ECOPHYSIOLOGY OF *PSEUDOTSUGA MENZIESII* GROWN AT HIGH OR LOW NITROGEN SUPPLY OR IN ASSOCIATION WITH *SHEPHERDIA CANADENSIS*

by

Rachel Emma Westfall

B.Sc., Brandon University, 1992

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

December 1994

APPROVAL

Name: **Rachel Emma Davies Westfall**

Degree: **Master of Science**

Title of Thesis:

ECOPHYSIOLOGY OF PSEUDOTSUGA MENZIESII GROWN AT HIGH OR LOW NITROGEN SUPPLY OR IN ASSOCIATION WITH SHEPHERDIA CANADENSIS.

Examining Committee:

Chair: Dr. M. Moore, Assistant Professor

Dr. Geoffrey R. Lister, Associate Professor, Senior Supervisor
Department of Biological Sciences, SFU

Dr. Robert C. Brooke, Associate Professor
Department of Biological Sciences, SFU

Dr. Edith Camm, Instructor
Department of Biological Sciences
University College of the Fraser Valley
Public Examiner

Date Approved Nov. 30, 1994

PARTIAL COPYRIGHT LICENSE

I hereby grant to Simon Fraser University the right to lend my thesis, project or extended essay (the title of which is shown below) to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users. I further agree that permission for multiple copying of this work for scholarly purposes may be granted by me or the Dean of Graduate Studies. It is understood that copying or publication of this work for financial gain shall not be allowed without my written permission.

Title of Thesis/Project/Extended Essay

Ecophysiology of Pseudotsuga menziesii grown at high or low nitrogen supply or in association with Shepherdia canadensis.

Author: _____
(signature)

Rachel Westfall
(name)

December 9, 1994
(date)

ABSTRACT

Effects of nitrogen nutrition on conifer growth and physiology were investigated through observation of seasonal variations in physiological indicators. *Pseudotsuga menziesii* (Douglas-fir) seedlings in one group (H) were provided with an ample supply of mineral nitrogen. Second and third groups (L and S) were limited to a trace supply of mineral nitrogen. *Shepherdia canadensis*, an actinorhizal shrub nodulated by *Frankia*, was planted with the third group of Douglas-firs (S). Over the course of 12 months, seedling height, collar diameter and silhouette area were monitored. Assays were made of foliar nitrogen and carbon concentrations, chlorophyll fluorescence, apparent photosynthesis (APS) and dark respiration (RD).

Seasonal variation was apparent in every parameter measured. Foliar nitrogen and carbon concentrations trended towards high values in winter and lower values in summer, as did quantum requirement (QR). Apparent photosynthesis (APS) rates, dark respiration (RD) rates, initial fluorescence (F_O), variable fluorescence (Fvar) and steady-state fluorescence (F_T) were highest in summer and lowest in winter. F_O was not independent of photochemical activity, as previously reported, nor was it correlated with plant size. The proportion of light absorbed by the sample in the fluorometer sphere (I_{ABS}) was used as a normalization factor instead of F_O.

H plants contained significantly higher concentrations of foliar nitrogen than did L and S plants. This paralleled higher RD rates and F_T values for the H plants, along with notably lower F_O, Fvar and QR. The S plants tended to be intermediate.

In the final four months, all three treatments differed significantly in growth rates and flushing times. APS rates were usually highest for the H and S plants.

Of the fluorescence parameters, F_T was the best indicator of the physiological status and health of the seedlings. According to F_T, the H plants were healthiest, and the S plants were slightly better off than the L plants.

ACKNOWLEDGEMENTS

My thanks and best wishes to those who have guided and encouraged me. They include Geoff Lister, Bob Brooke, Mei-Keng Yang, Maija Siekkinen, Sharon Gillies, Ken Lertzman, Kerry Delaney and Shawn Wilson.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Nutrition is one of numerous factors affecting plant growth and physiology. Due to its great importance in determining plant growth rates and likelihood of survival, it has been the subject of intense scrutiny by many researchers. This study focuses on the effects of nitrogen nutrition on conifer growth and physiology.

Nitrogen is an essential element in plant nutrition, and is potentially a limiting factor for plant growth. Most of a plant's nitrogen is contained in the foliage. It is obtained by plants from the soil in the forms nitrate and ammonium, which are subsequently metabolized into organic nitrogenous compounds. Soil nitrogen is replenished by biological activity, such as the decomposition of organic material, nitrification and mineralization. The latter processes are dependent upon the activity of nitrophilic bacteria; some types occur in symbioses with plants, and others are free-living in the soil.

The establishment of young plants is highly dependent on, among other factors, their nutritional status and the availability of soil nutrients. Typical forestry practices involve the planting of nursery-produced tree seedlings on clearcut sites. The likelihood of such seedlings surviving to maturity is greatly influenced by their nutritional requirements and how well these requirements are met in the nursery and on-site (Rook 1991). On sub-optimal sites, the chances of seedling survival can by enhanced by addition of nitrogenous fertilizers to the soil, in inorganic or organic forms. However, this comes at some cost, and is not self-sustaining; even with slow-

releasing fertilizers, subsequent applications are often necessary. In addition, competing vegetation must be controlled, as it too benefits from the fertilizer and may outcompete the slow-growing tree seedlings.

Many plants form symbiotic associations with nitrogen-fixing bacteria. The roots of some species are nodulated by *Rhizobium*, whereas others (known as actinorhizal species) are nodulated by *Frankia*. Nodulated plants tend to do well on nitrogen-limited sites, and their presence, growth and decomposition can make the nutritional environment more favorable for other plants. In silvicultural projects involving nitrogen-deficient sites, it may be beneficial to promote the growth of such plants in order to assist the desirable tree seedlings. A healthy, self-sustaining ecosystem could result, with fewer weedy species. Silviculturalists would need to invest less time and resources, as compared to the fertilization and brush control approach.

A study was conducted regarding the effects of nitrogen nutrition on the growth and photosynthetic physiology of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings, and whether the presence of the actinorhizal shrub (Racette and Torrey 1989) soopolallie [*Shepherdia canadensis* (L.) Nutt.] would affect that nutrition. The main objectives of this thesis are to present, discuss and interpret the results of that investigation.

CHAPTER 2

EFFECTS OF NITROGEN DEFICIENCY

2.1 Visual Symptoms Of Deficiency

When nitrogen availability becomes limiting to plant growth and function, it results in reduced foliar nitrogen content. The short-term consequences of this nutrient deficiency often include visual deficiency symptoms, such as chlorosis, an apparent yellowing of leaf tissue. In some cases, needles become purple tipped; necrosis of these needles occurs at the end of the growing season (Timmer 1991).

Approximately 75% of the nitrogen in green cells is located in the chloroplasts (Stocking and Ongun 1962). Thus it is no surprise that a decrease in nitrogen availability often results in chlorosis, which is due to degradation and reduced synthesis of chlorophyll. Researchers have observed linear relationships between nitrogen content and chlorophyll content of leaves (Linder 1980). However, the relationship differs between species, and is only consistent under controlled environmental conditions (Rook 1991).

Chlorosis is symptomatic of nitrogen deficiency, but not diagnostic. It can result from numerous conditions, including inadequate or imbalanced nutrition, drought, and unfavorable temperatures (Linder and Rook 1984). When working with hydroponic systems, Ingestad (1982) found that chlorosis disappears from plants when

growth rate and nitrogen availability are at equilibrium, regardless of the level of nutrient supply.

For this study, a reliable indicator of plant nutrient status was needed, in order to facilitate the interpretation of plant growth and physiology and how they are affected by nitrogen availability. Although reduced chlorophyll content of leaves and chlorosis are symptoms of nitrogen deficiency, neither is a reliable measure of plant nutritional status. It was necessary to measure leaf nitrogen concentration directly. Chlorosis was regarded simply as a side effect of treatment, not as a definitive factor.

2.2 Growth Effects

Plant nitrogen concentration is correlated with growth rate. Over a full range of concentrations, this relationship is not linear (Bigg and Schalau 1990). When nutrient availability is extremely low, there is a constant amount of nutrient in the tissue, which is diluted by plant growth. At a higher, but still deficient level of nutrient supply, growth and nutrient concentration are correlated, with a trend towards greater productivity as nutrient concentration increases. This trend continues until the point of luxury consumption, when the nutrient is no longer the limiting factor for growth. Above the level of luxury consumption lies toxicity. The antagonistic effects of oversupply can include cell damage due to changes in pH or salinity, or precipitation of another element which is relatively short in supply (Bigg and Schalau 1990). Competition for active transport carrier molecules in the roots can occur between ions

of similar charge, so oversupply of one element can result in a shortage of another element in the plant.

Most investigations of nitrogen nutrition have compared well supplied plants to poorly supplied plants. The framework for the present study followed that line of reasoning as well. Douglas-fir seedlings were provided with either a non-limiting nitrogen supply (H), permitting luxury consumption, or a limiting supply (L), by which nitrogen deficiency was expected to limit plant growth. Upon that framework a third variable (S) was supported; some of the nitrogen limited seedlings were grown in association with soopolallie, an actinorhizal shrub. My intention was to learn how this third set of seedlings would place along the continuum of effects of nitrogen supply.

Various studies have revealed the following effects of nitrogen deficiency on conifers (Rook 1991):

a reduction of the basal diameter: stem height ratio;

a reduced shoot: root ratio;

reduced stem bushiness;

fewer leaves;

shorter needles; and

reduced leaf area.

In species with preformed initials, such as Douglas-fir, the effects of nutrition on the amount of foliage will not become apparent until the new initials develop (Linder and Rook 1984). Several months to a year following treatment, nitrogen deficient plants will have less foliage, due to fewer needles and smaller leaf size, than well fed plants.

In the present study, stem height and collar diameter were measured in order to confirm or refute the findings of other researchers, and in order to compare the treatment sets.

2.3 Physiological Effects: Carbon Dioxide Exchange

Plant growth rates are directly affected by rates of photosynthesis. In addition, photosynthetic rates are well correlated with foliar nitrogen status (Brix 1981). Therefore the influence of foliar nitrogen status upon growth rate probably results from the effects of nitrogen availability upon photosynthesis.

Rates of CO_2 assimilation (a measure of apparent photosynthesis) under saturating light conditions increase as plant nitrogen status moves from deficient to optimum, and decrease with supraoptimal to toxic concentrations. Brix (1981) reported that the maximum reduction of CO_2 assimilation rates of Douglas-fir was 20% at suboptimal and supraoptimal nitrogen supply.

Carbon fixation (CO_2 assimilation) requires the enzyme Ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO). RuBisCO contains almost 50% of the soluble nitrogen in the leaf, or about 28% of total leaf nitrogen (Makino *et al.* 1984). In an investigation of the effects of nitrogen nutrition on structure and function of the photosynthetic apparatus, Andreeva *et al.* (1971) found that changes in the synthesis and activity of RuBisCO may regulate photosynthetic rates. A close

relationship between leaf nitrogen content and RuBisCO activity has since been confirmed many times (Hak and Natr 1987b).

Foliar respiration rates are usually found to decrease with decreasing N status (Brix and Ebell 1969; Kawahara *et al.* 1976; Kuriowa 1960). However, nitrogen deficiency has also been linked to higher rates of mitochondrial respiration in the light (Marek 1984, Marek and Frank 1984). Dykstra and Gatherum (1967) and Hak and Natr (1987a) found no effect of nitrogen deficiency upon respiration rates.

The present study investigated the effects of nitrogen supply upon rates of photosynthesis and respiration. It allowed me to relate the photosynthetic performance of the third treatment, S, to the H and L treatments. It also provided me with the opportunity to compare the seasonal patterns of CO_2 exchange between treatments, a subject which has not been well documented. CO_2 exchange rates were compared to chlorophyll fluorescence, a relatively new tool for physiological assessment.

2.4 Physiological Effects: Chlorophyll Fluorescence

Chlorophyll *a* fluorescence is used to assess the amount and activity of photosynthetic apparatus. The effects of nitrogen status on chlorophyll fluorescence have thus far been the focus of little study.

Kolber *et al.* (1988) investigated photosynthetic energy conversion in marine unicellular algae. They found that maximum variable fluorescence yield (Fv) at saturating flash intensities decreased with increasing nitrogen limitation. This indicated a reduction in the photochemical energy conversion efficiency of Photosystem II (Kolber *et al.* 1988). A loss of functional Photosystem II reaction centers was evidenced in nitrogen-starved algae by reduced levels of two constitutive proteins. Apparent absorption cross-sections of Photosystem II increased; this characteristic is also common in shade-acclimated plants. Fluorescence decay kinetics were affected by nitrogen starvation; an apparent decrease in the transfer of electrons from Q_A to Q_B was observed. Greene *et al.* (1991,1992) also found that nitrogen limitation leads to increases in the effective absorbtion cross-section of Photosystem II in marine algae. They attributed this phenomenon to the loss of functional Photosystem II reaction centres; antennae which would normally supply excitation to several reaction centres had only one functional trap (Falkowski *et al.* 1994).

Da Silva and Arrabaca (1992) investigated the characteristics of variable chlorophyll fluorescence of a C_4 pasture plant. In nitrogen-starved plants, the time of the transition from initial (F_O) to peak (P) fluorescence was short, as compared to that of control plants. This indicated that nitrogen starvation lowered the ratio between intercepted irradiance and electron flux out of the plastoquinone pool (da Silva and Arrabaca 1992). The P:F_O ratio was lower in the nitrogen deficient plants. Photochemical quenching (reduced fluorescence after P) was lower in nitrogen deficient plants than in control plants, indicating a less efficient photochemical energy conversion (and therefore a greater loss of energy in the form of fluorescence).

Correlations between fluorescence and apparent photosynthesis have been found (ie. Vidaver *et al.* 1989, 1991). Steady-state fluorescence, F_T, was positively correlated with CO_2 assimilation (Hipkins and Baker 1986). In addition, the fluorescence parameter Fvar/Fmax has been correlated with spring recovery of apparent photosynthesis (Lundmark *et al.* 1988).

One of the main purposes of the present study was to increase the understanding of the relationships between nitrogen nutrition and patterns of chlorophyll fluorescence. I intended to discover which fluorescence parameters varied with nutrient status and to describe and the nature of that variation. These parameters would then be used to assess the nutritional and physiological status of individuals in the S treatment group. Seasonal variations in fluorescence were monitored, in order to identify patterns typical of the species as well as indicators of physiological change such as flushing, maturation and the onset of dormancy. Correlations between fluorescence parameters and CO_2 exchange rates were sought.

CHAPTER 3

THE DOUGLAS-FIR NUTRITION STUDY

From July 1993 to June 1994, I investigated the role of nitrogen nutrition in the growth and physiology of Douglas-fir seedlings, and how the presence of soopolallie, a nitrogen-fixing shrub, affected that nutrition. Acclimation of the plants to the various nutrient regimes began in May 1993.

3.1 General Materials and Methods

3.1.a Origin of Plants.

Rooted stems of soopolallie were collected in April 1993 from a clearcut site south of Lillooet (50°40'N, 122°20'W, 1000 m elevation). Logging debris had washed over a number of large, established plants, pinning stems to the ground. Adventitious roots had subsequently grown from the stems, providing me with a number of relatively small, shallow rooted, even-aged plants. The plants, dormant at the time of collection, were transplanted into 1-litre milk cartons containing sand and perlite (3:1).

Interior Douglas-fir seedlings were provided in April 1993 by the B.C. Ministry of Forests. These were nursery-grown seedling 'plugs' (1+0) which had spent the winter in cold storage (temperature near 0°C, in the dark, packaged to prevent moisture loss). The seeds originated from 49°30'N 120°42'W (Coalmont Road), elevation 1000 m, seedlot 800900. In the first week of May, to prepare the seedlings for this project, I washed the soil from their roots and planted them individually in 1-litre milk cartons containing sand and perlite (3:1).

3.1.b Experimental Treatment and Design

The plants were placed outdoors in a raised plot bed at Simon Fraser University, Burnaby, British Columbia (49°N, 123°W, 365 m elevation), with the cartons sunk into sawdust to provide a cool, moist environment for the roots. Every two weeks, each plant was provided with 125 ml of fertilizer solution. One third of the Douglas-firs were provided with high nitrogen solution (detailed in Section 3.2), while the remaining Douglas-firs and the soopolallies were provided with low nitrogen solution (Section 3.2).

At the end of May, once the plants were no longer dormant, they were transplanted in pairs into numbered plastic pots (34 cm tall, 10 cm square) containing sand, perlite and vermiculite (3:1:1). Each pot contained either two high nitrogen fed Douglas-firs (hereafter referred to as H), two low nitrogen fed Douglas-firs (L), or one low nitrogen fed Douglas-fir and one soopolallie (S). The extent of the new root system of each soopolallie was recorded. The pots were sunk into the sawdust of the

raised plot bed in random design, approximately 10 cm apart, with a row of border trees around the perimeter. Application of fertilizer was increased to 250 ml every two weeks to compensate for the increased planting density.

One Douglas-fir from each H and L pot was selected for study and analysis; the other Douglas-fir (sometimes stunted, oversized, or missing its apical shoot due to frost damage) was tagged. The latter seedling was present in order to provide competition for water and nutrients, in place of the soopolallie in the S pots. The Douglas-firs in the S pots were left untagged.

The following measurements were made of all selected individuals: stem height, collar diameter (below the bottom lateral shoot, and above the soil line), and number of new shoots. Stem height and collar diameter were remeasured the following winter and the next summer. These values provided an indication of growth rates.

Three sample sets were randomly selected from the numbers on the pots. Only those pots which contained soopolallies with insufficient new root growth were excluded from the selection. Each sample set contained five pots from each treatment (H, L, and S) for a total of fifteen pots.

Once shoot extension and needle expansion were complete (early July), and plants were forming buds for a second flush, fertilization was increased to 250 ml per pot every week. This compensated for the increased plant size and high rates of metabolic activity (Ingestad 1982). By this time, the plants had been growing in association for one month, and had been given time to adjust to the nutrient supply. On July 9, physiological and chemical analysis began.

3.1.c Assessment of Growth and Physiology

Analysis of growth, nitrogen content, chlorophyll fluorescence, and gas exchange characteristics took place approximately every two weeks throughout the 1993 growing season and the spring of 1994. A timeline of analysis dates and other relevant information is provided in Table 3.1. Two alternating sample sets were selected for analysis. The third sample set was held in reserve, to provide replacement plants in case any of the others were damaged. (No replacements were needed in the course of the study.) This system of analysis allowed the progression of any single plant to be monitored over the study period, yet allowed the plants four weeks recovery time between trips to the lab. Alternating between two sample sets (as opposed to making repeated measures of only one set) provided greater overall frequency of sampling, without causing undue stress to the individuals in the sample sets.

For each analysis, the plants of the sample set were removed from the raised plot bed and transported into the lab. The top 20 cm of each untagged Douglas-fir was identified by the removal of several centimeters of needles and branches below that point. The plants were placed in a plastic tent, which kept the relative humidity between 80 and 90% and the temperature at 20+-2^{o}C. Approximately 400 μmol/m^2/s photon flux density (PFD) illuminated the plants from above, from two 500 watt Dicrolite quartz-tungsten-halogen lamps (Sylvania 500T3/Q/CL/120V), contained in a lamp housing with a cold mirror reflector and a front hot mirror. A 10 cm deep water bath was placed between the lamps and the plant canopy, to reduce the heat load.

This pre-treatment occurred for a minimum of 30 minutes. It ensured that the plants were acclimatized to the same light and humidity conditions on every analysis date, before fluorescence measurements were taken. This precaution was taken in response to reports that light and humidity conditions prior to dark acclimation can strongly influence variable fluorescence (Vidaver *et al.* 1991; Mohammed *et al.* in press).

The plants were then sequentially transferred into a dark box, where they remained for 20 to 25 minutes. A chlorophyll fluorescence induction curve was obtained for each dark-acclimated plant. The full procedure is detailed and discussed in Section 3.3.

Following the fluorescence assay, each plant underwent a light acclimation sequence, as described in Section 3.4.a. The light-acclimated plant was transferred to a closed-circuit gas circulation system for determination of CO_2 exchange rates at 800, 480, 200 and 0 $\mu mol/m^2/s$ PFD, as detailed in Section 3.4.c.

After CO_2 exchange was assayed, two or three needles were plucked from the middle of the apical shoot (current year's growth) and dried in an oven at 50^o C for approximately 3-4 days. Tissue nitrogen content was determined for these samples with a Carlos Erba HCN Combustion Gas Autoanalyzer. Full procedure is detailed in Section 3.5.

Measurements of stem height and collar diameter were recorded. The first time the plants were assayed, and following any subsequent growth periods, their shoot silhouette areas were determined. This procedure is discussed in Section 3.6.

Table 3.1
Timeline of events during the study period.
A(x) refers to analysis (x).
The two sample sets are identified as red and blue.

MONTH	DAY	NOTES	COMMENTS
APRIL '93		soopolallies collected	
MAY		conifers potted, placed outside	
JUNE	1	fed	
	11	trees repotted in pairs	
	15	fed	first signs of chlorosis
	17	pots randomized	(L and S treatments)
	27	fed	
JULY	6	fed	
	7	A1 (red)	
	13	fed	
	20	fed	some plants flushing
	21	A2 (blue)	(second flush)
	27	fed	
	28	A3 (red)	
AUG	3	fed	
	10	fed	
	11	A4 (blue)	substantial root
	17	fed	growth apparent
	24	fed	(from bottom of pots)
	25	A5 (red)	
	31	fed	
SEPT	7	fed	
	8	A6 (blue)	
	14	fed	
	21	fed	
	22	A7 (red)	
	28	fed	

Table 3.1 (continued)

MONTH	DAY	NOTES	COMMENTS
OCT	6	A8(blue)	
	12	fed	
	20	A9 (red)	
NOV	3	A10 (blue)	
	9	fed	
	17	A11 (red)	evidence of dormancy
DEC			
JAN '94			
	19	A12 (blue)	some needles reddening (L treatment)
FEB			
MARCH			
	9	A13 (red)	loss of dormancy-
	21	fed	soopolallies
	30	A14 (blue)	
APRIL	5	fed	Douglas-firs
	13	A15 (red)	are
	19	fed	beginning
	4	A16 (red)	to flush
MAY	5	fed	
	11	A17 (blue)	
	24	fed	
	25	A18 (red)	
	31	fed	
	7	fed	
JUNE	8	A19 (blue)	

Following analysis, the plants were placed back under the humidity-controlled tent. At the end of the day, they were returned to the raised plot bed, where they were re-randomized.

3.2 Plant Nutrient Supply

The high nitrogen solution (A, Table 3.2) contained 280mg N/L, 112mg P/L, and 280mg K/L (Table 3.3), in addition to the macronutrients Ca, Mg, and S, and the full range of micronutrients (200 mg/L FeDTPA, and other micronutrients in the proportions outlined by Machlis and Torrey 1956). This solution was formulated to provide a nonlimiting supply of all the essential elements (Donald 1991), including both nitrate and ammonium as sources of N. The 10:4:10 ratio of nutrient supply (N:P:K) approximates the fertilizers used as 'growers' in various container seedling nurseries (Donald 1991).

The low nitrogen solution (B, Table 3.2) differed from Solution A by containing only 14mg N/L (Table 3.3), while the other nutrients were supplied at the same rates as in Solution A. Solution B was formulated to provide a suboptimal (trace) supply of N (Rook 1991), as both nitrate and ammonium.

Care was taken to acclimatize the plants to a consistent rate of nutrient availability. In order for a constant nutrient supply to be maintained in a hydroponic or pot culture system, the rate of application must match the growth rate (Ingestad 1982). Nutrient supply should therefore increase exponentially at times of exponential

plant growth. This imitates the increase in nutrient availability which occurs in a natural ecosystem, where root growth results in the penetration of an exponentially increasing volume (Ingestad 1982). However, shoot growth of conifers tends to occur in bursts; their nutritional status before and during bud development has a greater impact on future growth rates than does the nutrient supply at the time of flushing (Rook 1991). As a result, nutrient supply rates for this study were based on the level of photosynthetic activity and the size of the plants, not on the apparent rate of growth.

Fertilization dates are identified in Table 3.1. During times of plant growth and high metabolic activity, fertilization frequency increased; the frequency was reduced as plants became dormant in the autumn. Fertilizer application ceased through the winter, and began again in the spring.

Table 3.2
Concentration of chemicals (mg/l) in the fertilizer solutions.

compound	Solution A	Solution B
NH_4NO_3	400.0	20.0
$Ca(NO_3)_2$	530.0	26.5
KNO_3	360.0	18.0
KH_2PO_4	490.0	490.0
$CaCl_2$	0.0	342.5
$MgSO_4$	480.0	480.0
KCl	0.0	253.8
FeDTPA	200.0	200.0
H_3BO_3	2.86	2.86
$MnCl_2{\cdot}4H_2O$	1.81	1.81
$ZnCl_2$	0.11	0.11
$CuSO_4{\cdot}5H_2O$	0.08	0.08
$Na_2MoO_4{\cdot}2H_2O$	0.025	0.025

Table 3.3
Elemental breakdown (mg/l) of the fertilizer solutions.

	N	P	K	Ca	Mg	S
Solution A	280	112	280	130	97	128
Solution B	14	112	280	130	97	128

Figure 3.1
The Integrating Fluorometer.

A = Fluorometer power and interface unit
B = 20 cm diameter integrating sphere
C = diffusion cone (in front of the shutter, filters and lamp)
D = I detector (measures light intensity in the sphere)
E = F detector (measures intensity of fluorescence in the sphere)
F = IBM- compatible PC, equipped with the Fluorometer A/D board
G = connecting cable

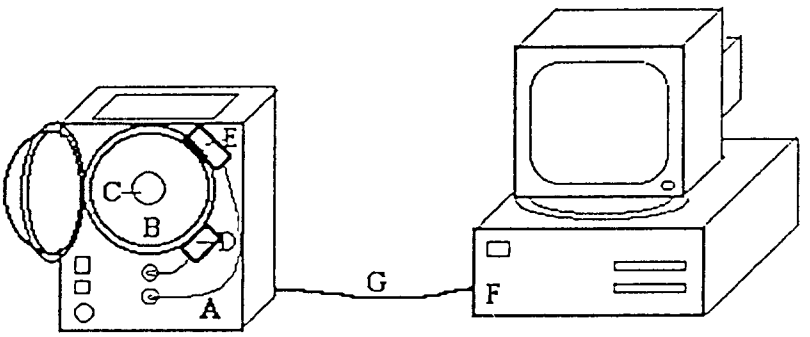
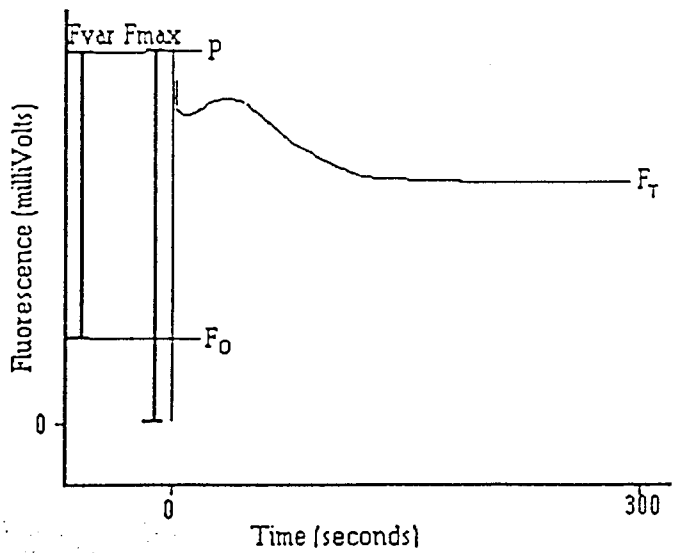


Figure 3.2
Sample fluorescence induction (Kautsky) curve.



3.3 Chlorophyll Fluorescence

Chlorophyll fluorescence induction curves are useful in the investigation of plant photosynthetic apparati and physiology. The subject is reviewed by Krause and Weiš (1991), and by Vidaver *et al.* (1991).

Fluorescence assays were conducted with an Integrating Fluorometer System (Pacific Fluorotec Corporation). The system is illustrated in Figure 3.1. The system's components included an integrating sphere, an instrument base unit, and a Computer Boards CIO-AD16 A/D board. A complementary software program, Fluoroview (version 0.8), coordinated the data collection.

The 20 cm diameter integrating sphere was comprised of two hemispheres, hinged together; its interior was painted with several layers of Kodak White Reflectance Coating (Eastman Kodak Co., Rochester, N.Y.). The sphere contained a central mounting collar through which excitation light entered. A diffusion cone was suspended in front of the mounting collar; it served to scatter the incoming light throughout the sphere and to prevent any direct excitation light from striking the sample. A sphere excitation light (I) detector and a fluorescence (F) detector fitted into two ports on the sphere and were wired into the instrument base unit. The F-detector port contained two far red-transmitting optical filters, in order to isolate fluorescence emissions from the excitation light.

The instrument base unit contained a lamp (Sylvania ELC 24 Volt, 250 watt), an excitation light optical filter (green-transmitting), a shutter, a cooling fan, and an infrared-transmitting cold mirror. It also contained the power switch and the interface

unit, which passed data along to an IBM compatible computer (in which the A/D board was installed).

Operation of the fluorometer was controlled entirely through the Fluoroview program. The program required an initial ten minute warmup period, to allow the lamp's light output to stabilize. Each scan set (5 scans) was preceded by an empty sphere scan, which permitted the adjustment of the illumination light intensity to the target level. For this study, 200 $\mu mol/m^2/s$ PFD was provided. (This was the maximum light intensity permitted by the program, although the Integrating Fluorometer System was capable of producing up to 800 $\mu mol/m^2/s$ PFD.) The shutter was closed, and the top 20 cm of a dark-acclimated seedling was placed in the sphere. The shutter was opened and the seedling was illuminated for 300 seconds. For the first 15 msec, the fluorescence signal was sampled at 5000 Hz, in order to catch the fast induction kinetics occurring in response to illumination. The sampling rate was dropped to 100 Hz for the next 3 seconds, then 20 Hz for the next 20 seconds, and finally 1 Hz for the remaining 277 seconds. A total of 1052 data points were collected for each scan.

A sample induction (Kautsky) curve is presented in Figure 3.2. The FluoroView program produced a data sheet listing a number of values, including:

I_{ABS}, the amount of illumination light absorbed by the plant in the sphere;

F_O, the initial fluorescence;

Fmax, maximum chlorophyll fluorescence;

Fvar, the peak variable fluorescence;

t, the time of maximum fluorescence;

Fvar/Fmax, the ratio of the induced to the maximum fluorescence at the time of peak fluorescence (P);

F_T, steady state fluorescence, in this case defined as Fv at t = 300 sec.

Curves could be plotted and printed out directly from the data collection program. The data were stored in ASCII files; curves could be extracted and transferred to a data manipulation program for further analysis and display.

The program normalized the curves automatically, as specified by Vidaver *et al.* (1989). Data were normalized according to the formula,

$$Fv = (F - F_O) * F_O^{-1}$$

where

Fv = the normalized variable fluorescence at time t,

F = the fluorescence (in mV) at time t, and

F_O = the O-level fluorescence (in mV).

F_O is the initial fluorescence level; it is proportional to the instantaneous excitation light intensity, but is not related to photochemistry. It originates from energy migration processes in the pigment antenna (Photosystem II) (Papageorgiou 1975; Lavorel and Etienne 1977; Duysens and Sweers 1963). It represents the fluorescence level when all intersystem intermediates are oxidized (Papageorgiou

1975; Lavorel and Etienne 1977), and theoretically contains no variable fluorescence component. Consequently, F_O was subtracted from the raw fluorescence data, leaving only the induced fluorescence component of the Kautsky curves.

F_O may be proportional to the total amount of fluorescence emitting chlorophyll *a* molecules of the sample (Dube 1990). As a result, for similar types of tissue, F_O should be indicative of the amount of photosynthetic tissue in the sphere. This justifies the use of F_O as a normalization factor for plant size, which was accomplished in the above formula by dividing the induced fluorescence by F_O. In this study, the suitability of F_O as an indicator of plant size was assessed.

A measure of the amount of light absorbed by the sample, I_{ABS}, may be a more appropriate estimate of plant size. Normalization by I_{ABS} is supported by the statement that "as with any photochemical process, a [fluorescence] yield can be defined as the light emitted divided by the light absorbed" (Gregory 1989, p103). In addition, use of I_{ABS} as a substitute for dry weight for normalization of photosynthetic parameters (CO_2 exchange rates) was proposed and used by Dube (1990). He found that I_{ABS} had a better correlation with shoot fresh weight than did F_O. A comparison of silhouette area with the F_O and I_{ABS} values obtained in this study (see Chapter 6) indicated that I_{ABS} was by far the more suitable normalization factor for plant size. As a result of this evidence, the fluorescence induction curves obtained in this study were renormalized according to the formula:

$$Fv = (F - F_O) * (I_{ABS}/100)^{-1}$$

where

Fv = the normalized variable fluorescence at time t,

F = the fluorescence (in mV) at time t,

F_O = the O-level fluorescence (in mV), and

$$I_{ABS} = I_O - I_S$$

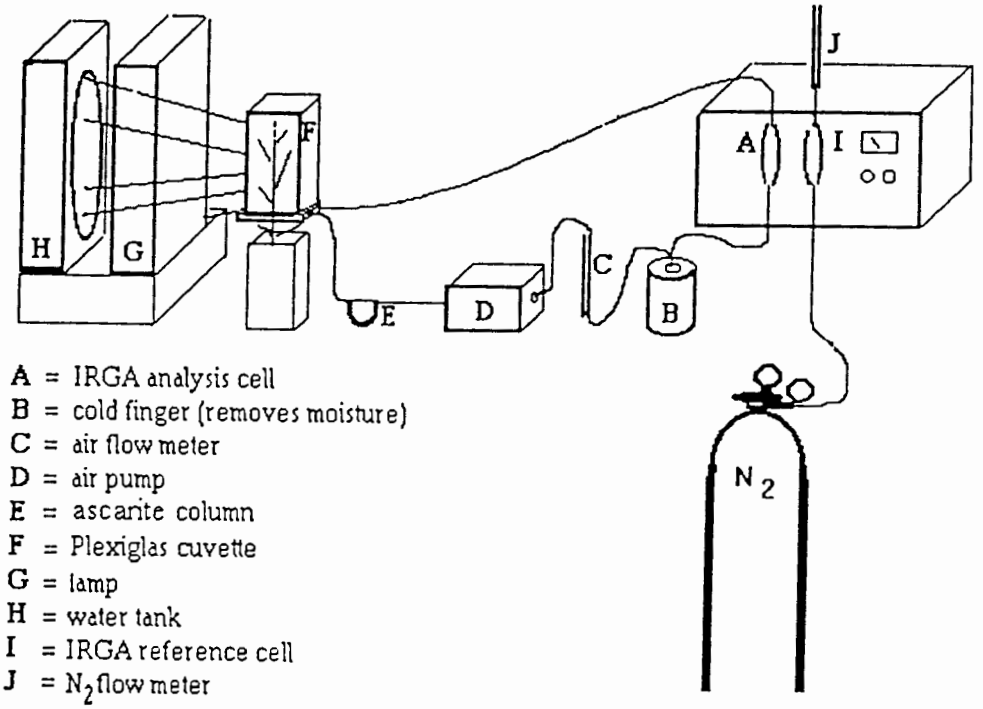
where

I_{ABS} = the light absorbed (in mV) by the sample,

IO = the light intensity (in mV) in the empty sphere, and

IS = the light intensity in the sphere (in mV) in the presence of the plant, immediately after shutter opening.

Figure 3.3
The closed-circuit gas exchange system.



A = IRGA analysis cell
B = cold finger (removes moisture)
C = air flow meter
D = air pump
E = ascarite column
F = Plexiglas cuvette
G = lamp
H = water tank
I = IRGA reference cell
J = N_2 flow meter

3.4 Carbon Dioxide Exchange

CO_2 uptake and release at saturating light intensities provide an estimate of apparent photosynthesis (APS), indicative of the plant's net carbon gain under consistent laboratory conditions. APS is a measure of total photosynthetic carbon gain, minus carbon losses due to photorespiration and dark respiration. It is useful for assessing a plant's metabolic performance as it varies with treatment, season and environment. The rate of CO_2 release in the dark provides an estimate of dark respiration, which is indicative of growth and maintenance costs of tissue (at laboratory temperature).

The rate of CO_2 uptake or release at two photosynthesis-limiting light intensities can be used to determine quantum efficiency, which indicates the relationship between incident PFD and CO_2 uptake and light intensity.

3.4.a Light Acclimation

Each plant was sequentially placed before a warmup lamp (400 watt Poot Elektra type PC 1078/N lamp, Lucalox bulb LU 400/40) at 300 $\mu mol/m^2/s$ PFD for 15 min. A plastic bag was loosely placed over the plant to maintain a high relative humidity, preventing water stress, stomatal closure, and altered photosynthetic gas exchange. Only one plant was exposed to 300 $\mu mol/m^2/s$ PFD at a time; the waiting

plants were placed back under the humidity-controlled tent. After 15 min., the light-acclimating plant was moved closer to the light source, to increase the illuminating PFD to 600 μmol/m^2/s. 15 min. later, the plant was moved forward again, where it was exposed to 800 μmol/m^2/s PFD for 30 min. This photosynthetic induction period is standard procedure for photosynthetic gas exchange assessment. It is intended to prevent adverse effects (such as photoinhibition) which can result from sudden exposure to high light intensities.

3.4.b The Gas Exchange Monitoring System

CO_2 exchange was measured using a method modified from Lister *et al.* (1961). The standard procedure is outlined in sections 3.4.c and 3.4.d.

The closed circuit gas exchange monitoring system is illustrated in Figure 3.3. The sample chamber is positioned before a 400 watt Poot Elektra type PC 1078/N lamp, Lucalox bulb LU 400/40. A cold water bath reduced the heat reaching the plants from the lamps.

3.4.c Calibration of the Instruments

The Beckman model 865 infrared gas analyzer (IRGA) and chart recorder (Metrohn labograph E478) were calibrated at the start of each day's measurements; they were often recalibrated at the end of the day, to check for any drift that might have occurred. .Bottled nitrogen gas (N_2; Linde gases, calibrated against a Matheson standard), used as a reference gas, was flushed through both the reference and the sample cells of the Beckman model 865 infrared gas analyzer (IRGA). Next, N_2 was flushed through the reference cell only, and an analyzed gas mixture containing 348 ppm CO_2 v/v in air (Matheson calibrated gases) was circulated through the sample cell (flow rate 3.8 liter min^{-1}; this matched the flow rate in the experimental procedure, nullifying any pressure effects). The gas mixture was disconnected from the system, and the closed air circuit was completed. Lab air pressure and temperature were recorded.

3.4.d Assessment of Gas Exchange

The top 20 cm of each light-acclimated plant was sealed into the Plexiglass cuvette, and connected into a closed gas circulation system (Figure 3.3). The IRGA measured changes in the concentration of CO_2 in the system. An air pump maintained a constant gas flow rate of 3.8 liter min^{-1}. At 800 $\mu mol/m^2/s$ illumination, CO_2 content of the air in the sealed system was measured as it fell from 380 to 330 ppm. (Preliminary analyses of CO_2 uptake rates at various light intensities indicated that the

light saturation point for these plants was between 550 and 600 μmol/m^2/s PFD.) The speed of the chart recorder was noted, so the resulting line could be converted into a CO_2 uptake rate. The circuit was briefly broken, and fresh air brought the CO_2 content back up above 380 ppm. The measurement was repeated several times if necessary, until a minimum of two consecutive readings produced lines of similar slope.

A neutral density filter (cheesecloth) was then placed between the plant and the lamps, to reduce the light intensity to 480 μmol/m^2/s. The CO_2 content of the air was brought back above 380 ppm, and a single measurement was made. An additional neutral density filter (chromatographic paper) was then added to reduce the light intensity to 200 μmol/m^2/s; the CO_2 content of the air was brought back up, and a measurement was made. Finally, an opaque cover was placed over the Plexiglass cuvette for measurement of the dark respiration rate (from 330 to 380 ppm CO_2).

The plant was removed from the cuvette, and the procedure was repeated for each plant in turn. Any change in room temperature was noted. (The temperature rarely fluctuated by more than 2°C on any given day).

3.4.e Analysis of Data

The raw gas exchange data is in a form from which slopes (μl/l CO_2/min) are easily obtained. Conversion of slopes into values of mgCO_2/plant/hour involves the formula;

$$F = (\mu l/l\ CO_2/min)/10^3 * [2.304] * 273/T * P/760 * 1.963 * 60$$

where

F = flux of CO_2 (mg/plant/hour),

P = barometric pressure (mm Hg),

T = lab temperature (K),

[2.304] = the volume of gas in the circuit (L), and

1.963 = conversion factor for ml to mg.

Gas exchange data can be normalized by division by a suitable estimate of plant size. Dry weight has been used in the past as a standard normalization factor for plant size. However, it was not well suited to this study, in which a nondestructive measure was required.

Dube (1990) used I_{ABS} as a normalization factor for rates of gas exchange. Although I_{ABS} provided a good estimate of plant size, it was obtained with a diffuse sample illumination, whereas CO_2 exchange rates were obtained with a unidirectional light source. Due to the complex anatomy and opaque leaves of conifer shoots and the self-shading that results, a measure of the illuminated surface area was a more appropriate correction factor for plant size.

Smith *et al.* (1991) and Carter and Smith (1985) indicated that photosynthesis measured on the basis of silhouette area may provide the best indication of photosynthetic capacity, as compared to using dry weight or total leaf area. Given

that evidence, estimations of silhouette area were used in this study to normalize CO_2 exchange rates (see Section 3.6).

3.4.f Calculation of Quantum Requirement

Quantum requirement is an estimation of the number of photons striking the plant surface for every molecule of CO_2 fixed in photosynthesis. It can be calculated from APS rates at two sub-saturating light intensities, with the following formula:

QR = change in PFD ($\mu mol/m^2/s$) / change in CO_2 fixation rate ($\mu mol/m^2/s$)

where

change in PFD = 480-200 $\mu mol/m^2/s$,

change in CO_2 fixation rate = (rate in $\mu mol/m^2/s$ at 480 $\mu mol/m^2/s$) -

(rate in $\mu mol/m^2/s$ at 200 $\mu mol/m^2/s$), and

1.584 = conversion factor from mg $CO_2/dm^2/hour$ to $\mu mol\ CO_2/m^2/s$.

3.5 Nitrogen Content Determination

The amount of nitrogen available to plants is often correlated with the resultant foliar nitrogen content. However, foliar nitrogen concentration varies between leaves of a given plant. Sun leaves tend to have a lower nitrogen concentration than shade leaves, due to high fiber and carbohydrate content (Boardman 1977). In addition,

older leaves (ie. more than one year old) may have a relatively low nitrogen content, due to lesser demand for nutrients (Mooney and Gulmon 1982; Field 1983). Older needles tend to be depleted of mobile nutrients such as nitrogen, which have been translocated to actively growing tissue. As a result of these factors, certain species show a characteristic pattern of nitrogen distribution in the foliage. The highest nitrogen levels in the foliage of Douglas-fir, for example, are found in the upper crown (van den Dreissche 1974).

These factors were taken into consideration before foliage was sampled for nitrogen content analysis. Needles of similar origin (ie. stem position and age) were sampled every time. Current-year needles from midway along the apical shoot were sampled in 1993. After the plants had flushed in 1994, apical shoot needles from both 1993 and 1994 were sampled for comparison.

Each oven-dried sample (2 or 3 needles) was cut into small pieces, from which two subsamples of 1.500 to 1.800 mg were taken. Their precise weights were recorded, and the samples, wrapped in small tin containers, were analyzed with an HCN Autoanalyzer (Carlos Erba). The instrument performed a flash combustion of each sample at +950^{o}C, then produced a light absorption curve for each element: H, C, and N.

A PC coordinated the data collection and calculated the nitrogen, hydrogen and carbon concentration of each sample. This involved a comparison of the absorption curve of the combustion products of each sample to the absorption curve of a known amount of the organic analytical standard Acetanilide

($C_6H_5 \cdot NH \cdot COH_3$=135.17gmw; BDH Chemicals Ltd., Poole, England). The calculations involved the following formulae:

A K-factor was calculated for each element from the following equation:

$$K = (\%t * Ws) * I^{-1}$$

where

%t = the theoretical percentage of the standard,

Ws = the weight of the standard (mg), and

I = the standard integral.

The percentage of each element in a sample was determined with the following equation:

$$\% = (K * I) * W^{-1}$$

where

K = the K- factor of each element,

I = the sample integral, and

W = the weight of the sample (mg).

A blank test was used immediately after the standard was analyzed to ensure that the instrument was running cleanly and properly. Any integrals obtained in the blank test were subtracted from the K-factor and percentage calculations. The blank

test was performed by running an empty tin container through a complete analytical cycle.

3.6 Silhouette Area Determination

The complex architecture of conifer foliage makes it extremely difficult to estimate surface area of the foliage nondestructively. In fact, this complexity may make leaf area an unsuitable parameter for plant size correction; when light is provided on a single plane, self-shading can result in large discrepancies between total surface area and the leaf area which actually intercepts the light. These discrepancies are greatly reduced with the use of an integrating sphere, which allows for diffuse, instead of unidirectional, illumination. For this reason, a parameter which is measurable in the integrating sphere and is well correlated to plant size is greatly desirable.

In previous studies, two parameters, F_O and I_{ABS}, have been linked to the amount of tissue in the integrating sphere (Dube 1990). In order to determine which, if either, of these parameters is a suitable estimate of sample size, estimations of sample silhouette area were made for comparison.

The top 20 cm of each shoot was carefully photocopied onto graph paper. The images were trimmed, and the foliage was blacked out with ink (when necessary). The images were photocopied onto overhead sheets, from which silhouette area values were calculated using a LI-3000 portable area meter (LI-COR, Lincoln, NB).

CHAPTER 4
RESULTS AND DISCUSSION:
SEASONAL PATTERNS

Chemical and physiological assessments revealed various seasonal patterns, which are discussed in this chapter. Seasonal variation occurred in foliar nitrogen and carbon concentrations, chlorophyll fluorescence parameters and CO_2 exchange rates. Weekly high and low temperatures are shown in Figure 4.1. They provide background information about the climatic trends of the study period, which assists the understanding of this seasonality.

4.1 Foliar Nitrogen Concentration

Average foliar nitrogen concentration for each treatment on each analysis date is illustrated in Figure 4.2. The May and June data (1994) are averages from old and new foliage. Nitrogen concentration did not remain constant throughout the study period; the variation showed some seasonality. There was a trend towards increasing nitrogen concentration as the tissue matured and approached dormancy in Autumn 1993. A drop in nitrogen concentration occurred at the time of flushing (April 1994).

This decline can be explained by the dilution of nitrogen; nutrients were shared between old and new foliage. Other researchers have shown that nitrogen translocated from year-old foliage of Douglas-fir is important for flushing and shoot extension (Webb 1975; Camm 1993).

The nitrogen concentrations of old and new needles before, during and after flushing are shown in Figure 4.3a to e. Note the greater discrepancies between old and new needles on May 10 (d), as compared with the two later dates (b,e). At first, nitrogen levels were highest in the new needles, and were notably reduced in the older foliage of most samples. This illustrates the mobilization of nitrogen from old to new needles.

In all three treatments, nitrogen distribution between the old and new foliage approached an equilibrium quite soon after needle expansion, as illustrated in Figure 4.3e. The nitrogen concentration of old needles did not recover and reach the level found in the new foliage; rather, an intermediate level was approached in both age classes. This suggested that the new foliage was no longer a stronger nutrient sink than the old foliage once needle expansion was complete. It also indicated that the plants' nutritional demands were not fully met by uptake from the soil. The nutrient supply was not sufficient to maintain the existing nitrogen concentration of the foliage, even for the H treatment. This shortfall did not result in any visible symptoms of deficiency, contrary to the predictions of Ingestad (1982).

Figure 4.1
Weekly high and low temperatures for the study period.
Weeks run from Wednesday to Tuesday.

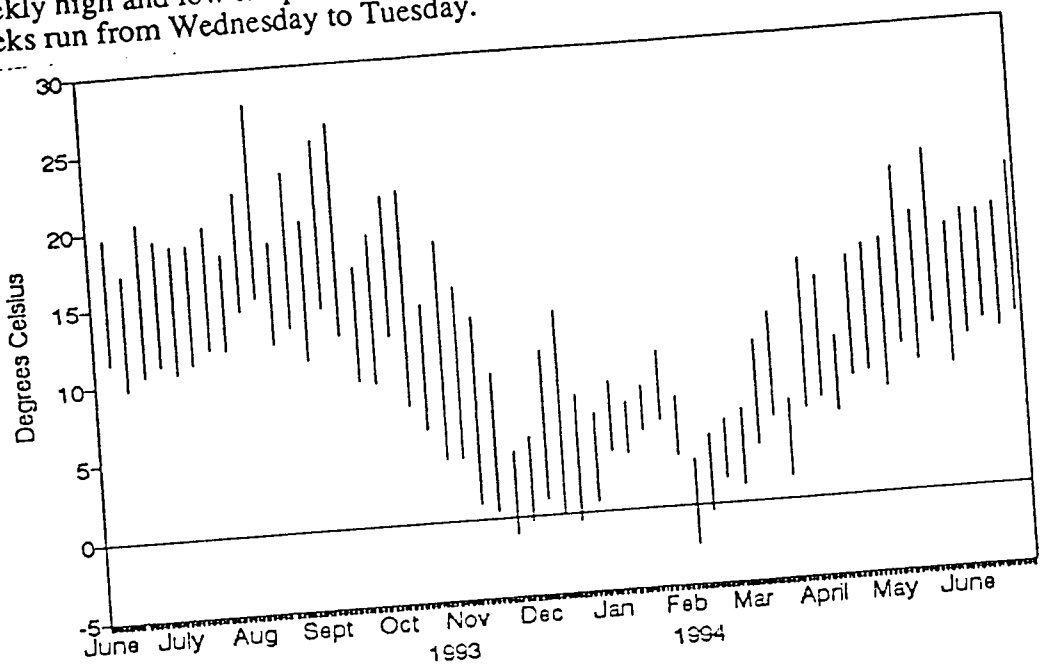


Figure 4.2
Average foliar nitrogen concentration (% of dry weight) for each treatment on each analysis date (n=5).

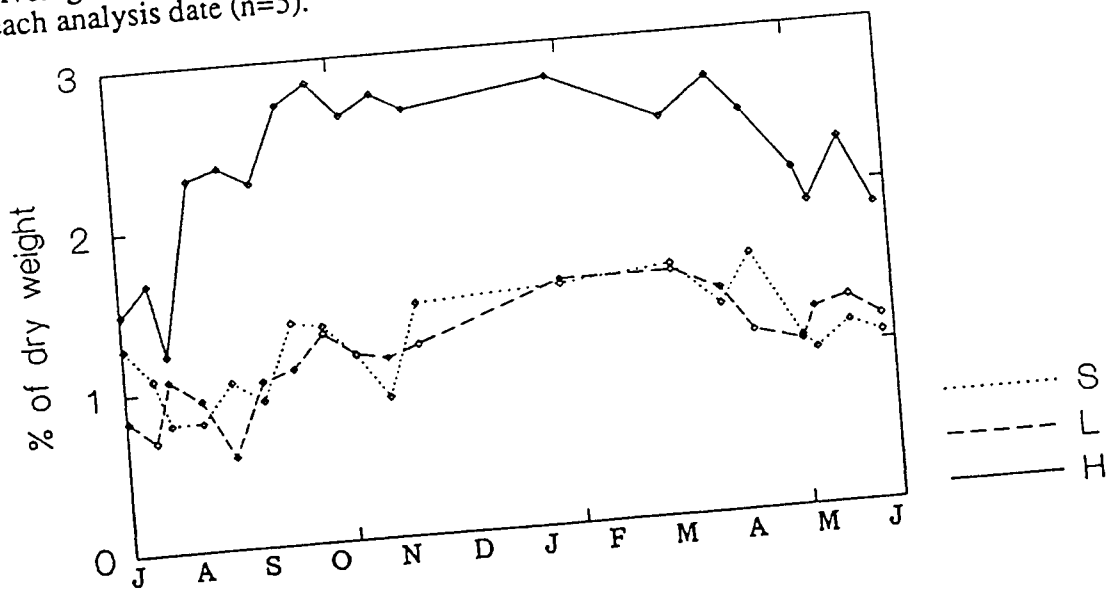
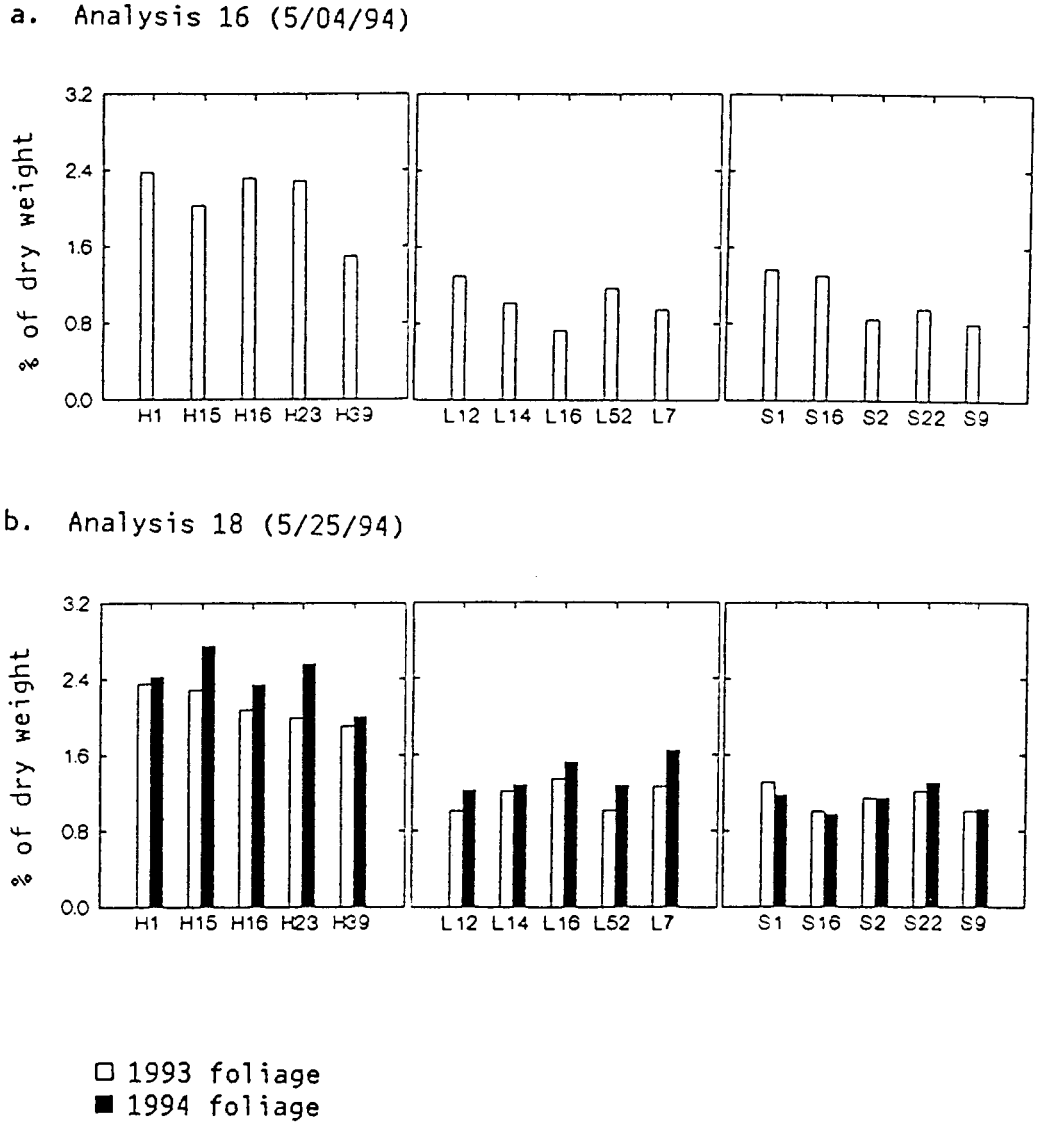
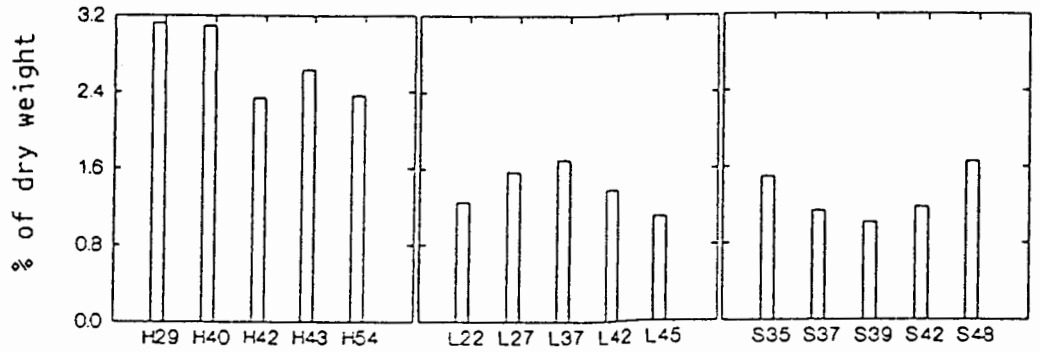


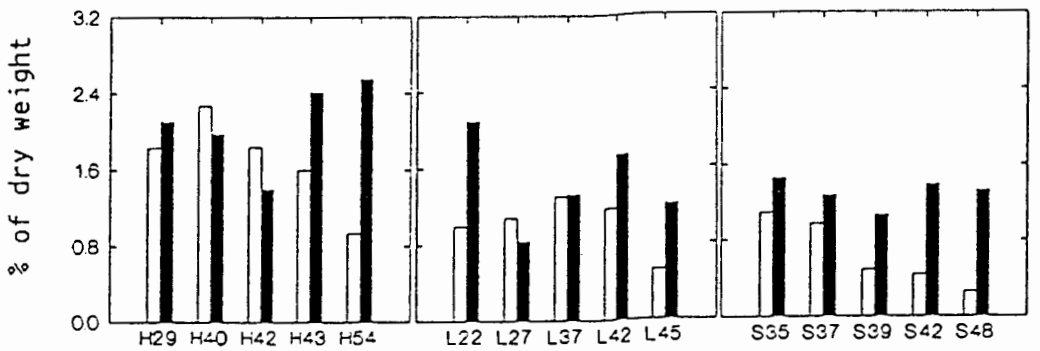
Figure 4.3a-e
Concentration of nitrogen (% of dry weight) in old and new needles of each sample in the spring of 1994.



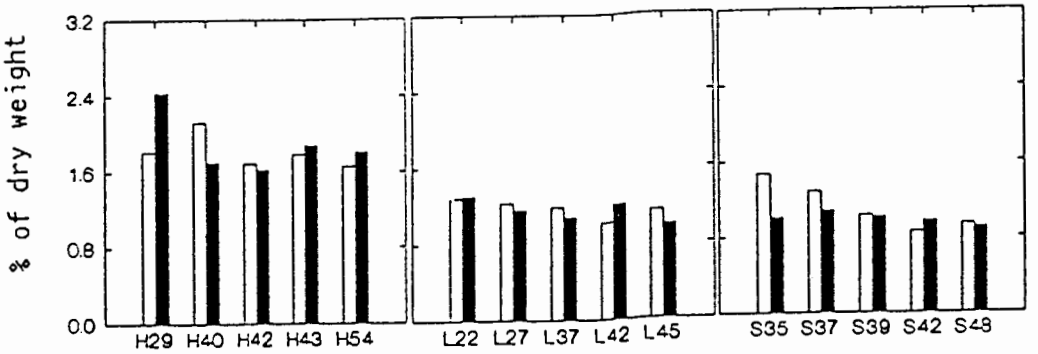
c. Analysis 14 (3/30/94)



d. Analysis 17 (5/11/94)



e. Analysis 19 (6/08/94)



1993 foliage
1994 foliage

Figure 4.4
Average foliar carbon concentration (% of dry weight) for each treatment on each analysis date (n=5).

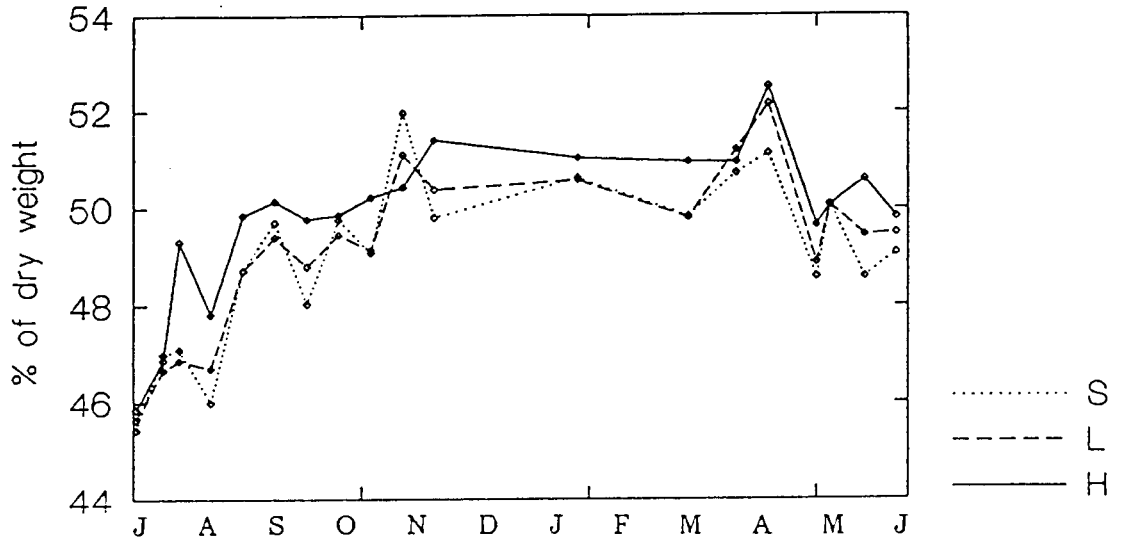
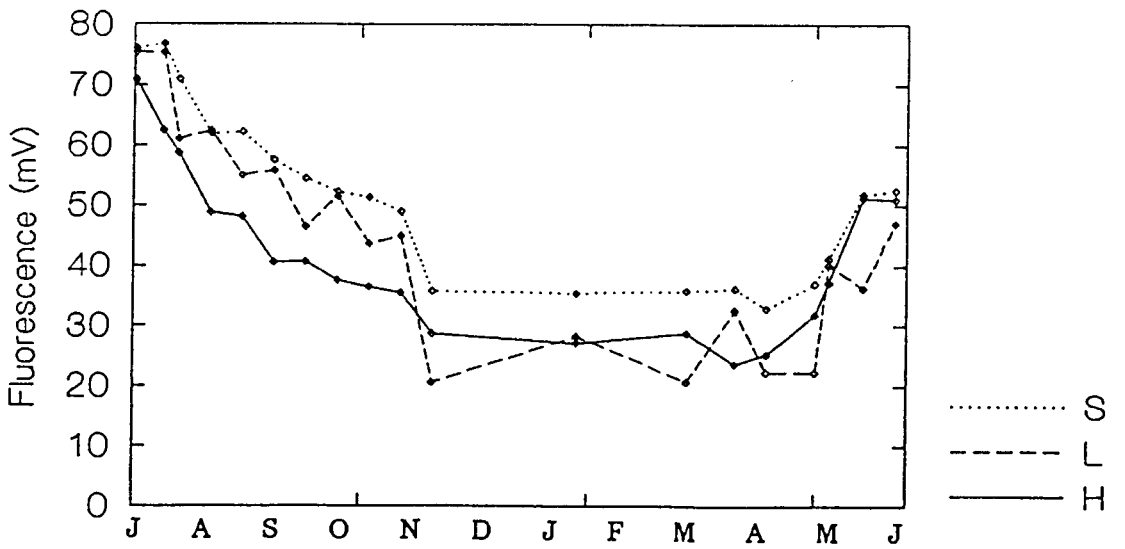


Figure 4.5
Average initial fluorescence (F_O; mV) values for each treatment on each analysis date (n=5).



Carbon concentration did not show a strong seasonal pattern; it trended slightly towards higher levels in the foliage during winter (Figure 4.4). One might have predicted a higher carbon concentration in tissue that was highly photosynthetically active, since the products of photosynthesis could have accumulated in the foliage. Apparently, newly fixed carbon was not stored in substantial quantities in the needles of these plants. It is likely that carbon was quickly exported to the stems, roots or newly forming tissue.

4.2 Initial Fluorescence

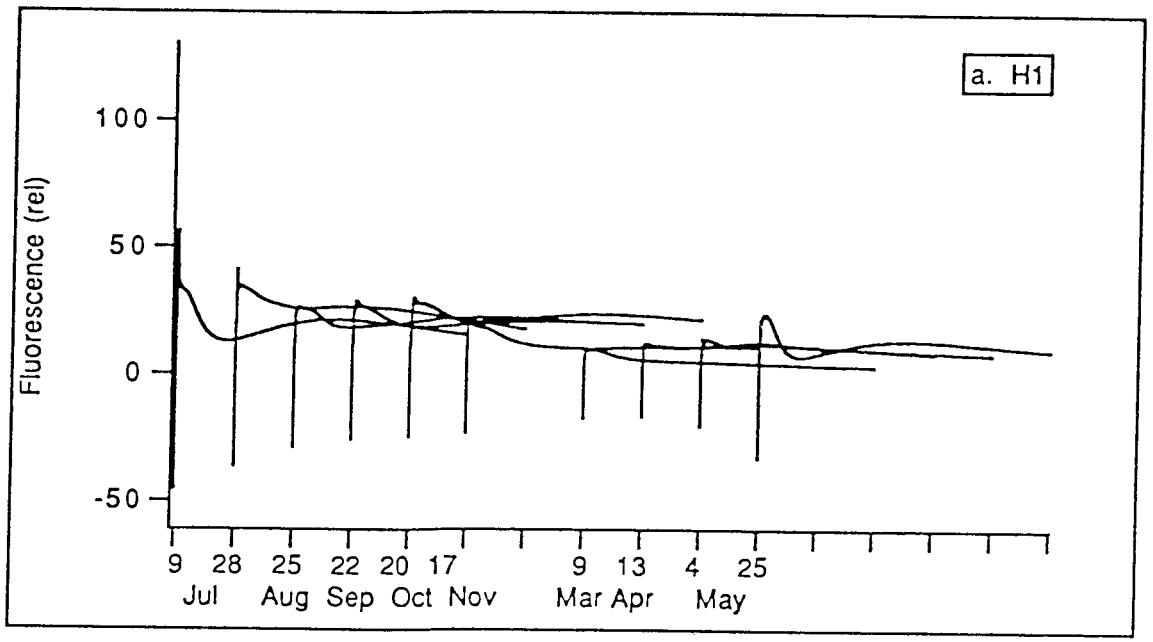
Initial fluorescence (F_O) decreased throughout the 1993 growing season, and fell to a minimum while the plants were dormant in winter (Figure 4.5). F_O values decreased by more than 50% between July and November 1993, and they started to recover when the plants began to flush in the spring.

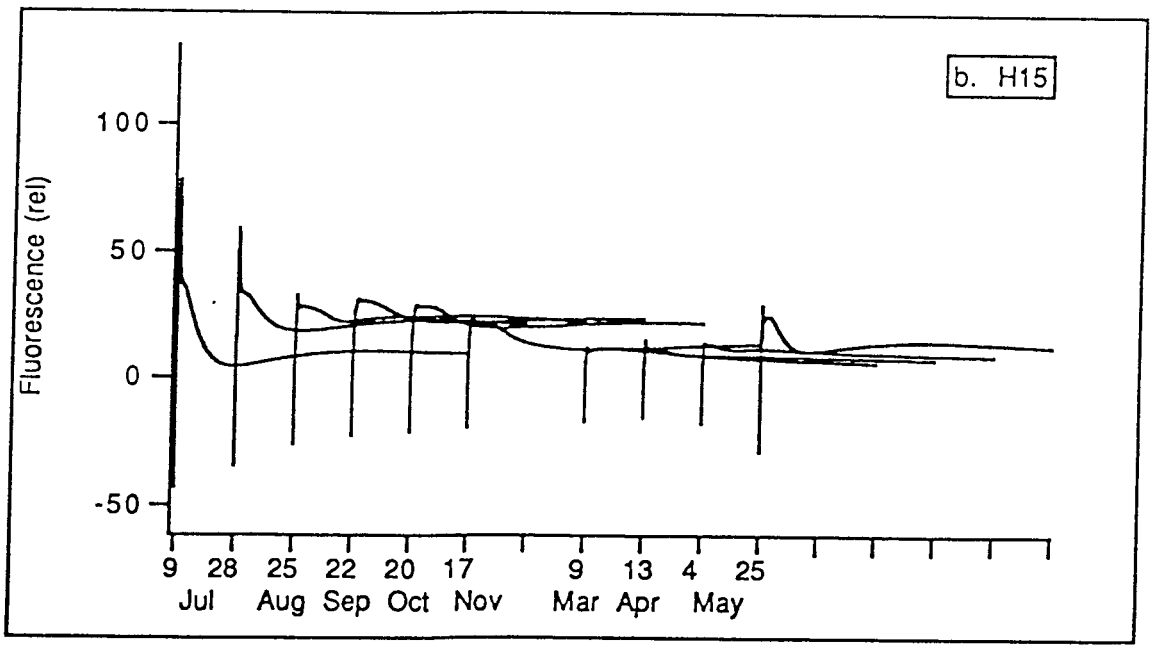
These findings bring the physiological significance of F_O into question. F_O is commonly used as a base-level fluorescence indicator. It is assumed that F_O is directly proportional to the number of chlorophyll *a* molecules in the sample (Dube 1990). It contains no variable fluorescence component and involves no photochemical activity. If these assumptions are correct, then F_O would remain relatively stable with time, in healthy plants of constant size.

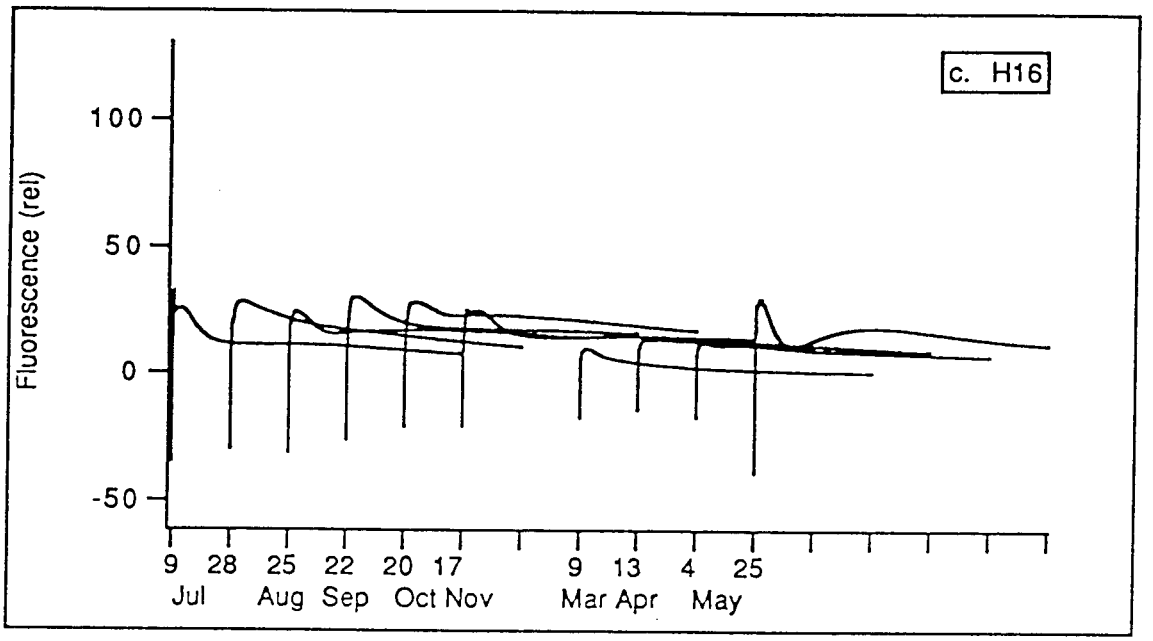
There are several possible explanations for the observed drop in F_O. Firstly, when the tissue was young and inefficient at light harvesting, a high degree of energy loss in the forms of heat and fluorescence may have occurred. As the tissue matured, it may have trapped light more efficiently, resulting in less apparent initial fluorescence. This hypothesis is supported by the increase in F_O which became apparent when the plants flushed in the spring of 1994. However, several H trees flushed for a second time in the summer of 1993 (including H16, H42 and H54). This flush was not accompanied by an apparent rise in F_O (Figure 4.5 and Figure 4.6c,r,t). Also, it is difficult to support this hypothesis without contradicting the assumption that F_O involves no photochemistry. Presumably, for the inefficiency of light harvesting to affect F_O, photochemical activity would have to be involved.

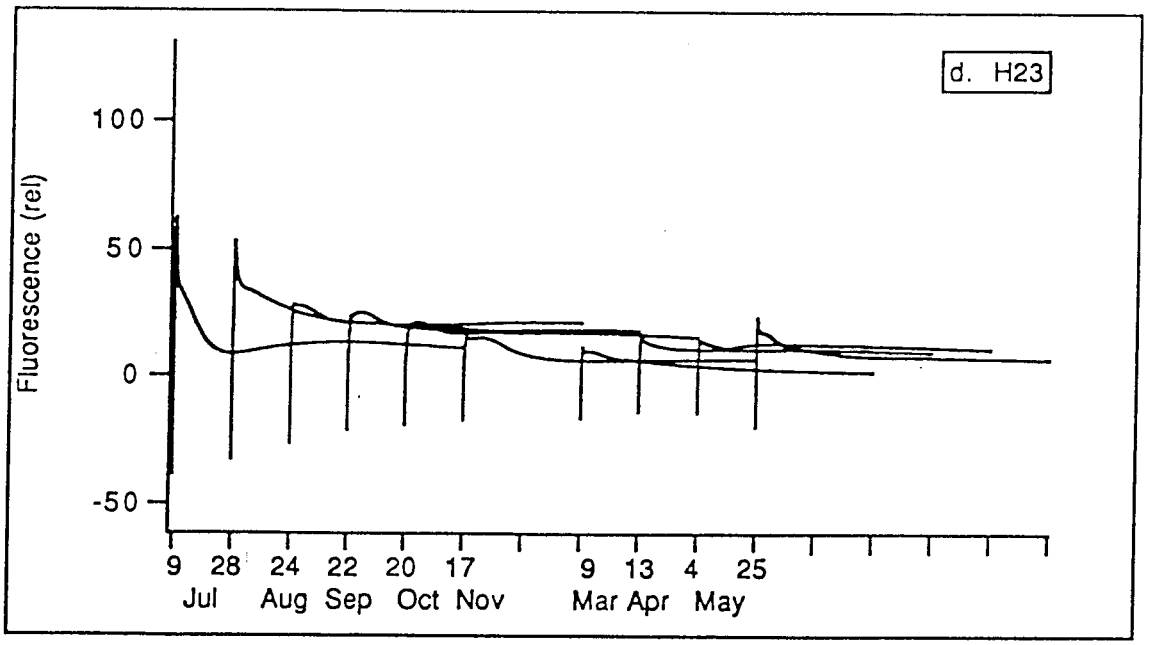
A second explanation relates to the maturation of tissue and the production of a thickened cuticle, epicuticular waxes and accessory pigments. F_O may drop because less light passes through the tissue to excite chlorophyll molecules, and less fluorescence may pass through the tissue to escape and reach the fluorescence detector. However, the lack of a rise in F_O to accompany the second flush of 1993 provided evidence against this hypothesis. The reduction in F_O may have been a result of the development of photon protection pigments. Photon protection through xeanthin synthesis is known to result in decreased F_O (Franklin *et al.* 1992; Osmond *et al.* 1993). A photon protection system is vital for plants which experience high light levels and sub-zero temperatures; the development of such a system is a necessary part of cold-hardiness induction. A rise in F_O is indicative of photon damage and chronic photoinhibition (Osmond et al. 1993).

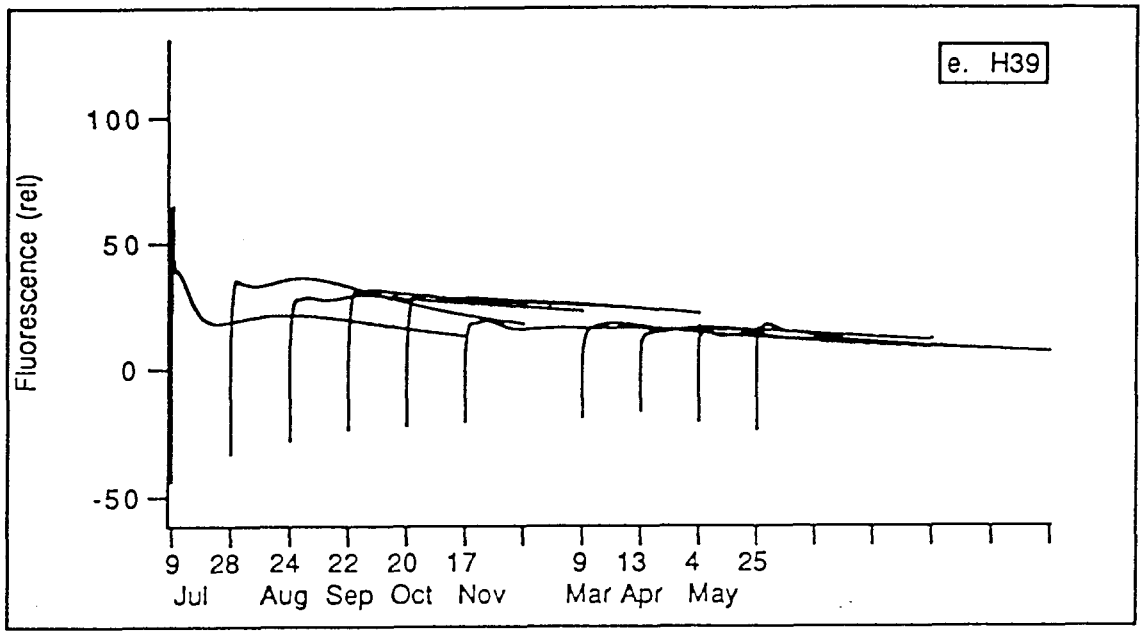
Figure 4.6a-ad
Fluorescence induction (Kautsky) curves for each sample at each analysis date.
Each induction curve is five minutes long.
The curves are staggered by 60 seconds for every month between analyses, in order to emphasize seasonal variations.
Graphs **a-o** are the red sample set; **p-ad** are the blue sample set.

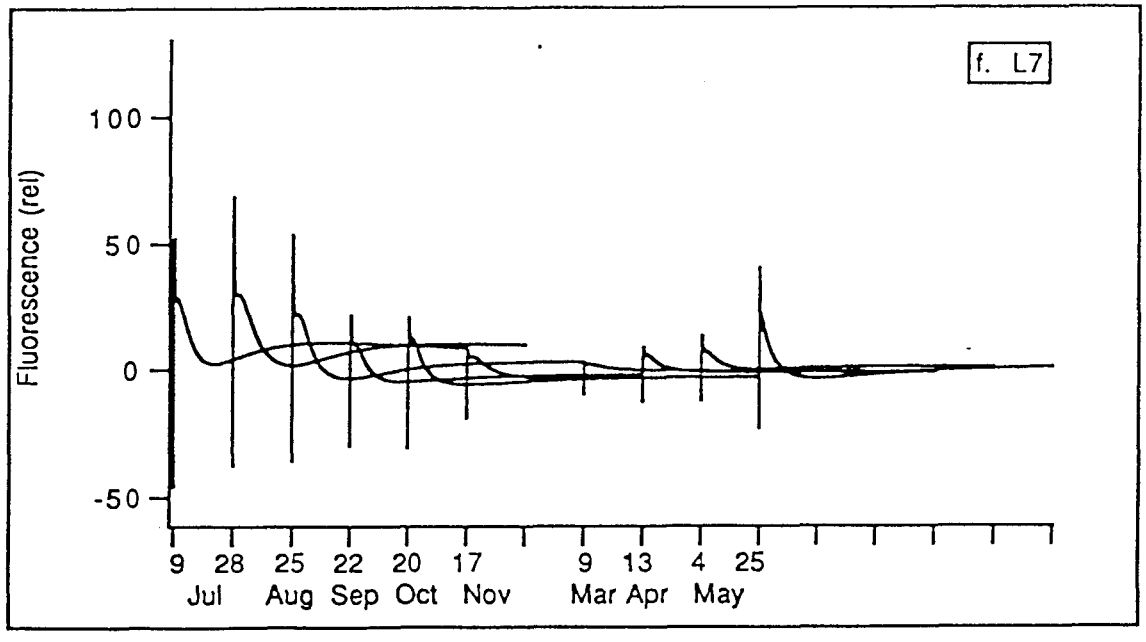


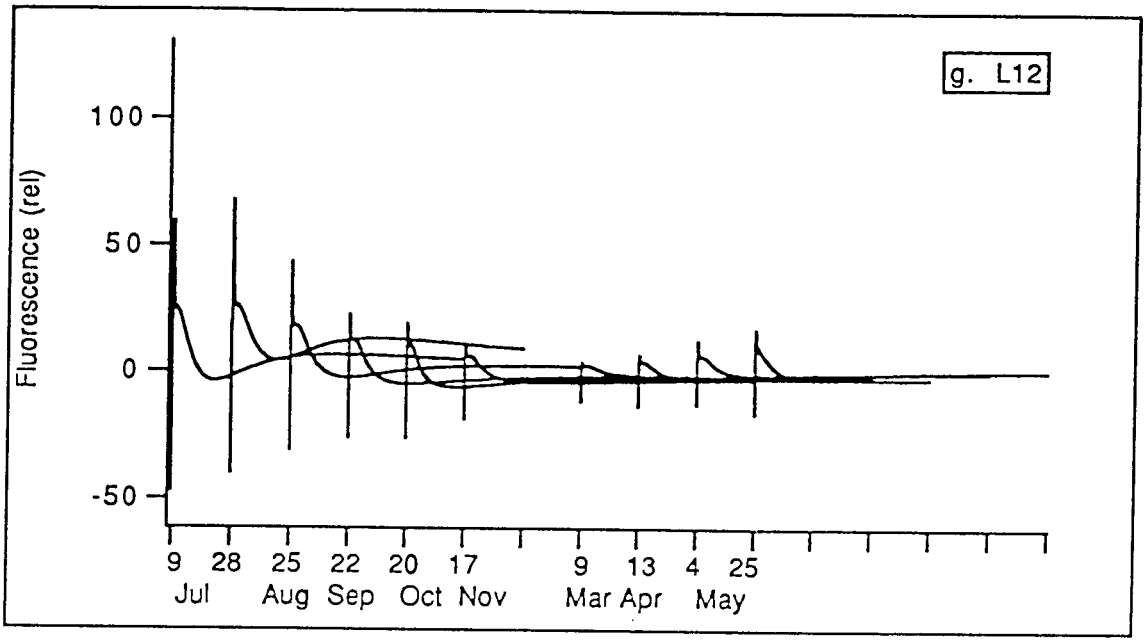


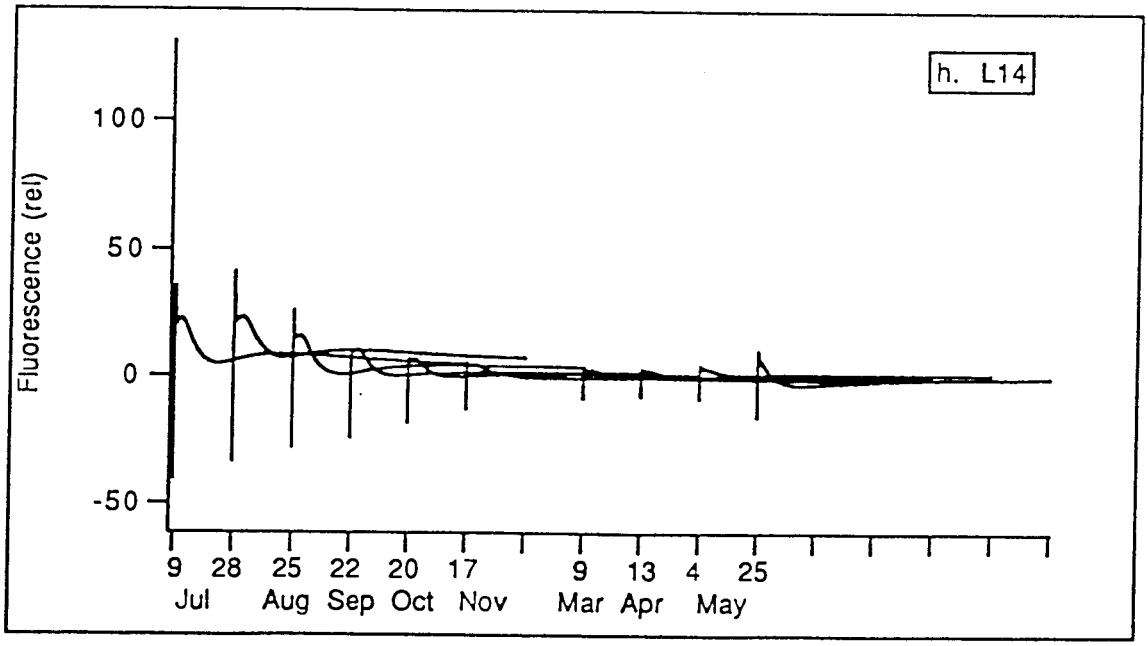

c. H16
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

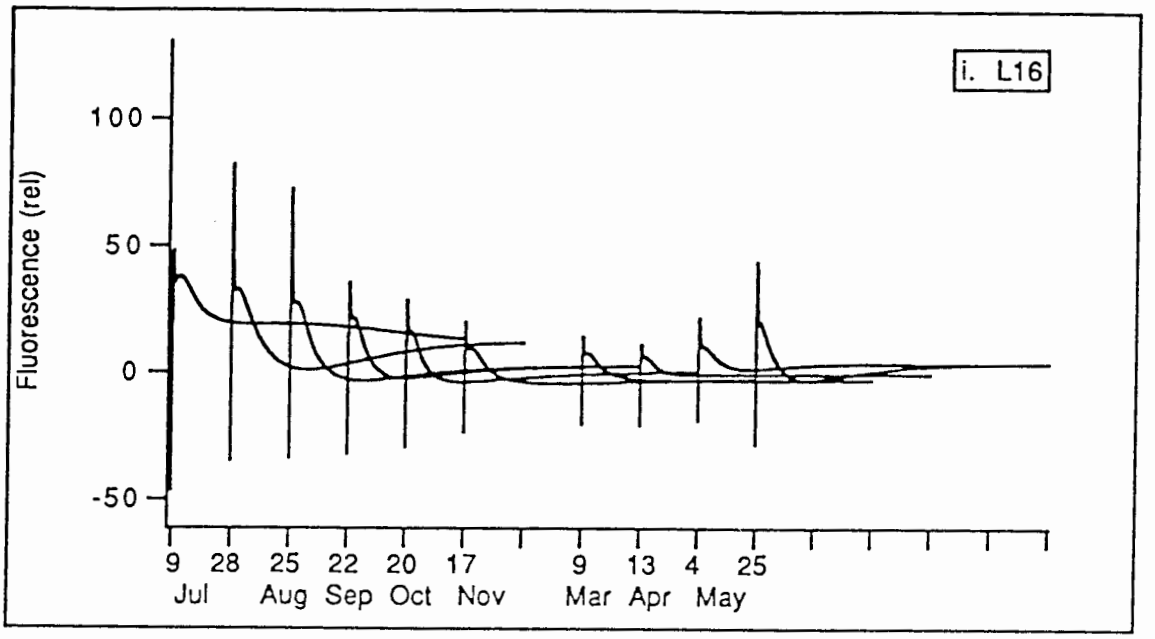

d. H23
Fluorescence (rel)
100
50
0
-50
9 28 24 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

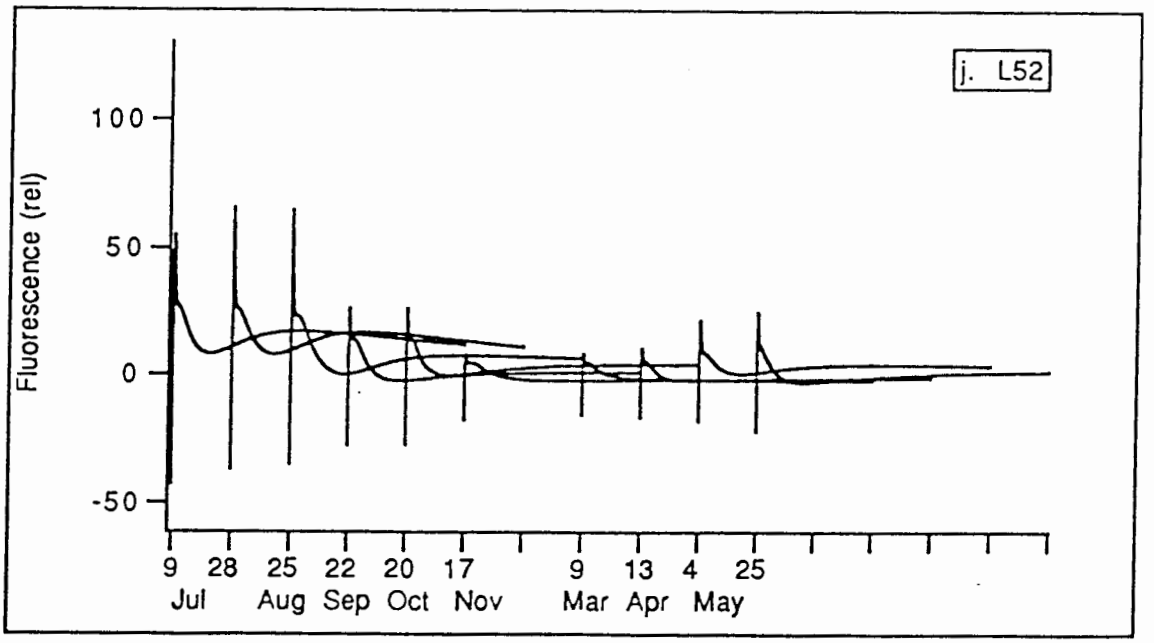

e. H39
Fluorescence (rel)
100
50
0
-50
9 28 24 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

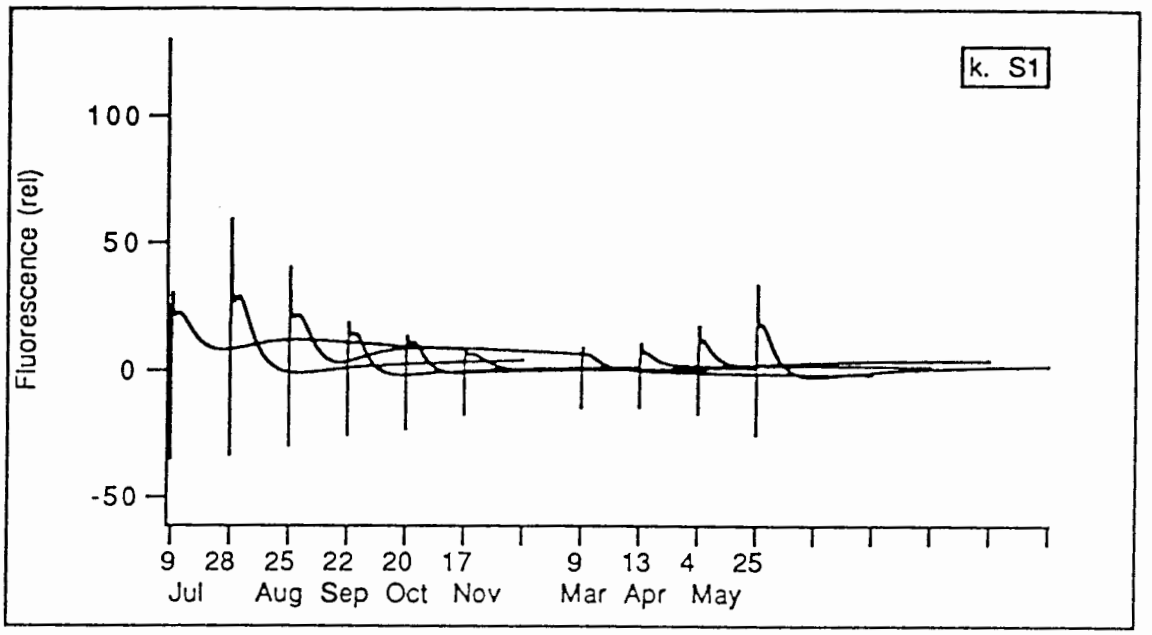

f. L7
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

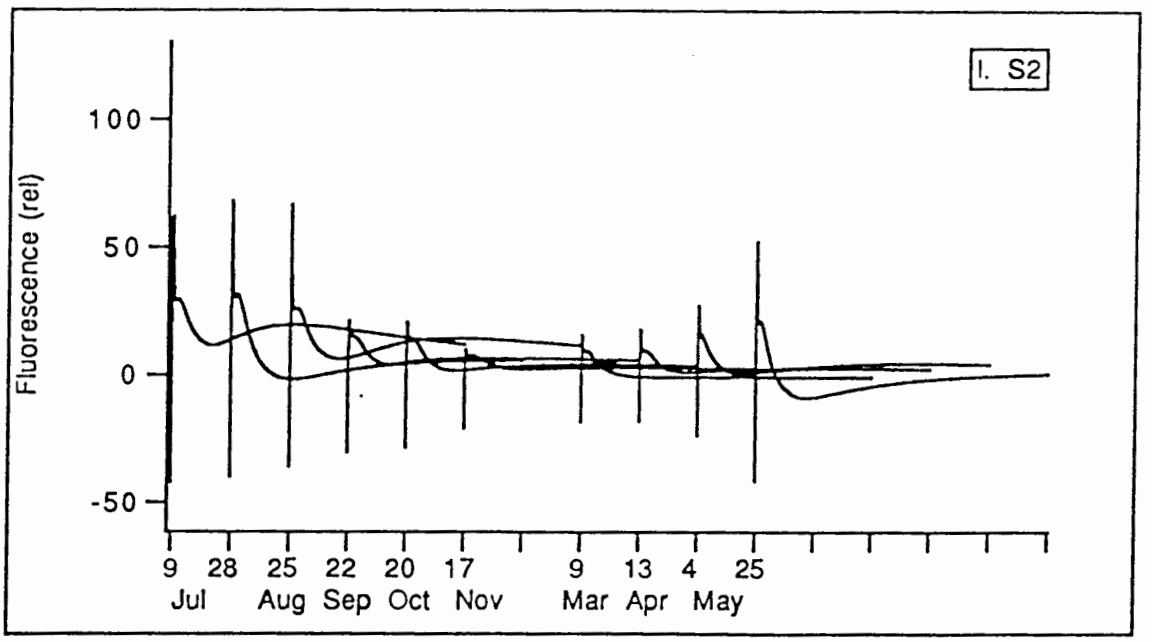

g. L12
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

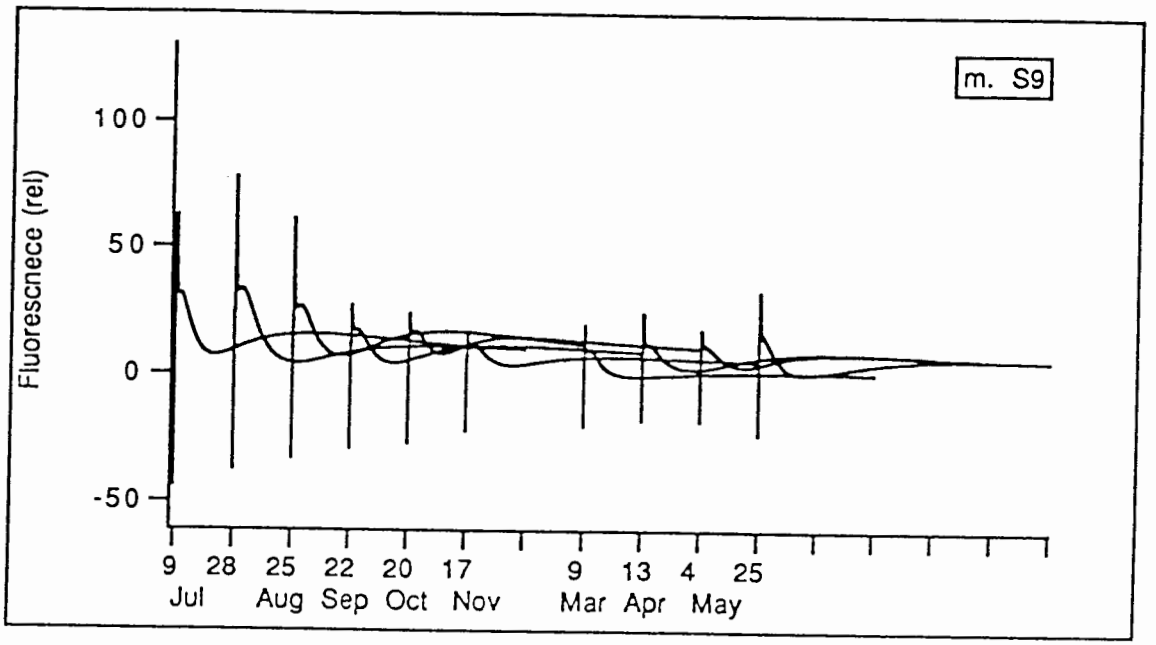

h. L14
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

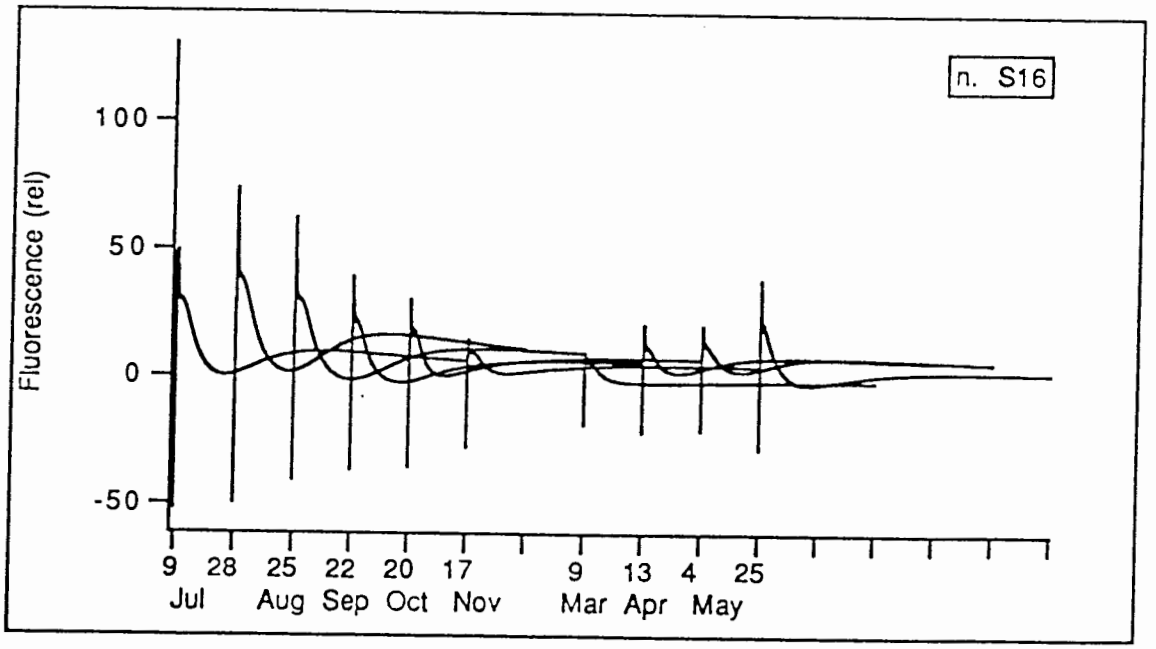

i. L16
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

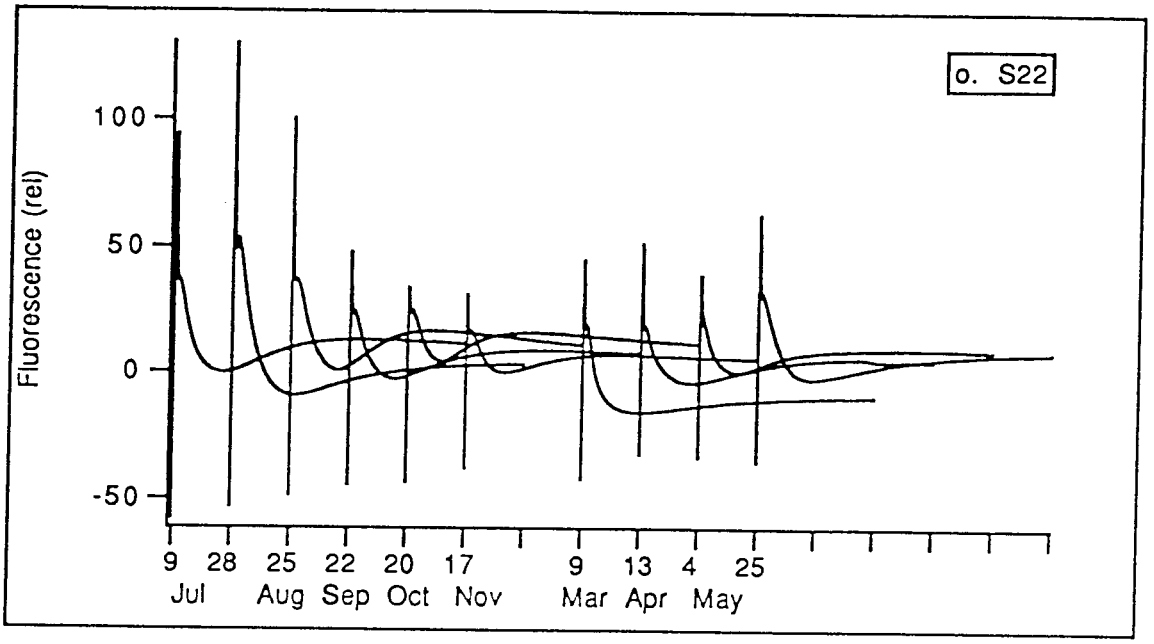

j. L52
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

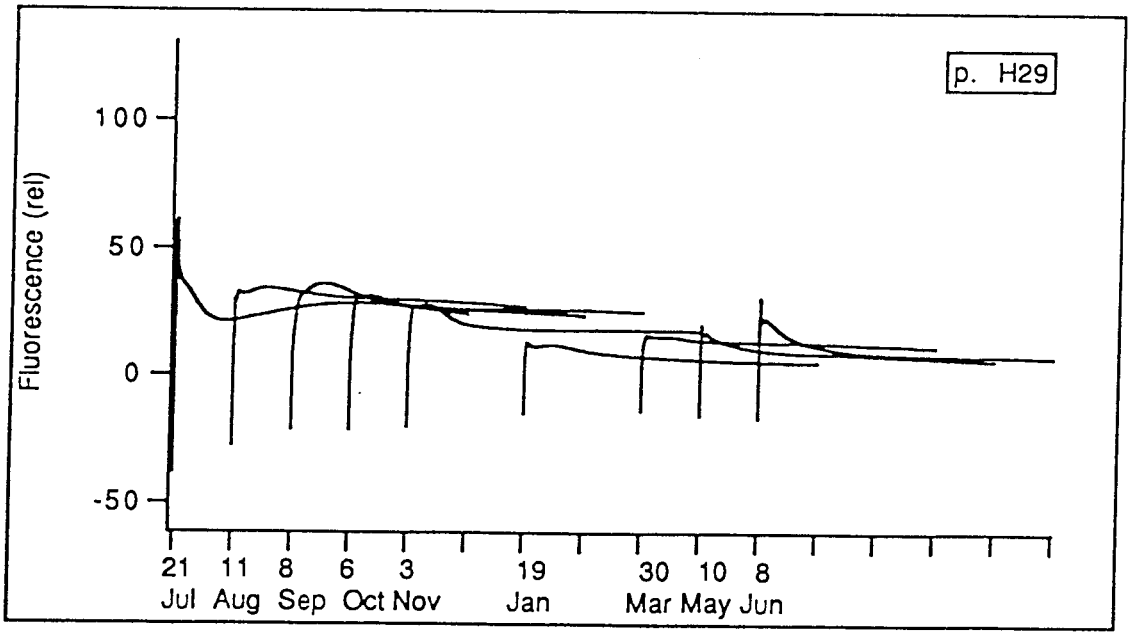

k. S1
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

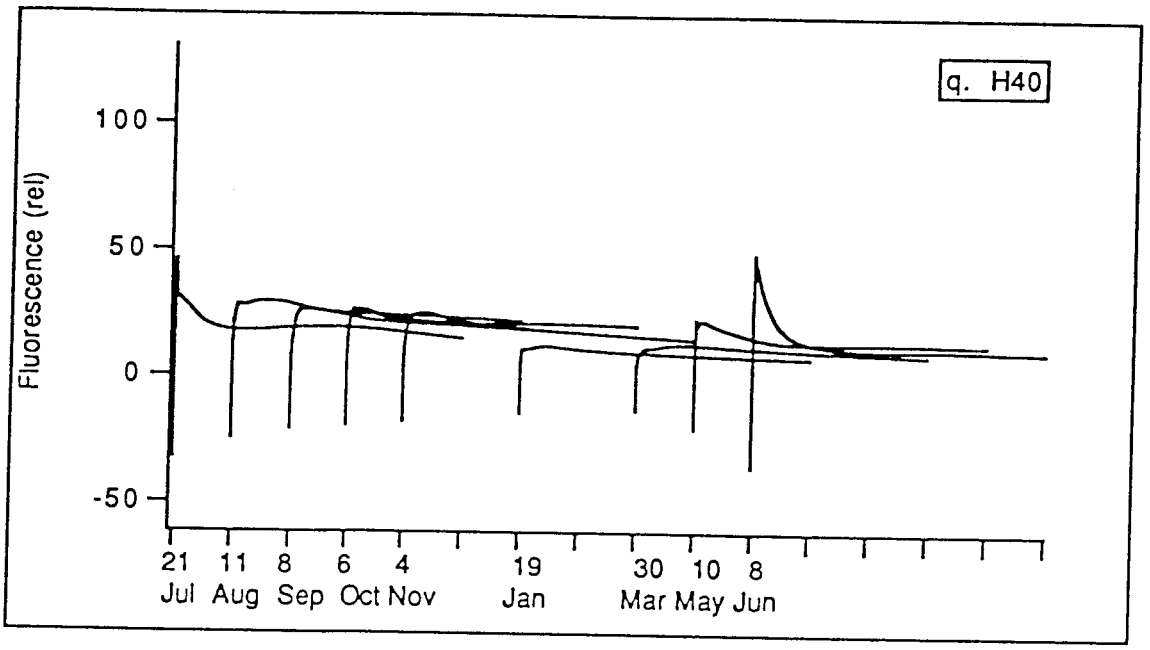

l. S2
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

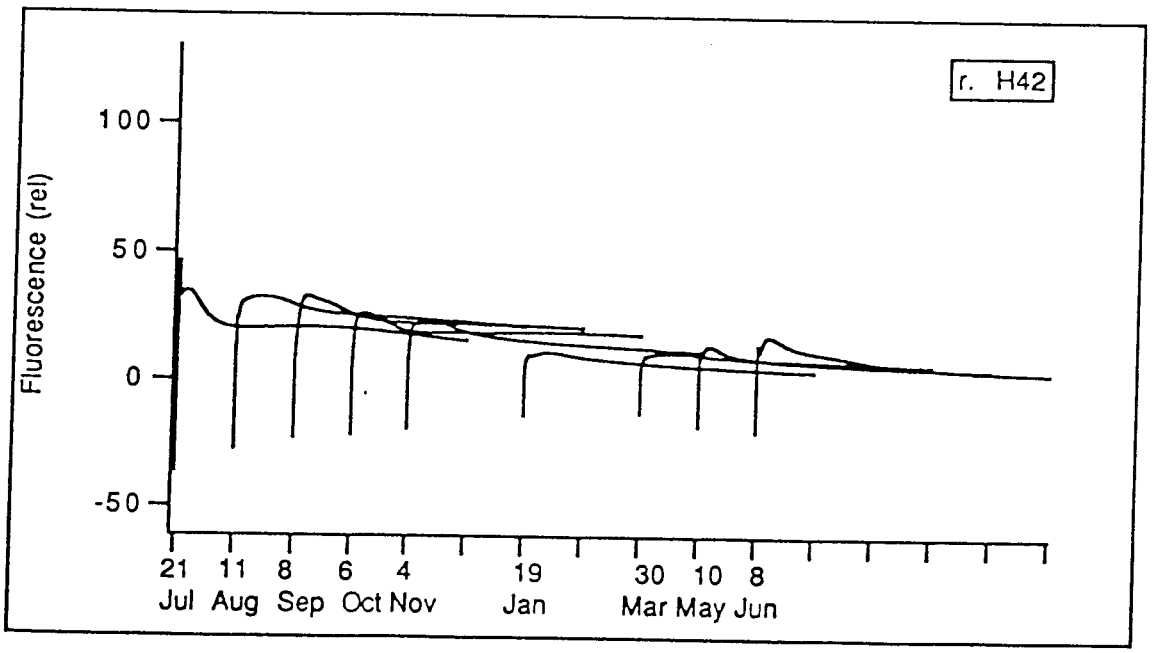

m. S9
Fluorescnece (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

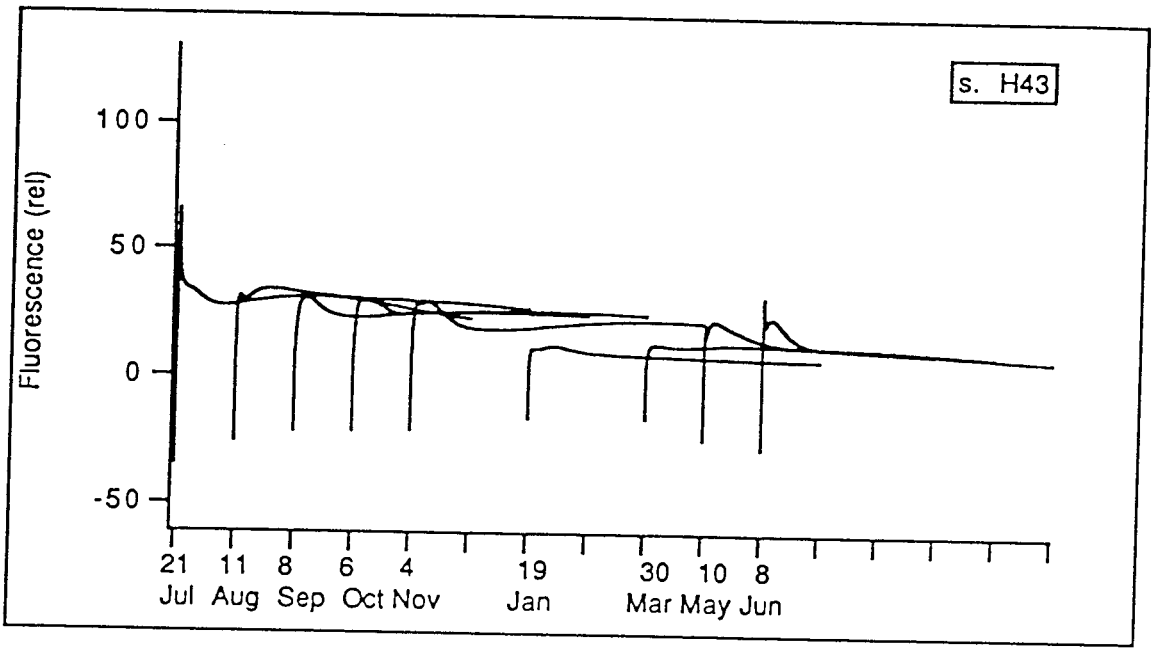

n. S16
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

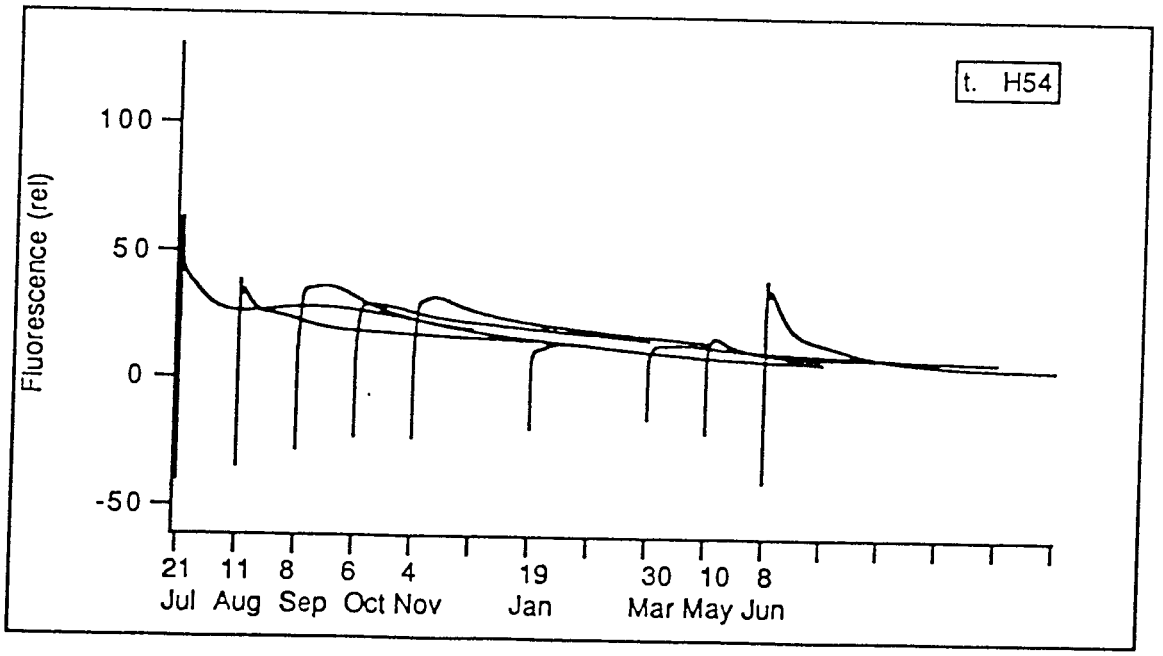

o. S22
Fluorescence (rel)
100
50
0
-50
9 28 25 22 20 17 9 13 4 25
Jul Aug Sep Oct Nov Mar Apr May

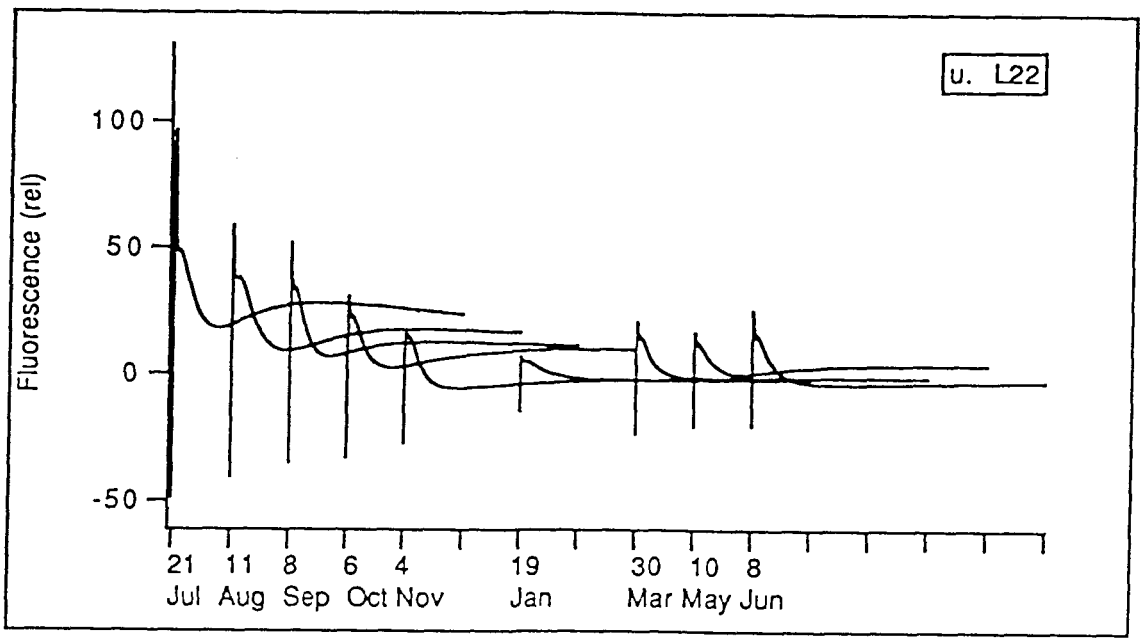

p. H29
Fluorescence (rel)
100
50
0
-50
21 11 8 6 3 19 30 10 8
Jul Aug Sep Oct Nov Jan Mar May Jun

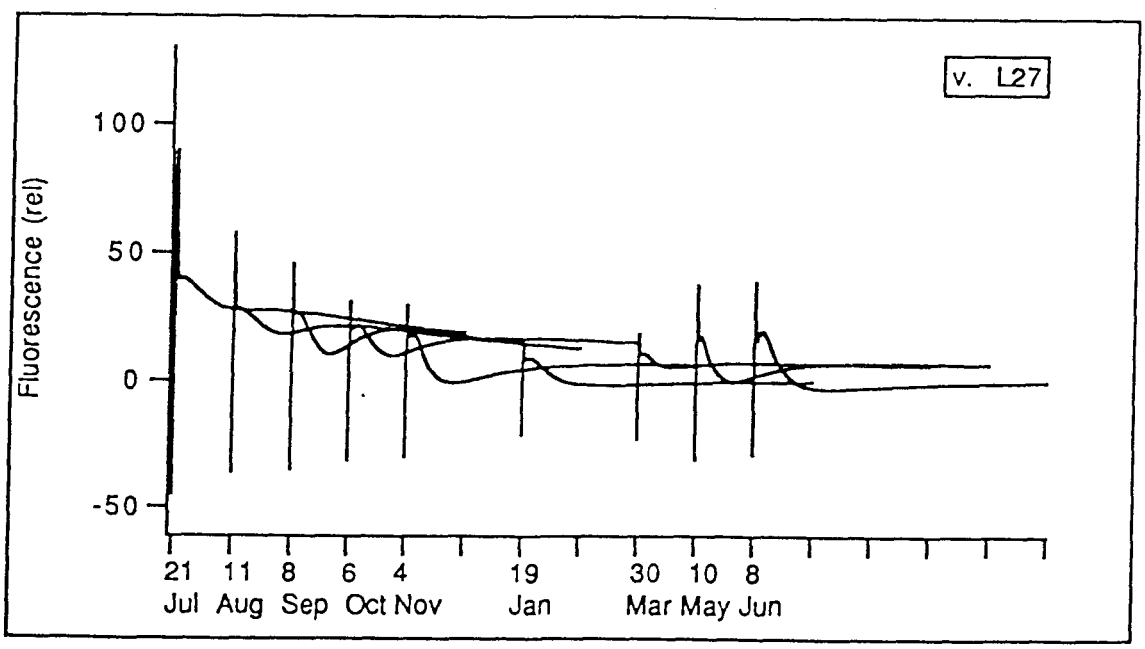

q. H40
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun

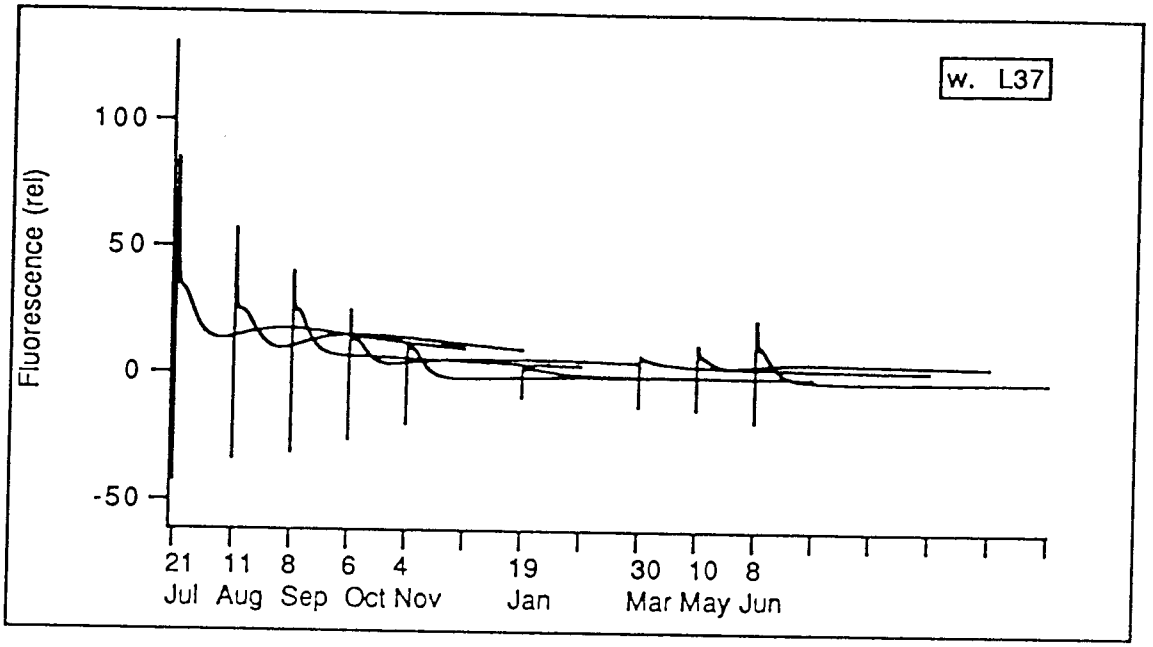

r. H42
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun

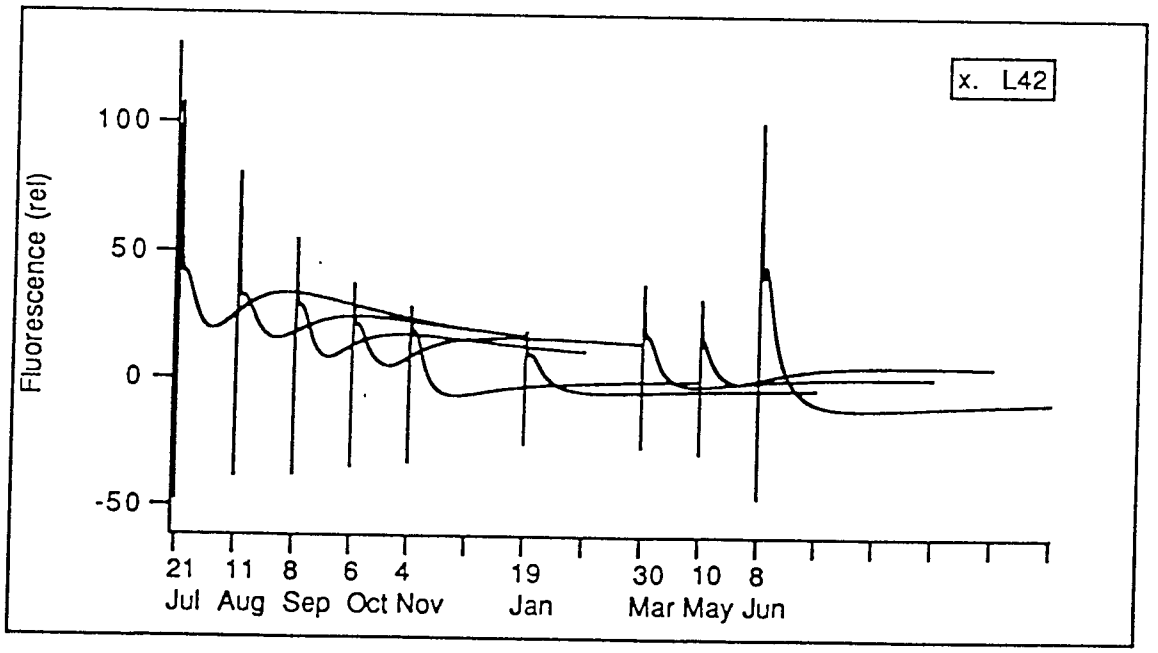

s. H43
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun

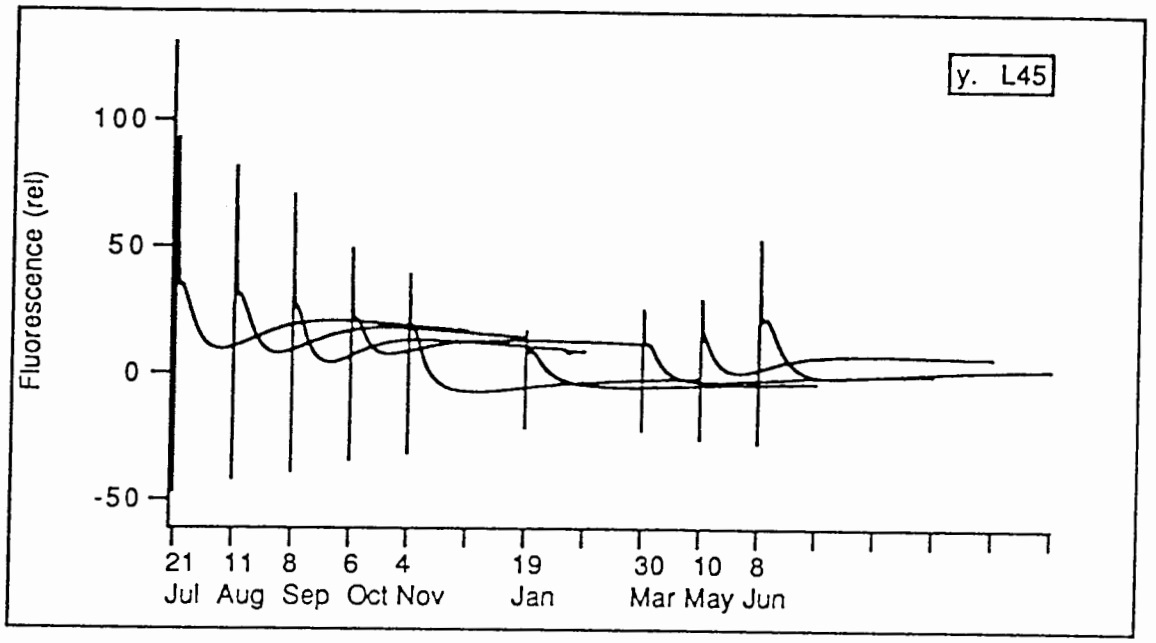

t. H54
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun

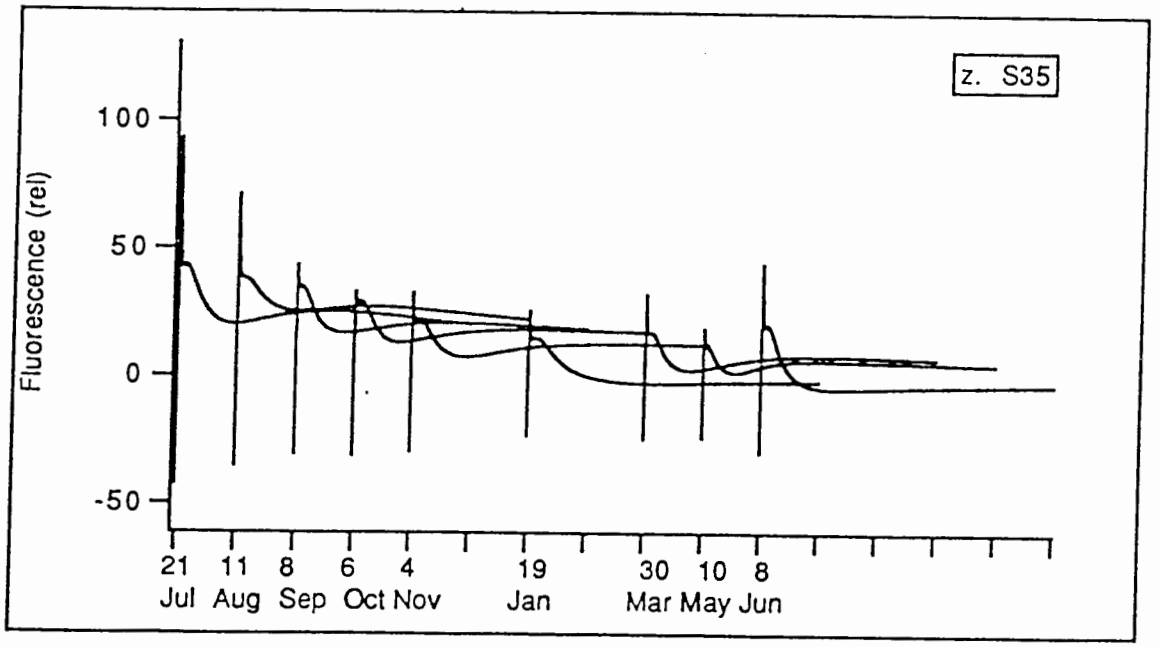

u. L22
Fluorescence (rel)
100
50
0
-50
21 11 8 6 4 19 30 10 8
Jul Aug Sep Oct Nov Jan Mar May Jun

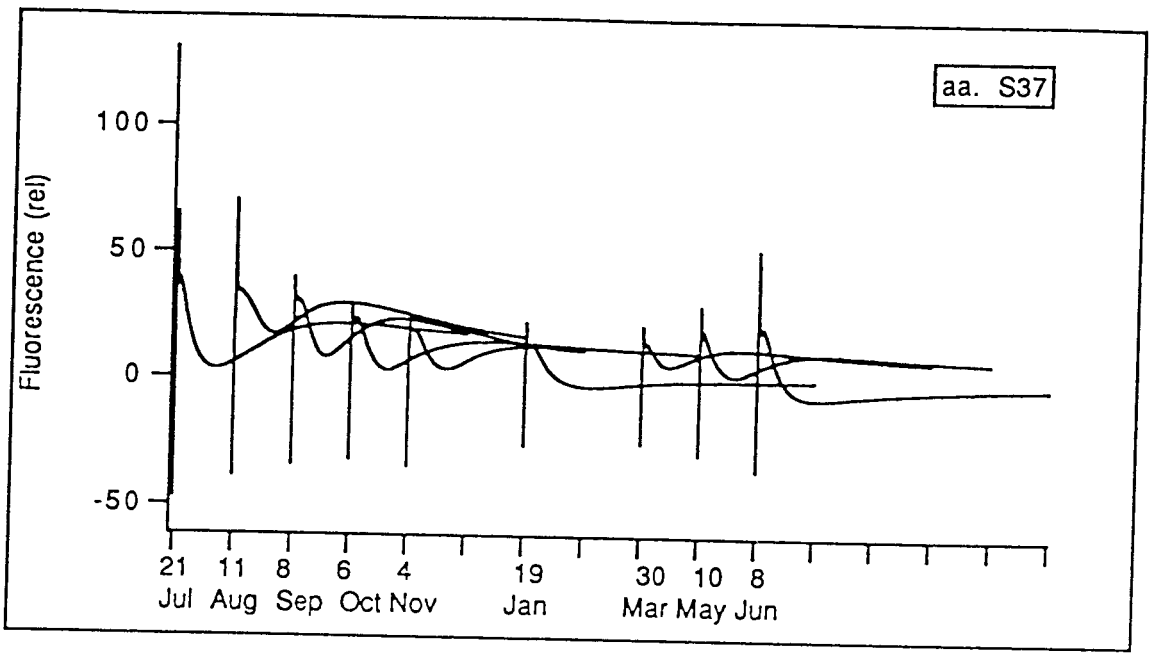

v. L27
Fluorescence (rel)
100
50
0
-50
21 11 8 6 4 19 30 10 8
Jul Aug Sep Oct Nov Jan Mar May Jun

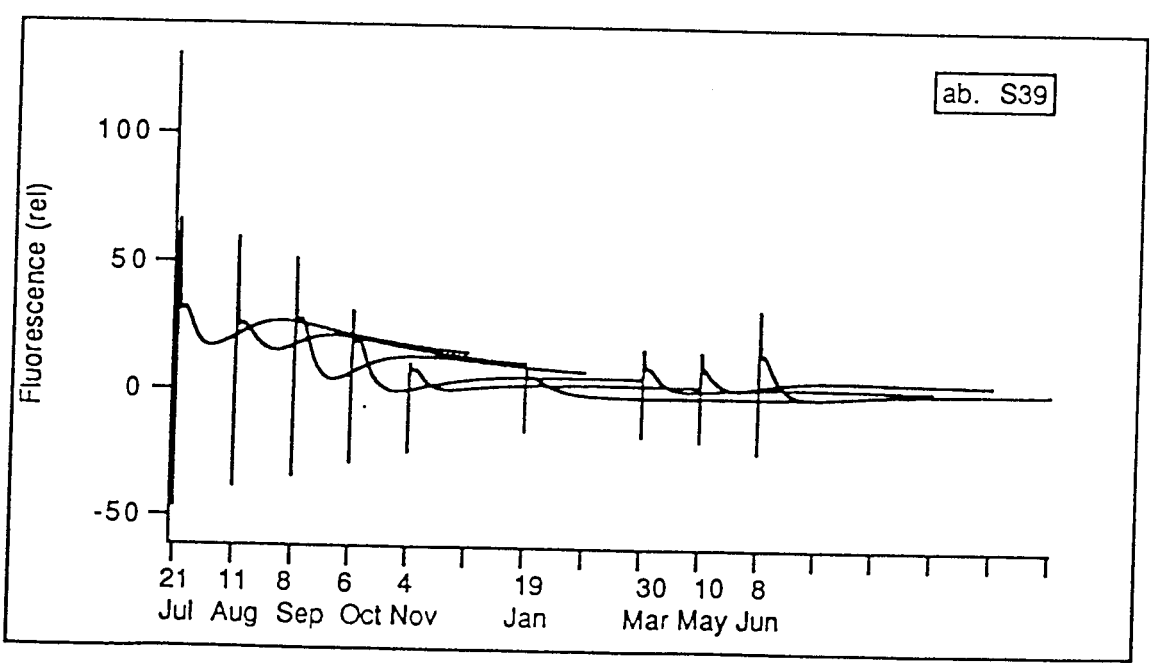

w. L37
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun

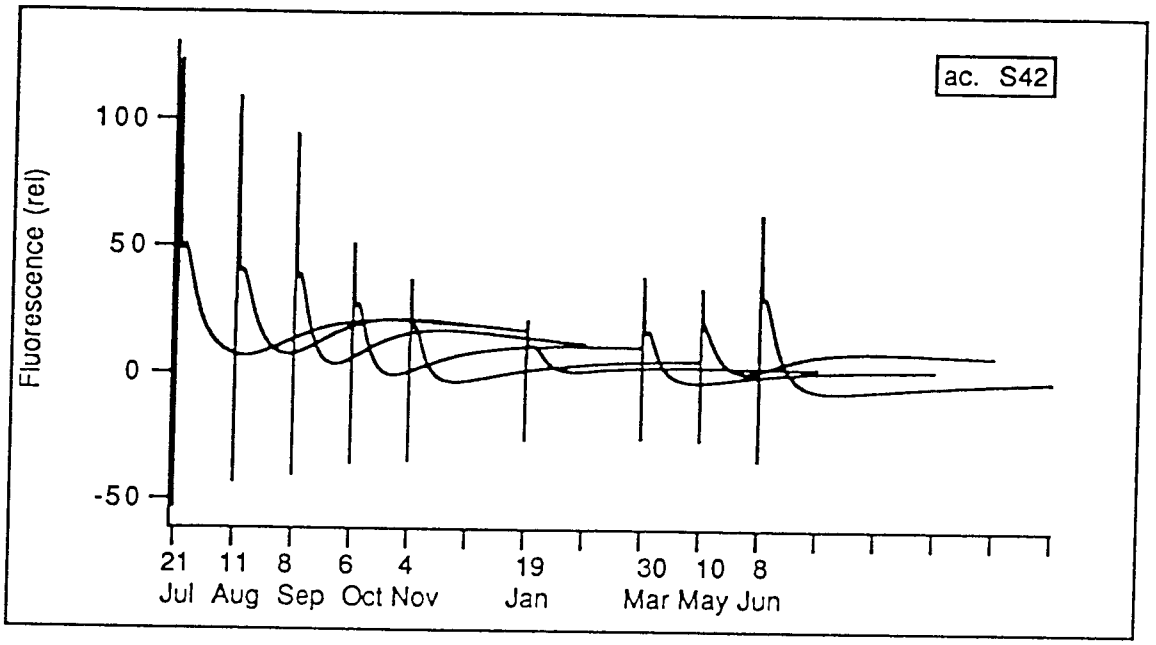

x. L42
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun


y. L45
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun


z. S35
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun


aa. S37
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun


ab. S39
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun


ac. S42
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun

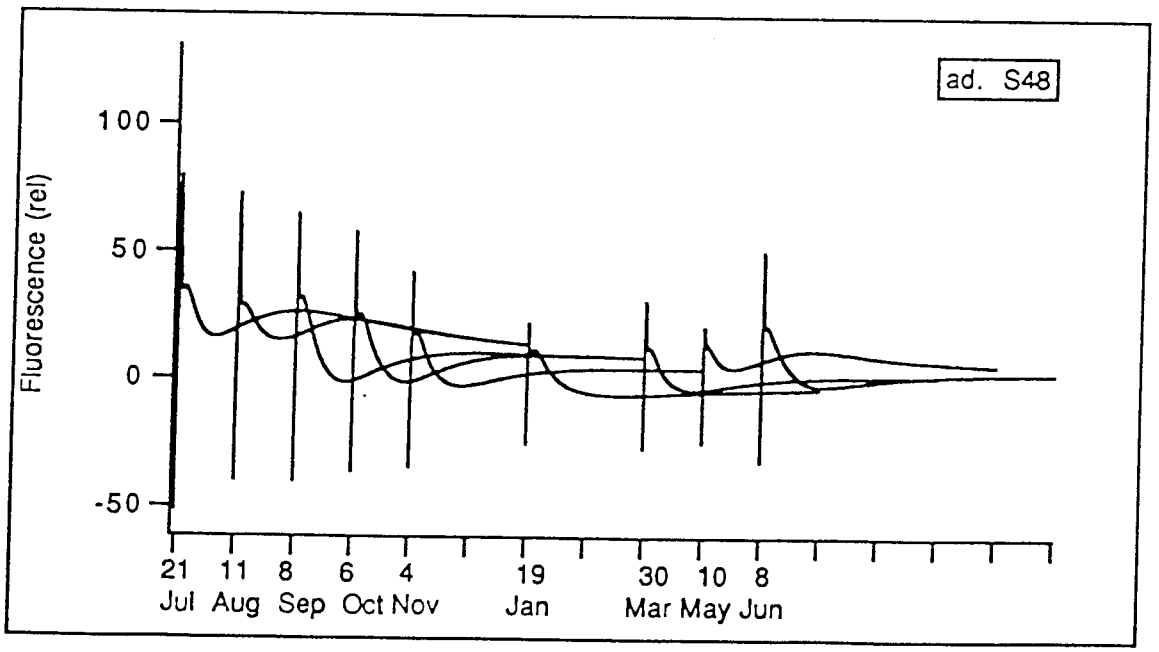

ad. S48
Fluorescence (rel)
100
50
0
-50
21 Jul
11 Aug
8 Sep
6 Oct
4 Nov
19 Jan
30 Mar
10 May
8 Jun

Figure 4.7
Average peak variable fluorescence (Fvar; relative units) values for each treatment on each analysis date (n=5).

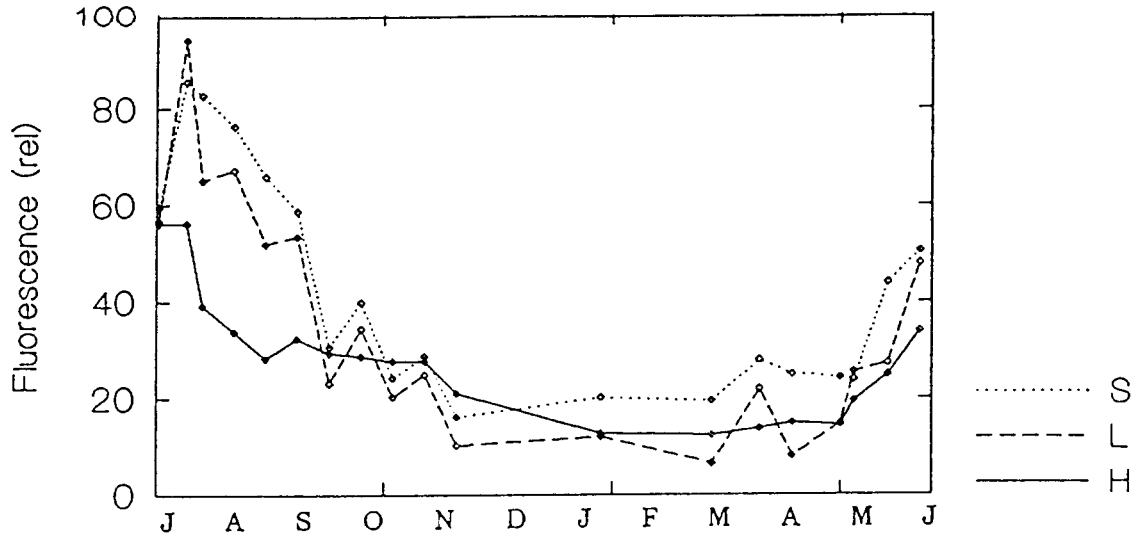
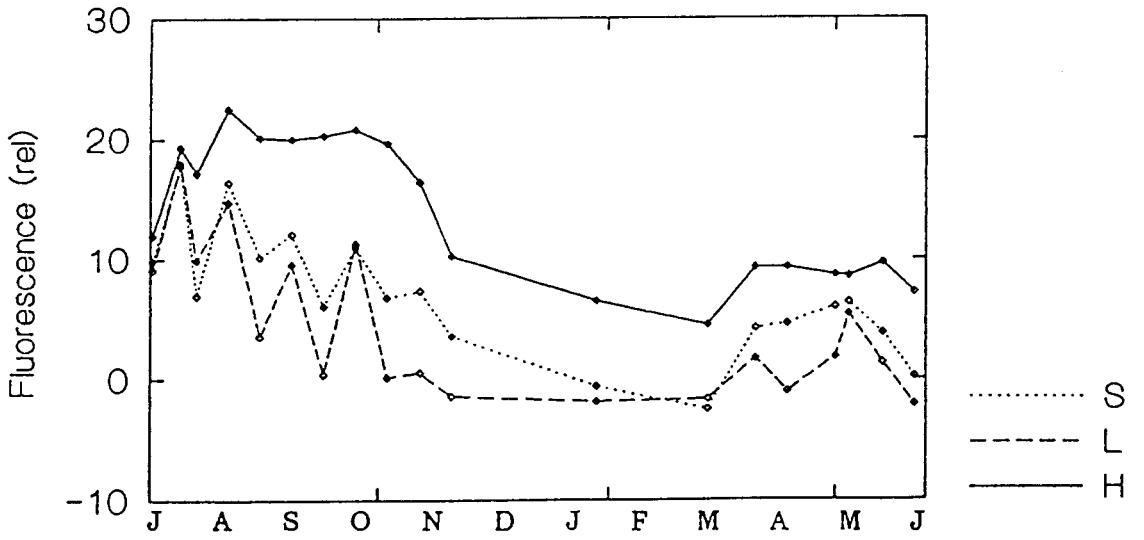


Figure 4.8
Average steady-state fluorescence (F_T; relative units) for each treatment on each analysis date (n=5).



The spring-time rise in F_O was probably due to the high proportion of vulnerable new tissue which did not yet have protective pigments in place.

A third possibility lies in our understanding of F_O. Some fluorescence systems generate an F_O value by illuminating the sample with very low intensity light [ie. the PAM system, described by Lichtenthaler and Rinderle (1988)]. Such low light intensities are not capable of inducing variable fluorescence, so the plant's response provides a baseline fluorescence level. The Kautsky curve is then generated by illuminating the sample with pulses of high intensity light. However, other fluorescence systems, such as the integrating fluorometer used in this study, illuminate the sample with light of a single intensity (ie. 200 $\mu mol/m^2/s$). F_O is calculated from the initial response of the tissue to the light, presumably before photochemical activity begins. When researchers learned how to interpret F_O values obtained with one system, it was assumed that results from the other system could be interpreted the same way. It is possible that these two methods for estimating F_O do not really measure the same thing. Some variable fluorescence component may be included in the F_O values obtained with the integrating fluorometer, as a result of the higher intensity induction light. Further research is needed regarding the origin of F_O values obtained with the integrating fluorometer.

4.3 Variable Fluorescence

Maximum variable fluorescence (Fvar) is a value indicating the highest level of fluorescence reached during a scan, minus the baseline fluorescence (F_O). Usually, Fvar is calculated from the P level fluorescence, which occurs about one second after the sample is illuminated. In this study, some of the H treatment curves had no significant P, so Fvar was calculated from a later part of the Kautsky curve (note the unusual curves in Figure 4.6a-e, p-t).

Fvar followed a seasonal pattern (Figures 4.6a-ad, and Figure 4.7) similar to that of F_O. This similarity suggests that F_O and Fvar have fundamentally similar origins. Since Fvar is known to reflect photochemical activity, F_O was probably reflecting some aspect of photochemistry as well. In the H treatment, both F_O and Fvar declined steadily until April 1994, when they began to increase. This indicated that the seasonal maxima for both fluorescence parameters occurred in the early summer, shortly before analysis began in 1993 and after it ceased in 1994. However, in the L and S treatments, the seasonal maxima for Fvar were reached slightly later than the seasonal maxima for F_O. This indicated that F_O and Fvar were not parallel measures of the same thing. Since the experimental conditions were not applied until June 1993, the increase in Fvar of nitrogen-deficient individuals was possibly a response to nutrient stress. Sharp P peaks (Figure 4.6f-o, u-ad) may represent a stress response (Brooke, 1994, Pers. Comm.). They are indicative of a strong imbalance between photon capture and electron transport. This trend became less noticeable around mid-September 1993. The fall in Fvar suggested that the plants may have

become accustomed to the nutrient supply. This decline was superimposed on the seasonal trend, resulting in an overall decrease in fluorescence levels. The significance of differences between treatments is discussed further in Chapter 5.

4.4 Steady-state Fluorescence

Steady-state fluorescence (F_T) followed a unique seasonal pattern (Figures 4.6a-ad, and Figure 4.8). In the H samples, it increased in July 1993 and remained at a plateau for the rest of the summer. It dropped in the autumn and remained relatively low for the rest of the study period. In the L and S samples, F_T dropped steadily throughout 1993; it showed a slight recovery in the spring of 1994 before falling again. (Differences between the red and blue sample sets produced the 'saw-toothed' effect in Figure 4.8. Despite those differences, the two sample sets followed a similar seasonal trend.) The significance of the differences between treatments is discussed in Chapter 5.

The seasonal trends in F_T appear to give a rough indication of the physiological (photosynthetic) activity of the plants, with the possible exception of late spring 1994. Unlike the parameters F_O and Fvar, F_T remained high throughout the summer and was highest in the most productive (H treatment) plants. The application of F_T as a physiological indicator is discussed in greater detail in Chapter 6.

4.5 Apparent Photosynthesis and Dark Respiration

Average apparent photosynthesis (APS) rates at a saturating light intensity (800 μmol/m^2/s PFD) are presented, by treatment and by analysis date, in Figure 4.9. APS rates trended upwards throughout July 1993, peaking in mid-August. They returned to July levels, where they remained for the next two months. A second peak occurred in the H and S treatments early in October, following a hot spell (Figure 4.1). The rates then declined, indicating the cessation of the growing season and onset of winter dormancy. Dormancy appeared to be triggered by photoperiod. Short or decreasing photoperiods are important for dormancy induction of Douglas-fir seedlings (McCreary *et al.* 1978). In their natural environment, these plants would experience long, cold winters (relative to the climate at Simon Fraser University). Warm weather in January did not induce flushing; there was no evidence to suggest that the plants were dormant for a shorter time than they would have been in their natural environment. Since the two locations have similar daylength cycles, dormancy was apparently triggered and maintained by photoperiod.

CO_2 exchange rates were not assessed throughout the winter, since the plants would have warmed up in the lab. The decision to halt assessments was made in mid-November, when APS rates of several plants were assessed twice in one day, and they had increased markedly the second time. This was due to the effects of temperature of enzyme activity rather than release from dormancy, since dormancy was apparently controlled by photoperiod and is not so easily reversible. Nonetheless, it reduced the reliability of the data, so assessments were postponed until the following spring.

APS rates increased around the end of March, then fell in April and May, when the plants flushed. The new tissue was photosynthetically inefficient, and respiration rates were high. APS rates began to recover in May and June for some samples (Figure 4.9; also see Figures 4.11a-ad).

Average dark respiration (RD) rates for each treatment on each analysis date are displayed in Figure 4.10. RD rates peaked in mid-August 1993 for all treatments and again in early October for the H and S treatments. Peaks in APS also occurred at those times (Figure 4.9). These increases in RD rates therefore corresponded with high levels of metabolic activity. RD rates declined in the late autumn, and rose again in the spring of 1994. Shortly after the plants flushed, production of new tissue and photosynthetic apparatus resulted in a peak in RD rates. The rates began to decline somewhat at the end of the study, as the new growth matured.

4.6 Quantum Requirement

CO_2 exchange rates for each sample at four light intensities (800, 480, 200 and 0 $\mu mol/m^2/s$ PFD) are illustrated in Figures 4.11a-ad. Negative values indicate net CO_2 output. Where no bar is visible for 800 $\mu mol/m^2/s$ PFD, the CO_2 exchange rate was equal to that of 480 $\mu mol/m^2/s$ PFD. (This was a fairly rare occurrence; 480 $\mu mol/m^2/s$ PFD was usually non-saturating.) Where no bar is visible for 200 $\mu mol/m^2/s$ PFD, the CO_2 exchange rate was 0. Quantum requirement (QR) was calculated from the APS rates at two non-saturating light intensities (480 and 200

$\mu mol/m^2/s$). QR values are indicative of how many photons of light must strike the plant in order for it to fix one molecule of CO_2. High numbers indicate low photochemical conversion efficiency, and vice versa. Figure 4.12 illustrates average QR values for each treatment on each analysis date.

There was a general trend towards high values in the winter and lower values in the summer. The lowest values in 1993 occurred at times of peak photosynthesis (ie. August 11 and October 6). At those times, the same amount of photosynthetic tissue was operating as at non-peak times, and at the same light intensity; thus the tissue was evidently functioning more efficiently at peak times. The plants deviated from this pattern in early spring (1994). The reestablishment of photosynthetic activity and the preparation for flushing appear to have resulted in extremely high QR, particularly in the L and S treatments (Figure 4.12). High QR was also evident in the H treatment in late spring, when light-saturated APS rates were at a minimum (Figure 4.9).

Figure 4.9
Average light-saturated (800 µmol/m²/s) photosynthetic rates (APS; $mgCO_2/dm^2/hour$) for each treatment on each analysis date (n=5).

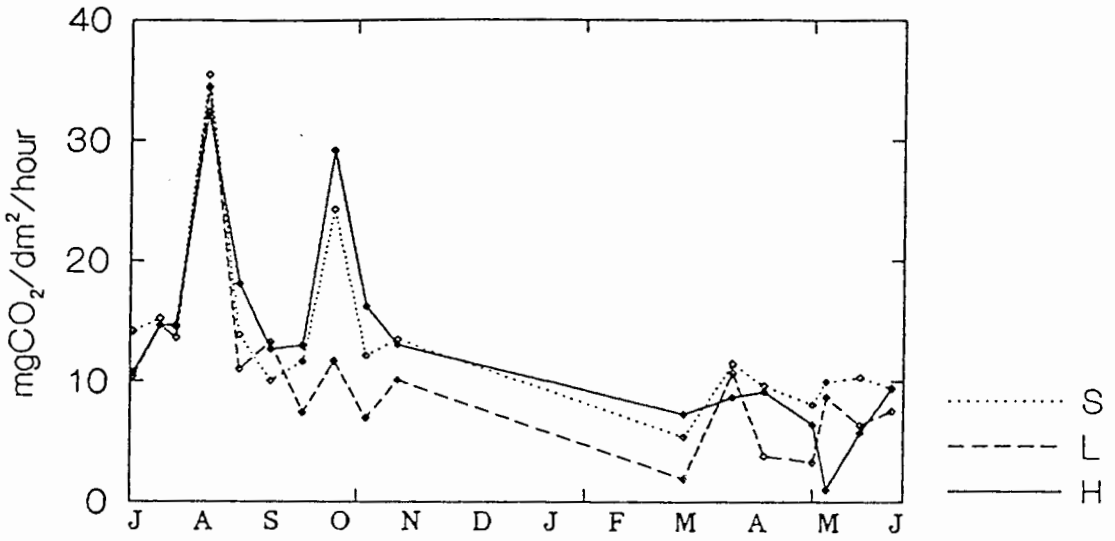


Figure 4.10
Average dark respiration rates (RD; $mgCO_2/dm^2/hour$) for each treatment on each analysis date (n=5).

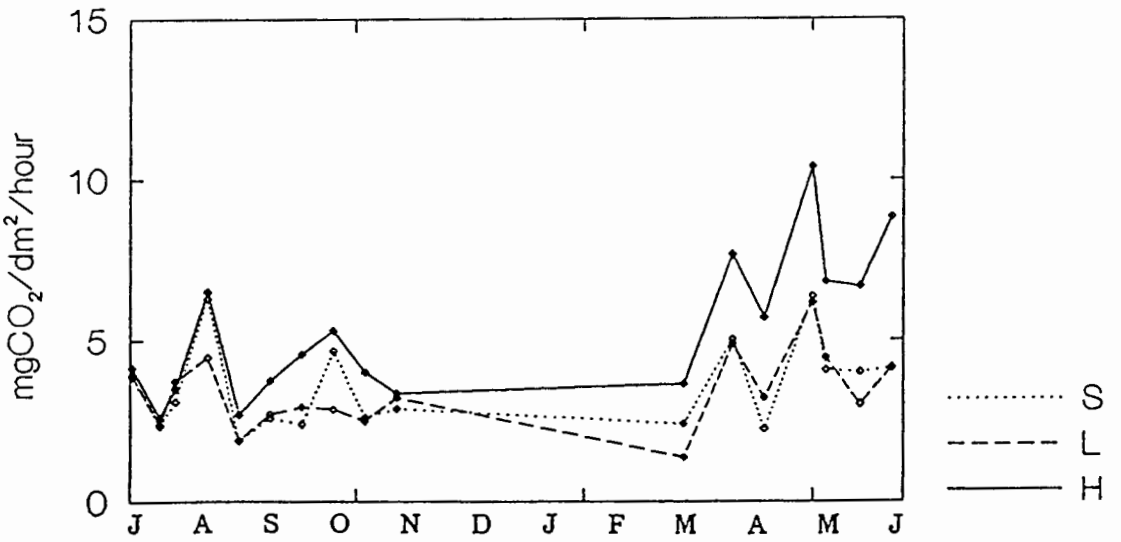
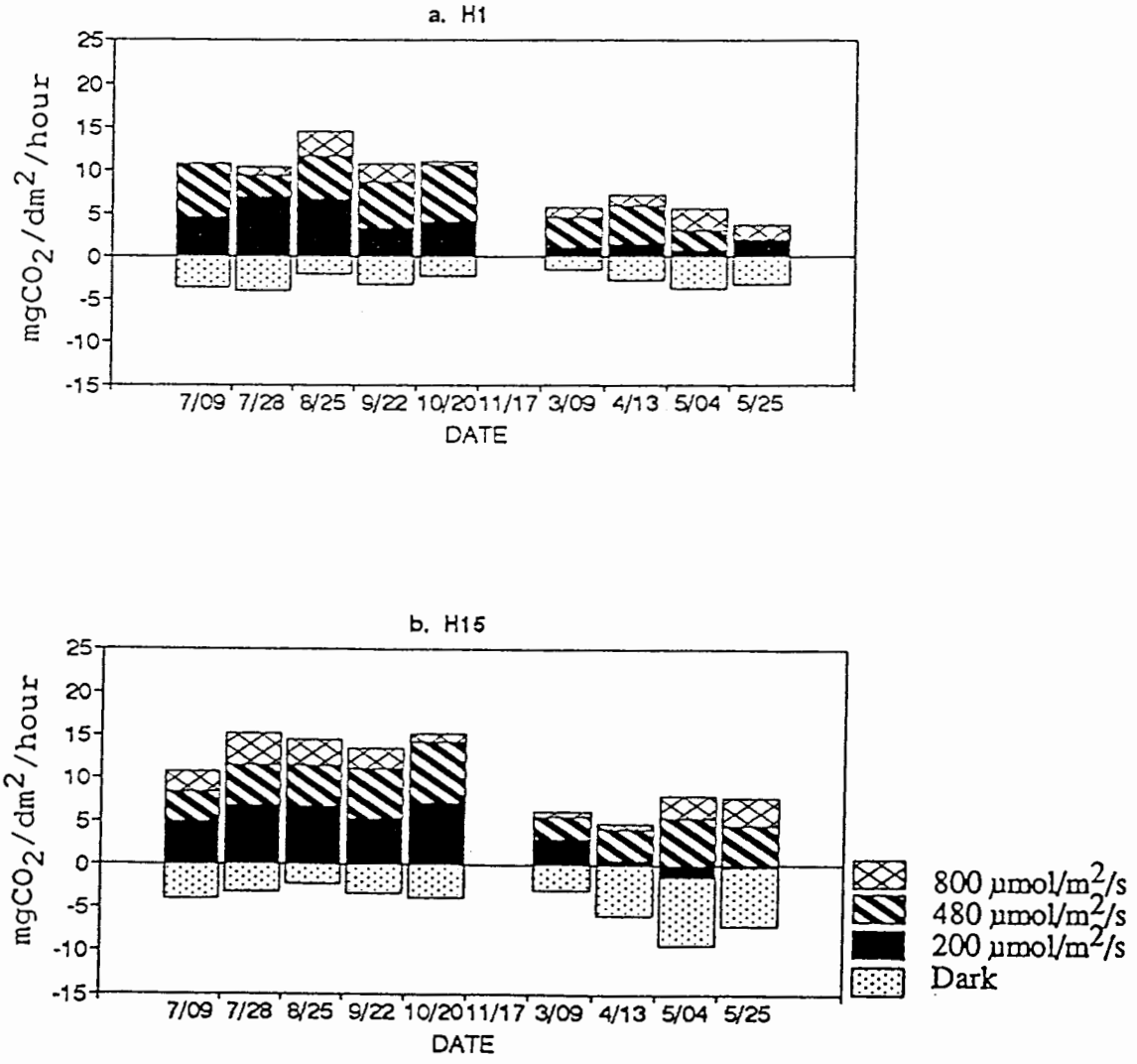
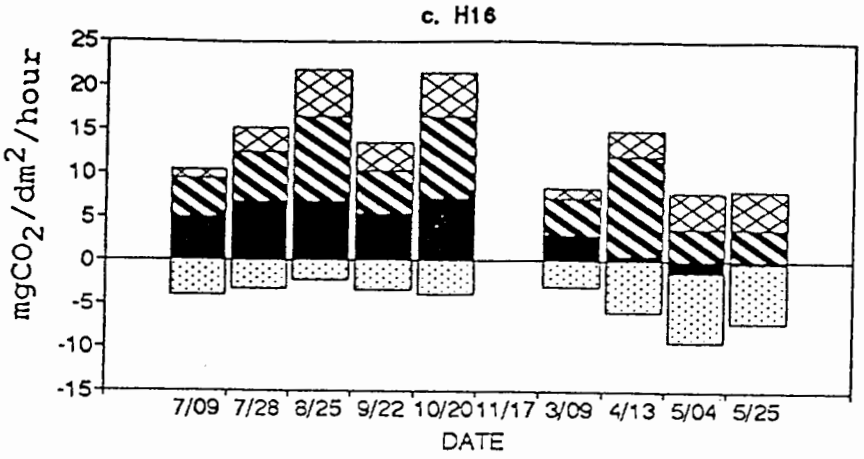
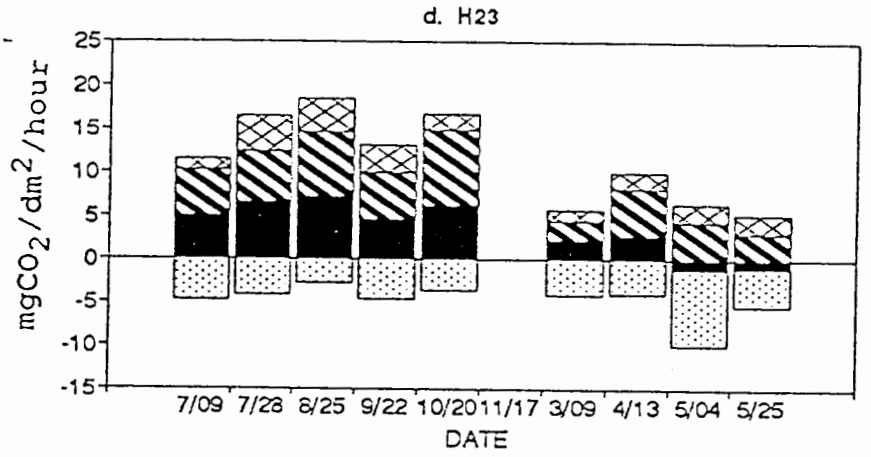
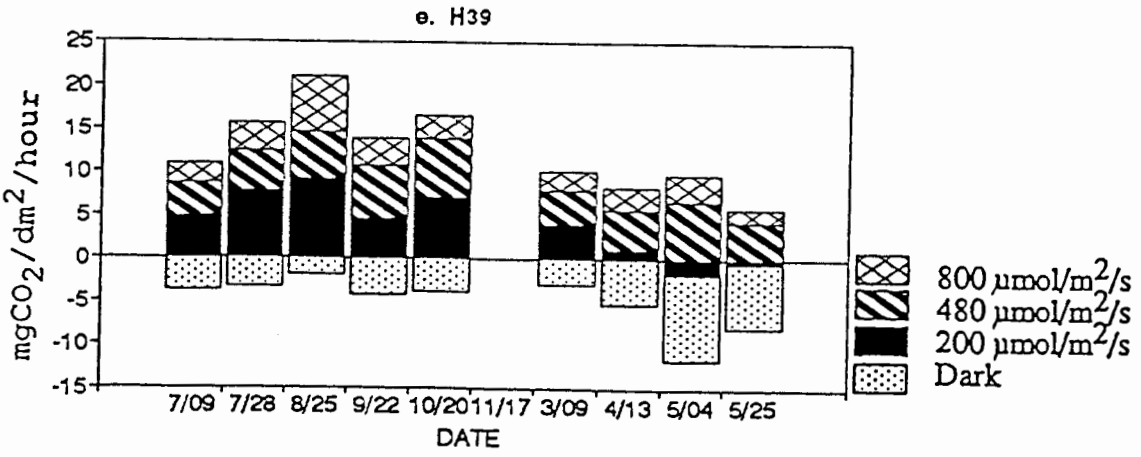


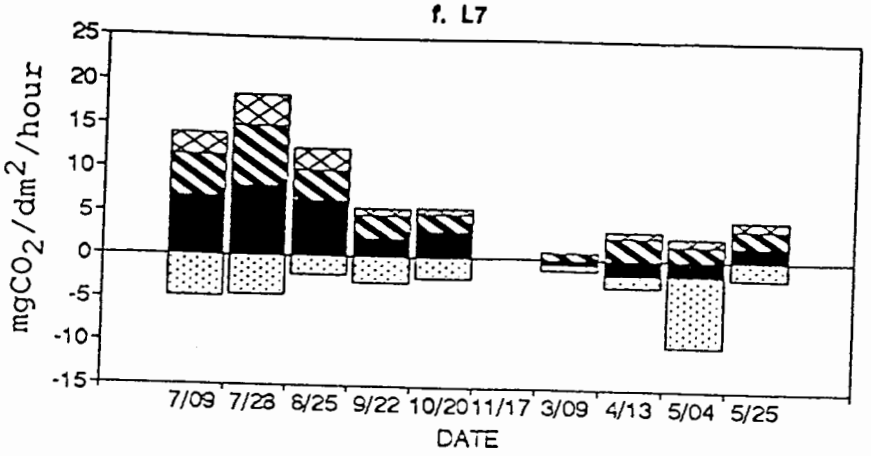
Figure 4.11a-ad
Carbon dioxide gas exchange rates (APS and RD; $mgCO_2/dm^2/hour$) at several light intensities, for each sample on each analysis date (n=5).
Graphs **a-o** are the red sample set; **p-ad** are the blue sample set.

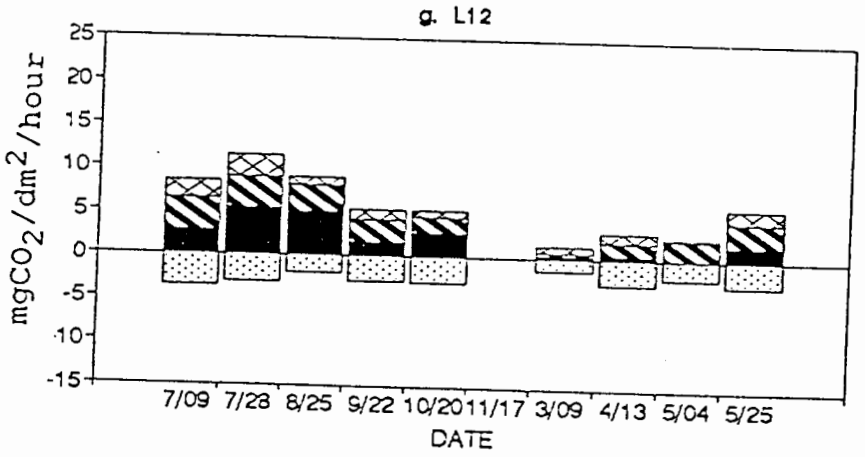


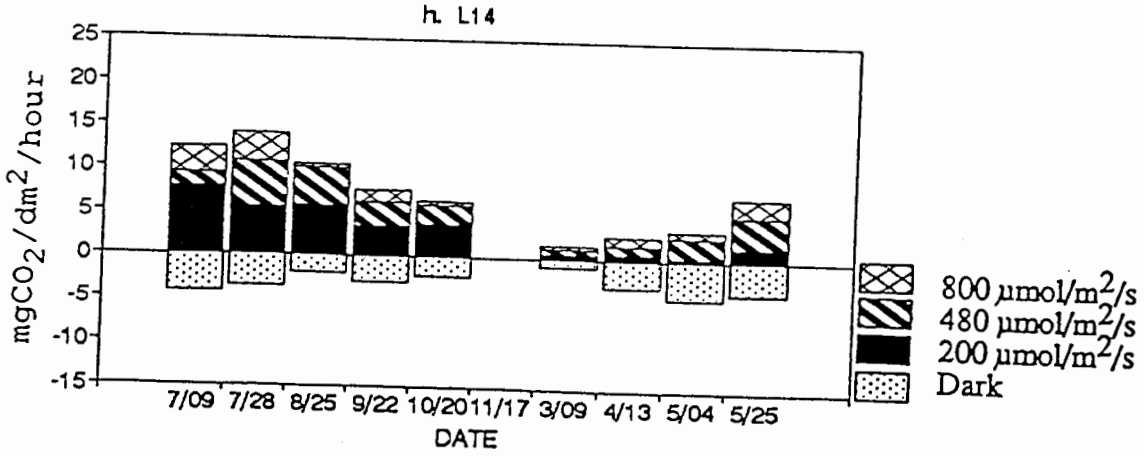

c. H16
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE

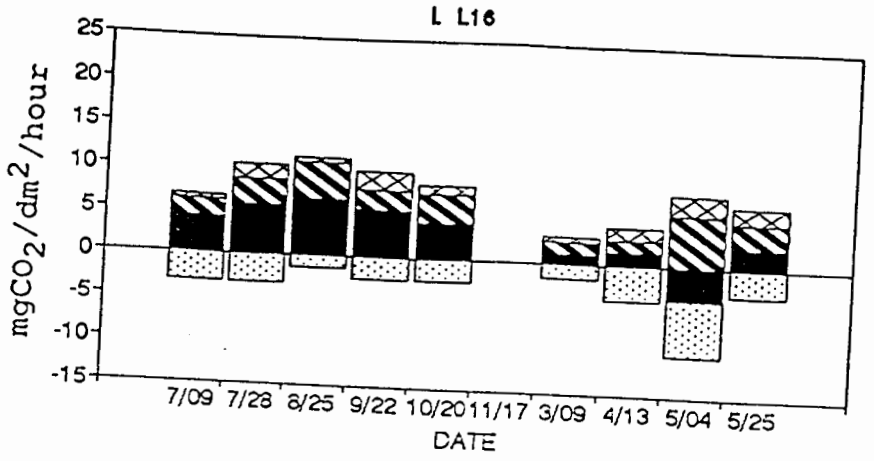

d. H23
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE

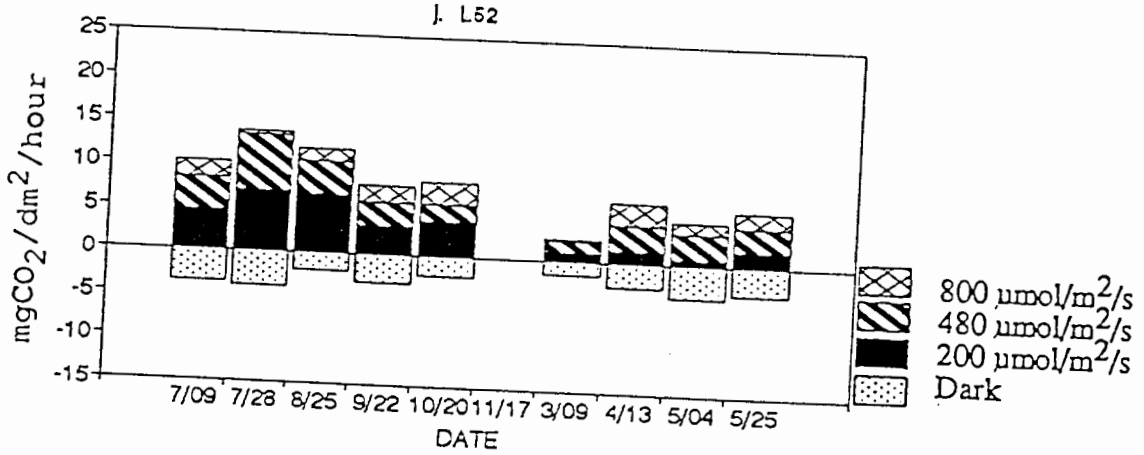

e. H39
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark

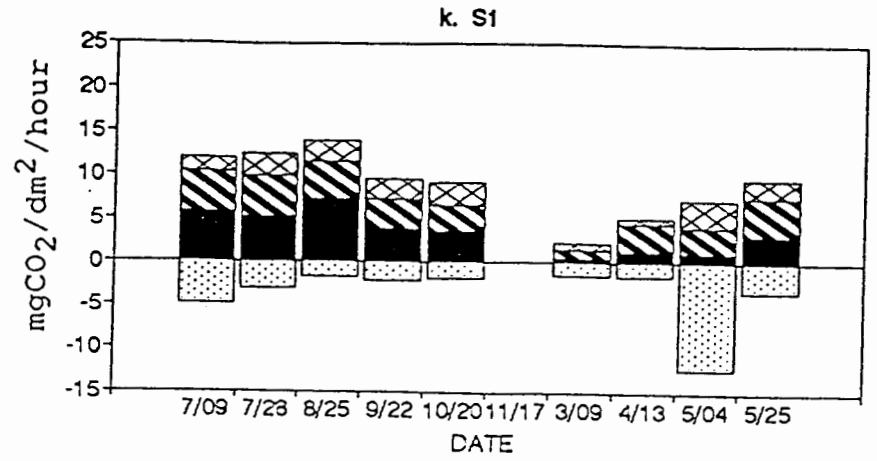

f. L7
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE

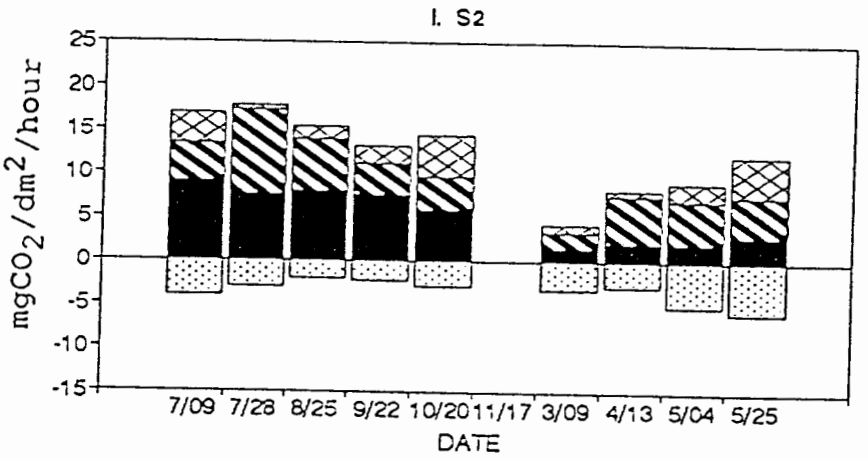

g. L12
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE

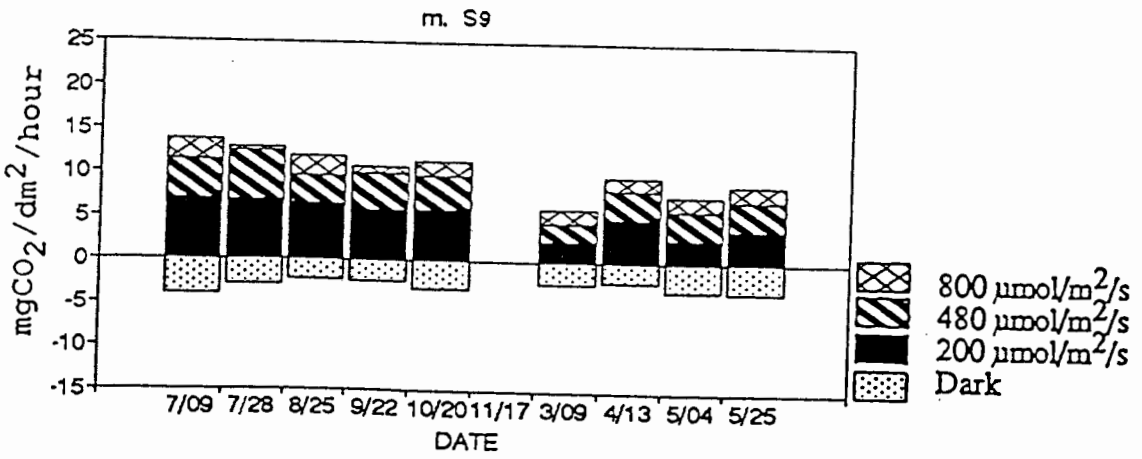

h. L14
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark

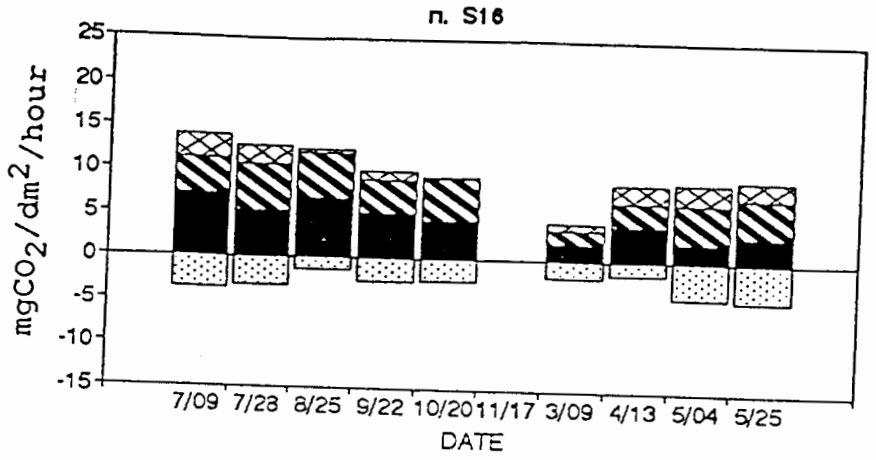

L L16
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE

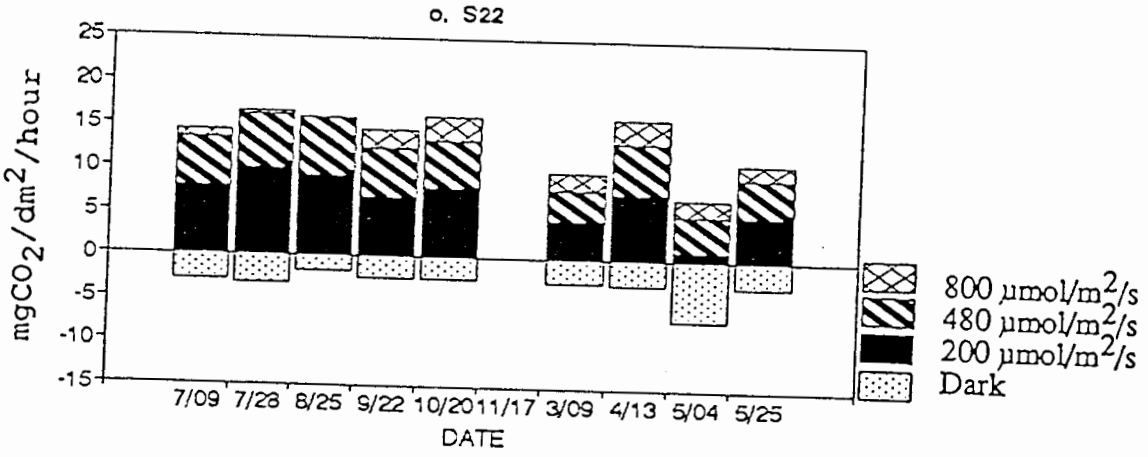

J. L52
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark


k. S1
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE

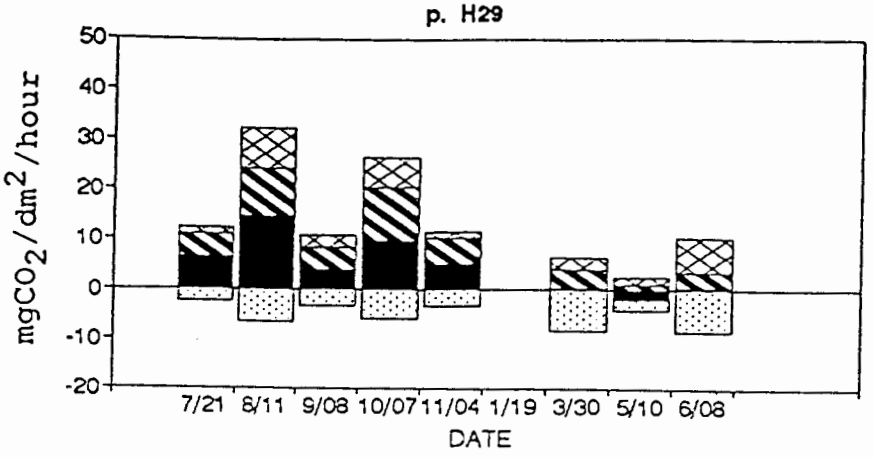

l. S2
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE


m. S9
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark

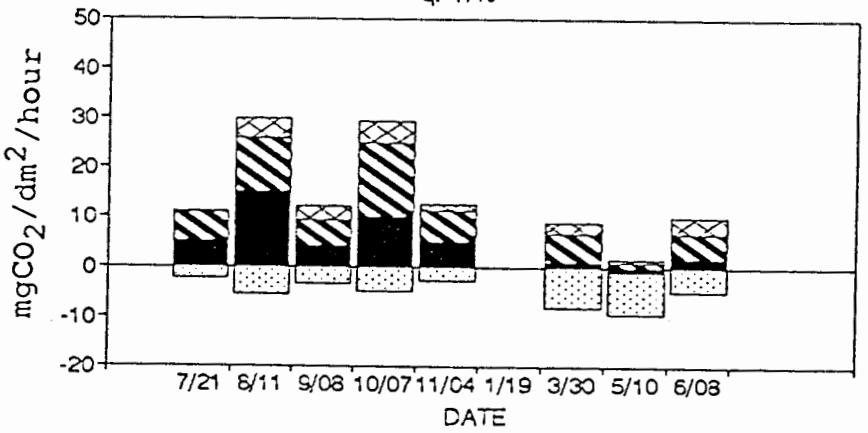

n. S18
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE


o. S22
25
20
15
10
5
0
-5
-10
-15
mgCO2/dm2/hour
7/09 7/28 8/25 9/22 10/20 11/17 3/09 4/13 5/04 5/25
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark

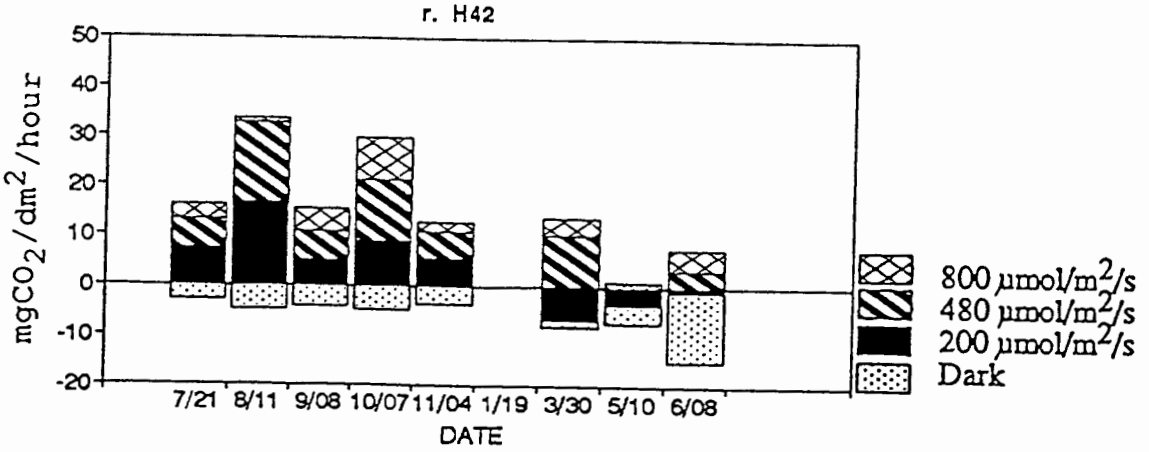
p. H29

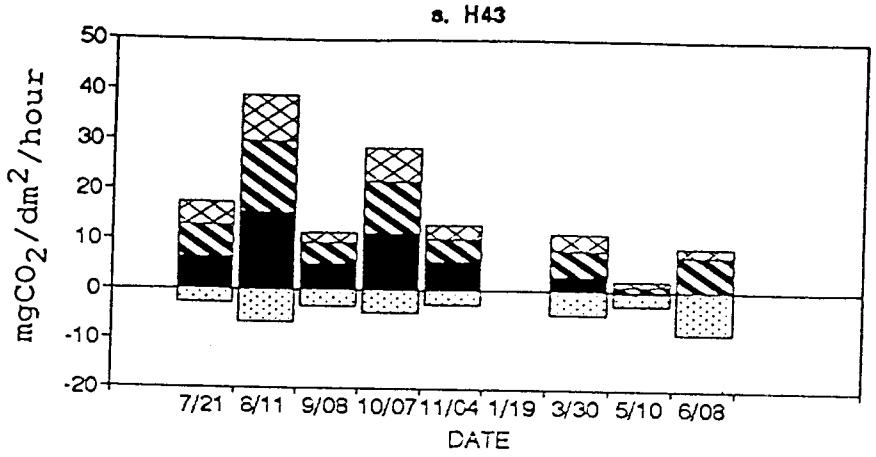


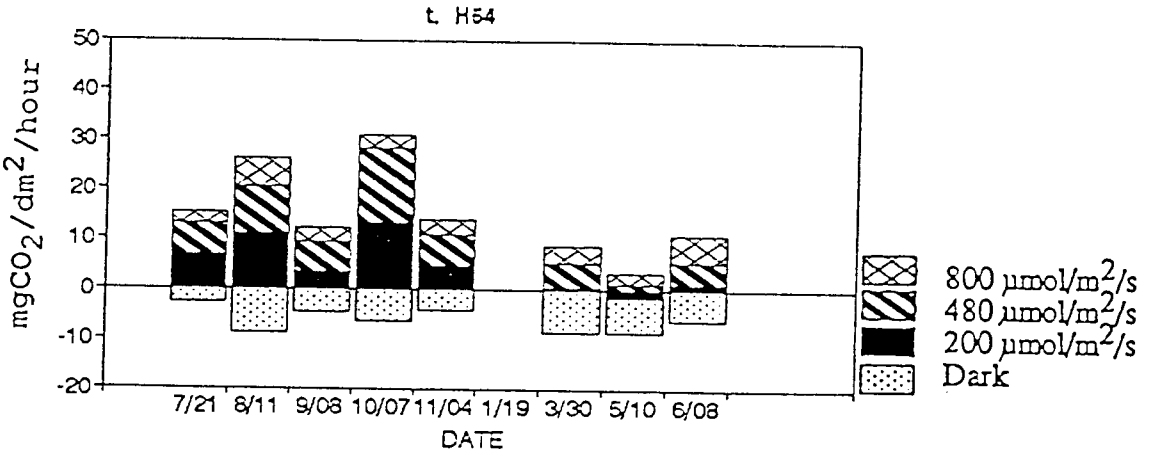
q. H40



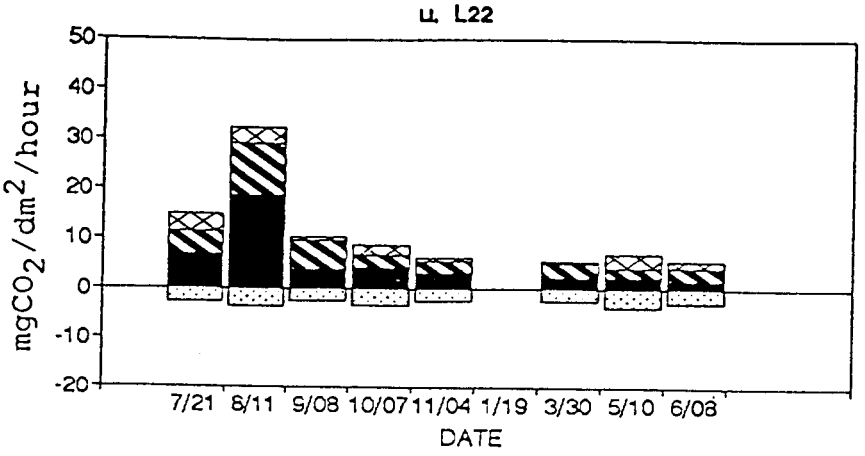
r. H42



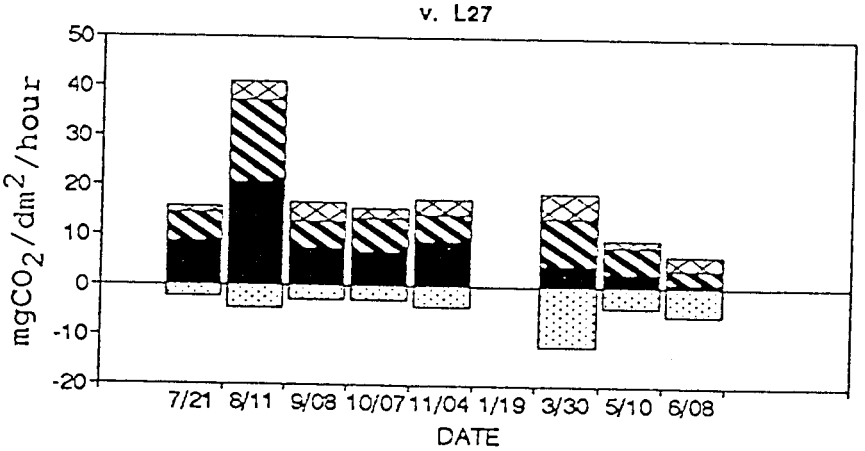

a. H43
50
40
30
20
10
0
-10
-20
mgCO2/dm2/hour
7/21 8/11 9/08 10/07 11/04 1/19 3/30 5/10 6/08
DATE


t. H54
50
40
30
20
10
0
-10
-20
mgCO2/dm2/hour
7/21 8/11 9/08 10/07 11/04 1/19 3/30 5/10 6/08
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark

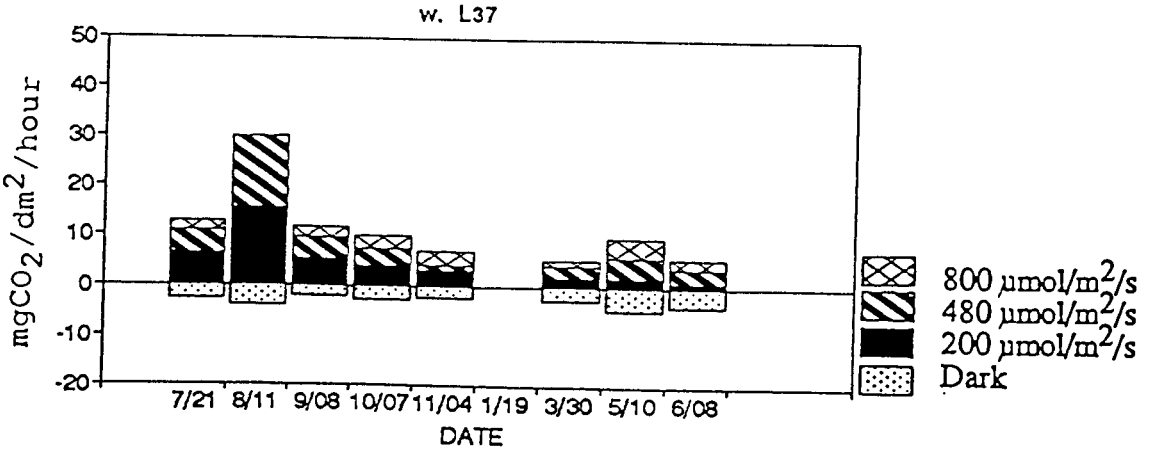
u. L22



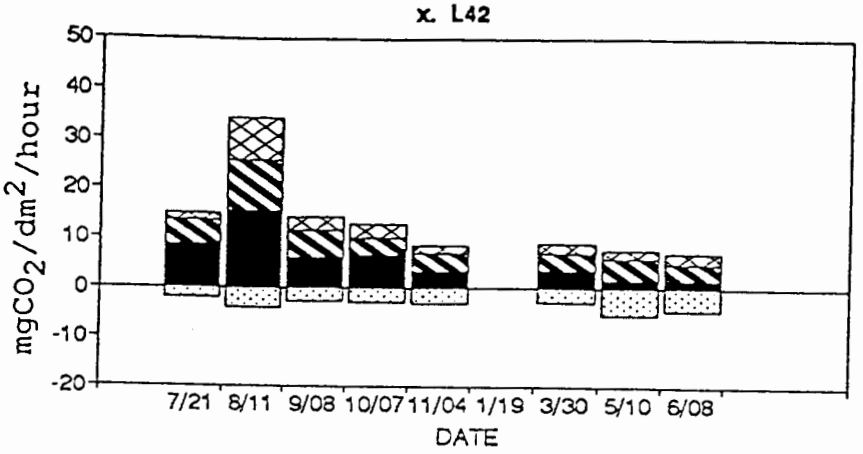
v. L27



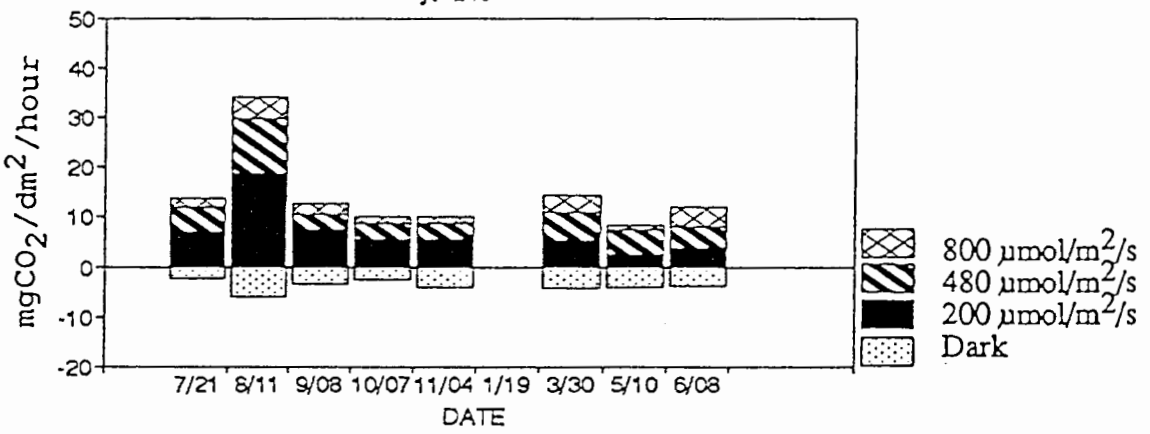
w. L37



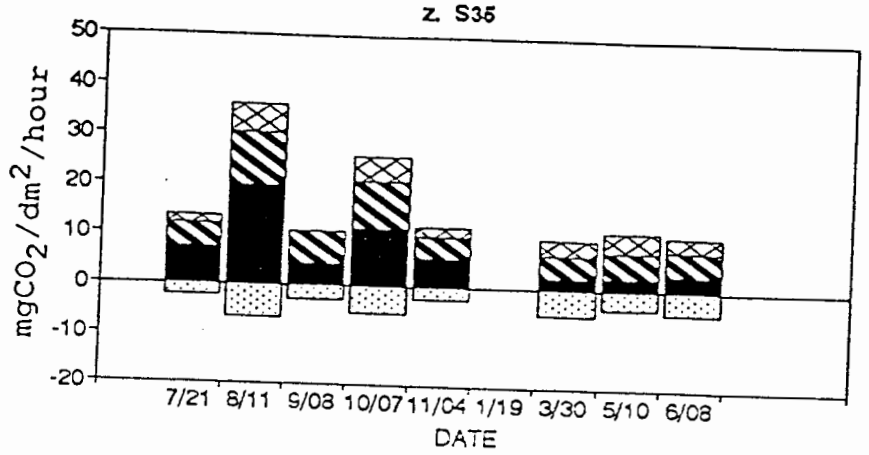
x. L42


50
40
30
20
10
0
-10
-20
mgCO2/dm2/hour
7/21
8/11
9/08
10/07
11/04
1/19
3/30
5/10
6/08
DATE

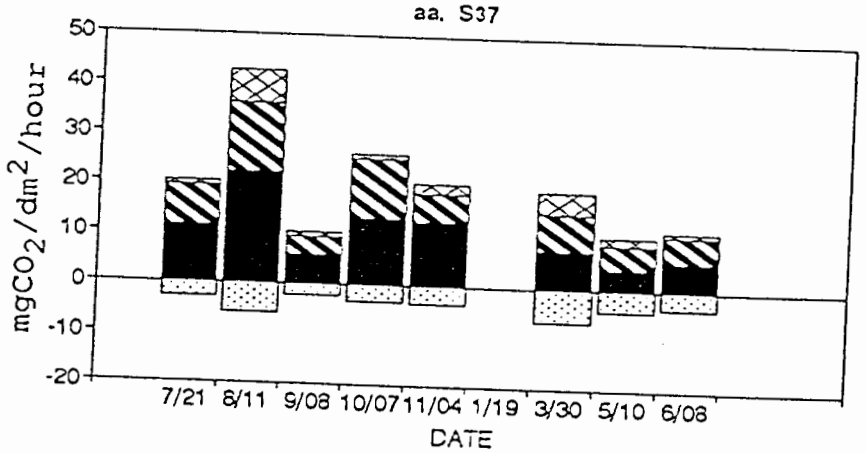
y. L45


50
40
30
20
10
0
-10
-20
mgCO2/dm2/hour
7/21
8/11
9/08
10/07
11/04
1/19
3/30
5/10
6/08
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark

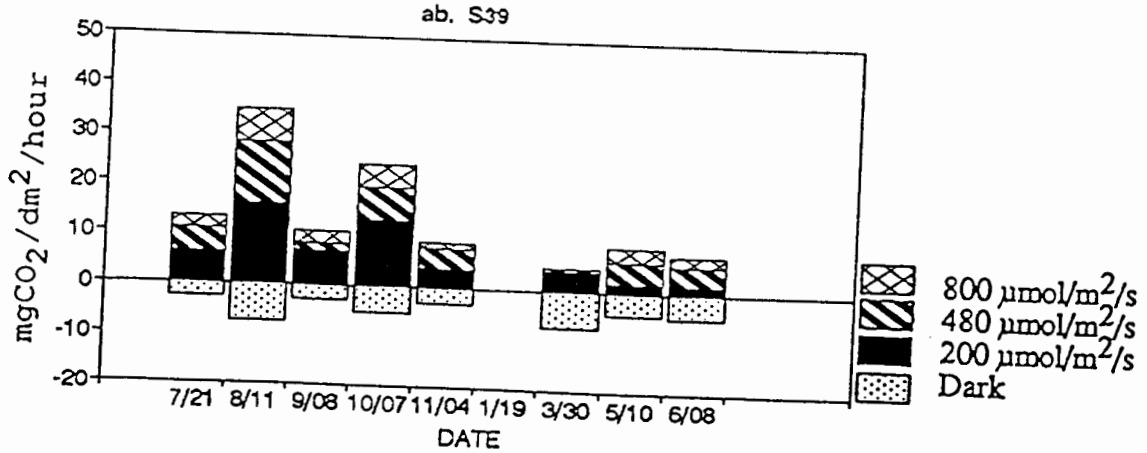
z. S35

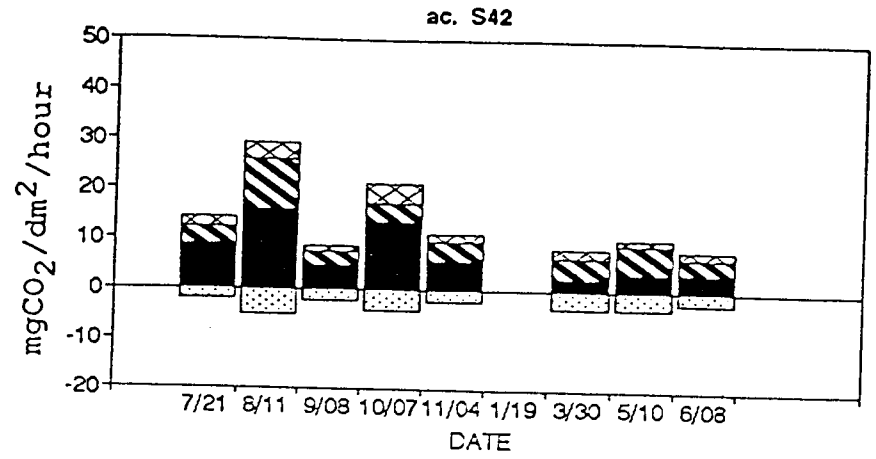


aa. S37



ab. S39




ac. S42
50
40
30
20
10
0
-10
-20
mgCO2/dm2/hour
7/21 8/11 9/08 10/07 11/04 1/19 3/30 5/10 6/08
DATE

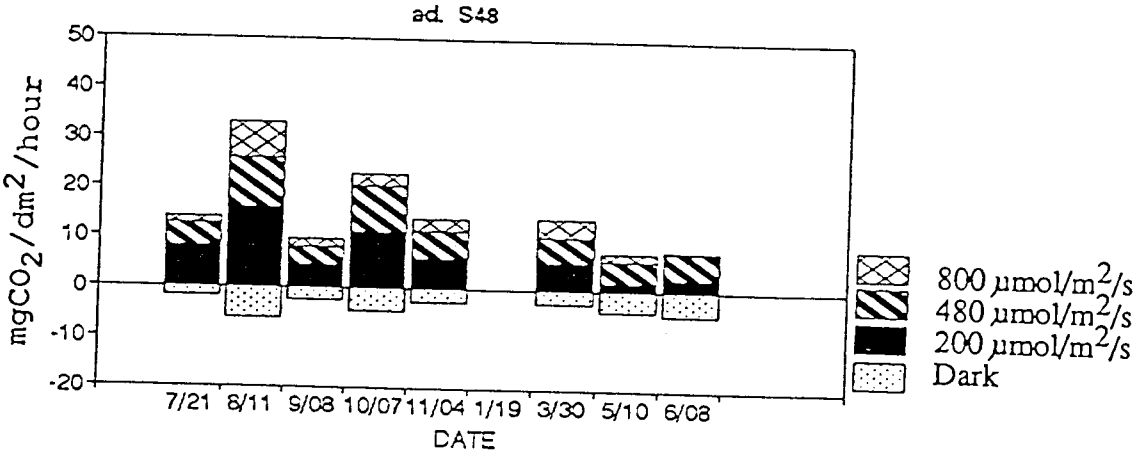
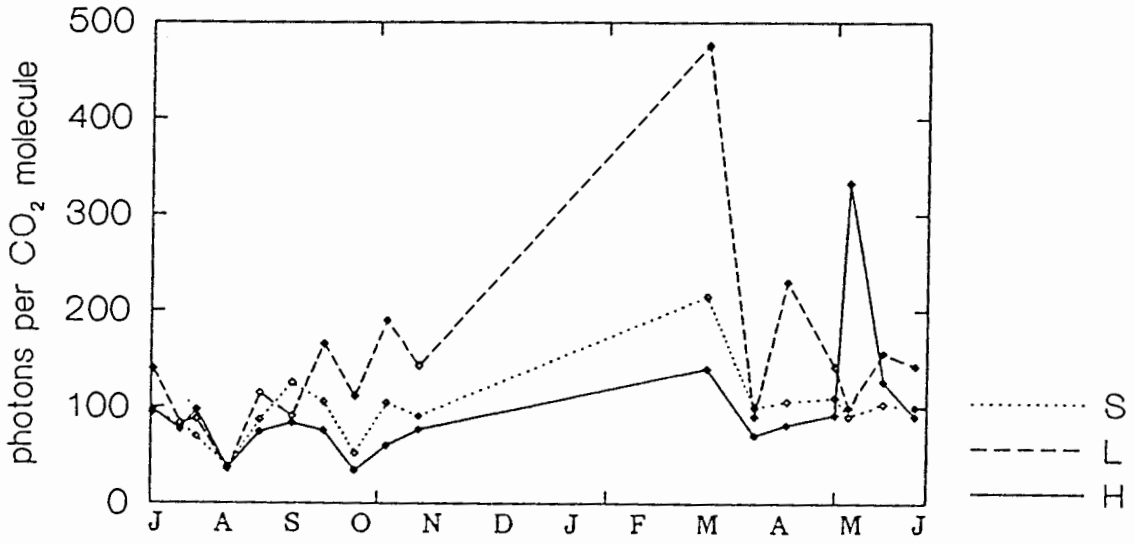

ad. S48
50
40
30
20
10
0
-10
-20
mgCO2/dm2/hour
7/21 8/11 9/08 10/07 11/04 1/19 3/30 5/10 6/08
DATE
800 µmol/m2/s
480 µmol/m2/s
200 µmol/m2/s
Dark

Figure 4.12
Average quantum requirement (QR; photons per molecule of CO_2) for each treatment on each analysis date (n=5).



CHAPTER 5
RESULTS AND DISCUSSION:
COMPARISON OF TREATMENTS

Differences between treatments occurred in growth rates, flushing times, foliar nitrogen concentrations, chlorophyll fluorescence parameters and CO_2 exchange rates. These differences are discussed in this chapter. Means and standard deviations were calculated for each treatment on each analysis date. ANOVA and post hoc tests (Tukey's procedure) were used to determine significance of differences between treatments. These procedures were indicated by Devore (1991) and conducted using Systat for Windows (version 5, Systat Inc., 1992).

5.1 Foliar Nitrogen Concentration

Table 5.1 summarizes the statistical analysis of nitrogen concentration data. In most cases, there was no significant difference between the L and S treatments, whereas the H treatment foliage contained significantly higher nitrogen concentrations than the other two treatments. The similarity between the three treatments at the first few analysis dates was due to their recent introduction to the various nutrient supply

regimes; the tissue was not yet at equilibrium with the nitrogen supply. (Before the first flush in 1993, the seedlings had an average foliar nitrogen concentration of 2.29%). The similarity between the L and S treatments could indicate one of two things. The presence of soopolallie in the S pots may not have significantly affected the nitrogen supply to the associated conifers. On the other hand, the nitrogen concentrations of foliage from L and S treatments may have been at a minimal level for survival. Nitrogen was the key limiting factor for plant growth, and growth would have occurred only at a level allowing the nitrogen concentration to remain sufficient. If the nitrogen supply was greater for the S treatment than for L, this would be evidenced by higher growth rates in the S individuals, not by higher tissue nitrogen concentrations. This possibility is further discussed in Section 5.2.

5.2 Stem Growth Rates

Stem heights and collar diameters were measured for all samples in the population initially (spring 1993), after one season's growth (autumn 1993), and after the initial growth period of 1994 (summer). Changes in size over time are indicative of stem growth rates. The data and results of statistical analysis are presented in Table 5.2.

Table 5.1
Foliar nitrogen concentration (% of dry weight).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	1.49	a**	0.14	0.83	b	0.16	1.28	a	0.12
7/21	2	1.67	a	0.24	0.69	b	0.21	1.08	b	0.31
7/28	3*	1.23	a	0.32	1.07	ab	0.05	0.79	b	0.20
8/11	4	2.30	a	0.31	0.93	b	0.34	0.80	b	0.30
8/25	5*	2.37	a	0.53	0.58	b	0.27	1.04	b	0.20
9/08	6	2.26	a	0.27	1.03	b	0.33	0.91	b	0.31
9/22	7*	2.73	a	0.20	1.09	b	0.12	1.38	b	0.28
10/06	8	2.86	a	0.34	1.30	b	0.23	1.34	b	0.35
10/20	9*	2.64	a	0.10	1.16	b	0.10	1.15	b	0.20
11/03	10	2.75	a	0.23	1.12	b	0.22	0.88	b	0.40
11/17	11*	2.65	a	0.22	1.19	b	0.26	1.44	b	0.13
1/19/94	12	2.78	a	0.42	1.52	b	0.12	1.49	b	0.16
3/09	13*	2.48	a	0.25	1.52	b	0.10	1.56	b	0.29
3/30	14	2.71	a	0.38	1.39	b	0.24	1.29	b	0.27
4/13	15*	2.48	a	0.15	1.11	b	0.17	1.59	c	0.36
5/04	16*	2.10	a	0.36	1.03	b	0.22	1.05	b	0.26
5/11	17	1.89	a	0.20	1.22	b	0.30	0.97	b	0.22
5/25	18*	2.27	a	0.21	1.28	b	0.16	1.13	b	0.13
6/08	19	1.85	a	0.18	1.15	b	0.09	1.05	b	0.15

* Indicates 'red' sample set. All other analyses involved the 'blue' sample set.

** Mean values within a row with the same letter are not significantly different (α=0.05).

Table 5.2
Stem height and collar diameter.
Population means and standard deviations (sd).

		H	n=60	sd	L	n=52	sd	S	n=48	sd
shoot	spring '93	23.4	a*	3.07	24.2	a	3.21	24.2	a	3.82
length	autumn '93	33.0	a	4.38	29.7	b	3.95	30.0	b	4.72
(cm)	summer '94	55.8	a	6.91	37.0	b	4.21	41.5	c	5.49
collar	spring '93	4.2	a	0.59	4.0	a	0.62	4.0	a	0.53
diameter	autumn '93	7.9	a	1.14	5.4	b	0.84	5.7	b	0.84
(mm)	summer '94	11.0	a	1.41	6.2	b	0.85	6.9	c	0.97

*Mean percentages within a row that have the same letter are not significantly different (α=0.05).

There were no significant differences between the initial (spring 1993) stem heights and collar diameters of plants in the three treatment groups (Table 5.2). By the end of the 1993 growing season, H plants had significantly greater heights and collar diameters than L and S plants. Some plants flushed for a second time in 1993 (lammas growth); most were of the H treatment group. Extra autumn growth in conifer seedlings in response to high nitrogen supply has been mentioned by other researchers (ie. Coutts and Philipson 1980). The greater size of the well-fed plants was indicative of the fertilizer effects of mineral nitrogen. By mid-summer 1994, significant differences were also apparent between the L and S groups. H plants were (on average) the largest; L plants were smallest, and S plants were intermediate.

These results supported the 'dilution of nitrogen' hypothesis introduced in Section 5.1. Although the L and S samples contained similar concentrations of tissue nitrogen, the higher growth rate of the S plants (in 1994) indicated that they may have had access to a greater supply of nitrogen. Nitrogen was the limiting factor for growth; the plants increased in size until the nitrogen concentration of the photosynthetic tissue was at a minimum allowable level.

5.3 Time of Flush

The date of the commencement of flushing in the spring of 1994 was recorded for each sample in the population. All of the Douglas-firs began flushing between April 10 and May 2. Mean flushing dates are presented in Table 5.3.

There were significant differences between flushing times for the three treatments. H plants tended to flush earliest, followed by S plants, and L plants tended to flush latest. When photosynthetic data was considered as well (Section 5.7), it became apparent that the H and S plants entered dormancy later in the autumn and recovered sooner in the spring, as compared to the L plants.

The dissimilarity between treatments in both timing and length of the dormant period was indicative of some of the physiological effects of nitrogen nutrition. Nutrition can affect dormancy induction (Rook 1991); an oversupply of nitrogen in the autumn can delay dormancy induction in conifer seedlings (Brix and van den Dreissche 1974; Coutts and Philipson 1980). The differences between treatments apparently resulted from the plants' nutritional status, though the physiological basis of these differences are not known.

Table 5.3
Mean flushing days (April 1994).

	H	n=60	L	n=52	S	n=48
day	15	a*	23	b	20	c

*Mean values are significantly different (α=0.05)

5.4 Initial Fluorescence

Table 5.4 summarizes the statistical analysis of initial fluorescence (F_O) data. Throughout the summer and autumn of 1993, F_O was notably lower in the H samples than in the L and S samples. The difference was most often significant between the H and S treatments; the S samples tended to have higher F_O values than the L samples in the red sample set (analysis dates indicated by * in Table 5.4).

These results did not support the current literature concerning the anatomical and physiological basis of F_O (ie. Vidaver *et al.* 1991). The H plants were dark green, whereas the L and S plants were pale green or yellow (chlorotic); the H plants evidently contained higher concentrations of chlorophyll. In addition, the H plants were generally larger and bushier than the others; their greater size is indicated by I_{ABS} in Table 5.5. Logically, they should have produced the highest F_O values. Perhaps the darker colour of the H tissue led to a higher degree of fluorescence absorption by the plants. However, it is more likely that some component of variable fluorescence was included in the estimations of F_O, since the H samples displayed lower fluorescence peaks as well. Some variable fluorescence may have artificially inflated all of the F_O values; this would have affected the L and S data most of all, as a result of their elevated P level fluorescence (Table 5.6 and Figure 5.1 a-s). The lower F_O values of the H samples probably reflected greater photochemical efficiency, perhaps in the form of better coordination between PSII and PSI.

Assuming that there was a photochemical component of F_O, there is another possible explanation for the differences between treatments. Inside the sphere, the ratio of photons to chlorophyll molecules was lowest for the largest tissue samples. For the large H plants, the intensity of illumination was not sufficient to overextend the light harvesting capabilities of the tissue, so the fluorescence output was not high. As for the other treatments, the smaller size of the plants resulted in a higher ratio of photons to chlorophyll molecules. The system was pushed, so more energy was given off in the form of fluorescence.

In the winter and the spring of 1994, the F_O values of the L treatment in the red sample set became the lowest overall. The S treatment remained slightly (but not significantly) higher than the H treatment. The L and S values appear to have dropped farther during dormancy than the H values. This was not due to greater photochemical efficiency on the part of the L and S plants; rather, it related to reduced photochemical capacity. The photochemistry typically 'shuts down' in winter to protect plants from photon damage (Vidaver et al. 1989). This process was implicated by the drop in F_O values of H samples; in the L and S plants, it may have been superimposed by reduced photochemical capabilities resulting from nitrogen deficiency.

Table 5.4
Initial fluorescence (F_O; mV).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	70.88	a**	6.79	75.40	a	4.22	76.00	a	12.81
7/21	2	62.51	a	4.62	75.28	b	4.68	76.75	b	8.56
7/28	3*	58.73	a	4.39	61.08	a	4.00	70.90	a	12.77
8/11	4	48.89	a	7.69	62.42	b	5.92	61.92	b	5.77
8/25	5*	48.17	a	2.30	55.03	ab	3.78	62.22	b	10.47
9/08	6	40.61	a	5.48	55.86	b	3.61	57.57	b	8.38
9/22	7*	40.69	a	2.48	46.48	ab	3.62	54.51	b	10.69
10/06	8	37.56	a	2.21	51.53	b	5.80	52.37	b	6.57
10/20	9*	36.50	a	3.38	43.70	ab	6.76	51.35	b	11.69
11/03	10	35.50	a	3.23	44.97	b	7.60	49.09	b	7.48
11/17	11*	28.78	a	2.70	20.70	a	6.98	35.79	a	15.61
1/19/94	12	27.21	a	3.98	28.33	a	9.82	35.45	a	7.26
3/09	13*	28.78	a	2.70	20.70	a	6.98	35.77	a	15.57
3/30	14	23.72	a	2.41	32.58	ab	8.59	36.15	b	6.49
4/13	15*	25.27	a	2.51	22.36	a	7.25	32.96	a	9.56
5/04	16*	31.91	ab	5.29	22.34	a	6.41	37.06	b	9.00
5/11	17	37.19	a	5.89	40.15	a	12.04	41.22	a	6.11
5/25	18*	51.25	a	9.58	36.36	a	9.09	51.88	a	11.50
6/08	19	50.99	a	16.90	47.03	a	16.80	52.49	a	7.64

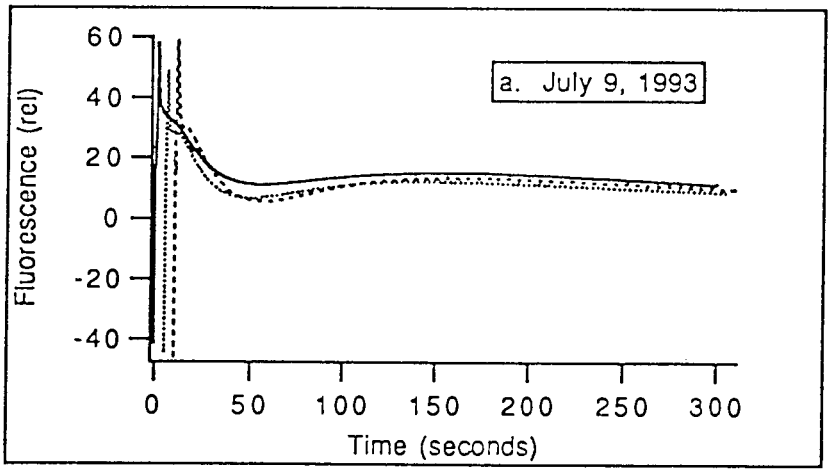
Table 5.5
Light absorbed by the plant in the sphere (IABS; umol/m^2/s).
Means (n=5) and standard deviations (sd).

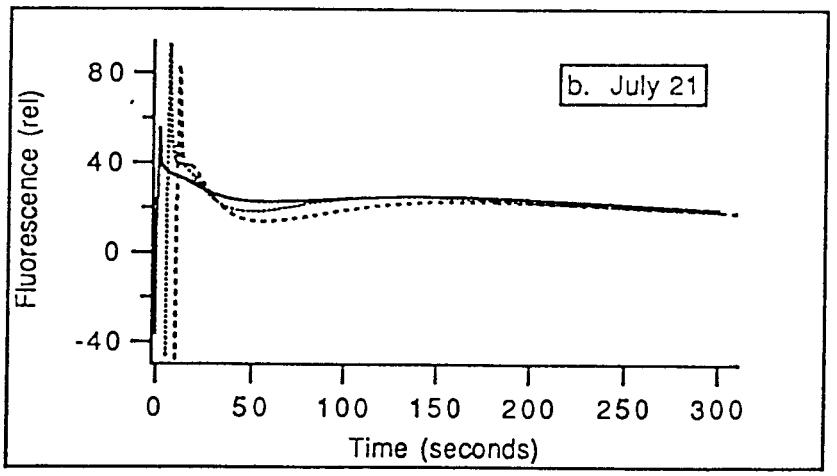
date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	175.0	a**	3.7	172.0	a	7.9	168.0	a	7.1
7/21	2	176.7	a	4.5	167.1	a	6.5	164.1	a	5.7
7/28	3*	178.5	a	3.2	172.0	a	6.3	171.1	a	8.0
8/11	4	179.0	a	2.7	168.2	b	9.4	163.9	b	5.1
8/25	5*	176.4	a	5.5	173.8	a	5.3	171.4	a	7.8
9/08	6	181.7	a	2.3	166.6	b	9.1	166.4	b	4.6
9/22	7*	177.9	a	6.5	173.0	a	5.2	170.7	a	8.2
10/06	8	181.6	a	2.4	167.9	b	8.4	166.3	b	4.8
10/20	9*	176.7	a	6.4	173.3	a	6.4	170.8	a	8.1
11/03	10	181.3	a	3.5	166.7	b	9.9	163.5	b	5.7
11/17	11*	176.0	a	6.4	172.8	a	5.8	170.5	a	8.2
1/19/94	12	181.5	a	3.9	166.9	b	10.4	163.5	b	6.4
3/09	13*	175.6	a	6.1	172.3	a	7.2	169.9	a	8.3
3/30	14	179.6	a	2.3	163.8	b	11.7	162.5	b	5.8
4/13	15*	175.5	a	5.6	171.5	a	7.5	170.4	a	8.2
5/04	16*	184.7	a	3.4	173.3	b	7.7	175.2	b	8.2
5/11	17	194.5	a	1.7	175.4	b	3.2	176.2	b	3.9
5/25	18*	184.9	a	15.0	179.9	a	2.6	176.0	a	6.1
6/08	19	189.6	a	6.6	174.9	b	7.1	176.7	b	5.0

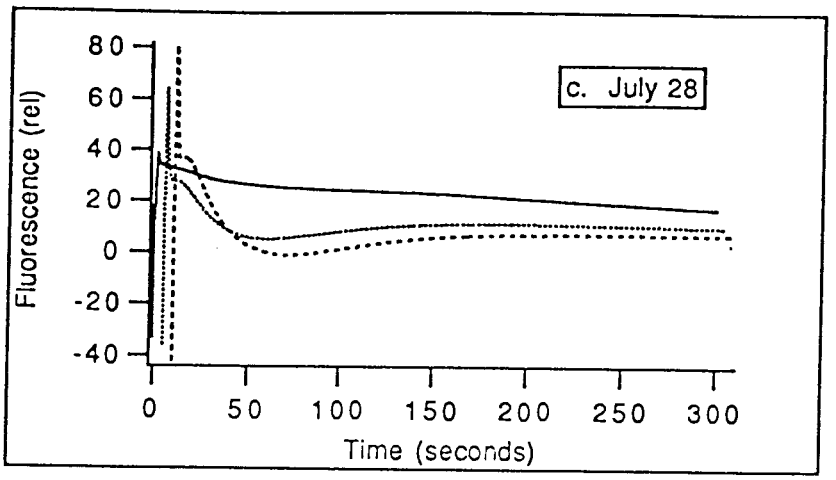
* Indicates 'red' sample set. All other analyses involved the 'blue' sample set.

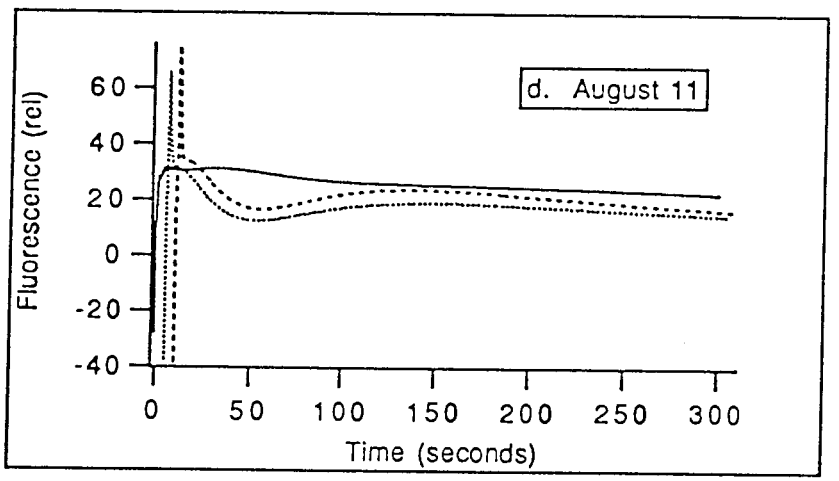
** Mean values within a row with the same letter are not significantly different (α=0.05).

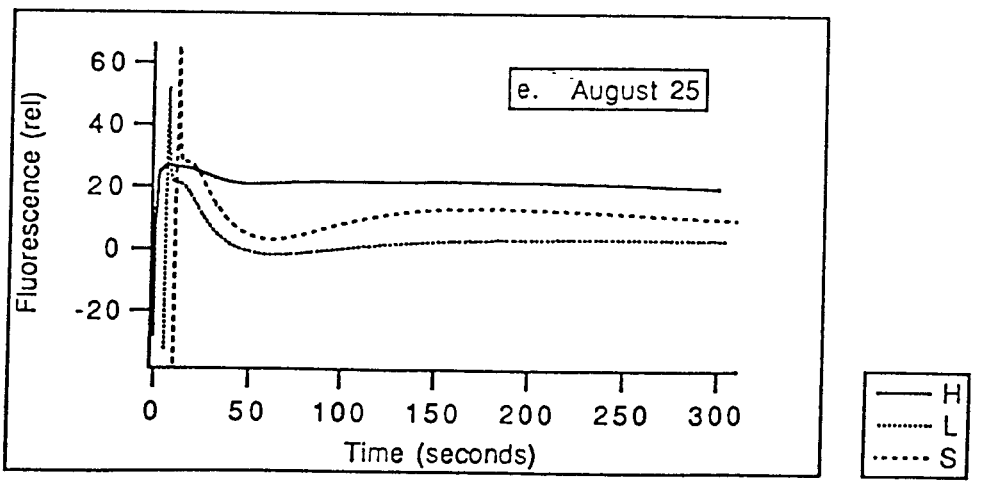
Figure 5.1a-s
Average fluorescence induction (Kautsky) curves for each treatment, by analysis.
Curves are 5 minutes long.
Curves are staggered by 5 seconds for enhancement of initial fluorescence dynamics.

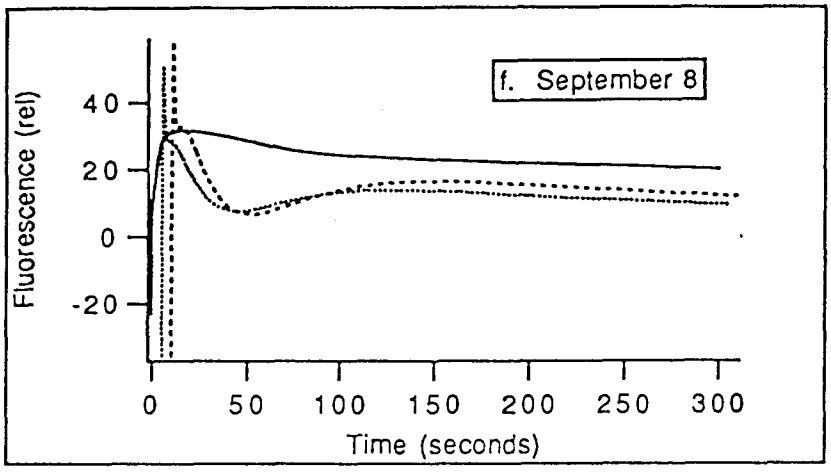


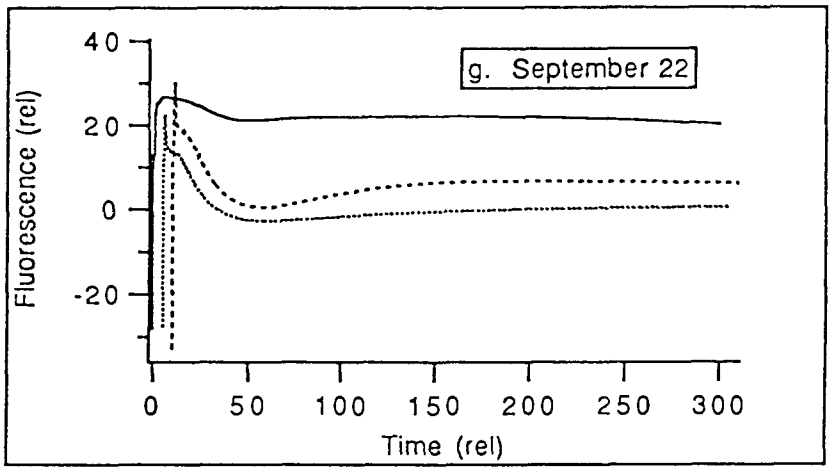


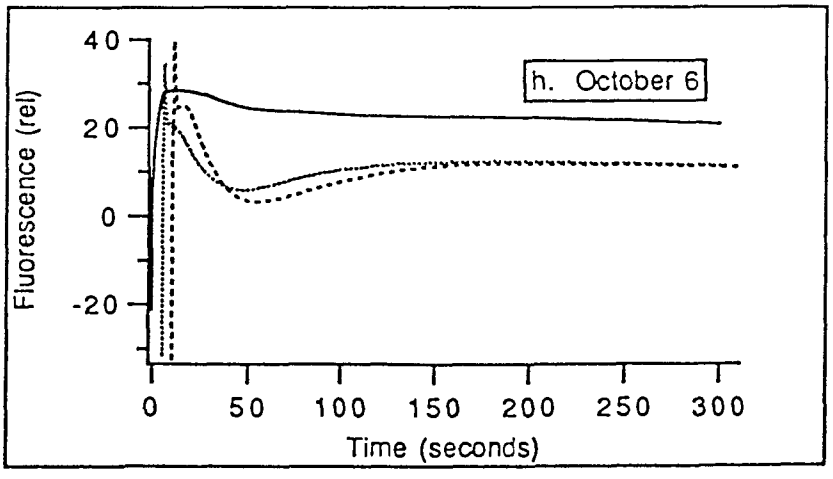

c. July 28
Fluorescence (rel)
80
60
40
20
0
-20
-40
0
50
100
150
200
250
300
Time (seconds)

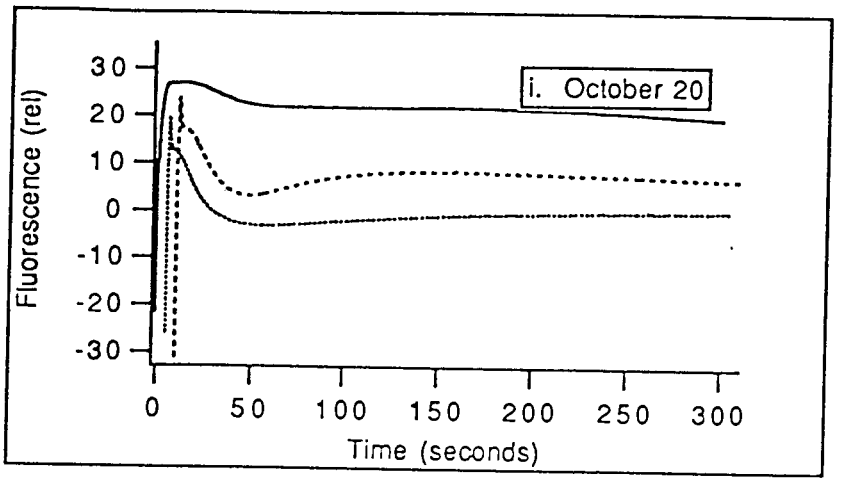

d. August 11
Fluorescence (rel)
60
40
20
0
-20
-40
0
50
100
150
200
250
300
Time (seconds)

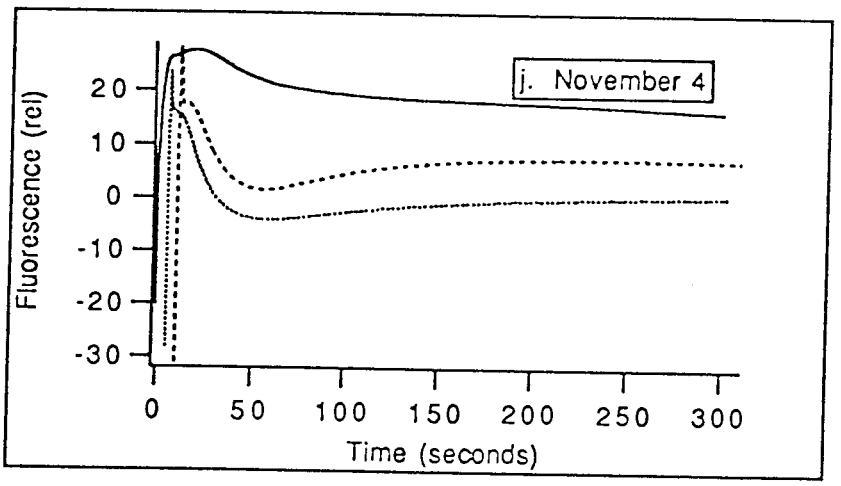

e. August 25
Fluorescence (rel)
60
40
20
0
-20
0
50
100
150
200
250
300
Time (seconds)
H
L
S

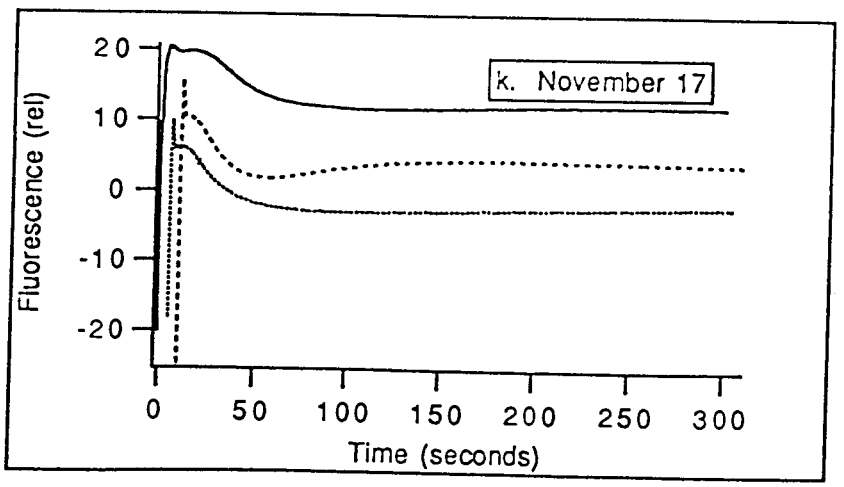

f. September 8
Fluorescence (rel)
40
20
0
-20
0
50
100
150
200
250
300
Time (seconds)

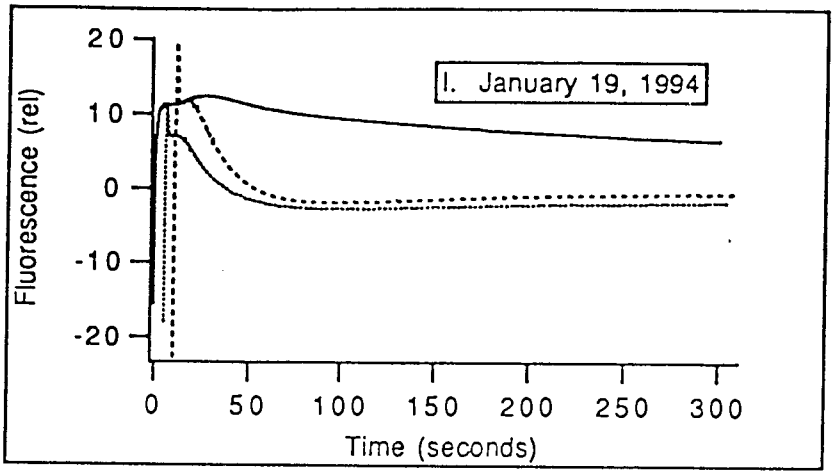

g. September 22
Fluorescence (rel)
40
20
0
-20
0
50
100
150
200
250
300
Time (rel)

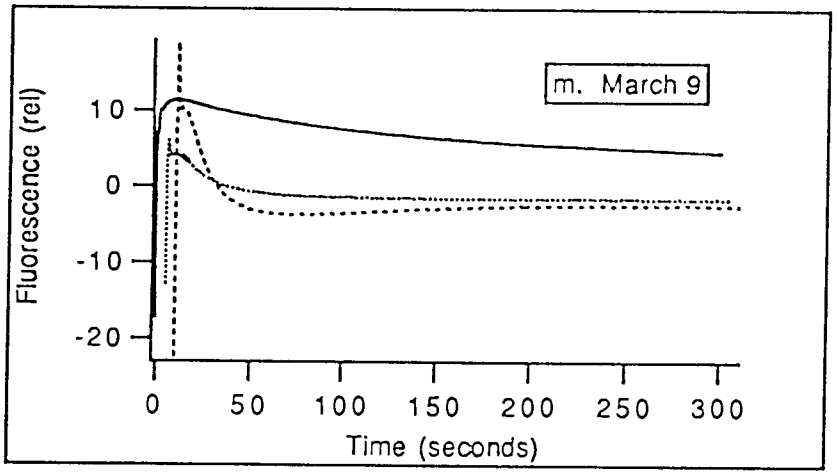

h. October 6
Fluorescence (rel)
40
20
0
-20
0
50
100
150
200
250
300
Time (seconds)
H
L
S

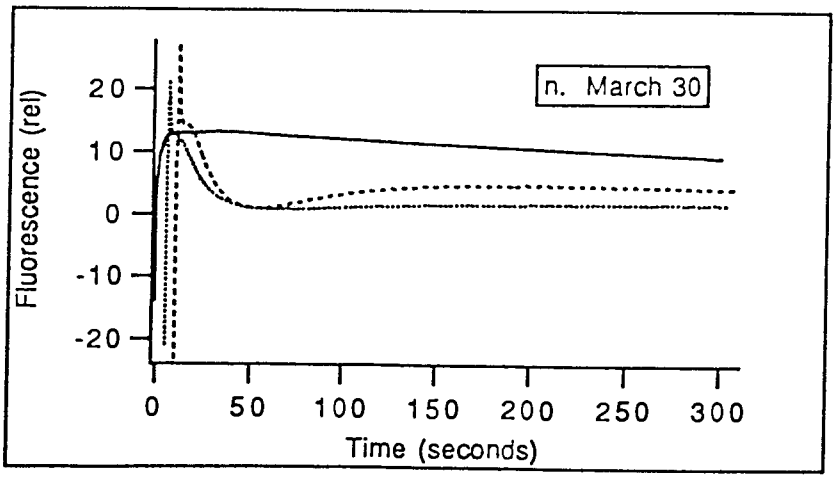

i. October 20
Fluorescence (rel)
30
20
10
0
-10
-20
-30
0
50
100
150
200
250
300
Time (seconds)

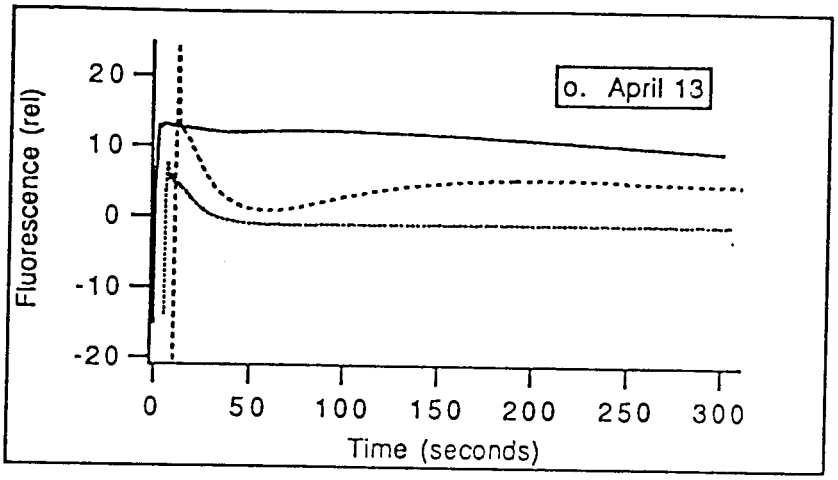

j. November 4
Fluorescence (rel)
20
10
0
-10
-20
-30
0
50
100
150
200
250
300
Time (seconds)

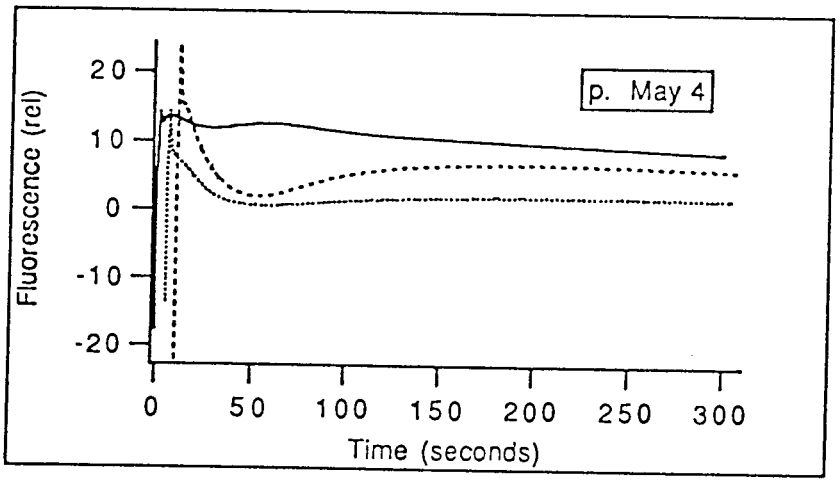

k. November 17
Fluorescence (rel)
20
10
0
-10
-20
0
50
100
150
200
250
300
Time (seconds)
H
L
S

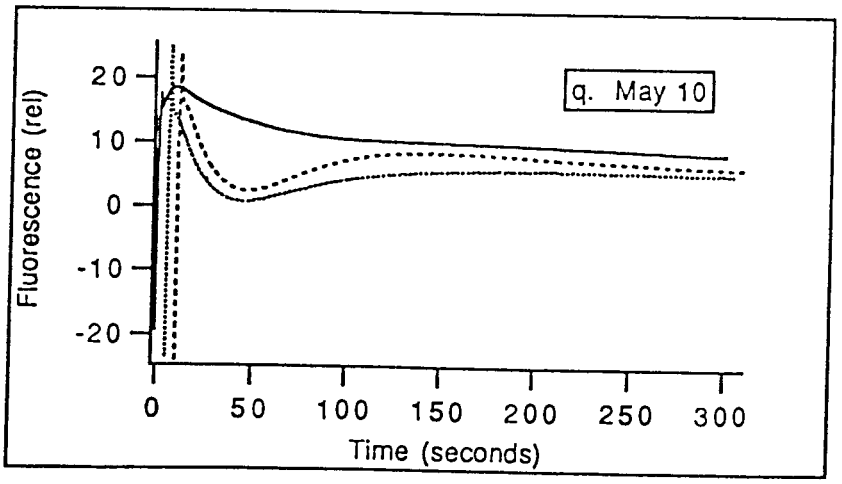

l. January 19, 1994
Fluorescence (rel)
20
10
0
-10
-20
0
50
100
150
200
250
300
Time (seconds)

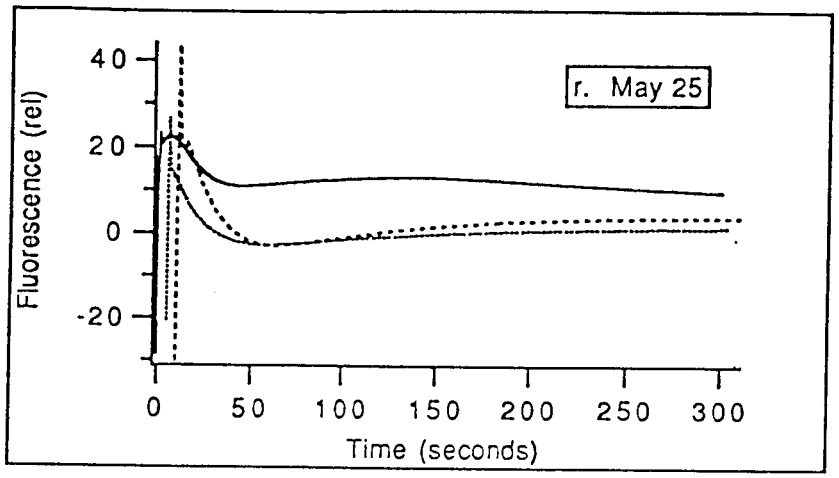

m. March 9
Fluorescence (rel)
10
0
-10
-20
0
50
100
150
200
250
300
Time (seconds)


n. March 30
Fluorescence (rel)
20
10
0
-10
-20
0
50
100
150
200
250
300
Time (seconds)
H
L
S


o. April 13
Fluorescence (rel)
20
10
0
-10
-20
0
50
100
150
200
250
300
Time (seconds)


p. May 4
Fluorescence (rel)
20
10
0
-10
-20
0
50
100
150
200
250
300
Time (seconds)


q. May 10
Fluorescence (rel)
20
10
0
-10
-20
0
50
100
150
200
250
300
Time (seconds)
H
L
S


r. May 25
Fluorescence (rel)
40
20
0
-20
0
50
100
150
200
250
300
Time (seconds)

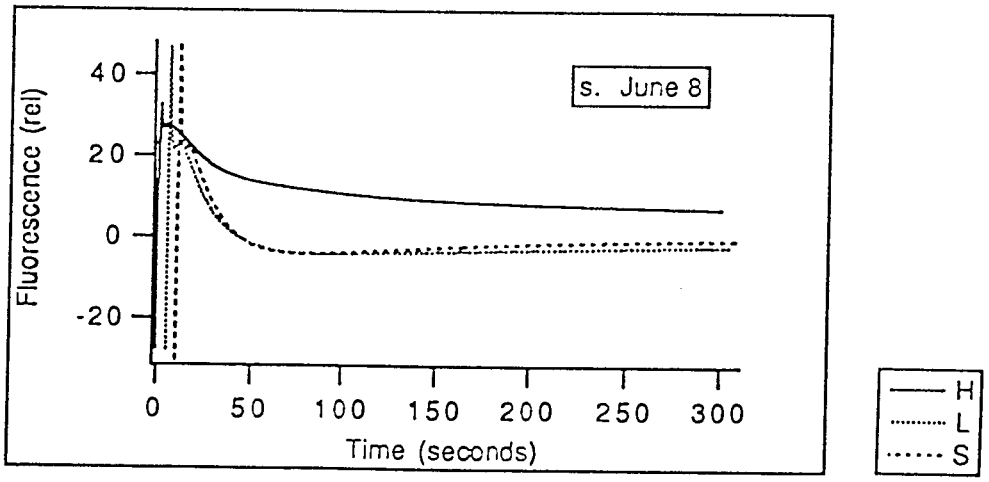

s. June 8
Fluorescence (rel)
40
20
0
-20
0
50
100
150
200
250
300
Time (seconds)
H
L
S

Table 5.6
Peak variable fluorescence (Fvar; relative units).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	56.11	a**	18.24	56.63	a	15.53	59.51	a	23.36
7/21	2	56.17	a	9.45	94.15	b	8.57	85.50	b	24.00
7/28	3*	39.07	a	16.80	65.00	ab	14.79	82.59	b	30.33
8/11	4	33.71	a	3.19	67.14	b	12.74	76.26	b	18.67
8/25	5*	28.23	a	3.14	51.91	ab	18.14	65.90	b	21.81
9/08	6	32.37	a	4.18	53.50	ab	12.02	58.77	b	21.78
9/22	7*	29.37	a	2.68	23.11	a	9.17	30.65	a	12.37
10/06	8	28.65	a	2.22	34.44	a	9.20	39.92	a	13.27
10/20	9*	27.63	a	3.54	20.27	a	8.26	24.19	a	8.03
11/03	10	27.68	a	3.41	24.89	a	10.88	28.68	a	12.62
11/17	11*	21.01	a	3.91	10.20	a	5.59	16.06	a	8.89
1/19/94	12	12.64	a	1.09	11.97	a	6.92	20.08	a	6.07
3/09	13*	12.34	a	3.30	6.43	a	4.85	19.44	a	14.79
3/30	14	13.69	a	1.70	21.81	ab	10.93	27.83	b	8.54
4/13	15*	14.83	ab	1.93	7.95	a	3.20	24.91	b	15.73
5/04	16*	14.44	a	1.60	14.61	a	7.20	24.24	a	9.09
5/11	17	19.39	a	3.66	25.41	a	10.90	23.78	a	7.54
5/25	18*	24.71	a	5.03	27.25	a	14.40	43.92	a	13.14
6/08	19	33.85	a	11.35	47.89	a	31.97	50.48	a	12.66

Table 5.7
Time of peak fluorescence (seconds).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	1.23	a**	0.13	1.15	a	0.15	1.21	a	0.15
7/21	2	1.23	a	0.14	1.32	a	0.15	1.23	a	0.18
7/28	3*	16.40	a	28.60	1.16	a	0.09	1.03	a	0.11
8/11	4	24.51	a	14.12	1.24	b	0.17	1.14	b	0.10
8/25	5*	18.38	a	32.84	1.02	a	0.24	1.05	a	0.07
9/08	6	18.39	a	8.80	0.96	b	0.13	0.92	b	0.09
9/22	7*	11.14	a	8.12	1.91	b	2.40	1.08	b	0.09
10/06	8	13.41	a	5.01	1.07	b	0.11	1.00	b	0.08
10/20	9*	9.95	a	5.59	0.96	b	0.13	0.72	b	0.30
11/03	10	18.19	a	2.18	0.94	b	0.15	0.98	b	0.07
11/17	11*	6.17	a	8.03	0.92	a	0.13	1.03	a	0.15
1/19/94	12	20.20	a	10.87	0.92	b	0.15	0.90	b	0.12
3/09	13*	8.03	a	12.52	0.93	a	0.12	0.85	a	0.10
3/30	14	44.36	a	34.89	1.15	b	0.34	0.97	b	0.09
4/13	15*	16.16	a	29.06	2.03	a	2.08	1.02	a	0.12
5/04	16*	1.07	a	0.16	1.12	a	0.10	1.00	a	0.11
5/11	17	6.13	a	4.82	0.96	b	0.18	0.89	b	0.07
5/25	18*	4.42	a	3.76	0.79	a	0.07	0.84	a	0.05
6/08	19	3.42	a	5.12	0.93	a	0.12	0.93	a	0.08

* Indicates 'red' sample set. All other analyses involved the 'blue' sample set.

** Mean values within a row with the same letter are not significantly different (α=0.05).

Some dieback (bronzing of needle tips) was observed in the early winter, particularly on L plants. The resulting tissue loss affected plant size, so it probably affected fluorescence levels as well; F_O values were not corrected for plant size.

In the spring of 1994, there was no consistent significant difference between the F_O values for the three treatments. However, the data were overestimated for the H samples, since these values were not corrected for plant size, and the H plants were even larger and bushier than before (as indicated by greater I_{ABS} values in Table 5.5). Therefore it is apparent that the pattern of the previous summer was maintained (lower F_O per unit of tissue in the H samples than in the others).

5.5 Variable Fluorescence

Like F_O, maximum variable fluorescence (Fvar) was lower for the H samples than for the L and S samples in the summer of 1993 (Table 5.6). Contradictory evidence was presented by Kolber *et al.* (1988) and Da Silva and Arrabaca (1992). Both found that nitrogen deficiency reduced Fvar.

The results from this study are somewhat misleading. It is apparent from Figure 5.1a-s and Table 5.7 that peak fluorescence occurred much later in most of the curves form H samples than from L and S samples. Fvar values for the H treatment did not usually represent P-level fluorescence; rather, they came from some point later in the fluorescence curve (M_1 or M_2). In Figure 5.1a-c, p, and possibly r and s, Fvar represented the P-level fluorescence. For all other analyses, the maximum

fluorescence level came later in the curve. Consequentially, the H data were not indicative of the imbalance between photon capture and electron transport, as would be expected with normal fluorescence induction curves. Instead, the peaks were likely due to some later (and normally proportionately less substantial) photochemical imbalance. The sharp peaks of the L and S samples indicated a backlog of electrons in PSII. The cooperation between PSII and PSI was impaired in the dark, and it took a few moments for PSI activity to become reestablished in the light. As mentioned in Chapter 4, such sharp peaks may be indicative of stress (Brooke, 1994, Pers. Comm.). The near-disappearance of P peaks for the H samples indicated that a backlog of electrons was not occurring to the same extent. The photochemistry was pushing the biochemistry; limitations were somewhere later along the pathway, so fluorescence was elevated at M and F_T, but not at P. The plants' biochemical capacity was exceeded somewhere downstream, so it took a while for a backlog of electrons to occur and for the fluorescence to rise.

By the end of September 1993, no significant differences remained between the treatments (Table 5.6). On average, the S samples consistently produced slightly higher Fvar values than the L samples throughout the entire study. Both the S and L treatment values fell in the autumn and remained at a relatively low level throughout the winter. The H values also fell, but proportionately less than those of the other treatments. As a result, the L values were the lowest of all treatments at that time. In the spring, both the L and S values rose proportionately more than the H values.

The lack of an initial P peak in many of the H curves was a curious phenomenon. In order to gain a better understanding of it, a series of induction curves

were obtained for the H and L samples at several light intensities. The results are presented in Table 5.8a and Figure 5.2a-b. A subsequent series of curves were obtained for the red sample set (all three treatments) at higher light intensities. Results are shown in Table 5.8b and Figure 5.3a-c.

At higher light intensities a prominent P peak appeared in the Kautsky curves of the H samples. There was no corresponding increase in P for the L and S treatments. These results suggested a reason for the lack of P in many H samples at the 200 $\mu mol/m^2/s$ illumination level. If the electron transport capacity was not saturated by the excitation light, there would have been no backlog of excitation energy in the chlorophyll molecules of Photosystem II and the subsequent electron transport chain, and hence no P peak would have occurred. Higher light intensities produced and intensified this backlog, resulting in a notable increase in P.

Why did the H plants require higher intensity light for the saturation of electron transport capacity, as compared to the other treatments? The nitrogen-limited plants probably had greater photosynthetic unit (PSU) sizes than the adequately fed plants (ie. more light-capturing molecules were connected to a single Photosystem II complex). As a result, a bottleneck in electron flow was created when the dark-acclimated tissue was illuminated. Until electron transport could occur at the rate of photon capture by the pigment molecules, energy loss (in the form of heat and fluorescence) was elevated. This evidence supported Kolber *et al.* (1988) and Greene *et al* (1991, 1992) who found an increase in the apparent absorption cross-section of Photosystem II in nitrogen-deficient cells.

Table 5.8a-b
The effects of light intensity upon fluorescence induction (a) of H and L treatments (n=3) on 1/27/94, and (b) of all three treatments (n=5) on 3/24/94.

a

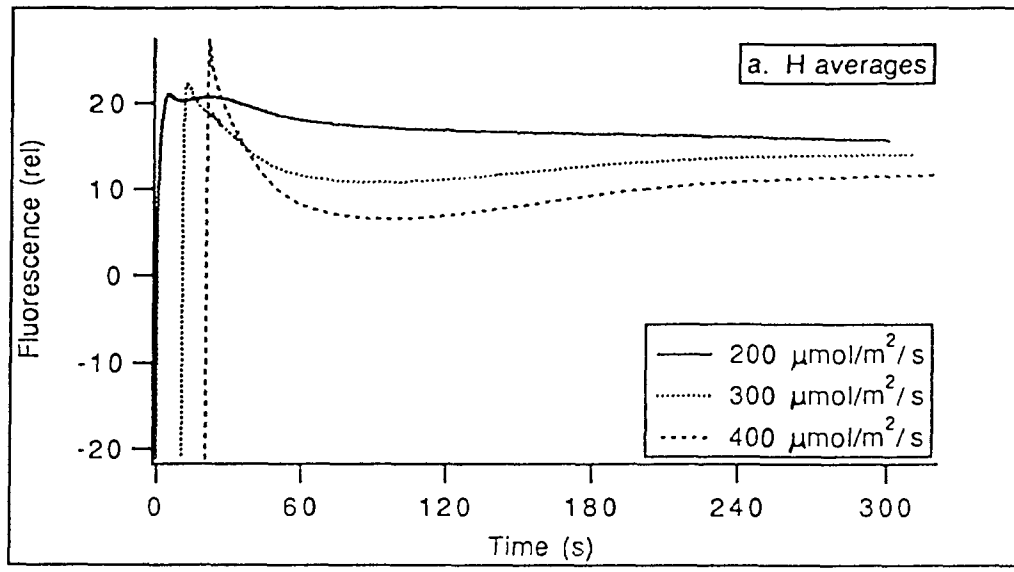
variable	illumination ($\mu mol/m^2/s$)	% increase (light)	H average	% increase*	L average	% increase*
Fo (mV)	200		36.23		30.86	
	300	50	53.09	47	46.94	52
	400	33	71.61	35	63.92	36
IABS ($\mu mol/m^2/s$)	200		178.2		166.3	
	300	50	264.8	49	246.4	48
	400	33	349.5	32	329.5	34
FO/IABS	200		0.204		0.187	
	300	50	0.201	-1	0.192	3
	400	33	0.205	2	0.195	2
Fvar (rel)	200		22.33		6.39	
	300	50	22.48	1	4.46	-30
	400	33	27.87	24	5.92	33
Fmax (rel)	200		42.66		24.94	
	300	50	42.53	0	23.51	-6
	400	33	48.35	14	25.32	8
time of peak fluorescence (seconds)	200		19.29		0.83	
	300	50	1.83	-91	0.54	-35
	400	33	0.89	-52	0.52	-5

b

variable	illumination ($\mu mol/m^2/s$)	% increase (light)	H average	% increase*	L average	% increase	S average	% increase
Fo (mV)	200		28.78		20.70		35.77	
	480	140	64.44	124	51.43	148	87.26	144
	700	46	105.03	63	87.18	70	145.47	67
IABS ($\mu mol/m^2/s$)	200		175.6		171.5		170.4	
	480	140	410.7	134	408.8	138	397.8	133
	700	46	598.7	46	593.6	45	583.9	47
FO/IABS	200		0.164		0.121		0.215	
	480	140	0.157	-4	0.126	4	0.219	2
	700	46	0.175	12	0.147	17	0.249	14
Fvar (rel)	200		12.34		6.43		19.44	
	480	140	10.70	-13	10.43	62	16.68	-14
	700	46	23.93	124	7.54	-28	19.39	16
Fmax (rel)	200		28.73		18.56		40.97	
	480	140	26.39	-8	23.01	24	38.61	-6
	700	46	41.48	57	22.22	-3	44.30	15
time of peak fluorescence (seconds)	200		8.03		0.93		0.85	
	480	140	3.47	-57	0.78	-16	0.71	-17
	700	46	0.80	-77	0.60	-23	0.55	-22

* Percent increase in each average value, over the average value obtained at the previous light intensity.

Figure 5.2a-b
Average fluorescence induction (Kautsky) curves for H and L treatments (n=3) at several light intensities.
Curves are staggered by 5 seconds for enhancement of initial fluorescence dynamics.
The data were collected from a random sample on January 27, 1994.



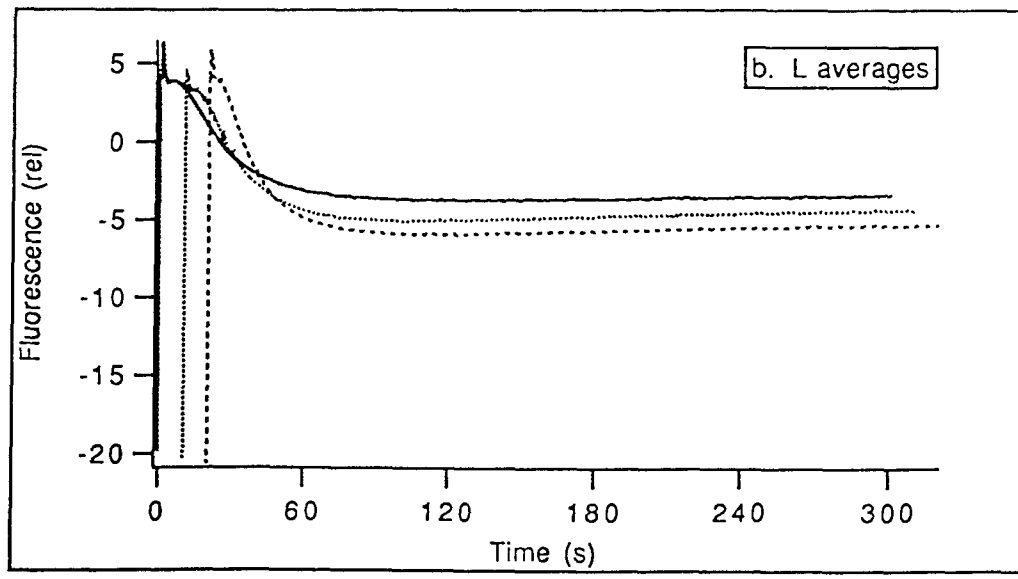


Figure 5.3a-c
Average fluorescence induction (Kautsky) curves for H, L, and S treatments (n=5) at several light intensities.
Curves are 25 seconds long, and they are staggered by 1 second for enhancement of initial fluorescence dynamics.
The data were collected from the red sample set on March 24, 1994.

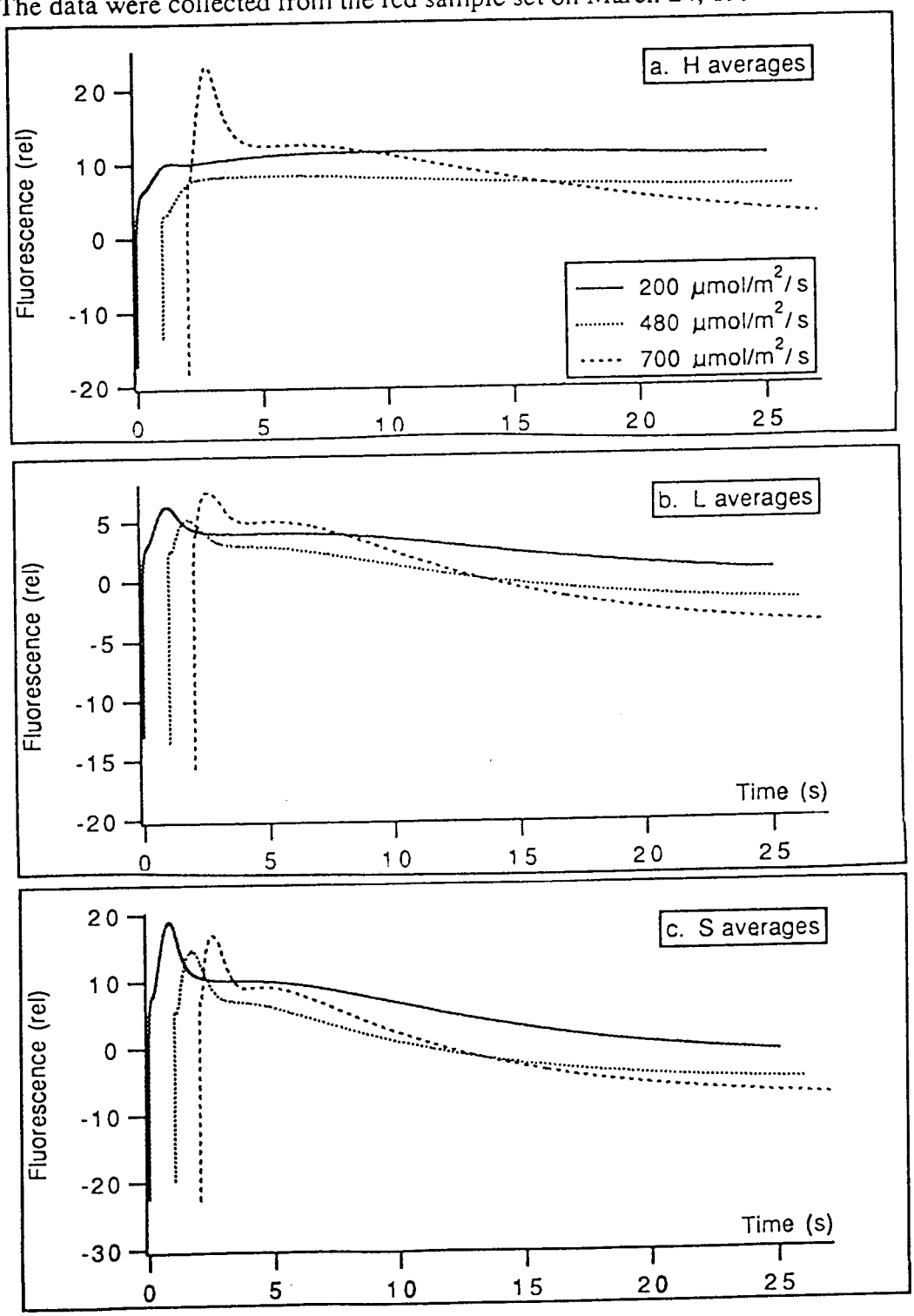


Table 5.9
Steady-state fluorescence (F_T; relative units).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	11.93	a**	2.38	9.11	a	4.32	9.88	a	2.52
7/21	2	19.29	a	4.09	17.99	a	5.14	17.78	a	2.02
7/28	3*	17.14	a	3.87	9.84	b	1.68	6.91	b	3.57
8/11	4	22.48	a	4.92	14.68	b	3.02	16.38	ab	4.05
8/25	5*	20.15	a	3.41	3.56	b	1.77	10.14	c	2.13
9/08	6	19.99	a	4.38	9.51	b	3.71	12.04	b	4.20
9/22	7*	20.33	a	3.43	0.44	b	2.04	6.08	c	2.89
10/06	8	20.81	a	4.01	11.30	b	4.14	10.96	b	4.20
10/20	9*	19.65	a	3.05	0.18	b	2.73	6.78	c	4.62
11/03	10	16.39	a	3.66	0.56	b	3.50	7.31	b	4.19
11/17	11*	10.27	a	2.92	-1.40	b	0.76	3.59	c	2.13
1/19/94	12	6.48	a	1.50	-1.85	b	1.65	-0.61	b	2.32
3/09	13*	4.53	a	3.94	-1.61	b	0.85	-2.46	b	4.01
3/30	14	9.29	a	1.63	1.74	b	2.98	4.19	b	3.21
4/13	15*	9.28	a	1.43	-1.00	b	1.03	4.62	c	2.59
5/04	16*	8.64	a	1.57	1.82	b	1.67	6.03	a	1.93
5/11	17	8.76	a	3.10	5.42	a	1.57	6.38	a	1.55
5/25	18*	9.63	a	2.83	1.32	b	1.54	3.79	b	3.07
6/08	19	7.19	a	2.38	-2.11	b	3.91	0.22	b	2.54

* Indicates 'red' sample set. All other analyses involved the 'blue' sample set.

** Mean values within a row with the same letter are not significantly different (α=0.05).

If H plants had smaller PSU sizes and therefore fewer pigment molecules per reaction centre, less of a backlog in electrons would have occurred before cooperation between the Photosystems could be restored.

Being larger and darker green, H plants certainly had higher chlorophyll contents than L and S plants, resulting in a lower ratio of photons to chlorophyll molecules when the tissue was illuminated inside the sphere. The 200 $\mu mol/m^2/s$ light intensity was probably not sufficient to over-extend the light harvesting capabilities of the tissue, so the fluorescence output was not high; a backlog of electrons did not occur in the first second or two. At the higher light intensities, the light harvesting capabilities and electron transport capacity were over-extended, and a backlog of electrons occurred at Photosystem II. This resulted in marked P-level fluorescence at 400 $\mu mol/m^2/s$ in Figure 5.2a and 700 $\mu mol/m^2/s$ in Figure 5.3a.

5.6 Steady-state Fluorescence

Steady-state fluorescence (F_T; Table 5.9) was significantly higher for the H samples than for the L and S samples on most analysis dates. The L values tended to be low, and the S values were intermediate.

Some L and S values dropped below zero, indicating that F_T was lower than F_O. This suggested that F_O did indeed contain some component of variable fluorescence; it also suggested that the plants were more photochemically efficient when light-acclimated than when dark-acclimated. As a result, after five minutes in

the light, there was less energy loss in the form of fluorescence than immediately after shutter opening. F_O evidently did not remain constant (though it is assumed to when it is used to normalize Kautsky curves). Since F_O is believed to reflect energy migrations within the pigments of Photosystem II (Lichtenthaler and Rinderle 1988), perhaps it is reduced as a source of fluorescence when those pigments are active in photon capture and electron transport. The nature of F_O's changeability during light adaptation was investigated by Bilger and Schreiber (1986). F_O decreased when dark-acclimated tissue was placed under conditions of saturating light and absence of CO_2. This phenomenon was interpreted as a result of physical changes in the light harvesting complex of Photosystem II (Bilger and Schreiber 1986). They found that photochemical and energy-dependent quenching of variable fluorescence led to negative Fv values if the quenching of F_O was not accounted for. Fv was affected proportionately by the reduction of F_O.

Regardless of the problems with F_O, the results of this study suggested that F_T was a good indicator of the physiological status and health of the individuals. In the H samples, F_T was low when the plants were flushing (a time of high respiration rates and low net carbon gain). F_T was highest when the tissue was mature and the plants were highly photosynthetically active, and lowest when the plants were dormant. This pattern was also apparent in the L and S samples, but it was overlaid by the impacts of nutrient stress which apparently resulted in significant decreases in F_T. This effect was most marked in the L samples (Figure 4.8).

In this study, F_T tended to be higher for the H samples than for the L and S samples. Photochemical quenching after the peak was therefore much more marked

for the L and S samples than for the H samples. In contrast, Kolber *et al.* (1988) and da Silva and Arrabaca (1992) both found that nitrogen deficiency resulted in a decrease in photochemical quenching after the peak.

5.7 Apparent Photosynthesis and Dark Respiration

Table 5.10 summarizes the statistical analysis of apparent photosynthesis (APS) data. The rates were initially similar for all treatments. Differences between treatments became apparent around the end of September 1993, when rates for the L samples were lower than those of the H and S samples. The L rates were thereafter consistently lower than the S rates. The L rates were occasionally higher than the H rates; this difference was significant on May 11, 1994 (analysis 17). This was due to the extensive new shoot growth of H plants; they were disproportionately affected by increased dark respiration rates (Table 5.11).

Throughout the study period, dark respiration (RD) values tended to be highest for the H samples (Table 5.11). This difference became significant in early autumn 1993; the pattern remained consistent throughout the winter and spring.

Table 5.12 (PSRD) was created to illustrate the magnitude of the impact of RD upon APS rates. It describes rough estimates of total photosynthesis. These are only rough estimates for two reasons. Firstly, dark respiration is inhibited by light, particularly in photosynthetic tissue (Lister 1993, pers. comm.). This inhibition probably occurred to a greater extent in the mature tissue than in newly flushed tissue,

since the photosynthetic function of the new tissue was not fully established. As a result, total photosynthesis was slightly overestimated for the L and S treatments as compared to the H treatment. Secondly, photorespiration, which significantly impacts APS rates, was not included in the calculations. Since the assessments were made at constant light, temperature, pressure and CO_2 and O_2 concentrations, it can be assumed that photorespiration introduced proportionately the same amount of error into all of the estimates.

The PSRD estimates closed the gap a little between the L and H samples on May 11 (analysis 17). However, the H data were still significantly lower. It must be concluded that the illuminated tissue (almost entirely newly flushed needles) was photosynthesizing at a very low rate. It took until June for photosynthetic activity to recover.

5.8 Quantum Requirement

Quantum requirement (QR) tended to be higher for the L and S samples than for the H samples (Table 5.13). This difference was initially quite small, but it became marked in the autumn and winter of 1993; QR became particularly high for the L plants. In the spring of 1994, the differences between treatments were once again less prominent. QR was actually highest for the H treatment on May 11. This was probably due to the high proportion of photosynthetically inefficient young new tissue in those plants.

The higher QR of L and S samples was indicative of the damaging effects of nitrogen limitation upon photosynthesis. The photosynthetic response to light intensity was apparently impaired in the L and S plants (though more so in the L plants), perhaps as a result of chlorosis. Fewer chlorophyll molecules per unit leaf area would almost certainly have reduced the light harvesting capbilities of those leaves.

Table 5.10
Average light-saturated (800 µmol/m²/s) photosynthesis rates (APS; $mgCO_2/dm^2/hour$).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	10.74	a**	0.60	10.42	a	2.97	14.19	b	1.76
7/21	2	14.67	a	2.65	14.66	a	1.08	15.22	a	2.94
7/28	3*	14.68	a	2.42	13.65	a	3.13	14.53	a	2.52
8/11	4	32.38	a	4.73	34.44	a	4.03	35.46	a	4.85
8/25	5*	18.15	a	3.51	11.05	b	1.38	13.91	b	1.77
9/08	6	12.70	ab	1.81	13.28	a	2.41	10.10	b	0.91
9/22	7*	13.02	a	1.23	7.40	b	1.88	11.68	a	2.09
10/06	8	29.21	a	1.82	11.73	b	2.70	24.33	c	2.10
10/20	9*	16.24	a	3.76	6.96	b	1.48	12.17	a	3.06
11/03	10	13.05	a	0.84	10.12	a	4.52	13.53	a	4.38
3/09/94	13*	7.27	a	1.97	1.89	b	1.12	5.39	a	2.86
3/30	14	8.72	a	1.96	10.76	a	5.86	11.49	a	5.74
4/13	15*	9.14	ab	3.78	3.82	a	2.06	9.63	b	4.03
5/04	16*	6.43	a	1.06	3.30	b	1.63	8.09	a	1.02
5/11	17	1.03	a	0.71	8.69	b	1.13	9.98	b	1.64
5/25	18*	5.71	a	1.83	6.37	a	1.09	10.34	b	1.35
6/08	19	9.45	a	1.74	7.55	a	2.62	9.42	a	1.85

Table 5.11
Average dark respiration rates (RD; $mgCO_2/dm^2/hour$).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	4.15	a**	0.53	3.91	a	0.60	3.88	a	0.69
7/21	2	2.59	a	0.28	2.40	a	0.26	2.36	a	0.47
7/28	3*	3.50	a	0.70	3.73	a	0.58	3.10	a	0.19
8/11	4	6.53	a	1.44	4.48	b	0.91	6.31	ab	0.90
8/25	5*	2.70	a	1.06	1.91	a	0.29	1.88	a	0.35
9/08	6	3.78	a	0.60	2.73	b	0.45	2.61	b	0.42
9/22	7*	4.59	a	1.36	2.95	b	0.31	2.42	b	0.16
10/06	8	5.33	a	0.73	2.87	b	0.29	4.68	a	0.87
10/20	9*	4.03	a	0.84	2.51	b	0.34	2.61	b	0.48
11/03	10	3.37	a	0.66	3.23	a	0.88	2.88	a	0.54
3/09/94	13*	3.65	a	1.01	1.38	b	0.27	2.41	b	0.67
3/30	14	7.67	a	1.56	4.89	a	4.11	5.04	a	1.93
4/13	15*	5.71	a	1.83	3.22	b	0.47	2.25	b	0.64
5/04	16*	10.39	a	1.46	6.17	a	3.81	6.35	a	3.60
5/11	17	6.83	a	2.79	4.45	a	0.69	4.07	a	0.33
5/25	18*	6.68	a	1.02	3.01	b	0.64	4.01	b	1.13
6/08	19	8.83	a	4.07	4.16	b	1.08	4.12	b	1.05

* Indicates 'red' sample set. All other analyses involved the 'blue' sample set.
** Mean values within a row with the same letter are not significantly different (α=0.05).

Table 5.12
Apparent photosynthesis (at 800 µmol/m²/s) plus dark respiration (PSRD; $mgCO_2/dm^2/hour$).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	14.89	a**	1.03	14.34	a	3.55	18.07	a	1.61
7/21	2	17.26	a	2.90	17.06	a	1.03	17.58	a	3.19
7/28	3*	18.18	a	3.02	17.38	a	3.69	17.63	a	2.51
8/11	4	38.91	a	4.19	38.92	a	4.42	41.77	a	5.18
8/25	5*	20.85	a	4.21	12.96	b	1.40	15.79	b	1.79
9/08	6	16.47	a	2.20	16.02	ab	2.69	12.70	b	1.25
9/22	7*	17.61	a	2.04	10.34	b	1.79	14.11	c	2.15
10/06	8	34.54	a	1.99	14.60	b	2.72	29.01	c	2.34
10/20	9*	20.27	a	4.55	9.47	b	1.34	14.78	ab	3.26
11/03	10	16.42	a	1.28	13.35	a	5.27	16.41	a	4.73
3/09/94	13*	10.92	a	2.33	3.27	b	1.26	7.81	a	3.25
3/30	14	16.39	a	1.06	15.65	a	9.65	16.54	a	5.84
4/13	15*	14.84	a	5.14	7.04	b	2.03	11.88	ab	4.45
5/04	16*	16.82	a	2.24	9.47	b	3.76	14.44	ab	3.12
5/11	17	7.86	a	2.74	13.15	b	1.36	14.05	b	1.48
5/25	18*	12.39	ab	2.44	9.38	a	1.70	14.35	b	2.21
6/08	19	18.28	a	2.64	11.71	b	2.78	13.54	b	1.94

Table 5.13
Average quantum requirement (QR; photons per molecule of CO_2).
Means (n=5) and standard deviations (sd).

date	analysis	H		sd	L		sd	S		sd
7/07/93	1*	101.12	a**	21.50	165.11	a	77.70	95.28	a	10.68
7/21	2	75.81	a	9.35	86.91	a	9.13	91.65	a	26.91
7/28	3*	106.91	a	43.01	96.66	a	35.62	73.75	a	17.86
8/11	4	38.33	a	8.80	36.45	a	7.55	39.22	a	5.63
8/25	5*	76.50	a	13.83	116.19	b	16.31	92.73	ab	28.19
9/08	6	85.04	a	12.54	94.25	a	27.50	141.07	a	65.83
9/22	7*	77.04	a	11.96	166.28	b	8.67	108.50	c	19.09
10/06	8	35.47	a	6.07	122.21	b	35.89	62.55	a	32.83
10/20	9*	61.45	a	10.83	198.34	b	39.95	108.55	c	24.53
11/03	10	80.52	a	10.80	191.55	a	150.13	96.04	a	16.02
3/09/94	13*	150.11	a	41.22	567.64	b	277.81	250.40	a	109.40
3/30	14	80.35	a	24.43	119.32	a	59.77	194.89	a	241.26
4/13	15*	90.55	a	27.95	265.76	b	105.12	115.91	a	36.27
5/04	16*	97.09	a	23.71	169.31	a	67.76	113.49	a	22.10
5/11	17	383.46	a	128.62	115.06	b	48.82	89.89	b	9.95
5/25	18*	137.38	a	46.84	162.39	a	37.55	105.03	a	14.36
6/08	19	102.34	a	29.76	142.31	a	25.95	101.70	a	23.13

* Indicates 'red' sample set. All other analyses involved the 'blue' sample set.
** Mean values within a row with the same letter are not significantly different (α=0.05).

CHAPTER 6
RESULTS AND DISCUSSION:
CORRELATIONS

In this chapter, correlations are sought between plant silhouette area and F_O or I_{ABS}, in order to determine which is the more suitable normalization factor for fluorescence induction (Kautsky) curves. Correlations between foliar nitrogen concentration and physiological parameters are investigated. Evidence of relationships between chlorophyll fluorescence, CO_2 exchange rates and quantum efficiency are also examined, in order to promote an understanding of the physiological significance of chlorophyll fluorescence. Table 3.1 is a useful guide for seasonal information (such as timing of flushes and dormancy).

6.1 Chlorophyll Fluorescence: Indication of Plant Size

As discussed in Chapter 3, researchers have found correlations between plant size (in terms of tissue volume, dry weight, leaf area or silhouette area) and either F_O or I_{ABS} (ie. Dube 1990).

In this study, I_{ABS} remained constant for each shoot sample throughout the study period, except during periods of growth (Table 6.1). In contrast, F_O declined markedly between analyses throughout 1993; it rose again after the plants flushed in 1994 (Figure 4.5). These results suggested that I_{ABS} was probably a better indicator of plant size than F_O.

I_{ABS} was reasonably well correlated with silhouette area (Figure 6.1; $r^2 = .624$ for analyses 1 and 2), considering that I_{ABS} is a 3-dimensional indicator of plant size and silhouette area is two-dimensional. (Silhouette area may underestimate the amount of tissue in large samples with a high degree of overlap, but then again, so might I_{ABS} when the integrating sphere is very full.) F_O (at any single analysis date) was not correlated with silhouette area (Figure 6.2; $r^2 = .008$ for analyses 1 and 2) and was evidently not a suitable measure of plant size.

These results justified the use of I_{ABS} as a size correction factor for normalization of fluorescence data (refer to Chapter 3 for details of how it was used).

Table 6.1a-b
Light absorbed by the plants in the sphere (IABS; µmol/m²/s) on each analysis date. (a) is the red sample set, and (b) is the blue sample set.

a	7/07	7/28	8/25	9/22	10/20	11/17	3/09	4/13	5/04	5/25
H01	170.2	177.8	175.8	176.4	175.2	173.2	173.8	173.3	183.1	168.2
H15	171.8	174.4	176.4	176.6	174	174.7	172.5	172.9	183.2	195.5
H16	177.2	176.8	167.8	168.6	168.2	167.4	167.9	168.7	182.7	168.9
H23	177.8	181.8	182	185.2	184	182.6	182.2	181.3	184	198.1
H39	178	181.8	180.2	182.8	182	182.1	181.5	181.5	190.7	193.7
L07	163.6	165.4	170.6	170.4	169.2	170.2	170.2	165	166.4	177.2
L12	177	176	176.4	175.6	176.8	173.3	174.1	175.3	177.6	182.7
L14	183.6	180.8	181.2	180.8	182.4	182	182.5	182	184.2	181
L16	167.4	167.9	167.6	167.6	166	166.2	162.4	163.9	166.1	181.4
L52	171	170	173	170.6	172.2	172.3	172.2	171.5	172.2	177.1
S01	178.8	178.6	179	178.8	178.8	178.2	177.5	177.3	183.4	183.2
S02	163.6	166	166.8	164.4	165.4	166	166.1	167.3	177	166.2
S09	171.4	175.4	173.2	174.4	174.2	173.5	173.7	173.5	175.6	177.1
S16	170.4	176	177.6	176.2	176.2	176.2	174.9	176.3	178.4	176.8
S22	160.6	159.6	160.2	159.8	159.4	158.4	157.1	157.4	161.6	176.7

b	7/21	8/11	9/08	10/06	11/03	1/19	3/30	5/11	6/08
H29	180.2	180	183.6	183.6	186	185.9	183.3	197.6	199.3
H40	182.6	180.6	182.6	182.3	184	184.6	178.4	194.4	182.2
H42	174	177	178.4	178.3	177.8	176.3	177.7	193.5	191.5
H43	174.8	175.6	180.2	180.1	178.6	179.8	178.2	193.6	190.2
H54	171.8	182	183.6	183.9	180.2	180.7	180.6	193.6	184.7
L22	170.2	174.8	174.4	175.7	173.9	174.1	171.7	178	181.4
L27	159.6	156.2	155.6	156.7	153	152.8	148.2	175.6	178.5
L37	175.4	177	176	175.5	176.7	177.8	175.9	178.6	179.9
L42	168.8	172.8	168	169.5	169.8	170.2	168.1	173.9	165.3
L45	161.6	160.2	158.8	162	160.2	159.4	155	170.7	169.6
S35	167	166	167.8	167.4	166.7	167.8	165.3	182.8	179.5
S37	154.6	155	159.4	159.1	153.7	152.4	152.3	172.3	168.3
S39	163.4	164.2	164.2	164.1	163.2	163.2	163.1	175.7	178.9
S42	166.8	166.8	169.4	169.1	166.6	166.8	166.1	174.5	180.9
S48	168.8	167.4	171	171.6	167.5	167.2	165.9	175.7	175.7

Figure 6.1
Regression of IABS against silhouette area for analyses 1 (7/07/93) and 2 (7/21/93).

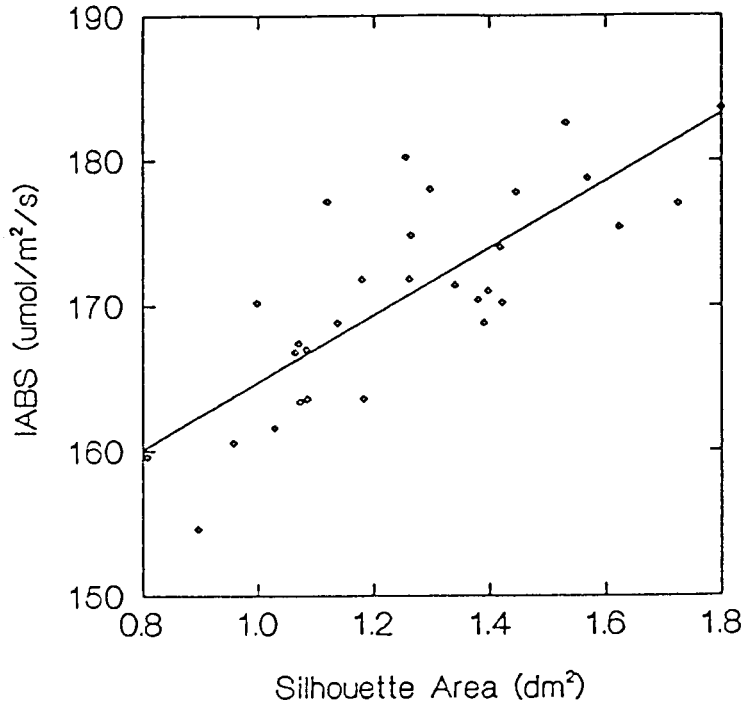


Figure 6.2
Scatterplot of F_O against silhouette area for analyses 1 (7/07/93) and 2 (7/21/93).

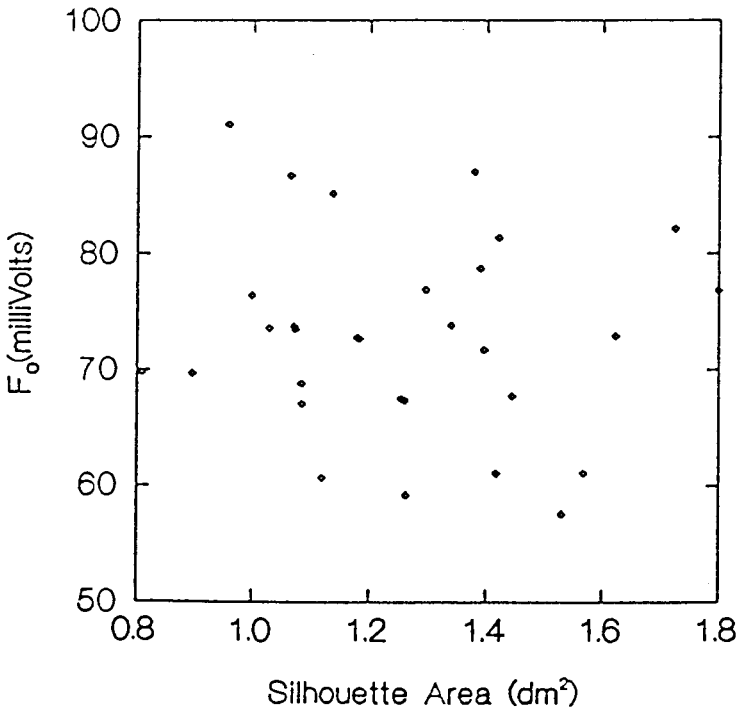


Table 6.2
Nitrogen concentration and fluorescence.
Pearson correlation coefficients for each treatment group (all analyses; n=95) and for each analysis (all treatments; n=15).

GROUP	FO vs Nitro	Fvar vs Nitro	FO/IABS vs Nitro	Fmax vs Nitro	Ft vs Nitro
H	-0.632	-0.512	-0.554	-0.571	0.103
L	-0.534	-0.563	-0.525	-0.561	-0.357
S	-0.395	-0.405	-0.359	-0.406	-0.272
7/07/93	-0.204	-0.048	-0.16	-0.081	0.441
7/21	-0.73	-0.617	-0.739	-0.668	0.231
7/28	-0.454	-0.541	-0.352	-0.548	0.351
8/11	-0.813	-0.86	-0.876	-0.882	0.715
8/25	-0.5	-0.63	-0.142	-0.611	0.863
9/08	-0.894	-0.677	-0.897	-0.759	0.862
9/22	-0.536	0.1	-0.505	-0.149	0.928
10/06	-0.804	-0.472	-0.85	-0.656	0.906
10/20	-0.585	0.351	-0.555	-0.089	0.867
11/03	-0.648	-0.011	-0.719	-0.331	0.815
11/17	0.11	0.578	-0.051	0.348	0.886
1/19/94	-0.328	-0.249	-0.443	-0.343	0.845
3/09	0.01	-0.036	-0.014	-0.027	0.569
3/30	-0.604	-0.537	-0.685	-0.606	0.763
4/13	0.105	0.153	0.063	0.128	0.789
5/04	0.033	-0.307	-0.059	-0.21	0.575
5/11	-0.205	-0.322	-0.376	-0.353	0.45
5/25	0.305	-0.295	0.217	-0.126	0.802
6/08	-0.063	-0.377	-0.171	-0.33	0.743

6.2 Nitrogen Concentration: Correlations with Physiological Activity

Many fluorescence parameters were well correlated with needle nitrogen concentration. F_O, Fvar, Fmax and F_O/I_{ABS} all tended to show a strong negative correlation with nitrogen concentration (Table 6.2). At most analysis dates, these parameters were lowest for the H treatment. In contrast, F_T generally showed a strong positive correlation with nitrogen concentration (Table 6.2).

These results indicated that F_T, rather than any of the other various fluorescence parameters, was perhaps the best indicator of the plants' photosynthetic capabilities. The H plants were visibly larger and darker green, and contained higher concentrations of nitrogen (Table 5.1). Their greater size and presumably higher chlorophyll content were not reflected by higher F_O values, contrary to expectations (Vidaver *et al.* 1989), nor did the fluorescence induction responses (Fmax and Fvar) reflect the amount of active chlorophyll. Instead, variable fluorescence was indicative of the plants' physiological status and their photosynthetic unit (PSU) sizes (see Chapter 5).

Of all the fluorescence parameters, only F_T was significantly higher in the H plants (Figure 5.1a-s, Table 5.9). As a result, the possibility of a correlation between F_T and photosynthetic (APS) rates was investigated. This relationship is discussed in Section 6.3.

6.3 Carbon Dioxide Exchange Rates: Correlations with Chlorophyll Fluorescence

Hipkins and Baker (1986) reported a relationship between F_T and CO_2 assimilation rates. Vidaver *et al.* (1989, 1991) also reported correlations between fluorescence and APS rates (but not specifically F_T). Since F_T is representative of chlorophyll fluorescence at the time of steady-state photosynthesis, it came as no surprise that F_T and apparent photosynthesis (APS) rates were correlated (Table 6.3). In fact, fluorescence quenching (the decline from P to F_T) is indicative of potential photosynthetic activity. The ratio of fluorescence quenching to steady-state fluorescence has been used as a measure of that potential (Lichtenthaler and Rinderle 1988). In this case, however, that ratio was not useful. It was roughly inversely proportional to F_T. Whenever F_T approached zero, the ratio was drastically overinflated. Also, because Fvar was not always measured at P for the H samples, the calculation of fluorescence quenching (Fvar-F_T) was not always accurate.

Correlations by analysis date between F_T and APS were strongest when gas exchange was measured under saturating light (800 $\mu mol/m^2/s$). They were somewhat weaker at the two lower light levels. Correlations for each treatment were very high at all three light levels (Table 6.3).

The ratio of fluorescence decrease to steady-state fluorescence was not correlated with APS (Table 6.3). In contrast, F_T was sometimes positively correlated with APS (when considering all samples on each analysis date). However, this

relationship was lost at certain points in the season, particularly when young (newly flushed) tissue was involved (Table 6.3). At those times, APS was artificially lowered by high respiration rates. The correlations were slightly better when the APS rates were corrected for dark respiration (RD; see PSRD in Table 6.3).

RD rates were also strongly correlated with F_T, but only on some analysis dates (Table 6.3). Correlations were strongest in the autumn and spring, and weakest in the summer of 1993. This inconsistency suggested that the relationship between F_T and RD was coincidental, and was due to the connection between APS and RD rates (Table 6.3) rather than any physiological connection between F_T and RD.

High correlations between F_T and APS or PSRD were also apparent for each treatment across all analysis dates (see groups H, L and S in Table 6.3). The correlations were better for each treatment than for all treatments combined. This may be due to the variation in factors impacting APS rates (such as respiration); variation was lower within treatments than between treatments at any given time. For instance, a large reduction of APS rates in the H samples caused some of the correlations to weaken in the spring of 1994 (Table 6.3). Since this reduction was not mirrored in the L and S samples, the APS rates of the H samples became the lowest, and the correlation coefficients became negative. This imbalance was not reflected in the H correlation coefficient, which considered all analyses together and did not include the other treatments.

Figure 6.3a-c illustrates the similarity between seasonal patterns of F_T and APS rates. The similarities between seasonal patterns in APS rates and F_T are quite apparent.

Other fluorescence parameters were not well correlated with CO_2 exchange rates (Table 6.4). Although Fvar/Fmax was reputedly correlated with spring recovery of APS in another case (Lundmark *et al.* 1988), no such pattern was apparent in this study. Nor were F_O, Fvar, Fmax or F_O/I_{ABS} consistently correlated with CO_2 exchange rates (Table 6.4).

6.4 Chlorophyll Fluorescence: Correlations with Quantum Requirement

There was a weak but moderately consistent negative correlation between F_T and quantum requirement (QR) throughout the study period (Table 6.5). This was accompanied by weak negative correlations between QR and F_O, Fvar and F_O/I_{ABS}.

The negative correlation between QR and F_T (Table 6.5) is perhaps best explained by the connections between QR and APS rates. Plants with high QR had shallow slopes of APS against light intensity. As a result, they had lower APS rates at subsaturating light intensities than did plants with low QR. Since F_T was indicative of the plants' photochemical capabilities at the illumination level in the sphere (200 $\mu mol/m^2/s$), then it follows that F_T was lower for plants with high QR.

The existence of correlations between QR and F_O or F_O/I_{ABS} (Table 6.5) supported the suggestion that F_O may have been affected by photochemical activity, though they may have been coincidental. Although F_O declined throughout 1993 (Figure 4.5) whereas QR increased (Figure 4.12), both displayed lower values in the H treatment than in the L and S treatments. This seasonal pattern suggested that F_O (as measured with the integrating fluorometer) was not entirely independent of photochemical activity.

Table 6.3
Steady-state fluorescence (F_T) and CO_2 exchange rates.
Pearson correlation coefficients for each treatment group (all analyses; n=85) and for each analysis (all treatments; n=15).

GROUP	FT vs PSRD	FT vs PS(800)	FT vs PS(480)	FT vs PS(200)	FT vs RD	PS(800) vs RD	(Fv-FT)/FT vs APS
H	0.535	0.678	0.688	0.693	0.468	-0.207	-0.204
L	0.644	0.675	0.660	0.684	-0.065	0.195	0.036
S	0.541	0.582	0.562	0.580	-0.057	0.331	0.005
7/07/93	-0.27	-0.211	0.104	0.368	0.344	0.192	0
7/21	0.195	0.204	0.002	-0.119	-0.042	0.454	-0.216
7/28	-0.08	-0.09	-0.073	0.117	0.006	0.557	0.223
8/11	0.173	0.136	-0.082	-0.219	-0.128	-0.104	-0.033
8/25	0.687	0.714	0.574	0.447	-0.319	0.612	-0.573
9/08	0.244	0.12	-0.024	-0.328	-0.529	0.489	-0.236
9/22	0.828	0.767	0.565	0.120	-0.564	0.348	0.203
10/06	0.545	0.549	0.371	0.087	-0.443	0.841	-0.434
10/20	0.779	0.765	0.870	0.769	-0.706	-0.774	-0.132
11/03	0.498	0.521	0.327	0.085	-0.158	0.507	0.543
3/09	0.295	0.267	0.427	0.415	-0.306	0.724	0.379
3/30	0.435	0.157	-0.267	-0.477	-0.66	0.248	-0.215
4/13	0.67	0.599	0.550	0.347	-0.481	0.306	0.311
5/04	0.618	0.583	0.410	0.117	-0.357	0.01	0.3
5/11	-0.277	-0.455	-0.501	-0.557	-0.502	-0.631	0.67
5/25	0.271	-0.154	-0.452	-0.566	-0.642	-0.137	0.124
6/08	0.649	0.41	-0.049	-0.399	-0.461	-0.126	0.03

Table 6.4
CO_2 exchange rates and fluorescence.
Pearson correlation coefficients for each treatment group (all analyses; n=85) and for each analysis (all treatments; n=15).

GROUP	FO vs APS	FO vs RD	Fv/Fm vs APS	Fv/Fm vs RD	Fvar vs APS	Fvar vs RD	FO/IABS vs APS	FO/IABS vs RD	Fmax vs APS	Fmax vs RD
H	0.165	0.299	0.465	0.183	0.293	0.387	0.177	0.343	0.266	0.383
L	0.543	0.062	0.534	-0.14	0.57	0.025	0.573	0.036	0.584	0.029
S	0.372	0.16	0.238	0.017	0.406	0.082	0.417	0.147	0.423	0.103
7/07/93	0.076	0.726	-0.17	0.433	-0.02	0.263	0.179	0.763	0.029	0.709
7/21	-0.09	0.508	-0.01	0.237	-0.05	0.51	0.093	0.429	-0.02	0.512
7/28	-0.05	0.348	0.026	0.13	0.172	0.079	-0.001	0.243	0.154	0.124
8/11	-0.26	0.176	-0.16	0.048	0.066	0.274	-0.014	0.163	0.051	0.256
8/25	-0.2	0.362	-0.48	0.57	-0.31	0.458	-0.088	0.144	-0.28	0.432
9/08	-0.37	0.579	0.101	-0.65	-0.35	0.477	-0.238	0.587	-0.33	0.523
9/22	0.053	0.459	0.584	-0.4	0.522	-0.04	0.099	0.408	0.389	0.144
10/06	-0.6	0.454	0.363	-0.36	-0.23	0.138	-0.602	0.515	-0.4	0.296
10/20	-0.19	0.459	0.654	-0.74	0.491	-0.38	-0.125	0.42	0.232	0
11/03	0.121	0.071	0.379	-0.17	0.322	-0.21	0.226	-0.025	0.327	-0.16
3/09/94	0.741	-0.32	0.766	-0.48	0.717	-0.29	0.701	-0.293	0.715	-0.29
3/30	0.33	0.505	0.123	-0.03	0.206	0.445	0.465	0.415	0.306	0.446
4/13	0.631	0.216	0.774	-0.1	0.755	0.127	0.627	0.196	0.731	0.153
5/04	0.726	-0.1	0.054	0.129	0.475	0.095	0.644	-0.044	0.557	0.039
5/11	0.251	0.164	0.032	0.121	0.337	0.246	0.417	0.268	0.379	0.262
5/25	0.484	-0.47	0.409	0.651	0.668	0.26	0.505	-0.394	0.664	0.034
6/08	0.343	0.266	0.318	0.545	0.259	0.441	0.325	0.332	0.287	0.424

Figure 6.3a-c
Average light-saturated (800 µmol/m²/s) photosynthesis (APS; $mgCO_2/dm^2/hour$) and steady-state fluorescence (F_T; relative units) for each treatment on each analysis date (n=5).

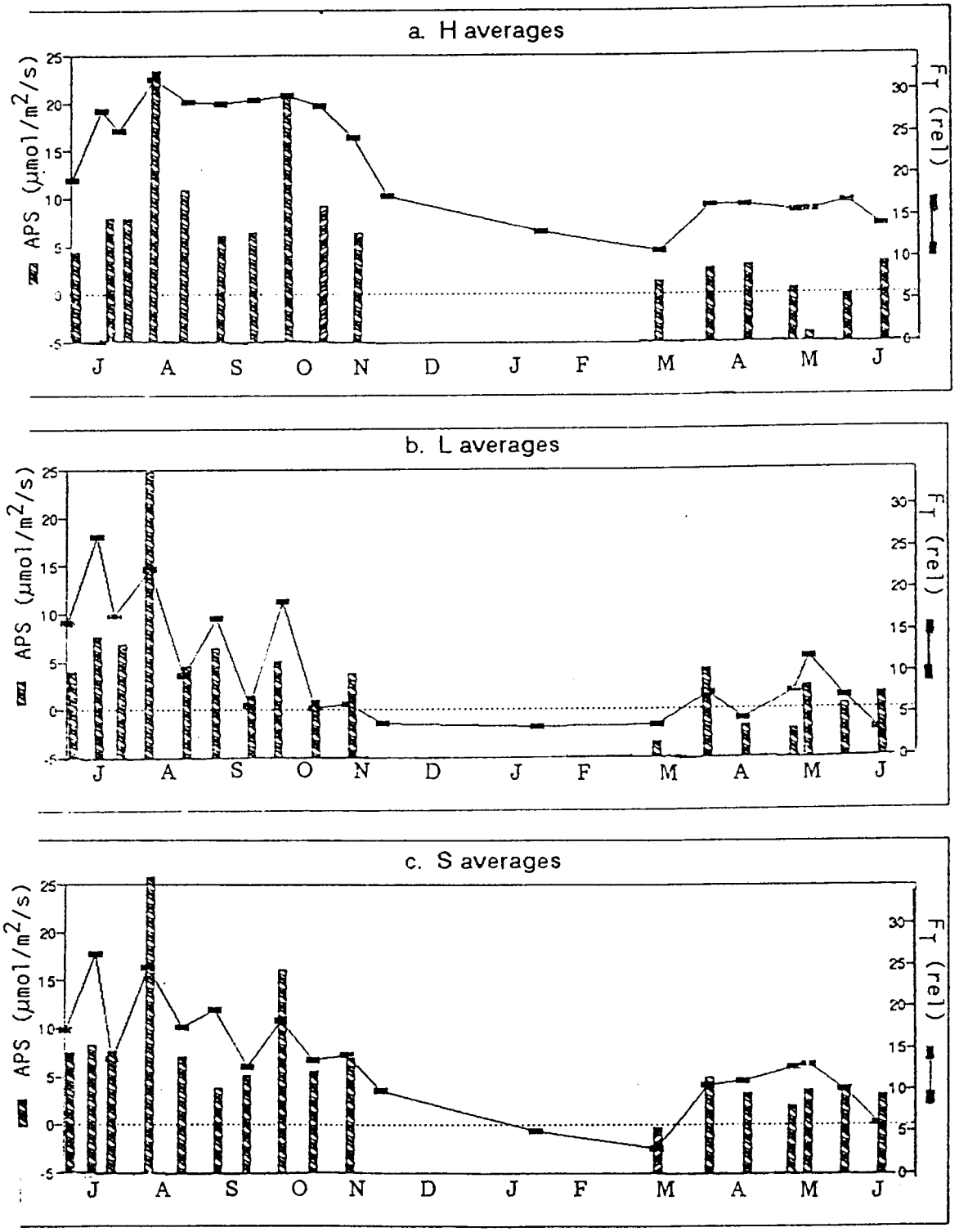


Table 6.5
Quantum requirement and fluorescence.
Pearson correlation coefficients for each treatment group (all analyses; n=85) and for each analysis (all treatments; n=15).

GROUP	FO vs QR	FO/LABS vs QR	FT vs QR	Fvar vs QR
H	-0.046	-0.099	-0.416	-0.213
L	-0.465	-0.464	-0.414	-0.444
S	-0.279	-0.253	-0.406	-0.251
7/07/93	-0.034	-0.024	0.413	0.211
7/21	0.587	0.599	-0.110	0.455
7/28	-0.222	-0.265	0.263	-0.541
8/11	0.148	0.182	-0.064	0.095
8/25	0.551	-0.011	-0.610	0.458
9/08	0.308	0.296	-0.321	0.215
9/22	0.229	0.244	-0.803	-0.299
10/06	0.431	0.466	-0.418	-0.156
10/20	0.123	0.111	-0.833	-0.485
11/03	-0.235	-0.208	-0.477	-0.453
3/09	-0.220	-0.153	-0.384	-0.146
3/30	-0.150	-0.094	-0.307	-0.108
4/13	-0.191	-0.142	-0.633	-0.332
5/04	-0.327	-0.281	-0.554	-0.094
5/11	-0.128	-0.295	0.266	-0.232
5/25	-0.149	-0.114	0.005	-0.114
6/08	0.020	0.065	-0.493	0.193

CHAPTER 7
CONCLUSIONS

This study illustrated the seasonal patterns in physiological activity which typified the seedlings under study. Seasonal variations were apparent in every physiological parameter measured. Foliar nitrogen and carbon concentrations trended towards high values in winter and low values in summer. This was indicative of the plants' greater nutritional demands during times of high metabolic activity, and the transport of newly fixed carbon out of the rapidly photosynthesizing tissue. Chlorophyll fluorescence parameters (including F_O, Fvar, and F_T) were highest in summer and lowest in winter. None of these parameters were independent of photochemical activity, although F_O should have been, by definition. Seasonal variation in F_O may have been due to maturation of the tissue and formation of accessory pigments which protected against photon damage. F_T was a particularly good indicator of the physiological activity of the tissue, as illustrated by its correlation with photosynthetic (APS) rates. APS and dark respiration (RD) rates were also highest in summer and lowest in winter, as one would expect. Quantum requirement (QR) was highest in winter.

Differences between treatments were observed for many parameters. H plants contained significantly higher concentrations of foliar nitrogen than did L and S plants. This led to higher RD rates and F_T values for the H samples, along with notably lower

F_O, Fvar and QR. S plants tended to be intermediate. In 1994, all three treatments differed significantly in shoot length, collar diameter and flushing times. H plants grew more and flushed earlier on average than L plants, while S plants were intermediate. APS rates tended to be higher for H and S samples than for L samples.

The higher RD rates of H samples indicated greater metabolic activity, and their higher F_T values suggested more photochemical activity and better overall health. Their low Fvar values were attributed to the non-saturation of electron transport apparatus in the chloroplasts during fluorescence induction. Their lower QR indicated higher photochemical conversion efficiency (a steeper slope of apparent photosynthesis against light intensity). While APS rates of H and S samples were usually fairly similar on a silhouette area basis, the actual rates per plant were higher for the (larger) H samples. The low APS rates of L samples were likely due to the poor health of the photosynthetic tissue (which was visually quite evident).

These findings have some implications towards future physiological research, particularly in the area of chlorophyll fluorescence. Researchers should not use F_O (as defined by the integrating fluorometer system) as a normalization for plant size without first checking its validity for the plant material under study. In the meantime, the non-photochemical parameter I_{ABS} is recommended for use in normalization formulae.

The results of this study also have implications towards forestry and silvicultural practices. The nitrogen-deficient plants benefitted from the presence of soopolallie, as indicated by physiological and growth responses. Since winter dieback was lessened, their chances of survival were probably enhanced. The presence of

nitrogen-fixing shrubs on extremely low nutrient sites is therefore likely to improve the chances of successful establishment of young conifers. Their presence should be encouraged, and brush control measures should be selective or should not be taken at all.

REFERENCES

Alm, D., J.W. Ycas, and J.D. Hesketh. 1989.
Variation in the photosynthetically active component of leaf organic nitrogen in *Amaranthus retroflexus* (L.). Photosynthetica **23**: 154-165.

Andreeva, T.F., T.A. Avdeeva, M.P. Vlasova, Nguen-Tchyu-Tchyok, and A.A. Nichiporovich. 1971.
Vliyanie azotnogo pitaniya rastenii na strukturu i funktsiu fotosinteticheskogo apparata. [Effect of nitrogen nutrition of plants on the structure and function of the photosynthetic apparatus.] Fiziol. Rast. **18**: 701-707.

Bigg, W.L. and J.W. Schalau. 1990.
Mineral nutrition and the target seedling. In: R. Rose, S.J. Campbell, and T.D. Landis (Eds.), Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations, August 13-17, 1990, Roseburg, Oregon. USDA Forest Service Gen. Tech. Rep. **RM-200**: 139-160.

Bilger, W. and U. Schreiber. 1986.
Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. Photosynth. Res. **10**: 303-308.

Boardman, N.K. 1977.
Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. **28**: 355-377.

Brix, H. 1981.
Effects of nitrogen fertilizer source and application rates on foliar nitrogen concentration, photosynthesis, and growth of Douglas-fir. Can J. For. Res. **11**: 775-780.

Brix, H. and L.F. Ebell. 1969.
Effects of nitrogen fertilization on growth, leaf area and photosynthesis rate in Douglas-fir. For. Sci. **15**: 189.

Brix, H. and R. van den Dreissche. 1974.
Mineral nutrition of container-grown tree seedlings. In: R.W. Tinus, W.I. Stein, and W.E. Balmer, Eds., Proc. North Am. Containerized Forest Tree Seedling Symp., Publ. no. 68. Great Plains Agricultural Council: 77.

Brooke, R.C. 1994.
Pers. Comm.

Camm, E. 1993.
Photosynthetic responses in developing and year-old Douglas-fir needles during new shoot development. Trees **8**: 61-66.

Carter, G.A. and W.K., Smith. 1985.
Influence of shoot structure on light interception and photosynthesis in conifers. Plant Physiol. **79**: 1038-1043.

Chapin, F.S. and K. Van Cleve. 1989.
Approaches to studying nutrient uptake, use and loss in plants. Chapman and Hall, London: 185-207.

Coutts, M.P. and J.J. Philipson. 1980.
Mineral nutrition and tree root growth. In: D. Atkinson, J.E. Jackson, R.O. Sharples, and W.M. Weller, Eds., Mineral Nutrition of Fruit Trees. Butterworths, London: 123.

da Silva, J.M. and M.C. Arrabaca. 1992.
Characteristics of fluorescence emission by leaves of nitrogen starved *Papsalum dilatatum* Poir. Photosynthetica **26**: 253-256.

Devore, J.L. 1991.
Probability and Statistics for Engineering and the Sciences. Third Edition. Brooks/Cole, USA.

Donald, D.G.M. 1991.
Nursery fertilization of conifer planting stock. In: R. van den Dreissche (Ed.), Mineral Nutrition of Conifer Seedlings. CRC Press, Inc., Florida: 135-167.

Dube, S.L. 1990.
Apparatuses and methods for enhancement and detection of photosynthetic competence in tissue cultured plantlets. Doctoral Thesis, Simon Fraser University, Burnaby, British Columbia.

Duysens, L.N.M. and H.E. Sweers. 1963.
Mechanisms of two photochemical reactions in algae as studied by means of fluorescence. In: E. Ashida (Ed.), Studies on Microalgae and Photosynthetic Bacteria. University of Tokyo Press, Tokyo.

Dykstra, G.F. and G.E. Gatherum. 1967.
Physiological variation of Scotch pine seedlings in relation to provenance and nitrogen. Iowa State J. Sci. **41**: 487.

Falkowski, P.G., R. Greene, and Z. Kolber. 1994.
Light utilization and photoinhibition of photosynthesis in marine phytoplankton. In: N.R. Baker and J.R. Bowyer, eds., Photoinhibition of Photosynthesis. BIOS Scientific Publishers Ltd., Oxford: 407-427.

Field, C. 1983.
Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the carbon allocation program. Oecologia (Berl.) **56**: 341-347.

Franklin, L.A., G. Levavasseur, C.B. Osmond, N.J. Henley, and J. Ranus. 1992.
Two components of onset and recovery during photoinhibition of *Ulva rotundata* Blid. Planta **186**: 399-408.

Greene, R.M., R.J. Geider, and G.R. Falkowski. 1991.
Effect of iron limitation on photosynthesis in a marine diatom. Limnol. Oceanogr. **36**: 1772-1782.

Greene, R.M., R.J. Geider, Z. Kolber, and P.G. Falkowski. 1992.
Iron-induced changes in light harvesting and photochemical energy conversion processes in eucaryotic marine algae. Plant Physiol. **100**: 565-575.

Gregory, R.P.F. 1989.
Biochemistry of Photosynthesis. Third Edition. John Wiley and Sons Ltd., Great Britain.

Gulmon, S.L. and C.C. Chu. 1981.
The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry matter allocation in the chaparral shrub, *Diplacus aurantiacus*. Oecologia (Berl.) **49**: 207-212.

Hak, R. and L. Natr. 1987a.

Effect of nitrogen starvation and recovery on gas exchange characteristics of young barley leaves. Photosynthetica **21**: 9-14.

Hak, R. and L. Natr. 1987b.

Effect of nitrogen starvation and recovery on carbon fluxes in photosynthetic carbon reduction and oxidation cycles in young barley leaves. Photosynthetica **21**: 15-22.

Hak, R. and L. Natr. 1988.

Photosynthesis, photorespiration and CO_2 photocompensation concentration of barley leaves under nitrogen starvation. Photosynthetica **22**: 335-340.

Haynes, R.J. 1986.

Uptake and use of nitrogen by plants. In: R.J. Haynes (Ed.), Mineral Nitrogen in the Plant-Soil System. Academic Press, Florida: 303-378.

Hipkins, M.F. and N.R. Baker. 1986.

Photosynthetic Energy Transduction. IRL Press, Washington, D.C.: 51-100.

Ingestad, T. 1982.

Relative addition rate and external concentration; driving variables used in plant nutrition research. Plant, Cell and Environ.: 443-453.

Ingestad, T. and A.B. Lund. 1979.

Nitrogen stress in birch seedlings I. Growth technique and growth. Physiol. Plant. **45**: 137-148.

Kawahara, T., K. Hatiya, I. Takeuti, and A. Sato. 1976.
Relationship between respiration rate and nitrogen concentration of trees. Jpn. J. Ecol. **26**: 165.

Kolber, Z., Z. Jonathan, and P. Falkowski. 1988.
Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in Photosystem II. Plant Physiol. **88**: 923-929.

Krause, G.H. 1988.
Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol. Plant. **74**: 566-574.

Krause, G.H. and E. Weis. 1991.
Chlorophyll fluorescence and photosynthesis: the basics. Ann. Rev. Plant Physiol. **136**: 472-479.

Kuriowa, S. 1960.
Ecological and physiological studies on the vegetation of Mt. Skigamore IV. Some physiological functions concerning matter production in young *Abies* trees. Bot. Mag. (Tokyo) **73**: 133.

Lavorel, J. and A.-L. Etienne. 1977.
In vivo chlorophyll fluorescence. *In*: J. Barber (ed.), Primary Processes of Photosynthesis, Elsevier/North-Holland Biomedical Press, 203-268.

Linder, S. 1980.
Chlorophyll as an indicator of nitrogen status of coniferous seedlings. N. Z. J. For. Sci. **10**: 166-175.

Linder, S. and D.A. Rook. 1984.
Effects of mineral nutrition on carbon dioxide exchange and partitioning of carbon in trees. In: G.D. Bowen and E.K.S. Nambiar (Eds.), Nutrition of Plantation Forests. Academic Press, London: 211-236.

Lister, G.R., G. Krotkov, and C.D. Nelson. 1961.
A closed-circuit apparatus with an infra-red CO_2 analyzer and a Geiger tube for continuous measurement of CO_2 exchange in photosynthesis and respiration. Can. J. Bot. **39**: 581-591.

Lister, G.R. 1993.
Pers. Comm.

Lundmark, T., J.-E. Hallgren, and J. Heden. 1988.
Recovery from winter depression of photosynthesis in pine and spruce. Trees **2**: 110-114.

Machlis, L. and J.G. Torrey. 1956.
Plants in Action: A Laboratory Manual of Plant Physiology. W.H. Freeman and Co.

Makino, A., T. Mae, and K. Ohira. 1984.
Effect of nitrogen, phosphorus or potassium on the photosynthesis rate and ribulose-1,5-biphosphate carboxylase content in rice leaves during expansion. Soil Sci. Plant Nutr. **30**: 63-70.

Marek, M. 1984.
The effect of nitrogen nutrition and photon fluence rate on the oxygen dependence of CO_2 compensation concentration and mitochondrial respiration in the light in young barley leaves. Photosynthetica **18**: 43-49.

Marek, M. and R. Frank. 1984.
Effect of nitrogen supply on net photosynthetic rate in barley leaves. Photosynthetica **18**: 219-225.

McCreary, D., Y. Tanaka, and D.P. Lavender. 1978.
Regulation of Douglas-fir seedling growth and hardness by controlling photoperiod. For. Sci. **24**: 142.

Mohammed, G.H., W.D. Binder, and S.L. Gillies. In press.
Chlorophyll fluorescence: A review of its practical forest nursery applications and instrumentation. 70 pp.

Mooney, H.A. and S.L. Gulmon. 1982.
Environmental and biological constraints on leaf structure and function in reference to herbivory. BioSci. **32**: 198-206.

Natr, L. 1970.
Gas exchange of barley leaves as influenced by mineral nutrient deficiency. Scientia Agriculturae Bohemoslovaca **2**: 211-218.

Natr, L. 1972.
Influence of mineral nutrients on photosynthesis of higher plants. Photosynthetica **6**: 80-99.

Natr, L. 1973.
Influence of mineral nutrition on photosynthesis and the use of assimilates. In: J.P. Cooper (Ed.), Photosynthesis and Productivity in Different Environments. Cambridge University Press, London: 537-555.

Papageorgiou, G. 1975.
Chlorophyll fluorescence: An intrinsic probe of photosynthesis. *In*: Govindjee (ed.), Bioenergetics of Photosynthesis, Academic Press, New York: 319-371.

Racette, S. and J.G. Torrey. 1989.
Root nodule initiation in *Gymnostoma* (Causarinaceae) and *Shepherdia* (Eleagnaceae) induced by Frankia strain HFPGpI1. Can. J. Bot. **67**: 2873-879.

Rook, D.A. 1991.
Seedling development and physiology in relation to mineral nutrition. In: R. van den Dreissche (Ed.), Mineral Nutrition of Conifer Seedlings. CRC Press, Inc., Florida: 85-111.

Ryle, G.J.A. and J.D. Hesketh. 1969.
Carbon dioxide uptake in nitrogen-deficient plants. Crop Science **9**: 451-454.

Smith, W.K., A.W. Schoettle, and M. Cui. 1991.
Importance of the method of leaf area measurement to the interpretation of gas exchange of complex shoots. Tree Physiology **8**: 121-127.

Stocking, C.R. and A. Ongun. 1962.
The intracellular distribution of some metallic elements in leaf. Am. J. Bot. **49**: 284-289.

Systat for Windows: Statistics, Version 5 Edition. 1992.
USA, Systat Inc., 750 pp.

Timmer, V.R. 1991.

Interpretation of seedling analysis and visual symptoms. In: R. van den Dreissche (Ed.), Mineral Nutrition of Conifer Seedlings. CRC Press, Inc., Florida: 113-133.

van den Dreissche, R. 1974.

Prediction of mineral nutrient status of trees by foliar analysis. Bot. Rev. **40** (3): 347-394.

van den Dreissche, R. 1991.

Effects of nutrients on stock performance in the forest. In: R. van den Dreissche (Ed.), Mineral Nutrition of Conifer Seedlings. CRC Press, Inc., Florida: 229-260.

Vidaver, W., W. Binder, R.C. Brooke, G.R. Lister, and P.M.A. Toivonen. 1989.

Assessment of photosynthetic activity of nursery grown *Picea glauca* (Moench) Voss seedlings using an integrating fluorometer to monitor variable chlorophyll fluorescence. Can. J. For. Res. **19**: 1478-1482.

Vidaver, W.E., G.R. Lister, R.C. Brooke, and W.D. Binder. 1991.

A manual for the use of variable chlorophyll fluorescence in the assessment of the ecophysiology of conifer seedlings. B.C. Ministry of Forests, Victoria, B.C. FRDA Report 163; ISSN 0835-0752. 60 pp.

Webb, W.L. 1975.

Dynamics of photoassimilated carbon in Douglas fir seedlings. Plant Physiol. **56**: 455-459.