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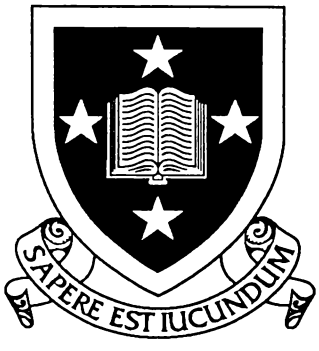
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Some Aspects of the Ecophysiology of *Paulownia* spp.



A thesis

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Paulownia elongata in flower

Abstract

Members of the *Paulownia* genus have recently received widespread attention in New Zealand for their potential as special purpose timber trees and application in agroforestry systems. There has been only limited prior investigation of the ecophysiology of *Paulownia*. This study aimed to quantify aspects of gas exchange; biomass, nutrient and carbohydrate partitioning; and propagation and seedling establishment techniques in field grown and pot grown *Paulownia* trees, in Hamilton, New Zealand.

Gas exchange measurements were undertaken using either a closed system LICOR 6200 unit or an open system Walz CMS 400 unit. Rates of gas exchange of *Paulownia* leaves under saturating light were high, with A_{max} in fully mature, non-senescent leaves ranging from 12 μmol m^{-2} s^{-1} to 25 μmol m^{-2} s^{-1}, with g_s between 0.2 mol m^{-2} s^{-1} and 0.8 mol m^{-2} s^{-1}. The relationship between A and g_s was variable which resulted in C_i ranging from 190 μl l^{-1} to 280 μl l^{-1} under ambient CO_2 concentrations. High rates of transpiration were maintained in the large leaves to facilitate evaporative cooling under high radiation load. Leaf water potentials remained high throughout the day and, on the occasions when a midday depression of gas exchange rates was found, there was little evidence that it was a response to water stress. Circumstantial evidence suggested some form of negative feedback was involved in decreasing gas exchange rates in during the latter part of the day. Measurement of the AC_i response found low levels of stomatal limitation of photosynthesis, with *l* typically between 12% and 30%.

The plants exhibited a number of characteristics of obligate sun plants, with the $PPFD_{sat}$ of the leaves generally above 1000 μmol m^{-2} s^{-1}. The pattern of biomass allocation, with a large proportion of the perennial tissues present as roots and the large investment in foliage, particularly in the establishment phase, was another feature typical of sun plants and enabled the plant to maintain a high growth rate. Growth rates were enhanced by the regular application of liquid fertilizer and frequent watering, and were higher in the warmer conditions of a glasshouse environment than outside. However, the temperature regime during leaf development had no effect on values of A_{max}.

The temperature regime during growth did not affect the allocation of nutrients. In the field grown trees, the highest concentrations of all nutrients, except zinc, were found in the annual tissues, with foliar levels of nutrients in *Paulownia* similar to those reported for other timber species. Nitrogen levels in fully mature *Paulownia* leaves were generally between 2% and 2.5%, and within this range there was little correlation between nitrogen content and A_{max}. Nutrient levels in perennial tissues showed little change throughout each season, although

nitrogen levels did increase in woody tissues at the end of each season. The levels of soluble sugars were high in all tissues of the *Paulownia* trees studied, in comparison with those found in other trees. The perennial root material stored large amounts of soluble sugars over the dormant season, with an average of 30% of the dry weight of the lateral roots found as soluble sugars at the last harvest of each season.

The high soluble sugar content of these roots probably facilitated the growth of new root and shoot material when root cuttings were taken. The position of the root cutting in the parent root system had no effect on the regeneration capacity of the cutting. Cuttings with diameters less than 5 mm were less likely to regenerate than larger cuttings. *Paulownia* seedlings were found to suffer from transplant shock and generally grew more vigorously after transplanting if they were coppiced, rather than planted intact.

Thus it was concluded that high rates of gas exchange and growth could be achieved by *Paulownia* trees if grown under conditions of high light, consistently warm temperatures, with frequent watering and the application of high rates of nutrients.

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List of Abbreviations

A	Assimilation rate
A_{max}	Maximum assimilation rate under non-limiting conditions
A_{sat}	Assimilation rate at saturating light
A_o	Assimilation rate when $C_i = C_a = 350$ ul l^{-1}
A_{2000}	Assimilation rate when $C_i = 2000$ µl l^{-1}
Ab	Absorbance
ABA	Abscisic acid
AC_i	Assimilation versus internal CO_2 concentration
C_a	Ambient CO_2 concentration
C_i	Internal CO_2 concentration
Chp	Chlorophyll
DBH	Diameter breast height
DW	Dry weight
E	Transpiration
FW	Fresh weight
G	Absolute growth rate
g_s	Stomatal conductance
l	liter
l	Stomatal limitation
*l*1	Stomatal limitation calculated by method of Farquhar and Sharkey (1982)
*l*2	Stomatal limitation calculated by method of Jones (1983)
NAR	Net assimilation rate
PPFD	Photosynthetic photon flux density
$PPFD_{sat}$	Photosynthetic photon flux density that saturates photosynthesis
PS I	Photosystem I
PS II	Photosystem II
Q_e	Quantum efficiency at low light
R	Relative growth rate
R_d	Dark respiration
RGC	Root growth capacity
RH	Relative humidity
R:S ratio	Root to shoot ratio
Rubisco	Ribulose-1,5-bisphosphate carboxylase-oxygenase
RuBP	Ribulose-1,5-bisphosphate
s.e.	Standard error
SLW	Specific leaf weight
TM	Trademark
TNC	Total nonstructural carbohydrates
VPD	Vapour pressure deficit
WUE	Water use efficiency
r	Light compensation point
ψ_p	Leaf water potential

Chapter 1
Introduction to the Genus *Paulownia*

1.1 History

The angiosperm genus *Paulownia* is native to eastern Asia, particularly mainland China, Taiwan and Korea. Members of the genus have been cultivated by the Chinese people for many centuries with the earliest written records about the use of the timber dating back to 600 B.C. when King Yui was purported to have been buried in a coffin made from *Paulownia* wood (Zhu *et al.*, 1986). Two hundred years later *Paulownia* was mentioned in the writings "Zhuang Tze" as the tree that the phoenix alighted upon, while the book "Shang Shu" (300 B.C.) records that the people in Shucho supplied the king with *Paulownia* timber. This reference and others published in the same era indicate that *Paulownia* trees were extensively cultivated over 2000 years ago (Zhu *et al.*, 1986). In the first Chinese encyclopedia of natural and cultural objects, Erh-ya, which was published in the third century B.C., the wood of *Paulownia* is listed amongst that of other useful woody plants (Hu, 1959).

More recently, in 1049 A.D., Ch'en Chu wrote a comprehensive treatise covering aspects of *Paulownia* propagation, cultivation, silviculture, ecology, utilization as well as folklore and poetry surrounding the trees (Hu, 1959). His descriptions were based on close observation of the trees and many of his findings and conclusions have been verified by workers this century. Ch'en referred to *Paulownia* by it's Chinese name of T'ung and classified two species by different morphological features as Pia-hua T'ung (white flowered *Paulownia*) and Tz'u-hua T'ung (purple flowered *Paulownia*) (Hu, 1959). It appears however, that the name T'ung was a general term used also to refer to other similar woody plants as Ch'en employed it in the description of a number of other species which he recognized as being unrelated to *Paulownia*, with the botanist Li Shih-chen (1590) also discussing the range of plants covered by the generic umbrella term T'ung, including *Paulownia* (Hu, 1959).

At some stage over this period, *Paulownia* was introduced into Japan where the tree became known as kiri and integrated into Japanese culture as well. In fact the first European record of *Paulownia*, written by Kaempfer in 1712, was a result of the time he spent in Japan for some years at the end of the 17th century. Not until 1784 was the first full botanical description published and this was by Thunberg who described *P. tomentosa* as *Bignonia tomentosa* in his work Flora Japonica (Hu, 1959). The genus was first accorded the name *Paulownia* by Siebold and Zuccarini in 1835. Siebold was a naturalist and medical officer who worked for the Dutch East India Company in Japan from 1822 to 1829. He decided to name the genus in honour of Princess Anna Paulownia, daughter of Czar Paul I of Russia,

who was the wife of Prince Willem who later became King Willem II of the Netherlands (Preston, 1983). This has led to the tree being known as the Empress tree or Royal Princess tree in the United States (Preston, 1983), although Stearns (1944) attributes the name Empress tree to the story that *Paulownia* was the favourite tree of a Japanese Empress who ordered the wide spread planting of the trees in the royal gardens at Tokyo.

The early descriptions of the genus were generally of the species now considered to be *P. tomentosa*. However, as the 19th century progressed and further explorations of China were made, other species were discovered by European explorers who sent seeds back to their home countries. One such adventurer, Robert Fortune, collected seed from the coast of China which was originally classified as *Campsis fortunei* by Seemann in 1867 (Hu, 1959) but was later reclassified as *P. fortunei* (Hemsley, 1890). The species *P. fargesii* also takes its name from the original seed collector, Frenchman P. Farges, who sent material of many plants back to the Museum d'Histoire Naturelle in Paris (Franchet, 1896).

Gradually *Paulownia* was distributed throughout Europe and further abroad for cultivation as an ornamental, with exchanges of plant material occurring between interested horticulturalists. Osborn (1929) reported the first *P. fargesii* flowers in England, on a 21 year old tree at Kew Gardens. At that time, *P. tomentosa* was relatively common in England but only flowered in the warmer, southern parts of the country. The earliest report of *Paulownia* in New Zealand dates back to 1865. One tree in Motueka is believed to be about 100 years old, although its stem has been destroyed three times and regrown each time (I. Barton, pers. comm.). It is believed that the first *Paulownia* seeds arrived in the United States in the 1840s, where they were widely planted in the eastern and southern states (Preston, 1983).

The genus may well have remained in the realm of the horticulturalist, to be grown as an attractive specimen tree, had it not been for a serendipitous event in 1973. A Japanese businessman being driven through the Blue Ridge country of Virginia (USA) suddenly exclaimed excitedly about a nearby tree in flower and, on closer inspection, decided it was indeed a 'kiri' tree. Up until that time, the Japanese were unaware of the large number of mature *Paulownia* trees growing across southern USA. Nor did the American land owners have any inkling that this weed tree, impossible to get rid of once established, had value to anyone (Preston, 1983). Virtually overnight, a new export market was discovered with the peak of mature log exports reaching approximately 30,000 m^3 in 1979, with high prices paid for slow grown, fine grained timber (Preston, 1983). However, the resource of mature trees grown slowly over fifty or more years was finite and it was this realization that there was a lucrative market for *Paulownia* timber in Japan that sparked interest in growing the trees as an alternative timber crop. *Paulownia* is now grown commercially, on a very short rotation,

in several South American countries (Larson, 1980) with interested parties in United States, Australia and New Zealand seriously investigating the potential of the trees as a viable industry, primarily to supply the Japanese market. The trees do have a range of other potential roles though, both as live trees and as timber, as will be discussed below (sections 1.4 and 1.5).

1.2 Taxonomy and Botany

1.2.1 To which family should *Paulownia* belong?

Despite two hundred years of spasmodic study, the question of which family *Paulownia* belongs to has yet to be unequivocally resolved. In the first instance, Thunberg's classification of *P. tomentosa* as *Bignonia tomentosa* in 1784 placed the genus in the Bignoniaceae family along with the similar looking tree *Catalpa*, but Siebold and Zuccarini reclassified the genus into Scrophulariaceae (the foxglove family) in 1835 (Hu, 1959).

The definition of plants as belonging to a particular genus, family or higher classification category has primarily been based on reproductive biology in the past. The recent advances in genetic techniques have enabled researchers to more clearly define relationships between plants. It may eventuate that only a detailed examination of the genetic characteristics of *Paulownia* and of other members of both the Bignoniaceae and Scrophulariaceae families will be able to resolve the dilemma of where to place the genus. As far as I am aware, this work has not been undertaken at this stage and so the classification remains primarily based on reproductive features.

The primary characteristic involved in assigning *Paulownia* to the Scrophulariaceae family is the presence of endosperm in the seed, a feature of this family (Armstrong, 1985). In the original assignation of the genus to this family, Siebold and Zuccarini considered the campanulate calyx to also be a distinguishing feature, suggesting a close relationship with *Lophosperma* (Hu, 1959). A detailed analysis of development of the seed from the time of the microspore and megaspore initiation through until germination by Millsaps (1936) found several similarities between *Paulownia* and other members of Scrophulariaceae. For example, the ovary was of the typical bilocular scrophulariaceous type. More recently Armstrong (1985) undertook a full investigation of various aspects of the reproductive structures of a number of species in both families and several species which are considered to be intermediate between the families. He found that features of the embryo, seed wing, presence of endosperm, cotyledon venation and morphology supported the placement of *Paulownia* in Scrophulariaceae.

Holding the opposing view, that *Paulownia* should be placed in the Bignoniaceae family, Campbell (1930) suggested that the small size of the endosperm and the large embryo indicated that *Paulownia* is a slightly aberrant member of the Bignoniaceae family as its woody nature and leaf morphology was more similar to other family members. Analysis of chromosome numbers and morphology, anatomy and embryological development led Westfall (1949) to call for the reclassification of the genus into this family. Chemical analysis of the anthocyanins found in the corolla of *Paulownia* showed that the complement of glucosides were more akin to those in species of Bignoniaceae than of Scrophulariaceae (Scogin, 1980).

Thus, the question remains unresolved at this time. There is one solution noted by Hu (1959) who reported that some years earlier Nakai created a separate family, Paulowniaceae, containing the one genus. However, after much examination of the problem Hu decided to leave the genus in Scrophulariaceae, as the tribe Paulownieae, and the recent treatise by Zhu *et al.* (1986) also places *Paulownia* in this family.

1.2.2 Classification of species within the genus

Parallel with the difficulties in assigning the genus to a particular family, the naming and definition of species within *Paulownia* has not been a straightforward matter. Thunberg's use of the specific name 'tomentosa', from the hairy nature of the leaves, was not continued by Siebold and Zuccarini who renamed the same species *P. imperialis* (Hu, 1959). Indeed this species has been known under at least ten different names, with different generic, specific and varietal labels confusing the issue. The correct appellation for the species is now considered to be *Paulownia tomentosa* (Thunb.) Steud. (Hu, 1959).

Unfortunately the naming confusion has not been confined to *P. tomentosa.* At the time of the publication of his monograph in 1959, Hu reported that 18 species and 3 varieties of *Paulownia* had been legitimately published. In an attempt to clarify the issue, he recognised six species : *P. tomentosa, P. glabrata, P. elongata, P. fortunei, P. kawakamii* and *P. fargesii.* The more recent work of Zhu *et al.* (1986) recognised all of those species excluding *P. glabrata* and included *P. australis, P. catalpifolia, P. albiphloea* and *P. taiwaniana*, making a total of nine species. It is this latter classification that I have used in the work reported here.

Part of the reason for the lack of consensus on the naming issue related to nature of the material collected by botanists in the field and sent back to herbaria in their home countries

for examination. Material was collected on expeditions carried out within a particular time frame, rather than from trees readily accessible at all times of the year. Thus a spring expedition would have been able to collect flowers and old seed pods, but no leaves, whereas one in summer could have collected foliage and developing fruit, but not flowers. This led to incomplete specimens being received by the herbaria, making identification difficult (Hu, 1959). Some collectors sent seeds home, but the length of time that elapsed before the plants were reproductively mature also compounded the problem. In addition, the relative plasticity within the genus of a number of features, particularly flower colour, added to the confusion.

In general, members of the *Paulownia* genus are described as deciduous trees with grey, brown or black bark that is lenticellate when young, but becomes increasingly cracked as the tree grows. They have a pseudo-dichotomous branching pattern with non-viable terminal buds (the exception being *P. fortunei* which sometimes exhibits growth from the terminal bud in the succeeding season). The branches, leaves and calyx are variously hairy. The opposite leaves are simple, entire, stipuleless with a long petiole. The flower buds are produced in summer, arranged in a cymose inflorescence that may be pedunculate or subsessile and the buds are pedicellate. The calyx is fleshy, campanulate and unequally five-lobed. The flowers open in the spring before the leaves are evident, with a large purple to cream corolla of five lobes and are lightly scented (Fig. 1). The four stamens are about half as long as the corolla, with the pistil as long or longer than the stamens. The bilocular ovary is superior with numerous ovules. The flower is insect pollinated. The numerous, small winged seeds are produced in loculicidal capsules which are generally ellipsoid in shape. Upon fruit dehiscence in late autumn the seeds are wind dispersed (Lee, 1935; Hu, 1959; Zhu *et al.*, 1986). The mature *P. elongata* tree in full leaf at the University of Waikato creche is shown in Fig. 2.

Within the genus, the species are identified by the length of the cymes and the presence or absence of peduncles, the depth of the calyx lobe, the shape of the corolla, capsule and placenta, and the thickness of the pericarp. Other characteristics such as hair anatomy, leaf size, fruit, flower and inflorescence size are also useful diagnostic features, but show variation within species as well as between species (Zhu *et al.*, 1986). For a full description of the morphology and taxonomy of the genus, the reader is referred to the references cited and also to Franchet (1896), Millsaps (1936) and Armstrong (1985), with Zhu *et al.* (1986) presenting a dichotomous key for the species they recognise.



Fig. 1.1. Inflorescence of *P. elongata*



Fig. 1.2. Specimen *P. elongata* tree in leaf in the grounds of the University of Waikato creche

1.3 Natural Distribution and Ecology of the Species

The effect of human colonisation over many centuries throughout China makes it difficult to determine the natural range and distribution of *Paulownia* in its native habitat, although Zhu *et al.* (1986) reported that in natural stands the trees grew along rivers and in open valleys. It is a minor element of the deciduous mesophytic forest of Eastern Asia, growing in association with species of *Acer*, *Castanea*, *Fraxinus*, *Pinus*, *Quercus* and *Tilia* (Hu, 1959). In cultivation *Paulownia* is grown extensively in the central and eastern parts of China from latitudes of 18°N to 40 °N (Zhu *et al.*, 1986). The range of each of the species is determined by environmental conditions with *P. fortunei*, *P. fargesii* and *P. kawakamii* being better suited to the southern warmer regions of China, preferring the sub-tropical climate, while *P. tomentosa* is the most cold hardy species (Zhu *et al.*, 1986).

All species of *Paulownia* are shade intolerant with seedlings establishing primarily on open ground. This, coupled with the ability to regrow from adventitious root buds after fire or other disturbances, makes the trees important pioneer species (Hu, 1959). Other environmental pre-requisites for good tree growth include a fertile, deep and well draining soil. Heavy, poorly drained soils can lead to anaerobic conditions that will kill *Paulownia* trees within days (Zhu *et al.*, 1986). The trees are tolerant of a wide range of soil pH from 4.5 to 8.5, depending on the species.

1.4 Utilization of *Paulownia* Trees During Growth

1.4.1 Agroforestry

The integration of *Paulownia* trees with the production of other crops and livestock has been widely practised in the northern central plain area of China for the past forty years at the instigation of government agencies (Zhu *et al.*, 1986). In reality it is merely an extension of ancient farm forestry techniques applied by peasants over the centuries. The climate in the northern central plain is influenced by the monsoon wind from east Asia, with 80% of the 500 - 900 mm of annual rainfall occurring between mid-June and September. In the two months prior to the monsoon, the region is prone to hot drying winds with wind speeds reaching 3 m sec^{-1}, which are associated with air temperatures over 30°C and low RH (less than 30%). This has resulted in loss of grain crop yields of up to 40%, and severe natural disasters such as sandstorms and droughts, as well as severe soil erosion (Zhu *et al.*, 1986).

Since the 1950's, extensive planting of shelter belts has been undertaken, primarily using

Paulownia trees, poplars and elms. These shelter belts run for many kilometres in all directions, intersecting to form blocks of arable land, between 6 and 17 hectares in size, completely surrounded by trees. This planting pattern has produced the so-called "forest net" system. An addition to this large scale planting has been the development of intercropping techniques with *Paulownia* and a range of other crops. The spacing between the trees is determined by the primary objective of the land use - tree production, crop production, or an equal mix of the two (Zhu *et al.*, 1986). *Paulownia* can be successfully intercroppped with a range of crops including wheat, maize, millet, vegetables, soya bean, cotton and even bamboo. In the first three to five years of tree growth under a planting regime of 80 - 400 trees per hectare, yields of crops in an intercropping arrangement can be up to 20% greater than crops planted in treeless control plots (Zhu *et al.*, 1986).

The main reasons for yield increases in the annual crops is the climatic amelioration as a result of the trees. The reduction in wind speed helps to increase the air temperature in autumn, but decreases the peak summer temperatures. The evapotranspiration rate is also lowered. In addition, the soil moisture in the top 50 cm of the soil is higher (Zhu *et al.*, 1986). All of these conditions combine to provide a more favourable microclimate for crop growth. The open canopy of *Paulownia* allows sufficient light through to understorey crops and the exploitation of deeper soil layers by the tree limits competition with the roots of the crops (Zhu *et al.*, 1986). In addition to the higher crop yields, the farmer can use the foliage as fodder for livestock, the prunings as fuel and can harvest the tree when mature. The prolific flowers are apparently a very attractive source of nectar to the bees (Hayes, 1978) and so honey production is another benefit from these trees for the farmer.

The multipurpose nature of the tree for providing fuel, fodder, shade, timber and nectar has led to the encouragement of planting *Paulownia* on "four sides" - around homes, within villages, along roadsides and along water ways. Extensive planting of *Paulownia* (and other tree species) throughout many areas of China has enabled the country to begin to meet the demands of the large population for both food and wood products and, at the same time, reverse soil degradation and erosion (Wilson, 1985).

1.4.2 Revegetation of strip-mined land

The observation that *P. tomentosa* seedlings have become well established on several surface mines in southeastern Kentucky (Cunningham and Carpenter, 1980) has led to research into the use of the *Paulownia* as part of the range of vegetation used to replant areas that have been strip mined. The trees have naturally colonised the drier, southern facing slopes on old sites and the edges of recently mined sites (Tang *et al.*, 1980). One of the problems

associated with the revegetation of old mining sites is the very acid soils. However, a study with *P. tomentosa* seeds showed that germination and subsequent seedling growth were not particularly tolerant of acidic soils. Germination rates were lower at pH 4 than at pH 5, and primary root growth inhibited at pH 5. It was concluded that the *Paulownia* trees that do establish on mine sites may only do so in the least acidic microsites or may be more acidophilic ecotypes (Turner *et al.*, 1988).

It is possible that *Paulownia* has a role to play in the revegetation of disturbed sites, as the pioneer strategy of the plant makes it ideal in many wasteland situations. Larson (1980) has reported that mature trees are often found along state and federal highways in the southern states of USA about five to fifteen years after major highway construction.

1.4.3 Medicinal uses

The long centuries of cultivation of *Paulownia* in China have resulted in the plant being woven into the folklore and fact of everyday life. One aspect of this interaction between man and plant is the use of various parts of the plant in traditional Chinese medicines. Hu (1959) gives several recipes to cure ailments ranging from baldness and greying hair, to swollen feet, liver and bile illnesses, fever, delirium and bruises. These concoctions involve tinctures prepared from the bark, wood, leaves, flowers and fruits of the trees. Fruit extracts are also believed to relieve coughs, asthma and blood pressure problems (Zhu *et al.*, 1986). It is not known at this stage if any of these claims have been substantiated by thorough research. In addition to supposedly curing the ills of the individual, *Paulownia* can have a role to play in the wider society as it tolerates polluted atmospheres and grows in areas where other trees are excluded because of air pollution (Zhu *et al.*, 1986).

1.5 Properties and Utilization of the Timber

1.5.1 Timber properties

Paulownia wood has a very low density. The density of air dried timber from various sites in China ranged from 260 - 300 kg m^{-3} (Zhu *et al.*, 1986), while the density of New Zealand grown *P. tomentosa* and *P. elongata* were measured at 340 and 335 kg m^{-3} respectively (Haslett *et al.*, 1992). In comparison, *Pinus radiata* timber has a density of about 480 kg m^{-3} (Barton, 1986) while the density of a range of *Eucalyptus* species is between 390 kg m^{-3} and 580 kg m^{-3} (J. Bathgate, pers. comm.).

The wood from *Paulownia* is unsuitable for situations requiring a lot of strength, such as house framing. In a number of strength and hardness tests, radiata timber was found to be twice as strong as *Paulownia* timber, with three times the surface hardness, but *Paulownia* wood was stronger than that from balsa (Haslett *et al.*, 1992).

The timber can be air dried or kiln dried and is very stable with little deformation occurring during the drying process. Its rapid rate of drying, low volume shrinkage and high dimensional stability make it a very easy timber to dry (Olson *et al.*, 1989). However, the presence of the large pith in the centre of the timber does affect the yield of sound wood, particularly in younger trees. Once dried, machining the wood presents no problems and it is an easy wood to turn and work with, although care must be taken not to indent the surface (Haslett *et al.*, 1992).

Other properties of the wood include its low thermal and electrical conductivity, indicating that the timber has better insulating capacity than other timbers. In addition it has good resonance properties which make it invaluable for the production of traditional musical instruments in China and Japan (Zhu *et al.*, 1986).

1.5.2 Uses of *Paulownia* wood

In both China and Japan there is a tradition of planting a *Paulownia* tree at the birth of a daughter. When she marries, the tree is cut down and the wood made into furniture for her new home, or sold to pay for the celebration and the dowry (Preston, 1983; Zhu *et al.*, 1986). In Japan this tradition involves the production of a *tansu* - bridal chest - in which the Japanese woman stores her kimono. These chests are made from the highest quality *Paulownia* wood and can be very intricate with carvings, jewels and gold decorating the chest. It is primarily the demand for these *tansu* that has sparked the international interest in *Paulownia* timber as the Japanese cannot meet the demand for the wood internally and must import it (Preston, 1983). *Paulownia* wood is used for several reasons. Its light colour and straight grain are attractive and drawers made from the wood run smoothly. The wood is said to protect the clothes inside from mildew which is a problem in the humid climate of Japan, and it also provides protection from insect attack - particularly moths, and appears to be fire resistant with stories of charred, but intact, *tansu* remaining in houses otherwise destroyed by fire (Simmons, 1979; Zhu *et al.*, 1986).

The wood is also used in the production of traditional *geta* sandals in Japan, and in the

manufacture of furniture, bee hives, musical instruments, packaging material, packing boxes, gift boxes, ceilings, window and door frames, beer barrels, utensils, bowls, handicrafts and even Japanese fighter planes in World War Two (Stearns, 1944; Zhu *et al.*, 1986). In addition, the dimensional stability of the wood and the fact that it can be sliced very thinly and takes glues very well makes it useful in the production of veneers and plywood (Zhu *et al.*, 1986; Haslett *et al.*, 1992). The light honey colour of the veneer can be retained or the wood can be stained to give a darker finish.

It is possible to make paper from the wood pulp and the short fibres produce a very strong, high quality paper (Zhu *et al.*, 1986), but the cost of production and the limited market for paper of that quality make it unlikely that it will ever be a significant pulp species in this country (J. Bathgate, pers. comm.). A sample of paper made from young *Paulownia* stem wood by students in the University of Waikato technology laboratory is presented below (Fig. 1.3).



Fig. 1.3. Paper made from the stems of three year old *P. tomentosa* trees growing at the Matangi experimental site.

1.6 The *Paulownia* Market and Industry

The development of a *Paulownia* industry in the USA, Australia, South America and New Zealand over the last ten to twenty years is a direct result of the high prices paid by Japanese importers for high quality timber. China continues to grow the trees for use internally, as well as exporting *Paulownia* products to about twenty different countries, including Japan (I. Barton, pers. comm.). From 1964 to 1990 there has been a increase in the volume of *Paulownia* imported by Japan as the ability of the country to meet the demand for the wood declined due to diminishing stands of *Paulownia* (Hardie *et al.*, 1989). The volume of sawn timber imported increased steadily to about 80,000 m^3 while the importation of logs peaked at

80,000 m^3 in 1979, but has been stabilized at about 30,000 m^3. Most of the early timber was imported from China and Chinese Taipei, but the United States, Brazil and Korea have become more important sources of timber in recent years (Glass, 1992). However the size of this market is small when compared with that for other timbers with the volume of exports of logs and sawn timber of all species from New Zealand reaching over 4.6 x 10^6 m^3 in 1992 (New Zealand Forestry Statistics, Ministry of Forestry, Wellington, 1993).

For any country wanting to establish a sustainable and profitable *Paulownia* industry, there are some key issues to be addressed. It is important to realize that the high prices paid for *Paulownia* timber by the Japanese have been for slow grown, old trees growing naturally (Preston, 1983). These are preferred because of the higher density of the timber and the prices decrease as the rate of tree growth increases *i.e.* as the ring size increases (Graves, 1989). Therefore there needs to be a balance between having a rapid turnover of trees (10- 20 years) which will produce lower value timber, or taking longer to produce millable trees that will be worth more. As yet the point of balance in the equation is unknown and is compounded by the time scale of the operation (B. Glass, pers. comm.). It is also important to consider the high cost of establishing and maintaining a plantation, in comparison with growing *Pinus radiata*, for example. However, taking into account these costs and estimating the returns for 20 year old plantation grown trees with DBH of 40 - 50 cm at 1989 prices, Hardie *et al.* (1989) calculated that the grower would make a substantial profit. But they cautioned that the increase in supply as more trees were produced could drive prices down to the point where returns became uneconomic.

At this time in New Zealand, there are no large scale plantings of *Paulownia*. There is much interest in the tree, particularly from land owners interested in farm forestry. It is envisaged that the planting of *Paulownia* will continue to be on a small scale, with the timber produced either being exported to Japan and other markets, or being used domestically in the cabinet making industry and for veneers and plywood. The timber can be considered as a viable alternative to the use of some of our native timbers in such situations. As well, the tree can have a role in agroforestry situations on New Zealand farms as one of a range of genera planted to benefit livestock and other crops.

1.7 *Paulownia* Research to Date

The relatively recent recognition of *Paulownia* as a genus of interest means that there has been little research published in the scientific literature of the Western world. Work has concentrated on such aspects as the factors influencing seed germination and propagation of

the trees by both sexual and vegetative means (including tissue culture); anatomical and morphological investigations of all parts of the tree, including the timber; management and silviculture of the trees; and some work on the chemical extractives. The problems of accessing information published in Chinese journals makes it difficult to know what work has be done by their researchers. It does appear that much of their effort has been concentrated on the genetic improvement, particularly the breeding of trees with good form, good timber quality and resistance to the witches broom disease (I. Barton, pers. comm.) and on various aspects of the intercropping management of the trees.

In terms of the physiology of the plant, very little work has been published. It is believed that the Chinese forestry research organisations have not had access to sophisticated equipment until recently and are restricted to the use of porometers (*e.g.* a LICOR 1600). Some work on the nutrition of seedlings has been published by Jia and Ingestad (1984), while mycorrhizal associations of *P. taiwaniana* have been studied by Donald *et al.* (1988).
In New Zealand, the research carried out by the Forest Research Institute Ltd. has been limited to the evaluation of different species in a range of sites, growth rates of the trees, investigation of the properties of some New Zealand grown timber and market research. Agresearch Ltd. has also undertaken trials with *Paulownia* with reference to using it as a cover crop for the shade tolerant myoga ginger (J. Follett, pers. comm.). Other students at the University of Waikato are currently involved in studies on the fertilizer requirements of *Paulownia* trees and the extraction of chemicals from the different tissues of the tree.

1.8 The Aim of the Work Reported Here

The research reported in this thesis was undertaken to provide a basis for the understanding of some aspects of the ecophysiology of *Paulownia* plants, including gas exchange, nutrient and biomass allocation, and vegetative propagation. As a first analysis of many of these aspects, it does not set out to be a definitive statement upon the physiology of the genus, but instead provides an initial base upon which future research can build. One of the problems with the fledgling *Paulownia* industry in this country is the abundance of dubious information about spectacular tree performance and characteristics of tree growth that are based on the Chinese experience. Direct translation of those results into the New Zealand situation is a very risky business, particularly when the different climatic and soil conditions are considered. Thus this research set out to investigate scientifically the reality of *Paulownia* performance in the New Zealand context. The results of this work can only be a benchmark for *Paulownia* growth in other areas of the country as the influences of site and soil conditions will affect the behaviour of the tree.

Chapter 2
Gas Exchange in *Paulownia*

2.1 Introduction

2.1.1 Gas exchange in C_3 plants

2.1.1.1 Photosynthesis

The conversion of light energy incident upon foliage into chemical energy via the process of photosynthesis is of the utmost importance for the life of the plant and for those species that are ultimately dependent upon plant material for their own sustenance. The photosynthetic process can be divided into the light reaction and the dark reaction. The light reaction involves the conversion of light energy into chemical energy, through a chain of electron transfers involving chlorophylls a and b, and accessory pigments, and various electron carriers. The ATP and NADPH thus produced are used in the dark reaction to reduce CO_2 to carbohydrates through a series of steps controlled by enzymes. One of the most important enzymes in this process is RuBP carboxylase-oxygenase (Rubisco) which catalyses the reaction binding CO_2 to the initial acceptor RuBP (ribulose-1,5-bisphosphate). For a more detailed analysis of the photosynthetic process, the reader is referred to Larcher (1980) and Sharkey (1985).

Occurring in tandem with the process of CO_2 reduction is the oxygenation of RuBP - photorespiration. This process is catalysed by Rubisco operating in oxygenase mode, and results in the production of PGA and phosphoglycolate which can eventually be used to produce glycine and serine with the release of CO_2 (Larcher, 1980). The ratio of photosynthesis to photorespiration is related to the partial pressures of CO_2 and O_2, with the affinity of Rubisco for CO_2 being higher than that for O_2. Under conditions of 21% O_2, 0.03% CO_2, strong light and temperatures between 20°C and 30°C, C_3 plants can lose between 20% and 50% of recently photosynthetically acquired CO_2 through photorespiration (Larcher, 1980).

From an analysis of the steps within the photosynthetic process, it is possible to determine some of key factors which could limit the rate of photosynthesis when they are available in insufficient quantity. These include light (discussed in section 2.1.2.1), chlorophyll, H_2O, P_i, CO_2, RuBP, various enzymes - particularly Rubisco, and the various electron chain and other intermediates. The rate of photosynthesis (A) is controlled so that a limitation in one of these essential factors slows the whole process to a level concomitant with the supply of that factor

(Sharkey, 1985). Photosynthesis in a plant growing in conditions of adequate moisture and nutrient availability is not usually limited by the amount of chlorophyll, water, P_i or the intermediates. The way in which CO_2 supply, RuBP supply and Rubisco activity affect the rate of photosynthesis is discussed in section 2.1.8, in the description of AC_i curves.

2.1.1.2 The functioning of stomata

In order for CO_2 to be reduced in the chloroplast it must diffuse into the leaf from the outside air and then into the mesophyll cell, where it is dissolved in the aqueous cellular solution, and from there diffuses into the chloroplast . The rate of diffusion is dependent on the resistances at each surface (Sharkey, 1985). The major point of entry of CO_2 into the leaf are the stomata and they play a large role in determining the internal leaf concentration (C_i) of CO_2. The resistance presented to gaseous diffusion at the stomata is a function of how open they are, which is generally documented as stomatal conductance - g_s, the inverse of resistance.

The degree of stomatal opening is a result of the difference in turgor between the guard cells and adjacent epidermal cells. The active transport of K^+ ions into the guard cells increases their turgor which leads to opening of the stoma. The change in ionic balance in these cells is rectified by the movement of inorganic anions (*e.g.* Cl^-) into the guard cells, or the production of organic anions such as malate, or the release of protons from the guard cells (Larcher, 1980). Stomatal closure occurs when the turgor decreases through the loss of K^+ ions from the guard cells. It has also been postulated that Ca^{2+} ions are involved in the stomatal movements, but in an antagonistic fashion to the influence of K^+ ions (Mansfield *et al.*, 1990).

Stomata respond to a number of external and internal cues. Light tends to cause the opening of stomata, with light in the blue part of the spectrum being most effective, which may be an adaptation to the light quality prevailing at dawn (Farquhar and Sharkey, 1982). Low C_i also promotes stomatal opening, and this effect, in concert with the response to light, causes stomata to open in the morning (Larcher, 1980). Artificially high C_i decreases g_s as a result of the leaf being able to minimize transpiration without decreasing photosynthetic rate (Farquhar and Sharkey, 1982). The vapour pressure deficit also affects g_s, with a high VPD causing a decrease in g_s, as do low leaf water potentials. Phytohormones are also important regulators of stomatal function with cytokinins promoting stomatal opening and ABA causing closure. The response of stomata to various stresses (*e.g.* low water potentials, flooding) may be mediated by the action of ABA (Farquhar and Sharkey, 1982).

2.1.1.3 Relationship between photosynthesis, transpiration and stomatal conductance

The opening of the stomata, to allow CO_2 to diffuse to the sites of carboxylation, inevitably leads to the diffusion of water (and oxygen) from the intercellular spaces, out into the ambient air. Therefore a balance must be struck by the leaf between carbon gain and water loss. Although transpiration may be seen as a negative side effect of carbon dioxide uptake, it does serve two useful purposes. Firstly transpiration exerts an effect on the water column throughout the plant such that the loss at the leaf surface causes a flow in the transpiration stream which transports solutes (primarily mineral nutrients) and hormones through the plant. The second effect is one of heat exchange - evaporation of the water from the surface of the mesophyll cells into the internal air spaces has a cooling effect in the leaf. This is important under conditions of high temperature and high light intensity and may explain why some leaves keep their stomata open even when transpiration rates are high. If the stomata were to close, the leaves could over heat.

The rate of transpiration (E) is primarily influenced by the vapour pressure deficit between the leaf and the ambient air. The internal air spaces of the leaf are considered to be saturated (Sharkey, 1985) and so will always have a higher concentration of water vapour than the air outside the leaf. The driving force of transpiration is therefore a result of the difference in vapour pressure between the two areas.

Although many workers have found a good linear relationship between A and g_s, (*e.g.* Wong *et al.*, 1979), Farquhar and Sharkey (1982) caution against apportioning causation to either variable. In situations where environmental factors lead to a decrease in photosynthetic rate, the concomitant decrease in g_s may not always be sufficient to account for the entire decline in A. Instead these workers suggests that the leaf operates in such a way as to keep the ratio of $\delta E/\delta A$ constant. This has been found to be the case when the effect of decreasing humidity was studied in leaves of *Nicotiana glauca* and *Corylus avellana* (Farquhar *et al.*, 1980).

2.1.2 Environmental influences on gas exchange parameters

The influence of environmental factors on gas exchange and stomatal conductance may be an instantaneous one, affecting rates at the time of measurement, or may occur over the longer term. The effects of the different variables on photosynthetic rate can be mediated directly, through changes in stomatal conductances and other sites of resistance to CO_2 diffusion into

the chloroplast, or in changes to the photosynthetic machinery such as enzyme activity or chlorophyll content. These variables have been studied over a wide range of species and only a few salient examples will be given here. Although each of the factors is discussed separately, it is generally the interaction of environmental effects that is more important in determining the net rates of gas exchange in the plant. The interaction of temperature and gas exchange will be discussed in Chapter 3, and so is not included at this point.

2.1.2.1 Light

Light is perhaps the most obvious variable to influence gas exchange rates, with a direct effect on the light reaction of photosynthesis. Photosynthetic rates, g_s and E tend to increase as the light level increases, up until the point when light saturation occurs. At this point, further increases in available light do not significantly increase A. The light level at which this occurs differs between species and as a result of the light environment during plant growth. Studies with a range of seedlings of rainforest trees have generally found that plants grown at lower light levels reach saturation at low light levels. In addition, seedlings raised in the higher light environments have higher values of A_{max} in comparison with those raised under low light (*e.g.* Kwesiga *et al.*, 1986; Turnbull, 1991; Thompson, Huang and Kriedemann, 1992). This relationship between light environment during growth and subsequent A_{max} (on an area basis) has been found in a wide range of species from ryegrass (Charles-Edwards *et al.*, 1974) to the pioneer tree species *Nauclea diderrichii* (Riddoch *et al.*, 1991), and chaparral shrub *Diplacus aurantiacus* (Gulmon and Chu, 1981).

It is not only the quantity of light at any one time that impinges on subsequent gas exchange rates, but the also the length of the photoperiod and the quality of light (range of wavelengths available). Seedlings of *Paulownia tomentosa* grown under an eight hour photoperiod had higher maximum photosynthetic rates and light saturation levels than seedlings grown at either 16 or 24 hour photoperiods (Carpenter *et al.*, 1983). Artificial modification of the red:far-red ratio during growth, to simulate natural shading effects in a forest situation, has been shown to change photosynthetic characteristics in the leaves of rainforest seedlings (Kwesiga *et al.*, 1986; Turnbull, 1991). The mechanisms by which the light environment affects the form and function of leaves will be discussed below (section 2.1.3).

2.1.2.2 Plant nutrient status

With the majority of the mineral elements having some role to play in the photosynthetic

pathway (*e.g.* N and Mg in chlorophyll, P in RuBP) then clearly the nutrient status of the plant is reflected in the rates of gas exchange, when other environmental factors are non-limiting. The strongest correlation is generally between the nitrogen content of the leaf and photosynthetic rate. This often linear relationship can be explained to a large extent by the observation that Rubisco accounts for approximately 50% of the leaf protein (Beadle *et al.*, 1985). There also tends to be a linear relationship between leaf nitrogen content and chlorophyll content (Kramer and Kozlowski, 1979). A recent review by Evans (1989) explores the relationship between photosynthesis and nitrogen and, in particular, the partitioning of the nitrogen between the soluble proteins and thylakoid membrane proteins.

In leaves of *Diplacus aurantiacus* grown in conditions of low nitrogen, A_{max} was lower than in leaves of plants grown at high nitrogen and there was a linear relationship between leaf nitrogen content (by weight) and photosynthetic rate measured on a weight basis (Gulmon and Chu, 1981). Tuohy *et al.* (1991) found a similar relationship between leaf nitrogen content and A, both measured on an area basis, in several woody nodulating legumes, non-nodulating legumes and non-legumes. These workers did not find a correlation between leaf P content and A_{max} in any of the tree species studied. A study on several rainforest seedlings grown at either high full nutrients or low full nutrients, at different light levels, found that generally rates of photosynthesis, dark respiration and light saturation points were higher in the trees grown with higher nutrient levels (Thompson, Huang and Kriedemann, 1992). Under high light conditions, leaves of all the species had higher N, P and chlorophyll levels when grown at higher nutrient concentrations.

The interaction of nutrient status and gas exchange may also be mediated by changes in other aspects of the leaf physiology and anatomy, apart from the direct influence on the photosynthetic machinery. An increase in specific leaf weight with decreasing nutrient availability has been found in several species (*e.g.* Gulmon and Chu, 1981; Thompson, Kriedemann and Craig, 1992). The number and functioning of stomata can be influenced by nitrogen nutrition with more stomata found on nitrogen - deficient apple leaves (Kramer and Kozlowski, 1979), while the movement of stomata may be impaired on N - deficient leaves (Larcher, 1980). Stomatal function is also dependent on adequate levels of phosphate and potassium and these two minerals are also involved in the energy transfer reactions of photosynthesis *e.g.* the ADP - ATP system (Kramer and Kozlowski, 1979). The various micronutrients are involved directly and indirectly with leaf gas exchange. Iron and copper are important in the electron transfer systems and these elements, in addition to manganese and zinc, influence the activity of several enzymes involved in the photosynthetic system (Kramer and Kozlowski, 1979). Deficiencies of iron and manganese can also decrease leaf area.

2.1.2.3 Ambient CO_2 levels

As ambient levels of CO_2 are increased over a short time period, photosynthetic rates tend to increase until the photosynthetic system is saturated. This is coupled with an increase in C_i and a decrease in g_s and E. The longer term increase in CO_2 levels has recently become a subject of many studies on a range of plant species as concerns about the so called "greenhouse effect" have led to investigation of how higher CO_2 levels affect gas exchange and other parameters in plants (Beadle *et al.*, 1985). In a complex series of experiments with willows (*Salix x dasyclados*) investigating nutrient and CO_2 regimes during growth, Silvola and Ahlholm (1992) found that plants grown at 1000 $\mu l\ l^{-1}$ tended to have lower photosynthetic rates than plants grown at either 300, 500 or 700 $\mu l\ l^{-1}$. This result was independent of the CO_2 level at the time of measurement. However, the photosynthetic rate of all plants (regardless of the CO_2 level during growth) increased with higher CO_2 levels during measurement such that A_{max} was approximately three times higher when measured at 1000 $\mu l\ l^{-1}$ than when measured at 300 $\mu l\ l^{-1}$. There was no significant difference in the biomass accumulation of the willows grown at the different CO_2 concentrations over the course of the experiment.

2.1.2.4 Plant water status

Application of a water deficit situation to plants leads to a decrease in gas exchange, primarily through a decrease in g_s and in protoplasm hydration and, if applied over a long enough period, a decrease in leaf area (Kramer and Kozlowski, 1979). The first response to a water deficit is the decrease in g_s to the extent that rates of both E and A are decreased, but this first critical point can quickly be reversed by the application of sufficient water (Larcher, 1980). However, if the deficit situation continues, the stomata close completely (the second critical point) and protoplasm hydration is reduced. Watering of the plant at this time does not result in an immediate return to previous gas exchange rates. If the water stress continues for a long enough period, gas exchange rates may never recover to the same level as before the water stress, despite the application of sufficient water to meet the plants requirements at those previous rates of gas exchange (Larcher, 1980). The effects of a water deficit on the photosynthetic machinery may include a decrease in electron transport and in cyclic and non-cyclic phosphorylation, and a decline in enzyme activity. Little work has been done, however, on changes to these aspects of the gas exchange system under field conditions of gradually declining water availability (Beadle *et al.*, 1985). While the application of a water deficit can occur through decreasing soil moisture availability, the opposite effect of soil flooding can also cause a decline in gas exchange parameters. This response is a result of poor aeration in the root zone which leads to impaired root performance and thus lead to a water deficit

developing in other parts of the plant (Kramer and Kozlowski, 1979). Different species have different tolerances to flooding with Dang *et al.*, (1991) showing that flooding of black spruce and tamarack decreased photosynthetic rate, but that swamp birch was able to maintain the same rates of A despite the flooding. A small preliminary experiment with potted *Paulownia fortunei* seedlings showed that total immersion of the root system in water led to a decline in g_s within 21 hours on average, and total closure within 43 hours (Ede, unpublished).

The water vapour content of the air also has a large effect on the instantaneous rate of gas exchange. A series of experiments with *Nicotiana glauca* and *Corylus avellana* showed a linear relationship between vapour pressure deficit (VPD) and gas exchange parameters with increasing VPD levels causing a decrease in A and g_s. Transpiration rates increased as VPD increased at lower deficits, but there was no further increase at higher VPD levels (Farquhar *et al.*, 1980). Increasing VPD also decreased g_s and water use efficiency in woody species growing in a peatland environment (Dang *et al.*, 1991).

2.1.2.5 Effects of man-made chemicals

In addition to the environmental influences discussed above, gas exchange parameters can be affected by the by-products of humankind. Since the advent of the industrial revolution, man has been able to greatly modify the environment, which has often been to the detriment of other species. Perhaps the most pertinent example is the wide spread destruction of forests by acid rain. However, on a more local scale, soil and air pollutants can influence plant functioning. The absorption of heavy metals with soil moisture has been shown to decrease photosynthetic activity by interfering with chloroplast activity (Beadle *et al.*, 1985). Particulate air pollutants affect gas exchange by clogging stomata and by decreasing the light available to the leaf (Kramer and Kozlowski, 1979). Gaseous air pollutants such as ozone, nitrogen oxides, sulphur dioxide and fluorides also decrease photosynthesis by a variety of mechanisms with fluorides interfering with the Hill reaction and SO_2 affecting chloroplast function (Kramer and Kozlowski, 1979; Beadle *et al.*, 1985).

Analysis of the response of shoots of different ages to changes in the ozone concentration in *Picea abies* (Norway spruce) showed that current-year shoots had higher light compensation points under the highest ozone concentration, but A was unaffected. However, A declined as the ozone level increased in two-year-old shoots (Wallin, Skarby and Sellden, 1992). From the results of these experiments it was concluded that ozone increased respiration rates in current-year shoots; decreased chlorophyll content (and hence efficiency of light capture) as

the shoots aged; and had a greater impact on photosynthesis under high light conditions than low light.

2.1.3 Sun and shade plants

There are many physiological and anatomical differences between leaves that develop under conditions of high light (sun leaves) and those developing in shady conditions. Plants can be loosely categorised into sun and shade plants depending on their preferred light environment, although many species show considerable plasticity and can survive in a range of light environments. Indeed, large plants with a dense canopy develop both sun and shade leaves depending on the leaf position. Most plants show some ability to acclimatize to a change in light conditions, but shade plants are less able to match the rates of gas exchange of sun plants when transferred to high light, whereas sun plants adapt more easily to lower light levels (Bjorkman, 1981).

In general, gas exchange parameters are decreased in shade plants in comparison with sun plants. Leaves grown under high light tend to have higher rates of A, E and g_s (particularly at high light), higher light compensation points, light saturation points, rates of dark respiration, total amounts of chlorophyll and a higher proportion of chlorophyll *a* (Boardman, 1977; Bjorkman, 1981). Shade adapted leaves have lower light compensation points because of a lower rate of dark respiration and so are able to use low light levels (0 - 100 μmol m^{-2} s^{-1}) more efficiently than sun leaves. However the effect of light levels during growth on the initial slope of the light response curve (Q_e - the ratio of moles CO_2 assimilated : moles of photons received) appears to differ between species, with some workers reporting higher values for Q_e in shade adapted leaves compared with sun leaves, while in other species no differences were found (Boardman, 1977; Turnbull, 1991).

Sun leaves tend to be smaller and thicker than shade leaves. The increase in specific leaf weight at high light levels reflects an increase in the number and degree of organization in the palisade and spongy mesophyll cell layers (Boardman, 1977). Leaves of strawberry (*Fragaria vesca*) grown at increasing light levels (from 22 - 650 μmol m^{-2} s^{-1}) were found to be thicker with a higher specific leaf weight at the higher light regimes. This was a direct result of an increase in the mesophyll cell volume and surface area, as a proportion of both leaf volume and surface area. The leaves from the low light had one poorly organised palisade layer and large air spaces, those grown at medium light had one or two palisade layers while those grown at high light had three layers of palisade cells (Chabot and Chabot, 1977). Similar results have been reported for many other species, including seedlings of

rainforest trees (*e.g.* Thompson, Kriedemann and Craig, 1992). Shade leaves also tend to be homobaric, with the bundle sheath extensions not extending from the upper epidermis to the lower, while sun leaves are heterobaric with leaf sections enclosed by the vertical extensions of the bundle sheath from epidermis to epidermis, isolating each section (areole) (Sharkey, 1985).

In addition to the increased amount of photosynthetic tissues in sun leaves, the various components of the photosynthetic pathway are also enhanced in sun leaves. The total amount of chlorophyll tends to be higher in plants grown under higher light levels, but the proportion of chlorophyll *a*:*b* changes, decreasing as the light level decreases. This can be explained by analysis of the light spectrum available to plants growing in naturally shady conditions such as the forest floor, which is higher in far-red, near infrared and green light as a result of the absorption of light by leaves higher in the canopy. The higher proportion of chlorophyll *b* allows the understorey plants to make full use of the available light and is coupled with an irregular arrangement of large grana stacks within the chloroplast to increase the efficiency of light collection (Boardman, 1977).

A significant part of the decrease in photosynthesis in shade leaves can be attributed to the decreased levels of Rubisco in these leaves (Boardman, 1977; Bjorkman, 1981). In addition, levels of the components of the electron transport chain such as cytochrome *f*, cytochromes *b* and ferredoxin, and levels of the enzyme ATP synthase have been shown to be higher in leaves grown at higher light than in shade leaves (Boardman, 1977; Bjorkman, 1981). Thus leaves grown under conditions of high light have an increased biochemical capacity for photosynthesis compared with those grown under low light.

2.1.4 Changes in gas exchange with leaf ontogeny

From the time of initiation until abscission there is a sequence of development in both the anatomy and physiology of the leaf that is common to most plant species. Initially there is a rapid period of cell division and expansion as the leaf grows, during which time the leaf is a net sink for photosynthetic resources. The rate of dark respiration in the developing leaves is very high at this stage, reflecting the high cost of construction of new tissue, but declines as the leaf matures (Larcher, 1980). As the leaf reaches full expansion, the photosynthetic machinery develops such that the leaf becomes a net exporter of photosynthates. In many plants the dry weight (as expressed as the specific leaf weight) continues to increase throughout the life of the leaf, even after expansion has ceased (Sestak, 1981; Field and Mooney, 1983).

If a leaf can be considered to be physiologically mature at the time when A_{max} is highest, then at this time the other factors impinging upon gas exchange rates are also maximal *e.g.* nitrogen and chlorophyll content of the leaves, g_s, light required to saturate photosynthesis, and the activity of Rubisco. The rate of increase in these factors is quite steep as the leaf grows, and peaks at full maturity. From then until senescence there is a slow decline in the gas exchange parameters (Sestak, 1981). The number and size of chloroplasts increases as the leaf grows, as a result of the activity of chloroplast DNA. The DNA contained in the chloroplast codes for a number of chloroplast components including ribosomal RNA, the large subunit of Rubisco and some structural proteins and other enzymes (Thomas and Stoddart, 1980). Expression of chloroplast DNA is confined to the early stages of leaf development and this results in the cessation of chloroplast construction as the leaf reaches full expansion. In addition, the amount of Rubisco is also maximal at this time as there is no further production of this enzyme once full leaf expansion is reached (Thomas and Stoddart, 1980).

The time at which full physiological maturity is expressed in the leaf tends to occur slightly before, or coincide with, the point of full leaf expansion in most herbaceous plants. In cotton, Constable and Rawson (1980) found that A_{max} was highest in leaves that were 75% - 90% fully expanded (13 -15 days after unfolding) and that this correlated with maximum rates of transpiration, g_s and light level needed to saturate photosynthesis. The rates of dark respiration were highest in leaves at five days after unfolding when the increase in leaf dry weight was maximal. Similar results have been reported for soybean, with A_{max} occurring at about 90% - 100% of full leaf size (*e.g.* Woodward, 1976; Lugg and Sinclair, 1981). However, some reports indicate that in a number of woody plants, physiological maturity occurs after the time of full leaf expansion. This has been found in coffee (Yamaguchi and Friend, 1979), the chaparral shrub *Lepechinia calycina* (Field and Mooney, 1983), red maple, sugar maple and northern pin oak (Reich, Walters and Ellsworth, 1991) and in kiwifruit when full rates of photosynthesis were not achieved until 2 - 4 months after full expansion (Buwalda *et al.*, 1991). In poplar, A_{max} corresponded with the point of full leaf expansion, but the maximum level of chlorophylls *a* and *b* in the leaves occurred later (Ceulemans and Impens, 1979).

In plants that retain their foliage for more than one year, the rate of increase in gas exchange parameters is slower, as is the decline from maximum rates to senescence (Field and Mooney, 1983). Foliar material that is in its first season of growth (once it is fully mature) tends to have the highest rates of gas exchange, but leaves that are several years old are still able to make a positive photosynthetic contribution (Larcher, 1980). In kauri, for example, photosynthetic rates were highest in leaves that were one, two and three years old and then decreased markedly in older leaves (Clarkson, 1992). Kauri leaves took eight months to

reach physiological maturity, but were retained on the trees for up to fifteen years. Studying trees in the Amazon basin, Reich, Uhl, Walters and Ellsworth (1991) found that leaves that lived for 12 months or less tended to have lower specific leaf weights, higher A_{max}, g_s, and N content than long lived leaves, which were also tougher. The trees with short-lived foliage were faster growing and found in resource-rich environments. Those leaves that lived for one to five years were lower in nutrients, but had a higher investment in defence mechanisms to protect against damage by herbivores and environmental factors (Reich, Uhl, Walters and Ellsworth, 1991).

The changes in the various gas exchange parameters with leaf ontogeny generally occur in parallel with one another. Thus although both A and g_s increase as the leaf matures and then decrease, the internal CO_2 concentration has been found to remain stable indicating the relative rate of change is similar for A and g_s (*e.g.* Constable and Rawson, 1980; Field and Mooney, 1983). This correlation also means that the water use efficiency (A/E) is not affected by leaf age. The efficiency in the use of other resources are also often independent of leaf age. Field and Mooney (1983) found that in *Lepechinia calycina* the change in the rate of photosynthesis was closely correlated with the change in the nitrogen content of the leaf once it was mature, so that nitrogen-use efficiency did not vary with leaf age. Quantum efficiency was also found to be independent of leaf age in this plant, and a similar result was found in cotton (Constable and Rawson, 1980).

2.1.5 Seasonal interactions with gas exchange

It is relatively easy to define a seasonal trend in gas exchange in evergreen conifers. Maximum rates occur in early summer, decline through autumn to low rates over winter and increase in spring again as temperature and light conditions become more favourable (Kramer and Kozlowski, 1979). In deciduous woody perennials, the seasonal trend can obscured by the effect of leaf age on gas exchange, particularly in those species which produce only a single cohort of leaves at the beginning of the season. With plants that produce leaves continuously throughout the season (such as *Paulownia*) it is possible to study leaves at the same level of physiological maturity at different times in the season.

Generally the seasonal trend of gas exchange rates in deciduous trees that produce a single cohort of leaves is similar to that of evergreens - increasing during spring, after bud burst, to peak during summer and decline again in autumn prior to abscission. In red maple, sugar maple and northern pin oak, Reich, Walters and Ellsworth (1991) found that both photosynthetic rate and leaf specific weight followed this pattern, but the extent of the

changes, at different times in the season, varied between the species. Leaf nitrogen content was high in young leaves and only declined later in the season.

The chaparral shrub *Lepechinia calycina* produces leaves throughout the season, but the final size of leaves changes depending on when during the season the leaf is initiated. Leaves that are initiated early or late in the season are smaller and expand more slowly than those produced in the middle of the season (Field and Mooney, 1983). Gas exchange parameters were reasonably constant throughout the season when measured on an area basis, but A and N content decreased during the season on a leaf weight basis as the specific leaf weight increased. Water use efficiency was relatively constant throughout the season, but quantum efficiency decreased at the end of the season in this shrub. In kiwifruit, leaves initiated later in the season tended to have higher specific leaf weights, N and chlorophyll contents, and quantum efficiency compared with similarly aged leaves produced earlier in the season (Buwalda *et al.*, 1991). However, there was no seasonal effect on rates of photosynthesis or dark respiration, but earlier leaves tended to have higher stomatal conductances. The last leaves produced in the season saturated at much lower light levels than the other leaves.

2.1.6 Diel changes in gas exchange

Over a 24 hour period (the diel time frame) the rates of gas exchange vary, primarily in response to light. Thus at night the predominant flux of CO_2 is out of the leaf into the surrounding air as a consequence of respiration. As the day dawns and the light level increases, photosynthesis, transpiration and stomatal conductance all increase to generally peak in the mid to late morning. On bright, sunny days many plants display a midday depression of gas exchange rates, followed by a full or partial recovery of rates at some point later in the afternoon (Kramer and Kozlowski, 1979). With the onset of darkness at dusk, respiration again becomes the major contributor to the gas exchange process, with the rate of respiration proportional to temperature. In order for the plant to grow satisfactorily, the net amount of carbon respired overnight must be less than that fixed by photosynthesis, and so nighttime temperatures can play an important role in determining growth rates.

The rate of increase in photosynthesis early in the morning can also be influenced by temperature independently of the increase in light, with temperatures lower than about 15°C inhibiting photosynthesis (TGA Green, pers. comm.). An interaction of environmental parameters may explain the commonly observed midday depression of gas exchange rates, with conservation of water within the plant becoming the primary influence under conditions of high light, high temperature, decreasing humidity and increasing VPD. Measurements of leaf or branch xylem water potential have shown that the water potential is highest at dawn,

decreasing through the morning to a midday low, and then slowly increasing later in the day (*e.g.* Downton *et al.*, 1987; Dang *et al.*, 1991). Comparison of gas exchange rates in grapevines that had been watered either 2 or 18 days prior to measurement showed higher rates of A, g_s and C_i in the recently watered vines and a much smaller decrease of rates in the afternoon (Downton *et al.*, 1987).

It is postulated that the stomata close in order to reduce the water loss rate during the middle of the day, and in doing so limit photosynthesis. However Downton *et al.* (1987) observed that the decrease in A could only be partly attributed to the the decrease in g_s, as has been discussed by Farquhar and Sharkey (1982). The maintainence of a constant C_i in the sclerophyll *Quercus suber*, for example, despite decreases in A and g_s as air temperature increases and RH decreases, has been attributed to a decrease in photosynthetic capacity, in carboxylation efficiency and an increase in the CO_2 compensation point (Tenhunen *et al.*, 1984).

It is possible that lower afternoon rates of gas exchange are a result of internal parameters. Phytohormones may play a role with ABA, for example, responding to increased soil drying during the first hours of the day and interacting with both the photosynthetic process and stomatal function to decrease gas exchange (*e.g.* Downton *et al.*, 1987). However later work by these authors (Downton *et al.*, 1988a and 1988b) on a range of species suggests that non-uniform stomatal closure can fully account for the apparent non-stomatal limitation of photosynthesis in response to both endogenous and exogenously applied ABA. Working with grapevines leaves exposed to several hours of high light (1450 μmol m^{-2} s^{-1}) or moderate light (750 μmol m^{-2} s^{-1}) Correia *et al.* (1990) found a greater afternoon decrease in rates of gas exchange in the high light leaves than in those under moderate light, despite provision of ample water. They suggested that the high light treatment impaired electron transport processes in the mesophyll chloroplasts which, in turn, impacted on proton movements and so indirectly affected partitioning of ABA, leading to an ABA-triggered decrease in g_s. This would then explain the observed decreases in both photosynthesis and stomatal conductance under these conditions.

The other major internal influence on gas exchange is negative feedback by the accumulation of the products of photosynthesis which are not able to be translocated from the foliage to other parts of the plant at the same rate as they are produced (J. Flore, pers. comm.). Comparison of gas exchange in leaves of a number of herbaceous species that had been girdled to prevent translocation of photosynthates showed that in leaves that were starch accumulators, A and g_s were depressed by girdling to a greater extent than in leaves that accumulated soluble sugars (Goldschmidt and Huber, 1992). The situation was less clear in *Prunus domestica* with feedback inhibition possibly causing a decrease in gas exchange in

fruited and defruited trees (Gucci *et al.*, 1991). However, both Roper *et al.* (1988), working on *Prunus avium*, and Tenhunen *et al.* (1984), working with *Quercus suber*, discounted accumulation of photosynthates as an explanation for diurnal variations in gas exchange in these trees, instead suggesting that environmental and ontogenic factors were more important.

In many species, the diurnal pattern of gas exchange described above cannot be fully explained by either environmental influences or by internal limitations. For example, Kuppers *et al.* (1986) observed lower gas exchange rates in the afternoon with eucalypt trees and *Acacia melanoxylon*, but neither internal nor external factors could fully account for this effect. It may eventuate, with further research, that a complex interaction of factors are involved and these could easily differ between species and between environments.

2.1.7 Light response curves

A plot of photosynthesis against photosynthetic photon flux density (PPFD) produces a hyperbolic curve with an initially steep slope which decreases as the light increases. The resultant light response curve can give a great deal of information about the leaf under study. The most obvious variable is the maximum photosynthetic rate achieved by the leaf and is denoted as A_{max} *i.e.* the photosynthetic rate at saturating light. The value of A_{max} varies between plants of the same species, between species, with leaf age and as a result of the growth conditions. The light intensity required to saturate A is known as the $PPFD_{sat}$ and tends to vary primarily as a result of the light history of the leaf. Thus a leaf adapted to shade conditions may saturate with only 100 - 200 μmol quanta m^{-2} s^{-1}, whereas a sun leaf requires in excess of 400 μmol quanta m^{-2} s^{-1}, and often much higher levels, before photosynthesis is light saturated (Larcher, 1980). The photosynthetic process at $PPFD_{sat}$ becomes limited by enzymatic processes and the availability of CO_2, rather than by light (Larcher, 1980).

Photosynthesis declines as light levels decline, until a point is reached when the net change in CO_2 concentration is nil. This light level is termed the light compensation point (r). At this point the release of CO_2 from respiratory processes just balances the uptake of CO_2 by photosynthesis. Higher respiration rates will increase the value of r, so it is influenced by external temperature as well as factors internal to the leaf. Shade leaves tend to have lower compensation points because of their lower rates of dark respiration (Schaedle, 1975). The rate of CO_2 release when PPFD is zero is the rate of dark respiration - R_d. Dark respiration occurs at all times in the plant as a consequence of metabolic processes and the rate of R_d depends on the species under study and upon the temperature, increasing with an increase in temperature.

The slope of the light response curve at very low light levels (0 - 100 μmol quanta m^{-2} s^{-1}) is linear as the rate of CO_2 uptake is directly proportional to the light level. In this range, the speed of the light reactions are the limiting stage of the photosynthetic process (Larcher, 1980). The slope of this linear portion of the graph is known as the quantum yield or quantum efficiency - Q_e, and is measured as μmol CO_2/μmol PPFD. The value of Q_e is determined primarily by the intrinsic characteristics of the leaf being studied and tends to remain unchanged by conditions of growth or leaf age. However, it has been found that the temperature and intercellular CO_2 concentration at the time of measurement do affect Q_e (Ehleringer and Bjorkman, 1977). Q_e did not differ between a range of C_3 and C_4 plants, when measured at 30°C and 21% O_2, but increased in the C_3 plants at 2% O_2 and at high C_i. The maximum value for Q_e was found at 15°C in the C_3 plants and decreased as temperature increased up to 40°C, but temperature, O_2 concentration and C_i had no effect on quantum efficiency in the C_4 plants. These interactive effects of temperature, oxygen and internal CO_2 concentrations on Q_e in C_3 plants are indicative of a change in rates of photorespiration. These workers concluded that the balance between the oxygenase and carboxylase activities of RuBP carboxylase-oxygenase enzyme influence the value of Q_e.

2.1.8 AC_i curves

Measurement of gas exchange parameters under conditions of changing external CO_2 concentrations (C_a) can give valuable information about the interactions of various limitations upon the photosynthetic rate. Plotting the data as A against C_i (an AC_i curve) produces an exponential curve with an initially steep slope which then flattens with a decreasing rate of increase in A as C_i increases (Farquhar and Sharkey, 1982). The point at which A is zero is the CO_2 compensation point where the fixation of CO_2 is balanced by the release of CO_2. The value of the compensation point may vary with changes in internal and external conditions such as leaf aging or drought stress, but in tree foliage has generally been found to be between about 20 and 100 μl l^{-1} (Schaedle, 1975).

Under conditions of low internal CO_2 concentrations, the photosynthetic process is limited by the activity of Rubisco. Thus, increasing the amount of CO_2 leads to a theoretically linear increase in A as the enzyme is saturated with respect to RuBP. This part of the graph is known as the demand function (Farquhar and Sharkey, 1982). The gradient of the graph decreases at the inflexion point and thereafter the increase in C_i causes a lower rate of increase in A. Under these conditions, the supply of RuBP becomes limiting as regeneration of the acceptor is not fast enough to meet the requirements of the system. In this part of the graph external factors such as light, temperature and water status influence the response of the

system by affecting the rate of RuBP regeneration, while at low C_i these factors have little effect on A (Farquhar and Sharkey, 1982).

Thus the inflexion point of the AC_i curve is where the change occurs from Rubisco to RuBP limitation. In the theory proposed by Farquhar and Sharkey (1982) this inflexion point is at the value of C_i when C_a is 350 $\mu l\ l^{-1}$ (C_i at approximately 220 $\mu l\ l^{-1}$) and is known as the operating point. The line that can then be drawn from 350 $\mu l\ l^{-1}$ on the x axis to the operating point is the supply function (refer Fig 2.15a). The equation describing this line is $A=g_c(C_a-C_i)$ where g_c is the conductance to CO_2 diffusion. However the practical application of the model to the AC_i response of a number of C_3 plants has found that the operating point can often be lower on the curve than is predicted by the theory, *i.e.* lower than the inflexion point (*e.g.* Tenhunen *et al.*, 1984; Kuppers *et al.*, 1986; Thompson, Huang and Kriedemann, 1992). This result, of a lower rate of photosynthesis in reality as opposed to that predicted by the model, is at least partly due to other factors (both internal and external) that impinge upon the photosynthetic pathway. Growing plants under different nutrient or light regimes has been shown to affect both the initial slope of the AC_i curve and the capacity for RuBP regeneration. This means that, although the value of the operating point changes, its relative position on the AC_i curve does not change (Long and Drake, 1992).

The model can be applied to define the proportions of the different limitations upon the photosynthetic process. The limitations imposed by the mesophyll capacity for photosynthesis result from the constraints upon the activity of Rubisco and upon RuBP regeneration, which can be determined biochemically. Stomatal limitation affects the amount of CO_2 available to the photosynthetic system. The maintainence of a constant C_i under conditions causing a decrease in g_s and A suggest that stomatal limitations to A are less than those imposed upon E, and less than initially thought (Farquhar and Sharkey, 1982). Calculation of the limitation imposed by g_s (l) can be estimated in two ways:

The first uses the equation $l=(A_o-A)/A_o$ where A_o is the assimilation rate when resistance to CO_2 diffusion is zero (*i.e.* when $C_i = C_a = 350\ \mu l\ l^{-1}$), and A is the normal rate of photosynthesis when C_a is 350 $\mu l\ l^{-1}$ (Farquhar and Sharkey, 1982).

The second method determines l as the proportion of the resistance imposed by the stomata to all the resistances in the system. The equation then becomes $l=r_g/(r_g+r_*)$, where r_g is the inverse of the slope of the supply function and r_* the inverse of the slope of the tangent to the curve at the operating point (Jones 1983).

Although neither method is more correct than the other, the second method is considered

more appropriate by Jones (1983) because it does not rely on the artificial situation of an infinite conductance. However, it is very sensitive to any factors that cause small changes in the operating point and thus change the value of r_*. The calculations of *l* from the AC_i curves undertaken on *Paulownia* (section 2.4.5) used the method of Farquhar and Sharkey (1982) primarily, but *l* was also calculated by the resistance method of Jones (1983) as a comparison of techniques.

Other parameters can be determined from the AC_i graph including the A_{max}, which is the maximum rate of photosynthesis when the system is saturated with CO_2, and the carboxylation efficiency which is the slope of the graph at any defined point. It is also possible to plot C_a vs C_i which results in a linear graph.

2.2 Materials

The trees used in the measurement of photosynthesis included three *P. tomentosa* (Japan) trees from the experimental plot at Matangi, described in section 4.2.1, and a mature *P. elongata* tree growing in the grounds of the creche of the University of Waikato. This tree was estimated to be between 30 and 40 years old. It flowered each season and produced viable seed.

2.3 Methods

2.3.1 Use of the LI-6200 portable photosynthesis system

In the 1990 - 1991 season, a LI-6200 portable photosynthesis system (LICOR, Lincoln, Nebraska, USA) was used to study diurnal patterns in photosynthetic rates on the *P. elongata* tree three times during the season. This unit used a closed system to calculate photosynthetic rate and other related factors. The leaf under study was enclosed in a 1000 ml perspex chamber and the air within system was continually pumped through the chamber and through the LI-6250 CO_2 IRGA (LICOR) operating in absolute mode. The IRGA measured the change in concentration of CO_2 over time and from this the microprocessor was able to calculate the photosynthetic rate. The rate at which water was lost from the leaf into the airstream was measured by the amount of flow diverted through desiccant to keep the humidity of the airstream constant, and thus the stomatal conductance could be calculated. From these parameters, the calculations could then be done to compute the internal CO_2 concentration (C_i). A thermocouple placed on the lower leaf surface recorded leaf temperature while air temperature and humidity within the chamber were also measured, and a quantum sensor (LICOR) measured the PPFD incident on the outside of the chamber.

Analyses of the diurnal patterns of photosynthesis using the LI-6200 were carried out in December, 1990, and in February and March, 1991. On each occasion six replicate leaves were measured. These leaves were generally the first fully expanded leaf on each branch at that time. Measurements were taken at 2 hourly intervals through the day from first light until dusk. Concomitant analyses of the leaf water potential (ψ_p) of similar leaves were taken with a pressure bomb as first described by Scholander *et al.* (1965), with the first measurement being made just before dawn. At each time of measurement, the leaves were quickly removed from the tree and placed immediately in the pressure bomb so the readings taken were as accurate as possible. Two leaves, similar to those undergoing gas exchange analysis, were sampled at each time. The leaf water potential was also measured in the following seasons

and at the Matangi block during diel experiments, but on an irregular basis. The large leaves and hollow petioles of the *P. tomentosa* leaves made it difficult to assess water potential using the pressure bomb.

During one of the diurnal experiments, the light environment below the canopy was assessed using a quantum sensor (LICOR).

In addition to these diurnal measurements, analysis of the rate of photosynthesis with respect to leaf age was carried out in February and March, 1991, by measuring the rates of all the leaves on selected branches. Some measurements were also taken on trees 35 and 43 at the Matangi block, with one leaf of each pair of leaves on the trees being sampled once in the late morning (11:00 hours) and again in the afternoon (15:00 hours) on 9 February. On 20 March the rates of photosynthesis, nitrogen content and chlorophyll content were measured on the same leaves on tree 35 prior to it being harvested.

2.3.2 The Walz CMS 400 photosynthesis system

During the 1991-1992 and 1992-1993 seasons, photosynthetic measurements were carried out using a climate-controlled mini-cuvette system (CMS 400, Walz Mess- und Regeltechnik, Effeltrich, FRG). This was an open system allowing gas exchange (both CO_2 and H_2O) to be continuously measured with part of a leaf enclosed in a temperature controlled cuvette. The air being pumped into the machine from outside first passed through a condenser with the dew point preset by the operator (generally about 6°C). This ensured that the air passing through the system had a particular humidity. The air stream was then split into two, with the flow in both streams regulated by mass flow controllers (Tylan, Carlson, USA). One stream (the measuring stream) then passed over a portion of the leaf enclosed in a perspex cuvette, while the second air stream (reference) passed through a compensation vessel such that the path length for the two streams was the same. The parallel streams then passed through a differential H_2O IRGA (Rosemount Binos 100, Wilhelm - Rohn - Strasse 51, D - 6450 Hanau 1, Germany) which determined the difference in the water vapour content between the two air streams (hence allowing the calculation of E and g_s). Most of that water was then removed by a condenser (set at a dewpoint of 2°C) before the air entered a CO_2 IRGA (Rosemount, Binos 100) operating in differential mode which measured the difference in CO_2 content of the measuring and reference streams. From there the reference stream of air passed through an absolute CO_2 IRGA (Rosemount, Binos 100) to measure the actual CO_2 content before venting to waste. From the measurements made by the two CO_2 IRGAs, the photosynthetic rate (A) and leaf internal CO_2 concentration (C_i) could then be calculated. A diagrammatic representation of the system is given in Fig. 2.1, while Fig. 2.2 shows the machine in use in the field at Matangi.

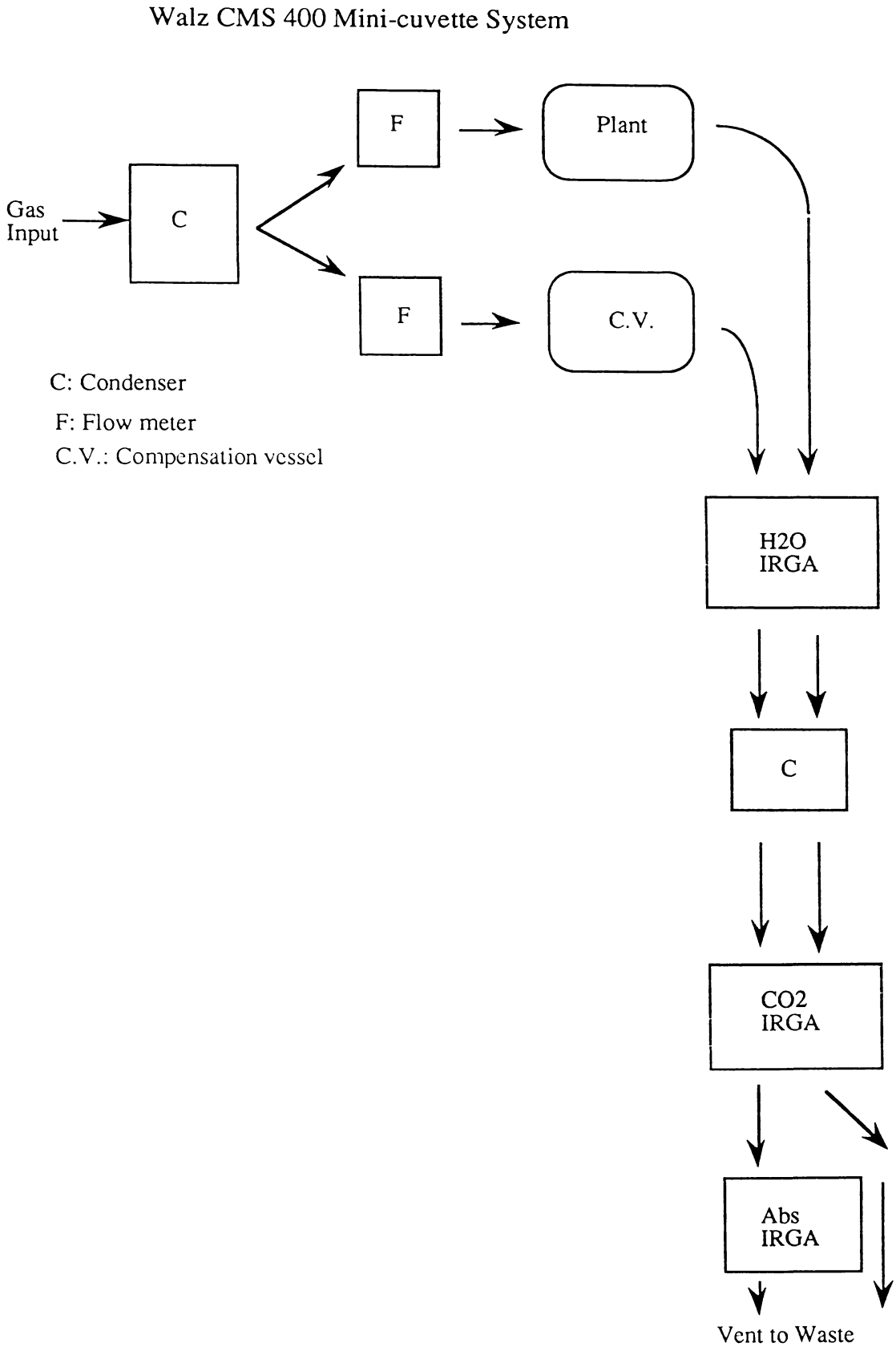


Fig. 2.1. Simplified diagram of air flow through Walz CMS 400 photosynthetic system.

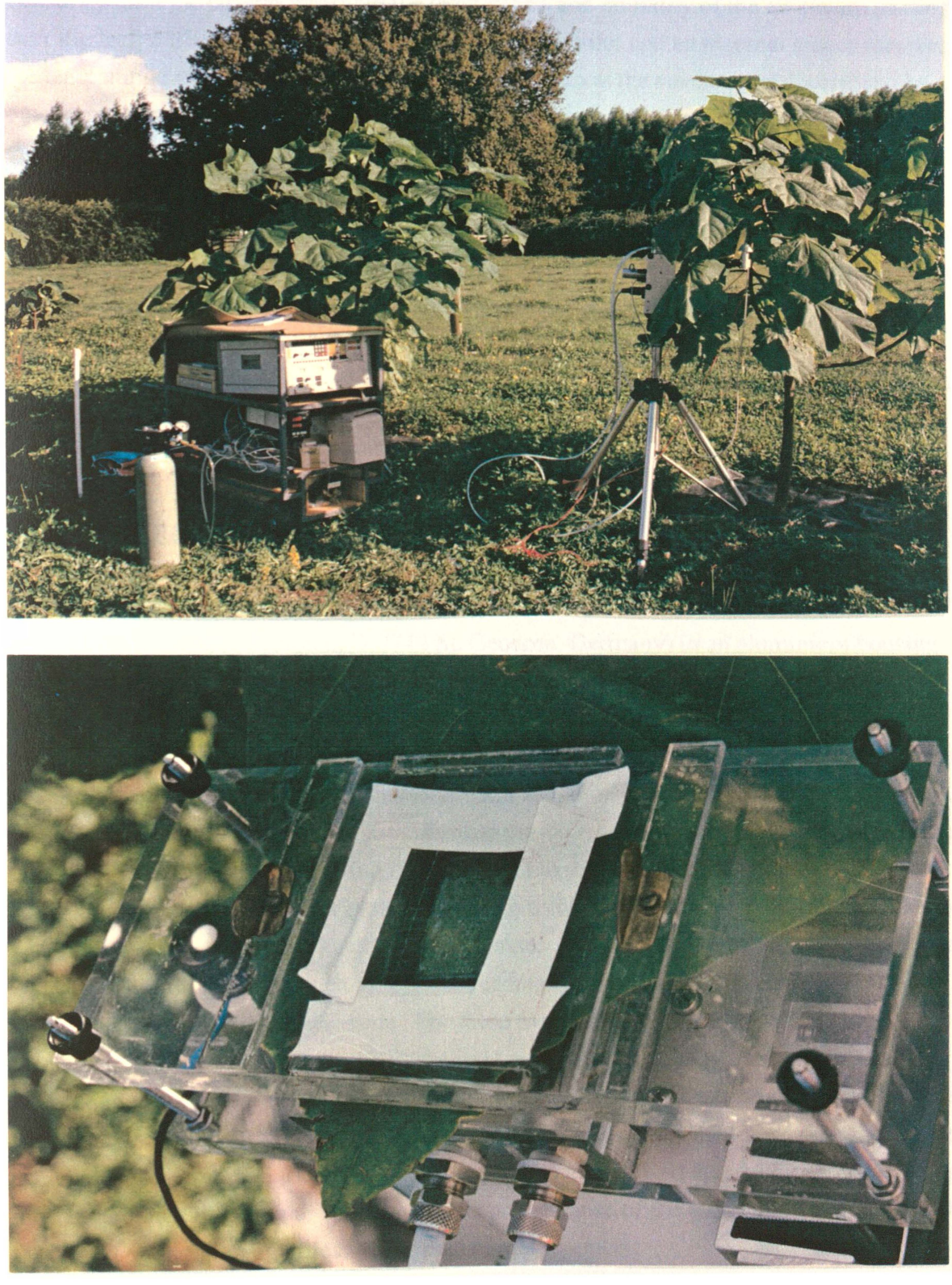


Fig. 2.2. The Walz CMS 400 unit in operation on *P. tomentosa* leaves at Matangi.

Sensors within the cuvette measured the temperature and humidity of the air stream passing over the leaf, a thermocouple measured the leaf temperature and an external sensor measured the temperature of the external air. The light incident upon the cuvette was determined by a quantum sensor situated under the cuvette lid, while a second quantum sensor, situated within the cuvette, measured the amount of light passing through the leaf.

The data thus collected was downloaded to a laptop computer (Arima, ACT386sx) every 10 seconds and averaged at 1 or 5 minute intervals. The calculations of gas exchange parameters used the equations presented by von Caemmerer and Farquhar (1981).

The differential IRGAs were zeroed twice daily and the absolute IRGA was zeroed daily and calibrated with a gas of a known CO_2 content on a weekly basis.

2.3.2.1 Light Response Curves

In order to carry out light response curves, a portable light source consisting of a 12V, 2.5W lamp (Typ 612, Papst-Motoren, D-7742 St. Georgen, Germany) in an aluminium housing was attached to the cuvette directly above the enclosed leaf portion. A heat filter directly beneath the bulb prevented overheating of the leaf. The amount of light available to the leaf was varied with a series of neutral density filters which were calibrated with a quantum sensor (Table 2.1). The KG_3 filters were made of glass while the remaining filters were made up of one, two or three layers of 50% neutral density plastic atop one another. The light apparatus was covered in a black cloth during measurements to prevent extraneous light reaching the leaf. The air entering the Walz system was set to a constant temperature of 22°C and the flow rates used were between 2000 and 3000 ml/minute. At the change of each light level the plant was left to equilibrate until such time as the reading for stomatal conductance was steady before the measurements were made. For those measurements made in November and December 1991, and in January 1992, the light response curves were carried out beginning with the lowest light level and proceeded with increasing light. The later measurements (from February, 1992 onwards) started with full light and decreased the light level over the course of the experiment. A small trial on leaves of the creche tree showed that the direction of the light change had little effect on the photosynthetic performance of the leaf. Thus the data of all the curves is comparable regardless of the date of measurement.

Measurements of the light response curve of the same leaves were taken at approximately monthly intervals, beginning in November, from the *P. elongata* tree at the creche and trees

Table 2.1. PPFD available to leaf with each filter combination

Filter Combination	PPFD (μmol m^{-2} s^{-1})
No filter [1]	2140
1 x KG_3	1660
2 x KG_3	1290
1 x layer 50% neutral density filter	970
1 x KG_3 + 1 x layer	750
2 x layer	450
3 x layer	210
no light	0

[1]all filter combinations included the IR filter.

43 and 72 at Matangi (*P. tomentosa*). This allowed the effect of leaf age on light response to be studied. In addition, the first fully expanded leaf on the branches under study was added to the group of leaves being sampled each month, enabling comparisons to be made with leaves of the same physiological age at different times during the season.

The results from the light response curves were plotted, and curves were fitted by non-linear least squares regression, using the GENSTAT V 5.0 statistical software (Buwalda *et al.*, 1991). The curve fitting resulted in the equation for an asymptotic exponential curve $A = A_{max} - B.a^{(PPFD)}$ where A - assimilation rate; A_{max} - assimilation rate at light saturation; PPFD - irradiance (photosynthetic photon flux density); B and a - empirical parameters (a<1) (Buwalda *et al.*, 1991). This equation provided an excellent fit for the majority of the light response curves. From the equation it was possible to make the following calculations (I. Gravett, pers. comm.):

Dark respiration: $R_d = A_{max} - B\ a$ (*i.e.*PPFD = 0)

Quantum efficiency: $Q_e = B.\ln a$

Light saturation: $PPFD_{sat} = \ln (0.05\ A_{max} / B) / \ln a$

Light compensation point: $r = \ln (A_{max} / B) / \ln a$

The water use efficiency of the leaves under saturating light was calculated by A/E (umol $mmol^{-1}$).

2.3.2.2 Measurement of Diel Patterns of Gas Exchange

Because the Walz CMS 400 employed an open system to measure gas exchange, it was possible to leave a portion of leaf enclosed in the cuvette for several hours at a time. Generally, the area of the leaf enclosed was between 0.0009 and 0.0012 m^2 (9 - 12 cm^2). The temperature of the air passing through the cuvette was set to track the ambient temperature, while the dew point was set at between 6°C and 8°C. The flow rate overnight was generally about 1000 ml min^{-1}, but during the day was increased to up to 4000 ml min^{-1}, depending on the rate of transpiration.

Leaves were sampled on a monthly basis from the *P. elongata* tree at the creche and from trees 43 and 72 at Matangi. In each instance two or three replicates were measured, these generally being the first fully mature leaves.

2.3.2.3. AC_i Curves

The effect of CO_2 concentration on the rate of photosynthesis was studied to determine the amount of stomatal limitation on the photosynthetic process. This was assessed by changing the CO_2 level of the air being pumped into the Walz. The air was first pumped through a 'Carbosorb' (BDH Chemicals, Prod. 33111) line to scrub the air of all CO_2, and then a set flow of pure CO_2 was added to the CO_2 - free air (Tylan 20, with FC 260 mass flow controller). After mixing in a buffer vessel, the air was pumped into the Walz and proceeded as normal. The absolute IRGA measured the CO_2 concentration which ranged from 1500 µl l^{-1} to 70 µl l^{-1}. With this experimental set-up it was not possible to precisely determine the CO_2 compensation point. The light level over the leaf was kept constant at 1660 µmol m^{-2} s^{-1} by placing the light (described above) over the leaf with one KG_3 filter in place. As with the light response curves, the system was left at each CO_2 level until the stomatal conductance readings stabilized.

The data collected needed to be recalculated because the differential IRGA was set to operate in air with approximately atmospheric CO_2 levels (340 µl l^{-1}) and the zero point shifted with significantly higher or lower CO_2 concentrations, leading to incorrect calculations. These recalculations were undertaken by multiplying the zero shift, recorded at the time of measurement, by the sensitivity factor of the IRGA at the CO_2 concentration being used. Curve fitting of the data was done using a general exponential fit (KaleidaGraph 3.0, Synergy Software). Constructs were then used to calculate A at chosen C_i or C_a values so A and A_o could be determined to calculate *l* by the method of Farquhar and Sharkey (1982). A_{2000} was estimated from the graph at C_i = 2000 µl l^{-1}. Calculation of *l* by the method of Jones (1983) involved calculating r_g and r_*. By taking C_a as 350 µl l^{-1} and determining C_i and A at

that point, r_g could be then be computed by (350 - C_i)/A. r_* was determined by C_i/A_*, where A_* was the difference between A and the point of intersection on the y axis of the tangent to the operating point. Stomatal limitation could then be calculated as $r_g/(r_g+r_*)$.

AC_i curves were carried out in February and March, 1991, at both sites on two replicate, first fully mature leaves. During the 1992 - 1993 season, these curves were undertaken on leaves on trees 43 and 72 at the Matangi block from December, 1992 through to March, 1993. Each month in this period, AC_i curves were carried out on first fully mature leaves on both trees to study seasonal trends. In succeeding months, the leaves on tree 43 only were remeasured to determine the change in response as the leaves aged.

2.3.2.4 Measurements of gas exchange in season 3

In the 1992 - 1993 growing season, a limited number of measurements of gas exchange were carried out on trees 43 and 72 at Matangi. In addition to the AC_i curves described above, monthly measurements of A under conditions of full light were undertaken on first fully mature leaves of the two trees. These data were collected from November, 1992 until March, 1993. Some leaves were assayed from month to month to investigate the effect of leaf age on gas exchange. Diel analysis of gas exchange were also carried out on a small number of leaves in December, January and March. A temperature response curve was undertaken in February on tree 43, under cool and cloudy ambient conditions.

No measurements were made on the *P. elongata* tree at the creche.

2.3.3 Determination of leaf parameters

Throughout each of the three seasons, foliar material was analysed for nitrogen content, chlorophyll content and stomatal density at the same time as gas exchange measurements were made. Analysis of leaf material for N content was either carried out by AgResearch Ltd (section 4.4) or in the university laboratory, by Mr P Stock, using Kjeldahl digestion techniques. The density of stomata was analysed by counting stomatal pores on a collodion peel of the leaf surface under 400x magnification. In one instance, an eye piece micrometer was used to assess the size of stomata.

The technique used to determine the chlorophyll content of leaf discs (of known area) punched from whole *Paulownia* leaves was based on the method of Arnon (1949) with some modifications. Each disc was ground in a mortar and pestle, and the tissue then washed

into a 10 ml conical, plastic tube with 10 ml of extraction buffer. The extraction buffer contained a 4:1 (v/v) mixture of acetone and 0.05M tris (tris[hydroxymethyl]-aminomethane). The tube was capped and placed in the refrigerator overnight, in a light proof container to prevent photo-decomposition.

After extraction, the solution was centrifuged at 4000 rpm for 3 - 5 minutes to separate the chlorophyll extracted in the liquid phase from the ground leaf tissue. The supernatant was tipped into a second container and made up to a known volume (*e.g.* 20 ml) with extraction buffer. After mixing, an aliquot was assayed spectrophotometrically at 664 nm and 647 nm. The spectrophotometer (Shimadzu UV - 160, Kyoto, Japan) was zeroed with a blank of extraction buffer. The results were then entered into a computer programme which calculated the concentrations of chlorophyll *a*, chlorophyll *b* and the combined concentrations using equations 1 - 4 (Arnon, 1949).

Equation 1: chp $a = 12.7 * Ab_{664} - 2.7 * Ab_{647}$ (mg l^{-1})

Equation 2: chp $b = 22.9 * Ab_{647} - 4.7 * Ab_{664}$ (mg l^{-1})

Equation 3: $a + b = (20.2 * Ab_{647} - 8.0 * Ab_{664}) *$ volume / 1000 (mg)

Equation 4: Total = $((a + b)$ / area).10 (g m^{-2})

The foliar material used in the determination these parameters was either that used directly in the measurement of gas exchange, or leaves of a similar ontogeny. For those leaves on which gas exchange parameters were measured in consecutive months, it was not possible to perform destructive analyses, hence the use of similar leaves.

In addition to these measurements, assessments were made of the rate of initiation of new leaves and the aging of existing leaves on the creche tree and several trees at Matangi in season 2 by tagging leaves at monthly intervals. At the time of each monthly measurement of gas exchange, observations were made about the health and growth status of the trees.

2.4 Results

2.4.1 Aspects of tree phenology

2.4.1.1 *P. elongata*

The mature *P. elongata* tree at the creche showed similar growth patterns over each of the three seasons. The tree flowered in late September - mid October, while leafless, with leaf production beginning during October. The new leaves were always produced on the end of a branch, which meant that the tree did not carry any shade leaves as all the leaves received direct sunlight at some time during the day. Branch extension was an ongoing process throughout the season even in this mature specimen with leaf initiation continuing until approximately early March. During the early part of the season, branches tended to have six - eight leaf pairs, of which the oldest three - five pairs were fully mature. Later this increased to ten - twelve pairs as flower bud formation commenced. Senescence and abscission of the leaves was a continuing process throughout the season, with the remaining leaves abscising at the onset of the first frosts of autumn - generally during late April or May.

Tagging of leaves enabled an assessment to be made of the lifetime of the foliage, which averaged 4 - 5 months. One leaf that was tagged in late November, when it was in the second pair and still immature, was still on the tree at the last analysis in late April, albeit in a advanced state of senescence. Once the leaves were dropped from the tree they decomposed quickly, within a few days.

The development of the fruit continued throughout the season until the woody seed cases were mature in May. At this time the fruit dehisced to release the thousands of seeds to the wind, but the seed cases remained on the tree. It was not possible to determine how long seed cases were retained, but in some instances it was at least one year and probably longer. Concomitant with this fruit ripening was the development of the following season's flower buds. Visible signs of initiation of the flower buds began in February and continued for the remainder of the season. It was noticed in late January that the apices of some branches were dead and that leaf initiation had ceased. These branches did not produce flower buds. In those branches retaining active apices, inflorescences developed in the axils of the new leaves, with very small leaves subtending each bud. These leaves were dropped as the inflorescence grew. The initiation of flower buds led to a change in the morphology of the new leaves developing at the same time. The flowering apex had several pairs (2 - 5) of very small, hairy leaves and although the number of leaf pairs increased at this time, it is likely that the total photosynthetic area of each branch was no greater than earlier in the season, when the leaves

expanded more rapidly.

There appeared to be a relationship between flower bud production and the branch position in the canopy. Although the canopy was such that only the exterior ends of the branches produced leaves, there were some branches that were slightly further inside the canopy than others. The leaves on these branches were shaded for longer than those on more exterior branches, but still received adequate sunlight due to the sparse leaf arrangement and effect of wind on the outer branches. However it was noticed that these interior branches failed to produce flower buds with the result that the tree only flowered on the outermost branches. It is possible that the assimilate production of the inner branches was insufficient to support production of flower buds, or that floral initiation required a high light level for a sustained period.

Once abscission had occurred, the branches with the flower buds retained them until spring in a semi-developed state. At this time, the dry weight of the buds was approximately half that of the mature, open flowers (Ede, unpublished). The flowering branch thus produced did not elongate in the following spring. Instead branch elongation occurred from a point at the base of the previous season's flowering branch, at an acute angle.

The very open structure of the canopy of the mature *Paulownia* tree allowed a significant quantity of light to penetrate to plants growing beneath it. The grass sward under the creche tree was not significantly different from that growing in the open under full sun. The human activities associated with the habitat seemed to have more impact on the undergrowth than the presence of the tree. Measurement of the light beneath the canopy carried out on a sunny day found 1900 μmol m^{-2} s^{-1} in full sun, 1550 μmol m^{-2} s^{-1} in the sunflecks on the ground and 90 μmol m^{-2} s^{-1} in full shade under the tree. The occurrence of sunflecks would be expected to be quite high as the sparse canopy of large leaves moved constantly in the wind.

2.4.1.2 *P. tomentosa*

The seasonal development observed in the *P. tomentosa* trees at Matangi was influenced by their juvenile nature. In the first season of growth from root stumps, a single unbranched leader developed with 10 - 20 pairs of very large leaves. On healthy, fast growing trees at the height of the season the leaves were up to 0.6 m in length, with a similar breadth. Height growth ceased during March and the leaves dropped during April and May.

In the following seasons, branches developed in the axils of the previous season's leaves on the upper half of the trees. The leaves that developed on these branches and on the leader

were generally smaller than the large leaves of season 1, with the leaves produced from the buds that overwintered being very small with short, poorly defined petioles. These persisted for about 3 months. The lifetime of leaves produced in September and October was influenced by the impact of the grass grub beetles (section 4.3.2.1C). Later leaves tended to live for between 3 and 5 months.

In season 2 branch and stem elongation ceased during late February and leaf drop occurred in late April after a severe frost. Some of the larger trees had initiated flower buds late in season 2 but none of these survived the winter. Bud burst began in late September in season 3 and at the end of October new branches had elongated by about 30 cm with 3 - 4 small leaf pairs present on each branch. Growth in the middle and later stages of season 3 was poor and in several trees both branch and stem apices had ceased growth in late January. Thus the initiation of new leaves occurred for a much shorter time than in either of the previous seasons. A lot of the leaves that remained on the trees in February and March looked quite yellow and unhealthy, with leaf drop occurring gradually over April and May. In comparison with the leaves produced in season 2, leaves in season 3 were smaller, thicker, lighter green and hairier. Several of the larger trees produced substantial quantities of flower buds that were carried over the winter to flower in the following spring.

2.4.2 Gas exchange in season 1

2.4.2.1 Diurnal patterns of gas exchange on *P. elongata* leaves

The results of the diurnal experiments on two representative leaves of the *P. elongata* tree at the creche are presented in Tables 2.2, 2.3 and 2.4. The light level incident on the leaves was not generally saturating until about midday due to the orientation of the leaves studied (facing north to northwest) and, excluding cloud cover, remained so until early evening. The air temperature increased through the morning in each experiment, but remained reasonably steady in the afternoon at about 25°C.

The diurnal pattern of A followed a similar trend to the environmental factors with lower photosynthetic rates being recorded in the morning compared with the afternoon. There was no evidence of a midday depression of gas exchange in any of the experiments. In December and February, the maximum values of A were measured in the early to mid afternoon and stayed reasonably constant until light levels became limiting in the early evening. With the experiment undertaken in March, cloud cover in the mid afternoon depressed A so that the maximum rate was recorded later (16:40 hours). The daily maximum levels of A were lowest in December and increased later in the season.

Table 2.2. Diurnal patterns of gas exchange parameters measured on leaves of *P. elongata* on 18 December, 1990.

	Time (hours)	Air Temp. (°C)	PPFD (μmol m^{-2} s^{-1})	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	ψ_p (-MPa)
Leaf 1	06:30	10.9	60	1	-[1]	347	0.24[2]
	08:20	13.9	160	3	0.21	320	0.29
	10:20	19.2	270	5	0.08	237	0.58
	12:30	25.6	2440	14	0.16	191	0.56
	14:20	25.0	2110	13	0.17	196	0.57
	16:20	24.4	1060	13	0.14	184	0.66
	18:20	22.3	1600	12	0.18	229	0.53
	20:30	16.7	20	0.1	0.05	346	0.33
Leaf 2	06:30	11.0	70	1	0.18	351	
	08:20	14.4	140	4	0.16	305	
	10:20	19.7	1310	8	0.09	193	
	12:30	25.9	2120	16	0.21	198	
	14:20	26.5	2670	13	0.15	186	
	16:20	24.8	1250	11	0.13	191	
	18:20	24.0	940	13	0.15	193	
	20:30	16.5	20	0.1	0.04	352	

[1] g_s not determined

[2] leaf water potential is the average of two replicate leaves of similar ontogeny to those undergoing gas exchange measurements

The pattern of stomatal conductance through the day was less clear cut. With the experiment carried out in December (Table 2.2), the measurements of g_s during the morning were quite variable and did not exhibit a trend of increasing values, unlike A. During the afternoon, the values of g_s were more constant (as was A) until early evening when the low values indicated the closure of the stomata overnight. In both the February and March experiments, the level of g_s measured was more closely correlated with the photosynthetic rate through the course of the day. The values measured in March (Table 2.4) were higher than those in either of the two previous experiments with a maximum value of 0.54 mol m^{-2} s^{-1} recorded for the

Table 2.3. Diurnal patterns of gas exchange parameters measured on leaves of *P. elongata* on 4 February, 1991.

	Time (hours)	Air Temp. (°C)	PPFD (μmol m^{-2} s^{-1})	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	Ψ_p (-MPa)
Leaf 1	08:30	14.6	100	2	0.15	308	0.08
	10:15	19.2	530	11	0.13	192	0.14
	12:20	22.6	780	16	0.21	199	0.23
	14:20	23.8	1630	20	0.25	187	0.28
	16:15	25.2	2230	18	0.20	181	0.26
	18:10	24.7	1540	15	0.18	181	0.28
	20:00	19.0	50	1	0.05	299	0.13
Leaf 2	08:30	14.6	110	4	0.10	287	
	10:15	19.4	420	10	0.13	221	
	12:20	22.9	670	14	0.20	216	
	14:20	24.8	2500	20	0.32	215	
	16:15	26.7	2190	17	0.21	190	
	18:10	24.0	1290	18	0.21	176	
	20:00	19.0	40	1	0.05	308	

reading at 16:40 hours on leaf 2. This corresponded to a value of 25 μmol m^{-2} s^{-1} for A which was the highest level recorded in any of the diurnal experiments through the season.

Under normal conditions in C_3 plants, it is generally accepted that when the internal CO_2 concentration drops below 200 - 220 μl l^{-1}, it is an indication of an increase in the stomatal limitation on photosynthetic rate due to some stress, often water stress. In the diurnal experiments carried out in December and February, the low g_s values and moderate to high photosynthetic rates led to very low C_i values for much of the day. Thus the rates of photosynthesis were probably limited by the supply of CO_2 to the mesophyll cells. During the March experiment, the C_i levels were much higher because the g_s values were also higher and so the CO_2 supply was unlikely to be limiting to A (Table 2.4).

Analysis of the predawn leaf water potentials, as a measure of the water status of the tree, showed that the factor increasing the stomatal limitation on A was not water stress. Leaf

water potentials measured at all times throughout the day and the season were high. There was a diurnal trend with ψ_p decreasing with to a minimum in the mid to late afternoon, then recovering slightly, but the lowest value was only -0.66 MPa (Table 2.2).

Table 2.4. Diurnal patterns of gas exchange parameters measured on leaves of *P. elongata* on 12 March, 1991.

	Time (hours)	Air Temp. (°C)	PPFD (μmol m^{-2} s^{-1})	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	ψ_p (-MPa)
Leaf 1	08:25	14.9	50	2	-[1]	345	0.13
	10:25	19.7	1160	8	0.24	283	0.17
	12:20	24.7	1800	12	0.33	272	0.23
	14:40	25.4	260	7	0.14	253	0.37
	16:40	27.3	1750	18	0.33	233	0.42
	18:30	23.2	880	17	0.26	232	0.26
Leaf 2	08:40	15.4	60	2	-[1]	342	
	10:30	19.9	110	3	0.19	319	
	12:25	25.3	1560	12	0.26	260	
	14:50	25.1	250	7	0.19	278	
	16:40	28.2	1770	25	0.54	236	
	18:40	23.0	1150	16	0.31	245	

[1] g_s not determined

2.4.2.2 Influence of leaf age along branches of *P. elongata*

Analysis of the effect of leaf age on photosynthetic activity (Table 2.5) showed an increase in A and g_s as the leaves matured which peaked at the fourth leaf pair. Gas exchange parameters were similar for the fifth and sixth pairs, then decreased as the leaves aged further. The nitrogen content of the leaves did not show a clear trend with leaf age, but the chlorophyll content was highest in the fifth leaf and lowest in the youngest and oldest leaves. Thus, in this case, the highest chlorophyll content did not occur in the leaf with the highest photosynthetic rate. The results from this experiment and a similar one carried out in February on two branches from this tree suggested that the leaves were physiologically

mature when they were about two - three months old (leaf pair 4 or 5) and maintained maximum levels of gas exchange for some time, before the onset of senescence led to a decease in activity. It should be noted, however, that even very young leaves made a positive photosynthetic contribution under the light conditions at measurement.

Table 2.5. The effect of leaf age on gas exchange and related parameters of leaves 1 - 8 on branch 1 of *P. elongata* (4 March , 1991).

Leaf number	PPFD (μmol m^{-2} s^{-1})	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	N (% DW)	Chlorophyll (g m^{-2})
1	2030	6	0.08	216	2.06	0.39
2	1930	9	0.10	191	2.25	0.44
3	2020	12	0.14	195	2.41	0.54
4	1900	21	0.42	239	2.20	0.52
5	1750	18	0.28	226	2.28	0.71
6	1890	14	0.28	248	2.28	0.55
7	1530	9	0.22	264	2.00	0.59
8	920	7	0.10	236	2.09	0.38

2.4.2.3 Photosynthesis in *P. tomentosa* trees in season 1

Gas exchange was measured on trees 35 and 43, at Matangi, in February in both the morning and mid afternoon, but the results were so similar that only the data from the morning experiment are given (Table 2.6). The air temperature during the measurements ranged from 27°C to 29°C. The rates of photosynthesis in fully mature leaves were very similar to those recorded for the creche tree at the same time (Table 2.3), but g_s was much higher which led to higher C_i values. It is not possible to clarify whether this difference in g_s was an affect of tree age, species or site conditions.

There was a clear effect of leaf age in tree 35 with the youngest leaf having the lowest rates of gas exchange. Leaf pairs two to five had higher rates of A and g_s, with pair three having the highest rates. As the leaves aged from pair six, rates of A decreased but g_s remained at similar levels to the younger leaves. Although the rates of photosynthesis were similar in leaves on tree 43, the values of g_s were higher. There was a less obvious age effect on rates of gas exchange , with only the two oldest leaf pairs showing a decline in A. The high levels of

A and g_s in the first leaf pair of tree 43 reflect the increased physiological maturity of this leaf in comparison with leaf one on tree 35.

Table 2.6. Effect of leaf age on gas exchange parameters in the first season of growth of *P. tomentosa* (9 February, 1991).

Leaf number	PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	A ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	C_i ($\mu l\ l^{-1}$)
Tree 35				
1	1570	4	0.12	277
2	2070	13	0.38	271
3	2040	17	0.43	246
4	1750	11	0.36	284
5	2070	14	0.38	267
6	1710	7	0.27	296
7	1670	9	0.34	290
8	1820	6	0.37	304
9	1550	6	0.33	301
Tree 43				
1	2100	11	0.53	291
2	1800	16	0.42	274
3	1830	14	0.50	275
4	1930	18	0.54	269
5	1840	12	0.45	287
6	2020	16	0.56	272
7	1950	7	0.43	301
8	1800	4	0.69	326

Remeasurement of various parameters on tree 35 in March showed a similar trend in A with leaf age, but with the maximum rates occurring in leaf pairs three to seven (Table 2.7). A decreased with age from pair eight, but g_s was highest in pairs seven and eight. There was less of a correlation between A and g_s at this time. There was little change in the chlorophyll content of the leaves as they aged, until leaf pairs 10 and 11 which were visibly senescent. The nitrogen content was highest in pair two to six, but began to decrease from leaf pair seven onwards and was considerably lower in the senescent leaves. This is despite the high photosynthetic rate of pair seven. There was little correlation between foliar N level

and A in those leaves with N levels above about 2%, but below that level A tended to decrease as foliar N content decreased.

Table 2.7. The effect of leaf age on gas exchange and related parameters of leaves on tree 35 in its first season of growth (20 March, 1991).

Leaf number	PPFD (μmol m^{-2} s^{-1})	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	N (% DW)	Chlorophyll (g m^{-2})
1	1610	8	0.13	240	1.92	0.31
2	1660	12	0.24	244	2.70	0.35
3	1800	16	0.31	236	2.55	0.34
4	1440	13	0.29	249	2.69	0.35
5	1900	17	0.45	269	2.29	0.38
6	1920	13	0.39	267	2.33	0.41
7	1120	20	0.59	268	1.97	0.42
8	1890	12	0.57	293	-[1]	0.39
9	2070	8	0.42	296	1.88	0.45
10	1550	6	0.23	285	1.69	0.22
11	1370	-0.5	0.11	350	1.49	0.19

[1]not determined

The effect of leaf age on gas exchange in young trees has been found in other species, with immature leaves and senescent leaves having lower rates of photosynthesis than leaves of an intermediate age. Analysis of gas exchange in several leaves of one year old seedlings of *Acacia auriculiformis*, for example, showed a similar effect of age, with rates of A, E, g_s and water use efficiency being highest in leaf pairs four - nine (Pathre *et al.*, 1990). The eighth leaf pair had the highest rates of photosynthesis (15 μmol m^{-2} s^{-1}) and transpiration (4.5 mmol m^{-2} s^{-1}). Rates of A in the first two leaf pairs were approximately 25% of those of pair eight, while rates in pair 20 were only 20% of the maximum rate.

2.4.3 Light response curves in season 2

2.4.3.1 Effect of Direction of Light Change

A study of the effect of the direction of light change on the light response curve was undertaken on two fully mature leaves of the *P. elongata* tree. As the results were similar for both, only one set of data is presented (Table 2.8). The gas exchange parameters measured did not change with the direction of light change, which initially decreased from full light to zero light and then increased again. Therefore light response curves undertaken in either direction were taken to be comparable with one another.

Table 2.8. Light response experiment undertaken on leaf 1 of creche tree in February, 1992, with decreasing and increasing light levels.

PPFD (μmol m^{-2} s^{-1})	A (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	VPD (kPa)
2140	16	5.0	0.37	296	1.8
1290	15	4.1	0.32	285	1.6
780	14	3.6	0.29	286	1.5
470	11	3.1	0.24	282	1.6
220	7	2.2	0.15	285	1.6
0	-0.1	0.7	0.04	362	1.7
220	7	2.0	0.15	275	1.6
470	11	2.8	0.22	272	1.6
1290	15	4.3	0.34	271	1.6

2.4.3.2 Effect of leaf age

The results of the monthly measurements of light response curves on leaves from the time of full expansion until leaf death on trees 43 and 72 at Matangi and the *P. elongata* tree at the creche are given in Tables 2.9 and 2.10, and Figs. 2.3 - 2.6. These results are of typical leaves, with the tabulated values of A_{max}, g_s, C_i and WUE (A/E) being those calculated under conditions of full light. Replication of age related light response curves of leaves was limited by the availability of the Walz equipment (10 days per month), the length of time required to undertake a full light response curve (two - three hours) and the number of other experiments carried out each month. The decision to study gas exchange rates on three trees (one mature

Fig. 2.3a.

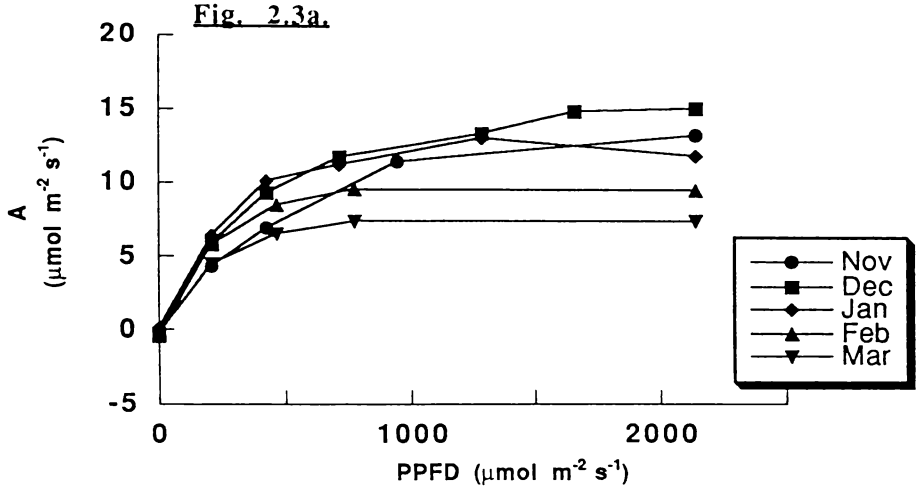


Fig. 2.3b.

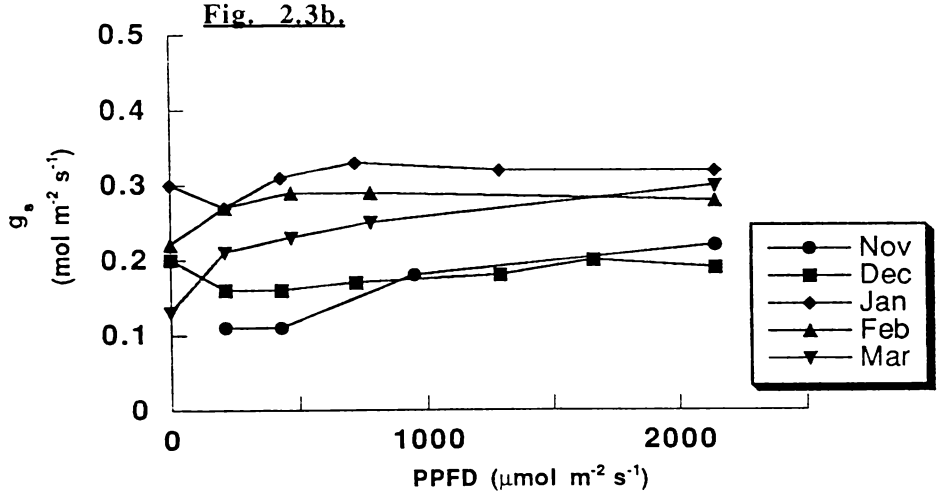


Fig. 2.3c.

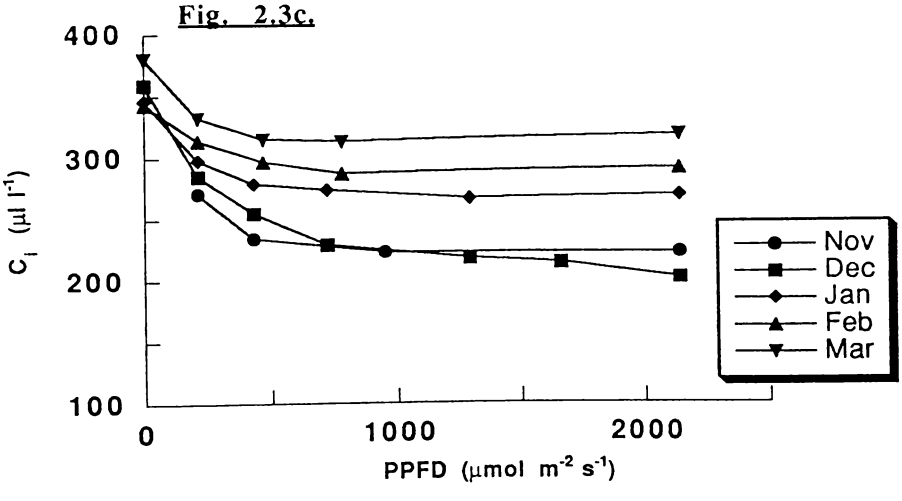


Fig 2.3a, b, c. Changes in the light response curve of a. photosynthesis; b. stomatal conductance; c. internal CO_2 of leaf 1 on tree 43 from November, 1991 to March, 1992.

Fig. 2.4a.

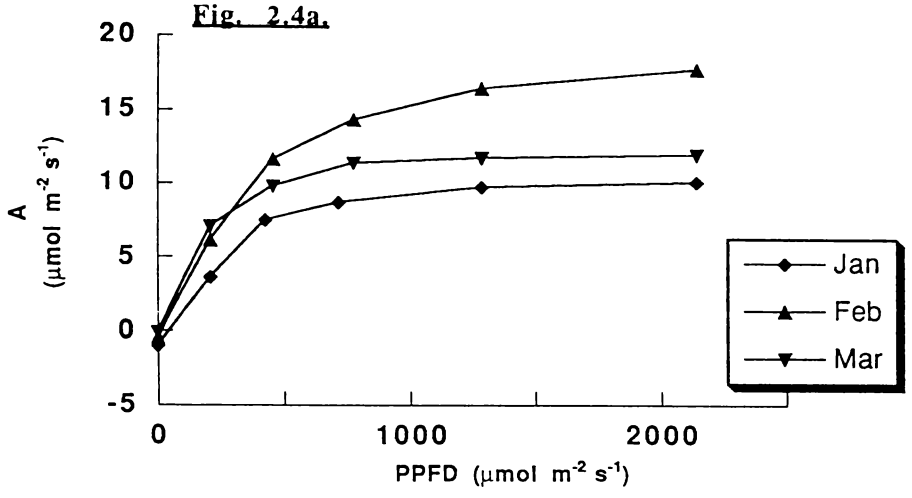


Fig. 2.4b.

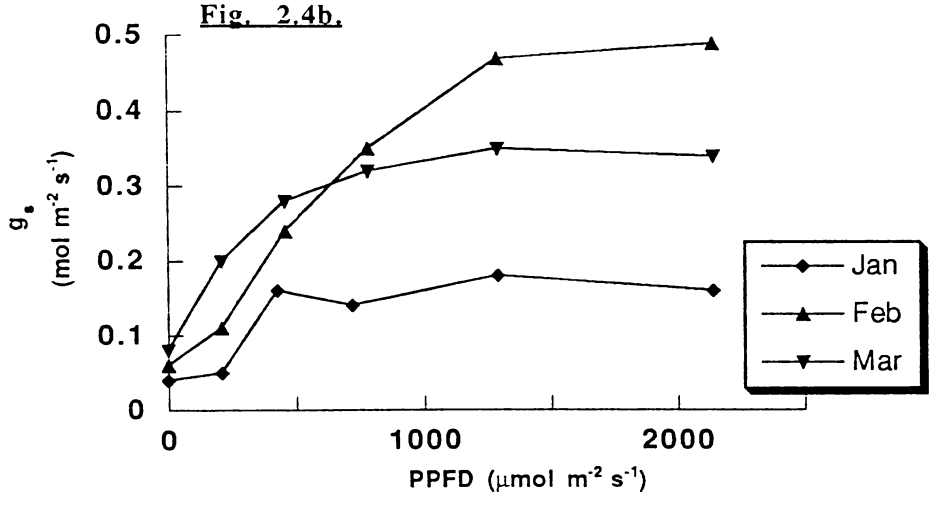


Fig. 2.4c.

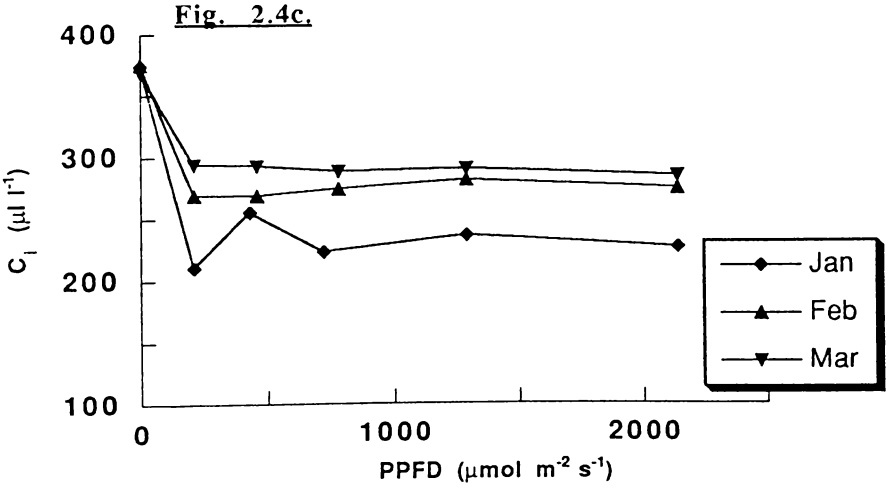


Fig 2.4a, b, c. Changes in the light response curve of a. photosynthesis; b. stomatal conductance; c. internal CO_2 of leaf 2 on tree 72 from January, 1992 to March, 1992.

Fig. 2.5a.

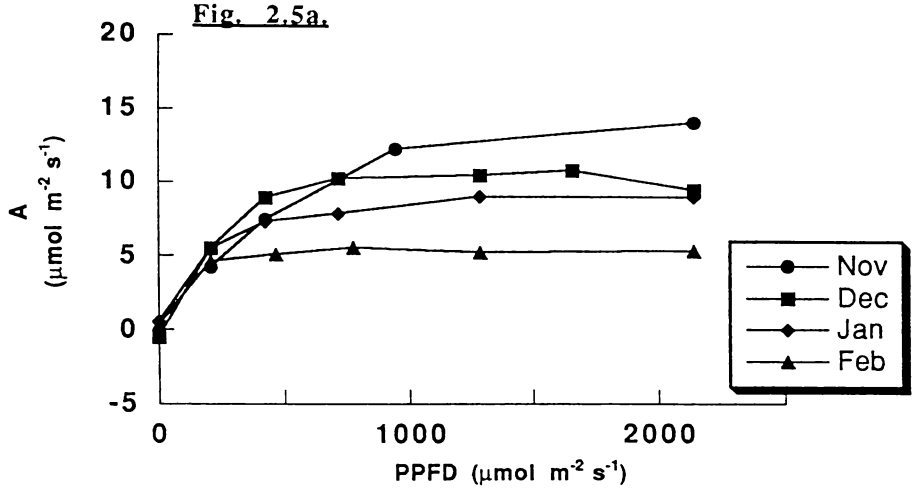


Fig. 2.5b.

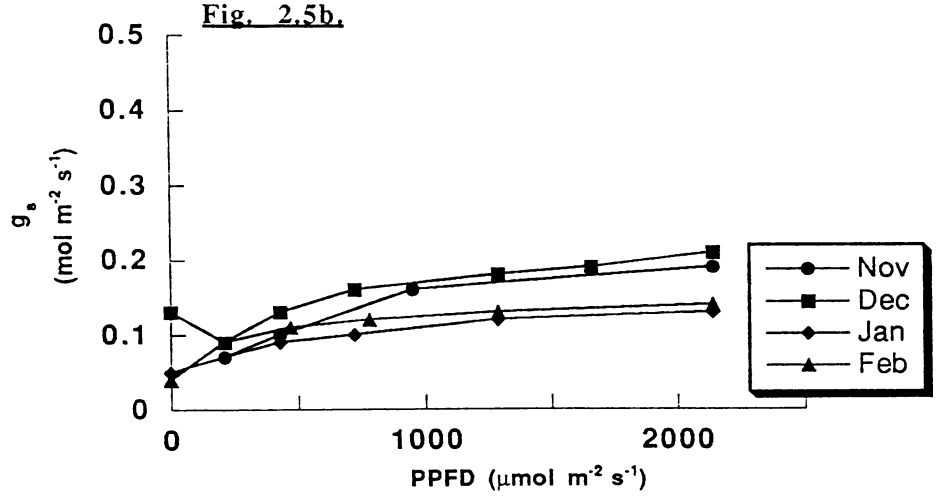


Fig. 2.5c.

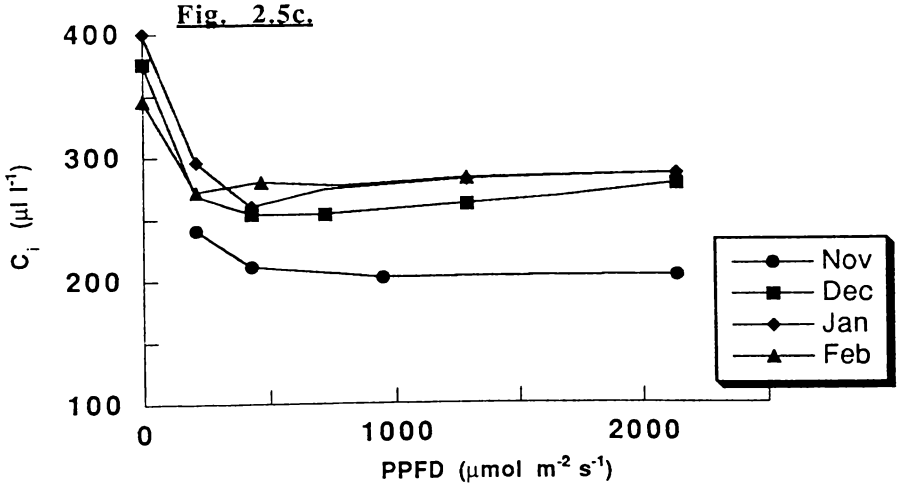


Fig 2.5a, b, c. Changes in the light response curve of a. photosynthesis; b. stomatal conductance; c. internal CO_2 of leaf 1 on the creche tree from November, 1991 to February, 1992.

Fig. 2.6a.

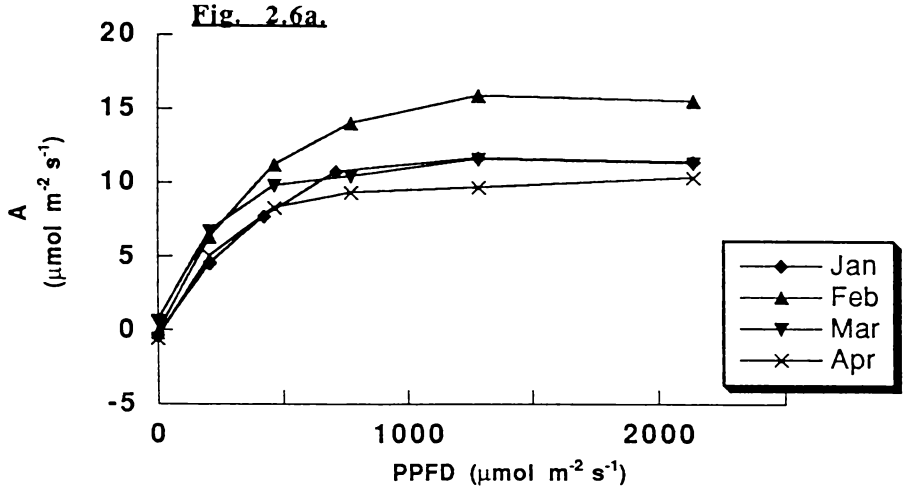


Fig. 2.6b.

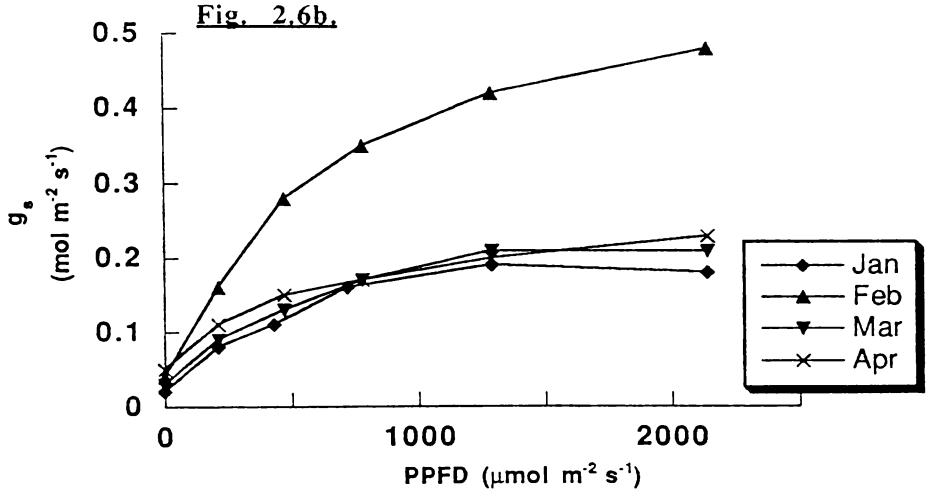


Fig. 2.6c.

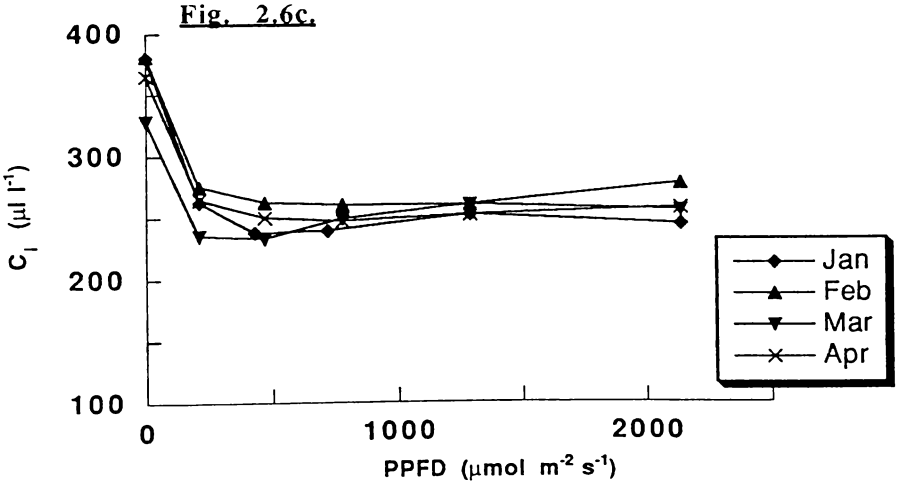


Fig 2.6a, b, c. Changes in the light response curve of a. photosynthesis; b. stomatal conductance; c. internal CO_2 of leaf 3 on the creche tree from January, 1992 to March, 1992.

and two juvenile) meant that the number of leaves able to be measured in all the experiments - light and CO_2 response curves and diel variation - was less than that needed to give full replication. Hence the results presented here are only a preliminary examination of gas exchange in *Paulownia* and have not been subjected to any statistical analysis beyond curve fitting.

Table 2.9. Gas exchange parameters determined from light response curves on leaves of tree 43 and tree 72 as they aged through season 2

Leaf	Month	A_{max} (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	$PPFD_{sat}$ (μmol m^{-2} s^{-1})	WUE (μmol mmol^{-1})	Q_e	r (μmol m^{-2} s^{-1})
Tree 43								
1	Nov	14	0.22	224	1710	3.6	0.024	5
	Dec	15	0.19	184	1300	3.0	0.035	6
	Jan	12	0.32	264	880	2.2	0.042	1
	Feb	10	0.28	268	700	2.2	0.041	1
	Mar	7	0.30	287	690	1.7	0.034	9
2	Dec	16	0.40	255	1360	3.2	0.037	19
	Jan	18	0.36	238	1740	2.9	0.030	8
	Feb	18	0.69	270	1400	2.6	0.040	7
	Mar	15	0.36	251	1020	3.2	0.040	16
3	Jan	13	0.17	190	1450	3.6	0.025	21
	Feb	18	0.77	278	2060	2.5	0.027	16
	Mar	14	0.41	262	1160	2.6	0.035	3
Tree 72								
1	Nov	13	0.18	210	1240	3.4	0.036	51
	Dec	11	0.23	235	1170	2.7	0.031	11
	Jan	8	0.18	253	500	2.5	0.051	4
2	Jan	10	0.16	222	1130	3.0	0.031	37
	Feb	18	0.49	255	1390	3.0	0.040	13
	Mar	12	0.34	262	750	2.5	0.048	2

A). *P. tomentosa* trees

The trends in the gas exchange parameters observed for leaves on both trees 43 and 72 were similar, with the highest values of A_{max} occurring approximately one month after the leaf was considered to be fully expanded (Table 2.9). A leaf was considered to be the first fully expanded leaf on the branch under study if the length and breadth dimensions were indistinguishable from the next oldest leaf. Although the size of these leaves was similar to older leaves, the texture was often softer and hairier, with older leaves becoming increasingly more brittle.

The responses of A, g_s and C_i to all light levels studied are shown in Fig. 2.3 for leaf 1 of tree 43 as it aged from November, 1991 to March, 1992, while Fig 2.4 shows the same parameters for leaf 2 of tree 72 as it aged. Photosynthetic rates at low light levels (below 500 μmol m^{-2} s^{-1}) varied less with leaf age than rates at higher light levels.

Stomatal conductance tended to increase as light levels increased, but showed less of a trend with increasing leaf age than A_{max}. There was little correlation between A_{max} and g_s and the relationship varied between leaves, which led to wide variation in C_i between leaves and within the same leaf at different times of measurement. WUE tended to decrease as the leaves aged.

The light level at which photosynthesis was saturated ($PPFD_{sat}$) was highest when A_{max} was greatest for all leaves except leaf 1 of tree 43. Light levels of 1000 μmol m^{-2} s^{-1} or more were needed to saturate photosynthesis in those leaves which were still maturing or had just become fully mature. The light compensation point (r) varied between leaves, but was generally highest in each leaf at the time of the first measurement. Q_e tended to increase as the leaves aged showing that the older leaves were able to photosynthesise more rapidly when light levels were low, than were the younger leaves.

Analysis of the rate of dark respiration by the curve fitting technique was not very accurate and so individual values for R_d are not given. Generally the rate of respiration was less than 1 μmol m^{-2} s^{-1}, but the rate was often higher in younger leaves, particularly on tree 72. Rates decreased as the leaves matured, as has been observed in several species *e.g. Acacia* (Pathre *et al.*, 1990), poplar (Ceulemans and Impens, 1979) and coffee (Yamaguchi and Friend, 1979). At the very end of the life of a leaf, R_d sometimes increased as a result of the senescence process and this was observed in leaf 2 of tree 43.

Comparisons of other leaf parameters between leaves of different ages were made on one occasion, with leaves of tree 72. The younger leaves had higher rates of A, higher foliar ntirogen levels and higher stomatal density than the older leaves. However, the chlorophyll

content of the older leaves was higher.

B). *P. elongata* tree

Comparison of the results measured on the leaves of the *P. elongata* tree (Table 2.10) with those of the *P. tomentosa* trees discussed above, showed similar trends occurring in the mature tree. In leaves 3 and 4, the values of A and g_s were highest one month after full leaf expansion, at all light levels (for leaf 3 data see Fig 2.6). This was not observed in leaves 1 and 2, with g_s increasing independently of A at the February measurement in leaf 2, while leaf

Table 2.10. Gas exchange parameters determined from light response curves on leaves of the *P. elongata* tree as they aged through season 2.

Leaf	Month	A_{max} (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	C_i (μl l^{-1})	$PPFD_{sat}$ (μmol m^{-2} s^{-1})	WUE (μmol mmol^{-1})	Q_e	r (μmol m^{-2} s^{-1})
1	Nov	14	0.19	199	1620	3.8	0.028	24
	Dec	10	0.19	252	700	4.8	0.048	13
	Jan	9	0.13	210	790	3.4	0.030	19
	Feb	5	0.14	265	340	2.0	0.043	7
2	Dec	12	0.18	222	1120	4.3	0.039	60
	Jan	13	0.22	218	940	3.0	0.043	7
	Feb	13	0.45	275	750	2.3	0.052	8
	Mar	12	0.24	253	810	3.2	0.045	3
	Apr	3	0.05	245	110	3.2	0.104	9
3	Jan	12	0.19	218	1220	3.0	0.031	15
	Feb	16	0.42	263	1190	3.1	0.042	9
	Mar	11	0.21	231	770	3.1	0.041	14
	Apr	10	0.23	253	870	3.3	0.038	17
4	Feb	12	0.19	220	1420	3.2	0.026	6
	Mar	17	0.33	229	1080	3.8	0.046	3
	Apr	11	0.21	233	820	3.0	0.036	14

1 showed full physiological maturity at the first time of measurement with the highest photosynthetic rate recorded then. The internal CO_2 levels were lowest when the leaves were youngest as tended to be the case in the *P. tomentosa* leaves, with this effect quite pronounced in leaf 1 at all light levels (Fig. 2.5c). The values of $PPFD_{sat}$, WUE and R_d all tended to decrease with increasing leaf age, while Q_e increased as the leaves aged. Light compensation point varied independently of leaf age. There were many exceptions to these generalisations, however, as the overall trends were less clear in the leaves of this tree than in the leaves of the *P. tomentosa* trees.

It is interesting to note that for many of the variables measured, particularly A_{max}, g_s, C_i and $PPFD_{sat}$, the average values were higher for the trees at Matangi than for leaves of the *P. elongata* tree. However, there was considerable overlap between the data sets. Comparison of tree 43 with tree 72, for leaves of the same maturity at the same point in the season, generally showed that rates of A_{max}, g_s and $PPFD_{sat}$ were higher in tree 43 than in tree 72.

2.4.3.3 Change in A_{max} through the season

A). A_{max} in first fully expanded leaves

Comparisons were made of leaves at the same point of maturity, but produced at different times during the season. Because of the difficulty in precisely assessing the point at which a leaf became physiologically mature, and the fact that measurements were made on a monthly basis rather than more frequently, the values tabulated in Tables 2.11 and 2.12 were for first fully expanded leaves measured each month. Thus the rates presented are not the maximum rates attained by the leaves. The leaves used in these tables were not always the same leaves as those tabulated above, nor presented in Figs. 2.7 and 2.8. Branch expansion ceased in mid February in the *P. tomentosa* trees so there were no new leaves produced after this time. Production of leaves continued for longer in the *P. elongata* tree, hence the inclusion of a new leaf at the time of the March measurement.

Analysis of the rate of photosynthesis on the leaves produced at different times showed little change in all trees from November to January (Tables 2.11 and 2.12). In February, A_{max} increased markedly in tree 43 and to much lesser extent in tree 72 and one of the examples in the creche tree. Values of g_s also increased in the same leaves, as did $PPFD_{sat}$ in the *P. tomentosa* leaves, but other gas exchange parameters changed little.

Table 2.11. Gas exchange parameters on first fully expanded leaves of trees 43 and 72 over season 2.

Month	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	C_i ($\mu l\ l^{-1}$)	$PPFD_{sat}$ ($\mu mol\ m^{-2}\ s^{-1}$)	WUE ($\mu mol\ mmol^{-1}$)	Q_e	r ($\mu mol\ m^{-2}\ s^{-1}$)
Tree 43							
Nov	14	0.22	224	1712	3.6	0.024	5
Dec	13	0.38	263	1187	2.7	0.038	43
Jan	15	0.28	231	1555	2.9	0.030	16
Feb	22	0.71	263	2384	3.2	0.028	3
Tree 72							
Nov	13	0.18	210	1241	3.4	0.036	51
Jan	10	0.16	222	1125	3.0	0.031	37
Feb	15	0.43	267	1575	2.6	0.030	27

Table 2.12. Gas exchange parameters on first fully expanded leaves of *P. elongata* tree over season 2.

Month	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	C_i ($\mu l\ l^{-1}$)	$PPFD_{sat}$ ($\mu mol\ m^{-2}\ s^{-1}$)	WUE ($\mu mol\ mmol^{-1}$)	Q_e	r ($\mu mol\ m^{-2}\ s^{-1}$)
Nov	14	0.19	199	1616	3.8	0.028	24
Dec	12	0.18	222	1122	4.3	0.039	60
	13	0.23	242	1217	5.3	0.038	57
Jan	12	0.19	218	1224	3.0	0.031	15
	13	0.21	220	1112	3.3	0.035	3
Feb	16	0.38	253	1417	3.1	0.034	15
	12	0.19	220	1421	3.2	0.026	6
Mar	13	0.20	204	968	4.2	0.041	4

B). Seasonal trends in leaves of all ages

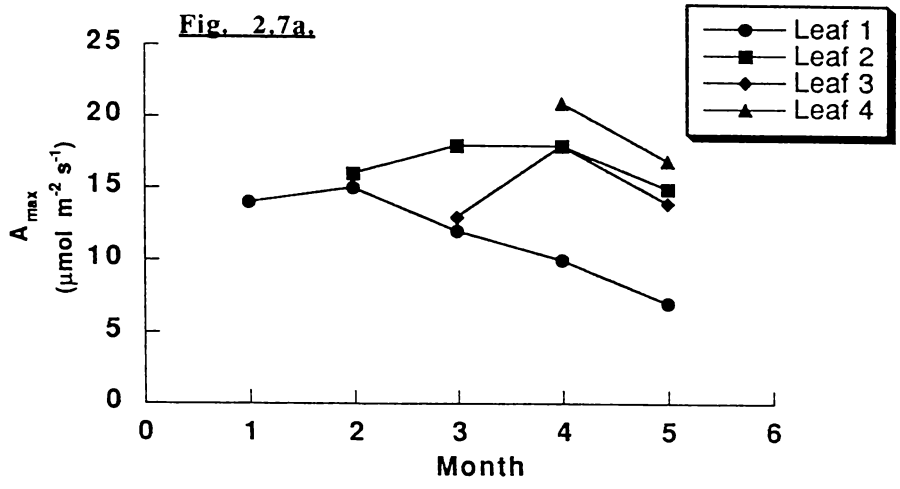
When comparisons were made over all leaf ages on the three trees it could be more clearly seen that leaves initiated later in the season had higher rates of photosynthesis and stomatal conductance, under conditions of full light, than those initiated earlier (Figs. 2.7 and 2.8). For example, leaves 2 and 3 of tree 43 had higher values for A_{max} and g_s when fully mature (second month of measuring) than leaf 1 at the same stage of maturity. In tree 72, rates of A_{max} and g_s in the second month of measurement were much higher in leaf 2 than leaf 1 at any stage, while leaves 3 and 4 from the creche tree had higher rates of gas exchange when fully mature than leaves 1 and 2. In all trees the highest values of g_s were obtained in February (month 4), and in trees 43 and 72 the same was true for A_{max}.

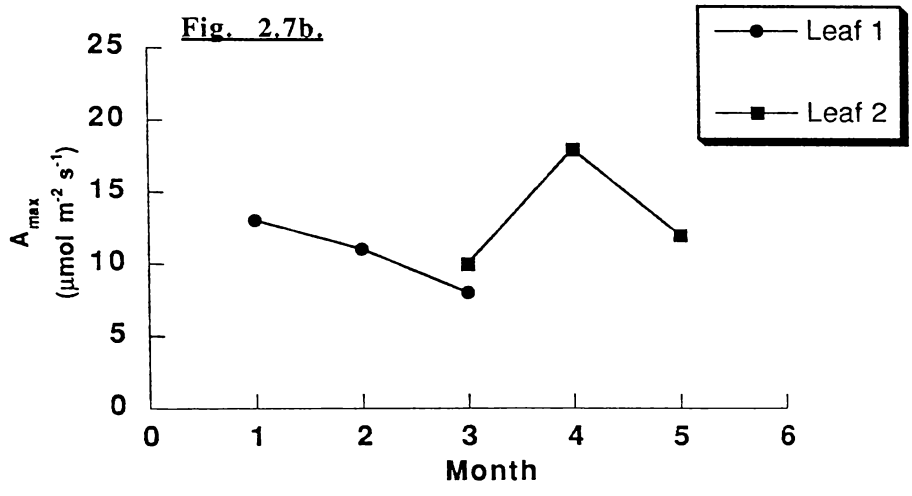
These gas exchange results were correlated with various leaf parameters measured at the same time, on first fully mature leaves (Table 2.13). In leaves of *P. tomentosa*, leaf nitrogen and chlorophyll content and stomatal density were highest in February. Chlorophyll levels changed little between the leaves measured at other times, but nitrogen levels and numbers of stomata gradually increased through the season, with %N decreasing in leaves measured in March. Thus there appeared to be a strong relationship between leaf structural characteristics and gas exchange.

Table 2.13. Leaf variables measured on first fully mature leaves for all trees, over season 2. Standard errors are given in parentheses.

Month	*P. tomentosa*			*P. elongata*		
	N (% DW)	Chlorophyll ($g\ m^{-2}$)	Stomatal Number (mm^{-2})	N (% DW)	Chlorophyll ($g\ m^{-2}$)	Stomatal Number (mm^{-2})
Nov	-[1]	0.33 (0.008)	602 (16.6)	-	0.34 (0.018)	407 (11.5)
Dec	1.90 (0.080)	0.34 (0.003)	-	2.33 (0.005)	0.42 (0.013)	-
Jan	2.16 (0.070)	0.34 (0.006)	615 (22.0)	2.18 (0.119)	0.54 (0.015)	507 (12.2)
Feb	2.34 (0.094)	0.49 (0.016)	715 (17.9)	2.10 (0.029)	0.53 (0.015)	608 (15.3)
Mar	2.05 (0.115)	0.36 (0.014)	-	1.96 (0.061)	0.49 (0.016)	-
Apr	-	-	-	1.41 (0.032)	0.35 (0.007)	-

[1] - not determined





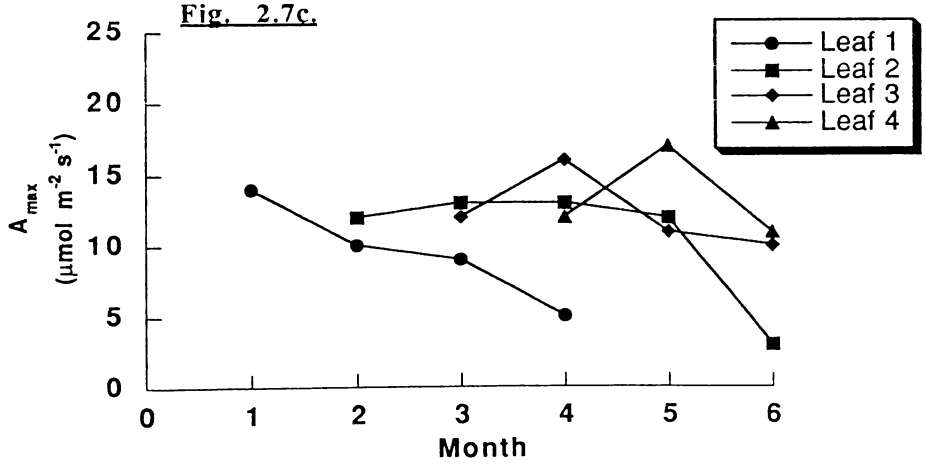
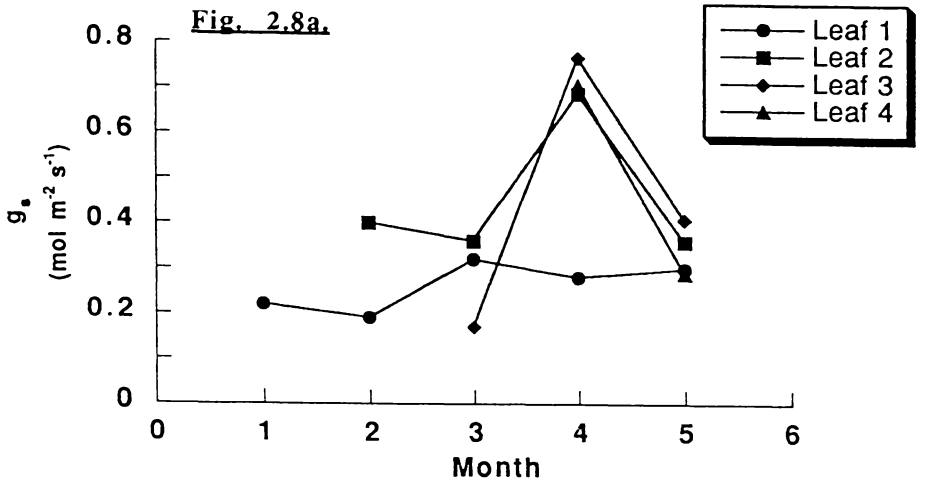
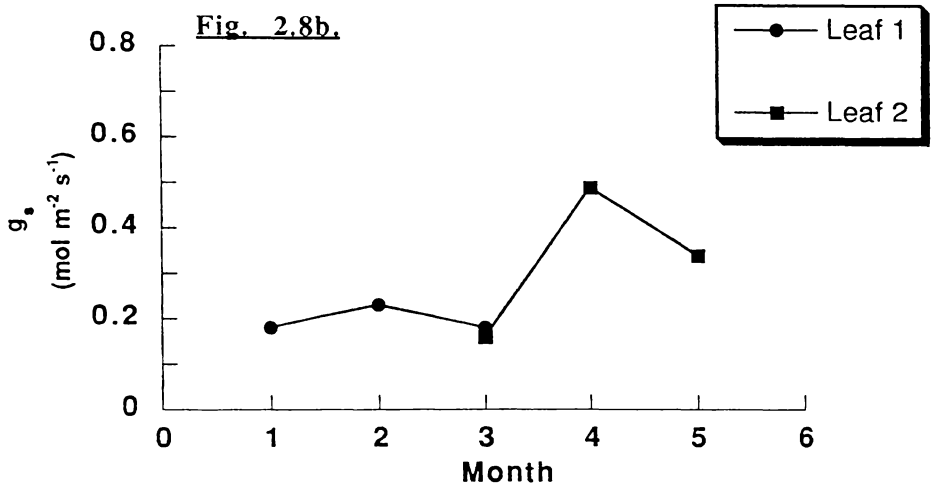


Fig 2.7a, b, c. Photosynthetic rate, at full light, of leaves of a. tree 43; b. tree 72; c. creche tree, produced at different times through season 2. Month 1: Nov, 1991; 2: Dec, 1991; 3: Jan, 1992; 4: Feb, 1992; 5: Mar, 1992; 6: Apr, 1992.





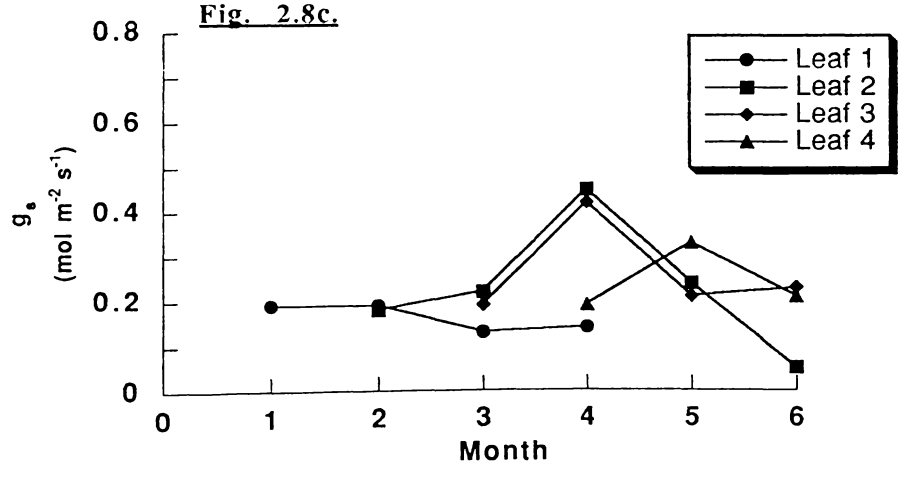


Fig 2.8a, b, c. Stomatal conductance, at full light, of leaves of a. tree 43; b. tree 72; c. creche tree, produced at different times through season 2. Month 1: Nov, 1991; 2: Dec, 1991; 3: Jan, 1992; 4: Feb, 1992; 5: Mar, 1992; 6: Apr, 1992.

The seasonal pattern of leaf parameters from the creche tree were less obvious. Stomatal density increased from November to February, but was always lower for these leaves than those from Matangi. The nitrogen content decreased through the season, while chlorophyll content increased until January, and then began to decrease again in March. The nitrogen and chlorophyll contents were similar in these leaves to the levels in the *P. tomentosa* leaves. Taking into account the observation that the seasonal change in gas exchange parameters of first fully expanded leaves was less obvious in leaves of the *P. elongata* tree than those of the *P. tomentosa* trees (Table 2.11 and 2.12), it is perhaps not surprising that the leaf characteristics showed less seasonality in the creche tree.

2.4.3.4 Equilibration period required during response curve measurements

During the measurement of both light and CO_2 response curves, the rate at which the experiments could be completed was limited by the time needed for the value of stomatal conductance to stabilize after the change in either light or CO_2 level. This lag was observed in light response curves regardless of the direction of the light change. In many instances the change in light or CO_2 caused an immediate increase in g_s, followed by a slow decline and then a gradual increase again to a stable level. This process generally took at least 20 minutes, and often up to 30 minutes, but there were times when a stable reading was not achieved. The value was considered to be stable when g_s remained relatively constant for 3 - 5 minutes. Although a similar pattern was often observed for A, the lag time was much shorter and did not limit the experimental process.

It appears that this equilibration period required before rates of A and g_s stabilize varies between species. It is not significant in either kiwifruit (J. Meekings, pers. comm.) or in nashi pears (L. La Franchie, pers. comm.), nor in *Populus tremuloides* and *P. fremontii* when undertaking a light response curve with a number of steps of decreasing light (Roden and Pearcy, 1993). However Turnbull (1991) reported that an equilibration time of 15 - 25 minutes was required at each light change in seedlings of rainforest trees and in Norway spruce the process took 15 minutes (Wallin, Skarby and Sellden, 1992). In a series of experiments studying sunflecks on two rainforest species which had been kept at very low light levels, Chazdon and Pearcy (1986) found that between 26 and 48 minutes were needed before steady-state rates of gas exchange were reached. In both species, the increase in g_s with increased light lagged behind increases in A. About 20 minutes were needed for g_s to stabilize in the two species of poplar mentioned above when light levels increased from 20 to 1700 $\mu mol\ m^{-2}\ s^{-1}$, but rates of A were stable within 8 minutes (Roden and Pearcy, 1993). Environmental factors influence the equilibration time required by leaves to some extent, with

sun leaves of *Piper spp.* equilibrating more quickly than shade leaves, while VPD affected the rate attained at equilibrium (Tinoco-Ojanguren and Pearcy, 1993).

2.4.4 Diel experiments

2.4.4.1 Comparison of gas exchange rates in the morning and afternoon

The data collected in each diel experiment from dawn until approximately midday was compared with that collected in the afternoon to determine if there was any evidence of the midday or afternoon depression of gas exchange rates in these *Paulownia* trees as found in other species (section 2.1.6). There was no observable difference in rates between the morning and afternoon periods in leaves on the creche tree in November, December, January and February in season 2 (Fig. 2.10b). However in March and April there was evidence of a slight decline in rates in the afternoon (Table 2.14).

Table 2.14. Maximum rates of gas exchange parameters, under saturating light, on selected leaves of *P. elongata* (creche tree) and *P. tomentosa* (trees 43 and 72) measured during diel experiments in seasons 2 and 3.

Tree	Date	Time	A (μmol m^{-2} s^{-1})	g_s (μmol m^{-2} s^{-1})	E (μmol m^{-2} s^{-1})	C_i (μl l^{-1})	VPD (kPa)
Creche	Nov 91	pm[1]	11	0.5	3	270	1.0
Creche	Mar 92	am	17	0.3	3	260	1.8
		pm	13	0.2	3	260	1.7
43	Jan 92	am	16	0.6	6	270	-
		pm	14	0.5	6	270	-
43	Feb 92	am	17	0.5	5	270	1.5
		pm	14	0.4	6	270	2.0
72	Nov 91	am	12	0.3	3	260	1.2
		pm	11	0.3	4	250	2.0
72	Jan 92	am	13	0.2	3	230	1.8
		pm	12	0.2	3	230	2.0
72	Dec 92	pm[1]	16	0.3	5	250	1.9

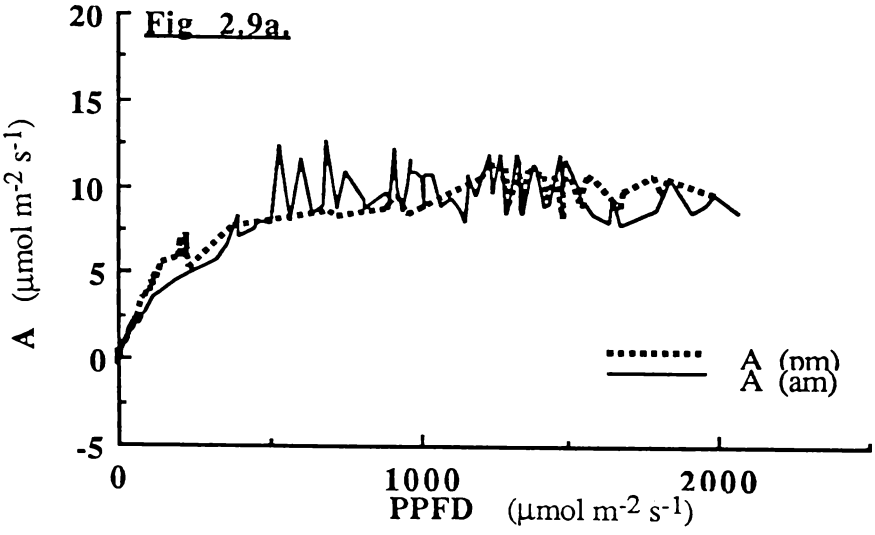
[1]Morning data is not available because light levels were not saturating.

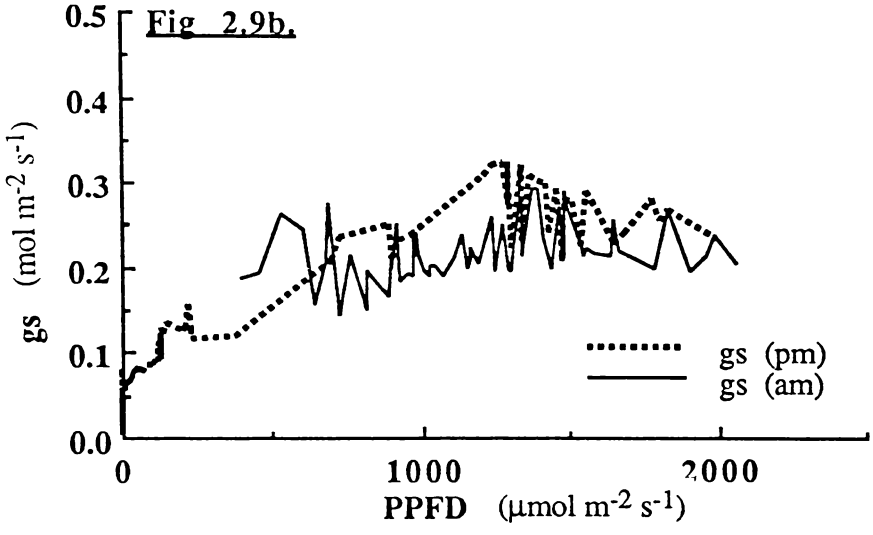
The situation was very similar for trees 43 and 72 in season 2, with tree 72 showing no difference in rates throughout the entire season (Table 2.14, Fig 2.9) while afternoon rates did show a slight decrease in some leaves of tree 43 in January, February and March. The trends in A and g_s were similar to one another, while C_i tended to remain relatively constant throughout the day (Table 2.14, Fig 2.9). The decline in A_{max} and g_s was generally less than 20% and there were many data points common to both data sets (Fig 2.10a). Overall, the trend of an afternoon depression in gas exchange rates in season 2 was weak.

These results correspond with those gained in the other analyses carried out on these trees in season 2. Measurement of light and CO_2 response curves were undertaken right throughout the day and there were few indications during those experiments of afternoon depression, with some very high rates of A and g_s recorded during the afternoon. In February, for example, leaf 2 of tree 72 (Table 2.9) had a photosynthetic rate of 18 μmol m^{-2} s^{-1}, with a g_s of 0.49 mol m^{-2} s^{-1}, despite the mid to late afternoon timing of the experiment. It is possible that results from some afternoon analyses are lower than if analysis had been completed in the morning, but the overall effect on the interpretation of the data presented here is minimal.

Although fewer diel experiments were conducted on trees 43 and 72 in season 3, it was clear from these and from the results of the other experiments undertaken during the season that the situation was very different from that described above, for season 2. Gas exchange rates tended to be highest in mid-morning (9 - 11am) and started to decrease before midday. The difference between photosynthetic rates in the morning and in the afternoon was greater than in the previous season. This decline was reflected not only in the decrease in rates at high light, but also in the lower gradient of the initial slope of the light response curve (Fig 2.10c). The lower rates of A at high light levels in Fig 2.10c are primarily a result of increased leaf temperature (discussed below, section 2.4.4.3).

The depression in gas exchange rates does not appear to be directly related to the water status of the plant. Water potentials of leaves measured throughout the day at the creche were lower earlier in the season when there was no evidence of afternoon depression (Table 2.15). In March, 1992, ψ_p at the creche did not change throughout the day, despite the change in gas exchange rates. Although the ψ_p of leaves from *P. tomentosa* trees at Matangi (season 3) were slightly lower, on average, than those from the *P. elongata* tree (season 2) and decreased from mid-morning, it is unlikely that the values measured were low enough to exert a significant influence on gas exchange rates. In comparison with leaf water potentials in other species, the values measured for *Paulownia* were high. In grapes for example, a 50% decrease in A through the day was correlated with a decrease in ψ_p from -0.9 MPa to -1.3 MPa (Downton *et al.*, 1987), while in *Quercus suber* the midday depression was





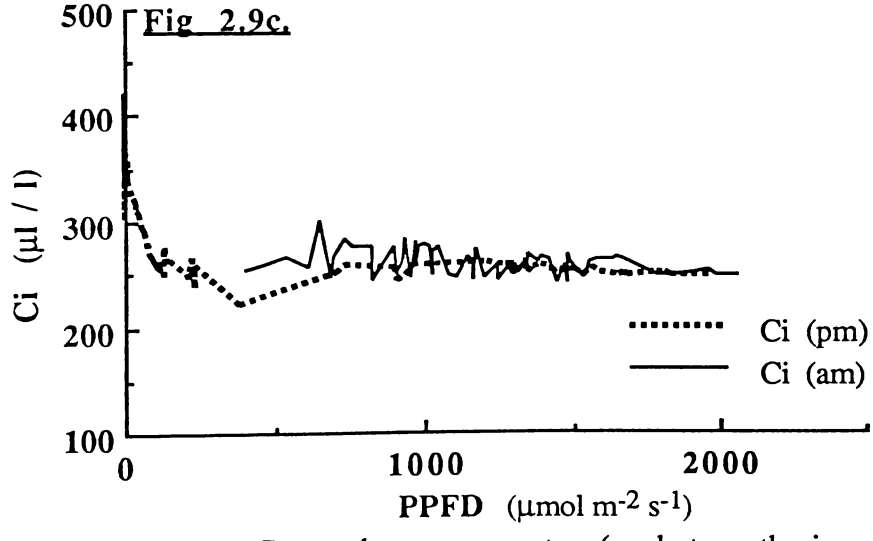
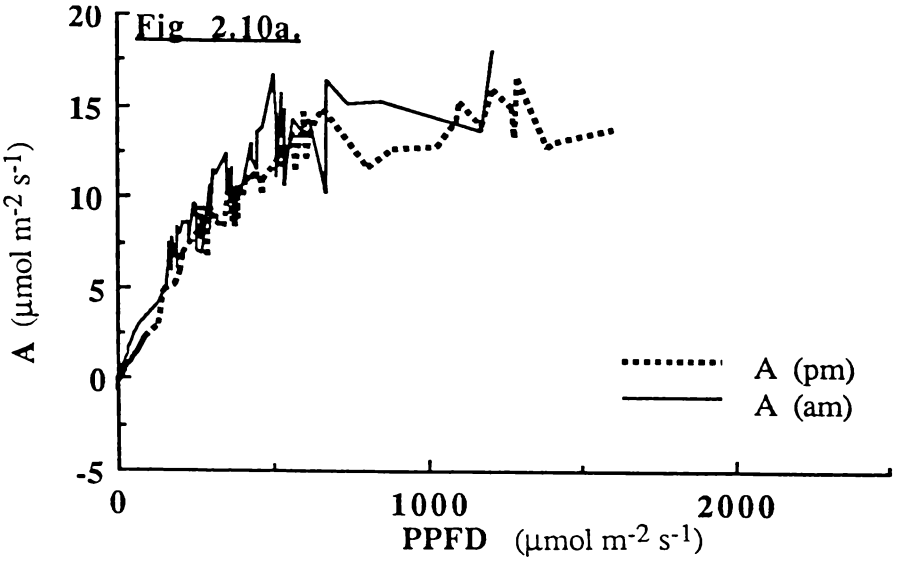
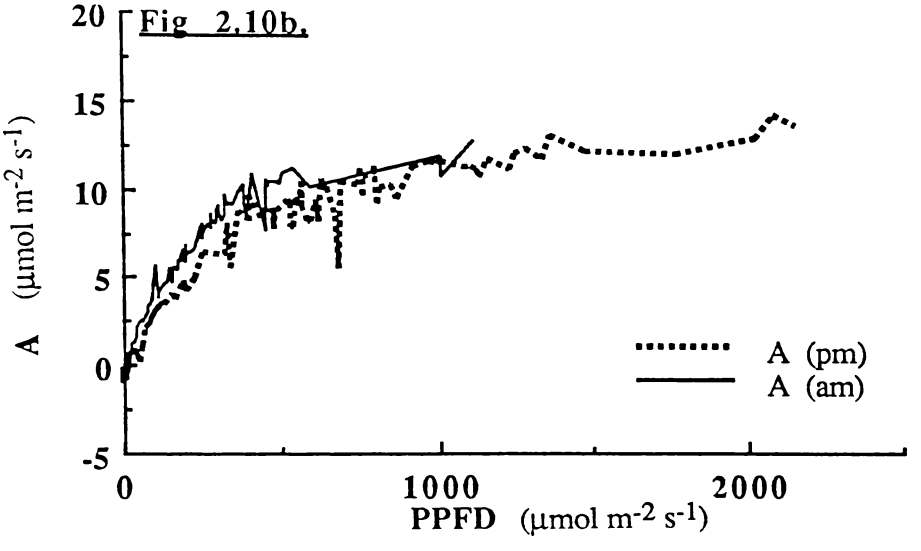


Fig 2.9a, b, c. Gas exchange parameters (a. photosynthesis; b. stomatal conductance; c. internal carbon dioxide concentration) of a leaf of tree 72 in November, 1991, measured in the morning or afternoon of a diel experiment.





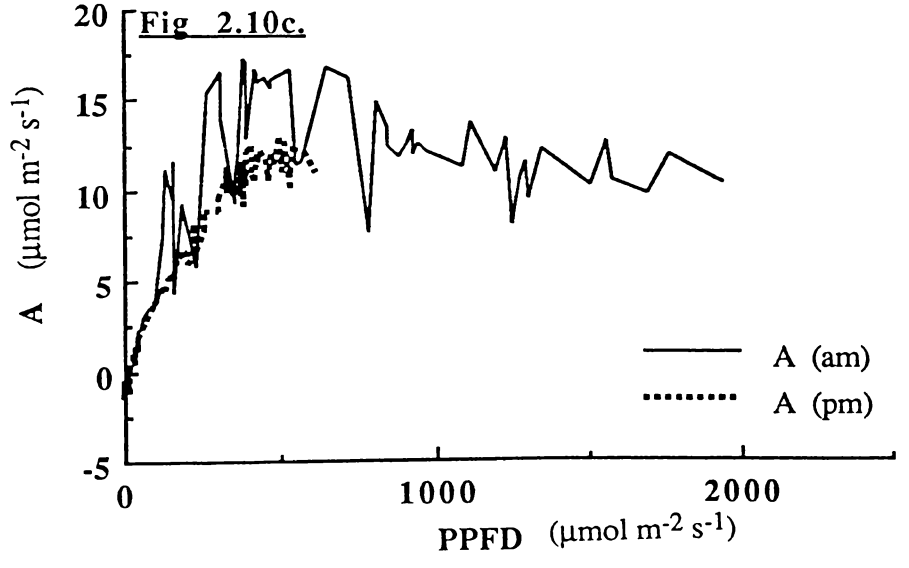


Fig 2.10a, b, c. Change in photosynthetic rate with light, measured in the morning and afternoon of diel experiments on leaves of a. tree 43, February, 1992; b. creche tree, November 1991; c. tree 72 January 1993.

Table 2.15. Leaf water potentials, measured with a pressure bomb, at the creche in season 2 and at Matangi in season 3.

Site	Date	ψ_p (-MPa)				
		Dawn	Mid-morning	Midday	Mid-afternoon	Late afternoon
Creche	Nov	0.25	0.50	0.46	0.37	0.30
	Jan	0.21	0.21	0.46	0.37	0.31
	Feb	0.17	0.26	-[1]	-	-
	Mar	0.12	0.18	0.18	0.17	0.22
Matangi	Jan	-	0.35	0.48	0.47	0.51
	Feb	-	0.33	0.46	0.55	-

[1]not determined

associated with a decrease in ψ_p from -2.6 MPa to -3.5 MPa (Tenhunen *et al.*, 1984).

Further evidence that the plants were not responding to a water stress effect with a decrease in gas exchange in the afternoons is given by the change in the rates of transpiration. E did not decrease as g_s decreased but either remained the same throughout the day or increased slightly with the increase in VPD (Table 2.14). There was little correlation between VPD and g_s, but higher vapour pressure deficits caused an increase in E by increasing the gradient between the interior and exterior of the leaf.

From the data collected it is not possible to ascertain if assimilate feedback inhibition was occurring (section 2.1.6). However, leaves measured on tree 43 and the creche tree later in season 2 had higher rates of A_{max} in the morning than rates measured at any time of the day in leaves analysed earlier in the season, while late season afternoon rates were similar to those found early in the season (Table 2.14). Tree 72 showed no evidence of an afternoon depression, but the highest photosynthetic rates measured at any time of day throughout the season were lower than the morning rates of the two remaining trees later in the season. From these results it could be suggested that rates of photosynthesis above 15 - 16 μmol m^{-2} s^{-1} during the first hours of the day did cause an inhibition of photosynthesis later in the day, whether by feedback inhibition or by another mechanism. As feedback inhibition is also related to sink strength, it is possible that the increased incidence of afternoon depression of gas exchange rates in season 3 was related to the lower growth rates of the trees and thus

lower sink strength, compared with the situation in season 2.

2.4.4.2 Gas exchange over 24 hours

The rates of gas exchange over each diel time period conformed to the expected pattern discussed earlier (section 2.1.6). In the absence of light, CO_2 was released from the leaf as a consequence of dark respiration. Stomatal conductance was very low at night with values ranging from 0.01 to 0.1 mol m^{-2} s^{-1}. As the light intensity and air temperature increased at the beginning of the day, values of the various gas exchanges parameters increased, with fluctuations occurring over the day primarily as a response to light (Fig. 2.11).

Comparison of photosynthetic rates on a day with periods of high light with rates on a cloudy day when the light level peaked at about 800 µmol m^{-2} s^{-1} showed higher maximum rates on the sunny day (Fig. 2.12). There was a very strong relationship between A and PPFD, with the fluctuations in light levels on the sunny day causing large changes in A. However, mathematical analysis of the area under the graph would probably find little difference between the total carbon fixed per day with an average rate of assimilation of about 10 µmol m^{-2} s^{-1} maintained for nine hours on the cloudy day and a slightly higher rate averaged over a shorter time on the sunny day. These results suggest that significant net assimilation of carbon can occur even on days when light levels are limiting to photosynthesis.

The values for the remaining parameters were also similar to those found in the response curve experiments. Thus A_{max} was generally between 10 and 19 µmol m^{-2} s^{-1}, with values for g_s up to 0.7 mol m^{-2} s^{-1}. The range of internal CO_2 concentrations and water use efficiencies was the same as in the other experiments. The differences between the trees in each experiment were consistent *e.g.* the creche tree generally had lower values of $PPFD_{sat}$ in the light response curves (Table 2.10) and this can be observed in the diel data (Fig. 2.10b). The trend of tree 72 and the creche tree having lower gas exchange rates than tree 43 in season 2 was observed across all experiments.

The seasonal influence on rates found with the light response curves (section 2.4.3.3) was less obvious in the diel data. The values for A_{max} and g_s were lowest on tree 43, in season 2, in November at 13 µmol m^{-2} s^{-1} and 0.4 mol m^{-2} s^{-1} respectively, but were similar for the remaining months with A_{max} ranging from 17 - 20 µmol m^{-2} s^{-1}, and g_s from 0.4 - 0.7 mol m^{-2} s^{-1}. The range of maximum values was less for tree 72 and the creche tree, with the highest rates found in February for tree 72 (A_{max} - 15 µmol m^{-2} s^{-1}, gs - 0.6 mol m^{-2} s^{-1}) and in March for the creche tree (A_{max} - 17 µmol m^{-2} s^{-1}, g_s - 0.3 mol m^{-2} s^{-1}). There were insufficient data to clarify the seasonal trends in season 3.

Fig 2.11a.

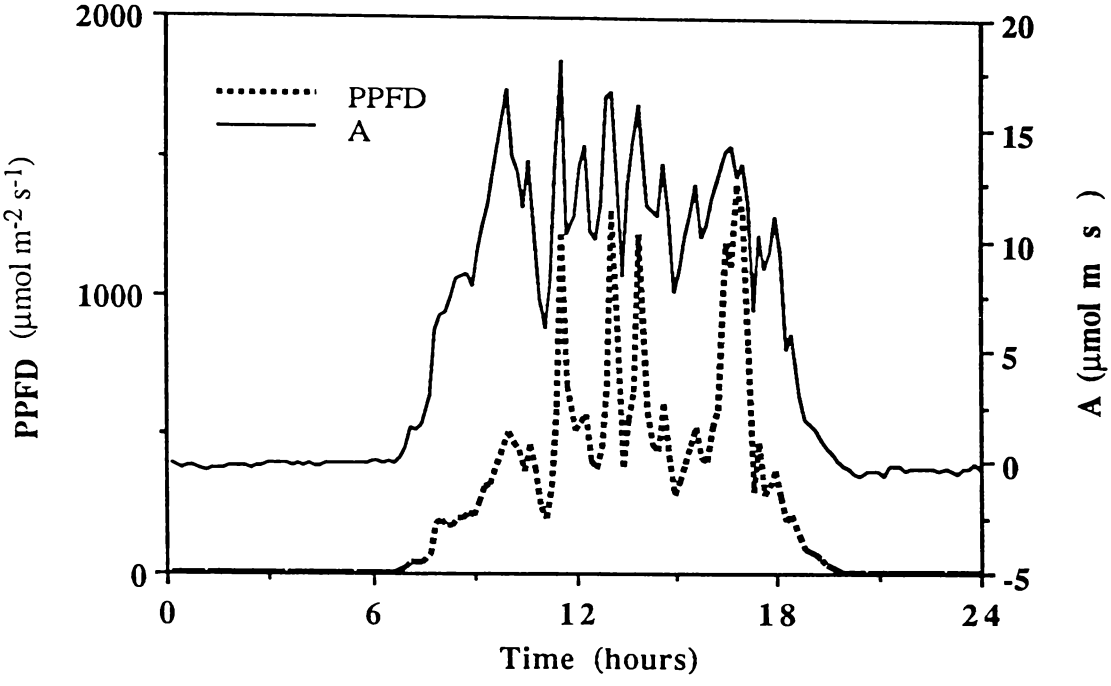


Fig 2.11b.

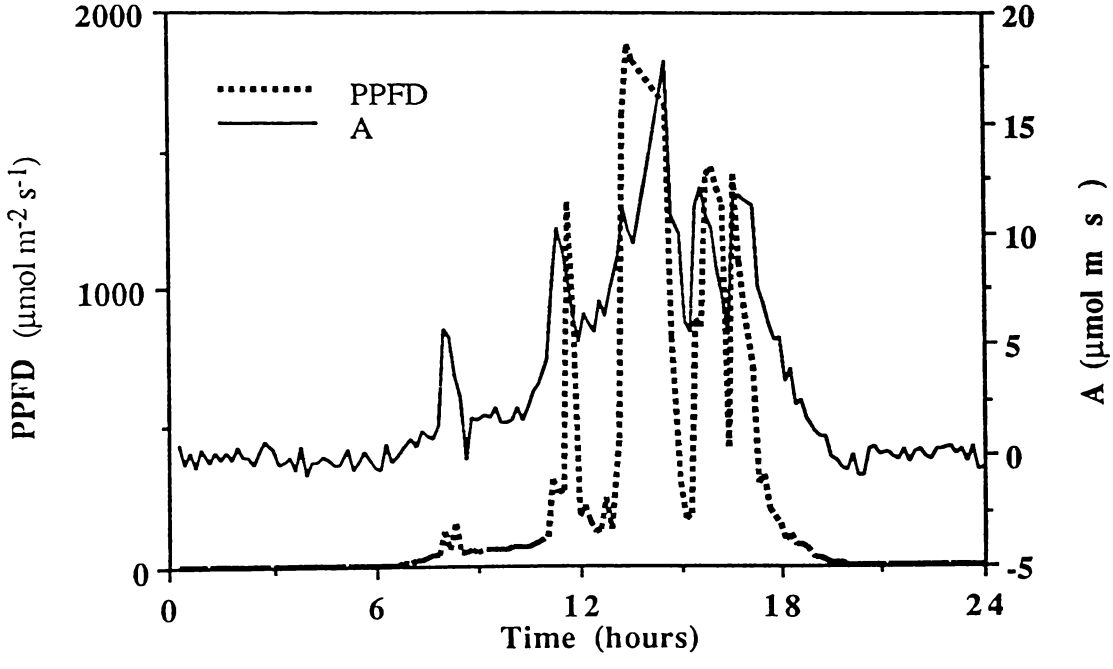
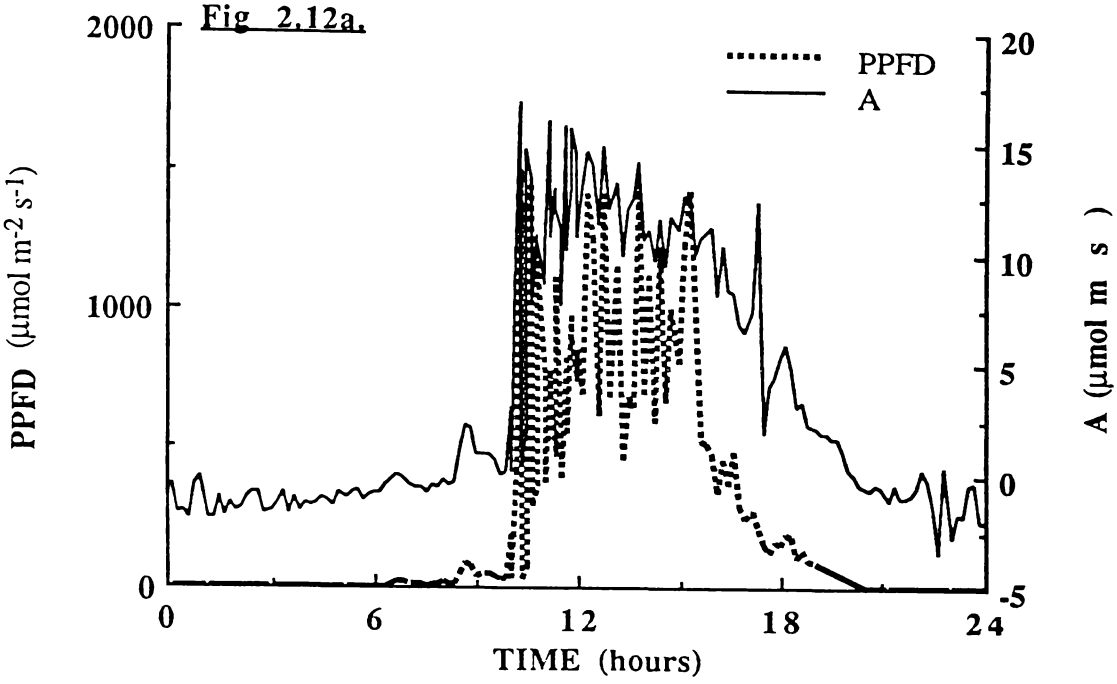


Fig 2.11a, b. Diel variation in PPFD and photosynthesis, from midnight to midnight, in a. leaf on tree 43 in February, 1992; b. leaf on the creche tree in February , 1992.



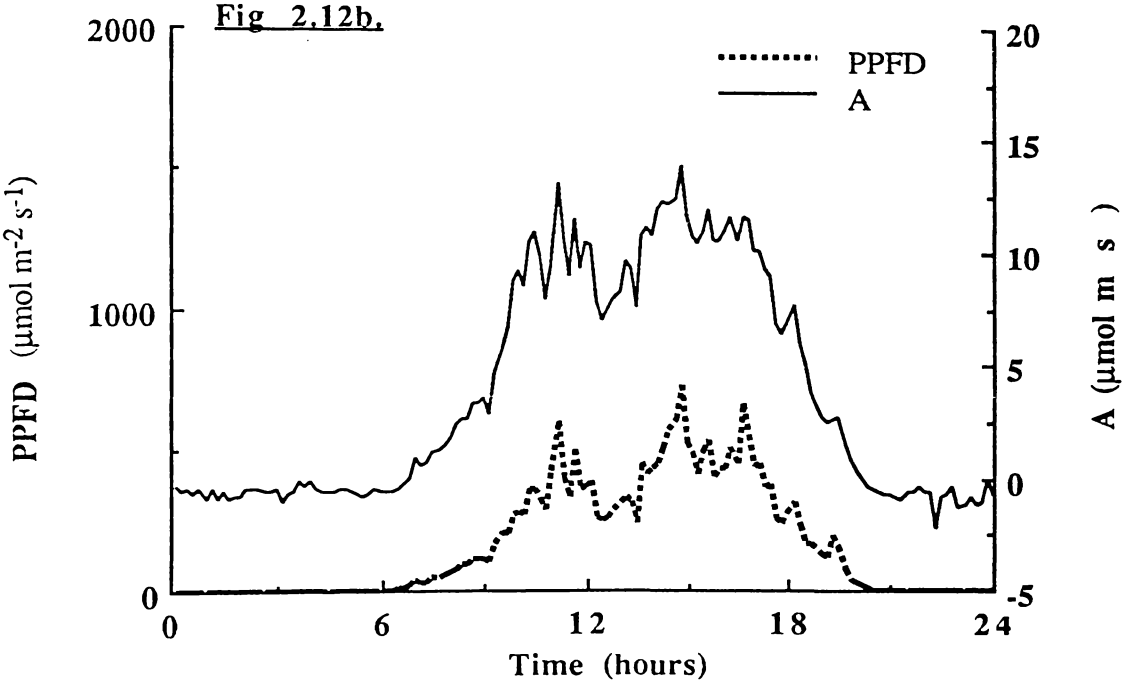


Fig 2.12a, b. Diel variation in PPFD and photosynthesis, from midnight to midnight, in a leaf on tree 43 in January, 1992 on a. a sunny day; b. a cloudy day.

The rates of leaf respiration estimated for all the trees in both seasons were low in comparison with the rates of CO_2 fixation. Respiration in the two *P. tomentosa* trees in season 2 ranged from 0.2 - 0.6 μmol m^{-2} s^{-1} and up to 0.8 μmol m^{-2} s^{-1} in the third season, while the creche tree had R_d rates of 0.4 - 0.7 μmol m^{-2} s^{-1}. This range of values is the same as that determined by the light response curves. These rates are comparable with those measured on kiwifruit (0.83 μmol m^{-2} s^{-1}) using the same experimental design (Buwalda *et al.*, 1991).

2.4.4.3 Limitations in the data from the diel experiments

The results from the diel experiments, presented above, are representative of the data, but are not replicates. Several problems were encountered in the process of obtaining the data, with the first limitation being imposed by the weather. In order to collect a good set of data of diel gas exchange under optimal environmental conditions, it was necessary to have reasonable light levels for most of the daylight period. This proved to be a difficult task on a number of occasions and some sets of results were not particularly useful as light levels did not rise above 500 μmol m^{-2} s^{-1}. The lack of appropriate weather was compounded in season 2 by the limited availability of the Walz equipment. There were also instances when windy conditions led to leaf damage and prevented data collection as the leaves couldn't be left for any length of time in the cuvette without tearing.

However, on days when the light levels were high a second problem arose. The area of leaf held in the cuvette tended to overheat which led to a significant decrease in gas exchange rates (Fig 2.13). In the first experiments the material used to seal the leaf into the cuvette was black closed cell foam and after exposure to high light for some hours, this tended to scorch the leaves. The situation improved when this was replaced with white foam, but even when the leaves were placed in a cuvette with an open top and the flow of air over the leaf was maximal, overheating was still a problem during some experiments. The temperature of the leaf was often 5 - 6 °C higher than the external temperature. Gas exchange rates tended to decrease when the leaf temperature increased above about 27°C, with a dramatic decrease as the temperature increased above 30°C. This result is believed to be an artifact of the measurement system, to some extent, as in their native habitat *Paulownia* leaves would be exposed to long periods of high light and temperatures between 25°C and 35 °C (Zhu *et al.*, 1986). However, a similar result has been found in the Mediterranean sclerophyll *Quercus suber* which, despite having a temperature optimum for photosynthesis between 30 and 35°C, had markedly reduced rates of photosynthesis at 37°C compared with rates at 29°C (Tenhunen *et al.*, 1984).

The remaining limitations were a result of the set up of the Walz equipment. The differential IRGA was calibrated daily at the ambient CO_2 level at the time (generally between 330 and 360 $\mu l\ l^{-1}$). The sensitivity and zero of the IRGA was stable when the CO_2 level was within $\pm$ 50 ppm of that calibration concentration. Overnight, however, CO_2 concentrations tended to rise and hence influence both the sensitivity and zero shift of the IRGA. Added to this was the slight difference in path length of the measuring and reference gas streams which magnified any sudden changes in the CO_2 levels. Thus the reading from the differential IRGA was quite 'noisy' overnight (Fig 2.14, also Fig 2.11b). The rate of CO_2 emission from the leaves was very low (generally less than 1 $\mu l\ l^{-1}$, but occasionally up to 2.5 $\mu l\ l^{-1}$) and so under fluctuating CO_2 conditions the signal from the plant was lost in the noise of the IRGA. Thus the values for respiration given above are those calculated from data when the CO_2 concentration did not rise significantly overnight (probably because of wind) and are only approximate estimates to give a 'ball park' figure for R_d.

A further problem was encountered with the diel experiments when the temperature at night dropped close to or below the dew point temperature. This caused condensation in the gas lines which seriously affected calculations of g_s and E, but did not affect A (or R_d). As the temperature increased during the morning, the lines dried and usually by mid morning, the values for g_s and E were correct. This is why the morning data graphed in Figs. 2.9b and 2.9c do not include data at very low light levels.

2.4.5 AC_i curves

Changing the ambient CO_2 concentration led to a change in the various gas exchange parameters, with A increasing as C_a and C_i increased, and g_s and E decreasing (Fig. 2.15). The shape of the AC_i curves was generally similar to that predicted by the Farquhar and Sharkey model (1982), but the slopes of the supply and demand functions varied somewhat between leaves. The operating point was always lower on the response curve than predicted by the theory (Fig 2.15a), with the photosynthetic rate apparently limited by enzyme activity when C_a was at 350 $\mu l\ l^{-1}$ (normal air).

The stomatal limitation (l) was between 12% and 44%, averaging 21%, when calculated by the Farquhar and Sharkey method. Calculation of l by the Jones method generally led to higher values, ranging from 14% to 54% (Tables 2.16 - 2.18). Working with leaves of *Prunus domestica*, Gucci *et al.* (1991) found a similar situation where l varied depending on the method used to calculate it and was higher when calculated by the Jones method (46% cf. 31% - 34% by the Farquhar and Sharkey method). It is not possible to determine from the *Paulownia* data which of the two methods in more correct, or more applicable under the

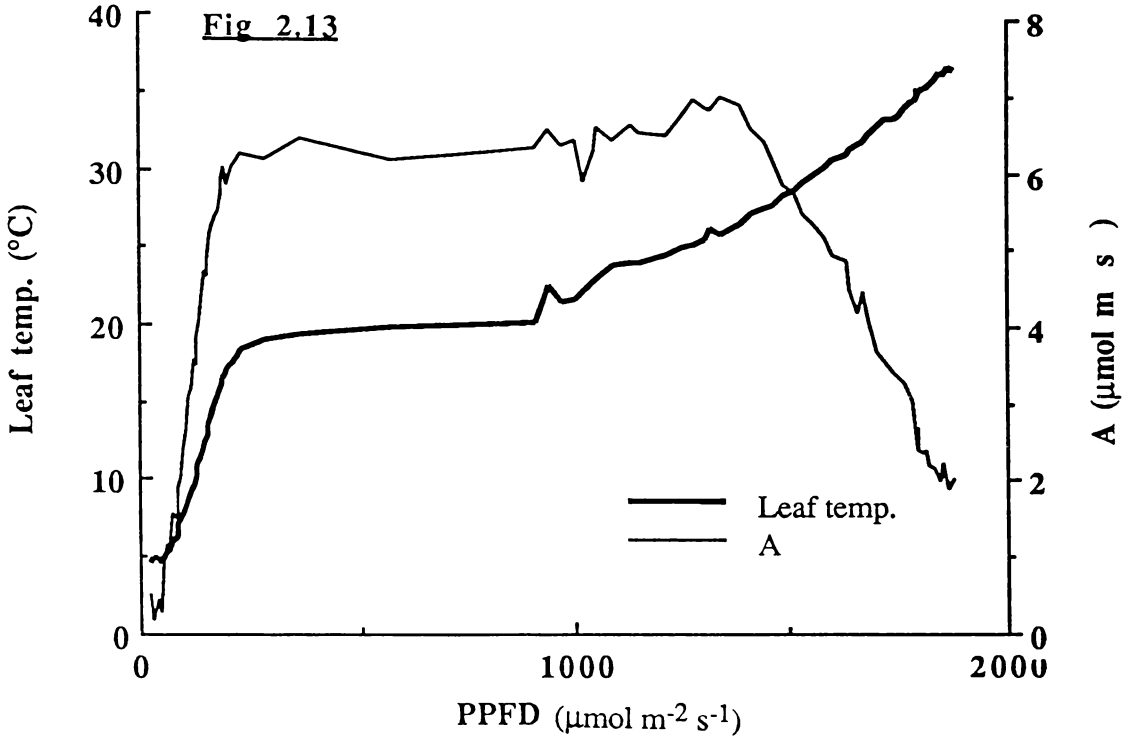


Fig 2.13. Effect of PPFD on leaf temperature and photosynthetic rate of a leaf on tree 72 in December, 1991.

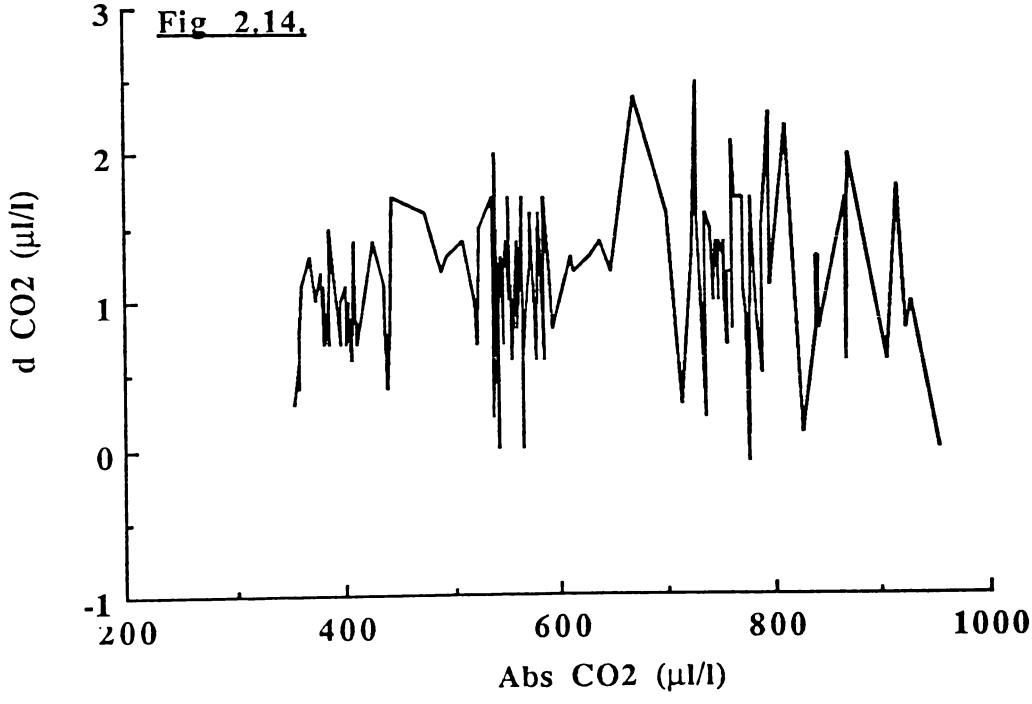
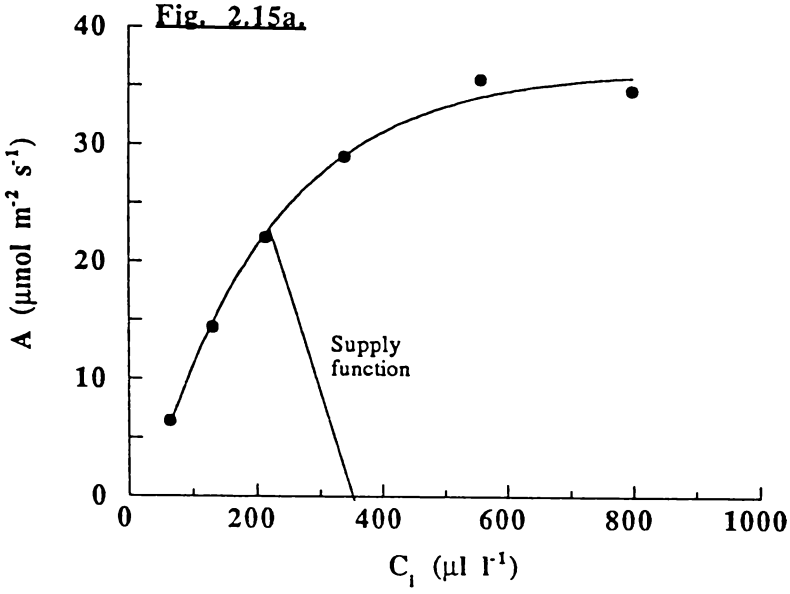
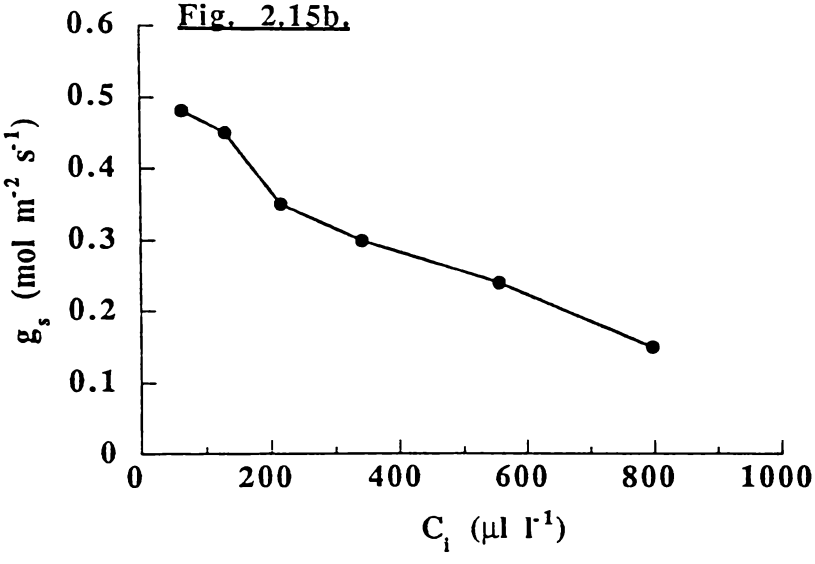


Fig. 2.14. Changes in the differential CO_2 signal as ambient CO_2 changes overnight.





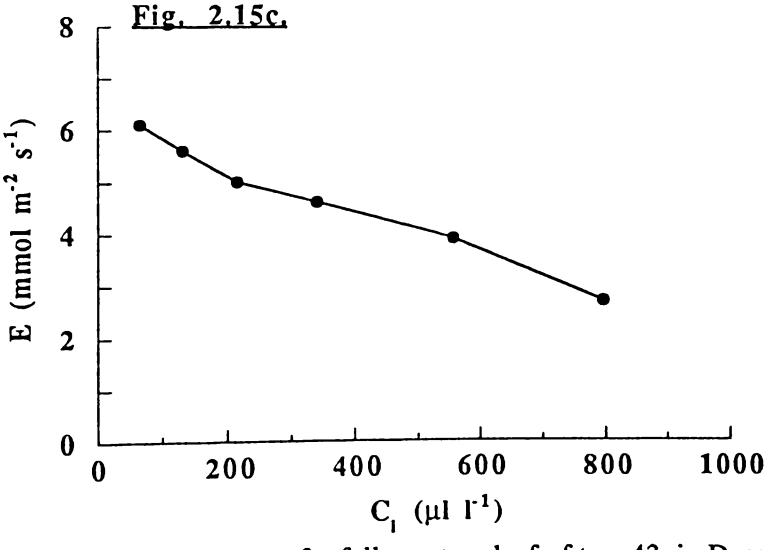


Fig 2.15a, b, c. Response of a fully mature leaf of tree 43, in December, 1992, of a. photosynthesis; b. stomatal conductance; c. transpiration to the increase in internal CO_2 concentration, under saturating light.

conditions of measurement. Thus the values for *l* are presented only as a general estimation of the extent of the stomatal limitation upon photosynthesis.

However, the range of values found for *l*, calculated by either method, was comparable with the results from other species, with *l* in grapes found to be between 21% and 40% (Downton *et al.*, 1987) and in Norway spruce from 18% to 44%, depending on foliage age and exposure to ozone (Wallin, Ottosson and Sellden, 1992). At low VPD in *Piper spp.*, *l* was between 10% and 30%, increasing to 40 - 60% at higher VPD (Tinoco-Ojanguren and Pearcy, 1993), while water stress increased *l* from 42% to 54% in *Quercus stellata* but decreased it in *Acer saccharum* from 34% to 19%, with no effect found in *Q. alba* and *Jugulans nigra* (Ni and Pallardy, 1992).

Table 2.16. Parameters calculated from the AC_i curves in season 2 on leaves from *P. elongata* and *P. tomentosa* trees, with *l*1 the stomatal limtiation calculated by the Farquhar and Sharkey method, and *l*2 by the Jones method.

Tree	Leaf Pair	Date	A ($\mu mol\ m^{-2}\ s^{-1}$)	A_o ($\mu mol\ m^{-2}\ s^{-1}$)	*l*1 (%)	C_i ($\mu l\ l^{-1}$)	A_{2000} ($\mu mol\ m^{-2}\ s^{-1}$)	*l*2 (%)
Creche	7A	Feb	11.4	13.0	12.3	274	14.4	16.1
Creche	7B	Feb	14.0	16.0	12.5	287	25.4	14.5
Creche	7	Mar	12.4	18.4	32.6	209	23.4	31.4
Creche	8	Mar	16.3	20.5	20.5	225	26.4	25.8
43	4	Feb	22.9	27.9	17.9	267	41.9	16.8
43	5	Mar	14.5	17.5	17.1	250	23.0	18.6
72	5	Feb	18.3	21.0	12.9	287	34.4	13.7
72	4	Mar	14.2	16.3	12.9	279	24.4	14.2

It was also possible to determine the rate of photosynthesis at CO_2 saturation by extrapolating the curve with the asymptotic concentration of C_i being standardized at 2000 $\mu l\ l^{-1}$. The photosynthetic rate at that point (A_{2000}) varied from 13 - 42 $\mu mol\ m^{-2}\ s^{-1}$ (Tables 2.16 - 2.18). The average ratio of $A:A_{2000}$ was 0.57 which was higher than that calculated from data on *Quercus suber* leaves, but in the same range as that from a number of other species (Tenhunen, 1984).

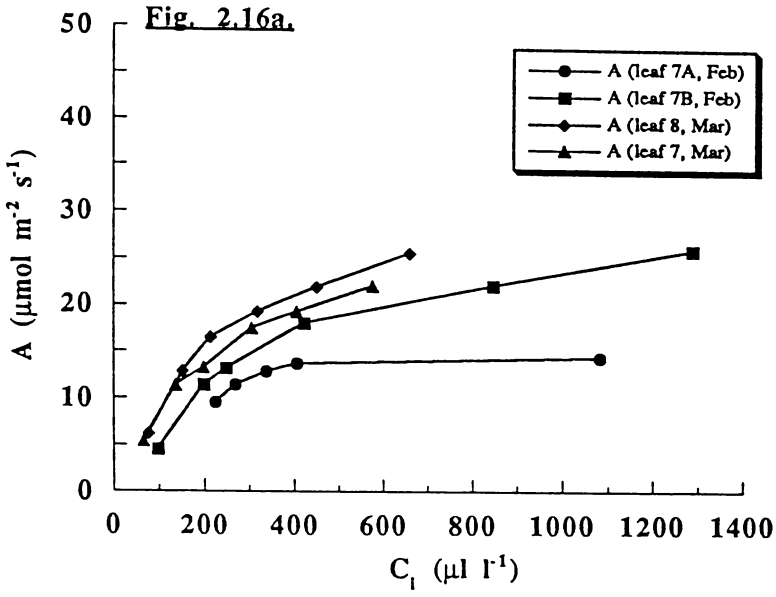
Analysis of the data from leaves of the creche tree in season 2 showed different responses between leaves, despite the fact that in February a pair of leaves (7A and 7B) was sampled. The values of A, A_o, *l* and C_i were reasonably similar between these two leaves (Table 2.16) but A_{2000} was much higher in leaf 7B which was a reflection of the differences in the curvature of the two AC_i plots (Fig 2.16a). In March the stomatal limitation was higher, compared with the February data, and this coupled with slightly higher rates of A led to lower internal CO_2 concentrations. The rate of transpiration was lower in March as a result of decreased g_s.

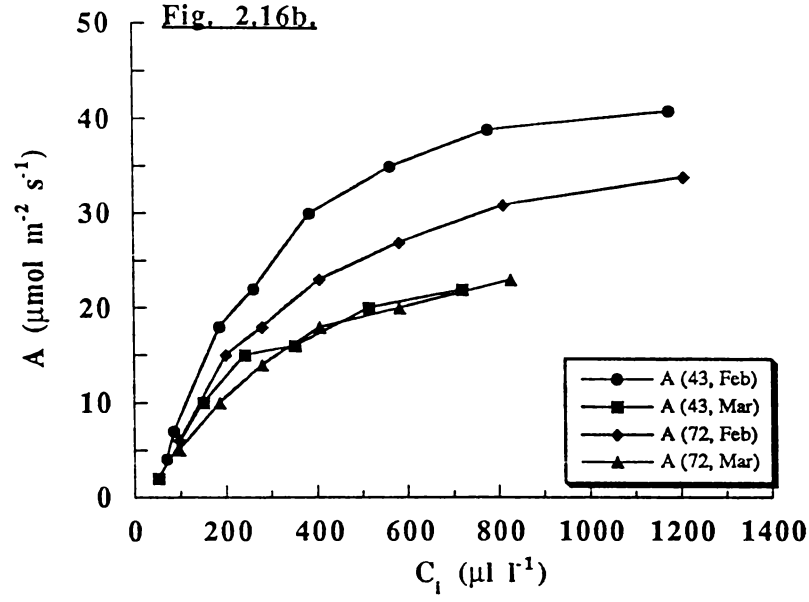
Leaves from the two *P. tomentosa* trees measured in February in season 2 had higher A, A_o, A_{2000} and C_i than leaves studied in March, but *l* did not change over the same period (Table 2.16). E and g_s were also lower in March. There was little difference in many of the gas exchange parameters measured on the two trees in March, but the rates of photosynthesis at all C_i levels were higher on tree 43 than on tree 72 in February (Fig 2.16b).

In season 3, first fully mature leaves from tree 72 had the lowest photosynthetic rates and highest *l* (hence lowest C_i) in February (Table 2.17, Fig. 2.16c). Over the season the rates of the various gas exchange parameters reflected those found in other experiments in this season, with maximum rates found in March (Table 2.19). Comparison of the data from tree

Table 2.17. Parameters calculated from the AC_i curves in season 3 on first fully mature leaves from trees 43 and 72, with *l*1 the stomatal limtiation calculated by the Farquhar and Sharkey method, and *l*2 by the Jones method.

Tree	Leaf Pair	Date	A (μmol m^{-2} s^{-1})	A_o (μmol m^{-2} s^{-1})	*l*1 (%)	C_i (μl l^{-1})	A_{2000} (μmol m^{-2} s^{-1})	*l*2 (%)
43	3	Dec	20.5	26.6	22.9	222	33.4	26.0
43	4	Dec	22.6	29.2	22.6	225	37.1	33.0
43	4	Jan	18.9	22.9	17.5	243	34.9	25.0
43	3	Feb	13.6	18.6	26.9	217	32.9	28.0
43	3	Mar	16.6	19.4	14.4	273	27.9	16.0
72	3	Dec	16.8	21.2	20.8	238	28.8	23.2
72	2	Jan	16.0	20.6	22.3	216	26.4	27.9
72	4	Feb	13.6	18.2	25.3	207	23.2	32.8
72	3	Mar	18.3	23.8	23.1	239	33.4	26.6





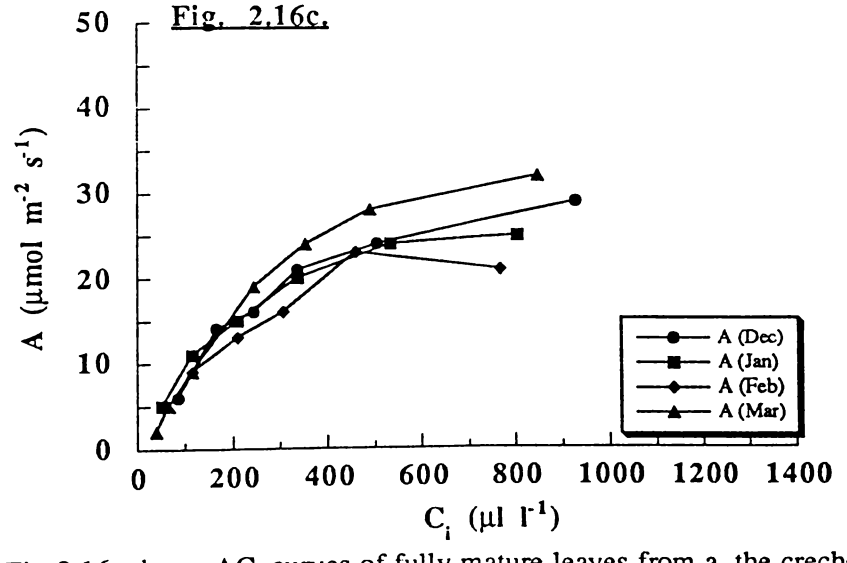


Fig 2.16a, b, c. AC_i curves of fully mature leaves from a. the creche tree in season 2; b. trees 43 and 72 in season 2; c. tree 72 in season 3.

72 in February and March in season 2 with that from season 3 showed that, on average, the rates of photosynthesis at all C_i were similar (despite the reversal of the monthly trends), but the stomatal limitation imposed upon photosynthesis was twice as high in season 3 as in the previous season and this led to lower C_i values when C_a was 350 $\mu l\ l^{-1}$.

Analysis of the gas exchange data for leaves from tree 43 in season 3 showed higher rates of A and A_o earlier in the season, with the lowest values measured in February, as for tree 72 (Table 2.17). At this time *l* was maximal and so C_i was low. The shape of the AC_i curve of the February leaf showed little change in gradient at higher CO_2 concentrations, however, (Fig. 2.17c) which led to a saturating rate of A similar to those calculated earlier in the season. Stomatal conductance and transpiration rates were lowest for the February and March measurements at all CO_2 concentrations except the highest. The values of A, A_o and A_{2000} were much higher in February, 1992 than in February, 1993, but the values for these parameters in March were higher in season 3 than in season 2. Stomatal limitation was higher in February in season 3, but at the March measurements was higher in season 2.

As the leaves aged through the season there was a definite decline in A, A_o and A_{2000}, while *l* tended to increase (Table 2.18). Rates of photosynthesis were highest at all CO_2 concentrations when the leaves were first fully mature (Figs. 2.17a and 2.17b). The decline with leaf age was more pronounced in leaf 1 from tree 43, which may have been influenced by the low values recorded generally for February. During the analysis on leaf 1 in February, g_s remained at 0.06 mol $m^{-2}\ s^{-1}$ at all CO_2 levels with E at 1.3 - 1.4 $\mu mol\ m^{-2}\ s^{-1}$. The leaf was the ninth pair on the branch at this time and was beginning to show signs of senescence with some necrosis and chlorosis visible.

However, the aging process in leaf 2 led to an opposite stomatal response with g_s being highest at higher CO_2 levels in March than at any other time and lowest in February. There was little difference in E between the March and January analyses, with E generally being lower in February. It is possible that the factors that led to the overall decline in gas exchange parameters for all the experiments in February, 1993, depressed rates in this leaf beyond the level that would be expected due to leaf age.

The small number of data points used to produce each AC_i fit, particularly at low C_a values, meant that it was not possible to accurately quantify the CO_2 compensation point nor the carboxylation efficiency at low CO_2 concentrations. However on the two occasions that the ambient CO_2 concentrations were lowered to 50 - 60 $\mu l\ l^{-1}$, the rate of net photosynthesis was close to zero. It was concluded from these data that the CO_2 compensation point of *Paulownia* was probably about 50 $\mu l\ l^{-1}$, which is similar to that for other woody species.

Fig. 2.17a.

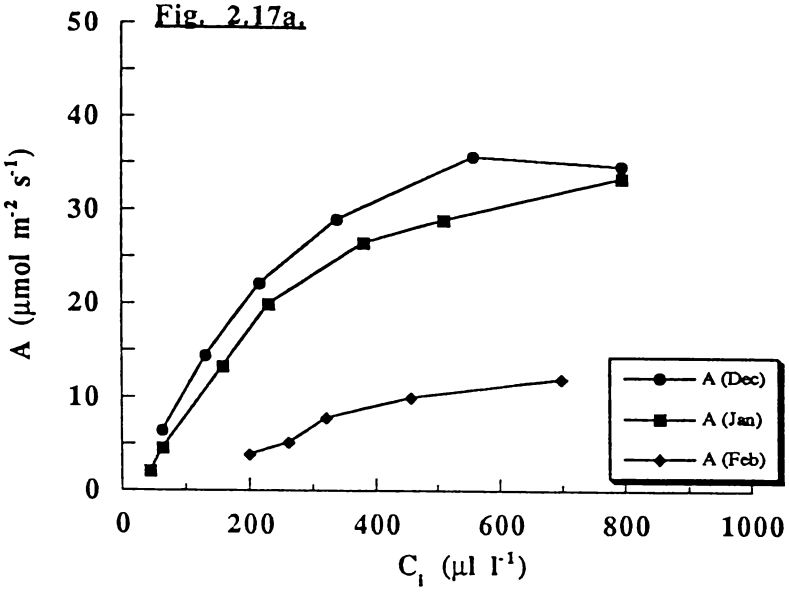


Fig. 2.17b.

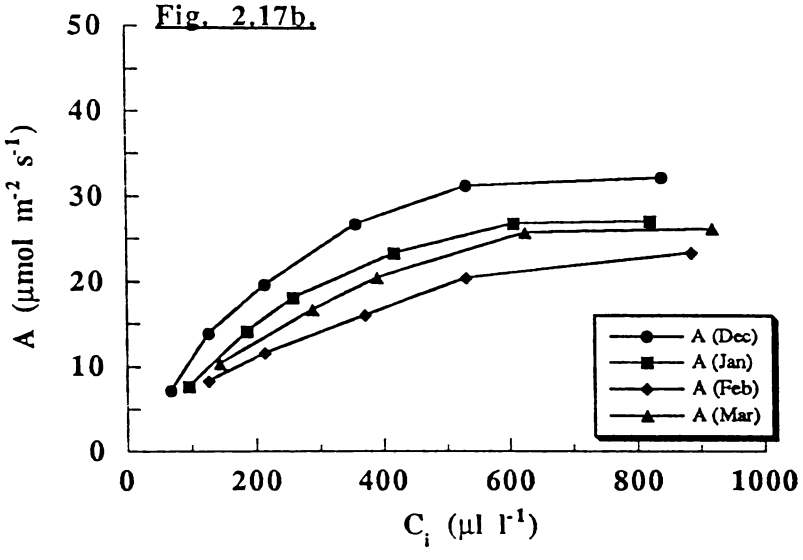


Fig. 2.17c.

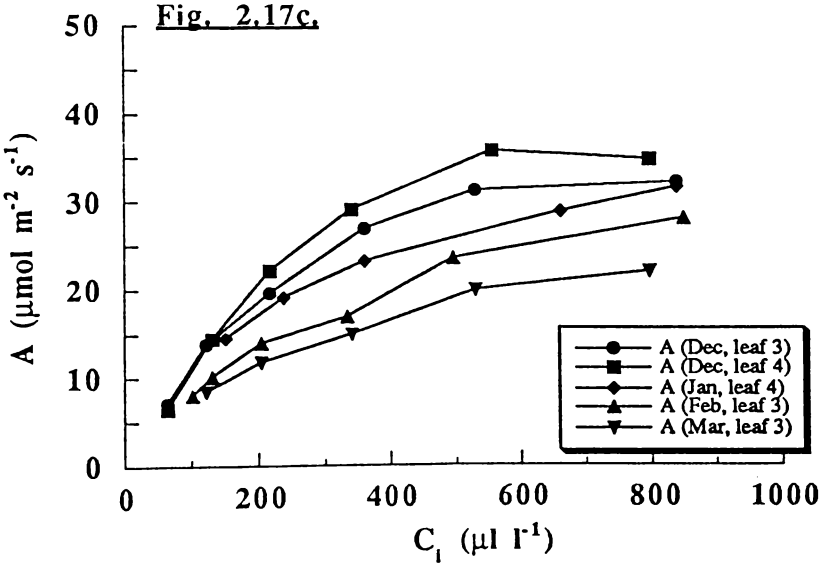


Fig 2.17a, b, c. AC_i curves of leaves from tree 43 in season 3 - a. leaf 1 from December, 1992 to February 1993; b. leaf 2 from December, 1992 to March, 1993; c. first fully mature leaves measured through the season.

Table 2.18. Parameters calculated from the AC_i curves as leaves from tree 43 aged through season 3, with *l*1 the stomatal limtiation calculated by the Farquhar and Sharkey method, and *l*2 by the Jones method.

Tree	Leaf Pair	Date	A ($\mu mol\ m^{-2}\ s^{-1}$)	A_o ($\mu mol\ m^{-2}\ s^{-1}$)	*l*1 (%)	C_i ($\mu l\ l^{-1}$)	A_{2000} ($\mu mol\ m^{-2}\ s^{-1}$)	*l*2 (%)
43	1	Dec	22.6	29.2	22.6	225	37.1	33.0
43	1	Jan	19.8	25.0	20.8	241	35.0	25.0
43	1	Feb	4.5	8.0	43.8	217	13.2	54.0
43	2	Dec	20.5	26.6	22.9	222	33.4	26.0
43	2	Jan	18.3	21.6	15.3	260	28.6	18.0
43	2	Feb	12.0	16.0	25.0	222	26.2	28.0
43	2	Mar	11.8	15.7	25.0	211	25.7	29.0

2.4.6 Gas exchange rates at full light on *P. tomentosa* in season 3

2.4.6.1 Seasonal trends

In addition to the diel and CO_2 response curve experiments presented above, measurements of gas exchange parameters under saturating light were made on trees 43 and 72 in season 3. Each month first fully mature leaves were used and some of these leaves were remeasured at subsequent samplings to determine the aging of leaves through the season.

The seasonal trend of gas exchange in first fully mature leaves was different from that found in season 2 and the two trees were different from one another (Table 2.19). The rate of photosynthesis was low in tree 43 in November, but was reasonably constant each month after that. Other parameters to show a similar trend were leaf chlorophyll content and g_s. In tree 72, A_{max} was variable from month to month with the highest measurement of 24 $\mu mol\ m^{-2}\ s^{-1}$ made in March. Chlorophyll content and g_s were also highest at this time. There was no obvious correlation between leaf nitrogen content and A_{max} in either tree. Despite the changes in g_s and VPD throughout the season, the rate of transpiration was remarkably constant at between 4 and 5 $\mu mol\ m^{-2}\ s^{-1}$.

In terms of the leaf structure, some seasonal trends were apparent. The specific leaf weight (SLW) was relatively constant between the leaves from both trees when measured in January and February, but increased slightly at the final analysis in March. This may have been a reflection of the increased age of leaves sampled in March. The early cessation of leaf initiation (section 2.4.1.2) meant that the leaves defined as first fully mature at that time were probably older than leaves thus defined earlier in the season. In tree 72 a slight trend of decreasing stomatal numbers was observed from December through to February, but there was insufficient data to give a pattern in tree 43. This result was opposite to that noted in season 2 when stomatal numbers increased in leaves that were initiated later in the season (Table 2.13).

Table 2.19. Gas exchange of first fully expanded leaves of *P. tomentosa* trees in their third season, measured monthly, under conditions of full light. Standard errors are in parentheses.

Month	A (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	$C_c - C_i$ (μl l^{-1})	Chlorophyll (g m^{-2})	N (% DW)	SLW (g m^{-2})	Stomata (mm^{-2})
Tree 43								
Nov	13(0.1)	3.6(0.15)	0.21(0.015)	122(6.6)	0.39(0.009)	-[1]	-	-
Dec	17(0.9)	3.9(0.45)	0.29(0.024)	124(9.4)	0.44(0.013)	2.50(0.141)	-	611(17.1)
Jan	21(2.1)	5.3(0.40)	0.37(0.030)	121(4.5)	0.48(0.020)	2.24(0.110)	93(3.3)	-
Feb	17(0.9)	3.5(0.12)	0.26(0.022)	131(13.3)	0.47(0.025)	2.16(0.030)	98(7.5)	566(27.7)
Mar	19(0.8)	4.2(0.13)	0.34(0.012)	114(1.7)	0.49(0.013)	2.23(0.195)	107(4.2)	-
Tree 72								
Nov	14(1.5)	4.3(0.35)	0.25(0.015)	111(5.6)	0.35(0.007)	-	-	-
Dec	15(1.0)	4.6(0.20)	0.36(0.036)	95(6.1)	0.45(0.012)	2.24(0.050)	-	606(18.9)
Jan	14(1.6)	3.9(0.40)	0.27(0.043)	108(4.3)	0.42(0.022)	2.10(0.129)	88(4.5)	572(21.1)
Feb	19(1.1)	4.1(0.18)	0.30(0.023)	128(5.7)	0.53(0.016)	2.19(0.087)	87(3.3)	515(12.0)
Mar	23(0.6)	5.1(0.26)	0.45(0.024)	118(5.6)	0.64(0.013)	2.17(0.043)	94(2.9)	-

[1]not determined

The data presented in both Tables 2.13 and 2.19 are the stomatal densities on the abaxial (lower) leaf surface only. The number of stomata found on the adaxial surface of the leaves ranged from 0 to 40 mm^{-2}. Thus it was considered feasible to ignore the stomatal population of the upper leaf surface in the interpretation of the gas exchange data. On one occasion the size of the stomata were measured and the average pore length was 14.7 (s.e. - 0.62) μm, while the length of the whole stomatal aperature was 21.7 (s.e. - 0.44) μm long. Comparison of these data with that tabulated by Kramer and Kozlowski (1979) for a

wide range of deciduous tree species indicated that the stomata of *Paulownia* leaves are smaller than those in many other species, but more numerous.

2.4.6.2 Daily trends

On a daily basis, the midday depression of gas exchange rates was significant in leaves on both trees in season 3 (see also section 2.4.4.1). A_{max} was between 21% and 36% lower when measured in the afternoon then when measured in the morning while g_s decreased by 37% on average. Transpiration rates tended to decrease by 1 unit from 4 - 5 μmol m^{-2} s^{-1} to 3 - 4 μmol m^{-2} s^{-1}. The actual morning values of A, for those leaves analysed in both the morning and afternoon, varied between 16 and 22 μmol m^{-2} s^{-1}, and in the afternoon between 12 and 15 μmol m^{-2} s^{-1}. The corresponding g_s values were 0.30 - 0.43 mol m^{-2} s^{-1} in the morning and 0.19 - 0.28 mol m^{-2} s^{-1} in the afternoon.

The diurnal pattern of change in the gas exchange rates may be more easily explained by the assimilate feedback theory than by a change in the water status of the plants. The rates of A_{max} in the morning were probably high enough to contribute to negative feedback. There was some increase in leaf water potential (Table 2.15) in leaves through the day, but the data relating to differences in VPD, RH, leaf and air temperature at the two times of gas exchange measurement were equivocal. VPD and leaf temperature were higher on some occasions during the afternoon measurement but it is not clear how much this was a cause or an effect of reduced gas exchange. Air temperature tended to be higher in the afternoon, while RH was generally reasonably constant between 50% and 60%. Thus it is possible that plant water status did play some role in the afternoon depression of gas exchange, but no decision about the primary cause of the afternoon depression can be drawn from these data.

2.4.6.3 Leaf aging

The limited data available of the change in gas exchange rates in season 3 showed no clear trends. Only three leaves from tree 72 were remeasured, and then only once, with the resultant pairs of monthly measurements showing either a increase in rates as the leaf aged, or a decrease or no change. The data from tree 43, although more comprehensive, was no more conclusive with two leaves measured for two consecutive months showing an increase in rates. A third leaf, analysed over three months showed a peak in values in the second month (January). The final leaf was measured from December until March with rates of A_{max} varying from 22 - 18 - 12 - 17 μmol m^{-2} s^{-1} from month to month, while g_s varied from 0.39

- 0.46 - 0.18 - 0.46 mol m^{-2} s^{-1}. The increase in gas exchange rates in March in this leaf reflected the overall trend of higher rates in March (Table 2.19) for first fully mature leaves. This result of individual leaves showing the same pattern of seasonal rates, regardless of leaf age, was also found in two of the leaves from tree 72.

2.4.6.4 Temperature response curve

The results presented in Table 2.20 showed that the rate of photosynthesis was unchanged with increases in the air temperature from 15°C to 25°C, but decreased at 30°C and by 35°C was only about 30% of the maximal rate. This result confirmed the observations made in the diel experiments when A decreased as leaf temperature increased (section 2.4.3.3). A_{max} was also lower at 10°C, but not to such an extent. Transpiration increased as the air temperature increased up to 35°C (as VPD increased), at which point the stomata were virtually closed. Stomatal conductance decreased with the increase in temperature but reached a plateau between 20°C and 25°C before declining again at higher temperatures. These results confirm that the leaf temperature used in the light and CO_2 response curves (22°C) was appropriate in the measurement of maximum rates of gas exchange.

Table 2.20. Gas exchange parameters from a temperature response curve of gas exchange measured on a mature leaf of tree 43 in February, 1993.

Air temp. (°C)	Leaf temp. (°C)	A (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	C_i (μl l^{-1})	C_a (μl l^{-1})	Ext. temp. (°C)	VPD (kPa)
10	12.7	14	0.45	1.3	412	482	13.5	-
15	16.7	17	0.38	2.2	338	433	14.1	0.8
20	21.3	16	0.20	2.4	273	422	15.2	1.4
22	22.9	16	0.22	3.0	249	391	17.7	1.6
25	25.4	16	0.20	3.5	231	381	18.4	2.0
30	29.9	13	0.14	3.8	205	376	20.9	2.9
35	36.1	6	0.05	2.5	182	360	21.2	4.8

2.4.7 Relationship between A and g_s

Under the conditions used in the AC_i response experiments, the relationship between C_a and C_i was remarkably constant for all concentrations of CO_2, with the C_i:C_a ratio being 0.7 (Fig. 2.18). This indicates that the rate of photosynthesis and the stomatal conductance were balanced in such a way as to maintain this ratio. Thus at high C_a, g_s was low, but A was high, and as C_a decreased, A also decreased but g_s increased (Fig. 2.15).

This tight coupling of A and g_s was not observed in other experiments at ambient CO_2 levels (Fig. 2.19). The photosynthetic rate did tend to increase as g_s increased, but in a number of instances the same value of A occurred with a range of g_s values, while at other times variation in A corresponded with the same stomatal conductance. For example leaf 2 of tree 43 had a photosynthetic rate of 18 μmol m^{-2} s^{-1} in both January and February, 1992, but g_s rose from 0.36 mol m^{-2} s^{-1} to 0.69 mol m^{-2} s^{-1} over the same period (Table 2.9). This rise in g_s resulted in an increase in C_i but a decrease in WUE as the increased transpiration was not accompanied by an increase in A. In leaf 1 of *P. elongata* measured in March, 1992, (Table 2.4) the rates of photosynthesis corresponding to a particular g_s were 50% - 100% higher in the afternoon than in the morning, despite slightly lower light and water potentials in the afternoon. The data plotted for the creche tree leaves in Fig 2.19a (taken from Tables 2.2 - 2.4) may reflect the fact that the experiments were undertaken using ambient light levels and so the stomata may still have been responding to a recent change in PPFD. However, the data for the remaining two graphs were collected under conditions of full light and controlled temperature.

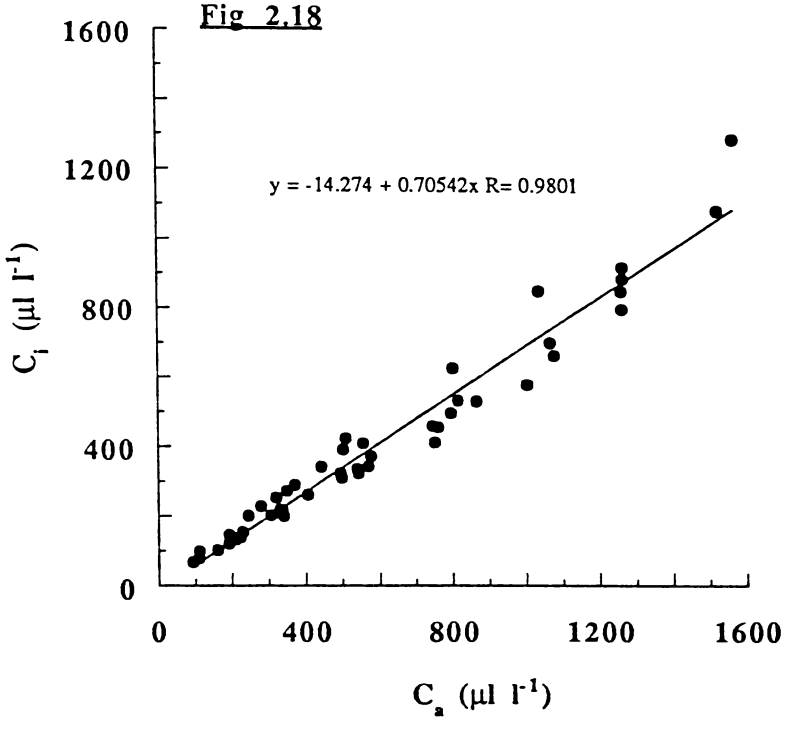
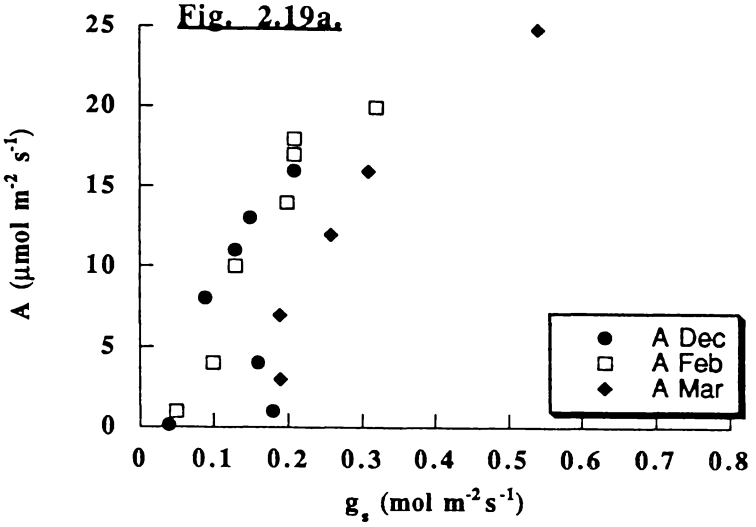
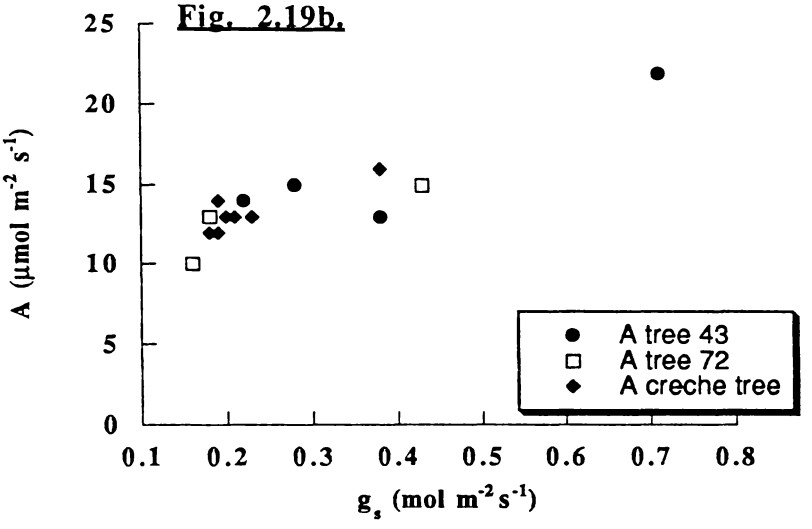


Fig 2.18. Internal CO_2 concentration against ambient CO_2 concentration during a number of AC_i curve experiments in seasons 2 and 3.





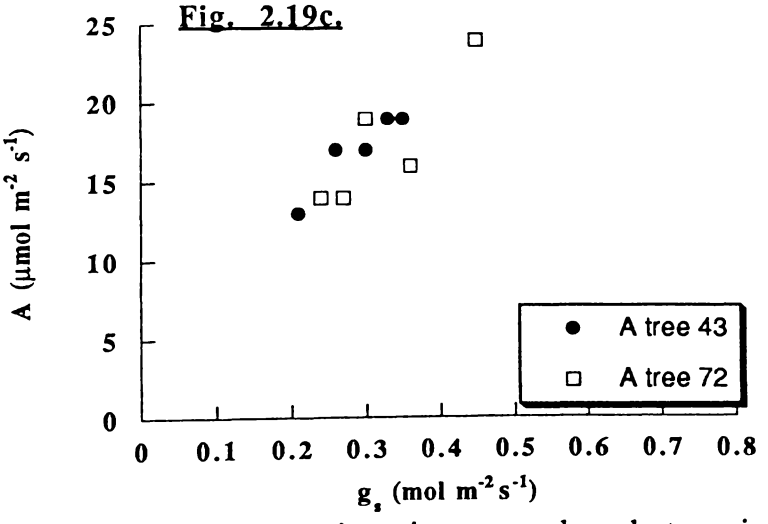


Fig 2.19a,b,c. Photosynthesis against stomatal conductance in a. - leaves of creche tree in season 1; b. - first full expanded leaves of all trees in season 2; c. - first fully mature leaves of trees 43 and 72 in season 3

2.5 Discussion

2.5.1 The light requirements of *Paulownia*

The results of the gas exchange measurements made in the light response curves, and confirmed by the diel experiments, indicate that *Paulownia* leaves show many of the characteristics of sun leaves. The values of $PPFD_{sat}$ for fully mature leaves from both *P. elongata* and *P. tomentosa* trees were generally above 1100 μmol m^{-2} s^{-1} (Tables 2.9 - 2.12). This is comparable to values for $PPFD_{sat}$ on a number of other woody perennials grown in full sun conditions which ranged from 800 μmol m^{-2} s^{-1} in kiwifruit (Buwalda *et al.*, 1991) to > 1500 μmol m^{-2} s^{-1} in *Acacia nigrescens* (Tuohy *et al.*, 1991).

The high light requirement of the photosynthetic process was coupled with a high rate of photosynthesis at saturating light. On two occasions values of 24 - 25 μmol m^{-2} s^{-1} were measured for A (Tables 2.4 and 2.19), while A_{max} was commonly between 16 and 18 μmol m^{-2} s^{-1} in the light response curves for fully mature leaves. Comparison of these rates of A with those of other species show that *Paulownia* has a higher than average rate of photosynthesis for sun leaves of woody species.

Young plants of tropical tree species grown at high light intensities tend to have low rates of photosynthesis (ranging from 4 - 9 μmol CO_2 m^{-2} s^{-1}), with $PPFD_{sat}$ generally below 500 μmol m^{-2} s^{-1} (Kwesiga *et al.*, 1986; Riddoch *et al.*, 1991). The tropical cashew tree also has quite low A_{max} of 8 μmol m^{-2} s^{-1}, but high rates of transpiration, 10 - 12 mmol m^{-2} s^{-1}, thus WUE is very low (Balasimha, 1991). Woody legumes growing in Zimbabwe have been found to have higher rates of A_{max}, averaging about 12 μmol m^{-2} s^{-1}, although A_{max} in the species of *Acacia* studied was 20 μmol m^{-2} s^{-1} (Tuohy *et al.*, 1991). Seedlings of different families of *Robinia pseudoacacia* (black locust) can have very high rates of photosynthesis - up to 30 μmol m^{-2} s^{-1} in some cases (Mebrahtu and Hanover, 1991).

Light response curves in a number of conifers have shown lower rates of gas exchange than measured in *Paulownia*. Current season's foliage on Norway spruce (*Picea abies*) has an A_{max} of 9 μmol m^{-2} s^{-1} and a $PPFD_{sat}$ of about 600 μmol m^{-2} s^{-1}, while rates in older foliage tend to be lower (Wallin, Skarby and Sellden, 1992). *Pinus radiata* had an A_{max} of 6 μmol m^{-2} s^{-1}, when grown in Canterbury, but saturated at high PPFD (about 900 μmol m^{-2} s^{-1}) with the light compensation point between 20 and 30 μmol m^{-2} s^{-1} (Benecke, 1980). Foliage of all ages in kauri is saturated at approximately 500 μmol m^{-2} s^{-1}, but A_{max} decreases from 13 μmol m^{-2} s^{-1} in current season's leaves to 6 μmol m^{-2} s^{-1} in four year old leaves (Clarkson, 1992).

One published study has investigated the light response curves in *Paulownia* plants. Working with very small seedlings of *P. tomentosa* grown under varying photoperiods, Carpenter *et al.* (1983) found A_{max} and $PPFD_{sat}$ were highest in the leaves grown under an 8 hour photoperiod as opposed to longer photoperiods. However, A_{max} was very low at only 4 μmol m^{-2} s^{-1}, although R_d was similar to the rates estimated for leaves discussed above. This low rate of A_{max} may have been related to the growth conditions, age or provenance of the plants.

Comparison of the rates of photosynthesis and stomatal conductance between the two *Paulownia* species studied showed that A and g_s were generally higher on the leaves from the *P. tomentosa* trees over the range of experiments undertaken, than on leaves from the *P. elongata* tree. It is not possible at this stage to determine if these differences were due to site, species or age. It would be of interest to measure gas exchange in a range of species growing in the same environment, and to analyse gas exchange in established specimens of different species to further clarify this issue.

It has been shown that rates of gas exchange can vary significantly between species of the same genus. Cottonwood (*Populus fremontii*) was found to have an A_{max} of 24 μmol m^{-2} s^{-1}, $PPFD_{sat}$ of 1320 μmol m^{-2} s^{-1}, light compensation point of 103 μmol m^{-2} s^{-1}, and R_d of 2.89 μmol m^{-2} s^{-1} (Roden and Pearcy, 1993). Comparative values for *P. tremuloides* (aspen) grown under the same conditions were A_{max} - 15 μmol m^{-2} s^{-1}; $PPFD_{sat}$ - 840 μmol m^{-2} s^{-1}; light compensation point - 71 μmol m^{-2} s^{-1}; and R_d - 1.75 μmol m^{-2} s^{-1}. The quantum efficiency for both species was the same, at 0.026 and 0.027 mol CO_2 mol^{-1} PPFD, respectively.

Despite the high light levels required to saturate photosynthesis in *Paulownia* leaves, significant amounts of carbon are able to be assimilated at lower light (*e.g.* Figs 2.4a, 2.6a and 2.12b). Thus at 500 μmol m^{-2} s^{-1} the leaves can photosynthesise at 10 μmol m^{-2} s^{-1}, which is similar to A_{max} in some species, while at half that light level (250 μmol m^{-2} s^{-1}) A is still 5 μmol m^{-2} s^{-1}. That appreciable carbon assimilation occurs in light conditions that are below saturating is an important feature for growth of the tree in this country. In many areas of New Zealand there are few days during the growing season without cloud cover at some stage during the day. This is a particular feature of the Waikato climate, with afternoon cloudiness a common occurrence in summer. As the data in Tables 4.1 - 4.3 show, even during the longest days, the average time when PPFD is above 1000 μmol m^{-2} s^{-1} is only between 5 and 6 hours per day.

The high values for $PPFD_{sat}$ and A_{max} in sun leaves result from the increase in the amount of

photosynthetic machinery in these leaves compared with shade leaves. Thus there are more layers of palisade cells and higher levels of the biochemical components of the photosynthetic pathway within the cells and so it takes more light to saturate the system. Extensive anatomical and biochemical analyses were not undertaken on the *Paulownia* leaves studied, but analyses were made of the chlorophyll and nitrogen contents of the leaves. In general, chlorophyll levels were lower than those found in fully mature kiwifruit leaves which were as high as 1.2 g m^{-2}, but nitrogen levels in the leaves from the two plants were similar (Buwalda *et al.*, 1991).

The typical responses of sun leaves to very low light conditions include a higher light compensation point and dark respiration rate than shade leaves. The values for both these parameters given in the results were determined from the fitting of the asymptotic exponential curve and so are open to some interpretation as an error in the fit in that area of the curve would make a significant difference to the values calculated. The range of values for r (from 1 to 60 μmol m^{-2} s^{-1}) makes it difficult to assess whether the light compensation point in *Paulownia* leaves is typical of sun leaves or not. A comparison of seedlings of shade tolerant and shade intolerant tropical tree species gave values of r ranging from 11 μmol m^{-2} s^{-1} for the shade tolerant tree grown at low light levels, to 10, 14 and 25 μmol m^{-2} s^{-1} for the light demanding species grown at high light (Kwesiga *et al.*, 1986). A similar range of results in r for shade tolerant and intolerant rainforest trees grown under a different light regimes was found by Thompson, Huang and Kriedemann (1992). In both these studies there were no clear trends of decreased rates of R_d in the shade tolerant plants compared with the sun plants when grown under the corresponding light regimes. The rates of R_d ranged from 0.3 to 2.0 μmol m^{-2} s^{-1}, which were similar to the range found in *Paulownia* (approximately 0.8 μmol m^{-2} s^{-1}). Thus although the theory of sun leaves having a higher light compensation point, due to the higher rate of dark respiration, may be correct for herbaceous plants it is difficult to prove in reality for some tree species. The values calculated for the quantum efficiency were also variable and showed no trend with increasing leaf age.

The conclusion drawn from measurement of the gas exchange parameters that the leaves of *Paulownia* are sun leaves is confirmed by observation of the growth form of both the mature and juvenile trees. The occurrence of leaves only around the exterior of the canopy of the mature tree and the lack of leaves in the interior ensures that all leaves receive direct sunlight at some stage during the day. On the young trees, the large leaves have very long petioles (between 10 and 40 cm depending on the size of the leaf) which extend the leaf out from the branch or stem. The long internodes also ensure that the leaves are well spaced from one another on the juvenile trees. Leaves in *Paulownia* are oppositely arranged with every second pair of leaves sited in the same plane (decussate arrangement). This combination of petiole length, internode length and opposite leaf arrangement minimizes shading of the lower

leaves by those higher on the branch. In both the mature and juvenile trees leaf abscission occurs throughout the season as the leaves age. When leaves do get to the stage of being significantly shaded by others, senescence is generally well advanced with the result that their contribution to the assimilation of the whole tree is already limited.

The light requirements of sun plants mean that they are found in open habitats and are often pioneer species. For example in the rainforest species discussed above, the shade intolerant tree *Toona australis* is an early successional, deciduous tree, while the shade tolerant species are late successional trees (Thompson, Huang and Kriedemann, 1992). Although little is known about the distribution of *Paulownia* in its natural habitat in China, due to the centuries of cultivation by man, it has been observed in the United States that seedlings of *Paulownia* do colonise disturbed land (Larson, 1980). Tang *et al.* (1980) has postulated that it would be a useful tree in the revegetation of mining wastelands. It is unlikely that the small seeds of *Paulownia* would be able to colonize areas with existing vegetation as the seeds have a light requirement for germination and also the small radicle and limited food supply in the seed makes rapid establishment after germination imperative. Thus the areas where new seedlings could establish would be limited to open areas which have the high light levels that suit the subsequent growth of the tree.

2.5.2 The interaction of gas exchange parameters and water relations

The maximum rates of photosynthesis and stomatal conductance are generally measurable on most types of plants early in the day, if light is saturating and the plant is not under severe water stress. Later in the day, both A and g_s tend to decline in response to a number of factors. However the decline in g_s has a greater effect on the rate of transpiration than on the rate of photosynthesis. This is because the relationship between g_s and E tends to be linear, even at high g_s, while the A vs g_s relationship is often best described by a saturation curve. In addition, the diffusive pathway for CO_2 is longer than that for water, with this effect being more noticeable in windy conditions which cause the rapid turnover of the boundary layer (Bannister, 1976). As mentioned earlier (section 2.1.1.3), the result of a larger decrease in g_s and E in comparison with the decrease in A, means that C_i remains relatively constant and WUE increases slightly.

The relationship between g_s, E and VPD is not a simple one when measured in a leaf cuvette with a small volume. Although a decrease in g_s would be expected to produce a lower E, a feedback via the chamber water vapour pressure (which is a product of the inlet air water vapour and the water vapour added by E) moderates changes in E. Thus, some data in Table

2.14 show an increase in E from morning to afternoon measurements as VPD increases, despite a decrease in g_s.

Both external factors and the internal water status of the plant influence the extent of stomatal opening. The water vapour pressure of the air outside the leaf and rate of change of that air (*i.e.* windiness) affect g_s. It is now believed that stomata can sense the vapour pressure gradient and respond appropriately by a "feed forward" mechanism yet to be fully elucidated (A. Lakso, pers. comm.). Internally, the water potential of the leaf influences stomatal opening. In general, stomata on leaves of both deciduous and coniferous trees start to close when ψ_p is about -0.5 to -1.0 MPa (Larcher, 1980). In kiwifruit, g_s began to decline at -0.65 MPa with g_s only 25% of maximum at -0.9 MPa, by which stage the vines were wilting (Dick, 1987). This was paralleled by a decrease in A and E. Analysis of gas exchange in nine year old *Pinus radiata* trees in Canterbury showed a depression of A, g_s and E under drought conditions when the twig xylem predawn potential was -1.1 MPa. Watering alleviated the water stress but full photosynthetic capacity was not regained until 10 days after watering, when the predawn potential was -0.6 MPa. Changing the VPD also influenced gas exchange with photosynthesis declining at > 2% $mbar^{-1}$ from 8 - 18 mbar (Benecke, 1980).

On a diurnal basis, ψ_p is generally highest just before dawn, decreasing through the morning to reach a minimum about mid afternoon, before increasing again in late afternoon and evening. In grapes Downton *et al.* (1987) found the minimum ψ_p at 14:00 was -1.5 MPa, while in plums it was -1.8 MPa (Gucci *et al.*, 1991) and in three peatland species ψ_p was between -1.5 MPa and -2.8 MPa in mid afternoon (Dang *et al.*, 1991). This led to a decrease in A and g_s in grapes in the afternoon, little change in plum and a slight decrease in the peatland species. The leaf water potentials measured on leaves from both the creche tree and trees at Matangi were generally very high and showed only a very weak diurnal trend (Tables 2.2 - 2.4, 2.15). In December, 1990, ψ_p decreased from -0.2 MPa at predawn to -0.7 MPa in the afternoon which may have contributed to the low gas exchange rates measured on that day. During the two later diurnal experiments in that season, ψ_p was between -0.3 MPa and -0.4 MPa in the afternoons so was unlikely to play a major role in limiting gas exchange. In seasons 2 and 3, the lowest leaf water potentials measured were about -0.5 MPa.

The high values and lack of diurnal trend of ψ_p in *Paulownia* leaves are indicative of a plant with a very low resistance to water movement. Coupled with the higher than average values for stomatal conductance and transpiration often measured, this result indicates that *Paulownia* has a root system able to absorb large quantities of water with little resistance, which is then quickly moved through the plant to be transpired at the leaf surface. The plant hydraulic resistance is a result of the interaction between environment and the inherent genetic

makeup of the plant, with many stone fruit crops having high root resistances. Root resistance is result of internal anatomy, rather than externally observable features such as root size or density (A. Lakso, pers. comm.).

The advantages to the plant of this low resistance strategy are twofold. Firstly the plant is able to take up nutrients in the soil solution quickly and hence meet the demand for minerals placed upon the tree by the fast rate of growth of new tissue. The second important benefit is the cooling effect the high transpiration rate has on the leaves. The large surface area of the leaves makes them an effective solar panel for absorbing not only the light required for photosynthesis, but also heat which is not easily dissipated by convection (Bannister, 1976). Closure of the stomata, even under water stress, could lead to overheating and internal damage. The plant attempts to solve this problem by maintaining g_s at a level high enough to allow transpirative cooling while decreasing the water gradient by having the abaxial surface of the leaves covered with fine hairs (particularly on the mature tree) to increase the effect of the boundary layer.

This cooling effect may partly explain the difference in the values of the gas exchange parameters between the creche tree and Matangi trees in season 1. The very large leaves of the *P. tomentosa* trees had higher values for g_s than leaves from the *P. elongata* tree, while maintaining similar or lower photosynthetic rates, particularly in February (cf Tables 2.3 and 2.6; Tables 2.4 and 2.7). From the results of the temperature response curve (Table 2.20) and several diel experiments (2.4.4.3), it does appear that gas exchange in *Paulownia* leaves is very sensitive to temperatures above about 28°C - 30°C.

Despite the high rate of water loss from the leaves, the water use efficiency is in a similar range to that reported for other woody perennials, due to the high rates of A in *Paulownia*. WUE ranged from 2 - 5 μmol CO_2/mmol H_2O in the light response experiments undertaken on both *P. elongata* and *P. tomentosa* leaves, with there being no clear difference in ratios between the two species (Tables 2.9 - 2.11). This compared with a WUE of 5 μmol CO_2/mmol H_2O in kiwifruit, which was similar to that in herbaceous species (Dick, 1987), 1.5 μmol CO_2/mmol H_2O in *Lepechinia calycina* (Field and Mooney, 1983) and ratios between 1 and 4 μmol CO_2/mmol H_2O in swamp birch, black spruce and tamarack (Dang *et al.*, 1991).

The water relations strategy in *Paulownia* trees makes it unlikely that water stress on a diurnal basis contributes significantly to the midday or early afternoon depression of gas exchange rates observed late in season 2 and throughout season 3. The lowest leaf water potentials were measured in December, 1990, when there was scant evidence of an afternoon depression (Table 2.2). Leaf 2 did show slightly lower rates of gas exchange in the afternoon

than at midday, but leaf 1 did not, and examination of the data from three other leaves measured at the same time showed that A and gs were highest at 14:00 hours. There was no evidence of a depression in rates in the early afternoon compared with those measured in the late morning in any of the gas exchange experiments carried out in season 1.

2.5.3 The negative feedback hypothesis

So if water stress does not appear to be implicated in the causation of the diurnal trend of gas exchange, what other factors could be involved? A second possibility is the depression of gas exchange rates by the accumulation of photosynthates, which causes a negative feedback on the photosynthetic process. As mentioned in section 2.4.4.1, there is no direct evidence for a negative feedback response operating in the leaves studied, but the circumstantial evidence does suggest it may be a major factor. In season 1, during the diurnal experiments on the creche tree, light was limiting in the morning and gas exchange rates were low. These rates increased as the light levels increased and were generally maintained while light was saturating. Thus the production of photosynthates during the first hours of the day would have been quite low and the overall levels of products would not have built up to potentially limiting levels until much later in the day, when gas exchange rates dropped anyway as a result of the decrease in light. In tandem with this result is the observation that A_{max} was higher in the mornings when depression of rates was observed than in the morning of days when there was no change in rates over the day.

There is much debate in the literature on the mechanism of feedback inhibition of photosynthesis, if indeed it exists at all. The complex regulation of the enzyme systems involved in the carbon reduction cycle ensures a workable balance is maintained between the production of sucrose, starch and the regeneration of RuBP. This involves regulation of the utilization of triose phosphates and of the concentration of inorganic phosphate (Sharkey, 1985). It is possible that high photosynthetic rates could cause the accumulation of products that alter this balance within the chloroplast and thus cause feedback inhibition, but Sharkey (1985) considers this unlikely.

There are differences observed between plants with respect to negative feedback which could be related to the type of sugar products primarily utilized in storage and translocation. Goldschmidt and Huber (1992) postulated that the inhibition of gas exchange was related to the soluble sugar metabolism in the extrachloroplastic compartment and that the mechanism may involve a metabolite that impinges on the photosynthetic process in some way, rather than a direct inhibitory effect of the accumulation of starch, sucrose or other sugars. Sink

strength can be an important factor in regulating translocation of photosynthates. This has been studied on comparable fruiting and defruited plants. In grapes, the depression of gas exchange rates occurred earlier in the day on defruited vines than those bearing fruit which led to a 22% decrease in the amount of carbon fixed (Downton *et al.*, 1987). However, in two species of the *Prunus* genus contrasting results were found. In *P. domestica*, defruiting led to a midday depression of gas exchange that was absent in fruiting trees, a slight increase in leaf starch and no change in sorbitol or sucrose levels (Gucci *et al.*, 1991). But Roper *et al.* (1988) found no change in A on defruited trees of *P. avium* compared with fruiting, on either a daily or seasonal basis. Leaf carbohydrate levels were lower on the defruited trees. It is interesting to speculate as to whether the role of sorbitol as the main transport sugar in these plants affects feedback in any way. What is clear is that different plants under different environmental conditions can produce opposite results when treated in a similar manner.

In order to more fully understand the possible mechanisms and metabolites involved in feedback inhibition in all plants, as well as in *Paulownia*, it will be necessary to carry out some detailed biochemical analyses in concert with measurements of gas exchange. Gucci *et al.* (1991) suggest that measurement of triose phosphates, inorganic phosphates and the rates of carbohydrate translocation from the leaves are needed to shed further light onto this issue. It may also be necessary to measure phytohormone levels, particularly ABA, to get a fuller understanding of all the interactions that can cause the midday depression of gas exchange rates under some conditions and not others.

The possibility of feedback regulation of gas exchange rates, through translocation and sink effects, highlights one of the limitations of the experimental procedure used in this research. The area of leaf analysed by the Walz CMS system was either 0.0012m^2 or 0.0020 m^2, depending on the cuvette size used and the LICOR 6200 analysed about 0.0040 m^2 of the leaf. The large leaf size, particularly of the juvenile *P. tomentosa* trees, meant that only a small fraction at the edge of the leaf was enclosed in the cuvette, probably between about 10% and 20%. Thus other areas of the leaf, and indeed the whole tree, were responding to uncontrolled environmental conditions that affected their functioning. This would then influence the behaviour of the area of the leaf under study in ways difficult to quantify using the present system. It is believed that interaction between sections of the leaf is less in heterobaric leaves than homobaric leaves and although detailed anatomical analysis of *Paulownia* leaves was not undertaken, the pattern of venation of both leaf surfaces and the sun leaf nature of the leaves suggest that they are heterobaric. Thus the area of the leaves studied probably enclosed a number of leaf sections that interacted to a certain extent, but it was not possible to determine this interaction, nor that resulting from the functioning of the remainder of the leaf and plant.

2.5.4 Other limitations upon the rate of photosynthesis

In addition to those those factors that influence the overall rate of gas exchange, there are a number of factors that affect the instantaneous rate of photosynthesis. The supply of CO_2 to the chloroplast, the activity of Rubisco, the amount of chlorophyll, the regeneration of RuBP - all are important in determining the final value of A. It is possible to apportion the limitations upon A to those imposed by the mesophyll and to stomatal limitation (*l*) as outlined in section 2.1.8.

In the *Paulownia* leaves studied, stomatal limitation was never the major limitation on the rate of A (Tables 2.16 - 2.18). In season 2, *l*1 ranged from 12% in leaves from the creche tree in February to about 27% in March, while the *P. tomentosa* leaves showed no change in *l*1 over the two months (17% in tree 43 and 13% in tree 72). In season 3 *l*1 showed more change through the season and with leaf age, and was generally higher than in season 2. It is likely that the difference in *l*1 between the two seasons is related to the greater depression of gas exchange rates from late morning onwards. The period of time needed to complete an AC_i experiment was between 2.5 and 4 hours, depending on the rate of stabilisation of the g_s value (section 2.4.3.4). Gas exchange was always measured at high CO_2 levels first, which were decreased over the course of the experiment. Even if the experiment started at 8.30am, often by the time measurements were made at ambient CO_2 levels and below, the depression of gas exchange had begun. Thus the rates measured in the experiments at lower CO_2 levels were affected by the controlled changes in the environment and the uncontrolled changes in the functioning of the plant. This is illustrated by a leaf measured in March, 1993. A was measured at 18 μmol m^{-2} s^{-1} at 10.15am on one day during the full light experiments (C_a was 367 μl l^{-1}), with g_s at 0.33 mol m^{-2} s^{-1}, E at 4.0 mmol m^{-2} s^{-1} and a C_i of 248 μl l^{-1}. During an AC_i experiment the following day (at 12.05pm) at a C_a of 340 μl l^{-1}, A was 12 μmol m^{-2} s^{-1}, g_s- 0.17 mol m^{-2} s^{-1}, E - 2.9 mmol m^{-2} s^{-1} and C_i - 213 μl l^{-1}.

It is possible to conclude from these results that a portion of the midday depression of gas exchange rates in season 3 can be attributed to an increase in the stomatal limitation, which led to a decrease in the availability of CO_2 for photosynthesis. Thus the factor or factors involved in the depression of rates acted on the opening on the stomata. A similar result was found in grapevines carrying fruit, with the afternoon depression in gas exchange resulting in a twofold decrease in g_s. However, the change in *l* only explained between 21% and 40% of the change in A, depending on the experiment, as the carboxylation efficiency also decreased in the afternoon (Downton *et al.*, 1987). Various other parameters have been found to influence *l* in a range of plants including light and nutrient levels during growth (*e.g.* Riddoch *et al.*, 1991); VPD levels (*e.g.* Tenhunen *et al.*, 1984; Tinoco-Ojanguren and

Pearcy, 1993); and water stress (*e.g.* Ni and Pallardy, 1992). But the carboxylation efficiency at low CO_2 concentrations was found to be lower under unfavourable conditions in these examples also.

This observed decrease in carboxylation efficiency reflects a decrease in the capacity of the mesophyll cells to fix CO_2. The mesophyll limitation upon photosynthesis was generally found to be greater than 50% in the examples cited above and in the *Paulownia* leaves studied. The major component of the non-stomatal limitation of the instantaneous rate of A is generally considered to be the rate of regeneration of the RuBP (Sharkey, 1985). But in the experiments reported here and in work reported by others (*e.g.* Ticha *et al.*, 1980; Tenhunen *et al.*, 1984; Riddoch *et al.*, 1991; and Tinoco-Ojanguren and Pearcy, 1993) where the operating point of the AC_i curve is on the linear portion of the graph, the rate of A continues to increase as C_i increases in a relatively linear way until very high C_i levels *e.g.* 600 $\mu l\ l^{-1}$. Beyond this point A continues to increase, but at a lower rate. This result implies that the activity of Rubisco is limited by CO_2 concentration even when CO_2 levels are twice those normally present in ambient air, and not by RuBP regeneration. In addition to this is the observation that the C_a:C_i ratio is linear at all CO_2 concentrations indicating a strong coupling of stomatal function and photosynthetic rates over the entire range of CO_2 concentrations. It is interesting to speculate as to why a wide range of plants show similar responses to artificially elevated CO_2 rates which are well beyond the range encountered in their natural habitats, and why they produce sufficient RuBP to meet the demands of photosynthesis at these elevated rates. It is one area of research that needs further study, particularly with the current concern of permanently elevated CO_2 levels due to human activity.

These results pose another question about the gas exchange strategy in *Paulownia.* If the stomatal limitation upon A is low and the supply of RuBP is not limiting and the leaves can operate with C_i values as low as 200 $\mu l\ l^{-1}$, then why do the leaves often photosynthesise at such a rate that C_i is much higher (*e.g.* 270 $\mu l\ l^{-1}$)? There must be other limitations upon the rate of A under favourable environmental condition, apart from the supply of CO_2 and RuBP. These leaves may be limited by triose phosphate utilization as discussed by Sharkey (1985) or perhaps by the amount and/or activity of Rubisco, or by some other unknown factor. That C_i can be so high indicates that the values of g_s are higher than required for the concomitant photosynthetic rate at that time. This situation may simply be a reflection of the need of the large leaves to lose heat (section 2.5.2).

On a longer term basis, the instantaneous rate of photosynthesis can be limited by nutritional and other factors influencing the concentration of key molecular components of the process. The most commonly studied nutrient is that of foliar nitrogen. In leaves from both the creche tree and Matangi trees over all three seasons, foliar N concentrations tended to range from

2.0% - 2.5%. Within this range there was no clear correlation between N and A, but in instances when the amount of nitrogen decreased to below 2%, A also tended to decrease. The other frequently studied correlation is that between leaf chlorophyll content and A. Here there was a clearer relationship with A tending to increase as chlorophyll level increased, particularly in season 3 (Table 2.19).

2.5.5 Changes in gas exchange with leaf ontogeny

In order to fully understand the change in leaf parameters with aging it is necessary to study individual leaves as they age (chronological aging) and to study a number of leaves of one age at one, or more, times in the season (age-structure dynamics) (Field and Mooney, 1983). This ensures that the data accounts for the seasonal influences on leaf aging as well as making comparisons between leaves responding to the same environmental factors operating at one time. Comparison of these two types of data sets from the light and CO_2 response curves from the *Paulownia* leaves in seasons 2 and 3 show that the pattern of leaf aging in *Paulownia* is similar to that documented for many other species (section 2.1.4).

The differences between species in terms of the relationship between leaf age and gas exchange lie in the rate of leaf expansion and achievement of physiological maturity, and in the maximum values of the various parameters. Thus in *Paulownia*, full leaf expansion takes about two months from emergence, but the leaves do not photosynthesise at the maximum rates until they are approximately three months old (Tables 2.9 and 2.10). The large size of the leaves probably accounts for the length of time required to full expansion as in other deciduous hardwood trees, leaf expansion often occurs much more rapidly. In poplar, for example, physiological maturity corresponded with the completion of leaf expansion at 12 - 14 days after leaf emergence (Reich, 1984), while in a range of families of black locust (*Robinia pseudoacacia*) the maximum rates of photosynthesis were measured 20 - 30 days after leaf emergence which was slightly after full leaf expansion (Mebrahtu and Hanover, 1991). However, in both coffee (Yamaguchi and Friend, 1979) and kiwifruit (Buwalda *et al.*, 1991) leaf expansion took about 1 month, but the rate of physiological maturation was much slower with A_{max} occurring three months after leaf emergence in coffee and at three - five months in kiwifruit. Thus it is obvious that plants have a range of leaf development strategies that may reflect the size, number and longevity of the leaves.

The age at which a leaf becomes a net exporter of photosynthates also varies between species. In season 1 measurement of the rates of gas exchange was undertaken of leaves of all ages down a branch of *P. elongata* and on *P. tomentosa* trees (Tables 2.5 - 2.7). The first leaves that were able to be analysed using the LICOR 6200 were probably about one month old,

although leaf 1 on tree 43 was probably older than that. These leaves had photosynthetic rates ranging from 4 - 11 $\mu mol\ m^{-2}\ s^{-1}$ which indicates that even before leaf expansion is completed, the leaves are able to contribute to the overall carbon fixation of the tree. It is not possible to determine from these results the exact point at which the leaves changed from being net consumers of photosynthates to net producers, but it had occurred by the end of the first month after leaf emergence. In other species, the rate of photosynthesis outweighed the rate of respiration within days of leaf emergence. For example, in black locust the rate of photosynthesis was greater than that of respiration when the leaves were ten days old (Mebrahtu and Hanover, 1991), while in cashew trees this occurred after only eight days (Balasimha, 1991).

As the rate of photosynthesis increases as the leaf matures, peaks and then slowly declines as the leaf continues to age, so other related parameters show similar trends in many species (section 2.1.4). In *Paulownia* leaves of different ages measured at one time in the season (Tables 2.5 - 2.7) the relationship between A measured under saturating light and g_s, N and chlorophyll content was weak. There was a slight tendency for these parameters to be lowest in the very youngest and very oldest leaves, but there were a wide range of values corresponding to A_{max} in the leaves of intermediate ages. C_i was low in the first three leaves on the *P. elongata* branch, indicating that g_s was imposing a limitation on A, but in the *P. tomentosa* trees the pattern of change in C_i with leaf age was less clear.

The chronological comparisons of gas exchange in the same leaves throughout season 2 were similar to those in season 1, with only weak trends in the relationships between A_{max} and g_s, C_i, $PPFD_{sat}$, WUE, Q_e and r with leaf age (Tables 2.9 and 2.10). There was more variation of values between leaves of the same age than during the ontogeny of any one leaf. The destructive nature of analysing for N and chlorophyll content meant that only limited data is available on the change in these factors with leaf age. The N content of older leaves (eighth pair) was lower than in younger leaves (fifth pair), as were stomatal numbers, but chlorophyll concentration was higher. This corresponded with a higher rate of A in the younger leaves. The decline in A has been observed to occur more quickly in *Nicotinia* leaves than the decline in chlorophyll (Sestak and Catsky, 1962), while in poplars Ceulemans and Impens (1979) reported that A_{max} occurred at leaf plastochron index 8, while maximum leaf chlorophyll content occurred at leaf plastochron index 13. Thus chlorophyll content does not always follow the same pattern as A_{max} during leaf ontogeny.

These results indicate that as the leaf ages the factors that cause the change in A impact independently on g_s, so that the values of these two parameters do not change at the same rate (Fig. 2.19). This can be seen in the comparison of AC_i curve parameters measured in

consecutive months on the same leaves (Table 2.18). As the leaves age A and A_o decrease to a greater extent than the increase in *l* and in leaf 2, the direction of change is not always consistent. Thus the increase in the stomatal limitation on photosynthesis explains only a portion of the observed decrease in A. In leaf 1, the proportion of change in the photosynthetic rate at CO_2 saturation (A_{2000}) was similar to that for A and A_o, while in leaf 2 A_{2000} decreased less than A and A_o.

In leaves of *Phaseolus vulgaris*, Ticha *et al.* (1988) found that A_{max} occurred at 11 days after sowing, while C_i was at a minimum 15 - 17 days after sowing. The decrease in A from day 11 to day 12 correlated with an increase in *l* and a decrease in g_s. Later in the life of the bean leaf, *l* decreased as the mesophyll limitation increased during senescence. Thus in bean leaves, as in *Paulownia* leaves, A and g_s vary independently of one another, with the rate of decrease in A from day 11 to day 17 less than that of g_s. This result is similar to that found in poplar leaves where g_s showed a bimodal response to leaf age and WUE changed as the leaves aged (Reich, 1984). In contrast, the variation in A and g_s in cotton leaves as they aged were such that C_i and WUE were constant over the life of the leaves (Constable and Rawson, 1980). In *Lepchinia calycina*, WUE, light use efficiency and nitrogen use efficiency were also found to be constant over the lifetime of the leaves (Field and Mooney, 1983). The strong correlation between A and foliar N content is common to many species *e.g.* kiwifruit (Buwalda *et al.*, 1991) and oak and maples (Reich *et al.*, 1991) and Field (1983) discussed this as a response to maintaining the distribution of nitrogen throughout the plant that maximizes photosynthetic carbon gain. He found that in *Lepchinia calycina*, 80% of the difference in A_{max} among fully expanded leaves could be explained by nitrogen content, and that the decrease in N as the leaves aged was related to the decrease in light available to these leaves due to the growth of new leaves.

There appeared to be a relationship between gas exchange and leaf node in the *P. tomentosa* trees in their first year of growth (Tables 2.6 and 2.7). The leaves analysed were arranged at right angles on the stem to the leaves above and below, so the first, third, fifth leaves etc. were all on the same vertical plane, and were the second, fourth and sixth leaves etc. were at 90° to them. Both sets of leaves were north facing, with one set being more northeast facing and the other northwest facing. It is not possible to determine which set of leaves were oriented which way. In both trees 35 and 43 at both measurement times, alternate leaves had higher rates of photosynthesis than either the leaf that was immediately younger or immediately older. This pattern was not caused by light at the time of analysis as it was always saturating. There was less of a consistent trend in g_s and C_i and no pattern in nitrogen or chlorophyll content. The observed pattern may have been a response to the light environment during growth, with one set of leaves receiving more light during the day, or light of a slightly different composition at either end of the day. It is also possible that wind was a factor with

the more western facing leaves probably subjected to more wind than the other set of leaves. This effect was probably confined to the very large leaves in the first year of growth, but it was not tested for in subsequent seasons. Certainly, there was no pattern of gas exchange relating to leaf position observable on the branch of the mature *P. elongata* tree (Table 2.5) and Reich (1984) found no effect of nodal position on g_s in poplar leaves.

2.5.6 Seasonal changes in gas exchange

Photosynthetic rates tended to be higher in the latter period of each of the three seasons studied, for both the *P. elongata* tree and the *P. tomentosa* trees. In season 1, the values of A_{max}, g_s and ψ_p were generally higher in February and March than in December (Tables 2.2 - 2.4) for leaves on the *P. elongata* tree. In both fully expanded and fully mature leaves in season 2, A and g_s were highest in February (Tables 2.11 and 2.12, Figs 2.6 and 2.7) under the conditions of light response experiments, as were N and chlorophyll levels in leaves from the Matangi trees (Table 2.13). Data from the diel experiments showed that A_{max} and g_s were highest in February on tree 72, but there was little difference in the values from tree 43 from December through to March, which were all higher than those measured in November. The highest rates of A and g_s were measured in March on leaves from the creche tree during diel experiments in season 2, with little difference in the values over the remaining months.

The trends of gas exchange parameters on the Matangi trees were somewhat different in season 3 (Table 2.19). Rates of A and g_s were lowest in November in both trees when measured at full light, as were leaf chlorophyll contents. In tree 43, values for these parameters were relatively constant over the remaining months. There was no clear trend of a seasonal increase in A or g_s in tree 72, with low rates in January and very high rates in March when chlorophyll levels were also high. The values of A and A_o from the AC_i experiments on trees 43 and 72 were similar, with the lowest rates measured in February when l was highest (Table 2.17). Thus the trend of high rates of gas exchange measured on these trees in February in season 2 was not repeated in season 3, but there were some experiments in which the rates reached their peak in March.

The changes in the values of other gas exchange and leaf parameters did show some correlations with the seasonal changes in A and g_s, but not on a consistent basis over the three seasons.

The overall pattern of generally lower rates of gas exchange early in the season which increased over the course the season may be a response to various environmental parameters. The temperature of the soil at 10 cm and 30 cm was considerably lower in September and

October in seasons 2 and 3 (Tables 4.2 and 4.3) than later in the growing season, which is normal (Table 4.4). It is possible that these lower soil temperatures during the development of the leaves that were assayed in November and December influenced the maximum photosynthetic rates these leaves were able to attain. Lower soil temperatures are known to increase the root resistance to water uptake, so leaves developing at that time may have have done so when both water and nutrients within the plant were limited. The extent of influence of both soil and air temperature on gas exchange is examined in Chapter 3, while a full discussion of whole plant functioning is given in Chapter 6.

It is interesting to note that in season 2, soil moisture decreased to moderately low levels in December, but remained reasonably constant throughout the rest of the season (Table 4.2). Gas exchange rates were a little lower in some leaves at that time when compared with leaves in November. In season 3, a more severe soil moisture limitation was apparent from late December until late February. This corresponded to a decline in A and an increase in l in February during AC_i experiments (Table 2.17), and a deviation in the seasonal trend observed at full light in both trees. It is likely that although soil moisture levels do not appear to play a major role in determining gas exchange rates on a diurnal basis, longer term soil moisture deficits do limit photosynthetic rates by decreasing stomatal opening at all times during the day. A similar response to water stress is common in many species. Analysis of gas exchange in grapes that had been watered either 2 days or 18 days prior to measurement found that the recently watered vines maintained higher rates of A and g_s throughout the day than those watered 18 days earlier (Downton *et al.*, 1987). They suggested that the response may be mediated by ABA accumulation.

The higher rates of gas exchange in the latter part of each season in the mature *P. elongata* at the creche may have been related to the increase in the size and number of sinks at that time. As noted earlier (section 2.4.1.1), fruit development occurred throughout the season, with the production of the next season's flower buds starting about mid season. Both these processes would create large sinks as large numbers of fruit and flowers were produced annually. Thus it is possible that higher rates of photosynthesis were needed to sustain the development of both reproductive structures from February onwards.

Comparison of the seasonal trends observed in *Paulownia* with those reported for other species is complicated by the fact that many studies are undertaken on leaves that are produced only at the beginning of the season as a single cohort. Thus seasonal variation in gas exchange is compounded by changes in leaf age as the season progresses. Some work has been done on the gas exchange of leaves over several seasons in deciduous, woody species. Reich, Walters and Ellsworth (1991) found little change in gas exchange and leaf

parameters in leaves of mature oak and maple measured over four consecutive seasons. However, in grapevines studied over two seasons, the diurnal pattern of photosynthesis differed between seasons. This change was related to changes in air temperature and humidity between the seasons (Slavtcheva, 1990). In a series of defruiting experiments undertaken on plum trees in two consecutive seasons, it was found that the size of the decline in gas exchange as a result of the defruiting treatment differed between the seasons (Gucci *et al.*, 1991).

In the mature *P. elongata* tree at the creche, the gas exchange rates measured in seasons 1 and 2 were similar (Tables 2.2 - 2.4, Figs. 2.7c, 2.8c and 2.10b). This is despite the fact that the leaves analysed in season 1 were generally first fully expanded leaves rather than first mature leaves and that a different gas exchange measuring system was used. Rates of A and g_s were slightly higher in the first season, particularly later in the season. $PPFD_{sat}$ was estimated to be approximately 800 $\mu mol\ m^{-2}\ s^{-1}$ from the diurnal curves undertaken in season 1 with ambient light levels, which was a little lower than that calculated from the light response curves in season 2. The estimation of r as approximately 20 $\mu mol\ m^{-2}\ s^{-1}$ from the diurnal experiments was within the range calculated in season 2.

Comparisons of the gas exchange analyses undertaken on the *P. tomentosa* trees over the three seasons are complicated by the immature nature of the trees. However, the rates of gas exchange were all within the same range over the three years and over the range of experiments. The exact pattern of seasonal trends was different between seasons 2 and 3, as discussed above. The nitrogen content of the leaves produced each season did not show any change as the trees aged, but chlorophyll contents were slightly higher in leaves in season 3 than in the previous two seasons.

In season 3, the highest rates of A were correlated with somewhat lower stomatal conductances than in the previous seasons. This led to higher water use efficiencies, which tended to increase as the season progressed (Table 2.19). The stomatal limitation of photosynthesis was also generally higher in the latter part of season 3 than at a comparable time in season 2. From these results it can be postulated that the stomata of the leaves on the trees in their third season of growth were regulated to a greater extent than stomata on leaves in previous seasons. This is possibly correlated with the decrease in leaf size from season to season and the corresponding decrease in the need to keep the g_s value high to dissipate heat.

2.5.7 Summary of gas exchange in *Paulownia*

It is possible to conclude from the results of the various gas exchange experiments discussed

here that *Paulownia* is light demanding tree, capable of very high rates of gas exchange under favourable environmental conditions. The leaves operate in such a way that there is little stomatal limitation of photosynthesis, with high values of g_s measured on several occasions. This is correlated with high rates of transpiration, and it is postulated that the leaves operate with high g_s and E in order to maintain leaf temperature at a level that does not adversely affect gas exchange processes. Photosynthetic rates are more limited by mesophyll capacity, but the mechanisms of that limitation were not determined.

Despite the very high rates of transpiration and the maintainence of high leaf water potentials at all times throughout the day, the water use efficiency of the leaves is comparable with that of other perennial, woody species. Water stress on a diurnal basis does not affect gas exchange rates to a large extent, but in the longer term soil moisture levels do regulate the functioning of the leaves. Gas exchange rates over the day appear to be regulated, to a greater degree, by some form of negative feedback inhibition.

Thus, the high light requirement, high water use and high gas exchange rates exhibited by *Paulownia* are indicative of a seral plant, adapted to open habitats rather than to dense forest situations.

Chapter 3
The Influence of Temperature During Development on Photosynthetic Rate, Plant Biomass and Nutrient Allocation

3.1 Introduction

3.1.1 General effects of temperature on plant growth

The temperature regime is just one of the many environmental factors that affects plant growth rate, biomass and nutrient partitioning, photosynthesis and other plant processes. The complex interaction of these environmental variables - light quantity and quality, photoperiod, nutrient availability, air and soil temperatures, CO_2 levels and water availability - on the functioning of plants is still poorly understood.

The effect of temperature on plant growth and photosynthesis has been studied in a wide range of species from pasture grasses and clover (Davidson, 1969), tall fescue (*e.g.* Treharne and Nelson, 1975), wheat (*e.g.* Dale, 1982b), tomato (Gent, 1986), wild strawberry (Chabot and Chabot, 1977) through to Engelmann spruce (Delucia 1986), radiata pine (*e.g.* Rook, 1969), jojoba (Wardlaw *et al.*, 1983), banana (Turner and Lahav, 1983), *Nothofagus spp.* (Read, 1990), eucalypts (*e.g.* Slayter and Ferrar, 1977) and many other plants. Generally for each species there is an optimum temperature range for growth which is related to the plants natural habitat, hence tropical species tend to grow better at higher temperatures than those plants found in alpine or arctic environments. However, there are also maximum and minimum limits for each species, beyond which growth is retarded and physical damage may occur.

Reviewing a series of experiments undertaken to compare species endemic to coastal California with others found in Death Valley, Bjorkman *et al.* (1980) observed that the temperature at which the highest growth rates and rates of photosynthesis for each species was measured, correlated closely with the plant's original habitat. Subjecting the coastal species to the temperature regimes of Death Valley led to their death, while growth of the Death Valley plants was inhibited in the coastal environment (Bjorkman *et al.*, 1980).

Most plants have the ability to acclimatise to changes in temperature with time, and indeed must do so in order to deal with the changing seasons (Berry and Bjorkman, 1980). This acclimation process results in different temperature optima for growth and photosynthesis, depending upon the new temperature regime. The process may be quite rapid - in radiata

pine seedlings, for example, rates of photosynthesis and respiration responded to significant changes in both day and night temperatures within two days (Rook, 1969). In *Encelia california*, a shrub of the Asteraceae family, acclimation of gas exchange parameters started to occur after 24 hours treatment at warm temperatures in previously cold treated plants (Mooney and Harrison, 1970), while in *Nothofagus spp* acclimation to a different temperature regime was complete within eleven days (Read, 1990).

3.1.2 The influence of temperature on whole plant functioning

Temperature influences on the various plant processes can be broken down into the effects on whole plant functioning (*e.g.* allocation of biomass and partitioning); on tissue development; and on development at the cellular level. At the whole plant level, studies on the allocation of biomass in a range of grasses showed that the optimum temperature for foliage growth (when the R:S ratio was lowest) differed between species depending on their natural habitat. The R:S ratio increased at growth temperatures above and below the optimum (Davidson, 1969). In tall fescue, Treharne and Nelson (1975) showed that the R:S ratio was higher in plants grown at 10°C than those grown at 25°C. However, in jojoba plants Wardlaw *et al.* (1983) found that the R:S ratio varied little with changing growth temperature regimes. Plants grown at mean temperatures below 20°C had specific leaf dry weights greater than those from plants grown at higher temperatures and this was correlated with an increase in leaf thickness with decrease in growth temperature.

The R:S ratio in grapevine cuttings, grown at a range of temperatures and light intensities, was relatively constant at about 0.15 between 20°C and 30°C, for both medium and high light levels. However, the distribution of biomass between leaf and stem tissue changed so that the leaf weight per plant was greatest at the highest temperature, while stem weight decreased with increasing temperature (Buttrose, 1968). Working with banana plants at a range of day/night temperatures, Turner and Lahav (1983) found that the growth of leaf matter was greatest at the 33°C/26°C regime, but that the greatest whole plant dry weight was produced at intermediate regimes - 25°C/18°C and 21°C/14°C. The R:S ratio was highest at intermediate temperatures, when it was more than double that at the warmest temperature combination tested (37°C/30°C). In contrast, the accumulation of dry matter in cotton increased with an increase in growth temperature from 20°C to 33°C, while the R:S ratio decreased (Winter and Koniger, 1991). The nature of the biomass allocation response to growth temperature by plants seems to be very species specific. One generalisation that is possible is that as growth temperature increases within the range tolerated by a particular plant, the R:S ratio decreases indicating an increased allocation of biomass to shoot material (Farrar, 1988).

Carbohydrate partitioning also changes at different growth temperatures. In jojoba plants

lower temperature regimes (18°C/13°C) resulted in increased starch levels in both young and mature leaves. In young leaves glucose levels were higher at lower temperatures, but in mature leaves temperature had no effect. Sucrose levels in young leaves were unaffected by temperature, but were higher in mature leaves at higher temperatures (30°C/25°C) (Wardlaw *et al.*, 1983). The starch level of whole tomato plants changed with temperature and the proportion of TNC present as starch changed from 80% - 90% at growth temperatures above 13 °C to 50% at 9°C (Gent, 1986). A similar response to very low temperatures was observed by Delucia (1986) in the root tissue of Engelmann spruce. The total level of carbohydrates was the same in roots held at 20°C as those chilled for five days at 0.7°C, but the relative level of starch decreased on chilling and the concentration of soluble sugars increased. The needles and stems of the same plants showed less response to the chilling treatment, with the needles maintaining similar concentrations and ratios. However, after chilling, the starch concentration increased slightly in the stem as the soluble sugar content dropped.

3.1.3 Temperature and leaf development

Under normal growing conditions the expansion of the leaf is dependent upon the rate and duration of cell division and the enlargement of the cells (Dale, 1982a; Ong and Baker, 1985). The final size of the lamina will be a result of the interaction of the various environmental and internal influences on these cellular processes. Development of the leaf blade is uneven, with a basipetal gradient in cell divisions resulting in divisions ceasing first in the tip of the lamina and last at the base (petiolar end). Division continues in the palisade layer for a longer period than in the epidermis, whereas cell enlargement is greater in the epidermal cells than in palisade cells (Dale, 1982a). Observations of the extension of leaves in two species of the grass *Phleum* have shown that extension is completed more rapidly in plants growing at 20°C than those growing at 10°C. This is coupled with an increase in leaf area, in the numbers of epidermal cells and in the frequency of cell divisions at the higher temperature. The resultant leaves are thinner, with smaller cells, but with higher specific leaf areas (Woodward, 1979). In the first four leaves of wheat plants, leaf areas were not affected by temperature of growth, but those leaves grown at 20°C had more rapid leaf extension, but for slightly shorter duration, than leaves grown at 15°C (Dale, 1982b). The number of cells in the first two leaves was higher at 20°C than at the lower temperature, but in leaves three and four was unchanged.

Interactions between light level, temperature regime and various anatomical and physiological processes have been investigated in the woodland strawberry (Chabot and Chabot, 1977). Under conditions of low light (25 μmol m^{-2} s^{-1}), leaves were thin with poorly developed

mesophyll cells and no palisade layer. The leaves became thicker with increasing light levels, with an increased specific leaf weight and normal cellular development with mesophyll cells making up a higher percentage of the leaf volume. This, in turn, led to higher mesophyll volume: leaf surface area ratios and mesophyll surface area: leaf surface area ratios. Similar results were found in experiments with light levels on cellular development in *Plectranthus* (Nobel *et al.*, 1975). Growth of the strawberry leaves proceeded normally at moderate temperature regimes (20°C/10°C and 30°C/20°C) but was disrupted at high temperatures (40°C/30°C) and low temperatures (10°C/2°C). At these two extremes the leaves were thicker than at the two moderate treatments, but the relationships between mesophyll cell size and leaf area were less clear cut than for the light treatments. Photosynthetic rates were highest for the plants grown at the moderate temperature regimes and least in the cold treated plants (Chabot and Chabot, 1977).

In a similar series of experiments on six populations of ryegrass, Charles-Edwards *et al.* (1974) found that photosynthesis, expressed as a rate per unit leaf volume or per unit mesophyll cell volume, increased with increasing growth temperature from 11°C up to 24°C, but decreased again in plants grown at 28°C. Mean leaf thickness tended to decrease with increasing growth temperature as did the number of vascular bundles and mesophyll volume. A significant correlation, found between leaf thickness and mesophyll cell volume, indicated that the difference in leaf thickness in different environments was a result of changes in the size of mesophyll cells, rather than a change in cell numbers.

3.1.4 The effect of temperature on the photosynthetic apparatus

At the biochemical level, the effect of temperature on the rate of photosynthesis at any one instant is the result of temperature interactions with all the components of the photosynthesis mechanism (Berry and Raison, 1981). These components include the quantity and functioning of the various enzymes involved, the activity of the electron transport chain, the uptake and diffusion of CO_2, the integrity of the chloroplast membranes, and the ratio of carbon fixation to photorespiration.

Analysis of the temperature response of the electron transfer chains in the chloroplast membranes has shown a remarkable similarity to the whole leaf photosynthesis temperature response, suggesting that the determination of the temperature optimum for the photosynthetic rate of the leaf is a result of the influence of temperature on the chloroplast membrane, when photorespiration is inhibited (Berry and Raison, 1981). Bjorkman *et al.* (1980) showed that adaptation to high temperature conditions was related to the thermal stability of the chloroplast membrane reactions, especially those of PS II. They also found

that some of the enzymes associated with the carbon fixation pathway were inactivated at temperatures that reduce photosynthesis, *e.g.* Ru5P kinase and NADP malate dehydrogenase. Other enzymes, such as Rubisco and PEP carboxylase, were found to be more thermo-stable. Changes in the activity of fructose-1,6,-bisphosphate phosphatase in plants of *Nerium oleander* appeared to be closely correlated with changes in the photosynthetic rate of plants moved from moderate temperature regimes (20°C) to higher temperatures (45°C), and in plants moved from high to moderate temperatures (Bjorkman *et al.*, 1980). There was no difference found in the temperature response curve of some enzymes involved in photosynthesis, photorespiration and dark respiration isolated from leaves of tall fescue grown at 10°C or at 25°C (Treharne and Nelson, 1975). The activities of Rubisco and glycolate oxidase did not differ between temperature treatments, but in leaves grown at 10°C the activities of malate dehydrogenase and isocitrate dehydrogenase (dark respiration enzymes) were higher than in leaves grown at 25°C, which was reflected in the increased respiration rate for the cold treated leaves. These observations indicate that the qualitative characteristics of the enzymes were unchanged by temperature treatment, but that the quantitative features were affected.

In order for the whole photosynthetic apparatus to function effectively, a steady supply of CO_2 is required. There are two major sites of resistance to the diffusion of CO_2 into the leaf - the first is at the stomata and the second is at the mesophyll cells. Treharne and Nelson (1975) found that the number of stomata on leaves of tall fescue grown at 10°C was lower than on leaves grown at 25°C. Stomatal resistance at all temperatures measured (13°C, 24°C, 29°C, 34°C) was lower for leaves grown at 25°C. For leaves from both growth treatments, stomatal resistance decreased with temperature up to 29°C, and then increased sharply at 34°C. The resistance to CO_2 diffusion, both through stomata and intracellularly, was found to increase with decreasing growth temperature in *Eucalyptus pauciflora*, and this was coupled with decreasing photosynthetic rates (Slayter and Ferrar, 1977). Similarly in *Encelia*, Mooney and Harrison (1970) observed decreasing stomatal and mesophyll resistances as plants were moved from growth temperatures of 15°C/2°C to 30°C.

As can be seen from the above discussion, the complex interaction of temperature on plant growth and functioning is multi-faceted, impinging on gross anatomical development through to sub-cellular processes. This complexity is further increased by the fact that it is not just the mean temperature of growth that influences plant development, but the day/night regime is also important. Jojoba plants grown at the same daytime temperature of 23°C, but with decreasing night temperatures (18°C, 13°C and 8°C) showed a decrease in shoot extension and an increase in specific leaf weight (Wardlaw *et al.*, 1983). Working with wild strawberry plants grown at the same mean temperature but with different day/night regimes

(20°C/20°C, 30°C/20°C and 35°C/15°C), Chabot and Chabot (1977) observed increased leaf thickness and specific leaf weight with increased temperature amplitude. In addition to this, the soil temperature during growth not only influences root development, but also above ground tissue growth as investigated in Engelmann spruce (Delucia, 1986) and in apples (Tromp, 1977), for example.

3.1.5 The role of temperature in the growth of *Paulownia*

Paulownia is a genus of temperate regions and the optimum range for growth and photosynthesis lies between 20°C and 30°C and may vary between the species. The deciduous nature of the tree allows it to withstand the winter frosts and snow of its native habitat (Zhu *et al.*, 1988).

The effect of temperature on the growth, photosynthetic rates and nutrient content of *P. tomentosa* (Japan) trees in their first season was studied in this experiment. It had been observed in the 1991 - 1992 season that fully expanded leaves on the trees at the Matangi experimental site had different photosynthetic rates, depending on when they developed. Thus, leaves maturing in November had lower maximum rates of photosynthesis than leaves maturing in February. It was the hypothesis that the temperature during leaf development affected the maximum rate of photosynthesis attainable by the mature leaf, that was tested in this experiment.

3.2 Materials and Methods

Root stumps of *Paulownia tomentosa* (Japan) were planted, on 4 September 1992, in large (100 l) pots in commercial potting mix. Of the seven stumps planted, three remained outside for the duration of the experiment and four were moved into an adjacent glasshouse on the campus of the University of Waikato. Plants in the glasshouse sprouted within ten days, whilst those outside required 17 - 19 days for shoot emergence. Multiple shoots arose on each of the plants and when one of these had reached approximately 30 cm tall, the other shoots were removed to leave a single leader.

Environmental variables, other than temperature, were kept as similar as possible between the two groups of plants. Each set of pots was kept well watered and, from November onwards, each pot was watered weekly with 2 l of a complete commercial fertilizer solution (N - 96 ppm, P - 68 ppm, K - 320 ppm, Ca - 185 ppm, S - 69 ppm, Mg - 56 ppm, Na - 2 ppm, Mn 1.9 ppm, Zn - 0.11 ppm, Cu - 0.13 ppm, Fe - 0.16 ppm, B - 0.40 ppm). In addition, 100 g of Osmocote™ slow release fertilizer was added to the pots in early November in an attempt to ensure that the plants were not limited by nutrients. All the trees were sprayed in October with Captan™, a fungicide and Attack™, an insecticide; in November with Basudin™, an insecticide; and in December the inside trees were sprayed with Orthene™, an insecticide. The insecticidal sprays were necessary to combat the whitefly infestations that were particularly bad in the glasshouse.

There were differences in the environment between the two positions of the plants that were less controllable - primarily wind and light. The outside plants experienced windier conditions than those inside, while those trees inside the glasshouse had less light available. Generally the light levels inside were about 50% - 60% of those outside. The RH of the two environments was similar.

The daily minimum and maximum temperatures in each environment were recorded and monthly averages of these were calculated (Table 3.1). Gas exchange rates were measured on fully expanded leaves as described previously (section 2.3.2). Measurements were made at the beginning of each month, from November, 1992 until the termination of the experiment in January, 1993. Each leaf was measured under standard conditions of full light (2100 μmol m^{-2} s^{-1}), an air temperature of 22°C, a dew point of 6°C and ambient concentration of CO_2 (340 - 370 μl l^{-1}). Two to four leaves were measured on each of the outside trees each month, and on three of the inside trees. Some leaves were measured in successive months. The length, width and petiole length was measured for each leaf that was sampled and a small section of the leaf was removed for analysis of chlorophyll content (for method see 2.3.3),

nitrogen content, specific leaf weight and DW/FW ratio. Tree height was measured each month and relative and absolute growth rates were calculated.

The experiment concluded in early January 1993 because the height of the inside trees (approximately 3 m) threatened the roof of the glasshouse. At this time each of the six sampled trees was harvested. This involved cutting the stem at the base and measuring the length, width, area and petiole length of each leaf and the fresh and dry weights of the stem and leaf components. The fresh and dry weights of the roots were also determined after the roots had been washed clean. There was some minor loss of root material in the washing process, but this was consistent between all the trees. Most of the tissue was oven-dried at 70°C for dry weight determination. Some material from four of the trees was freeze-dried for subsequent nutrient analysis and soluble carbohydrate analysis as per the methods in section 4.4.1 and 4.5.1.

3.3 Results

3.3.1 Temperature regimes during growth

The daily minimum and maximum temperatures for the two environments, averaged on an approximately monthly basis, are given in Table 3.1. The temperatures in the glasshouse increased from the first month to the second, but stayed constant for the remainder of the experiment. Those temperatures recorded outside continued to increase for the first three months (September through to November), but were then constant.

Table 3.1. The average minimum and maximum temperatures (°C) measured inside the glasshouse and outside in the open (standard errors are given in parentheses).

	Inside Glasshouse			Outside Glasshouse		
Month	Minimum	Maximum	Range	Minimum	Maximum	Range
1[a]	14 (0.28)	21 (0.20)	10-24	6 (0.35)	17 (0.30)	2-20
2	16 (0.25)	25 (0.61)	13-32	9 (0.54)	20 (0.47)	4-24
3	16 (0.23)	25 (0.49)	12-30	12 (0.43)	23 (0.32)	7-27
4	16 (0.21)	26 (0.47)	13-30	12 (0.59)	24 (0.40)	5-28

[a] Month 1 goes from 4 September, 1992 to 10 October, 1992; month 2 from 11 October to 10 November, 1992; month 3 from 11 November to 8 December, 1992; month 4 from 9 December, 1992 to 5 January, 1993.

The temperatures recorded inside the glasshouse were always higher than those recorded outside, especially the minimum temperatures. Early in the experiment the average maximum temperatures were also higher in the glasshouse, but the difference in maximum temperatures between the two environments decreased as the experiment progressed. The range of temperatures, from the lowest minimum in the month to the highest maximum, was greater outside than inside, as the glasshouse environment tended to even out temperature extremes with artificial heating and cooling systems. The highest maximum each month, however, was recorded inside the glasshouse.

3.3.2 Plant growth

The roots planted in the glasshouse sprouted about 7 - 9 days earlier than those outside, suggesting an initial temperature effect on root development. The weight of the root stump planted had no effect on the rate of shoot emergence or the subsequent growth of the plant.

Table 3.2. Growth parameters of *P. tomentosa* grown inside and outside the glasshouse.

	Inside Trees			Outside Trees		
	1	2	3	1	2	3
Initial root wt [a]	0.14	0.13	0.12	0.12	0.19	0.12
Height: Nov	1.17	1.03	1.19	0.46	0.42	0.41
Height: Dec	1.95	1.96	2.05	1.19	1.10	1.12
Height: Harvest	2.90	2.39	2.85	1.84	1.65	1.77
DBH	40	32	40	37	28	30
Collar Diameter	53	58	55	55	49	54
Leaf Area	8.3	5.6	6.8	6.1	4.4	5.8
Specific leaf wt	74.4	71.9	58.9	64.1	83.9	72.5
Fresh Weight						
- Above ground	5.18	3.66	4.16	4.32	3.24	3.88
- Below ground	3.84	3.60	4.05	2.69	2.76	3.22
Dry Weight						
- Above ground	1.51	1.00	1.16	0.90	0.78	0.92
- Below ground	0.79	0.63	0.88	0.53	0.48	0.56
Total Biomass	2.30	1.63	2.04	1.43	1.27	1.48
R:S Ratio	0.52	0.63	0.76	0.59	0.62	0.61

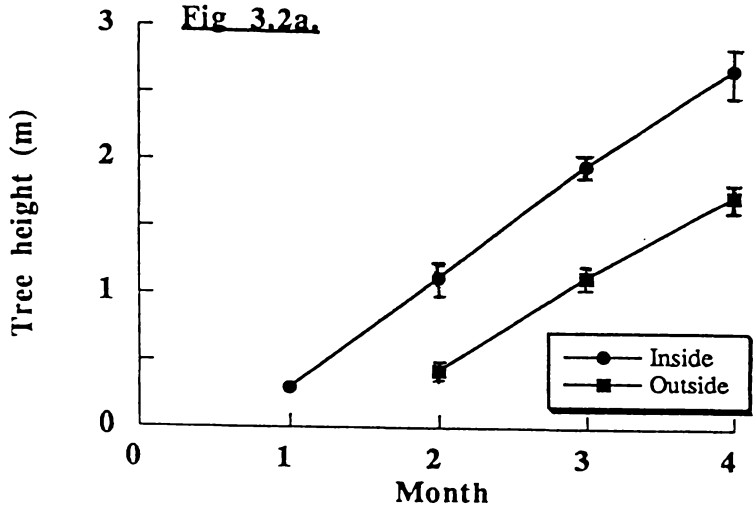
[a] Units for weight - kg; height - m; diameter - mm; leaf area - m^2; specific leaf weight - g m^{-2}.

At the conclusion of the experiment the trees inside were approximately 2.7 m tall in comparison with those grown outside that averaged 1.75 m in height (Fig 3.1). Although there was a difference in height between the two sets of trees, the diameter at the base of the stem was similar for all six trees. This may have been a result of the outside trees developing thicker stems as a result of exposure to stronger winds than the inside grown trees. However, a comparison of the ratio of stem dry weight to height showed that the stems of inside grown trees were heavier for a given length, than outside grown trees. The DBH (diameter breast height) measurements were greater for the inside grown trees because the stems were taller and so the stem tissue at 1.37 m was older (and thus thicker) for these trees.

The absolute growth rate of the height of the inside plants (G=0.79 m $month^{-1}$) was higher than that of the outside plants (G=0.66 m $month^{-1}$), but relative growth rates were similar for the two environments. R from October to January for the inside trees was 0.73 m m^{-1} $month^{-1}$, while from November to January, R was 0.70 m m^{-1} $month^{-1}$ for the outside trees. The absolute growth rate was constant through the season for both sets of trees (Fig. 3.2a),



Fig. 3.1. *P. tomentosa* trees after four month's growth. The tree on the left was grown inside the glasshouse, the tree on the right was grown outside the glasshouse. Scale: author height 1.72 m.



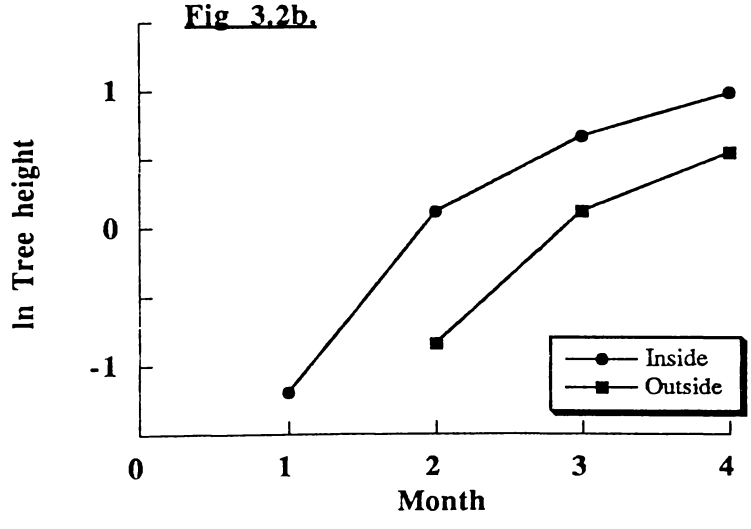


Fig. 3.2a, b. Growth of P. tomentosa trees inside and outside the glasshouse.
a. - height of trees; b. ln transformed height data.
Month 1 - Oct; 2- Nov; 3 - Dec; 4 - Jan.

but R declined as the season progressed (Fig. 3.2b). Comparison of R for each month showed that the outside trees tended to be about a month behind the inside trees (Fig. 3.2b). From October to November, for example, R was 1.04 m m^{-1} $month^{-1}$ for the inside trees, while it was 0.97 m m^{-1} $month^{-1}$ for the outside trees from November to December.

Two months after planting the trees inside had an average of 8 leaf pairs with the fully expanded leaves being approximately 0.6 m in length and 0.6 - 0.7 m wide. At the same time, those trees growing outside had 5 - 6 pairs of smaller leaves (approximately 0.3 m x 0.3 m). The number of leaf pairs increased to between 10 and 12 in the inside trees by December (of which 5 were new pairs), and the average size of leaves increased slightly with some as large as 0.8 m x 0.8 m. The outside trees averaged 8 leaf pairs at this time, with 4 of those pairs having been initiated in the previous month, with sizes up to 0.6 m x 0.6 m. At the final measurement of photosynthetic rate, just prior to harvest in early January, the trees grown inside had 12 - 13 leaf pairs (3 - 4 new pairs), while those outside averaged 10 pairs (4 new pairs). The sizes of the fully expanded leaves of both sets of trees were similar to those measured in December.

The trees grown inside accumulated more biomass, both above and below ground, over the experimental period than those grown outside. The allocation of the biomass varied between individuals more than between treatments, so the R:S ratio was not affected by growth temperature. Although the leaf areas were generally higher for inside grown trees, the LAI (leaf area index) for all the trees was reasonably constant, being between 2 and 3. As the results for the specific leaf weight show, the leaves in the two environments did not differ significantly in thickness. The water content of the root tissue did not change between growth regimes (at about 80%), but the DW:FW ratio showed that water content of the above ground tissues was higher in the outside grown trees at 77%, than those trees grown inside (72%). This was probably a result of the inside trees having a greater amount of lignified stem tissue than the outside trees.

At the time of harvest the trees were severely pot bound, with root tissue exploiting the entire volume of the pots, and protruding beneath the pots. In comparison with trees harvested from the field (section 4.3.2.2), there were less woody roots per tree in the pot grown plants. This was compensated for by an increased volume of non-woody roots (loosely termed 'fine' roots) which were generally yellowish in colour, often with bright orange tips. Many of these fine roots were in fact quite thick and fleshy with diameters up to 3.5 mm. The nature of the soil environment, with regular supplies of water and nutrients, may have precluded the lignification of root tissue and instead favoured the proliferation of roots capable of greater absorption. There were no observable differences in the composition of root tissues of trees from the two different environments.

3.3.3 Nutrient and soluble sugar content of tissues

The levels of the various nutrients found in the tissues of inside trees 1 and 3, and of outside trees 1 and 2 were averaged (Table 3.3). The concentrations of N, P, S, Ca and B were generally higher in the lamina tissue while the level of K was highest in the petioles. The concentration of potassium in all tissues was high which was a reflection of the amount of potassium in the liquid fertilizer fed to the trees each week. The highest levels of the remaining nutrients were found in the fine roots, the primary site of nutrient uptake in these trees.

Most of the nutrients were found in higher or similar concentrations in the various tissues of the inside trees when compared with the outside trees. Ca was, however, found in higher levels in the tissues of the outside trees, except in the fine roots and levels of several nutrients were higher in stems of the outside trees. The difference in the temperature regimes that the two sets of trees were grown under was unlikely to be enough to cause significant changes in nutrient concentrations between the trees. Although colder temperatures do cause an increase in nutrient levels in many plants (Clarkson *et al.*, 1988) the temperatures studied by most workers are much lower, on average, than the regime the outside trees were grown in.

Table 3.3. Nutrient levels in *P. tomentosa* tissues after four months growth either inside or outside the glasshouse.

Position	Tissue	N	P	S	Mg	Ca	K	Mn	Zn	Cu	Fe	B
		(% DW)						(ppm DW)				
Inside	Lamina	3.15	0.31	0.23	0.31	0.73	1.61	90	24	9	154	70
	Petiole	0.74	0.24	0.14	0.17	0.49	2.74	79	22	6	44	17
	Stem	0.51	0.12	0.07	0.08	0.08	0.64	14	26	4	20	12
	Root	0.78	0.20	0.09	0.28	0.15	2.14	42	37	8	48	14
	Fine root	1.21	0.22	0.19	0.55	0.42	2.80	94	39	26	207	19
Outside	Lamina	2.69	0.25	0.18	0.27	0.77	1.30	87	20	9	151	50
	Petiole	1.02	0.18	0.06	0.31	0.84	2.59	73	16	5	45	21
	Stem	0.60	0.14	0.06	0.09	0.16	0.74	12	26	4	28	10
	Root	0.75	0.18	0.09	0.18	0.19	1.29	18	25	13	62	10
	Fine root	1.20	0.24	0.13	0.42	0.34	2.09	60	33	21	158	14

There were large differences in the soluble sugar content of all tissues between the two sets of trees (Table 3.4). The outside trees had significantly higher concentrations than the inside

trees, with the levels in the fine root twice as high in the outside trees. When the biomass of the trees is taken into consideration, the outside trees had much higher total amounts of soluble sugars on a per tree basis. Because of the technical difficulties with the starch analysis (section 4.5.2.5), the results of the starch content of the tissues are not given, but there were no perceivable differences in starch levels between tissues from the trees in the two environments.

This result is similar to that found for a wide range of species, with the general observation that the concentration of total non-structural carbohydrates increases in various tissues as temperature decreases (Farrar, 1988), although the partitioning of the TNC between starch and sugars may vary between tissues and species. The difference between the two environments may have been a response to root temperature as a result of the cooler soil temperatures outside. Rook and Whitehead (1979) reported that the concentrations of soluble sugars increased in the needles of *Pinus radiata* when the roots were grown at 3°C as compared with 15°C, but that sugar levels were unchanged when grown at a regime of 17°C/5°C day/night temperatures as when grown at a constant 17°C.

Table 3.4. Soluble sugar levels (% DW) in *P. tomentosa* tissues after four months growth either inside or outside the glasshouse.

Tissue	Inside Glasshouse	Outside Glasshouse
Lamina	8.3	13.7
Petiole	12.3	13.5
Stem	16.5	26.4
Root	20.7	27.9
Fine root	11.8	25.8

3.3.4 Photosynthesis and related parameters

3.3.4.1 Gas exchange rates compared between the two environments

The photosynthetic rate, measured on a leaf area basis, varied more between individual trees in each environment than between environments (Tables 3.5 - 3.7). The highest rates of photosynthesis were measured on the outside grown trees in November and December with fully mature leaves of outside tree 2 reaching rates of 23 μmol CO_2 m^{-2} s^{-1}. For the first two months of measurement, A and E were generally higher in the trees grown outside than the inside grown trees, as were the stomatal conductances. Rates of gas exchange measured in January were similar between the trees in the two environments. It is possible that the higher

rates of gas exchange in the outside trees were a result of the increased light levels during growth, as leaves grown under lower light regimes tend to have lower maximum rates of A, even when measured at full light. However, the range in values of the various parameters at the three measurement times makes this unlikely.

When the gas exchange data for the two environments are compared taking into account the one month lag in growth (section 3.3.2), then the rates were not different in the two environments. Thus the photosynthetic rates measured in December on the leaves of the outside trees were similar to those measured in November on the inside trees; and in January the rates of the outside leaves were similar to those in December on the inside leaves.

The cuvette used to measure gas exchange sampled a very small part of the leaf (0.0010 m^{-2}). Taking into account the total photosynthetic area, which was larger for the inside grown trees at each time of measurement, the amount of photosynthates produced by the inside trees would have been higher than the outside trees. This is despite the higher photosynthetic rates of the outside trees. Indeed, recalculation of the photosynthetic data on

Table 3.5. Gas exchange and leaf parameters of leaves grown inside and outside the glasshouse, in November, 1992.

Tree	Leaf	E ($mmol\ m^{-2}\ s^{-1}$)	A ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	$C_a - C_i$ ($\mu l\ l^{-1}$)	Chlorophyll ($g\ m^{-2}$)	N (% DW)	DW/FW
In 1	Pair 2	2.5	9	0.13	127	0.59	5.75	0.27
In 1	Pair 3	3.9	16	0.26	124	0.67	[1]	-
In 1	Pair 4	3.7	15	0.25	123	0.64	-	-
In 1	Pair 5	3.9	15	0.26	115	0.62	-	-
In 2	Pair 3	5.0	19	0.39	106	0.52	-	-
In 2	Pair 4	5.1	16	0.48	78	0.47	5.05	0.22
In 3	Pair 3	5.0	18	0.32	118	0.53	-	-
In 3	Pair 4	5.0	19	0.38	110	0.56	4.60	0.27
Out 1	Pair 2	5.8	22	0.46	108	0.53	4.71	0.21
Out 2	Pair 2	7.2	23	0.73	87	0.54	4.91	0.21
Out 2	Pair 3	5.0	18	0.38	103	0.62	-	-
Out 3	Pair 2	4.9	20	0.45	103	0.58	-	-
Out 3	Pair 3	5.3	18	0.51	87	0.57	-	-

[1] not determined

Table 3.6. Gas exchange and leaf parameters of leaves grown inside and outside the glasshouse, in December, 1992.

Tree	Leaf	E ($mmol\ m^{-2}\ s^{-1}$)	A ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	$C_a - C_i$ ($\mu l\ l^{-1}$)	Chlorophyll ($g\ m^{-2}$)	N (% DW)	DW/FW	SLW ($g\ m^{-2}$)
In 1	Pair 4	2.2	10	0.11	166	0.72	4.52	0.29	50
In 1	Pair 5	3.1	13	0.16	153	0.76	4.16	0.34	57
In 1	Pair 6	3.0	13	0.15	163	0.77	-[1]	-	-
In 1	Pair 7	2.7	11	0.14	149	0.74	-	-	-
In 2	Pair 4	3.8	18	0.23	159	0.78	5.05	0.29	44
In 2	Pair 5	4.5	20	0.32	134	0.64	4.93	0.29	46
In 2	Pair 7	3.5	13	0.20	127	0.69	-	-	-
In 3	Pair 6	3.9	15	0.20	146	0.72	3.88	0.31	51
In 3	Pair 8	3.6	14	0.20	135	0.66	-	0.25	43
Out 1	Pair 4	4.2	17	0.28	126	0.79	4.18	0.28	55
Out 1	Pair 6	4.2	17	0.29	117	0.74	3.72	0.21	46
Out 2	Pair 3	4.9	23	0.36	138	0.77	3.03	0.36	78
Out 2	Pair 4	4.3	22	0.29	155	0.66	2.53	0.38	97
Out 2	Pair 6	3.9	15	0.26	117	0.69	-	-	-
Out 3	Pair 3	2.5	11	0.14	154	0.82	4.83	0.26	45
Out 3	Pair 4	4.6	20	0.33	128	0.70	3.45	0.31	71
Out 3	Pair 5	4.2	18	0.30	122	0.62	-	-	-

[1] not determined

a leaf weight basis showed that the rates were slightly higher for the inside leaves than the outside, and decreased significantly between December and January for both sets of trees. The average rates were (in $\mu mol\ g^{-1}\ s^{-1}$): inside, December - 338, January - 226; outside, December - 290, January - 186.

The value of the CO_2 differential calculated for each of the leaves varied as a result of the relationship between A and g_s, but tended to be slightly lower in the outside trees. Some of the values were very low (below 120 $\mu l\ l^{-1}$) which indicated that the supply of CO_2 to the photosynthetic apparatus was not limiting in any way and thus the stomatal limitation upon photosynthesis was low.

Table 3.7. Gas exchange and leaf parameters of leaves grown inside and outside the glasshouse, in January, 1993.

Tree	Leaf	E ($mmol\ m^{-2}\ s^{-1}$)	A ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mol\ m^{-2}\ s^{-1}$)	$C_a - C_i$ ($\mu l\ l^{-1}$)	Chlorophyll ($g\ m^{-2}$)	N (% DW)	DW/FW	SLW ($g\ m^{-2}$)
In 1	Pair 7	3.3	16	0.21	148	0.76	-[1]	0.33	80
In 1	Pair 8	3.4	16	0.22	140	0.74	3.17	0.30	73
In 1	Pair 9	2.9	18	0.15	217	0.73	3.15	0.33	79
In 2	Pair 6	3.6	14	0.20	134	-	-	0.30	71
In 2	Pair 7	4.2	20	0.28	140	-	-	0.29	67
In 2	Pair 8	4.6	18	0.39	97	-	-	0.29	70
In 3	Pair 8	3.5	14	0.17	151	0.67	-	0.28	62
In 3	Pair 9	2.7	11	0.16	126	0.59	2.83	0.28	64
In 3	Pair 10	3.3	14	0.17	159	0.66	3.00	0.27	60
Out 1	Pair 6	2.6	13	0.14	170	0.58	2.80	0.20	69
Out 1	Pair 7	3.5	15	0.19	149	0.50	-	0.22	66
Out 1	Pair 8	2.9	10	0.14	130	0.62	2.48	0.21	64
Out 2	Pair 5	2.7	12	0.13	178	0.69	2.76	0.29	93
Out 2	Pair 6	3.3	17	0.20	163	0.69	-	0.29	97
Out 2	Pair 7	3.6	14	0.19	140	0.63	-	0.29	96
Out 3	Pair 5	3.3	18	0.21	167	0.64	-	0.26	78
Out 3	Pair 6	3.6	17	0.25	138	0.66	2.48	0.26	72
Out 3	Pair 7	3.5	15	0.22	136	0.67	2.76	0.26	81

[1]not determined

The leaf parameters measured differed little between the two environments. The ratio of leaf dry weight to fresh weight varied slightly between individual trees, but averaged about 0.30 overall. The specific leaf weight was higher, on average, for leaves grown outside indicating that these leaves were heavier for a given area, perhaps due to the windier nature of their environment. Nitrogen and chlorophyll contents of the various leaves showed little relationship to either environment, rates of gas exchange or, indeed, to each other. There was a weak trend of higher N in the inside leaves at each time of measurement, but there were several exceptions to this pattern. The highest concentration of nitrogen (5.75%) was found in an young leaf on inside tree 1 in November, which had a low rate of photosynthesis due to its immaturity. This leaf was 51 cm long in November and 70 cm long in December. In November and January, leaves on inside tree 1 had the highest average chlorophyll levels but the values of A for this tree were the lowest for any tree in November, and were only average

in January.

The influence of growth temperature on the stomatal numbers of leaves from the two environments was significant at the 5% level (by Student's t-test) when measured in December. The average number of abaxial stomata on inside leaves was 549 (s.e. = 12.3) stomata mm^{-2} and for outside leaves was 490 (s.e. = 17.4) stomata mm^{-2}. This result was similar to that found in leaves of tall fescue. When grown under very different temperature regimes (10°C and 25 °C) Treharne and Nelson (1975) found that the leaves grown at the lower temperature had less stomata.

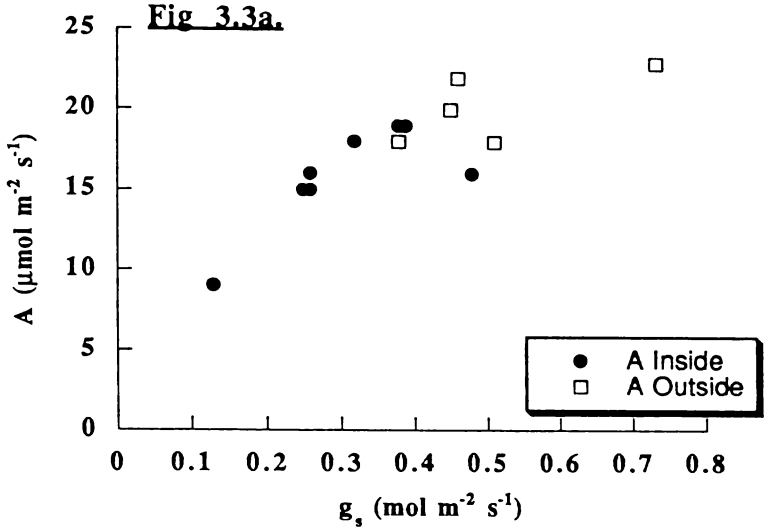
3.3.4.2 Variation in gas exchange and leaf parameters with tree age and leaf age

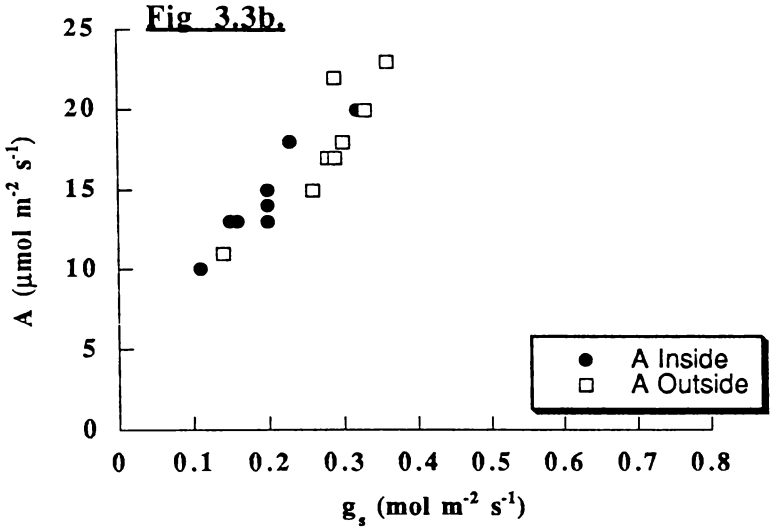
For the outside trees A, E and g_s were highest in November and decreased as the trees aged. The rate of decrease in stomatal conductance was higher than the decrease in A, which led to potentially limiting CO_2 differentials. Thus for a leaf with a photosynthetic rate of 18 μmol m^{-2} s^{-1} in November, g_s was between 0.4 and 0.5 mol m^{-2} s^{-1}, but in January the g_s associated with the same value of A was 0.2 mol m^{-2} s^{-1} (Tables 3.5 and 3.7). This difference in the rates of decrease in A and g_s over the time period studied resulted in an improved water use efficiency (as defined by A/E). The WUE efficiency was about 20% higher in January than in November in leaves on the trees grown outside.

The range of photosynthetic rates of the leaves grown inside were similar over the three months measured, but g_s and E declined slightly in the last two months. This decline resulted in an increase in WUE and in the CO_2 differential. If gas exchange measurements had been made on these leaves in October (when the tree was at the same stage of growth as the outside trees in November), it is possible that the photosynthetic rates would have been higher. The correlation between A and g_s at each time of measurement was weak, as it was with the outside leaves (Fig. 3.3).

Nitrogen levels in the leaves from both environments tended to decrease as the trees aged, but chlorophyll levels in all leaves increased from November to December. The chlorophyll content of the leaves grown in this experiment were much higher than in leaves of *P. tomentosa* grown in the field at Matangi, while nitrogen levels also tended to be higher (Tables 2.7 and 2.13).

For both sets of leaves, the SLW increased from December to January. This result and the decrease in N content and gas exchange as the trees aged may have been related to the observation that towards the end of the experiment the trees were becoming limited by space.





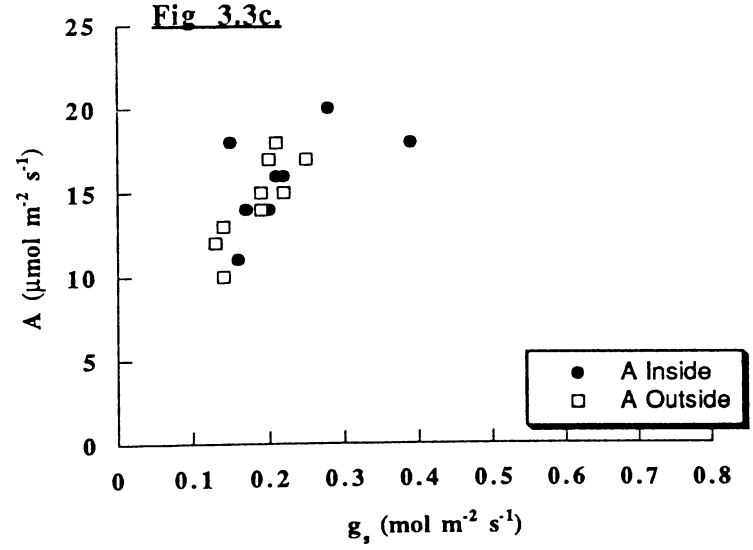


Fig. 3.3a,b,c. Photosynthetic rate against stomatal conductance for leaves on trees grown inside or outside the glasshouse a. - in November, 1992; b. - in December, 1992; c. - in January, 1993.

Despite the continued application of nutrients, growth began to slow such that the one tree remaining in the glasshouse in February had increased in height by only 17 cm over the previous month. It may have been possible to maintain growth rates and photosynthetic rates by increasing either the concentration or rate of application of the nutrient solution, as suggested by Jia and Ingestad (1984), but even this may not have halted the decline. As mentioned in 3.3.2, the whole volume of the pots had been exploited by the roots systems in both the inside and outside trees and so the amount of root was close to the maximum possible in the containers used. The restrictions placed upon root growth by this lack of space probably exerted a negative feedback on the growth and photosynthetic activity of the shoot.

Comparisons of leaves of different ages at the same measurement time indicated that the rates of photosynthesis and chlorophyll content were not different in fully mature leaves, regardless of their ages. There was some decline in A in the oldest leaves measured as a result of the decrease associated with senescence. E, g_s and the CO_2 differential varied independently of age in both environments, as did the DW/FW ratio and SLW. In the leaves that were assayed for N in December, younger leaves tended to have higher N levels than older leaves.

Measurements of the various parameters in the same leaves as they aged from November to December showed a decline in all cases in A, E and g_s. However, the chlorophyll content in all the leaves increased over the same period. In those leaves that were mature when measured in December and remeasured in January, a similar decline in gas exchange parameters was observed but in this instance chlorophyll levels also decreased. The SLW increased in all leaves from December through to January. The nitrogen content measured in a number of leaves decreased as they aged over both the two monthly periods. The only increase in leaf activity from one month to the next was in two leaves measured in December before they were fully mature. Remeasurement of gas exchange parameters in January showed an increase in A, E and g_s.

3.4 Discussion

3.4.1 The effect of temperature regime on gas exchange and related parameters

There was no effect of the temperature during the development of a leaf on the subsequent maximum rate of photosynthesis (measured on an area basis) attainable by the mature leaf, in this experiment. This result disproves the original hypothesis the experiment set out to investigate. Photosynthetic rates of leaves grown inside were slightly higher when calculated on a weight basis, than rates on outside leaves as a result of the higher specific leaf weights for the outside leaves. From these results it is clear that, although temperature does play a large role in plant growth, it is not a dominant factor in determining the photosynthetic capacity of the leaves of *P. tomentosa*. A similar result has been reported for jojoba grown at a range of different temperature regimes (Wardlaw *et al.*, 1983) while Delucia (1986) reported that increasing the root temperature in Engelmann spruce from 10°C to 20°C had no effect on A. The temperature regime of the growth conditions had little effect on any of the gas exchange or leaf parameters measured in the *Paulownia* experiment. It would be expected that an effect would be found if the difference between the two regimes was greater, with one set of conditions being closer to the lower limit of temperature tolerance in *Paulownia*, as has been reported for many other species in the literature.

A full discussion of the various factors important in determining the rates of growth and gas exchange in *Paulownia* is given in Chapter 6.

The differences in gas exchange and leaf characteristics that were observed in this experiment were a result of tree and leaf aging. For both sets of leaves, the values of E, g_s and leaf N content decreased over the experiment period. The value of A was initially high in the outside leaves but decreased as the trees aged, despite the increase in air temperature over the same period. This result is the opposite to that gained in the field which led to the proposal of the original hypothesis, further lending weight to the suggestion that temperature is not a primary controlling influence in photosynthetic capacity.

It is likely that as the trees grew over the experimental period, the root system was unable to provide the water and nutrients at the levels needed to maintain the rates of gas exchange at the high levels measured initially. The increase in WUE in both sets of trees as they aged suggests that, despite the frequent watering of the pots, the amount of water that the roots could take up was insufficient to meet the demand of high transpiration rates. Rates of transpiration of between 5 and 7 mmol m^{-2} s^{-1} in the outside trees in November corresponded with trees that were 43 cm tall with 5 - 6 leaf pairs. As the trees grew, the leaf area increased as a result of an increase in both the number and the size of leaves. To maintain the same

transpiration rates with an increasing leaf area would have required a concomitant increase in the size of the root system. But the size of the pots precluded this, and so it is likely that gas exchange rates decreased as a result of this limitation.

It would have been interesting to measure the gas exchange rates of the inside trees in October to determine if the difference in rates between the two sets of trees each month was due to phenological factors. If indeed the outside trees were approximately a month behind the inside trees in maturity, it would be expected that photosynthesis of the inside leaves in October would have been higher than those measured later. However, the range of rates of photosynthesis across all the trees illustrates the genetic variability that affects the physiological functioning of each individual. The small number of replicates used in this study means that it is not possible to conclude with any certainty that the initially higher rates of photosynthesis in the outside trees was due only to phenology, under the environmental conditions studied.

In both sets of leaves, A was lower before full maturity, peaked at the time of full maturity and then declined as the leaf aged. This latter decline was detectable before any obvious signs of senescence were visible on the leaf. Generally leaf chlorophyll content follows the same pattern as A, as the leaf ages (Sestak, 1977). However, in this experiment the chlorophyll content in all leaves was highest at the December measurement, and increased in the leaves that had previously been assayed in November. In some cases this increase was accompanied by an increase in leaf size, indicating that these leaves were not fully mature in November, despite the high photosynthetic rates measured (*e.g.* tree 1 out, leaf pair 2). But in the second leaf pair of outside tree 2, the increase in chlorophyll from November to December was not accompanied by an increase in leaf size. This particular leaf may have been fully expanded but not fully mature at the time of the November measurement. The overall increase in chlorophyll in December did not lead to an increase in the photosynthetic rates of the leaves, and there was no clear relationship between leaf chlorophyll content and A. It is not clear what caused the December peak in chlorophyll content but it may have been a response to nutrient availability increasing in the preceding weeks as the root systems of the plants increased in size and nutrient uptake. A similar result of a lag in the peak of chlorophyll concentration with respect to A_{max} has been observed in poplar leaves (Ceulemans and Impens, 1979). The interpretation of this result given by these workers was that the extra amount of chlorophyll served to enhance only the light capturing ability of the leaf, not the conversion of the light energy into chemical energy, and so was not translated into higher photosynthetic rates.

3.4.2 Growth of *P. tomentosa* trees under two temperature regimes

It is well documented for a range of species that plant growth rates increase as the temperature of the environment increases, within the temperature range tolerated by the plant. This is a result of the interaction of temperature with a range of processes within the plant including cell initiation and expansion, and the effect of temperature on the functioning of a myriad of enzyme systems. For temperate species, the upper limit of this temperature induced promotion of growth occurs at about 30°C, while many processes are inhibited below 10 °C. The temperature regimes studied in this experiment were both within the range tolerated by *P. tomentosa*. The values of the various growth parameters including total biomass accumulation, absolute growth rates, tree height and DBH were all greater for the trees grown in the warmer environment.

It is not clear whether the plants grown inside the glasshouse were responding to a higher average temperature over each 24 hour period, to a higher minimum (night) temperature, or to a longer period each day that was sufficiently warm as to allow photosynthesis. The trees growing outside in early spring had to contend with some very low night temperatures (as low as 2°C) which would have slowed the growth rates and also decreased the soil temperature in comparison with the plants growing inside. Low day/night temperature regimes and lower soil temperatures have both been found to decrease growth rates in other species. For example, rates of shoot extension were approximately four times lower in jojoba plants grown at either 23°C/13°C or 23°C/8°C day/night regimes than those plants grown at 23°C/18°C (Wardlaw *et al.*, 1983). With respect to soil temperature, Tromp (1977) found that apple shoots had higher growth rates when the air/soil regime was either 24°C/24°C or 19°C/19°C than when it was 24°C/10°C or 19°C/10°C. The growth of root, shoot and leaf tissue was found to be higher in seedlings of *Ceanothus greggii* grown under hydroponics when both the nutrient solution and air temperatures were warmer (Larigauderie *et al.*, 1991). In their experiment, the extent to which the growth rates of the different tissues increased varied with depending on the solution/air temperature regime. Thus it is possible that the growth rates of the *Paulownia* trees grown outside were lower than the inside trees because of the lower night temperatures and/or lower soil temperatures.

Initially, at least, the faster growth of the inside trees was a result of the faster rate of shoot emergence, which was related to the soil temperature. As the first shoots emerged at least a week earlier inside than outside, they were able to grow over that time and develop a viable photosynthetic area more quickly. This then meant that the inside plants were able to further develop as new leaf and root growth provided the substrates necessary for growth. This led to a compounding effect. Comparison of the relative growth rates of the trees at a similar phenological stage showed that the rates were slightly higher for the inside trees. Although

this difference in growth rates was small it was probably sufficient to explain the difference in final tree size between the two environments, when coupled with the earlier shoot development of the inside trees.

Although the leaf area of the inside trees was greater, this factor alone did not cause the higher biomass accumulation. The amount of biomass at harvest, when divided by the leaf area at harvest, was higher in the inside trees, thus the net assimilation rate was higher. As the rate of photosynthesis, on a leaf area basis, was generally lower for the trees growing inside, the time available for assimilation must have been greater. The warmer temperatures at the beginning and end of each day probably prolonged the period available for photosynthesis in the glasshouse. The cool morning temperatures outside (Table 3.1), particularly in the early part of the experiment, would have decreased the photosynthetic rate at that time even if light was not limiting (cf Table 2.20). This resulted in the fixation of more carbon by the trees inside. Respiration rates in the two environments were not measured, but it would be expected that respiration would occur at a higher rate inside due to the warmer temperatures. Thus the extra amount of carbon fixed inside must have been more than that lost by the higher respiration rates.

The trees inside the glasshouse had more and larger leaves, overall, than those growing outside. Without a full anatomical examination of the two sets of leaves, it is not possible to state whether the rate or duration of cell division and/or expansion was higher in the inside leaves, nor whether there were any differences in the size and number of cells in the different cells layers as a result of the temperature regimes. As was discussed in the Introduction (section 3.1.3) the temperature during leaf growth does play a major role in influencing the development of the various cell layers. Temperature can also affect the different aspects of leaf morphology such as length, breadth and final area in different ways (Dale, 1982a).

3.4.3 Influence of temperature on nutrient and soluble sugar partitioning

Temperature of growth has been shown to affect nutrient concentrations in some woody plant tissues. In apple trees grown at 10°C, 17 °C or 26°C at low and high relative humidity levels, concentrations of K in woody tissues and new growth increased with increasing growth temperatures. The situation with Ca and Mg was less clear cut, with Ca increasing when calculated with respect to whole plant weight, but decreasing at low RH levels when calculated on a g dry weight basis (Tromp, 1977). A series of experiments on several woody species grown at a range of soil temperature from 4°C to 41°C found increased root and shoot growth at moderate temperatures (generally between 12°C and 32°C, depending on the species) (Barr and Pellett, 1972). Foliar nutrient concentrations of N, P and K were highest in most species when grown at the intermediate soil temperatures, with different patterns of nutrient levels

occurring in different species. In some instances, the range of soil temperatures from 10°C to 35°C had no effect on nutrient levels in the leaves, and for the majority of the species/temperature interactions between 20°C and 30°C the soil temperature did not affect foliar nutrient levels (Barr and Pellett, 1972). This latter result was similar to that found in the *Paulownia* experiment where there was little difference in nutrient levels throughout the plants between the two environments.

It would have been interesting to be able compare plant tissues harvested earlier in the season (at the November measurement time, for example) with those harvested in January to determine if the plants were becoming nutrient limited at all. The declining foliar nitrogen concentrations over the experimental period do suggest that the nutrient status of the trees was changing and so it could be argued that the concentrations of all nutrients in all the tissues would have been higher in November. There may have been more of a difference between the trees from the two environments at that time.

Although the nutrient contents in the trees in the two environments were relatively similar, the soluble sugar concentrations in the tissues were markedly different, with higher levels in all tissues of the outside trees. The accumulation of carbohydrates in plants grown at low temperatures has been observed for several plants and is a result of growth being more sensitive to lower temperatures than photosynthesis and also the differential sensitivity of the enzymes of carbohydrate metabolism to temperature (Farrar, 1988). The outside grown trees in this experiment had similar, or higher photosynthetic rates than the inside trees (Tables 3.5 - 3.7) but lower absolute growth rates over the course of the experiment. Thus it is possible that a large proportion of the photosynthates produced were stored in the various tissues of the outside trees rather than being directly used to support new growth, whereas the inside trees directed a higher proportion of the photosynthates produced into growth. The difference in the temperature regime between the two sets of trees was therefore enough to affect the way the trees allocated the products of photosynthesis, but not enough to influence rates of nutrient uptake.

3.4.4 Overall effects of growth temperature on *Paulownia*

The temperature regimes studied in this experiment were not particularly dissimilar, in comparison with much of the work reported in the literature. The outside environment represented field conditions for the trees in the first half of a normal growing season, while the trees inside the glasshouse grew in temperature conditions that could be expected during the warmest months of a good summer in the Waikato district. The result of growing *Paulownia* trees under these sets of conditions showed that temperature plays an important role in determining tree growth and the partitioning of the products of photosynthesis, but that temperature is not a primary determinant in the allocation of biomass (as seen by the R:S ratios) in the trees, the rate of nutrient uptake or the rate of photosynthesis.

Chapter 4
Growth, Nutrient Levels and Carbohydrates in *P. tomentosa* Trees

4.1 Introduction

4.1.1 Growth analysis

4.1.1.1 Rates of growth in *Paulownia*

Anecdotal evidence abounds regarding the extremely rapid growth rate of *Paulownia*, especially of young trees. Examples include trees grown from tissue cultured ex-plants in Australia that reached in excess of 6 m in their first growing season (Lucas, 1989); and coppiced *P. tomentosa* trees growing to approximately 5 m in 4.5 months in Maryland, USA (Preston, 1983). In China, the fastest recorded growth of a tree grown from seed is 7.56 m in one season, while a coppiced root produced a stem of 10.38 m in height with a 14.0 cm root collar diameter in the Szechun Province in 1977 (Zhu *et al.*, 1986). Heights of older trees ranged from 11 m for 5 year old *P. elongata* trees (with an average timber volume of 0.12 m^3), 17.5 m for 13 year old *P. elongata* through to 22.0 m for a *P. fortunei* that was 11 years old. The latter tree had a DBH. of 75.1 cm and a timber volume of 3.7 m^3 (Zhu *et al.*, 1986).

From the work carried out in China by Zhu and others, and from early observations of tree growth in New Zealand, it appears that there are marked differences in growth rates between the nine species of *Paulownia* with *P. fortunei* and *P. elongata* growing more quickly than *P. tomentosa* in most environments (I. Barton, pers. comm.). One factor contributing to the differences in growth between the species may be different patterns of allocation of new biomass to root and to shoot tissue (G. Rogers, pers. comm.). In addition, *P. tomentosa* often flowers after three or four years of growth, whereas *P. fortunei* does not begin to reproduce until age six or seven and may be able to invest more resources into stem growth for a longer period of time. Data regarding the long term growth rates of *Paulownia* trees in a range of soil and environmental situations are not available at this stage, so the results presented here represent only the beginning of the research process needed to clarify the various issues concerning growth and development of *Paulownia* trees.

4.1.1.2 Techniques of growth analysis

Many of the tools for the quantification of plant growth rates were developed early this

century (for a full historical account see Evans, 1972). These include the equations for calculating absolute and relative growth rates; unit leaf rate (also known as net assimilation rate); leaf area ratio; specific leaf area; and leaf weight ratio (Evans, 1972; Hunt, 1978). The classical approach to growth analysis involves the calculation of the relative growth rate (R), while the functional approach fits curves to various data sets (Brand *et al.*, 1987). Despite the length of time these techniques have been available to plant biologists, there is still much discussion in the literature about the application of the correct set of equations to accurately describe plant growth. This is perhaps a consequence of attempting to fit precise mathematical models to biological subjects, the growth of which are influenced by many soil, genetic and environmental interactions that are, as yet, poorly understood.

Among the various improvements suggested by different workers is the demographic analysis of plant growth whereby parts of the plant ('modules') are treated as members of a population with distinct life cycle stages such as birth, reproduction and death. Bazzaz and Harper (1977) used this approach with *Linum usitatissimum* to study the effect of environmental parameters on various stages of the leaf life cycle. The advantages of this approach include the non-destructive nature of the observations, which can be carried out on individuals in controlled environments or in their natural habitat, and the ease with which observations on growth rates can be related to plant fecundity (McGraw and Wulff, 1983). Another approach that has been widely used is yield component analysis whereby the overall growth of the plant is subdivided into its yield components, reflecting the morphological sequence of plant development. This technique has been taken a step further with the integration of traditional growth analysis with yield component analysis to produce a further model termed 'sequential plant growth analysis' (Jolliffe *et al.*, 1982). However, as Hardwick (1984) has observed, the definitive method for analysing plant growth has yet to be discovered due to the complexity of the biological systems involved and, indeed, may not even exist.

Much of the work reported concerning plant growth rates has involved the study of herbaceous species, often annuals. However, it is also important in the management of perennial plants to know those factors that influence the rates of development of the various structures that are of interest to the manager *e.g.* stem tissue in forestry, fruit in horticulture. There are numerous problems associated with the growth analysis of perennial plants that are not encountered by those studying annual plants. The most important of these is the time scale needed to fully investigate the growth of the plant under study (often several years). A second major hurdle is the increasing size of the plants after some years growth, which is usually overcome by subsampling plant tissues. In the study presented here, these constraints were overcome by harvesting whole trees, but only during the first three years of growth.

A further problem when attempting to fit growth models designed for herbaceous plants to

woody species is that the annual increment of tissue declines over time as a proportion of the total biomass of the plant (Brand *et al.*, 1987). These workers have suggested an alternative parameter with which to assess growth - the relative production rate. The data used to calculate the relative production rate are the annual increments of the variable under study, stem volume for example, which removes the effect of the accumulated past growth. This takes into account the increasing amount of biomass present in the perennial tissues as the tree grows over time.

4.1.1.3 Biomass accumulation and partitioning

The growth of any plant is a result of a complex series of interactions between genotype and environmental factors. In order to survive, a plant must produce a viable root system for nutrient and water uptake and for anchorage, as well as a viable shoot system for the support of photosynthetic and reproductive structures. The allocation of biomass to the different structures will be influenced, to some extent, by internal factors as discussed by Schulze (1982). He observed that when the relative water content of a plant is low, root growth is promoted, while higher levels promote shoot growth. External factors that influence the partitioning of biomass have been studied in a wide variety of plants and these variables include nutrient levels, soil moisture levels, soil and air temperature and the light environment (a fuller discussion is given in section 3.1.2). The commercial grower of *Paulownia,* primarily interested in maximising stem wood production, needs to know those factors that affect biomass partitioning in the plant. The ideal *Paulownia* tree would be one that produced the greatest stem volume for the least investment in root and reproductive tissues.

The accumulation of biomass is directly related to the photosynthetic activity (primarily in leaves) of the plant. The amount of investment of the carbon resources of the plant into new foliage determines the continuing rate of biomass accumulation, thus a balance must be struck within the plant between production of foliage and of other tissues (Schulze, 1982). The carbon that is fixed by photosynthesis must then be converted to the myriad of other compounds essential to the development of the plant. The cost of production of some of these compounds in terms of glucose units ('glucose equivalents' - McDermitt and Loomis, 1981) has been calculated by several workers. In *Pinus taeda*, for example, the production of 1 g of lipid cost 3.019 g of glucose, with 1 g of lignin costing 1.897 g of glucose and overall the production of 1 g of typical shoot biomass cost 1.574 g of glucose (Chung and Barnes, 1977).

4.1.2 Mineral nutrition

4.1.2.1 Essential mineral elements

Thirteen mineral elements are generally considered as being essential for healthy plant growth. Six of these are macronutrients (N, P, K, S, Mg, Ca) which are required in amounts ranging from 0.01% to approximately 2% of tissue dry weight. The remainder are micronutrients (also known as trace elements) and are required in ppm levels and include Mn, Zn, Cu, Fe, Cl, B and Mo. An element is only considered essential if plant growth and reproduction is inhibited or curtailed in its absence and its role cannot be completely filled by another element (Marschner, 1986). The functions of the essential elements range from structural roles (*e.g.* Mg in chlorophyll, N and S in amino acids) to enzyme activation through to regulation of osmotic and ionic balances within the cells, with most elements fulfilling a range of roles.

Species of plants vary in the amounts of the various nutrients needed to sustain growth and also in their requirement for other elements. Natrophilic plants can use sodium in place of potassium, for example, in osmoregulation in the vacuole. Many plants that require sodium for growth are halophytes and live in soils with high levels of sodium (Marschner, 1986). Some groups of grasses show a growth requirement for silicon while nitrogen fixing plants need cobalt for the fixation process (Marschner, 1986). Other elements present in the soil may be taken up by plants, but the presence of a particular element in plant tissues does not mean that it is essential.

4.1.2.2 Nutrient uptake and translocation

The point of entry into the plant for mineral nutrients is the root system. In soils with adequate soil moisture levels, nutrients such as Mg, Na, Ca, S (as SO_4^{2-}) and N (as NO_3^-) arrive at the root surface by convection (mass flow). For the nutrients Cu, Fe, Mn, Zn, N (as NH_4^+) and P ($H_2PO_4^-$), travel through the soil is by diffusion along a concentration gradient. The remaining nutrients - K, Mo and B - move through the soil by both diffusion and convection (Bowen, 1984). Many interactions which affect the availability of nutrients for plants occur within the soil environment. Soils with high clay contents tend to retain nutrients more than sandy soils as the charged clay particles attract the ionic nutrients and release them slowly to the soil solution. This is particularly a problem with allophane, a particle type with a strong affinity for phosphate and which limits the availability of phosphate to plants. Sandy soils, on the other hand, generally tend to have lower nutrient levels as the nutrients are easily leached from the soil.

The absorption of nutrients from the soil solution into the root cells is by active uptake for most of the ionic nutrients (Bowen, 1984). Entry of water and solutes occurs most freely in fine, unsuberized roots and root tips, but uptake by suberized and woody roots does occur, especially at fissures and exit points of branch roots (Clarkson and Hanson, 1980). Quantification of water and solute uptake in apple roots has indicated that significant absorption must occur in brown and woody roots, as well as in fine roots, to meet the plants requirements (Atkinson, 1983). As the ability of the root to absorb nutrients from the soil solution with which it is in contact is generally not limited, the extent to which the plant can exploit the soil resources is a direct consequence of the area of the root system available for absorption. Thus the growth of root hairs and mycorrhizal associations which greatly increase the surface area of unsuberized tissue in direct contact with the soil improve the potential of the plant to maximise nutrient uptake. It is well documented that mycorrhizal associations are important in phosphate nutrition of plants, but they may also improve Zn and S nutrition as well (Clarkson and Hanson, 1980).

Nutrients are primarily absorbed by the roots as ions *e.g.* K^+, Ca^{2+}, Mg^{2+}, Cl^-, Na^+, Zn^{2+}, Mn^{2+}, with nitrogen generally taken up as either nitrate (NO_3^-) or as ammonium (NH_4^+), P as phosphate ($H_2PO_4^-$), S as sulphate (SO_4^{2-}) and Mo as MoO_4^{2-}. Cu is either absorbed as Cu^{2+} or as a Cu-chelate. Iron is also primary absorbed as a Fe (III)-chelate, while boron enters the root as boric acid - $B(OH)_3$ (Marschner, 1986). The long distance transport of nutrients from the site of absorption to the remainder of the plant generally occurs in the xylem, where they are passively carried in the transpiration stream (van den Driessche, 1984). The major exception to this is ammonium which is assimilated in the roots to non toxic amino acids, amides and other low molecular weight compounds by glutamine synthase and glutamate synthase (Marschner, 1986). These compounds are then translocated in the xylem. Redistribution of many of the nutrients occurs via the phloem but analysis of the contents of phloem from a number of plants indicates that B, Mo, Ca and nitrates are either absent or present only in very low levels in the phloem sap (Marschner, 1986; van den Driessche, 1984).

4.1.2.3 Nutrient cycling through ecosystems

In a complex ecosystem composed of perennial woody species, perennial herbaceous plants and annuals there is much competition for the soil resources. The deeper roots of trees are able to utilize nutrients at greater depths than the shallow roots of grasses and other annuals, which tend to root in the top 10 - 20 cm of the soil. These nutrients may then be immobilized in the woody tissues of the trees for several years, or may be released back to the soil

environment through litterfall, crown leaching by rainfall or through fine root turnover (Miller, 1984). The nutrients stored in woody tissue may be remobilized when required for the growth of new tissue, especially in deciduous trees when new leaf growth in early spring places a high demand for nutrients on the root system that is also undergoing renewed development. The storage of nutrients in root material is critical in those plants that are able to be coppiced, such as *Paulownia*, since they must have sufficient reserves to develop both new shoot and root material (van den Driessche, 1984).

The recycling of nutrients back to the soil environment occurs most obviously through leaf fall. Other aerial tissues, including branches, bark flakes and reproductive structures add to the litterfall from trees (Miller, 1984). Less obvious and, until recently, little considered is the recycling of biomass and nutrients through the turnover of fine root material. It is very difficult to quantify the change of fine root biomass over time in the field situation. Changes in the soil environment, particularly decreasing soil moisture, can lead to the rapid demise of fine roots and fluctuations in fine root biomass.

Work with apple trees using root observation chambers showed that all roots throughout the season were initially produced as unsuberized, white root which turned brown after 3 - 4 weeks. Some of the brown roots that survived then underwent secondary thickening to become part of the permanent root system (Atkinson, 1983). In a field study in a young Scots pine (*Pinus sylvestris*) stand, Persson (1980) found that the bulk of the fine roots (both biomass and necromass) of the range of species studied were present in the top 10 cm of the soil at any one time. Frequent soil sampling over the growing season resulted in the estimation of fine root turnover in *P. sylvestris*, *Calluna vulgaris* and *Vaccinium vitis-idaea*, to a depth of 30 cm, at 214, 35 and 162 g m^{-2} respectively.

Turnover of fine root material not only releases nutrients to the surrounding soil (which may then be reabsorbed by the same or neighbouring plants), but also increases the investment the tree must make in the root system. Thus the 'cost' of fine roots to the tree, in terms of both nutrient and biomass allocation, is higher than would be assumed from analysis of total tree biomass and nutrient content made at one or two points in the season (Bowen, 1984).

4.1.2.4 Nutrition in *Paulownia*

Some limited studies of nutrition in *Paulownia* have been published. A study into the potential of *P. fortunei* leaves as feed for animals found foliar crude protein levels of 17% dry weight with 2.4% Ca, 0.9% K, 0.64% Mg, 0.16% P, 700 ppm Fe, 83 ppm Mn, 36 ppm B, 26 ppm Zn and 13 ppm Cu (Song, 1988). Other workers found that the relative amounts of

nutrients differed between *Paulownia* tissues, with the foliage containing 45% of the total nitrogen in the tree; the roots containing between 40% and 56% of the tree's total P; the trunk having 40 - 57% of the tree's calcium while potassium was found in the highest concentrations in the branch and root tissue (Liu and Liu, 1988). The *Paulownia* forest studied absorbed 320 kg nutrients ha^{-1} yr^{-1}, of which 104 kg was Ca, with lesser amounts of N, K (70 - 100 kg), P and Mg (26 - 27 kg) absorbed (Liu and Liu, 1988).

A modified hydroponics system was used by Jia and Ingestad (1984) to study the relative addition rates of different nutrients to seedlings of *P. tomentosa.* They found that maximum growth occurred when nutrients were added in the ratio 100 N: 75 K: 20 P: 8 Ca: 9 Mg. There was a linear relationship between the relative nutrient addition rate for all nutrients and the relative growth rate of the plants, with the highest addition rate of 27.5 % day^{-1} producing a relative growth rate of 26.1 % day^{-1}. The plants grown under these conditions had a nitrogen content of 4.2% dry weight (Jia and Ingestad, 1984). The concept of a linear relationship between nutrient addition rate and relative growth rate has been tested for a number of species in hydroponics systems, and has been developed further in forestry trials. Ingestad (1987) recommends that small amounts of appropriate fertilizer be added often through the growing season to forest plantations in order to produce maximum growth rates by maintaining constant internal nutrient levels.

4.1.3 Carbohydrates

4.1.3.1 Total non-structural carbohydrates in woody plants

The role of various carbohydrates in the functioning of a wide diversity of plants has been well documented. The conversion of the products of the photosynthetic pathway to a range of other compounds is critical to a plant's survival. Among the compounds produced are monosaccharides such as fructose and galactose; sugar alcohols (*e.g.*sorbitol); the disaccharide sucrose; and polysaccharides such as starch, cellulose and hemicelluloses. This discussion will focus on the non-structural carbohydrates important in woody plants.

Non-structural carbohydrates may be defined as those that do not play a role in the cellular structure. The analysis of total non-structural carbohydrates (TNC) generally involves assaying for starch, soluble sugars and sometimes sugar alcohols. Starch is considered to be the major storage carbohydrate in woody plants, but debate continues as to whether hemicelluloses (components of plant cell walls) also have a role as storage carbohydrates (*e.g.* Loescher *et al.*, 1990). In most plants sucrose is the major transport sugar, but in many members of the Rosaceae family (*e.g.* apples, peaches, plums) is replaced by the sugar

alcohol sorbitol (Hansen, 1977; Gucci *et al.*, 1991), while mannitol is important in plants of other families including Scrophulariaceae (Loescher *et al.*, 1990). It was not possible to ascertain from the literature if *Paulownia* was included in the original analysis of members of Scrophulariaceae. It seems unlikely as I have been unable to find any reference to this point in the *Paulownia* literature published since the analyses were reported in 1958 (Loescher *et al.*, 1990).

4.1.3.2 Seasonal dynamics of the partitioning and storage of TNC

The partitioning of carbon into the different carbohydrates is influenced by the rate of CO_2 assimilation, the metabolic demands of the various plant organs and environmental and seasonal factors. At times of rapid assimilation, the excess carbohydrates produced may be stored until remobilized when demand for carbon exceeds the supply from photosynthesis. Storage compounds include soluble sugars as well as starch with the relative importance of each varying between species and between tissues; roots, for example, tend to have higher concentrations of glucose and fructose than of sucrose, while the converse occurs in shoots (Loescher *et al.*, 1990).

The synthesis of starch from glucose occurs by a number of similar pathways which involve glucose-1-phosphate and either phosphorylase or pyrophosphorylase and transglucosylase (Kramer and Kozlowski, 1979). The resultant starch polymer is made up of chains of α–1,4 linked glucose molecules with numerous side branches (amylopectin) and of unbranched chains of amylose. The size of the polymer precludes translocation of starch between cells so it is manufactured on site in the cells and stored in starch grains (Kramer and Kozlowski, 1979). Starch grains are only found in living tissue and are abundant in leaf material and in the ray and axial parenchyma cells of the sapwood, the pith, the parenchyma and albuminous cells of the bark, and the living cells of the phloem (Kramer and Kozlowski, 1979).

A seasonal pattern of carbohydrate partitioning has been well documented in deciduous tree species. During the dormant season, the level of stored carbohydrates decreases slowly as a result of respiration in the living cells. At the resumption of growth in spring, reserves in both the root and shoot are remobilized to support the development of new foliage and new root material (Kramer and Kozlowski, 1979; Loescher *et al.*, 1990). As the latter authors note, these stored reserves are especially important in the development of flowers in species that flower before full leaf production. This is the case in *Paulownia*, although the metabolic requirements are lower as the flower buds are carried over the winter in a reasonably advanced state of development. Remobilization of reserves is also critical to the development of new shoots in trees that are coppiced (such as *Paulownia*). Studies on coppiced trees have found

that the level of starch reserves in the root at the end of the growing season is a major factor determining the growth of new shoots the following season (*e.g.* Kays and Canham, 1991). The early season translocation of carbohydrates from root tissue to developing shoot tissue has been found to occur in the xylem in a number of species, as the activity of the phloem is very limited in late winter/early spring due to low temperatures (Loescher *et al.*, 1990). As the season progresses the foliage becomes a source, rather than a sink, of carbohydrates which then tend to be translocated in the phloem. When the canopy of the tree has developed so that the CO_2 fixation rate becomes more than sufficient to meet the needs of the plant, surplus carbohydrates can be stored. The exact timing of this point will depend on a variety of environmental factors, as well as the species and phenology of the tree. In whole apple trees, for example, Kandiah (1979a) found that the levels of TNC were lowest in May (late spring), but by July were higher than in the previous December and continued to increase for the remainder of the growing season. A similar pattern of TNC levels in the roots and shoots of sweet cherry has been reported by Roper *et al.* (1988).

The relative importance of the different tissues as storage sites depends not only on the absolute concentration of carbohydrates in the tissue (% DW), but also on the relative size of each tissue with respect to the tree as a whole. Thus, although the roots of a mature tree may have higher concentration of storage carbohydrates than the stem and branches, the greater above ground biomass means that overall more carbohydrates are stored above than below ground (Kramer and Kozlowski, 1979).

4.2 The Experimental Site

4.2.1 Physical aspects of the site

The trees used in the analyses of growth and nutrient allocation were located at Matangi, Hamilton (37° 48' S, 175° 21' E; NZSM 260 Sheet S14 195753) on a privately owned block of land. Prior to the planting of the trees, the land had been grazed by beef cattle for some years, but it is believed that the paddock had, at one stage, been used for the growing of berry crops (perhaps 7 - 10 years ago). The paddock was surrounded on three sides by hedges and shelter belt trees, while the fourth (western) side was more exposed but protected to some extent by a stand of mature eucalypts midway along the boundary. The trees were positioned in the block so as to minimize interactions with the surrounding shelter, but some of the trees planted out in the propagation experiment (Chapter 5) may have faced root competition with the willow trees on the southern boundary. The block was predominantly flat and extended approximately 90 m in the north/south direction and 35 m in the east/west direction. A topographical survey was undertaken using a Sokkisha Set 2 total station theodolite (which measured angle and distance), with the data being stored by a SDR 22 field recorder and processed by Sokkisha SDR mapping and design software (Fig. 4.1).

The soil was classified as a Horotiu sandy loam (R. Chapman, pers. comm.) and had between 20 - 40 cm of topsoil over a sandy subsoil, making it very free draining. The pH of the soil was 6.1 with moderate amounts of the major nutrients available, although the levels of phosphate (11 - 14 ppm) and potassium (3 ppm) were lower than would be acceptable in an agricultural situation (Soil Fertility Service, AgResearch Ltd., Ruakura, Hamilton). Horotiu sandy loam soils tend to be low in potassium and in phosphates, the latter mineral being limited by the high allophane content of these soils (R. Chapman, pers. comm.). The nitrate level in the soil was 5 - 6 ppm, with 7 - 9 ppm of NH_4^+, 14 - 24 ppm of S, 6 - 9 ppm of Zn and 1.4 - 2.6 ppm of Cu present.

4.2.2 Planting design

In late winter of 1990, the block was prepared for planting by spot spraying, at 3 m spacings, in rows 3 m apart, with Roundup™. On 15 August, 1990, 120 roots of *Paulownia tomentosa* of Japanese origin (obtained from the nursery of Mr. Ian Barton, Hunua, South Auckland) were planted in hand dug holes approximately twice the size of the roots. The fresh weights of the roots ranged from 38.8 g to 473.4 g with an average of 146.7 g. These roots had been grown from seeds received from Japan. It is not known whether all the seeds

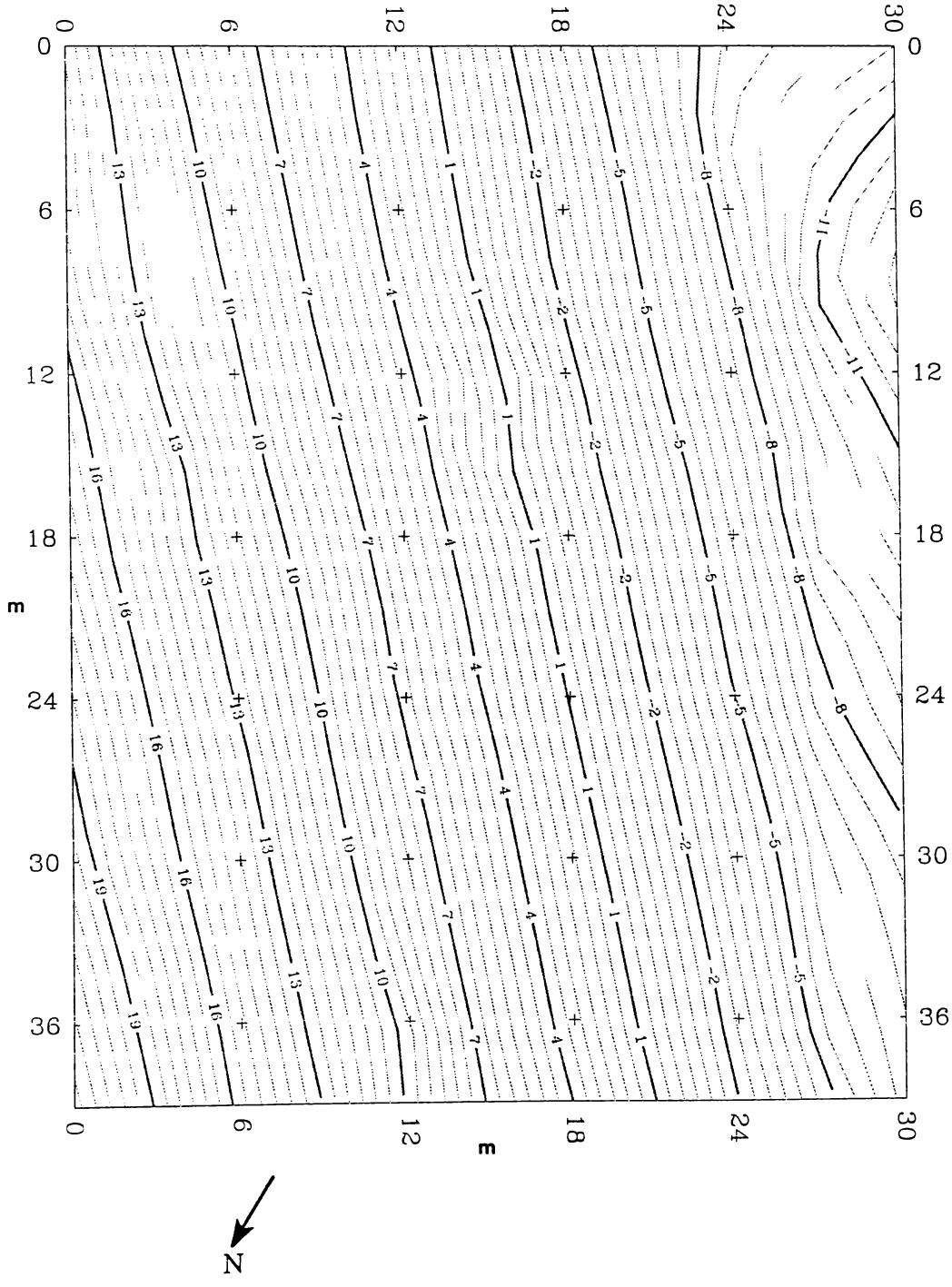


Fig. 4.1. A contour map of the experimental site at Matangi. Contour interval is 0.5 cm.

were from a single tree or from a number of trees. Thus the root material used in these experiments was not clonal, but possessed an unknown amount of genetic variability.

The first shoots began to appear approximately three weeks after planting and by mid September, more than 75% of the roots had produced visible shoots. As the shoots emerged, the plants were covered with open ended plastic containers to protect them from frosts. This was not a totally adequate solution and a number of shoots were killed by frosts. However, as each root produced multiple shoots, this did not pose a serious problem. In late November the best shoot on each plant was selected and the remainder of the shoots removed.

The planting design (Fig. 4.2) allowed for 48 trees to be harvested over the three seasons of the experimental period with each of these trees surrounded by guard trees (permanent trees) on four sides. Hence the trees at the ends of each row and the entirety of the outer rows were considered to be guard trees, as well as alternate trees within the rows. Due to the heterogenous sizes of the root material available at the time of planting, the allocation of the

Fig. 4.2. Positioning of trees in experimental block at Matangi. Those trees marked in bold were harvested over the course of the study (see Table 4.5)

	117		118		119		120		
108	**109**	110	**111**	112	**113**	**114**	115	116	
	99	**100**	**101**	**102**	103	**104**	105	**106**	107
	90	**91**	92	**93**	94	**95**	96	**97**	98
81	**82**	**83**	84	85	86	87	88	89	
	72	**73**	74	75	76	**77**	78	**79**	**80**
	63	**64**	65	**66**	67	68	69	**70**	**71**
		59		60		61		62	
	50	**51**	52	**53**	54	55	**56**	**57**	58
41	**42**	43	**44**	**45**	**46**	47	**48**	49	
32	**33**	34	**35**	36	**37**	38	**39**	40	
	23	24	25	**26**	27	28	29	30	31
14	**15**	16	**17**	**18**	19	20	**21**	22	
5	**6**	7	**8**	9	10	11	**12**	13	
	1		2		3		4		

roots to the 120 positions was not strictly random. There were 22 small roots (generally less than 100 g in weight), which were in fact root cuttings rather than whole root stumps, and these were randomly allocated to guard positions around the edges of the planting. The remaining 98 roots were randomly assigned to the remaining positions within the block.

The design split the planting area into two identical plots which originally received different fertilizer treatments. Trees 1 - 58 (at the more northern end of the block) were to receive no fertilizer, while trees 63 - 120 were to receive two applications per season. The trees between these two plots (59 - 62) acted as guard trees and increased the buffer zone between the fertilized and non-fertilized areas. At the outset it was envisaged that of the four trees dug out at each harvest time, one pair would come from the fertilized plot and one pair from the unfertilized plot.

4.2.3 Maintainence and silviculture

During the first season of growth, two applications of approximately 50 g of solid fertilizer were made to the appropriate trees. The fertilizer was a mixture of urea prills and a commercially available garden fertilizer giving a N:P:K ratio of 12:5:4.3 with added trace elements and Mg, S and Ca. This mixture was broadcast around two sides of each tree and carefully dug in to just below the soil surface with a hand fork. The applications were made at the end of November, 1991 and mid March, 1992. It was decided early in the second season to abandon the fertilizer aspect of the trial as trees in the unfertilized plot were showing signs of nutrient deficiencies. Thus, for seasons two and three, all the remaining trees in the experimental block were given 50 g of the same fertilizer in November and again in March. In addition, liquid fertilizer in the form of a balanced hydroponics solution (section 3.2) was applied twice to the trees during the middle of the 1992/1993 season as many of the trees were not looking as healthy as anticipated.

Other maintainence of the block was carried out as required. This involved various forms of non-chemical weed control including mowing the block, hand weeding around the tree trunks, using a line trimmer to reduce weeds and placing hay or weed mat at the base of the trees. The weed mat was reasonably successful at suppressing weed growth but did tend to cause the soil underneath to dry out more as it presented a semi - permeable barrier to water entering the soil. The major problem with the weed mat, however, was the resident pukeko population which enjoyed foraging under it so that the matting was displaced and rendered useless. As a result of these difficulties the weed mat was only used extensively during the second season and during the third season many of the trees were of sufficient height that only occasional

weeding was required.

Silvicultural maintainence of the trees included pruning of lower branches at the end of the third season, tipping out branches during the growing season to prevent them becoming too long and early season stem trimming. This was necessary because the leaves on *Paulownia* trees occur in opposite pairs, hence the axillary buds occur in pairs down the stem. Generally at the beginning of the season, new growth begins at two or three nodes down the stem and both buds at that point may shoot which would lead to a branched trunk. It is therefore important to remove the top few centimetres of stem, cutting on an angle so as to retain the strongest new shoot. Apart from this, most of the trees were left alone. It is interesting to note that at the end of season three, several of the larger trees were carrying quantities of flower buds.

4.2.4 Measurement of climate and soil variables

A CR21 Micrologger (Campbell Scientific Inc., Logan, Utah) was used to continuously log various weather and soil parameters each season. During season 1 (1990 - 1991) the datalogger was operational from mid November until May, while in seasons 2 and 3 data were collected from September until May. The information was logged at intervals of one minute and averaged every thirty minutes; these averages were stored on cassette tape for subsequent down-loading to a Macintosh computer and further data analysis.

During the 1990 - 1991 season, the parameters measured included PPFD, using a quantum sensor (LI 190 SB, LICOR, Nebraska); air temperature and relative humidity using probe type 201; rainfall with the model RG2501 rain gauge; and soil moisture with a type 221 soil moisture block made of gypsum. In the second and third seasons, rainfall was measured with a plastic rain gauge which was emptied periodically after the rainfall had been recorded. In addition to the other probes, two thermistors (probe type 101) were placed at 10 cm and 30 cm depth in the soil to measure soil temperatures during these two seasons.

The data thus collected were generally analysed by averaging the variables on a monthly basis, based on the calendar months (Tables 4.1 - 4.3). However, when the Walz CMS system was used on the trees (November, 1991 - April, 1992 and November 1992 - April 1993) the data for the month immediately preceding the first photosynthetic measurement was averaged. This meant that for those periods the data were averaged from the middle of one calendar month through to the middle of the next calendar month. Each set of monthly data was further sub-divided into data collected during the daylight hours and that collected at night. The data collected from the quantum meter was presented as the average number of hours each day when the quantity of PPFD was above saturation for *Paulownia* (taken to be

Table 4.1. Climate and soil parameters averaged over season 1 (1990 - 1991) at Matangi experimental site.

Dates	Time	Air Temp (°C)	RH (%)	Soil Moist. (-MPa)	Light (μmol m^{-2} s^{-1})	Light > 1000 (hours)	Rainfall (mm)
20 Nov -	day	17.4	87.8	0.06	767	5.0	28
30 Nov		7 - 23				1 - 8.5	
	night	11.6	96.4	0.06			6
		6 - 16					
1 Dec -	day	19.6	80.4	0.15	870	6.1	26
31 Dec		5 - 28				1 - 9.5	
	night	13.7	93.3	0.15			1
		5 - 19					
1 Jan -	day	19.9	78.1	0.68	781	4.9	39
31 Jan		3 - 27				0 - 9.5	
	night	13.4	90.6	0.74			11
		3 - 21					
1 Feb -	day	21.1	83.3	0.08	721	4.2	20
28 Feb		6 - 29				0 - 8.0	
	night	15.6	91.4	0.08			18
		6 - 24					
1 Mar -	day	19.0	77.5	0.12	721	3.7	16
31 Mar		5 - 25				0 - 7.0	
	night	12.9	88.9	0.13			35
		5 - 20					

Table 4.2. Climate and soil parameters averaged over season 2 (1991 - 1992) at Matangi experimental site.

Dates	Time	Air Temp (°C)	Soil 10 (°C)	Soil 30 (°C)	RH (%)	Soil Moist. (MPa)	Light (μmol m^{-2} s^{-1})	Light > 1000 (hours)	Rainfall (mm)
26 Aug-	day	14.7	12.0	11.6	74.7	0.07	587	2.5	144
30 Sept		-5.5 - 29						0 - 7.0	
	night	7.9	12.1	12.0	92.2	0.07			
		-7 - 15							
1 Oct-	day	15.2	13.7	12.6	79.1	0.07	701	3.7	45
31 Oct		2 - 22						0 - 7.0	
	night	10.4	13.7	12.9	88.3	0.07			
		2 - 16							
1 Nov-	day	15.3	15.1	13.1	77.3	0.07	732	4.1	92
28 Nov		3 - 22						0 - 8.0	
	night	10.5	15.2	13.5	86.4	0.07			
		3 - 16							
29 Nov-	day	19.3	17.7	15.3	71.6	0.28	845	5.7	43
29 Dec		3 - 27						0- 8.5	
	night	13.0	17.7	15.9	84.4	0.32			
		3 - 20							
30 Dec-	day	20.3	19.4	15.7	74.5	0.07	704	4.2	87
24 Jan		6 - 29						0 - 8.5	
	night	14.3	19.4	16.1	84.4	0.07			
		6 - 21							
25 Jan-	day	20.9	19.3	15.0	76.0	0.08	702	4.0	113
23 Feb		5 - 29						0 - 8.5	
	night	15.3	19.4	15.3	85.3	0.08			
		6 - 21							
24 Feb-	day	18.3	17.9	12.1	74.4	0.09	689	3.0	51
26 Mar		1 - 28						0 - 7.5	
	night	12.4	17.9	12.3	84.5	0.09			
		1 - 21							
27 Mar-	day	15.1	14.0	8.3	75.5	0.08	553	2.2	88
4 May		-3 - 23						0 - 5.0	
	night	8.8	13.9	8.5	84.8	0.08			
		-3 - 18							

Table 4.3. Climate and soil parameters averaged over season 3 (1992 - 1993) at Matangi experimental site.

Dates	Time	Air Temp (°C)	Soil 10 (°C)	Soil 30 (°C)	RH (%)	Soil Moist. (MPa)	Light (μmol m^{-2} s^{-1})	Light > 1000 (hours)	Rainfall (mm)
3 Sep - 30 Sep	day	12.1 0 - 18	10.3	10.7	65.2	0.07	520	1.6 0 - 5.0	70
	night	7.2 -0.3 - 12	10.7	10.7	71.8	0.07			
1 Oct - 31 Oct	day	14.0 2 - 22	12.1	12.2	64.3	0.07	522	2.3 0 -7.0	139
	night	9.2 2 - 16	12.3	12.3	82.5	0.07			
1 Nov - 18 Nov	day	16.8 5 - 22	16.0	15.0	64.2	0.09	544	2.6 0 - 6.0	30
	night	12.6 5 - 17	16.1	15.0	70.9	0.10			
19 Nov - 13 Dec	day	18.5 6 - 25	17.8	17.3	60.5	0.09	569	3.0 0 - 7.5	103
	night	14.1 5 - 18	18.1	17.3	68.3	0.09			
14 Dec - 13 Jan	day	18.9 2 - 27	17.8	17.5	56.6	0.20	748	5.0 0 - 7.5	32
	night	12.7 2 - 19	18.2	17.6	68.2	0.21			
14 Jan - 21 Feb	day	20.3 2 - 32	18.1	17.9	48.9	0.80	713	4.5 0 - 8.0	-[1]
	night	13.4 2 - 21	18.5	17.9	65.3	1.00			
22 Feb- 16 Mar	day	20.8 6 - 32	18.6	18.3	49.1	0.07	627	3.1 0 - 6.0	-
	night	13.9 5 - 21	18.9	18.3	66.3	0.07			
17 Mar- 31 Mar	day	17.4 -0.5 - 24	16.0	16.4	47.9	0.07	466	1.3 0 - 5.0	-
	night	11.2 -1 - 18	16.3	16.4	61.4	0.07			
1 Apr- 31 Apr	day	17.0 -1 - 27	14.5	15.0	45.0	0.07	448	0.8 0 - 2.5	-
	night	9.3 -1.5 - 17	14.8	15.1	66.6	0.07			

[1] not determined

1000 μmol m^{-2} sec^{-1} from the results of light response curves). The results were compared with monthly averages of similar variables measured at the Ruakura research site for the past thirty years (Table 4.4).

The standard errors for the monthly averages of the meteorological data in Tables 4.1 - 4.3 were highest for air temperature, ranging from 16% of the monthly average to 56% across all three seasons. Soil temperatures were less variable, with a range of s.e. from 2% to 20% of the monthly averages. With all the temperature data, there was little difference between the variability of the daytime averages and the nighttime averages. However, RH was more variable during the day (s.e. range 11% - 36%) than at night (s.e. range 4% - 12%). The soil moisture data varied little when the soil was well watered with very low standard errors calculated, but standard errors increased markedly when the soil began to dry out with the maximum s.e. being 64% of the monthly average in one instance.

The average monthly air temperature was similar in all three seasons, although season 3 was slightly cooler overall than the other two seasons. The warmest month in each season was February with an average temperature of 21 °C. Direct comparison of air temperatures with the data from Ruakura was not possible as only minimum and maximum air temperatures were provided. These data did show, though, that the average minimum and maximum temperatures in season 3 were generally slightly lower than the 30 - year averages each month. Air temperatures were close to the average in season 1 and for the beginning of season 2, but were lower in the latter half of this season (data not shown).

The data relating to soil temperature was directly comparable between the Ruakura station and the experimental site. In general the soil at 10 cm was slightly warmer in season 2 than in season 3, with values of the 30 - year average from the Ruakura site between those of the two seasons at the experimental site. The situation was different for the data for the soil temperature at 30 cm when the values were higher from the Ruakura site (which may reflect a different soil type at that depth) than at the experimental site. The temperatures at that depth were higher in season 3 than in season 2, particularly in the latter part of the season. This difference between the two seasons may be a reflection of the soil moisture level which was lower for a longer period (December through to February, Table 4.3) in the third season. In season 2, the soil moisture level was reasonably constant, decreasing significantly only in December (Table 4.2). The situation in season 1 with respect to soil moisture was more similar to season 3, with low levels in December, January and March.

The daily average number of hours when the light levels were saturating was consistently higher in season 1 than in season 2, and generally lowest each month in season 3. The hours

Table 4.4. Climate variables measured at the Ruakura Research Station, Hamilton, averaged on a monthly basis, from 1963 to 1992. (Data obtained from NIWA Ltd., Kelburn, Wellington).

Month	Max. Temp. (°C)	Min. Temp. (°C)	Soil Temp. 10 cm (°C)	Soil Temp. 30cm (°C)	Sunshine (hours)	Rainfall (mm)	RH (%)
Sept	16.2	6.5	10.5	12.0	147	102	81
Oct	17.9	8.0	13.0	14.2	180	92	79
Nov	20.0	9.8	15.3	16.4	195	92	78
Dec	22.0	11.4	17.4	18.4	223	100	78
Jan	23.7	12.5	18.7	19.9	232	87	79
Feb	24.2	12.8	18.7	20.1	197	79	82
Mar	22.8	11.7	17.0	18.9	187	85	82
Apr	19.8	8.8	14.0	16.4	131	89	85
May	16.5	6.2	10.9	13.3	131	104	89

of saturating light were particularly low at the beginning and at the end of season 3, with January and February the only two months when the light was saturating for a similar time in all three seasons. This result related to the number of sunshine hours recorded at Ruakura at the beginning of season 3 (especially October, November and December, 1992) being considerably lower than the 30 - year average, while values for the same period in 1991 were higher than the average (data not shown).

There was a large difference between the seasons in the average relative humidities for both the daytime and nighttime data. The first season generally had the highest humidity each month. The relative humidities recorded in season 3 were significantly lower than those recorded in seasons 1 or 2. Although not measured directly, wind seemed to be more prevalent in season 3 which would tend to decrease the RH. The RH data from the Ruakura site were the average humidity measured daily at 9 am, for the past 22 years. The monthly averages at Ruakura over the third season were similar to the 22 - year average, but those in seasons 1 and 2 were generally higher (data not shown).

4.3 Biomass Accumulation and Partitioning

4.3.1 Materials and methods

4.3.1.1 Tree growth

For each of the three growing seasons studied, measurements of tree growth were carried out on a monthly basis from November until May. Tree height from the base of the stem to the apex was recorded, as was the stem diameter at the base. This diameter was measured using digital callipers but the fact that many of the stems were irregularly shaped possibly led to the underestimation of stem diameter. A comparison of measurements with callipers and a diameter tape in the latter part of season three indicated that the results using callipers could be up to 10% less than those from the tape. However, in order to maintain consistency between measurements made over the three years, only those results using the callipers will be presented. Other morphological characteristics were measured at different times over the three seasons including leaf number, leaf length, branch length, DBH and overall appearance of the trees.

The growth of many of the trees over the three seasons was acceptable, but a number did not grow as well as expected. This resulted in some of the smaller trees being accidentally coppiced by the tractor during mowing. During season one, three trees (25, 88, 103) were replaced with plants that had grown from roots that were left over at the time of planting. These roots had been planted into long planter bags and grown up in the glasshouse for four months. At the beginning of season two, many of the smaller plants were either intentionally coppiced, to match those done accidentally, or replaced altogether with new roots.

4.3.1.2 Whole plant harvests

In each season, whole plants harvests were carried out in November (after approximately two months of new growth), mid January, late March and in May at the end of the season. The exact dates of each harvest are given in Table 4.5. At each harvest, four trees were dug up. Initially, eight pairs of trees in matching positions in the two plots (unfertilized and fertilized) were selected and randomly assigned harvest dates in the first two seasons. The selection of trees for harvest in season three was left until the beginning of that season, when suitable pairs of trees were randomly assigned to the four harvest dates. The large variability in growth patterns across the experimental block led to some modifications of the original harvesting scheme, *e.g.* the May harvest of the season 1 involved trees that were originally

Table 4.5. The dates at which whole tree harvests were undertaken and the identity of each tree harvested.

Harvest	Date	Trees Harvested
1	31.10.90	6, 15, 70, 79
2	10.01.91	12, 21, 64, 73
3	18.03.91	35, 44, 95, 104
4	27.05.91	5, 14, 71, 80
5	4.11.91	33, 42, 97, 106
6	7.01.92	37, 46, 93, 102
7	12.03.92	8, 17, 66, 77
8	11.05.92	39, 48, 91, 100
9	23.11.92	50, 51, 101, 111
10	22.01.93	18, 26, 99, 109
11	24.03.93	56, 57, 82, 83
12	27.05.93	45, 53, 113, 114

guard trees so as to leave the allocated trees for later harvests if necessary. The actual positions of each of the harvested trees are given in Fig. 4.2.

Each tree was carefully dug out by hand in order to recover as much of the root matter as possible. The soil around the trees was first loosened with a large garden fork, then the top soil layers sorted through for fine roots that had broken off and finally the larger lateral roots were dug out to release the tree. This process was very rapid in the first season as the trees were still relatively small, but as the trees grew so did the time taken to harvest them. Larger trees harvested in the second and third seasons generally took four people about 3 - 4 hours to completely dig out. Total recovery of all the root material was not possible with these larger trees, but it is estimated that between 80% and 95% of both the fine roots and the woody, lateral roots were harvested, with the actual percentage recovery depending on the tree involved. It would have been possible to recover more of the root biomass, but because the carbohydrate content of the trees was to be ascertained, then a trade off had to be made between the time involved in recovering the last 10% - 20% of the root material and minimizing the changes in carbohydrate status which the various tissues underwent as the tree was harvested. As soon as the trees were brought back to the laboratory, all parts of the plant were refrigerated to minimize further carbohydrate conversions.

Processing of the harvested trees included measuring the height and basal diameter of the stem, the leaf area and the fresh and dry weights of each tissue. As can be seen from Fig. 4.3 leaves destroyed by grass grub beetle (discussed in section 4.3.2.1C) had little lamina tissue and were therefore not subjected to leaf area measurements. The tissue of the older trees were generally divided into lamina, petiole, stem, branches (when present), root stump, lateral (woody) roots, and fine roots. The size of the first few trees harvested in season one precluded such subdivision, so more appropriate tissue categories were employed. Before being weighed, the root material was thoroughly washed to remove soil particles, and then blotted dry. All the tissues were freeze-dried, in preparation for carbohydrate and other nutrient analysis, then ground to pass through a 1.1 mm diameter mesh. As the trees increased in size, only subsamples of each tissue type were dried. In order to subsample in an unbiased way, the larger, woody tissue were chipped in a tree shredder that produced fragments up to 5 cm in length.

The separation of the root material into the three classes was somewhat arbitrary at the extremes of each class. The root stump was defined as the large mass of root material found directly below the stem. In the first season, this generally corresponded to the the root material originally planted, but as the trees grew, then the stump became more than that. However, it was from this root mass that the lateral roots arose and it was difficult at times to distinguish between stump matter and the beginnings of lateral roots. The lateral roots were classified as such by their woody nature. They grew both horizontally and vertically through the soil profile and were often branched. As the lateral roots decreased in size, it became difficult to differentiate between thin lateral roots and thick 'fine' roots. Those roots which had a fleshy periderm, with no woody thickening, were considered to be 'fine' roots, but some of the larger fine roots had diameters of up to 4 mm with very thick periderms. The smaller fine roots were usually less than 2 mm in diameter and many only about 0.7 mm. They were reasonably easy to distinguish from the roots of the surrounding herbaceous plants by their colouring which ranged from cream with orange markings through to completely bright orange. Because of these classification difficulties, the results of total root biomass for each tree are more significant than biomass of each root type, but the results of the various nutrient analyses are valid for the three types.



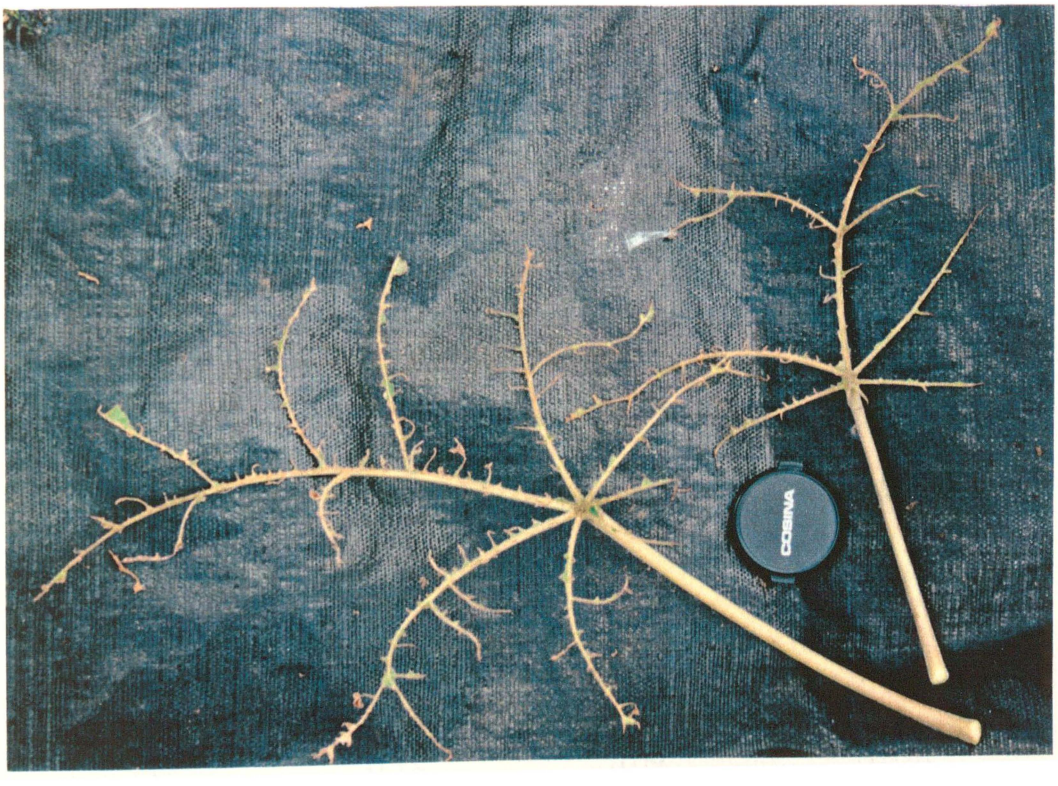


Fig. 4.3. Damage done to leaves by grass grub beetles.

4.3.2 Results

4.3.2.1 Growth patterns over three seasons

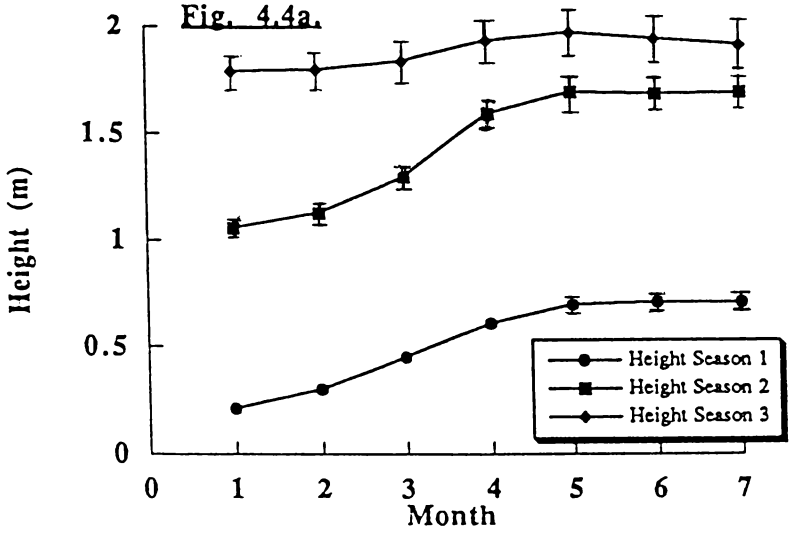
A). Growth rates of the good trees

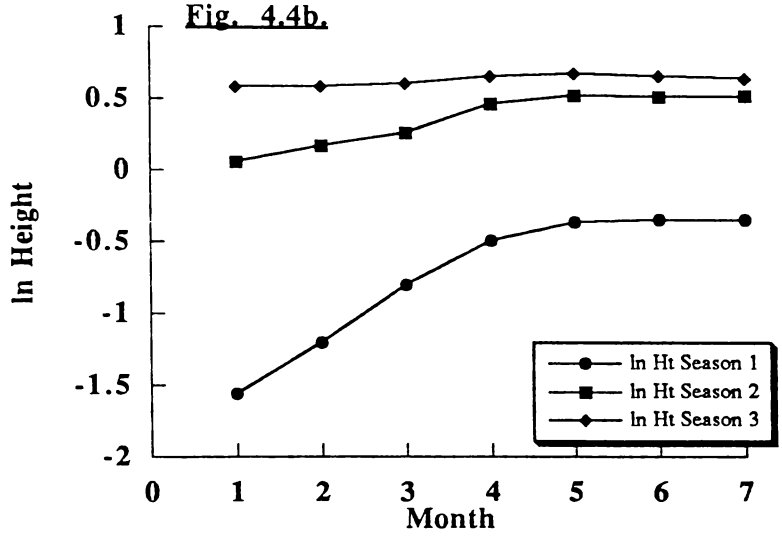
The average relative growth rates (R) for the growth parameters of height and diameter at the base of the stem decreased over the three seasons (Figs 4.4 and 4.5). The value of R for height was constant in each season for the period between November to February, after which time the increase in tree height declined. The period of constant relative growth of basal diameter was one month longer, with the values of R (calculated by functional growth analysis techniques) for the two growth parameters similar over that period. R for height was 0.36 m m^{-1} $month^{-1}$, 0.13 m m^{-1} $month^{-1}$ and 0.027 m m^{-1} $month^{-1}$ for the three seasons respectively, while for diameter R was 0.31mm mm^{-1} $month^{-1}$, 0.15 mm mm^{-1} $month^{-1}$ and 0.048 mm mm^{-1} $month^{-1}$ for seasons 1 to 3. The values for R (calculated by classical growth analysis techniques) over the whole season are given in Table 4.6 and are lower than those calculated using only the linear period of growth because they take into account the slower stem growth at the end of the season.

The absolute levels of growth (Fig 4.4a and 4.5a) were highest in season 2 (Table 4.6) when the average heights of the trees increased by 0.99 m (to 1.70 m in May, 1992). Over the same period the average basal diameter increased by 27.1 mm (to 52.3 mm). In comparison, during season 1, the average height of the trees increased by 0.71 m and the diameter by 25.2 mm. The overall growth of the trees was noticeably less during season 3 with several trees

Table 4.6. The absolute and relative growth rates for stem height and basal diameter over the entire growing season, for the first three seasons of *Paulownia* tree growth.

Parameter	Season 1	Season 2	Season 3
G (stem height) (m $month^{-1}$)	0.083	0.12	0.028
G (stem diameter) (mm $month^{-1}$)	2.74	3.39	1.59
R (stem height) (m m^{-1} $month^{-1}$)	0.20	0.11	0.015
R (stem diameter) (mm mm^{-1} $month^{-1}$)	0.18	0.09	0.026





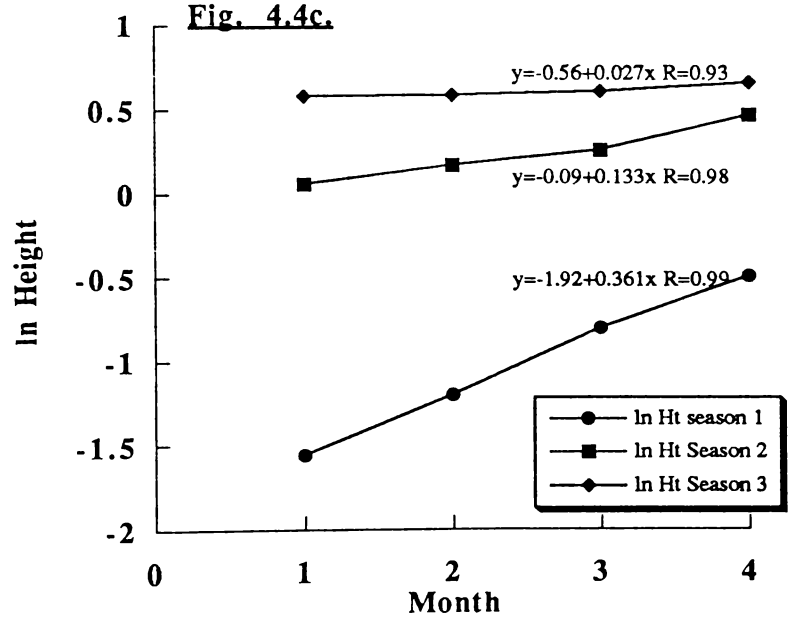
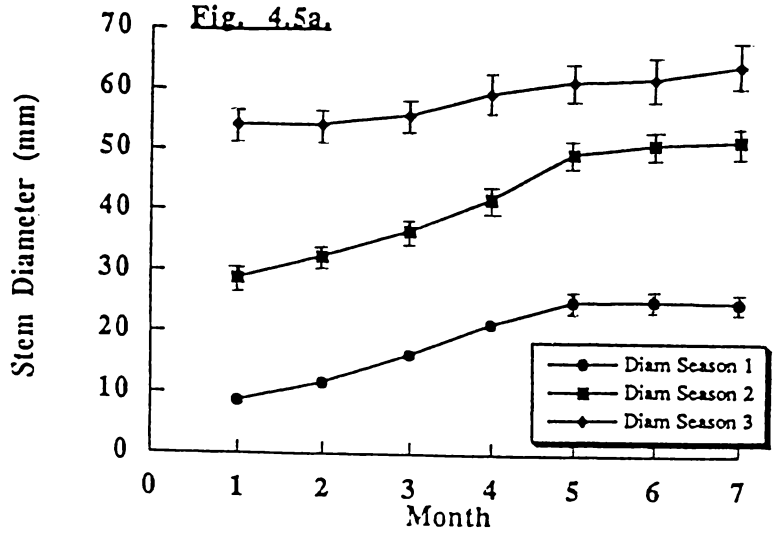
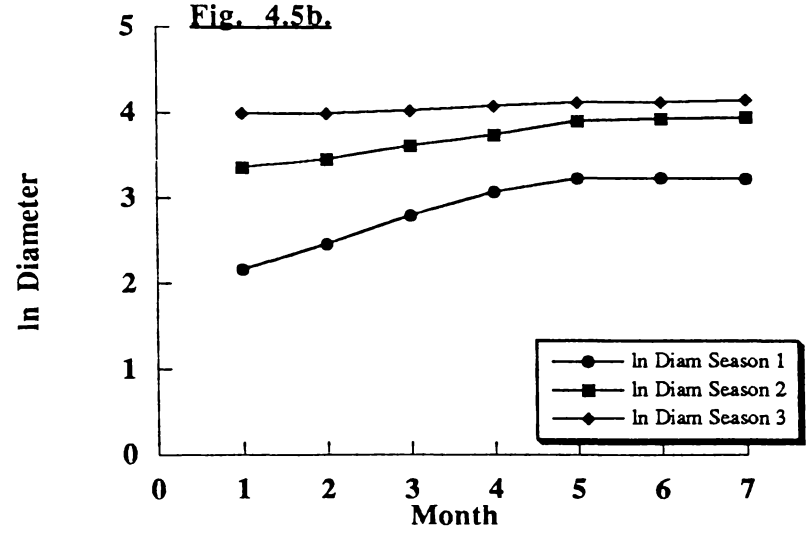


Fig. 4.4a, b, c. Increase in height over P. tomentosa trees over three seasons. a. - untransformed height data (Nov - May); b. ln transforme height data (Nov - May) ; c. ln transformed height data (Nov - Feb). Month 1 - Nov; 2- Dec; 3 - Jan; 4 - Feb; 5 - Mar; 6 - Apr; 7 - May.





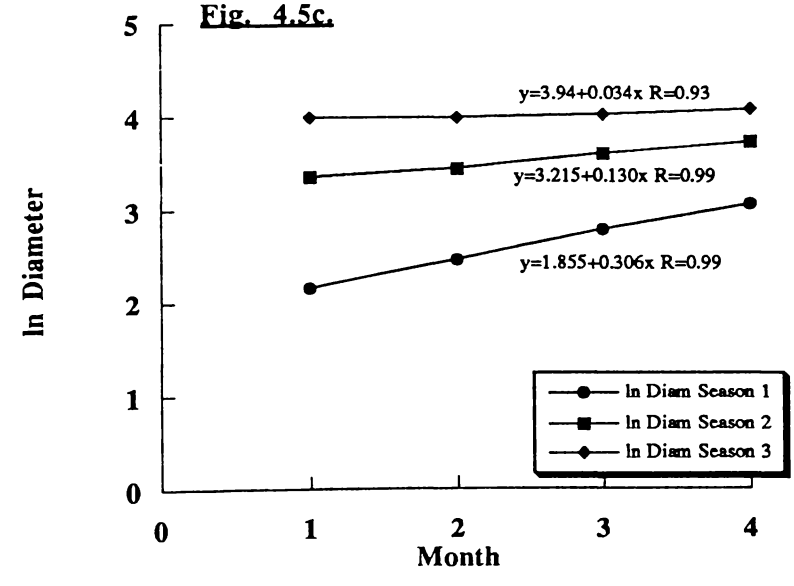


Fig. 4.5a, b, c. Increase in diameter over P. tomentosa trees over three seasons. a. - untransformed diameter data (Nov - May); b. ln transformed diameter data (Nov - May) ; c. ln transformed diameter data (Nov - Feb). Month 1 - Nov; 2- Dec; 3 - Jan; 4 - Feb; 5 - Mar; 6 - Apr; 7 - May.

gaining very little in height over the season. The absolute growth rate (G) of stem height was four times lower in season 3 than season 2, and G for stem diameter was half that in season 2. Consequently the average height at the end of the season was 1.92 m (an increase of 0.22 m) and the average diameter of the trees was 65.0 mm (an increase of 12.7 mm). This overall growth trend was reflected in the growth of individual trees. Tree 10, for example, was 1.34 m tall with a diameter of 44.1 mm in May, 1991; 3.25 m tall with a diameter of 83.9 mm in May 1992 (the tallest tree in the plot); and 3.47 m tall with a diameter of 101.0 mm in May 1993.

The data used to calculate the averages presented in Figs. 4.4 and 4.5 and Table 4.6 only included those trees that were not in the poor growing area (discussed below), and in seasons 2 and 3 included only those trees that had been growing for the entire length of the study. This excluded the trees that were coppiced or replaced at the beginning of season 2.

B). The poor growing area

It was obvious very early in the experimental period that the growth of some trees was markedly less than that of the remainder of the plants (Fig 4.6). These trees were only 20 - 40 cm tall at the end of the first season, with diameters averaging about 10 mm. The foliage of these trees was also small and pale green to yellow in colour. Some of the poorly performed trees were isolated but many of the slower growing trees were in an area in the middle of the block on the western side (Fig 4.7). A number of reasons were advanced as to why the plants in this area grew poorly and, although these will be discussed here, it is not known at this stage which factor or combination of factors prevented healthy growth.

The first factor was the genotype of the planted material. Although all the trees were of the same species and origin (*P. tomentosa* Japan), the planting stock was not clonal and so inherent differences in the genetic make-up of the roots planted certainly would influence growth rate. This may account for the isolated trees that grew poorly, but the randomization of the root material before planting meant that the chances of a clump of trees occurring with poor inherent growth rates were very small. Similarly, the size of the roots at planting was unlikely to cause the observed patchiness as the roots planted in the poor area were of varying weights which, on average, were no different from roots planted elsewhere. Thus it was deemed unlikely that the plant material was the primary cause of the poor performance.

The second possible cause was the physical nature of the soil environment. Results from the



Fig. 4.6. Comparison of tree from the good growing area (top photo) with tree from poor growing area (lower photo) after six month's growth.

Fig. 4.7. Layout of experimental plot at Matangi indicating the area of poor tree growth (numbers marked in bold) and those trees either coppiced or replaced during seasons 1 and 2 (underlined).

	117		118		119		120		
108	109	110	111	112	113	114	115	116	
	99	100	101	102	103	104	105	106	107
	90	91	92	93	**94**	**95**	**96**	97	98
81	82	83	84	**85**	**86**	**87**	**88**	89	
	72	73	74	**75**	**76**	**77**	**78**	79	80
	63	64	**65**	66	**67**	**68**	**69**	70	71
		59		**60**		**61**		62	
	50	51	52	53	54	55	56	57	58
41	42	43	44	45	46	47	48	49	
32	33	34	35	36	37	38	39	40	
	23	24	25	26	27	28	29	30	31
14	15	16	17	18	19	20	21	22	
5	6	7	8	9	10	11	12	13	
	1		2		3		4		

topographical survey (Fig 4.1) confirm the visual impression that the poor area was slightly lower than other areas of the site. A possible result of this depression could be a higher water table. Results from a soil survey done in this area did show some evidence of flooding with gleying of the soil in the B_1 horizon visible (R. Chapman, pers. comm.). This may have affected growth, but it seems unlikely as during the first season there was a drought in the early summer and so it would be expected that the water table would be lower than the zone of new root growth. The water table was about 1.5 m below the soil surface over most of the site (R. Chapman, pers. comm.). However, even a transitory rise in the water table to the level where the roots were temporarily under water would adversely affect tree growth as *Paulownia* roots are very sensitive to flooding.

The chemical properties of the soil could also have affected growth. The soil in the poor area was a Bruntwood loam as opposed to the majority of the soil in the site which was a Horotiu sandy loam, but it is unlikely that this difference is enough to explain the growth differences.

What may be significant is the previous land use of the site, which has included berry fruit cultivation as well as beef cattle grazing (R. Chapman, pers. comm.). Physical evidence of this horticultural usage was found by way of black polythene buried in the soil in different places. It is possible that, during the cultivation of the berry crops, extensive use was made of chemical pesticides and that there are residual amounts of persistent phytotoxic compounds concentrated in the lower lying poor area. Alternatively, one of the pathogens associated with berry and cane crop growth is *Phytophthora* which can live in the soil for many years after the removal of the crop plants (D. Klinac, pers. comm.). This fungus is not specific in its choice of host material and so could possibly be involved in the retardation of the *Paulownia* growth rates.

Another biological feature of the soil that may be involved is the presence of reasonable numbers of soil nematodes which were identified by Dr. R. Watson of AgResearch Ltd. He found several species of nematodes in soil from both the poor area and other areas of the experimental site. These included *Heterodera* and *Pratylenchus* which were present in the highest concentrations in soil from the poor area, and *Meloidogyne* which was found more in the soil from areas of good tree growth. Both *Pratylenchus* and *Meloidogyne* can invade plant roots of a number of species (R. Watson, pers. comm.). It is likely, therefore, that nematode infection of *Paulownia* roots occurred in the experimental site, but whether this caused the poor performance of the trees in the poor area is as yet unclear. It has been reported that infection by *Meloidogyne incognita* has caused severe growth retardation in *P. kawakamii* nursery stock in Taiwan (Ying, 1975).

To further investigate the cause of the poor performance of trees this area, a preliminary experiment involving the application of the nematicide Nemacur™ and the fungicide Ridomil™ to plots in the poor area containing new *Paulownia* roots was carried out in season 3. Due to incorrect preparation of the planting material, this experiment did not yield any results. It needs to be repeated properly to shed further light on this problem. In addition, analysis of the soil for chemical residues may also prove to help solve the problem.

C). Constraints on overall tree growth

One further problem encountered as a result of the soil biota was severe leaf damage caused by grass grub beetles (Fig. 4.3). The soil at the experimental site had a very high concentration of the larvae of grass grub (*Costelytra zealandica*), which may have inhibited root development in some trees (K. Ross, pers. comm.). However, the major effect of this insect was the emergence, in late spring, of the flying adult beetles which chewed any leaves in their flight path. Many leaves on the taller and medium size trees were skeletonized by the

beetles. Smaller trees were less affected as they were probably below the height of the flight path. *Paulownia* leaves were not the only targets of these beetles - they also decimated the leaves on a nearby young chestnut plantation.

There was a little damage done in the first season with some leaves being eaten in mid October, but this was minimal due to the small size of the trees. In late October of the second season, many of the trees succumbed virtually overnight with large numbers of leaves being affected. In season 3, the damage was spread over a longer time period (perhaps as a consequence of the cooler spring) starting in late October and continuing through the early part of November. The major result of this leaf damage was the reduction in photosynthetic area available to the plant early in the growing season. This photosynthetic area was gradually replaced, but the continuing damage in season three may have contributed to the low growth rates for the season. It is suggested that in future seasons the trees are protected by spraying the leaves with a synthetic pyrethroid insecticide that would kill any beetles eating them (K. Ross, pers. comm.).

4.3.2.2 The harvested trees

A). Season 1

The first harvest of season 1 was carried out after only two months of growth. Consequently, most of the biomass was concentrated in the root stump tissue which had changed little since planting. The new shoot and root material constituted only a small proportion of the biomass and were not able to be separated into their components, hence the tabulation categories in Appendix 1. During the season, the development of new shoot tissue (lamina, petiole and stem) was more rapid than that of new root material as shown by the R:S ratios for the second harvest (Table 4.7). This result indicates that the reserves within the root stump were initially allocated to aid the rapid development of a functioning (photosynthesising) shoot, rather than to the growth of new roots. However, by the latter half of the season the biomass in the lateral root tissue had increased until it was greater than that in the stump. At the end of the season more biomass was present in the perennial roots than in the perennial shoot material. The stem remained unbranched throughout this season.

There was much variation between the growth and allocation patterns of biomass among the 16 plants harvested in season 1 (Table 4.7, Appendix 1) with very few general trends observable. For example tree 73 had a stem length of 0.6 m at harvest which weighed 39.8 g, while tree 71 was the same height with a slightly larger basal diameter, but was almost twice as heavy (66.6 g). Tree 21, meanwhile, was slightly taller at 0.65 m but much lighter

Table 4.7. Tree size and growth parameters of trees harvested in season 1 (1990 - 1991).

Harvest	Tree	Height (m)	Diameter (mm)	Leaf Area (m^2)	R:S Ratio	Biomass Total (g)	Root (g)	Shoot (g)
1	6	0.17	10	0.08	4.83	23.7	19.1	4.1
1	15			0.11	13.43	80.8	75.2	5.6
1	70			0.19	4.27	38.3	31.0	7.3
1	79	0.23	9	0.11	7.09	70.3	61.6	8.7
2	12	0.39	15.7	0.24	0.96	66.7	32.7	34.0
2	21	0.65	20.9	0.88	0.77	218.7	94.9	123.8
2	64	0.55	19.4	0.92	0.63	191.2	73.8	117.4
2	73	0.60	22.4	1.03	0.74	257.9	109.3	148.6
3	35	0.98	34.5	2.02	0.79	697.0	307.3	389.7
3	44	0.71	28.4	1.10	1.89	608.1	397.5	210.6
3	95	0.34	8.8	0.05	4.83	67.0	55.5	11.5
3	104	0.55	22.6	0.66	1.51	280.6	169.0	111.6
4	5	0.44	19.6		5.31	191.8	161.4	30.4
4	14	0.41	14.5		2.54	69.0	49.5	19.5
4	71	0.60	25.8		3.40	367.2	283.8	83.4
4	80	0.53	23.2		3.15	360.0	273.2	86.8

than either of trees 71 and 73. There was no clear relationship between tree height and basal diameter of the stem, nor between tree size and leaf area. Leaf area of the plants generally increased with the age of the plants which resulted in an increase in shoot biomass. But overall those plants harvested at the end of the season were no taller, on average, than those harvested in January or March and had less biomass in stem and root material than the plants harvested in March.

These results reflected the uneven growth patterns discussed above (section 4.3.2.1B). The results of the one tree (95) harvested from the poor growing area illustrated the low growth rates of trees in this patch. After 6 months of growth, tree 95 was only 34 cm tall with a total biomass of 67 g. Most of this biomass was concentrated in the stump tissue, with little development of either new root or shoot material having occurred. The leaves of this plant

were small and yellow with necrotic patches. The only new root tissue was emerging from the base of the planted root (at a depth of 15 cm) and was predominantly small woody roots and a few fine roots.

In healthy trees, the emergence of new root tissue from the root stump occurred along the entire length of the planted root, while new shoots were generally initiated in the upper portion of the root. In the case of tree 70, some new roots developed from the base of the shoot. By January many of the larger laterals roots were 30 - 40 cm long, with maximum diameters ranging from 7 mm to 15 mm. These larger roots were branched and grew out at a variety of angles from the parent root to exploit the greatest soil area. Some roots initially grew horizontally and then bent to grow in a more vertical plane to depths of at least 30 cm. The development of the root system was influenced by the fact that these trees were propagated from root stumps. These stumps persisted, and increased in biomass over the three seasons studied, precluding the development of a deep tap root. Instead, a number of small to medium sized lateral roots arose from the lower parts of the stump, growing vertically. Thus directly under the stem of the tree there was between 10 cm and 20 cm of root stump (with a diameter up to 100 mm) and at the base of this stump were several thinner roots descending straight down. The same pattern of rooting was observed with all trees during the three seasons of harvests.

During the first season, new fine roots developed at the end of small lateral roots in highly branched groups. Fine roots were found throughout the soil profile in March, down to about 30 - 40 cm, but were present in the highest concentrations in the upper 20 cm of the soil. By the harvest in May, the number of fine roots had decreased considerably as the trees were entering the dormant season. For those trees harvested in March and May, lateral roots extended 60 - 70 cm horizontally from the parent root, and to depths of 50 - 60 cm. The largest roots were about 20 mm in diameter, although one root on tree 35 had a diameter of 32 mm.

B). Season 2

The relationship between height and diameter was stronger for trees harvested in season 2 than in season 1, with both variables increasing at each harvest (Table 4.8). The shorter trees (97, 93 and 39) also generally had lower biomasses in each tissue category (Appendix 2), smaller leaf areas, but higher R:S ratios than concurrently harvested trees. Tree 77 was growing in the poor area (section 4.3.2.1B), and displayed the lack of development of new root and shoot material characteristic of trees in that area.

Table 4.8. Tree size and growth parameters of trees harvested in season 2 (1991 - 1992).

Harvest	Tree	Height (m)	Diameter (mm)	Leaf Area (m^2)	R:S Ratio	Biomass Total (g)	Root (g)	Shoot (g)
5	33	1.22	35	0.85	1.44	647	382	265
5	42	1.67	49	2.08	1.08	1288	668	619
5	97	0.82	28	0.70	1.59	268	165	104
5	106	1.04	32	1.37	1.34	549	314	235
6	37	1.67	50	2.63	0.91	1557	742	815
6	46	1.42	42	1.94	0.80	818	363	455
6	93	0.81	24	0.75	1.17	329	178	151
6	102	1.51	43	2.95	0.67	111	449	668
7	8	2.23	62	7.19	0.68	2987	1213	1774
7	17	1.53	40	1.66	0.64	858	334	524
7	66	2.06	53	3.90	0.90	2125	1007	1117
7	77	0.28	9	0.07	2.65	59	43	16
8	39	0.86	27		2.22	280	193	87
8	48	1.85	47		2.18	1881	1290	591
8	91	1.63	59		1.49	1895	1133	762
8	100	1.67	59		1.24	2019	1118	901

The amount of foliage (both area and mass) increased with each harvest through the season, with tree 8 having a leaf area of 7.2 m^2 with a dry weight of 0.6 kg in March. The low leaf area and mass at the beginning of the season was a partly reflection of the damage done to the foliage by the grass grub beetles and partly a result of the sizes of leaves produced at different times in the season. The first leaves produced each season were smaller with less well defined petioles than those leaves that developed subsequently. These small leaves stayed on the trees for two to three months. The longevity of the subsequent leaves ranged from two to five months, with some leaves tagged in early December still present on the trees in April.

Total biomass generally increased over the season as did root and shoot biomass. Branches developed on the trees during this season. In Appendix 2 the stem material was classified as

either old stem (material present from the previous season) and new stem (material produced in the current season). A comparison of trees that were the same height at the start of season 2 but harvested at different times through the season is given in Fig 4.8. This shows that the total biomass of the trees increased by a factor of four from the November harvest to the May harvest. The allocation of biomass to the different perennial tissues was slightly different between individuals, but generally more root material was present at the end of the season than shoot material. It is interesting to note that trees of the same height at harvest but harvested at different stages of the season had quite different amounts of tissue. Trees 42, 37 and 100 were all 1.67 m tall with similar diameters at harvest, but the root biomass, shoot biomass and total biomass values increased with age. This is despite the fact that tree 100 was leafless at harvest. This indicates that the allocation of biomass differed between trees, with tree 100 investing more resources into branch and root growth rather than stem extension.

The horizontal and vertical extensions of the root system increased markedly between seasons 1 and 2. Trees harvested in November had lateral roots out to a distance of 1.5 m from the collar, and to a depth of 1.0 m. Trees harvested in January and March had roots extending for similar distances, while the roots of trees harvested in May tended to be longer and deeper (1.5 m). One root was followed for 3.2 m just below the soil surface before it branched into two much smaller roots. The diameter of the thickest roots increased through the season from 28 mm to 60 mm. It was possible to observe growth rings in the larger woody roots from the May harvest, with a translucent layer present between the outer growth ring and the bark.

Within the soil profile the majority of fine roots were present in the upper soil layers (0 - 10 cm). This may have been a consequence of fertilization with the solid fertilizer supplying nutrients primarily to the upper soil layers. Fine roots were present in smaller quantities down the profile, with those found in the sand (below about 30 - 40 cm) generally thicker but with less pigmentation than those found at a lesser depth. Several trees showed signs of nematode infection in the fine roots.

C). Season 3

The low relative growth rates in season 3 discussed above (section 4.3.2.1A) were reflected in the heights of the trees harvested in this season. Although the trees were generally taller than those harvested in the previous season, there was only a weak trend of increasing height with increasing age of the trees through the season (Table 4.9). The linear relationship between height and diameter was obscured by some trees of the same height having quite different diameters (*e.g.* 57 and 83), while trees with the same diameter were often different

Table 4.9. Tree size and growth parameters of trees harvested in season 3 (1992 - 1993).

Harvest	Tree	Height (m)	Diameter (mm)	Leaf Area (m^2)	R:S Ratio	Biomass Total (g)	Root (g)	Shoot (g)
9	50	2.27	65	0.58	0.77	2355	1023	1331
9	51	2.10	65	2.00	0.77	2373	1030	1343
9	101	1.68	58	2.90	1.03	2485	1258	1227
9	111	2.64	90	5.43	0.91	4692	2232	2460
10	18	1.48	52	6.08	0.54	2212	774	1438
10	26	2.25	70	9.60	0.57	4008	1454	2554
10	99	1.60	44	2.24	0.71	1214	502	712
10	109	2.31	69	7.97	0.72	4266	1788	2478
11	56	2.33	68	4.12	0.87	4050	1881	2169
11	57	2.05	64	6.13	0.87	3584	1664	1920
11	82	2.22	76	5.77	0.87	4962	2311	2651
11	83	2.02	80	3.86	1.01	4147	2087	2060
12	45	1.76	68		1.45	3536	2095	1442
12	53	1.45	53		1.12	1491	789	702
12	113	1.43	48					740
12	114	1.92	61					1127

heights (*e.g.* 50 and 51; 56 and 45).

The total biomass in each tree and the biomass of the different tissues tended to increase through the season, but there was much variation between individuals (Appendix 3). The trees harvested in March had the highest average biomass in each of the perennial tissues, although the amount of foliar tissues was less than in January. Once again, the effect of the grass grub beetle is reflected in the low leaf area and biomass values for the trees harvested in November. The allocation of resources to new branch material increased through the season. In the first two harvests, the new branch material was still green and not fully lignified so was treated separately from the previous year's branch tissue. By the March harvest, the extension of branches had ceased and the new material was woody, so it was decided to treat all the branch material as one category. With respect to the new stem material, there was generally so

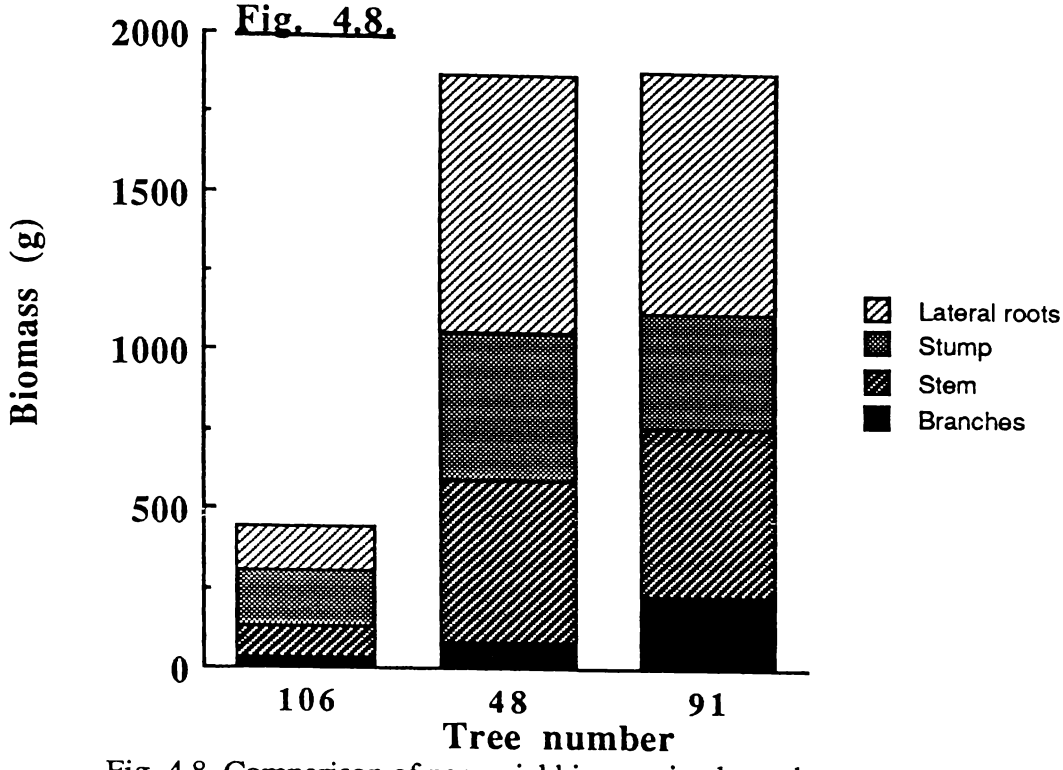


Fig. 4.8 Comparison of perennial biomass in plants that were the same height at the beginning of season 2. Tree 106 harvested in November, 1991, trees 48 and 91 harvested in May, 1992

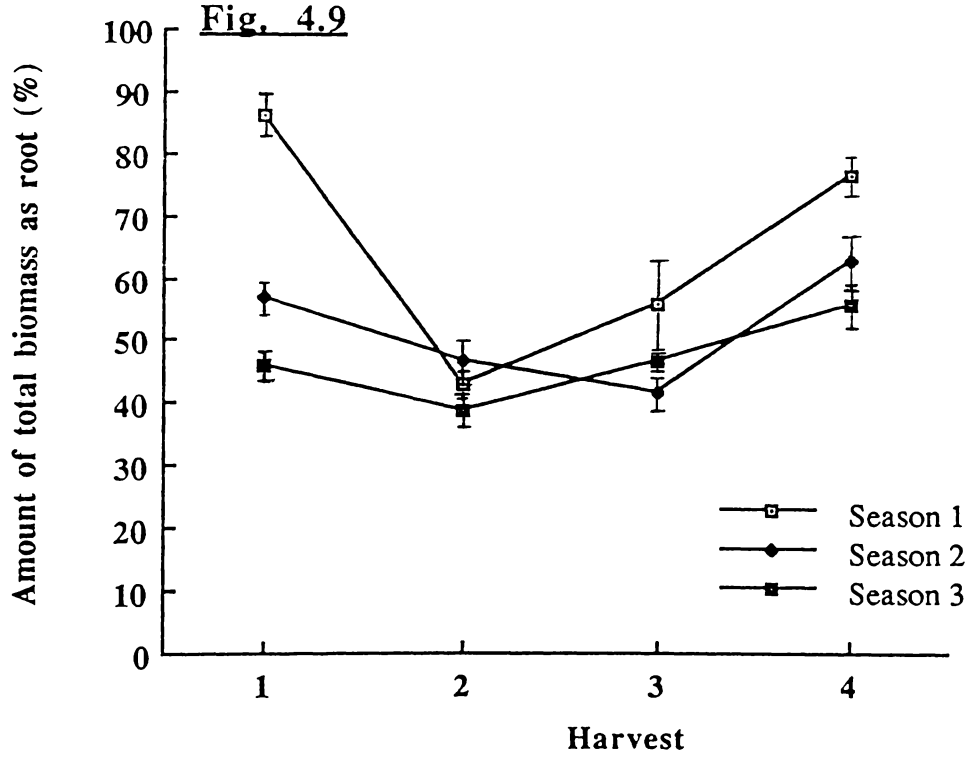


Fig. 4.9. Amount of total biomass present as root biomass at each harvest over three seasons.

little of it in comparison with the existing stem that it was considered simpler to combine the two groups for analytical purposes.

Another contribution to the increase in total biomass over the season was the increasing size of the perennial roots with increases in both the stump and lateral root components. The extension of the woody root system was such that lateral roots were commonly followed out to at least 1.5 m from the stump, and were still growing vertically at depths of more than 1 m. The longest root found was 2.35 m, growing in a horizontal direction. The diameter of the thickest lateral roots ranged from 35 mm to 75 mm. Due to the spacing of the trees (3 m in each direction) there were several instances where roots from a neighbouring tree were uncovered in the course of the harvest.

The biomass of fine roots was greatest at the January and March harvests. Those fine roots that were found still attached to the tree during the digging process were generally concentrated in highly branched masses arising from thin, twiggy lateral roots that grew horizontally from the stump of the tree. These roots extended the range of the fine roots to more than 1 m from the tree stump. The majority of the fine roots were found in the upper soil layers, but some were present in the B horizon. As was the case in season 2, there was some evidence of nematode infection.

D). Biomass partitioning over the three seasons

As the trees grew, a decreasing proportion of the biomass was present as root tissue as evidenced by the decreasing R:S ratios (Tables 4.7 - 4.9). There was a seasonal trend in the partitioning of total biomass with the proportion made up by root biomass highest at the beginning and end of each season (Fig 4.9). The high R:S ratio in November was a result of the incomplete foliage development at that stage and in May, reflected the loss of foliage from the trees. In seasons 1 and 3, the lowest R:S ratios occurred in January when the trees were undergoing their maximum height growth (Fig 4.4a). At the time of the March harvest, stem and branch growth had practically ceased and so the photosynthates still being produced were used to increase the size of the root system in preparation for storage over the dormant season. In season 2, the lowest R:S ratio was recorded at the March harvest as the strong growth of the previous two months had increased the biomass of foliage, stem and branch material at a greater rate than root tissue.

E). Analysis of growth parameters with respect to tree biomass

A comparison of the growth rates of various parameters of the trees over the three seasons is given in Table 4.10. It is interesting to note that although R decreased for all classifications of biomass as the trees grew, the absolute growth rate (G) increased from season 1 to season 2, with the values for G in seasons 2 and 3 being the same. This indicates that the same amount of biomass (in grams) was added to the trees in season 3 as in season 2. However, the net assimilation rate (NAR) decreased as the trees aged as the growth rate declined with respect to amount of leaf area available.

Table 4.10. Absolute and relative growth rates of *Paulownia* tree biomass of trees harvested in the first three seasons of growth.

Parameter	Season 1	Season 2	Season 3
G (total biomass) ($g\ month^{-1}$)	216.3	302.7	302.5
R (total biomass) ($g\ g^{-1}\ month^{-1}$)	0.22	0.13	0.085
R (root biomass) ($g\ g^{-1}\ month^{-1}$)	0.20	0.14	0.09
R (shoot biomass) ($g\ g^{-1}\ month^{-1}$)	0.27	0.10	0.083
NAR (total biomass) ($g\ m^{-2}\ month^{-1}$)	216.3	124.1	81.0

F). Moisture content of the tissues

The water content in the various tissues was reasonably similar between all the trees harvested over the three seasons. The above ground material tended to have a lower moisture content at the end of the season. For the stem tissue, this is a reflection of the lignification process with the concomitant decrease in water content compared with herbaceous stem tissue. Woody tissue generally had a lower water content than herbaceous material. The lateral roots had a higher moisture content than other woody tissues because the washing process meant they were wetter at the time of the fresh weight determination. The data presented in Table 4.11 represent averages for the tissue types taken over the three seasons.

Table 4.11. Average Moisture Content of *Paulownia* Tissues (% Fresh Weight)

Lamina	Petiole	Stem	Branch	Stump	Laterals	Fine roots
71	81	64	70	66	76	87

4.4 Nutrient Levels in *Paulownia* Tissues

4.4.1 Materials and methods

Analyses for the inorganic nutrients of the freeze dried tissue samples obtained from the harvests described above, were carried out by the Plant Analysis Service of AgResearch Ltd., at the Ruakura Agricultural Centre, Hamilton. The analyses determined the total amounts of the following nutrients in each tissue: N, P, S, Mg, Ca, Na, K, Mn, Zn, Cu, Fe, B and Mo (first season samples only).

4.4.2 Results

4.4.2.1 Analysis of the raw data of the nutrient contents

The data presented in Tables 4.12 - 4.22 are the nutrient contents of each tissue type averaged over the four trees sampled at each harvest, except in harvests 3 and 7. Included in these two harvests were trees from the poorly growing area (section 4.3.2.1 B), and so the averages were calculated from three replicates only. The nutrient levels in the two poorly performed trees will be discussed below (section 4.4.2.9). The size of the trees at harvest 1 were such that the above ground material (foliage and stem) was bulked together for nutrient analysis and the roots were separated only into fine roots and the remaining roots. The raw data of the nutrient content of each tissue type from each tree is tabulated in Appendices 4 - 6.

The standard error of the mean is included in the tables where there the mean was calculated from three or more replicates. There were several instances where only one or two trees at a harvest had sufficient tissue of a particular type to allow nutrient analysis to be undertaken. This generally happened with fine root material, but in harvest 4 two of the trees were without leaves and in harvest 12 only two of the trees were totally excavated. The standard error is also not presented in the instances when the average is less than 0.10% DW.

Examination of the raw data showed that there was often large variation among the nutrient levels in the petiole tissue at each harvest. For example the petiole tissue of tree 82 had five times the P content of petioles from the other trees harvested at that time (March, 1993) and the calcium content of petioles in harvests 6, 7 and 10 varied by a factor of 1.5 - 3 among replicate trees (Tables 2.13 and 2.15). This variability may have been a reflection of the role of the petiole in the translocation of substances to and from the lamina. At the time of harvest in these trees there may have been higher quantities of the nutrients being translocated for a variety of reasons, rather than high nutrient levels in the structure of the petiole tissues.

Table 4.12. Nitrogen Content of *Paulownia* Tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	2.94					0.93		2.20
2	2.25(0.17)	0.77(0.06)	0.61(0.03)			0.44(0.01)	0.68(0.03)	
3	2.42(0.25)	0.75(0.09)	0.54(0.08)			0.55(0.10)	0.84(0.15)	1.35
4	1.29	0.40	0.76(0.02)			0.68(0.04)	1.02(0.07)	1.36
5	2.29(0.21)	0.89(0.07)	0.36(0.03)	1.02(0.10)		0.25(0.04)	0.56(0.13)	
6	1.75(0.07)	0.55(0.02)	0.23(0.03)	0.44(0.02)		0.21(0.02)	0.25(0.03)	1.83(0.10)
7	2.11(0.12)	0.50(0.06)	0.32(0.04)	0.52(0.04)		0.31(0.04)	0.44(0.08)	1.44(0.08)
8			0.31(0.07)	0.52(0.03)		0.38(0.04)	0.53(0.03)	1.00
9	2.67(0.06)	0.95(0.06)	0.24(0.02)	0.40(0.03)	1.05(0.09)	0.23(0.03)	0.46(0.10)	1.98
10	1.96(0.15)	0.59(0.07)	0.30(0.06)	0.30(0.02)	0.50(0.06)	0.23(0.02)	0.32(0.04)	1.57(0.14)
11	1.92(0.06)	0.50(0.03)	0.30(0.03)	0.50(0.03)		0.36(0.02)	0.46(0.02)	1.41(0.08)
12			0.40(0.02)	0.61(0.03)		0.40	0.59	1.31

Table 4.13. Phosphate Content of *Paulownia* Tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	0.33					0.08		0.21
2	0.17(0.02)	0.09	0.09			0.07	0.09	
3	0.21(0.02)	0.10(0.01)	0.08			0.07	0.11(0.01)	0.14
4	0.08	0.05	0.10(0.01)			0.08	0.10(0.01)	0.13
5	0.22(0.03)	0.14(0.02)	0.06	0.16(0.03)		0.04	0.06	
6	0.18(0.01)	0.10(0.01)	0.03	0.07		0.02	0.02	0.25(0.01)
7	0.19(0.02)	0.09	0.04	0.08		0.04	0.09	0.17(0.01)
8			0.05	0.08		0.06	0.07	0.13
9	0.25(0.02)	0.15(0.01)	0.03	0.05	0.17(0.02)	0.03	0.05	0.24
10	0.17(0.01)	0.09	0.04	0.04	0.08	0.03	0.04	0.23(0.02)
11	0.15(0.01)	0.07	0.03	0.06		0.04	0.05	0.17(0.02)
12			0.05	0.07		0.04	0.05	0.12

Table 4.14. Potassium Content of *Paulownia* Tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	1.30					0.51		1.85
2	0.73(0.12)	1.16(0.37)	0.61(0.13)			0.50(0.06)	0.84(0.11)	
3	1.34(0.08)	2.33(0.55)	0.56(0.09)			0.52(0.08)	1.06(0.15)	2.28
4	0.54	0.24	0.51(0.05)			0.47(0.02)	0.79(0.0.8)	2.05
5	0.95(0.05)	1.46(0.06)	0.42(0.03)	1.21(0.10)		0.31(0.03)	0.80(0.07)	
6	1.08(0.22)	1.48(0.59)	0.35(0.01)	0.84(0.09)		0.30(0.02)	0.78(0.10)	1.48(0.25)
7	0.67(0.16)	0.53(0.11)	0.27(0.01)	0.60(0.02)		0.35(0.11)	0.62(0.08)	0.98(0.12)
8			0.27(0.04)	0.59(0.01)		0.40(0.03)	0.71(0.07)	1.21
9	1.24(0.11)	2.06(0.23)	0.23(0.02)	0.52(0.05)	1.31(0.07)	0.23(0.03)	0.95(0.09)	1.00
10	0.91(0.18)	1.31(0.37)	0.34(0.09)	0.35(0.03)	0.84(0.12)	0.32(0.05)	0.76(0.14)	2.22(0.34)
11	0.93(0.18)	1.04(0.37)	0.30(0.05)	0.64(0.03)		0.42(0.03)	0.84(0.05)	2.12(0.19)
12			0.38(0.02)	0.64(0.06)		0.35	0.69	1.91

Table 4.15. Calcium Content of *Paulownia* Tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	0.51					0.10		0.51
2	0.93(0.07)	1.59(0.06)	0.32(0.04)			0.13(0.01)	0.23(0.01)	
3	1.63(0.03)	3.09(0.31)	0.20(0.02)			0.12(0.01)	0.13(0.01)	0.48
4	3.20	5.09	0.22(0.01)			0.14(0.02)	0.14(0.02)	0.52
5	0.97(0.16)	1.34(0.18)	0.23(0.03)	0.57(0.07)		0.14(0.01)	0.20(0.02)	
6	1.43(0.19)	2.18(0.42)	0.16(0.02)	0.39(0.03)		0.11(0.01)	0.14(0.02)	0.47(0.02)
7	1.40(0.23)	2.80(0.44)	0.12(0.01)	0.27(0.03)		0.14(0.04)	0.13(0.02)	0.60(0.05)
8			0.12(0.01)	0.23(0.03)		0.16(0.02)	0.15(0.01)	0.51
9	1.45(0.01)	1.79(0.05)	0.14(0.03)	0.26(0.03)	0.66(0.05)	0.12(0.02)	0.23(0.04)	0.61
10	1.33(0.15)	2.44(0.28)	0.16(0.03)	0.18(0.02)	0.35(0.02)	0.13(0.02)	0.15(0.02)	0.51(0.03)
11	2.21(0.14)	4.15(0.02)	0.13(0.02)	0.25(0.01)		0.13(0.01)	0.13(0.01)	0.53(0.05)
12			0.16(0.01)	0.24(0.01)		0.14	0.14	0.49

Table 4.16. Magnesium Content of *Paulownia* Tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	0.20					0.08		0.35
2	0.21(0.02)	0.29(0.05)	0.10(0.01)			0.06	0.17(0.02)	
3	0.15(0.03)	0.32(0.05)	0.07			0.06	0.11(0.01)	0.46
4	0.22	0.57	0.10(0.00)			0.07	0.10(0.01)	0.39
5	0.14(0.01)	0.13(0.02)	0.07	0.11(0.01)		0.05	0.09	
6	0.17(0.01)	0.22(0.05)	0.05	0.09		0.05	0.10(0.01)	0.49(0.04)
7	0.18(0.01)	0.37(0.01)	0.04	0.10(0.01)		0.05	0.07	0.46(0.05)
8			0.05	0.10(0.01)		0.06	0.10(0.02)	0.38
9	0.20(0.01)	0.22(0.01)	0.04	0.08	0.15(0.01)	0.04	0.14(0.02)	0.48
10	0.18(0.01)	0.20(0.02)	0.05	0.06	0.09	0.05	0.10(0.02)	0.48(0.02)
11	0.14(0.02)	0.30(0.06)	0.05	0.10(0.01)		0.06	0.11(0.01)	0.53(0.08)
12			0.07	0.11(0.01)		0.05	0.11	0.41

Table 4.17. Sulphate Content of *Paulownia* Tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem		Branch	New Br.	Stump	Laterals	Fines
1	0.16						0.08		0.13
2	0.16(0.01)	0.07	0.05				0.04	0.05	
3	0.18(0.02)	0.12(0.01)	0.05				0.05	0.06	0.13
4	0.15	0.08	0.06				0.05	0.07	0.13
5	0.14(0.02)	0.06	0.03	0.06			0.02	0.04	
6	0.13(0.01)	0.08	0.01	0.02			0.01	0.01	0.15(0.01)
7	0.14(0.01)	0.12(0.03)	0.01	0.03			0.02	0.02	0.16(0.02)
8			0.01	0.02		0.02	0.02	0.10	
9	0.19(0.01)	0.10(0.01)	0.02	0.04	0.09	0.02	0.04	0.17	
10	0.16(0.01)	0.11(0.01)	0.02	0.02	0.05	0.03	0.03	0.15(0.01)	
11	0.19(0.01)	0.26(0.04)	0.04	0.06			0.05	0.05	0.17(0.02)
12			0.05	0.07			0.05	0.06	0.13

Table 4.18. Iron Content of *Paulownia* Tissues (ppm DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	479					392		1939
2	220(48.6)	195(65.3)	105(41.6)			488(31.3)	1296(85.1)	
3	149(7.4)	76(9.5)	66(10.4)			420(140.8)	855(242.2)	1071
4	303	115	100(16.7)			312(60.6)	453(120.5)	1048
5	217(34.0)	222(27.9)	239(101.7)	72(13.6)		333(44.4)	535(129.8)	
6	140(15.8)	88(8.1)	153(34.6)	49(6.3)		343(59.9)	449(90.4)	1378(505.6)
7	118(24.5)	76(10.9)	74(14.3)	93		231(57.8)	558(83.2)	1443(243.6)
8			87(13.2)	125(42.4)		162(33.1)	405(20.0)	3246
9	182(14.7)	138(31.2)	214(58.3)	264(90.6)	71(5.1)	512(184.9)	1265(96.6)	2056
10	112(12.5)	134(25.4)	101(33.0)	107(26.4)	56(10.8)	527(181.2)	671(145.0)	1680(250.1)
11	153(7.3)	96(8.4)	171(79.1)	181(29.2)		574(48.1)	487(84.0)	935(107.2)
12			109(36.5)	98(24.3)		376	406	2648

Table 4.19. Manganese Content of *Paulownia* Tissues (ppm DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	41					19		126
2	41(4.9)	37(5.7)	12(0.8)			27(6.3)	82(9.5)	
3	59(4.5)	90(22.1)	26(10.5)			12(2.6)	37(11.0)	156
4	115	170	19(4.7)			15(4.0)	28(9.8)	173
5	49(8.3)	17(3.3)	22(4.7)	16(3.9)		19(3.3)	36(9.3)	
6	34(2.0)	41(6.6)	13(2.5)	18(1.5)		12(2.1)	19(1.7)	156(26.3)
7	61	109	727			9	25	289
8			8(2.3)	20(4.2)		10(2.1)	22(3.0)	79
9	37(3.4)	20(1.8)	9(1.1)	17(3.5)	17(0.9)	22(8.3)	68(16.6)	222
10	42(6.0)	58(4.9)	10(2.7)	13(1.0)	15(2.2)	18(4.5)	32(9.6)	237(60.8)
11	58(6.0)	103(12.0)	11(2.9)	23(1.5)		25(5.3)	29(5.6)	182(10.1)
12			11(0.6)	24(5.3)		11	22	105

Table 4.20. Zinc Content of *Paulownia* Tissues (ppm DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	39					58		63
2	30(2.2)	41(3.3)	59(4.1)			51(4.8)	44(3.4)	
3	26(2.1)	49(6.2)	49(6.2)			42(2.1)	34(0.7)	68
4	22	73	53(2.5)			45(3.6)	32(5.2)	82
5	43(8.8)	31(6.4)	63(4.2)	54(8.9)		40(3.5)	32(4.9)	
6	28(2.7)	36(1.6)	41(4.1)	42(3.4)		38(4.8)	27(2.8)	72(3.8)
7	22(1.8)	47(5.5)	31(3.5)	45(4.6)		41(1.5)	33(4.7)	92(5.9)
8			28(1.9)	38(2.7)		41(3.8)	29(1.9)	44
9	39(3.4)	26(1.8)	32(3.9)	44(3.1)	52(1.4)	33(4.8)	33(4.1)	83
10	23(2.3)	30(1.5)	35(6.3)	40(3.1)	39(5.4)	36(1.2)	34(5.4)	72(5.4)
11	22(0.9)	42(2.6)	34(4.4)	42(3.2)		35(2.3)	30(2.1)	71(5.3)
12			32(1.3)	40(1.9)		37	31	51

Table 4.21. Copper Content of *Paulownia* Tissues (ppm DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	19					24		38
2	14(1.9)	10(1.9)	19(0.3)			24(5.6)	17(0.9)	
3	10(0.9)	7(0.9)	16(1.8)			15(1.2)	14(2.4)	56
4	7	5	17(2.6)			18(2.7)	17(2.8)	50
5	13(1.5)	10(2.1)	21(5.0)	14(2.3)		29(6.6)	20(5.1)	
6	15(2.1)	9(0.9)	17(1.4)	19(2.3)		22(1.0)	20(4.7)	40(1.8)
7	15(2.0)	9(1.2)	13(2.7)	19(5.6)		25(3.8)	21(5.0)	47(0.9)
8			13(1.3)	15(0.7)		18(1.1)	20(3.0)	30
9	18(2.3)	13(0.6)	21(2.9)	21(2.9)	24(2.3)	30(7.0)	22(3.8)	57
10	15(1.6)	11(1.7)	13(2.0)	15(0.5)	21(4.0)	21(1.7)	17(2.9)	37(3.1)
11	11(0.8)	9(0.9)	13(1.0)	16(0.8)		19(2.0)	24(6.6)	28(2.8)
12			13(2.8)	14(3.0)		35	30	53

Table 4.22. Boron Content of *Paulownia* Tissues (ppm DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	25					14		23
2	27(1.9)	23(1.0)	13(1.0)			12(2.6)	16(0.9)	
3	48(2.8)	27(1.4)	10(0.3)			10(0.6)	10(1.2)	17
4	81	26	12(1.1)			9(1.4)	10(0.6)	16
5	27(4.1)	17(1.3)	11(0.9)	12(1.4)		7(0.9)	9(1.2)	
6	28(3.1)	21(1.5)	9(0.4)	11(0.3)		8(0.8)	9(0.9)	17(1.6)
7	43(6.0)	25(2.7)	8(0.3)	14(3.7)		10(1.4)	10(0.3)	18(0.6)
8			9(1.1)	12(0.0)		10(0.4)	9(0.7)	22
9	31(2.4)	18(1.5)	5(0.6)	8(1.0)	12(1.7)	7(1.7)	10(2.3)	20
10	28(3.5)	26(1.7)	8(1.0)	6(0.9)	10(0.9)	7(1.2)	9(1.4)	19(1.0)
11	48(4.1)	22(0.4)	9(1.8)	11(0.6)		10(1.5)	10(2.0)	20(1.0)
12			13(0.8)	15(0.9)		13	12	27

The variation in trace element concentrations between replicate trees was also high in some instances. The copper content of the stump root of tree 37 was three times higher than levels in stumps of the trees harvested concurrently, but the remaining tissues of tree 37 had Cu levels that were similar to the other trees (Table 2.21). Zinc levels in the stem of tree 50 harvested in November, 1992, were four times higher than levels in the stems of the remaining trees harvested at that time (Table 2.20). The molybdenum concentration in one of the unplanted roots (section 4.4.2.2) was 4.2 ppm in comparison with Mo concentrations of between 0.02 ppm and 0.23 ppm in other unplanted roots, and between 0.1 ppm and 0.8 ppm in the tissues harvested from the trees in season 1. The only exception to this range was the new root material of tree 95 which had a Mo concentration of 1.36 ppm. In the case of tree 17 (harvested in March, 1992) all the tissues analysed had extremely high levels of manganese in comparison with all other trees harvested over the three seasons. These variations in particular tissue micronutrient concentrations may reflect localization of high soil concentrations of these nutrients in the area where the tree was growing which led to higher uptake rates. However, it is not clear why some tissues concentrated the nutrients to a greater extent than other tissues.

The application of fertilizer to the southern half of the plot had no perceptible effect on the nutrient contents of the trees harvested from this area. The variation of nutrient levels within

the pairs of trees was as great as the variation between the pairs for all of the nutrients and tissues types. This result suggests that there was sufficient nutrients in the planted root and available in the soil to sustain the trees in the first season of growth. The slow rate of breakdown of the fertilizer applied may also have influenced the result by increasing the soil concentrations of nutrients slightly, but for a long period. The results may have been different if a liquid fertilizer had been applied at high concentrations as the growth of *Paulownia* trees have been found to respond quickly to applications of high strength liquid fertilizer (K. Avery, pers. comm.). The applications of hydroponic nutrient solution to the trees in season 3 were probably of insufficient concentration to markedly influence the nutrient levels in the tissues of the trees at the last two harvests.

4.4.2.2 Comparison of nutrient levels with unplanted root material

At the time of planting the experimental block, more roots were available than were needed and three of these surplus roots were analysed for nutrient levels. In addition, roots of *P. tomentosa* (Japan) were obtained from the Barton nursery in July, 1991 and July, 1992 for nutrient analysis. The results from these analyses (Table 4.23) gave an indication of the baseline nutrient levels in the roots at the time of planting and were similar to the levels present in the woody roots at harvest 1 (Table 4.7). For nitrogen and potassium, the levels in the harvested roots were slightly lower than those in the unplanted roots, indicating translocation of these nutrients to developing tissues early in growth. The level of molybdenum increased in the harvested roots, in comparison with the unplanted roots, indicating that the plants were actively taking up Mo from the time of planting instead of relying on the stores within the planted root material.

Comparisons between nutrient levels in these unplanted roots with levels in root stump material sampled at each of the final harvests for the three seasons showed different patterns for different nutrients. Nitrogen levels were higher in the unplanted roots than any of the harvested roots, while concentrations of P, S, K, and Mn were similar in roots harvested at the end of season 1 as in the unplanted roots but decreased in comparison at the final harvests of seasons 2 and 3. The concentration of Mg was similar between the two sets of roots, except in season 3 when the harvested material had lower levels than the unplanted roots. The concentration of calcium in harvested roots was higher at all harvests than in the unplanted roots, as was that of Mo (but data from season 1 only available). The remaining trace elements - Zn, Cu, Fe and B - were present in similar concentrations in both the harvested and unplanted roots.

Table 4.23. Nutrient levels in *P. tomentosa* (Japan) root stumps grown in the Barton nursery (South Auckland) and harvested during the dormant season in 1990, 1991 and 1992. Data are presented as average concentration per dry weight with standard errors in parentheses.

N (%)	P (%)	S (%)	Mg (%)	Ca (%)	K (%)	Mn (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)	B (ppm)	Mo (ppm)
1.19	0.11	0.08	0.09	0.08	0.85	23	52	24	338	10	0.11
(0.10)	(0.01)	(0.009)	(0.005)	(0.007)	(0.09)	(3.4)	(5.1)	(5.2)	(45.6)	(1.1)	(0.03)

The differences between the two sets of roots probably reflect the differences in soil nutrient levels between the two sites. The Barton nursery is situated on a heavy soil with a high clay content which would tend to have higher nutrient levels than the sandy soil of the experimental site. It is not known whether the concentrations of the various nutrients in the root material during the dormant season are important in the ability of the roots to produce strong growth of new shoots in the spring. This may be a factor influencing the usefulness of roots for propagation.

4.4.2.3 Concentration of sodium in *Paulownia* tissues

The results presented in Appendices 4 - 6 indicated that *Paulownia tomentosa* is a typical natrophobic plant with sodium being restricted to the fine root tissue. The range of concentrations of sodium in fine root material over the three seasons of harvesting was from 0.37% DW to 0.68% DW, while the concentrations in every other tissue type was never above 0.05% DW and generally about 0.02% DW. It can be concluded from these results that in *P. tomentosa* sodium cannot replace potassium in its various metabolic roles (as it can in natrophilic plants) and that this tree would be intolerant of soils with a high salt content (Marschner, 1986).

4.4.2.4 Distribution of nutrient in different tissues

There were several distinct patterns of distribution of the nutrients in different parts of the harvested trees. Concentrations of nitrogen, boron, phosphate and sulphate were generally highest in the lamina and fine root material, slightly lower in the petiole tissue and lowest in the perennial tissue. In comparison the highest levels of magnesium, potassium and

manganese were recorded in the fine roots, with the concentrations decreasing in the petiole, then in the lamina and lowest in the woody root, stem and branch tissues. A third set of nutrients, the trace elements zinc, copper and iron, were found in the highest levels in the fine roots with decreasing amounts in other tissues. With Cu and Fe, other root material (laterals and stump) had lower levels than the fine roots, but higher than any of the above ground tissues. Zn, however, was present in higher concentrations in the branch, stem and petiole tissue than in the woody root material, with the lowest concentrations being found in lamina. The final pattern of allocation was shown by calcium which was present in the highest concentration in petiole tissue, with decreasing amounts in lamina material, then less again in fine roots and the lowest concentrations found in the perennial tissue both above and below ground.

Nutrient allocation patterns in *Paulownia* were similar, but not identical to, those reported for other plants. Working with cottonwood, Baker and Blackmon (1977) found that for N, Ca and Mg the highest levels were found in the leaf material and lesser concentrations were present in root material, with the lowest levels in the branch and stem tissues. However, P and K were found in the highest concentrations in root tissue, with less in the leaf material and lowest amounts in branch and stem tissue, as for the other nutrients. In juniper plants of varying stages of maturity, N, K and Ca were found in the highest concentrations in leaf material, followed by levels in the fine roots and then stem and other root tissues. The remaining nutrients (P, Mg, Zn, Cu, Fe, Mn, B, Mo) were present in the highest levels in fine roots, then in leaves, then stems and branches, with the lowest concentrations in lateral roots (Miller *et al.*, 1990). This compares with the work done on kiwifruit where the majority of the nutrients (N, P, K, Ca, Mg, S, Fe, Mn, B) were found in the highest concentrations in the leaf material, followed by levels in the root tissue, with concentrations in the above ground woody matter (shoots, canes, stem and leader) being lower (Buwalda and Smith, 1987).

In a series of studies on the above ground tissues of the timber trees *Pinus radiata*, *Acacia dealbata*, *Eucalyptus regnans* and *E. saligna*, Frederick *et al.* (1985a and 1985c) found that in all cases the highest concentrations of N and P were found in the foliage. In *A. dealbata*, Ca, Mg and Mn were also found in the highest levels in the foliage with the order of the decreasing concentration in the remaining tissues (twigs, branches, stem bark and stem wood) varying between the elements. Foliage of *P. radiata* also had the highest levels of K, Mn, Mg and Zn and again the order of decreasing concentration within the woody tissues differed between the elements. Ca, however, was most concentrated in the branch tissue of radiata. With the two species of eucalypt the distribution of some of the elements were similar with Cu and Zn being present in the highest levels in the twigs in both species. For each element in each of the four species (except K in *E. regnans*) the lowest concentrations were found in the stem wood tissue. The absolute concentrations of each

nutrient in the different tissues of the range of plants noted above were generally of the same order of magnitude as for *Paulownia*.

4.4.2.5 Seasonality of nutrient allocation

Some trends in the change of nutrient concentrations during the season in different tissues were observed. The concentration of Ca, for example, in the lamina and petiole increased through the season, was either static or decreased slightly in the perennial tissues over the season and showed no clear pattern in the fine root material. The trace elements Mn and B also increased in foliar tissues over the season but showed less clear trends in concentration changes in the other tissues, with boron levels remaining relatively static in other tissues. Nitrogen, by comparison, tended to decrease in the lamina, petiole and fine root materials as the season progressed, while increasing in levels in the perennial tissues towards the end of each season. This reflected the conservative nature of nitrogen nutrition whereby the senescence process withdrew nitrogen from tissues before abscission.

The concentrations of S and Mg increased as the season progressed in petiole tissues only, with levels in most other tissues quite stable. Zn also tended to increase in petioles over the season, but declined in lamina and branch tissue. The copper content of most of the tissues declined over the season, as did the iron content in perennial root tissue. Trends in Fe concentrations in other tissues were less clear, as were the changes over the season in levels of potassium in the various tissues. The levels of the remaining element, P, declined in lamina, petiole, fine root and branch tissue and were stable in the stem, stump and lateral roots.

An intensive study of seasonal accumulation of foliar nutrient levels in kiwifruit (Smith, Clark and Henderson, 1987) showed three patterns of change through the season. Potassium levels in the leaves declined through the season, whereas concentrations of N, P, Cu and Zn remained relatively constant once leaf expansion was completed after about 8 weeks of growth. The third set of elements included Ca, Mg, S, Mn, Fe and B which all showed increases in foliar concentration as the season progressed. These results for Ca, Mn and B were similar to those found in *Paulownia*.

4.4.2.6 Changes in nutrient concentrations in tissues between seasons

As the perennial tissues aged over the three seasons, the concentrations of some nutrients declined. This was most apparent in the stem tissue with levels of N, Ca, Mg, P, S, K, Mn and Zn decreasing as the stems aged. In the root stump levels of N and Zn

decreased, while in the lateral roots there was a decrease in the levels of N and P. The concentration of other nutrients was relatively stable over time. The rate of change in nutrient levels was not always the same between seasons, with nitrogen highest in all tissues in season 1 but declining to more stable levels in seasons 2 and 3, for example.

The observed changes in the concentrations may have been the result of increased wood production in the perennial tissue, especially in the expanding stem, which required increased levels of organic compounds (*e.g.* lignin, hemicelluloses etc.) but few extra inorganic compounds.

The analysis of the nutrient contents of the foliar and fine root materials between the seasons showed little change. A study of the foliar nutrient concentrations in *Eucalyptus regnans* aged 4, 7, 10, 13 and 17 years gave similar results with no change in levels of K, Ca, Cu and Zn over time. The levels of the remaining nutrients (N, P, Mg and Mn) varied independently of each other and of age (Frederick *et al.*, 1985b). The biomass of the older stands did tend to accumulate more nutrients on a total kg/ha basis, although there were some variations between sites.

4.4.2.7 Foliage nutrient levels as predictors of plant growth

A series of analyses carried out using Microsoft Excel 4.0 (Microsoft Corporation, Redmond, WA, USA) regressed various growth parameters of the harvested trees against the concentration of 12 nutrients in the lamina at the time of harvest. The results of these analyses indicated that the foliar levels of calcium were strongly related to tree height, tree diameter, leaf area, R:S ratio, total tree biomass and shoot biomass. The foliar levels of sulphur and boron were more weakly correlated with height, diameter, shoot biomass and total biomass, with sulphur concentrations also correlated with the R:S ratio and boron with the leaf area.

These relationships may be a consequence of the transportation mechanisms of these nutrients rather than a direct causal effect. Calcium, boron and sulphate (SO_4^{2-}) are all primarily transported in the xylem (Marschner, 1986) with very little redistribution occurring via the phloem. Thus plants with high rates of carbon assimilation will have high rates of transpiration which would lead to high foliar concentrations of those minerals from their incorporation into the transpiration stream. These three nutrients will tend to stay in the leaves, rather than being translocated to other areas of the plant. This is illustrated by a comparison between trees 8 and 17 which were both harvested in March, 1992. Tree 8 was 2.23 m tall with foliar concentrations of 1.59% Ca, 0.15% S and 50 ppm B. Tree 17 was 1.53 m tall and had foliar concentrations of 0.95% Ca, 0.13% S and 31 ppm B.

It is also possible, though, that the converse is true. The relationship between foliar concentrations of calcium, sulphate and boron and tree growth may reflect the soil microsites of these trees. If the soil has higher nutrient concentrations in some areas, then the trees would be expected to grow more vigorously. Thus the increased tree vigour may be an effect of increased nutrient levels in the soil, and thus in the foliage, than an effect of it. It is not possible to determine the precise nature of the relationships from these data.

The only other relationship that was significant at the 5% level was that between Fe and the total leaf area of the tree with an R^2 value of 0.15. As 80% of the iron in leaves is present in the chloroplasts (Marschner, 1986), this relationship may be a reflection of the increased photosynthetic capacity of trees with large leaf areas.

In general the relationships between foliar nutrient levels and the growth parameters studied was weak, so the use of foliar nutrient levels as a predictor of tree growth rates would be very limited.

4.4.2.8 Nutrient levels in senescent leaves

Comparison of the nutrient levels in the lamina tissue in healthy leaves in the latter part of the season and senescent leaves showed that only nitrogen, phosphate and potassium were withdrawn in any quantity from senescing leaves (Table 4.24). Other nutrients were present in similar amounts in the two types of leaf, with some (*e.g.* Ca, Fe and B) found in higher levels in both sets of senescing leaves than in the comparable healthy leaves. It is possible that

Table 4.24. Nutrient concentrations in healthy and senescent leaves.

Leaf Type	N	P	S	Mg	Ca	K	Mn	Zn	Cu	Fe	B
	(% DW)						(ppm DW)				
Healthy (March 1990)	2.42	0.21	0.18	0.15	1.63	1.34	59	26	10	149	48
Senescent (May 1990)	1.65	0.13	0.17	0.17	3.24	0.84	72	30	24	157	96
Healthy (March 1991)	1.91	0.17	0.13	0.17	0.95	0.37	133	19	13	87	31
Senescent (March 1991)	1.22	0.09	0.14	0.22	1.51	0.24	104	22	14	113	42

further withdrawal of nutrients occurs in the last stages of senescence, just prior to abscission, but it does seem from these results that the leaves of *P. tomentosa* contain significant quantities of all nutrients at the time of leaf drop. A similar result has been reported for kiwifruit where the levels of N, P and K decreased in leaves at the end of the season, but the foliar concentrations of the remaining nutrients were relatively stable (Smith, Clark and Henderson, 1987).

4.4.2.9 Nutrient levels in the plants harvested from the poor growing area

Tree 95 was harvested in March, 1991, and tree 77 in March, 1992. Comparison of the nutrient concentrations of the tissues of these trees with levels in trees harvested concurrently showed that generally the nutrient levels were similar in most tissues between the trees that grew well and those that grew poorly. The foliar levels of most of the nutrients were lower in trees 77 and 95 and stem tissue in these two trees had lower levels of K, Mg and some micronutrients. Overall there seemed to be insufficient difference in the nutrient levels to explain the poor growth of trees 77 and 95. The lower levels of some nutrients was more likely to be an effect of poor growth than a cause. It is possible that the high Mo level (1.36 ppm) in the new roots of tree 95 influenced growth, but it seems unlikely that this level of molybdenum would be phytotoxic given the wide range of concentrations of Mo found in different plants (Marschner, 1986). Mo concentrations were not analysed in season 2 there is no data for Mo levels in tree 77.

4.5 Carbohydrate Levels in *Paulownia* Tissues

4.5.1 Materials and methods

Spectrophotometric techniques were used to determine the non-structural carbohydrate contents of the tissues. The amounts of structural carbohydrates (*e.g.* cellulose, hemicelluloses) and lignin were not determined.

4.5.1.1 Analysis for soluble sugar content

Using the method of Haslemore and Roughan (1976), soluble sugars were assayed after extraction into methanol. Pre-weighed samples (100 mg or less) of freeze dried tissue were extracted (in 11 ml eppendorf tubes with water-tight lids) in 10 ml of 62.5% methanol in a 55°C water bath for 15 minutes. After centrifugation for 5 min at 4000 rpm, 4 ml of the supernatant was added to a second series of tubes containing 0.1 ml of a saturated aqueous solution of lead acetate, which cleared the solution of non-carbohydrate interfering material that would affect the spectrophotometric reading. After shaking, and standing for 10 min, 5 ml of chloroform was added to the tubes, which were then shaken vigorously. Centrifugation, as before, allowed clear separation of the aqueous and chloroform layers, the latter containing unwanted fatty acid based components.

From the aqueous layer of the tubes, 50 µl was sampled and added to a third series of tubes containing 5% phenol (to precipitate the proteins). These were then chilled in an ice bath and 4 ml of chilled 98% sulphuric acid added. The tubes were shaken immediately and, after standing, the absorbances read at 485 nm, using a phenol - sulphuric acid blank. The need for chilling the tubes during this last stage of the process arose after the samples from the first season gave quite variable results within replicates.

The process was replicated at two points, with two samples of each tissue of each harvested tree being extracted, and then two 50 µl subsamples were taken, so that the results for each tissue were the average of four replicates. Sucrose standards (4 ml) were incorporated at the lead acetate stage and treated in exactly the same manner as the extracted samples. A plot of standard concentration against absorbance was linear, passing through the origin, up to 2.4 absorbance units. The results were calculated as percentage of soluble sugar after correction for a water blank and real dry weight. The samples tested were approximately 96% dry so corrected by a factor of 1/0.96.

Identification of the compounds present in the methanol extract was not attempted so it is not

known which sugars were extracted from the *Paulownia* samples, nor the proportion of each sugar. In addition to the presence of the expected sugars (*e.g.*sucrose, glucose and fructose) sugar alcohols and other compounds may also have been extracted by the extraction in methanol. It is not clear if these compounds would have remained intact through the analytical procedure, but it is likely that sugar alcohols would have remained sufficiently intact to form part of the assayed soluble sugar component (R. Newth, pers. comm.).

4.5.1.2 Variability among replicates during analysis for soluble sugars

The determination of the soluble sugar content of *Paulownia* tissue was not as straight-forward as for the tissues analysed in the original study by Haslemore and Roughan (1976). Although their technique was initially employed without modification, it was necessary in later analyses to undertake the final stages of the procedure using chilled reagents to minimize variability between replicates (section 4.5.1.1). The results of the four replicates for each tissue were often variable, with one replicate having twice the absorbance level of other replicates in some instances. The variation between the replicates was not confined to that between the initial samples taken, as there were also large differences often found between the results for two replicates taken at the later stage of the analysis. The variations followed no clear patterns, with similarly variable results found for all the tissue types sampled, and appeared to be operator independent. It was not possible to determine the exact cause of the high variability, even though each step of the procedure was studied. It was initially thought that the rapid caramelization of the sugars during the addition of the sulphuric acid to the phenol mixture at the end of the procedure may have been an important factor (R. Newth, pers. comm.), but the chilling of the reagents and the prompt mixing of the solution immediately after the addition of the acid failed to significantly improve the replicate variation problem.

Despite the highly variable results for the individual tissue samples, it was interesting to note that the average sugar concentrations of each tissue, calculated from the four replicates, were very similar between trees harvested at the same time. Thus lamina material from the four trees harvested each time had very similar average sugar levels, as illustrated by the low standard errors in Table 4.25, despite the large variation within the results of the lamina for each tree.

4.5.1.3 Starch Determination

The residual pellet of tissue after the methanol extraction was then analysed for starch content

using enzymatic degradation (R. Newth, pers. comm.). The remaining extraction mixture was discarded, then the pellet was washed in 62.5% methanol and briefly centrifuged. The supernatant was again discarded and the pellet transferred to a glass vessel with a known amount of water. The early analyses used a 250 ml flask with 25 ml water, but later analyses used a 28 ml universal bottle with 10 - 12 ml of water. The use of this smaller vessel allowed a more rapid turnover of samples as more bottles could be fitted into the water baths at the latter stages of the process.

The water/pellet mixture was then autoclaved at 120°C for 1 hour as were starch standards and a water blank. After the solutions had cooled to below 60°C, 10 ml of 0.05% amyloglucosidase reagent was added and the bottles were incubated at 55°C in a waterbath for one hour. The amyloglucosidase reagent consisted of 500 mg solid enzyme in 1 l of acetate buffer (25 ml of 5 M acetic acid + 25 ml of 5 M sodium acetate in 1 l water) at pH 4.5.

Duplicate 1 ml aliquots from the solutions were then placed in 11 ml eppendorf tubes and 2 ml of glucose oxidase reagent was added. After mixing, the tubes stood in a water bath for 1 hour at 30°C. Glucose standards also underwent this same treatment. The glucose oxidase reagent included 1 ml of o-dianisidine and 1 ml of glucose oxidase/peroxidase solutions diluted to 100 ml with tris buffer. The tris buffer solution was made up of 30.5 g tris (hydroxymethyl) aminomethane in 42.5 ml of 5 M HCl diluted to 500 ml with water, with 330 ml glycerol then added (pH 7.0). The 1% aqueous o-dianisidine solution was prepared weekly, as was the enzyme mixture which involved dissolving 100 mg of glucose oxidase and 10 mg peroxidase in 10 ml tris buffer.

Immediately after incubation, 5 ml of 5 M HCl was added to the tubes. The solutions were mixed and the absorbances read at 540 nm using incubated enzyme solution plus HCl as a blank. The results were then calculated as percentage starch, taking into account the value for the water blank, the same dry weight correction as for the sugar analysis, the dilution factor at the autoclaving stage and the conversion factor of 0.9 for glucose to starch.

4.5.1.4 Possible enzyme inhibition in the starch analytical procedure

In each of the starch analyses carried out standards of both starch and glucose were incorporated. In addition a sample of distilled water was included in the analysis before the autoclaving stage, and treated in the same manner as the remaining samples. The absorbance measured for this water blank was then subtracted from the values of the samples. This corrected for the starch content of the amyloglucosidase solution which resulted from the use of starch as a filler in the enzyme preparation from the manufacturer. The value of the

absorbance for this water blank typically varied between 0.1 and 0.3 units when the enzyme solution was prepared daily. The analyses of the tissues harvested in the first season often had higher values for the blank as the enzyme solution was not prepared daily and over time the enzyme reacted with the starch filler.

It was noticed throughout most of the analyses that several of the samples had much lower absorbances than the water blank (*e.g.* 0.03 units in some cases) and these solutions were visibly less coloured than that of the water blank. This anomaly led to the formation of the hypothesis that some compound (or compounds) in the *Paulownia* tissue was being extracted by the analytical process and was then inhibiting the action of either of the two enzymes, or preventing the detection of glucose at the final stage by some other means. To test this hypothesis, a number of *Paulownia* samples that had previously been analysed and found to contain no starch were reanalysed after being suspended in an aqueous solution of 100 ppm starch prior to autoclaving. These were compared with samples suspended in water, as was the normal technique. The absorbance values of the samples with the added starch were higher than those in water, but were lower than the values for a 100 ppm starch standard tested at the same time. The absorbance of the starch standard was 0.7 units, while the values for the tissues plus starch samples ranged from 0.08 to 0.54 units, with several of these samples having lower absorbances than the water blank (0.25 units).

These results confirmed that the *Paulownia* tissue was somehow interfering with the analytical procedure. This means that the results from the starch analysis are unreliable and that many of the *Paulownia* samples tested may, in fact, have reasonable levels of starch. Time constraints prevented further investigation of this problem and it may eventuate that analysis by a different technique will be required to shed more light on this matter.

4.5.2 Results

4.5.2.1 Concentration of soluble sugars in different tissues

The perennial root material generally had the highest levels of soluble sugars, (Table 4.25) with the lateral roots containing a higher concentration than the root stump. Visual inspection of the thicker lateral roots revealed a white layer of tissue just under the bark of the root which was a little watery on cutting and had a slightly sweet taste (C. Beard, pers. comm.). The remainder of the root tissue was darker in colour with a different texture. It may be that this white layer was the major site for storage of soluble carbohydrates in the lateral roots. The above ground perennial tissues had variable levels of sugars at different times, but the concentrations tended to average about 15% of the dry weight.

Table 4.25. Soluble sugar content of *Paulownia* tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	20					14		8
2	12(0.6)	20(0.7)	19(0.6)			16(0.5)	27(1.3)	
3	13(0.4)	13(1.6)	23(1.5)			25(1.9)	40(2.6)	13(1.5)
4	6	6	17(1.1)			23(1.7)	32(2.0)	7(0.6)
5	11(0.9)	32(2.1)	10(0.5)	21(1.0)		15(1.8)	14(0.9)	8(1.7)
6	10(0.5)	19(1.0)	12(0.7)	15(1.5)	14(0.5)	15(0.9)	21(1.3)	10(1.2)
7	13(0.3)	14(1.3)	15(0.5)	16(0.3)	19(1.6)	16(4.3)	25(2.4)	5(0.0)
8			15(0.9)	17(1.3)	17(0.3)	20(1.7)	27(2.1)	7(1.1)
9	10(0.7)	21(2.2)	7(0.6)	7(1.1)	14(1.8)	12(0.9)	12(1.8)	5(1.9)
10	11(0.3)	19(1.1)	12(0.8)	13(0.8)	17(1.1)	17(2.2)	27(2.7)	16(2.1)
11	10(0.7)	10(1.1)	12(0.8)	16(2.1)		18(1.3)	23(0.9)	12(3.0)
12			13(0.9)	14(0.5)		18	27	11

Table 4.26. Starch content of *Paulownia* tissues (% DW). Standard errors are in parentheses.

Harv.	Lamina	Petiole	Stem	Branch	New Br.	Stump	Laterals	Fines
1	0.36(0.072)					0.01(0.008)	0	
2	0.16(0.031)	0.01(0.013)	0			0.03(0.024)	0.04(0.013)	
3	0.30(0.015)	0.02(0.015)	0.13(0.068)			0.28(0.073)	0.36(0.048)	0.08(0.025)
4	0	0	0			0.17(0.029)	0.29(0.019)	0.04(0.018)
5	0.29(0.027)	0.06(0.054)	0			0	0	0
6	0.26(0.061)	0	0			0	0	0
7	0.17(0.032)	0	0.03(0.027)	0.05(0.007)		0.04(0.031)	0.12(0.067)	0
8			0	0		0.06(0.019)	0.16(0.019)	0
9	0.13(0.049)	0	0	0	0	0	0	0
10	0.13(0.038)	0	0	0	0	0	0.04(0.022)	0
11	0	0	0	0.01(0.006)		0.17(0.010)	0.28(0.021)	0
12			0	0		0.11	0.24	0

In the tissues that were produced annually, the level of soluble sugars was highest in the petioles and lowest in the fine roots. The lamina material had comparatively low levels of soluble sugars (6% - 13%), considering it is the major site of production of these compounds. This may be a result of conversion of the sugars into storage compounds in the leaf or of rapid translocation of the sugars to other areas of the tree. It is likely that the primary reason is rapid translocation as the petioles tend to have higher sugar levels, and also the concentration of starch is not particularly high in lamina tissue in comparison with foliage of other species (section 4.6.6.1).

4.5.2.2 Seasonal changes in soluble sugar concentrations

In the lamina tissue there was little change in the concentration of soluble sugars through each season, with the exception of the final harvest for season 1 (Table 4.25). At this time the leaves were senescing with the concomitant withdrawal of carbohydrate and nitrogenous compounds occurring. This very low level of sugars (6%) was also noted in the petiole tissue at that time, but generally sugar levels in the petioles decreased during the season, particularly in the latter half of the season. Sugar concentrations in the fine root tissue tended to peak in mid season (January), with lesser amounts found at either end of the season.

In the perennial root tissues the concentration of soluble sugars was generally highest at the end of each season and lowest at the time of the November harvests. This resulted from the storage role of the roots which involved the translocation of excess carbohydrates to the roots during the latter part of the season, and the remobilization of these reserves at the beginning of the growing season in September. An exception to this trend was observed in the March harvest of season 1 when the sugar levels in the lateral root material was much higher than in roots harvested in May (40% in March compared with 32% in May). This may have been a reflection of the diameters of the roots at the time of harvest as it has been shown by other workers that TNC decreased with an increasing diameter of lateral roots *e.g.* in *Acer saccharum* (Kolb and McCormick, 1991). Over the three years studied the levels of sugars were highest in the first season in root tissue, but were similar in the second and third seasons.

The seasonal trends of sugar levels in branch and stem tissue were less clear. In seasons 2 and 3 sugar concentrations were lowest at the beginning of the season, probably resulting from the remobilization of stored sugars to growing points (as for the perennial roots). Herbaceous branch material tended to have higher levels in comparison with woody branches as was shown by the levels in the new branch tissue in season 3 and the data for branch tissue at harvest 5 which was predominantly herbaceous at that time. The higher sugar levels in stem tissue in season 1 was also a reflection of the lower proportion of stem material that was

woody, compared with later harvests.

4.5.2.3 Starch concentrations in *Paulownia* tissues

The results from the analyses of starch levels in the different tissues must be viewed with caution due to the problems of the technique encountered (section 4.5.1.4). It is not possible to draw any conclusions from the data and so it will be discussed only briefly.

The amount of starch in all the *Paulownia* tissues analysed over the three seasons was very low (Table 4.26). The highest levels occurred in lamina and lateral root tissues, with less in stump material and very little in the petiole, stem, branch and fine root material. In many instances it was not possible to detect any starch at all in the tissues. In those with detectable starch levels, no clear trends were observable through the season although there tended to be higher levels in the perennial root system at the March and May harvests each season. This may have been as a result of storage in the roots of reserves which could be mobilized at the beginning of the next growing season.

4.6 Discussion

4.6.1 Growth rates

The growth of the *P. tomentosa* trees in the first three years after planting as root stumps was influenced by a range of internal and external factors. During the initial establishment phase the growth of stem and foliar tissue was rapid as the reserves in the root were mobilized to produce a self-sustaining structure. The relative growth rates of both stem height and diameter were greater during this time than at any other time in the three years studied. These growth rates declined later in the first season as the trees allocated more photosynthate to root growth. Although in season 2 the relative growth rates were lower than in the first season, absolute growth rates were higher and many trees more than doubled in height and stem diameter over the course of the season. The average increase in tree height of almost 1 m and in diameter of 27 mm were indicative of the growth rates this species is capable of under good growing conditions. Unfortunately, these rates were not sustained in the third season, when both relative and absolute growth rates were significantly lower for stem height and diameter. Comparison of R for stem height and for shoot biomass indicated that more of the above ground biomass was allocated to branch extension than leader extension in season 3 than in season 2. The relative growth rates of total biomass, root biomass and shoot biomass all decreased over the three seasons as did the net assimilation rate (NAR).

Analysis of the data gathered on the various climatic and soil parameters indicated that the third season was different from the second in a number of ways that may have influenced tree growth. The decreased number of hours with saturating levels of PPFD in season 3 may have led to decreased carbon assimilation resulting in less photosynthate available for growth in season 3 than in season 2. This was despite the greater photosynthetic area produced by the trees harvested in season 3, as a result of increased branching. The resultant decrease in carbon assimilation was reflected in the lower NAR in season 3 compared with season 2.

The differences in the average air and soil temperatures (both daytime and nighttime) between seasons 2 and 3 do not seem to be consistent enough or large enough to account for the dramatic difference in growth rates between the two seasons. The effect of temperature on *Paulownia* growth has been discussed above (Chapter 3). Early spring (September and October) was cooler in the third season which may have influenced the growth rates at this time, but it is unlikely that this effect would last all season. The cooler temperatures may have slowed down the development of new foliage material after the damage done by the grass grub beetles and, if so, might have decreased growth rates for an extra one or two months. Although the average monthly air temperatures in season 3 recorded at Ruakura were lower

than the 30 - year averages, the difference between the temperatures at Ruakura for season 2 and season 3 were minimal.

It is more likely that the low growth rates in season 3 were influenced by the effect of climatic variables on the water relations of the plants. The soil moisture level was lower in December, January and February in this season, in comparison with season 2 as a result of lower rainfall over this period. In addition, the average daily RH was much lower in season 3 than in either of the two previous seasons (Tables 4.1 - 4.3). In season 2 the greatest growth occurred during periods of still, warm and humid days when the vapour pressure deficit (VPD) was low. These conditions allowed the plants to maintain high rates of photosynthesis without increasing the transpiration rate to unsustainable levels. Warm, humid days were rare in season 3 and, in addition, the warmest parts in the growing season were much windier. Although the A_{max} was not significantly different between seasons 2 and 3 (Chapter 2), it was measured using the Walz climate controlled cuvette at saturating light and a constant temperature (22°C). Under the field conditions the leaves were growing in, it is likely that the interaction of the lower soil moisture levels and increased wind meant that the amount of time that the leaves were able to photosynthesise at high rates in season 3 was less than that in the previous season. This would explain the decrease in NAR.

Thus, it is likely that the climatic variables that affected the growth rates in season 3 were a combination of light and water interactions that led to a decrease in the time during which leaves were able to photosynthesis at high rates, rather than the direct influence of air or soil temperature on growth rates. However, as will be discussed below (section 4.6.5), other factors may also have influenced the rates of tree growth during the three seasons.

Over the three seasons, both the absolute and relative growth rates of the *P. tomentosa* trees at the Matangi site were less than the spectacular rates measured for other *Paulownia* trees in different locations, as discussed earlier (section 4.1.1.1). This was probably a result of the interaction between the environmental conditions of the site and the inherently slower growth rates of *P. tomentosa* (Japan) in comparison with other *Paulownia* species and provenances. It is believed that this provenance has been selected for its slow growth rate by the Japanese growers who prefer *Paulownia* timber with a high density (I. Barton, pers. comm.).

The rate of increase in biomass in the young *P. tomentosa* trees was less than that reported for outside grown seedlings and cuttings of species of poplar, beech and maple which had relative growth rates between 0. 3 g g^{-1} $month^{-1}$ to 1.2 g g^{-1} $month^{-1}$ (Pollard and Waring, 1968). In the first two years, the height of *Eucalyptus regnans* stems increased at 0.07 m m^{-1} $month^{-1}$ (G was 0.075 m $month^{-1}$ for the same period) (Wilcox and Thulin, 1979). Growth was considerably more rapid in *Populus deltoides*, with G being 0.6 m $month^{-1}$ for

stem height and 4.3 mm month^{-1} for stem diameter in the first season of growth (Baker and Blackmon, 1977). From these data, it can be seen that the growth rates of *P. tomentosa* were in a similar range to those of some other trees that are considered to grow quickly.

4.6.2 Biomass partitioning

The partitioning of biomass to the different *Paulownia* tissues changed as the trees aged. Initially the root reserves were mobilized to produce primarily stem and foliage material. Once a viable shoot had been established, the trees began to develop a lateral root system in the latter part of the first season. This seasonal trend of a greater allocation of biomass to the shoot system earlier and to the roots later in the season was repeated in seasons 2 and 3, although the absolute values of biomass allocated to each tissue differed between the seasons. The relative growth rates of both the root and shoot tissues were highest in season 1 and lowest in season 3. The value of R for shoot material was high in season 1 because of the very low shoot biomass at the first harvest. New shoot material was produced more quickly than root material in season 1, but in season 2 trend this was reversed. During season 3, the relative growth rates of the root and shoot material were very similar.

Comparison of the R:S ratios of the perennial material at the end of each season showed a decline which indicates that an increasing amount of biomass is present as stem and branch tissue as the trees age. However, the average R:S ratio at the final harvest in season 3 of 1.3 is still high in comparison with similarly aged trees of different species. *Populus deltoides*, for example, grown from unrooted stem cuttings had a R:S ratio of approximately 0.5 after the first season of growth (Baker and Blackmon, 1977). In *Eucalyptus tereticornis* the R:S ratio was 0.27 in two year old trees, and increased to 0.40 after three years growth as the stump root increased in size by a factor of 10, but decreased to 0.36 at age four and continued to decrease as the trees aged through to eight years old (Bargali *et al.*, 1992). In kiwifruit the proportion of total biomass (including foliage and fruit) that was present as root material decreased from 55% at age one, to 41% at age five (Buwalda and Smith, 1987). However, in 6 year old vines root material made up more than half of the perennial tissues of the vine and most of that root tissue was less than 20 mm in diameter (Smith *et al.*, 1992).

A number of genotypic and environmental factors influence the partitioning of biomass between root and shoot tissue. As mentioned previously (section 4.1.1.1) there are differences in partitioning between species, both within and between genera. It seems likely that *P. tomentosa* allocates much of its biomass resources to root growth early in development, which may explain why the relative growth rate of total biomass was higher each season than R for stem height and diameter. It is possible that one of the reasons *Paulownia*

can be so readily propagated from root material is the large accumulation of biomass in the roots. In a study of the partitioning of biomass in a invasive conifer species (*Juniperus occidentalis*), Miller *et al.* (1990) found that in juvenile and small-adult trees the R:S ratio was 0.75 and 0.67 respectively which was higher than for conifers of similar ages. These plants allocated more biomass to foliage and to root tissue at the expense of branch and trunk material which improved their competitive advantage in the semi-arid communities into which they invaded.

When a tree is very young, the nature of the original propagule has a large influence on the R:S ratio. In those trees grown from stem cuttings (*e.g. Populus deltoides* mentioned above) the initial R:S ratios would be lower than in trees propagated from root cuttings, as is the case in *Paulownia*. For seedlings, the size and structure of the seed would influence the initial development of root and shoot material.

When either water or nutrients are limiting in the soil environment, trees tend to increase the amount of biomass in the root system at the expense of the shoot system to increase the soil volume able to be exploited (Sutton, 1990). In a series of experiments with *Quercus rubra* and *Liriodendron tulipifera* grown over 2 years, Kolb and Steiner (1990) found that increased competition for soil resources led to an increased amount of root material in both species, while shading led to an increase in leaf material. The average R:S ratios of these seedlings over all treatments was 0.45 for *L. tulipifera* and 0.71 for *Q. rubra*. The role of soil and air temperatures in influencing R:S ratios has been discussed in the previous chapter.

As the trees aged, the proportion of total biomass present as foliage material decreased, from 43% in season, to 23% in season 2 and 21% in season 3. This reflected the increase in the perennial material in the latter two seasons rather than a decrease in leaf material, with increased proportions of the total biomass present as stem and branch tissue and as woody root material. The decrease in foliage as a proportion of total biomass with increasing age is a common feature of woody plants as the perennial framework increases in biomass over a number of seasons.

4.6.3 Nutrient levels in *Paulownia* tissues

For the majority of the nutrients studied in these *P. tomentosa* trees, the highest concentrations were found in the annual tissues (the lamina, petiole and fine roots) as is the case in most woody perennials. The lower nutrient concentrations in the perennial tissues are a result of the process of lignification which increases the organic components of the tissue in

proportion to the inorganic components. The roles of the different annually produced tissues also affect their nutrient concentrations with leaves, for example, needing high levels of nitrogen, iron, magnesium, chlorine, manganese and copper due to direct involvement of these elements in photosynthesis. Fine roots tend to have high nutrient levels as their role is primarily that of uptake of water and nutrients.

The one element that was generally found in higher concentrations in perennial tissue than in foliage was zinc. There were some variations within and between seasons, but zinc levels in stem and branch tissue tended to be higher than levels in lamina material. A similar situation was found in young kiwifruit vines where perennial material had higher zinc levels than leaf tissue (Buwalda and Smith, 1987).

There was little difference between nutrient levels in each tissue type between seasons. This lack of difference in the nutrient levels in foliar material between seasons 2 and 3 was surprising. Compared with the healthy looking leaves on the trees in season 2, the leaves in season 3 were noticeably less green. It was suspected that the early cessation of apical growth and the poorer health of the leaves in the third season was related to a nutrient deficiency problem, perhaps compounded by the lower soil moisture levels. However, applications of a liquid nutrient solution (section 3.2) and watering the trees did nothing to improve leaf colour or to prolong apical growth. It may have been a matter of "too little, too late". But the results of the nutrient analyses of the foliage, and other tissues, did not show any significant difference in any of the nutrients, suggesting that other factors were more important. This lack of relationship between foliar nutrient levels and visible indications of plant health and vigour was indicated by the regression analyses (section 4.4.2.7).

The role of the perennial components of the trees as storage sites for nutrients over the dormant season seems to be limited to nitrogen only. In stems, branches and roots the concentration of nitrogen tended to be higher at the end of each season than during the season. The levels of other nutrients in the woody tissues did not show a strong seasonal pattern. This result is not unexpected when consideration is made of the limited withdrawal of nutrients from the leaves during senescence, with only moderate amounts of N (34%), P (42%) and K (36%) being withdrawn prior to leaf drop. In comparison with other hardwood species, *Paulownia* tends to translocate lower amounts of nutrients from foliage at senescence. In *Betula alleghaniensis* and in two species of *Eucalyptus* between 54% and 67% of the nitrogen was withdrawn, while in these and other trees up to 80% of the P and up to 60% of the K were translocated out of older leaves prior to leaf fall (van den Driessche, 1984). It is interesting to note that in some of these species the levels of Ca, Fe and Zn were higher in the old leaves, as was found to be the case in *Paulownia* also.

The low rates of nutrient retranslocation from *Paulownia* leaves during senescence mean that when the leaves abscise they still have reasonably high nutrient contents which probably partly accounts for the rapid decomposition rates observed for these leaves. The other implication of this strategy is that the tree does not attempt to conserve nutrients between seasons. This implies that the nutrient supply within the tree is sufficient to support growth early in the next season and that the development of the absorbing root system is rapid enough to meet the demands of that new growth. It is probable that the water relations of the plant (high transpiration rate and low resistance to water movement within the plant) mean that the tree is absorbing large quantities of soil moisture and hence, soil nutrients, during the period of active growth. In a species with more conservative water relations the amount of water passing through the plant each day is likely to be less, so less nutrients would be absorbed and so the plant would probably have a more conservative nutrient strategy as well. This can be seen in the examples of the eucalypt species (van den Driessche, 1984) which tend to inhabit semi-arid areas.

4.6.4 Comparison of foliar nutrient levels with other tree species

A comparison of the nutrient concentrations in the foliage of the *P. tomentosa* trees harvested in November, 1992 with a range of other tree species grown for their timber (Table 4.27) shows that the concentration of nitrogen in *Paulownia* leaves is high when compared with species of eucalypt and softwoods (pine and kauri), but similar to that in the poplar species and the nitrogen fixers grown in Zimbabwe (*L. leucocephala* and *C. cunninghamiana*). The only tree with significantly higher foliar nitrogen was the nitrogen fixing *Acacia* grown in New Zealand. The lower levels of nitrogen in the eucalypts and the softwoods may be a reflection of the longevity of the foliage in these trees which are all evergreen. The levels of the other macronutrients in *P. tomentosa* foliage were generally similar to those reported for the other species, with the exception of potassium which was higher in these leaves and in the leaves of poplar. From the limited data available on the micronutrients it can be seen that the levels of these were similar between tree species except for Mn which was higher in the New Zealand grown pine, eucalypt and *Acacia* than in the leaves of either of the two *Paulownia* species.

The range of foliar concentrations of all the nutrients in *P. tomentosa* was not dissimilar to the range of values tabulated by Drechsel and Zech (1991) for over thirty different species of broad-leaved tropical trees, including both legumes and non-legumes. Included in this data set were the range of concentrations that were considered to be deficient, adequate or high (potentially toxic) for each nutrient in the various species.

Table 4.27. Foliar nutrient levels in a range of forestry tree species.

Species	N	P	K	Mg	Ca	S	Mn	Zn	Cu	Fe	B
	(% DW)						(ppm DW)				
P.tomentosa	2.67	0.25	1.24	0.20	1.45	0.19	37	39	18	182	31
P. fortunei [a]	2.7	0.16	0.9	0.64	2.4		83	26	13	700	36
Populus deltoides[b]	2.9	0.2	1.9	0.35	1.5						
Eucalyptus grandis[c]	1.30	0.08	0.28	0.28	1.24						
E. camaldulensis[c]	1.09	0.08	0.27	0.28	1.99						
E. tereticornis[c]	1.09	0.06	0.28	0.39	1.17						
E. regnans[d]	1.61	0.15	0.74	0.23	0.54		891	9	8		
Pinus radiata[d]	1.63	0.19	0.98	0.10	0.25		219	45			
Agathis australis[e]	0.67	0.07	0.49		0.89	0.10				130	
Acacia dealbata[d]	3.78	0.16	0.93	0.22	0.86		133	18	9		
Leucaena leucocephala[c]	2.33	0.26	0.26	0.52	1.60						
Casuarina cunninghamiana[c]	2.42	0.26	0.32	0.37	1.41						

[a]Song (1988); [b]Baker and Blackmon (1977); [c]Sanginga *et al.* (1991); [d]Frederick *et al.* (1985a); [e]Peterson (1961).

Only general comparisons between the nutrient data from different authors can be made as the exact levels on all the nutrients reported will have been influenced by a number of soil and silvicultural factors. Whether or not the sites had been fertilized, and with what type of fertilizer, impacts on foliar nutrient concentrations. In addition the age of the trees and of the foliage tested differed between studies, although the majority of the studies were on young trees. Comparison of the nutrient levels between the two *Paulownia* species show differences that not only result from the fact that different species were studied, but also that they were grown on different sites (one in New Zealand and one in China) and that the age of the trees and of the foliage when tested were also unlikely to have been the same.

4.6.5 Nutrient levels and growth rates

The results from the experiment discussed in Chapter 3, and from field trials on the effect of different fertilizers on the growth of young *P. fortunei* trees near Hamilton (Paterson, pers. comm.) indicate that the growth rates of *Paulownia* are significantly affected by the levels of nutrients available. This relationship has also been noted by various commercial growers of *Paulownia* (K. Avery, pers. comm.). The value of R for the height of trees grown outside the campus glasshouse from November to January (0.71 m m^{-1} $month^{-1}$) was twice that of trees growing in the field at Matangi from November to January in the first season (0.35 m m^{-1} $month^{-1}$). This result is unlikely to be due to differences in planting stock or aerial environment (temperature, light, RH etc.) as these variables were similar for both two sets of trees. The major difference between the two environments was the regular watering and weekly provision of nutrients to the trees at the campus site, which probably accounted for the more rapid growth of those trees.

The nutrient concentrations in the foliar and stem tissues of the trees grown on campus were compared with concentrations in trees of a similar age, harvested from Matangi. P and K levels were higher in stem and leaf tissues of the campus trees, while N and Mn levels were higher in the foliage of these trees than in the foliage of the Matangi trees. There were no difference between the two sets of trees in the concentrations of Mg and S in any tissue. The levels of Ca and the micronutrients Zn, Cu and Fe were higher in the trees harvested from Matangi. The concentration of B was higher in the lamina material from the campus trees, but similar in the petiole and stem tissues from the trees at the two sites.

The study by Jia and Ingestad (1984) discussed earlier (section 4.1.2.4) highlights the linear relationship between seedling growth rate and nutrient addition rate which has been found in several species of forest trees. They argue that maximum growth rates are achieved only when the internal nutrient status of the plants is maintained at a stable (and high) level. It has been long known that plant growth rates are slowed when one or more nutrients are limiting, regardless of the status of other environmental factors (Clarkson and Hanson, 1980). A decrease in the level of availability of a particular nutrient will slow growth rates until the requirements of the plant are able to be met by the new nutrient levels, with this rate of change being fastest when it is nitrogen that becomes limiting in the soil environment (Clarkson and Hanson, 1980).

It is, therefore, possible that in addition to the limitations imposed on the growth rates of the *P. tomentosa* trees in their third season by climatic factors (section 4.6.1), the nutrient supply in the soil may have been insufficient to sustain higher growth rates. Variation of soil nutrient levels across the experimental site may also have played a role in the large differences in

growth rates between trees. Further investigation of this hypothesis requires the addition of high levels of readily available nutrients to the trees in the coming season and maintainence of high soil moisture levels to overcome any limitation on growth imposed by these factors.

4.6.6 Carbohydrate levels

4.6.6.1 TNC levels in above ground tissues

It appears from the results of the analyses of total nonstructural carbohydrates (TNC) undertaken in this study that young *P. tomentosa* trees maintain high levels of soluble sugars in all tissues but very low starch levels (Tables 4.25 and 4.26). The foliar concentrations of soluble sugars in *P. tomentosa* averaged about 11% in the lamina, but ranged from 6% to 32% in petiole tissue at different times. The low soluble sugar levels in both the lamina and petiole at harvest 4 (May, 1991) reflect the advanced senescent state of these leaves. These levels are comparable to those reported for other deciduous hardwood species. In *Prunus domestica*, soluble sugar levels ranged from about 7% to 15% (Gucci *et al.*, 1991), with similar levels found in *Prunus avium* leaves (Roper *et al.*, 1988). In both these species, sorbitol was the most abundant soluble sugar and starch made up less than 2% of the dry weight of the leaf at any time. The effect of defruiting these trees on TNC levels was less than the seasonal variations on control trees. Tschaplinski and Norby (1991) studied the effect of fertilizer treatment on soluble sugar levels in seedlings of sycamore (*Platanus occidentalis*) at different times in the season. They found the highest levels at the end of the season (16%) regardless of fertilizer regime, with sucrose comprising about 50% of the total sugars. The starch levels in kiwifruit leaves were found to be highest (7%) early in the season, decreasing to 1% later (Smith *et al.*, 1992). However, it was possible to observe the effect of a strong sink in these plants with starch levels in fruit rising to over 30% as the season progressed.

The evergreen nature of the foliage of many gymnosperms can affect TNC levels. In *Pinus radiata* Cranswick *et al.* (1987) found that about 8% - 10% of the dry weight of needles of all ages was composed of soluble sugars and cyclic sugar alcohols. But starch levels increased from 1% in current seasons needles to 2.6% in those aged over two years. These starch levels are lower than those found in needles of Douglas fir which ranged from 1% to 6% throughout the season (Webb and Kilpatrick, 1993).

In the above ground perennial tissues in *Paulownia*, TNC levels were variable within and between seasons. The soluble sugar concentrations ranged from 7% in the first harvest in season 3, in both stem and old branch tissue, to 23% in stem tissue in March of the first

season. Overall the levels were higher than those reported for other species, with the soluble sugar levels in first season stems of sycamore reaching 8% at the end of the season (Tschaplinski and Norby, 1991). In *Pinus radiata* wood aged between zero and three years, TNC levels were never higher than about 2% (Cranswick *et al.*, 1987). In the perennial above ground tissues of kiwifruit, starch levels varied from 1% - 6% through the season (Smith *et al.*, 1992).

4.6.6.2 TNC levels in below ground tissues

The perennial root tissues of the *P. tomentosa* roots had very high levels of soluble sugars. Concentrations in the lateral roots in the middle and end of each season ranged from 20% to 40% (Table 4.25), while levels in the stump were between 15% and 25% over the same periods. Kramer and Kozlowski (1979) caution against over-emphasizing the role of roots in carbohydrate storage as although often the concentrations are higher than in stem and branch tissue, the greater relative proportion of the above ground biomass means that the total storage pool is greater above than below ground. However, in the trees studied here the R:S ratio was generally high (Tables 4.7 - 4.9). Thus in absolute amounts, the root material had more soluble sugars than the stem and branch tissue.

Although it is not possible to accurately quantify starch concentrations in the roots from the results of the analyses on *P. tomentosa*, it seems unlikely that starch is present in large amounts in these tissues. Even if the calculated concentrations (Table 4.26) were underestimated by a factor of 10, the maximum starch levels would be less than 4% and so would not contribute greatly to the level of TNC. However, in many other woody species, both gymnosperms and angiosperms, the greatest proportion of TNC in root material is made up of starch. In the roots of 18 year old Jonathan apple trees Tromp (1983) found between 11% and 12% starch, while soluble sugars comprised only 3% - 5% of the root dry weight. He also measured hemicellulose concentrations, which were about 25%. Starch levels in fibrous kiwifruit roots reached 30% at some points in the growing season, and in coarse roots averaged between 10% and 12% (Smith *et al.*, 1992). In a series of experiments studying resprouting from root stumps in four hardwood species, Kays and Canham (1991) found that the timing of coppicing affected starch reserves in the roots. The starch concentrations were generally highest in the uncut controls at between 7% and 9%.

In the woody roots of mature *Pinus radiata*, starch levels were between 1% and 2%, with soluble sugars present in slightly higher levels (Cranswick *et al.*, 1987). In ponderosa pine root tissue, the highest starch level was found to be 9% (Omi and Rose, 1991). However in

seedlings of *Pinus caribaea*, starch levels ranged from 17% to 35% depending on the watering and root pruning regime imposed on the plants. Starch levels in the needles of these plants were also high (23% - 44%), but were lower in stem tissue (15% - 24%) (Abod and Sandi, 1983).

In the fine roots of *P. tomentosa* TNC levels were lower than in the perennial root tissues. The role of the fine roots is primarily one of water and nutrient uptake and so there would be little storage of carbohydrates in these tissues. The rapid turnover of these roots, in comparison with the perennial roots, would also preclude storage and so the levels of TNC probably reflect the requirements of these roots for their own metabolism and growth. Similar results have been found in other species with starch concentrations in the fine roots of kiwifruit (between 1% and 5%) being much lower than those in fibrous roots (Smith *et al.*, 1992). In seedlings of loblolly pine (*Pinus taeda*) the starch levels in fine roots were between 6% and 11% with soluble sugars levels at about 3%. In coarse roots of the same plants, both starch and sugar levels were almost twice as high (Hallgren *et al.*, 1991). These workers found that the effect of drought stress on TNC levels in root and shoot tissue was influenced by the provenance of the seedlings. In *P. radiata* trees, in contrast to the other species discussed, TNC levels were similar in the fine and coarse roots (Cranswick *et al.*, 1987).

4.6.6.3 Seasonal trends in TNC

The typical seasonal pattern of carbohydrate allocation in deciduous trees discussed earlier (section 4.1.3.2) was found in the perennial tissues of the *P. tomentosa* trees studied. Soluble sugar levels were generally lowest in the perennial root and shoot material at the first harvest of each season. Starch levels also appeared to be lowest in the perennial roots early in the season, but the data are inconclusive. Although it is sometimes assumed that the reserves of carbohydrates mobilized at the beginning of the growing season provide the structural components for the new tissues, it has been found in apple trees that only a small proportion of reserves are actually used in this way. More than 75% of the stored carbohydrates are used in respiration to provide energy for the development of the new tissues (Hansen, 1977).

The timing of the *P. tomentosa* harvests provided a limited "snapshot" impression of the patterns of carbon allocation over the season. In order to more fully investigate allocation, increased numbers of harvests at shorter time intervals can be undertaken, as with the kiwifruit work discussed above (Smith *et al.*, 1992). It is also possible to use labelled tracers (either ^{13}C or ^{14}C). Labelling leaves on one year old apple trees with ^{14}C in autumn, spring and summer, Kandiah (1979b) was able to follow photosynthate allocation patterns. He found

that in autumn, the carbon fixed initially tended to be translocated to woody regions below the point of labelling, particularly the roots. Some of this labelled carbon was remobilised in spring and was found in the new shoots. The label applied in spring tended to remain in the labelled shoots, while that applied in summer was more widely distributed, particularly in regions below the point of labelling. It would be very interesting to undertake a similar experiment with *Paulownia* (perhaps using ^{13}C) to determine if the direction of carbon movement was influenced by seasonal factors. It would be expected that carbon is translocated from the roots to the shoots early in the growing season, with movement in the opposite direction towards the end of the season.

4.6.6.4 Further research into TNC in *Paulownia*

The results of the research undertaken in this study on the TNC levels in *P. tomentosa* trees in their first three years of growth has raised a number of interesting questions. In addition to the quantification of the soluble sugars, it would be interesting to determine the proportions of each type of sugar and whether mannitol is present in significant quantities, as in other members of Scrophulariaceae (section 4.1 3.1). It may be found, with further work, that the allocation patterns of the various soluble sugars change throughout the season or are different between the different tissues.

The very high soluble sugar levels in the perennial root tissues raises an intriguing question of protection. Why are the roots not predated by soil fauna and microfauna, given the very strong sugar solution contained within? It would be expected that such a rich food source would suffer from some attack, but there was no evidence of damage to any of the roots harvested throughout the three seasons. Hallgren *et al.* (1991) noted that insect and fungal attack of roots may be more likely when soluble sugar levels were high. Nematodes were obviously present in the fine root material, but did not appear to affect woody roots. Anecdotal evidence suggests that the wood of *Paulownia* has some insecticidal properties. One of the reasons that *Paulownia* wood is so highly sought after for the traditional *tansu* in Japan (section 1.5.2) is that it keeps moths away from the clothes stored inside. It is possible that there are chemicals within the stem and root wood that prevent attack by insect and by micro-organisms. There is currently a research project under way at the University of Waikato to determine if any of the chemical extractives from the wood display any properties associated with this characteristic.

It has also been noted by Sang (1980) that the leaves on *Paulownia* trees secrete a mucus that traps cotton aphids, which helps to decrease the levels of this pest in cotton fields. It is likely

that the insecticidal property in this case is more of a physical effect than a chemical one. It was noticed during the course of the research described here that at the junction of the lamina and petiole on the adaxial leaf surface, a sticky secretion was evident. This was more noticeable on leaves of the *P. elongata* tree at the creche than on the *P. tomentosa* trees. As the season progressed, this secretion turned black - perhaps as a result of fungal infection? On a number of occasions flying insects were observed apparently feeding on this secretion. No attempt was made to characterise the secretion in this study, but it would be interesting to determine its chemical composition. This may help to shed some light as to why the leaves secrete the material.

There is also a need to determine further the possibility of enzyme inhibition during the starch analysis, by chemicals within the *Paulownia* tissues. It may eventuate that some of the chemicals involved in the protection of the sugar supply are extracted in the starch analysis process and interfere with functioning of either of the two enzyme systems used. The starch analysis method employed here is widely used apparently without problems (*e.g.* Roper *et al.*, 1988; Gucci *et al.*, 1991; Hallgren *et al.*, 1991; Kays and Canham, 1991; Omi and Rose, 1991; Smith *et al.*, 1992). However, Webb and Kilpatrick (1993) do mention that they undertook tests on Douglas fir tissue to determine if there was any inhibition of the enzymes during starch analysis. Their tests were negative. But to undertake such tests, they must have been aware of a potential problem. Unfortunately they give no reference to the reasons for taking such precautions and I have been unable to find any further literature that mentions this problem with this method of starch analysis.

Chapter 5
The Propagation Of *Paulownia*

5.1 Introduction

5.1.1 Propagation by seed

The potential for propagation of *Paulownia* by sexual means is enormous with a mature tree capable of producing up to 20 million seeds per season (Carpenter and Smith, 1979). Each pollinated flower gives rise to a single capsule that containing about 2000 seeds (Millsaps, 1936). The seeds are very light with a kilogram containing between four and six million individual seeds (Zhu *et al.*, 1986), ranging in size from 2 - 10 mm long and 1 - 4 mm wide (Hu, 1959). The shape is such that the central seed portion is surrounded by two 'wings' to facilitate airborne dispersal.

Under ideal conditions, the seeds germinate within 7 - 10 days. Several workers have studied environmental influences on the germination of *Paulownia* seeds and the subsequent growth of the seedlings. Some of the earliest work investigated the effect of red and far-red light, and the phytochrome mediated response to different irradiance regimes on germination (Borthwick *et al.*, 1964). Working with several seed lots of *P. tomentosa* collected over different seasons, they found a strong light requirement in all seeds. Only two seeds, out of over 5000, germinated in the dark, regardless of the temperature or length of incubation. The classic phytochrome response was observed with alternating flashes of far-red and red light only resulting in germination (between 65% and 100%) when the series of flashes ended with red light. In a series ending with far-red light, a maximum of 3% of the seeds germinated. There was, however, wide variation in the length of time of illumination needed by different seed lots to achieve high levels of germination. Studies into the effect of the length of the photoperiod on the growth of seedlings of *P. tomentosa* showed that increasing the photoperiod significantly increased the growth of the seedlings (Downs and Borthwick, 1956; Immel *et al.*, 1980) and that this effect was enhanced at higher temperatures (Downs and Borthwick, 1956).

The viability of *Paulownia* seeds is relatively short compared with other species. The major requirement of storage conditions to maintain viability is a cool temperature. Zhu *et al.* (1986) advocated cool, dry conditions under which seeds remained viable for up to at least three years. Carpenter and Smith (1979) compared dry storage with stratification in a 1:1 mix of peat moss and sand, at 4°C. The germination percentage and rate of germination was slightly higher with the stratified seed, but under both systems the seeds remained viable for

up to three years. Light was found to stimulate germination regardless of temperature and stratification treatments in a study by Barnhill *et al.* (1982). Some germination still occurred in the dark under the higher temperature regimes, with or without stratification, but at lower rates than in the light. Stratification improved the germination rate at the lower temperature (5°C - 15°C), especially in the light. These workers concluded that, in the wild, *Paulownia* seeds could germinate successfully in late autumn, but are more likely to germinate in the spring after natural stratification when conditions for subsequent growth would be more favourable.

The effect of substrate acidity on seed germination and seedling development has been investigated by Turner *et al.* (1988), with respect to growing *Paulownia* on sites previously strip mined. It was found that seeds of *P. tomentosa* would not germinate below pH 4.0 and that the primary roots of those that germinated between pH 4.0 and pH 5.0 stopped elongating after 10 days. The roots of seeds germinated at higher pH levels elongated more quickly, and for a longer period. The numbers of seedlings emerging in soil at pH 4.5 was less than in soils at pH 5.5 and pH 6.5, and overall seedling growth was depressed in the more acid soil.

Seeds of *Paulownia* can be directly sown into nursery beds which decreases the time and labour involved in germinating the seeds in the glasshouse and transplanting them later, and also avoids transplant shock. It is important that the nursery beds are well prepared with good drainage, adequate fertilizer and a pH between 5.0 and 8.0 (Stringer ,1986). Experimenting with different pretreatments of the seed, including soaking and mixing the seed with Terrasorb (a moisture holding compound), Beckjord *et al.* (1984) found that unsoaked seeds resulted in higher seedling densities 125 days after seed sowing than the soaked seeds. The addition of Terrasorb decreased seedling density of both the soaked and unsoaked seeds, while those covered by shade cover survived better than those left uncovered. Stringer (1986) also studied the effect of shade cover and found it to enhance seedling survival as the delicate radicles of the germinating seed are extremely prone to desiccation. A concomitant thinning trial with one year old seedlings resulted in the greatest plant height being recorded for plants thinned to 20 plants m^{-2} compared with plants at 10 m^{-2}, 100 m^{-2} and an unthinned control (Stringer, 1986).

The advantages of propagating *Paulownia* from seed include the genetic variability between seeds which can allow the development of a breeding programme to select trees with desirable characteristics. The sheer number of seeds produced annually and the ability of the seeds to remain viable for about three years mean that a ready supply of material is always available. And, as Zhu *et al.* (1986) noted, in areas where witches broom (a disease caused by a

mycoplasm) and other diseases are present, the seed is pathogen free. However, in order to produce clonal material of known quality, vegetative propagation is required.

5.1.2 Vegetative propagation

The most common method of vegetative propagation of woody perennials in commercial situations is by stem cuttings. The three main types of stem cuttings from trees and shrubs are hardwood, semi-hardwood and softwood cuttings and, given favourable conditions, cuttings from many species will root easily to produce new plants. There are several advantages in propagating from stem material including the low cost of the process, the ease with which large numbers of cuttings can be produced and, in comparison with root cuttings, the ready availability of sufficient quantities of suitable parent material. The majority of tree species used in forestry situations can be propagated vegetatively by stem cuttings.

For a small number of timber species such as aspens (*Populus spp.*) and *Robinia pseudoacacia*, it is possible to propagate from root cuttings but it is uneconomic on a large scale (Wright, 1962). Hartmann and Kester (1983) list several species that propagate readily from root material and included are members of the Rosaceae family *e.g. Rubus spp.*, *Malus spp.* and *Rosa spp.*, as well as *Ulmus carpinifolia*, *Actinidia deliciosa* var. *deliciosa* (kiwifruit) and others. *Paulownia* plants establish very easily from root cuttings. In a commercial situation in New Zealand, the root systems of plants used in propagation are usually harvested in July, cut into portions of the appropriate size and treated with fungicide. The root cuttings are then shipped to customers in damp sawdust, ready for planting in August (I. Barton, pers. comm.). Trees can be coppiced (cut back to ground level) if they do not grow well in the first two or three years, or if they have poor form. This usually results in the production of a strong healthy leader in the following season that will grow more quickly because the root system is already established. It is theoretically possible to maintain a plantation indefinitely because of this feature of the trees. At harvest, only the above ground material is removed leaving a large root system which should be capable of producing new shoots and eventually harvestable stems. Whether or not the growth from old root systems will sustain more than one harvest of stems is not known at this stage.

The ability of a root cutting or root stump to produce shoot material involves the production of adventitious buds. These buds may arise from a range of tissues within the root and are probably stimulated as a response to wounding (Hartmann and Kester, 1983). The production of new root tissue may also be adventitious or may be the result of the development of latent root initials already contained within the parent root (Hartmann and Kester, 1983). In *Haplopappus ravenii*, for example, Blakely *et al.* (1972) found that new

root primordia developed at the margin of the vascular cylinder, and in *Malus spp.*, adventitious root primordia were found to arise in association with the vascular cambium, especially the xylem ray parenchyma (Siegler and Bowman, 1939).

Both root cuttings and stem cuttings exhibit strong polarity in that roots will almost always develop at the true basal end of the cutting, while the new shoot material arises at the end that was uppermost in the original plant (the proximal end), even if the cutting is inverted (Ting, 1982; Hartmann and Kester, 1983). This polarity effect is still observed when sequential cuttings are taken from the same root, so that of the two cut surfaces that were originally joined, one will produce shoots and the other roots. A series of experiments carried out on root segments of *Taraxacum* showed that the application of auxins (IAA, IBA or NAA) to either end of the root section promoted rooting, while shoot development only occurred at the proximal end and then only if no additional auxins were applied (Warmke and Warmke, 1950). However, rudimentary shoots did develop at the distal end if the internal level of auxin was decreased by washing or by the application of auxin inhibitors. Investigations of the internal concentration of auxin in *Cichorium* root segments by the same authors showed that at the time of cutting, the concentration of auxin was the same at each end of the segment, but after 48 - 96 hours was higher at the distal (root producing) end. They concluded that it was a gradient of auxin in the root section that lead to the development of the shoot at the proximal end and root at the distal end. This gradient is a result of the acropetal transport of auxin in roots (Robinson, 1975). This natural phenomenon is exploited commercially in the horticulture and forestry industries where the production of rooted stem cuttings is facilitated by the dipping of the cut stems in rooting hormone made up of auxins (often IBA) to promote root development. Struve (1990) noted that treating both seedlings and stem cuttings of scarlet oak with IBA led to an increase in the number of roots regenerating, rather than an increase in the rate of regeneration or a decrease in the time to initiation of regeneration.

Both the ability to regenerate new tissue of a different type (which involves dedifferentiation) and the polarity exhibited by cuttings are mediated by auxins but the exact mechanisms remain unclear (Hartmann and Kester, 1983). It is likely that interactions between cytokinins and auxins, and the carbohydrate status of the parent material influence regeneration ability (*e.g.* Robinson, 1975). It has also been shown, in aspens, that at early stages of shoot development gibberellic acid (GA_3) is inhibitory to shoot growth, but promotes shoot elongation at later stages of development (Schier, 1973).

There has been much work done on the use of tissue culture as a technique for vegetative propagation in *Paulownia*. The advantages of tissue culture include the production of large numbers of clonal plants from very small amounts of material from trees selected for

particular traits such as disease resistance, good growth form or rapid growth rates. Tissue cultured plants of *P. elongata* have been produced on a commercial basis in New Zealand (K. Avery, pers. comm.).

5.1.3 Raising seedlings in hydroponics

The application of hydroponic systems to plant production on a commercial scale has mainly been limited to annual flower and vegetable crops under glasshouse conditions, *e.g.* lettuce (Care, 1991). However various hydroponic techniques can be used to study nutrition and growth in seedlings of perennial plants. Jia and Ingestad (1984) used a root misting system to investigate the nutrient requirements of *Populus simonii* and *Paulownia tomentosa,* as well as *Betula verrucosa* and *Alnus incana* seedlings (Ingestad, 1981). Using the same system, McDonald *et al.* (1986) studied the growth rates of *Betula pendula* under different nutrient regimes. Aerated pots have been used extensively in New Zealand to study the nutritional requirements of kiwifruit and, to a lesser extent, tamarillo and avocado (S. Miller, pers. comm.). The nutrient film technique (NFT) employed in the experiments reported here involves continuously flowing the appropriate nutrient solution over a portion of the root mat.

The advantages of using various hydroponic techniques in studying the nutritional requirements of plants include the flexibility to precisely control the total amount of nutrients available to the plants or to vary the proportions of the different nutrients, without the complication of other nutrient reserves within the growing medium. It is possible to study the effects of both nutrient deficiencies and toxicities on a range of plants under controlled environmental conditions. These techniques can also be used to study the tolerance of root systems to different levels of anoxia by altering the aeration in the root zone.

5.1.4 The aims of the experiments undertaken in this study

As the use of root material is the most common method of vegetative propagation in the production of large numbers of *Paulownia* trees (Zhu *et al.*, 1986; Graves and Stringer, 1989), it was decided to study some of the factors involved in determining the development of new tissues. Some work investigating the effect of root cutting length and diameter has been reported by Rin (1975), but little other research into factors influencing the viability of root material appears to have been done. A preliminary experiment involved assessing the rate of emergence of new tissue and the site of emergence for both roots and shoots for root cuttings planted in the field. Root cuttings were then taken from field grown *P. tomentosa* trees to study the effect of size of cutting and position of cutting in parent root system on the

development of new root and shoot tissue. Most of the cuttings were planted as they would be in the field - that is upright, in the same vertical direction as they were in the parent root system, with 1- 2 cm of potting mix covering the uppermost surface. But a small number of cuttings were grown under a number of other treatments to study the effect of orientation, hormone application and exclusion of light on root and shoot growth.

The second set of experiments compared the growth of seedlings of different species of *Paulownia* in hydroponics at two nutrient levels. The effect of the nutrient regime on the development of shoot and root material was measured after two months in the hydroponics system.

The third set of experiments set out to study transplant shock in *P. tomentosa* and *P. elongata* seedlings. One of the problems associated with raising *Paulownia* plants from seeds or root material is that unless they are planted or sown at the desired spacing in the plantation, they must be transplanted at some stage. This is more often a consideration when dealing with seedlings which are generally germinated and grown initially in protected environments (*e.g.* a glasshouse) whereas root cuttings can easily be planted directly into their final field positions. Young *Paulownia* roots appear to be sensitive to disturbance and the plants suffer transplant shock when the seedlings are pricked out, repotted or transplanted into the field if insufficient care is taken in handling the fine roots. This results in the temporary cessation of stem growth. Stem growth also declines if the plants become pot-bound. The rapid growth of the seedlings means that this can happen quickly and it is sometimes necessary to repot seedlings on a fortnightly basis. *P. tomentosa* appears to be more susceptible to this problem than other species (Ede, unpublished).

In order to study this problem seedlings were either raised in the hydroponics system used in the previous experiment or grown in pots with commercial potting mix. The two contrasting growing mediums were employed to determine if the more rapid root development, particularly of non-woody roots, in hydroponics was an advantage to the subsequent growth of the plants in the field. At the time of transplanting into the field, half of each set of seedlings was planted intact and the remaining half were planted as roots only. The hypothesis under investigation was that the growth of the intact plants would be checked for some time after transplanting, but those planted as roots only would quickly send up new shoots. It was anticipated that at the end of the growing season that the heights of the two sets of plants would not be different as the coppiced plants would grow more quickly and match the overall size of the intact plants.

5.2 Regeneration of Tissues from Root Material

5.2.1 Materials and methods

In the preliminary experiment to study the growth of tissue from root material, 33 roots stumps of *P. tomentosa* (Japan) and *P. tomentosa* (China) were used. The fresh weight of the stumps was measured before planting out, on 13.9.90, at the Matangi site. Fresh weights ranged from 4.7 g to 91.0 g, averaging 40 g. At weekly intervals for the following 12 weeks, 3 of the root stumps were harvested and examined for signs of initiation of new shoot and root tissues. The sampling of the three replicates was determined by the random placement of the root stumps at planting time.

The root material for the glasshouse studies of root propagation were harvested in mid August, 1991, from the nursery of Mr. Ian Barton, Hunua, South Auckland. The entire root systems of 12 *P. tomentosa* (Japan) plants were dug up. These plants were one year old, grown from root cuttings from the previous season. The fine roots were trimmed off the woody lateral roots and discarded. Each root system was composed of a main root stump with numerous lateral root branches, some of which had secondary branches. Only cuttings from the lateral roots and lowest parts of the root stump were used in the glasshouse trials.

The position of each cutting was recorded, *i.e.* the vertical distance from the root collar, the horizontal distance from the root collar, the primary orientation of the root branch it was taken from (*i.e.* vertical or horizontal with respect to the soil surface), as well as the length and diameter (at the midpoint) of the cutting. The cuttings were then planted (in 15 cm diameter pots) under the conditions described in Table 5.1, with the normal planting position being represented by the vertical treatment. As the primary objective of the experiment was to determine the effect of the position in the parent root system and size of the root cutting on new tissue development, the majority of the cuttings were planted vertically. For the other treatments it was important to use cuttings of similar size and root position to limit confounding effects, and this limited the number of replicates available.

The rate of emergence of shoots above the soil surface was recorded for the following two months. The plants were harvested in mid October, 1991, and the height of the tallest shoot was measured as well the position on the root cutting from which the shoot emerged. The extent of new root development was scored on a scale of 0 - 4, with 0 indicating that no new roots were produced while a score of 4 reflected extensive development of new fine roots.

Table 5.1. Planting position of root cuttings from field grown *P. tomentosa* roots.

Description of Position	No. of Reps
Vertical - normal planting position, with cutting upright in pot, below soil	79
Above - cutting upright but uppermost surface above soil	4
Inverted - cutting inverted	9
45° - cutting upright, but planted on 45° angle	9
45°, inverted - cutting inverted, planted on 45° angle	3
Horizontal - cutting planted horizontally	18
Dark - cutting planted vertically, whole pot kept in the dark	13
Dark, inverted - inverted cutting kept in dark	3
Deep - cuttings planted vertically very deeply in pots	14
Short - cuttings only 2 cm in length, planted vertically	12
Hormone, inverted - cuttings inverted, root hormone applied to proximal end	6

5.2.2 Results

5.2.2.1 Regeneration from roots growing in the field

Fine root development began in the first week after planting in the preliminary experiment with field grown roots. For the first five weeks after planting, small numbers of fine roots were the only visible signs of regeneration in these roots, but in the sixth week shoots from a number of the remaining roots were visible above ground. The three roots harvested at that time all had shoots beginning to emerge from the root. Root 16 had two shoots which were 2 cm long and emerged from the root at 2 cm below the upper surface. Root 17 had 5 shoots varying in length from 0.5 - 6.5 cm that arose from an area 0.5 - 2.5 cm down from the upper root surface. In the final root, the 9 shoots were up to 2 cm in length and emerged at depths of 0.5 - 5.5 cm on the root. In each of these roots, new roots of 2 - 4 cm in length were present, having emerged generally from the lower two thirds of the parent root length.

In later weeks, the extent of root development slowly increased so that towards the end of the experiment fine roots up to 8 cm in length were in evidence in some plants. These were orange in colour and sometimes branched, although smaller white roots were also found. The tallest shoot by this stage (11 weeks after planting) was 27 cm tall with 8 leaves. There appeared to be little correlation between fresh weight of the original planted root and the rate

or extent of regeneration of new tissue. New shoots emerged from the upper portion of the parent root, with new roots emerging slightly lower down, but the sites of root emergence were above those of shoot emergence in some plants. There was no obvious sign on the parent root as to where emergence of root or shoot material would occur. Shoot development seemed to occur at a faster rate than new root development which may be a reflection of the amount of nutrients and moisture stored in the parent roots. These roots were generally about 60% water. The dry weight:fresh weight ratio did not change significantly over the course of the experiment.

5.2.2.2 Effect of root cutting size and position on tissue regeneration

Planting the cuttings horizontally or on a 45° angle did not affect any of the regeneration variables measured. The depth of planting also had no effect on regeneration with deeply planted cuttings and the cuttings incompletely buried all showing similar rates of regrowth. Keeping the pots enclosed in black polythene bags to exclude light led to etiolated shoots which were taller than average. These shoots tended to emerge slightly more quickly than those in other treatments.

Inverting the cuttings increased the failure rate, regardless of whether the cutting was treated with rooting hormone or grown in the dark. In those inverted cuttings that did produce new root and shoot material, the root material was produced from the true basal end of the cutting (the lower end in the original root system) and shoot material from the proximal end (Fig. 5.1). The rate of shoot emergence was a little slower in the inverted shoots than in normally planted cuttings.

The position and orientation that a root cutting occupied in the original root system had no effect on the ability of that cutting to produce new shoot and root material when planted normally (Figs. 5.2b and 5.2c, Table 5.2). The diameter of the root cutting had little effect on regenerative capacity (Fig. 5.2a). However, the very thinnest cuttings (less than 3 - 5 mm) tended to produce less vigorous shoot regrowth, although the root scores and days to emergence were not affected. The length of the cutting had no effect when greater 5 cm, but the very short cuttings (2 cm) did not regenerate as well as longer cuttings. These short cuttings had a very high failure rate (when no regeneration occurred) of 67% and those shoots which did develop were shorter than those from other cuttings, being 6 cm tall on average. In all the cuttings planted vertically, the new shoots tended to arise within the upper 2 cm of the cutting. New root material was often produced from the lower surface of the cutting, but also arose from other sites on the cuttings and from the base of the new shoots in some instances.

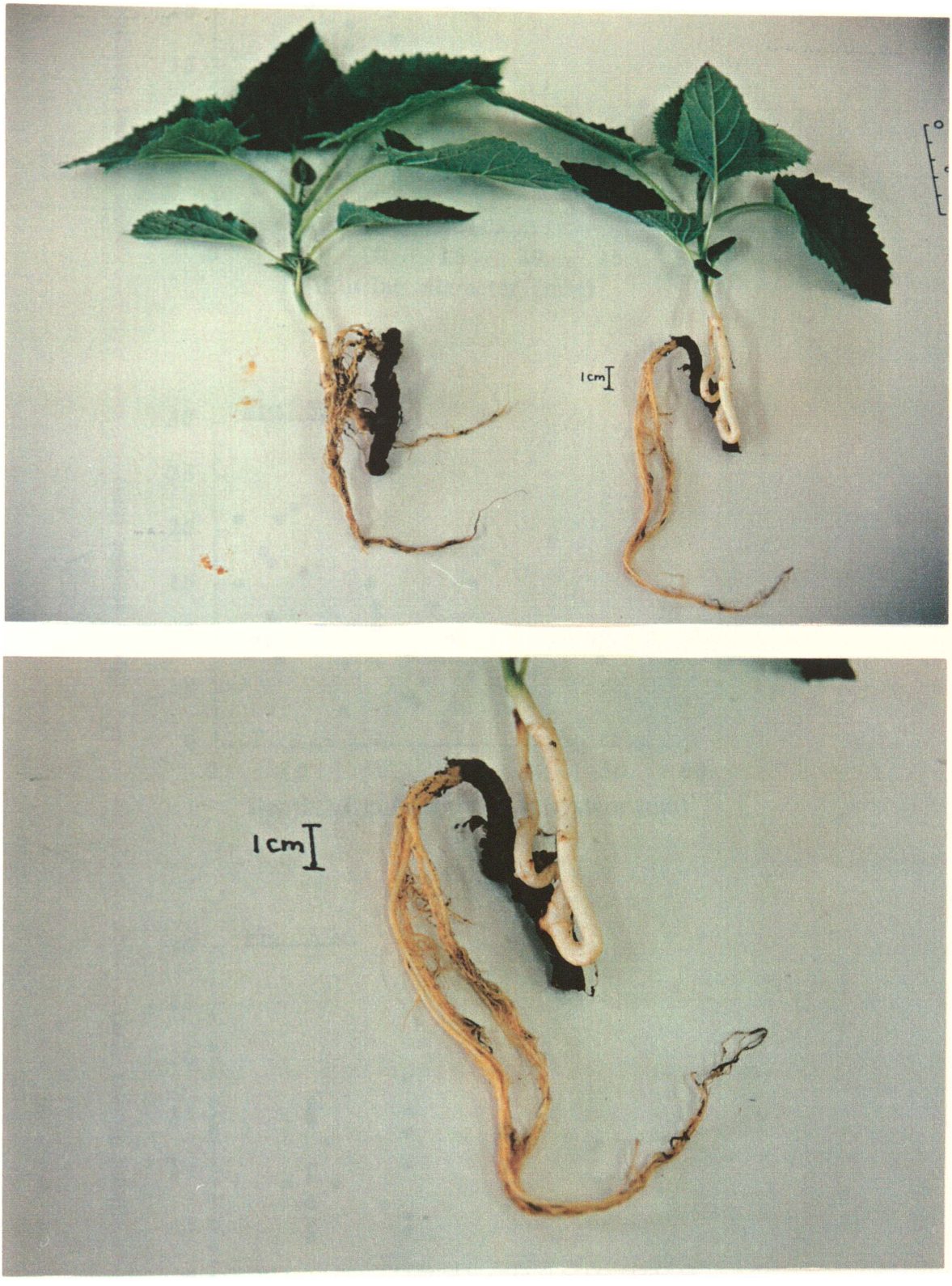
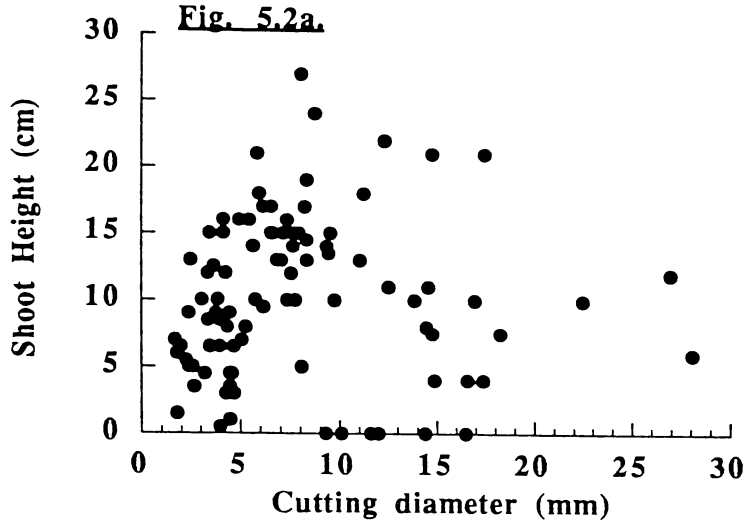
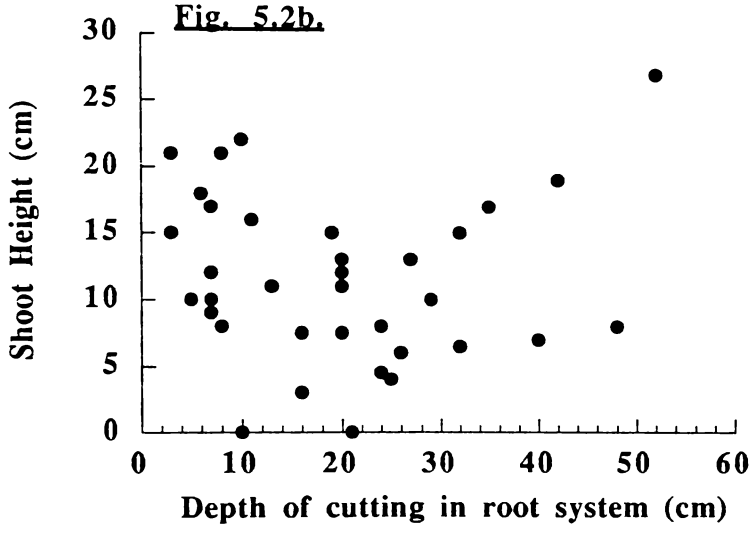


Fig. 5.1. Emergence of new root and shoot material from cuttings that were inverted at planting. Note that the shoot emerges from true proximal end of the cutting and the root from the true distal end of the cutting.





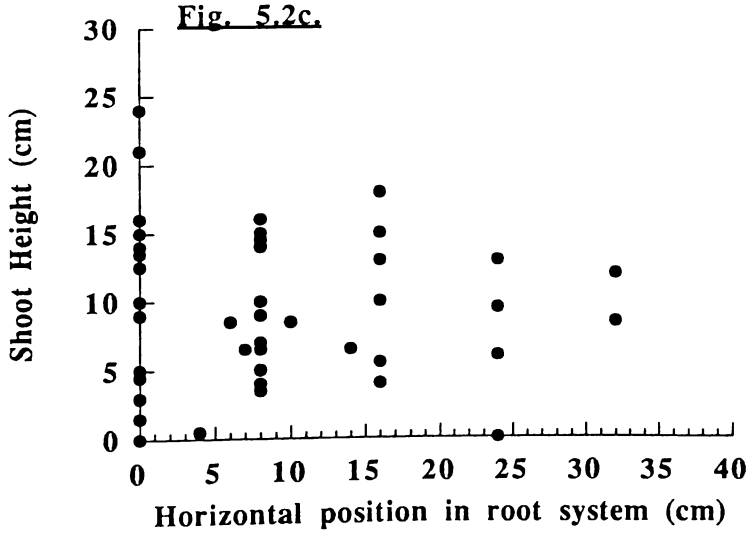
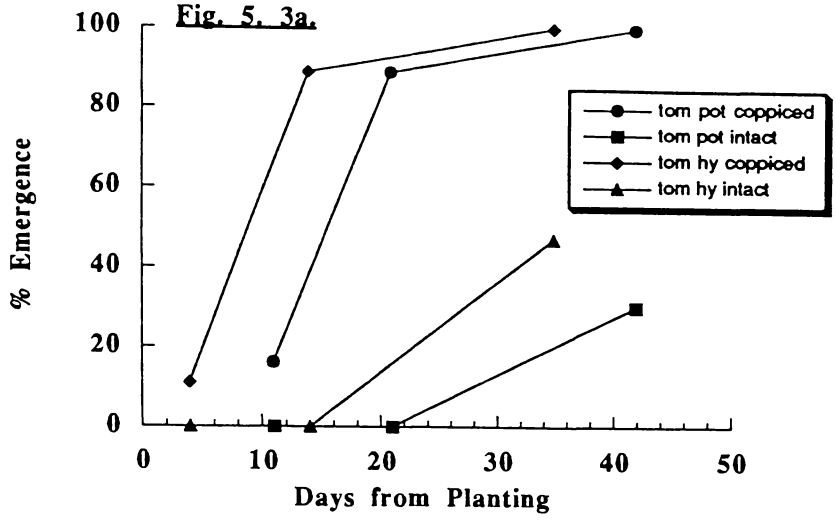
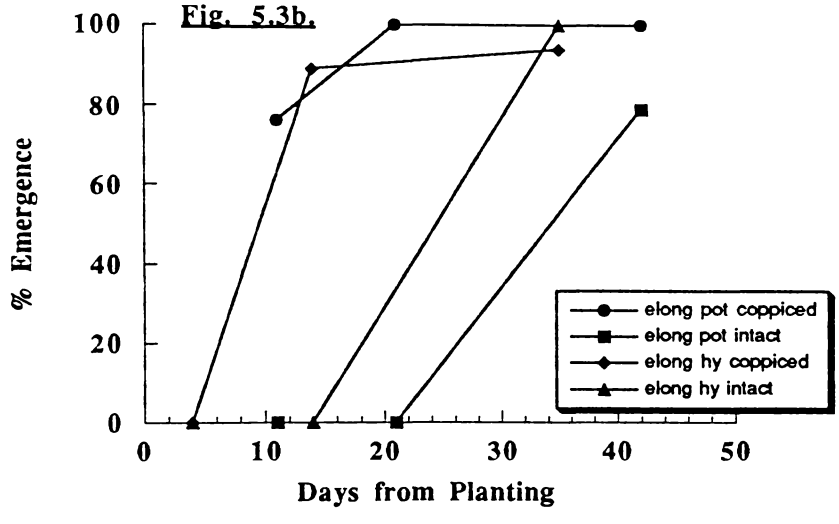


Fig. 5.2a,b,c. Height of new shoot from root cutting as a function of a. cutting diameter; b. depth of root cutting in original root system; c. horizontal distance from root collar in original root system.





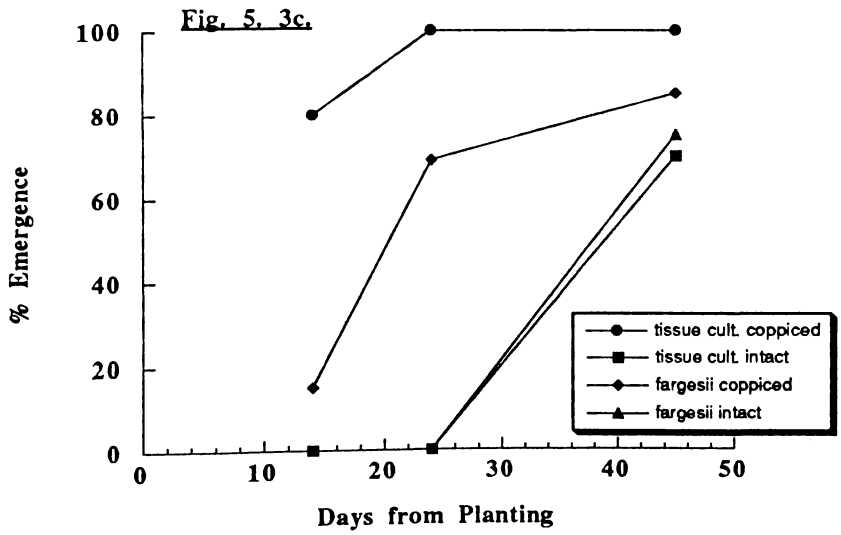


Fig 5.3a, b, c. Emergence of new growth in plants of a. P. tomentosa; b. P. elongata; c. P. fargesii and tissue cultured P. elongata grown either in pots or in hydroponics (hy), planted as intact plants or coppiced.

Table 5.2. Effect of original position of root cutting in parent root system, with respect to root collar, on the capacity of the cutting to regenerate new root and shoot tissues.

Distance from Collar (cm)	Cutting Diameter (mm)	Number of Shoots	Shoot Height (cm)	Days to Emergence	Root Score	Failure Rate (%)
Vertical						
0 - 10	7.3	2	13	23	3	18
11 - 30	7.2	2	10	23	4	0
30+	7.8	2	14	21	4	0
Horizontal						
0 - 5	6.2	1	9	24	3	6
6 - 15	5.1	1	9	22	3	33
16 - 25	8.5	2	8	20	3	14
25+	4.4	1	10	20	3	33

5.3 Growth of Seedlings in Hydroponics

5.3.1 Materials and methods

Seeds of *P. tomentosa*, *P. fortunei* (white flowering), *P. fortunei* (purple flowering), *P. fargesii* and *P. elongata* (creche tree) were germinated in vermiculite, in seedling trays, in late August, 1991. When the plants had 2 pairs of true leaves they were transplanted into plastic pots (8 cm high, 4.5 cm wide) filled with vermiculite and placed in the hydroponics system. The base of each pot was sufficiently open as to allow the growth of roots through the base and into the hydroponics channel.

At each nutrient level, five channels of hollow polyvinyl chloride (PVC) piping were used. These channels were rectangular, with eleven holes drilled in the top surface, at 25 cm intervals, allowing the pots to be placed into the system. The flow of nutrient solution was maintained by a Terada SL 100 submersible pump, which pumped the solution from the reservoir to the top of each channel. As the channels were elevated at this end to give a slope of 1 in 10, the solution flowed under gravity down the channels, over the plant roots, to the bottom of the system where it drained back into the reservoir tank. Conductivity and pH probes tested the incoming solution and automatically replenished it with fresh nutrient solution if necessary (Care, 1991). It was important that, while the roots received appropriate nutrient levels in the solution, they were also well aerated. To achieve this, the depth of the solution was manipulated so that the upper surface of the root mat remained in the air, but was kept moist by a film of nutrient solution (Cooper, 1979).

This system did result in a gradient of nutrient levels down the channels. To overcome this, each channel was treated as a replicate and the positioning of the plants of the different species in the channels was predetermined randomly. The first and last plants in each channel acted as buffer plants and were not included in the analysis, so only nine plants per channel (45 per nutrient level) were measured in the final analysis of growth. The sets of five channels used in each nutrient treatment were adjacent to one another in the glasshouse, so the other environmental factors (*e.g.* light, air temperature) were the same for each treatment.

Nutrient solutions were made to the required strength by manipulating the quantities of stock solutions being fed into the reservoir tank. The two stock solutions (A and B) were produced by dissolving the raw chemicals in the appropriate amount of warm water according to the recipe from the supplier (Bio - Tissue Products, Hamilton, New Zealand). These stock solutions were then mixed and diluted with deionised water in the reservoir tank to give the final solution at each nutrient level. The levels of the nutrients in the solution at CF 30 were

N - 131 ppm, P - 92 ppm, K - 435 ppm, Ca - 252 ppm, Mg - 76 ppm, S - 94 ppm, Na - 3 ppm, Mn - 2.58 ppm, B - 0.54 ppm, Fe - 0.22 ppm, Cu - 0.18 and Zn - 0.15 ppm. In the CF 15 solutions these concentrations were halved.

One set of plants were grown at CF 15 (1.5 mS cm^{-1}) and the second at CF 30 (3.0 mS cm^{-1}), both at pH 6 and a flow rate of 2 l min^{-1}. The plants were grown at glasshouse temperatures with a 16 hour day, with the natural photoperiod extended by the use of mercury vapour lamps. After two months the plants were harvested and the following variables measured -

shoot fresh weight and dry weight
shoot length
leaf area
collar diameter
root fresh weight and dry weight
length of the longest root.

5.3.2 Results

At the end of the experiment, there was wide variability in the health and vigour of individual plants at both nutrient levels, as can be seen by the raw data presented in Appendix 7. In order to test whether there were any significant differences between the means of each growth parameter of the five species types at two nutrient levels, the data was processed by SuperANOVA (Abacus Concepts, Inc., 1989). This programme employed analysis of variance (ANOVA) procedures followed by the Scheffe's S test. Because the effect of nutrient level for each growth parameter was significant at the 0.1% level, with the plants growing more vigorously at CF 30 than at CF 15, the data at each nutrient level were analysed separately.

At the lower nutrient level, the greatest overall growth was achieved by the purple fortuneii seedlings, followed by the fargesii and white fortuneii seedlings, while the tomentosa and elongata plants did not grow well at all (Table 5.3). However, at the higher nutrient level, the white fortuneii seedlings grew better than the others on average, especially with respect to collar diameter and shoot length. The remaining species did not show a clear trend in growth performance, with the purple fortuneii plants, for example, producing comparatively tall shoots on average, but recording the smallest leaf area and stem weight means at CF 30.

For every growth parameter measured, except root length, the lowest mean value recorded

Table 5.3. Growth parameters of *Paulownia* seedlings grown in hydroponics at two different nutrient strengths.

Species	Reps	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)	Collar Diam. (mm)	Shoot Length (cm)	Root Length (cm)	Leaf Area (m^2)	Root:Shoot Ratio
CF 15										
tomentosa	5	4.4	0.8	3.4	0.3	3.4	5.6b[1]	18.8b	0.021	0.38
purple fort.	6	15.7	2.3	9.6	0.8	4.4	17.8a	59.5a	0.066	0.35
white fort.	9	10.6	1.5	6.5	0.6	3.9	11.3ab	29.0b	0.046	0.40
fargesii	7	10.7	1.6	5.8	0.5	3.6	10.9ab	35.6b	0.044	0.31
elongata	8	4.0	0.6	3.1	0.2	3.7	8.0b	21.2b	0.021	0.33
CF 30										
tomentosa	9	59.3	9.4	44.2	3.7	8.1ab	20.3b	52.4	0.266	0.39
purple fort.	7	36.8	6.3	19.6	1.6	5.7b	29.4ab	58.3	0.146	0.25
white fort.	8	84.0	15.5	42.8	4.1	9.8a	40.4a	66.7	0.322	0.26
fargesii	8	44.1	7.0	27.2	2.1	6.6b	24.4ab	56.4	0.148	0.30
elongata	7	64.4	11.9	37.3	2.8	6.7b	28.1ab	49.9	0.217	0.24

[1]Differences between means in the same column, at each nutrient level, are significant at the 5% level if followed by a different letter. There is no difference between means in the remaining columns.

under the high nutrient conditions was still higher than the highest recorded at CF 15. The health of the plants at CF 30 was far superior to that at CF 15. With respect to root length, it appears that the maximum length possible in the nutrient film technique system used here is between 60 and 70 cm. The distance between pots in the system is only 25 cm so any roots longer than that become entangled in those of the next plant which probably affects the maximum length attainable. However, it is obvious that although the maximum length of root was not any greater at the higher nutrient level, the number of roots increased compared with CF 15 as both the fresh and dry weights indicate. The investment by the plant in root material as a proportion of the whole plant was higher for both types of fortuneii seedlings and elongata at CF 15 compared with CF 30 as shown by the root/shoot ratio. With the two remaining species, there was no difference between the two nutrient levels in their root/shoot ratios.

5.4 Transplanting Experiment

5.4.1 Materials and methods

Seeds from the *P. elongata* tree in the grounds of the University of Waikato creche (section 2.2) and from a *P. tomentosa* tree growing on the property of Mr. I. Barton (Hunua, South Auckland) were sown onto potting mix and covered with a light layer of vermiculite, on 10 July, 1991. They were germinated in the glasshouse. Three weeks later 50 seedlings of each species were pricked out into small pots filled with potting mix, while 55 seedlings of each species were pricked out into hydroponics pots. These pots were half filled with vermiculite and topped up with potting mix. At the time of pricking out, the seedlings generally had one pair of true leaves. Despite being regularly watered with nutrient solution, the seedlings in the hydroponics pots did not remain healthy, so a second batch of seedlings were pricked out later and used subsequently.

Two months after sowing (11 September, 1991) the seedlings growing in potting mix were potted up into 14 cm pots. Those in the hydroponics pots were placed in the hydroponics system at that time at a nutrient level of CF 30 and pH 6 - 6.2. Of the potted seedlings, 42 of each species were transplanted into planter bags (size pb 8) on 13 November, 1991. The *P. tomentosa* seedlings transplanted were generally smaller than the *P. elongata* plants, with darker green leaves and quite woody root systems. A number of the *P. tomentosa* plants had stopped growing, with dormant apices, while the *P. elongata* plants were still actively growing.

The hydroponically grown plants were potted up into planter bags of the same size, filled with potting mix, on 18 November, 1991. A number of the larger plants suffered root loss as they were being removed from the hydroponics due the large size of their root mass and this caused some temporary transplant shock. A lesser number of the *P. tomentosa* plants had ceased growth in the hydroponics compared with the potted seedlings. Some had quite woody root systems. Both groups of plants were left in the glasshouse, after potting up, for a further three weeks before being shifted to the shade house to harden off. The potted seedlings were transplanted into the field on 9 December, 1991, after one week in the shade house. The hydroponic seedlings were transplanted a week later, after also spending one week in the shade house.

At the time of planting at the Matangi experimental site (section 4.2.1), each group of seedlings was divided in two with one set being planted intact, while the stems of the second set were cut off and discarded, so only the root systems were planted out. The lengths of the

discarded stems were measured. A number of other seedlings that had been raised in pots in the glasshouse were planted out at the same time. These included tissue cultured *P. elongata* (obtained from K. Avery, New Plymouth) and *P. fargesii* all grown from Chinese sourced seed. These plants were also either planted intact or as roots only. However, many of these plants had been growing for some time in pots without frequent repotting and so had been pot-bound and ceased stem growth.

The emergence of new shoots and the general health and vigour of the plants were scored at various intervals over the growing season. At the end of the season, on 9 April, 1992, the height and diameter of each plant was measured.

5.4.2 Results

The overall growth rates of the *Paulownia* seedlings in this experiment were low with *P. tomentosa* seedlings from all treatments, for example, averaging only 30 cm in height at the end of the season (Table 5.4). Analysis of variance was undertaken, as described above, to compare the treatments within each species. No significant difference was found in the height or diameter of *P. tomentosa* seedlings at the end of the season as a result of initial growing system (pot versus hydroponics) or as a result of planting method (intact versus coppiced). Thus those seedlings which had been coppiced were able to reach the same height as those planted intact, primarily because the intact seedlings grew very little, if at all, after planting. The difference in number of actively growing plants at the end of the season reflected the treatment at the time of transplanting. All of those plants which were coppiced regrew, while only a small number of the intact seedlings from both the pot grown and hydroponically grown sets of plants showed any signs of new growth after transplanting.

The situation was different with the *P. elongata* seedlings, with those pot grown seedlings that were planted intact being significantly taller than those coppiced at the time of planting. This was primarily a result of the height of the intact plants at the time of planting as the average increase in height was only 5 cm over the season and only a third of these plants showed active growth. In comparison, all of the coppiced seedlings grew. The diameters of the two sets of seedlings were not different at the end of the season. For the seedlings grown in hydroponics, both the final average height and diameter were significantly greater on the intact plants than the coppiced plants. However, the increase in height through the season was the same for both transplanting treatments and there was no difference between the percentage of actively growing plants as a result of treatment at transplanting time.

The growing technique also had an influence on the sizes of these plants. At the time of

Table 5.4. Comparison of growth parameters of *P. tomentosa* and *P. elongata* seedlings at the time of transplanting and at the end of the season. Seedlings were initially grown in either pots or hydroponics, and transplanted intact or coppiced.

	P. tomentosa				*P. elongata*			
Growth Medium	Initial Ht (cm)	Final Ht (cm)	Final Diam. (mm)	% Active[1]	Initial Ht (cm)	Final Ht (cm)	Final Diam. (mm)	% Active
Pot grown								
intact	22	25a[2]	10a	25	56	61a	16a	32
coppiced	0	32a	12a	100	0	42b	13a	100
Hydroponics								
intact	24	31a	11a	35	27	52a	18a	100
coppiced	0	32a	11a	100	0	24b	8b	89

[1] % active refers to the percentage of plants actively growing in the latter part of the season

[2] Pairs of numbers in a column within each growing treatment are not significantly different from one another at the 5% level (by Scheffe's S test) if followed by the same letter.

transplanting the pot grown seedlings were taller than those grown in hydroponics. This effect was not seen at the end of the season in the intact plants with the differences in the heights and diameters of the seedlings as a result of different growing technique not significant at the 5% level. However, in those seedlings which had been coppiced the pot grown plants were significantly larger.

The effect of transplanting treatment on the final height and diameter of the pot grown tissue-cultured *P. elongata* seedlings was not significant, but fewer of the intact plants showed active growth in the field than the coppiced plants (74% compared with 100%, respectively). The rate of new growth from the coppiced *P. fargesii* plants was very slow (final height of 21 cm), probably as a result of the severity of the pot-bound nature of the roots. These roots had ceased growth at the time of planting and so it took them some time to respond to the new soil environment. As a result of this, the difference between the height and diameter of the intact and coppiced plants was significant, with the intact plants being larger. There was no difference between treatments in the proportion of plants actively growing.

Comparison of the rates of emergence of new growth from both intact and coppiced plants showed that new growth emerged more rapidly on the coppiced plants (Fig. 5.3). At 5 - 6

weeks after planting, there was little difference in the proportion of plants showing new growth as a result of transplanting treatment in any of the *P. elongata* or *P. fargesii* seedlings. In *P. tomentosa*, however, there was a lower proportion of those plants left intact showing signs of new growth than in coppiced plants. The system used to initially grow the seedlings had no effect on rates of emergence of new growth across the species tested. At the same time as emergence of new growth was assessed, the overall health and vigour of the plants were scored. At each time of scoring, in all species across all treatments, except *P. elongata* grown in hydroponics, the coppiced plants were healthier and more vigorous than those planted intact. In the *P. tomentosa* plants there was no difference due to initial growing treatment.

5.5 Discussion

5.5.1 Propagating from root cuttings

The development of new shoot and root material in the roots planted in the field followed the same pattern as that in glasshouse grown roots of *P. tomentosa* studied by Beckjord (1984), with shoot growth occurring more rapidly than new root growth. The rate of new tissue production was higher in the glasshouse grown roots in his study than in the roots grown at Matangi, probably because the temperatures were higher and watering was more frequent.

The size of the roots planted in the field had no discernable effect on regeneration capacity, as was found to be the case with the majority of the root cuttings studied in the second experiment (Table 5.2, Fig. 5.2a). However, very short and very thin cuttings did not produce vigorous new growth and, in many cases, did not regenerate at all. The effect of cutting size on capacity to regenerate tissue is probably related to the nutrient and carbohydrate resources and phytohormone levels within the cutting. The cuttings used in this experiment were smaller than would normally be used in a commercial situation. Working with cuttings of *P. taiwaniana* ranging in length from 5 cm to 15 cm, and in diameter from 5 mm to 34 mm, Rin (1975) found that the largest cuttings (15 cm long, 25 - 34 mm in diameter) produced the most vigorous growth. The average height of the stems after one years growth from these cuttings was approximately eight times greater than stems produced from cuttings that were 5 cm long and 5 - 9 mm in diameter. These large cuttings were similar in size to cuttings supplied commercially in New Zealand.

In *P. tomentosa*, cuttings taken from any part of the root system are capable of regenerating new tissues. This implies that the root material throughout the entire root system is physiologically similar. For the commercial grower of *P. tomentosa*, this means that all parts of the root system can be used to propagate the tree as long as the cuttings are large enough. In discussing regeneration from roots of rosaceous plants, Robinson (1975) observed that the position in the root system from which a cutting was taken had less effect on regenerative capacity than the age of the whole parent root system. Thus the roots of young trees were able to regrow more readily than roots from old trees. The new tissue grown from root cuttings is juvenile in nature, and can itself be used for propagating. This is exploited in the commercial production of *Paulownia*, with root cuttings being planted in one season to produce root material for sale in the following season. As a result of this process, the root material available for use in the experiments reported here was all from juvenile plants and the effect of age of parent root stock on regeneration capacity was not studied.
Root cuttings in a number of species are traditionally planted horizontally, rather than

vertically. In roses, for example, Marston (1973) found higher rates of regeneration in horizontally placed cuttings than vertically planted cuttings. However, fewer saltcedar roots placed horizontally produced shoots than in vertically placed cuttings (Wilkinson, 1966). In many plants orientation does not affect regrowth and this appears to be the case with *P. tomentosa* . Those cuttings placed horizontally, on a 45° angle or vertically showed no difference in subsequent root and shoot growth. Raising the upper part of the cutting also had no effect on growth, but Robinson (1975) reports that incomplete burial of cuttings is essential for regrowth in a number of plants.

In all the *P.tomentosa* cuttings studied, regardless of the treatment applied, shoots arose at the proximal end of the cutting, generally within 2 cm of the cut surface. Root emergence occurred along a greater length of the cutting but was restricted to the more basal region. Inversion did not change the polarity of the cuttings, and the application of rooting hormone to the proximal surface did not stimulate root production in the inverted cuttings. This strong polarity is common in both stem and root cuttings in many plants.

That auxin levels are involved in the regulation of polarity has been discussed above (section 5.1.2), but it is likely that other factors are also important and relate to the plants response to gravity. An explanation of polarity must take into account the fact that when an intact root (or shoot) is cut sequentially, the two surfaces that were originally joined in the intact root are now capable of producing either root tissue or shoot tissue, depending on their relative positions in the new cutting. The influence of any gradient within the new cutting must be the result of relative amounts rather than absolute amounts of the relevant factor. It is likely that polarity is regulated at a cellular level. It has been suggested that the micro-structures of the cells walls are aligned in such a way as to sense orientation (Bleasdale, 1984), while the fact that a cell maintains an electrical polarity forms the basis of a second theory (O. Lange, pers. comm.). In both cases the cell acts in a similar manner as a bar magnet, which can be divided into smaller pieces but will always maintain its polarity.

It would be possible to test these theories, but an attempt to reverse the electrical gradient in *Paulownia* roots using an electrophoresis system did not succeed. The cuttings failed, probably because they were too small. The electrophoresis system used was only able to handle very small cuttings. Working with stem cuttings of grapevines, Cristoferi *et al.* (1988) found that centrifuging the cuttings in an inverted position, then inverting the cuttings upon planting did not affect the rooting pattern of the cuttings, when compared with uncentrifuged, inverted cuttings. It may be possible to influence polarity if plants from which cuttings were to be taken were grown on a slowly rotating platform in such a way that they were unable to respond to gravity.

In the *Paulownia* roots studied, the polarity effect appeared to operate on the longitudinal axis only. In horizontally planted cuttings, shoots arose from all positions around the proximal end of the cutting. This result is the same as that found in apples where transverse polarity did not exist, despite a strong longitudinal polarity (Robinson and Schwabe, 1977).

5.5.2 Nutrient concentrations and growth of *Paulownia* seedlings

The results of this preliminary experiment examining the effect of nutrient concentration on the growth of *Paulownia* seedlings in the NFT system of hydroponics indicate that *Paulownia* plants need a relatively high nutrient level to grow well. Those plants grown at CF 30 were significantly more vigorous than those grown at CF 15. It would be interesting to experiment further with a wider range of nutrient concentrations to study growth at even higher nutrient regimes. Other plants commonly grown in the NFT system include lettuces grown at between CF 12 - CF 25 (Care, 1991) and tomatoes at CF 20 (M. Auger, pers. comm.). Cooper (1979) recommends a minimum CF value of 20 to be used with all crops in NFT. However the nutrient levels used for kiwifruit grown in aerated pots can be as high as CF 80 (8 mS cm^{-1}), which is higher than nutrient levels used for other woody perennials such as tamarillo and avocado in the same system (S. Miller, pers. comm.).

The biomass accumulated by the seedlings under the high nutrient regime was 6 - 7 times greater than that produced with low nutrients. With respect to stem biomass, the major contribution to this increase was made by the leaves, with leaf area being 5 times greater in the high nutrient treatment, while stem length and collar diameter were only twice as great. The difference in biomass between the two treatments is a direct reflection of the nutrients available to the plants as every other variable was the same for both treatments. Thus the seedlings responded to the higher nutrient availability by increasing their growth rates.

That *Paulownia* seedlings grow well in hydroponics systems with optimum nutrient concentrations has been demonstrated by Jia and Ingestad (1984). Using a system whereby the plant roots were hung in the air and sprayed with the circulating nutrient solution, they recorded relative growth rates of over 25% day^{-1}. They also determined that the optimum ratio (by weight) of macronutrients in the circulating solution was 100 N: 75 K: 20 P: 8 Ca: 9 Mg at a relative addition rate of 27% day^{-1}. However the actual concentration of nutrients was lower in their experimental system (from CF 0.5 - CF 5). In comparison, *Populus* reached a relative growth rate of 19% day^{-1} with an optimum nutrient ratio of 100 N: 70 K: 14 P: 7 Ca: 7 Mg (Jia and Ingestad, 1984), while the optimum ratio for *Betula* was 100 N: 65 K: 13 P and for *Alnus* was 100 N: 50 K: 18 P (Ingestad, 1981). The relative growth rate of *Betula* was similar to that of *Paulownia*, while that of *Alnus* was more comparable with

Populus (Ingestad, 1981). The rapid growth rate of *Paulownia* found by these workers was reflected by the optimum ratio of nutrients with the demand for P, K, Ca and Mg being highest in *Paulownia* compared with the other three species. These results indicate that it may be the ratio of nutrients in the circulating solution, rather than the total concentration, that determines the rate of growth for hardwood seedlings in hydroponics systems. The results of the experiment reported here for *Paulownia* growing at CF 15 indicate that indeed there was some nutrient limitation on growth. The fact that in three of the species types the root/shoot ratio was higher at CF 15 than at CF 30 suggests that nitrogen may have been limiting at the lower nutrient level.

The relationship between the fresh weight and dry weight of roots and of shoots was reasonably constant between species types and nutrient levels. The dry weight of roots averaged 8% - 9% of the fresh weight. Similar ratios of dry weight to fresh weight were recorded for roots of alder and birch grown in a root misting system (Ingestad, 1981). The roots of many plants grown in hydroponics systems tend to be high in water content as the role of the root is primarily water and nutrient uptake. The support offered by the structure of the hydroponics system delays the development of woody roots and after two months in hydroponics, no lignification of *Paulownia* roots was observed. In comparison, lignification of the roots of pot grown *Paulownia* is already occurring after two months. With respect to the water content of the shoot, this ranged from 82% to 86%, with the dry weight/fresh weight ratio averaging 16%. Most of the shoot development was herbaceous, but in some plants the lower part of the stem was starting to become woody after two months.

There remains much to be discovered about the nutrition of *Paulownia spp.* Various hydroponics systems, including NFT, aerated pots and even sand culture can be used to study the effect of nutrient levels, ratios of different nutrients in solution and the effects of deficiency or toxicity of individual nutrients on the growth of seedlings. Zinc toxicity in beans, soybeans and corn growing in aerated pots has been investigated (Rauser, 1973), while Smith, Clark and Holland (1987) have quantified the requirement of kiwifruit for chlorine by growing plants in aerated pots and have also studied the interaction between chlorine levels, light and nitrogen availability on kiwifruit growth (Smith *et al.*, 1988). Similar experiments need to be done with *Paulownia* and then tied in with field trials of fertilisers, as has been done for kiwifruit (S. Miller, pers. comm.). That many growers have noticed an increased growth of *Paulownia* upon the application of a variety of fertilisers on a range of soils suggests that these trees will respond well to the application of the correct nutrients at the right time. A study by Beckjord and McIntosh (1983) on the effect of fertilizers on the growth of pot-raised seedlings of *P. tomentosa* transplanted into the field (a sandy loam soil, pH 5.9) led to a recommendation of the application of 800 lb $acre^{-1}$ (approximately 900 kg

ha^{-1}) of 25:10:10 (N:P:K) fertilizer at planting.

5.5.3 Transplanting of *Paulownia* seedlings

The coppicing of *Paulownia* plants prior to transplanting, as recommended by Graves and Stringer (1989), did generally lead to the production of more vigorous and healthy growth of new shoot material. In *P. tomentosa* in particular, the fact that there was no difference in final stem height or diameter between any of the treatments indicated that the growth of the coppiced shoots during the four months in the field was equivalent to the total stem growth of intact seedlings over nine months. From this result it can be recommended that *P. tomentosa* plants be coppiced just prior to planting out and that the loss in stem height from such a treatment will be regained during the season.

The higher rates of stem growth of pot grown *P. elongata* after coppicing were not sufficient to match the overall growth of the intact plants, but this may have been a result of the short growing season available to the coppiced plants. The tissue cultured plants showed no difference in height and diameter between the intact and coppiced plants at the end of the season. Thus it is possible to make the same recommendation for *P. elongata* as for *P. tomentosa*, that coppicing prior to planting will result in the growth of a more vigorous shoot than transplanting intact plants.

These results are similar to those found by other workers studying *Paulownia*. Coppicing seedlings of *P. tomentosa* after one season of growth led to a four fold increase in stem height and total plant weight in the second season of growth, regardless of fertiliser treatment (Beckjord and McIntosh, 1983). This was probably a result of the increased size of the root system in the second year which was able to sustain higher growth rates. The sizes of the plants in this experiment were small, with stem height averaging 12 cm at the end of season 1 and 50 cm at the end of season 2.

The reasons as to why coppiced plants grow more rapidly and why intact plants suffer from transplant shock are not fully understood. Working with *Eucalyptus camaldulensis* seedlings, Blake (1980) found that the rate of stem elongation (absolute growth rate) was three times higher in coppiced plants than in intact plants, with there being no difference in height between the treatments three months after coppicing. There was no difference in leaf water potential between treatments, but the number of stomata, the stomatal conductance and transpiration rates were all higher in the leaves of the new shoots that arose after coppicing, partly due to their more juvenile nature. The changes that result from coppicing such as an altered root:shoot ratio, loss of apical dominance and reduction in growth inhibiting

substances have been suggested as reasons for the increased growth rate of shoots (Blake, 1980).

The lack of continuing growth in the intact plants in the research described here is a reflection of the susceptibility of this plant to transplant shock. Although some of the pot grown *P. tomentosa* and *P. fargesii* plants had ceased growth when planted out which may explain their lack of stem increase, most of the hydroponically grown *P. tomentosa* and all the *P. elongata* seedlings still had active apices at the time of transplanting. The hydroponically grown *P. elongata* plants were the only set of plants to show no signs of transplant shock, which may have been a result of the different nature of the root mass to those produced by pot grown plants.

It is important when transplanting cuttings or seedlings from any species that there is minimal disturbance of the roots and that the plant is quickly able to produce new roots in its new soil environment. Investigations into the development of new roots in stem cuttings of coniferous species have led to the description of the parameter - Root Growth Capacity (RGC) (Sutton, 1990). This is a measure of the ability of a root system to extend existing roots and to initiate new roots, particularly after transplanting (Sutton, 1990). However, although this parameter has been extensively quantified in a number of species, there is still no complete explanation of the principle factors that influence the value of RGC. It is not possible to determine the rooting potential of a plant by observation of external features, but it is known that treatment during handling of the plant does affect rooting at planting time as does the physiological condition of the plant at the time of lifting (Sutton, 1990).

In deciduous hardwood species, the success of transplanting has been linked to cuttings having a fibrous root system with highly branched laterals (Struve, 1990). Fibrous root systems tend to have intact roots tips which can elongate rapidly upon transplanting. This may partly explain the result for the hydroponically grown *P. elongata* seedlings as the root systems were composed of numerous fleshy (non-woody) roots, whereas the roots of many of the *P.tomentosa* seedlings in the hydroponics had begun to visibly lignify at the time of potting up. Thus the use of hydroponics to raise seedlings is only advantageous if the roots remain non-woody until the plants are transplanted.

The low overall growth rates for the *Paulownia* plants after transplanting may have resulted from the change in environmental parameters. Both the pot grown and hydroponically grown plants were well watered during growth in the glasshouse, with the pots being watered on a daily basis. At the time of planting out (early to mid December, 1991) the soil moisture levels were lower than at any other time in that season (Table 4.2). Although the plants were watered in at the time of planting, it is possible that the irregular watering from manual

irrigation and from rainfall delayed root establishment in the field. The late planting of the trial also affected the final size of the coppiced shoots. It is more usual to plant *Paulownia* roots out in late August or early September. By planting two months later, the time available for the coppiced roots to match the stem heights of the intact plants was reduced.

Chapter 6
Discussion and Conclusions

The aim of this study was to gain better understanding of the functioning of *Paulownia* by quantifying aspects of growth and gas exchange. It was considered important to know how various environmental factors would influence the plant so an appropriate management strategy could be utilised to grow the tree successfully as a timber crop or in agroforestry situations.

6.1 The Dynamics of Form and Function in *Paulownia*

The analysis of the form of a plant in terms of the costs and benefits to the overall functioning of the plant has been applied to the characteristics of sun and shade plants by Givnish (1988). In terms of allocation of biomass and resources, *Paulownia* shows many of the typical traits of an obligate sun plant that is an effective coloniser of disturbed, open habitats. The maximal gas exchange rates and $PPFD_{sat}$ for photosynthesis are high in comparison with other woody, deciduous species. The growth rate is also high and there are several features that represent effective strategies to enhance success in both establishment and later growth.

The first of these is the continued abscission of leaves throughout the season, on both mature and juvenile trees. This ongoing senescence process means that, when a leaf becomes shaded by younger leaves to the extent that photosynthesis is severely restricted, the leaf abscises. Prior to abscission, there is a limited withdrawal of nutrients from the leaves (section 4.6.3) but, when abscised, the leaves still contain significant amounts of nutrients, which can be recycled through the soil system. This continued production and loss of leaves throughout the season leads to a very open canopy, with light levels beneath the tree sufficient for the growth of many other plants (section 2.4.1.1).

A second feature of the growth of *Paulownia* that influences light interception is the lack of branching in the first season of growth. Under good growing conditions in the glasshouse, *P. tomentosa* (Japan) was able to grow 3 m in height in four months (Chapter 3), although the tallest tree in the field at the end of season 1 was only 1.5 m. The plant appears to allocate biomass to height growth to guarantee access to light, rather than allocating resources to branch production. To overcome this lack of lateral spread, large leaves (typically 0.05 - 0.2 m^2) are produced with very long petioles (more than 0.5 m in some instances), so that the

canopy spreads about a metre from the trunk. This is an effective first season strategy since a large photosynthetic canopy is established with good carbon budget effects. The cost to the plant, in terms of biomass and resources, is minimized because large leaves with long petioles are produced rather than woody branches.

Another significant feature of the growth of *Paulownia*, particularly in its first season, is the use of hollow cylinders for organs with support roles. The stem is hollow, but this is a feature of woody tissue only in its first year of growth (branches are also hollow when produced). As the tree ages the diameter of this hole remains constant so that its proportion, in relation to the entire stem diameter, diminishes. The petioles are also hollow on young trees. On the mature *P. elongata* tree the majority of petioles were solid, but some were hollow. This employment of hollow structures allows the plant to grow taller and to spread out laterally at a lower cost in terms of biomass than if the tissue were solid, without losing a significant amount of strength. Also wood that is produced has a very low density, so any comparison of the rate of increase in plant height with other fast growing species such as eucalypts must take this in account.

Because of the continual abscission of leaves throughout the season the load borne by the branches alters little through the season. By losing leaves that are no longer productive, the branch carries the minimum load and decreases the likelihood of damage in windy conditions. Branches, therefore, can be relatively thin in comparison with those of other trees.

Givnish (1988) also draws attention to the high rates of biomass partitioning to leaves, roots and reproductive structures in sun plants. A large investment in root material is necessary to support leaves that are expensive to produce in relation to nutrient levels. Sun leaves tend to have higher levels of the various components of the photosynthetic machinery than shade leaves, in order to sustain higher rates of photosynthesis (section 2.1.3), but this comes at a cost. The foliar concentrations of nitrogen, for example, in *Paulownia* are reasonably high in comparison with other non-leguminous species (Table 4.27). The second aspect of the high rates of photosynthesis in sun leaves is the concomitant rates of water loss through transpiration. This is a significant feature in *Paulownia* leaves which often have high rates of transpiration. Thus a large root system is required by these plants to meet the demands from the leaves for both nutrients and water. Indeed, the investment by *Paulownia* in perennial tissues, even after three years of growth, shows a greater amount of biomass in root material than shoot material (Table 4.9).

6.2 Interaction of Gas Exchange, Growth and Climatic Variables

6.2.1 Air temperature

The optimum mean daily temperature for stem growth of *Paulownia* in China is between 24°C and 29°C (Zhu *et al.*, 1986). The longer the period that temperatures remain in this range, the higher the growth rate of the trees. Over the three seasons studied at Matangi, the highest daytime mean temperature, of 21°C, occurred in February each year (Tables 4.1 - 4.3), although temperatures up to 30°C occurred on some days. The highest mean maximum temperature (24°C) usually occurs in January and February at the Ruakura research station which is about 8 km from the Matangi field site (Table 4.4). Thus although there are times during the growing season that air temperatures in the Waikato do reach 24°C and higher, the overall daily averages are lower than those of a Chinese season. It is very likely that this factor had some influence on the growth rates of the *P. tomentosa* trees at Matangi.

The comparison of growth rates and gas exchange rates of trees grown inside and outside the glasshouse on the University of Waikato campus (Chapter 3) showed greater rates of growth when air temperatures were more akin to those found under a continental climate. However, there was no difference in gas exchange rates between the two environments, despite the initial hypothesis that the temperature regime during leaf development affected subsequent rates of gas exchange. The higher growth rates of the inside grown trees appeared to be the result of a longer period available for photosynthesis due to the warmer temperatures; a compounding effect of growth beginning earlier; and the lower rate of storage (higher rate of utilization) of photosynthates. From these results, it can be concluded that the growth rates of field grown *P. tomentosa* trees at Matangi were likely to be limited by temperature, but gas exchange rates were unlikely to be influenced by temperature to a large extent. The high levels of soluble sugars in the tissues harvested from the field grown trees (Table 4.25) probably reflect the cooler than optimum air temperatures. It would be interesting to compare soluble sugar levels in field grown trees grown under a warmer temperature regime than that studied here.

6.2.2 Soil moisture levels

Analysis of the results from the various experiments undertaken in this study showed that on a daily basis, fluctuations in soil moisture had little effect on rates of gas exchange. The low diurnal variation of leaf water potential and high rates of transpiration (up to 7 mmol H_20 m^{-2} s^{-1}) indicated that the trees were not conservative in their use of water. This would be

expected because of the need to regulate the temperature of the large leaves (section 2.5.2). In fact, on some occasions in high summer, the need to regulate leaf temperature seemed to become predominant. In AC_i experiments, *Paulownia* was found to maintain the C_i:C_a ratio at close to 0.7 (Fig. 2.8). This meant that g_s was set at the level required to generate this ratio for the existing photosynthetic rate. However, C_i values were found on some occasions to be higher than expected (260 - 280 $\mu l\ l^{-1}$, instead of 245 $\mu l\ l^{-1}$) indicating a higher g_s than required for CO_2 exchange. In those situations the plant had become a "water-spender" and seemed to have increased its transpiration rate in order to improve the evaporative cooling of the leaf. Large leaves, exceptionally large in the case of *Paulownia*, cannot be effectively cooled by heat convection under high radiation load and therefore require increased transpiration through increased g_s.

However, in the longer term, lower soil moisture levels did limit tree growth, with the early cessation of growth of branch and stem apices in season 3 probably at least partially caused by the low soil moisture levels in the middle of that season (Table 4.3). Comparison of the change in average leaf areas of the harvested trees from January to March in season 2 showed an increase of 70%, while in season 3 the leaf area of trees harvested in March was 20% less than the leaf area of trees harvested in January (Tables 4.8 and 4.9). This decrease in leaf area would have decreased the amount of water required by the plants in the latter half of season 3. Analysis of changes in biomass partitioning over the same harvesting periods in seasons 2 and 3 show that the R:S ratio was higher in March in season 3 than in season 2. This was a result of the decrease in leaf area and of an increase in the allocation of biomass to new roots. In season 2, the amount of new root material increased by 64% from January to March, but in season 3 the increase was 76% over the same time period. This reallocation of biomass is a typical response of plants under water stress. In season 3, values of g_s and E were lower than in the previous season which increased WUE and *l*. Thus the trees were operating with a more conservative water strategy than during season 2.

It has been reported that long term water stress reduces the photosynthetic capacity on many species (Schulze, 1982). Although tree 72 showed a higher A_{max} in March (season 3) than in preceding months, possibly from an improved water situation, in general there was little difference in the A_{max} of trees 43 and 72 over the same months in seasons 2 and 3.

It is interesting to note that in season 3 there were two trees that did not appear to be affected by the soil moisture deficit. Tree 63 maintained healthy green leaves and continued to grow until late in season 3. The form of this tree was unusual in that it had a highly branched structure that resulted in a very dense leaf canopy that prevented some leaves from receiving sunlight. It is not known why this tree grew as it did during the third season. It may have

been situated in a position with an adequate supply of water and nutrients that did not diminish during the season. The second tree to show growth throughout the season was tree 25. Although the leaves were significantly paler green than those of tree 63, more akin to the leaves of the remaining trees, both the stem and branch apices of this tree continued to elongate until March. This tree was one of the three transplanted in season 1 and was planted in the good growing area. The remaining two trees were transplanted into the poor area. After being transplanted intact in December, 1990, tree 25 did not grow any taller for the remainder of that season. However, the leaves remained on the tree so it is likely that the photosynthates produced were allocated to developing a strong root system in the new soil environment. The tree did grow in seasons 2 and 3, and it is possible that the root system developed in the latter half of season 1 was more extensive than that of the trees planted originally. This could explain the continued growth of the tree when those neighbouring trees ceased growth in season 3. It appears from these two trees that microsite effects and individual plant growth forms can have significant impact on the growth of trees in adverse situations.

Overall *Paulownia* seemed relatively tolerant of short periods of water deficiency despite the requirement to cool the large leaves, a requirement that declined as leaves became smaller in older plants. However, there was clearly a need to establish an extensive, healthy root system in order to maintain a good water supply. Root system allocation became a priority when environmental water stress increased, such as in the third season.

6.3 Interaction Between Gas Exchange, Growth and Nutrition

6.3.1 Nutrition in *Paulownia*

It can be concluded from the results of the glasshouse experiment with *P. tomentosa* trees (Chapter 3) and the hydroponics experiment with several species of *Paulownia* seedlings (section 5.3.2) that the stem growth of *Paulownia* plants is enhanced upon the addition of sufficient quantities of complete nutrients, as is found in most plant species. Root growth tends to decrease, which was reflected in the lower R:S ratio in the pot grown trees compared with similarly aged field grown trees and in the seedlings grown at CF 30 compared with those at CF 15. This reallocation of biomass with changes in nutrition is also common to many species. Working with 10 year old *Populus grandidentata* growing on a fertile site and a less fertile site, Briggs *et al.* (1986) found that the above ground production rate on a leaf weight basis was more than twice as high on the fertile site as on the less fertile site. Their estimation of root biomass from soil respiration data indicated that there was little difference in the absolute amount of below ground biomass in trees from the two areas, which meant that the R:S ratios were higher on the less fertile site. It is interesting to note that, despite the large difference in biomass accumulation rates between the two sites, photosynthetic rates were only 48% higher in leaves from the trees growing on the fertile site. Studies on two coniferous species led Axelsson and Axelsson (1986) to conclude that improved nutrition of forest stands increased stemwood production by decreasing allocation of dry matter to fine roots, rather than increasing foliage biomass or photosynthetic rates.

It is possible that the growth of the trees at Matangi in season 3 were limited by nutrients as well as by soil moisture. The two effects could interact with a decrease in water uptake by the plants when water is limiting also decreasing nutrient uptake. The higher R:S ratio of the trees harvested in March in season 3 compared with those harvested in March, season 2, showed that the trees were responding to a water stress and/or a nutrient stress. The results from the nutrient analyses of the tissues harvested from the trees in season 3 did not show any significant differences in the levels in these tissues when compared with those harvested in the previous season (section 4.6.3). However, although nutrient concentrations were not different in tissues between the two seasons, the change in the relative amounts of biomass in the above ground tissues from the January to March harvests (Appendices 2 and 3) indicate that the total uptake of nutrients was lower in season 3, during that period.

It is difficult to determine from the experiments undertaken in this study whether rates of gas exchange were affected by different levels of nutrition. The rates of photosynthesis

measured on leaves of the outside pot-grown trees in November and December (Tables 3.5 and 3.6) were higher than would be expected in the field grown trees of the same age. Gas exchange rates were lower in the early part of all seasons for both the *P. tomentosa* trees and *P. elongata* tree. The photosynthetic rate measured on the *P. tomentosa* trees towards the end of their first season of growth (Tables 2.6 and 2.7) were similar to those measured in January, 1993, on the pot grown trees (Table 3.7). Thus it is likely that the improved nutrition of the pot grown trees, in comparison with those grown in field, did facilitate higher rates of gas exchange in these leaves in the early stages of growth.

The decline in growth rates and gas exchange rates in the pot grown trees both inside and outside the glasshouse during the course of the experiment was probably due to a decrease in the levels of nutrients available to the plants. Although the trees were watered weekly with a complete nutrient solution, the strength of that solution did not change as the trees grew. Thus the amount of nutrient provided for the biomass already present decreased over the experiment. That the plants were becoming nutrient limited is also suggested by the severely pot bound nature of the root systems at harvest. As full nutrient analyses were only undertaken at harvest, it is not possible to confirm this hypothesis, but it is significant that foliar nitrogen concentrations decreased as the experiment progressed (Tables 3.5 - 3.7). To determine if the trees were becoming nutrient limited, it would have been necessary to increase the concentration or frequency of the nutrient treatment, as well as providing substantially larger growth containers.

In his discussion of the nutrition of forest trees, Ingestad (1987) suggests that as the relative growth rate of a plant is directly related to the relative addition rate of nutrients, and proportional to the internal nutrient concentrations within the tissues, then small frequent doses of fertilizer would result in the highest growth rates in forests. Although his hypothesis of daily adding about 6 kg N ha^{-1} to maximise tree growth in the first season is unlikely to be practicable in a commercial forest, some experimental results do support his theory with a system that is more practical. Tschaplinski and Norby (1991) found little difference in the rates of growth and gas exchange, and leaf nitrogen and chlorophyll contents in the leaves of *Platanus occidentalis* (sycamore) when fertilized once at the beginning of the season with 150 or 450 kg N ha^{-1} as compared with fertilization three times throughout the season with 37.5 kg N ha^{-1}. Thus it would be more economically viable to fertilize with the lower rate of nitrogen, but to do so more frequently. It is likely that the twice yearly application of 50 g of solid fertilizer (approximately 3.6 kg N ha^{-1}) to the *P. tomentosa* trees was inadequate. To improve the growth rates of the trees at Matangi, the frequency and amount of fertilizer needs to be increased.

Overall it is clear that *Paulownia* is a tree that needs careful management and monitoring of its nutrient regime.

6.3.2 Nitrogen

There has been much work done on the relationship between the application of nitrogen fertilizers and the subsequent growth, gas exchange and foliar nitrogen levels in a range of plant species. Field (1983) and Evans (1989) have reviewed different aspects of the nitrogen - gas exchange relationship. Generally, increased foliar concentrations of nitrogen leads to increased rates of photosynthesis, until saturation is reached. This has been found in a diverse range of trees and shrubs including *Diplacus aurantiacus* (Gulmon and Chu, 1981), *Eucalyptus spp.* (*e.g.* Mooney *et al.*, 1978; Sheriff, 1992), *Gmelina arborea* (Cromer *et al.*, 1993) and some conifers (Linder and Rook, 1984).

In *P. tomentosa* and *P. elongata* A_{max} tends to be correlated strongly with increasing foliar nitrogen content up to about 2% N. In leaves with higher nitrogen content there appears to be little correlation with photosynthetic rate. However, the pot grown *P. tomentosa* trees that were well watered and regularly received complete nutrient solution had very high foliar nitrogen levels and high rates of gas exchange. Uncertainty therefore remains about the interaction between A_{max} and leaf nitrogen content at nitrogen levels greater than 2%.

In addition to increasing rates of gas exchange, improved nitrogen nutrition leads to increased growth rates in many trees. This may be a result of increased leaf area due to the development of larger leaves, an increased number of leaves or an increase in the longevity of leaves (Linder and Rook, 1984). In *Betula pendula*, for example, both plant relative growth rate and leaf area per plant were found to be linearly related to plant nitrogen concentration in a hydroponics system. A sudden decrease in nitrogen had a greater affect on the development of new leaf area than on the photosynthetic rate of existing leaves, which led to an increase in the starch content of the plants (McDonald *et al.*, 1986). The decrease in growth rate at lower nitrogen levels in *Gmelina arborea* (Cromer *et al.*, 1993) was found to be primarily a result of the decrease in the final size of leaves. Comparison of the potted *P. tomentosa* trees grown outside the glasshouse and the field grown trees showed little difference in the range of leaf sizes, but data is not available on longevity of leaves. The leaves on the trees grown inside the glasshouse were generally larger than those grown outside (section 3.2.2) indicating that other environmental factors are important in determining leaf size in *Paulownia*.

The emphasis on growth in height and the investment in leaves rather than wood by the plant is probably a major complicating factor in interpreting the nitrogen nutrition in *Paulownia*.

6.3.3 Other nutrients

A soil deficiency in several elements other than nitrogen can lead to lower plant growth rates and gas exchange rates. However, these have been studied to a lesser extent than nitrogen and so our understanding of the relationships between these nutrients and plant functioning in tree species is poorer.

Phosphate has a role, for example, in determining leaf area as well as in gas exchange. Cromer *et al.* (1993) found in *Gmelina arborea* that decreasing the P level led to a decrease in leaf area by depressing the rate of leaf expansion, with only a small decrease in final leaf size. In other species, the effect of P on plant leaf area is mediated through a change in leaf size (Linder and Rook, 1984). The roles of P in photosynthesis in the conversion of ADP to ATP, in several of the key compounds of the process (*e.g.* RuBP), and the production of triose phosphates in the photosynthetic carbon reduction cycle could all be affected by a deficiency in P. It appears, however, that phosphate levels in leaves are generally in excess of the requirements of photosynthesis (Sharkey, 1985; Leuning *et al.*, 1991). It is unlikely that the P levels in the leaves of *Paulownia* trees studied in this work were limiting to growth or gas exchange as they were high in comparison with those in other species (Table 4.27).

The linear relationship between chlorophyll content of leaves and Fe and Mn levels has been noted in several species (Linder and Rook, 1984). When severely iron deficient plants of *Populus euramericana* were treated with iron chelate, Keller and Koch (1964) found a large increase in foliar levels of chlorophylls a and b, and accessory pigments. Photosynthetic rates also increased after the addition of the iron. Again, it is unlikely that iron was limiting in the *Paulownia* trees studied here.

Although it is not possible to fully explain the mechanisms by which higher rates of nutrient availability lead to higher growth rates in *Paulownia*, it does appear from the results discussed here that we have yet to determine the upper limit for nutrition in these plants. Further hydroponics studies with higher concentrations of nutrients and field trials with different amounts and proportions of fertilizers need to be undertaken before a more complete picture of the nutrient requirements of this tree can be gained.

6.4 Relationship Between A_{max} and Tree Growth

There was an emphasis in this research on measuring the CO_2 exchange and A_{max} in *Paulownia* leaves. The measurement of gas exchange rates using the Walz CMS system set A_{max} as the photosynthetic rate of a leaf under controlled, defined conditions. This produced a benchmark value that allowed comparison between leaves. However, in reality this instantaneous measure of photosynthetic rate on a portion of a leaf is unlikely to reflect the actual assimilation rate of the whole plant over time. This can be illustrated by the higher growth rate of the potted *P. tomentosa* trees in the glasshouse despite the leaves on these trees having lower values of A_{max} at the first two measurement times than leaves on the trees growing outside the glasshouse. The trees at Matangi maintained similar A_{max} values in both season 2 and 3 but showed very different growth rates. Thus growth rate is a reflection of the photosynthetic rate of all leaves, integrated over time and the value of A_{max} gives an indication of the range of photosynthetic rates that the leaves may attain. The lack of direct correlation between values of A_{max} and rates of growth has been found in many plants (McGraw and Wulff, 1983, Briggs *et al.*, 1986), although the generalisation probably remains true that high growth rates need high photosynthetic rates. As well, the difference in allocation strategies between plants complicates the relationship between A_{max} and growth, with growth often being defined in woody species as stem growth.

It is possible to study the carbon balance of an entire tree with whole plant cuvette systems, as has been done with kiwifruit (Buwalda *et al.*, 1992). Technical difficulties prevented this type of experiment from being undertaken on the *Paulownia* trees. It is certainly one aspect of further research that could produce some very interesting results. Such experiments would give an indication of rates of respiration of non-photosynthetic tissues, and total diel carbon budgets. It is important to qualify extrapolations of measurements made on single leaves under defined conditions to how the whole plant is performing in the natural environment. As Jarvis (1986) cautions, the gas exchange and growth interactions of a mature tree or a whole forest stand cannot be easily assessed from the study of these parameters on isolated leaves or on small seedlings grown under ideal conditions.

6.5 Relating the Role of Root Reserves to Propagation Capacity

Root material from *Paulownia* propagates very readily to quickly produce viable root and shoot tissues (Chapter 5). This capacity is related to the phytohormone balance within the roots and the storage of large reserves of carbohydrates and, to a lesser extent, nutrients. The range of nutrient concentrations within roots of *P. tomentosa* (Tables 4.12 - 4.22 and 4.23) were similar to those found in the root material of other plants *e.g.* kiwifruit (Smith and Clark, 1992), the roots of which can also be propagated from, and western juniper (Miller *et al.*, 1990). However, the storage of high levels on soluble sugars in *Paulownia* roots (Table 4.25) is unusual (section 4.5.2.1) and it may be this store of a readily translocated food supply that allows the rapid development of new tissues from root material.

It has been found in other species that the propagation capacity of root cuttings is related to the levels of stored carbohydrates. In four hardwood species, the starch reserves in the roots in autumn were manipulated by a variety of cutting treatments to remove shoot material. In the following season, those root systems with lower starch reserves produced fewer and less vigorous sprouts (Kays and Canham, 1991). With other species, it has been found that cold treatment improves the ability of roots to propagate as the starch reserves are transformed into soluble sugars (Robinson, 1975) and that the interaction of hormone activity and carbohydrates may stimulate new tissue production when the balance of hormones is such that starch hydrolysis is favoured. If indeed the concentration of soluble sugars is important to the regeneration ability of root material, then the tendency of *Paulownia* roots to store sugars as opposed to starch may explain the readiness of these roots to propagate.

6.6 The Growth and Management of *Paulownia* in New Zealand

The success of the extensive planting of *Paulownia* in China for both timber production and in agroforestry systems may not be easily reproducible in this country. China has a continental climate, with rainfall being plentiful during the warm summer months in the areas where *Paulownia* is grown. Even in areas where the annual precipitation is less than 500 mm, *Paulownia* will grow without irrigation as two thirds of the rain falls between June and September, when the trees are undergoing their fastest growth (Zhu *et al.*, 1986). Summers in many places in New Zealand tend to be drier than other seasons of the year, with summer drought often a problem in eastern areas. The oceanic climate also means that although the coldest temperatures during winter are much higher than those in mainland China, the temperatures in summer are lower. The changeable nature of the weather patterns in New Zealand result in short periods of settled warm weather during the growing season, which are punctuated by cooler periods. Frosts are even possible in late spring and early summer, with a mild frost being recorded in the Waikato in early January of 1991, which damaged the growth of young *Paulownia* trees. This combination of unseasonal frosts, dry summers and generally cooler summer temperatures combine to limit the growth of *Paulownia* trees in the New Zealand situation.

It can be concluded from the discussion above regarding the climatic and soil influences on the growth of *Paulownia* that maximum growth rates will only be achieved when the trees are grown in an unshaded, consistently warm site, with a good water supply (either provided naturally or by irrigation) and given large amounts of nutrients. It is unlikely that the Waikato district is an ideal site for these trees to realise their full growth potential as the summers are not hot enough. It may prove that an area such as Central Otago with its more continental climate may be more suitable, provided sufficient water and nutrients are available. However, it must be borne in mind that if these trees are to be grown primarily for the Japanese timber market, then rapid growth rates are not necessarily desirable. As discussed earlier (section 1.6) the financial equation is still in the balance. So, although growth rates in the Waikato are not particularly rapid, in the end this may result in the trees being more valuable.

There is one other problem that was highlighted in this study from the trees growing in the field at Matangi and that is the variability between individuals. At the end of season 3, the tallest tree was 3.5 m tall with a basal diameter of 100 mm, while the smallest trees in the good growing area were only approximately 1.0 m tall with diameters of 40 mm. For the commercial grower, interested in producing a high quality timber crop, the selection of clonal material with the appropriate characteristics for the growing district is essential. There is still much work to be done on selecting trees with good form and growth rates for the various growing districts in New Zealand.

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Appendix 1. Dry weights (g) of tissues of trees harvested in season 1 (1990 - 1991).

Tree	Above	Lamina	Petiole	Stem	Stump	Laterals	Fine Roots	New Roots
6	4.1				19.0			0.6
15	5.6				74.7			0.5
70	7.3				30.1			0.9
79	8.7				60.8			0.9
12		22.1	3.3	8.6	25.1			7.6
21		78.0	17.5	28.3	68.5			26.4
64		70.1	15.6	31.7	50.9			22.9
73		84.5	24.3	39.8	30.9			78.4
35		194.9	44.4	150.4	149.6	153.3	4.4	
44		108.0	21.2	81.4	192.0	200.0	5.5	
95		4.7	0.5	6.3	53.8			1.7
104		63.0	10.0	38.6	44.6	122.0	2.4	
5				30.4	59.3	101.7	0.4	
14				19.5	30.8	18.3	0.4	
71		13.7	3.1	66.6	112.0	171.0	0.8	
80		36.0	7.3	43.5	75.9	196.0	1.3	

Appendix 2. Dry weights (g) of tissues of trees harvested in season 2 (1991 - 1992).

Tree	Lamina	Petiole	Branch	New Stem	Old Stem	Stump	Laterals	Fine Roots
33	75	18	32		140	161	221	0.40
42	143.8	29.5	57.0		389	221	447	0.28
97	46.5	9.4	14.9		32.8	78	85.8	0.66
106	80.7	19.7	34.9		99.4	171	143	0.43
37	253.7	53.4	100.9	38.2	369	239	500	3.0
46	152.9	29.8	69.8	14.2	188	105	254.6	3.4
93	67.0	12.1	19.1		53	77	98	2.5
102	238.8	49.7	81.4	47.4	250.4	138	310	1.1
8	598.2	131.3	226.8	309.6	507.9	351.9	857.2	4.2
17	146.6	18.9	74.0	43.7	240.4	119.4	212.5	2.1
66	338.1	67.7	120.4	199	392	277	725	5.3
77	8.4	0.5		3.4	3.9	38.1	4.6	0.3
39				35.3	51.7	72.9	119.9	0.6
48			81.0	169.9	340.3	472.4	815.6	1.6
91			230.8	139.5	391.6	367.7	761.6	3.5
100			309.4	56.9	535.0	304.1	810.8	2.8

Appendix 3. Dry weights (g) of tissues of trees harvested in season 3 (1992 - 1993).

Tree	Lamina	Petiole	Old Branch	New Branch	Stem	Stump	Laterals	Fine Roots
50	55	22	308	62	884	275	747	0.9
51	215	59	229	96	745	253	773	4.2
101	178	54	402	76	518	441	815	1.8
111	360	77	512	122	1389	796	1435	1.0
18	518	85	191	149	495	273	484	17.0
26	710	119	487	269	969	718	730	6.1
99	194	28	139	69	282	157	327	18.0
109	686	108	411	342	931	545	1219	24.0
56	424	63	705		977	598	1268	15.0
57	498	81	590		751	799	842	22.5
82	506	98	1051		996	621	1679	11.0
83	324	58	762		916	724	1352	11.0
45			625		817	745	1347	2.5
53			319		383	256	532	0.9
113			419		321			
114			409		718			

Appendix 4. Nutrients in *Paulownia* tissues harvested in season 1.

tree	N	P	S	Mg	Ca	Na	K	Mn	Zn	Cu	Fe	B	Mo
	%	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm
6 root	1.40	0.09	0.11	0.09	0.11	0.02	0.67	24	76	35	619	16	0.80
6/15 above	3.20	0.37	0.15	0.22	0.51	0.02	1.46	55	38	22	537	23	0.12
15 root	0.90	0.09	0.07	0.08	0.11	0.03	0.52	20	46	28	291	17	0.71
all - fine root	2.20	0.21	0.13	0.35	0.51	0.66	1.85	126	63	38	1939	23	0.25
70/79 above	2.68	0.29	0.16	0.18	0.50	0.03	1.14	27	41	16	421	26	0.07
70 root	0.70	0.05	0.06	0.06	0.10	0.03	0.48	18	56	19	316	10	0.26
79 root	0.72	0.08	0.06	0.08	0.08	0.03	0.37	13	52	15	340	11	0.28
12 lamina	1.89	0.14	0.14	0.16	0.79	0.02	1.00	29	26	13	144	24	0.08
12 petiole	0.70	0.09	0.05	0.18	1.57	0.02	2.07	28	38	9	109	25	0.05
12 stem	0.66	0.11	0.05	0.09	0.42	0.02	0.98	12	64	18	50	14	0.16
12 stump	0.43	0.06	0.04	0.05	0.13	0.03	0.65	45	51	40	571	6	0.06
21 lamina	2.14	0.15	0.15	0.22	1.01	0.03	0.80	38	29	10	161	27	0.12
21 petiole	0.66	0.08	0.06	0.28	1.51	0.02	1.39	26	41	7	59	21	0.06
21 stem	0.53	0.07	0.04	0.09	0.27	0.01	0.58	10	67	18	41	10	0.08
21 stump	0.43	0.07	0.04	0.07	0.15	0.02	0.52	26	56	18	451	18	0.30
21 laterals	0.67	0.10	0.05	0.19	0.24	0.12	1.10	102	50	16	1179	17	0.17
64 lamina	2.68	0.21	0.19	0.26	1.08	0.02	0.45	48	36	19	216	32	0.30
64 petiole	0.93	0.11	0.10	0.39	1.76	0.02	0.42	48	35	7	291	24	0.04
64 stem	0.66	0.09	0.05	0.11	0.33	0.02	0.39	12	52	19	105	14	0.06
64 stump	0.47	0.10	0.04	0.07	0.13	0.03	0.43	17	60	15	430	10	0.04
64 laterals	0.74	0.10	0.06	0.18	0.24	0.16	0.69	81	46	19	1458	16	0.09
73 lamina	2.28	0.16	0.15	0.19	0.83	0.02	0.68	50	28	15	358	24	0.17
73 petiole	0.77	0.09	0.07	0.29	1.50	0.02	0.75	45	50	15	322	21	1.13
73 stem	0.59	0.08	0.04	0.09	0.24	0.02	0.49	14	51	19	222	12	1.50
73 stump	0.42	0.04	0.04	0.06	0.11	0.02	0.41	20	38	24	501	14	0.26
73 laterals	0.62	0.08	0.04	0.13	0.21	0.08	0.74	64	37	17	1252	14	0.70
35 lamina	2.02	0.16	0.15	0.21	1.68	0.01	1.25	54	30	11	154	44	0.30
35 petiole	0.63	0.10	0.13	0.40	2.58	0.01	1.27	53	39	9	95	28	0.15
35 stem	0.47	0.07	0.04	0.06	0.17	0.01	0.44	10	41	15	58	10	0.68
35 stump	0.42	0.06	0.03	0.05	0.11	0.02	0.41	10	39	17	241	10	0.33
35 laterals	0.58	0.08	0.05	0.10	0.12	0.03	0.77	34	35	18	925	11	0.50
35/44 fine	1.06	0.12	0.11	0.41	0.47	0.39	1.87	135	64	83	1022	19	0.84
44 lamina	2.37	0.24	0.18	0.14	1.61	0.02	1.29	56	25	10	132	53	0.44
44 petiole	0.68	0.10	0.10	0.23	3.07	0.02	2.70	88	47	7	57	28	0.15
44 stem	0.47	0.07	0.04	0.07	0.19	0.01	0.51	22	45	19	51	10	0.16
44 stump	0.48	0.07	0.04	0.06	0.11	0.02	0.49	17	42	16	745	11	0.37
44 laterals	0.87	0.12	0.06	0.11	0.14	0.03	1.19	57	33	15	1300	12	0.41

Appendix 4 ***(continued)***

tree	N %	P %	S %	Mg %	Ca %	Na %	K %	Mn ppm	Zn ppm	Cu ppm	Fe ppm	B ppm	Mo ppm
95 leaf	1.10	0.07	0.09	0.08	0.55	0.02	0.59	10	17	7	126	25	0.73
95 stem	0.46	0.04	0.03	0.03	0.23	0.02	0.32	7	15	8	82	13	0.07
95 stump	0.38	0.03	0.04	0.04	0.13	0.03	0.45	16	27	23	426	10	0.16
95 laterals	0.80	0.06	0.09	0.19	0.35	0.35	1.19	35	48	16	745	13	1.36
104 lamina	2.86	0.22	0.21	0.11	1.59	0.02	1.49	68	23	8	160	46	0.38
104 petiole	0.93	0.11	0.13	0.33	3.63	0.02	3.01	128	60	6	75	24	0.52
104 stem	0.69	0.09	0.06	0.09	0.25	0.01	0.75	45	61	13	90	9	0.32
104 stump	0.74	0.08	0.07	0.08	0.13	0.01	0.67	9	46	13	275	9	0.42
104 laterals	1.07	0.12	0.08	0.12	0.13	0.02	1.21	20	35	10	339	8	0.17
104 fine	1.64	0.16	0.14	0.50	0.49	0.38	2.69	177	71	28	1119	15	0.28
5 stem	0.80	0.09	0.06	0.10	0.21	0.02	0.45	12	56	21	96	14	0.30
5 stump	0.78	0.09	0.06	0.08	0.18	0.03	0.52	23	49	14	492	11	0.50
5 laterals	1.19	0.11	0.08	0.08	0.13	0.02	0.64	23	35	18	374	10	0.13
all - fine	1.36	0.13	0.13	0.39	0.52	0.39	2.05	173	82	50	1048	16	0.34
14 stem	0.71	0.08	0.05	0.10	0.21	0.02	0.39	10	53	21	72	9	0.09
14 stump	0.60	0.05	0.05	0.08	0.16	0.03	0.46	20	51	24	272	5	0.38
14 laterals	0.84	0.13	0.06	0.14	0.20	0.05	0.96	55	45	24	807	11	0.18
71 lamina	1.38	0.09	0.15	0.28	3.14	0.04	0.68	150	26	8	294	85	0.24
71 petiole	0.44	0.05	0.09	0.72	5.12	0.05	0.28	223	92	5	76	22	0.08
71 stem	0.78	0.11	0.06	0.09	0.22	0.02	0.61	29	46	13	84	13	0.11
71 stump	0.67	0.08	0.05	0.06	0.11	0.02	0.44	9	35	13	235	8	0.34
71 laterals	1.02	0.09	0.06	0.09	0.13	0.03	0.85	23	24	13	365	9	0.09
80 lamina	1.19	0.07	0.14	0.16	3.26	0.04	0.40	88	17	6	311	76	0.34
80 petiole	0.35	0.05	0.07	0.41	5.06	0.03	0.19	116	54	5	153	30	0.12
80 stem	0.76	0.10	0.06	0.09	0.24	0.01	0.58	25	57	11	148	11	0.38
80 stump	0.65	0.08	0.05	0.07	0.12	0.02	0.47	7	44	21	248	11	0.26
80 laterals	1.01	0.08	0.07	0.07	0.11	0.02	0.70	9	23	12	266	8	0.12
old leaves lam	1.65	0.13	0.17	0.17	3.24	0.04	0.84	72	30	24	157	96	0.27
old leaves pet	0.49	0.06	0.08	0.30	4.00	0.03	0.85	72	36	5	49	27	0.08
stump 1	1.29	0.12	0.09	0.09	0.09	0.02	0.87	16	52	26	224	10	0.23
stump 2	0.93	0.10	0.06	0.09	0.10	0.02	0.82	19	61	16	353	12	0.11
stump 3	1.53	0.18	0.10	0.11	0.10	0.02	1.22	41	77	32	338	16	4.20
stump 4	1.53	0.12	0.11	0.10	0.07	0.02	1.05	30	48	26	581	9	0.06
stump 5	0.94	0.08	0.07	0.07	0.05	0.02	0.58	18	45	24	275	8	0.02

Appendix 5. Nutrients in *Paulownia* tissues harvested in season 2.

tree	N	P	S	Mg	Ca	Na	K	Mn	Zn	Cu	Fe	B
	%	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm
33 lamina A	2.41	0.26	0.15	0.13	1.00	0.02	0.98	48	45	11	193	31
33 lamina B	2.51	0.26	0.15	0.13	0.94	0.01	0.99	46	45	11	177	31
33 petiole	0.93	0.16	0.07	0.13	1.31	0.02	1.42	21	32	8	95	19
33 branch	1.09	0.20	0.07	0.13	0.58	0.02	1.28	19	65	16	75	13
33 stem	0.32	0.06	0.02	0.06	0.18	0.01	0.39	17	55	19	173	11
33 laterals	0.63	0.06	0.03	0.08	0.16	0.03	0.62	23	24	13	302	8
33 stump	0.26	0.04	0.01	0.05	0.11	0.02	0.26	23	35	30	306	6
42 lamina	2.55	0.27	0.18	0.16	1.42	0.02	0.98	65	67	15	140	35
42 petiole	1.07	0.17	0.07	0.18	1.86	0.02	1.58	24	49	15	96	20
42 branch	1.26	0.21	0.08	0.12	0.72	0.03	1.45	26	73	19	59	15
42 stem	0.39	0.07	0.03	0.08	0.24	0.02	0.44	17	58	19	194	11
42 laterals	0.88	0.08	0.06	0.10	0.22	0.04	0.84	58	43	25	617	11
42 stump	0.35	0.06	0.02	0.06	0.15	0.02	0.38	24	46	34	368	9
97 lamina	1.68	0.13	0.11	0.12	0.72	0.02	0.79	27	29	15	249	16
97 petiole	0.77	0.10	0.04	0.10	1.05	0.02	1.32	12	24	11	206	15
97 branch	0.82	0.11	0.04	0.10	0.58	0.02	1.00	11	41	11	109	10
97 stem	0.29	0.04	0.02	0.06	0.19	0.02	0.37	18	68	10	57	8
97 laterals	0.27	0.03	0.02	0.09	0.19	0.03	0.93	18	24	9	355	7
97 stump	0.19	0.02	0.02	0.04	0.14	0.02	0.29	10	32	11	225	5
106 lamina	2.45	0.22	0.13	0.14	0.76	0.02	1.02	58	31	9	294	25
106 petiole	0.78	0.11	0.04	0.12	1.15	0.02	1.50	11	20	5	92	15
106 branch	0.89	0.13	0.04	0.09	0.41	0.02	1	9	37	9	46	9
106 stem	0.42	0.06	0.03	0.08	0.31	0.02	0.49	36	72	34	530	12
106 laterals	0.44	0.06	0.03	0.10	0.23	0.03	0.79	44	38	31	865	10
106 stump	0.20	0.03	0.03	0.04	0.16	0.02	0.31	17	45	42	433	6
37 lamina	1.70	0.18	0.14	0.20	1.86	0.02	1.02	39	30	17	114	36
37 petiole	0.56	0.11	0.11	0.33	3.15	0.02	1.18	48	40	10	82	22
37 branch	0.49	0.08	0.03	0.10	0.45	0.02	0.77	17	45	24	53	11
37 new stem	0.42	0.08	0.02	0.08	0.36	2.00	0.77	13	34	19	41	11
37 old stem	0.28	0.05	0.01	0.05	0.19	0.01	0.35	11	48	16	249	10
37 laterals	0.24	0.04	0.01	0.09	0.10	0.05	0.64	22	32	33	609	9
37 stump	0.25	0.04	0.01	0.05	0.13	0.02	0.30	18	51	60	517	10
37 fine	1.70	0.24	0.14	0.57	0.43	0.49	1.39	118	67	43	849	16
46 lamina	1.92	0.20	0.13	0.17	1.07	0.02	1.64	35	28	20	115	25
46 petiole	0.59	0.11	0.05	0.12	1.14	0.02	3.11	34	34	11	96	17
46 branch	0.45	0.07	0.02	0.09	0.33	0.02	1.08	22	46	21	52	11

Appendix 5 *(continued)*

tree	N %	P %	S %	Mg %	Ca %	Na %	K %	Mn ppm	Zn ppm	Cu ppm	Fe ppm	B ppm
46 new stem	0.45	0.07	0.02	0.08	0.33	0.02	1.09	22	45	22	35	11
46 old stem	0.18	0.03	0.01	0.05	0.13	0.01	0.37	18	35	17	139	8
46 laterals	0.19	0.03	0.01	0.12	0.15	0.04	0.97	15	28	16	386	10
46 stump	0.17	0.02	0.01	0.06	0.10	0.02	0.34	9	38	20	267	7
46 fine	1.72	0.27	0.16	0.46	0.49	0.25	2.02	216	69	40	2544	20
93 lamina	1.60	0.15	0.11	0.16	1.18	0.02	0.58	30	21	10	180	22
93 petiole	0.55	0.08	0.06	0.22	2.07	0.02	0.34	55	33	7	106	21
93 branch	0.39	0.06	0.02	0.10	0.43	0.01	0.68	18	32	13	31	11
93 stem	0.37	0.02	0.02	0.06	0.21	0.02	0.33	16	47	20	138	9
93 laterals	0.28	0.00	0.01	0.08	0.14	0.04	0.56	17	19	11	222	7
93 stump	0.22	0.00	0.01	0.03	0.13	0.02	0.25	11	33	22	261	7
93 fine	2.06	0.24	0.15	0.44	0.49	0.36	1.03	134	81	37	741	15
102 lamina	1.78	0.19	0.13	0.15	1.62	0.02	1.09	32	34	14	149	30
102 petiole	0.49	0.09	0.08	0.20	2.36	0.02	1.29	26	35	8	69	24
102 branch	0.43	0.06	0.02	0.08	0.36	0.02	0.81	15	45	18	60	12
102 new stem	0.55	0.11	0.03	0.09	0.41	0.03	0.98	15	40	20	50	14
102 old stem	0.22	0.02	0.01	0.04	0.12	0.01	0.35	7	32	13	84	9
102 laterals	0.29	0.02	0.02	0.12	0.17	0.05	0.93	21	28	19	579	11
102 stump	0.20	0.01	0.01	0.05	0.09	0.02		10	29	24	328	9
8 lamina A	2.07	0.20	0.14	0.19	1.56	0.02	0.71	49	24	18	107	50
8 lamina B	2.12	0.23	0.15	0.19	1.61	0.02	0.72	50	25	19	95	50
8 petiole	0.43	0.10	0.11	0.38	3.37	0.02	0.60	85	55	11	62	22
8 branch	0.46	0.07	0.02	0.11	0.32	0.01	0.61	18	51	25	37	10
8 new stem	0.34	0.06	0.02	0.07	0.17	0.01	0.36	10	33	18	84	8
8 old stem	0.29	0.05	0.01	0.05	0.13	0.01	0.29	7	31	17	62	8
8 laterals	0.34	0.06	0.02	0.06	0.14	0.02	0.53	26	33	31	558	9
8 stump	0.26	0.03	0.02	0.04	0.16	0.01	0.30	7	43	28	129	11
8 fine	1.45	0.18	0.13	0.54	0.66	0.56	0.81	301	91	49	1233	17
17 lamina	1.91	0.17	0.13	0.17	0.95	0.02	0.37	133	19	13	87	31
17 old lamina	1.22	0.09	0.14	0.22	1.51	0.02	0.24	104	22	14	113	42
17 petiole	0.63	0.09	0.18	0.37	1.96	0.02	0.31	330	37	7	97	22
17 branch	0.54	0.07	0.03	0.09	0.22	0.01	1	41	36	8	35	10
17 new stem	0.67	0.10	0.04	0.09	0.20	0.01	0.56	32	44	10	69	12
17 old stem	0.41	0.02	0.02	0.04	0.13	0.01	0.26	22	37	8	102	9
17 laterals	0.60	0.15	0.03	0.10	0.15	0.05	0.77	82	41	15	699	10
17 stump	0.30	0.03	0.01	0.02	0.08	0.01	0.18	23	38	18	238	7

Appendix 5 ***(continued)***

tree	N %	P %	S %	Mg %	Ca %	Na %	K %	Mn ppm	Zn ppm	Cu ppm	Fe ppm	B ppm
17 fine	1.57	0.16	0.20	0.39	0.50	0.56	0.93	1107	103	47	1176	18
66 lamina	2.32	0.18	0.15	0.19	1.65	0.02	0.93	71	22	13	165	47
66 petiole	0.44	0.07	0.08	0.37	3.08	0.02	0.67	133	50	8	69	30
66 branch	0.57	0.09	0.03	0.09	0.28	0.01	0.64	35	48	24	208	21
66 new stem	0.41	0.07	0.02	0.07	0.18	0.01	0.47	19	31	16	171	10
66 old stem	0.27	0.04	0.01	0.02	0.09	0.01	0.25	7	25	13	58	8
66 laterals	0.38	0.06	0.02	0.05	0.09	0.02	0.55	24	25	18	416	10
66 stump	0.39	0.06	0.03	0.08	0.19	0.02	0.56	10	42	30	325	11
66 fine	1.30	0.16	0.14	0.45	0.63	0.76	1.20	277	83	46	1920	19
77 leaf	0.72	0.09	0.07	0.14	0.67	0.02	0.35	38	12	5	142	19
77 new stem	0.42	0.06	0.04	0.08	0.31	0.01	0.57	34	25	8	31	11
77 old stem	0.34	0.03	0.03	0.07	0.36	0.02	0.13	12	27	7	36	12
77 laterals	0.31	0.03	0.02	0.15	0.25	0.03	0.90	56	35	11	314	9
77 stump	0.25	0.02	0.02	0.05	0.17	0.02	0.35	17	39	11	142	9
39 new stem	0.53	0.08	0.03	0.09	0.02	0.01	0.44	16	45	14	51	12
39 old stem	0.47	0.07	0.02	0.07	0.15	0.01	0.33	14	38	12	79	12
39 laterals	0.50	0.08	0.03	0.12	0.16	0.03	0.90	30	29	13	347	11
39 stump	0.48	0.08	0.02	0.07	0.20	0.02	0.47	14	35	17	131	10
48 branch	0.39	0.07	0.03	0.09	0.17	0.01	0.57	26	41	16	53	12
48 new stem	0.58	0.09	0.03	0.10	0.23	0.02	0.64	25	42	19	229	14
48 old stem	0.32	0.05	0.02	0.05	0.10	0.01	0.31	10	30	16	123	10
48 laterals	0.50	0.06	0.02	0.08	0.16	0.03	0.60	20	29	27	420	8
48 stump	0.31	0.04	0.02	0.05	0.15	0.02	0.43	7	41	20	161	10
91 branch	0.60	0.09	0.02	0.09	0.23	0.02	0.60	21	33	14	124	12
91 new stem	0.52	0.08	0.02	0.09	0.21	0.02	0.48	13	31	12	106	12
91 old stem	0.25	0.04	0.01	0.04	0.11	0.01	0.27	5	22	10	60	8
91 laterals	0.48	0.06	0.02	0.08	0.12	0.03	0.64	16	24	17	412	9
91 stump	0.35	0.05	0.01	0.05	0.13	0.02	0.37	5	35	15	101	9
91 fine	0.91	0.13	0.08	0.47	0.57	0.60	1.49	81	47	30	5832	28
100 branch	0.58	0.09	0.02	0.11	0.28	0.02	0.59	12	41	16	197	12
100 new stem	0.45	0.08	0.02	0.09	0.24	0.02	0.46	6	37	13	50	11
100 old stem	0.18	0.02	0.00	0.04	0.11	0.01	0.17	4	30	13	85	7
100 laterals	0.62	0.08	0.02	0.10	0.15	0.04	0.69	22	33	22	439	8
100 stump	0.36	0.05	0.01	0.06	0.17	0.02	0.34	12	51	19	254	11
100 fine	1.08	0.13	0.12	0.29	0.45	0.75	0.93	76	41	30	659	15
stump 6	1.21	0.09	0.06	0.09	0.08	0.02	0.83	18	41	23	234	8
stump 7	0.90	0.08	0.04	0.08	0.08	0.02	0.58	20	38	19	362	8

Appendix 6. Nutrients in *Paulownia* tissues harvested in season 3.

tree	N	P	S	Mg	Ca	Na	K	Mn	Zn	Cu	Fe	B
	%	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm
50 lam	2.85	0.31	0.20	0.18	1.34	0.02	1.08	36	41	17	157	34
50 pet	1.08	0.18	0.12	0.24	1.87	0.03	1.63	23	26	14	104	21
50 new br	1.31	0.21	0.10	0.16	0.68	0.02	1.24	17	53	24	57	16
50 old br	0.46	0.06	0.04	0.09	0.26	0.03	0.53	22	44	29	523	9
50 stem	0.23	0.03	0.02	0.04	0.18	0.02	0.19	10	132	28	340	3
50 stump	0.25	0.03	0.02	0.04	0.15	0.02	0.23	24	32	40	366	9
50 laterals	0.73	0.07	0.06	0.19	0.34	0.08	1.20	109	44	32	1473	16
51 lam	2.60	0.25	0.19	0.18	1.32	0.02	1.17	40	33	24	212	33
51 pet	0.79	0.13	0.09	0.02	1.72	0.02	1.89	22	21	13	96	20
51 new br	0.95	0.15	0.08	0.16	0.74	0.02	1.18	19	48	30	74	8
51 old br	0.33	0.04	0.02	0.07	0.17	0.02	0.41	11	36	20	122	5
51 stem	0.21	0.03	0.02	0.03	0.07	0.01	0.20	6	25	14	82	4
51 stump	0.16	0.02	0.01	0.03	0.06	0.02	0.16	8	26	18	249	4
51 laterals	0.34	0.04	0.03	0.15	0.19	0.05	0.95	71	29	24	1155	7
51 fine	1.70	0.18	0.15	0.48	0.65	0.34	0.58	255	70	57	3045	21
101 lam	2.59	0.23	0.18	0.19	1.37	0.02	1.17	28	35	14	156	24
101 pet	0.99	0.15	0.10	0.23	1.88	0.02	2.01	15	25	11	120	16
101 new br	0.97	0.15	0.09	0.15	0.69	0.02	1.33	15	53	22	73	13
101 old br	0.38	0.05	0.04	0.08	0.28	0.02	0.49	11	44	21	158	7
101 stem	0.22	0.03	0.02	0.04	0.14	0.01	0.26	10	34	20	155	6
101 stump	0.21	0.03	0.02	0.04	0.11	0.02	0.24	10	27	18	372	5
101 laterals	0.33	0.04	0.04	0.12	0.21	0.04	0.81	28	25	16	1055	6
101/111 fine	2.26	0.29	0.19	0.48	0.57	0.50	1.42	189	96	57	1067	18
111 lam	2.65	0.22	0.20	0.23	1.75	0.02	1.55	44	48	15	202	34
111 pet	0.95	0.13	0.10	0.19	1.69	0.02	2.70	21	30	13	230	15
111 new br	0.97	0.17	0.08	0.14	0.51	0.02	1.50	18	54	19	81	11
111 old br	0.40	3.06	0.04	0.09	0.31	0.02	0.63	24	51	15	253	9
111 stem	0.28	0.04	0.03	0.04	0.17	0.01	0.28	11	38	20	77	5
111 stump	0.29	0.04	0.02	0.06	0.15	0.02	0.30	44	47	44	1060	11
111 laterals	0.42	0.05	0.03	0.11	0.17	0.04	0.83	65	35	16	1377	11
18 lam	1.66	0.15	0.14	0.19	1.35	0.01	0.51	46	19	11	94	28
18 pet	0.48	0.08	0.09	0.26	3.16	0.02	0.47	72	33	9	125	29
18 new br	0.43	0.07	0.04	0.09	0.33	0.01	0.58	11	34	18	45	9
18 old br	0.32	0.04	0.02	0.08	0.17	0.01	0.32	12	37	14	89	6
18 stem	0.25	0.04	0.01	0.05	0.11	0.01	0.23	7	29	13	120	6
18 stump	0.19	0.03	0.01	0.04	0.09	0.02	0.20	9	33	17	235	4

Appendix 6 ***(continued)***

tree	N %	P %	S %	Mg %	Ca %	Na %	K %	Mn ppm	Zn ppm	Cu ppm	Fe ppm	B ppm
18 laterals	0.33	0.05	0.04	0.10	0.19	0.04	0.63	56	38	24	850	10
18 fine	1.24	0.18	0.15	0.50	0.54	0.71	1.50	189	67	36	1746	17
26 lam	2.01	0.16	0.17	0.18	1.71	0.02	1.00	57	28	16	117	36
26 pet	0.55	0.08	0.14	0.17	2.37	0.02	1.40	56	28	9	72	26
26 new br	0.48	0.07	0.05	0.08	0.38	0.02	0.88	15	35	17	35	11
26 old br	0.29	0.04	0.03	0.05	0.19	0.02	0.42	16	38	14	171	9
26 stem	0.24	0.03	0.02	0.03	0.13	0.01	0.33	10	28	10	59	6
26 stump	0.25	0.04	0.03	0.05	0.15	0.02	0.41	30	38	25	1056	9
26 laterals	0.39	0.04	0.03	0.12	0.18	0.05	1.11	38	30	18	981	12
26 fine	1.77	0.23	0.15	0.48	0.55	0.45	2.14	403	84	45	2166	21
99 lam	2.34	0.20	0.17	0.17	1.00	0.02	1.34	35	25	18	145	19
99 pet	0.78	0.10	0.11	0.18	1.79	0.02	2.23	49	27	16	195	21
99 new br	0.67	0.10	0.06	0.11	0.39	0.02	1.14	21	55	33	59	12
99 old br	0.27	0.03	0.02	0.04	0.14	0.01	0.31	13	35	16	122	5
99 stem	0.48	0.05	0.04	0.08	0.25	0.02	0.59	18	54	19	186	10
99 stump	0.23	0.03	0.02	0.04	0.12	0.02	0.30	16	38	22	422	7
99 laterals	0.35	0.05	0.03	0.10	0.14	0.05	0.81	17	46	15	379	6
99 fine	1.46	0.21	0.12	0.51	0.51	0.38	3.11	117	59	38	981	19
109 lam	1.81	0.16	0.14	0.18	1.26	0.02	0.79	30	19	13	91	27
109 pet	0.53	0.08	0.11	0.18	2.44	0.03	1.14	56	32	11	145	26
109 new br	0.42	0.06	0.03	0.08	0.31	0.02	0.74	13	32	16	85	8
109 old br	0.33	0.04	0.02	0.06	0.22	0.02	0.35	12	49	15	46	6
109 stem	0.23	0.03	0.02	0.02	0.13	0.01	0.20	6	28	11	40	9
109 stump	0.23	0.03	0.04	0.05	0.15	0.02	0.36	15	35	21	393	9
109 laterals	0.22	0.03	0.03	0.06	0.10	0.03	0.49	16	21	10	474	7
109 fine	1.80	0.28	0.18	0.42	0.45	0.44	2.12	241	76	30	1827	17
56 lam	2.07	0.16	0.19	0.10	1.81	0.03	1.38	46	23	13	133	51
56 pet	0.57	0.07	0.37	0.26	3.89	0.03	2.05	88	47	11	104	23
56 br	0.49	0.07	0.06	0.09	0.24	0.02	0.66	25	50	17	86	11
56 stem	0.31	0.04	0.05	0.05	0.14	0.02	0.32	11	45	14	402	12
56 stump	0.37	0.04	0.05	0.07	0.13	0.02	0.50	31	38	25	484	11
56 lat	0.49	0.06	0.05	0.12	0.13	0.05	0.95	43	34	39	633	12
56 fine	1.56	0.19	0.20	0.62	0.63	0.36	2.15	198	81	36	674	17
57 lam	1.95	0.15	0.19	0.18	2.28	0.02	0.58	49	22	10	168	55
57 pet	0.43	0.07	0.24	0.44	4.17	0.03	0.41	80	45	10	107	22
57 br	0.46	0.06	0.06	0.11	0.26	0.02	0.61	20	42	14	205	11

Appendix 6 ***(continued)***

tree	N %	P %	S %	Mg %	Ca %	Na %	K %	Mn ppm	Zn ppm	Cu ppm	Fe ppm	B ppm
57 stem	0.19	0.02	0.03	0.04	0.08	0.01	0.18	4	26	10	54	7
57 stump	0.30	0.04	0.05	0.06	0.13	0.02	0.37	19	31	17	589	10
57 laterals	0.40	0.06	0.06	0.13	0.15	0.05	0.87	31	30	30	628	9
57 fine	1.47	0.20	0.20	0.68	0.58	0.61	1.58	179	74	26	877	21
82 lam	1.82	0.15	0.17	0.15	2.37	0.02	0.71	66	19	10	154	50
82 pet	0.47	0.36	0.18	0.33	4.33	0.03	0.63	133	37	7	71	22
82 br	0.48	0.05	0.06	0.09	0.23	0.01	0.56	20	35	15	85	9
82 stem	0.37	0.03	0.04	0.07	0.16	0.01	0.41	18	37	14	141	5
82 stump	0.37	0.04	0.04	0.06	0.14	0.02	0.38	36	39	18	702	13
82 laterals	0.48	0.04	0.06	0.11	0.12	0.03	0.77	23	31	14	314	13
82 fine	1.38	0.17	0.14	0.43	0.47	0.39	2.31	197	73	28	1006	21
83 lam	1.83	0.14	0.20	0.11	2.38	0.02	1.06	70	23	10	157	36
83 pet	0.52	0.06	0.24	0.18	4.20	0.03	1.06	111	37	8	102	21
83 br	0.58	0.06	0.07	0.10	0.25	0.01	0.71	25	39	17	94	12
83 stem	0.33	0.03	0.04	0.05	0.14	0.01	0.29	9	28	14	87	12
83 stump	0.38	0.03	0.04	0.06	0.13	0.02	0.42	13	30	16	519	6
83 laterals	0.47	0.03	0.04	0.09	0.10	0.03	0.75	17	24	11	371	4
83 fine	1.21	0.13	0.12	0.37	0.45	0.37	2.44	155	56	23	1183	21
45 br	0.56	0.07	0.06	0.10	0.25	0.02	0.50	14	36	15	74	14
45 stem	0.38	0.04	0.05	0.06	0.15	0.01	0.33	7	29	12	82	13
45 stump	0.36	0.03	0.05	0.05	0.13	0.02	0.38	13	34	29	512	13
45 laterals	0.58	0.04	0.05	0.08	0.12	0.03	0.66	17	29	22	407	11
45 fine	1.21	0.10	0.10	0.34	0.46	0.67	2.09	105	41	47	3246	31
53 br	0.68	0.08	0.07	0.13	0.25	0.02	0.59	18	45	22	85	17
53 stem	0.47	0.06	0.05	0.07	0.18	0.02	0.34	11	35	21	217	15
53 stump	0.43	0.04	0.05	0.05	0.14	0.02	0.32	9	40	41	240	12
53 laterals	0.59	0.05	0.06	0.13	0.16	0.04	0.71	27	33	37	404	12
53 fine	1.41	0.14	0.15	0.48	0.52	0.74	1.73	104	61	59	1643	22
113 br	0.59	0.07	0.07	0.10	0.22	0.02	0.72	25	40	10	62	13
113 stem	0.36	0.03	0.05	0.06	0.14	0.01	0.41	15	33	12	60	13
114 br	0.59	0.07	0.07	0.10	0.24	0.02	0.76	38	38	9	169	14
114 stem	0.40	0.05	0.06	0.07	0.15	0.01	0.43	11	31	8	75	11

Appendix 7. Growth parameters of *Paulownia* seedlings grown in hydroponics.

Species	CF	Shoot FW (g)	Root FW (g)	Collar (mm)	Shoot ht (cm)	Root length (cm)	Leaf area (cm)	Shoot DW (g)	Root DW(g)
purple fort	15	5.7	4	3.6	12.5	58	254.1	0.7	0.3
purple fort	15	10.1	6.5	4	14.5	64	477.8	1.4	0.5
purple fort	15	20.2	13.1	5	18	45	976.2	3.7	1
purple fort	15	36.2	22	6.2	24	75	1383	5.3	1.7
purple fort	15	12.8	7.6	4	21	77	435.4	1.6	0.7
purple fort	15	9.2	4.6	3.4	17	38	409.5	1.1	0.4
tom	15	11.2	11.2	6.5	7	27	502.4	2.5	1.1
tom	15	5.5	3.4	3.7	6	23	264.5	0.6	0.3
tom	15	1.4	0.8	2.4	4.5	12	74.9	0.3	0.1
tom	15	2.2	1.2	2.2	6	19	109.4	0.3	0.1
tom	15	1.5	0.6	2.3	4.5	13	71.6	0.1	0.04
white fort	15	29	23	4.9	14	27	1254.8	5.4	2
white fort	15	7.1	3.4	4.1	11	23	340.3	0.8	0.2
white fort	15	29.5	14.5	5.8	20	58	1248.2	4.2	1.3
white fort	15	3.6	2.8	3.6	8	27	132.7	0.4	0.2
white fort	15	3.1	1.8	2.6	9	31	134.6	0.3	0.1
white fort	15	12.5	7.3	4	17	32	540	1.6	0.6
white fort	15	3.5	1.9	2.9	5.5	12	151.6	0.4	0.2
white fort	15	2.9	1.8	3.6	8.5	38	129.3	0.3	0.2
white fort	15	4.1	1.8	3.9	9	13	175.8	0.4	0.2
farg	15	5	2.5	2.9	10	28	232.4	0.6	0.2
farg	15	2.4	2.2	2.2	6	48	110.2	0.4	0.2
farg	15	5.1	3.1	4.1	11.5	40	192.7	0.6	0.3
farg	15	18.1	9	5	13	34	768.5	2.7	0.9
farg	15	4.7	1.8	2.2	9	17	201.2	0.6	0.2
farg	15	5	3.5	3	7	27	196.8	0.9	0.3
farg	15	34.7	18.2	5.5	20	55	1402.9	5.7	1.6
elong	15	2.9	1.6	3.4	6.5	25	124.5	0.4	0.1
elong	15	10.3	8.5	5.2	12.5	49.5	525.4	1.6	0.6
elong	15	3.2	7	4	11	16.5	313.4	0.8	0.3
elong	15	4.7	2.5	4	8	17	194	0.6	0.1
elong	15	1.6	0.4	3	5.5	10.5	81.9	0.4	0.3
elong	15	2.6	1.5	3	6	14.5	119.5	0.3	0.1
elong	15	2.4	1.4	3.1	6	21.5	130.2	0.3	0.08
elong	15	4.4	1.8	3.8	8.5	15	222.6	0.6	0.1
purple fort	30	33.6	12.3	5.4	45	45	1128.2	4.4	1
purple fort	30	39.6	17.3	6	40	44	1595.2	6.5	1.6
purple fort	30	57.1	29.7	6.7	21	47	2270	10.7	2.5

Appendix 7 ***(continued)***

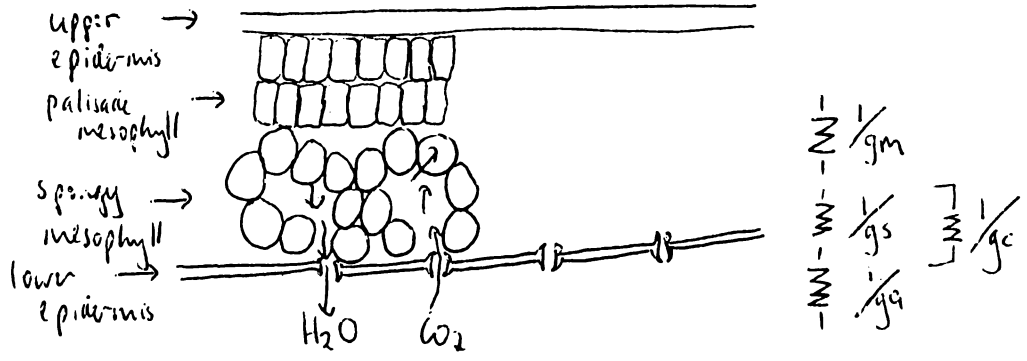
Species	CF	Shoot FW (g)	Root FW (g)	Collar (mm)	Shoot ht (cm)	Root length (cm)	Leaf area (cm)	Shoot DW (g)	Root DW(g)
purple fort	30	29.6	15.8	5	22	26	1253.9	5.7	1.2
purple fort	30	11.1	6.2	4	10	57	512.9	2.4	0.6
purple fort	30	55	40.6	7.8	34	99	2187	8.7	3.2
purple fort	30	31.6	15.6	5.1	34	90	1306.4	5.4	1.3
tom	30	61.1	36.4	7.4	31	51	2526.4	9.8	2.9
tom	30	34.7	18.9	6	15	47	1362.8	5	1.6
tom	30	136.5	73.9	10.6	37	51	4439	19.7	6.2
tom	30	53	64.3	9.6	12.5	45	3827	10.6	5.5
tom	30	22.3	13.9	7.8	9	39.5	890.7	4	1.4
tom	30	17.7	13	4.7	12	33	878.2	2.8	1.3
tom	30	31.9	26.9	6.8	12	61.5	1454.6	5.1	2.7
tom	30	66.3	50.8	9.4	22	64	2540.6	11.1	4.2
tom	30	110.2	100	10.3	32	80	6000.4	16.2	7.5
white fort	30	121	70.9	12.5	52	32.5	4766	23.7	6.6
white fort	30	39	25.2	6.8	22	46	2915.6	5.6	1.8
white fort	30	67.8	31.4	8.6	41	66	2492.4	10.8	3.3
white fort	30	74.9	46.5	8.2	41	89	2715.6	12.9	3.6
white fort	30	70.7	32.6	10.6	31	76	2457	11.6	3.6
white fort	30	84.4	39.2	9.2	37	45	3171.8	15.2	4
white fort	30	134.4	60.4	12	55	95	4444.4	28.1	6.6
white fort	30	79.8	36.4	10.6	44	84	2780.6	15.9	3.6
white fort	30	57.9		10.5	45	65	1853.4	11.6	2.3
farg	30	24.2	11.5	9.3	23	38.5	908.2	3.8	1
farg	30	64.8	22.6	6.3	34	70	2194.6	8.9	1.8
farg	30	69.2	63.8	7.9	27	84	2542.6	13.4	4.3
farg	30	15.6	8.2	4.7	18	52	589.6	2.1	0.7
farg	30	7.1	4.1	5	11	35	217.8	1.3	0.4
farg	30	82.4	31.2	6.3	40	85	2309.6	11.5	2.7
farg	30	77.5	69.8	8.1	28	48	2659	13.3	5.1
farg	30	11.8	6.1	4.8	14	39	451.4	1.5	0.5
elong	30	83	53.9	7.6	35	67	2767	12.5	3.7
elong	30	82.5	72	8.3	29	56.5	2680	13.8	5.1
elong	30	114.6	50.2	8	37	51	3802	29.6	3.9
elong	30	74.2	27.6	7.5	37	25	2480.2	11.5	2.2
elong	30	65.8	33.9	7.6	32	75	2039.2	10.5	2.7
elong	30	19.4	18	4.4	11	40	955.4	3.7	1.5
elong	30	11.5	5.5	3.6	16	35	495.8	1.5	0.5

Addendum

Ad.1 The Relative Importance of the Boundary Layer

The diffusion pathway of gases through a leaf involves a series of conductances, where the conductance is the inverse of resistance. As shown in Fig. Ad.1, the outermost of these is the conductance resulting from the boundary layer of the leaf. The thickness of the boundary layer is a function of leaf size and wind speed (Fig. Ad.2, Table Ad.1). The boundary layer conductance (g_a) is often not constant across a leaf, varying from the edge to the middle of the leaf. The stomatal conductance (g_s) is controlled by the operation of the guard cells which respond to internal and environmental cues. It is possible for gases to diffuse through the cuticle but in many leaves the cuticular conductance (g_c) is so small that diffusion through the cuticle is negligible. Within the leaf, CO_2 must reach the sites of carboxylation within the mesophyll cells. The conductances of that pathway are often collectively called the mesophyll conductance (g_m).

Fig. Ad.1. Pathway of gaseous diffusion in a *Paulownia* leaf where g_a is the boundary layer conductance, g_s the stomatal conductance, g_c the cuticular conductance and g_m the mesophyll conductance.



Typical values for each of these conductances in *Paulownia* leaves have been either calculated or measured. g_a was calculated by the equation presented by Gates and Papian (1971) such that

$$1/g_a = k_2 . W^{0.02} . D^{0.35} / V^{0.55} \qquad \text{Eqn. Ad.1}$$

where g_a is the boundary layer conductance, measured in cm min^{-1}

k_2 is a constant, value of 35×10^{-3} when W and D > 10 cm

W is the dimension of the leaf at the wind angle x 0.7, measured in cm

D is the opposite leaf dimension, measured in cm

V is the wind speed, measured in cm sec^{-1}.

Values for g_a were calculated for a range of *Paulownia* leaf sizes and wind speeds (Table Ad.1). The smallest leaves (20 cm x 20 cm) were typical of many leaves on the trees at the Matangi field site in seasons 1 and 2, and the majority of the leaves in season 3. In the first two seasons, some trees had several leaves in the second size category, while the largest leaves (60 cm x 60 cm) were generally confined to the trees used in the glasshouse experiment (Chapter 3) and a very few leaves on the field grown trees in season 1. The wind speeds used in the calculations represent conditions ranging from a very light wind, most likely to occur in the morning of a fine day in the Waikato, to moderately windy conditions which would commonly occur in the study area. The field grown trees were often subjected to higher wind speeds (above 30 km hr^{-1}). The g_a values calculated by this method were higher than those calculated by Grace (1977) using an alternative technique, as shown in Fig. Ad.2.

Table Ad.1. Boundary layer resistance ($1/g_a$), conductance (g_a) and thickness (δ_a) for leaves of different sizes in different wind conditions.

Leaf size (m)	Wind Speed ($m\ s^{-1}$)	Wind Speed ($km\ hr^{-1}$)	$1/g_a$[1] ($s\ m^{-1}$)	g_a ($mol\ m^{-2}\ s^{-1}$)	δ_a[2] (mm)
0.2 x 0.2	1	3.6	75	0.53	1.8
0.2 x 0.2	3	10.8	41	0.98	1.0
0.2 x 0.2	5	18.0	31	1.28	0.8
0.4 x 0.4	1	3.6	114	0.35	2.5
0.4 x 0.4	3	10.8	60	0.67	1.5
0.4 x 0.4	5	18.0	46	0.87	1.1
0.6 x 0.6	1	3.6	144	0.28	3.1
0.6 x 0.6	3	10.8	78	0.51	1.8
0.6 x 0.6	5	18.0	59	0.68	1.4

1 - determined by the equation presented by Gates and Papian (1971)
2 - determined by the equation presented by Nobel (1983)

The calculation of the thickness of the boundary layer employed Eqn. Ad.2 (Nobel, 1983).

$$\delta_a = 4.0\sqrt{L / V} \qquad \text{Eqn. Ad.2}$$

where δ_a is the thickness of the boundary layer next to a flat leaf, measured in mm
L is the mean length of the leaf in the downwind direction, measured in m
V is the wind speed, measured in $m\ s^{-1}$.

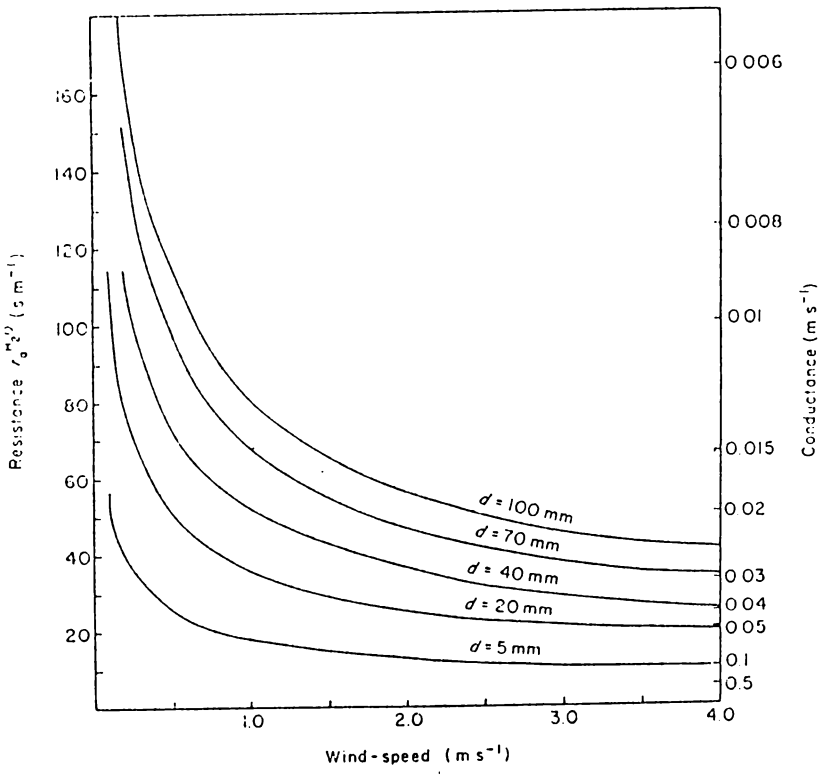


FIG. Ad.2 Boundary layer resistance calculated from $Sh = 0{\cdot}6\,Re^{0{\cdot}5}$, for various leaf dimensions, d, (length parallel to wind direction). The calculation is for a single surface: if it is required to treat the leaf as two surfaces with a total area equal to the plan area, then the calculated value must be halved. (Grace, 1977)

The boundary layer conductance used in the calculations of the data from the Walz photosynthesis unit was that of the cuvette used to enclose a portion of the leaf. The value of g_a for the cuvette was measured at 1.123 mol m^{-2} s^{-1} (Buwalda, pers. comm.) which was higher than the conductances calculated for all the leaf sizes at all wind speeds except the smallest leaf at the highest wind speed. The g_a for the cuvette was equivalent to that for a 20 cm x 20 cm flat leaf in a wind of 4 m s^{-1}, a 40 cm x 40 cm leaf in a wind of 8 m s^{-1} (29 km hr^{-1}), and a 60 cm x 60 cm leaf in a wind of 12 m s^{-1} (43 km hr^{-1}). Thus as leaf size increased, the cuvette g_a was a greater overestimation of leaf g_a. This overestimation would most affect the data from the pot grown plants (Chapter 3) as these trees had the largest leaves. For the leaves on the field grown trees, the g_a used in the calculations assumed moderately windy conditions. These calculations suggest that the boundary layer conductance for leaves inside the cuvette was greater than the values estimated for leaves growing in the field under most conditions. It is important, therefore, to exercise caution when extrapolating measurements of gas exchange made inside the cuvette directly to conditions occurring naturally in the field. Errors would be greater as leaf size increases in field conditions.

g_s was measured using the Walz CMS 400 photosynthesis system and typically ranged between 0.2 and 0.5 mol m^{-2} s^{-1}. The cuticular conductance was determined by measuring water loss from detached leaves over several hours. g_c in these leaves averaged 1.5×10^{-4} (s.e. - 0.4×10^{-4}) m s^{-1} and hence it is unlikely that there is any significant diffusion through the cuticle in *Paulownia* leaves. From the A/C_i experiments it was possible to estimate g_m for CO_2 diffusion into the sites of carboxylation at between 0.05 and 0.13 mol m^{-2} s^{-1}, assuming that g_m is four times less than g_s.

It is possible to determine the relative effects of g_a and g_s on leaf temperature by applying energy balance equations. The energy absorbed by a leaf must be exactly balanced by the energy released and stored by the leaf (Eqn. Ad.3, Grace, 1983).

$$R = \lambda.E + C + S + G + P \qquad \text{Eqn. Ad.3}$$

where R is the net heat absorbed by the leaf

λ is the latent heat of vaporisation and E is the evaporation rate

C is the heat loss by convection (sensible heat loss)

S is the rate of physical heat storage in the leaf

G is the conduction of heat down the petiole

P is the storage of heat within the chemical bonds of the compounds within the leaf.

It is possible to simplify this to $R = \lambda.E + C$ if the net energy balance of the leaf is considered over a time period of minutes, rather than seconds (Grace, 1983).

The boundary layer conductance to heat transfer is important to the rate of heat loss by convection as shown by Eqn. Ad.4. Both the boundary layer and stomatal conductances to water loss are involved in the loss of heat by evaporation (Eqn. Ad.5). If it is assumed that the transfer conductances for sensible heat and water vapour are the same, then g_a becomes the same in both equations.

$$C = pc_p.(T_l - T_a) / (1/g_a^{heat}) \qquad \text{Eqn. Ad.4}$$

where pc_p is the volumetric heat capacity of air (in J m^{-3}, where p is the density of air in kg m^{-3} and

c_p is the specific heat of air in J kg^{-1})

T_l is the leaf temperature (in °C)

T_a is the air temperature (in °C)

g_a^{heat} is the boundary layer conductance to heat transfer (Grace, 1983).

$$\lambda E = pc_p.VPD / \gamma(1/g_s + 1/g_a) \qquad \text{Eqn. Ad.5}$$

where VPD is the vapour pressure deficit between the inside of the leaf and the air (in mbar)

γ is the psychrometric constant (0.66 mbar $°C^{-1}$)

g_s is the stomatal conductance to water loss

g_a is the boundary layer conductance to water loss (Grace, 1983).

From Eqn. Ad.4 and Eqn. Ad.5 it can be seen that transpiration, heat transfer from the leaves and leaf temperature are dependent on the ratio of the conductances g_s and g_a. As g_s increases in relation to g_a, transpiration (λ.E) becomes more dependent on the value of g_a. However, as g_a increases the convective heat loss (C) from the leaf also increases. When g_a is small, as is the case for large leaves in conditions of low wind speed, convective heat loss is reduced which results in an increase in leaf temperature relative to air temperature. As g_a increases, transpiration is determined to a greater extent by g_s. In a leaf with dimensions of 60 cm x 60 cm, in a light wind (1 m s^{-1}) g_a is 6.9 x 10^{-3} m s^{-1}. A typical g_s would be 7.5 x 10^{-3} m s^{-1} (= 0.3 mol m^{-2} s^{-1}), so that g_s contributes approximately 50% of the conductance to water loss by evaporation. An increase in stomatal aperture to the extent that g_s increases by 50% (to 15 x 10^{-3} m s^{-1}) leads to an overall increase in total conductance of 25%. Thus it is likely that even in large leaves on a still day, the increase in g_s is significant in the control of the energy balance of the leaf. As wind speeds increase and leaf sizes decrease, the g_a increases and so increases in g_s become even more significant in the energy balance of the leaf.

Ad.2 Whole Tree Carbon Budget

The extrapolation of instantaneous photosynthetic rates of portions of single leaves within the canopy of a juvenile *Paulownia* tree to an evaluation of the carbon budget of the whole tree requires that estimations be made of the total carbon assimilated by all the leaves over time; of the rates of maintenance and growth respiration of different organs; and of the carbon partitioning between organs.

In order to estimate the carbon assimilated on a daily basis, it is necessary to know the solar angle, the radiation incident on the canopy, radiation attenuation through the canopy, the radiation response of photosynthesis and the leaf area (Buwalda, 1991). The radiation response of each leaf will vary according to leaf age. It is possible to calculate the daily carbon assimilated by specific trees if a number of assumptions are made. In March of season 2, the average time each day when the incident radiation was above saturation for photosynthesis in *Paulownia* leaves was three hours (Table 4.2). The photosynthetic rate for healthy, fully mature leaves at that time was approximately 16 µmol m^{-2} s^{-1}. The leaf area for tree 8 in late March was 7 m^2, with a leaf area index (LAI) of about 3. It may be assumed that for 3 hr each day 40% of the leaves had a photosynthetic rate of 16 µmol m^{-2} s^{-1}, and that the remaining 60% of the leaves A was 8 µmol m^{-2} s^{-1}. For the remaining 9 hr of the day, the assumption is made that for both sets of leaves, photosynthesis proceeded at half the maximum rate. The estimated amount of carbon assimilated on a March day by tree 8 in season 2 is then 90 g C d^{-1}. A similar calculation for tree 109 in January, 1993, with a leaf area of 8 m^2, LAI of 2, maximum photosynthetic rate of 17 µmol m^{-2} s^{-1}, and 5 hours of saturating radiation per day produces an estimate of 154 g C d^{-1}. These estimates are about twice as large as those given by Buwalda (1991) for seasonal carbon assimilation in kiwifruit. The assumptions made in the calculations for *Paulownia* assimilation are very broad and a more accurate estimation would require

integration of the radiation levels over a typical day and measurement of A over a wide range of leaves. The employment of a whole plant cuvette would greatly assist in the procurement of such data.

Measurement of the respiration rates were not made in this study, apart from an estimation of lamina maintenance respiration at about 1 μmol m^{-2} s^{-1}. It is possible to estimate maintenance respiration rates of both perennial and annual organs based on the nitrogen content, biomass and temperature (Buwalda, 1991):

$$R_{mi} = m_i.C_i.e^{ri.(T-20)} \qquad \text{Eqn. Ad.6}$$

where R_{mi} is the daily carbon requirement for maintenance respiration of organ *i*

m_i is a maintenance respiration coefficient (g C g^{-1} C d^{-1}) for organ *i*

C_i is the current biomass of organ *i* (measured in g)

r_i is the temperature response coefficient for organ *i*

T is the mean daily temperature (measured in °C)

The relationship between *m* and nitrogen concentration is assumed to be linear, at 20°C, such that:

$$m_i = 0.4\ N_i\ d^{-1} \qquad \text{Eqn. Ad.7}$$

where N_i is g N g^{-1} C (Buwalda, 1991).

Applying these equations to the data presented in Table 4.12, it would be expected that the maintenance respiration rates of the lamina would be higher than that for any other organ. Fine root rates would be slightly lower and the rates of other organs would generally be between 10% and 40% of the rate for the lamina. By determining the amount of biomass in each organ relative to that in the lamina and the relative nitrogen concentrations of the different organs (Appendices 2 and 5) and assuming a maintenance respiration rate of 1 μmol m^{-2} s^{-1} in the lamina tissue, it is possible to calculate the daily amount of carbon respired by a whole tree. For tree 8 in March of season 2, that amount was 44 g C d^{-1}, which is approximately 50% of the carbon assimilated by the tree.

In order to determine the amount of carbon respired as a result of tree growth, it is necessary to know the growth rates of different organs. As can be seen from the data in Appendices 1 - 3, the variability between the *Paulownia* trees harvested in this experiment precluded the determination of growth rates of the various organs. It is also important that such calculations also take into account the turnover of annual tissues during the season. In *Paulownia* trees both the fine roots and leaf tissues do not survive the entire season. Analysis of total biomass of these tissues at any one time in the season is likely to underestimate the growth rates of these organs as it ignores the ongoing processes of initiation, maturation, senescence and death.

The partitioning of the carbon remaining after maintenance respiration will be influenced by sink strength. Actively growing organs will be strong sinks, but it is also important to consider the storage roles of different organs. The balance between growth and storage will change over the season with

most available carbon being consumed by growth of new tissues early in the season. Later in the season, in autumn, growth rates decline and much carbon is translocated to the perennial organs for storage. At this time it is expected that the total amount of carbon within the tree would increase.

The correlation between photosynthetic rate measured on a leaf by leaf basis and overall tree growth will be poor, if such a high percentage of carbon assimilated is lost as maintenance respiration. In order to more fully understand the dynamics of the relationship between assimilation and growth, it would be necessary to more comprehensively study all the components of the carbon balance equation.

Ad.3 Statistics

Standard errors have been calculated for the data presented in Tables 2.13 and 2.19. It should be noted, however, that in some instances that only two replicates were available for statistical calculations. Error bars (representing $\pm$ 1 s.e.) have been added to Figs. 3.2, 4.4, 4.5 and 4.9, and are based on at least three replicates.

In the first major set of experiments, those measuring photosynthetic rates under varying environmental conditions in leaves of different ages, insufficient replicates were measured to allow for statistical analysis of the the data (refer to Section 2.4.3.2.). Thus the significance of the differences between treatments was not able to be measured. Future studies on photosynthetic rates in *Paulownia* leaves would need to be designed in such a way as to incorporate sufficient replicates as to allow for statistical analysis. In order to gain an overall picture of photosynthesis in this tree, a large number of well replicated experiments need to be undertaken studying leaf age effects, tree age and species effects, seasonal changes and environmental influences.

The biomass and nutrient experiment was initially planned in conjunction with a statistician who recommended the two fertilizer treatments with two replicates being harvested from each treatment at each harvest time. However, there was large variability between the growth rates of the trees within the field trial and it was decided at the beginning of season 2 to discontinue the fertilizer treatment (Tables 4.7 - 4.9, Appendices 1 - 3). It was decided that presentation of the data from all the individual trees harvested was more meaningful than averaging results from widely different individuals.

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