EFFECTS OF MINERAL NUTRIENT SUPPLY ON NEEDLE GROWTH AND PHOTOSYNTHESIS IN SITKA SPRUCE (*PICEA SITCHENSIS* (BONG.) CARR.)

by

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DECLARATION

I hereby declare that the work described in this thesis has been carried out by myself, except where specific reference is made to other sources, and that it has not been submitted in whole or in part, for any other degree.

John W. Chandler
Edinburgh, 1989

"Are you a botanist Dr. Johnson?"

"'No sir, I am not a botanist;' and (alluding no doubt to his nearsightedness) 'should I wish to become a botanist, I must first turn myself into a reptile.'"

Samuel Johnson 1709–1784.

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ABBREVIATIONS

A	Absorbance
a	Projected leaf area (cm^2)
ABA	Abscisic acid
ANOVAR	Analysis of variance
°C	Degrees celsius
C	Carbon
Ca	Calcium
C_c	Partial pressure of CO_2 in air leaving cuvette, corrected for the diluting effect of water vapour picked up in the leaf cuvette
C_e	Partial pressure of CO_2 in air entering cuvette (µbar)
C_i	Intercellular partial pressure of CO_2 (µbar)
Ci	Curie
C_o	Partial pressure of CO_2 in air leaving cuvette, as measured by the analyser (µbar)
C_p	Specific heat at constant pressure (=1.012 J g^{-1} K^{-1})
c.p.m.	Counts per minute
d.f.	Degrees of freedom
d.p.m.	Decays per minute
Dry wt.	Dry weight
e_l	Saturated vapour pressure at leaf temperature (bar)
e_o	Vapour pressure of water of air in cuvette (bar)
e_s	Saturated vapour pressure at cuvette air temperature (bar)
E	Transpiration rate (mol H_2O m^{-2} s^{-1})
EDTA	Ethene diamine tetracetic acid
Fr.wt.	Fresh weight
g	Gramme
G_b	Boundary layer conductance to water vapour (mol m^{-2} s^{-1})
G_M	Mesophyll conductance to CO_2 (mol m^{-2} s^{-1})
G_S	Stomatal conductance to CO_2 (mol m^{-2} s^{-1})
$G_{s\ water}$	Stomatal conductance to water vapour (mol m^{-2} s^{-1})
h	Hour
h_c	% relative humidity in cuvette
H	Radiation absorbed by the leaf (J m^{-2} s^{-1})
H	Hydrogen
ha	Hectare(s)
K	Potassium
K_M	Michaelis-Menton constant
l	Litre(s)
LSD	Least significant difference
λ	Latent heat of vaporisation of water (45032 J mol^{-1} at 0°C, decreasing to 42906 J mol^{-1} at 50°C)
m	Metre(s)
M	Molar
M_a	Molecular weight of air (28.97)
Mg	Magnesium
n	Number

N	Nitrogen
Na	Sodium
O	Oxygen
P	Phosphorus
P_a	Atmospheric pressure (bar)
PEPC	Phosphoenol pyruvate carboxylase
pH	Negative log of the hydrogen ion concentration
P-I	Photosynthesis-irradiance
P_N	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1})
Pp	page
p.p.m.	Parts per million
S	Sulphur
Q	Photon flux density incident on cuvette (μ mol m^{-2} s^{-1})
$r_{b\ water}$	Boundary layer resistance to water vapour (m^2 s mol^{-1})
r_M	Mesophyll resistance to CO_2 (m^2 s mol^{-1})
r_S	Stomatal resistance to CO_2 (m^2 s mol^{-1})
$r_{s\ water}$	Stomatal resistance to water vapour (m^2 s mol^{-1})
RUBISCO	Ribulose 1,5-bisphosphate carboxylase/oxygenase.
RuBP	Ribulose bisphosphate.
RuBPC	Ribulose 1,5-bisphosphate carboxylase.
s	Second(s)
S.D.	Standard deviation
S.E.	Standard error
σ	Stefan Boltzman constant (5.7 x 10^{-8} W m^{-2} k^{-1})
spp	Species
t_a	Cuvette air temperature (°C)
Δt	Temperature difference between leaf and air(°C)
t_l	Leaf temperature (°C)
V	Volume flow rate of dry air into cuvette (cm^3 s^{-1})
v/v	Volume per volume ratio
W	Watts
W-(f)	Mass flow of dry air per unit leaf area (mol m^{-2} s^{-1})
w/v	Weight per volume ratio (as percentage)
y	Year
%	Percent
</>	Less than/greater than
*	Significance at $p < 0.05$
**	Significance at $p < 0.01$
***	Significance at $p < 0.001$

Prefixes

d	deci (10^{-1})
c	centi (10^{-2})
m	milli (10^{-3})
μ	micro (10^{-6})

CONVERSION OF UNITS

For net photosynthetic rate;

$$1\ \mu mol\ CO_2\ m^{-2}\ s^{-1} \equiv 1.584\ mg\ CO_2\ dm^{-2}\ h^{-1}$$

For stomatal resistance to CO_2;

$$2.44\ m^2\ s\ mol^{-1} \equiv 1\ s\ cm^{-1}$$

EXPLANATION OF TERMS

Coefficient of variation – The standard deviation expressed as a percentage of the mean.

Whorl number – Whorls are counted basipetally from the tree top; growth at the apex of a tree in any one season consists of a leading shoot and an apical whorl of branches (whorl 1), with increasing whorl numbers being branches arising from each node moving basipetally down the trunk. The whorl number of a particular branch increases by one every year, as a new apical whorl is produced.

Terminal shoot of whorl x ≡ First order lateral of whorl x

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ABSTRACT

The aims of this project were to study the effects of mineral nutrient supply on the growth, and development of photosynthetic capacity of needles of Sitka spruce (*Picea sitchensis* (Bong.) Carr.).

Needle development was studied in field material, using healthy mature trees (Controls), and trees deficient in P, K or all nutrients. Needle dimensions, fresh weight, projected surface area, and dry weight:fresh weight ratio were determined, according to needle position on the shoot, throughout the growth season, and needle growth was also characterised in terms of cell size and number. Variation in material was great, but cell division lasted for about 3 weeks following bud burst in early May, with cell expansion continuing for a further 3 weeks until mid June. The greatest reduction in all variables measured was with P deficiency. Cell size and cell number were equally important in controlling final needle size, although despite slight treatment differences in cell size, differences in final needle size with needle position and nutrition were determined by differences in cell number. Final needle size was partly predetermined by primordium cell number, as a result of nutrient conditions during primordium initiation, but refertilisation of N-deficient potted trees showed that limitations to needle growth could be overcome by supplying nutrients during the period of needle expansion.

The development of photosynthetic capacity was studied using healthy 2-3 year old potted seedlings (Controls), and seedlings where deficiency of Mg, K, P, N or all nutrients had been induced by differential fertilisation, and following restoration of these trees to full nutrient supply. Some morphological characteristics of needle development were also monitored, for comparison with field trees. Net photosynthetic rate was studied throughout the season, as well as pigment content, activity of ribulose 1,5-bisphosphate carboxylase, stomatal conductance to CO_2, and the intercellular partial pressure of CO_2. Most photosynthetic variables showed an increase throughout the season, with a slight or more obvious decrease in August/September. Some treatment differences existed for the photosynthetic variables, and N-deficiency caused the largest inhibition to photosynthesis and growth, with fewer effects on needle growth being found with other nutrient deficiencies than in the field. The differences in photosynthetic variables and the correlations between them,

are discussed with reference to their importance in determining and limiting photosynthetic capacity. Major limitations vary with time in the season, and with treatment, but stomatal conductance and activity of ribulose 1,5-bisphosphate carboxylase appear to be important towards the end of the season.

The effect of N nutrition on needle growth and photosynthesis was studied in more detail, by refertilising N-deficient potted trees with a range of N concentrations. Ribulose 1,5-bisphosphate carboxylase activity limited photosynthetic rate, but chlorophyll content was of major importance. The 'plasticity' of photosynthetic development in response to nutrient supply was shown by refertilisation of N-deficient and totally nutrient deficient trees, which caused free growth of shoots to occur, and a rapid increase in photosynthetic capacity of current and one year old needles.

CHAPTER 1. INTRODUCTION

1.1 Sitka Spruce as a Species and a Crop.

Sitka spruce (*Picea sitchensis* (Bong.) Carr.), is an evergreen conifer native to N. America. It is the largest and most imposing of all the spruces, often growing to a height of 60m in the British Isles, and up to 80m in America (Morton and Lewis, 1949), and living for up to 750 years (Malcolm, 1987). The natural range of Sitka spruce extends for about 20° of latitude of N. America, from northern California to southern Alaska, and is restricted to a narrow coastal belt characterised by low elevations and moist soils (Harris, 1978). Sitka spruce is fast growing under these conditions, often forming leading shoots up to 1.5m long in a single season (Dallimore and Jackson, 1954), and producing a large volume of timber per unit area. Sitka spruce was introduced into Great Britain in 1831 (Fletcher and Faulkner, 1972), although widespread planting did not occur until after the first World War. Between 1911 and 1920, 200 ha of Sitka spruce were planted in Great Britain, and between 1961 and 1970, this figure was 86,100 ha, representing 5.3% and 41.7% respectively of the total area planted by the Forestry Commission (Fletcher and Faulkner, 1972). In 1984, 4,592 ha of Sitka spruce were planted in Britain, representing 68% of the total area planted (Steele, 1987), making it the most widely planted tree species in the United Kingdom, and particularly in Scotland; in 1980, the total area of Sitka spruce in Scotland was 48% of the total coniferous high forest area (Low, 1987). It has also been planted extensively in northern Europe (Harris and Ruth, 1970). Sitka spruce is a valuable timber crop, and was used for aircraft construction during the First World War, and also for building rowing boats, oars, and for the sound boards of pianos (Hyde, 1961). Although the timber is strong for its weight, it has a low density, and is not very suitable for constructional purposes. However, it is one of the best all-purpose pulping species in the world (Cahalan, 1987), and has been used in Britain for pulp for the paper industry since the 1960's, and also in the manufacture of particle board and fibreboard (Brazier, 1987).

1.2 Productivity and Nutrition.

Sitka spruce seedlings are fairly slow growing, but after a few years, stands are extremely productive, even on poor upland soils, and the highest mean stem volume increments are 50-100% larger than those achieved by Norway

spruce in continental Europe (Christie and Lines, 1979). Sitka spruce is one of the highest yielding tree species in Britain, and published productivity values under normal management treatment are between 6 and 24 m^3 ha^{-1} y^{-1} stemwood (Malcolm, 1987). Ford (1982) estimated net annual above-ground dry matter production for a 17 year old stand in southern Scotland to be over 25 t ha^{-1} y^{-1}, which approaches the highest values for north temperate forests, or even well tended agricultural crops (Cannell, 1987).

Sitka spruce can tolerate a wide range of soil conditions, but as it is often grown on poorly fertile upland soils, the major factor limiting productivity is insufficient amounts of one or more mineral nutrients. Sitka spruce seedlings in a plantation are relatively nutrient-demanding (Miller, 1981), and phosphate is often needed (Dickson, 1971). Binns (1962) showed that potassium is also required on poorer peat soils, and in the presence of heather, Sitka spruce seedlings suffer a check in growth, which is due to inadequate nitrogen uptake (Weatherell, 1953). Early growth of Sitka spruce has been improved due to planting it along side "nursing" species (larch or Lodgepole pine), which causes a greater availability of nitrogen in the soil. The fertiliser needs of young Sitka spruce in Britain have been reviewed by McIntosh (1981), who lists whether application of N, P or K is required for a particular age of tree on a particular soil. Once canopy closure has occurred, no fertiliser inputs are usually required, due to efficient nutrient recycling, including recovery of nutrient from dying tissue, and from the developing litter layer (Miller, 1981).

Ingestad (1971) has stressed that the nutrient requirement of trees is satisfied when all the essential minerals are present in the plant in optimum proportion, and the ratio of the nitrogen sources (NH_4^+ or NO_3^-) in the nutrient solution is at an optimum. Hocking (1971) surveyed the literature on nutrition studies in conifers, and found concentrations giving good growth were over a wide range, as follows:

Element	Concentration (p.p.m.)
N	25-250
P	1-200
K	50-500
Mg	15-90

Hocking (1971, 1972) developed a nutrient solution based on studies of

nutrient requirements of Lodgepole pine and White spruce in sand culture, and found that the optimum concentrations were the same for both species; 112 p.p.m. N, 31 p.p.m. P, 156 p.p.m. K, and 48 p.p.m. Mg. Van den Driessche (1968) compared the growth responses of Sitka spruce and Douglas fir in sand culture, to 5 concentrations of N, P and K. In terms of relative growth rate, and net assimilation rate, optimum concentrations for Sitka spruce were found to be between 50 and 200 p.p.m. N; 15-30 p.p.m. P and < 200 p.p.m. K. The foliar nutrient concentrations of healthy Sitka spruce seedlings from one to three years old, grown at a range of sites in S.E.England are reported by Benzian and Smith (1973).

The balance of mineral elements is important, as well as the optimum concentration, and from sand culture experiments it is clear that the N/P ratio should be > 1, and the P/K ratio always < 1, and the best proportions for conifers seems to be; N, 4-10; P, 1; K, 1.5-2.0; Ca, 5-10; Mg, 1-4, and S, 2-4 (Brix and Van den Driessche, 1974), although Ingestad (1959), who studied the growth of *Picea abies* at four to six different concentrations of each major mineral nutrient, found the absolute requirement varied in the order N > K > P > Mg > Ca. The source of nitrogen is also important, and for most conifer seedlings, ammonium nitrogen was found to cause greater growth than nitrate nitrogen, (McFee and Stone, 1968; Benzian, 1965; Ingestad and Molin, 1960), however, mixtures of both nitrogen sources may cause greater growth than either alone (Christersson, 1972). Leyton (1952) found little effect on dry weight production of Sitka spruce seedlings whether ammonium or nitrate was used, but in either case the optimum pH was between 4 and 5. The cyclic variation in N, P, K and Ca expressed as a dry weight percentage in needles of *Pinus sylvestris* and *Picea abies* has been shown by Tamm (1955), with a decrease in spring and early summer in all needle age classes. This was attributed partly to an increase in dry matter of the needles during spring and early summer, and a decrease in the amount of dry matter at the end of the summer, and also to some retranslocation of nutrients. Fluctuating soil nutrient levels may be compensated for by translocation of nutrients from older needles to the required sink, and the content of N, P and K in needles decreases with needle age (Tamm, 1955). Although much work has been done to maximise early growth of Sitka spruce in the field using fertiliser application, and in determining the optimum nutrient requirements, the decrease in productivity due to nutrient deficiency has not been quantified at the basic level of growth

of the needle, and its photosynthetic functions, neither has the role of mineral nutrients as yield determining factors.

Productivity is dependent on the rate of leaf area production per tree, and this is determined by the number of shoots, the number of needles per shoot, needle size, and longevity. The high productivity of Sitka spruce after canopy closure is partly due to the large number of shoot apices, and therefore a large amount of foliage which is quickly generated. Numerous branch buds are produced from cortical tissues between the leaves (Cannell and Bowler, 1978), the number of which are proportional to the length of the branch (Cannell, 1974). The seasonal needle duration is 6-8 years (Cannell, 1987), which also causes the total amount of foliage to increase rapidly. The number of needles per shoot is determined by the rate of needle production at the apex, and the seasonal duration of needle production.

1.3 Bud Development and Apical Growth.

The annual growth cycle of vegetative buds and shoots of Sitka spruce, has been described by Owens and Molder (1976). They classify three phases of bud and shoot development, as described by Parke (1959) for *Abies concolor*: A resting phase including winter dormancy, a phase of shoot elongation and bud scale initiation, and a phase of needle primordium initiation. The timing and duration of these phases in Sitka spruce has been shown to differ with provenance and environmental conditions: Burley (1965) found needle production and elongation occurred for longer in more southerly provenances, which caused greater height growth than in more northerly provenances. This was also found by Lines (1963). A clinal pattern of latitudinal variation in height growth and bud set was also found by Birot and Christophe (1983), and phenological differences (including time of flushing and cessation of growth) were shown in provenance trials (Lines and Mitchell, 1965). Differences in timing, duration and rate of apical bud formation were also found by Cannell and Willet (1975), and Cannell (1978). The rate of shoot extension was also found to vary with daytime temperature (Ford et al., 1987a, b).

The period of shoot and needle growth in Sitka spruce is short, and Cannell (1987) reports it to occur for four to six weeks in June/July. Owens and Molder (1976) working in British Columbia also report bud burst to occur in early June, with shoot elongation being complete by mid-July. The time of

bud burst is dependent on photoperiod (Burley, 1965, 1966). Expansion of needles usually occurs from primordia laid down the preceding season, except in some southerly provenances, where some primordia may be initiated before the onset of bud scale initiation in the spring (Burley, 1965). Following winter dormancy of the bud, Owens and Molder (1976) found the first cell divisions to occur about six weeks prior to bud burst. During this period of early cell division, initiation of bud scales for the over-wintering bud for the next growth season occurs, and continues at a constant rate until the cessation of shoot elongation. Apical development then changes to needle initiation, which occurs in an "early" and "late" stage. The "early" stage lasts for about six weeks, during which time about half the final number of needles are rapidly initiated, and the "late" stage lasts for about three months, during which the remaining half of the needle primordia are initatiated at a slower rate.

Cannell and Willett (1975) report that for Sitka spruce growing in Scotland, 15% of the needle primordia are formed by mid-July, and 85% by the end of September. Needle primordia are initiated sequentially, in spiral phyllotactic patterns, and some features of apical growth are summarised in Table 1.1 (reproduced from Cannell, 1987).

Table 1.1

Species	Minimum Plastochron Duration (h)	Maximum Apical Dome Diameter (mm)	Minimum Mean Cell Generation Time (h)
Picea sitchensis seedlings	3.5-4.5	0.45-0.50	50-60
Picea abies seedlings	5.7	0.32	45-78
Pinus contorta seedlings	8	0.70	120
Trifolium spp.	86	0.17	16-64
Pisum sativum	46	0.15-0.26	28
Silene coeli-rosa	70-140	0.11	20

Sitka spruce seedlings have a very short plastochron duration, compared with other conifer species, and herbaceous plants, and also a large apical dome diameter, although the mean cell generation time of the apical tissue is fairly high (see Table 1.1). The minimum plasotchron duration in leaders of mature trees in mid summer may be as short as 1.2 h, with a maximum apical dome diameter of 1.6 mm, in mid-summer (Baxter and Cannell, 1978). Sitka spruce

can produce primordia so quickly, because of the large apical dome, and because the primordia are small (Cannell, 1978). The apical bud therefore, is an area of very intense metabolic activity during the summer, and it is not known how subsequent cellular growth of the needle proceeds in relation to apical growth.

1.4 Gymnosperm Leaf Growth.

Studies on vegetative development in Sitka spruce and gymnosperms in general, have been limited to bud development at the shoot apex ;see review by Johnson (1951); Gregory and Romberger (1972 a,b) for *Picea abies*; Riding (1972) for *Pinus radiata*; Owens and Molder (1973) for Western hemlock; Powell (1974) for *Abies balsamea*; Gabilo and Morgensen (1978) for *Pinus monophylla*; and Sucoff (1971) for *Pinus resinosa*, but the determinants of needle size for Sitka spruce have not been studied, and indeed, studies of needle growth past the primordial stage in gymnosperms are few, by comparison with those on the development of the angiosperm leaf (see Humphries and Wheeler, 1963; Dale and Milthorpe, 1983; Dale, 1988). Leaf development in two members of the Taxodiaceae were studied by Cross: *Taxodium distichum (1940)*, and Cunninghamia lanceolata (1942). Leaf primordia of *Taxodium distichum* are initiated by apical and sub-apical initials, and laminar growth is initiated by marginal and sub-marginal initials. Increase in length of the primordium is by apical and intercalary growth until leaves reach 2-3 mm in length, after which extension growth proceeds by intercalary cell divisions. In *Cunninghamia lanceolata*, increase in leaf length is largely due to cell divisions in the basal half or third of the leaf. Growth in width by cellular divisions ceases early in ontogeny, but the basal part of the leaf remains meristematic to give cells for increase in length, until the leaf is completely mature. More recently, Owens (1968) studied the initiation and development of needles of Douglas fir in British Columbia. He found that needle growth following dormancy, at the end of March, was mostly due to cell elongation, with most of the cells forming the lamina of the needle being present when the needle was 400 µm long. He suggests that some cell divisions do occur after this early stage, throughout most of the needle, and most frequently in the basal intercalary meristem. Apical and subapical initials, which were active during needle initiation did not divide after the breaking of dormancy. Vanden-Born (1963) studied the histochemistry of enzyme distribution in shoot tips of *Picea glauca*, and states that needle growth occurs rapidly at first, by activity of primary meristems, and

then more slowly, due to activity of a basal meristem. This conclusion was based on observations that peroxidase activity, usually associated with rapidly dividing cells, or cells about to divide, was particularly noticeable at the base of developing needles.

In pine, needle growth is from a basal intercalary meristem within the protective sheath at the very base of the needle. This was shown by Kienholz (1934), who marked elongating needles of Red pine, White pine and Pitch pine at 2 mm intervals with Indian ink, and remeasured the intervals over a time period. Kienholz (1934) also reviews reports that in some species of pine, the needle basal meristem may be active during the second year. Riding and Aitken (1982) - for *Pinus radiata*, and Cannell et al. (1976) support the idea of needle growth in pine by a basal intercalary meristem. Evidence for the basal meristem in pine does not include detailed sectioning past the primordial stage, and none of the studies on needle growth in any gymnosperm include data on cell number changes, so that the cellular basis of conifer needle growth is largely unknown. Apart from the anatomy of the apical regions of Sitka spruce already mentioned, few other anatomical studies exist. Marco (1939) examined needle anatomy of spruce species, including Sitka spruce, and mentions the presence of a thick cuticle, and the thick walls of the epidermal cells, which have teeth to project into, and embed within, the cuticle. The mesophyll cells are arranged in uniseriate layers, perpendicular to the main axis of the needle, with air spaces between the layers. Many aspects of the growth of Sitka spruce have been studied (see Harris and Ruth, 1970; Phelps, 1973), although little work has been done on vegetative bud development, and no work on needle development.

1.5 Sitka Spruce and Photosynthesis.

The high productivity of Sitka spruce stands may be attributed to some photosynthetic properties of the canopy; because needles are retained for a long period, canopies have high leaf area indices, of 8-12, which intercept nearly all the non-reflected incoming radiation (Norman and Jarvis, 1974), and only reflect 10-15% of the total incoming radiation. The regular arrangement of branches and shoots enables an effective penetration of light into the canopy, so that a large leaf area receives radiation between light compensation and saturation, to give a high quantum efficiency. Also, a considerable amount of the total annual photosynthesis occurs in the winter months, and the dry

weight of one year old seedlings in Scotland can almost double between late September and mid-April (Bradbury and Malcolm, 1978). This is because a positive carbon balance is maintained at air temperatures as low as -5°C (Ludlow and Jarvis, 1971). The optimum temperature for photosynthesis is about 18°C for field trees, and 20-22°C for glasshouse seedlings (Ludlow and Jarvis, 1971). During the winter, this optimum temperature declines to about 12°C by April, and stomatal conductances are not reduced until temperatures fall to below 0°C (Neilson and Jarvis, 1975). Needles of Sitka spruce are adapted to make efficient use of low flux densities, resulting from shade from other parts of the canopy, or shading from other needles on the same shoot. This is achieved because shaded needles are arranged more horizontally on the shoot, and have lower light compensation points and dark respiration rates than unshaded needles (Leverenz and Jarvis, 1979). Sitka spruce has typical C_3 photosynthesis, where the early stable products in the Calvin-Benson cycle are C_3 acids, and the primary carboxylating enzyme is RuBPC. A detailed description of the characteristics of the photosynthetic processes, including photorespiration, and stomatal and mesophyll resistance has been made by Ludlow and Jarvis (1971). Saturated photosynthetic rates are at irradiances of between 150 and 200 W m^{-2}, and the slope of the response curve is largely determined by r_M, which is larger than r_S at all irradiances. The response of net photosynthesis to irradiance is linear up to about 3 W m^{-2} and between about 3 W m^{-2} and 20 W m^{-2}, the slope being greater at the lower irradiances. The change in slope is due to the Kok effect, which is a light-induced depression of dark respiration and an increase in photorespiration; known to occur in Sitka spruce (Cornic and Jarvis, 1972), and which can reach values equivalent to about 30% of current net photosynthesis (Neilson, 1977). The carbon dioxide compensation concentration was found to be 40-50 µl l^{-1}. The maximum rate of photosynthesis with optimum temperature and non-limiting radiation was found to be between 15 and 18 mg CO_2 dm^{-2} h^{-1} (9.5-11.4 µmol CO_2 m^{-2} s^{-1}) (Ludlow and Jarvis, 1971), and 14.0 to 14.5 mg CO_2 dm^{-2} h^{-1} (8.8-9.2 µmol CO_2 m^{-2} s^{-1}) (Fry and Phillips, 1977; Krueger and Ruth, 1969). These values are high for a conifer (Larcher, 1969). The rate of dark respiration was found to be about 4-6% of the net photosynthetic rate, depending on the provenance, and minimum values of stomatal and mesophyll resistance to CO_2 diffusion are 1.8 cm s^{-1} (4.39 m^2 s mol^{-1}) and 6 cm s^{-1} (14.64 m^2 s mol^{-1}) (Ludlow and Jarvis, 1971). It is known that conifer needles reach their maximum rate of photosynthesis by the end of their first season's growth, and

then rate diminishes with increasing age (Freeland, 1952). Fry and Phillips (1977) showed a variation in photosynthetic capacity with season and age in 4 species of conifer, including Sitka spruce, and found that for Sitka spruce, photosynthetic rate in new and one year old needles showed a peak in August, and one year old needles had lower photosynthetic capacities than current needles. Ludlow and Jarvis (1971) measured net photosynthetic rate, and stomatal and mesophyll resistances to CO_2 transfer for developing shoots at four times throughout the growth season, and also at different times to these for one, two and three year old needles. They found photosynthetic rate rose as the shoot elongated, with a maximum in August. This increase was attributable initially, to a large drop in mesophyll resistance, and subsequently to decreased stomatal resistances. Older needles showed a gradual decrease in net photosynthetic rate with increasing age, due to a gradual increase in stomatal and mesophyll resistances, with no seasonal variation in any of the variables. Their study was limited, in that the variables were measured in developing shoots, only from mid-June, when many needles were fully expanded, until September, and changes were not correlated with changes in any other photosynthetic variable, such as RuBPC activity, or chlorophyll content.

The structure of Sitka spruce chloroplasts and the amounts and proportions of chlorophyll a and b are similar to those found in other C_3 species (Jarvis, 1981), and the seasonal variation in chlorophyll and carotenoid content, specific leaf area and dry weight fraction in Sitka spruce was studied by Lewandowska and Jarvis (1977). They found an increase in chlorophyll content in developing needles until September, then a slight decrease to a winter minimum. Although they found the increase in chlorophyll until September was associated with an increase in the dry weight ratio, and a decrease in the specific leaf area, no data was obtained before June, during the period of most rapid needle expansion, and they did not show how changes in chlorophyll and carotenoid content were related to changes in any other photosynthetic variable. Activity of RuBPC has successfully been extracted and assayed in Scots pine (Gezelius 1975; Gezelius and Hallen, 1980), and also in Corsican pine, Japanese larch and Sitka spruce (Beadle and Jarvis 1977), and Sitka spruce by Beadle et al. (1983), who also quote activities for Douglas fir and Lodgepole pine. Beadle et al. (1983) showed that the K_M of RuBPC in Sitka spruce was similar to that found in herbaceous species, and the ratio of RuBPC:PEPC activity was about 35:1,

indicating that PEPC made a very small contribution to carbon fixation. Gezelius and Hallen (1980) showed a seasonal variation in RuBPC activity over three seasons, with a peak in July to September in all age classes of needle studied. They also found very little difference in activity between one and two year old needles in most seasons. Studies therefore, have shown changes in some photosynthetic variables with conifer needle development and season, but there is a large gap in comprehensive knowledge of the development of photosynthetic capacity and the factors controlling photosynthesis in gymnosperms, and particularly in Sitka spruce.

1.6 Photosynthesis and Nutrition.

Compared with effects of nutrient deficiency on photosynthesis and its components in herbaceous plants (see review by Natr, 1972), effects in trees are less well documented (see Keller, 1967), and studies have concentrated on the effect of fertilisation, rather than deficiency. For example, application of N and P to *Pinus radiata* seedlings greatly increased biomass (Waring, 1980), and application of N to mature *Pinus radiata* trees showing no signs of N deficiency caused productivity to double over a seven year period (Woolon and Will, 1975; Crane, 1981), which must have been due to an increased assimilation rate. Nitrogen and phosphorus are the most important nutrients in determining forest productivity (Helms, 1976). Helms (1964) did not find any effect on photosynthetic rate per unit dry weight in current growth the year following addition 560 Kg ha^{-1} NH_4NO_3 to Douglas fir. This finding was also supported for Douglas fir by Brix and Ebell (1969), who found no effect on net photosynthetic rate per unit leaf area on current and one year old shoots the year after addition of 448 Kg ha^{-1} NH_4NO_3. However, Brix (1971) subsequently showed that the photosynthetic rate in the same experiment did increase in shoots from July in the first season following fertiliser addition in the spring, until the following July.

A significant relationship between degree of N fertilisation and photosynthetic rate has been found for Douglas fir (Brix, 1981), for *Pinus radiata* (Squire, 1983), and for *Picea abies* (Keller, 1972). Photosynthetic rate and foliar content of N and P increased following fertilisation of *Pinus pinaster* with N and P (Keay et al., 1968), and a positive relationship has also been found between foliar phosphorus content and photosynthetic rate, for *Eucalyptus pilularis* (Mulligan and Patrick, 1985); for *Pinus radiata* (Sheriff et al., 1986;

Conroy and Barlow, 1986). Keller (1972) found a 20% reduction in net photosynthetic rate of *Populus nigra* under conditions of mild phosphorus deficiency, but for 3 species of Eucalyptus, photosynthetic rate did not decrease with P deficiency, even though the foliar concentration of N was reduced (Mulligan, 1989). For two of the three species, photosynthetic rate increased following P deficiency, due to an associated increase in foliar N levels. The effect of potassium on photosynthetic rate has been studied by Zech et al. (1969), who found a positive effect of K fertilisation in pine. Keller (1972) found little effect of K supply on photosynthetic rate in an unnamed species of spruce, but with *Populus nigra*, photosynthesis was correlated with K supply, when P_N was expressed per unit dry weight, but not per unit leaf area (Keller, 1967).

Nutrient effects on the partial processes of photosynthesis in gymnosperms are very few. Cizkova (1981) studied the chlorophyll content of spruce, larch and pine seedlings under N, P, K and Ca deficiencies. N deficiency caused the largest decrease in chlorophyll content, and reductions were also found with K and P deficiency, whilst Ca deficiency caused a slight increase in total chlorophyll content. Gezelius (1986) found that a concentration of 2.5 p.p.m. N caused a lower needle chlorophyll content and activity of RuBPC, compared with 10 and 50 p.p.m. N treatments, for Scots pine, although the specific activity of RuBPC per unit protein did not change between treatments.

Despite this research, there still exists a lack of quantitative data on nutrient deficiency effects on productivity, and the components of the photosynthetic process affected by nutrition. Because nutrients may affect photosynthesis in many ways, by acting on metabolic processes, such as protein synthesis, or "directly" on stomata, or chloroplast structure and function, any investigation into the effects of mineral nutrients on photosynthetic productivity must focus on the biochemical processes of photosynthesis, the partial processes, and effects on assimilatory tissue at the level of the leaf and cell.

1.7 Aims and Objectives.

The literature review highlights areas where knowledge of conifer growth and effects of mineral nutrient deficiency on development and productivity are lacking. The aims of this project were to study growth of needles, these being the primary unit of productivity, and the development of photosynthetic function of needles, as affected by mineral nutrition. In more detail, the aims fall into two areas:

1) To study needle development in Sitka spruce in the field, in terms of morphological variables and cellular parameters, and to investigate the effect of deficiency of the major mineral nutrients on these.

2) To study the development of photosynthetic capacity of needles, in terms of biochemical and physiological components, and the effects of mineral nutrient deficiency on these.

Initial studies were done on plants of Sitka spruce growing in the field. This was because a field site of mature trees growing under a range of nutrient deficient conditions was already available, and because of the importance of Sitka spruce as a commercial crop, knowledge and implications of nutrient effects on productivity and development were considered to be of great importance. The measurements of photosythetic variables were carried out in a controlled environment, using 3 year old seedlings which had been grown in pots and subjected to carefully controlled nutrient deficiencies. The use of young potted plants was partly due to practical considerations of sampling and measuring photosynthesis under standard, controlled conditions, and also due to the need to study nitrogen-deficient trees which were not available in the field. During this part of the study, some morphological aspects of needle growth were also measured, to try to correlate photosynthetic development and the nutrient effects, with those found for material in the field.

CHAPTER 2. MATERIALS AND METHODS

2.1 Material.

The material used throughout these studies was Sitka Spruce, Picea sitchensis (Bong.) Carr., Queen Charlotte Island Provenance, either as mature trees at a field site planted by the Forestry Commission, or as two year old transplants in pots.

2.2 Source of Material and Initiation of Experiments.

2.2.1 Field Experiment 1987.

The experimental field site is in the Glentress Forest at Eddleston, Leadburn, 19km south of Edinburgh. The site is an upland raised bog, with 7 m deep peat overlying boulder clay, at an altitude of 285 m. The peat contains low basal levels of potassium, phosphorus and nitrogen. Two series of plots were planted with two year old Sitka Spruce, by the Forestry Commission in 1967 and 1973. Both the 1967 and 1973 series of trees contained a plot of Control trees, referred to as "Controls", or "+All", which were fertilised with all major nutrients, and also plots where deficiency of either P, K or Mg had been induced, by supplying all major nutrients apart from one (Table 2.1). Trees from these plots will be referred to as "-P", "-K" or "-Mg" throughout this thesis. The series of plots planted in 1967 also contained trees which received no fertiliser. These trees will be referred to as "-All". As part of management practice, fertilisers were applied as top dressings annually to both series of plots for the first few years, and subsequently every three years to the trees planted in 1967, and every four years to the trees planted in 1973.

A nitrogen deficient plot was also set up in the 1967 series, but the trees showed no visible deficiency symptoms, and foliar analysis results showed deficiency was not extreme, probably because the application of other nutients caused a release of bound nitrogen in the soil (pers. Comm. M.P.Coutts). Because of this, no -N treatment was available, and it was decided to sample trees from the Control plot, and -P, -K and -Mg treatments from the 1973 planting, and -All trees from the 1967 planting.

Table 2.1 Type and amount of fertiliser applied to each treatment in the field.

Fertiliser	Application (Kg Ha^{-1})	% Nutrient in fertiliser	Rate of fertiliser application (Kg Ha^{-1})	Plots receiving fertiliser
Urea	61.7	45% N	27.8 N	+All,-P,-K -Mg
Ground Mineral Phosphate	107.9	12.6% P 36% Ca	13.6 P 38.8 Ca	+All,-K,-Mg
Sulphate of Potash	66.8	41.5% K	27.7	+All,-P,-Mg
Ground Limestone	159.3 46.3	45% Ca	71.6 20.8	-P +All,-K,-Mg
Epsom Salts	185.0	10% Mg	18.5	+All,-K,-P

2.2.2 **Pot Experiment 1988.**

To investigate nutrient deficiency effects on photosynthesis, pot-grown material was used. This enabled measurements to be made under standard conditions of light and temperature, as material could be accommodated in a controlled environment room. Nutrient deficient material could be produced in a more controlled way than in the field, with the added advantage that nitrogen-deficient trees could also be produced. It was thought that using two year old transplants would simplify sampling compared with the field, with material being easier to handle and manipulate, and possibly showing less tree-to-tree variation than observed in the field. Use of clonal trees would have reduced the variation further, but such material was not available.

In May 1987, 120, 2 year old Sitka Spruce seedlings (Q.C.I. provenance) produced by the Forestry Commission and referred to by them as 1+1 transplants, were potted in 8" diameter pots in Edzell grit. The experiment was split into 6 treatments, each of 20 trees. The treatments were deficiencies in; nitrogen, "-N", magnesium, "-Mg", potassium, "-K", and phosphorus, "-P", or no nutrient application, "- All", and a fully fertilised Control, known as "Controls", or "+All". The pots in each treatment were colour-coded according to treatment, and rows of each treatment were randomised into four blocks with six rows, with five trees in each row. The trees were kept at the Forestry

Commission Northern Research Station in a sheltered place until they were sampled.

The basic nutrient solution was that of Hocking (1972) - (Table 2.2). The solution was made up by adding 4 cm^3 of each of two stock solutions to 1 dm^3 water, and was modified for each deficiency treatment as shown. Each tree was given 250 cm^3 of the appropriate nutrient solution twice-weekly, with additional applications of water when necessary throughout the 1987 growth season. Watering continued over the winter, and the trees were protected from frost by covering with a fine mesh cage. Nutrient application ceased at the beginning of September 1987, and recommenced at the beginning of May 1988. By the end of the 1987 growth season, trees were beginning to show deficiency symptoms, by yellowing of the needles, or by a purple discoloration of the needle tips, particularly in the -N and -All treatments. In order to investigate the effect of reversing the deficiency treatments, each treatment apart from the Controls was divided into two groups of ten trees. One group continued to receive the deficiency nutrient regimes as before, with nutrient application resuming in May 1988, whilst the other group began to receive full Hockings solution. The sub-groups receiving full nutrients are denoted "-MgR", "-KR", "-PR", "-NR", and "-AllR".

Table 2.2 Chemical Composition of Hocking's Nutrient Solution.

Stock solution 1:

Chemical name	Formula	Quantity
Distilled water	H_2O	1 litre
Sulphuric acid (1% v/v)	H_2SO_4	5 cm^3
Ammonium chloride	NH_4Cl	53.5 g
Potassium sulphate	K_2SO_4	43.5 g
Potassium phosphate	K_2HPO_4	43.5 g
Boric acid	H_3BO_3	0.563 g
Molbdenum trioxide	MoO_3	0.0035 g

Stock solution 2:

Chemical name	Formula	Quantity
Distilled water	H_2O	1 litre
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	118.0 g
Magnesium chloride	$MgCl_2.6H_2O$	101.5 g
Manganous chloride	$MnCl_2.4H_2O$	0.25 g
Zinc chloride	$ZnCl_2$	0.025 g
Cupric chloride	$CuCl_2.2H_2O$	0.0125 g
Ferric chloride	$FeCl_3.6H_2O$	0.675 g

For the -N treatment: NH_4Cl was omitted from stock one, and $Ca(NO)_3)_2$ was replaced in stock two by The same quantity of $CaCl_2$.
For the -P treatment: K_2HPO_4 was omitted from stock one, and was replaced by the same quantity of KCl.
For the -K treatment: K_2SO_4 and K_2HPO_4 in stock one were replaced by similar quantities of Na_2SO_4 and Na_2HPO_4.
For the -Mg treatment: $MgCl_2$ was omitted from stock two.

2.2.3 **Pot Experiment 1989.**

In addition to the nutrient experiment carried out in 1988, an experiment was performed during the 1989 growth season to investigate more specifically the effect of refertilisation of nitrogen-deficient trees. This was done with respect to effects on photosynthesis and some needle morphological characteristics. During 1988, 40 pot trees were planted as for the 1987 experiment. Five of the trees received full Hocking's solution (Controls), and 35 trees received a nitrogen deficient solution (as for -N trees in 1988). The trees

were looked after in the same way as those in 1987. In May 1989, the 35 nitrogen-deficient trees were randomly separated into 7 treatments, each of 5 trees. The treatments were a range of nitrogen concentrations, with trees in each treament receiving Hocking's solution, with the amount of nitrogen varied in an exponential series, (Table 2.3).

All trees, including the Controls, were randomised by treatment, into 8 rows, each of 5 trees, and kept in the open behind the Botany department. Control trees received full Hocking's solution, and the treatments received their respective solutions from May 1989, with the same rate of application as for trees in the 1988 experiment.

Table 2.3 The amounts of nitrogenous compounds in stock 1 and 2 of Hocking's solution, used in the nitrogen concentration experiment, 1989.

Treatment	Amount of NH_4Cl in stock 1 (g added to 1 l water)	Amount of $Ca(NO_3)_2.4H_2O$ in stock 2 (g added to 1 l water)
zero N	----	----
7 p.p.m. N	3.34	7.38
14 p.p.m. N	6.69	14.75
28 p.p.m. N	13.38	29.50
56 p.p.m. N	26.75	59.00
112 p.p.m. N	53.50	118.00
224 p.p.m. N	107.00	236.00

2.3 Sampling Procedure.

2.3.1 Shoots.

There are significant variations in needle morphological characteristics associated with canopy position, and whether needles are from shoots with a 'sun' or 'shade' shoot morphology; needles from sun shoots from the upper part of the canopy are more or less vertically inclined and are uniformly distributed around the shoot, whilst shade shoots in the lower part of the canopy are nearly horizontal, and are orientated in a single plane (Busgen and Munch, 1929). It was therefore important to standardise a procedure for sampling shoots, and in field material, needles were sampled from a mid-canopy position, which was whorl 7 from the trees planted in 1973, to give

shoots which did not show extreme sun or shade shoot morphology. Although the "-All" trees were slightly older, material was again sampled from whorl 7, which was again a mid-canopy position. Terminal shoots from first order laterals were sampled in all cases, using extendable pole cutters when necessary. In the -P, -K and -All treatments, trees from the edge of the plot were avoided, but such trees had to be sampled in Control and -Mg treatments, as the close spacing and large size made penetration to other trees impossible. With pot material, differences in needle morphology due to canopy position were not as important, and needles during the 1988 growth season were sampled from terminal shoots from the terminal whorl of 1987 growth (whorl 2).

2.3.2 **Needles.**

More needles are present on the top, adaxial surface of the shoot than on the bottom, abaxial surface, so that along the ontogenetic spiral, the stem unit length, defined as the distance on the shoot between needles, i.e. the needle internode length (Cannell et al., 1976), is shorter on the top, adaxial side of the shoot than on the lower, abaxial side (Groom, 1907). In any season's growth, needle length and width vary along a shoot, such that the most basal needles are the widest, and those near the tip of the shoot are the narrowest, with little difference along most of the shoot. The longest needles are found at about 25% to 50% of the distance from the shoot base (Table 2.4).

Table 2.4 Lengths and widths (mm) of needles at different positions on Control shoots, and for different canopy positions. Values are means ± S.E.

	Shoot Quarter							
	Distal Quarter		Second Quarter		Third Quarter		Proximal Quarter	
	Length	Width	Length	Width	Length	Width	Length	Width
Whorl 1	18.52 ± 3.66	2.32 ± 0.17	17.93 ± 3.84	2.28 ± 0.23	16.88 ± 3.68	2.21 ± 0.25	15.35 ± 2.69	2.07 ± 0.30
Whorl 5	19.98 ± 6.82	1.72 ± 0.05	20.43 ± 6.74	1.68 ± 0.04	19.25 ± 5.75	1.67 ± 0.02	17.45 ± 4.82	1.61 ± 0.08
Whorl 9	18.12 ± 1.45	1.35 ± 0.17	20.51 ± 1.60	1.33 ± 0.16	20.17 ± 1.35	1.33 ± 0.15	19.09 ± 1.35	1.27 ± 0.13

A study of needle growth must therefore involve a sampling regime which takes into account the distribution and variation in needle characteristics along and around a shoot.

Needles could be sampled in several different ways e.g.

a) From particular distances along the shoot, or from fractions of the length.

b) by following needles sequentially along the phyllotactic spiral from shoot base to tip.

The second method was selected, as it was the most reproducible for shoots of different lengths from different treatments, and depended less on the developmental state of the shoot. Many spirals could be chosen (Fig. 2.1 A), varying in the number of turns around the shoot, and the number of needles in each. Needle length varies with position in any spiral, such that needles on the abaxial side of the shoot may be up to 2 mm longer than those on the adaxial side (Fig. 2.2), as found for *Picea abies* by Frey and Ivask (1983). This effect becomes less pronounced moving basipetally through the canopy, so that at whorl 9 the difference is only about 1 mm (Fig. 2.2). Differences in needle length of up to 1 mm due to adaxial or abaxial orientation of the needle on sun and shade shoots were also found for Sitka spruce by Leverenz and Jarvis (1980). Therefore, the tightest spiral (A) (Fig. 2.1 A) was not chosen, as it was affected the most by variations between adaxial and abaxial sides of the shoot, as well as distal/proximal differences due to developmental state. The intermediate spiral (B) was chosen, as it provided more needles than the loose spiral (C), so giving a better indication of needle development according to needle position, but without being greatly affected by adaxial/abaxial positional differences.

Figure 2.1 A

Diagram showing three possible phyllotactic spirals on stems of Sitka spruce.

Figure 2.1 B

Diagram showing the measurements made for median longitudinal sections of dormant buds from field shoots, in February 1988;

A = Diameter of the apical dome.
B = Bud height from the crown region.
C = Width of the bud core at the position of the most proximal primordium.

Figure 2.1 C

Diagram showing the phyllotactic spiral followed in the bud for measurement of needle characteristics, in Control field buds prior to bud burst 1988, and for the estimation of primordium cell number in dormant field buds in 1987.

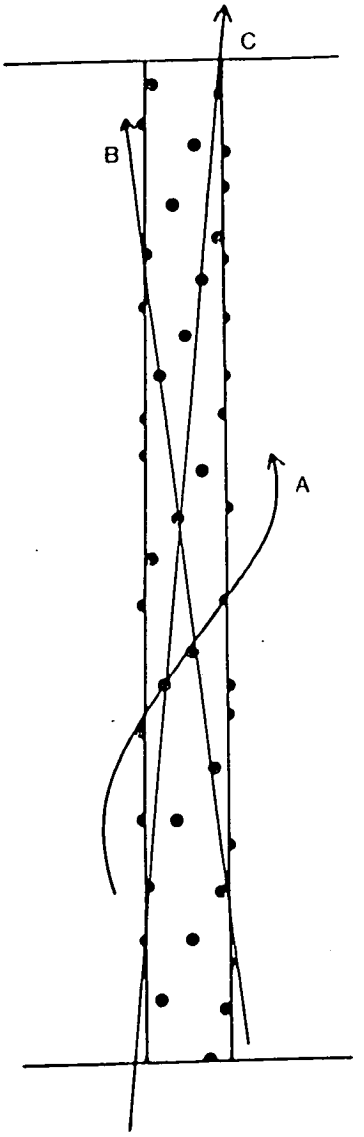


Figure 2.1A

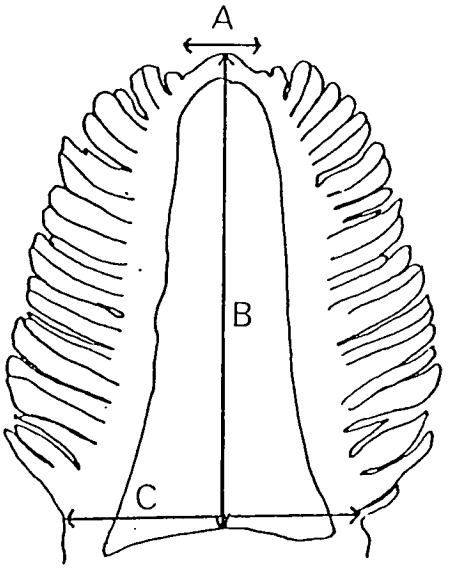


Figure 2.1B

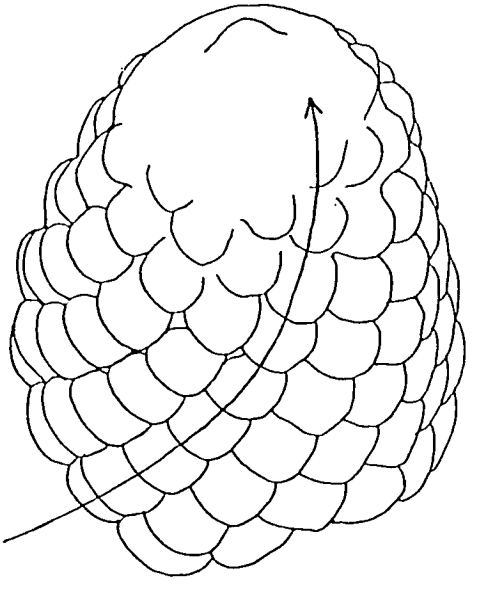
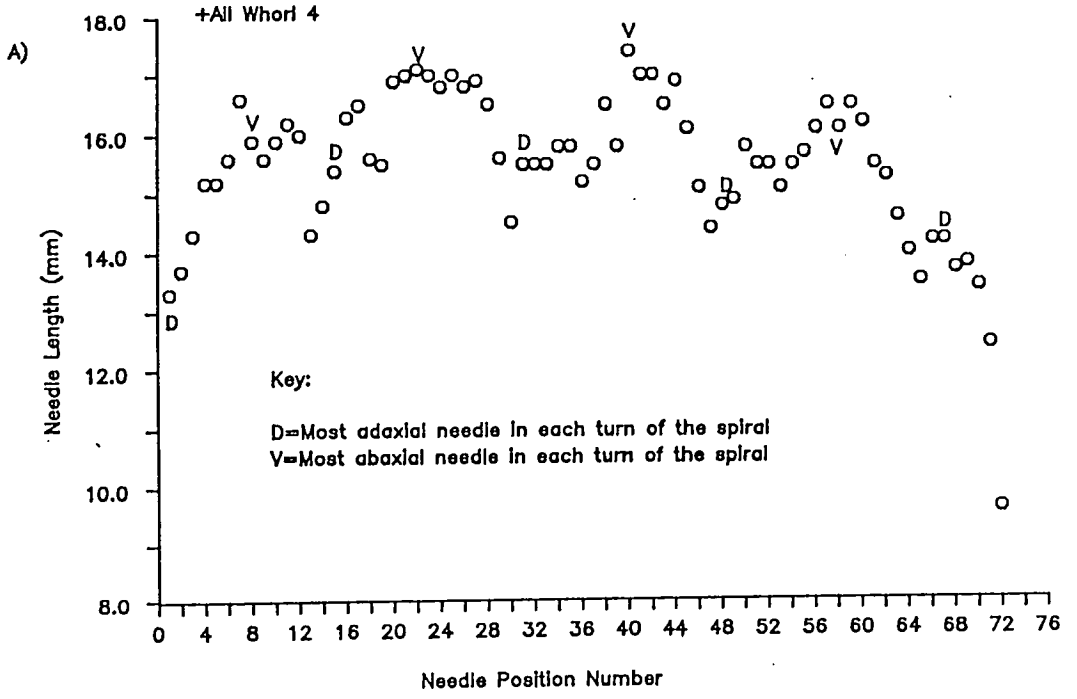
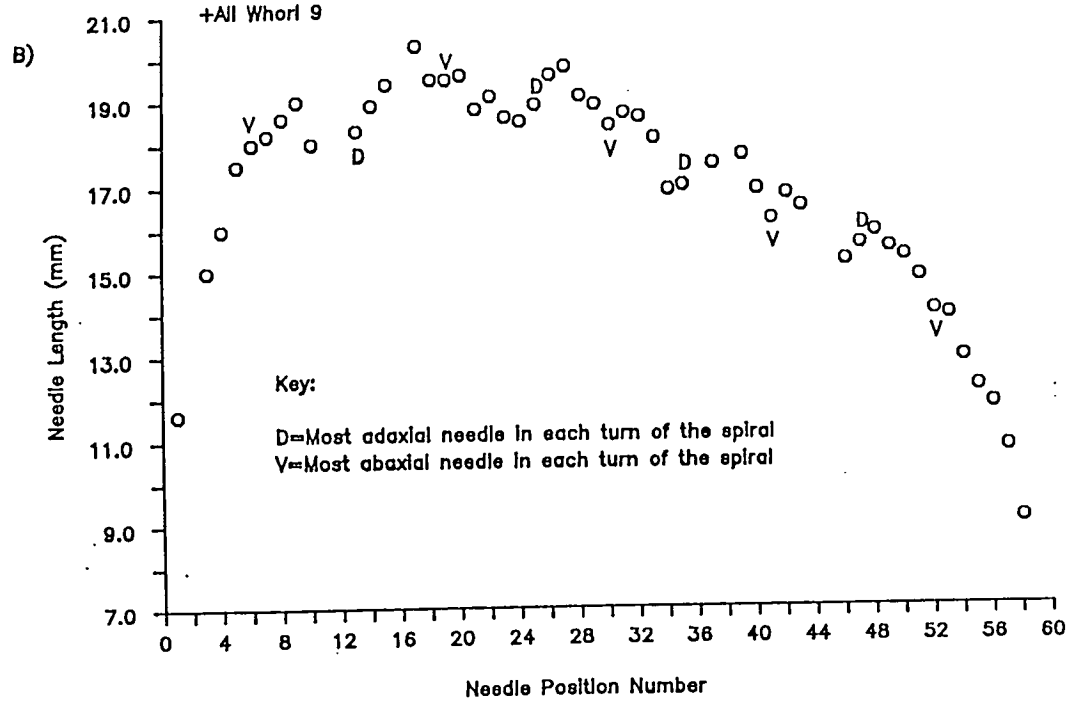


Figure 2.1C

Figure 2.2 Variation in needle length following phyllotactic spiral A from shoot base to shoot tip, for a Control shoot from A) the top half of the canopy (whorl 4), and B) the bottom half (whorl 9) in the field.





2.4 Sampling Regime.

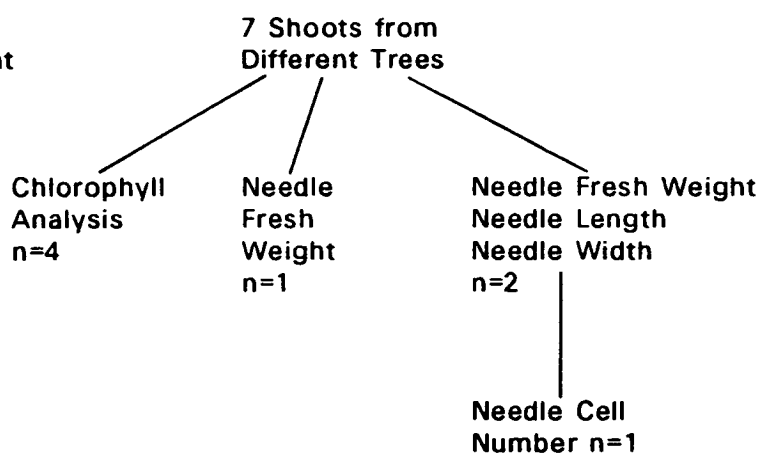
2.4.1 Field Material 1987.

Following bud burst, samples were taken weekly, but as it was not possible to sample every treatment at every sampling time because of the work load, only two of the five treatments were sampled at any one time, with different treatments being sampled each time. Each treatment was sampled four times, on the following dates:

Treatment	Sample Number and Date			
	1	2	3	4
+All	May 4	May 18	June 10	June 22
-Mg	May 4	May 25	June 10	June 29
-K	May 11	May 25	June 15	June 29
-P.	May 11	June 1	June 15	July 8
-All	May 18	June 1	June 22	July 8

Seven shoots per treatment were sampled each time, from different trees chosen at random. Four shoots were used for chlorophyll determinations. Needles from the remaining shoots were excised following spiral B (Fig. 2.1 A) Initially three, and later, two series of needles were removed from each of the three shoots, to give replicate needles for each position. Results are presented as mean values, to minimise adaxial/abaxial positional differences. Fresh weight, length and width measurements were made for needles from two of the three shoots, and needles from one of these were used for cell counts. Lack of time did not allow these data to be replicated. Needle fresh weights alone were measured for the remaining shoot.

Allocation of Shoots for different Measurements.



In addition to the sampling times shown in the table above, one bud from the Control treatment was also sampled on April 27, prior to bud burst, for needle length, width, fresh weight and cell number. Each measurement was made on five needles from each position. Following July 8, the treatments were sampled in the same rotation, until the beginning of November, with six, and later four, shoots being sampled for chlorophyll estimation only.

2.4.2 **Field Material 1988.**

On four dates prior to bud burst 1988, which occurred just before May 7, and once immediately following, one bud was removed from a mid canopy position from each of two Control trees in the field. On April 7, the bud was too small to remove primordia from each position along the phyllotactic spiral, so the bud was cut horizontally, perpendicular to the its main axis, to divide the bud into six equal portions according to total bud height. Ten primordia were removed at random from each portion, and kept on damp filter paper to await measurements. At the remaining sampling times, needles were studied along a phyllotactic spiral (Fig. 2.1 C), but this did not correspond to that chosen following bud burst 1987, as in the bud, the phyllotactic spirals are more compact and it was not possible to trace spirals easily seen on the elongating shoot, back to the bud. The number of needles studied for each position from each bud, was 5 at April 15; 4 on April 20; and 3 on April 27 and May 7. For each needle at each sampling time, including April 7, length and width measurements were made, and for each bud, the 3 to 10 needles for each position were macerated, and the total cell number was divided by the number of needles to give mean cell number per needle.

2.4.3 **Pot Material 1988.**

Material was sampled on a weekly basis but with only some of the treatments studied in any one week, so that analysis of all treatments was spread over two weeks; during the first week, the -P, -Mg and -K treatments were sampled, and also their fully fertilised complements, -PR, -MgR and -KR. In week two, -N, -All, -NR and -AllR treatments were analysed. The Controls were sampled every week for purposes of comparison, and also to give a more detailed time-course for the measured parameters. Two trees were used from each treatment at each sampling time, chosen at random, but ensuring that all trees were used once. As each treatment only consisted of 20 trees, and the

study lasted for 20 weeks, each tree was sampled twice, again choosing trees randomly, but ensuring that all trees were sampled no more than twice. Every fortnight, 24 trees; 14 for study in week one and 10 for week two, were removed from the Forestry Commission Northern Research Station, and kept outside the Botany department. Every Friday all the trees for study during a particular week were transferred to a growth room, and kept at continuous 20°C on a 16 h light / 8 h dark cycle. The trees remained in the growth room for one week exactly, during which time soluble protein was extracted and assayed for RuBPC activity on Monday/Tuesday, chlorophyll analyses were made on Tuesday/Wednesday, photosynthetic rate was measured on Thursday, and needle areas calculated on Friday.

Transfer began on Friday April 29th, and measurements on May 2nd, continuing until September. Trees from all treatments were sampled further at the end of October and the beginning of November.

2.4.4 **Pot Material 1989.**

On August 4 1989, 3 trees from each of the 8 treatments were randomly selected and placed in a controlled environment room at a continuous temperature of 20°C, with a 16 hr light/8 hr dark period. One terminal shoot was sampled from whorl 2 (the previous season's apical whorl) from each tree, in the same way as in 1988, to measure needle pigment content, RuBPC activity, and net photosynthetic rate. Mean needle length, projected area, number and fresh weight per shoot were also measured for the same shoot used to determine the photosynthetic rate. Some shoots from all treatments apart from zero and 7 p.p.m. N showed "free growth", with new needle primordia being laid down during the season. Therefore, shoots in these treatments contained needles at a wide range of developmental states, as did shoots from -NR and -AllR treatments in 1988. It was decided to sample needles from the shoot mid-point for estimation of chlorophyll content and RuBPC activity, as although these needles were not the most mature, the values obtained represented a mean for the whole shoot, and therefore provided a good comparison with the photosynthetic rate, which was a value for the whole shoot. Because some trees showed free growth, in order to estimate needle cell number, needles expanding from primordia initiated in 1988, and those needles arising from new primordia initiated during 1989 had to be considered. Two needles were taken from the shoot mid point, from

trees in zero, 7 and 14 p.p.m. N treatments not showing free growth, and mean needle fresh weight and cell number were estimated. The mean distance of the shoot mid point from the base of the shoot in these treatments was used as the point from which two needles were taken from the other treatments. These needles were also used to estimate mean fresh weight and cell number, and are those which were initiated in 1988. From the trees showing "free growth", two needles were taken from a point two-thirds (three-quarters in the case of the Controls) of the distance along the shoot from the base. These needles were initiated during the 1989 season, and were used for fresh weight measurements and cell number counts, for estimation of cell volume. The exact position from which needles were taken was not important, since results from the 1987 experiment showed that the relationship between needle cell number and ~~position~~ size was linear, irrespective of needle position on the shoot. However, the above procedure ensured that needles initiated during both 1988 and 1989 were sampled, for comparison of mean cell size between needles initiated in two different nutrient regimes.

2.5 Shoot and Needle Characteristics.

2.5.1 Shoot Length.

From bud burst until the cessation of shoot elongation in July 1987, shoots in the field were measured from the base of the bud scales to the tip of the most distal needle.

2.5.2 Needle Fresh Weight, and Dry Weight:Fresh Weight Ratio.

The fresh weight of needles from each position on the shoot (Fig. 2.1 A) was taken during the 1987 season, (see flow chart, section 2.4.1). Weights were taken to 0.1 mg as quickly as possible following needle removal. The dry weight:fresh weight ratio was determined throughout the 1987 and 1988 seasons by taking 25 needles from near the mid-point of the shoots used for chlorophyll analysis, and oven drying them at 60°C for 96h (1987 needles), or at 105°C (for 1988 needles). The difference between drying needles at these two temperatures was found to be no more than 1.8%.

2.5.3 Estimation of Number of Primordia in the Bud.

Needle primordia are laid down sequentially in spiral phyllotactic patterns,

mostly with a divergence angle close to the Fibonacci angle. This gives contact parastichies (spirals of primordia) numbering in the series 1,2,3,5,8,13....., with 50% of apices having clockwise generative spirals of primordia, and 50% having anticlockwise spirals (Cannell, 1978). This regular phyllotaxy enabled numbers of primordia in dormant buds to be estimated after bud-scale removal, by multiplying the number of primordia per parastichy by the number of contact parastichies in the bud.

2.5.4 **Measurement of Dimensions of Dormant Buds.**

Dormant buds from terminal whorl 7 shoots from Control, -K, -P and -All treatments were studied in February 1988. After removal of the bud scales, the bud was cut longitudinally in half using a razor blade under a dissecting microscope (x20 objective), and a thin hand section taken from one of the cut faces. The section was stained in safranin for a few minutes, rinsed in methanol and dehydrated in ethanol for about an hour before mounting on a slide in Canada Balsam. Comparison of dehydrated and non-dehydrated sections showed shrinkage of linear dimensions to be a mean of about 16%. Using a binocular dissecting microscope (x20) objective, the diameter of the apical dome was measured, the dome being defined as the area of undifferentiated tissue distal to the last discernable primordium (Baxter and Cannell, 1978). Also measured was bud height from the crown region to the tip of the apical dome, and the width of the bud core at the point of the most proximal primordium (see Fig. 2.1 B).

2.5.5 **Measurement of Primordial and Needle Dimensions, and Needle projected surface area.**

For primordia, and needles from the 1987 growth season, length and width at the widest part were measured to the nearest 0.1 mm using a hand lens with a 250 x 0.1 mm eyepiece graticule. Projected surface areas for needles sampled during 1987 were calculated from length and width measurements using the equation developed by Steele (1987):

$$\text{Projected Area} = b \times (\text{Length} \times \text{Width})$$

-where coefficient $b = 0.75243 + 0.00439 \times$ ratio of needle length to width. Steele derived this equation by using photographs of needles from needle samples from trees of different ages and from different sampling positions, to

estimate projected area, then calculating b from the relationship

$$b = \text{Area} \div (\text{Length} / \text{Width})$$

Needle dimensions and projected areas from shoots of pot grown trees during the 1988 season were determined by Image Analysis, using a Quantimet Image Analyser (Cambridge Instruments Ltd.):

Following mesurement of photosynthetic rate, all needles were removed from each shoot, and were stuck in rows, on a sheet of Transpaseal adhesive film, ensuring that the flat surface of the needles was uppermost. The needles were then analysed in groups of about 100 needles.

2.6 Analysis of Needle Nutrient Content.

2.6.1 Preparation of Samples.

Needles were analysed from field trees in February 1987, and from pot trees in April 1988 and in November 1988, for content of nitrogen, phosphorus, potassium, calcium and magnesium (see Tables 2.5 to 2.7). Analyses were carried out for some samples, by the chemical analysis laboratory, Site Studies, Forestry Commission, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH. The procedures used were as follows.

Needles were removed from terminal shoots at a mid-canopy position (whorl 7) from field material, and from whorl 2 shoots which had expanded during 1986, from pot trees. Two to four replicate samples were taken for each nutrient treatment. Needle samples were large enough to give at least 100 mg dry weight following oven drying at 105°C for 48 h. Dry needles were ground in a mill, and 100 mg of material was put in a test tube. Digestion of the samples was effected by adding 1 cm^3 conc. sulphuric acid to each tube, folowed by 2, 0.4 cm^3 aliquots of hydrogen peroxide, with a short interval between additions, and then refluxing the tubes for 30 min at 320—350°C. The samples were then left to cool. Another 0.4 cm^3 aliquot of hydrogen peroxide was then added and the samples reheated to 350°C for 10 min. These last two steps were repeated until all digest solutions appeared colourless. When cool, each resulting Kjeldahl digest solution was made up to a volume of 15 cm^3 with distilled water, and was then ready for chemical analysis.

2.6.2 Analysis of Potassium, Calcium and Magnesium in Kjeldahl Digest Solutions.

Needle potassium, calcium and magnesium content was determined using a DC plasma emission spectrometer (Spectraspan 3): The digest solution was sucked into a nebuliser to produce an aerosol, which was passed via a jet into an argon plasma. The atoms in the sample became excited, and emitted radiation (in the visible or U.V. range), at wavelengths specific to each element present. The light emitted by the K, Ca and Mg in the solution was determined spectrometrically, and the concentration of the elements in the original samples was calculated, on a dry weight basis.

2.6.3 Analysis of Nitrogen and Phosphorus in Kjeldahl Digests.

The concentration of nitrogen and phosphorus was determined using continuous flow (segmented flow) analysis. Nitrogen was determined as ammonium nitrogen using the indo-phenol method (the reaction of ammonium ions with sodium phenate and sodium hypochlorite, catalysed by sodium nitroprusside), and phosphorus was analysed as phosphate, using the phosphomolybdenum complex method, with ascorbic acid as the reducing agent. Eight standards were run in duplicate, with nitrogen and phosphorus concentrations ranging from 0 to 3.5% dry weight. The colour change in the standards and samples due to each assay was measured colourimetrically, and the results drawn on a chart recorder. This colourimetric output was then analysed by a Trivector computer which measures the peak heights and calculates a concentration of N or P on a dry weight basis, by comparison with the calibration curves from the standards. A standard plant tissue digest sample (usually Sitka Spruce foliage) was run at the start and finish of each batch of samples when analysed, to check the reproducibility of the digestion step.

2.6.4 **Foliar Analyses for Field Material.**

Table 2.5 Nutrient analyses of needles from terminal shoots from whorl seven, for all field treatments, in February 1988. Values are means ± S.E. for four replicate shoots (% dry weight).

Treatment	**Nutrient**				
	N	**P**	**K**	**Mg**	**Ca**
+All	1.00[d] ± 0.042	0.16 ± 0.006	0.64 ± 0.069	0.09 ± 0.005	0.36 ± 0.048
-Mg	1.03[d] ± 0.027	0.20 ± 0.005	0.69 ± 0.029	0.08 ± 0.006	0.39 ± 0.054
-K	1.03[d] ± 0.043	0.18 ± 0.020	0.17[d] ± 0.008	0.10 ± 0.007	0.37 ± 0.019
-P	0.94[d] ± 0.077	0.06[d] ± 0.004	0.86 ± 0.092	0.07 ± 0.007	0.37 ± 0.019
-All	0.76[d] ± 0.076	0.06[d] ± 0.006	0.40[d] ± 0.079	0.08 ± 0.007	0.23 ± 0.039

Key: d indicates a deficient concentration (see Binns et al., 1980; Forestry Commission leaflet number 76, p16).

Foliar results (Table 2.5) show that the -Mg set of trees was not deficient in Mg; presumably due to a sufficient amount of Mg being present in the soil. All treatments had slightly deficient foliar concentrations of N, according to deficient concentrations published by the Forestry Commission. However, these published values are for young stands of 0.3-3.5 m in height, and therefore may only provide a rough guide to the nutritional state of older trees. The Controls showed no N deficiency symptoms, and a concentration of 1.00 % dry weight in field trees is not interpreted as being deficient. Nitrogen concentration in -All trees however, probably was deficient.

2.6.5 **Foliar Analyses For Pot Material.**

Table 2.6 Nutrient analyses of needles from terminal shoots from whorl two of pot trees, from all treatments, in April 1988, prior to bud burst. Values are means ± S.E. for four replicate shoots. (% dry weight).

Treatment	**Nutrient**				
	N	**P**	**K**	**Mg**	**Ca**
+All	1.28 ± 0.040	0.14 ± 0.003	0.76 ± 0.033	0.09 ± 0.058	0.41 ± 0.036
-Mg	1.34 ± 0.042	0.16 ± 0.005	0.76 ± 0.029	0.04 ± 0.005	0.44 ± 0.023
-K	1.23 ± 0.063	0.23 ± 0.018	0.42[d] ± 0.023	0.19 ± 0.019	0.53 ± 0.038
-P	1.40 ± 0.051	0.11[d] ± 0.004	0.89 ± 0.133	0.08 ± 0.008	0.34 ± 0.023
-N	0.67[d] ± 0.042	0.14 ± 0.009	1.10 ± 0.065	0.09 ± 0.012	0.29 ± 0.030
-All	0.58[d] ± 0.017	0.09[d] ± 0.003	0.72 ± 0.007	0.08 ± 0.006	0.30 ± 0.019

Key: d indicates a deficient concentration (See Binns et al., 1980; Forestry Commission leaflet number 76, p16).

Table 2.7 Nutrient analyses of needles from terminal shoots from whorl two of pot trees, from all treatments, in November 1988, following cessation of growth. Values are means for bulk needle samples for -N and -All treatments, otherwise are means ± S.D. for two shoots from different trees, and four shoots for +All samples (% dry weight).

Treatment	Nutrient				
	N	P	K	Mg	Ca
+All	1.48 ± 0.432	0.25 ± 0.019	1.19 ± 0.144	0.14 ± 0.029	0.43 ± 0.135
-Mg	0.91[d] ± 0.071	0.21 ± 0.057	1.15 ± 0.085	0.07 ± 0.028	0.51 ± 0.106
-MgR	1.31 ± 0.170	0.24 ± 0.007	1.49 ± 0.276	0.13 ± 0.007	0.36 ± 0.021
-K	0.95[d] ± 0.021	0.24 ± 0.078	0.80 ± 0.028	0.16 ± 0.042	0.42 ± 0.148
-KR	1.55 ± 0.283	0.27 ± 0.071	1.39 ± 0.141	0.15 ± 0.014	0.41 ± 0.014
-P	0.92[d] ± 0.085	0.10[d] ± 0.021	1.27 ± 0.113	0.08 ± 0.007	0.25 ± 0.071
-PR	1.40 ± 0.184	0.19 ± 0.014	1.32 ± 0.085	0.10 ± 0.028	0.36 ± 0.057
-N	0.62[d]	0.21	1.72	0.11	0.30
-NR	1.57 ± 0.049	0.27 ± 1.70	1.63 ± 1.11	0.16 ± 0.11	0.53 ± 0.33
-All	0.96[d]	0.17	1.11	0.11	0.33
-AllR	1.39 ± 0.318	0.24 ± 0.028	1.67 ± 0.403	0.11 ± 0.000	0.44 ± 0.021

Key: d indicates a deficient concentration (see Binns et al., 1980; Forestry Commission leaflet number 76, p16).

Foliar results for pot trees (Table 2.6) show that trees in all deficiency treatments show expected deficiencies of elements, apart from -All trees, which were not P or K-deficient. The concentration of Mg in the -Mg set bordered on the published deficiency concentration of 0.03 % dry weight (Forestry Commission leaflet no.76), and is interpreted as being deficient.

Foliar results for new needles in November 1988 (Table 2.7) show that needles in -P and -K treatments also had a deficient concentration of N. Refertilisation of each treatment caused a restoration of optimal concentrations of elements, apart from the -KR treatment, where K deficiency was maintained.

2.7 Cell Number Estimation.

2.7.1 Needle Primordia.

Cell numbers in needle primordia were estimated using a method based on that of Sunderland and Brown (1956). After removal of the bud scales, the shoot apex was removed, and leaf primordia removed under a dissecting microscope (x20 objective), using a fine scalpel and fine tweezers, onto Petri-dishes containing water to prevent desiccation. To aid dissection of the primordia, horizontal cuts across the bud were made, (perpendicular to the main axis of the bud), after bud-scale removal, to divide the bud into quarters or sixths of the bud height, and primordia were then removed from each bud segment. From the population of primordia from each segment, a few were taken at random for cell counting. Alternatively, needle primordia were taken in series from the bud base to tip, from the phyllotactic spiral as shown in Fig. 2.1 C. Due to the small size of the primordia it was hard to excise them exactly at the point of attachment to the bud core, so up to five replicate primordia were taken from each segment, to minimise the resulting variation. Excised primordia were treated with acetic acid/ethanol (3:1,v:v) for 10 min, and then in N Hydrochloric acid for 8 min at 60°C, before being left in Feulgen reagent overnight, to stain the nuclei and render the counting of cells easier. The softened, stained primordia were then placed on a slide in a drop of glycerol, covered with a coverslip cut from a sheet of acetate, and squashed by gently rubbing the coverslip with the rounded end of a glass rod. The cells dispersed to a single layer, but covered a large area of the slide, so that the total cell number had to be counted by scanning the whole slide in a series of transects.

2.7.2 Needle Maceration and Cell Counting.

Needle cell numbers were estimated using a technique based on maceration in chromic acid (Sunderland, 1960; Brown and Rickless, 1949; Milthorpe and Newton, 1963; Maksymowych, 1963).

Initially, needles were soaked in 2 cm^3 of 5% (w/v) chromic acid solution

for 24 h, in a small sealed glass vial, and the tissue was then fragmented gently, by stirring with a metal dissecting needle. Further maceration was achieved by taking up the tissue fragments into a 5 cm^3 plastic syringe via a 5 cm long needle and expelling gently to shear the cells apart. This process was repeated 20 times, however, due to the large thickness of the cuticle and the presence of epicuticular wax, infiltration of chromic acid was reduced, and poor macerations resulted. Increasing the concentration of chromic acid used, from 5% to 10% (w/v) did not increase the degree of maceration, when a drop of the suspension was observed under a microscope. The length of time the needles were left in 5% chromic acid was increased from 24 h, to 48 h and to 72 h, but this only led to breakdown of separated cells, and not to an increase in extent of cell separation. Warming needles in 5% chromic acid at 30°C had no effect either, on the extent of cell separation, neither did increasing the concentration of acid, time of soaking or temperature together, in any combination. Therefore, work was concentrated on developing a pretreatment to break down the cuticle to aid acid penetration.

To dissolve cuticular wax, needles were soaked for 48 h in a solution of chloroform:methanol (1:1,v:v), then were rinsed thoroughly with water and soaked for a further 48 h in a solution of 5%, 10 M NaOH in methanol, to effect alkaline hydrolysis of the cuticle. The length of time the needles were left to soak in each solution was varied and 48 h was found to be the optimum duration. Following this pretreatment, needles were again rinsed thoroughly with water, and then macerated in 2 cm^3 of 5% chromic acid, under a slight vacuum to further aid penetration, and were fragmented as described earlier. The final cell suspension was fairly even, although it still contained some clumps of epidermal cells which were very hard to separate. A drop of the suspension was placed on a haemocytometer slide (Hawksley Crystallite modified Fuchs Rosenthal), and cell counts made for 6 replicate grids. The mean cell number contained per grid was then extrapolated to give the total number of cells in the suspension, and hence the cell number per needle, as two or three needles for each position in the spiral were used to give the suspension.

2.8 Estimation of Needle Chlorophyll Content.

Chlorophyll was measured in needles from the field plots during the 1987 growth season, and in needles from pot-grown trees during the 1988 growth season. Twenty-five needles were taken from the mid-point of extending terminal shoots from whorl 7 from trees in the field, and from whorl 2 (1987 apical whorl) of the pot trees in 1988.

Obviously the position of the shoot mid-point changed as the shoot elongated, so that needles were sampled from a progressively more distal position each time, until early July when shoot extension ceased. Chlorophyll levels during this period however, increased so rapidly that the effect of the slight difference in developmental state of the needles taken for chlorophyll analysis was minimised. At each sampling time, needles from four shoots from different trees were sampled from the field plots, and needles from two trees from each treatment of the pot experiment. The fresh weight of the needle sample was taken, before homogenising the needles in a chilled mortar, with about 5 cm^3 of 100% acetone and a little acid-washed sand. The brei was centrifuged for 10 minutes at 500 g and 4°C. The supernatant was removed and the residue reground in a further few cm^3 of acetone and centrifuged again. The supernatants were combined and diluted to a final known volume of 15 - 25 cm^3. Absorbance readings were taken on a Pye Unicam Spectrophotometer at 440.5 nm, 644 nm and 662 nm. The chlorophyll and carotenoid contents (mg l^{-1}) were calculated by substituting the absorbance values into the following equations (Holm, 1954):

$$\text{Chlorophyll a} = 9.78.A(662) - 0.99.A(644)$$
$$\text{Chlorophyll b} = 21.40.A(644) - 4.65.A(662)$$
$$\text{Carotenoids} = 4.69.A(440.5) - 0.267(\text{Chl a} + \text{Chl b}).$$

2.9 Assay of RuBPC.

2.9.1 Preparation of Needle Extracts.

For measurement of RuBPC activity and soluble protein content, a needle protein extract was prepared. The method was based on the single stage extraction used by Beadle et al. (1983) for Sitka Spruce and other conifers, which was a modification of the method of Gezelius and Hallen (1980).

The extraction medium contained the following:

Tris buffer (Trizma base, Sigma)	50 mM
Magnesium chloride (B.D.H.)	10 mM
E.D.T.A. (B.D.H.)	0.2 mM
Dithiothreitol (Sigma)	5 mM
Tween 80	1% (v/v)

The final pH of the medium was adjusted to 7.9 using 1 M HCl. The use of E.D.T.A. (0.2 mM) appears to be standard throughout the literature. The magnesium needed to maintain enzyme activity (Weissbach et al., 1956) was shown by Bassham et al. (1968) and Suguyama et al. (1968) to affect the enzyme pH optimum with varying concentration, and at 10 mM Mg^{2+} the pH optimum lay between 7.8 and 8.0, and so a mean of 7.9 was used. Dithiothreitol was selected as the sulphydryl group protective agent in preference to β-mercaptoethanol, as it is also an inhibitor of polyphenol oxidase. This is important in extracting enzymes from conifers, due to the large quantities of polyphenols present in the tissues. Tween 80 was used to help solubilise the membranes. The extraction medium was made freshly for each weekly set of extractions and kept at 2°C in the dark. Extraction of needles was performed in a cold room, where all equipment was left prior to use. 0.5 g fresh weight of needles was frozen in a small amount of liquid nitrogen, and ground with 10 cm^3 of the extraction buffer, and a little acid-washed sand, until a uniform homogenate was produced. This was then filtered through 8 layers of cheesecloth, and the residue reground in a further 10 cm^3 buffer solution, and refiltered. The combined homogenate was then centrifuged at 15,000 g at 1°C for 15 min, in a Sorvall RC-5B centrifuge. The pellet containing cell debris and sand was discarded, and the supernatant containing the carboxylases was decanted and stored on ice. Some of the supernatant was frozen in liquid nitrogen, and stored in eppendorf vials at -40°C to await protein determination. The remainder was assayed for RuBPC activity as quickly as possible.

2.9.2 **RuBPC Assay System**

The basic reaction which RuBPC catalyses is the conversion of Ribulose 1,5-bisphosphate and carbon dioxide to 3 Phosphoglyceric acid, in the presence of magnesium ions. The assay measures RuBPC activity by following the incorporation of $[^{14}C]O_2$ from $NaH[^{14}C]O_3$ into acid stable products. The

method used was based on that of Beadle et al. (1983), and experimental justification for the technique is given in section 2.10.

A stock solution of 3 mM RuBP (Sigma) was freshly prepared for each day's assays. A stock solution of labelled $NaHCO_3$ was made, containing 9.5 cm^3 150 mM cold $NaHCO_3$, and 0.5 cm^3 $NaH[^{14}C]O_3$ (1 mCi cm^{-3},Amersham), and kept in the dark at 2°C. Speed of assay of the crude extracts was important, and assays were carried out within 30 min of extract preparation. Reaction times were reduced to 1 min, to allow maximum linear rates to be obtained (Beadle et al., 1983). Duplicate assays were performed on each extract. The final reation mixture contained 0.1 M Tricine, 0.025 M $MgCl_2$, 6 mM Dithiothreitol, 0.2 mM E.D.T.A., 0.5 mM RuBP, and 0.05 M $NaH[^{14}C]O_3$ stock. The final reaction volume was 300 mm^3, composed of 50 mm^3 3 mM RuBP, 100 mm^3 of stock $NaH[^{14}C]O_3$, and 25 mm^3 of each of the four stock solutions of E.D.T.A., Dithiothreitol, $MgCl_2$ and Tricine. 50 mm^3 of the enzyme extract was preincubated with the sodium bicarbonate and the other four constituents in a water bath at 25°C for 10 min, as the enzyme must be activated by Mg^{2+} and CO_2 prior to assay (Jensen and Bahr, 1977). The reaction was started by the addition of 50 mm^3 of stock RuBP, and was stopped after 1 min by adding 0.2 cm^3 5N HCl to degrade excess bicarbonate. Sample vials were shaken, and 20 mm^3 aliquots of each reaction solution were pipetted onto 30 x 10 mm strips of Whatman No.1 filter paper, using 10µl microcaps (Shandon). Duplicate aliquots were pipetted onto different strips for each assay, and the strips left to dry for 3 h under a fume hood, along with the sample vials, to allow excess labelled CO_2 to be released safely. The dried paper strips were placed in Beckman scintillation vials containing 2 cm^3 scintillant [Analar toluene containing 5% (w/v) PPO (2,5 Diphenyloxazole), and 0.5% (w/v) POPOP (1,4 Di-[2-(5 phenyloxazolyl- benzene)])], and duplicate counts were made for 10 min in a ISL-3000 liquid scintillation counter.

2.9.3 **Calculation of Counting Efficiency.**

The counting efficiency of the scintillation counter could not be calculated by spotting a known amount of sodium bicarbonate stock solution onto filter paper and counting, as exchange of labelled CO_2 with atmospheric CO_2 would occur during drying of the filter paper. A 20 µl aliquot of $[^{14}C]$ glycine (1 µCi cm^{-3}) was therefore spotted onto filter paper which was then dried, and duplicate counts were made in the same scintillant, in the same way as for the

assay samples. This was performed twice, on three separate occasions, and a comparison of the counts per minute obtained, with the counts per minute expected from the specific activity, the counting efficiency of the scintillation counter was found to be 46%.

2.9.4 **Calculation of RuBPC Activity.**

To calculate the activity of RuBPC in the extracts, a comparison has to be made between the counts obtained, and the counts expected by the stock bicarbonate solution, corresponding to a known amount of substrate.

Stock bicarbonate solution:

$9.5\ cm^3$ of 150 mM $NaHCO_3$ and $0.5\ cm^3$ of $NaH[^{14}C]O_3$ (1 mCi)

The specific activity of the $[^{14}C]$-labelled sodium bicarbonate = 55 mCi $mmol^{-1}$

So the total amount of bicarbonate in $10\ cm^3$ stock solution =

1.425 mMol (unlabelled) + 0.01818 mMol (labelled) = 1.44318 mMol (Total)

Therefore every $0.1\ cm^3$ used in the assay, contains 14.4318 µmol bicarbonate, and 370,000 Bq (10 µCi) activity.

The d.p.s. of the 20 µl aliquot counted is equal to;

c.p.m. ÷ 0.46 (counting efficiency) ÷ 60 (per second)

Total assay volume = $0.5\ cm^3$, so d.p.s. x (0.5 / 0.02) = d.p.s. per assay.
(from a total d.p.s. provided by $0.1\ cm^3$ stock, of 370,000)

So total µmol substrate converted per min per assay =

(d.p.s. fixed per assay ÷ 370,000) x 14.4318

As $0.05\ cm^3$ of needle extract was used in each assay, from a total of $15\ ^3$ (= 0.5 g. needle mass), then the activity of the extract in µmol $CO_2\ g^{-1}$ fr.wt. min^{-1}

= activity per assay (µmol $CO_2\ min^{-1}$ x (15 ÷ (0.05 x 0.5)))

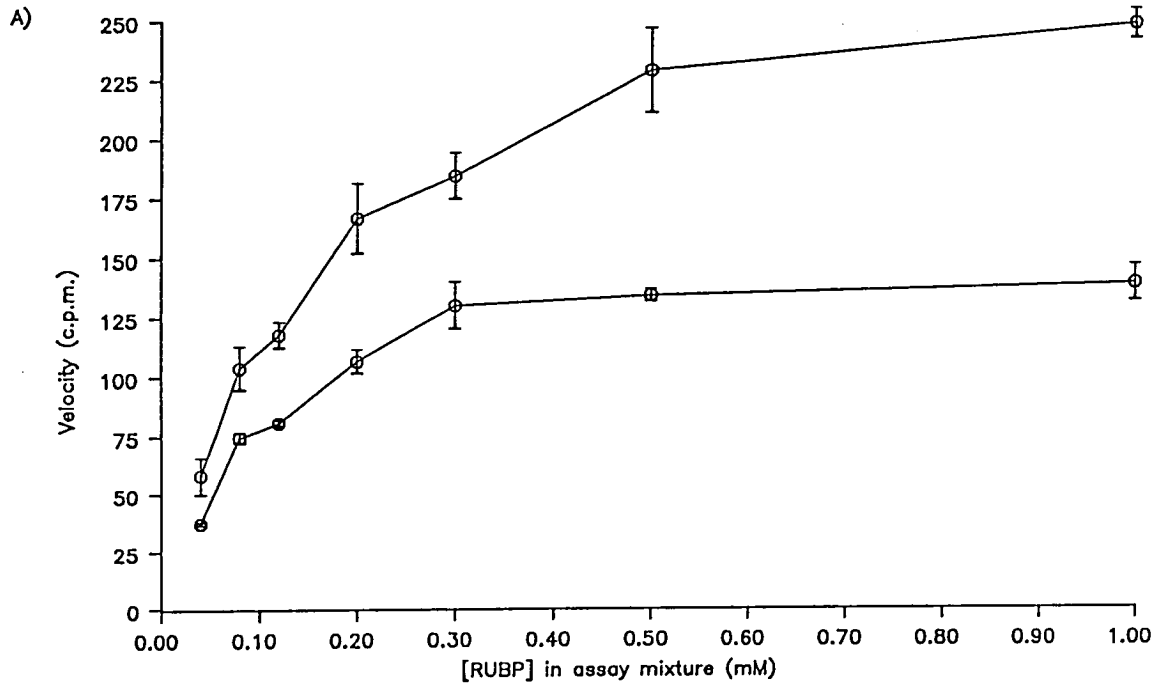
2.10 Kinetic Work With RuBPC.

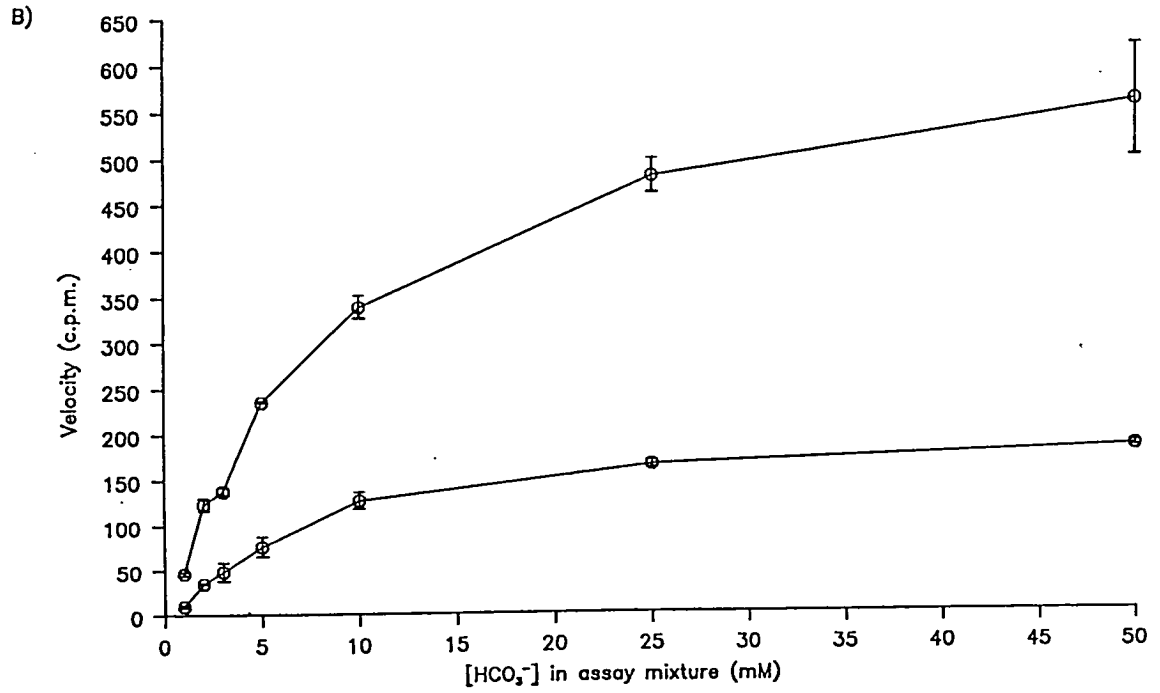
As in the study of any enzyme, it was important to check that the substrate concentrations used in the assay were on the plateau of the relationship between reaction velocity and substrate concentration, and therefore that the enzyme was operating under saturating conditions. The work of Beadle et al. (1983) was repeated to find optimal concentrations of RuBP and bicarbonate, using extracts from Control needles from mid-canopy in the field.

A) RuBP:- Using a constant bicarbonate concentration of 50 mM, the RuBP concentration in the assay was varied over the range 0.04 mM to 1 mM. Reaction velocity against RuBP concentration for two extracts of different activities (Fig. 2.3 A) show that the response of reaction velocity to RuBP concentration was hyperbolic, with a concentration of 0.5 mM used in the assay, falling on the plateau of the relationship.

B) Bicarbonate:- Using a constant RuBP concentration of 0.5 mM, the effect on reaction velocity was examined by varying the concentration of bicarbonate in the assay over the range 1 mM to 50 mM (Fig. 2.3 B). The kinetics of the reaction again showed a hyperbolic relationship between velocity and bicarbonate concentration, for two extracts of differing activities. A concentration of 50 mM HCO_3^-, used the assay, fell on the plateau of the graph, and was therefore not limiting enzyme activity.

Figure 2.3 A and B. Velocity of RuBPC reaction. A) against RuBPC concentration, with constant bicarbonate concentration (50mM). B) against bicarbonate concentration, with constant RuBP concentration (0.5 mM), for an extract of high and low RuBPC activity in each case. Values are means for two replicate assays ± S.D.





It was also necessary to check that an assay duration of 1 min represented a period of linear reaction velocity, and that a preincubation time of 10 min was needed, and that preincubation did not cause any CO_2 fixation:

C) The reaction velocity was examined against duration of assay in two extracts (Fig. 2.3 C). Reaction rate was essentially linear up to 1 min and then declined, confirming 1 min to be a suitable assay duration.

D) Preincubation of the enzyme extract with all constituents apart from RuBP, was carried out for 2 min, 5 min and 10 min, before the reaction was performed. Results showed that maximum velocity was achieved with a 10 min incubation period (Fig. 2.3 D).

E) In order to test whether the number of counts in control assays was affected by length of preincubation, control tubes were left for a range of times up to 10 min, before the addition of water. Preincubation time did not affect the counts (Fig. 2.3 E).

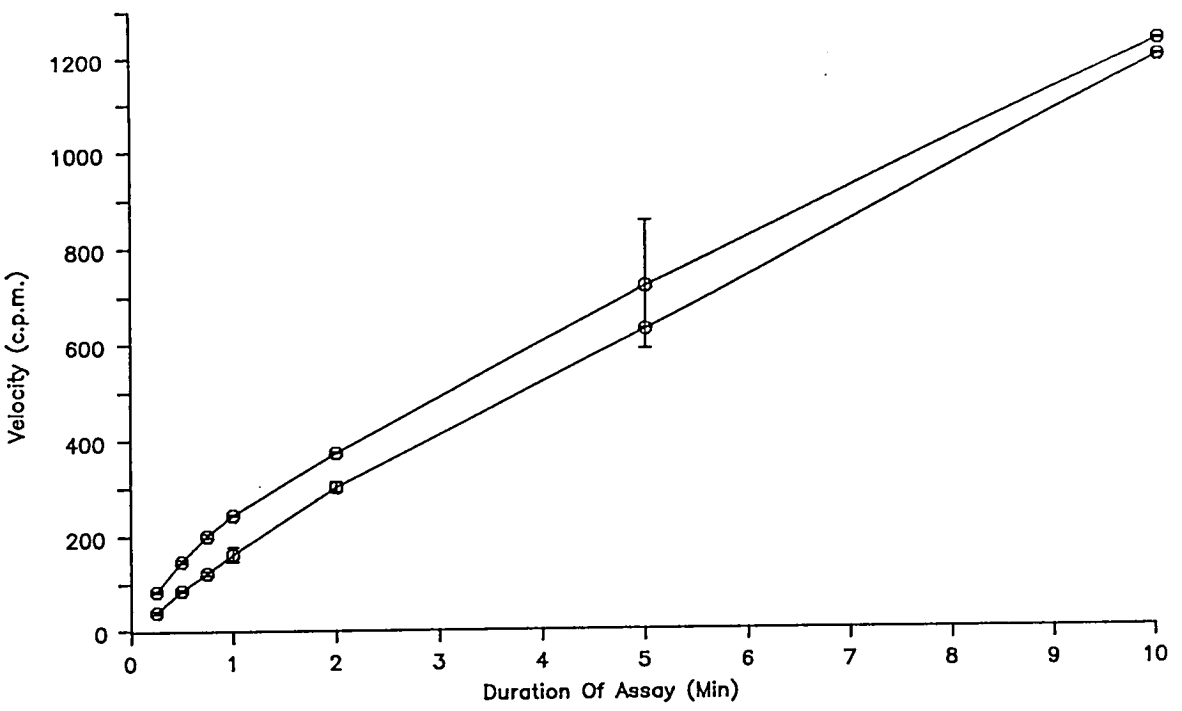
Figure 2.3 C

Reaction velocity plotted against duration of assay for two extracts. Each value is the mean of two assays ± S.D.

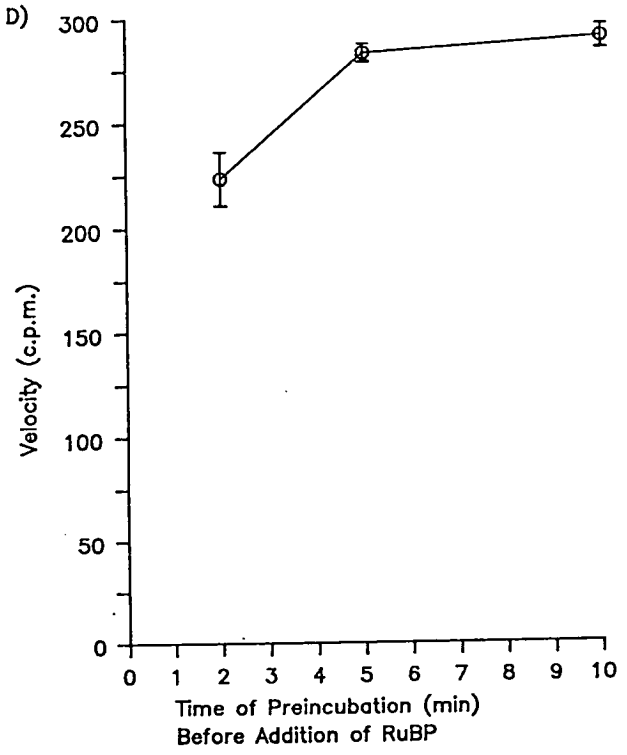
Figure 2.3 D and E

Reaction velocity plotted against time of preincubation of reaction constituents before the addition of D) RuBP, and E) Water.

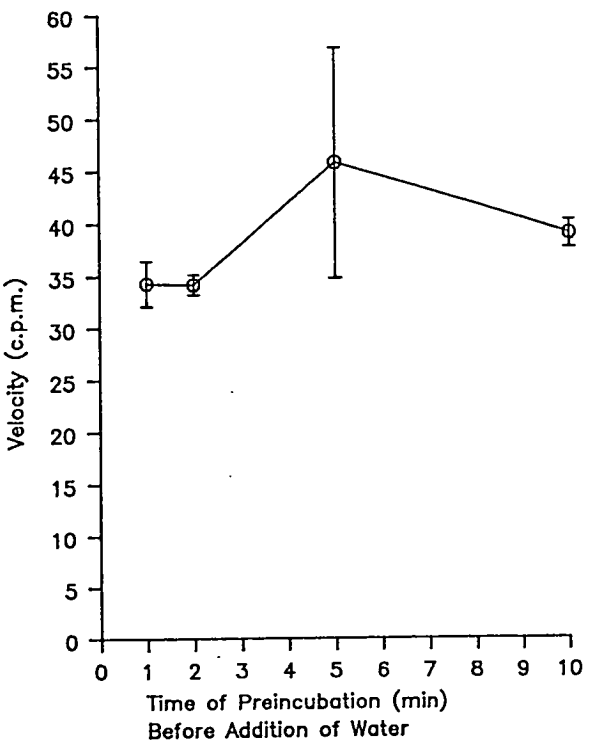
C)


Velocity (c.p.m.)
0
200
400
600
800
1000
1200
0 1 2 3 4 5 6 7 8 9 10
Duration Of Assay (Min)

D)

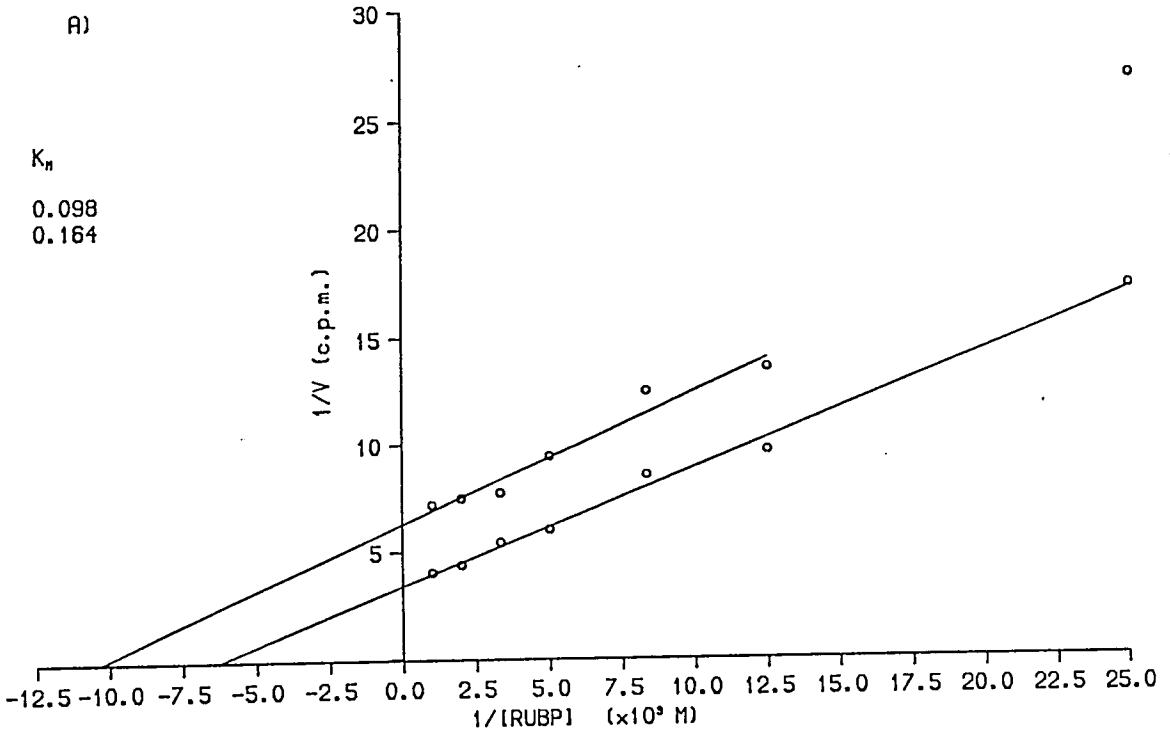

Velocity (c.p.m.)
0
50
100
150
200
250
300
0 1 2 3 4 5 6 7 8 9 10
Time of Preincubation (min)
Before Addition of RuBP

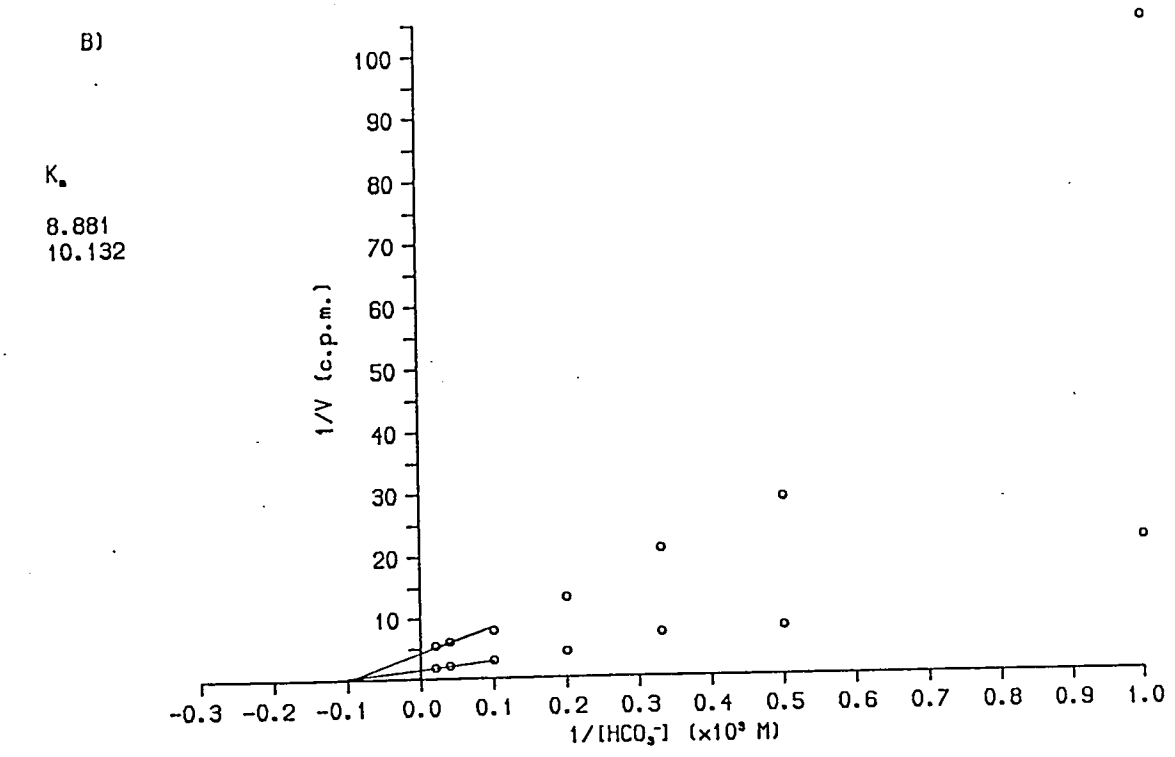
E)


Velocity (c.p.m.)
0
5
10
15
20
25
30
35
40
45
50
55
60
0 1 2 3 4 5 6 7 8 9 10
Time of Preincubation (min)
Before Addition of Water

In order to show that the enzyme assay did in fact measure RuBPC activity, the Michaelis-Menten constant, K_M of the two substrates was determined, and compared with the values in the literature. Double-reciprocal (Lineweaver-Burk) plots for data for the extracts are shown in Fig. 2.4 A and B. The points did not always fall on a straight line (Fig 2.4 A and B), with a tendency towards curvilinearity at low concentrations of RuBP or HCO_3^- when the enzyme activity was high. This suggests some positive allosteric interaction between substrate and enzyme may occur, at low substrate concentrations. This is the same conclusion as that reached by Beadle et al. (1983), working with Sitka Spruce. The K_M values have been calculated by drawing a straight line through these higher concentrations (Fig. 2.4 A and B), and assuming that the enzyme is fully activated at the higher bicarbonate concentrations i.e. > 3 mM and < 20 mM (Beadle et al., 1983; Gezelius and Hallgren, 1980). The K_M(RuBP) values of 0.10 to 0.16 mM compare well with a K_M(RuBP) of 0.12 mM (Beadle et al., 1983), and are slightly lower than the value of 0.18 mM reported for Scots pine (Gezelius, 1975). The K_M (HCO_3^-) values of 8.9-10.1 mM from this study, are about twice as high as that of 4.7 reported by Beadle et al. (1983) for RuBPC for Sitka spruce. The reason for this difference may be variation due to the small number of extracts used for the determination (n=2 in both cases). Alternatively, preincubation of the enzyme and assay constituents prior to assay may cause a transition from a low or intermediate K_M form to a high K_M form (Bahr and Jensen, 1974), so that the different K_M values for different extracts in this study, and the difference between these and those found by Beadle et al. (1983) may represent different activated states of the enzyme. The values of $K_M(HCO_3^-)$ obtained here are similar to those of 8-10 mM found for *Pinus sylvestris* by Gezelius (1975), who used a slightly higher concentration of Mg^{2+} than was used in this study, although at lower concentrations of Mg^{2+}, they found higher $K_M(HCO_3^-)$ values.

Figure 2.4 Lineweaver-Burk plots for the relationship between RuBPC reaction velocity and A) Concentration of RuBP. B) Concentration of HCO_3^-, for extracts with high and low RuBPC activity. Each point is the mean of two determinations.





2.11 Protein Estimation.

The estimation of the total soluble protein content in frozen extracts from needles from the pot trees was attempted, using a method similar to that of Sarjala et al. (1987), The deep-frozen samples were thawed at room temperature, and 0.2 cm^3 of the extract was added to 1.2 cm^3 of 10% trichloroacetic acid (T.C.A.) in and eppendorf vial. The vials were shaken and stood for 30 min. on ice, to precipitate the protein, before centrifuging for 10 min. at 11,000g. The supernatant was discarded, and 0.5 cm^3 0.1 M NaOH was added to the pellet to re-solubilise the protein. The vials were vortexed, and left to stand for a further 30 min. to effect thorough resolubilisation, before recentrifuging at 11,000g for 10 min. Protein content was measured using the method of Bradford (1976). Absorbance readings at 595 nm were first calibrated against a calibration curve, which was prepared using bovine serum albumen (B.S.A.) (fraction 5) in the extraction buffer, covering the range up to 50 mg per 0.1 cm^3 extraction buffer. The calibration samples were passed through the same procedure of precipitation and resolubilisation in 0.1M NaOH as the samples. However, the extraction buffer gave some background colour, even when it contained no added B.S.A., although a quantitative relationship existed between A_{595} and B.S.A. concentration. The reason for this background colour was not known, but was possibly due to the presence of Tween 80 in the buffer, as detergents are known to interfere with the Bradford reagent. A sample of the extraction buffer with no added protein was used as the reagent blank in the spectrophotometer, until it was realised that the samples gave a lower reading than the blank, due to a suppression of the background colour, presumably due to some component of the needle extract. The Bradford method was therefore unsatisfactory for the determination of the protein content of the samples, and the results were unreliable, and were not used for any calculations.

2.12 Measurement of Photosynthesis Using Infra-red Gas Analysis.

The uptake of CO_2 by shoots was measured using infra-red gas analysis. The system consisted of a LCA-2 battery operated portable infra-red gas analyser, a mass flow air supply unit, a leaf cuvette (Parkinson leaf chamber), and a data logger (all ADC Ltd.).

2.12.1 Use of the IRGA.

The equipment was used in an open system (Fig. 2.5), where the plant material was placed in a cuvette into which there was a measured flow of air of known water vapour pressure and carbon dioxide content. Gas exchange rates were then calculated from the flow rate and the concentration differences between inlet and exhaust air. The four components of the system and the settings used are outlined below;

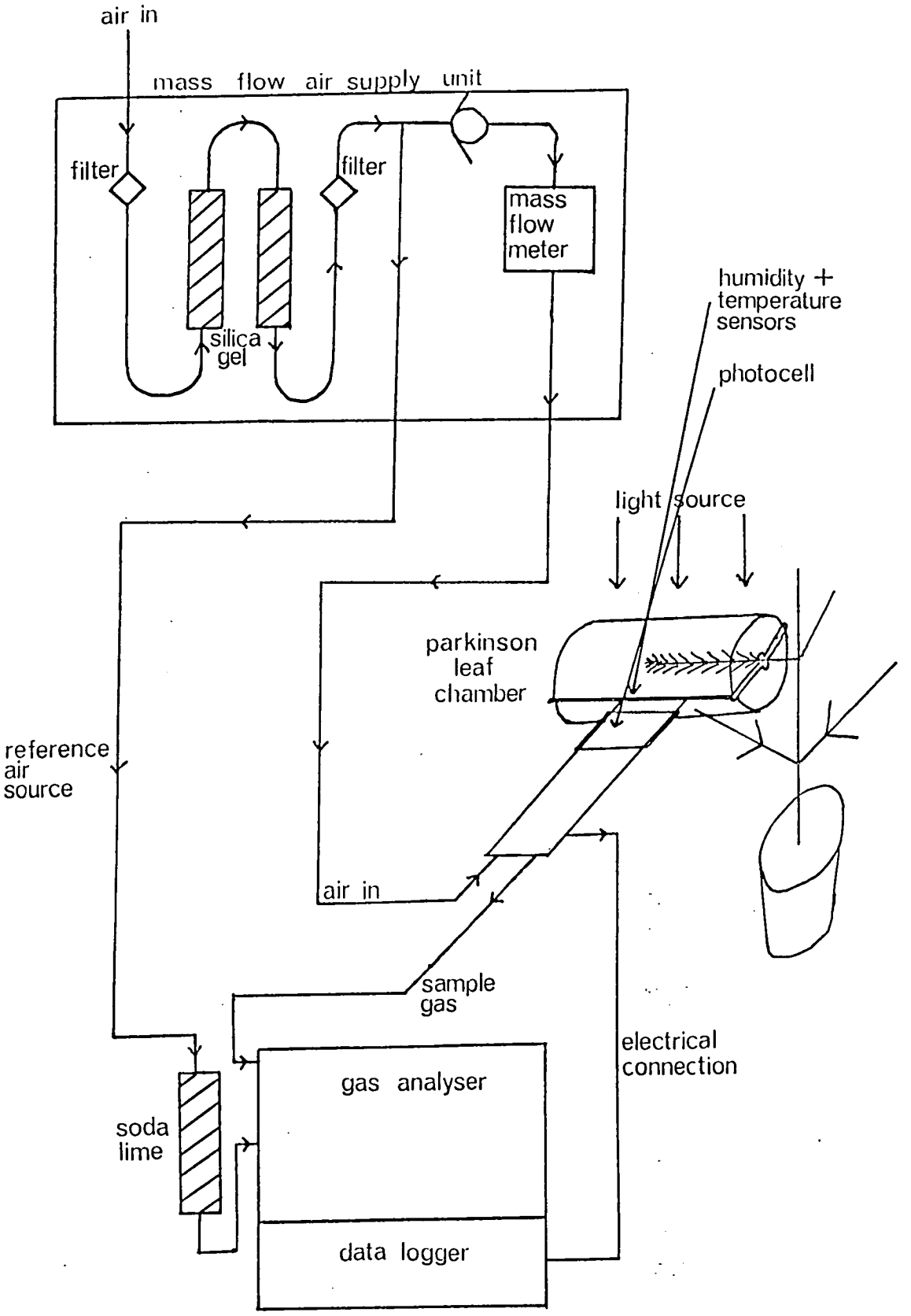
a) The mass flow air supply unit (ASUM) supplied dry air (passed through two columns of silica gel) to the Parkinson leaf chamber at a rate of 500 cm^3 min^{-1}. This flow rate was chosen as there is a minimum requirement of 150 cm^3 min^{-1} for the analyser, and the flow through the leaf chamber must be about 50 cm^3 min^{-1} greater. Also it is recommended flow be about 60 cm^3 min^{-1} for every 1 cm^2 projected leaf area in the cuvette. As total shoot area was likely to be up to several cm^2, a high flow rate was considered necessary.

b) The Parkinson leaf chamber contained humidity, light and temperature sensors, and these readings were passed directly to the data logger for the photosynthetic calculations.

c) The gas analyser was used in 'differential' mode, which allowed absolute concentrations of CO_2 in reference and sample gases, and the differential concentration to be displayed on the data logger. The analyser had a single infra-red beam passed through a filter to restrict radiation to the 4.3 µm absorption band. This radiation was passed through a measuring cell of the sample gas to a detector. In differential mode the time taken for alternation of gas in the measuring cell was 8 seconds: during the first half of the cycle the gas in the cell alternated every 2 seconds between sample gas from the reference source, and gas which has had CO_2 removed by soda lime. During the second half of the cycle, the alternation was between gas from the leaf chamber, and CO_2-free air. Signals from the detector were stored, and

Figure 2.5
The arrangement of IRGA apparaus in the open system for the measurement of photosynthetic rate.

Figure 2.5


air in
mass flow air supply unit
filter
filter
mass flow meter
silica gel
humidity + temperature sensors
photocell
light source
parkinson leaf chamber
reference air source
air in
sample gas
electrical connection
gas analyser
soda lime
data logger

compared as the measuring cell gas changed, to give absolute concentrations for sample and reference gases and the differential reading.

d) The data logger provided various options for the type of information displayed, and calculations made. Option 2 was selected as this allowed the flow-rate and leaf projected area, which must be input, to remain constant for all measurements, and to input these values only once. As the projected leaf area of the shoot was not known until destructive determination following the measurement of photosynthetic rate, a constant value of 10 cm^3 was input, as this was thought to be a reasonable estimate of the total needle projected area of fully extended shoots. Subsequently, the calculated values were adjusted after the actual projected areas had been determined.

2.12.2 **Calibration.**

Before use and at regular periods during use, the leaf chamber humidity sensor and gas analyser had to be calibrated;

2.12.2.1 **Parkinson Leaf Chamber Humidity Sensor.**

This was calibrated by firstly passing dry air, obtained by passing air through a column of Drierite, into the leaf chamber, and adjusting the appropriate potentiometer until the reading on the gas analyser was zero. Air of known vapour concentration was then obtained by passing air through a 30 cm x 5 cm column of $FeSO_4.7H_20$, with a maximum flow-through of 150 ml min^{-1}, before passing the air through the leaf chamber. The relative humidity at the measured air temperature was obtained from tables, and the span control potentiometer was adjusted to give the appropriate reading.

2.12.2.2 **Gas Analyser.**

1) Flow Adjustment.

The flow of air through the gas analyser had to be maintained above 150 cm^3 min^{-1}. The flow rate was gauged by a flow meter on the side of the analyser, and was altered to be above the necessary value by adjusting the flow set potentiometer.

2) Calibration for Carbon Dioxide.

(i) Zero adjustment: - For this the analyser was used in 'zero' mode, which only allowed gas which had been passed through soda lime to enter the measuring cell. The 'zero potentiometer' was then adjusted so that a vpm reading of zero was displayed.

(ii) Span calibration: - The analyser was used in 'reference' mode, which allowed air in the measuring cell to alternate between CO_2-free air and input air supplied from a gas mixer, containing air at a concentration of 350 ppm CO_2. The reading was adjusted accordingly.

(iii) The differential calibration: - This was checked by passing air with a CO_2 concentraion of 350 ppm from a gas mixer, through both inlet and reference ports of the analyser in 'differential' mode, and adjusting the reading to zero.

These calibrations were carried out about every 3 weeks of experiments, during which time the CO_2 differential calibration hardly ever changed, and the CO_2 zero and humidity adjustments needed recalibration.

2.13 Preparation of Material and Measurement of Photosynthetic Rate.

Measurements were made every Thursday after the trees had been put in the growth room the preceding Friday. Lights came on in the growth room at 9 a.m. and measurements were made from one hour later to allow time for maximum photosynthetic rate to be reached. Measurements took 2 hours which minimised effects due to diurnal fluctuations in rate. The input gas to the ASUM was taken from outside the growth room where all work was done, to avoid CO_2 from breath changing ambient CO_2 levels. The IRGA was switched on only a few minutes before use, as no warm-up time was necessary. Before each shoot was put into the leaf chamber, needles were removed from about 2 cm of the preceeding year's shoot to ensure none were inserted by accident and that a good seal was maintained. All tubing was kept short to minimise response times, and constant readings were obtained after about 30 sec - 1 min; the data were recorded and the calculated values from the data logger retrieved immediately. The shoot was then excised and projected surface area estimated.

2.14 Inputs into the Data Logger.

2.14.1 Manual inputs.

Manual inputs to the data logger include:

1. Volume flow rate of dry air into the cuvette (read from air supply unit).
2. Projected leaf area (arbitrary value of 10 cm^3).
3. Boundary layer resistance to water vapour (determined to be 0.495 m^2 s mol^{-1}).
4. Atmospheric pressure (bar).

The boundary layer resistance to water vapour was determined by suspending wet filter paper (2 cm^3 area) in the leaf position in the cuvette, and measuring the equilibrium relative humidity and cuvette air temperature at a flow rate of 5 cm^3 s^{-1}. The value of r_b was obtained from the % relative humidity at the measured temperature, by consulting tables (see Parkinson 1984).

A correction factor for the water response of the analyser is sometimes needed, when there is a different concentration of water vapour in the sample cell during the two halves of the measurement cycle. This may occur if the sample air is drier than the soda-lime equilibrium, so that during the CO_2 free part of the cycle, gas having passed through the soda-lime enters the measurement cell containing 1-2% water vapour, depending on the temperature of the soda-lime. This reduces the measured CO_2 concentration by about 1 vpm. The correction factor is a function of E_{MAX}, which is the analyser response to an infinite concentration of water vapour. It was not employed in the calculations, as the sample gas was not drier than the soda-lime equilibrium.

2.14.2 Automatic Inputs to the Data Logger From the Parkinson Leaf Chamber.

These include:

1. Relative humidity in cuvette (%).
2. Partial pressure of CO_2 in air entering cuvette(µbar).
3. Partial pressure of Co_2 in air leaving cuvette (µbar).
4. Photon flux density incident on cuvette (µmol m^{-2} s^{-1}).
5. Cuvette air temperature (°C).

2.15 Calculations.

The other calculated variables, which were performed by hand on the raw data for the first four weeks, and subsequently by the data logger, make use of the following equations (see abbreviations section for meaning of letters and symbols): A) The mass flow of air per unit leaf area through the cuvette;

$$W_f = ((V \times P_a) / ((273 + t_a)a)) \times 120.311 \text{ mol } m^{-2} s^{-1}$$

B) Assuming that dry air enters the cuvette, the transpiration rate from the leaf (E);

$$E = (e_o / (P_a - e_o)) \times W_f \text{ mol } m^{-2} s^{-1}$$
$$\text{where } e_o = e_s \times (h_c / 100)$$

C) To calculate the leaf temperature from the energy balance of the leaf: The energy absorbed by the leaf (H):

$$H = (Q \times 698 / 3190) \times ((0.8 \times 0.85) + (0.2 \times 0.6))$$

where (698/3190) converts the mol quanta (Q), to W m^{-2}; 0.8 is the fraction of visible light absorbed by leaves and 0.85 is the fraction transmitted through the chamber; 0.2 is the fraction of infra red absorbed by the leaves, and 0.6 the fraction transmitted through the leaf chamber. Then:

$$\Delta t = ((0.175 \times Q) - (\lambda E)) / 0.93 \times M_a \times (C_p / r_{b\ water} + 4\sigma(t_a + 273)^3$$

The leaf temperature: $t_l = t_a + \Delta t$

D) The stomatal resistance to water vapour:

$$r_s = ((e_l / e_o - 1) \div W) - r_b$$

The stomatal conductance to water vapour:

$$G_{s\ water} = 1/r_s$$

The stomatal conductance to carbon dioxide:

$$G_s = G_{s\ water} / 1.606$$

E) Before the calculation of photosynthetic rate, the analyser CO_2 concentration must first be corrected for the diluting effect of water vapour picked up in the cuvette:

$$C_c = (P_a \times C_o) \div (P_a - e_o)$$

F) The assimilation rate:

$$P_N = (C_e - C_o) \times W_f \text{ mol } m^{-2} s^{-1}$$

G) The intercellular concentration of CO_2:

$$C_i = (((G_c - E/2) \times C_c) - P_N) / (G_c + E/2)$$
where $1/G_c = 1.606/G_s = 1.37/G_b$
and where $G_b = 1/r_{b\ water}$

2.16 **Statistical Analysis.**

A major statistical analysis used in these studies was a single or double analysis of variance (ANOVAR), to determine the significance of differences among treatment means subject to one or two factors (Parker, 1979). The level of significance was determined from the variance ratio, using the tables in Kokoska and Nevison (1989).

With two factor analyses, significantly different means were distinguished by calculating the Least Significant Difference (LSD), and employing it to compare the difference between means. For one factor analyses, significantly different means were determined by using a Multiple Range Test; involving calculation of the Shortest Significant Range (SSR), and using it to test the difference between pairs of means according to their relationship in size order. Treatments with non-significant differences between means were then underlined.

Significant differences between treatment means for some variables were tested using 95% confidence limits for P=0.01, 0.05 and 0.001. Correlation coefficients were tested for significance using the tables in Parker (1979).

Linear, polynomial and geometric functions were fitted to some data using a curve fit programme by Paul Warme (Interactive Microware Inc., P.O.Box 771. State college, Pa 16801, America), on an apple II microcomputer.

CHAPTER 3. RESULTS. NEEDLE GROWTH: MORPHOLOGY.

This chapter is divided into sections dealing with morphological characteristics of needles measured during 1987 on field material, and 1988 on pot material. Also included are apical data for field material for 1988.

3.1 Bud Burst, Shoot Extension, and Needle Number Per Shoot.

In 1987 in the field, bud-burst of shoots in Control and -Mg trees occurred at the beginning of May, but was about two weeks later for -P, -K and -All shoots. In 1988, bud burst in Controls occurred at the end of April, but buds were smaller than those in the field, and shoots were only large enough to sample from May 2. Trees in the deficiency treatments flushed at about the same time as the Controls, apart from those in -All, -AllR, -N and -NR treatments, where bud burst was delayed by about two weeks.

Substantial stem extension occurred from May 18 onwards, for the 1987 experiment,. with shoot elongation being completed in all deficiency plots by the harvest on July 8 (Fig. 3.1), and by July 13 in the Controls. The fastest rates of extension, up to a mean of 5.6 mm d^{-1}, were found in Control shoots in early June, and these showed the greatest final length. The mean growth rate for the whole period of shoot extension was 3.27 mm d^{-1} (Table 3.1). Although the duration of growth was slightly different between treatments, final shoot length was related to extension rate, with shoots from the -P set of plants extending slowest (Table 3.1), and showing a final length about one third of the Controls. Shoots in the -All treatment grew slower initially, but attained a significantly greater final length than those in the -P set. The number of needles per shoot, for -P and -All treatments was similar in October, and significantly less than the values for the other treatments (Table 3.2). When the mean stem unit lengths were compared, -K and -All treatments were similar, with lower values than the Controls; the shortest shoots were those in the -P set, where needles were packed nearly twice as closely as on Control shoots (Table 3.2). Shoot extension was not measured in 1988, so no stem unit data may be derived.

Figure 3.1
Changes in shoot length for shoots from mid canopy, for all treatments in the field throughout the 1987 growth season. Each point is the mean of five values ± S.E.

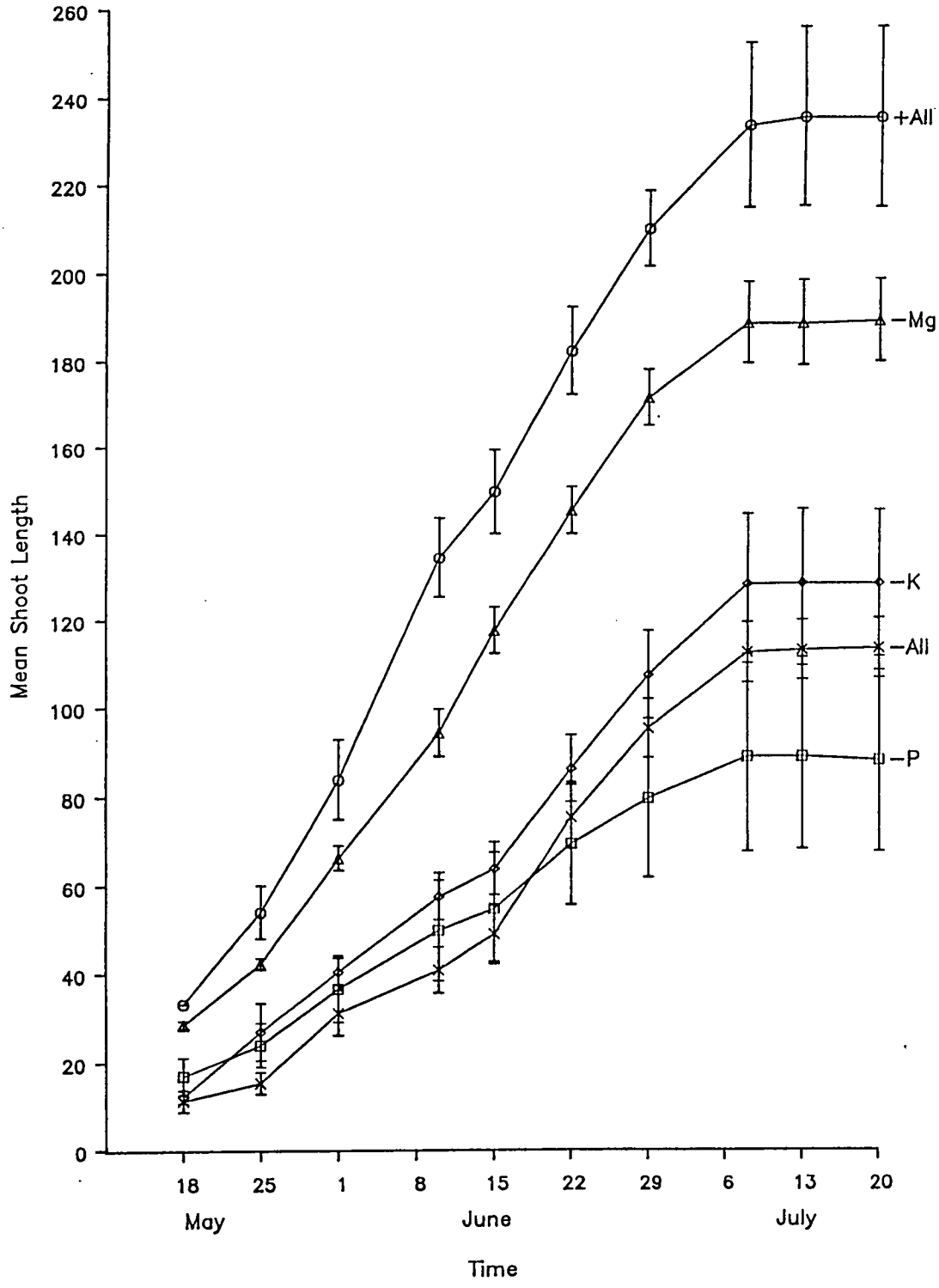


Table 3.1 Mean rates of shoot extension during the whole growth season for all treatments.

Treatment	Total Shoot Growth (mm)	Period of Growth (days)	Mean Extension Rate (mm d^{-1})
Control	236	71	3.32
-Mg	188	71	2.66
-K	128	57	2.25
-P	88	57	1.55
-All	112	57	1.97

Table 3.2 Mean lengths, needle numbers and stem unit lengths of first order shoots taken from mid-canopy. Trees were sampled in October 1987. n=10.

Treatment	Mean Shoot Length (mm) ± S.E.	Mean Needle Number Per Shoot ± S.E.	Mean Stem Unit Length (mm)
Control	212 ± 6	347 ± 19	0.61
-Mg	212 ± 10	331 ± 11	0.64
-K	164 ± 14	326 ± 20	0.50
-P	85 ± 9	240 ± 11	0.35
-All	114 ± 6	225 ± 15	0.50

The mean needle number per shoot in 1988 represents the total number of primordia initiated during the prece ding year, for all treatments apart from -N and -All. These two treatments showed "free" growth during the 1988 season, whereby new needle primordia were initiated, and expanded to give fully mature needles within the same season, as a result of favourable growth conditions following refertilisation. Since for all other treatments, the needle number per shoot was determined during 1987, when the deficiency treatments were applied, and before refertilisation of some plots, no difference is to be expected between refertilised and deficient sets. When 95% confidence limits were compared, -N, -NR, -All and -AllR treatments had significantly reduced needle numbers per shoot compared with the Controls (Table 3.3). Needle number of -N and -All shoots was also significantly lower than that for shoots in the respective -NR and -AllR treatments. This was due to the "free" growth of -NR and -AllR shoots, which began to show considerably higher needle numbers compared with -N and -All shoots, from as early as June (Table 3.4).

Table 3.3 Needle number per shoot for all treatments, averaged over the whole season 1988. Values are means ± S.E. n=35 for Controls and 17 or 18 for the other treatments. Asterisks show degree of significant difference from the Controls.

	+All	-MgR	-Mg	-KR	-K	-PR	-P	-NR	-N	-AllR	-All
Mean For Whole Season	182 ±8	181 ±9	196 ±11	167 ±6	182 ±10	211 ±13	185 ±11	116 ±10 **	60 ±3 ***	133 ±10 *	67 ±5 ***
% Reduction From Control									63%		67%

Table 3.4 Mean needle number per shoot during the 1988 season, for Control, -AllR, -All, -NR, and -N treatments. n=2.

Date	**Treatment**				
	Control	-AllR	-All	-NR	-N
May 23	248	82	95	68	59
June 6	186	85	58	85	64
June 20	210	105	68	89	55
July 4	154	94	74	94	54
July 25	198	143	64	109	74
Aug 15	130	138	67	138	72
Aug 22	182	181	79	143	59
Sept 5	211	202	58	180	53
Nov 7	146	169	46	136	47

3.2 Bud Dimensions of Field Material.

Buds sampled in February 1988 in the Control set were the largest in all dimensions, and those in the -P set by far the smallest (Table 3.5 A). No data were recorded for -Mg buds. Variation in the linear dimensions of the bud core was substantial, with larger differences seen in the calculated areas (Table 3.5 B). The ratio of basal to surface area varies from 0.35-0.36 for the Control and -K set, to 0.25-0.27 for the -P and -All treatments (Table 3.5 B), suggesting differences in shape of the bud between the two groups.

Table 3.5A Apical bud dimensions from first order lateral shoots from mid-canopy, for all nutrient treatments. Buds were sampled in March 1988. Data are means of 10 buds ± S.E.

Treatment	Dome diameter (mm)	Basal diameter (mm)	Bud height (mm)
Control	0.485 ± 0.042	2.045 ± 0.099	2.855 ± 0.0100
-K	0.470 ± 0.019	1.495 ± 0.097	2.145 ± 0.0113
-P	0.315 ± 0.026	1.035 ± 0.045	1.925 ± 0.094
-All	0.420 ± 0.013	1.310 ± 0.105	2.620 ± 0.127

Table 3.5B Derived apical data from Table 3.5A.

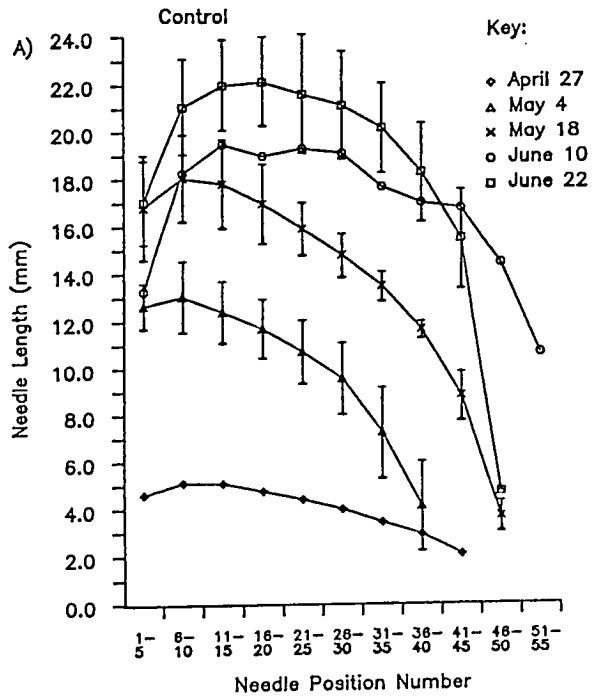
Treatment	Basal area (mm^2)	Flank surface area (mm^2)	B/F
Control	3.28	9.20	0.36
-K	1.76	5.04	0.25
-P	0.84	3.13	0.27
-All	1.35	5.39	0.25

3.3 Needle Extension.

3.3.1 1987 Experiment.

One week before bud-burst, mean needle length for five consecutive positions in Control buds varied from about 5 mm at the base of the bud, to 2 mm at the tip (Fig. 3.2 A). By the time of flushing, considerable needle elongation had occurred and the basal needles had reached lengths of up to 13 mm. The fastest mean extension rates were found for positions 1-5 over the flushing period, between April 27 and May 4, and reached values of about 1.15 mm d^{-1}. This rate was not maintained, and fell to 0.3-0.5 mm d^{-1} for needles at all positions between May 4 and May 18. The data for June 10 are anomalous, probably because of tree-to-tree variation, and if these data are not considered, the mean rate of needle extension between May 18 and June 22 fell further to less than 0.2 mm d^{-1}. Omitting the data from June 10, the region of most active needle growth from immediately prior to bud burst shifted distally along the shoot, from positions 1-5 between April 27 and May 4, to positions 11-15 between May 4 and May 18, and to positions 31-35 between May 18 and June 10. Basal needles ceased growth first, by May 18. At final size, on June 22, needle length varied with position, the longest being those at positions 15-25 which reached about 22 mm, with those needles

Figure 3.2 A to F, (and overleaf).
Changes in length of needles from whorl 7 shoots of field trees, from all treatments at times throughout the 1987 growth season, and according to needle position on the shoot. Values have been averaged for groups of five needle positions. Points are data for for one shoot, or means of two or three values from different shoots ± S.D. Standard deviations are printed alongside some graphs for some sample dates.



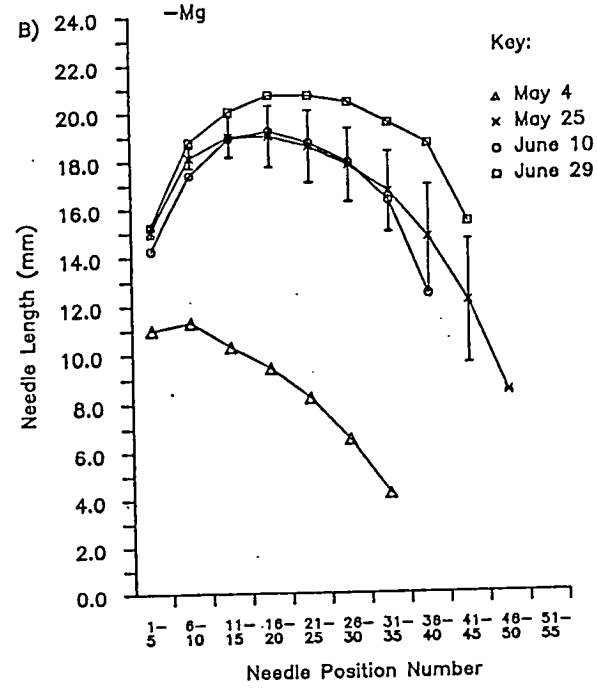


Table of standard deviations for the -Mg set

Date	Needle Position Number								
	1- 5	6- 10	11- 15	16- 20	21- 25	26- 30	31- 35	36- 40	41- 45
June 29	1.3	2.0	3.0	3.4	3.2	2.8	2.9	2.5	1.1

C)

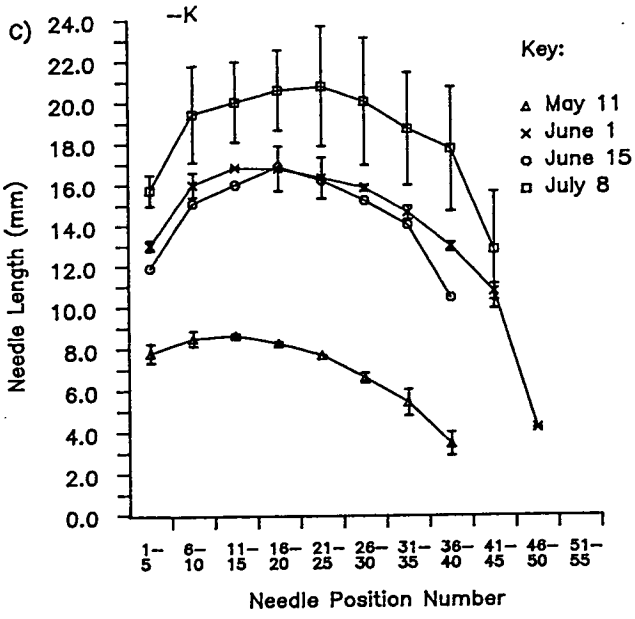


Table of standard deviations for the -K set

Date	Needle Position Number							
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40
June 15	0.3	0.4	1.0	1.4	1.0	0.1	1.3	2.3

D)

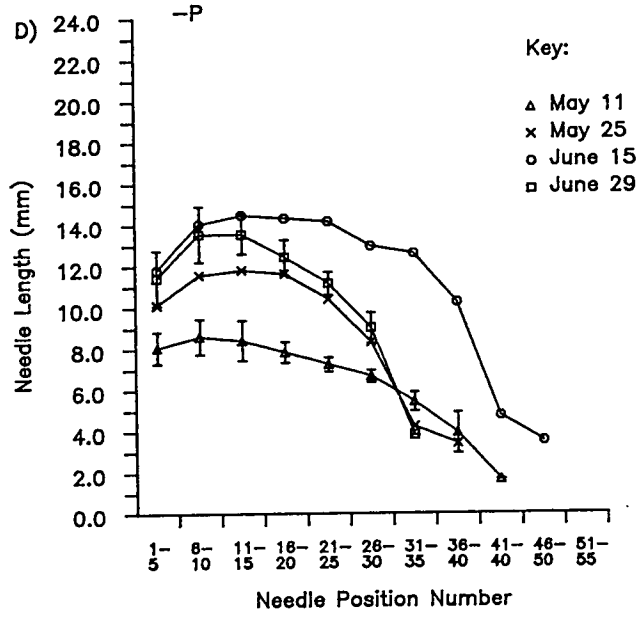


Table of standard deviations for -P set

Date	Needle Position Number									
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50
May 25	1.1	0.7	0.7	1.7	2.0	2.8	0.0	0.0	--	--
June 15	0.01	1.2	1.6	1.3	1.9	1.3	1.9	3.5	0.0	0.0

E)

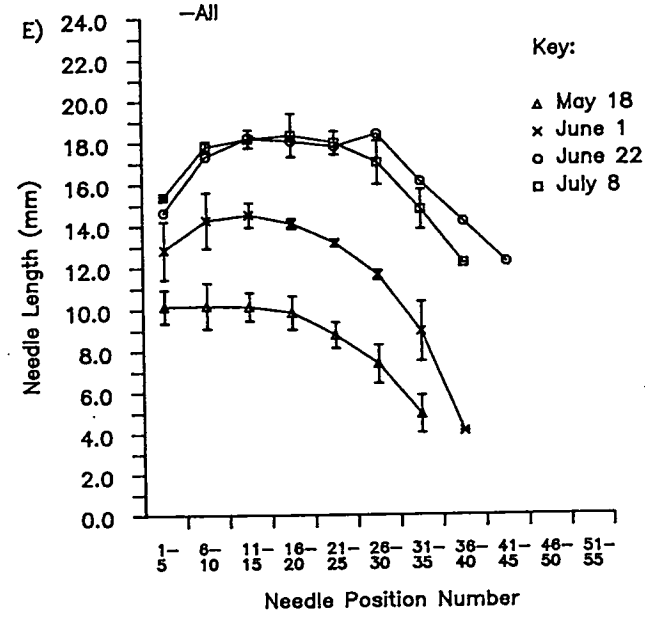


Table of standard deviations for the -All set

Date	Needle Position Number								
	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45
June 22	1.8	1.8	3.7	3.5	2.4	3.4	2.5	3.0	0.0

closest to the tip of the shoot about 12 mm shorter. The difference between replicates is substantial (e.g. Fig. 3.2 A), but within-harvest variation was less than the differences seen in successive harvests. Needle numbers per spiral also varied between trees, and ranged from 37 to 53.

Direct comparison of the treatment data is impossible, as harvest times vary, but the treatments are qualitatively similar in their pattern of needle development: The position where longest needles were situated was initially near the shoot base, with a progressive shift of this position distally with time, so that at the final harvest, in June or July, mean needle length was greatest near the shoot mid-point, at positions 16-20, or to within 5 positions of this (Fig. 3.2 B to E). Within-harvest variation for each treatment was again great, and tree-to-tree variation sometimes caused anomalous data, e.g. for -Mg and -K harvests 2 and 3 (Fig. 3.2 B and C), and for -P harvests 3 and 4 (Fig. 3.2 D) so that a smooth increase in needle length with time was not obtained. The number of needles per spiral also varied between shoots, so that a needle at a particular position at one sampling time could not be equated with a needle at the same position at another, in terms of similar relative position on the shoot. This makes estimates of rates of needle extension unreliable. However, the total increase in needle extension in -P and -All treatments of 7 to 8 mm between bud burst and final harvest, means that extension rate was reduced in these treatments compared with the Controls. The -Mg data are quantitatively similar to those of the Controls, in terms of timing of development and final needle size. The onset of needle extension in -P, -K and -All treatments was delayed; even though the first harvest in these treatments was one to two weeks later than those for the Control and -Mg treatments, the maximum needle length of 8-10 mm was shorter than that of Control and -Mg needles on May 4. Final needle length was significantly reduced in the -P and -All sets (Fig. 3.2 D and E), and needle lengths at the final harvest were in the order Control > -Mg = -K > -All > -P.

Needles in the -K set began extending later than the Controls, yet reached a similar length, although the final harvests were two weeks apart. In order to compare extension rates, it was important to know whether extension had ceased in each treatment by the time of the final harvest.

A comparison of the data for the final harvest for each treatment with length of needles from the shoot mid-point measured at a supplementary

sampling in October, (Table 3.6), show that the size ranking of treatments follows that at the final harvest. Needle lengths at the final harvest from -P, -K and -All treatments were larger than the October means (Table 3.6), because of sample variation, but the Control and -Mg data were not significantly different. The data in Table 3.6 confirm that needles in the -P treatment were smaller than those in the -All treatment.

Table 3.6. Mean needle dimensions from first order shoots from mid-canopy in the field, sampled in October 1987, for all nutrient treatments. Values are means ± S.E. for 10 shoots, where the value for each shoot is the mean of 20 needles from a mid shoot position.

Treatment	Mean needle length in October (± S.E.) (mm)	Mean needle width in October (± S.E) (mm)	length:width ratio
Control	21.79 ± 0.70	1.49 ± (3.55×10^{-2})	14.62
-Mg	20.44 ± 0.89	1.40 ± (3.48×10^{-2})	14.59
-K	19.40 ± 0.84	1.45 ± (6.80×10^{-2})	13.38
-P	11.89 ± 0.50	1.33 ± (4.41×10^{-2})	8.94
-All	15.62 ± 0.54	1.32 ± (4.51×10^{-2})	11.83

3.3.2 **1988 Experiment.**

No positional needle length data were obtained during 1988, but the mean length for needles from the whole shoot in the Controls increased from bud burst at the end of April, until the end of May (Fig. 3.3 A). Subsequently, the mean length was just over 10 mm (Table 3.7), with considerable sample variation. Data for -PR and -P treatments also showed an initial increase in mean needle length, until mid May. For the other treatments, mean length did not vary from the beginning of May (Fig. 3.3 B,C,E and F), suggesting extension to occur rapidly, within a week or two following bud burst. When the 95% confidence limits were compared, only needle lengths in -N and -All treatments were significantly lower than the Controls (Table 3.7). There was only a significant effect of refertilisation on mean needle length in -NR and -AllR treatments, where there was an increase in length of about 5 mm.

Figure 3.3 A to F

Length of needles from terminal whorl 2 shoots of pot trees throughout the 1988 season for each treatment. Needle length was averaged for all needles from the shoot, and each point is a mean of values from two different trees ± S.D. For treatments apart from the Control, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. The letter d shows where standard deviation bars for data from deficient and refertilised trees do not overlap at any one sample date.

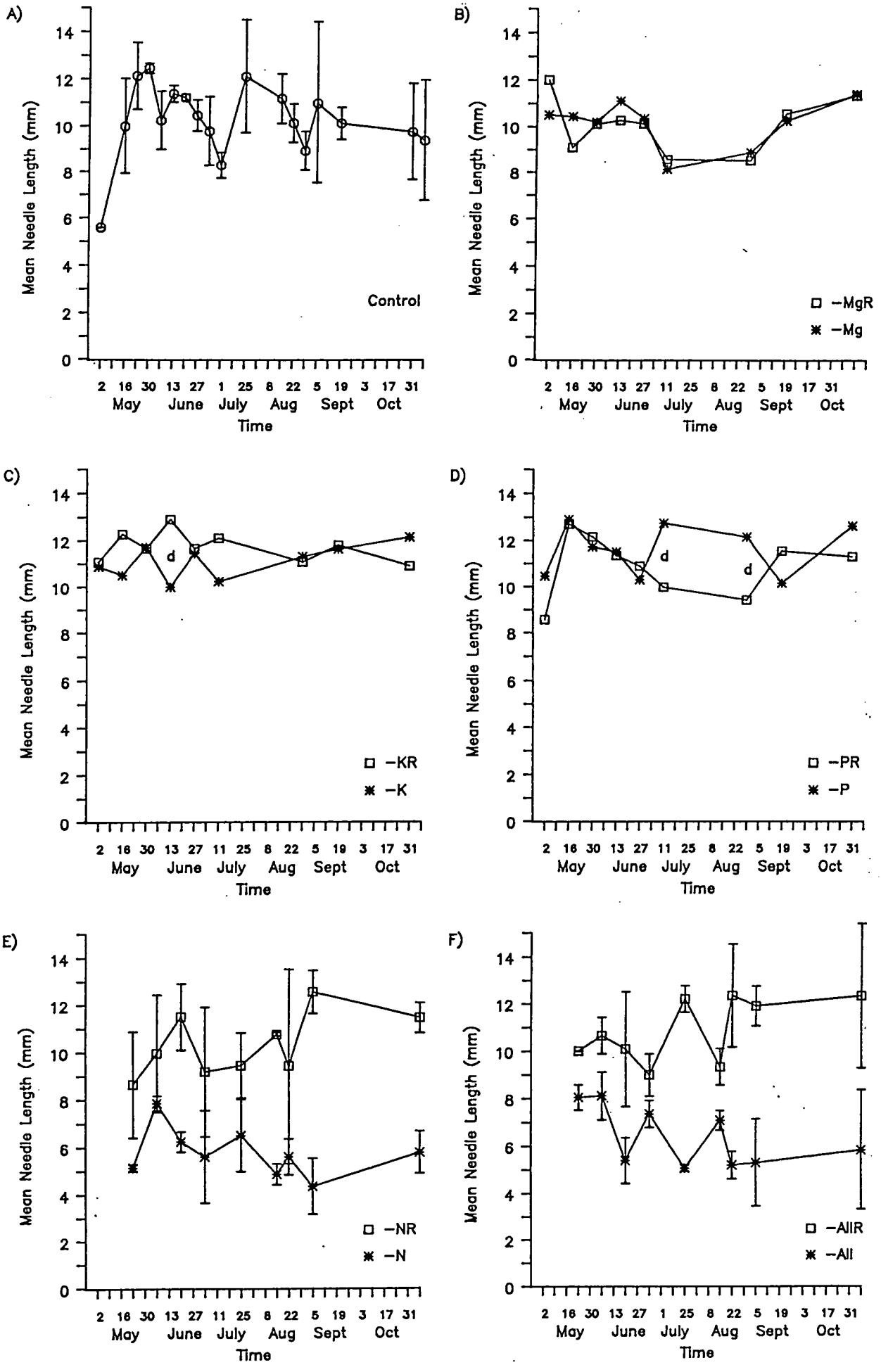

A)
Mean Needle Length (mm)
Control
2 16 30 13 27 1 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
B)
Mean Needle Length (mm)
−MgR
−Mg
2 16 30 13 27 11 25 8 22 5 19 17 31
May June July Aug Sept Oct
Time
C)
Mean Needle Length (mm)
d
−KR
−K
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
D)
Mean Needle Length (mm)
d
d
−PR
−P
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
E)
Mean Needle Length (mm)
−NR
−N
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
F)
Mean Needle Length (mm)
−AllR
−All
2 16 30 13 27 1 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

Table 3.7. Mean needle length for needles at all positions on the shoot, for all treatments from May 30 1988. Values are means ± S.E. N=30 for Controls; 14-16 for the other treatments. Asterisks show degree of significant difference from the Controls.

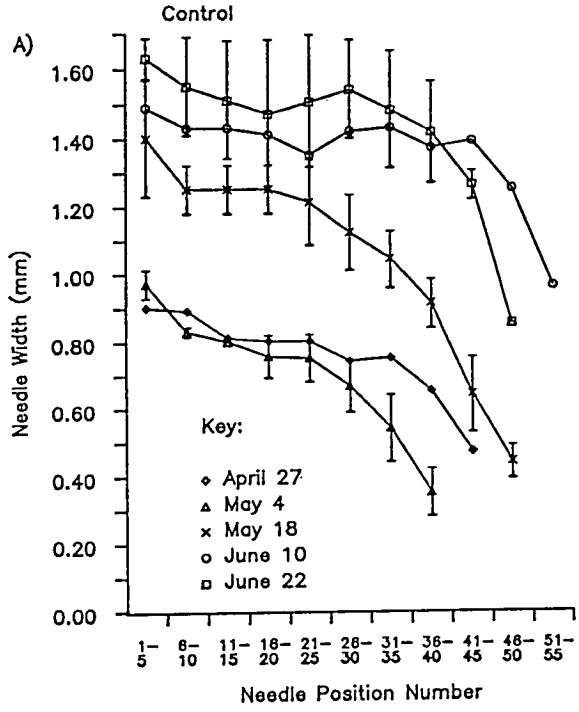
Treatment	+All	-MgR	-Mg	-KR	-K	-PR	-P	-NR	-N	-AllR	-All
Mean Needle Length (mm)	10.40 ±0.29	9.92 ±0.54	10.03 ±0.65	11.72 ±0.33	11.20 ±0.52	10.94 ±0.36	11.60 ±0.45	10.53 ±0.49	5.85 ±0.32 ***	10.98 ±0.46	6.18 ±0.38 ***

3.4 Needle Width, 1987.

For Controls, mean needle width immediately prior to flushing on April 27, ranged from 0.9 mm for the first five needles, to 0.5 mm for the smallest measurable distal needles (Fig. 3.4 A). Needle width only increased greatly after May 18, and then increased as the needles extended. The high rate of width increase between May 18 and June 10 subsequently fell. Needle width was greatest for needles at the base of the shoot, and by June 10 width declined slightly from positions 1-40, and sharply for needles at more proximal positions.

For the deficiency treatments, needle widths at the first harvest for each treatment were similar to those of the Controls on May 4 (Fig. 3.4 B to E). The most proximal needles were the widest, with a progressive decrease in width in all treatments, moving towards the shoot tip, with widths of the most distal needles being less than half that of proximal ones. The widths of needles from all deficiency treatments at the final harvest was not as high as those in the Controls, but variation between samples was sometimes greater than between harvests, so that comparisons of absolute size, or estimates of rates of growth are not useful. By October, sampling of a larger number of trees showed needles from Control, -Mg and -K treatments to be wider than those from -P and -All treatments (Table 3.6). The length:width ratio was also reduced in the latter two treatments.

Figure 3.4 A to E, (and overleaf).
Change in width of needles from whorl 7 of field trees, from all treatments at times throughout the 1987 growth season, and according to needle position on the shoot. Values have been averaged for groups of five needle positions. Points are data for one shoot, or means of two or three values from different shoots ± S.D. Standard deviations are printed alongside some graphs, for some sample dates.



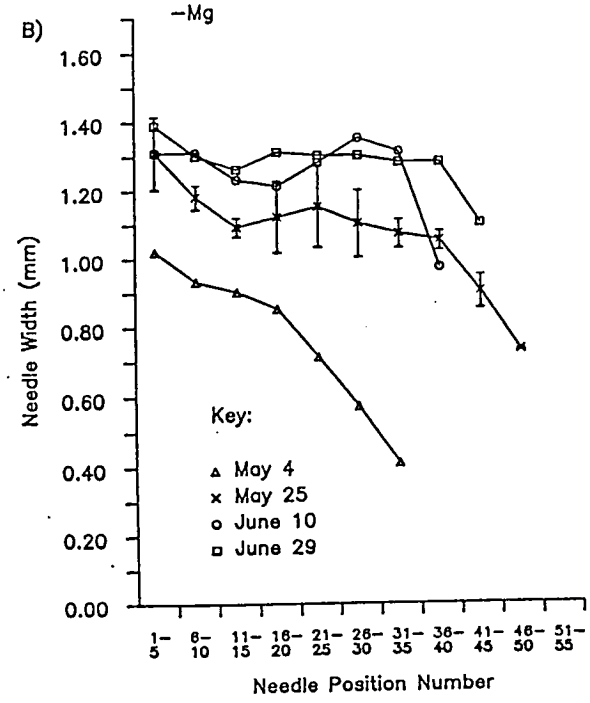


Table of standard deviations for the -Mg set

Date	Needle Position Number								
	1- 5	6- 10	11- 15	16- 20	21- 25	26- 30	31- 35	36- 40	41- 45
June 29	0.03	0.08	0.09	0.11	0.07	0.11	0.15	0.16	0.13

C)

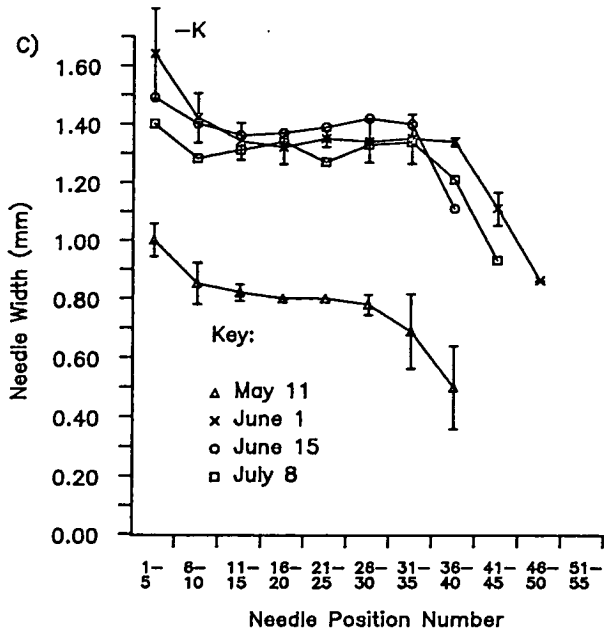


Table of standard deviations for the –K set

Date	Needle Position Number								
	1–5	6–10	11–15	16–20	21–25	28–30	31–35	36–40	41–45
June 15	0.17	0.19	0.13	0.08	0.06	0.18	0.24	0.26	—
July 8	0.11	0.03	0.09	0.08	0.03	0.15	0.10	0.13	0.04

D)

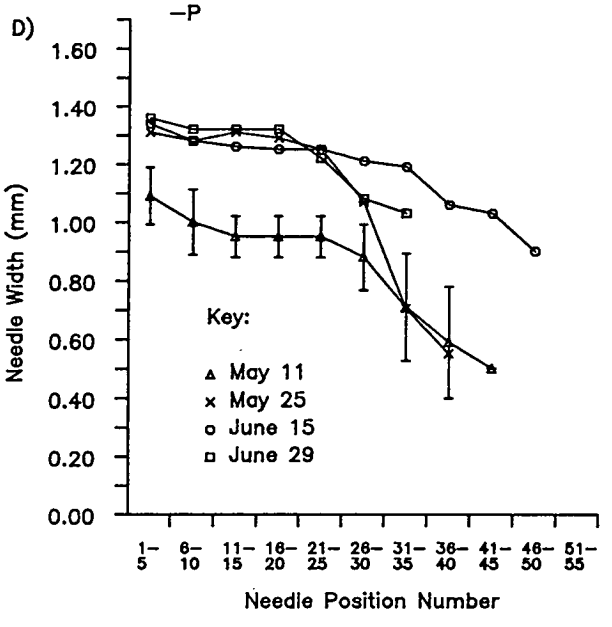


Table of standard deviations for the –P set

Date	Needle Position Number									
	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50
May 25	0.29	0.30	0.32	0.34	0.40	0.62	0.0	0.0	—	—
June 15	0.09	0.10	0.09	0.09	0.09	0.16	0.14	0.23	0.0	0.0
June 29	0.01	0.01	0.03	0.01	0.07	0.11	0.0	—	—	—

E)

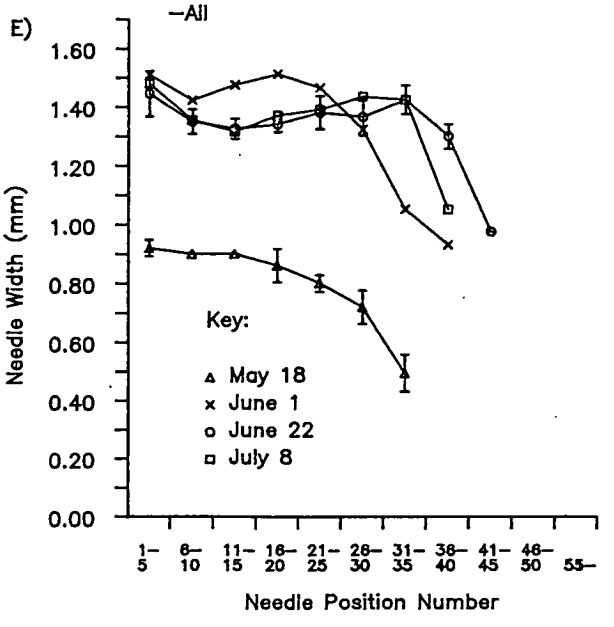


Table of standard deviations for the –All set

Date	Needle Position Number							
	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40
June 1	0.29	0.30	0.31	0.42	0.41	0.42	0.35	0.0
July 8	0.16	0.12	0.12	0.20	0.24	0.30	0.25	0.0

3.5 Needle Fresh Weight.

3.5.1 **1987 Experiment.**

Immediately following bud burst in the Controls, needle fresh weight increased most rapidly in basal needles, until May 18 1987, with the mean fresh weight of the most proximal needle remaining constant (Fig. 3.5 A). Maximum rates of mean fresh weight increase for the basal needles reached 2.7×10^{-4}g d^{-1}, between May 4 and May 18. Between May 18 and June 10, there was a shift in the region of greatest increase in fresh weight, from positions 1-5 to 26-30. Final maximum needle fresh weight for needles about 1/3 of the distance along the shoot was about 11.5×10^{-3}g.

At the first harvest for each deficiency treatment, needle fresh weight showed the same pattern with needle position as in the Controls (Fig. 3.5 B to E). The greatest increase in mean fresh weight was subsequently in needles towards the middle of the shoot. Needle fresh weight at the final harvest for -Mg and -All needles was slightly less than for the Controls, at around 10.1×10^{-3}g, but for -K needles the maximum was 8.8×10^{-3}g, and for -P needles, 6.7×10^{-3}g.

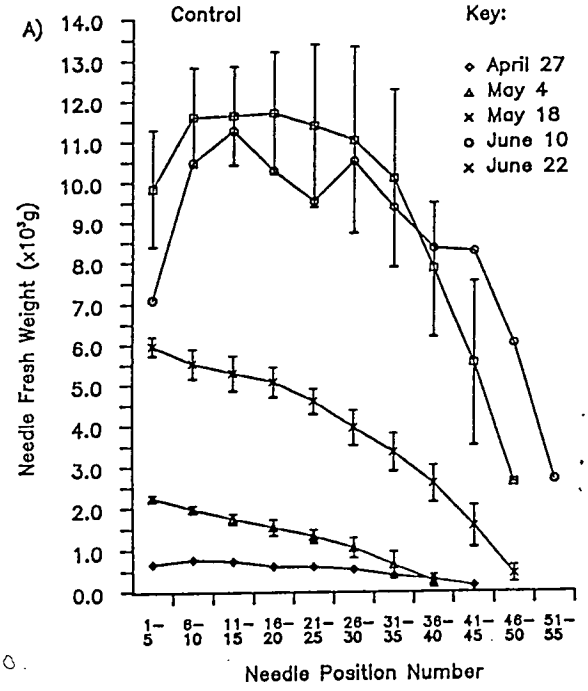
3.5.2 **1988 Experiment.**

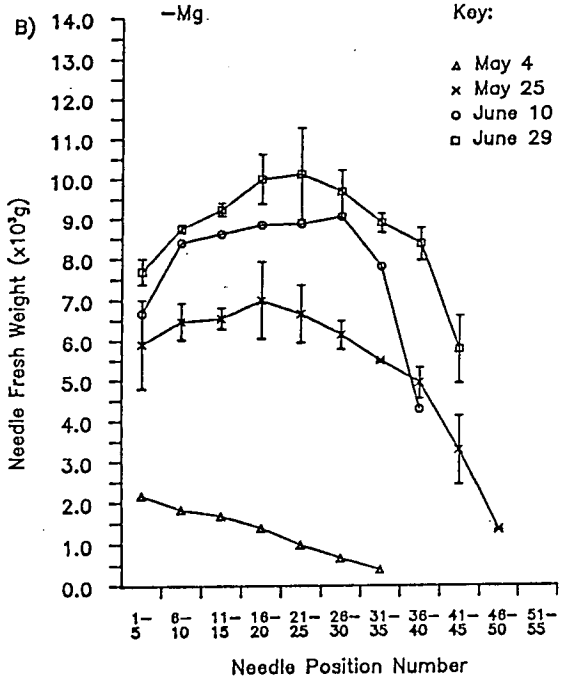
Mean needle fresh weight for all pot treatments after May 30 showed that needles in -N and -All treatments were significantly lighter than the Controls (Fig. 3.6 A to E), and -KR needles were significantly heavier than the Controls, when 95% confidence limits were compared (Table 3.8) The increase in needle fresh weight on refertilisation of -N and -All sets was also significant at $p < 0.05$.

Table 3.8. Needle fresh weight for all treatments, from May 30 1988. Values are means $\times 10^{3}$ ± S.E. n=30 for Controls, 13-16 for other treatments. Asterisks show degree of significant difference from the Controls.

	+All	-MgR	-Mg	-KR	-K	-PR	-P	-NR	-N	-AllR	-All
Needle Fresh Weight (g)	2.81 ±0.12	2.53 ±0.29	2.84 ±0.31	3.53 ±0.21 *	3.26 ±0.22	2.72 ±0.14	3.42 ±0.17	3.20 ±0.17	1.65 ±0.29 *	3.57 ±0.26	1.49 ±0.15 ***

Figure 3.5 A to E, (and overleaf).
Fresh weight of needles from whorl 7 shoots of field trees, from all treatments at times throughout the 1987 growth season, and according to needle position on the shoot. Values have been averaged for groups of five needle positions. Points are data for one shoot only, or means of two or three values from different shoots ± S.D. Standard deviations are printed alongside some graphs, for some sample dates.





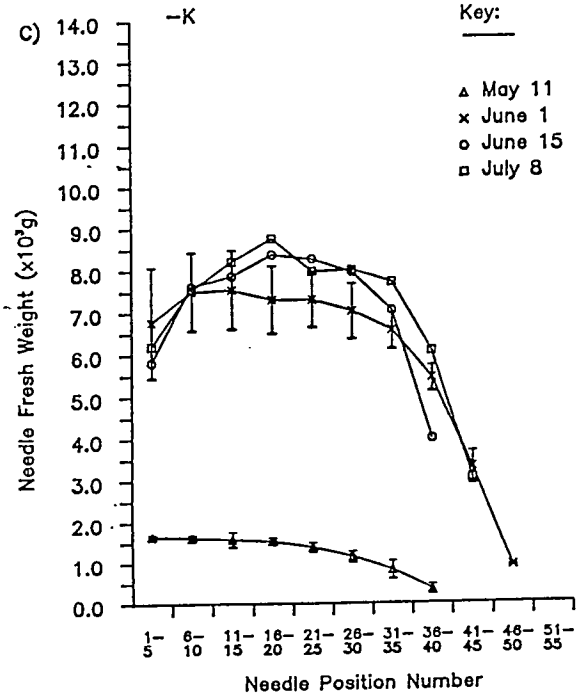


Table of standard deviations for -K set (x10³)

Date	Needle position number								
	1–5	6–10	10–15	16–20	21–25	26–30	31–35	36–40	41–45
June 15	0.6	1.7	1.6	1.3	1.4	1.8	2.5	2.4	--
July 8	1.0	0.6	0.2	0.4	1.3	0.5	0.5	0.8	0.6

D)

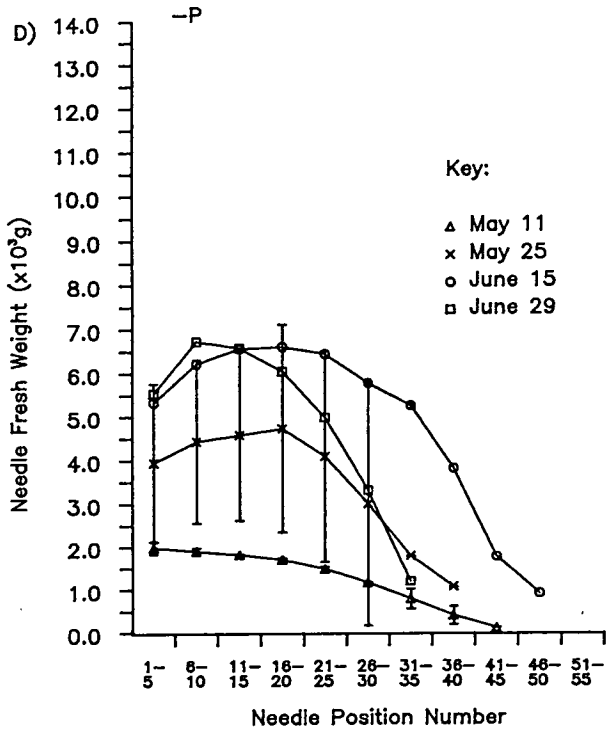


Table of standard deviations for −P set ($x10^3$)

Date	Needle position number									
	1−5	6−10	11−15	16−20	21−25	26−30	31−35	36−40	41−45	46−50
June 15	0.4	0.9	1.4	1.7	1.9	1.6	2.0	2.1	0.0	0.0
June 29	0.6	1.0	0.6	0.9	0.5	0.7	0.0	---	---	---

E)

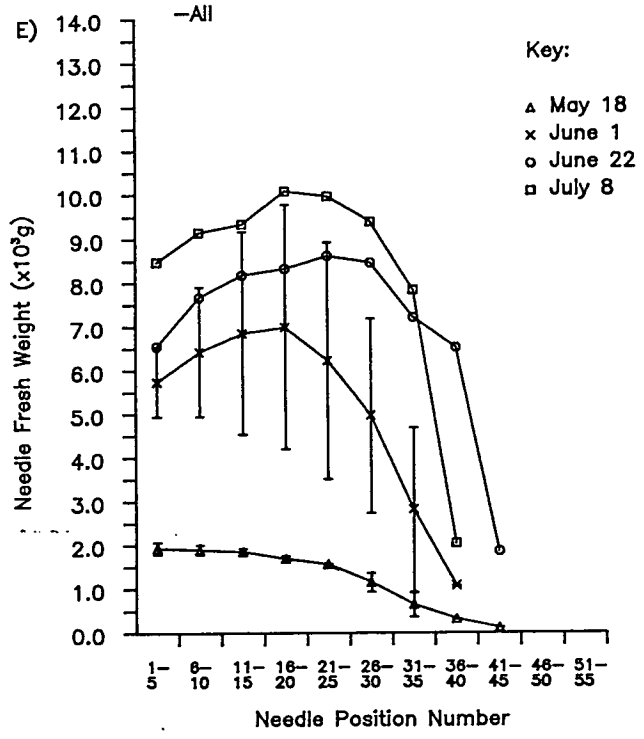
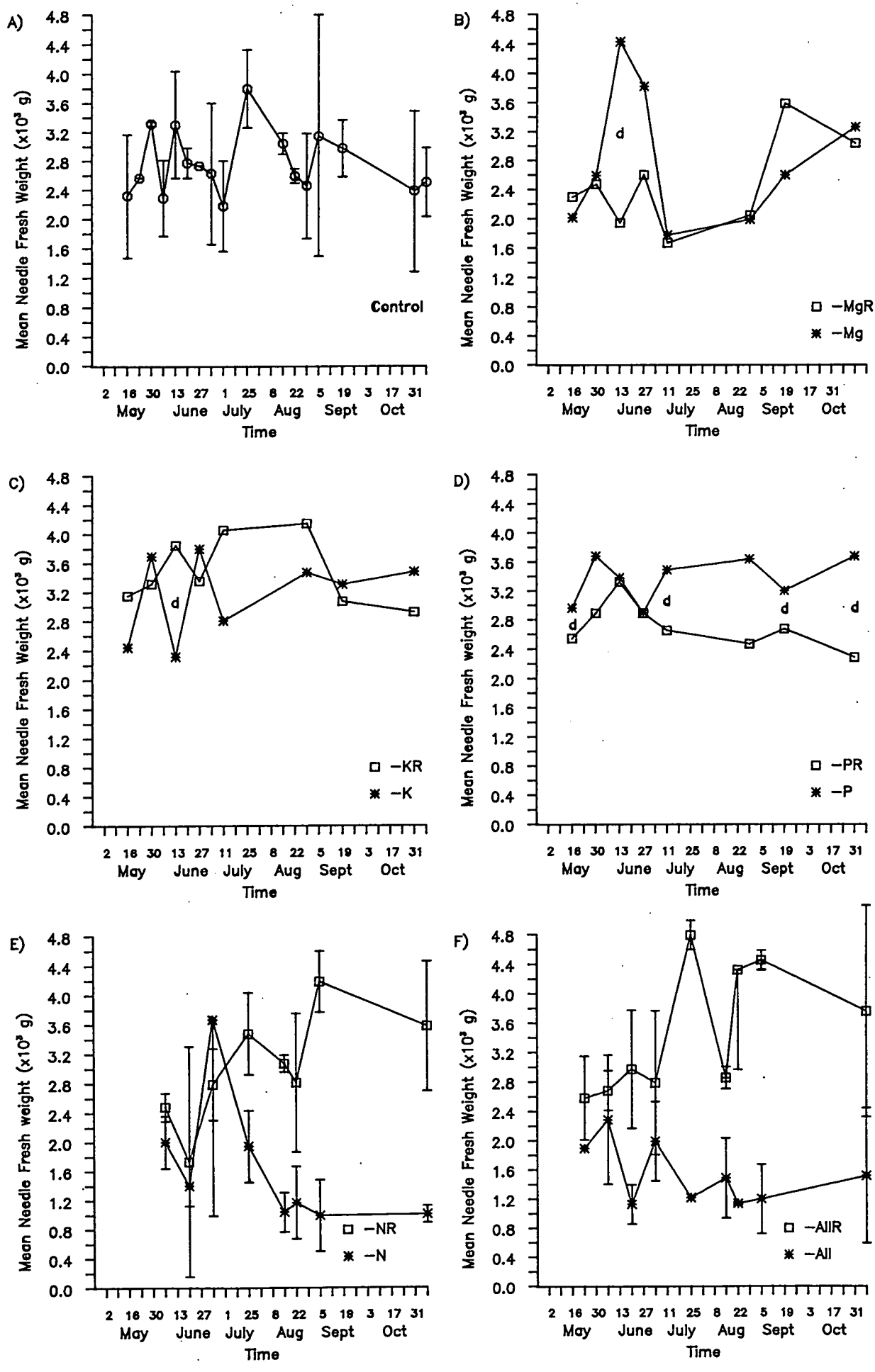


Table of standard deviations for −All set ($x10^3$)

Date	Needle position number								
	1−5	6−10	11−15	16−20	21−25	26−30	31−35	36−40	41−45
June 22	2.0	2.3	2.7	2.3	1.9	2.1	2.4	1.0	0.0
July 8	1.6	1.1	1.0	1.4	2.0	2.0	1.7	0.0	---

Figure 3.6 A to F
Fresh weight of needles from whorl 2 shoots of pot trees throughout the 1988 season for each treatment. Needle fresh weight has been averaged for needles at all shoot positions, and each point is a mean of values from two different trees ± S.D. For treatments apart from Controls, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. The letter d shows where standard deviation bars for data from deficient and refertilised trees do not overlap at any one sample time.


A)
B)
C)
D)
E)
F)
Mean Needle Fresh Weight (x10³ g)
Mean Needle Fresh weight (x10³ g)
Time
2 16 30 13 27 1 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Control
−MgR
−Mg
−KR
−K
−PR
−P
−NR
−N
−AllR
−All
d

3.6 Dry Weight:Fresh Weight Ratio.

The dry weight:fresh weight ratio for needles from field material in 1987, showed similar seasonal trends for all treatments, being at around 0.2 until early June, then rising sharply to a plateau of 0.38–0.48 by the end of July (Fig. 3.7). Analysis of variance on the data for harvests over the period September to early November (4 harvests with 4 values taken at random from the total number of replicates on each occasion) (Table 3.9), showed differences between nutrient treatments to be significant, with -P and -K needles having higher dry weight:fresh weight ratios than the Controls.

The seasonal change in the ratio for needles in 1988 from pot trees was similar to that in 1987, increasing in all treatments from a minimum of about 0.2 at bud burst, to a maximum by the end of July (Fig. 3.8 A and B), although the spread was slightly greater than that seen in 1987. The lag until early June seen in the ratio of needles from the field was not seen with needles from pot trees, where the ratio increased linearly with time. Analysis of variance on the last 4 harvests of the -MgR, -Mg, -KR, -K, -PR, and -P treatments (Table 3.10 A), and the Control data for these dates (2 values for each treatment), again showed significant differences to exist between treatments, with -P and -K needles having a significantly higher ratio than the Controls. The only significant difference in the dry weight:fresh weight ratio between deficient and refertilised trees involved K, with -KR needles having lower ratios than -K needles. A similar analysis of variance for -AllR, -All, -NR, and -N treatments in Fig. 3.8 A (4 harvests, 2 values for each), and the Controls (Table 3.10 B), showed treatment differences not to be significant.

Figure 3.7. Dry weight:Fresh weight ratio throughout the 1987 season, for all field treatments, 1987. Each point is the mean of 4 to 6 values.

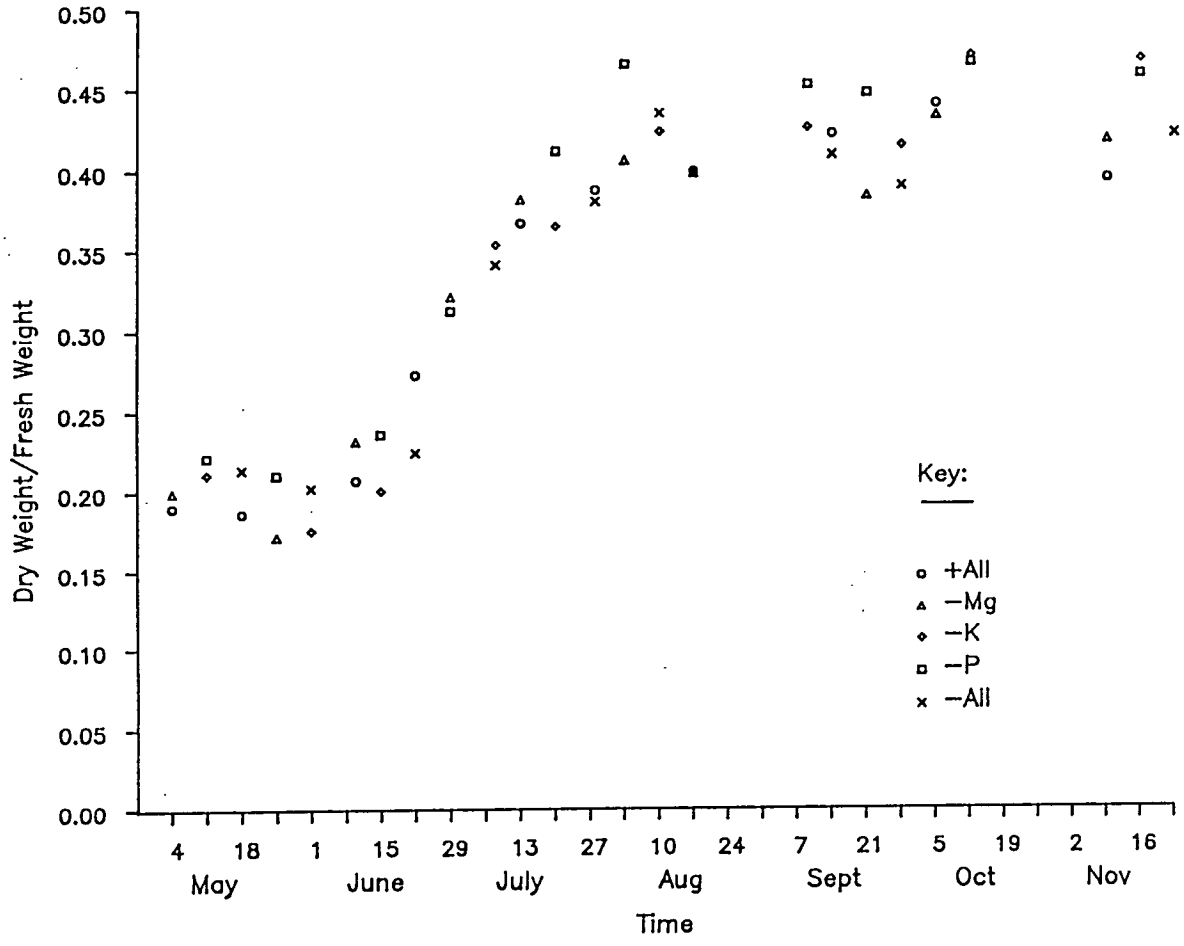


Table 3.9. ANOVAR on the data for the last 4 harvests for treatments in Fig. 3.7.

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	4	8.19×10^{-3}	14.50 ***
Time	3	6.68×10^{-3}	10.34 ***
Interaction	12	5.65×10^{-4}	0.87
Error	60	6.46×10^{-4}	

L.S.D. = 0.018

Means for the last 4 harvests:

Control	−Mg	−K	−P	−All
0.415	0.409	0.447	0.458	0.412

Figure 3.8A. Dry weight:Fresh weight ratio for needles from Control and some of the pot treatments throughout the 1988 season. Each point is the mean of two values.

Table 3.10A. ANOVAR for data for the last 4 harvests in Fig. 3.8A.

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	6	3.98×10^{-3}	3.43 *
Time	3	11.17×10^{-3}	10.13 ***
Interaction	18	1.16×10^{-3}	1.05
Error	28	1.10×10^{-3}	

L.S.D. = 0.039

Means for the last 4 harvests:

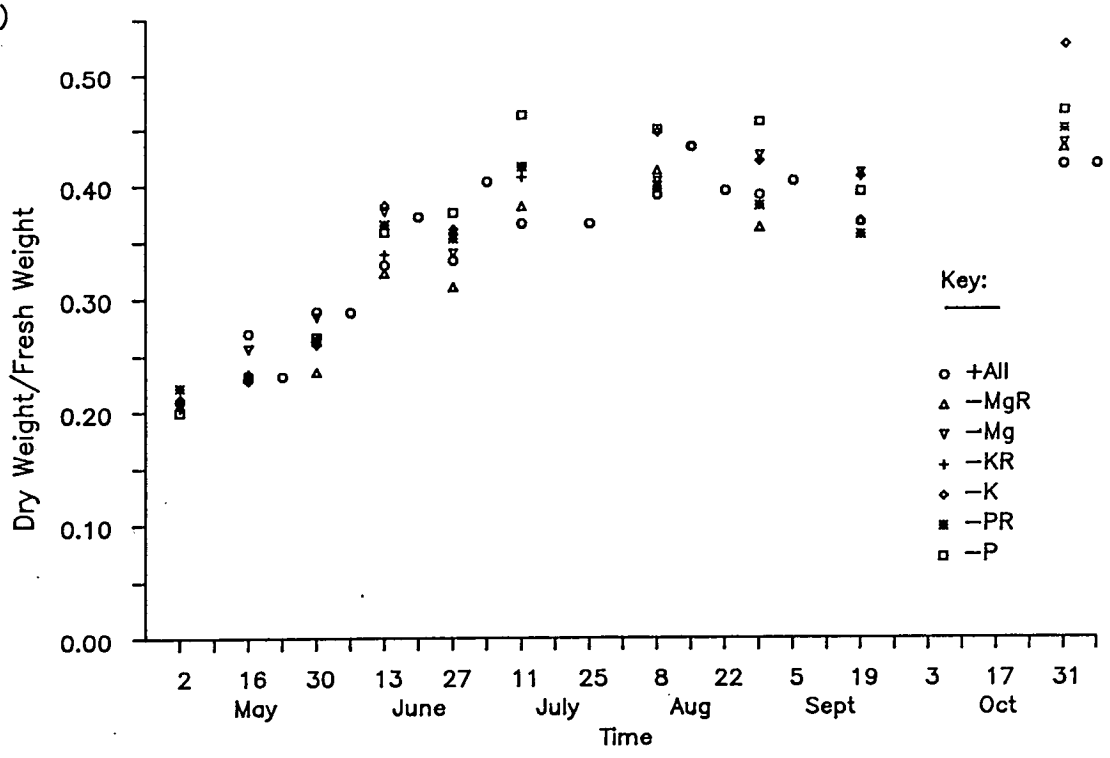
Control	**-Mg**	**-MgR**	**-K**	**-KR**	**-P**	**-PR**
0.393	0.420	0.395	0.452	0.395	0.433	0.407

Figure 3.8B. Dry weight:Fresh weight ratio for needles from Control and some of the pot treatments throughout the 1988 season. Each point is the mean of two values.

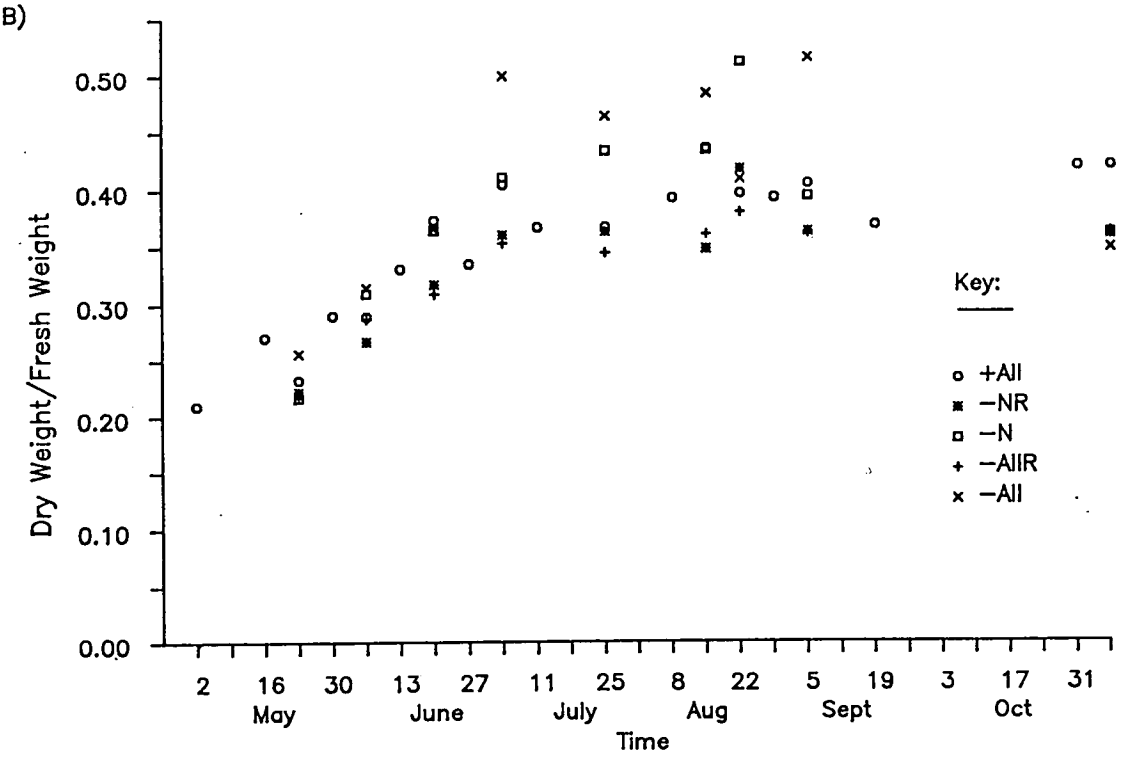
Table 3.10B. ANOVAR for data for the last 4 harvests in Figure 3.8B.

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	4	8.47×10^{-3}	1.97 n.s.
Time	3	5.33×10^{-3}	3.89 *
Interaction	12	4.29×10^{-3}	3.14 *
Error	19	1.37×10^{-3}	

A)


Dry Weight/Fresh Weight
0.00
0.10
0.20
0.30
0.40
0.50
2
16
30
13
27
11
25
8
22
5
19
3
17
31
May
June
July
Aug
Sept
Oct
Time
Key:
+All
−MgR
−Mg
−KR
−K
−PR
−P

B)


Dry Weight/Fresh Weight
0.00
0.10
0.20
0.30
0.40
0.50
2
16
30
13
27
11
25
8
22
5
19
3
17
31
May
June
July
Aug
Sept
Oct
Time
Key:
+All
−NR
−N
−AllR
−All

3.7 **Needle Projected Area.**

3.7.1 **1987 Experiment.**

For the Controls, mean projected area for harvests up to May 18 was greatest for basal needles, and decreased for needles towards the shoot tip (Fig. 3.9 A). In the period May 4 to May 18, projected area of basal needles increased from 3 mm^2 to 18 mm^2, whilst that of distal needles did not increase. Subsequently, the greatest increase in projected area was for needles at a mid-shoot position.

Mean needle projected area in the deficiency treatments showed the same pattern of increase as the Controls. Maximum projected area at the final harvest in June or July for -Mg and -K needles was slightly less than for the Controls, at about 22 mm^2. Needles from the -All treatment had slightly lower final maximum areas, at about 20 mm^2, and needles in the -P treatment had considerably lower areas, with a maximum of just over 14 mm^2 (Fig. 3.9 B to F).

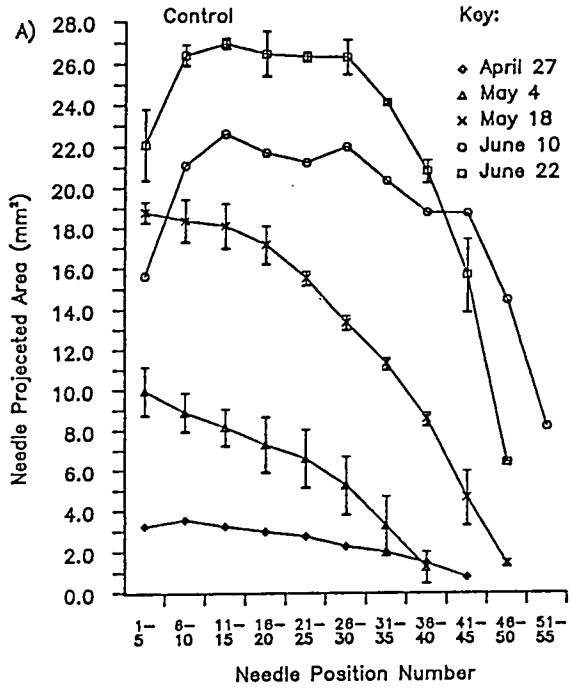
3.7.2 **1988 Experiment.**

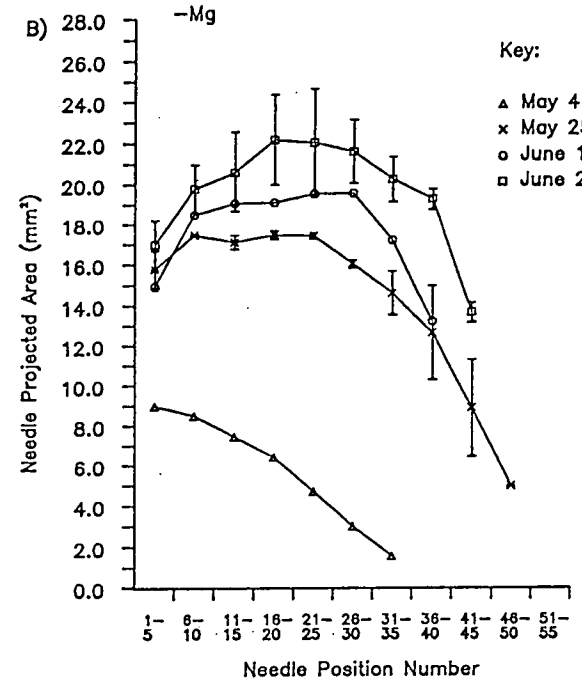
Mean needle projected area for Controls increased during the first week of May (Fig. 3.10), until attainment of final needle length, and reached a mean of about 7 mm^2. Projected area of -N and -All needles was significantly lower than for the Controls, (Fig. 3.10 E and F, and Table 3.11), and refertilisation of these treatments caused a significant increase in projected area (Table 3.11). Projected area of needles in -NR and -AllR treatments continued to increase until early September, which is the same pattern as for needle length (section 3.3.2), and is partly due to free growth. All other treatments showed no increase in needle projected area from early May (Fig. 3.10 B to D), with only the projected area of -KR needles being subsequently significantly different to that of the Controls, when 95% confidence limits are compared (Table 3.11).

Table 3.11 Mean needle projected area for all treatments from May 30 1988. Values are means ± S.E. n=30 for Controls; 13-16 for other treatments. Asterisks show significant difference from the Controls.

	+All	-MgR	-Mg	-KR	-K	-PR	-P	-NR	-N	-AllR	-All
Mean Needle Area (mm^2)	6.61 ±0.27	6.29 ±0.49	6.25 ±0.56	8.27 ±0.43 *	7.56 ±0.49	6.86 ±0.31	7.71 ±0.40	6.73 ±0.32	3.62 ±0.45 ***	7.41 ±0.42	3.50 ±0.25 ***

Figure 3.9 A to E, (and overleaf).
Projected area of needles from whorl 7 shoots of field trees, from all treatments, at times throughout the 1987 growth season, and according to needle position on the shoot. Values have been averaged for groups of five needle positions. Points are data for one shoot only, or means of two or three values from different trees ± S.D. Standard deviations are printed alongside some graphs for some sample dates.





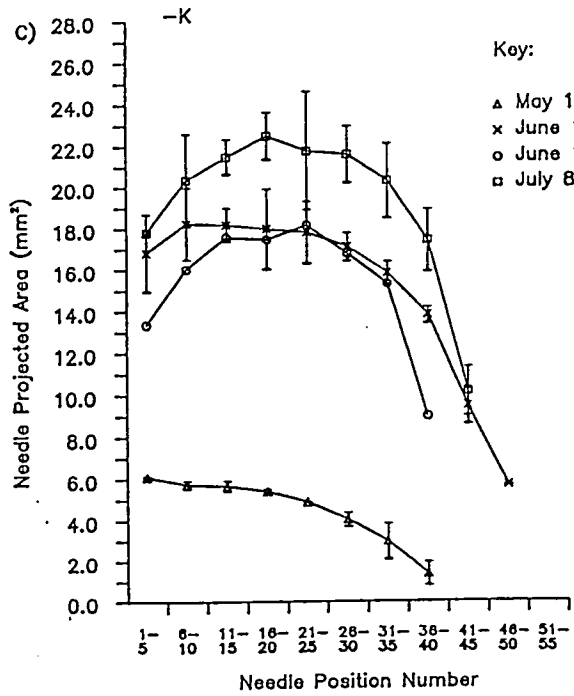


Table of standard deviations for -K set

Date	Needle Position Number								
	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45
June 15	1.1	2.6	2.7	2.2	1.9	1.5	3.9	3.9	—
July 8	0.04	2.3	0.9	1.1	2.9	1.4	1.8	1.5	1.2

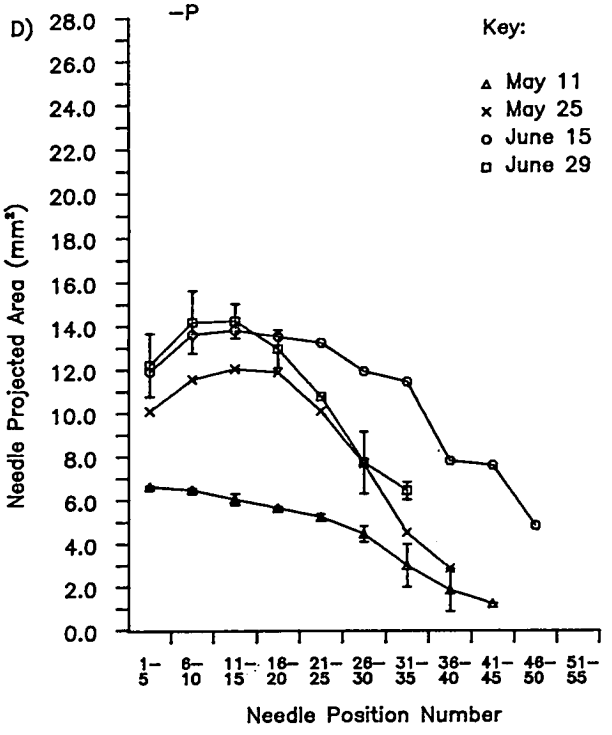


Table of standard deviations for the −P set

Date	Needle Position Number									
	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50
May 25	3.2	3.7	3.9	5.1	5.0	6.3	0.0	0.0	---	---
June 15	0.8	2.2	2.4	2.2	2.7	2.7	3.0	4.4	0.0	0.0

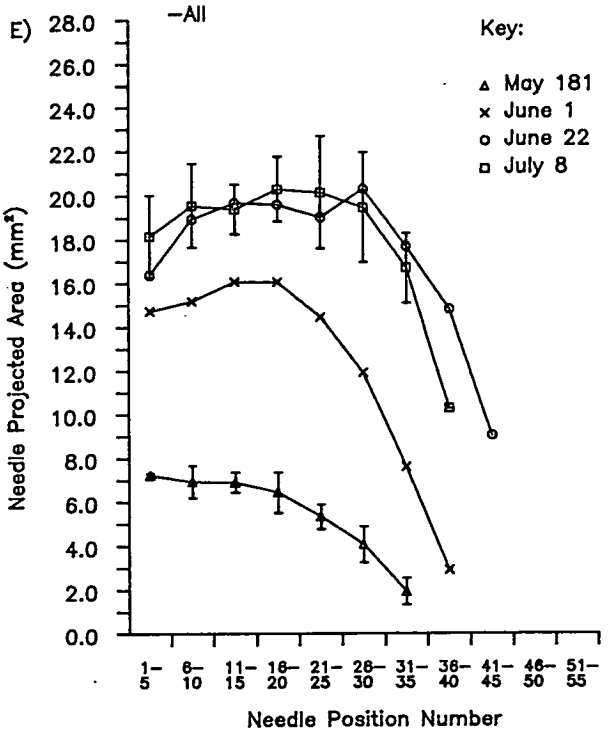
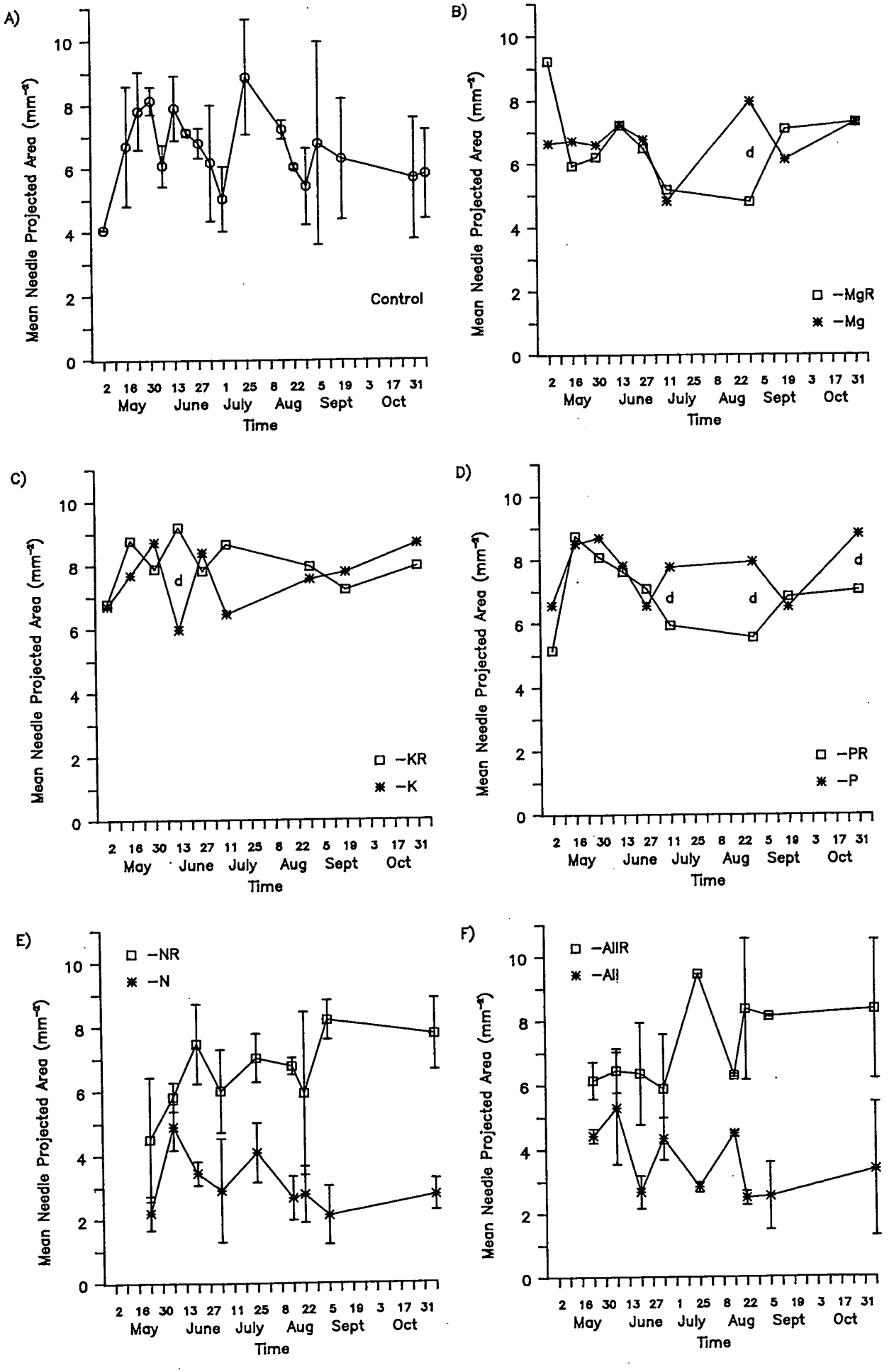


Table of standard deviations for the −All set

Date	Needle Position Number								
	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45
June 1	1.5	1.8	2.7	4.1	3.9	3.5	3.8	0.0	---
June 22	2.3	2.7	4.7	3.7	1.0	3.2	1.5	3.8	0.0

Figure 3.10 A to F

Projected surface area of needles from whorl 2 shoots of pot trees throughout the 1988 season for each treatment. Needle projected area was averaged for needles at all shoot positions, and each point is a mean of values from two different trees ± S.D. For treatments apart from the Controls, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. The letter d shows where standard deviation bars for data from deficient and refertilised trees do not overlap at any one sample time.


A)
Mean Needle Projected Area (mm⁻²)
Control
2 16 30 13 27 1 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
B)
Mean Needle Projected Area (mm⁻²)
□ –MgR
✱ –Mg
d
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
C)
Mean Needle Projected Area (mm⁻²)
□ –KR
✱ –K
d
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
D)
Mean Needle Projected Area (mm⁻²)
□ –PR
✱ –P
d
d
d
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
E)
Mean Needle Projected Area (mm⁻²)
□ –NR
✱ –N
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
F)
Mean Needle Projected Area (mm⁻²)
□ –AllR
✱ –All
2 16 30 13 27 1 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

3.8 Specific Leaf Area.

3.8.1 1987 Experiment.

Differences in specific leaf area for the field treatments in 1987 (Table 3.12) were not significant, even though mean needle fresh weight and projected area followed the same ranking with treatment as for needle length (Table 3.6).

Table 3.12 Mean projected area, fresh weight and specific leaf area for needles from all treatments sampled from first order shoots from mid canopy in October 1987. Values are means ± S.E. based on data for 10 shoots, where the value for each shoot is the mean of 20 needles from a mid shoot position.

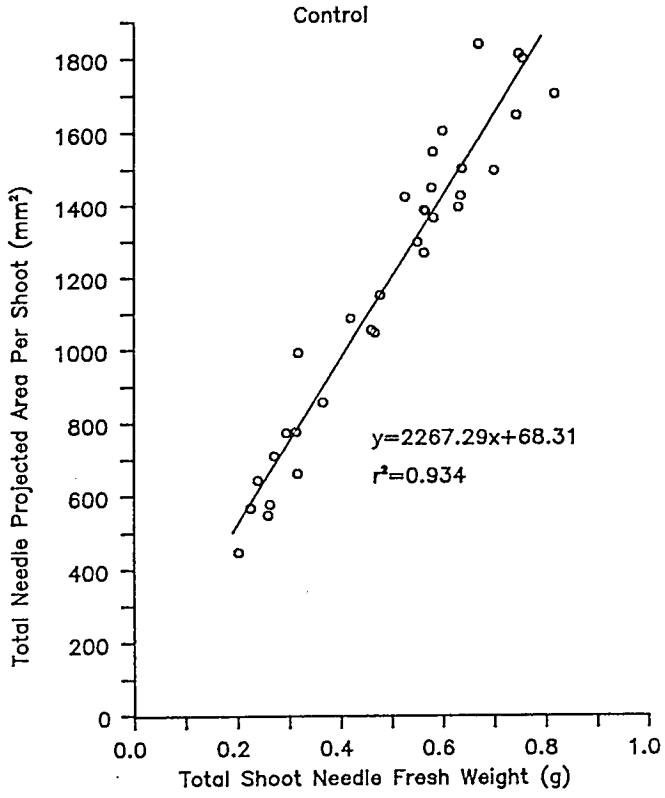
Treatment	Projected Area (mm^2)	Fresh Weight (g)	Specific Leaf Area ($cm^2\ g^{-1}$)
+All	26.35 ± 0.70	0.0144 ± 0.0005	18.43 ± 0.78
-Mg	23.42 ± 1.44	0.0122 ± 0.0029	19.41 ± 0.41
-K	22.83 ± 1.36	0.0120 ± 0.0009	19.24 ± 0.53
-P	12.56 ± 0.81	0.0068 ± 0.0005	18.79 ± 0.33
-All	16.58 ± 0.94	0.0085 ± 0.0008	20.15 ± 1.00

3.8.2 1988 Experiment.

For all pot treatments during the 1988 season, a good linear relationship was obtained between total needle projected area of the whole shoot, and total needle fresh weight of the same sample, with between 73% and 99% of the variation accounted for by the fitted lines (Fig. 3.11). This enables the projected area of a needle sample from any treatment to be made from the fresh weight of the sample. The linearity of this relationship suggests that the specific leaf area (cm^2 projected area g^{-1} fresh weight) did not alter during the season, after May 30. The mean specific leaf area for each treatment was calculated by dividing the total shoot projected area and total fresh weight by the number of needles per shoot, at each sampling time and averaging over the whole season. Data show that -NR and -AllR treatments had lower specific leaf areas than other treatments (Table 3.13), but only the -AllR treatment showed a significant difference from the Controls when 95% confidence limits were compared. None of the treatments showed any significant differences in specific leaf area on refertilisation.

Figure 3.11

The relationship between total needle projected area per shoot, and total shoot needle fresh weight, for terminal shoots from whorl 2 of pot trees. Values are for individual shoots, for all sample times throughout the 1988 season.



Equations for other treatments

Treatment	Equation	r^2
–Mg	y = 2231.98x + 144.5	0.941
–MgR	y = 1994.77x + 173.55	0.929
–K	y = 2220.81x + 126.01	0.817
–KR	y = 2028.95x + 194.68	0.824
–P	y = 2325.90x + 5.90	0.916
–PR	y = 2784.64x – 50.47	0.736
–N	y = 2077.53x + 24.31	0.979
–NR	y = 1884.29x + 90.67	0.986
–All	y = 1756.70x + 145.78	0.991
–AllR	y = 2073.52x + 24.57	0.965

Table 3.13 Specific leaf area (cm^2 g^{-1} fresh weight), for all treatments, from May 30 1988. Values are means ± S.E. n=30 for Controls and 13–16 for other treatments. Asterisks show degree of significant difference from the Controls.

	+All	-MgR	-Mg	-KR	-K	-PR	-P	-NR	-N	-AllR	-All
Specific Leaf Area.	23.74 ±0.33	25.34 ±1.29	23.76 ±1.10	23.26 ±0.75	23.63 ±0.65	25.71 ±0.97	22.65 ±0.46	21.68 ±0.72	24.50 ±0.72	21.14 ±0.48 **	23.87 ±1.23

3.9 Total Needle Projected Area Per Shoot.

3.9.1 **1987 Experiment.**

The final mean needle projected area for needles at all positions (from Fig. 3.9) was calculated for each treatment, and multiplied by the mean number of needles per shoot (Table 3.2), to estimate the total needle projected area per shoot. The estimate was highest for Control shoots, and lowest for -P shoots, with the other treatments having intermediate values (Table 3.14).

Table 3.14 Total needle projected area per shoot, for all treatments at the final harvest (June or July 1987).

Treatment	Total Needle Projected Area Per Shoot (cm^2)
+All	80.4
-Mg	64.9
-K	61.0
-P	27.0
-All	46.1

3.9.2 **1988 Experiment.**

Total needle projected area per shoot for Control trees in 1988 was about 14% of the value for 1987 (Table 3.15). In contrast to 1987 data, there was no effect of P or K deficiency on the total area, and the only treatments which had significantly lower total needle area than the Controls, when the 95% confidence limits were compared, were -N and -All. In these two treatments there was a significant effect of refertilisation ($p < 0.05$). In the other three deficiency treatments there was no significant effect of refertilisation on the total needle area (Table 3.15).

Table 3.15 Mean total needle projected area per shoot (cm^2) for all treatments, from May 30 1988. Values are means ± S.E. n=30 for Controls; or 18 for the other treatments.

	+All	-MgR	-Mg	-KR	-K	-PR	-P	-NR	-N	-AllR	-All
Total Area	11.64	10.82	12.32	13.62	13.75	14.51	14.65	7.95	1.87	10.04	2.46
	±0.76	±0.86	±1.50	±0.87	±1.27	±1.33	±1.59	±1.00	±0.20 ***	±1.18	±0.30 ***

Asterisks show degree of significant difference from the Controls.

3.10 Discussion.

3.10.1 Seasonal Development.

The data show shoot extension in 1987 to begin in early May and end in early July. This is some 2-3 weeks earlier than the period of extension found by Ford et al. (1987a,b), who investigated seasonal variation in shoot extension of 12 year old trees of *Picea sitchensis* grown in the Moffat forest about 60 km from Leadburn, at an altitude of 335 m. For the 1973 season they found shoot extension to begin in the latter part of May and to cease after 55-60 days, in late July-early August. However, the shoots of the Control set in 1987 in the present study grew substantially more than those at Moffat (Ford et al. 1987a,b), probably because of a higher seasonal mean temperature at Leadburn during the study.

There were only small effects of nutrient treatment on the timing of shoot elongation and of leaf expansion in 1987 and 1988. In 1987, the onset of shoot extension was delayed by about two weeks in the nutrient treatments and was completed by early July in all cases; there was no indication that the small delay in the onset of extension in the treatments was compensated for by a longer period of development. The shorter final length of the shoot in the deficiency treatments reflected partly this shorter extension period, but also a slower extension rate (Table 3.1). In 1988, the period of needle expansion was very short for all treatments, in comparison with that of 1987, and was complete by the end of May. This is because the needles in 1988 were much smaller than those of field trees in 1987, and had a slower extension rate. This short extension period for seedlings effectively increases the growth season, which is useful during seedling growth, to enable the apical bud to be

enlarged and to initiate more needle primordia than during the preceding year.

The final length of the Control shoots in 1987 was nearly double that of those of the other treatments and was not correlated with bud height, measured on field trees in 1988, as reported for *Pinus ponderosa* (Hanover, 1963), and *Pinus resinosa* (Rehfeldt and Lester 1966), but was more closely related to the diameter of the bud core (Table 3.5 A). Little (1970) also found shoot length to be correlated with shoot diameter as well as bud length, in *Pinus strobus*. Interpreting correlations for data obtained in different seasons has to be made with caution but bud diameter may be an index of the potential for development of vascular connections to the extending shoot which may control growth.

Nutrient deficiency also affected final needle number per shoot in 1987 and 1988 (Tables 3.2 and 3.3). In 1987, needle number was not correlated with shoot length, and mean stem unit length varied from 0.61 mm in the Control treatment, to 0.50 in -K and -All treatments, to 0.35 in the -P treatment. This crowding of the needles in the deficiency treatments suggests that stem elongation is affected more than needle initiation. This reduced stem elongation may be due to an effect of mineral deficiency in decreasing the cytokinin content of stems, as reported for leaves and roots of sunflower (Salama and Wareing, 1979). This is probably via an indirect effect on the roots. There was no relationship between bud surface area, treated as a cone, and final needle number, although these were measured in different seasons for this comparison. In 1988 the smaller needle number per shoot in -N and -All treatments is probably due to a smaller apical bud, although bud sizes were not measured for pot trees. Neither was it possible in 1988 to confirm an effect of nutrient deficiency in decreasing stem unit length, as found in 1987.

The acropetal developmental sequence of needles along the shoot has been reported for species of *Pinus* (Kienholz, 1934), for long shoots of *Larix laricina* (Clausen and Kozlowski, 1970), and *Larix decidua* (Frampton, 1960). The time interval between commencement of rapid expansion of the proximal and distal most needles is probably about 3 weeks although the widely spaced harvest intervals make it difficult to be more precise. It means that until the later harvests, the largest needles are always at the base of the shoot and even when extension is complete the most distal needles remain the shortest and narrowest; the most proximal needles are also shorter, but wider, than those

towards the middle of the shoot. This ontogenetic trend resembles that found for spikelet size in barley (Cottrell et al., 1982). In both cases the basal structures are initiated following cessation of production of a different kind of organ, in barley the leaves, and in Spruce the scale cataphylls. It is possible that closeness to the zone of transition influences the size of the spikelet or needle in the two cases.

The strong correlation between needle fresh weight and projected needle area for each treatment in 1988 is important, and enables needle weight from young trees to be of predictive use in estimating surface areas, as for *Pinus radiata* (Ohmart and Thomas, 1986). The b values change slightly between treatments, reflecting the differences in the relationship, caused by nutrition, although the specific leaf area, represented by these b values is only significantly reduced in the -AllR treatment, compared with the Controls. However, the difference in specific leaf area between -All and -AllR needles is not significant, nor those between other deficiency and refertilised treatments, even though significant differences exist between fresh weight and projected area of -N and -NR needles, and -All and -AllR needles. Therefore, needle weight upon refertilisation of -N and -All trees increases in proportion to increases in needle size. In contrast to the present data, Bhat et al. (1979) found increased N supply increased the leaf area ratio ($cm^2\ g^{-1}$ dry wt.) in rape. This difference is probably because conifer needles have a relatively small projected area and high weight, so that the specific leaf area is less sensitive to changes in either variable, compared with a broad leaved species. Needles in the -KR treatment are significantly heavier than the Controls, but needle projected area is not significantly greater, which may provide indirect evidence for a change in needle thickness between these treatments, but this is not supported by the 1987 data, leaving the possibility that the significance of the difference between fresh weight of -KR and Control needles is a Type 1 error. The value for specific leaf area for the Controls in 1987 of 18.4 $cm^2\ g^{-1}$ fr.wt. is lower than that of 24.1 $cm^2\ g^{-1}$ fr.wt. reported for needles of Sitka spruce at a similar canopy position by Lewandowska and Jarvis (1977), who also showed specific leaf area to decrease from 28.3 $cm^2\ g^{-1}$ fr.wt. to 20.0 $cm^2\ g^{-1}$ fr.wt. with decreasing canopy position, according to whether needles had a "sun" or "shade" shoot morphology. Needles sampled in October 1987 were from trees on the edge of the plot, and so had greater "sun" shoot morphology and hence a higher specific leaf area, than the shoots at a similar position within the

canopy, as sampled by Lewandowska and Jarvis (1977). The significantly higher dry weight:fresh weight ratio of -P and -K needles compared with the Controls in 1987 and 1988 is interesting, and reflects a change in the pattern of partitioning of carbon. This may be due to thicker cell walls, or a thicker cuticle, alterations in the amount of surface wax on the needle, or a higher amount of assimilates retained in the needle, possibly because of a reduction in translocation of assimilates out of the needle under nutrient deficient conditions.

The -Mg set of trees in the field in 1987 did not have deficient foliar amounts of Mg (see Table 2.5). The lower shoot extension rate than Controls (Table 3.1) is probably therefore due to variation in material, as shoot length for a larger sample in October (Table 3.2), showed no difference to the Controls. No significant difference was found between needle length and width of -Mg and Control needles (Table 3.6), or in any of the other variables measured, so that the -Mg field data may be considered as a second Control.

It is of interest that -P trees in 1987 had the lightest, shortest, narrowest needles, with the most reduced projected area and needle growth rate and the shortest shoots and smallest apical buds. These effects were all greater than for -All trees, suggesting that nutrient imbalance resulting from phosphorus deficiency reduces growth and therefore potential productivity more than that of potassium deficiency, or deficiency of P and K as represented by total nutrient deficiency, and that P is therefore the most important element of those studied, for growth. However, the needle characteristics measured in P-deficient trees in 1988 did not show the same reductions as in 1987, and neither did those resulting from K deficiency. This may be due to differences in tree age, with seedlings showing less extreme growth effects. Reduction in needle characteristics for -All trees was greater in 1988 than in 1987, as there was a greater effect of N deficiency in pot trees, as N deficiency is not regarded by the Forestry Commission as being present at the field site (pers. Comm. M.Coutts). The spectacular increase in needle length, fresh weight and projected area of -N and -All trees following refertilisation, and the increases in the morphological variables with increasing N supply, in 1989 support the findings of Brix and Ebell (1969), who found an increase in needle area with N fertilisation of Douglas fir.

3.10.2 **Free Growth.**

Free growth observed upon refertilisation of -N and -All trees in 1988 is interesting; Jablanczy (1971) reported a tendency for free growth in young seedlings of spruce and fir, which disappears between 5 and 10 years of growth. Nienstaedt (1966) found that capacity for free growth in White spruce disappeared after 4 years. Pollard and Logan (1974) suggested that free growth is a response to photoperiod, and serves to increase leader height and therefore seedling survival in early years of growth.

This photoperiodic free growth response was found to decline with age for *Pinus resinosa* and *Pinus strobus* (Watt, 1961), and for *Picea abies* (Balut and Zelawski, 1955). In the present study, free growth appears to be a response to favourable nutrient conditions, and particularly after a period of unfavourable conditions. It is unlikely to be a result of exposure to a 16 h photoperiod in the controlled environment room, as not all the treatments showed the response, and it was observed in -NR and -AllR trees prior to placing them in the controlled conditions. The increase in needle size, fresh weight and cell number due to free growth must have important implications in productivity during early years of growth.

CHAPTER 4. RESULTS. CELLULAR PARAMETERS OF NEEDLE GROWTH

The results in this chapter describe primordium cell number, and the cellular parameters of needle growth in field trees from all nutrient treatments, from bud burst to maturity. Also presented are results for the early growth of Control needles of field trees, prior to bud burst in 1988.

4.1 Needle Cell Number, 1987.

4.1.1 Primordium Cell Number.

Because of the labour involved in counting cells in primordia, only limited data are available (Tables 4.1 and 4.3). Mean values were obtained for primordial cell number of needles at proximal (basal), median proximal, median distal and distal (apical) positions in the dormant bud, for Control, -K and -P treatments (Table 4.1). Between 2 and 5 primordia were counted from each position (see Materials and Methods, section 2.7.1), from each of two buds. For each bud, two of the estimates of primordium cell number for each position were taken at random, and the average of the four values found. The largest primordia at the base of the bud had the largest cell numbers, with the distal primordia having the smallest. Analysis of variance (Table 4.2) on the data showed a small, but significant interaction between position and nutrient treatment, with cell number at the proximal (base) position greatest in the Control primordia and least in the -P treatment. Primordia from the -P treatment had fewer cells than the Controls at other positions as well, however, at the distal position, primordia from the -K treatment had fewest cells.

Table 4.1 Cell numbers in needle primordia from dormant buds from mid-canopy, for +All, -K and -P treatments, and at different positions in the bud. Values are means for two primordia from each of two separate buds, to 3 significant figures.

	Position of primordium in the bud.			
Treatment	Base	2nd Quarter	3rd Quarter	Apex
Control	6640^{a}	6450^{a}	4020^{d}	2930^{f}
-K	5390^{b}	4090^{d}	3615^{e}	1890^{g}
-P	3820^{c}	4090^{cd}	3480^{e}	2170^{g}

LSD (p=0.05): for position = 319
for treatment = 362

Similar letters in rows and columns indicate non-significant differences.

Table 4.2 Analysis of variance on primordium cell number data.

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	2	578190.5	6.56 **
Position	3	10555933	51.63 ***
Interaction	6	88138	4.31 **
Error	12	204453	

Primordium cell numbers were also counted from two Control buds, and one -P and -K bud, from mid canopy in the field, according to position in the bud (see Fig. 2.1 C, Materials and Methods). Data show the same decrease in cell number in primordia towards the bud apex (Table 4.3), with highest cell numbers in basal primordia for each treatment. Control buds also had higher primordium cell numbers than the deficiency treatments.

Table 4.3 Primordium cell number according to position of the primordium in the bud.

Position number of primordium in the bud*	Treatment			
	+All (Replicate Buds)	+All	-K	-P
1	---	---	4460 ± 1350	---
2	5340 ± 60	5590	---	3560
3	---	---	4380	---
4	4450 ± 370	---	---	---
5	---	5380	---	2660 ± 350
6	4840	---	---	---
8	4790	---	---	3170 ± 410
10	---	5090	---	2470 ± 400
12	5280	---	---	---
13	---	---	---	2250 ± 200
14	1900	---	---	---
15	Apex	Apex	Apex	---
16				Apex

Values are for one measurement only, or means ± S.D. of up to three replicate primordia from the same bud.
*For explanation of primordium position number, see Materials and Methods, section 2.7.1.

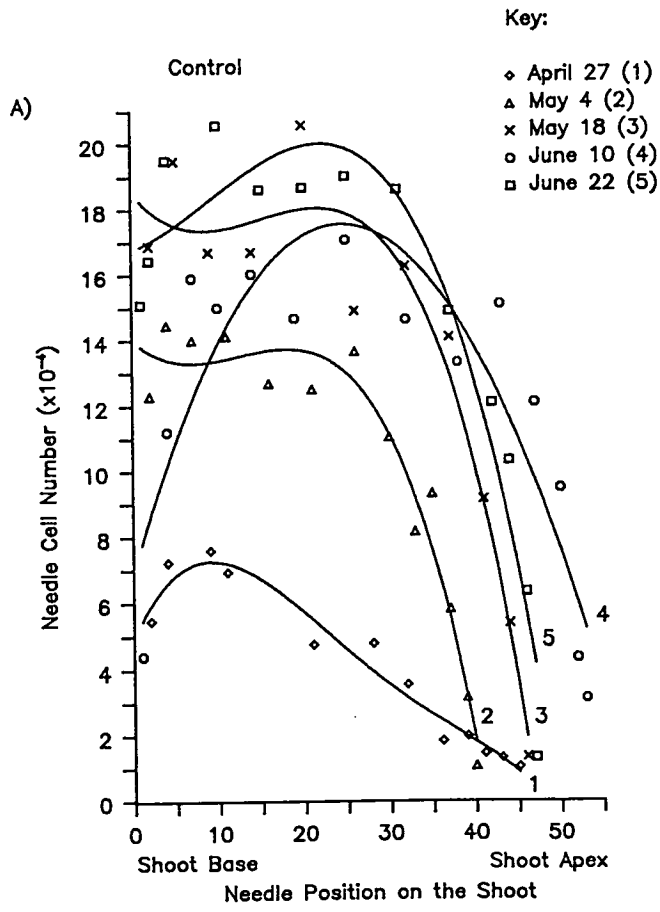
4.1.2 **Needle Cell Number 1987.**

Data for needle cell number varied according to position on the shoot, as did primordium cell number with position in the bud. Third or fourth order polynomial curves were fitted to the data for successive harvests (Fig 4.1) and accounted for not less than 52%, and usually over 80% of the observed variance.

One week before bud-burst, Control needles at the base of the bud were already showing a large increase in cell number, with values of between 50,000 and 70,000 cells per needle. This represents at least a tenfold increase over the values in Table 4.1, and can be accounted for by assuming at least 3-4 cycles of cell division of all cells prior to sampling. At bud-burst (May 4), cell number in these basal needles had reached 130,000, indicating that at least another 1-2 cycles of cell division had occurred. Mean cell cycle time was thus a maximum of 1 week at this stage. Subsequently cell number increased more slowly in these needles. For needles at positions along the rest of the bud, the rates of cell division following bud burst were slightly higher; between positions 20-35, there was a three-fold increase in needle cell number in one week, from April

27 to May 4. This is equivalent to between 1 and 2 cycles of cell division, with a mean cell cycle time of not more than 112 hours. Between May 18 and June 22, changes in needle cell number were seen for all except the distal needles; these may have continued to show cell division beyond this point, but data are lacking. For the Controls, final cell numbers for needles in the proximal half of the shoot were between 180,000 and 200,000. These values are higher than those for needles in the deficiency treatments (Figs 4.1 B to E). In all these treatments, needle cell number showed similar trends with position and time to that of the Controls. However, final needle cell number varied; needles in the -All and -K treatments did not vary significantly from each other, but needles in the -P set showed maximum cell number to be generally not more than 80,000.

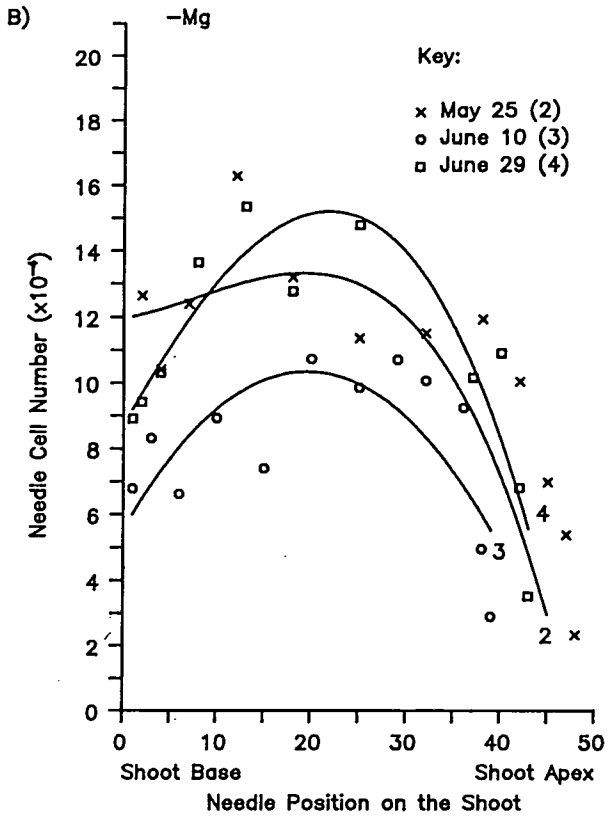
Figure 4.1 A to E. (and overleaf). Needle cell number according to needle position on the shoot, for each treatment in the field throughout the 1987 growth season. Each value is a mean, obtained by dividing the total cell number in each maceration solution by the number of needles macerated (n = 2 to 5), for one shoot only. For equations of the fitted curves see overleaf.

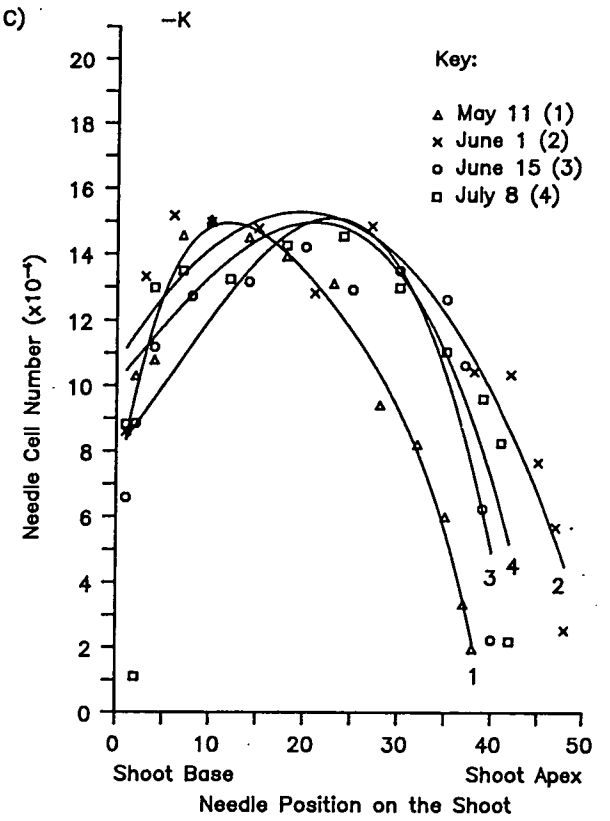


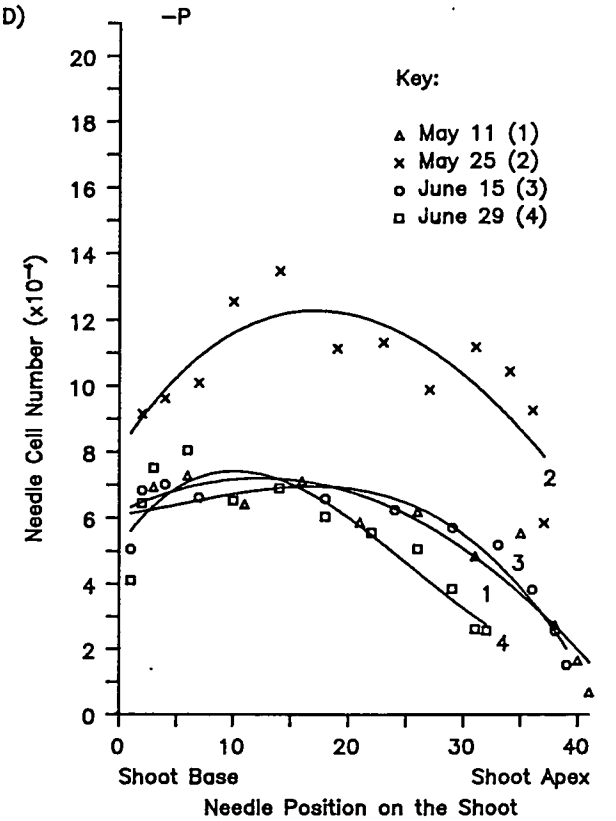
C

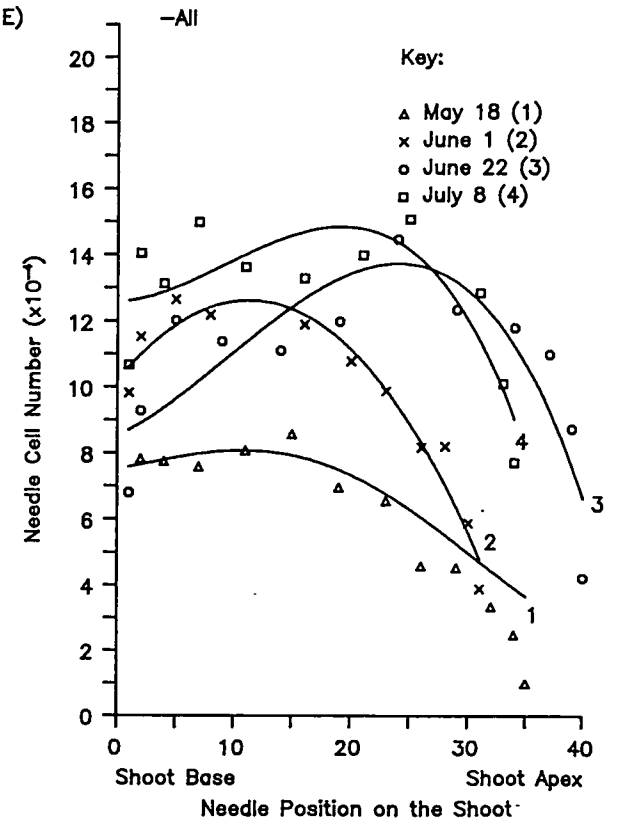
Equations of the fitted polynomial curves, and r^2 values for data in Fig. 4.1.

Treatment	Sampling Date	Fitted equation	r^2
Control	April 27	$y = 49167.4 + 5848.5X - 451.1X^2 - 0.09X^3$	0.96
	May 4	$y = 140148.4 - 2415.7X + 240.1X^2 - 6.4X^3$	0.95
	May 18	$y = 185547.4 - 3352.5X + 276.5X^2 - 6.1X^3$	0.94
	June 10	$y = 68865.3 + 8925.4X - 197.5X^2 + 0.4X^3$	0.91
	June 22	$y = 167458.5 + 1009X + 109.3X^2 - 4X^3$	0.92
-Mg	May 25	$y = 119742 + 440.4X + 65.4X^2 - 2.3X^3$	0.82
	June 10	$y = 55241.1 + 4968.9X - 127.4X^2$	0.52
	June 29	$y = 86926.1 + 5100.7X - 56.2X^2 - 1,8X^3$	0.84
-K	May 11	$y = 67282.1 + 17511.8X - 1256.4X^2 - 0.4X^3$	0.97
	June 1	$y = 107130.1 + 4593.9X - 110.9X^2 - 0.2X^3$	0.85
	June 15	$y = 80356.5 + 3602.1X + 93.3X^2 - 5.1X^3$	0.80
	july 8	$y = 101219.1 + 3498X - 2.4X^2 - 2.6X^3$	0.83
-P	May 11	$y = 61743.7 + 1657.7X - 67.6X^2 + 0.9X^3$	0.89
	May 25	$y = 80677.7 + 5196X - 175.8X^2 + 0.9X^3$	0.64
	June 15	$y = 60868.7 + 514.8X + 29.5X^2 - 1.8X^3$	0.91
	June 29	$y = 51570.8 + 4865.8X - 300.5X^2 + 3.9X^3$	0.82
-All	May 18	$y = 75194.3 + 602.3X + 41X^2 - 5.4X^3$	0.97
	June 1	$y = 102210.6 + 4221.2X - 181.5X^2 - 0.4X^3$	0.95
	June 22	$y = 85162.2 + 1673.1X + 132.2X^2 - 4.6X^3$	0.64
	July 8	$y = 125795 + 169.8X + 166.9X^2 - 6X^3$	0.69


B)
−Mg
Key:
× May 25 (2)
o June 10 (3)
□ June 29 (4)
Needle Cell Number (x10⁻⁴)
Shoot Base
Shoot Apex
Needle Position on the Shoot


C)
−K
Key:
△ May 11 (1)
× June 1 (2)
o June 15 (3)
□ July 8 (4)
Needle Cell Number (x10⁻⁴)
Shoot Base
Shoot Apex
Needle Position on the Shoot


D)
−P
Key:
△ May 11 (1)
× May 25 (2)
o June 15 (3)
□ June 29 (4)
Needle Cell Number (x10⁻⁴)
Shoot Base
Shoot Apex
Needle Position on the Shoot


E)
−All
Key:
△ May 18 (1)
× June 1 (2)
o June 22 (3)
□ July 8 (4)
Needle Cell Number (x10⁻⁴)
Shoot Base
Shoot Apex
Needle Position on the Shoot

4.2 Duration of Cell Expansion and final cell size, 1987.

For each sampling date for each treatment, the plot of needle cell number and fresh weight gave a highly significant linear relationship (Fig. 4.2 A to E, with the fitted lines accounting for between 50% and 97% of the observed variation (Table 4.4). For the Controls, the slope of the fitted line decreased at successive harvests, as mean fresh weight per cell increased to a value of about 7.0×10^{-8}g on June 10 (Fig. 4.2 A). For the -K and -All treatments, the slope of the relationship also decreased with each sampling time (Fig. 4.2 C and E), and also for the other deficiency treatments, (Fig.4.2 B,C,D), although these showed slightly more variation in material between sampling times. The time at which the slope of the relationship, b, ceased to change (Table 4.4), indicates when maximum needle cell fresh weight was reached, and hence when cell expansion, had ceased.

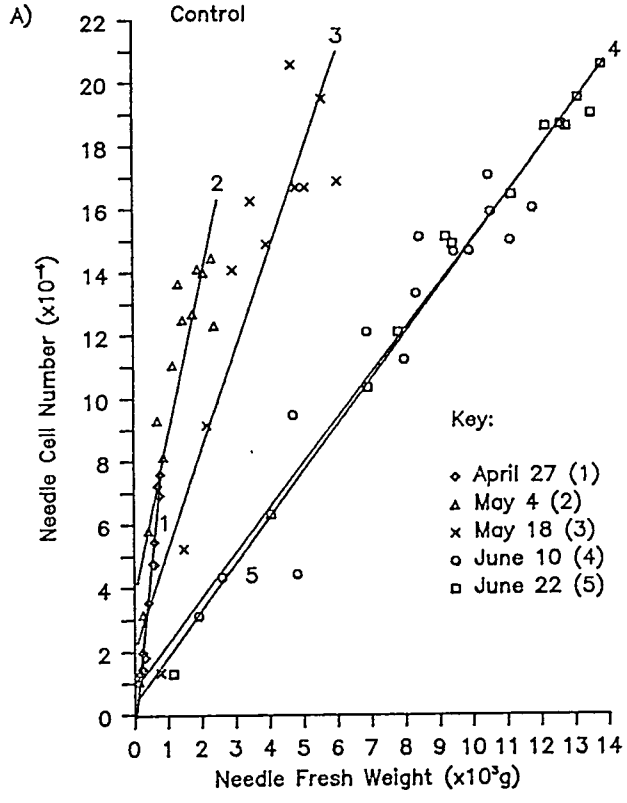
Table 4.4 Table of regression coefficients, b ($\times 10^{-7}$), for the relationship between needle cell number and fresh weight, for all treatments at each sampling time, (see Fig. 4.2).

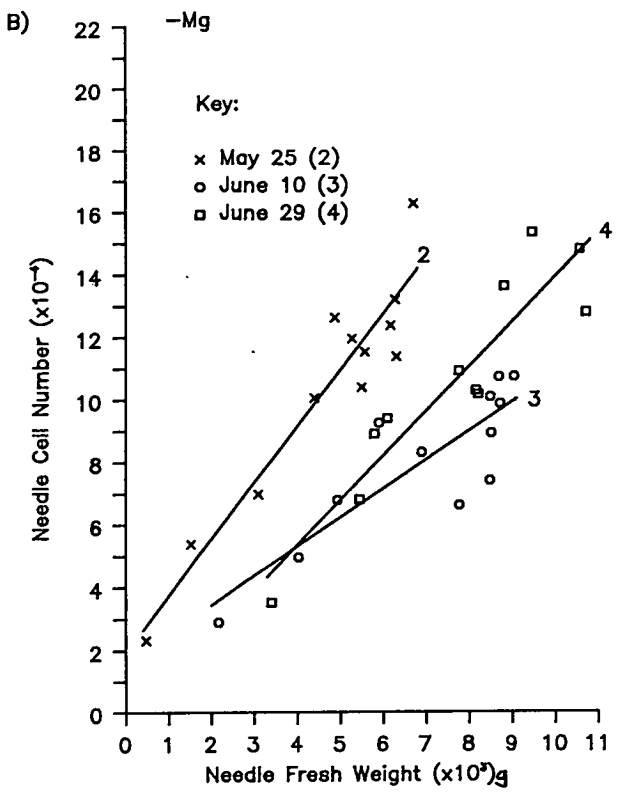
Sampling Time	Coefficient	Treatment				
		Control	**-Mg**	**-K**	**-P**	**-All**
1	b	9.998	---	---	---	---
	r^2	0.964	---	---	---	---
2 (1)	b	5.042	---	8.141	3.266	3.704
	r^2	0.793	---	0.946	0.878	0.937
3 (2)	b	3.163	1.808	1.993	8.757	2.143
	r^2	0.822	0.896	0.899	0.501	0.982
4 (3)	b	1.422	9.297	1.570	1.306	1.364
	r^2	0.897	0.73	0.97	0.949	0.929
5 (4)	b	1.4645	1.436	1.642	1.237	1.390
	r^2	0.989	0.86	0.909	0.916	0.766

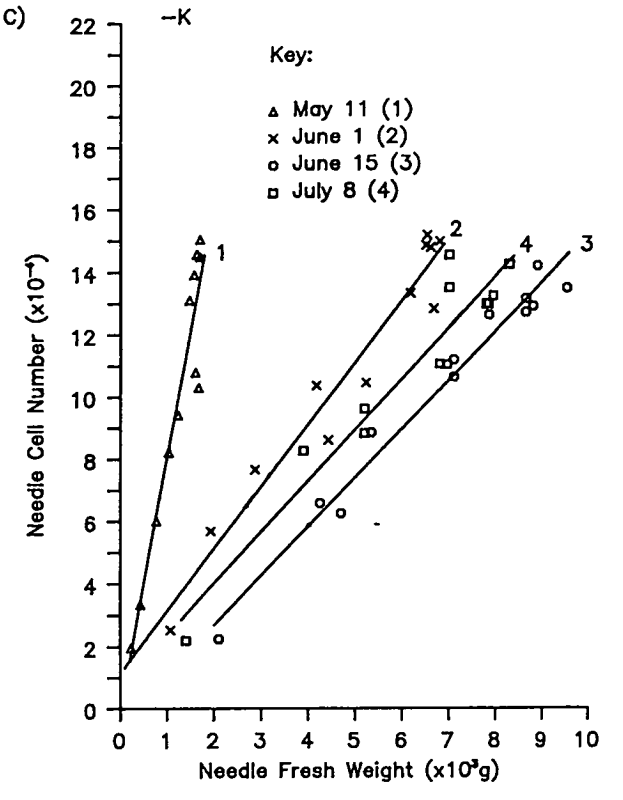
Numbers 1 to 5 refer to the order of sampling dates in the key of Fig. 4.2 A for the Controls, and numbers in brackets refer to the sampling dates in the keys to Fig. 4.2 B to F for the deficiency treatments.

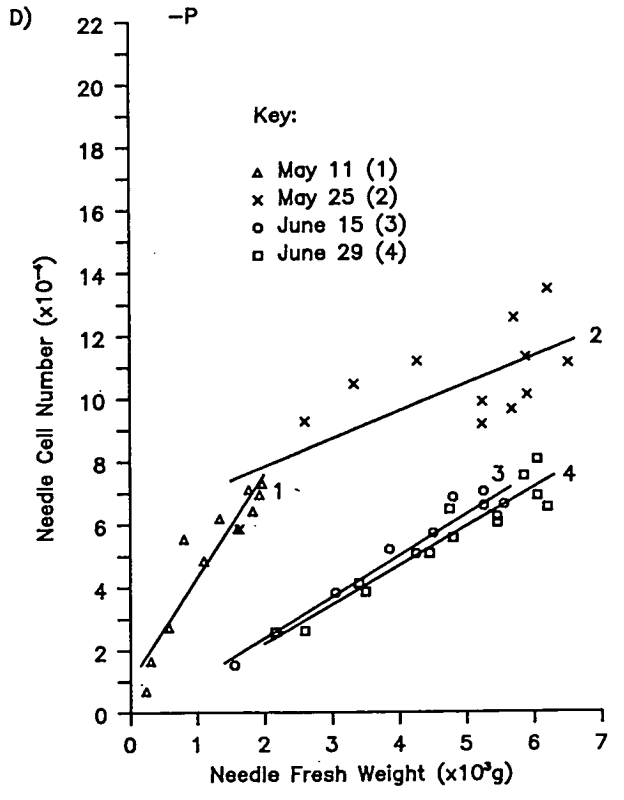
Figure 4.2 A to E

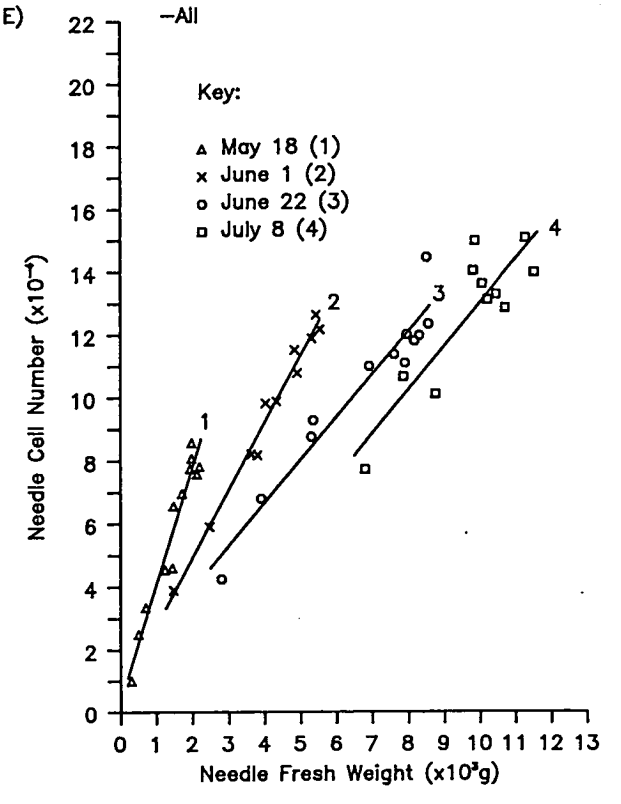
The relationship between needle cell number and fresh weight for all sampling times throughout the 1987 growth season, for each treatment in the field. Fitted lines are described by the coefficients in Table 4.4. The points at any one sample time represent the data in Fig. 4.1, for the same positions on the shoot.




B)
-Mg
Key:
× May 25 (2)
o June 10 (3)
□ June 29 (4)
Needle Cell Number (x10⁻⁴)
Needle Fresh Weight (x10³)g
1
2
3
4


C)
-K
Key:
△ May 11 (1)
× June 1 (2)
o June 15 (3)
□ July 8 (4)
Needle Cell Number (x10⁻⁴)
Needle Fresh Weight (x10³g)
1
2
3
4


D)
-P
Key:
△ May 11 (1)
× May 25 (2)
o June 15 (3)
□ June 29 (4)
Needle Cell Number (x10⁻⁴)
Needle Fresh Weight (x10³g)
1
2
3
4


E)
-All
Key:
△ May 18 (1)
× June 1 (2)
o June 22 (3)
□ July 8 (4)
Needle Cell Number (x10⁻⁴)
Needle Fresh Weight (x10³g)
1
2
3
4

The rate of increase in mean needle fresh weight per cell was similar for all treatments until early June (Fig. 4.3 A) Subsequently, values reached a plateau, apart from cells in the -All treatment which continued to increase in fresh weight. Mean fresh weight per cell at the final harvest (Table 4.5) varied in the order; -P > -All > -Mg > Control > -K. However, cells from the -Mg and -K treatments showed a lower mean fresh weight at the final harvest than for the preceeding one, indicating tree-to-tree variation; using the final data set as an indication of cell size (Table 4.5) must therefore be done with caution. Because the amount of dry matter per cell increases during needle development (Figs. 3.7 and 3.8), a more accurate estimate of cell size is obtained from the mean cell water content. The fresh weight values for the needles used for cell counting were therefore corrected for the proportion of dry weight (from the dry weight:fresh weight ratio). Mean cell water content increased in all treaments (Fig. 4.3 B) in a similar way to mean cell fresh weight; increasing at the same rate, until a maximum in mid June. Cells from the -All treatment did not increase in water content after this time, as they did in fresh weight.

When the mean cell water contents for needles at all positions are averaged for the last two sampling times (Table 4.6) it is seen that cells from needles in the -P set are still larger than those of the Controls, and cells from -K needles are smaller than the Controls, confirming the interpretation based on the fresh weight per cell data.

Table 4.5 Mean fresh weight and cell number of needles from all treatments at the final harvest.

Treatment	Mean fresh weight per needle ($x10^{3}$g)	Mean cell number per needle ($x10^{-5}$)	Mean Fresh weight per cell ($x10^{8}$g)	Fresh weight per cell expressed as % of Control
Control	9.8	1.47	6.67	---
-Mg	7.4	1.06	6.98	105
-K	6.3	1.10	5.73	86
-P	4.6	0.54	8.52	128
-All	9.8	1.27	7.72	116

Figure 4.3 A) Mean fresh weight per cell (x10^8g), B) Mean cell water content (x10^8g), for each treatment in the field, at each sampling time throughout the 1987 growth season. Values are means ± S.E. (n = 11–14) for the data for needles at all positions in Figure 4.2 at each sampling time.

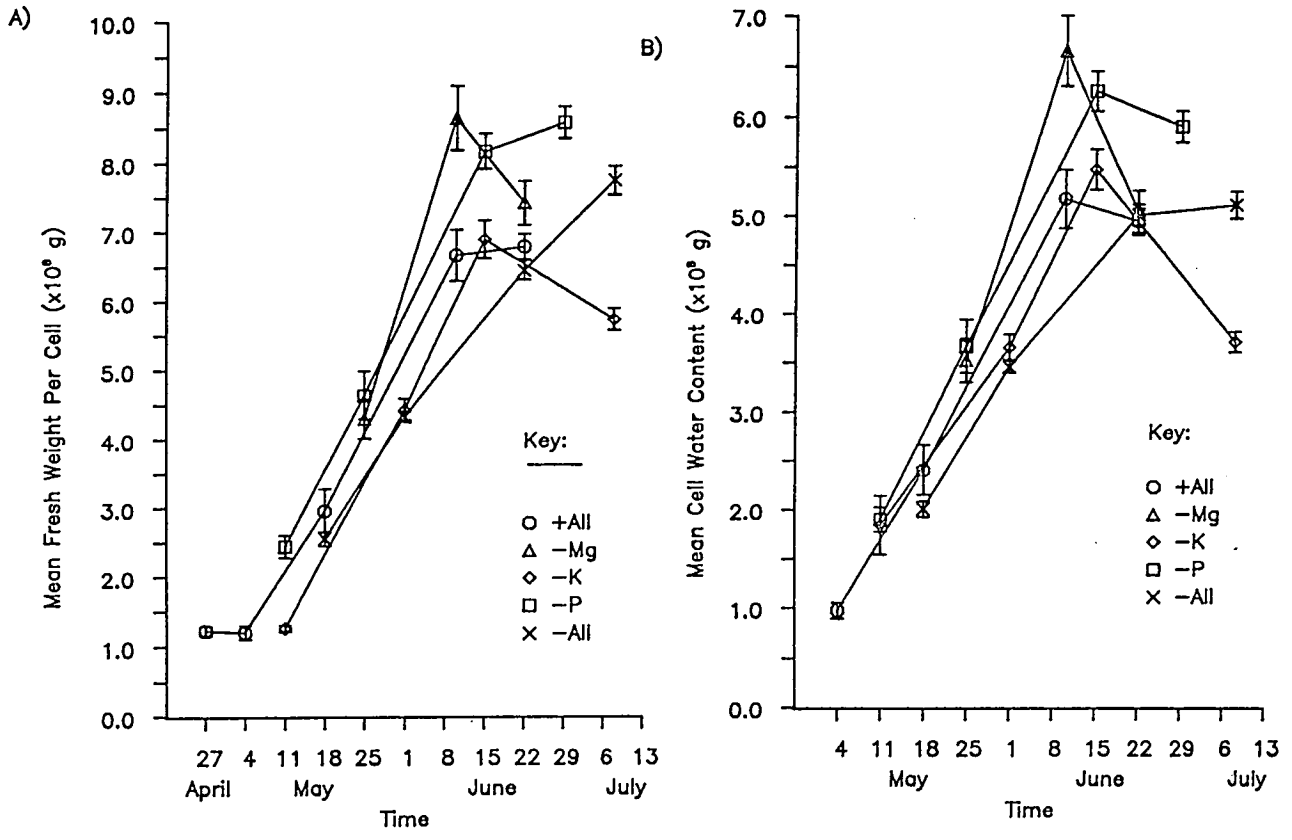


Table 4.6 Mean cell water content, for needles along the whole shoot, averaged for the last two harvests.

Treatment	Mean cell water content (x10^8g)	Expressed as % of control
Control	5.06	---
-Mg	5.85	116
-K	4.59	91
-P	6.08	120
-All	5.06	100

4.3 Early Needle Development in Field Material, 1988.

During the 1987 study it was found that at bud burst, Control needles had already extended several mm in length, and undergone considerable cell division. A more detailed analysis of needle growth prior to bud burst was therefore made in 1988, for needles from Control trees from the field, to give a fuller description of the pattern of increase in cell number and length during this early period. Position numbers refer to those in the spiral in Fig. 2.1 C.

4.3.1 Needle Size.

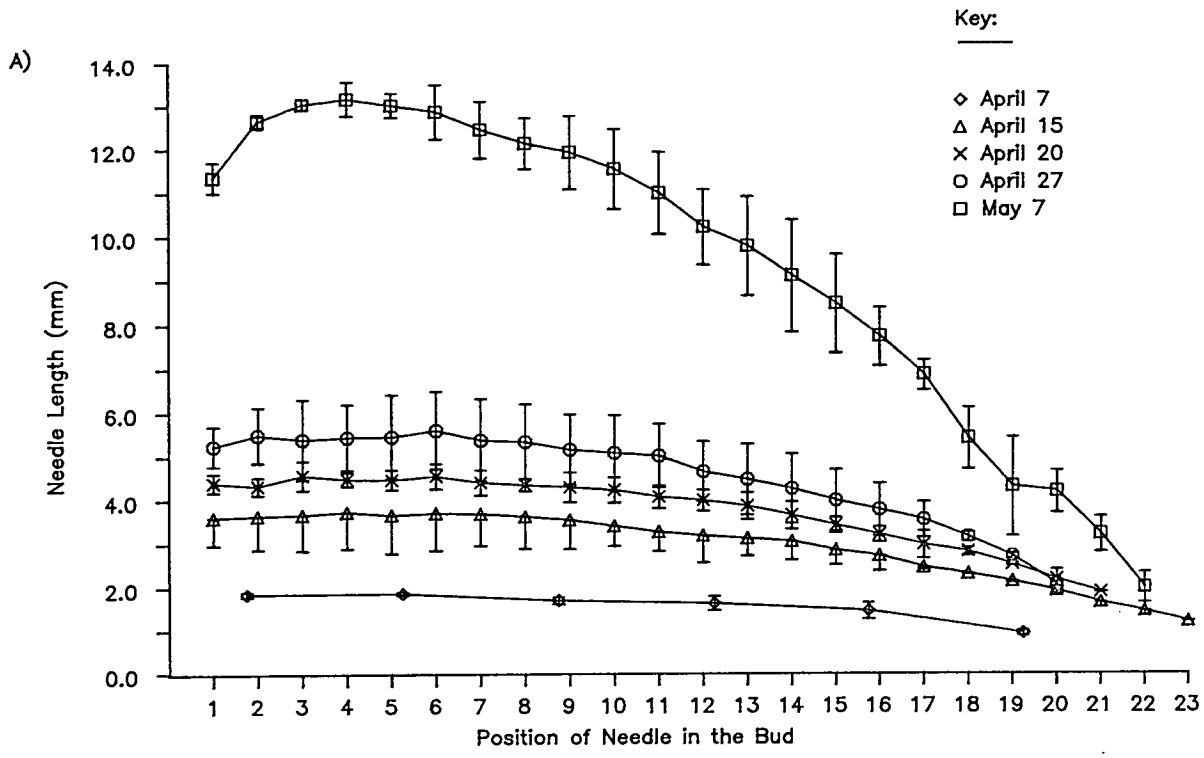
On April 7, one month before bud burst, mean needle length at all bud positions ranged from 0.9 to 1.9 mm (Fig. 4.4). The pattern of mean needle length increase with time was the same as that observed following bud burst in 1987; distal needles extended the least, and proximal ones the most, so that by April 27 distal needles were still about 2 mm in length, whilst needles at positions 1-11 were more than 5 mm long. Between April 15 and April 20, rates of extension between positions 1-10 varied from 0.14-0.18 mm d^{-1}, and continued at these values until April 27, subsequently increasing in the period up to and immediately following bud burst on May 7. Rates were now between 0.61 and 0.77 mm d^{-1} for needles at positions 4-10, but rates were less for the basal three needles, so that the needle at position four was the longest, over 13 mm, with progressively shorter needles towards the tip of the bud, these being less than 2 mm long.

At the time of first harvest (April 7; Fig. 4.4 B), the broadest needles were at the bud base, with those at the tip being about 30% narrower. By the time of bud burst (May 7), differences in width extension rate along the bud meant that the basal needles had reached a final width of more than 9 mm whereas those at the tip were less than 4 mm wide.

The needle length:width ratio was greatest for needles at positions 4 to 7 at all sampling times, and decreased in needles towards the shoot tip. With time, this difference became more pronounced (Fig. 4.5).

Figure 4.4 A and B

The change in A) Needle length; B) Needle width, for Control needles from terminal shoots from whorl 7 of field trees, at times prior to bud burst 1988, and according to needle position in the bud (see Fig. 2.1 C). Values for each position at each sampling time are means ± S.D. for two buds, where the value for each bud is the mean of 3–10 needles.


A)
Key:
April 7
April 15
April 20
April 27
May 7
Needle Length (mm)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Position of Needle in the Bud

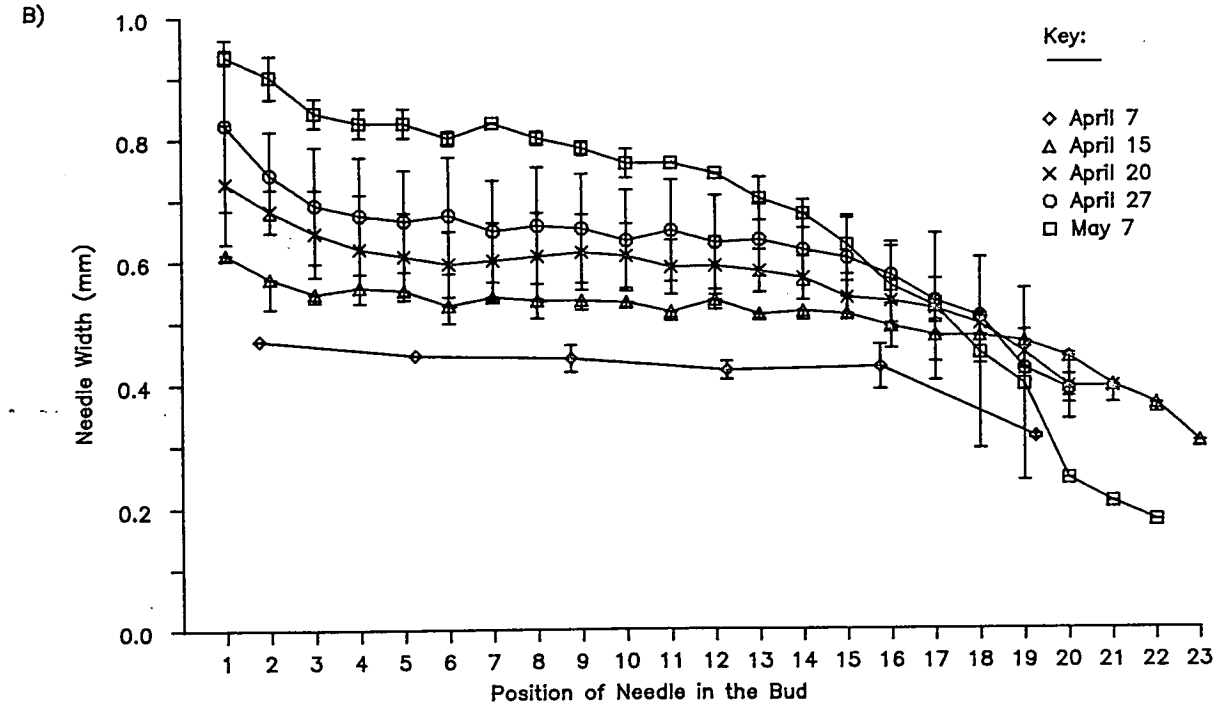
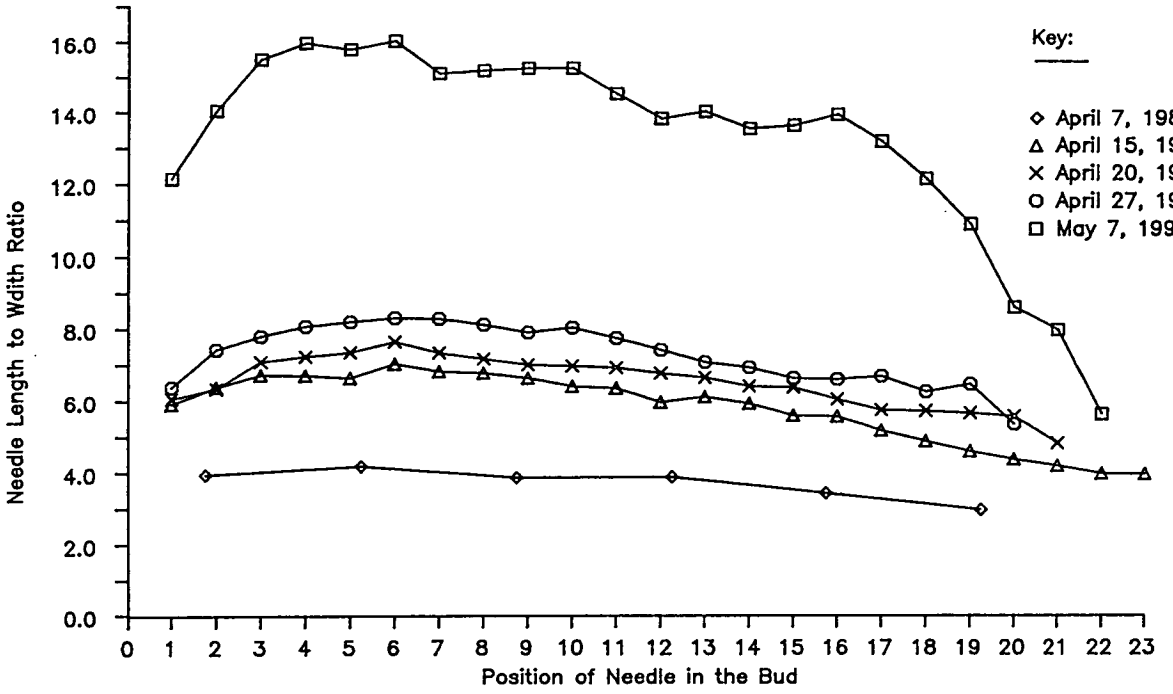
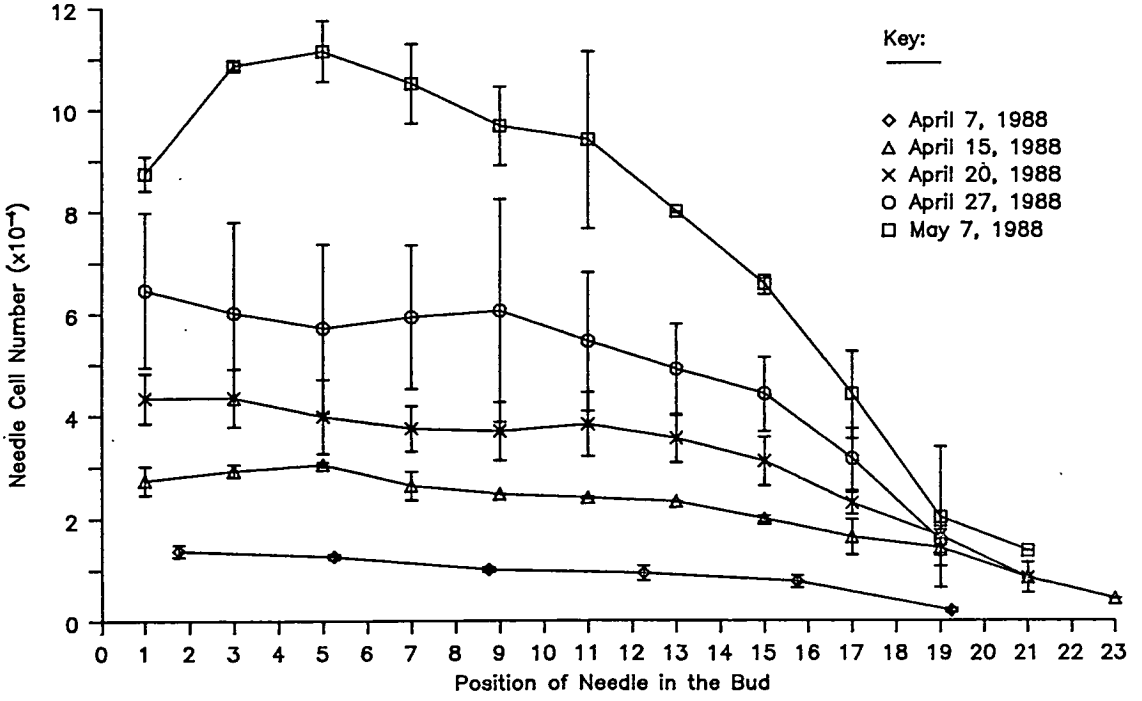

B)
Key:
April 7
April 15
April 20
April 27
May 7
Needle Width (mm)
1.0
0.8
0.6
0.4
0.2
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Position of Needle in the Bud

Figure 4.5
The change in length:width ratio for the data in Figure 4.3.

Figure 4.6
The change in cell number for Control needles from terminal shoots from whorl 7 of field trees, at several times prior to bud burst 1988, and according to needle position in the bud (see Fig. 2.1 C). Values for each position at each sampling time are means ± S.D. for two buds, where the value for each bud is the mean of 3–10 needles.


Key:
April 7, 1988
April 15, 1988
April 20, 1988
April 27, 1988
May 7, 1998
Needle Length to Width Ratio
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Position of Needle in the Bud


Key:
April 7, 1988
April 15, 1988
April 20, 1988
April 27, 1988
May 7, 1988
Needle Cell Number (x10⁻⁴)
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Position of Needle in the Bud

4.3.2 **Needle Cell Number.**

On April 7 mean needle cell number ranged from about 14,000 at the bud base, to less than 2,000 at the tip (Fig. 4.6). Cell number increased with time, and from April 27, maximum rates of increase in cell number occurred for needles at positions 3–7 (Table 4.7). Immediately following bud burst, cell number of needles at the shoot tip were still only about 13,000, whilst those at position 5 were over 110,000. The most basal needle had a smaller rate of cell number increase at each sampling time. Although the rate of cell number increase was more between April 27 and May 7 than previously, the time taken for the cell number to double, increased from about 8 days between April 7 and 15, to more than 10 days between April 27 and May 7.

Table 4.7 The rate of cell number increase for Control needles prior to bud burst 1988, according to needle position. (For total cell number for positions 2,6,10 and 14, see Table 4.8).

	Mean Rate of Cell Number Increase (Cells d^{-1})		
Needle Position Number in bud	April 15 – April 20	April 20 – April 27	April 27 – May 7
1	3199	3025	2278
3	2844	2370	4862
5	1866	2460	5451
7	2218	3113	4584
9	2436	3371	3616
11	2843	2309	3958
13	2815	1913	3088
15	1847	1855	2156
17	1312	1212	1249
19	470	---	445
21	---	---	---
23	---	---	---

The increase in cell number in needle halves, taken from the buds used for total cell number counts was also measured, to try to locate the area of major meristem activity. Needles at positions 2, 6, 10 and 14 were removed, and cut exactly in half according to length with a fine scalpel. Cell numbers in distal and proximal halves were counted (Table 4.8).

Data show similar proportions of cells in distal and proximal halves of the needles at all positions at each sampling time, with about 30% of cells in the distal half, and 70% in the proximal half.

Table 4.8 Cell number in distal or proximal halves of Control needles, according to position in the bud, prior to 1988 bud burst.

Needle Position Number		Cell number ± S.D and % proportion of the total, in each portion.					
		April 20		April 27		May 7	
	Needle Portion	Cell Number	% of Total	Cell Number	% of Total	Cell Number	% of Total
2	Distal	11950 ± 4050	29.5	20300	30.6	35450 ± 3150	34.0
	Proximal	28550 ± 3600	70.5	46000	69.4	68950 ± 1850	66.0
	Total	**40500**		**66300**		**104400**	
6	Distal	12700 ± 200	34.1	25000	34.7	35600 ± 1050	33.0
	Proximal	24550 ± 8300	65.9	47150	65.3	71700 ± 9500	67.0
	Total	**37250**		**72150**		**107300**	
10	Distal	10550 ± 1050	27.6	19750	32.0	31500 ± 7300	34.4
	Proximal	27700 ± 9850	72.4	41950	68.0	60200 ± 6350	65.6
	Total	**38250**		**61700**		**91700**	
14	Distal	9850 ± 2650	33.4	16100	30.7	22350 ± 2000	33.9
	Proximal	19650 ± 4500	66.6	36400	69.3	43600 ± 5250	66.1
	Total	**29500**		**52500**		**65950**	

4.4 Discussion

No published data are available for comparison of primordial cell numbers (Tables 4.1 and 4.2) with those of other gymnosperms or angiosperm trees. However, the range of primordial cell numbers recorded here compare with data of Sunderland and Brown (1956), who found cell number in the four youngest primordia at a mid-plastochron stage in *Lupinus albus* to vary from 1,630 to 7,400. No data are available for the number of cells contributing to the primordium at its inception but if the number is around 120 as found by Poethig and Sussex (1985) using clonal analysis for *Nicotiana*, then a minimum of 5-6 doublings of cell number must occur during primordial growth prior to dormancy. However, the plastochron is very short in Sitka Spruce, with up to 7 primordia per day being initiated in apices of shoots at a mid-canopy position during August (Cannell, 1978), resulting in 300 or more primordia in the bud (see Table 3.2, Chapter 3). Mitotic activity in the bud must therefore be intense, yet diffused over a large number of primordia. What is not known is

whether the duration of mitotic activity is similar for all primordia or whether first-formed primordia show a longer period of cell division with cessation being governed by environmental factors rather than ontogenetic ones.

Owens and Molder (1976), working on Sitka Spruce in British Columbia reported cell division to resume in primordia during March, and for the material here, cell number in the basal primordia had risen to about half the final value as a result of very rapid division immediately prior to bud burst (i.e. during April). Rapid rates of division were also seen early in expansion of the more distal needles. It must be stressed that the estimates of mean cell cycle times assume that all the cells in the needle remain meristematic and capable of division; if progressively fewer cells enter successive divisions (Dale, 1970; Milthorpe and Newton, 1963; Wilson, 1966), then mean cell cycle times will be shorter. At any time following the resumption of primordial growth, the ratio of cell number in basal and proximal halves of the needle was similar (Table 4.8), suggesting cell division to occur over the whole blade. These data do not support the existence of a basal meristem, as reported for *Picea glauca* (Fraser, 1962; Vanden-Born, 1963), *Pseudotsuga menziesii* (Owens, 1968), and *Pinus* (Kienholz, 1934), nor do they rule it out. It may be that such a basal meristem functions during the early stages of needle growth, and that generalised cell divisions, as occur in the marginal meristems of angiosperm leaves become more obvious with time, so that small changes in cell number due to activity of a basal meristem are not noticed amongst large generalised cell divisions.

The minimum mean cell generation time of not more than 112 h reported here, is much higher than published values for expanding leaves of perennial plants; 39-53 h for *Trifolium repens* (Denne, 1966); 22-52 h for *Xanthium* (Maksymowych, 1973; Maksymowych and Blum, 1966); 17 h for *Cucumis* (Wilson, 1966), and means that relatively few generations of cells are needed for the needle to reach final cell number.

The maximum number of about 200,000 cells per needle, attained in the Controls by Mid-May (see Fig. 4.1), was found some 3 weeks before the attainment of maximum mean cell fresh weight, indicating that, as in many dicotyledon species, the final period of leaf expansion is marked by cell expansion free from division (see Dale, 1976; 1988). This was found also for the other treatments, and the time scales for increase in cell number and size, and hence needle expansion, were similar despite final differences. As already

noted, the inevitable gaps between harvests mean that more precise timings cannot be given.

Evidence from many species suggest that the increase in cell number is exponential during early leaf development (Maksymowych, 1973; Milthorpe and Newton, 1963; Hannam, 1968; and Lyndon, 1968). The present data show this trend prior to bud burst (Fig. 4.6), but only for needles at positions 3 to 7. Needles at more distal positions show a decreasing rate of cell number increase, and the rate only increases following bud burst (Fig. 4.1).

For the Controls, mean cell size between April 27 and June 22 increased about 6-fold. This is not as great as the 22-fold increase seen during early expansion of the 2nd leaf of sunflower (Sunderland, 1960), or the 15-fold increase for primary leaves of *Phaseolus vulgaris*, (Murray, 1968; Verbelen and De Greef, 1979), and is probably a reflection of the difference in final leaf morphology between a lamina angiosperm leaf and a gymnosperm needle. The increase in cell number between April 27 and June 22, is less than 4-fold; not quite equivalent to the magnitude of increase in cell size. It is usually accepted that the cell number changes during leaf expansion are greater than those in cell size, so that leaf size is determined mainly by cell number (Humphries and Wheeler, 1963). However, superficial interpretation of the present data suggests that cell expansion is at least as important as cell number in determining the final size of any particular needle. This interpretation requires modification for differences in needle size at different shoot positions. The relationship between needle cell number and fresh weight (Fig. 4.2) is important; the values included in Fig. 4.2 are for needles taken along the length of the expanding shoot, and since the needles extend in acropetal sequence, at different developmental stages. Yet despite this, for each sampling date the data fall on a straight line, with no indication of any strong curvature. This must mean that mean cell fresh weight, given by the slope of the line, is similar for all sizes of needle sampled; it follows that the distal needles which commence expansion later, nevertheless have cells of similar sizes to proximal needles which are larger and which commenced expansion earlier. In other words, cell enlargement appears to be related more to calendar time rather than to needle position. The Control data for May 4, which show a slight curvature, suggest that early in the expansion period distal needles may contain cells up to 40% smaller than in proximal needles. Data for the cell number:leaf weight relationship for the other treatments are

qualitatively similar to those of the Controls (Fig. 4.2). Because mean cell size is constant in needles at all positions for each treatment, the final size of the needle is a function of cell number irrespective of position on the shoot. In shoots of *Liquidambar* and *Picea*, variation in needle stem unit length has also been attributed to differences in pith and cortical cell numbers rather than to cell length (Lam and Brown, 1974; Baxter and Cannell, 1978). Considering the differences in cell size between treatments (Tables 4.5 and 4.6), which are not as great as the differences in needle cell number and needle length, also suggests that it is cell number which causes differences in final needle size between treatments. In the case of P deficiency, mean cell size is increased, whilst it is the reduction in needle cell number which is more important, causing the final size of the needle to be reduced. In so far as data are available, the differences in final needle cell number reflect differences in primordium cell number (Tables 4.1 and 4.3). Since the time course of cell division between treatments was similar, as already stated, it is likely that the potential for needle expansion under nutrient deficient conditions is determined by controls acting during primordium initiation.

The mean cell size in the -K treatment was significantly smaller than that of the Controls. This could be a result of the suggested role of potassium as an osmoticum for leaf expansion (Mengel and Kirby, 1982). Marschner (1986) reports results of Arneke showing also, that cell size in leaves of K-deficient beans was significantly lower than in Controls supplied with potassium. In contrast, increasing the supply of potassium to leaf discs of spinach increased cell size (Marschner and Possingham, 1975). Mean cell size for the -P treatment was larger than for the Controls; this is interesting since deficiency of phosphorus was found to inhibit cell expansion in cotton plants (Radin and Eidenbock, 1984). The central role of this element in plant nutrition makes it difficult to explain the observed effects. Deficiency may lead to accumulation of solutes in the expanding needles with consequent increase in turgor and cell expansion; alternatively deficiency might affect wall characters (Tomos, 1985; Dale, 1988) leading to greater cell wall extensibility or a lower wall yield threshold, thus allowing greater enlargement. The dry weight:fresh weight ratio was significantly higher for needles in the -P treatment and could indicate thicker cell walls. Such a change would be unlikely to increase wall extensibility, although the possibility that deficiency affects extracellular components, such as surface wax, which would not affect wall properties

cannot be ruled out. Interpretation of the effect of nutrient supply on growth must take into consideration effects on the phytohormone balance. Mineral deficiency has been shown to decrease the cytokinin content of the leaves (N, P and K deficiency in sunflower - Salama and Wareing, 1979; P and N deficiency in *Betula pendula* - Horgan and Wareing, 1980; N deficiency in pumpkin - Goring and Mardanov, 1976), and this may inhibit cell division and cell expansion.

Until April 15, needles at all positions showed similar increases in all variables measured, but subsequently the data show the same trends in needle development with position as following bud burst (see Chapter 3); It is the needles near the shoot base which have the fastest extension rates, and show the largest increase in cell number, with the exception of the most basal two or three needles. These are the widest of all needles, but begin to show reduced extension rates and rates of increase of cell number by April 7. The positional differences in needle dimensions and cell number become more pronounced with time, so that by bud burst, the differences in size and cell number for needles at different positions is already established. This shows that to some extent, final differences in size and cell number are due to different rates of change of the variables, more than due to different durations of growth, although the data in chapter 3 show that the most distal needles undergo more rapid development slightly later in the season than proximal needles. These pronounced ontogenetic differences due to needle position on the shoot therefore appear to be established very early in development. To some extent this is a result of the differences in size and cell number found in dormant primordia according to position, where the most distal primordia are those last to be formed, during the preceding winter, in unfavourable growth conditions, and they might be expected to show a lower growth potential. However, this does not explain the smaller size of the most proximal needles. These are the first needles to be initiated at the shoot apex, following the cessation of bud scale formation, and they may represent an intermediate organ between a bud scale and a needle. This is supported by the low length:width ratio for these needles, which is more a characteristic of bud scales. The control of the switch from bud scale to needle production is not well known, but may be largely hormonal, so that basal needles may retain some hormonal inhibition to development, associated with the control of bud scale development.

CHAPTER 5. RESULTS. PHOTOSYNTHETIC DEVELOPMENT.

5.1 Introduction.

The data in Chapters 3 and 4 describe needle development and the effect of nutrient deficiency in field and pot-grown trees, in terms of morphological variables, and for field material also in terms of cell number and size. The aim of this chapter is to present results on the development of photosynthetic capacity. This was mainly studied in pot material, but needle pigments were also measured in field material in 1987. It is also aimed to show whether treatment differences exist in net photosynthetic rate, or some of its main components; chlorophyll content, RuBPC activity, or stomatal conductance to CO_2. The following sections examine how each photosynthetic variable measured changes throughout the season, and the effect of nutrient deficiency or re-fertilisation treatments. The correlations between photosynthetic rate, chlorophyll content and RuBPC activity are then presented.

5.2 Leaf Pigments.

Pigment data have been expressed per unit fresh weight, consistent with most of the literature in general, and for Sitka spruce in particular. On a leaf developmental basis, expression of pigment content on a projected area basis is useful, but in conifers, needle projected areas are small, and the specific leaf weight is high. It was therefore considered justified to express pigment content on a fresh weight basis. Data for projected area was not available for needles used for pigment analysis in 1987, so in order for comparison of the 1988 data with those of 1987, these too were expressed per unit fresh weight. RuBPC activity has also been expressed per unit fresh weight, to be consistent with the chlorophyll data, but has also been expressed per unit chlorophyll, to give more meaning to changes in terms of photosynthetic capacity, instead of developmentally.

5.2.1 **Total Chlorophyll Content.**

5.2.1.1 1987 Experiment.

Total chlorophyll content in Control needles decreased between the first and second harvests, then increased steadily, from 0.2 mg g^{-1} fr.wt. on May 18, to a maximum of over 1.2 mg g^{-1} fr.wt. at the beginning of October (Fig. 5.1 A).

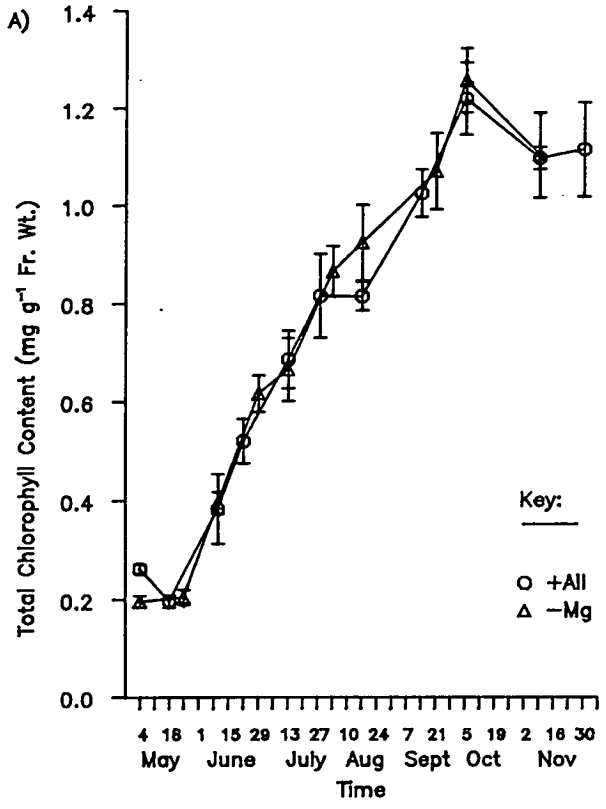
Two harvests in November gave slightly lower values, possibly indicating some breakdown, as reported during Winter by Lewandowska and Jarvis (1977) for Sitka spruce, and Linder (1972) for Scots pine and Norway spruce. Changes in chlorophyll content of needles in the -Mg set were virtually indistinguishable from the Controls throughout the season (Fig. 5.1 A). In the -P and -All treatments, needles showed similar rates of increase in chlorophyll content as the Controls, from early May to a maximum in October, although the similarity in the curves was less marked than for the -Mg set. For needles in -P and -All treatments (Fig. 5.1 C and D), a plateau in chlorophyll content was not reached, and it is not known whether levels continued to increase during December. Needles in the -P treatment had a significantly lower chlorophyll content than Controls until mid June, reflecting the delayed onset of needle development. After an initial rapid rise, needles from the -K treatment had significantly lower chlorophyll content than Controls during August and September (Fig. 5.1 B).

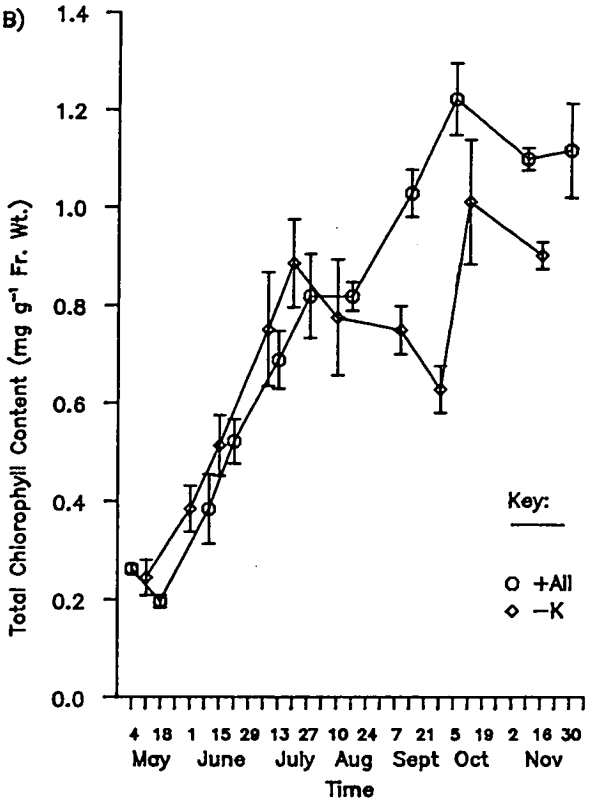
5.2.1.2 1988 Experiment.

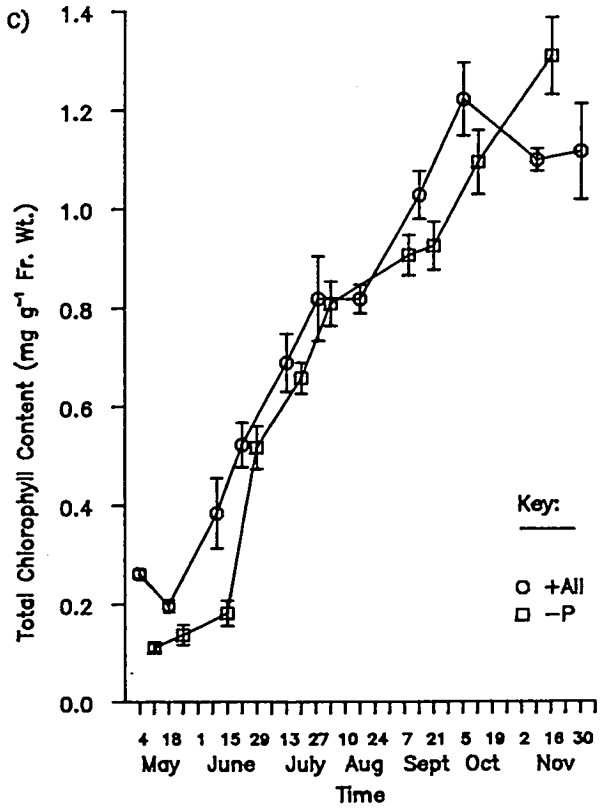
Total chlorophyll content (n=2) of Control needles in 1988 increased from 0.5 mg g^{-1} fr. wt. shortly after bud burst, to a maximum of about 2 mg g^{-1} fr. wt. at the end of October (Fig. 5.2 A) i.e. about 70% greater than comparable values from the field trees, with a faster rate of increase.

In all deficiency treatments, apart from -N,-All and possibly -K, total chlorophyll content increased steadily until the final harvest (Fig. 5.2 B to F). At the final sampling time, content in needles from the -Mg treatment was lower than the Controls, at about 1.25 mg g^{-1} fr.wt. Chlorophyll content in -N and -All needles increased to a maximum level of about 1 mg g^{-1} fresh weight in late August, and then decreased. The effect of refertilisation was small for the -K and -P sets, but three weeks after refertilisation on May 1, needles in -NR and -AllR treatments showed increased chlorophyll levels when compared with -N and -All needles (Fig. 5.2 E and F). This effect continued, and the difference became larger throughout the growth season, with refertilisation causing maximum levels to reach between 2.0 and 2.5 mg g^{-1} fr. wt. The effect of refertilisation on the -Mg set was much less spectacular.

Figure 5.1 A to D.
The change in total chlorophyll content for needles from terminal shoots from whorl 7 of field trees throughout the 1987 season, for each treatment. Each point is the mean ± S.E. of 4 to 6 determinations, each from different trees.


A)
Total Chlorophyll Content (mg g⁻¹ Fr. Wt.)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Key:
O +All
Δ −Mg
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time


B)
Total Chlorophyll Content (mg g⁻¹ Fr. Wt.)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Key:
O +All
◇ −K
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time


C)
Total Chlorophyll Content (mg g⁻¹ Fr. Wt.)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Key:
O +All
□ −P
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time

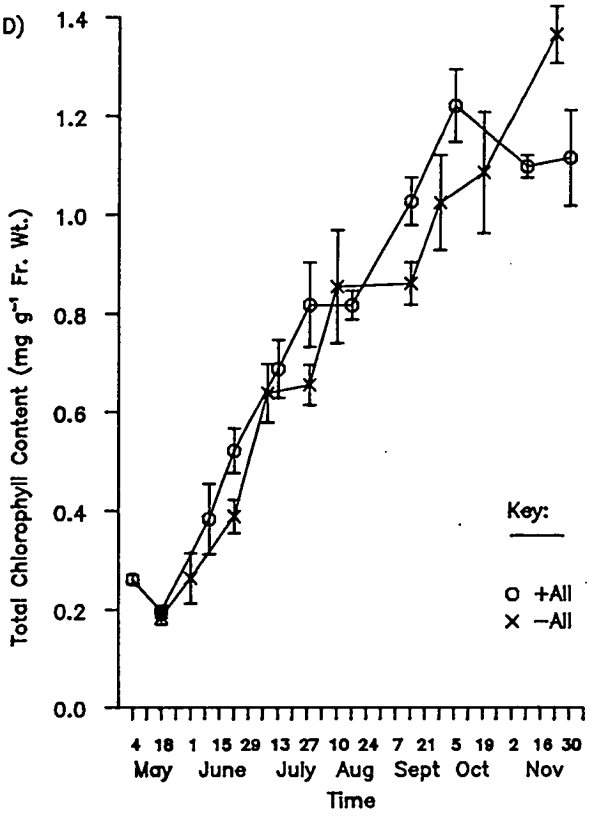
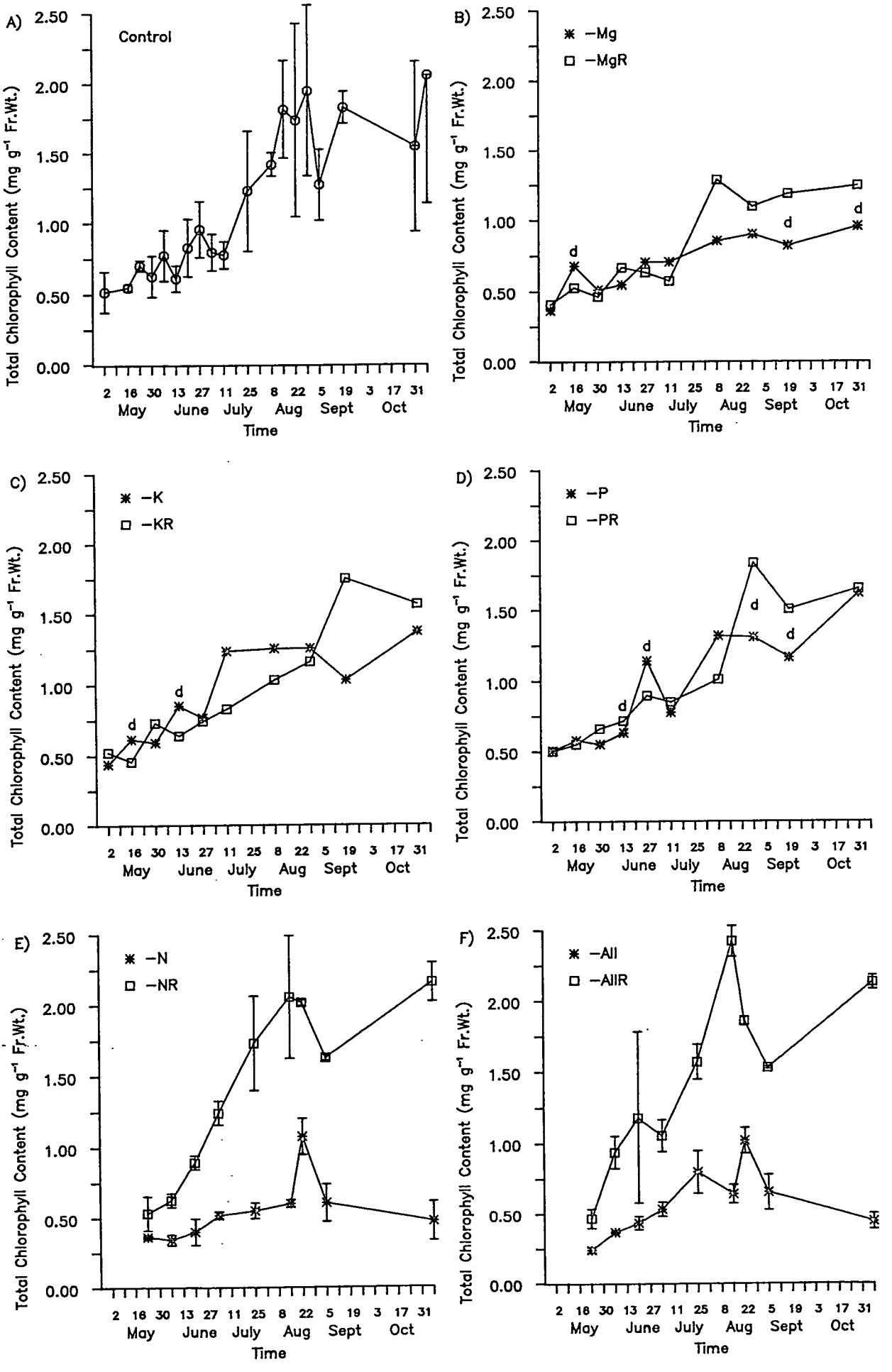

D)
Total Chlorophyll Content (mg g⁻¹ Fr. Wt.)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Key:
O +All
× −All
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time

Figure 5.2 A to F.

The change in total chlorophyll content for needles from terminal shoots from whorl 2 of trees from each pot treatment throughout the 1988 season. Each point is the mean of two determinations, each from different trees. For treatments apart from the Controls, closed symbols represent nutrient deficient trees, and open symbols respresent refertilised trees. Bars for some treatments represent ± the standard deviation, and the letter d for other treatments shows where standard deviation bars for data from deficient and refertilised treatments do not overlap at any one sample date.


A)
Control
B)
−Mg
−MgR
C)
−K
−KR
D)
−P
−PR
E)
−N
−NR
F)
−All
−AllR
Total Chlorophyll Content (mg g⁻¹ Fr.Wt.)
2.50
2.00
1.50
1.00
0.50
0.00
d
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

5.2.2 **Chlorophyll a/b Ratio.**

5.2.2.1 1987 Experiment.

The chlorophyll a/b ratio of Control needles increased rapidly following bud burst, nearly doubling between May 4 and May 18, and continuing to increase until mid June (Fig. 5.3 A). A summer maximum was maintained at a value of about three, which declined from early September until sampling ceased. The seasonal trend in the chlorophyll a/b ratio of needles in -Mg, -K and -All treatments was not significantly different to that of the Controls (Fig. 5.3 A, B and D), but for -P needles, the ratio was significantly lower than that of the Controls from mid June onwards (Fig. 5.3 C).

5.2.2.2 1988 Experiment.

The chlorophyll a/b ratio of Control needles was roughly constant throughout the whole of the season, varying between 2.0 and 3.0 (Fig. 5.4 A). No significant seasonal trend was seen either, in the ratio for needles for -Mg, -K and -P treatments, and values varied between about 1.75 and 3.00, with no effect of refertilisation (Fig. 5.4 B to F). In -N and -All treatments, needles had much reduced ratios from those of the Controls, but again, no seasonal trend was found. Refertilisation of these treatments caused a very rapid increase in the ratio, which increased until early July, and reached a plateau at a ratio more than twice as high as that of -N and -All needles, and slightly higher than that for all other treatments.

Figure 5.3 A to D.
The change in chlorophyll a/b ratio for needles from terminal shoots from whor; 7 of field trees throughout the 1987 season, for each treatment. Each point is the mean ± S.E. of 4 to 6 determinations, each from different trees.

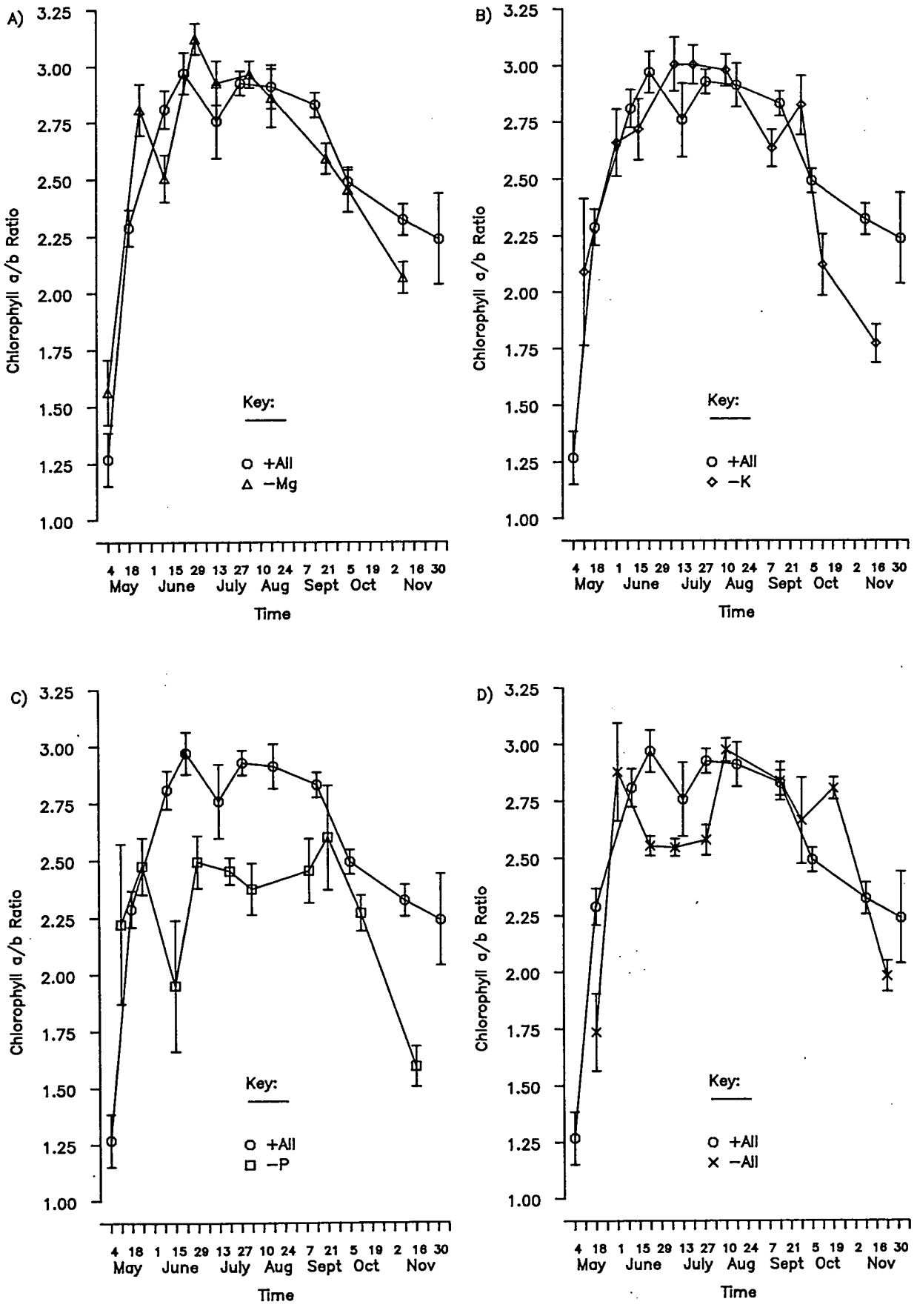
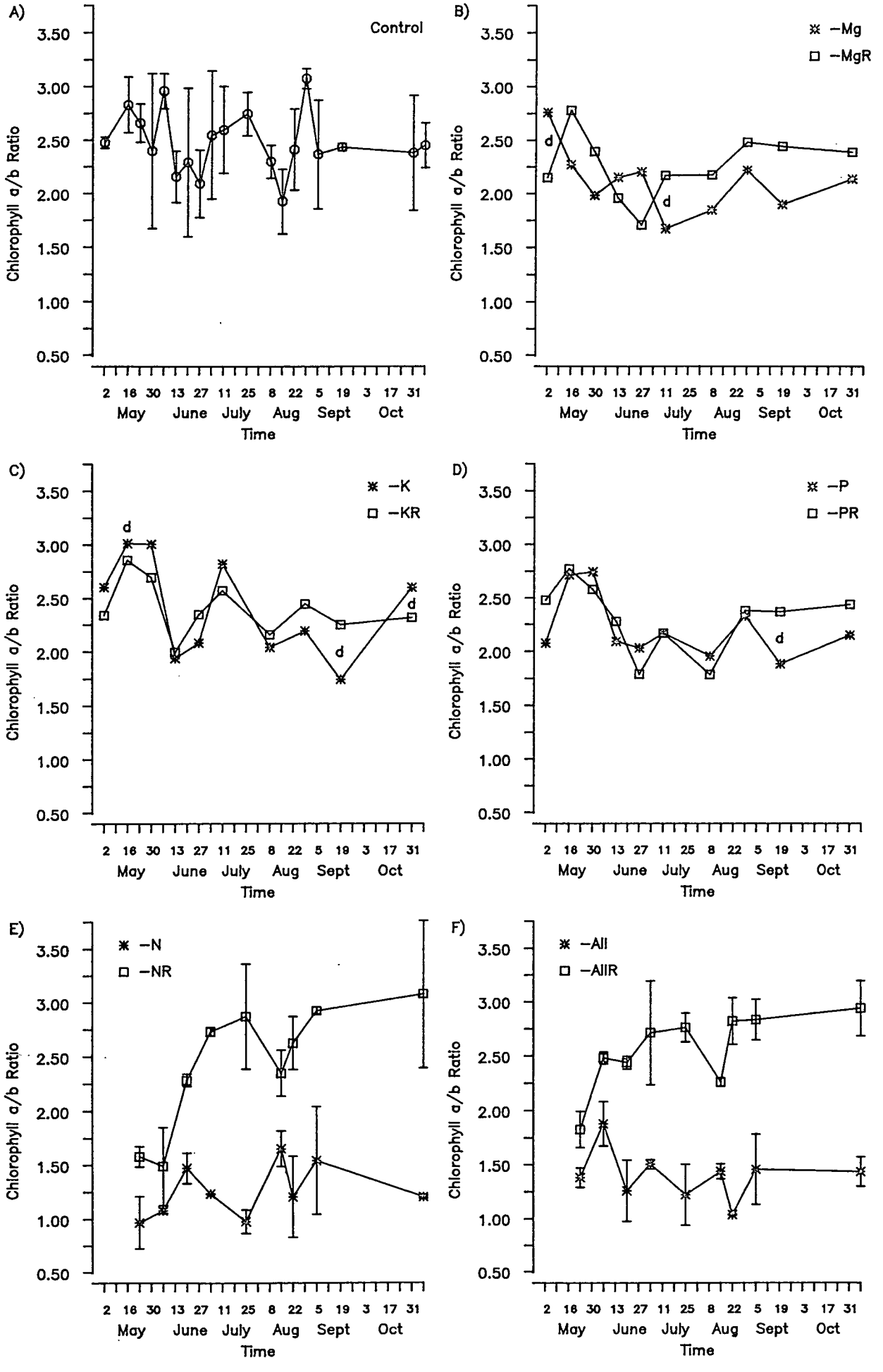

A)
B)
C)
D)
Chlorophyll a/b Ratio
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
Key:
+All
−Mg
−K
−P
−All
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time

Figure 5.4 A to F

The chlorophyll a/b ratio for needles from terminal shoots from whorl 2 of trees from each pot treatment throughout the 1988 season. Each point is the mean of two determinations, each from different trees. For treatments apart from the Control, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. Bars for some treatments represent ± the standard deviation, and the letter d for other treatments shows where standard deviation bars for data from deficient and refertilised treatments do not overlap at any one sample time.


A)
Control
B)
−Mg
−MgR
C)
−K
−KR
D)
−P
−PR
E)
−N
−NR
F)
−All
−AllR
Chlorophyll a/b Ratio
3.50
3.00
2.50
2.00
1.50
1.00
0.50
d
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

5.2.3 **Carotenoid Content.**

5.2.3.1 1987 Experiment.

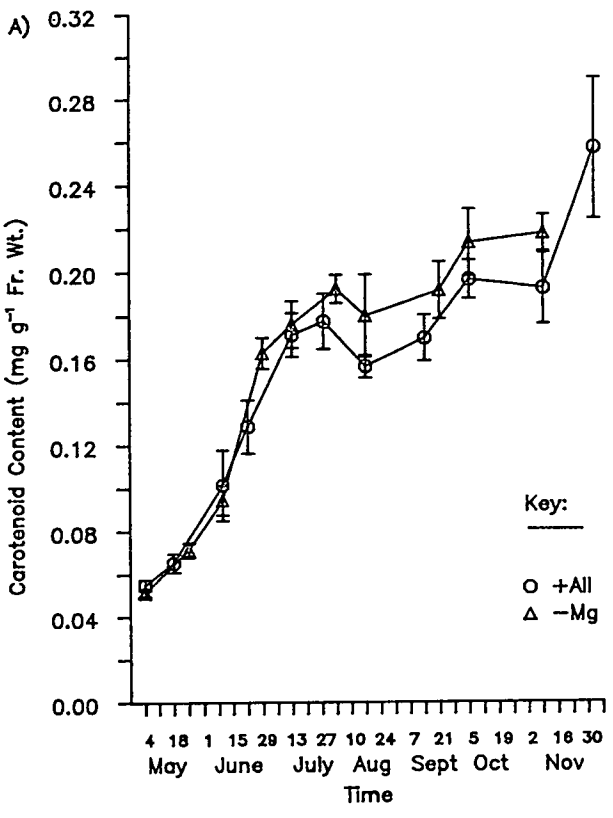
Carotenoid content of Control needles increased rapidly from 0.06 mg g^{-1} fr.wt. at the start of May to 0.18 mg g^{-1} fr.wt. at the end of July, and then increased more slowly until December, reaching a final value of 0.26 mg g^{-1} fr.wt. (Fig. 5.5 A). Carotenoid contents of needles in -Mg and -K treatments showed similar seasonal trends to those in the Control treatment (Fig. 5.5 A and B), but carotenoid content of -P and -All needles increased steadily to higher mean values than the Controls (Fig. 5.5 C and D), although the differences were not significant.

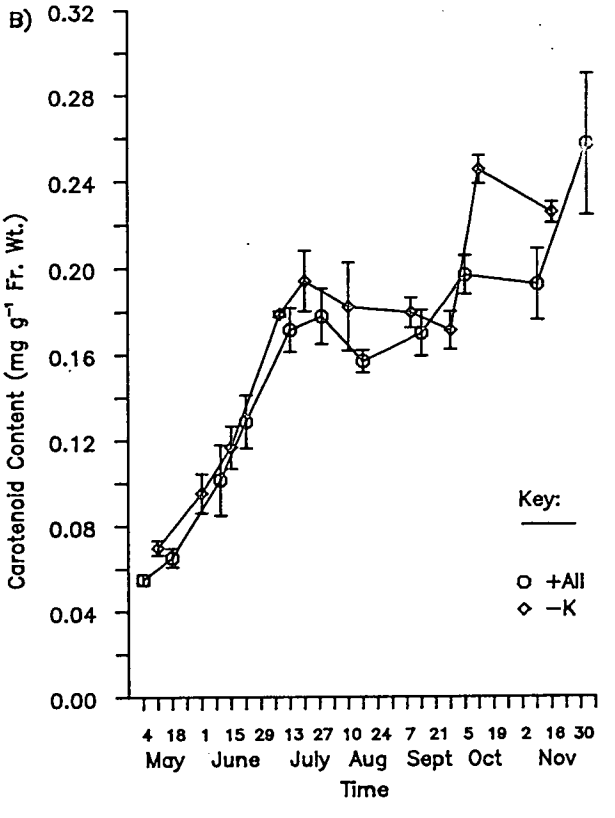
5.2.3.2 1988 Experiment.

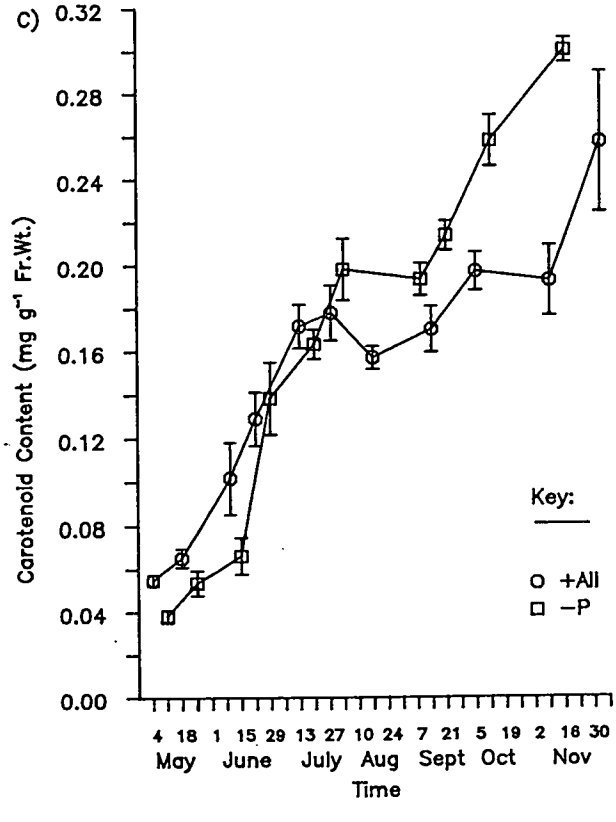
Carotenoid content of Control trees increased steadily from 0.13 mg g^{-1} fr.wt. at the start of May, until mid-August, when a plateau at about 0.4 mg g^{-1} fr.wt. was reached (Fig. 5.6 A). These values are much higher than for Control needles in the field in 1987 (Fig. 5.5). Needles in the deficiency treatments showed a similar change in carotenoid content as the Controls (Fig. 5.6 B to F), although in -KR needles, content increased until mid-September, and in -NR and -AllR needles, carotenoid content reached a peak in mid-August and then declined. The effect of refertilisation on carotenoid content in -Mg, -K and -P needles was not significant, but in the -NR and -AllR sets, values were higher than for -N and -All needles at all harvests, and the difference became larger throughout the season (Fig. 5.6 E and F). The carotenoid content of -Mg needles was equivalent to that of Control and -Mg needles in 1987, but for the other deficiency treatments, values were higher than for the same treatments in the field in 1987.

Figure 5.5 A to D

The carotenoid content of needles from terminal shoots from whorl 7 of field trees throughout the 1987 season, for each treatment. Each point is the mean ± S.E. of 4 to 6 determinations, each from different trees.


A)
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
Carotenoid Content (mg g⁻¹ Fr. Wt.)
Key:
O +All
Δ −Mg
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time


B)
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
Carotenoid Content (mg g⁻¹ Fr. Wt.)
Key:
O +All
◇ −K
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time


C)
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
Carotenoid Content (mg g⁻¹ Fr.Wt.)
Key:
O +All
□ −P
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time

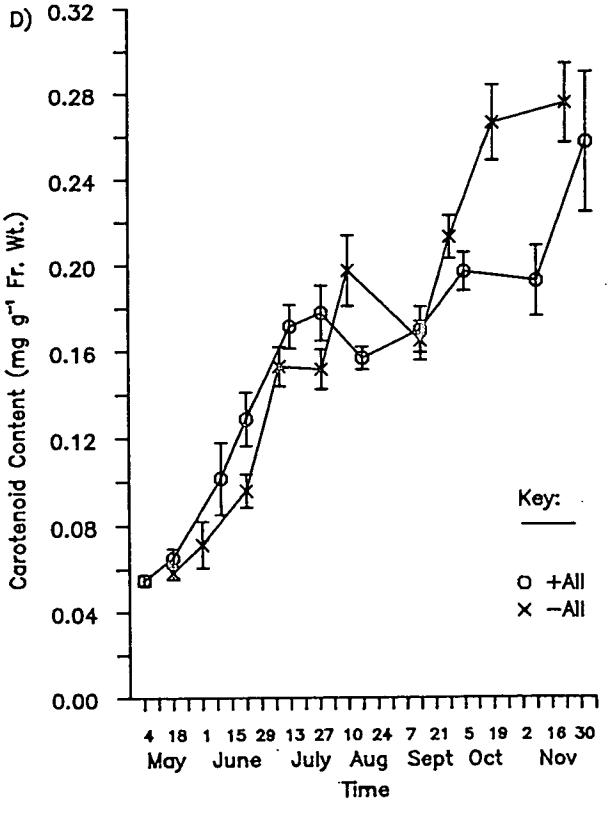
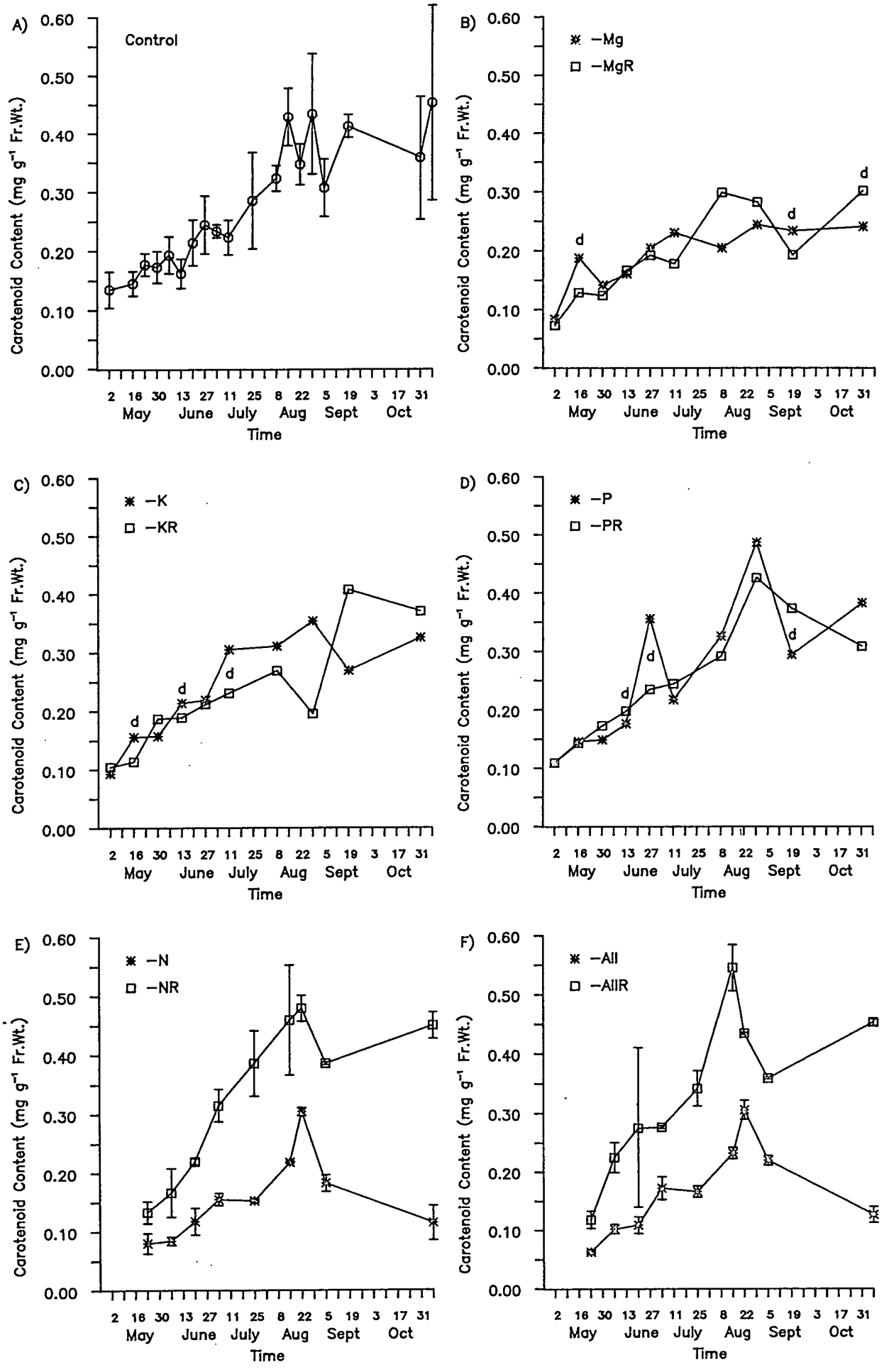

D)
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
Carotenoid Content (mg g⁻¹ Fr. Wt.)
Key:
O +All
× −All
4 18 1 15 29 13 27 10 24 7 21 5 19 2 16 30
May June July Aug Sept Oct Nov
Time

Figure 5.6 A to F

The carotenoid content of needles from terminal shoots from whorl 2 of trees from each pot treatment throughout the 1988 season. Each point is the mean of two determinations, each from different trees. For treatments apart from the Control, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. Bars for some treatments represent ± the standard deviation, and the letter d for other treatments shows where standard deviation bars for data from deficient and refertilised trees do not overlap at any one sample time.


A)
Control
B)
−Mg
−MgR
C)
−K
−KR
D)
−P
−PR
E)
−N
−NR
F)
−All
−AllR
Carotenoid Content (mg g⁻¹ Fr.Wt.)
0.00
0.10
0.20
0.30
0.40
0.50
0.60
d
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

5.3 RuBPC Activity.

5.3.1 Field Material 1987.

Following the experiments to check the conditions and concentrations used in the RuBPC assay procedure (see Materials and Methods, section 2.10), RuBPC activity was measured in needles from Control, -K and -P treatments in the field, in January 1988. This was in order to consolidate the technique as a prelude to applying it to pot trees throughout the 1988 growth season, and also to see whether treatment differences in RuBPC activity existed in field material. Only -P and -K plots were sampled, as it was thought these would show greatest treatment effects, as -P needles had shown the most extreme morphological effects in 1987, and -K needles had the lowest chlorophyll content of the treatments studied.

Table 5.1 Chlorophyll content, and RuBPC activity expressed per unit chlorophyll, per g fresh weight and per g dry weight, for needles from Control, -K and -P treatments in the field, sampled January 1988. Values are means ± S.D. (n=2).

Treatment	Total Chl Content	RuBPC activity		
	mg g^{-1} fr.wt.	µmol CO_2 g^{-1} fr.wt. min^{-1}	µmol CO_2 g^{-1} dry wt. min^{-1}	µmol CO_2 mg^{-1} Chl. min^{-1}.
Control	1.12 ± 0.003	6.65 ± 0.93	17.11 ± 2.44	5.95 ± 0.82
-K	0.69 ± 0.13	3.40 ± 0.81	8.39 ± 1.550	5.10 ± 2.12
-P	0.97 ± 0.06	1.82 ± 0.15	4.42 ± 0.550	1.88 ± 0.26

RuBPC activity, expressed per g. fr.wt. and per g. dry wt. was reduced in the -K treatment, and still more in the -P treatment (Table 5.1). However, needle chlorophyll content was not correlated with RuBPC activity, and when expressed per mg Chl., RuBP activity in needles from the -K plot showed no difference from the Controls, although the reduction in the -P plot was much greater.

5.3.2 **1988 Experiment.**

Sampling of treatments began at the beginning of May, at the start of 1988 needle growth, and in Control trees the seasonal trend for RuBPC activity followed a sigmoid curve, reaching a plateau in late September (Fig. 5.7 A). In -NR and -AllR treatments, activity increased from early June, as in the case of the Controls (Fig. 5.7 E and F); for the other treatments the rise in activity occurred 3-4 weeks later (Fig. 5.7 B to D). Maximum RuBPC activity in the refertilised sets was between 7.0 and 8.0 µmol CO_2 s^{-1} g^{-1} fr.wt., and was achieved between mid-August (-MgR) and mid-September (-PR). The maximum activity in all refertilised treatments was not different to that of the Controls. The peak in activity was followed by a sharp decrease in RuBPC activity, consistent with the autumn decline found in 1 and 2 year old needles of *Pinus sylvestris* by Gezelius and Hallen (1980). This contrasts with the plateau seen in the Controls, although if later harvests had been made a decrease may have been seen. With the exception of the -K treatment, the deficiency treatments showed much lower RuBPC activities than the refertilised treatments, especially -N and -All treatments, and the highest activities were again found in late summer/early autumn. For the -K treatment, maximum activity was only slightly less than in the -KR set. Comparing the maximum activities from Control, -K and -P treatments with the values obtained from the field (Table 5.1), the ranking with respect to treatment is the same, and the values are similar, even though the field material was assayed in the winter, possibly indicating higher values in the field in summer.

When RuBPC activity is expressed per mg chlorophyll, all deficiency treatments except -Mg and -K showed lower activities than the respective refertilised treatments (Fig. 5.8 B to F). The maximum value of RuBPC activity in all refertilised treatments was not different to that of the Controls, and was between 4.0 and 5.0 µmol CO_2 mg^{-1} Chl.

Figure 5.7 A to F

Activity of RuBPC per g fresh weight, in needles from terminal shoots from whorl 2 of trees from each pot treatment throughout the 1988 season. Each point is the mean of two determinations, each from different trees. For treatments apart from the Control, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. Bars for some treatments represent ± the standard deviation, and the letter d for other treatments show where standard deviation bars for data from deficient and refertilised treatments do not overlap at any one sample time.

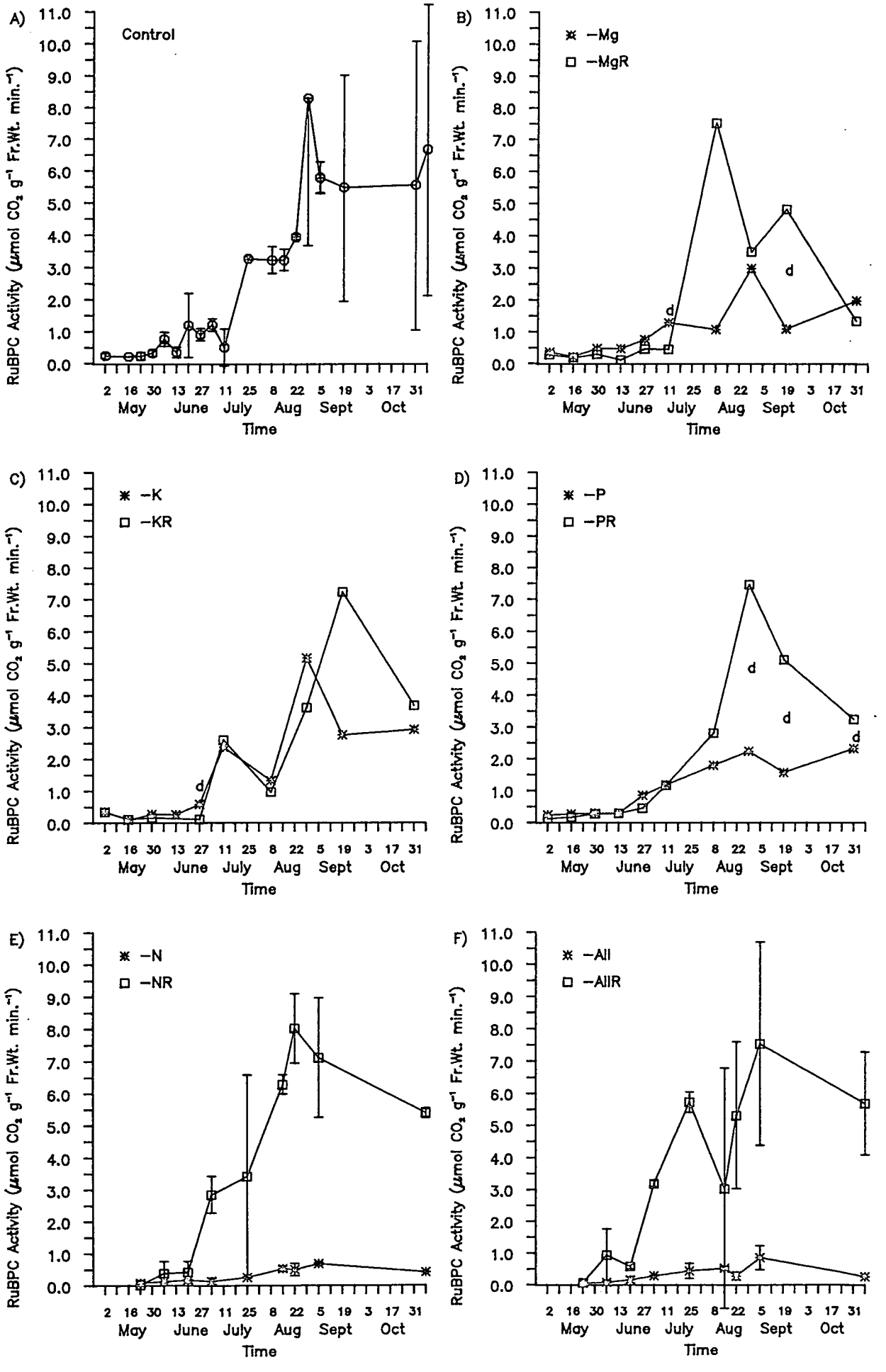
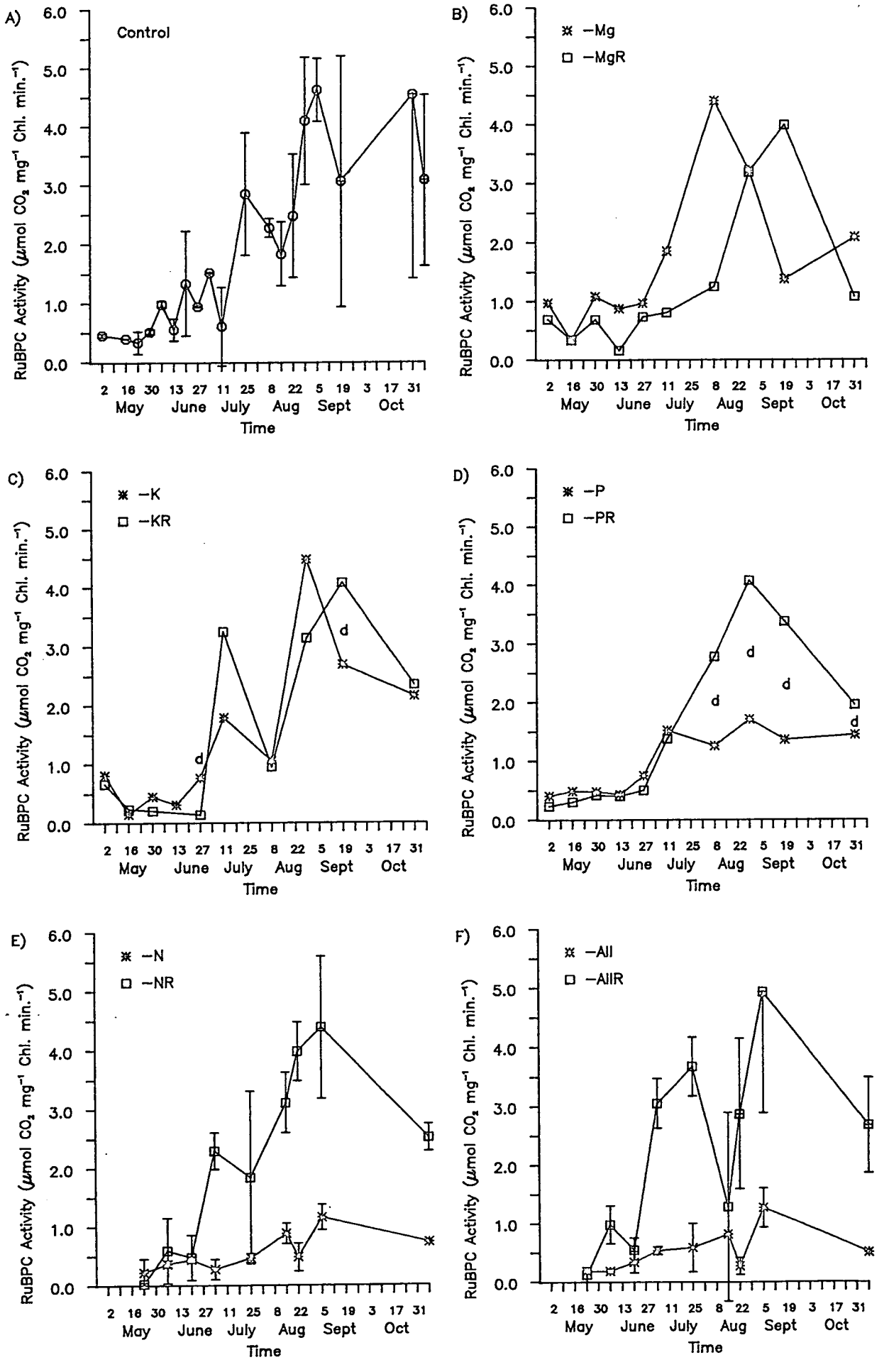

A)
Control
B)
−Mg
−MgR
C)
−K
−KR
D)
−P
−PR
E)
−N
−NR
F)
−All
−AllR
RuBPC Activity (μmol CO2 g-1 Fr.Wt. min.-1)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
d
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

Figure 5.8 A to F
The data in Fig. 5.7 expressed per unit chlorophyll. All symbols and details as for Fig. 5.7.


A)
Control
B)
−Mg
−MgR
C)
−K
−KR
D)
−P
−PR
E)
−N
−NR
F)
−All
−AllR
RuBPC Activity (μmol CO2 mg-1 Chl. min.-1)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time
d

5.4 Net Photosynthetic Rate, 1988.

For all treatments, net photosynthetic rate increased with time to a maximum in August, and then declined. Second or third order polynomial functions have been fitted to the data and all show values of r^2 of at least 0.53 (see overleaf). In Control shoots, P_N increased from about 1.5 µmol CO_2 m^{-2} s^{-1} at bud burst, to a maximum of about 7.0 µmol CO_2 m^{-2} s^{-1} in July and August (Fig. 5.9 A), and then decreased slightly by the end of November. All other treatments showed an increase in P_N from bud burst, to a summer maximum, followed by a decrease at the beginning of September (Fig. 5.9 B to F). Maximum mean values of P_N in shoots from -Mg, -MgR, -K, -KR, -P and -PR treatments were of the order of 5.0 to 7.0 µg CO_2 m^{-2} s^{-1}, and there were only slight differences in P_N between deficient and refertilised shoots of these treatments. However, maximum values of P_N in shoots from -AllR and -NR treatments were higher than for Controls, at about 9.0 µg CO_2 m^{-2} s^{-1}, and values for -All and -N shoots were lower than this at all harvests, apart from a peak in September. The maximum values of P_N were calculated from the fitted curves, and are compared with the actual maximum values (Table 5.2).

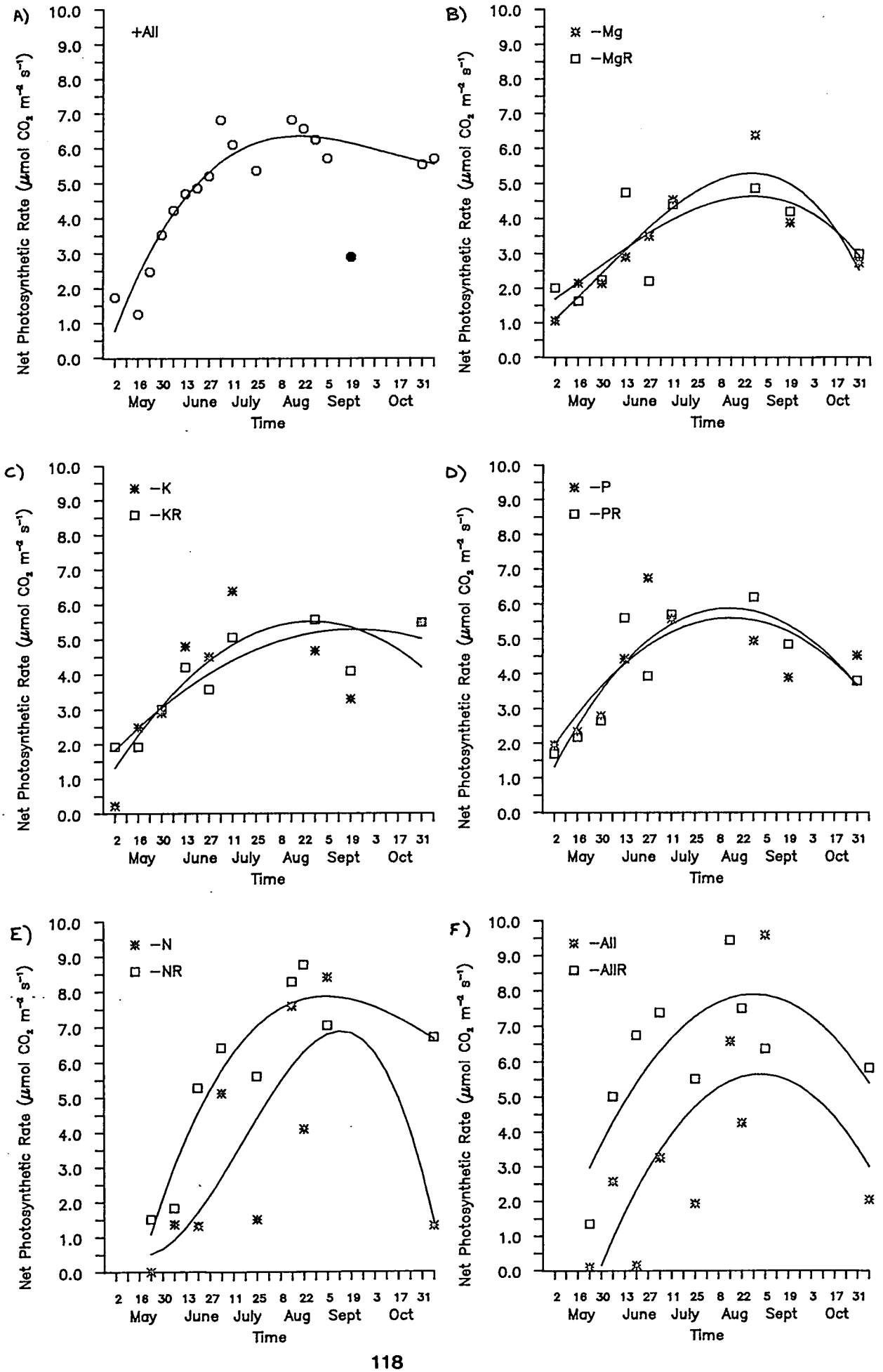
Table 5.2 A comparison of the maximum values of P_N (µmol CO_2 m^{-2} s^{-1}) from the fitted curves in Fig. 5.9 A to F, with the maximum actual values measured.

Treatment	Maximum Calculated Value	Maximum Actual Value
+All	6.36	6.83
-Mg	5.28	6.30
-MgR	4.63	4.85
-K	5.53	6.39
-KR	5.29	5.57
-P	5.60	6.74
-PR	5.88	6.19
-N	6.89	8.41
-NR	7.87	8.76
-All	5.66	9.59
-AllR	7.90	9.44

Figure 5.9 A to F

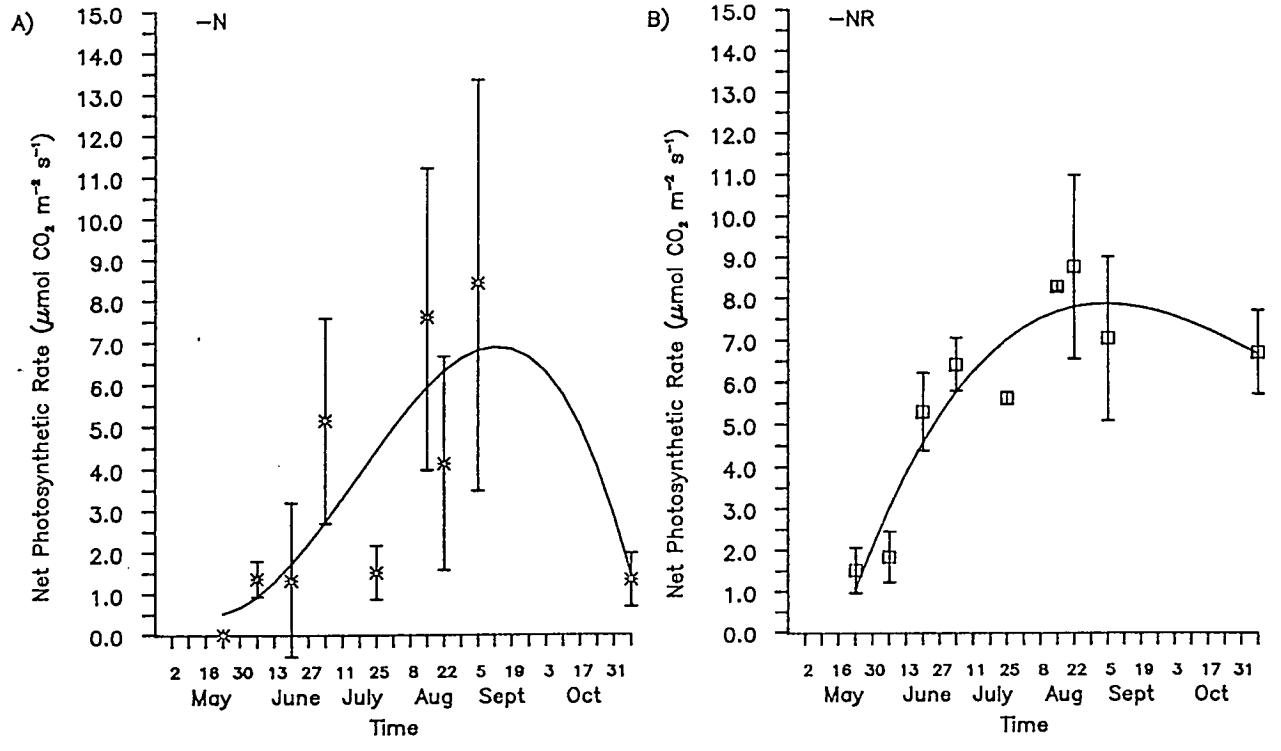
Net photosynthetic rate for terminal whorl 2 shoots from each pot treatment throughout the 1988 season. Each point is the mean of two determinations, each from different trees. For treatments apart from the Control, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. Equations of the fitted polynomial functions are shown below. For the For the Control data, a curve has been fitted excluding the data for Sept 19 (shown by a filled circle).

Treatment	**Equation**	r^2
Control	$y = -0.143 + 0.955\ x - 0.0443\ x^2 + 0.000623\ x^3$	0.89
-Mg	$y = 0.782 + 0.300\ x + 0.00913\ x^2 - 0.000661\ x^3$	0.85
-MgR	$y = 1.441 + 0.236\ x + 0.00351\ x^2 - 0.000378\ x^3$	0.60
-K	$y = 0.785 + 0.536\ x - 0.0152\ x^2$	0.59
-KR	$y = 1.464 = 0.359\ x - 0.00843\ x^2$	0.80
-P	$y = 1.424 + 0.520\ x - 0.0162\ x^2$	0.60
-PR	$y = 0.643 + 0.687\ x - 0.0242\ x^2$	0.80
-N	$y = 1.277 - 0.523\ x + 0.0933\ x^2 - 0.00266\ x^3$	0.66
-NR	$y = -4.010 + 1.497\ x - 0.0587\ x^2 + 0.000672\ x^3$	0.88
-All	$y = -4.652 + 1.110\ x - 0.030\ x^2$	0.53
-AllR	$y = -0.259 + 0.905\ x\ \ 0.0251\ x^2$	0.63


A)
+All
B)
−Mg
−MgR
C)
−K
−KR
D)
−P
−PR
E)
−N
−NR
F)
−All
−AllR
Net Photosynthetic Rate (μmol CO₂ m⁻² s⁻¹)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

Maximum values from the fitted curves are similar to actual maxima, except for -N and -All treatments. In these treatments, although the mean of the values is lower than for -NR and -AllR treatments, some values are exceptionally high. The standard deviations of the data in -N and -All treatments are much higher than those in -NR and -AllR treatments (see Fig. 5.10 A and B, for a comparison of -N with -NR). The high values of P_N observed in -N and -All treatments must be treated with caution; shoots from these treatments had very small projected areas, often < 1 cm^2, and < 5% of the area of -NR and -AllR shoots, and the IRGA readings were also very small, often 1 v.p.m. or less. Therefore, a small error in the analysis CO_2 concentration, due to a drifted zero calibration, or the usual fluctuation in the reading, would give an enormous error when the values are expressed on a m^2 basis. It is suggested that the low values in -N and -All treatments represent times at which the IRGA was newly calibrated, and high values represent times where the calibration had drifted, since the data points are alternately high and low on a monthly cycle, corresponding to when the IRGA was calibrated.

Figure 5.10 A and B
Data from Fig. 5.9 E replotted with ± S.D. for the two determinations.



5.5 Resistance Pathway to CO_2, 1988.

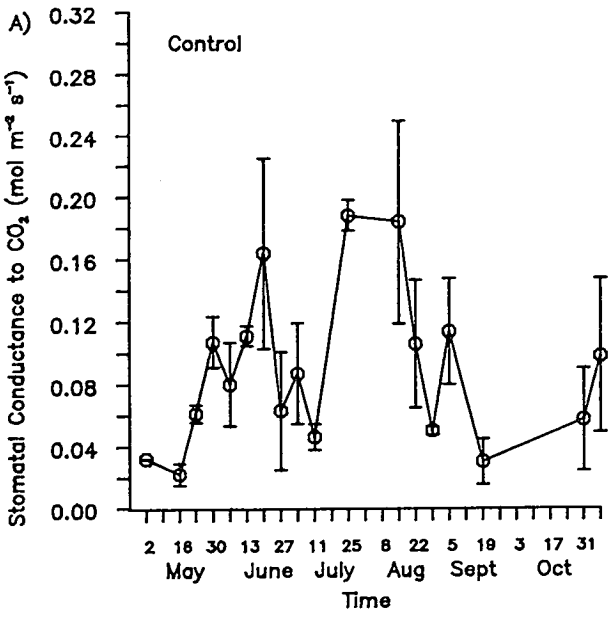
5.5.1 Stomatal Conductance to CO_2 Transfer.

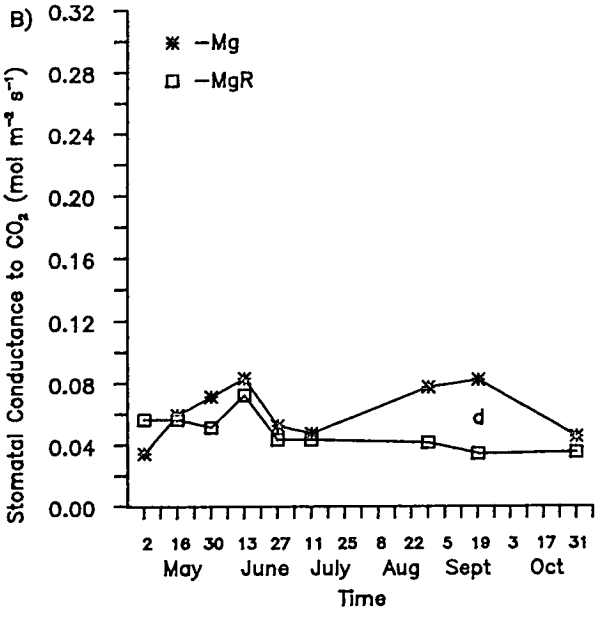
For the Controls, the stomatal conductance increased as the shoot extended, reaching a peak at the end of July and the beginning of August (Fig. 5.11 A). Maximum mean values were 0.19 mol m^2 s^{-1} (equivalent to 2.16 cm s^{-1}). From August the stomatal conductance declined until sampling ceased. In -Mg, -K and -P treatments, and the respective refertilised sets, the change in G_S with time was not as great as in the Controls (Fig. 5.11 B to F). Values remained fairly constant throughout the season although these treatments showed a rise in G_S in May and early June, during the period of most rapid needle extension. Data from the -K set showed a slight peak in G_S at the end of August. Refertilisation of -Mg, -K and -P trees had no effect on G_S, but refertilisation of -N and -All trees caused G_S to increase rapidly and linearly, from early June until November (Fig. 5.11 E and F). Final values in -NR and -AllR sets were between 0.20 and 0.31 mol m^2 s^{-1}, and were much higher than the Control values at the same time. Stomatal conductance in -N and -All treatments remained low throughout the whole season.

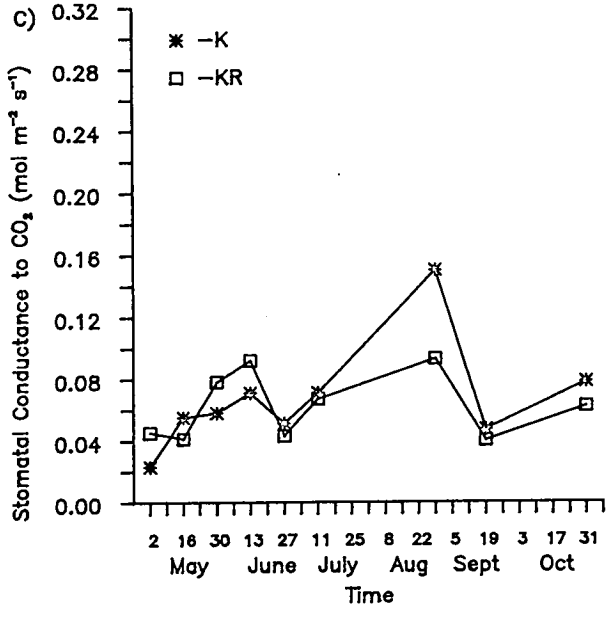
5.5.2 Intercellular Partial Pressure of CO_2.

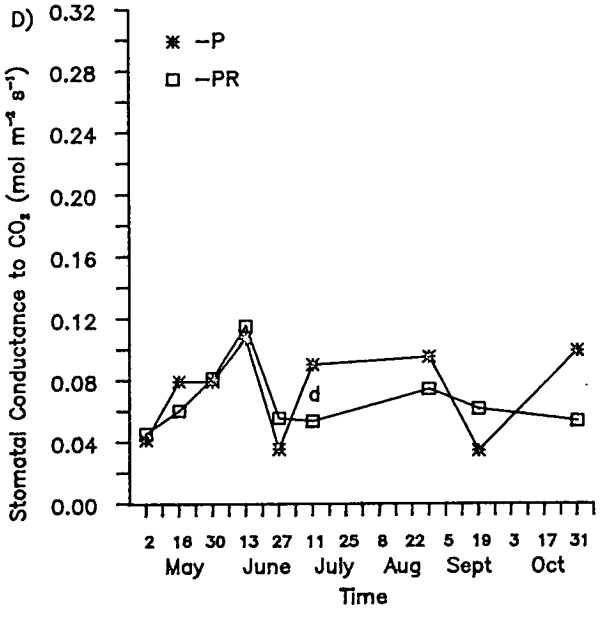
No treatment showed a significant difference in C_i from the Controls throughout the season (Fig. 5.12 A to F), although -N and -All treatments had slightly higher values than the other treatments (Fig. 5.12 E and F). Values ranged from 207 µbar to 380 µbar. The difference in C_i between -N and -NR treatments was greater than for other treatments, where there was no effect of refertilisation.

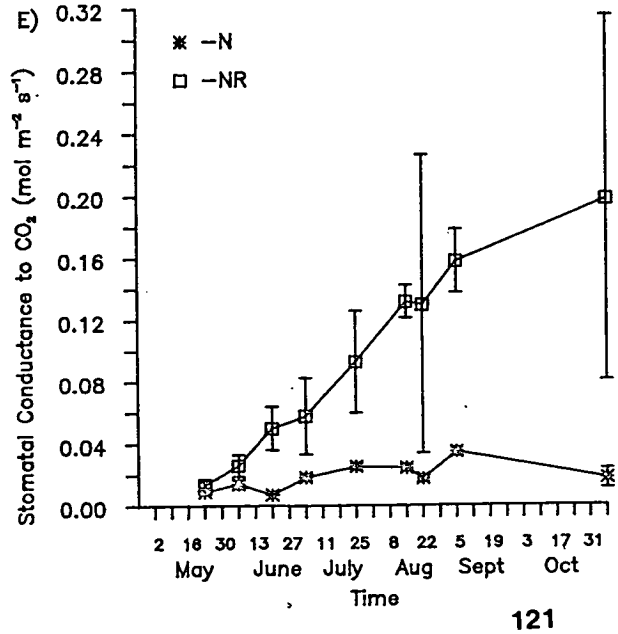
Figure 5.11 A to F.
Stomatal conductance to CO_2 for terminal whorl 2 shoots from each pot treatment throughout the 1988 season. Values are means for two determinations, each on different trees. For treatments apart from the Control, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. Bars for some treatments represent ± the standard deviation, and the letter d for other treatments shows where standard deviation bars for data from deficient and refertilised trees do not overlap at any one sample time.


A)
Control
Stomatal Conductance to CO_2 (mol m^{-2} s^{-1})
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


B)
−Mg
−MgR
d
Stomatal Conductance to CO_2 (mol m^{-2} s^{-1})
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


C)
−K
−KR
Stomatal Conductance to CO_2 (mol m^{-2} s^{-1})
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


D)
−P
−PR
d
Stomatal Conductance to CO_2 (mol m^{-2} s^{-1})
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


E)
−N
−NR
Stomatal Conductance to CO_2 (mol m^{-2} s^{-1})
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

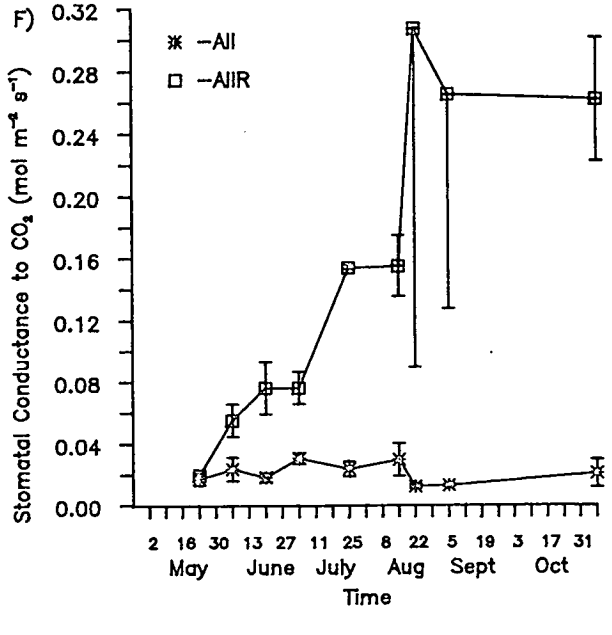
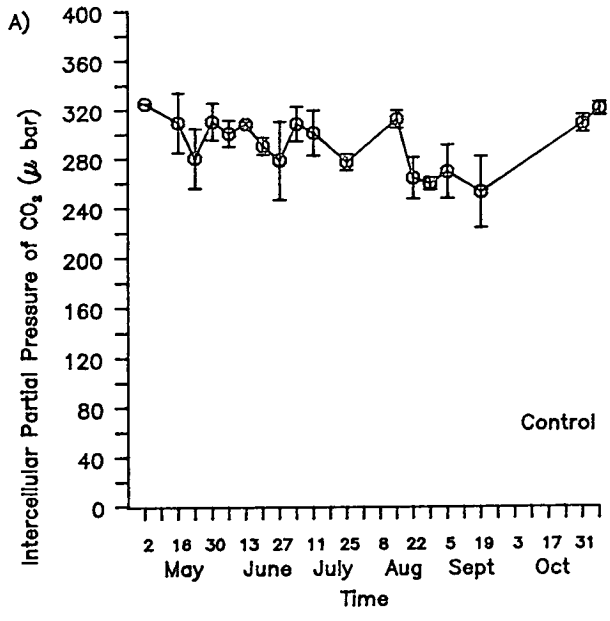
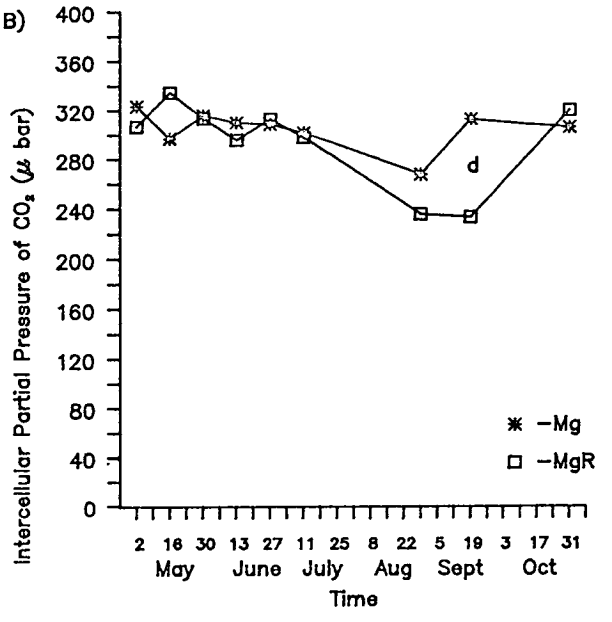
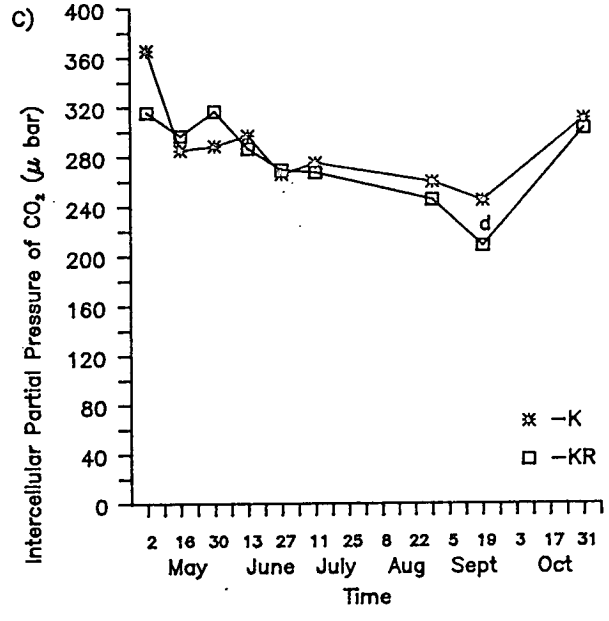

F)
−All
−AllR
Stomatal Conductance to CO_2 (mol m^{-2} s^{-1})
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

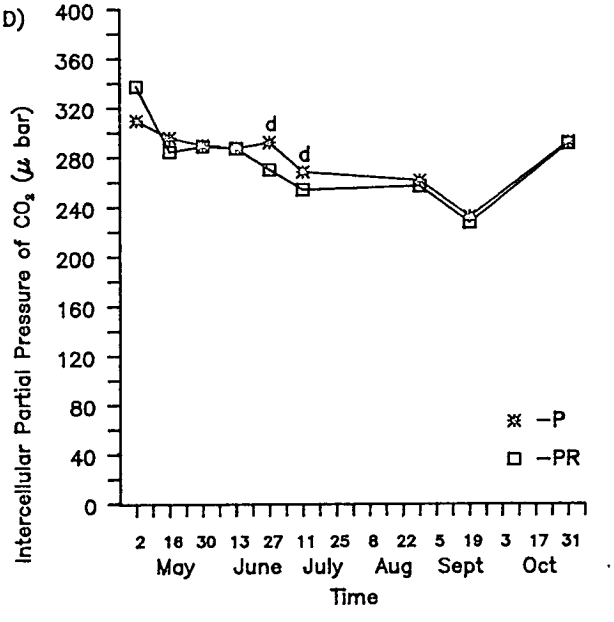
Figure 5.12

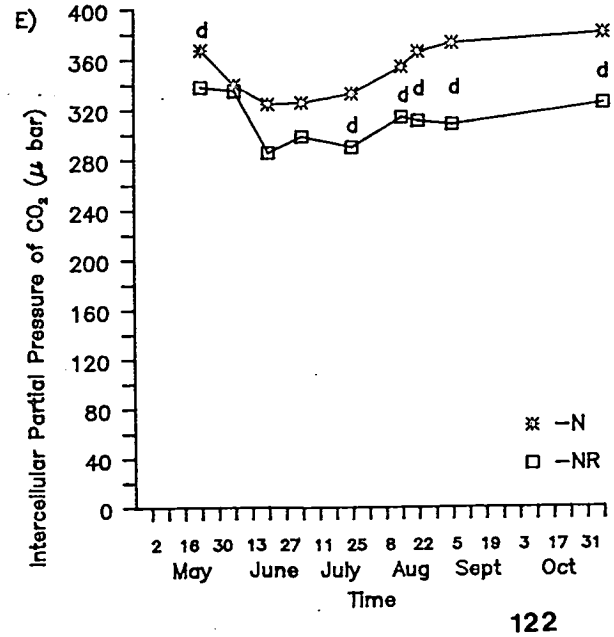
The intercellular partial pressure of CO_2 measured on terminal whorl 2 shoots from each pot treatment throughout the 1988 season. Each point is the mean of two determinations, each on different trees. For treatments apart from the Control, closed symbols represent nutrient deficient trees, and open symbols represent refertilised trees. Bars for some treatments represent ± the standard deviation, and the letter d for other treatments shows where standard deviation bars for data from deficient and refertilised treatments do not overlap at any one sample time.

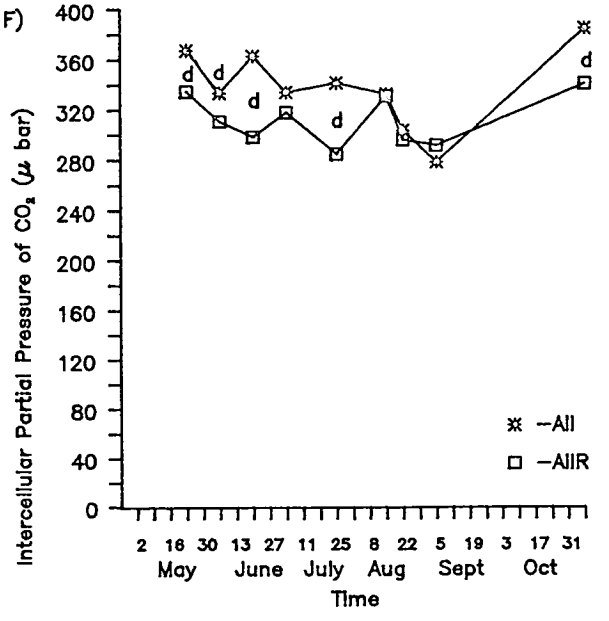

A)
Intercellular Partial Pressure of CO_2 (μ bar)
400
360
320
280
240
200
160
120
80
40
0
Control
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


B)
Intercellular Partial Pressure of CO_2 (μ bar)
400
360
320
280
240
200
160
120
80
40
0
d
−Mg
−MgR
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


C)
Intercellular Partial Pressure of CO_2 (μ bar)
400
360
320
280
240
200
160
120
80
40
0
d
−K
−KR
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


D)
Intercellular Partial Pressure of CO_2 (μ bar)
400
360
320
280
240
200
160
120
80
40
0
d
d
−P
−PR
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


E)
Intercellular Partial Pressure of CO_2 (μ bar)
400
360
320
280
240
200
160
120
80
40
0
d
d
d
d
d
d
d
−N
−NR
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time


F)
Intercellular Partial Pressure of CO_2 (μ bar)
400
360
320
280
240
200
160
120
80
40
0
d
d
d
d
d
−All
−AllR
2 16 30 13 27 11 25 8 22 5 19 3 17 31
May June July Aug Sept Oct
Time

5.6 Correlations Between Photosynthetic Variables, 1988.

5.6.1 Correlation Between Photosynthetic Rate and Chlorophyll Content.

Net photosynthetic rate was significantly correlated with total chlorophyll content (Fig. 5.13 for Control data), with the exception of three treatments (Table 5.3), although the highest value of r only reached 0.76, and the lowest, 0.19. Considering the relationship between P_N and chlorophyll a only gave higher values of r in -N and -All treatments, and effects on the coefficient in other treatments were small. The photosynthetic efficiency (P_N per unit chlorophyll) for the Control data inceased with increasing chlorophyll content for the first part of the season, and then declined at higher chlorophyll concentrations (Fig. 5.14).

Table 5.3 Correlation coefficients for the linear regression between P_N, expressed as mg CO_2 m^{-2} s^{-1}, and total chlorophyll or chlorophyll a content, expressed as mg m^{-2} projected area for all treatments. Values are for samples all samples between May and November. n=38 for Controls, and n=18 for all other treatments.

Treatment	Correlation Coefficient r	
	P_N and Total Chlorophyll	P_N and Chlorophyll a
+All	0.44 **	0.47 **
-Mg	0.46	0.49 *
-MgR	0.28	0.28
-K	0.76 ***	0.76 ***
-KR	0.62 **	0.62 **
-P	0.52 *	0.51 *
-PR	0.54 *	0.52 *
-N	0.19	0.29
-NR	0.68 **	0.70 **
-All	0.55 *	0.60 **
-AllR	0.55 *	0.56 *

Figure 5.13

The relationship between net photosynthetic rate and total chlorophyll content for Control pot trees, throughout the 1988 season. Each point represents one measurement for each variable, from one tree, for all sample times.

Figure 5.14

The relationship between photosynthetic efficiency (P_N per unit chlorophyll), and total chlorophyll content, for Control pot trees throughout the 1988 season. Each point represents data for one tree, for all sample times. Circles are for data to July 11 inclusive, and crosses represent subsequent data until early November.

Figure 5.13

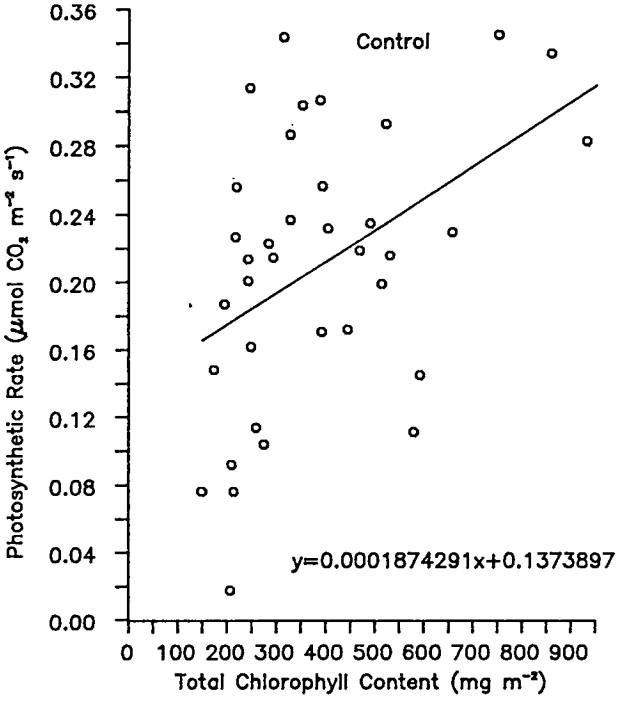


Figure 5.14

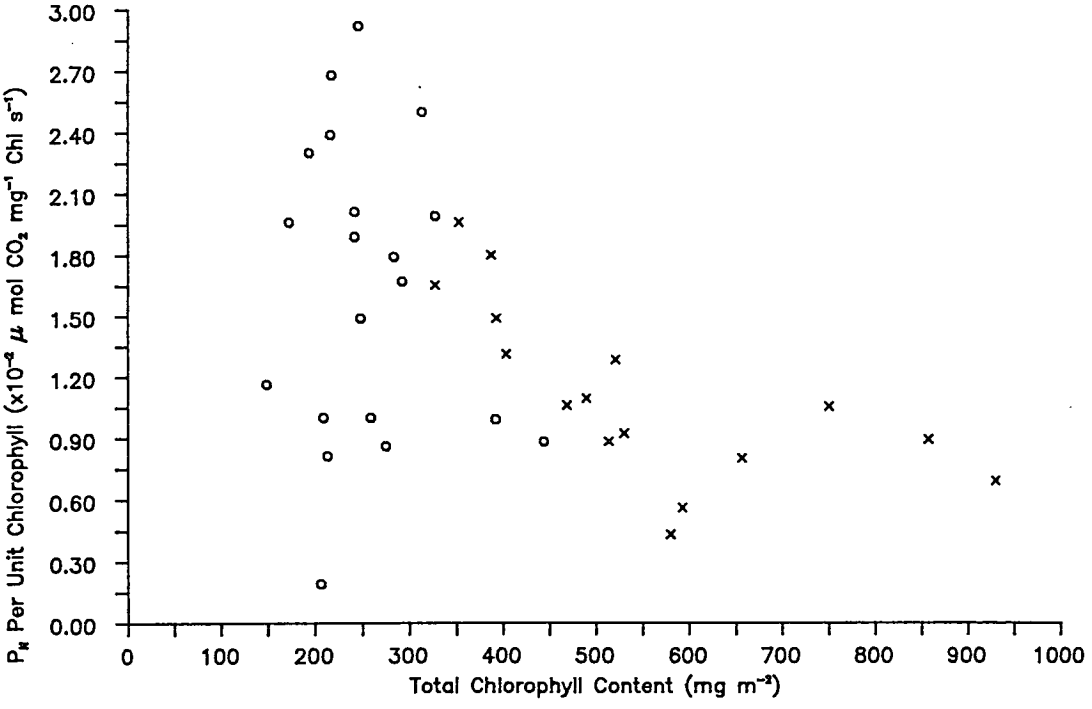
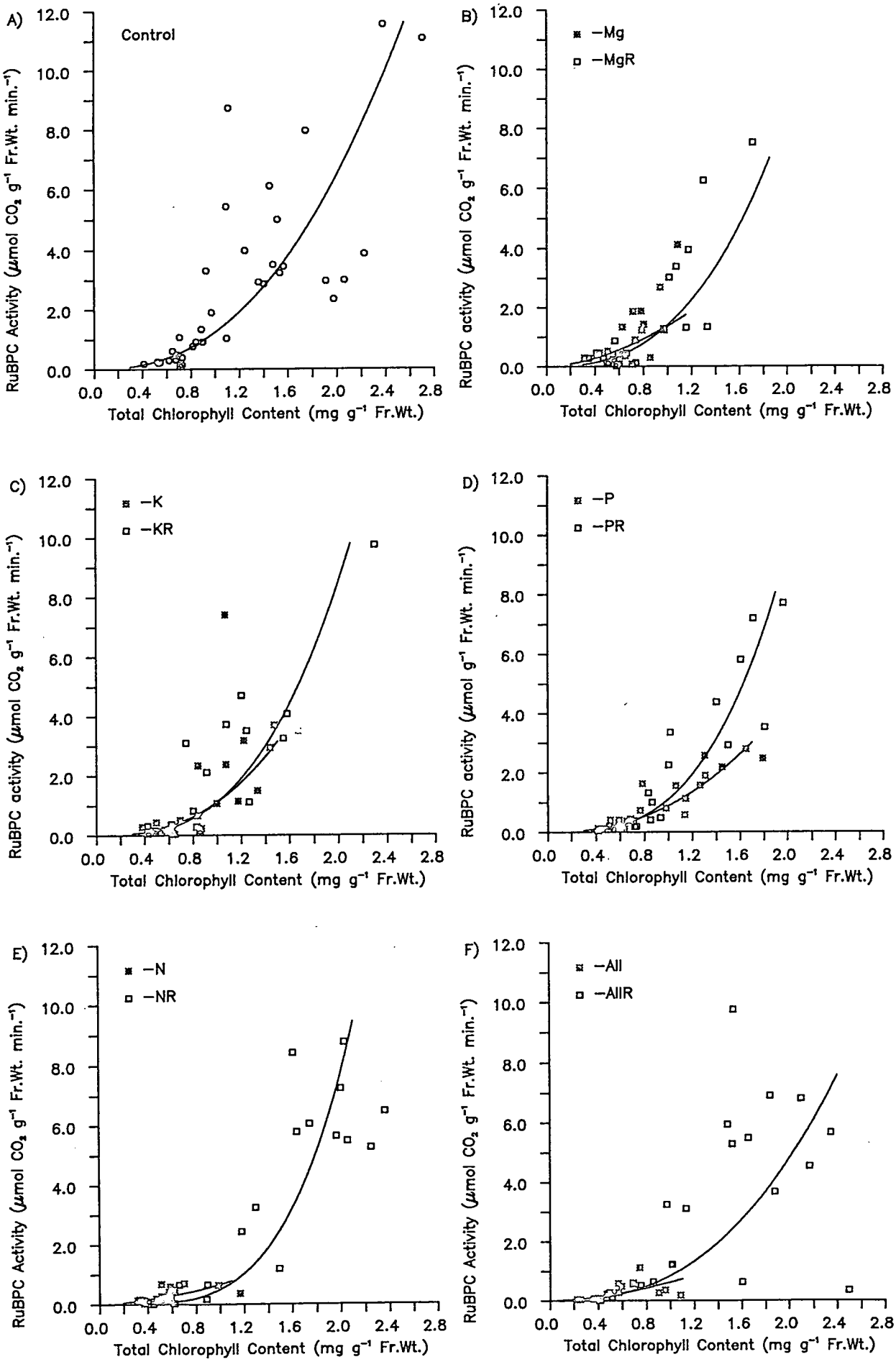


Figure 5.15 A to F.
The relationship between total chlorophyll content and RuBPC activity for each pot treatment throughout the 1988 season. Each point represents one measurement for each variable, from different trees, for all sample times. Equations of the fitted curves are as follows:

Control	$y = 1.2512\ X^{0.7423}$
-Mg	$y = 1.3708\ X^{1.7363}$
-MgR	$y = 1.4043\ X^{2.6088}$
-K	$y = 1.1179\ X^{2.5496}$
KR	$y = 1.1183\ X^{2.9263}$
P	$y = 0.8925\ X^{2.2885}$
PR	$y = 1.1051\ X^{3.0954}$
-N	$y = 0.6771\ X^{1.6415}$
-NR	$y = 0.5085\ X^{3.9380}$
-All	$y = 0.6300\ X^{1.6908}$
-AllR	$y = 0.8501\ X^{2.5040}$


A)
Control
B)
−Mg
−MgR
C)
−K
−KR
D)
−P
−PR
E)
−N
−NR
F)
−All
−AllR
RuBPC Activity (μmol CO2 g−1 Fr.Wt. min.−1)
RuBPC activity (μmol CO2 g−1 Fr.Wt. min.−1)
Total Chlorophyll Content (mg g−1 Fr.Wt.)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8

5.6.2 Correlation Between RuBPC Activity and Total Chlorophyll Content.

The relationship between RuBPC activity and total chlorophyll content can be described by a geometric curve, when both are expressed per g fresh weight (Fig 5.15 A to F). The correlation coefficients for the fitted functions were all significant at $p < 0.01$, apart from data for -All and -Mg treatments (Table 5.4), with values of r^2 ranging from 0.27 to 0.86.

Table 5.4 Correlation coefficients for the fitted curves in Fig. 5.15 A to F, relating RuBPC activity, expressed as µmol CO_2 g^{-1} fr.wt. min^{-1}, and total chlorophyll content, expressed as mg g^{-1} fr.wt., for all treatments. Values are for all samples between May and November.

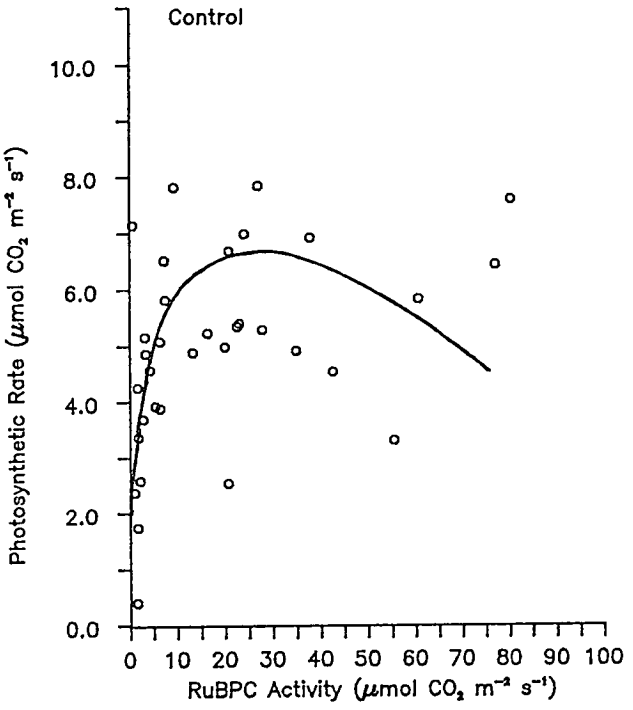
Treatment	n	Coefficient r
+All	37	0.86 ***
-Mg	19	0.52 *
-MgR	18	0.77 ***
-K	20	0.75 ***
-KR	18	0.81 ***
-P	19	0.87 ***
-PR	20	0.93 ***
-N	17	0.64 **
-NR	18	0.91 ***
-All	17	0.74 **
-AllR	18	0.74 ***

5.6.3 Correlation Between Photosynthetic Rate and RuBPC Activity.

The relationship between photosynthetic rate and RuBPC activity (both expressed on a m^{-2} s^{-1} basis), showed that for Controls, P_N increased rapidly with increasing in RuBPC activity up to about 15 µmol CO_2 m^{-2} s^{-1} (Fig. 5.16). At higher activities of RuBPC, P_N did not increase, and decreased at activities of RuBPC higher than about 40 µmol CO_2 m^{-2} s^{-1}. A curve has been fitted by eye to the data in Fig. 5.16, as mathematically fitted curves could not account for the sharp increase in P_N at low RuBPC activities, or the decrease in P_N at high RuBPC activities. The relationship between P_N and RuBPC activity for other treatments was qualitatively similar to that of the Controls.

Figure 5.16

The relationship between photosynthetic rate and RuBPC activity for terminal shoots and needles from whorl 2 of Control trees in the pot experiment. Values are for individual trees, for all sampling times throughout the 1988 growth season.



5.6.4 Correlation Between Net Photosynthetic Rate, and Stomatal Conductance to CO_2.

The correlation between P_N and G_S was only significant in -Mg and -NR treatments (Table 5.5), but the maximum value of r^2 was only 0.27.

Table 5.5 Correlation coefficients for the linear regression of P_N (µmol CO_2 m^{-2} s^{-1}), onto G_S (mol m^{-2} s^{-1}) for all treatments. Values include data for all samples between May and November.

Treatment	n	Coefficient r
+All	35	0.34
-Mg	17	0.48 *
-MgR	17	0.15
-K	17	0.41
-KR	17	0.43
-P	17	0.02
-PR	17	0.36
-N	18	0.34
-NR	18	0.52 *
-All	18	0.14
-AllR	18	0.34

5.7 The Effect of Canopy Position on Photosynthetic Components.

The effect of shoot position in the canopy on RuBPC activity, total chlorophyll content and carotenoid content was also studied, to confirm the importance of a standardised sampling procedure, and to see whether differences were as large as between treatments in the field. One shoot was taken from a "top" position (apical whorl), a "mid" position (whorl 6), and a "bottom" position (whorl 12), from each of two Control trees in the field, in March 1988.

Chlorophyll a, chlorophyll b and total chlorophyll content of needles increased at lower canopy positions (Table 5.6), although none of the differences were significant. Carotenoid content remained virtually constant, regardless of canopy position. RuBPC activity, expressed per g fresh weight and per g dry weight was non-significantly lower at the bottom position than mid or top positions. On a mg chlorophyll basis, there was a trend for RuBPC

activity to decrease in shoots towards the base of the canopy, but again, the positional differences did not reach significance.

Table 5.6 Chlorophyll a, chlorophyll a/b ratio, total chlorophyll and carotenoid content, and RuBPC activity, for different canopy positions in Control trees in the field, sampled in March 1988. Values are means ± S.D. n=2.

Variable	Canopy Position of Shoot		
	Top	**Middle**	**Bottom**
Total Chlorophyll (mg g^{-1} fr.wt.)	0.80 ± 0.12	1.05 ± 0.07	1.26 ± 0.12
Chlorophyll a (mg g^{-1} fr.wt.)	0.62 ± 0.09	0.79 ± 0.05	0.88 ± 0.09
Chlorophyll a/b Ratio	3.47 ± 0.09	3.10 ± 0.02	2.35 ± 0.04
Carotenoid Content (mg g^{-1} fr.wt.)	0.25 ± 0.12	0.27 ± 0.01	0.28 ± 0.03
RuBPC Activity (µmol CO_2 g^{-1} fr.wt. min^{-1})	6.51 ± 1.53	6.35 ± 1.43	3.09 ± 0.32
RuBPC Activity (µmol CO_2 g^{-1} dry wt. $min.^{-1}$)	15.06 ± 2.57	15.26 ± 4.42	7.70 ± 0.26
RuBPC Activity (µmol CO_2 mg^{-1} Chl. $min.^{-1}$)	8.37 ± 3.18	6.02 ± 0.97	2.91 ± 0.63

5.8 The Effect of Needle Age on Needles and Photosynthesis.

Using pot trees, the effect of needle age on the variables monitored in the 1988 experiment, was investigated using terminal shoots from whorl 7, which had expanded during 1987. Measurements were made on shoots from two trees, from +All, -N, -NR, -All and -AllR treatments, following measurements on, and removal of, the current season's growth. Time did not allow measurement of P_N, chlorophyll and carotenoid content, and RuBP activity to be carried out together, in addition to the main experiment, so these were each measured on a separate occasion (for dates, see Table 5.8).

5.8.1 **Needle Number Per Shoot.**

Variation in needle number for shoots expanded in 1987 was not due to treatment, because needle primordia on these shoots were initiated in 1986, prior to the start of the experiment in May 1987. Hence low needle numbers per shoot for the Controls were due to chance. Needle number in Control shoots in 1988 (i.e. number of primordia laid down in 1987), was significantly higher than in 1987 (Table 5.7), but in the -N and the -All sets, needle numbers were smaller. A significant increase in needle number, as a result of new primordia being initiated during the season, by "free growth" of the shoots was seen in -AllR trees and one -NR tree.

5.8.2 **Needle Length.**

Significant variations were found in mean needle length between replicates in both 1987 and 1988 needles (Table 5.7), and variation in needle length between successive growth seasons was also found. However, there was a large, significant reduction in mean needle length in -N and -All treatments in 1988, compared with lengths of needles expanded in 1987, which were initiated before the onset of deficiency treatments. Refertilisation had large effects on needle length, bringing values much closer to those of Control needles.

Table 5.7 Needle number per shoot and mean needle length, for each of two current, and one year old shoots, from +All, -N, -NR, -All and -AllR treatments, sampled July 25 1988.

	One Year Old Needles		**Current Needles**	
Treatment	Needle Number per shoot	Mean Needle Length (mm) ± S.E	Needle Number per shoot	Mean Needle Length (mm) ± S.E.
Control	55	10.90 ± 0.23	179	13.80 ± 0.17
	27	11.30 ± 0.42	217	10.40 ± 0.21
-NR	92	9.22 ± 0.20	79	8.45 ± 0.19
	80	10.70 ± 0.30	139	10.40 ± 0.28
-N	114	11.10 ± 0.18	75	5.43 ± 0.09
	109	12.70 ± 0.26	73	7.57 ± 0.17
-AllR	79	11.50 ± 0.31	146	11.80 ± 0.32
	85	9.41 ± 0.20	140	12.60 ± 0.37
-All	88	10.90 ± 0.19	78	5.16 ± 0.08
	83	10.50 ± 0.20	50	4.97 ± 0.10

5.8.3 **Photosynthetic Rate, Chlorophyll Content and RuBPC Activity.**

The difference in P_N, total chlorophyll content and RuBPC activity between one year old and current needles (Table 5.8) proved not to be significant. It was therefore considered justified to pool the data for these variables for both age classes of needle, in order to test treatment differences. Analyses of variance on these data (four values for each treatment (Tables 5.9–5.12)), showed significant treatment differences. These differences were further analysed by a Multiple Range Test (see after Tables 5.9–5.12), and showed that shoots in –N and –All treatments had a significantly lower photosynthetic rate, chlorophyll content and RuBPC activity than those in Control, –NR and –AllR treatments. For photosynthetic rate, –N shoots also had significantly lower values than –All shoots (see Table 5.9). There was no significant difference in the variables between –AllR and –NR, and Control treatments, although the mean values for –AllR and –NR shoots were higher than the Controls in every case. These data show that refertilisation of –N and –All trees caused an increase in photosynthetic rate and RuBPC activity, and a synthesis of chlorophyll in one year old needles, in addition to affecting development of current needles, and this increase gave higher values than for the Controls.

5.8.4 **Carotenoid Content.**

All treatments studied showed non-significantly higher carotenoid content in one year old needles compared with current needles (Table 5.8). The carotenoid content of needles in the –NR and –AllR treatments was higher than that of the Controls, for current and one year old needles, but again, the difference was not significant.

Table 5.8 Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), total chlorophyll content (mg g^{-1} fr.wt.), carotenoid content (mg g^{-1} fr.wt.) and RuBPC activity (μmol CO_2 g^{-1} fr.wt. min^{-1} and μmol CO_2 mg^{-1} Chl. min^{-1}) of current and one year old needles from Control, -NR, -N, -AllR treatments. Sample dates are weeks beginning as follows; P_N, July 25; Chlorophyll content, August 15; RuBPC activity, August 22 1988. Values are means ± S.D. (n=2).

Variable	Needle age class	Treatment				
		+All	-NR	-N	-AllR	-All
P_N	1987	4.37 ± 0.66	5.48 ± 0.43	1.25 ± 0.23	4.49 ± 0.59	3.34 ± 0.62
	1988	5.37 ± 0.04	5.61 ± 0.16	1.51 ± 0.65	6.01 ± 0.08	1.94 ± 0.39
Total Chlorophyll	1987	1.81 ± 0.35	2.05 ± 0.44	0.60 ± 0.03	2.42 ± 0.11	0.64 ± 0.07
	1988	1.30 ± 0.24	2.03 ± 0.13	0.98 ± 0.23	1.70 ± 0.53	0.62 ± 0.004
Carotenoid Content	1987	0.43 ± 0.05	0.46 ± 0.09	0.22 ± 0.002	0.55 ± 0.04	0.23 ± 0.01
	1988	0.22 ± 0.08	0.39 ± 0.10	0.15 ± 0.01	0.43 ± 0.03	0.22 ± 0.01
RuBPC Activity Per g fr.wt.	1987	3.95 ± 0.09	8.01 ± 1.08	0.50 ± 0.20	5.31 ± 2.29	0.27 ± 0.12
	1988	3.52 ± 4.12	4.37 ± 1.17	0.59 ± 0.32	4.26 ± 3.49	0.83 ± 0.38
RuBPC Activity Per mg Chlorophyll	1987	2.21 ± 2.70	2.12 ± 0.12	1.00 ± 0.58	1.79 ± 1.52	1.34 ± 0.73
	1988	3.10 ± 0.65	3.98 ± 0.80	0.50 ± 0.08	3.06 ± 0.40	0.57 ± 0.20

Tables 5.9–5.12

Analyses of variance on pooled data for current and one year old needles, for treatment effects on photosynthetic rate, chlorophyll content and RuBPC activity. following use of a Multiple Range Test. Treatments with non-significant differences are underlined. n.s. = not significant.

Table 5.9. Photosynthetic Rate (μmol CO_2 m^{-2} s^{-1}).

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	4	12.78	31.34 ***
Error	15	0.41	

Treatment	-N	-All	Control	-AllR	-NR
Mean	1.38	2.64	4.87	5.00	5.54

Table 5.10. Total Chlorophyll Content (mg g^{-1} fr.wt.).

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	4	1.83	16.53 ***
Error	15	0.11	

Treatment	-All	-N	Control	-NR	-AllR
Mean	0.63	0.79	1.55	2.04	2.06

Table 5.11. RuBPC Activity (μmol CO_2 g^{-1} fr.wt min^{-1}).

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	4	25.80	7.43 **
Error	15	3.47	

Treatment	-N	-All	Control	-AllR	-NR
Mean	0.55	0.55	3.73	4.78	6.19

Table 5.12. RuBPC Activity (μmol CO_2 mg^{-1} Chl. min^{-1}.)

Source of Variation	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	4	4.50	3.61 n.s.
Error	15	1.25	

5.8.5 Stomatal Conductance to CO_2, and Intercellular Partial Pressure of CO_2.

In current and one year old needles, C_i did not change significantly between treatments when the 95% confidence limits were compared. However, the difference between current and one year old -All needles was significant (Table 5.13). Values of G_S were also not significantly different between treatments following comparison of the 95% confidence limits in one year old needles, but in current needles the differences were the same as for Fig. 5.11. Significant reductions in G_S were found in one year old needles in Control and -AllR treatments, compared to current needles.

Table 5.13 Stomatal conductance to CO_2, G_S (mol m^{-2} s^{-1}), and the intercellular partial pressure of CO_2 (µbar), for current and one year old needles from Control, -NR, -N, -AllR and -All treatments. The sample date is the week beginning July 25 1988.

Variable	Needle age Class	Treatment				
		Control	**-NR**	**-N**	**-AllR**	**-All**
G_S	1987	0.188 ± 0.010	0.093 ± 0.033	0.026 ± 0.002	0.154 ± 0.001	0.024 ± 0.005
	1988	0.031 ± 0.002	0.051 ± 0.009	0.031 ± 0.001	0.037 ± 0.003	0.051 ± 0.006
C_i	1987	276.9 ± 6.7	289.4 ± 9.7	332.2 ± 11.5	341.3 ± 3.1	284.4 ± 3.8
	1988	328.2 ± 15.1	318.1 ± 10.0	340.1 ± 2.2	335.1 ± 1.7	330.4 ± 0.6

5.9 Discussion.

5.9.1 Leaf Pigments.

The Control data show a maximum chlorophyll content in field material in September, with a slight decline in November. This is in agreement with Linder (1972), who found seasonal variation in needle chlorophyll content, with a maximum in August and a minimum during the winter, for Scots pine and Norway spruce. The same seasonal trend was also found by Gerold (1959) for Scots pine, and by Lewandowska and Jarvis (1977), for Sitka spruce. The changes in plastid structure, underlying this winter decrease in chlorophyll content were studied by Senser et al. (1975) for *Picea abies*. They found that the thylakoid system became disorganised and deformed in the autumn and winter, and the number and size of grana were reduced. The maximum chlorophyll content of Controls in 1987, of 1.2 mg g^{-1} fr.wt. and 1.5 mg g^{-1} fr.wt. in 1988 is slightly lower than 1.6 mg g^{-1} fr.wt. reported for Sitka spruce by Lewandowska and Jarvis (1977), for needles at a similar canopy position. Linder (1972) quotes maximum values of 1.5 and 1.7 mg g^{-1} fr.wt. for needles of Scots pine and Norway spruce, and the data fall within the range of 0.76–2.19 mg g^{-1} fr.wt. reported for a range of 15 forest tree species by Wolf (1956).

The fall in chlorophyll a/b ratio needles late in the 1987 season, and the maximum value reached in the winter differ from data of Lewandowska and Jarvis (1977), who found an increase during the winter, and lower maximum values during the summer. Since total chlorophyll does not fall significantly at the end of the season, the fall in the ratio must be due to an increase in chlorophyll b and a balancing drop in chlorophyll a. The sharp increase in the ratio during early needle development, in June, indicates rapid chloroplast development. This was also shown by Valanne et al. (1981), who found that the a/b ratio increased during the development of chloroplast ultrastructure in leaves of Silver birch, and work with irradiation of etiolated radish has shown that chlorophyll a is intitally synthesised in preference to chlorophyll b (Lichtenthaler et al., 1981). Nilsen and Bao (1987) also found that the chlorophyll a/b ratio was low at leaf initiation in *Rhododendron maximum* and increased rapidly following leaf expansion due to an increase in chlorophyll a. The a/b ratio of Control needles in 1988 did not increase during early development, and remained constant all season. The reason for this is not

known, and it may be that the ratio increased rapidly during early needle elongation, prior to bud burst, and was therefore not detected. The chlorophyll a/b ratio reflects the distribution of chlorophyll between the various chlorophyll-protein complexes in the chloroplast (Leong and Anderson, 1984). As the decrease in total chlorophyll with N deficiency and total nutrient deficiency in 1988 was also associated with a decrease in the chlorophyll a/b ratio, the distribution of chlorophyll between the chlorophyll-protein complexes was also affected, so that there was a reduction in the amount of chlorophyll a in reaction centres in comparison to the amount of light harvesting chlorophyll b.

Carotenoid content of Control needles in 1987 and 1988 showed no sign of a decrease towards the end of the season, as reported by Linder (1972) for Scots pine and Norway spruce in August, and data are consistent with those of Lewandowska and Jarvis (1977) who observed a continued increase throughout the winter in needles of Sitka spruce. A winter maximum in carotenoid content was also found in needles of Pine and Fir (Sirotkin and Anufrieva, 1973), and needles of Scots pine (Gerold, 1959). Values in October and early November of 0.20 mg g^{-1} fr.wt. are slightly lower than the value of just over 0.30 mg g^{-1} fr.wt. reported for needles of Sitka spruce at a similar canopy position by Lewandowska and Jarvis (1977). The higher control values of carotenoid content at bud burst and in October in 1988, compared with those in 1987, may be because shoots from the seedlings were exposed to higher irradiances than field shoots, as they received less shading from other foliage. Carotenoid biosynthesis may therefore have been stimulated by higher light intensities, because of their capacity to act as antennae pigments, and their role to protect the chlorophylls from photobleaching (Krinsky, 1964).

The chlorophyll content of needles in the -Mg set in the field, in 1987 was not different to that of the Controls, probably for the reasons already discussed (Chapter 3), concerning adequate levels of Mg in the peat. Under more controlled pot conditions, the lower chlorophyll content seen in -Mg needles is consistent with findings of Dorenstouter et al. (1985), for sun and shade shoots of Poplar; Pandev et al. (1982) for sunflower; and Baszinski et al. (1980) for rape. Because magnesium deficiency has been shown to reduce protein synthesis (Bamji and Jagendorf, 1966), the reduction in chlorophyll content is probably more due to inhibited protein synthesis, rather than to a lack of Mg^{2+} for incorporation into chlorophyll molecules. A reduction in protein synthesis

explains why carotenoid content is also reduced with Mg deficiency, as also found by Baszynski et al., 1980 - for rape; Pandev et al., 1982 - for sunflower. The slight increase in chlorophyll content from July onwards upon refertilisation differs from findings of Peaslee and Moss (1966), who found that the decrease in chlorophyll content caused by magnesium deficiency in maize, could not be increased by readdition of Mg. This may be because the capacity to synthesise chlorophyll once Mg becomes available may be lost; this does not seem to be the case in Sitka spruce.

The effect of K deficiency on leaf pigments in the literature is variable. In seedlings of *Picea abies* and *Larix decidua* grown in K deficient nutrient solution for 200 days, total chlorophyll content was significantly lower than in the Controls, but there was no effect of K deficiency for *Pinus sylvestris* (Cizkova, 1981). No effect of K deficiency was seen on the chlorophyll content of rice (Kabaki et al., 1979). The 1987 data show a lower amount of chlorophyll in K deficient trees from August onwards. No effect prior to this may be due to the translocation of K^+ within the tree to the developing shoot to buffer deficiency. By August probably K^+ is becoming limiting in the new shoots. No effect on chlorophyll content was found with K deficiency in 1988, maybe also due to internal translocation of K^+, although this is unlikely in view of the deficient levels of K^+ in needles in the -K set (see Materials and Methods, Tables 2.6 and 2.7). An absence of an effect due to K deficiency in pot trees may also be due to a difference in severity of response compared with field trees, because of the young age of the trees.

Nitrogen deficiency, together with total nutrient deficiency caused the greatest reduction in chlorophyll content of all the treatments. A reduction of chlorophyll content with N deficiency is widely found in many species (*Atriplex* - Medina, 1971a; spinach - Evans and Terashima, 1987; sunflower - Pandev et al., 1982; three species of conifer - Cizkova, 1981). The increase in chlorophyll content seen with refertilisation of nitrogen-deficient trees in 1988 is supported by Ingestad and Kahr (1985), who observed regreening in needles of *Pinus sylvestris*, *Picea abies* and *Pinus contorta*, when seedlings grown in low nutrient solutions were resupplied with N, although no chlorophyll data are given.

Phosphorus deficiency did not cause a change in chlorophyll content for the Controls in 1987 or 1988. Deficiency of P usually causes an increase in

chlorophyll content on a leaf area or fresh weight basis, as it retards cell and leaf expansion more than chlorophyll synthesis (Hecht-Buchholz, 1967). Data show no change in chlorophyll content in comparison with the Controls, for trees in the -P set in 1987 or 1988. Results in 1987 (Tables 4.5 and 4.6) showed that P deficiency increased cell size, so must also cause an increase in chlorophyll synthesis per cell.

5.9.2 **RuBPC Activity.**

During leaf development of broad leaved species, RuBPC activity usually reaches a maximum at, or prior to, the attainment of maximum leaf area, and then declines (Callow, 1974; Gordon et al., 1978; Lloyd, 1976; O'Toole et al., 1977). Dickmann (1971) found RuBPC activity to increase sigmoidally during leaf ontogenesis in *Populus deltoides* throughout the first season. Work with conifers has concentrated on seasonal trends in RuBPC activity, rather than ontogenetic changes with needle development. The data here, show changes in activity with season, superimposed onto changes associated with needle growth.

The peak in RuBPC activity in August and September, occurred some time after shoot and needle growth had ceased. Gezelius and Hallen (1980) also found a peak in RuBPC activity in August in current needles of Scots pine, but only on a fresh weight basis, with no change or a decline, per unit dry weight, protein or chlorophyll. They also found a marked seasonal variation in RuBPC activity in older needles, with a peak in August and September when activity was expressed per unit dry weight, chlorophyll or protein, but not on a fresh weight basis. An increase in RuBPC activity could be due to increasing amounts of the enzyme, or an increase in the specific activity, or both. Gezelius and Hallen (1980) attributed the seasonal increase in RuBPC activity in older needles, to an increase in specific activity. This was because total soluble protein, including RuBPC, decreased between April and July, and was used as a nitrogen reserve for the developing shoot. In contrast, they found a decrease in specific activity in current needles in August. Unfortunately data are not available for changes in total soluble protein throughout the season, so these alternatives cannot be explored for Sitka spruce. It is known that RuBPC protein can accumulate throughout the summer, and during conditions of favourable N supply (Millard and Thomson, 1989 - for *Malus*; Millard, 1988 - for potato), so that the seasonal increase in RuBPC activity in all treatments apart

from -N and -All may partly be a response to N supply, as well as an ontogenetic increase. In wheat, however, not all the accumulated RuBPC protein was found to be functional (Lawlor et al., 1987b). Analysis of actual amounts of RuBPC present in the needles would therefore be useful in determining to what extent non-functional N-storage RuBPC is present. In trees, spring growth occurs before the uptake of N by the roots is optimal (Millard and Neilsen, 1989), so that stored N from older tissues is particularly important for early growth of young trees. This emphasises that the low RuBPC activity in -N trees is not only due to a lack of supplied nitrogen during needle expansion, but also due to a lack of N for storage during N-deficient conditions in the preceding year.

The reasons for the decline in RuBPC activity seen at the end of the season, and following leaf expansion in non-evergreen leaves, may be due to proteolytic enzyme(s) which may be specific to RuBPC Protein (Peterson and Huffaker, 1975; Peoples and Dalling, 1978). Changes in isoenzyme composition of the RuBPC protein may also occur (Shomer-Ilan and Waisel, 1975; Huner and Macdowall, 1976).

Maximum activities of RuBPC in Control needles of 6.5 µmol CO_2 g^{-1} fr.wt. $min.^{-1}$, which are equivalent to 950 µmol CO_2 g^{-1} dry wt. h^{-1}, are higher than the 600µmol CO_2 g^{-1} dry wt. h^{-1} found in current needles of *Pinus sylvestris* (Gezelius and Hallen, 1980), but lower than values of up to 1500 µmol CO_2 g^{-1} dry wt. h^{-1} found by Beadle and Jarvis (1977) for fully expanded current needles of two year old Sitka spruce. Maximum values of up to 1100 µmol CO_2 g^{-1} dry wt h^{-1} have been reported for mature trees of *Picea abies* (Schmeiden-Kompalla et al., 1989).

The reduction in RuBPC activity in all deficiency treatments is not surprising considering the key role of many elements in protein synthesis. The slight decrease in RuBPC activity per unit fresh weight in -K needles compared with the Controls, is probably partly due to the role of K^+ in protein translation (Evans and Wildes, 1971; Wyn Jones et al., 1979): Peoples and Koch (1979) showed the synthesis of RuBPC to be reduced with K deficiency, and also the specific activity on a unit protein basis. Refertilisation with K increased enzyme activity slightly, which would be expected if K^+ caused normal protein synthesis to resume. The activity of RuBPC per unit chlorophyll was not different in -K or -KR treatments to the Controls, which would support the hypothesis that K

deficiency inhibits protein synthesis, if chlorophyll synthesis is inhibited to the same extent as RuBPC protein. However, the chlorophyll content of -K needles was not different to that of the Controls.

Magnesium functions as a bridging element for the aggregation of ribosome subunits (Cammarano et al., 1972), so that with Mg deficiency the subunits begin to dissociate, and protein synthesis is impaired. The absence of a reduction in RuBPC activity with Mg deficiency from that of the Controls per unit chlorophyll also supports this view, as an inhibition of protein synthesis would affect the formation of chlorophyll to the same extent as that of RuBPC.

The reduction in RuBPC activity on a fresh weight and chlorophyll basis with nitrogen deficiency and total nutrient deficiency was larger than for any other treatment, and suggests that a lack of nitrogen causes a severe decrease in the amount of protein synthesised in the cell. A similar large reduction found with N deficiency in RuBPC activity was found with N deficiency in rice (Kabaki et al., 1979).

The lower activity of RuBPC with P deficiency was also apparent per unit chlorophyll, unlike with Mg deficiency. This suggests that the effect of P deficiency is not due to a general reduction in protein synthesis, since chlorophyll synthesis is unaffected. However, it has been shown that P deficiency reduces the rate of formation of ATP and NADPH (Tombesi et al., 1969), which in turn might be expected to reduce the metabolic processes of the cell, including the regeneration of RuBP and the synthesis of RuBPC protein, although this still does not explain why the synthesis of chlorophyll is unaltered. The failure of protein determination using the Bradford method was unfortunate, and means that it is not known whether effects on RuBPC activity were due to changes in amount of RuBPC protein, or the specific activity per unit protein. The suppression of colour of the Bradford reagent with the presence of the needle extract (see Materials and Methods, section 2.11) may have been due to interference by polyphenols, resins or tannins which are present in conifer needles in large amounts. The problems of studying enzyme characteristics and activities in conifers are recognised for this reason (Beadle et al., 1983), and the isolation of active proteins is difficult using conventional techniques (Loomis and Bataile, 1966). The presence of differing amounts of ploy phenols in different extracts may cause some proteolytic action, although 1% (v/v) Tween 80 (used here), was found to be the best protective agent

(Gezelius and Hallen, 1980). This may explain why data for RuBPC activity were variable between trees.

5.9.3 **Net Photosynthetic Rate.**

During leaf ontogeny in broad leaved species, P_N increases rapidly, reaching a maximum before full leaf area expansion is complete, and then declining during later developmental stages (*Nicotiana* - Wada et al., 1967; *Glycine* - Dornhoff and Shibles, 1974; *Pisum* - Bethlenfalvay and Phillips, 1977). The same developmental trend has been found in some tree species (*Populus* - Dickmann and Gordon, 1975; *Prunus* - Sams and Flore, 1982). For conifers, there is also a seasonal trend in P_N, which is also superimposed onto developmental changes in current needles, whereby P_N reaches a maximum in late summer, and then declines towards winter, increasing again in the following spring (Scots pine - Zelawski and Goral, 1966; *Picea sitchensis*, *Abies grandis*, and *Tsuga heterophylla* - Fry and Phillips, 1977). The data for all treatments show this seasonal trend, and maximum values for the Controls were up to 7.0 µmol CO_2 m^{-2} s^{-1}, equivalent to 11.09 mg CO_2 dm^{-2} h^{-1}. This is slightly lower than the maximum reported for Sitka spruce by Fry and Phillips (1977), and the 14.32 mg CO_2 dm^{-2} h^{-1} reported for two year old seedlings of Sitka spruce preconditioned in 31% shade (Krueger and Ruth, 1969), and much lower than the 18.00 mg CO_2 dm^{-1} h^{-1} measured by Ludlow and Jarvis (1971). This is almost certainly because the published values are for saturating irradiances (150-200 W m^{-2}, calculated as 0.61 - 0.91mmol m^{-2} s^{-1}; Ludlow and Jarvis, 1971), whilst the trees in 1988 were measured at irradiances of 0.41 to 0.71 mmol m^{-2} s^{-1}. Maximum values of P_N during the 1989 experiment were only 2.5 to 3.0 µmol CO_2 m^{-2} s^{-1} at light intensities of 0.27-0.29 mmol m^{-2} s^{-1}. Values of P_N of 2.0 µmol CO_2 m^{-2} s^{-1} were found in Controls, even in early May, before any needles had reached maturity, showing that photosynthesis of the shoot exceeded respiration. This differs from findings of other workers, that negative, or very low positive values of P_N occurred during early leaf expansion; Dickmann, 1971 - for *Populus deltoides*; Loach and Little, 1973 - for *Abies balsamea*; Bourdeau, 1959 - for Norway spruce and blue spruce. This difference may be because of the very short period of needle extension in Sitka spruce, compared with broadleaved trees and other conifer species, such that needles become photosynthetically independent from older needles early in their development.

The similarity of the decrease in P_N in the -All and -N treatments suggests that the effect of total nutrient deficiency is due to N deficiency, and also because the effect of treatment on -All trees is much greater than for any of the other treatments, apart from -N. A decrease in N supply is usually found to decrease P_N (Slobodskaya et al., 1970 - for pea, bean and sunflower; Yoshida and Coronel, 1976 - for rice; Kabaki et al., 1979 - for rice; Longstreth and Nobel, 1980 - for *Gossypium*; Ojima et al., 1965 - for soybean; Evans, 1983 for wheat; Evans and Terashima, 1988 - for spinach).

The lack of effect of Mg deficiency on P_N is in contrast to the data of Peaslee and Moss (1966), who found a decrease in P_N with Mg deficiency, which was due to chlorophyll deterioration.

A decrease in P_N has been reported for K deficiency for *Phaseolus vulgaris* (Ozbun et al, 1965); for *Zea mays* (Peaslee and Moss, 1966); for *Pinus* (Zech et al., 1969); for *Trifolium subterraneum* (Bouma, 1970); for *Beta vulgaris* (Terry and Ulrich, 1973); and for *Gossypium hirsutum* (Longstreth and Nobel, 1980). However, no influence of leaf potassium content on P_N was found for *Glycine* (Ojima et al., 1965), and for sugarcane, Hartt (1969; 1970) found that P_N did not decrease until K deficiency became severe. Re-application of K to K-deficient maize caused a recovery of P_N within 24 h (Peaslee and Moss, 1966), and within 10 days of refertilisation of K-deficient sugarbeet, P_N increased by 49% (Okanenko et al., 1965). In the present study, no difference in P_N from the Controls was found in the -K treatment, even though foliar analysis (Materials and Methods, Tables 2.6 and 2.7) showed the K content to be deficient in -K needles. This low foliar content of K shows that the high value of P_N in the -K set was not due to internal retranslocation of K^+ to the developing needle. It is possible that Sitka spruce seedlings have a high tolerance to K deficiency.

A deficiency of P did not decrease P_N, consistent with findings of Andreeva and Pessanov (1970). Other reports of the effect of phosphorus deficiency on P_N are variable: Ojima et al., (1965) found only a small decrease in P_N with P deficiency, and Natr and Purs (1970) found a decrease in barley, but only after several weeks. Lal and Subba Rao (1960) even found that P_N increased slightly in members of the *Graminae*, under conditions of P deficiency, but Longstreth and Nobel (1980) found a decrease in P_N for *Gossypium hirsutum*, attributable to an increase in r_M, which was similar to the conclusion of Terry and Ulrich (1973) for sugar beet.

5.9.4 Resistance Pathway to CO_2.

The ontogenetic trend of change in G_S in broad-leaved species is similar to that of net photosynthetic rate i.e. an increase to a maximum, then a decrease (Catsky et al., 1985). Changes in P_N may therefore be partly explained by, changes in G_S. The relationship was found by Rawson and Woodward (1976) for *Glycine max*, and by Jewiss and Woledge (1967) for *Festuca arundinacea*, both sets of workers showing that P_N and G_S reached maximum rates at the same time as the attainment of maximum leaf area. However, maximum photosynthetic rate was not found to be coupled to maximum G_S for *Phaseolus vulgaris* (Catsky et al., 1976), or for *Helianthus annuus* (Rawson and Constable., 1980).

For Sitka spruce, the most detailed study reported is that of Ludlow and Jarvis (1971), who measured r_S and r_M to CO_2 transfer at four times between June and September in current needles, of 20 year old forest trees. They attributed the rise in net photosynthetic rate during needle development to a large drop in mesophyll resistance initially, with only small changes in r_S. This is in contrast to the data obtained in this study, which show an obvious increase in G_S during May and early June, i.e. the period of rapid needle expansion, in all treatments apart from -N and -All. An increase in stomatal conductance is to be expected during leaf development, due to the increase in pore size as guard cells differentiate and become fully functional.

The maximum value of G_S in Controls, of 0.19 mol m^{-2} s^{-1} is equivalent to a resistance to CO_2 transfer of 2.16 s cm^{-1}, which is rather higher than the minumum value of 1.8 s cm^{-1} reported for Sitka spruce by Ludlow and Jarvis (1971). However, these values are lower than those for a range of other tree species (Holmgren et al., 1965), and may account for the higher values of P_N in Sitka spruce when compared with other tree species (Larcher, 1969). A reason for the decrease in G_S from late August, may be the build up of wax in the stomatal antechamber during late needle development. This has been shown to contribute over 30% of the total pathway resistance to CO_2 (Jeffree et al., 1971). It is possible that deficiency of Mg, K or P causes more wax to be deposited, or affect stomatal development and thereby limit conductance earlier in time than in the Controls. However, refertilisation of -Mg, -K and -P sets at the onset of needle growth had no effect on G_S; this would not be expected if needle anatomical development was affected by deficiency, unless by this stage

the number of stomata and the nature of their development was already established. The increase in G_S in -NR and -AllR treatments compared with -N and -All treatments, and to higher values than the Controls, is probably a reflection of the free growth observed in these treatments: new needles were initiated and continued to expand throughout the season, so that younger needles would have less wax than needles in other treatments. There may also be differences in needle anatomy; larger stomata, a higher density per unit area, or changes in the thickness of the cuticle which may affect the degree to which the stomata are sunken. Treatment differences in needle anatomy are likely, considering the significant effects on shoot and needle morphology outlined in chapter 3, and more data on anatomical differences due to treatment would be useful in interpreting the data. No data are available on changes in mesophyll resistance with treatment and time, which would be useful in interpreting the limitations operating on the availability of CO_2 to the cells. It is known that nitrogen deficiency causes an increase in concentration of ABA in plants (Goldbach et al., 1975), and a lower cytokinin concentration (Radin et al., 1982; Krauss and Marschner, 1982). Phosphorus deficiency also increases the sensitivity of stomata to ABA. These effects are important in causing stomata to close more rapidly (Mittelheuser and Van Steveninck, 1971), usually in conditions of water stress, but may also lower maximum pore dimensions, and hence decrease G_S, as seen in -P, -N and -All treatments. The role of potassium in causing the increase in turgor in the guard cells responsible for stomatal opening is well known (Humble and Raschke, 1971), and therefore K-deficiency may cause stomatal closure. This could explain the reduced stomatal conductance in -K trees, compared to the Controls, although no effect was observed following refertilisation with K.

Wong et al. (1985) found that decreasing the availability of N or P led to a reduction in both G_S and photosynthetic rate for *Zea mays* and *Gossypium hirsutum*. Refertilisation of N deficient *Zea mays* plants caused an increase in both these variables. The increases and decreases in P_N and G_S were in the same proportion, so that the intercellular partial pressure of CO_2 was unaffected. Raschke (1975) discusses the theory of feedback mechanisms controlling stomata, and Wong et al. (1979) suggest that stomata respond to the capacity of the mesophyll cells to fix CO_2, by maintaining the intercellular concentration of CO_2 at a constant level, which could be achieved by negative feedback by RuBP, ATP or NADPH.

The decrease in C_i upon refertilisation of -N and -All trees, although slight, is in contrast with findings of Longstreth and Nobel (1980) for *Gossypium hirsutum*, and implies that the increase in mesophyll conductance (G_M) is greater than that in G_S. This indicates that the response of G_M to nutrition may be more important than G_S in determining the overall diffusive resistance of CO_2 to the cells.

To assess the importance of the components processes of photosynthesis in determining P_N, a study of the possible correlations between them is necessary.

5.9.5 **Correlations Between Photosynthetic Variables.**

It is generally agreed that there is a relationship between photosynthetic rate and chlorophyll content; this was found to be linear for soybean (Buttery and Buzzell, 1977), and for leaves arranged at different positions on the stem of *Nicotiana tabacum* and *Brassica oleracea* (Sestak, 1963). The data for Controls (Table 5.3), and all treatments except -Mg, -MgR and -N show a significant linear relationship between total chlorophyll content and P_N per unit area. However, there are many reasons why a linear relationship is not always found; for example, during early leaf expansion, chlorophyll formation precedes measurable activity of the photosystems (Sestak et al., 1985). Wieckowski (1959; 1960 a,b; 1961) showed that in early leaf growth, the rate of pigment synthesis exceeds that of area increase, and subsequently the situation is reversed, so that correlations between chlorophyll content and photosynthetic rate depend on the basis on which both are expressed. A discrepancy in the correlation is sometimes observed, due to photosynthetic rate reaching a maximum earlier in leaf ontogeny than chlorophyll content (Hernandez-Gil and Schaedle; 1973 for *Populus*; Sestak and Bartos, 1963 for *Zea*). The present data show the reverse situation, with P_N reaching a peak before chlorophyll content, but this discrepancy still reduces the significance of the correlation. The correlation coefficients for the relationship between P_N and total chlorophyll content for the period up to August are higher than the calculated values using data from the whole season. The dependence of photosynthetic rate was found to be higher with respect to chlorophyll a content rather than chlorophyll (a+b) content, in only a few of the treatments (Table 5.3), but is usually reported to be more significant (Sestak, 1966; Okubo et al., 1975). The reason for this is that chlorophyll a plays a more leading role *in vivo* in photosynthetic

reactions than chlorophyll b (Witt et al., 1965). Explanations of the relationship between chlorophyll and photosynthesis involve the concept of the "photosynthetic unit", a hypothetical unit being defined as the amount of chlorophyll molecules per one molecule of the reaction centre of photosystem 1. The capacity of the photosynthetic apparatus must therefore be closely linked to the number of photosynthetic units present. During chloroplast development, the synthesis of the photosynthetic units is thought to proceed either by the rapid synthesis of reaction centres of the units, followed by the synthesis of bulk chlorophyll which surrounds the reaction centres, or by the synthesis of one complete unit after another (Heron and Mauzerall, 1972). In the first case, photosynthetic efficiency (P_N per unit chlorophyll) would be expected to increase with increasing chlorophyll content, as chlorophyll a is synthesised, then decrease as chlorophyll b is synthesised. An increase in the chlorophyll a/b ratio followed by a levelling off or a decrease would also be expected. In the second case, photosynthetic efficiency and chlorophyll content would be expected to increase in parallel, and the a/b ratio to remain constant. The data for 1988 show the chlorophyll a/b ratio to be constant, and therefore support the second interpretation, although a sharp increase in the ratio may have occurred prior to bud burst and the start of sampling. However, the photosynthetic efficiency in 1988 increased with increasing chlorophyll content in the first part of the season, for Control needles, which is inconsistent with the second theory. The increase in the chlorophyll a/b ratio in 1987 in field trees, followed by a levelling off would support the first interpretation. The process of photosynthetic unit development in Sitka spruce is therefore unclear. Possibly, chlorophyll content is not limiting P_N in 1988, so that photosynthetic efficiency increases for other reasons. It may be that in juvenile trees photosynthetic units develop sequentially, and there is a shift with increasing age, to development based on the first interpretation. Earlier measurement of the chlorophyll a/b ratio immediately prior to bud burst in pot trees might detect an increase, and resolve the apparent differences in interpretation between field and pot trees.

Although the photosynthetic capacity P_N and the number of photosynthetic units present (e.g. chlorophyll content) are fundamentally related, the correlation is improved when the conditions under which photosynthesis is measured, particularly light intensity are optimum (Sestak, 1963; Sestak and Bartos, 1963). The light intensities used in this study were not saturating, and

as needles are fairly thick, lower layers of chloroplasts may not be as saturated as upper layers. A higher correlation would therefore be obtained by expressing chlorophyll content on a fresh weight or a dry weight ("chlorophyll concentration") basis, rather than per unit area. The effects of nutrient deficiency on needle morphology, and particularly effects on needle or cuticle thickness, may be significant in determining the extent to which the chloroplasts are light saturated, thereby altering the P-I response curve. This could be important in conditions of low light intensity.

The relationship between total chlorophyll content and RuBPC activity during leaf ontogeny is similar to that reported for *Theobroma cacao* (Baker and Hardwick, 1973), when both are expressed per unit fresh weight. Because of the apparent geometric nature of the relationship, it appears that during needle expansion, chlorophyll synthesis exceeds the synthesis of photosynthetic enzymes. This is not surprising considering the higher priority that must be given to development of the light harvesting system. Following needle elongation, synthesis of photosynthetic enzymes is greater than that of chlorophyll, which is more likely to limit photosynthesis during this period of maximum photosynthetic capacity.

The data show a poor correlation between photosynthetic rate and RuBPC activity. This is interesting, since a correlation between maximum RuBPC activity and maximum photosynthetic rate has been found by many authors (Smillie, 1962 - Pea; Medina, 1971a - *Atriplex patula*; Tselniker 1981, - Aspen; and Steer, 1971 - *Capsicum*), and has led to the belief that RuBPC is a major limiting factor of photosynthesis. This view is also supported by the high abundance of RuBPC in the cell, contributing up to 33% of the total leaf protein (Collatz et al., 1979). The data show that for some treatments (-Mg, -MgR and -N), maximum photosynthetic rate does coincide with maximum RuBPC activity (per unit fresh weight) (compare Fig. 5.7 with Fig. 5.9), however, in other treatments, P_N reaches a peak slightly prior to the peak in RuBPC activity. During the needle expansion phase, until mid June, RuBPC activities in all treatments are very low and do not increase, although P_N increases rapidly in all treatments, indicating that during early needle development, RuBPC activity is not a limiting factor to P_N. A decline in RuBPC activity from September onwards is matched by a decrease in P_N, although the discrepancy in the timing of the peaks, with that of RuBPC being later, does not suggest that RuBPC activity limits P_N late in the season. Some authors agree that RuBPC

activity is not a limiting factor to P_N, at least during early leaf ontogeny (Blenkinsop and Dale, 1974; Thomas and Thorne, 1975). Baker and Hardwick (1973) found that a plot of RuBPC activity per unit photosynthetic rate against time showed enzyme activity to increase during leaf expansion, with no increase in photosynthetic rate. This is in contrast to the relationship found here during early needle expansion, but is similar to that found later in the season, when the increases in RuBPC activity are not matched by similar increases in P_N. The reason for the discrepancy in maximum rates of RuBPC activity and P_N, may be due to differences in carboxylating activities in leaf extracts and *in vivo* in the leaf. For example, assay of RuBPC *in vitro* may give lower measured activities than those occurring *in vivo*, due to incomplete extraction from the leaf, or higher activities *in vitro* due to providing optimum conditions for the reaction. It is therefore difficult to relate an enzyme activity to a reaction rate, and any interpretation of the correlation between RuBPC activity *in vitro* and P_N must bear this in mind. Some studies have shown that RuBPC is not fully activated *in vivo* (Perchlorowicz et al., 1981; Perchlorowicz and Jensen, 1983), but this was concluded for low concentrations of CO_2, where RuBPC was likely to be rate-limiting. Caemmerer and Farquhar (1981), Seeman et al., (1981), and Seeman and Berry (1982) concluded that RuBPC activity was consistent with *in vivo* photosynthetic rates, only if full activation of RuBPC is assumed.

In the present study, G_S increases in parallel with P_N for all treatments in May and early June, but subsequently, changes in G_S are not proportional to those in P_N, and the correlation between both variables (Table 5.5) is poor for most treatments. Although P_N and G_S vary independently, C_i remains fairly constant throughout the season and between treatments, and this supports the hypothesis of Wong et al. (1979), that the constant value of C_i is controlled by the stomata reponding to the capacity of the mesophyll cells to fix CO_2. Some workers report that G_S is the prime controlling factor in photosynthesis (McPherson and Slatyer, 1973; Pasternak and Wilson, 1973), whilst others maintain that photosynthesis is primarily controlled by G_M, (Fraser and Bidwell, 1974; Berry and Farquhar, 1978; Bierhuizen and Slatyer, 1964). The usual finding is that G_S and G_M change in parallel as leaves age (Wilson and Ludlow, 1970; O'Toole et al., 1977), so that the concentration of CO_2 in the intercellular spaces remains constant. The data here show that this is true for all treatments, so we may conclude that even though data for G_M are not

available, values of C_i reflect changes in both G_S and G_M, so that P_N is affected by similar changes in both. However, the rate for -NR and -AllR treatments show that this duality of response of G_S and G_M may be disrupted by nitrogen nutrition, with G_S increasing more than G_M, in response to favourable N supply. P_N for all treatments is probably limited to some degree by CO_2 diffusion, as well as by biochemical factors; because C_3 plants are not saturated in normal air (Pearcy and Ehleringer, 1984), every step in the diffusion pathway represents a concentration drop in CO_2 which decreases photosynthesis. Diffusional limitations completely limit P_N when the CO_2 concentration at the carboxylation sites falls below the CO_2 compensation point. Ludlow and Jarvis (1971) found that G_S and G_M in needles of Sitka spruce did not change in parallel, and during initial needle development, G_M increased more rapidly than G_S, and following needle elongation, G_S increased by much more than G_M. Evans (1983) has concluded that for wheat, the liquid-phase diffusion resistance to CO_2 usually imposes relatively small limitations on P_N, but when leaves develop exceptionally high biochemical capacities, limitations by liquid-phase resistance become important. Ludlow and Jarvis (1971) suggest that the liquid-phase transfer conductance component of G_M may be more important in needles of Sitka spruce as a rate limiting step in CO_2 availability than in broad leaves. This is because of the densely packed arrangement of large mesophyll cells, with a small internal volume of intercellular spaces for gaseous diffusion.

5.9.6 **Limitations on Photosynthetic Rate.**

From the data for the Controls in 1987 and 1988, the following pattern emerges for the development of photosynthetic capacity: chlorophyll synthesis precedes that of RuBPC, and is of primary importance in the establishment of photosynthetic capacity, by providing a light capture system. In field trees, chlorophyll a synthesis precedes that of chlorophyll b, so that the reaction centres of the photosynthetic units develop first, followed by the synthesis of light-harvesting chlorophyll. Pot trees however, provide some evidence for the sequential development of photosynthetic units, and this ontogenetic difference may be due to tree age.

Photosynthetic rate during needle expansion increases more rapidly than chlorophyll content or RuBPC activity, and is probably more limited by stomatal resistance to CO_2, which changes in parallel with P_N during needle development. Photosynthetic rate reaches a peak prior to that of RuBPC

activity and chlorophyll content, and is probably not limited by chlorophyll content. The limitation of P_N *in vivo* by RuBPC activity is more difficult to assess, due to the difficulty already mentioned, of relating an enzyme activity *in vitro*, to a reaction rate *in vivo*. It is possible that *in vivo*, P_N and RuBPC activity are both reduced by other limiting factors dependent on the season, such as levels of NADPH or ATP, or amounts of RuBP. Photosynthesis towards the end of the season is probably also limited by G_S.

In -NR and -AllR treatments, G_S and G_M (as inferred from a constant C_i), continued to increase until November, so cannot account for the decrease in P_N in September. Chlorophyll content also remained high, so again, the decrease in RuBPC at the end of the season is most closely related to the decrease in P_N. Bouma (1970) found that with N deficiency, P_N in *Trifolium subterraneum* decreased per unit area, but not per unit chlorophyll. When the plants recovered from N stress, an increase in P_N preceded an increase in chlorophyll content, indicating that a recovered chlorophyll content was not the sole reason for the fall in P_N. The present data show a rapid increase in P_N and chlorophyll content together, immediately following refertilisation with N, suggesting that chlorophyll content was limiting P_N in N-deficient trees. However, G_S also increased rapidly on refertilisation of -N trees, making it hard to determine which is the main N-induced factor limiting P_N. As it is, a combination of low RuBPC activity, low chlorophyll content, and high stomatal resistance to CO_2 transfer appear to be involved. However, Nevins and Loomis (1970) found a rapid increase in P_N to normal rates within 4 days of refertilisation of N-deficient sugar beet. They suggest that the rapidity of recovery indicates the remedying of a lack of nitrogenous components (including RuBPC), rather than long term morphological alterations of the leaf (i.e. changes in r_S). In contrast, the present study shows that the increase in RuBPC activity following refertilisation of -N and -All trees is not as great as that in chlorophyll content and G_S, and it is probably these which limit P_N under conditions of N deficiency. However, the rise in P_N to a maximum in August is not accompanied by an increase in G_S, and cannot be limited by it, which suggests that chlorophyll content is the more important factor in N-deficient conditions.

Interestingly, despite P_N not being affected by other deficiency treatments, a reduction in chlorophyll content was found in -Mg and -MgR treatments, a reduction in RuBPC activity in -Mg, -P and to a small extent, -K treatments,

and a reduction in G_S in all these treatments, compared with the Controls. This does not implicate a major limiting factor on the maximum value of $P_{N'}$ although as already stated, this maybe due to non-saturating light levels used. For most treatments, the decrease in P_N at the end of the season, is followed by a decrease in RuBPC activity, and it may be that either RuBPC activity is causal in decreasing $P_{N'}$ or P_N is causal in decreasing RuBPC activity. Alternatively, other factors may act within the needle, to affect P_N and RuBPC independently. Data for the -Mg set do not show a large drop in RuBPC activity or chlorophyll content concomitant with that in $P_{N'}$ which may be caused by the decrease in G_S from mid-September. In the -P treatment, the decrease in P_N is independent of any change in chlorophyll content, RuBPC activity or G_S, which also implicates the involvement of other factors, such as electron transport or limitations of other photosynthetic enzymes or substrates.

5.9.7 **Effect of Canopy Position on Photosynthesis.**

Differences in amount of chlorophyll and RuBPC activity with different canopy positions (Table 5.6) are related to whether shoots have "sun" or "shade" characteristics, and reiterate the importance of a standardised sampling regime, since differences due to canopy position are larger than some treatment differences (e.g. Fig. 5.1 and Table 5.1). The increased chlorophyll content with decreasing position in the canopy is consistent with findings of Lewandowska and Jarvis (1977), and is a typical response to shade conditions (Boardman, 1977). The decrease in the chlorophyll a/b ratio in "shade" shoots, suggests that the increase in total chlorophyll is largely due to an increase in the light-harvesting pigment, chlorophyll b, so providing a more efficient system of light capture in a light-limiting environment (Goodchild et al., 1972). This is also in agreement with Alberte et al. (1976), who suggest that "shade" needles may have fewer and larger photosynthetic units than "sun" needles, and it has been shown that "sun" needles have a larger capacity for electron transport through photosystem I and II than "shade" needles (Lewandowska et al., 1977; Lewandowska and Jarvis, 1978). The tendency of RuBPC activity to decrease at lower canopy positions (Table 5.6), especially per unit chlorophyll, is to be expected for "shade" leaves (Bjorkman, 1968), and it is supported by Beadle (1977), who found higher activities of RuBPC, on an area basis, in "sun" needles rather than in "shade" needles.

5.9.8 **Effect of Needle Age on Needles and Photosynthesis.**

It is reported that in leaves with a duration of more than one year, P_N decreases with leaf age (Field and Mooney, 1983). In conifers, the effect of age on P_N has been studied in 6 species by Freeland (1952), who found that P_N, expressed per needle, reached a maximum at the time of needle maturity in the first season, and then declined with increasing age, beginning with the second season. However, Stafelt (1924) concluded that for spruce, photosynthetic rate per g fr.wt. decreased with increasing needle age, but only because the weight of the needles increased with age, and the photosynthetic rate per number of needles increased with increasing age for five years before declining. The data show slightly higher rates of P_N in current needles rather than in one year old needles in all treatments studied, apart from -All. The significant differences in G_S in Table 5.13 do not account for the differences in P_N, although the C_i values also showed no significant differences between age classes apart from the -All treatment, where the significantly lower values found in one year old needles accord with the higher measured P_N. The data for G_S contrast with those of Ludlow and Jarvis (1971) who found G_S and G_M to decrease with increasing needle age. A general comment here is that the sample size of two trees was not large enough to detect small differences in the parameters measured, given the tree-to-tree variation which existed. The total chlorophyll content of Controls did not vary significantly between the two age classes, whereas Koch (1976) found chlorophyll a and b to increase per unit dry matter in spruce, from current to 5 year old sun and shade needles. Wood (1974), however, found the chlorophyll content per unit dry matter or needle area to decrease from 1 to 4 year old needles. Linder (1972) also found that total chlorophyll content per g fresh weight was higher in current needles of Scots pine and Norway spruce compared with one year old needles. The higher carotenoid content in 1 year old rather than current needles from all treatments (Table 5.8) is supported by Godnev et al. (1969) for *Picea pungens* and *Picea excelsa*, who found that a maximum content was not achieved for several years; whether greater values would have been found in years 2 onwards remains unknown. Gezelius and Hallen (1980) found slightly higher RuBPC activities in 1 and 2 year old needles of mature Scots pine than in current needles, on a dry weight basis, but successively lower values with increasing needle age per unit chlorophyll. Activity of RuBPC was not found to vary between needle age classes on a g fr.wt. or mg chlorophyll basis (Table 5.8),

again, possibly because the sample size did not allow small differences to show significance. It may be that differences in the photosynthetic variables with nutrient stress become more pronounced as trees become older. It is interesting that the increase in total chlorophyll content, P_N, and RuBPC activity following refertilisation of -All and -N treatments also occurs in 1 year old needles, and to the same extent as in current needles. This demonstrates how plastic the response of the photosynthetic apparatus is to changes in nutrient availability. It also shows that considerable synthesis of chlorophyll and photosynthetic enzymes can occur in fully developed chloroplasts, which are likely to have had fewer and more compartmentalised grana, as a result of N deficiency (Vesk et al., 1966; Thomson and Weier, 1962).

CHAPTER 6. RESULTS. THE EFFECT OF SUPPLIED NITROGEN ON NEEDLE CHARACTERISTICS AND PHOTOSYNTHESIS

6.1 Introduction.

Results from the 1988 experiment (Chapter 3), showed that the greatest reduction in all morphological and photosynthetic variables measured was in needles from -N and -All treatments, and that there were spectacular increases in these variables on refertilisation of these treatments. Sitka spruce is widely grown on nitrogen-deficient sites, and it was decided to study the effect of refertilising N-deficient trees, in more detail, and in particular, to attempt to quantitatively correlate the degree of response to the amount of supplied nitrogen. Trees were therefore supplied with a range of nitrogen concentrations (see Materials and Methods, section 2.2.3), and the same morphological and photosynthetic characteristics were measured as in the 1988 experiment. The sample size was increased to three trees per treatment, to allow more detailed statistical analyses to be performed, in particular, analyses of variance (see Materials and Methods, section 2.16). Results are shown below, with an analysis of variance table for each variable. Following each table is a list of the treatments, in ascending order of their means for that variable. Treatments having non-significant differences ($p < 0.05$) are underlined. In each case, degrees of freedom are 7 for treatments and 16 for error. In each figure, the Control value, for trees not subjected to any deficiency of nitrogen, and fertilised with Hockings solution in 1988 and 1989, is plotted as a broken line across all concentrations of N, for comparison.

6.2 Results.

6.2.1 Leader Length.

Mean leader length increased with increasing concentration of supplied nitrogen, to a maximum with 112 p.p.m. N (Fig. 6.1). With 112 p.p.m. and 224 p.p.m. treatments, the length of the leading shoot did not differ significantly from that of the Controls. Leader length was significantly reduced with zero and 7 p.p.m. nitrogen treatments, compared with the Controls. Trees in all treatments apart from zero, 7 p.p.m. and some trees in the 14 p.p.m. treatment showed free growth. Since harvest in these treatments occurred before free growth had ceased, leader lengths at the end of the season would be greater

in these trees, so that differences between Controls and trees in the most deficient treatments would be greater than shown in Fig. 6.1.

Leader Length (mm)

Source of Variation	Mean Square	Variance Ratio
Treatment	5235.80	5.66 **
Error	924.38	

Treatment	**7**	**0**	**14**	**28**	**56**	**+All**	**224**	**112**
Means	16.7	23.3	48.0	67.7	87.7	105.0	118.3	124.3

6.2.2 **Needle Number Per Shoot.**

Mean needle number per shoot increased with increasing N concentration (Fig. 6.2), and analysis of variance showed needle number per shoot with zero, 7 and 14 p.p.m. N to be significantly lower than that of the Controls. The value with zero N was equivalent to that in the -N treatment in 1988 (section Chapter 3, Table 3.3).

Needle Number Per Shoot

Source of Variation	Mean Square	Variance Ratio
Treatment	11099.8	14.93 ***
Error	743.4	

Treatment	**0**	**7**	**14**	**28**	**56**	**112**	**+All**	**224**
Means	52	63	88	141	176	180	188	205

6.2.3 **Needle Length.**

The response of needle length to treatment was more variable than that found for leader length; shortest needles were found in the zero and 7 p.p.m. treatments, and needles in zero, 7 and 28 p.p.m. treatments were all significantly shorter than the Controls (Fig. 6.3). Maximum needle length was obtained with 112 p.p.m. nitrogen. Mean needle lengths were longer in 1989 than in 1988; for example, needles in the zero, 112 p.p.m. and Control treatments were 7.6, 14.5 and 12.6 mm long, compared with lengths of 5.8, 10.3 and 10.5 mm for the -N, -NR and Controls in 1988. This probably reflects the warmer spring in 1989.

Needle Length (mm).

Source of Variation	Mean Square	Variance Ratio
Treatment	18.15	6.11 **
Error	2.97	

Treatment	**7**	**0**	**28**	**14**	**56**	**224**	**+All**	**112**
Means	7.31	7.62	9.54	10.22	11.50	11.67	12.60	14.53

6.2.4 **Needle Projected Area.**

Changes in needle projected area with nitrogen concentration showed the same pattern as needle length, with the exception of a higher projected area at 7 p.p.m. than with zero nitrogen (Fig. 6.4). All treatments up to and including 28 p.p.m. had significantly lower projected areas than Control needles. Because needle projected area with 14 p.p.m. was significantly lower than in the Controls, whilst needle length was not, implies that needle width was smaller with 14 p.p.m. than in the Controls. Values for zero, 112 p.p.m. N and Control treatments were up to 50% higher than for corresponding -N, -NR and Control treatments in 1988 (compare values in Fig. 6.4 with Table 3.11).

Needle Projected Area (mm^2).

Source of Variation	Mean Square	Variance Ratio
Treatment	11.21	5.84 **
Error	1.92	

Treatment	**0**	**7**	**28**	**14**	**224**	**56**	**+All**	**112**
Means	3.84	4.59	5.78	5.98	7.29	7.32	8.35	9.66

Figure 6.1

Leader length in early August 1989, plotted against concentration of nitrogen supplied to nitrogen-deficient pot trees. Each point is a mean of three values ± S.E., each from dfferent trees. The broken horizontal line represents the Control value (also a mean of three measurements), and the vertical bar on the right reresents the S.E. of the Controls.

Figures 6.2 to 6.4

Needle number per shoot, mean needle length per shoot, and mean needle projected area per shoot, for terminal shoots from whorl two of Nitrogen-deficient pot trees refertilised with a range of nitrogen concentrations. Replication and symbols are as for Figure 6.1.

Figure 6.1

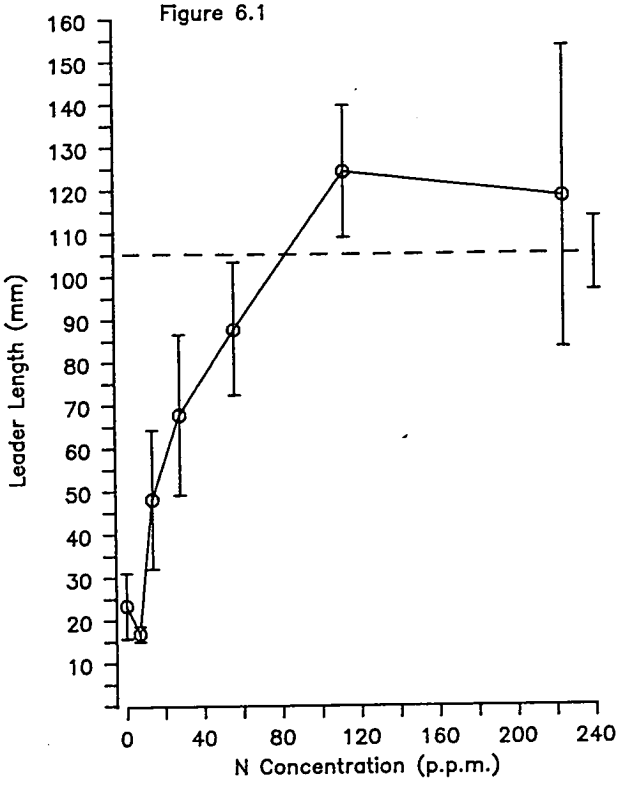


Figure 6.2

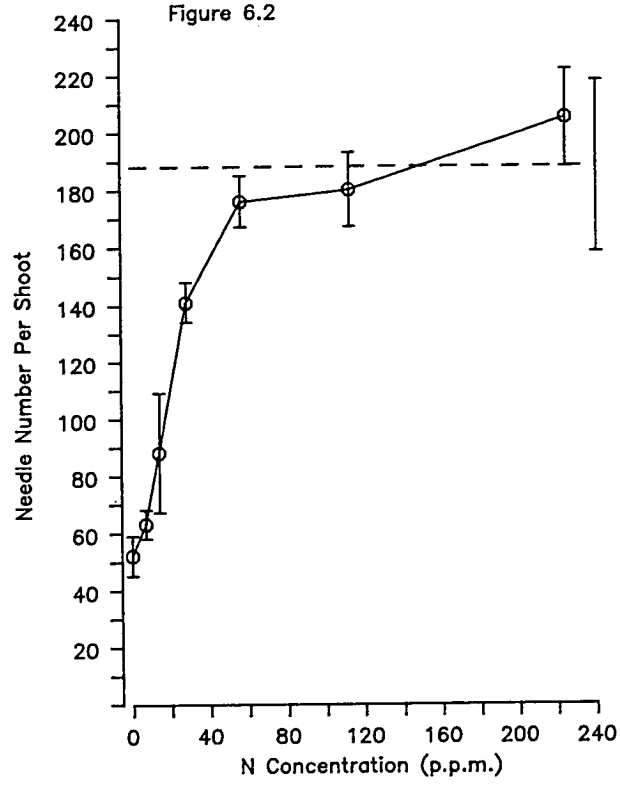


Figure 6.3

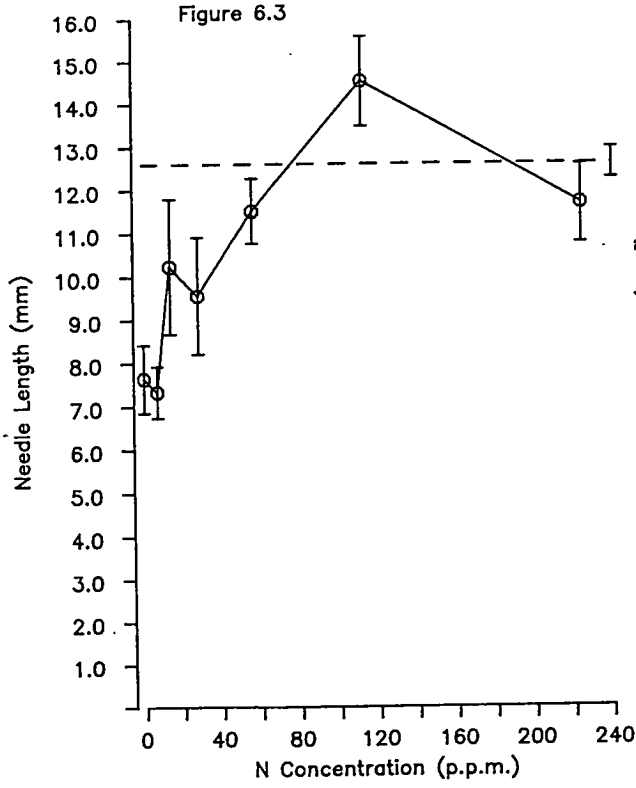
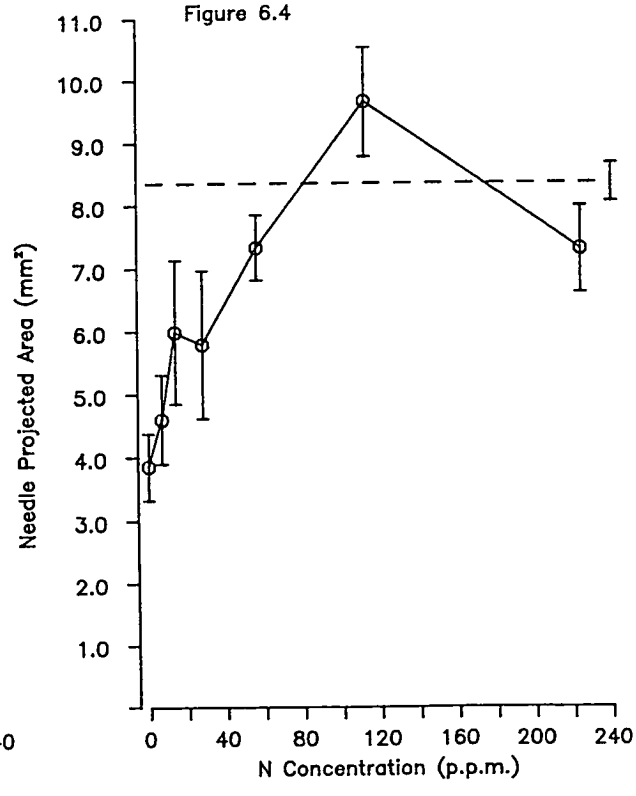


Figure 6.4



6.2.5 **Needle Cell Number, Fresh Weight and Cell Volume.**

For needles expanding from primordia initiated in 1988, cell number increased with increasing N concentration above 28 p.p.m. Needles with zero and 7 p.p.m. N had similar cell numbers to those with 14 p.p.m. N (Fig. 6.5.) The same pattern was seen for the change in fresh weight with N concentration, for the same needles initiated in 1988 (Fig. 6.6). For needles initiated in 1989, increases in cell number and fresh weight were found with increasing N supply (Figs. 6.5 and 6.6), apart from with 224 p.p.m. N, where the values were less than with 112 p.p.m. N. These treatment differences in cell number and fresh weight have not been tested for significance against the Controls, for needles initiated in 1988 or 1989, because the position on the shoot from where the needles were taken, differed between the Controls and the other treatments (see Materials and Methods, section 2.2.3), and this limited meaningful interpretations from an analysis of variance. Data for needle cell number and fresh weight for needles initiated during the 1989 season are higher than for those initiated in 1988, for all treatments studied apart from 224 p.p.m. N. Since needles initiated in different seasons had to be taken from widely different parts of the same shoot (see Materials and Methods, section 2.2.3), statistical analyses were not performed, as it was impossible to tell whether any differences were due to time of primordium initiation (and therefore the current nutritional environment), or merely due to positional effects of the needle on the shoot. The main aim was to see whether mean fresh weight per cell, which was not found to differ much with needle position on the shoot, in 1987, was affected in needles arising from free growth (and hence initiated during exposure to different degrees of nitrogen availability, rather than nitrogen deficiency).

Data show that for needles expanding in 1989, from primordia laid down in 1988 or 1989, there was no significant difference in mean cell volume (as estimated as mean fresh weight per cell) in any of the treatments, compared with the Controls (Fig. 6.7). The Control values of needle cell fresh weight for current needles initiated in 1988 or 1989 were almost identical, so that differences in other treatments between both sets of data were not significant. Therefore, even though increasing the degree of refertilisation increases needle length, this was not due to an increase in cell size, irrespective of whether the expanding primordium was initiated during the previous or current year.

Figures 6.5 to 6.7

Needle cell number (thousands), fresh weight (mg), and mean fresh weight per cell ($x10^{-8}$ g), measured in early August 1989, 1989, for needles from terminal shoots from whorl 2 of nitrogen-deficient trees refertilised with a range of nitrogen concentrations. Data are for needles expanded from preformed primordia (circles), or primordia initiated by free growth (crosses). Each point is the mean of three values, each from different trees ± S.E. Broken horizontal lines represent Control values (also means of three measurements), for needles expanded from preformed primordia (1988), or from newly initiated primordia (1989). The vertical bar on the right represent the S.E. of the Controls.

Figure 6.5

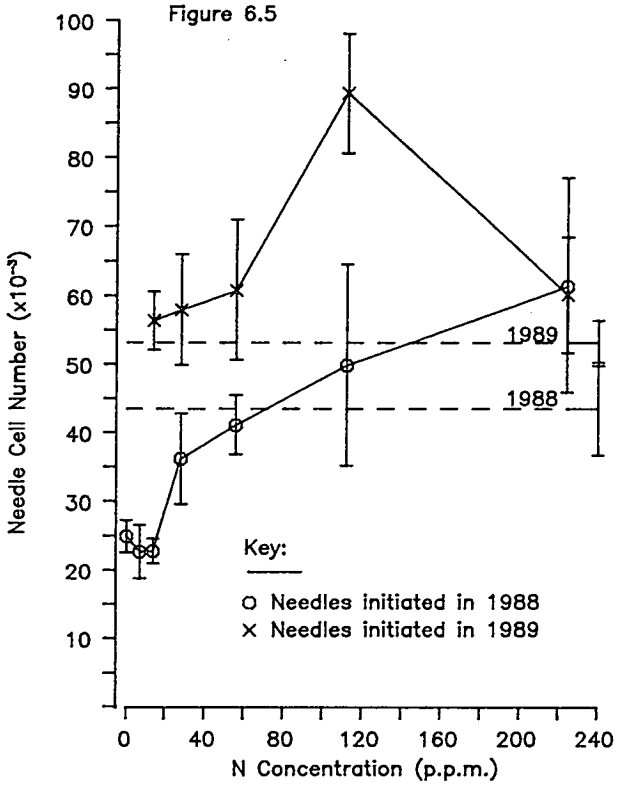


Figure 6.6

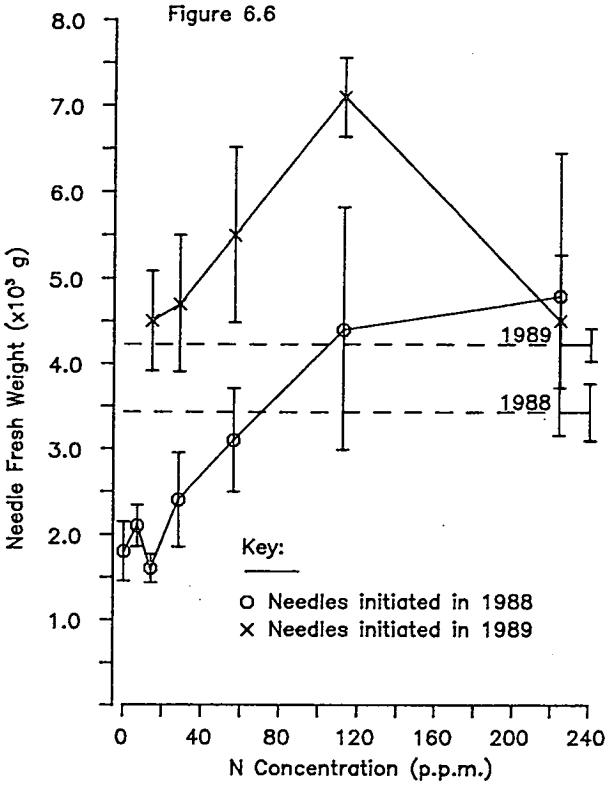
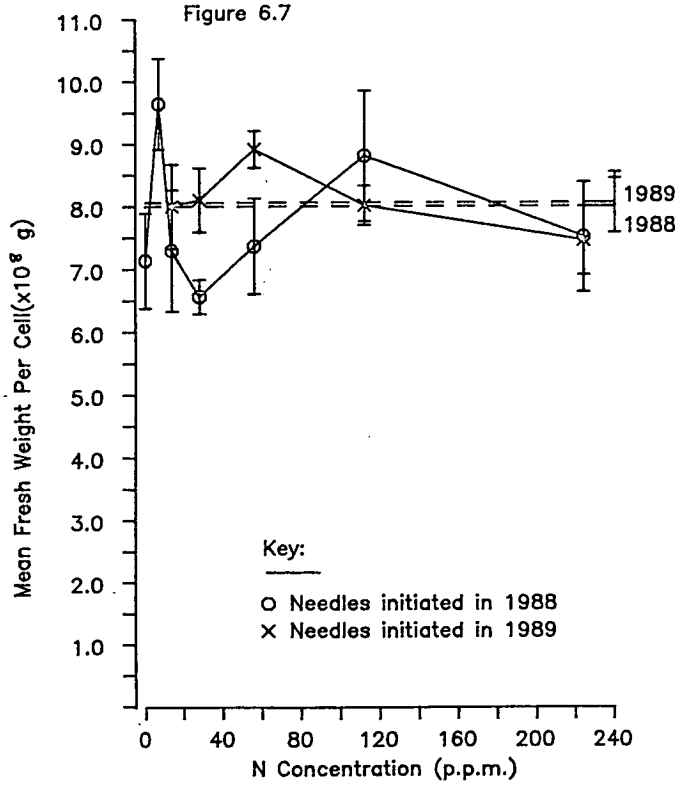


Figure 6.7



Mean Fresh Weight Per Cell:

a) Needles Initiated In 1988.

Source of Variation	Mean Square	Variance Ratio
Treatment	1.68	2.13 n.s.
Error	0.79	

b) Needles Initiated In 1989.

Source of Variation	Mean Square	Variance Ratio
Treatment	0.66	1.12 n.s.
Error	0.60	

6.2.6 Total Chlorophyll Content.

Total needle chlorophyll content increased with increasing N concentration, except that values for zero p.p.m. were slightly, but not significantly higher than with 7 p.p.m. N (Fig. 6.8). Values for needles in Control and 112 p.p.m. treatments were equivalent, but chlorophyll content with 224 p.p.m. was significantly higher than for the Controls. A total chlorophyll content of about 2.0 mg g^{-1} fr.wt. for Control and 112 p.p.m. treatments is equivalent to the values obtained for Control and -NR treatments in 1988 (Chapter 5, Fig. 5.2 A and F), but a mean of 1.24 mg g^{-1} fr.wt. for zero N is about twice the value for -N needles at the same time in 1988.

Total Chlorophyll Content (mg g^{-1} fr.wt.)

Source of Variation	Mean Square	Variance Ratio
Treatment	0.53	8.67 ***
Error	0.06	

Treatment	7	0	14	28	56	112	+All	224
Means	1.14	1.24	1.61	1.82	1.86	1.95	1.95	2.44

6.2.7 **Chlorophyll a/b Ratio.**

Although the chlorophyll a/b ratio increased between zero and 28 p.p.m. N, and the qualitative response of the ratio was similar to that shown by other variables (Fig. 6.9), no differences between treatments were significant. This contrasts with the significant reduction in chlorophyll a/b ratio seen in N-deficient needles in 1988 (Chapter 5, Fig. 5.4 E). Values for 112 p.p.m. N and Controls of about 2.9 were slightly higher than the values of 2.5-2.7 obtained in -NR and Control treatments in 1988 (Chapter 5, Fig. 5.4 E and A).

Chlorophyll a/b Ratio.

Source of Variation	Mean Square	Variance Ratio
Treatment	0.047	0.96 n.s.
Error	0.049	

6.2.8 **Carotenoid Content.**

Carotenoid content showed similar changes with N concentration as total chlorophyll content, although 56 p.p.m. N gave lower values than with 28 p.p.m.N (Fig. 6.10). Needles in the 224 p.p.m. treatment had a significantly higher carotenoid content than the Controls, and values with zero and 7 p.p.m. were slightly lower. The mean carotenoid content of needles in zero, 112 p.p.m. and Control treatments was between two and three times greater than the values for needles in -N, -NR and Control treatments in 1988 (Chapter 5, Fig. 5.6 E and A).

Carotenoid Content (mg g^{-1} fr.wt.)

Source of Variation	Mean Square	Variance Ratio
Treatment	0.11	10.57 ***
Error	0.01	

Treatment	**7**	**0**	**14**	**28**	**56**	**+All**	**112**	**224**
Means	0.54	0.62	0.81	0.89	0.86	0.94	0.95	1.15

Figures 6.8 to 6.11

Total chlorophyll content, chlorophyll a/b ratio, carotenoid content and RuBPC activity measured in early August 1989, in needles from shoots from whorl 2 of nitrogen-deficient pot trees refertilised with a range of nitrogen concentrations. Each point is a mean of three values, each from different trees ± S.E. The broken horizontal line represents the Control value (also a mean of three measurements), and the vertical bar on the right represent the S.E. of the Controls.

Figure 6.8

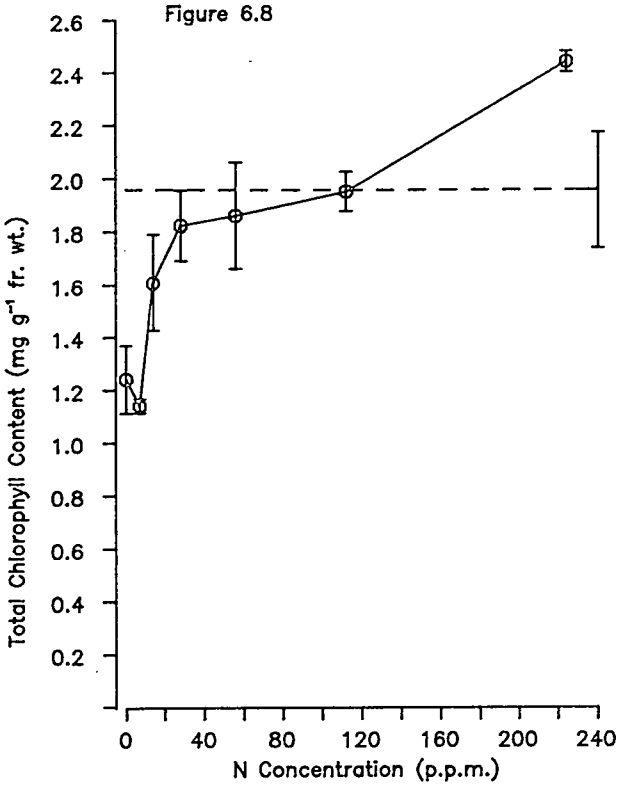


Figure 6.9

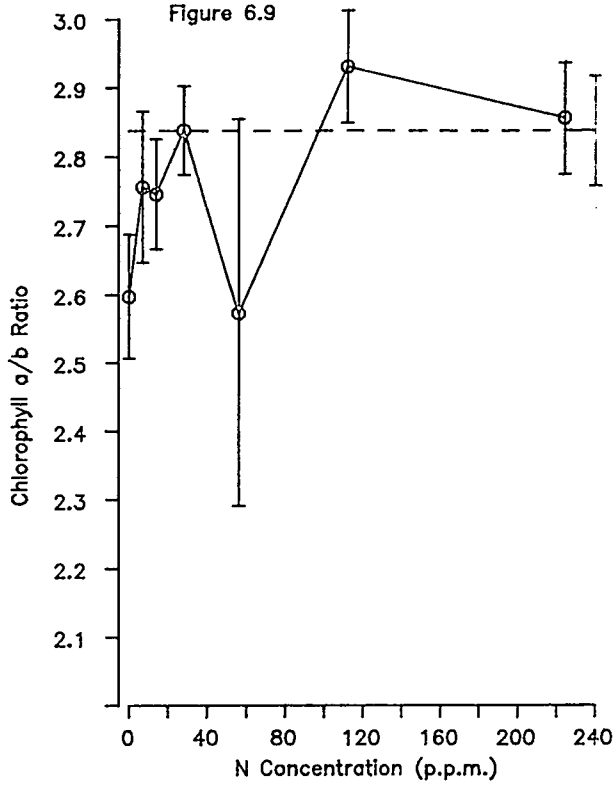


Figure 6.10

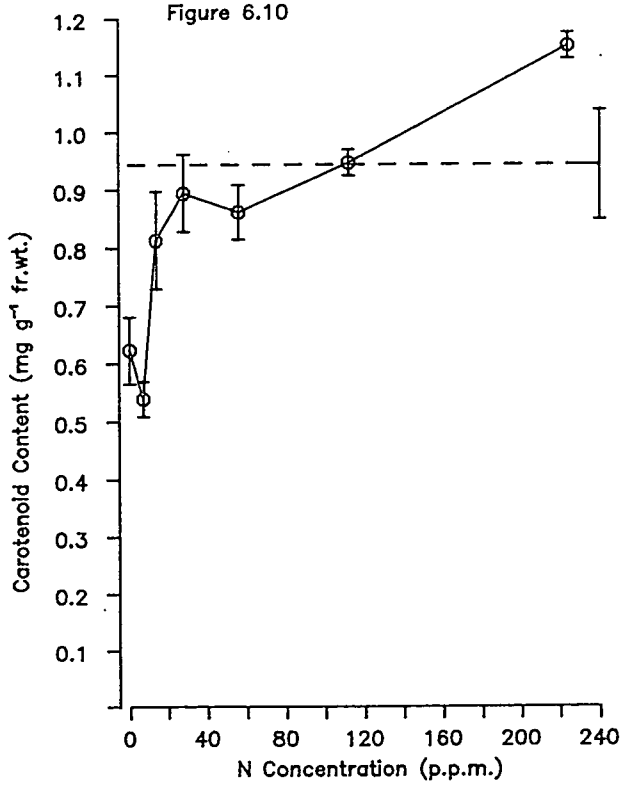
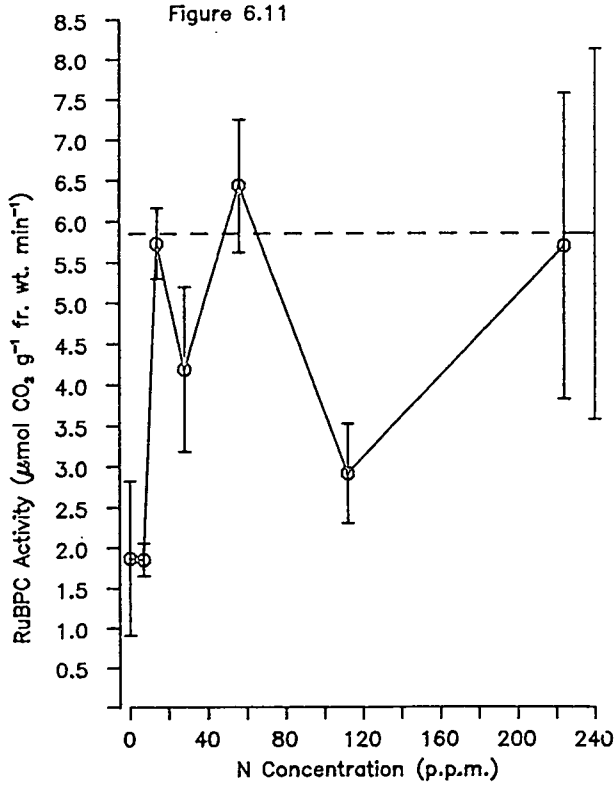


Figure 6.11



6.2.9 **RuBPC Activity.**

The data for RuBPC activity show considerable variation, with coeffecients of variation as large as 89% for the zero p.p.m. treatment. Analysis of variance on the data showed RuBPC activity per g fresh weight was not significantly different to the Controls in any treatment, but zero and 7 p.p.m. N gave lower mean RuBPC activity than other treatments (Fig. 6.11). RuBPC activity in the Controls was about half the value measured in 1988 (Chapter 5, Fig. 5.7 A), and activity with zero and 112 p.p.m. N were also much lower than that found for -N and -NR needles in 1988 (Chapter 5, Fig. 5.7 E). Because of the variation in chlorophyll content with varying N supply, analysis of variance showed RuBPC activity per unit chlorophyll did not differ with treatment.

RUBPC Activity.

a) μmol CO_2 g^{-1} fr.wt. $min.^{-1}$

Source of Variation	Mean Square	Variance Ratio
Treatment	10.65	2.38 n.s.
Error	4.47	

b) μmol CO_2 mg^{-1} Chl $min.^{-1}$

Source of Variation	Mean Square	Variance Ratio
Treatment	1.46	1.3 n.s.
Error	1.12	

6.2.10 **Net Photosynthetic Rate.**

P_N increased rapidly with increasing N supply up to 14 p.p.m., and a plateau was then reached (Fig. 6.12), probably because light was not saturating in the controlled environment room used. Only shoots with no supplied nitrogen had a significantly lower photosynthetic rate than the Controls, and the rate was negative, showing that respiration exceeded photosynthesis. Values for zero, 112 p.p.m. and Control treatments were much lower than for corresponding -N, -NR and Control treatments in 1988 (Chapter 5, Fig. 5.9 E and A). This also, is probably due to the lower light intensities used in 1989.

Net Photosynthetic Rate (μmol CO_2 m^{-2} s^{-1}).

Source of Variation	Mean Square	Variance Ratio
Treatment	4.13	4.68 **
Error	0.88	

Treatment	0	7	+All	28	112	14	56	224
Means	-0.65	1.82	2.43	2.53	2.57	2.71	2.79	2.88

6.2.11 Stomatal Conductance to CO_2 Transfer, and Intercellular Partial Pressure of CO_2.

Stomatal conductance increased with increasing N supply, reaching a plateau with concentrations above 112 p.p.m. N (Fig. 6.13). treatments with up to 28 p.p.m. N gave lower values of G_S than the Controls, although no differences between treatments were significant. The value of C_i decreased with increasing concentration of nitrogen (Fig. 6.14), and analysis of variance showed that values with zero p.p.m. N were significantly higher than for all other treatments.

Stomatal Conductance, G_S.

Source of Variation	Mean Square	Variance ratio
Treatment	4.01×10^{-3}	2.44 n.s.
Error	1.65×10^{-3}	

Intercellular Partial Pressure of CO_2, C_i (μ bar).

Source of Variation	Mean Square	Variance Ratio
Treatment	2446.88	9.81 ***
Error	249.33	

Treatment	+All	112	224	56	14	28	7	0
Means	293	297	301	304	322	324	342	378

Figures 6.12 to 6.14

Net photosynthetic rate, stomatal conductance to CO_2, and inter-cellular partial pressure of CO_2, measured in early August 1989 for terminal shoots from whorl two of nitrogen-deficient pot trees, refertilised with a range of nitrogen concentrations. Each point is the mean of three values, each from different trees ± S.E. The broken horizontal line represents the Control value, and the vertical bar on the right represents the S.E. of the Controls.

Figure 6.12

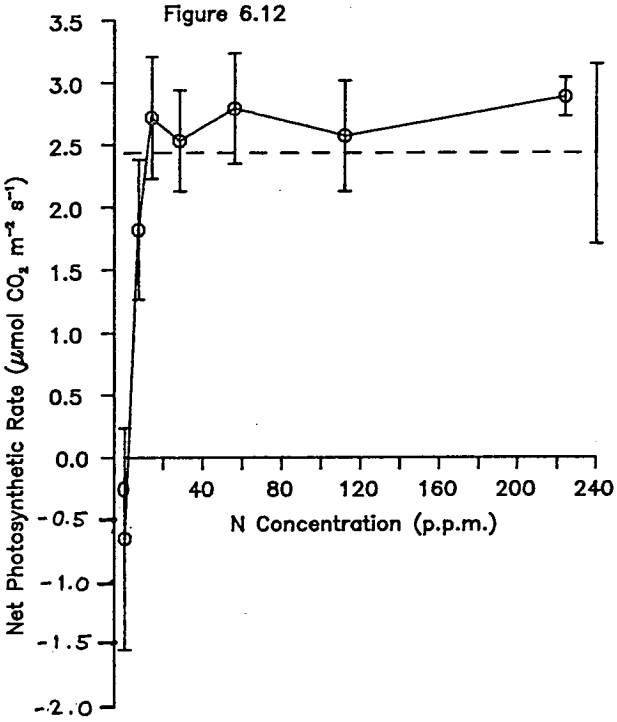


Figure 6.13

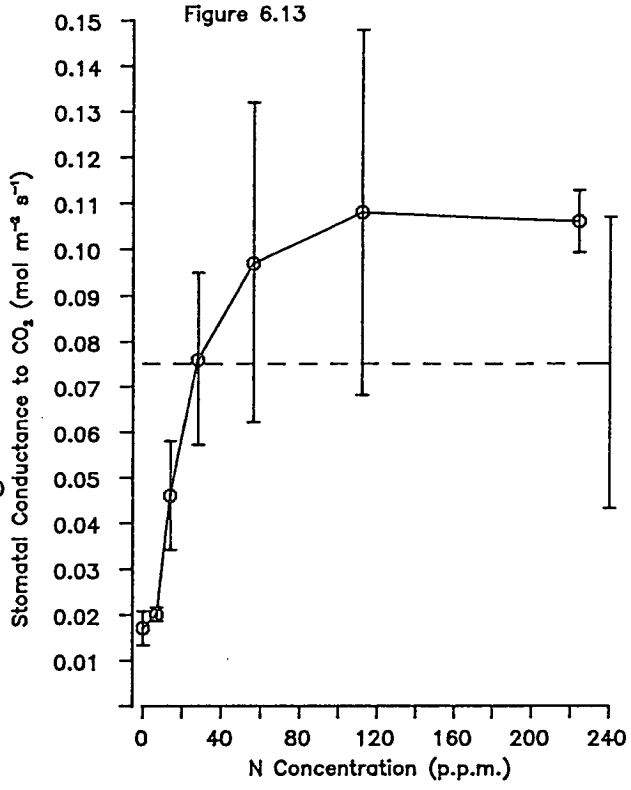
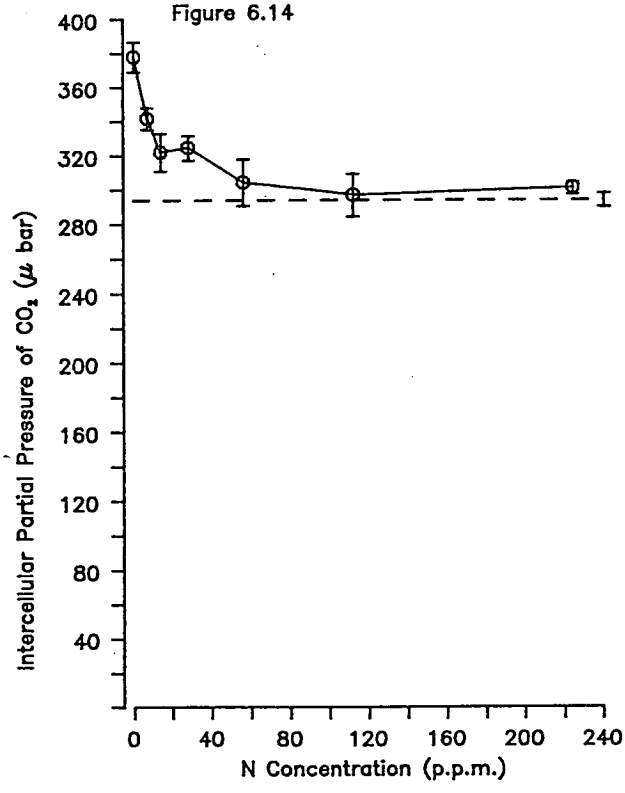


Figure 6.14



6.3 Discussion.

The effects on needle morphology in response to refertilisation of N-deficient trees in 1989 were the same as in 1988; namely a significant increase in needle length and projected area with increased N supply. Deficiency of nitrogen usually causes an inhibition of leaf growth (Radin, 1983), for a range of broadleaved species. Nitrate has more effect on leaf growth than any other major nutrient (Trewavas, 1985), and addition can cause final leaf area to increase over three-fold (Dale, 1982; Terry et al., 1983). For trees, it was found that an increase in N supply increased the rate of leaf area increase for N-deficient birch seedlings (Ingestad and Lund, 1979), and N fertilisation of Douglas fir caused an increase in needle length and width (Brix and Ebell, 1969). It is not known whether these effects are primarily due to an increase in cell size, or cell number of the leaf. Terry (1970) found that increased nitrogen supply in sugar beet increased mean cell size, as well as the number of cells per leaf and the rate of cell expansion. Radin and Parker (1979) also found that cell size was related to N nutrition in cotton plants, with smaller cells being the result of N deficiency. This is suggested to be a result of a decrease in hydraulic conductivity in the roots, which causes water deficit in expanding leaves (Radin and Boyer, 1982). In the present study however, no effect on mean cell size was found with any concentration of N, although higher N concentrations caused larger needles with more cells, The effect of nitrogen nutrition therefore, is on cell division rather than on cell expansion. Differences in needle size at different nitrogen concentrations is determined by cell number, regardless of whether needles were initiated during the previous season, during N-deficient conditions, or during the current season through free growth, during favourable nutrient conditions. This conclusion is the same as that in Chapter 4, that differences in needle size between treatments are primarily determined by differences in cell number. It is not known whether the greater needle cell number due to N addition is due to a faster rate of cell division, or a longer duration of the period of cell division. These alternatives can not be further distinguished, as cell number was not measured throughout the season.

The correlation between chlorophyll content and N concentration, was also shown for Poplar (Keller and Koch, 1962) and for *Gossypium* (Sage et al., 1987). The increases in the chlorophyll a/b ratio which accompanied the increase in total chlorophyll content with up to 28 p.p.m. N, is the same response found

following refertilisation of N-deficient trees in 1988. This suggests that nitrogen nutrition has an effect not only on chlorophyll content, but on the distribution of different chlorophylls, with a higher proportion of chlorophyll a than chlorophyll b being present at low N concentrations. These data are in contrast to data of Evans and Terashima (1987), in studies with spinach, who found that N nutrition had no effect on the chlorophyll a/b ratio. The present data contrast even more markedly with those of Gezelius (1986), who found that an increase in total chlorophyll content in needles of Scots pine seeedlings with 10 and 50 p.p.m. N compared with 2.5 p.p.m. N, was accompanied by a decrease in the chlorophyll a/b ratio. This difference in response of Sitka spruce and Scots pine seedlings to N supply cannot be explained, but may represent differences in the size and composition of the photosynthetic units between species. The increase in the chlorophyll a/b ratio with increasing N supply is the same response found in needles at low light intensities near the base of the canopy (Chapter 5, Table 5.6), and may be a general stress response to limiting conditions for photosynthesis, to provide a more efficient system for light capture. Carotenoid content of needles here were higher than in 1988 and 1987, and may be explained by the spring and summer in 1989 being much warmer and sunnier than in 1988 and 1987, so that needles were exposed to higher light intensities, which may have caused the synthesis of more carotenoids to protect the chlorophylls from photobleaching. It is interesting to note that for pigment data, and for many of the other variables studied, a concentration of 28 p.p.m. N was enough to restore values to similar levels as the Controls. The very low concentrations of N needed to limit growth and photosynthesis severely, suggests that Sitka spruce is tolerant to low N supply, and that it responds well to very small additions of N, which is an important feature for any management practice.

A strong correlation has been reported between RuBPC activity and N availability (Medina, 1971a - for *Atriplex*; and Wong, 1979 - for several C_3 and C_4 spp.; Mooney et al., 1983 - for 6 *Eucalyptus* spp.; and Field et al., 1983 - for 5 spp. of evergreen trees and shrubs). In the present study, RuBPC activity and N supply are poorly correlated, although the reduction in RuBPC activity with the lowest nitrogen concentration treatments, despite not being significant, agrees with findings of other workers (Gezelius, 1986 - for Scots pine; Sage et al., 1987 - for *Chenopodium album* and *Amaranthus retroflexus*). The decrease in RuBPC activity was not as great as that found for the -N treatment in 1988,

which may be due to variation in material, coupled with different prevailing climatic conditions in different summers. It is not known to what extent the decrease in RuBPC activity with increasing N deficiency is caused by a change in the specific activity of the enzyme, or merely lower amounts of enzyme, however, Gezelius (1986) found the specific activity of RuBPC in needles of Scots pine was slightly reduced with 2.5 p.p.m. N compared with that at 10 and 50 p.p.m. N.

It is known that the RUBISCO protein can act as a storage of N in many species, and plays an important role in N cycling within plants; increasing the N application to potato plants from zero to 25 g m^{-2} caused concentrations of RUBISCO in the leaves to increase by 120%, and during tuber growth, Rubisco-N was mobilised more readily than N from other proteins (Millard and Catt, 1988). In conditions of favourable N supply, the concentration of RUBISCO has also been shown to increase for leaves of *Triticum* (Lawlor et al., 1987a), and by more than that of other soluble proteins (Huffaker, 1982 - for *Hordeum vulgare*; Yamashita, 1986 - for *Morus alba*). The acumulation of RUBISCO in leaves was not associated with a change in the specific carboxylation activity for rice (Makino et al., 1984), or by a change in the rate of carbon assimilation for *Triticum aestivum* (Lawlor et al., 1987b). It has been suggested (Lawlor et al., 1987b), that for wheat, under conditions of high N supply, only half the RUBISCO catalytic sites are activated and functional, supporting the hypothesis that the protein is accumulated for storage of N, as well as having a catalytic role. From the data, it cannot be determined whether the concentration of RuBPC in the needles increases with increasing N supply, although activity does show some correlation with N supply, especially at low concentrations. Although the estimates of RuBPC activity with zero N are higher than for the -N treatment in 1988, those at 112 p.p.m. are lower than for -NR needles in 1988. This may be due to seasonal differences in material, or to the prevailing climate during needle development.

In view of the key role of RuBPC as a rate-limiting factor in photosynthesis (Medina, 1971a; Woolhouse and Batt, 1976; Avdeeva and Andreeva, 1973), and that P_N and RuBPC activity vary in parallel with nitrogen availability (Medina, 1971b - for *Atriplex hastata*), supported by this study, evidence is strong that P_N is determined by RuBPC activity. Alternatively, in nitrogen deficient conditions, P_N may determine RuBPC activity, by a regulation of nitrogenous compounds to reflect photosynthetic capacity, or both P_N and RuBPC may be

affected by other parameters in response to nitrogen deficiency.

A line of evidence indicating that RuBPC activity rather than other nitrogenous compounds controls P_N, is from studies where RuBPC protein was not a constant proportion of the total soluble protein, and yet P_N was better correlated with the amount of RuBPC than of total protein (Medina, 1971b; Wittenbach, 1983). From the present data is seems likely that a biochemical limitation on P_N by RuBPC activity exists, and can partly explain reduced P_N at low N concentrations. Field and Mooney (1986), working with a group of 21 spp. of trees, shrubs and herbs suggest that variation in RuBPC alone cannot account for the P_N/N relationship, as levels of many nitrogenous compounds must be changing in concert. They propose that limitation of P_N by N may also operate via an effect on RuBPC regeneration; the investment of N in the components of RuBPC regeneration is substantial, and possibly greater than that in RuBPC (Kirk and Tilney-Basset, 1978). A low allocation of N to carbonic anhydrase, which catalyses the interconversion of CO_2 and bicarbonate, to help deliver CO_2 to the sites of fixation, can decrease P_N by a reduction of CO_2 transport (Gowan, 1986). No data are available for Sitka spruce from this study, on effects of N supply on RuBPC regeneration, nor on the effect on liquid-phase transfer of CO_2, but possible effects on P_N via CO_2 diffusion can be considered.

Shimshi, (1967) found that N deficiency in wheat decreased stomatal aperture, which would be expected to decrease G_S, and an increase in stomatal and mesophyll resistance to CO_2 transfer has also been found as a result of N deficiency for several species (maize - Ryle and Hesketh, 1969; sugar beet - Nevins and Loomis, 1970; barley - Natr, 1970). The decrease in G_S with increasing N deficiency in the present study support these findings, and may be due directly to a reduction in the size of the stomatal aperture. However, Longstreth and Nobel (1980) found little effect of N deficiency on G_S in *Gossypium*, but G_M decreased with decreasing N concentration. The intercellular concentration of CO_2 also decreased with increasing nitrogen supply. This decrease is slightly larger than that found between the comparable -N and -NR treatments in 1988 (Chapter 5, Fig. 5.12 E), where C_i was only slightly reduced by nitrogen refertilisation and must mean that the efficiency of CO_2 transfer into the mesophyll cells increases with increasing nitrogen supply. It is therefore likely that the mesophyll conductance to CO_2, (G_M), increases in parallel with the increase in G_S with increasing N supply, but

to a greater extent, allowing C_i to decrease. This points to a greater importance of G_M than G_S in controlling CO_2 availability to the cells. This might partly explain the increase in P_N observed up to 14 p.p.m. N, and P_N at higher N concentrations is probably limited by light intensity, which was lower than for the 1988 experiment. The reason for the greater response of C_i to N supply in 1989 than in 1988 may be due to differences in experimental design; in 1989 the wide range of treatments, with a sample size of three, made it easier for differences to be statistically quantified, in comparison with 1988, where n was only two, and there were only two treatments, namely the presence or absence of nitrogen. The data for 1989 is also for one sample time in August, compared with the larger number of sample times in 1988, so that although G_M was found to be more important in 1989, it does not preclude the possibility that it is less important earlier in the season. Since needle development is dependent on environmental conditions during development (Terry et al., 1983), it is also possible that the different conditions in 1988 and 1989 caused slight differences in leaf architecture, affecting G_S and G_M.

A quantitative correlation has been found between foliar N concentration and P_N (Natr, 1975; Brix, 1981 - for Douglas fir; Keller, 1972 - for Norway spruce; Keller and Koch, 1962 - for poplar; Medina, 1971a - for *Atriplex*; Wong, 1979 - for several C_3 and C_4 spp.). The present data show a similar relationship between P_N and concentration of supplied N (Fig. 6.12), although at higher N concentrations, light levels in the controlled environment room probably limit P_N. A distinction must be made here between concentration of supplied N on which the correlation between N and P_N is based here, and foliar N concentration, on which most of the correlations in the literature are based, and for which data are not available in this study.

As 75% of the nitrogen in a leaf is contained in chloroplast proteins (Stocking and Ongun, 1962; Morita and Kono, 1975), it is more likely that N deficiency effects operate at the chloroplast level as well as altering the availability of CO_2 to the cells. Even though N deficiency decreases the amount of chlorophyll, the rate of electron transport, amount of thylakoid components, and the rate of oxygen evolution, each expressed per unit chlorophyll, are unaffected (Medina, 1971a for *Atriplex*; Evans and Terashima, 1987; Terashima and Evans, 1988 for spinach). This suggests that N deficiency decreases the amount of thylakoids without altering their properties. Although the data (Fig 6.11) show variable RuBPC activity with N treatment, the general

decrease in activity with decreasing N concentration shows the same trend as chlorophyll content, so that the ratio of RuBPC activity to chlorophyll content is independent of the N supply. In *Atriplex* (Medina, 1971a), and *Gossypium* (Wong, 1979), the ratio of RuBPC protein and chlorophyll content was found to decrease with increasing N stress, but in *Phaseolus* (Caemmerer and Farquhar, 1981), and *Triticum* (Evans, 1983; 1985), the ratio was independent of N nutrition. Because electron transport activity remains independent of N nutrition, but that of RuBPC decreases with increasing N stress, the efficiency of carboxylation decreases. So although low P_N in N-deficient trees is partly explained by low RuBPC activity, because the increase in activity on refertilisation, which was much more marked in 1988 (Fig. 5.7 E and F, section 5.3.2) is not as rapid as that in chlorophyll content (Fig. 5.2 E and F, section 5.2.1.2), it appears that the major limiting factor to P_N is chlorophyll content.

CHAPTER 7. GENERAL DISCUSSION.

7.1 Introduction and General Considerations

The aim of this thesis has been to study the effects of mineral nutrient deficiency on needle development and photosynthesis in field and pot-grown trees. This chapter discusses the major findings in general terms. Some general problems associated with this type of study are discussed first, then the role of nutrients in determining growth and productivity are considered.

Two of the problems in studying nutrient deficiency effects in trees are those of nutrient balance, and variation in material. Nutrient deficiencies can be induced in field and pot trees by withholding a particular element or elements, however, the transport and distribution of nutrients within the plant may complicate interpretation of results. For example, the nutrient content of roots may differ considerably from the content of leaves. Therefore, the effects on photosynthesis of an inadequate nutrient supply to the roots may not be predicted, and foliar nutrient analysis can be important in explaining differences in variables measured on leaves. It is also known that interactions between nutrients have important effects (Nichiporovich and Chen'-In', 1959; MacLeod and Carson, 1969). Natr and Purs (1969; 1970) found that N deficiency in barley caused an increase in P concentration in the leaves, and a beneficial effect of nitrogen supply on phosphate nutrition in barley roots was found by Humble et al. (1969). A close relationship between Fe and P metabolism is known (Machold and Scholz, 1969), so that an increase in P content in the tissue may bind more Fe, and induce P deficiency effects. It is therefore sometimes hard to explain a causal relationship between a particular nutrient deficiency and a change in a specific variable, since the effect of any one mineral element cannot always be isolated from that of other elements (Peck et al., 1969). In the field, differential application of fertiliser may alter the solubility of nutrients in the soil, or lead to ion antagonism. Nutrient deficiency effects may also vary with plant age and genotype (Odurukwe and Maynard, 1969). In current work, in the field, the -K and -P sets in 1988 were deficient in -K and -P respectively, and the -All set were deficient in N, P and K. However, needles in the supposed -Mg set were not deficient in Mg; presumably the soil contained adequate amounts of Mg. The foliar analyses for pot trees (Materials and Methods, Tables 2.5 to 2.7) show deficient foliar concentrations of elements in the

corresponding deficiency treatments, although -All needles had rather higher concentrations of K and Mg than in -K and -Mg treatments respectively. Also, needles in -P and -K pot treatments in November 1988 were N deficient, perhaps due to an inhibition of N uptake during growth.

Variation in material was great for all variables measured, in both pot and field trees. In the field, differences in needle characteristics with canopy position and position on the shoot were minimised or accounted for by the shoot and needle sampling procedure (see Materials and Methods). However, this procedure was not totally successful, and differences in some needle characteristics in the field between trees, exceeded those between successive harvests (e.g. Fig. 3.2 B, C, and D). Tree-to-tree differences in the field could partly be due to differences in local nutrient availability, or local soil characteristics within a plot, but is probably a natural consequence of sampling from a genetically heterogeneous population. The use of clonal material for the pot experiments might have reduced the variation, but such material was not available. The variation could have been reduced by increasing the sample size. However, this was limited by the need to measure many variables on many treatments, and time did not allow larger samples to be taken. A sample size of only two limited the type of statistical analysis which could be performed, and meant that differences in variables between treatments in 1988 were hard to detect.

7.2 Refertilisation and Free Growth.

Free growth, although noticeable in some individuals of most refertilised treatments in 1988, arose mainly through refertilisation of -N and -All trees in 1988, and in trees supplied with 14 p.p.m. N and more in 1989. In these refertilised seedlings in 1989, free growth increased shoot length, and needle number per shoot, and the newly initiated needles due to free growth were larger and heavier, with a greater cell number than needles expanding from existing primordia. Also, needles expanding from existing primordia and subject to higher nitrogen concentration treatments also showed increases in all the variables measured, over needles from nitrogen deficient trees. Unfortunately, data for chlorophyll content, RuBPC activity and photosynthetic rate were estimates for the whole shoot, or mean values, for needles taken from the shoot mid point. A comparative study of the photosynthetic characteristics of needles expanding from pre-formed primordia and those expanding from

primordia initiated by free growth, as was done for needle fresh weight and cell number in 1989, might well have shown interesting differences. Time did not allow such a study, and profitable future research might be to characterise these differences, to extend knowledge about the control of photosynthesis by fertilisation. Free growth provides an excellent system for such studies, as growth is indeterminate, and needle initiation and development occur without the usual temporal separation, and can therefore be easily manipulated by conditions applied for a short period of time. It is not known whether free growth would be caused by application of nitrogen later in the season, and more work should be done to determine whether the timing of application is critical.

It is impossible to distinguish to what degree the elevated photosynthetic rates in -NR and -AllR treatments in August are due to increased area arising from free growth, and how much they are due to a direct effect of refertilisation on expansion and development of existing primordia. The effect of refertilisation on needle development is very great, and spectacularly rapid, with most needle and photosynthetic characteristics increasing in comparison with -N and -All seedlings: Total chlorophyll and carotenoid content and the chlorophyll a/b ratio increased within 1-2 weeks of refertilisation, photosynthetic rate increased after about 2 weeks, G_S after about 4 weeks, and RuBPC activity after about 6 weeks. This order points to the primary importance of chlorophyll formation for photosynthetic development, although RuBPC seems to limit maximum photosynthetic rate later in the season, and maybe cause the seasonal changes seen following the August peak.

It is surprising that refertilisation effects on photosynthesis should be seen to a similar extent in one year old foliage. This indicates that photosynthetic productivity is not rigidly determined by the nutrient conditions in previous seasons, since the capacity to synthesise components of the photosynthetic apparatus exists long after the cessation of needle growth. It also suggests that the timing of N addition at the start of needle expansion may not be critical in determining the potential photosynthetic component of productivity, and this could be further investigated by refertilising N deficient trees at successively later times throughout the growth season. However, it is not known whether the degree of the refertilisation response diminishes with increasing age of the tree, and maybe the time of fertiliser application becomes more important in older trees. Although photosynthetic development was not

studied for a range of nitrogen concentrations, the response near the end of the season to a small concentration of added nitrogen was large, and even though 112 p.p.m. is a good estimate of the optimum concentration, 14-28 p.p.m. is enough to halve the difference between trees in zero and 112 p.p.m. N treatments in most variables measured in 1989. A concentration of 14 p.p.m. N was enough to give free growth, so that a large increase in potential productivity can be achieved for a relatively small amount of applied nitrogen.

7.3 Control of Needle Size.

Needle size is affected by nutrient deficiency and needle position on the shoot. Although cell size was shown to be as important as cell number in determining final needle size, differences in cell size seen with -P and -K treatments in the field were not as great as those in cell number. It is cell number alone which determines the difference in size between -N and -NR needles of pot trees, and the difference in needle size with position on the shoot in all field treatments. The significantly lower number of cells in primordia of -P and -K needles compared with the Controls is reflected in a lower final cell number, so that to some extent, potential needle cell number is predetermined at the primordial stage. This limitation to final needle cell number only exists under nutrient deficient conditions, and the refertilisation data show that a restoration of favourable nutrient conditions to N-deficient trees causes a greater number of cell divisions to occur in needles, than in N-deficient needles. This suggests that needle size, as determined by cell number, is a result of prevailing nutrient status during needle expansion, which can override any limitations due to nutrient conditions at the time of primordium initiation. Future work should investigate whether this N-induced increase in cell number is the result of a faster rate of cell division, or a longer duration of the period of cell division. No data are available on needle cell number responses to refertilisation of P and K deficient needles, and future work should further investigate the role of nutrition on cell division by determining cell number in P- and K-deficient needles following refertilisation. The effect on cell number of restoring N, P or K supply to -All trees at progressively later times during needle elongation could also be studied, to determine the stage at which final cell number can no longer be altered by refertilisation.

In the same way as the ability for free growth declines with increasing

seedling age (Watt, 1961; Balut and Zelawski, 1955), so might the plasticity of response of needle cell number to nutrient availability during expansion, and this should be studied further. Highest final cell number is found in needles at a mid-shoot position, whereas maximum primordium cell number is found in primordia at the base of the bud, so that positional differences in needle cell number are not completely determined by the number of cells in the primordium. Therefore, other controls must operate to determine the number of cell divisions occurring in a needle, according to its position on the shoot. These controls are probably not due to environmental (i.e. climatic) effects. As evidence for this, distal needles elongate later than proximal needles, when the temperature is warmer and the photoperiod is longer, however, they reach a smaller size. This may be because proximal needles act as a sink, so that more distal needles become increasingly stressed; for example, they might lack phosphorus for nucleic acid synthesis, and carbohydrates for cell walls. Carbohydrate stress would be expected to reduce cell size as well as cell number, and this is supported by data for Control needles in the field in early May 1987, which show a slight curvature in the relationship between needle cell number and fresh weight (Fig. 4.2 A), indicating that distal cells may be smaller than proximal cells. Subsequently, cell size is not altered by needle position on the shoot, and the hypothesis that early in shoot expansion distal needles are limited in cell division and expansion by carbohydrate supply and nutrient deficiency, and later by nutrient deficiency alone, is proposed. Therefore, as for differences in needle size in the nutrient deficient treatments, needle size according to position on the shoot is reduced due to limiting nutrient conditions during growth.

7.4 Photosynthesis.

A general point concerning the measurement of P_N for all treatments, is that it is expressed per unit projected area, based on the total needle projected area for the whole shoot. Due to a high degree of mutual shading, the projected outline area of a shoot is only about half the total projected area of the needles (Norman and Jarvis, 1974). Therefore, if the measured values of P_N are calculated per shoot projected area, values would be higher than those recorded here. The contribution of the stem to net photosynthesis of the shoot has been shown to be near enough to zero, at irradiances above 20–50 W m^{-2} (Ludlow and Jarvis, 1971), and can be neglected in interpretation of P_N.

The trend in all treatments for photosynthetic rate to increase until about August, and then to decrease, reflects a seasonal limitation to P_N mirroring the activity of RuBPC. Changes in RuBPC activity may cause the seasonal changes in P_N, or alternatively, P_N and RuBPC activity may both be limited by some other factor. Superimposed onto this seasonal fluctuation in P_N are the nutrient limitations observed with N deficiency, in -N and -All trees.

As the activity of RuBPC was studied during needle development, during which time the activity varied 40 fold or more, it was very important to show that the substrate concentrations used in the assay were not limiting for any treatment at any time throughout the season. This was established using Control extracts from the field, in March 1988 (see section 2.10), which showed higher activities than for Control pot trees. Future work should involve estimates of the amount of total protein in needles throughout their development, to relate activity to specific activity. This would show whether the seasonal change in activity is due to a direct adjustment of RuBPC synthesis, or of the specific activity, and whether the amount of enzyme increased as a store of nitrogen, in relation to nitrogen nutrition. The amount of RuBPC protein in relation to total protein chould also be investigated, by quantitatively assaying the bands obtained from electrophoresis of the extracts, to further answer these questions.

Because the photosynthetic rate in -P needles is not different to that of the Controls, and yet field data show the cells are larger, this must mean that photosynthetic capacity per cell is greater with phosphorus deficiency. This is not due to an increase in RuBPC activity per cell, which is reduced in the -P treatment, and is more related to chlorophyll content, which is also higher per cell than for the Controls. However, interpreting data from pot trees using those from field trees must be done carefully, as large differences in response of morphological variables with nutrient treatment were found between the two sets of material, and it may be that -P pot trees do not show an increase in cell size with P deficiency.

The development of assimilation capacity (Chapter 5, section 5.6.2), differs from that outlined for *Pinus sylvestris* (Kovalev and Malkina, 1985), in the only published study of the development of photosynthesis related to needle development in a gymnosperm. In this species the period of mesophyll growth and development of photosynthetic capacity could be divided into 4 sections:

1) From the beginning of needle growth to 20% of final needle length; dark respiration is high, and P_N is negative and limited by low chlorophyll content. Needle growth is supported by photosynthate imported from older needles.

2) Between 20% and 40% of final needle length; dark respiration decreases, and P_N becomes positive, although still limited by chlorophyll content. 50% of the mesophyll cells are mature at this stage.

3) Between 40% and 70% of final needle length; P_N reaches a maximum, and all mesophyll cells are mature.

4) Between 70% and 100% of final needle length; chlorophyll content reaches a maximum, P_N declines, due to an increase in r_s to CO_2.

Pine needles grow indeterminately, from a basal meristem (Kienholz, 1934), so that there is a gradient of maturity from the distal end of the needle, as in monocots, which means that photosynthetic maturity of the whole needle is reached more slowly than for Sitka spruce. The Control data for development of photosynthetic capacity in Sitka spruce can be roughly divided into three stages, summarised as follows:

1) Needle expansion, from bud burst to early June; P_N rapidly increases, together with G_S for CO_2, and chlorophyll content. Cells and needles reach final size.

2) Needle maturity, early June to September: P_N and G_S reach a maximum, RuBPC is synthesised rapidly, chlorophyll content continues to increase.

3) Needle ageing and close of growth season: September onwards; RuBPC activity reaches a maximum, then declines, chlorophyll content reaches a maximum slightly later, and declines slightly. P_N and G_S decrease.

4) Following the first growth season: Data for young pot trees show that for needles in their second growth season, carotenoid content is higher, P_N is slightly higher, and G_S lower, than for current needles. There is no difference in RuBPC activity, chlorophyll content, or C_i between current and one year old needles.

An interesting feature is that P_N is positive during early needle development, immediately following bud burst, unlike for *Pinus sylvestris*

(Kovalev and Malkina, 1985); *Populus deltoides* (Dickmann, 1971); *Picea abies* (Loach and Little, 1973), and chlorophyll content is not limiting at this time. This reduced dependence of developing needles on photosynthates from other organs, means that productivity of the whole tree at this time is very high, and a large amount of assimilates may be accumulated and stored, to use for apical bud development later in the season, when photosynthesis decreases. It is not known if the high P_N in young needles is a characteristic only found in seedlings, and whether developing needles of field trees are more dependent on older needles. Not enough data has been collected on photosynthesis in the field, and the summary above formulated for seedlings may not apply for older trees, with more pronounced reductions in photosynthetic capacity possibly being found in older needles. A further characterisation of the development of photosynthetic capcity in the field is therefore needed.

The implications of the lack of a principal meristem during needle expansion, with general cell divisions occurring along the length of the needle, are that the needle matures as a whole, and can extend more rapidly than if extension was dependent on a basal meristem, as in pines (Kienholz, 1934). This means needles reach a peak area for light interception early in the season, before optimum climatic conditions for photosynthesis. Although proximal needles on the shoot mature before distal ones in terms of size, it is not known whether there is the same acropetal trend in attainment of photosynthetic capacity, which should be an area for future research, together with the determination of the degree to which distal needles may be dependent on proximal ones for photosynthates. The significant increase in the dry weight:fresh weight ratio with P and K deficiency in pot and field trees is interesting, as it suggests a change in carbon allocation within the needle. It is not known whether this is due to an increase in the structural or soluble fraction, and an analysis of soluble carbohydrate in the needle throughout the season would show which is the case. Even though photosynthetic rate is unaffected in these treatments, further work on the fate of assimilates may show treatment differences, perhaps with -P and -K needles storing more photosynthates within the needle.

7.5 Productivity.

Productivity is dependent on the size of the shoot, since the number of branches is determined by the length of the shoot (Cannell, 1974), and also on the number of needles per shoot, needle size, and assimilation rate. Hence productivity may be reduced in nutrient deficient conditions, by a reduction in shoot and needle area, a reduction in P_N per unit area, or a reduction in both.

Field results from 1987 showed that -P trees have fewest needles per shoot, with the smallest projected area, and the shortest stem unit length. The importance of the stem unit length is considerable, with respect to the degree of mutual shading on the stem, which is a constraint to photosynthesis (Cannell, 1987). Phosphorus deficient shoots in the field therefore have the greatest limitations to light interception, and might be expected to show reduced photosynthetic rates. Results with seedlings in 1988 did not support this hypothesis, since photosynthetic rates in -P trees did not differ from the Controls. However, seedlings in 1988 did not show the same reductions in morphological characteristics found for field trees in 1987. This lack of response to P deficiency by young trees may indicate that a long period of growth in deficiency conditions is needed before morphological effects of P deficiency are established. In 1988, the largest limitation to growth and photosynthesis was due to N deficiency in -N and -All treatments. It is important to know whether P_N of P shoots in particular, in the field, is reduced relative to that of Control trees, in view of the more extreme morphological effects than seen for P-deficient seedlings, and also the decreased stem unit length, and increased mutual shading of the needles of field trees. It appears that inhibition of needle development with P deficiency is more marked in older trees than in seedlings, and it is not known whether P_N declines in parallel, as tree age increases. It may be that P_N in mature, P-deficient trees does not differ from that of Control trees per unit area. However, it is the amount of photosynthesis per shoot, rather than P_N, which is more important in terms of productivity for the tree, and this should be estimated for field trees. In seedlings, N-deficiency not only reduces P_N, but the size, area and number of needles, and shoot length, so that P_N per shoot is drastically reduced, more than that of any other treatment, to levels which barely support growth.

Data from chapter 3 show that in the field, deficiency of P and K cause the apical dome size to be reduced, so reducing the number of needle primordia

initiated for growth the following season (Cannell, 1978), thereby reducing potential productivity. In seedlings, this may be overcome by free growth, but in mature trees where free growth does not occur, productivity may be limited more by nutrient deficiency effects on shoot and needle morphology, than by $P_{N'}$ if P_N per unit area is not affected, as much. In 3/4 year old seedlings, needle and shoot morphology and photosynthesis are only significantly reduced with N deficiency, and although photosynthetic capacity may be increased by addition of N in subsequent seasons, it is not known whether a greater increase is found with addition at the start of the season, rather than later on. It may be that the timing of fertiliser application makes a large difference, despite the apparent plasticity of the response of photosynthetic components to nutrition. Data in Chapter 6 showed that needle and shoot growth are highly related to N concentration, and that needle size is determined by nutrient conditions during needle expansion regardless of previous nutrient conditions. Therefore, maximum productivity is achieved by fertilisation at the time of needle expansion, with a concentration of 14-28 p.p.m. N being adequate to give a substantial increase in needle size compared with nitrogen deficiency. However, with field trees, needle growth is also more dependent on nutrient conditions during primordium initiation, and it is not known whether the photosynthetic capacity of all needle age classes responds as well as in pot trees. Because nutrient requirements of stands decrease with increasing tree age (Miller, 1981), maximising productivity of young trees for the first few years is of principal importance, to ensure high yields of older stands. Therefore these data support the practice of annual fertiliser application, and especially nitrogen, just prior to needle growth in young trees. If the peak of RuBPC activity and chlorophyll content coincided with that in $P_{N'}$ assimilation rate might be improved, although there is no easy way of selecting for trees where this is the case, or inducing this to happen, so that the response to fertiliser application at the right time remains the best way to maintain productivity.

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