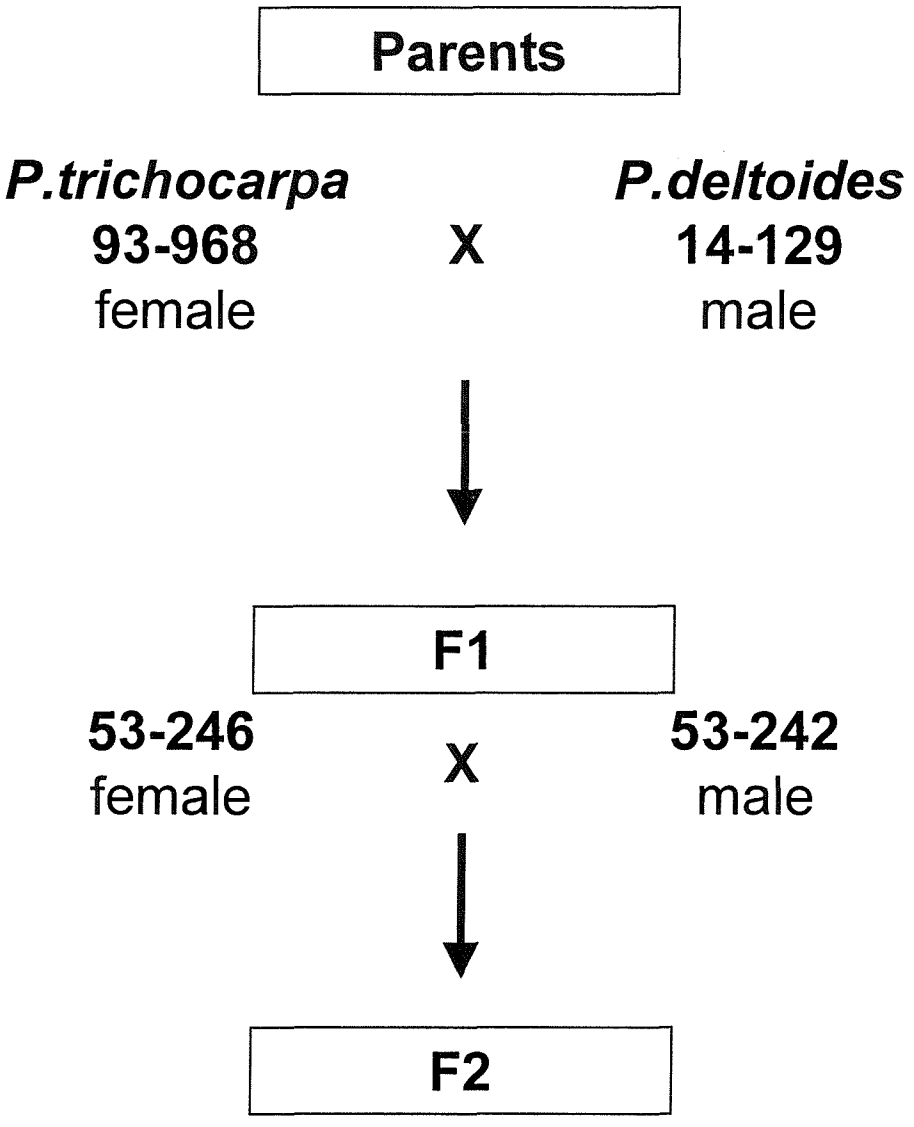
3 To identify gross morphological traits and to use them in a preliminary investigation to identify QTL.

Materials and Methods:

The pedigree used for QTL mapping was generated by the hybridisation of the maternal *P. trichocarpa* clone 93-968 from western Washington USA and the paternal *P. deltoides* clone ILL-129 from central Illinois USA in 1981. Two siblings, 53-246 and 53-242, from the resulting F1 family (Family 53) were mated to form an inbred F2 family (Family 331) (Bradshaw *et al.*, 1994) (Diagram 2.2).

This experiment was conducted in the UK in 16 open top chambers (OTC) at the Forestry Commission field site, Headley, UK, (Grid Ref. SU813382) (Taylor & Dobson, 1989) (Plates 2.1 & 2.2). In May 1999, 285 labelled F2 genotypes of Family 331 pedigree, the *P. trichocarpa* and *P. deltoides* parents and the F1 genotypes were established from unrooted hardwood cuttings derived from a stool bed at the University of Washington, Seattle, USA. Cuttings were grown in John Innes no. 2 (lime free) compost in clear-sided plastic tubes (50cm high, 15cm diameter) in a randomised complete block design. The clear sides were covered with removable black plastic to keep out the light, but allow access for visual root measurements (Plate 2.3).

Diagram 2.2 - The Breeding of Family 331



Family 331

A population of individuals from the same parents, exhibiting the widest possible range of phenotypes

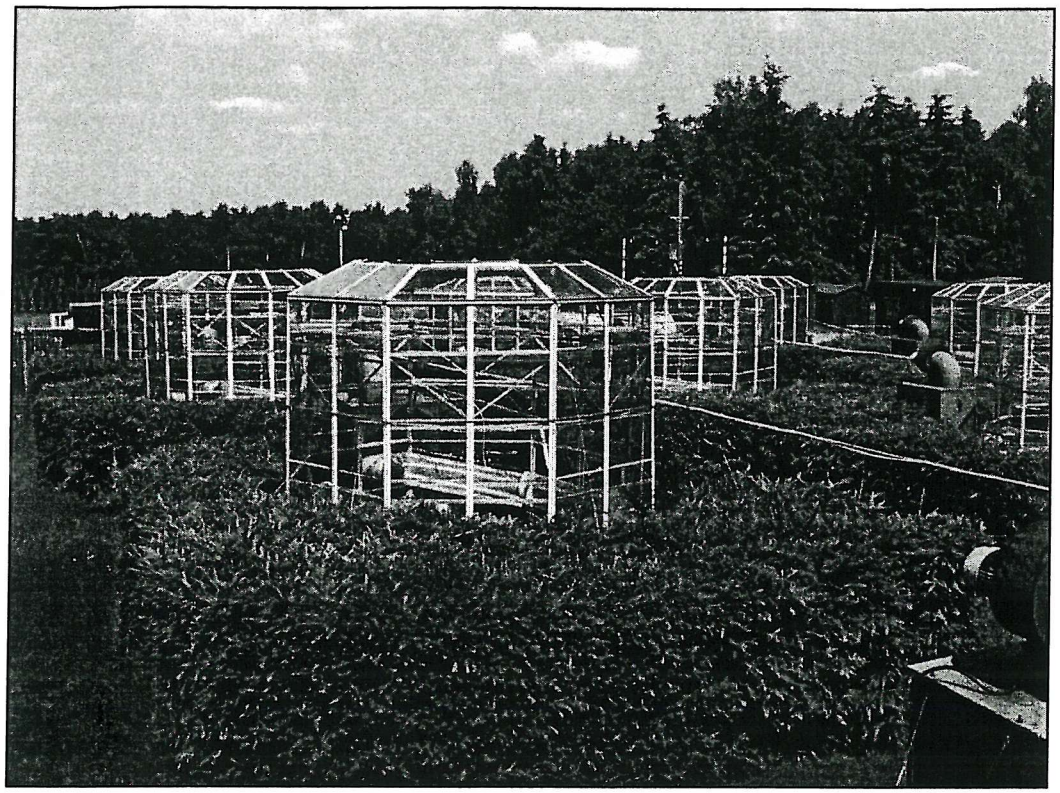


Plate 2.1: Some of the 16 open top chambers at the Forestry Commission's Headley nursery, near Alton, Hampshire.

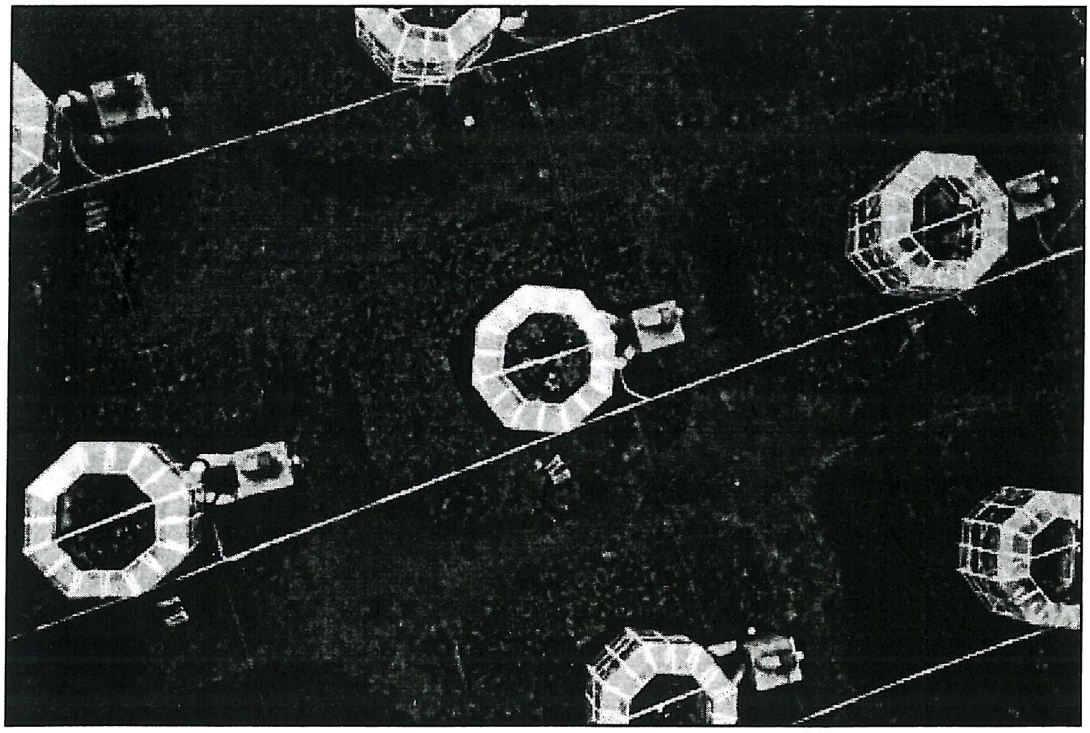


Plate 2.2: Ariel view of open top chambers showing CO_2 lines and air circulating machinery. Poplar trees of family 331 can also be seen growing inside chambers. (Photo taken using camera suspended from a kite).

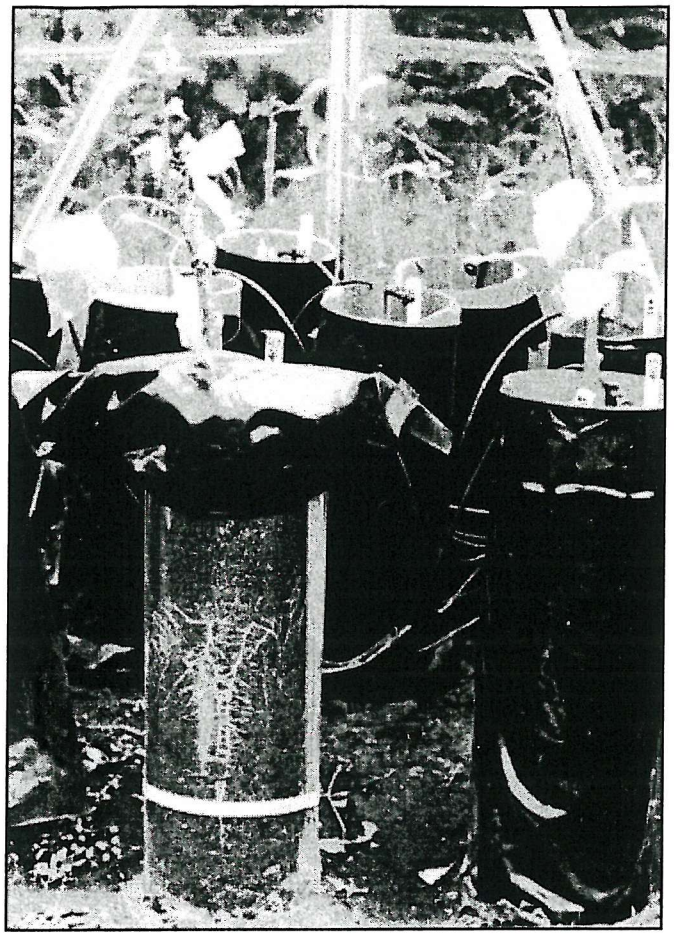


Plate 2.3: Rhizotrons planted with cuttings of poplar family 331. Black plastic covers maintained dark below ground environment, but allowed easy access for observation and measurement.

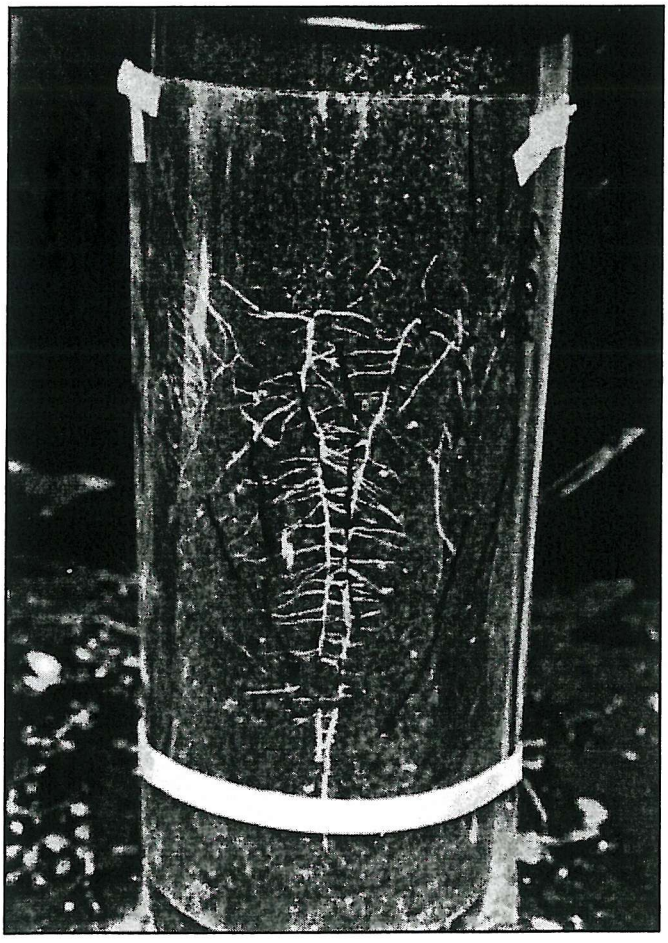


Plate 2.4: Close-up of Rhizotron window showing method of tracing roots using marker pen onto acetate sheets.

Prior to planting each cutting was dipped into a solution of TILE 250 EC fungicide (CIBA-GEIGY Agrochemicals, Cambridge, UK). Each chamber housed 36 genotypes that were placed in a circular pattern with a distance of 25 cm between the pots. The pots were buried to a depth of 10 cm for stability. A cutting of each genotype was planted in eight of these chambers which received ambient concentrations of CO_2 (aCO_2) and another cutting of each genotype was planted in the other eight chambers which received elevated concentrations of CO_2 (eCO_2) at a target concentration of 600μmol mol^{-1} CO_2. The mean CO_2 concentration recorded between May and September 1999 in the eCO_2 chambers was consistent, ranging between 588.29 (± 63.35) and 595.3 (± 83.52) μmol mol^{-1} CO_2. Similarly, consistent values were recorded in the aCO_2 chambers (range between 400.81 (± 39.00) and 408.08 (± 38.84) μmol mol^{-1} CO_2.

A 1-hp centrifugal pump mounted in an adjacent cabinet constructed of galvanised steel ventilated each chamber. Each pump drew in the surrounding ambient air, which was evenly distributed via a perforated polythene tube around the edge of each chamber. The ventilation rate was 75 m^3 min^{-1}, providing one complete air change every 15 seconds (Gardner, 1996). Across chambers, the mean monthly air temperatures (± SD) outside and inside the chambers respectively were in May: 13.2 (4.0), 16.4 (5.7) °C; June: 14.3 (4.4), 17.6 (6.1) °C; July: 18.1 (5.1), 21.3 (6.9) °C; August: 16.3 4.5), 19.0 (6.0) °C; and September: 15.3 (4.3), 17.4 (5.6) °C. Initially all chamber floors were treated with Stomp, a pre emergent herbicide MAFF No. 04183 (Cyanamid Novotec), applied with a Gloria sprayer at a dilution of 30ml/L. A slow release nitrogen fertiliser (5g osmocote – Grace-Sierra,

Nottingham, UK) was added to each pot after 67 days of growth. The trees were staked at 68 days after planting (DAP). Water was supplied to each pot via a drip irrigation system as required. Measurements of tree development and the physiological traiting of these genotypes occurred throughout the season. The root traits presented here are primary root elongation rate and root density.

Root growth rate:

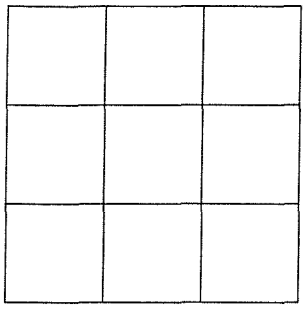
A4 size acetate sheets were attached to the clear plastic sides of the rhizotrons using PVC tape, and labelled with the code number corresponding to the genotype planted within. On 7th June 1999 (23 days after planting) the position of any primary root tips appearing in the clear plastic rhizotron window were marked on the acetate sheets using black permanent overhead projector marker pens. 3 days later this procedure was repeated, marking the new position of the root tips and tracing the path of the growing roots. On this occasion a blue marker was used in order to differentiate between marks made on the previous visit. This was continued at regular intervals– each time using a different colour marker pen, until 15th July by which time the roots had all grown the full length of the rhizotron windows (Plate 2.4). The acetate sheets were then removed and taken back to the laboratory for measurement. The root traces were measured using a self-made root-measuring wheel (appendix 1), and the mean daily growth rate was calculated for each root.

Primary and Secondary Root density:

A grid consisting of 9 squares, each measuring 1.27cm x 1.27cm was laser printed onto clear acetate. Root density was measured by placing the acetate grid against

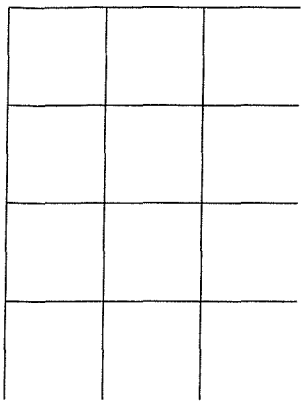
the rhizotron windows and counting the number of intersections of primary and secondary roots with the lines of the grid. The dimensions of the grid squares meant that number of intersections = root length (cm) (Marsh, 1991; Murray & Bristow, 1997).

The pattern of the grid used for measurement measured 11.43cm^2 and was printed as follows:



However, it was noted that the number of intersections of roots passing through this size grid does not give root length per 11.43cm^2. (For example a root passing vertically through the grid will measure 3cm in length, but will intersect 4 lines, therefore giving an apparent length of 4cm). In order for the root intersection method to work properly it is necessary to use a grid that is open on 2 sides. (cont. overleaf)

A grid that is open on 2 sides, but has the same total length of lines as the grid above is shown below:



This grid covers an area of 19.35cm^2 and so the count of root / grid intersections taken from the original grid gave total root length per 19.35cm^2. This adaptation had to be made from the method of Murray and Bristow since they used a grid larger than the total root area whereas in this experiment the roots extended beyond the boundaries of the grid.

Data analysis and QTL mapping:

The Data were analysed for QTL using interval mapping software MAPMAKER/QTL 1.1 (Lander *et al.*, 1987; Lander & Botstein, 1989). The RFLP, STS and RAPD based genetic map made from a total of 90 F2 individuals of the pedigree has been previously described (Bradshaw *et al.*, 1994; Bradshaw & Stettler, 1995). The data were tested for normality using a Shapiro-wilk test. A stringent threshold value of LOD (logarithm of the odds ratio or log likelihood in the output) score of 3.0 for declaring the potential existence of a QTL was used, corresponding to an approximate nominal significance level of P = 0.05 for the

entire genome (Bradshaw & Stettler, 1995). In the event that data for a particular trait followed a normal distribution, and had a LOD score of 3.0 or greater, the data set was then processed using composite interval mapping software QTL CARTOGRAPHER v.1.14. (Basten *et al.*, 1994; Basten *et al.*, 2000). 100 permutations were performed on each data set to identify positively the presence and location of any QTL ($P>0.05$) (Frewen *et al.*, 2000). A step by step description of QTL data analysis using the software and statistics described above is included in appendix 2.

Figure 2.1: Frequency distribution of primary root densities of poplar family 331 grown in elevated and ambient CO_2 showing distribution of Parents and F1 genotypes.

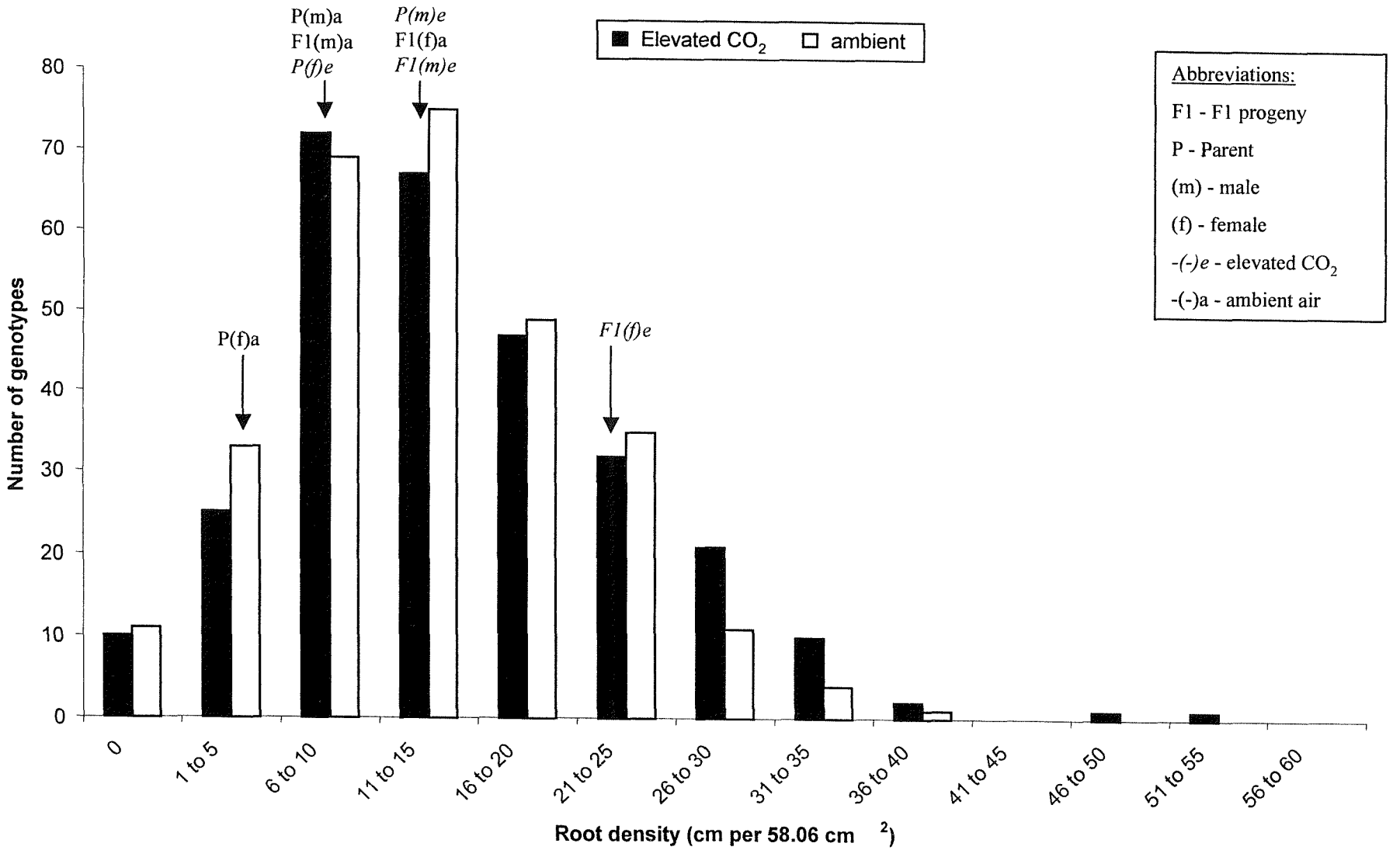


Figure 2.2: Frequency distribution of secondary root densities of poplar family 331 grown in elevated and ambient CO_2 showing distribution of Parents and F1 genotypes.

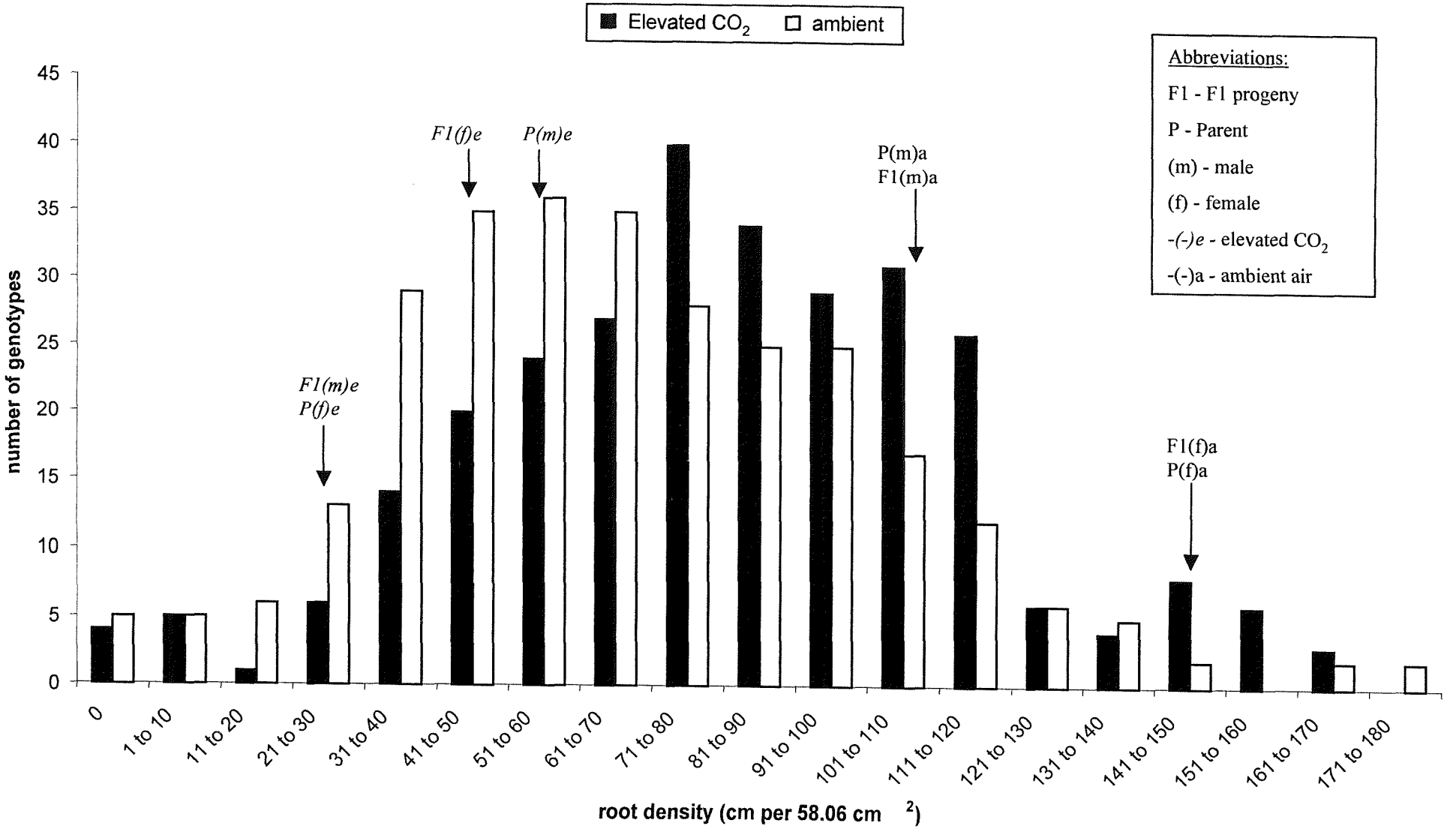
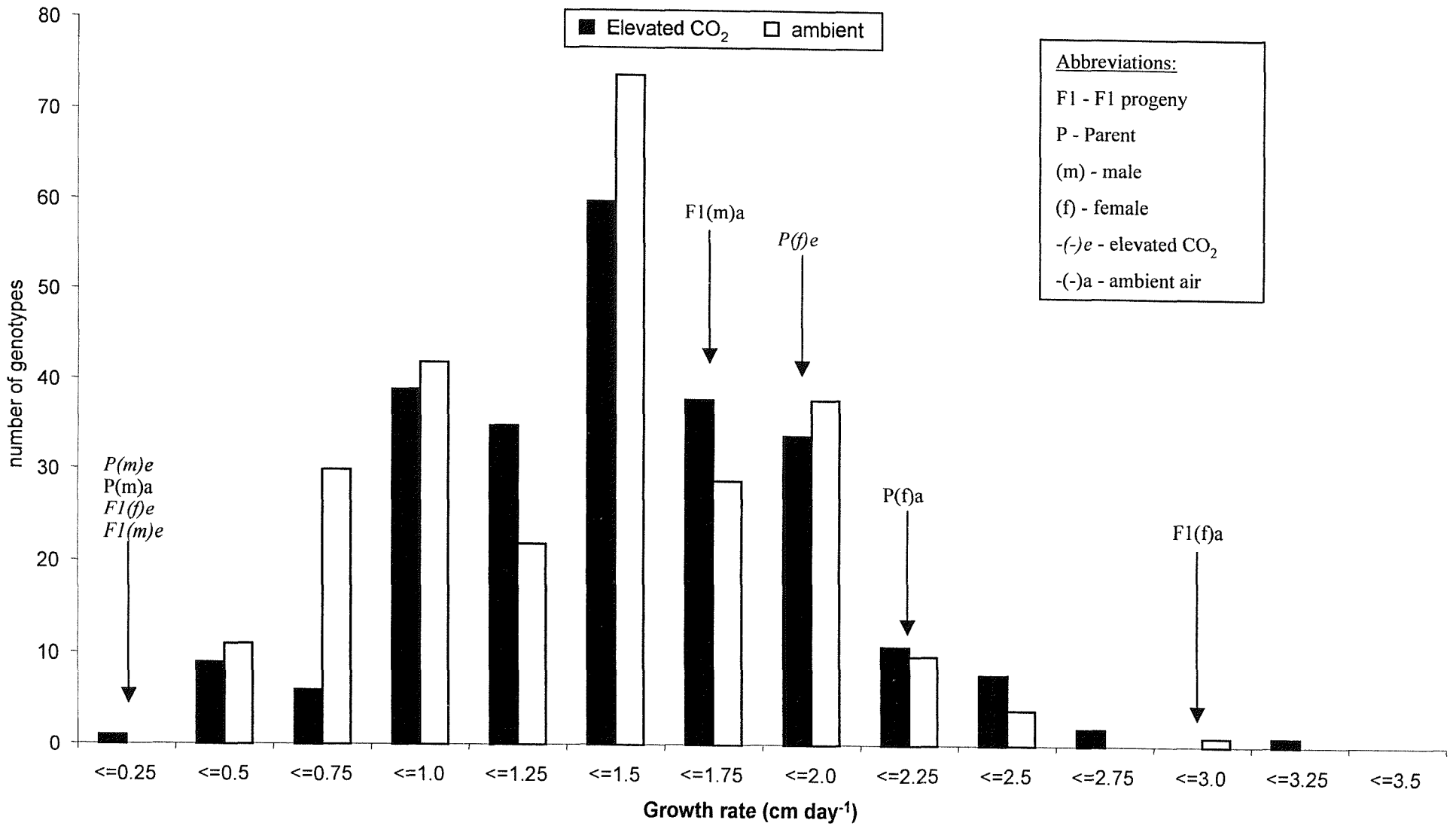


Figure 2.3: Frequency distribution of growth rates of fastest growing observed roots of poplar family 331 grown in elevated and ambient CO_2, showing distribution of Parents and F1 genotypes.



Results:

Figures 2.1, 2.2 and 2.3 show the frequency distribution of root growth rates and root densities across the whole population of family 331 with the values of the parent and F1 genotypes indicated by arrows. Each graph shows an effect of elevated CO_2 on the data set, but this is most pronounced in figure 2.2 where secondary root density appears to have increased under conditions of elevated CO_2. Table 2.1 shows the results of a Shapiro-wilk normality test. Only two of the six data sets (root growth rate in elevated CO_2 and secondary root density in elevated CO_2) followed a normal distribution:

Table 2.1: Phenotype data sets were tested to see if they followed a normal distribution using a Shapiro-wilk test. Data sets with a P value less than 0.05 were deemed to be significantly different to a normal distribution.

	Shapiro-wilk (P value)	Normal distribution (P>0.05):
Root growth rate (elevated CO_2)	0.0656	Yes
Root growth rate (ambient CO_2)	0.000418	
Primary root density (elevated CO_2)	0.0084	
Primary root density (ambient CO_2)	0.048	
Secondary root density (elevated CO_2)	0.19	Yes
Secondary root density (ambient CO_2)	0.0037	
[1]Tree height (elevated CO_2)	0.753	Yes

[1] Tree Height Data:

Other traits were measured during this experiment and these will form part of the Ph.D. thesis of Katherine Robinson. However, the author undertook the analysis of all of these traits during a visit to the Bradshaw Laboratory, University of Washington, Seattle, USA. Consequently, data from one of these traits (tree height) is presented here in order to demonstrate the successful identification of a QTL.

Table 2.2 shows the results of data processing using the Mapmaker QTL software (more than one LOD score indicates possible presence of more than one QTL):

	LOD scores:
Root growth rate (elevated CO_2)	None
Root growth rate (ambient CO_2)	3.23 5.12 3.43
Primary root density (elevated CO_2)	3.3
Primary root density (ambient CO_2)	2.26
Secondary root density (elevated CO_2)	2.01
Secondary root density (ambient CO_2)	None
[1]Tree height (elevated CO_2)	3.53

From table 2.2, only 3 traits had LOD scores of greater than 3.0. Only [1]Tree height (elevated CO_2) had a data set with LOD score greater than 3 and followed a normal distribution (table 2.1). Processing the data set for Tree height (elevated CO_2) using QTL CARTOGRAPHER software resulted in a Permutation significance level for alpha = 0.05 of 13.0085. This corresponded to a peak on Chromosome 4, located between markers 15 and 16.

Discussion:

From a Total of 90 traits measured during this experiment, 20 were identified by interval mapping using MAPMAKER QTL as having LOD scores greater than 3. Following a Shapiro-wilk test 7 traits were found to have a normal distribution and LOD scores greater than 3. Composite interval mapping using QTL CARTOGRAPHER located a positive QTL for one trait – Tree height. This is important in confirming the presence of a QTL originally identified by Bradshaw (1995) and testing the effect of environment on genotype. This suggests that this QTL is robust. Given that tree height is an important determinant of yield, this may provide a valuable marker for future selection.

The data support previous research that indicates root biomass is increased in elevated CO_2 (Rogers *et al.*, 1994). Secondary root density was especially enhanced by elevated CO_2, which implies that the extra carbon resource is utilised to increase nutrient uptake from the soil, rather than improving stability by extending the structural primary roots.

Previous attempts to identify QTL for root traits have been published and include root architectural traits and water acquisition in cultivated and wild type lettuce (Johnson *et al.*, 2000). Thirteen QTL that each accounted for 28-83% of the phenotypic variation between wild and cultivated varieties were detected by composite interval mapping. A close association was found between QTL for tap-root length and QTL for ability to resist drought in the wild species. Johnson *et al.* conclude that this information could be used to improve cultivated lettuce by increasing its resistance to drought. Ray *et al.* (1996), Price *et al.* (2000) and

Zheng *et al.* (2000) all used a wax- petrolatum layer system to simulate compacted soil in order to assess root penetration ability in rice. This resulted in the detection of 12 putative QTL by interval mapping for Zeng et al. (2000), 7 QTL reported by Price et al. (2000) and 39 reported by Ray et al. (1996). 3 replicates of each of 202 recombinant inbred lines were used in this study (Ray *et al.*, 1996). It is interesting to note the high numbers of QTL claimed to have been identified in these experiments compared to the results from the poplar experiment. The rice experiments only used interval mapping to detect QTL and it is likely that some of these occur purely by chance (Basten *et al.*, 2000). Further data processing using composite interval mapping would be likely to reduce the number of putative QTL in these cases. Johnson et al. (2000) used composite interval mapping to analyze their data so it is likely that there are in fact 13 QTL involved in the rooting characteristics of lettuce. Based on this evidence it would be reasonable to expect that QTL for root growth in poplar could be found once genetic marker information has been added to more of the progeny of family 331, enabling analysis to be carried out on the whole data set.

One of the potential uses of QTL analysis is that it will enable plant breeders to use marker-assisted selection for developing future plant varieties. The ability to use molecular techniques to screen an F2 progeny for particular traits and growth characteristics in a matter of hours, rather than several days, weeks, months or even years has obvious time and labour saving advantages (Tuskan, 1992). This is especially true for traits that are hard to observe such as root characteristics and insect and disease resistance, and traits in slow growing species such as most trees. Marker assisted selection has recently been used for improving resistance to corn

earworm (*Helicoverpa zea*) in maize (da Silva *et al.*, 2000), identifying extreme late bolting in Chinese cabbage (*Brassica rapa*) (Ajisaka *et al.*, 2001), and the molecular characterization of iron deficiency chlorosis in soybean (Lin *et al.*, 2000). However, marker assisted selection can only identify a relatively broad range of favorable genotypes, whereas plant breeders usually require just one or two of the best performing phenotypes. These are still best identified using traditional screening techniques and consequently marker assisted selection may be of limited value in replacing traditional screening methods (Kearsey & Farquhar, 1998).

Only one QTL was positively identified in this study, but it would be reasonable to expect many more traits than tree height to be governed by QTL and the question as to why more QTL were not identified can possibly be answered by the following points:

Many of the genotypes for which there was genetic marker information were not provided in the original shipment of Family 331 plant material delivered from the States. Furthermore, some of the genotypes that were delivered had no genetic marker information attributed to them, so out of approximately 300 genotypes screened, only around 46 of the data points were useful for QTL analysis. Further work is now in progress on the extended family 331, to apply florescent AFLP (Southampton University) and SSR markers (Oak Ridge National Laboratories, USA) to this population to ensure that these phenotypic data can be remapped at a later stage. This will allow data from this experiment to be processed again in the future with a better chance of identifying more QTL.

Some of the limitations in the design and execution of the experiment perhaps prevented the identification of more QTL. Firstly there was a shortage of plant material allowing only 2 cuttings of each genotype to be established in the first instance. Had more time been available it would have been desirable to plant the cuttings in an open nursery and allow them to establish themselves in the first year in order to provide a greater number of cuttings for experiments in subsequent years. Although care was taken to arrange the plants in order to limit any block effects between the treatments, it was impossible to guarantee that all plants were experiencing the same environmental conditions within the chambers. Plants, depending on their location within the chamber and the vigour of the surrounding genotypes, may have experienced variations in light, airflow and shading. Greater replication may have improved the results by allowing the environmental effects to be averaged out, however there would have inevitably been a trade off in extra cost, space and time.

If the parental genotypes were not significantly different for a particular trait then there would not necessarily have been any segregation for that trait in the F2 population and hence no chance of detecting any QTL. Ideally for QTL analysis the two parents should exhibit extreme and opposite characteristics for a particular trait (e.g. very large leaves and very small leaves). When these parents are crossed their F1 progeny should all exhibit traits half way between those of the parents (e.g. medium sized leaves). The F2 population should then exhibit phenotypes spanning the entire range of variation for that trait (e.g. very small leaves to very large leaves) and should also follow a normal distribution for that particular trait. The

parents of family 331 had originally been selected to breed a population segregating for the traits: Stem growth and form, and spring leaf flush (Bradshaw & Stettler, 1995), but no previous research had been done on these parents to characterise their root traits or their responses to elevated CO_2. Figures 2.1 to 2.3 show the distributions of the parent and F1 phenotypes in relation to the rest of family 331. In the case of root growth rate (fig 2.1) the distribution of the male and female parents follows this requirement (when grown under ambient conditions) and the male F1 progeny falls approximately mid-way along this distribution, but the F1 female lies outside this range. None of the other traits fulfil these distribution requirements, although since there is no replication there is approximately a 32% chance that these values fall outside one standard error of the likely mean (assuming a normal distribution) and so it is unwise to draw too much inference from these results

A final possibility for the lack of QTL detection is that there may not be any genetic variation for a particular trait. This would mean that there were no QTL present, and so no chance of detection regardless of parental characteristics or number of replicates. This is unlikely, as it would mean that all of the variation observed in these rooting characteristics was due only to environmental variations within the experiment, or to sampling errors during data collection rather that differences in the genotypes of each plant.

Chapter 3

Water-use in poplar grown in elevated CO_2 concentrations.

Introduction:

This chapter details an experiment to determine the net effect of increased atospheric CO_2 on water use in populus. This experiment goes further than previous research as the trees were in field conditions with an enriched CO_2 atmosphere instead of being subjected to the artificial conditions of a controlled environment.

The aims of this study were

1. To assess the effect of increased CO_2 on water use in poplar.
2. To compare the effects on different species of poplar
3. To identify any seasonal variation in the response to elevated CO_2 by taking measurements throughout the growing season.

Methods

Site and growth conditions:

The experimental plantation and FACE facility is located in central Italy, near the city of Tuscania (province of Viterbo, latitude 42°37.04 N, longitude 11°80.87 E, altitude 150m). The 9ha plantation was planted with *Populus* cuttings of three different species in six experimental plots at a planting density of 10,000 trees per ha (spacing of 1m x 1m) during the spring of 1999 (Plate 3.1). The species were *Populus alba* (clone 2AS-11), *P.nigra* (clone Jean Pourtet), and the hybrid, *Populus* x *euramericana* (*P.deltoides* x *P.nigra*, clone I-214). Within the plantation, six 314m^2 plots were treated either with ambient or enriched (target concentration, 550 +/- 70 μmol mol^{-1}) CO_2. The three enriched plots were equipped with octagonal shaped FACE rings enclosing a circular area of 20m diameter (Plate 3.2). Each ring had eight vertical telescopic masts, which could be erected up to 12m above the ground, supporting 16 horizontal polyethylene pipes (25mm in diameter) bearing a variable number (245 to 350 per side) of small holes of 0.3mm diameter. 16 on/off solenoid valves activated the directional control of the releasing pipes and an automated pressure regulator controlled the amount of CO_2 vented by the pipes. A computer in the centre of each FACE ring controlled the valves and pressure regulator. Two IRGAs, an anemometer and a wind direction sensor were the monitoring devices used to control the whole FACE system. Miglietta, Zaldei & Peressotti (2001) describe details of this system and its performance.

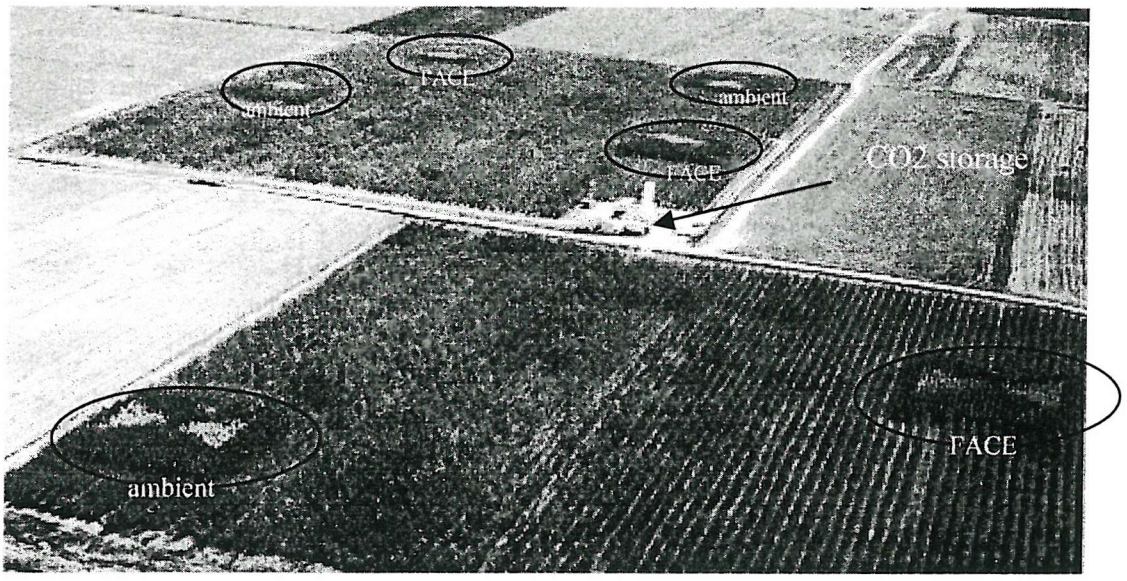


Plate 3.1: Ariel view of the POPFACE site showing all six rings and the CO2 storage facility. Photograph taken using a camera suspended from a kite. View is from the North East, looking South West.

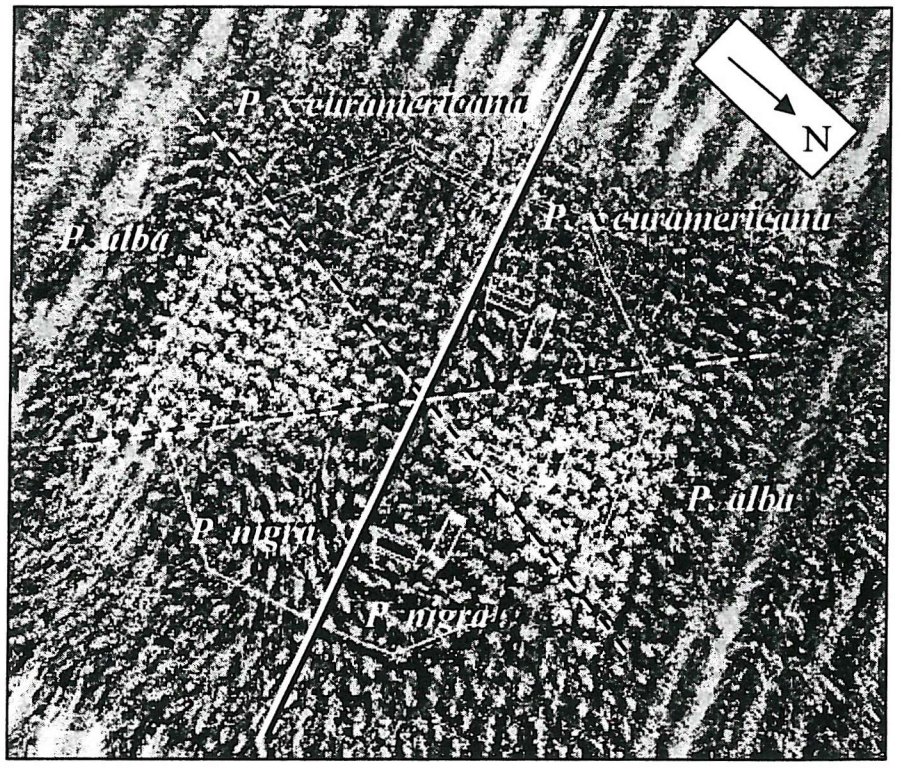


Plate 3.2: Kite Ariel Photograph of FACE ring 5, clearly showing arrangement of species. Scaffolding towers and CO_2 pipes can also be seen.

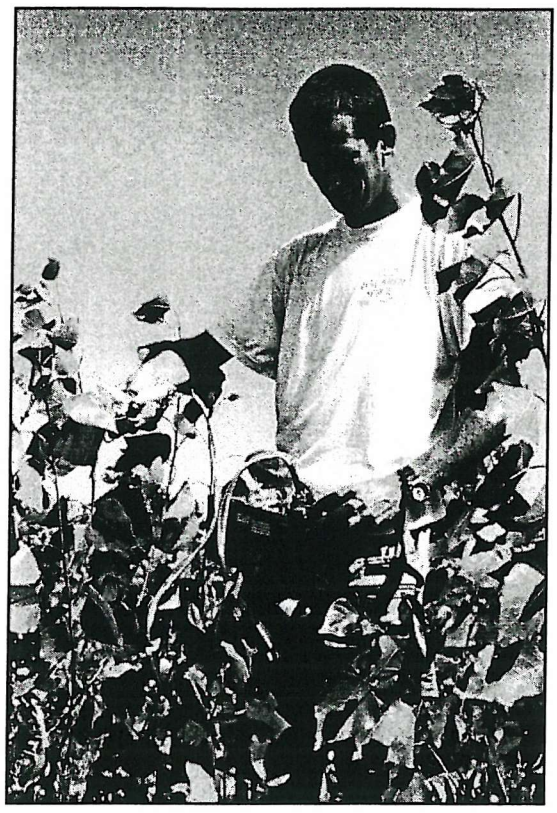


Plate 3.3: Making stomatal conductance measurements using a Licor 1600 steady state porometer.

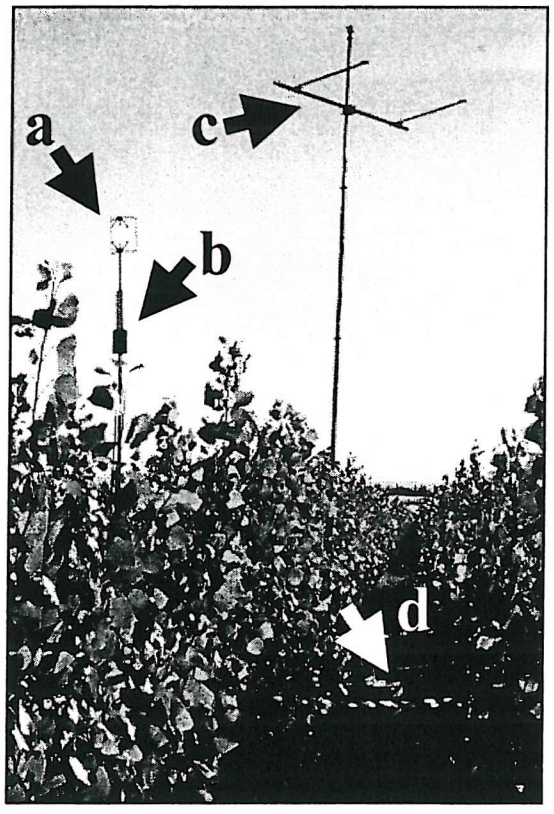


Plate 3.4: Eddycovariance was calculated from measurements of:

a - air turbulence (three axis sonic anemometer).

b - water vapor (closed path infra-red gas analyser (arrow indicates air intake)).

c - incident and reflected radiation (2 net radiometers).

d - soil heat flux (8 soil plates).

Measurement of leaf stomatal conductance:

Diurnal leaf level measurements were carried out using a LiCor 1600 steady state porometer (LiCor, Lincoln, NE, USA) (plate 3.3). Four FACE and four control trees were selected for each species, and four recently matured leaves on the main leader branch were selected for each tree. These were marked with plastic tags in order to enable the same leaves to be used for repeated measurements. A ladder or scaffolding was used in order to reach the top of the canopy. Starting at dawn, the transpiration and stomatal conductance of each labelled leaf was measured in quick succession. The measurements took approximately half an hour to complete, and were repeated approximately every 2 hours throughout the day until sunset.

Energy and water fluxes:

A collaborating research group from Italy (Franco Miglietta, CNR, Ist Agrometeorol & Anal Ambientale, Florence, and Alessandro Peresotti, DPVTA, University of Udine, Udine) made the following measurements in order to check the reliability of the sap flow gauges. Their eddy covariance technique measured canopy level water use by utilising sensors positioned above the canopy. This part of the experiment took place in an area of the guard plantation since the experimental plots were too small for accurate measurement using the eddy covariance technique. Sap flow gauges were positioned on individual trees within the 'footprint' area of the eddy covariance sensors in order to directly compare the results of each method.

Latent and sensible heat was measured with eddy covariance technique (Moncrieff *et al.*, 1997) every 30 minutes on a tower centred above the sap flow gauge

installation (plate 3.4). Eddy covariance measurements were collected according to EUROFLUX methodology (Aubinet *et al.*, 2000). A closed path system was used that comprised of a three axes sonic anemometer (Solent 1012R2, Gill Instruments, Lymington UK), a closed path infra red gas analyser (LiCor 6262, LiCor, Lincoln, NE, USA) and analysis software for real time and post processing analysis (Edisol, Edinburgh University, Edinburgh, UK). The sonic anemometer and air sampling tube were positioned 4.2m above soil level and 1.5m above vegetation height. The fetch in the direction of the prevailing winds was 1:95 and 1:20 in the opposite direction. After processing, data with a friction velocity less than 0.3 and with a fetch less than 1:80 were discarded from the calculation. Gaps were then filled with linear interpolation.

Net radiation was measured with two net radiometers (Q7, Rebs, Pullman, WA, USA) on a tower at 6m from the soil surface. Soil heat flux was measured with 8 soil plates (own made) placed across the plant row. Sensor signals were read every 10 seconds and averaged every 30 min with a data logger (21x Campbell Scientific, Logan, UT, USA)

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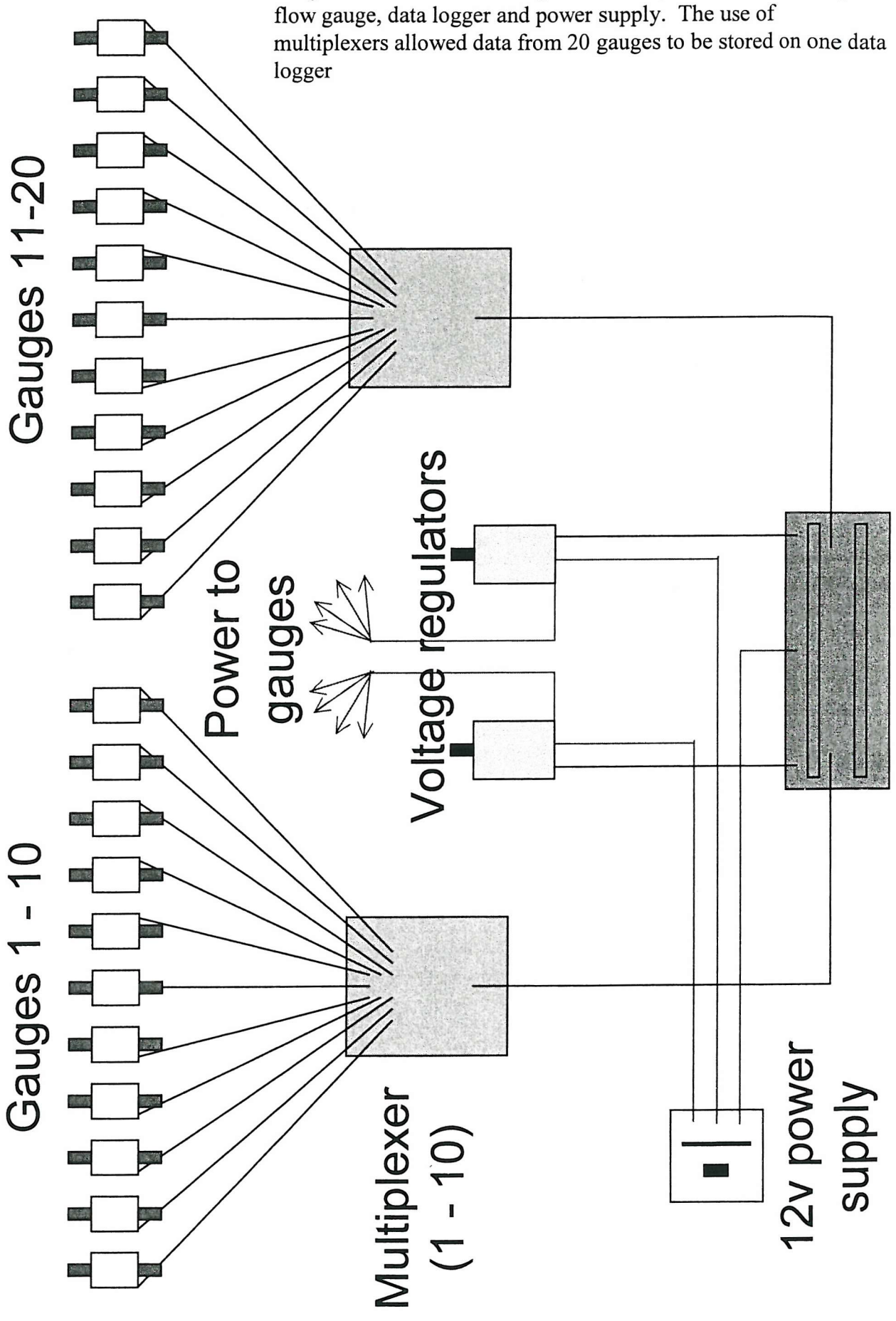
Diagram 3.2: Schematic diagram showing arrangement of sap flow gauge, data logger and power supply. The use of multiplexers allowed data from 20 gauges to be stored on one data logger



Sap Flow Measurements:

Two species of *Populus* (*P. euramericana* and *P. alba*) were used to investigate the effects of elevated CO_2 on total tree sap flow. Sap Flow was measured using the stem heat balance method (Sakuratani, 1981). Forty 30mm diameter stem heat balance gauges were used to determine main stem sap flow. These were made by hand at the University of Udine, Italy under the direction of Alessandro Peressotti. The gauges were connected to two Campbell CR10 data loggers via four Campbell multiplexers (Campbell Scientific, Logan, UT, USA). (10 gauges per multiplexer, 2 multiplexers per data logger). Each data logger was housed in a separate weatherproof box along with 2 multiplexers, 2 voltage regulators to supply power to the heaters, and a transformer to convert 220V AC to 12V DC. Mains power was supplied via extension cables from a 220V AC-power supply installed in each FACE ring. The gauges were connected to the multiplexers via individual 8 core cables of know resistance, each measuring 10m long. Backup power was provided by a 12V-car battery connected in parallel with the 220V transformer in order to maintain its charge when mains power was available (diagram 3.2). This arrangement allowed one FACE and one control ring to be measured simultaneously, with a maximum of 20 gauges fitted in each.

Prior to installation of the sap flow gauges the height at which each stem measured 30mm in diameter was determined using a height stick and vernier callipers. Trees with diameter heights at the mid-point of the distribution of data were selected for sap flow measurement (data not shown).

Sap flow gauges were installed onto the stems at diameters between 33 and 27 mm. The stems were prepared by removing any rough or loose bark, including lenticils. In some cases branches had to be removed in order to fit the gauges. Where this was necessary the diameter and dry weight of removed branches was recorded. The cork bodies of the gauges were carefully fitted around the stems, ensuring good contact between the stem, thermocouples and heater. The cork was clamped into position using metal "Bulldog" clips. The diameter of the stem at the top and the bottom of the cork gauge was carefully measured and used to calculate the mean diameter of the stem for use in the sap flow calculations. Finally the gauges were insulated with 2 layers of foam pipe lagging and covered with silver foil (plate 3.5). Once installed the gauges were connected to the data loggers and power supply, and left in place for a minimum of five days during which time the data loggers were downloaded daily (plate 3.6).

Many of the trees were greater than 30mm diameter at the base, which necessitated fitting the gauges further up the stem. Because of this, some trees had leafy branches below the main gauge that were contributing to whole tree sap flow. In order to quantify the sap flow in these branches a number of smaller gauges ranging from 3 to 11mm in diameter were used. Sap flow data were collected from these branches for a minimum of four days and then used to construct a relationship between mean sap flow and stem diameter. This relationship was used to estimate the quantity of sap flow in the branches below the main gauges, based on the number and diameters of the branches below each gauge. In order to account for daily variations in sap flow the mean daily sap flow was calculated for each main gauge then the daily variation above or below this mean was calculated

and expressed as a percentage +/- the mean. This percentage was then added or subtracted from the average daily sap flow of the branches to give an estimate of the branch sap flow corrected for daily fluctuations. There were no branches below the sap flow gauges used in the validation experiment with the eddy covariance technique.

Plate 3.5: A 30mm sap flow gauge in position on the main stem and 2 smaller gauges positioned on side branches. Silver foil was used as a final layer of insulation to reflect the hot Italian sun.

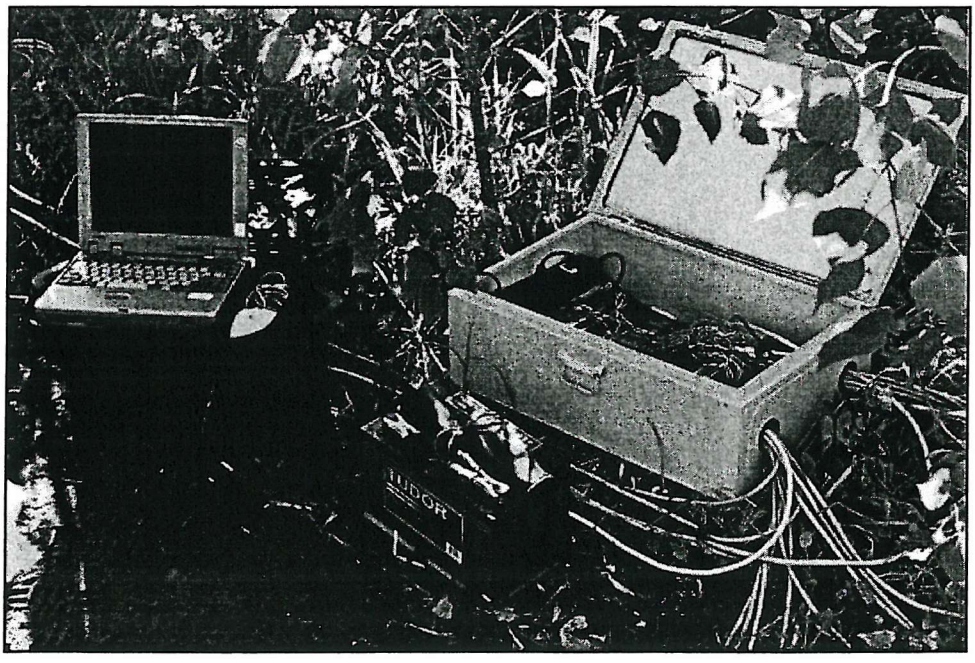


Plate 3.6: The data loggers and all wiring connections were stored in a weather-proof box. Power came from a mains connection, with a 12volt car battery as emergency backup. Data was downloaded daily to a laptop PC.

Each sap flow gauge was fitted with a flexible heater, which circulated the stem beneath the insulating cork. The temperature of the heater depended upon the voltage applied to it and its resistance. Since gauges of different diameters had heaters of different resistances it was important to provide the correct voltage. Too much power would have resulted in damage to the stem by overheating during periods of low sap flow, while too little power would not provide a sufficient temperature difference to enable sap flow to be calculated during periods of high flow. The minimum temperature difference required between the heater and stem during peak sap flow was 0.5°C, although 1°C was optimal. The voltages found to give optimal temperatures for each size of gauge varied between 6.5V (for 30mm gauges) and 1.9V (for 3mm gauges).

Sap flow measurement campaigns:

Two campaigns were undertaken, one between 12th June and 12th July and a second between 31st July and 19th August. During the first campaign, sap flow gauges were installed onto *P. x euramericana* trees in the plantation below the eddy covariance measurement apparatus and on *P. x euramericana* and *P. alba* in the experimental plots. The plantation trees were measured first for 7 days, followed by *P. x euramericana* for 7 days on the main stems and 7 days on the branches. *P. alba* were measured for the final 7 days of the first measurement campaign. The stems of *P. alba* were narrow enough to install the gauges below all the side branches and so it was not necessary to make branch measurements on these trees. During the second campaign, intensive measurements were made on *P. alba* trees.

Eighteen gauges were used in each treatment and on each occasion a 'pair' of rings (1 ambient, 1 FACE) was employed. Data were analysed using one way ANOVA with CO_2 as a factor. When it was necessary to remove some side branches in order to fit the gauges, the diameters of all removed branches, and any branches occurring below the sap flow gauge were measured at 1cm from their base using vernier callipers and later used in sap flow calculations.

Seasonal sap flow predictions:

Using LAI data collected at the same time as the sap flow measurements (Birgit Geilen, personal communication) a linear relationship was constructed linking sap flow to LAI. From this relationship it was possible to make preliminary estimates of seasonal sap flow using LAI measurements made by Geilen throughout the growing season.

FIGURE 3.1 – Effects of FACE on leaf water use:
Diurnal measurements of stomatal conductance (gs) made consecutively with sap flow measurements at POPFACE during summer 2000. Closed circles denote measurements made in the FACE treatment ([CO_2] 550ppm), open circles denote control points (ambient [CO_2]). Error bars show standard error. Significant ANOVA results: * P<0.05, ** P<0.001, *** P<0.005. n = at least 16 leaves per treatment.

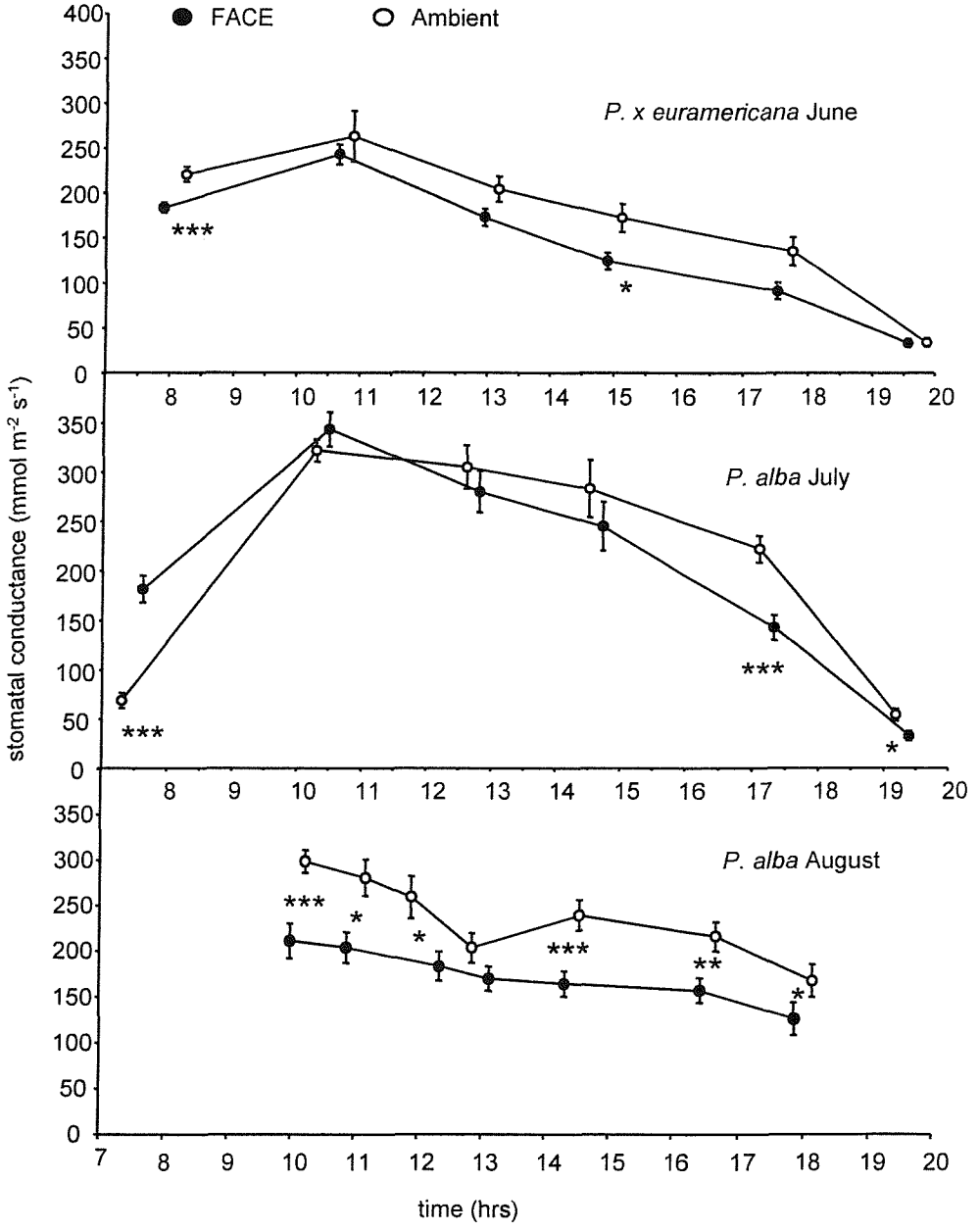


FIGURE 3.2 – Validation of Sap Flow Gauges using eddy covariance:
Figures show comparison of canopy transpiration calculated by eddy covariance or sap flow techniques measured in *P. x euramericana* plantation trees. Gauges were fitted at the base of trees, below all side branches. Sap flow was converted to latent heat by:
1 gh^{-1} $plant^{-1}$ = 1/3600 hs^{-1} x 2450 Jg^{-1} x ½ plant m^{-2} = 0.34 Wm^{-2}. 2a) Diurnal pattern of sap flow measured at 30-minute intervals. (●) denote Eddy covariance data, (O) denote sap flow gauge measurements. 2b) The relationship between water flux measurements obtained by eddy covariance and sap flow methods. Best fit relationship: $y = 0.9206x + 10.749$, $R^2 = 0.8956$.

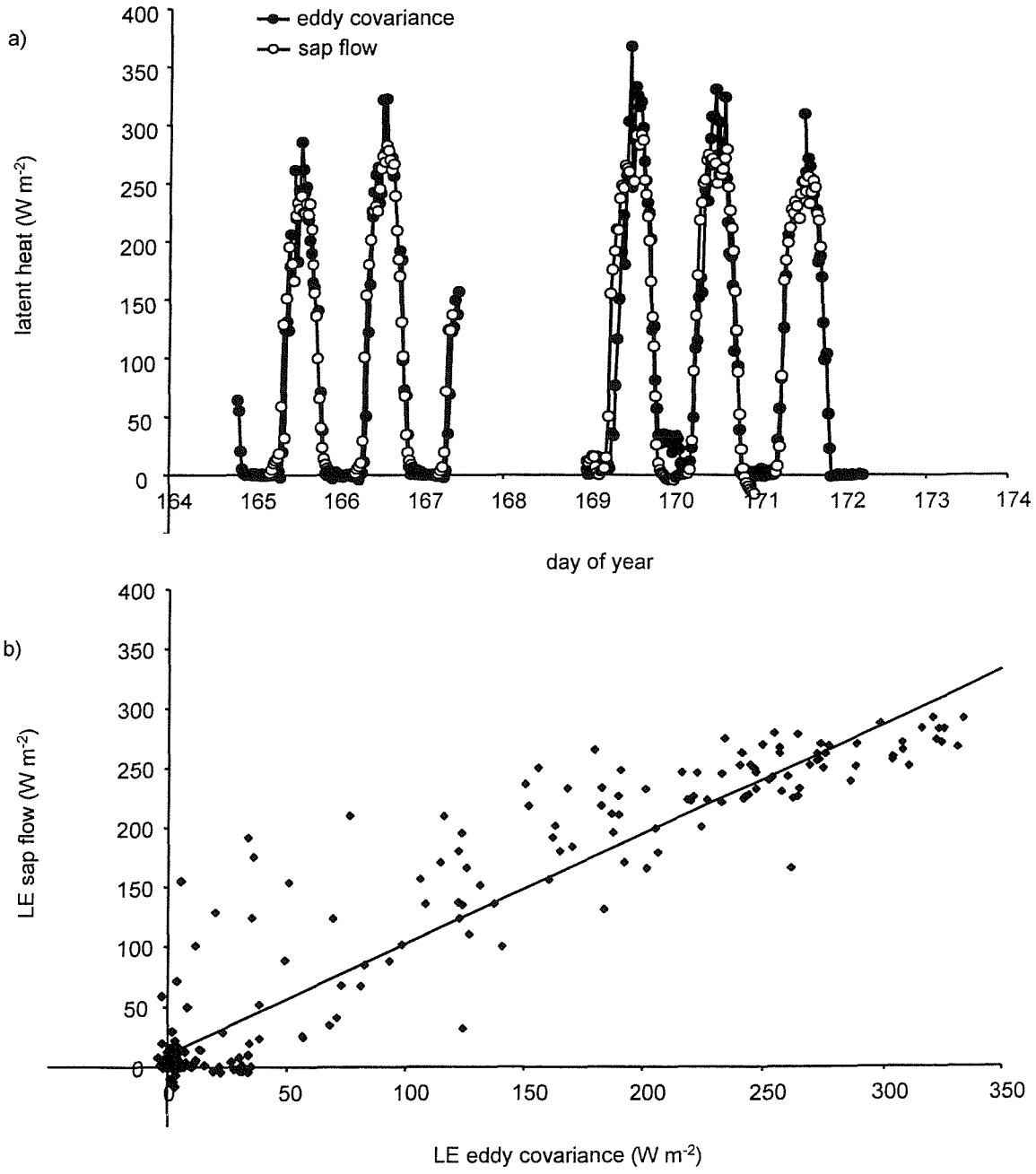


FIGURE 3.3 – Effects of FACE on sap flow measurements in P. alba (July 2000):
(●) FACE measurements ([CO_2] 550 ppm), (O) control points (ambient [CO_2]). n = 18 gauges, each plot is the mean.

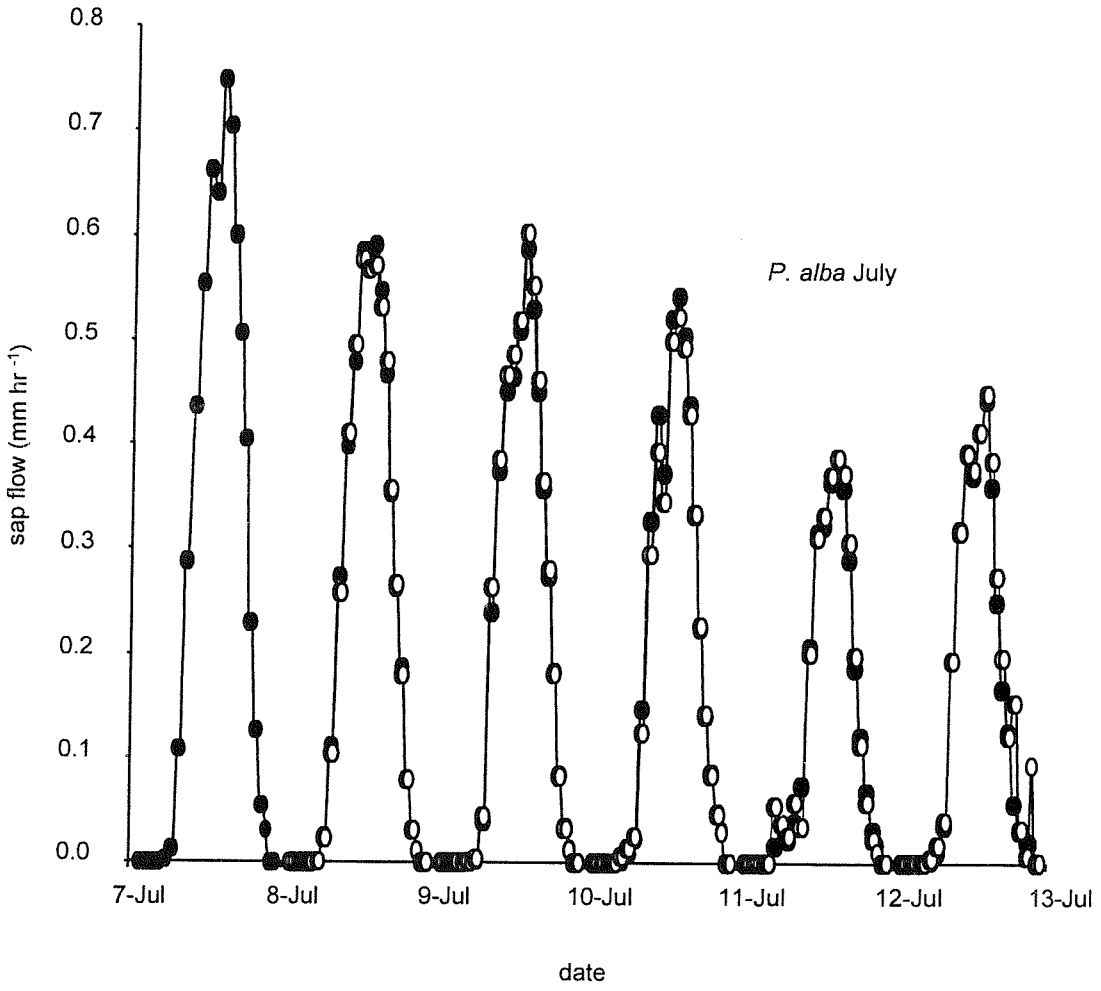


FIGURE 3.4 – Relationships between branch diameter (measured 1cm from base of branch) and sap flow for branches growing below the main sap flow gauge: Measurements are averaged over 5 days, taken in June (euramericana) and August (alba) during the 5 days following the main measurements. Branch gauges were not needed for P. alba in July as the stems were small enough to fit main gauges below all branches.

(●) FACE measurements ([CO_2] 550 ppm), (O) control points (ambient [CO_2]). 3a) *P. x euramericana.* Relationship (ambient and FACE): $y = 0.0219e^{0.2402x}$ $R^2 = 0.83$. Fig 3b: *P. alba.* Relationships: (FACE) $y = 0.0023x^2 - 0.0042x - 0.0519$, $R^2 = 0.66$. (Ambient) $y = 0.0009x^{2.41}$, $R^2 = 0.80$.

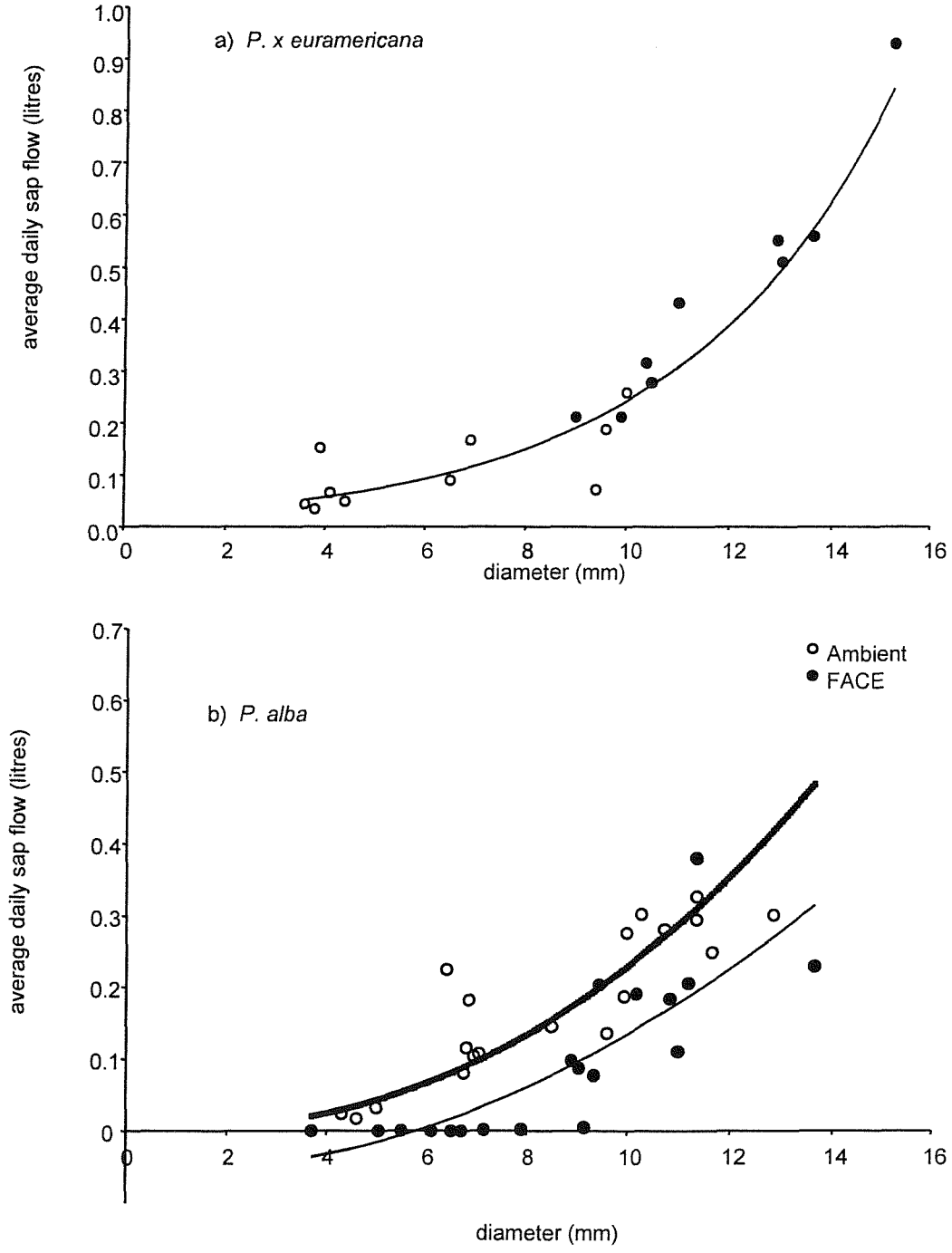
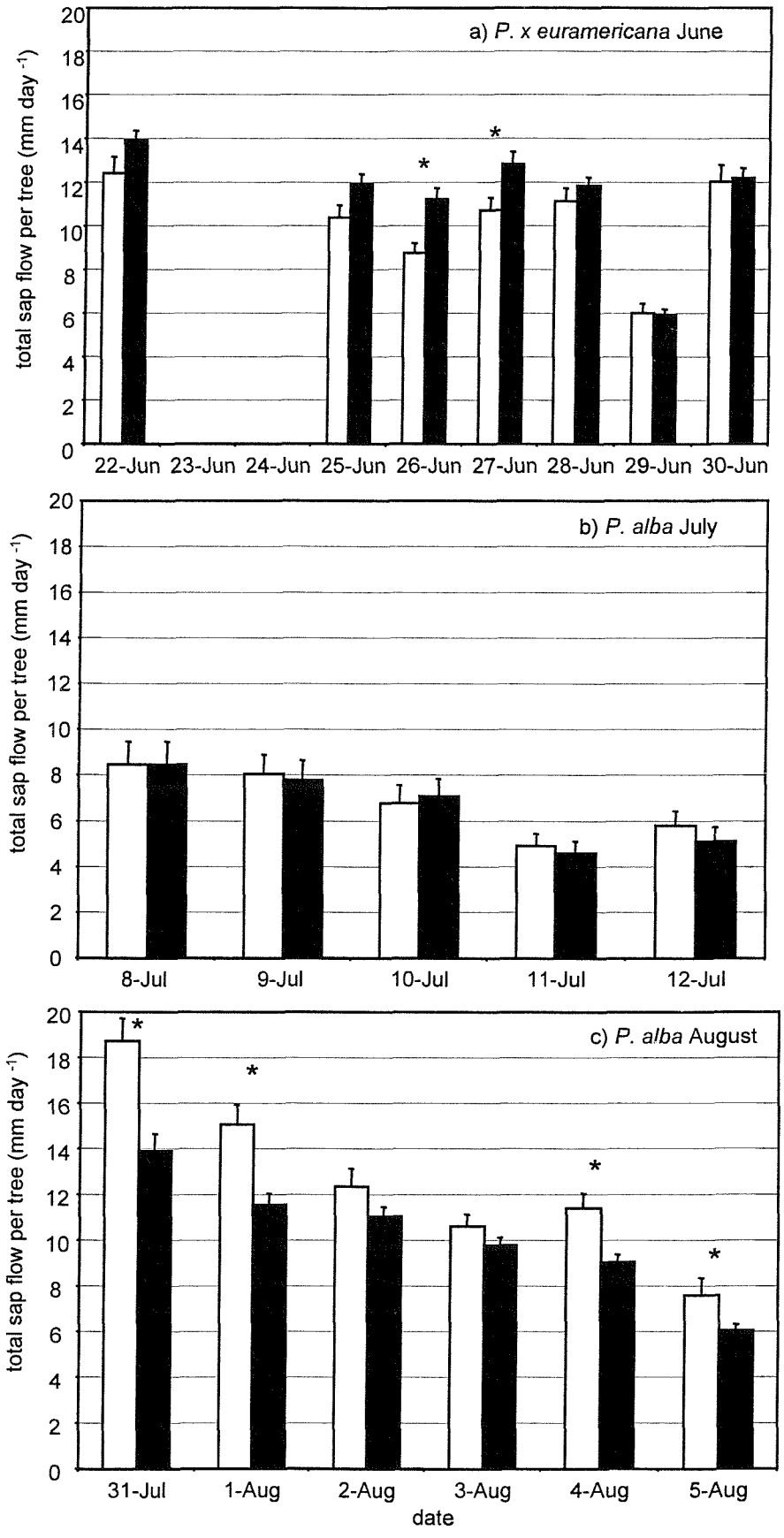


FIGURE 3.5: - mean daily sap flow per tree, corrected to include sap flow in branches growing beneath the main sap flow gauge:
Closed bars denote FACE data ([CO_2] 550ppm), open bars denote control data (ambient [CO_2]). Asterisk's denote significant differences: *P. x euramericana* June - significant difference on 26th and 27th June ($P<0.05$), *P. alba* July - no significant difference ($P>0.05$) *P. alba* August - significant difference on 31 July, 1st, 4th & 5th August ($P<0.05$). n = 18 trees.



RESULTS

Diurnal measurements showed that stomatal conductance of both genotypes was consistently decreased by FACE (Figure 3.1), although this decrease was not always statistically significant ($P>0.05$). Stomatal conductance in *P. x euramericana* was significantly decreased at 08:00 hrs and 15:00 hrs with reductions of 17% and 28% respectively, but was not significant at other times. Measurements made in July for *P. alba* showed significant reductions in the FACE treatment at 17:00 hrs (35%, $P<0.005$) and 19:00 hrs (39%, $P<0.05$), but no significant effect between 10:00 hrs and 15:00 hrs ($P>0.05$). *P. alba* measurements made in August 2000 showed significant decreases of between 24% (18:00 hrs, $P<0.05$) and 31% (14:30 hrs, $P<0.005$) at all time points except at 13:00 hrs where stomatal conductance in ambient trees was reduced to within 16% of the FACE trees ($P>0.05$).

Figure 3.2a shows the diurnal fluctuations in transpiration from an area of *P. x euramericana* growing outside of the experimental rings. On each day, transpiration measured by sap flow tended to be greater than that detected by the eddy covariance method during the morning and late afternoon, but always tended to be less than eddy covariance measurements during the mid-day period (typically between 12:00 hrs and 15:00 hrs). Figure 3.2b shows the relationship between the latent energy of water loss measured by sap flow and eddy covariance methods. The spread of data indicates that there was a better correlation between the two methods at high sap flow, and the trend described for figure 3.2a is also apparent,

although a straight line relationship ($y = 0.9206x + 10.749$) gave the best fit ($R^2 = 0.9$)

The effects of FACE on sap flow are shown for P. alba in figure 3.3. The figure shows a typical diurnal trace for whole tree sap flow, used to calculate daily sap flow m^{-2} (figure 5). During July 2000, for *P. alba*, no effect of FACE was observed

Figure 3.4 (a&b) shows the relationships between sap flow and branch diameter for both species. A single curve was found to be most appropriate to describe the relationship for *P. x euramericana* grown in both FACE and ambient conditions ($y = 0.0219e^{002402x}$, $R^2 = 0.83$). However, separate curves were required to describe the relationships for *P. alba* in the FACE ($y = 0.0023x^2 - 0.0042x - 0.0519$, $R^2 = 0.66$) and ambient ($y = 0.0009x^{2.4115}$, $R^2 = 0.8$) treatments. Zero flow was observed in some branches of *P. Alba*. This was attributed to the small number of leaves and their extremely shaded position at the bottom of these trees.

Figure 3.5 shows the total daily sap flow per tree including estimated sap flow for branches below the main gauges. Sap flow in *P. x euramericana* was consistently increased in the FACE treatment, but this increase was only significant ($P<0.05$) on the 26th and 27th June where the percentage increases in FACE were 28% and 19% respectively. Sap flow in *P. alba* in early July fluctuated between 5.0 and 8.5 mm per day, but there was no significant difference between the FACE and ambient treatments at that time. However, the second set of measurements made in August showed rates of sap flow up to 18 mm per day. They also show that sap flow was

significantly decreased in the FACE treatment on four out of six days with reductions of between 20% and 25%.

DISCUSSION:

For this mature poplar forest canopy, stomatal conductance was consistently reduced by the FACE treatment. Although this was not always significant at the 5 % level of probability, the result is in contrast with that for *Pinus taeda* reported from the FACTSI Duke forest FACE exposure where CO_2 was shown to have no significant effect on stomatal conductance or leaf-level water relations. Previous reviews of the effect of elevated CO_2 on gs have also reached conflicting conclusions. For example, a meta analysis of gs measurements made in 48 studies of woody plants by Curtis and Wang (1998) reported a modest and non-significant reduction of just 11% in elevated CO_2, whilst a more recent meta analysis (Medlyn *et al.*, 2001) on data from 13 long-term studies of woody species shows a significant (21%) decrease of gs in response to elevated CO_2. The main difference in the two data sets used for these analyses was the length of studies. 41 of the 48 experiments analysed by Curtis and Wang were exposed to elevated CO_2 for less than one year, in contrast to the long term experiments (18 months to 4 years) analysed by Medlyn and Barton. These two studies perhaps highlight the variability of gs data from experiments of less than one year old, but also suggest that the data reported here from POPFACE trees are likely to be representative of future responses to increasing atmospheric CO_2, since they were collected as the canopy closed and after exposure to CO_2 throughout the life of the tree. Stomatal conductance in *Populus* is considered large for a woody plant with several studies reporting gs between 300-400 mmol m^{-2} s^{-1} (Allen et al., 1999), whilst in the *Pinus*

study, gs was consistently below 90 mmol m^{-2} s^{-1}. This observation also supports the concept that trees are in general, less responsive to CO_2, since, in general, lower stomatal conductance is observed in woody versus herbaceous plants (Saxe *et al.*, 1998). Poplar may be an exception to this generalisation, since here, reductions in gs of 25 % in FACE were not uncommon.

The consequences of such reductions in gs for tree and stand-level water use have rarely been quantified. Here, whole tree sap-flow measurements were validated initially using assessments of water vapour flux from the surrounding plantation canopy. Previous validations have shown good agreement between sap flow and eddy flux (Rana and Katerji, 2000). Kelliher and Kostner (1992) found a disparity of 10-20 % between measurements made by sap flow and eddy covariance techniques. For POPFACE, 74 % of the data points showed a disparity of less than 10 %, whilst 82 % had a disparity of less than 20 %. Disparity in the study reported here was probably due to a community of weeds and grasses at the site, although these were negligible in the rings, because the trees formed a closed canopy. Transpiration from plantation weeds, especially at midday, probably resulted in canopy measurements recorded by eddy covariance being greater than those recorded with sap glow gauges. Despite this, the data confirmed that the sap flow gauges provided a good assessment of whole tree water-use and could be used as a base from which to scale measurements made on individual trees to that of the canopy.

Daily estimates of total tree water use, made using the sap flow gauges revealed an interesting pattern of effect. Early in the season, sap flow was enhanced in FACE, mid season there was no effect of FACE, whilst towards the end of the season, total

tree sap flow was significantly reduced by FACE. Since measurements were made on two different species of *Populus*, it is possible that these differences were species-specific. However, this seems unlikely. Much more likely, is that these effects were the result of changes in tree leaf area and LAI induced by the FACE treatment throughout the season. Measurements of leaf area index (LAI) made concurrently with the sap flow showed that leaf area was increased for both species grown in the FACE treatment (Birgit Gielen, personal communication, data not shown) but that the difference in LAI between the treatments tended to be markedly reduced as the season progressed.

In early season, leaf expansion and production may both be stimulated in FACE (Ferris et al., 2001), whilst differences in patterns of leaf growth and LAI begin to disappear by mid-July (Gielen, pers com). The effect of this is to change the balance between FACE-induced leaf-level effects on gs and FACE -induced tree-level effects on leaf area in determining tree water use. Estimates of the overall seasonal water use of the canopy in ambient and FACE treatments suggest that leaf-level reductions in gs are, in general, inadequate to provide a large 'ameliorative' effect of elevated CO_2 in future. However, clear species differences were seen here when estimates of seasonal water use were considered. In *P.x euramericana*, a consistent small stimulation in LAI throughout the season resulted in the 23 % increase in water use by this species, whilst for *P alba*, the combined effect of lower stomatal conductance and no effect on mid to late season LAI resulted in a 9% reduction in seasonal water use by this species in FACE. These data provide no evidence to suggest that there is an 'anti-transpirational' effect of

elevated CO_2 at the canopy level or that water use efficiency will be dramatically improved in poplar forests at future CO_2 concentrations.

General Discussion:

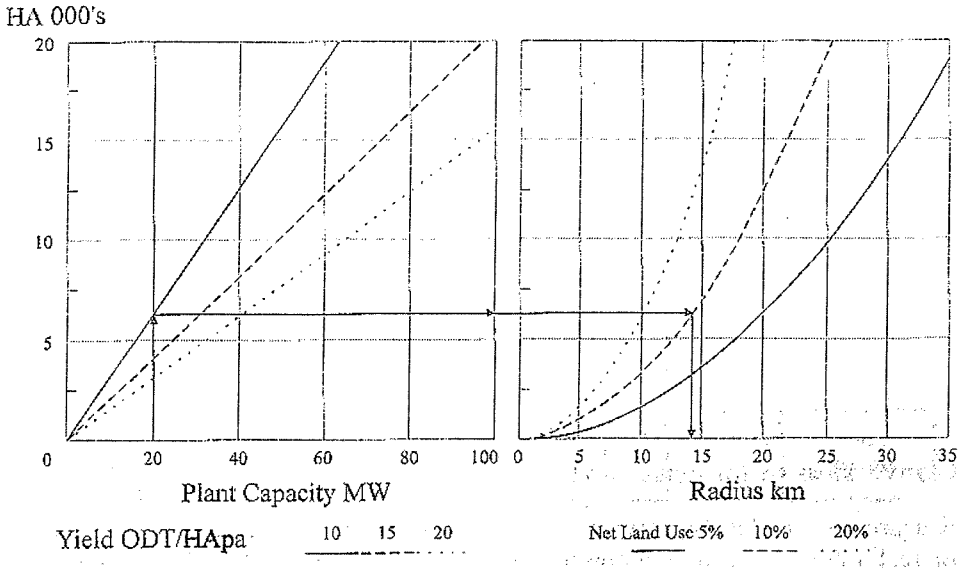
The three chapters of this thesis have dealt with diverse topics. They have explored how plant physiology relates to yield, how the genetic blueprint of a plant determines some of its more complex traits, and how some of these traits, especially those related to water use, may be affected by climate change in the future. The common thread linking these chapters together is the genus *Populus* which has been shown to be an important economic crop, and a potentially useful source of renewable energy for the future. This final discussion aims to address the implications of some of these findings and how they will impact in the future.

It is widely accepted that poplar has potential for use as a biomass crop for renewable energy production as an alternative to the unsustainable use of fossil fuels. However, this raises several questions: Will renewable energy from biomass be an economically viable alternative to fossil fuels? How much land will be needed to produce a significant quantity of energy, and is there enough land available for this purpose? How much water will be required to sustain these crops? The global climate is changing – how will these changes affect the suitability of Poplar as a biomass crop? Is it likely to become more or less feasible as time goes on? Is it worth investing in this technology for the future?

The viability of poplar as a biomass crop for renewable energy:

Several papers and books have been written that consider the economic viability of renewable energy from biomass (Hummel *et al.*, 1988; Hansen, 1991; Hall, 1994; Hall & House, 1995; El Bassam, 1998; Makeschin, 1999). Hall (1994) proposes the following model to help calculate the area of land required to supply a power plant of a specific output:

Figure 4.1: Land requirements for biomass power plants, (Hall, 1994)



Taking Hall's example it can be seen that a 20MW power plant would require 7,000 hectares of biomass crop yielding 10 oven dry tonnes (ODT) per hectare per year. Extrapolating this across to the second graph it can be seen that 10 percent of the land within a 14km radius of the plant would be required for growing that biomass crop. This formula not only allows calculation of the area of land required to supply a biomass power plant, but also gives an indication of the distance the crop may have to be moved allowing transportation costs to be considered.

Using this model with data from figure 1.1 of this thesis it is possible to assess the areas of land required to grow poplars as biomass crops for energy:

Hoogvorst, managed as short rotation coppice (SRC) and grown in conditions similar to those found at Cambridge yields approximately 17 ODT ha^{-1} yr^{-1}. This

would require just 3,500 hectares of land - a radius of 11km around a 20MW power plant (assuming 10% land use). However, 3,500 hectares of the same clone planted in an area similar to Brecon would yield approximately 12 ODT ha^{-1} yr^{-1} - only enough to supply a 14MW power plant. This calculation shows the importance of understanding how different environments influence yield, and how this needs to be taken into consideration when assessing the viability of renewable energy for biomass.

So, assuming that renewable energy from biomass could be economically viable, how much land would be required to produce a significant proportion of our energy from biomass?

The combined output of all UK power stations is in the region of 50,000MW (National Grid UK). Using Hall's model this equates to somewhere in the region of 10 million hectares of high yielding SRC poplar! The UK covers approximately 24.5 million hectares, 18.6 million ha of which is agricultural land (National Statistics Office, June 1999 figures). Therefore over half of the UK's agricultural land would need to be planted with high yielding poplar in order to produce the same amount of energy as our current power stations.

The UK government has made a commitment to provide 10 percent of the UK's energy requirements from renewable resources by the year 2010 (DTER 1999). Assuming UK energy requirements in the year 2010 will be 50,000MW, then 5,000MW needs to come from renewable resources. This equates to approximately 1 million ha of high yielding poplar, or just over 5% of current (1999) UK

farmland. To put this figure into context – in 1999 3% of all farmland was 'set-aside' land, therefore if all set-aside land were turned over to poplar this could potentially provide in the region of 3,000MW of power - 60% of the governments total commitment to renewable energy.

These estimates based on data from this thesis show that renewable energy from biomass could in theory be used to provide a significant proportion of the UK's energy requirements. However, the economic viability of such a venture is harder to assess since it depends on a host of factors that are in constant flux. For example the cost of competing energy sources (fossil fuels and other forms of renewable energy), production, processing and harvesting costs, and the potential profit obtainable from competing land use options.

Issues arising from the increased planting and distribution of poplar:

Assuming that renewable energy from biomass will increase in the future, there will necessarily be an increase in the large scale cultivation of biomass crops. It is likely that poplar will form a significant proportion of these crops and it is important to consider the implications of this for the future.

One immediate issue that became apparent early on in this project was the potentially devastating effect of pests and diseases on yield. Chapter 1 mentions the presence of rust (*melampsora* spp.) at one of the field sites and suggests that this may be responsible for the unexpectedly poor performance of what was known to be a high yielding genotype. The dangers of relying on large scale plantations of

genetically identical material are obvious as a single strain of disease such as rust could easily devastate the whole crop, which in the case of renewable energy crops would leave power stations without sufficient fuel. This danger is increased for short rotation crops such as poplar which are usually harvested after 3 years of growth, so increasing their exposure to potential pests and diseases.

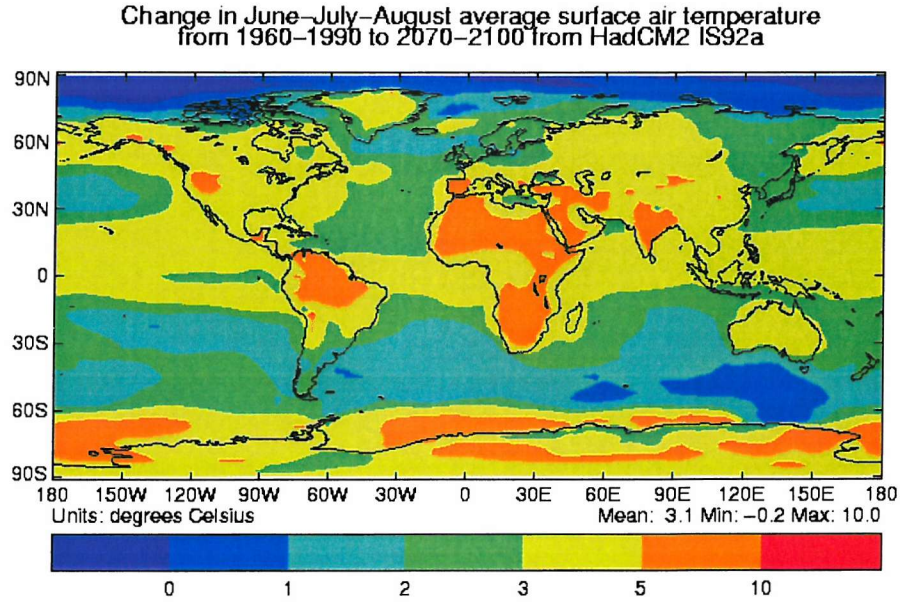
Good management practices such as planting a mosaic of different genotypes will reduce the potential damage caused by a single pest or disease, but from a scientific point of view this is one area where a better understanding of the genetic control of disease resistance and tolerance may help. In fact this is one area where marker assisted selection could be a valuable tool since potential disease resistance is not easy to identify by conventional selection techniques.

A second, longer-term consideration is how large scale plantations of poplar will perform in the future. Global climate change is a widely acknowledged phenomenon with environmental and economic implications for all crop plants. Figures 4.2, 4.3 and 4.4 show graphical predictions of how the world's climate may change in the future (based on a 'business as usual' scenario where emissions of CO_2 and green house gasses continue to increase).

Figures 4.2 – 4.4: Global climate change projections from "The Hadley Centre for Climate Prediction and Research"
(http://www.meto.govt.uk/research/hadleycentre/):

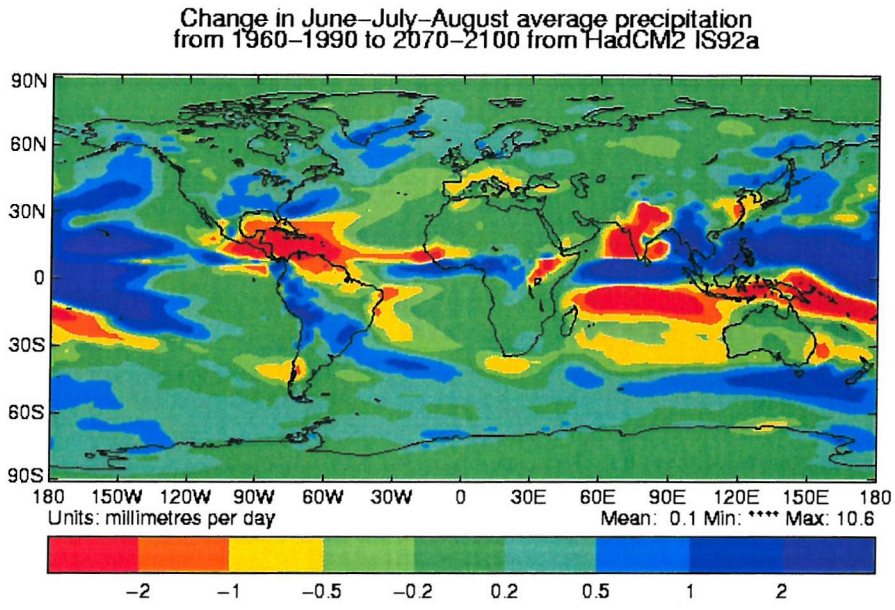
These figures show some key results from climate-change experiments conducted using Hadley Centre computer models of the climate system. The models are two versions of coupled atmosphere-ocean general circulation model (and HadCM3). The experiments assumed that future emissions of greenhouse gases would follow the IS92a scenario, in which the atmospheric concentration of carbon dioxide more than doubles over the course of the 21st century. This is a 'business as usual' scenario, which assumes mid-range economic growth but no measures to reduce greenhouse-gas emissions. Figures show average data for June, July & August – the majority of the growing season for poplar in the Northern Hemisphere.

Figure 4.2:



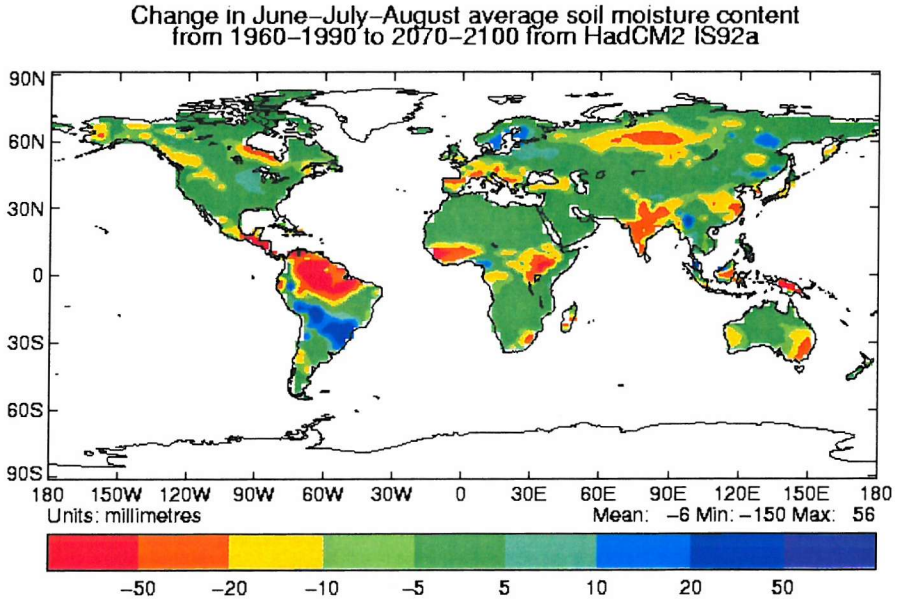
Hadley Centre for Climate Prediction and Research, The Met. Office

Figure 4.3:



Hadley Centre for Climate Prediction and Research, The Met. Office

Figure 4.4:



Hadley Centre for Climate Prediction and Research, The Met. Office

The global distribution of poplar covers wide areas of the Northern Hemisphere including Europe, North America and Asia (Ceulemans, 1990). Figure 4.2 shows predicted changes in average annual surface air temperature for June to August. According to this scenario the majority of the UK can expect to be up to three degrees warmer during the summer months while some areas of southern Europe, Asia and the USA could become up to ten degrees warmer. Meanwhile the predictions show that rainfall (figure 4.3) is likely to decrease slightly in mid Europe, and by up to one mm per day in the South, while the majority of North America looks set to experience a slight increase in rainfall. However, despite relatively small fluctuations in rainfall in these areas, the net effect of rainfall and increasing temperatures is predicted to result in significant reductions in soil moisture of up to 50mm in southern areas of Europe and Asia, and northern areas of North America and Eastern Europe (figure 4.4). Consequently, this projection of climate change predicts there is likely to be significantly less water available in areas that are currently considered suitable for poplar cultivation. This will increase the need for irrigation, which (assuming there is sufficient water supply to provide it) will increase the economic cost of producing biomass crops for energy. In fact, it is possible that the shortage of water could limit current agricultural practice and this may even lead to a change in the types of crops that are farmed in order to conserve water. For example, as temperatures increase and water availability falls it is likely that conditions will favour more water efficient C$ plants. Photosynthesis in these plants requires a higher ambient temperature, but does not involve photorespiration. Furthermore, their ability to fix carbon more efficiently requires fewer and smaller stomatal openings compared to C3 plants

making them ideally suited to warm dry conditions (Raven *et al.*, 1992). From a biomass perspective this could mean that C4 crops such as Miscanthus (*Miscanthus spp.*) might become more favourable for planting in areas previously considered suited to temperate C3 crops such as poplar and willow. However, water will always be a limiting factor to yield and therefore it will be important to take future availability of water into account when planning sites for renewable energy power plants.

There is another important factor to consider alongside predicted decreases in water availability. It is also widely recognised that the concentration of Carbon dioxide in the atmosphere is increasing. This not only contributes to global climate change as a greenhouse gas, but is also well known to increase productivity in plants. The mechanisms of this have been discussed in previous chapters, but it is interesting to consider that as the atmosphere becomes more and more polluted with CO_2, the more economical renewable energy from biomass is likely to become.

Improved crop yields may be a positive side to increased carbon in the atmosphere, but CO_2 is only one of the potential limiting factors to yield. If, as predicted, available water decreases as CO_2 increases, what will be the net effect on crop yields? To further confuse this issue the known effect of CO_2 induced stomatal closure must also be taken into account – is it possible that improved water use efficiency due to stomatal closure could allow increased yield coupled with decreased water use? Data presented in chapter 3 of this thesis suggests that this scenario is unlikely for poplar. However this experiment concentrated on discrete sample intervals and it would be interesting to monitor water use constantly

throughout the growing season and relate it to biomass yield at harvest to see how the water use to biomass yield ratio is affected by elevated CO_2.

The response of below ground biomass to elevated CO_2 is also likely to be an important aspect of how poplars will perform in the future. Data presented in chapter 2 of this thesis (figure 2.2) shows an increase in secondary root density of poplars grown in elevated CO_2. Increased below ground biomass is likely to become more important to plants as soil water content decreases and it is possible that increased CO_2 may help plants to counter a reduction in soil water availability in this way. The effectiveness of increased root biomass in helping to maintain viable yields in conditions of water stress will depend on a number of factors such as root architecture, foraging ability and root: shoot biomass ratios. As previously mentioned, the work in this thesis shows an increase in secondary root density under elevated CO2, implying that root architecture may become more branched, and so potentially improve water uptake from the soil. Previous research has identified that root: shoot ratios increase for poplar grown in elevated CO2 (Radoglou & Jarvis, 1990; Bosac *et al.*, 1995) which is an indication that current yield may be sustainable in the future, provided that increased below ground biomass goes some way towards offsetting reduced soil water availability.

Consequently there are a number of scenarios that may apply to poplar cultivation in the future:

1 Harvestable biomass yields remain constant as elevated CO_2 stimulates root growth enabling water uptake from a wider area.

2 Harvestable biomass yields increase due to elevated CO_2 stimulated growth and stomatal closure overriding the effect of reduced water availability.

3 Harvestable biomass yields decrease as plants allocate more carbon below ground to counter reduced water availability

4 Harvestable biomass yields decrease as reduced water availability overrides increased growth due to elevated CO_2.

As this discussion has shown it is extremely difficult to predict how future climate changes will impact on poplar cultivation. It has been demonstrated that poplar could be an economically viable crop for use in renewable energy power plants and that is has the potential to play a significant role in achieving the UK commitment of providing 10% of its energy requirements from renewable resources by the year 2020. The possible effects and consequences of global climate change have also been discussed based on 'business as usual' projected forecast. However, 'business as usual' is only one of a number of scenarios that could play out over time. Environmental issues are now high on the agenda in many societies and steps are being taken to reduce emissions ranging from reduced road tax for smaller cars burning cleaner fuel to a tax on industries emitting large quantities of carbon. This will hopefully lead to a reduction in the increase of CO_2 in the atmosphere, but it is unlikely to reverse or even stop the trend – so great is our dependence on fossil fuels. Alternatively other issues may give rise to increased emissions over and above current predictions. Economic drivers are extremely important in determining environmental policy as recently demonstrated by the United States who have disregarded their Kyoto commitments in order to help divert an

economic recession. This situation is likely to continue to occur as short term issues are given priority by short term elected governments.

However, regardless of the precise nature of climate change in the future, it is obvious that current levels of global energy consumption are unsustainable in the medium to long term. Consequently it is important that the knowledge base relating to alternative energy resources and their performance in changing climates is continually built upon to ensure a successful future for renewable energy. The work presented in this thesis has helped to increase the knowledge base for poplar and forms a basis for future work on the physiological and genetic basis of productivity and water use in Poplar in current and future climates.

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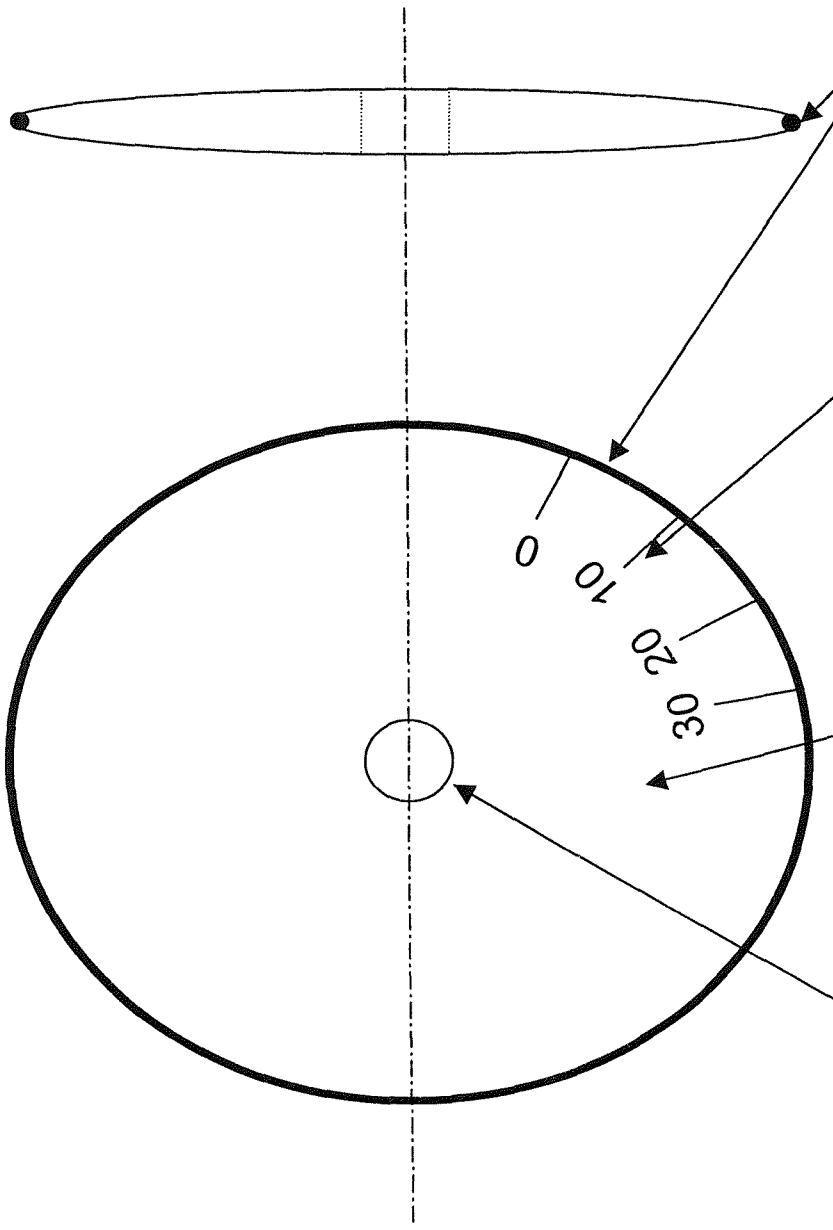
Appendix 1

Design of Root Measuring Wheel.

Root Measuring Wheel



Rubber O-ring to grip acetate sheet - sits in groove machined into rim of wheel.

Scale marked on to rim - minor scale in unlabelled 1 mm graduations, major scale in labelled 10mm graduations. Scale starts from 0 and increases in a clockwise direction as shown.

Ideal Ø = 63.7mm to give circumference of 20cm. Actual diameter will be dictated by size of available O-ring.

Construct a handle attached via central hole to allow free rotation of wheel, and one handed operation.

Appendix 2

Data Processing for QTL Analysis – a step by step account.

Data processing for QTL analysis.

Report by Stephen Bunn following a visit to Toby Bradshaw's lab at the University of Washington, Seattle, USA. May 2000.

1. Data Organisation

Assuming a set of data has already been collected regarding a particular trait (or traits) of family 331, it needs to be organised in a spreadsheet as follows:

	Genotype 1	**Genotype 2**	**Genotype 3**	**Genotype 4**	**...Genotype n**
Trait 1					
Trait 2					
Trait 3					
Trait 4					
...Trait n					

Give each trait a unique code. Use a '*' as the first character with up to seven further letters / numbers, but no spaces.

Enter a minus (-) sign into any cells within the spreadsheet that do not contain any data.

Take a look at the molecular marker spreadsheet (marker map.xls). Sheet 2 (column labels) shows the genotype names that correspond to the marker maps in sheet 1. If the data set includes data for genotypes not listed on the marker map spread sheet these must be deleted. Also, if there is no trait data for any of the genotypes in the marker map spread sheet each cell in the column must be filled with a minus (-) sign.

Copy all the cells from the marker map worksheet into a new workbook.

Paste the organised data set directly below the marker map in the new work book, ensuring the columns are correctly aligned. Omit any cells containing column headings, but be sure to include the trait codes in the left hand column.

Go to cell C2, and change the value to correspond to the number of traits to be analysed.

Save this new worksheet as a spreadsheet file, and then as a .txt file (tab delimited).

Using file explorer, change the file extension from .txt to .raw.

2. Using MAPMAKER software.

MAPMAKER can be downloaded via the internet from:

ftp://ftp-genome.wi.mit.edu/distribution/software/mapmaker3/

There is also a tutorial available on the web from:
http://linkage.rockefeller.edu/soft/mapmaker/

(note: MAPMAKER creates a lot of files with different file extensions. It is a good idea to give all of these files the same filestem (e.g. steveqtl.*xxx*) This way it is easier to keep track of all the files relevant to a particular analysis).

Save a copy of the fam331.pre file in the same directory as the .raw file. This file has been created to prepare the data for processing by MAPMAKER/QTL. It needs to be used each time this program is used to analyse family 331 data, so don't rename it or delete it!

Run MAPMAKER from a MSDOS window.

This will create a number of files, the most relevant of which is a .map file which is used by MAPMAKER/QTL, so it is a good idea to save all of these files in the same directory as the .raw and .pre file.

Run MAPMAKER/QTL from an MSDOS window.

At the command prompt type "photo *filename*" – this will create an output file in which all output from MAPMAKER QTL is recorded. (It will be called *filename*.out).

At the command prompt type "load *filepath/filename* (don't add a file extension – the program will automatically look for the *filename*.map file in the folder that you have specified).

At the next command prompt type "seq [all]"

At the next command prompt type "trait #" (where # is the number of the trait to be analysed – the traits are numbered in order relating to the original spreadsheet, where number 1 is the trait in the first row below the markers).

At the next command prompt type "scan" – the screen should run through columns of data and then stop.

At the next command prompt type "show peaks" – this will show the statistical analysis of the scanned data for that trait. Most useful info on this screen is the LOD score. Typically, lod scores of over 3.0 are worth further analysis.

Note: if the system crashes go back to run mapmaker QTL, type the same name for the output file, then repeat the steps detailed above.

3.Writing a batch file to process data through MAPMAKER.

The above method of running data through MAPMAKER is OK for one or two traits, but becomes very tedious when there are several traits to process. It will

also create a huge output file, most of which is unnecessary (e.g. processing 90 traits created an output file of over 2100 pages long!).

To save on time and work it is possible to write a batch file to run the process for you. The following is an example of a batch file:

load *drive:\folder\filename*
pr na on
seq [all]
tr 1
scan
photo *outputfilename*
show peaks
photo off
tr 2
scan
photo *outputfilename*
show peaks
photo off
.... repeat for as many traits as required...
save
quit

Save this as a .bat file

To run the batch file, load MAPMAKER QTL then type "run *filename*.bat"

Note: If MAPMAKER QTL crashes during the running of a batch file – examine the output file to determine the last trait that was processed – open the batch file in a text editor and delete the traits that have already been processed. Finally, run MAPMAKER QTL again and run the modified batch file to continue data processing from where it crashed.

4.Making sense of the output from MAPMAKER QTL

Typical output from MAPMAKER QTL:

```
LOD score peaks for scan 1.1 of trait 1 (lfareaE).
Sequence: [all]
No fixed-QTLs.
Scanned QTL genetics are free.
Peak Threshold: 2.00  Falloff: -2.00
```

```
QTL-Map for peak 1:
Confidence Interval:  Left Boundary= P1074-G12_15 (off end)
                     Right Boundary= P1321-H12_03 + 0.0

INTERVAL               LENGTH  QTL-POS  GENETICS    WEIGHT
DOMINANCE
P1074-G12_15           19.7    6.0      free       -2290.7 -4161.8

chi^2= 12.545 (2  D.F.)        log-likelihood= 2.72
```

```
mean= 12531.559  sigma^2= 4224224.038  variance-explained= 42.6%
```

The value of most interest from this output is "`log likelihood`" (also referred to as a lod score). If this value is greater than 3.0, then it is possible that there may be a QTL associated to this trait.

Scan through the output file and extract all traits with lod scores greater than 3.0. Put these into a spreadsheet for ease of reference. At this point it is also useful to record the following data for each trait:

Trait,
Trait number (the number corresponding to the order in which the traits were processed – ie first trait in MAPMAKER QTL is trait 1)
Trait abbreviation (7 letters used for mapmaker processing),
Lod score,
Left hand marker (first letter/number sequence given under `INTERVAL - P1074` in the example above),
Linkage group (found by locating marker on linkage map of populus genome (Populus IV, Bradshaw and Stettler 1994)).

5. To determine whether the traits follow a normal distribution:

In order to detect a QTL the trait data should follow a normal distribution. This can be tested using a Shapiro-Wilk test. This can be done easily using MS Excel combined with a stats analysis plug in which can be downloaded from http://www.analyse-it.com/ . This comes as a 30 day free trial, after which it has to be purchased.

To install Analyse-it software, select the download option from the web site and follow the instructions. The program will automatically install itself into MS Excel.

To analyse the data using Analyse-it, arrange the data in a spreadsheet as follows:

Genotypes:	Trait 1	Trait 2	Trait 3	Trait 4	...Trait n
Genotype 1					
Genotype 2					
Genotype 3					
Genotype 4					
...Genotype n					

This can be done easily by selecting the entire trait /genotype data from the spreadsheet prepared for MAPMAKER processing, and pasting into a new workbook using the Paste Special / transpose option on the edit menu.

Proceed with the following selections from the menu bar:

Analyse
Descriptive

Summary (continuous)

Select one trait from the drop down list in the dialogue box.
Check the following settings in the same dialogue box:
Confidence interval: 95%
Percentile plot: 95%
Normality test: Shapiro-Wilk

The output from the test will appear in a separate work sheet as a report showing several graphs and tables. Scroll to the bottom of this page to find the last table which looks like this:

	Coefficient	p
Shapiro-Wilk	0.9841	0.7529
Skewness	0.1084	0.7402
Kurtosis	-0.2865	0.7724

If the P value for Shapiro-Wilk is greater than 0.05, then the data set is not significantly different to a normal distribution and this trait can be analysed further using QTL CARTOGRAPHER. For ease of reference It is useful to record Shapiro-Wilk P values in the same spreadsheet used to record Lod scores.

6. Using QTL CARTOGRAPHER to confirm presence of QTL.

QTL cartographer is available via the internet from:
http://www.statgen.ncsu.edu/qtlcart/

This site also contains information and a tutorial for using QTL CARTOGRAPHER.

Download and unzip the files into a new folder. This will insert several .exe files into the folder.

QTL Cartographer requires the use of 2 output files from MAPMAKER QTL – the .map file and the .raw file. Make a copy of these two files in the QTL Cartographer folder.

Note: Just to confuse the issue, QTL Cartographer produces its own output .map file, so it is a good idea to rename the MAPMAKER .map file as a .mps file.

Step 1 – Use Rmap.exe to convert the MAPMAKER .mps file into a QTL Cartographer .map file:

Run Rmap.exe by double clicking the icon from windows explorer.
A list of options will appear . Proceed with the following steps:

Select option (Change filename stem) to change the name of all the files created for this analysis to something relevant (e.g. 331qtl)

Select option (Input file), and specify the name of the .mps file that you wish to convert.

Select option 0 (continue with these parameters).

This will create a new file in the folder called *filestem*.map

Step 2 – Use Rcross.exe to convert the MAPMAKER .raw file into a QTL Cartographer .cro file:
Run Rcross.exe by double clicking on the icon from windows explorer.
A list of options will appear as before.

Select option to change filename stem if necessary and use the same name as you did in Rmap.

Select option to Input file and specify the name of the MAPMAKER .raw file that you wish to convert

Select option 0 (continue with these parameters)

The program will run and create a new file called *filestem*.cro

Step 3 – Use Zmapqtl.exe to look for QTL in the data set.

Warning – Zmapqtl.exe takes a very long time to analyse a data set. It is a good idea to set the program running on a fast computer to run overnight when there will be no other software in use to slow it down.

Zmapqtl.exe looks at one trait at a time and tries to determine whether or not a potential QTL is significant by randomly distributing the markers for a set number of permutations to see if it can find any significant associations. If it can't, then it will confirm the location of the potential QTL.

Run Zmapqtl.exe by double clicking on the icon from windows explorer.
A list of options will appear as before.

Select option to change filename stem if necessary and use the same name as you did in Rmap.

If you have used the same filename stem throughout the previous steps then it will not be necessary to specify the input filename as it will be entered automatically. However it is a good idea to specify a new output filename which is relevant to the particular trait you want to analyse. The 7 figure code used in MAPMAKER is a good filename to use as it allows you to cross check your data easily. Make sure you include the .z file extension to the name so that you can easily recognise it as a Zmapqtl output file.

Select option (Trait to analyse). Enter the number of the particular trait that you want to analyse (refer to lod score table for easy reference).

Select option (Number of permutations). Enter 100 – this figure is suggested by Toby Bradshaw as one that will provide a reasonable statistic without taking too long to achieve.

Select option 0 (continue with these parameters)

The program will run for several hours before producing a new output file *trait.z*

7. Making sense of QTL Cartographer output:

Here is an example of a much shortened version of an output file from QTL cartographer:

```
#     959262292
        QTL Cartographer V. 1.10b, released May 1996
        This output file (thte15.z) was created by
I:\PROGRA~1\QTLCART\QTLCAR~1\ZMAPQTL.EXE...
        It is 09:44:52 on Thursday, 25 May 2000


The position is from the left telomere on the chromosome
-window       10.00       Window size for models 5 and 6
-background       5       Background parameters in model 6
-Model            3       Model number
-trait           15       Trait analyzed
-cross          RF2       Cross
   Test Site    * Like. Ratio Test Statistics   *     Additive        *     Dominance         * Misc. HT
 c  m position      H0:H3      H1:H3      H2:H3       H1:a       H3:a       H2:d       H3:d       H0:H1
H0:H2
-s
 1  1  0.0001      3.061     11.162      0.481     39.700    -14.574    -24.953    -24.562     -8.101
2.580
 1  1  0.0201      3.019     10.860      0.491     40.419    -14.564    -25.375    -24.858     -7.840
2.528
-e
         Comparisonwise p values for the shuffles
 c  m   pos       LR        p                n
-b
 1  1  0.0001  3.0611  0.2100             21
 1  1  0.0201  3.0192  0.2200             22
-b
 Performed 100 permutations of the phenotypes and genotypes
Here are the Experimentwise significance levels for different sizes
Permutation significance level for alpha = 0.1    : 12.6570
Permutation significance level for alpha = 0.05   : 13.0085
Permutation significance level for alpha = 0.025  : 14.0746
Permutation significance level for alpha = 0.01   : 14.3582
-end of shuffling results
```

First scroll to the bottom of the output file and make a note of the value given for "Permutation significance level for alpha = 0.05"

Next, scroll back to the top of the page and slowly scan down the fourth column of numbers "H0 : H3", looking for any values greater than the value given for "Permutation significance level for alpha = 0.05"

If you find one – congratulations you have found a QTL!!!

The chromosome on which the QTL lies is given in column 1 "C".
The nearest marker to the QTL is given in column 2 "M".

If you don't find one – it doesn't necessarily mean there are none there – just that you didn't manage to detect any (better luck next time!).

Appendix 3

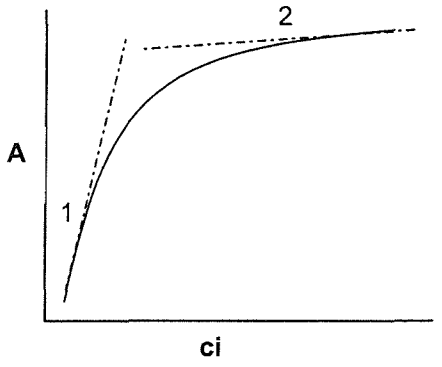
A/ci curves as an indicator of plant responses to elevated CO_2.

The first biochemical step in photosynthesis is the calvin cycle. This invloves the conversion of a single molecule of ribulose 1,5-bisphosphate (5C) (**RuBP**) into two molecules of 3-phosphoglycerate (3C). This takes place as follows:

$$C_5 \xrightarrow{CO_2} C_6 \xrightarrow{H_2O} C_3 + C_3$$

The catalyst in this reaction is ribulose 1,5-bisphosphate carboxylase (**Rubisco**).

Diagram 1.2: A typical Aci curve.



A : CO_2 assimilation rate

ci : intercellular CO_2 concentration

Aci curve

extrapolated

Stage 1.

Here the rate of assimilation increases linearly as intercellular CO_2 increases. This is because at this point the limiting factor in photosynthesis is the availability of CO_2. The gradient of this initial slope indicates the maximum rate of carboxylation of RuBP, and this is used to give the value $V_{c\,max}$ - an *in vivo* measurement of Rubisco activity ($mol.m^{-2}.s^{-1}$).

Stage 2.

Here the curve flattens off as all of the available RuBP is carboxylated by Rubisco. The limiting factor now is the rate of regeneration of RuBP from Fructose 6-phosphate (the end product of the Calvin cycle). This can be due to either the rate of regeneration of RuBP that may be supported by the availability of inorganic phosphate, or to the electron transport capacity of the thylacoid, known as J_{max}

Appendix 4

Calculation of sap flow using the constant power heat balance method:

The rate of sap flow (g h^{-1}) is calculated from the components of the energy balance equation:

$$\mathbf{Q_f = P - Q_v - Q_r} \quad \text{(W)} \qquad (1)$$

P (the power input to the heater) is calculated from Ohm's Law:

$$\mathbf{P = V^2 / R} \quad \text{(W)} \qquad (2)$$

where **V** = heater voltage
R = heater resistance (Ohms)

$\mathbf{Q_v}$ (the vertical heat loss by conductance) is calculated from Fourier's Law:

$$\mathbf{Q_v = ((dT_b - dT_a)) K_{st} A) / d_z} \quad \text{(W)} \qquad (3)$$

where $\mathbf{dT_a}$ and $\mathbf{dT_b}$ are the thermocouple a and b temperature gradients (°C)
$\mathbf{K_{st}}$ = stem thermal conductivity (W m^{-1} K^{-1})
A = stem area (m^2)
$\mathbf{d_z}$ = thermocouple junction spacing (m)
The thermal conductivity of wood is 0.42 W m^{-1} K^{-1} (Kellomaki and Wang, 1999).

$\mathbf{Q_r}$ (the radial heat loss) is calculated from:

$$\mathbf{Q_r = dT_r \times K_{sh}} \quad \text{(W)} \qquad (4)$$

where $\mathbf{dT_r}$ = thermopile radial temperature gradient (K)
$\mathbf{K_{sh}}$ = sheath conductance of the heater/ thermopile (W m^{-1} K^{-1}) solved during zero-flow by
$\mathbf{((P - Q_v)\text{-}C_p / 3600) \times dT_{sap}}$
where $\mathbf{C_p}$ = specific heat capacity of water (4.186 J g^{-1} K^{-1})
$\mathbf{dT_{sap}}$ = sap temperature differential (°C) = $\mathbf{(dT_a + dT_b) / 2}$

The rate of sap flow (g h^{-1}) is then calculated from:

$$\mathbf{Flow = (Q_f \times 3600) / (dT_{sap} \times C_p)} \qquad (5)$$