Physiological Ecology of Understorey Trees in Low Impact Silvicultural Systems

by

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Thesis

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Declaration

I hereby declare that this thesis is my own composition and has not been submitted for any other degree or qualification. The work described is my own expect where stated otherwise.

Sophie Bertin, June 2009

To Jean-Marie Bertin, my father

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Abstract

Continuous cover forestry (CCF), an alternative forest management approach to clearfelling, is increasingly being adopted in the UK. It aims at enhancing stand structural diversity and favouring natural regeneration and subsequent seedling growth below the existing canopy of plantation forests. One area of limited knowledge is the critical level of below-canopy light for the growth of naturally regenerating seedlings. In addition, plant growth beneath canopies is influenced by other factors (e.g. herbivory). *Picea sitchensis* (Bong.) Carr. (Sitka spruce) seedlings under canopies have been observed to be severely damaged by *Elatobium abietinum* (Walker) (green spruce aphid) attacks. The combined effects of light availability and insect attack on seedling growth are not well understood, however, this understanding is crucial in order to ensure successful management of regeneration within CCF systems.

A controlled experiment, which mimicked different natural understorey light levels along with repeated artificial aphid infestation, was conducted over two years to look at seedling performance through structural and physiological (chlorophyll fluorescence) measurements. Aphid population assessments showed significant increased population density under shaded conditions. Nevertheless, aphid impacts were mainly localised in extent while the impact of light was the major component that described seedling growth. Light was the primary factor affecting the whole-plant biomass, whereas aphids had only localised effects on the total dry weight of older needles and roots, and on leader extension growth. A significant interaction between light levels and presence/absence of aphid infestation was found for main leader extension growth of the seedlings during the second year of the experiment, with lower values at low light levels under infestation. Plant biomass allocation was affected strongly by light, while aphid presence did not result in significant changes. At the leaf physiology-level, the light environment was found to be the main driving factor affecting photosynthetic response, whilst aphid presence had only a short-term localised effect on photosynthesis.

The impact of light levels and aphid presence on seedling growth were also determined at an experimental field site where plots were located across two light regimes typical of CCF conditions in upland UK coniferous forests. The comparison of the photosynthetic response of the seedlings in both the controlled and field experiment highlighted the importance of considering the temporal heterogeneity of the light environment experienced by understorey seedlings in CCF stands, while aphid and aphid x light interaction effects could not be determined due to very low aphid levels during the year.

Finally, a pre-existing light model was parameterised to predict the understorey light environment required to promote successful seedling growth in CCF stands. Sensitivity and validation analyses were also performed.

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List of Abbreviations and Symbols

A	rETR NLME model parameter
A_N	Projected needle area (mm^2)
A_T	Total needle area per 3-cm shoot section
A_b	Hemi-surface branch area (m^2)
A_l	Hemi-surface leaf area (m^2)
A_s	Hemi-surface stem area (m^2)
A_w	Hemi-surface woody area (m^2)
B	rETR NLME model parameter
BA	Basal area (m^2 ha^{-1})
BL	Light model based on the Beer-Lambert law
BSAI	Branch hemi-surface area index
C	Controlled experiment
C	NPQ NLME model parameter
c	Ratio of branches covered by needles
$\mathrm{C}_{\mathrm{mass}}$	Dry weight of Carbon (mg g^{-1})
CCF	Continuous Cover Forestry
CF	Clumping factor
Chl a/b	Chlorophyll a/b ratio
C/N	Carbon/nitrogen ratio
C+0	Current-year foliage age-class
C+1	One-year old foliage age-class
C+2	Two-year old foliage age-class
D	NPQ NLME model parameter
d	Diameter at breast height (cm)
DFIN	Diffuse non-interceptance
DMF	N,N-dimethylformamide
DW	Dry weight
E_s	Aphid count
EA	Total aphids$\cdot$cm^{-2}
EN	Aphids$\cdot$100 needles^{-1}
F	Field experiment

F_{o}	Minimal fluorescence level from dark-adapted leaves
F_{m}	Maximal fluorescence level from dark-adapted leaves
F'_{m}	Maximal fluorescence level from leaves in light
F'_{q}	Difference in fluorescence between F'_{m} and F_{s} ($F'_{\mathrm{m}} - F_{\mathrm{s}}$)
$F'_{\mathrm{q}}/F'_{\mathrm{m}}$	Effective quantum yield of PSII ($(F'_{\mathrm{m}} - F_{\mathrm{s}})/F'_{\mathrm{m}}$)
F_{s}	Steady state fluorescence level from leaves in light
F_{v}	Variable fluorescence from dark-adapted leaves ($F_{\mathrm{m}} - F_{\mathrm{o}}$)
$F_{\mathrm{v}}/F_{\mathrm{m}}$	Maximum effective quantum yield of PSII ($(F_{\mathrm{m}} - F_{\mathrm{o}})/F_{\mathrm{m}}$)
h_{cr}	Tree height at the base of the live crown (m)
HL	High light treatment
IL	Intermediate light treatment
ISF	Indirect Site Factor
K	Extinction coefficient
L	Leader shoot
L_b	Hemi-surface branch area index ($\mathrm{m^2\,m^{-2}}$)
L_e	Hemi-surface area index of canopy elements cumulated from top of the canopy ($\mathrm{m^2\,m^{-2}}$)
L_l	Hemi-surface leaf area index ($\mathrm{m^2\,m^{-2}}$)
L_t	Hemi-surface plant area index ($\mathrm{m^2\,m^{-2}}$)
L_w	Hemi-surface woody area index ($\mathrm{m^2\,m^{-2}}$)
LAI	Leaf area index
LL	Low light treatment
LME	Linear mixed effect
$\mathrm{N_{mass}}$	Dry weight of Nitrogen ($\mathrm{mg\,g^{-1}}$)
NLME	Non linear mixed effect
NPQ	Non-photochemical quenching
$\mathrm{NPQ_{max}}$	Maximum NPQ
NRS	Northern Research Station
PAI	Plant area index
PPFD	Photosynthetic photon flux density ($\mathrm{\mu mol\,m^{-2}\,s^{-1}}$)
$\mathrm{PPFD_{sat}}$	PPDF level at which NPQ is 90 % of $\mathrm{NPQ_{max}}$
PSII	Photosystem II
RCD	Root collar diameter (mm)
REML	Mixed model approach
RLC	Rapid light response curve
RTM	Radiative transfer model
R/FR	Red/far-red ratio
rETR	Relative electron transport rate
$\mathrm{rETR_{max}}$	Maximum rETR ($\mathrm{\mu mol\,m^{-2}\,s^{-1}}$)
$\mathrm{rPPFD_{sat}}$	PPDF level at which rETR is 90 % of $\mathrm{rETR_{max}}$

S	Side shoot
SD	Stand density (trees ha^{-1})
SLA	Specific leaf area (m^2 g^{-1})
SSAI	Stem hemi-surface area index
W	Wald statistics
WAI	Woody area index
z	Height in the canopy (m)
4-CA	Canopy Conductance-Constrained Carbon Assimilation model
4-CA-RTM	Light sub-model of the 4C-A model
-, A-	Aphid uninfested treatment
+, A+	Aphid infested treatment
α	Woody-to-total area ratio
β	Clumping factor
τ	Transmittance

Chapter 1

Introduction

1.1 Context: Continuous Cover Forestry in Sitka spruce in the UK

Low impact silvicultural systems, an alternative forest management approach to clearfelling, are being increasingly adopted in the UK. These regimes are often termed Continuous Cover Forestry (CCF). Mason et al. (1999) state 'continuous cover is defined as the use of silvicultural systems whereby the forest canopy is maintained at one or more levels without clearfelling'. This silvicultural approach is not innovative and has a long-standing history in continental Europe, where it has been the subject of substantial amounts of applied forest research (e.g. Turckheim, 1999; Schütz, 2001).

However, the experience of CCF in continental Europe is of little relevance for application to UK conditions, because Bristish forestry is characterised by one predominant silvicultural system: clearfelling and replanting (Hart, 1995; Mason and Kerr, 2004; Pommerening and Murphy, 2004). The extensive afforestation programme that started in the 1920's in the UK has lead to a forest resource mainly composed of even-aged single-species coniferous plantations (Hart, 1995; Mason and Quine, 1995; Wilson et al., 1999; Aldhous, 1997). The most important commercial species in the UK is Sitka spruce (*Picea sitchensis* (Bong.) Carr.), an exotic species, which make up nearly 30 % of the total forest area (Anonymous, 2007).

The adoption of CCF systems requires changes in silvicultural and forest management practices, yet there is little experience in the UK in transforming even-aged coniferous plantations to continuous cover management (Mason and

Kerr, 2004). Initial studies have focused on Sitka spruce because of its commercial importance and large scale planting. Further field trials and exploratory experiments are required to develop appropriate silvicultural systems adapted to UK forest conditions. A primary goal for successful implementation of CCF systems is the establishment and growth of natural regeneration (Nixon and Worrell, 1999). However, two factors, light and insect attack, have been reported to limit the success of natural regeneration of Sitka spruce seedlings in even-aged Sitka spruce stands undergoing transformation to CCF in the UK, yet currently there is no guidance on how to tackle these problems.

1.2 Issues: Sitka spruce seedling growth in relation to light and insect attack

An adequate light environment is one of the most important factors controlling seedling survival and growth (Lieffers et al., 1999). To promote the establishment of natural regeneration, forest management practices need to create favourable light conditions. The choice of silvicultural system adopted for transforming even-aged stands to continuous cover is strongly influenced by the light requirement of the targeted species to be regenerated below the existing canopy. To encourage the growth of Sitka spruce seedlings, a species classified as a shade- or intermediate shade- tolerant species (Mason et al., 2004; Leverenz and Jarvis, 1980), current guidance derived from UK field studies (Hale, 2001; Page et al., 2001) and modelling work (Hale et al., 2004) recommends a minimum below-canopy light transmittance of $15 - 20\,\%$. However, because of the limited experience of transforming stands to CCF in the UK, there is a lack of data on light level assessments across stands that have a large variation in spatial and vertical structure and this impedes the formulation of definitive guidance regarding threshold light regimes necessary for Sitka spruce seedling growth.

Furthermore, field observations have suggested that green spruce aphid (*Elatobium abietinum* (Walker)), a sap feeding insect, could further constrain Sitka spruce seedling growth beneath the canopy in CCF systems. It is well established that high *E. abietinum* populations can cause severe defoliation and a reduction in biomass of Sitka spruce seedlings and young trees, impacting stand productivity over the long-term (Straw et al., 1998, 2000, 2002, 2005; Williams et al., 2005). These studies have examined growth performance at the whole-plant level of Sitka spruce seedlings grown under full light conditions. To date, no studies investigating the combined effect of below-canopy light and aphid infestation on the growth of Sitka spruce seedlings have been reported. Work in this direction is required to fully understand CCF seedling response and develop guidance for the successful management of CCF systems in UK conditions.

Therefore there is a need to conduct detailed measurements to understand the interaction between light and aphid upon biomass allocation and growth. Assessments of biomass allocation and growth response allow direct comparison with the existing studies and integrate the effects of light and aphid upon whole-plant growth. In addition, measures of seedling ecophysiology (i.e. chlorophyll fluorescence) offer the potential for rapid assessment of plant response and the opportunity to identify the effects of these factors on light utilisation and carbon capture. Chlorophyll fluorescence assessments enable the direct effects of below-canopy light environment on seedling physiology to be investigated.

1.3 Objectives and hypotheses of Thesis

The primary research question this study addresses is: ‘How is Sitka spruce seedling growth affected by the interactions between light environment and aphid population dynamics?’

The main objective is to investigate the combined effects of light availability and insect attack on seedling growth, by structural and physiological measures, in order to understand the effects upon regeneration in CCF systems. Specific objectives within the study are: 1) To determine the effect of light availability on Sitka spruce seedling growth response. 2) To assess the impact of aphid attack on Sitka spruce seedling growth response.

The study addresses the following hypotheses: 1) Low light environments cause reductions in Sitka spruce seedling growth. 2) Aphid induced defoliation causes reduction in growth increment in the year of attack and in the subsequent year. 3) Combined effects of low light and aphid induced defoliation will result in cumulative reductions in seedling growth.

1.4 Overview of Thesis

The thesis is designed as a series of four chapters, which are intended for publication as scientific papers. This approach implies some overlap and cross-referencing between the chapters but presents each chapter as a stand-alone publication.

Controlled and field experimental conditions, as well as modelling approaches were combined in this study. Here, the contents and purposes of each chapter are outlined.

Chapter 2. Interactive effects of light and green spruce aphid on growth of Sitka spruce seedlings

This chapter describes a controlled experiment designed to explore the effect of light availability and aphid infestation on potted Sitka spruce seedlings over two consecutive years, using shadehouses and artificial aphid infestation. The study focuses on the effect of light and aphid presence on seedlings at the whole-plant level, looking at the response of growth and allocation. It first presents the results of aphid densities and percentage needle loss for a number of set positions on the experimental trees over the course of the experiment. It then investigates the results of the destructive harvest conducted at the end of each growing season, and explores the light and aphid effect on needle and stem mass for each position and foliage age-classes, and on total root mass. Finally, it investigates the effect of light and aphid on allocation and growth.

Chapter 3. Sun - shade acclimated Sitka spruce seedlings: aphid impacts on foliar morphology and photosynthetic performance

This chapter presents the effect of light and aphid presence on the photosynthetic performance of the one-year old shoots of the seedling trees in the controlled experiment introduced in Chapter 2. It describes the chlorophyll fluorescence methodology adopted to measure the photosynthetic response functions to light throughout the experiment, and discusses the likely mechanisms underlying the observed results. The morphology, chlorophyll and nitrogen concentrations of needles from different positions and age-classes at the end of the growing season are also analysed in terms of seedling response to light environment and aphid presence.

Chapter 4. Physiological responses of Sitka spruce seedlings under variable light environments

This chapter explains the need to combine controlled and field experiments to look at the effect of light and aphid on seedling performance. It describes the design of a field forest experiment implemented to assess the growth and physiological responses of naturally regenerated Sitka spruce seedlings under different natural understorey light regimes and exposed to absence/presence of natural aphid outbreaks. The same chlorophyll fluorescence methodology is utilised in the field experiment as in Chapter 3 to allow for comparison of photosynthetic response between the field and controlled seedlings exposed to similar light levels, and the results are discussed comparatively. The study then links the growth and fluorescence results from the seedlings in the field experiment and discusses the differences in responses to the treatments imposed under 'natural' conditions.

Chapter 5. Modelling understorey light for seedling regeneration under Continuous Cover Forestry canopies

This chapter focuses on the prediction of the understorey light environment required to promote successful seedling growth in CCF stands. It uses data collected from the field experiment. It first explores the relationship between transmitted light assessed by hemispherical photographs and stand-level characteristics, and it discusses the potential for stand-level characteristics to act as predictors of transmitted light. It then presents the parameterisation and sensitivity of two radiative transfer models, a simple Beer-Lambert law model and a detailed pre-existing light model, and compares them over a range of selected inputs. Finally, it presents a validation of the two models by comparing modelled transmittance against measured transmittance from the hemispherical photographs, and by evaluating modelled transmittance with respect to observed seedling growth.

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Chapter 2

Interactive effects of light and green spruce aphid on growth of Sitka spruce seedlings

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Abstract

Light availability and infestation by the green spruce aphid (*Elatobium abietinum*) are key factors affecting Sitka spruce (*Picea sitchensis*) seedling growth, but their combined effect on seedling growth is unknown. This interaction needs to be understood to give guidance on regeneration potential in Continuous Cover Forestry (CCF), a system increasingly adopted in the UK as an alternative to clear-felling, and which aims to promote structural diversity and natural regeneration within managed forest stands. A controlled experiment in which light levels (high light: 100 % or low light: 24 %) and aphid infestation (absence/presence) were manipulated, was conducted over two years to look at effects upon seedling growth and biomass allocation. At the whole-plant level, light was the main driving factor influencing total seedling biomass and allocation. By the end of the experiment, seedlings grown under low light conditions produced about 50 % less biomass and allocated relatively more dry weight to above-ground components than seedlings grown under high light conditions. *E. abietinum* populations were significantly larger in low light conditions, with cumulative aphid densities $3-4$ times higher than in high light conditions. Defoliation rates of infested seedlings were directly related to aphid densities. In the second year, infested seedlings in the low light treatment lost, on average, 66 % of their older needles (one-year old and older), whereas seedlings in the high light treatment, which had lower aphid densities, suffered only 20 % defoliation. Leader extension growth of infested seedlings was reduced by $15-17$ % compared with uninfested seedlings under low light conditions, whereas there was no significant reduction ($2-3$ %) in leader extension under high light conditions. Total root dry weight at the end of the second year was significantly affected by aphid infestation, and the reduction ($14-18$ %) was similar in low and high light treatments despite large variation in aphid densities. Biomass allocation in infested trees was similar to that of uninfested trees within each light treatment, indicating that decreases in root biomass were accompanied by similar reductions in the above-ground parts of the seedlings. Results from the study showed that the response of seedlings to *E. abietinum* were dependant on the light environment.

Keywords: allocation, *Elatobium abietinum*, light regime, seedling growth, Sitka spruce

2.1 Introduction

Alternative management systems to clearfelling are being adopted in the UK as a means of increasing structural diversity and natural seedling regeneration of plantation forests (Mason and Kerr, 2004; Mason et al., 2004). These regimes are often termed Continuous Cover Forestry (CCF). Activity of CCF systems involves plantations of *Picea sitchensis* (Bong.) Carr. (Sitka spruce) because with a coverage area of 30 %, Sitka spruce represents the most important economic species in the UK (Anonymous, 2007). The adoption of CCF systems requires changes in silvicultural practices, yet there is little experience in the UK in transforming even-aged stands of coniferous plantations to continuous cover management (Mason and Kerr, 2004). Light is known to be one of the most important factors controlling seedling growth (Lieffers et al., 1999). However, the critical level of below-canopy light for growth of understorey seedling trees in CCF management is uncertain (Malcolm et al., 2001; Hale et al., 2004). Field studies carried out in the UK suggest that a minimum of $15 - 20\,\%$ transmitted light through the canopy should provide enough light to promote Sitka spruce seedling growth (Hale, 2001; Page et al., 2001). Plant growth can also be influenced by other factors such as insect herbivory (Kotanen and Rosenthal, 2000; Löf, 2000). Green spruce aphid (*Elatobium abietinum* (Walker)), a sap feeding insect, is the main defoliator of Sitka spruce in the UK (Carter and Halldórsson, 1998). The aphids feed on one-year old and older needles. High *E. abietinum* populations on Sitka spruce seedlings and young trees in the UK cause severe defoliation and a reduction in plant biomass, with an impact on stand productivity on a long-term scale (Straw et al., 1998a, 2000, 2002, 2005; Williams et al., 2005).

So far, all studies on the impact of *E. abietinum* on Sitka spruce seedlings have concerned growth performance at the whole-plant scale of either planted or potted young trees grown in full light conditions (Straw et al., 1998a, 2000, 2002, 2005; Williams et al., 2005). Therefore, the impact of aphid infestation on Sitka spruce seedlings beneath the canopy in CCF systems is uncertain. Under the conditions present in the understorey of CCF systems in the UK, high rates of defoliation by *E. abietinum* on Sitka spruce seedlings have been observed. Currently, there is no data for an effect on seedling performance, but there is a need to conduct detailed measurements to understand the impact of the interaction between light and aphid infestation on seedling establishment and consequently the potential for regeneration success within CCF systems.

In order to gain a better understanding of the independent and interactive effects of light availability and aphid attack on the performance of Sitka spruce seedlings, a controlled experiment was set up using potted trees in a nursery with light levels typical of those found in CCF systems and different rates of aphid infestation. The study was carried out over two growing season in order that cumulative effects could be understood. To allow direct comparison with previous studies (Straw et al., 1998a, 2000; Straw and Green, 2001; Straw et al., 2002, 2005; Williams et al., 2005), we looked at the integrated effects of light and aphid infestation upon seedling performance through biomass allocation and growth measurements at the whole-plant level. We predicted that the combination of low light and aphid attack would interact to cause poorer Sitka spruce seedling growth than uninfested trees grown in the open.

2.2 Material and methods

2.2.1 *Elatobium abietinum*

E. abietinum is anholocyclic (egg not fertilised) in the UK with apterous (wingless) female aphids maintaining populations by continuous parthenogenetic (asexual) reproduction on spruce throughout the year. Alatae (winged forms of aphids) are produced during the early summer population peak, with dispersal between trees or stands restricted almost entirely to this period. After the population peak, numbers decline and remain low until autumn, largely in response to a reduction in the nutritional quality of the phloem sap (Parry, 1974b, 1976; Day et al., 1998).

The aphids feed year round on the one-year old and older needles, and on the current season's needles in the autumn (Straw et al., 1998b). Chlorosis develops in the needles where the aphids have been feeding, leading to premature needle fall within 3-5 weeks (Parry, 1974a). However, the aphids rarely kill the trees, because of the immunity of the new foliage during the growing season (Jackson and Dixon, 1996; Straw et al., 1998a).

2.2.2 Site and plant material

The experiment was established in an open field at the Forestry Commission's Northern Research Station (NRS) near Edinburgh, in Scotland (55°86′N, 03°20′W). Two-year old bare-rooted Sitka spruce seedlings (QSS 1U1), that had been grown under full light conditions (i.e. 100 %), were obtained from the Forestry Commission Delamere nursery in February 2006. The seedlings were planted up in 4-L flexi-pots using a 2:1:1 peat, bark, perlite growing medium. They were subsequently re-potted in 8-L pots before the start of the experiment in 2007. At the time of potting in 2006 and in 2007, each tree was top-dressed with 20 g of controlled release granular fertilizer (Osmacote® Exact® Standard: $15\%N + 9\%P + 9\%K + 3\%MgO$, Scotts Ltd, UK). A Hemcore BioMat® Mulch was placed over the surface of each pot to minimise weed growth. Seedlings were watered to field capacity via a drip irrigation system.

During the summer months of 2006 and 2007 in Eastern Scotland, maximum temperatures were 18.9 and 16.3 °C and minimum temperatures were 9.6 and 8.7 °C, sunshine were 537.5 and 369.3 hours, and total precipitation was 184.7 and 366.5 mm, respectively (Met Office, 2008).

2.2.3 Experimental design

Four treatments were explored in this study: 1) high light (i.e. in the open, 100 % light) with no aphids (HL-); 2) high light with aphids (HL+); 3) low light (i.e. 24% light) with no aphids (LL-); 4) low light with aphids (LL+).

A split-plot design was used with two light levels as main plots, and two aphid infestation levels as subplots. In 2006, each subplot consisted of nine experimental trees placed at 0.5 m x 0.5 m spacing. Buffer trees, 16 around each subplot, were placed at the same spacing to attenuate the border effect and to help prevent aphids from walking between the infested and uninfested subplots. The study was replicated across four blocks for a total of 144 experimental trees (4 blocks x 2 light treatments x 2 aphid infestation levels x 9 trees per aphid subplot). Light availability was manipulated using shade cloth. Eight shadehouses covering 26 m^2 (1 x 4 x 6.5 m) were constructed on the site. Four of the shadehouses were covered with two different layers of shade net fabrics to simulate the low light treatment, whereas the other four shadehouses were left uncovered, and served as controls (i.e. 100 % light). The bottom sides of the shadehouses were left uncovered up to a height of 30 cm to allow air circulation. The shadehouses were placed 3 m apart to prevent treatment overlap. In April 2006, 400 Sitka spruce seedlings (i.e. 144 experimental trees and 256 buffer trees) were divided equally and randomly amongst the eight shadehouses. In 2007, the study was reduced to three blocks. Five experimental trees were kept in each subplot with 13 buffer trees. All trees were placed equidistant from each other. The height of the six selected shadehouses was raised from 1 to 2 m in April 2007 to ensure that the seedlings had enough space to grow.

The experiment in 2006 was initially set up with six light levels (Table 2.1) and two aphid infestation levels. Aphid densities were monitored through all light levels in the experiment in 2006 (see method below). In the present study only two light levels were explored, HL and LL, that corresponded to the light levels where the lowest and the highest aphid densities were recorded, respectively (Table 2.2 and Fig. 2.1).

Table 2.1. Light environment in the six shadehouses in 2006. Measurements were taken on an overcast day during the summer (see method below).

Light treatment	Shade cloth description	Transmittance (±SD) (%)
A (HL)	0 layer (open)	100
B	1 layer	61 ± 0.8
C	2 identical layers	38 ± 0.9
D (LL)	2 different layers	24 ± 0.7
E	3 identical layers	22 ± 0.9
F	4 identical layers	15 ± 0.5

Table 2.2. Cumulative aphid density (aphids·100 needles^{-1}) on one-year old leader and side shoots of the infested and uninfested trees in the six light treatments in 2006. Mean ±1 SEM shown for the whole population. See Table 2.1 for light treatments.

Light treatment	**Leader shoots**		**Side shoots**	
	Uninfested	Infested	Uninfested	Infested
A (HL)	1.9 ± 1.0	28.4 ± 5.3	1.1 ± 0.3	6.4 ± 1.2
B	0.2 ± 0.1	65.5 ± 9.1	0.3 ± 0.2	14.8 ± 2.6
C	0.2 ± 0.2	73.2 ± 13.3	0.7 ± 0.3	19.9 ± 3.1
D (LL)	0.5 ± 0.3	94.0 ± 9.4	0.3 ± 0.1	29.4 ± 3.4
E	2.8 ± 0.9	78.9 ± 10.0	0.5 ± 0.2	17.3 ± 2.6
F	2.1 ± 1.0	88.7 ± 11.5	1.0 ± 0.3	14.1 ± 1.7

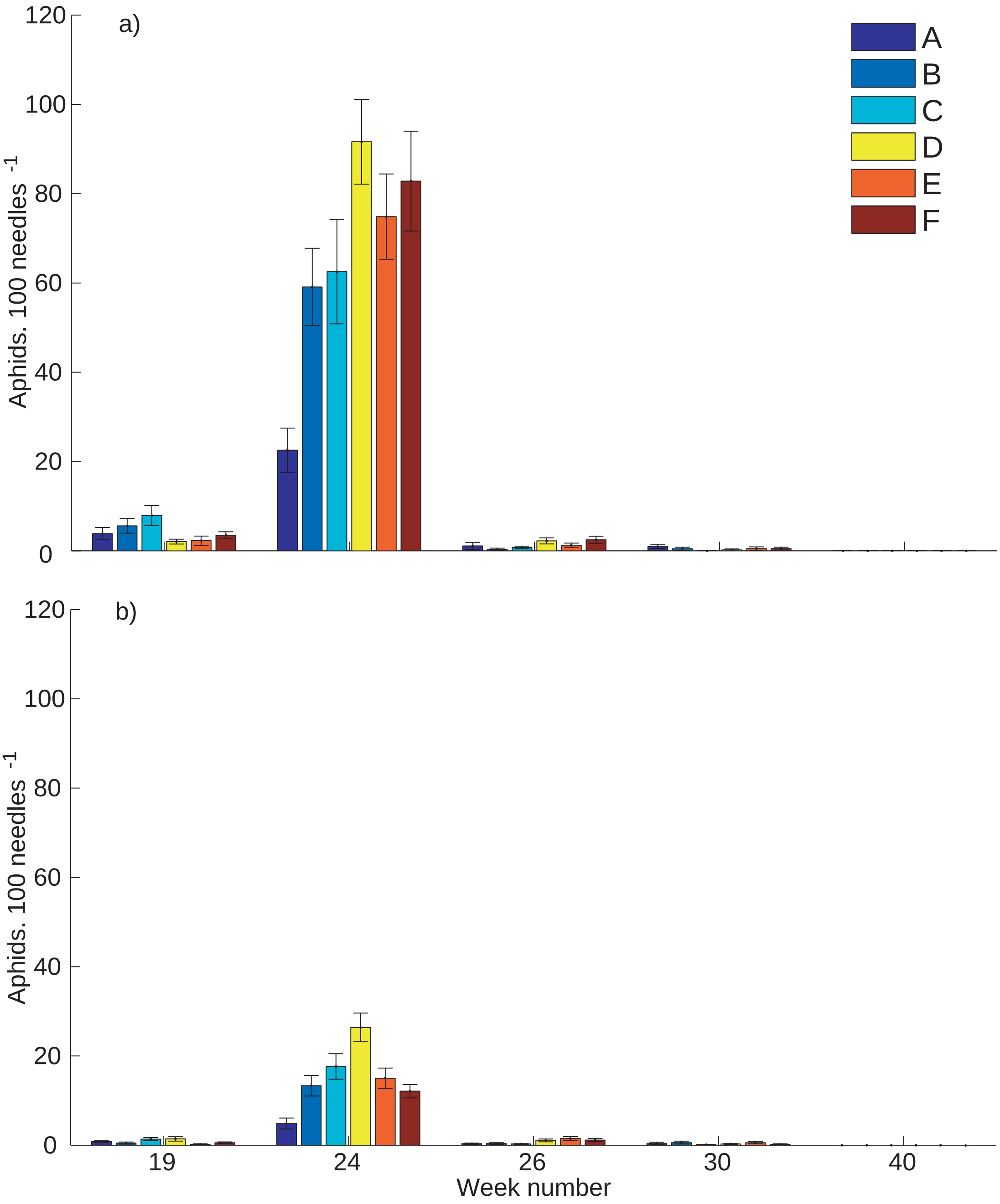

Fig. 2.1. Mean aphid densities (aphids·100 needles^{-1}) on one-year old shoots in the infested trees for the six light treatments for a) leader shoots and b) side shoots in 2006. Mean ±1 SEM shown for the whole population. See Table 2.1 for light treatments.

2.2.4 Micrometeorological measurements

Light environment in the shadehouses was assessed by measuring the percentage transmittance of diffuse photosynthetic photon flux density (PPFD, µmol m^{-2} s^{-1}) on a representative overcast day (15 August 2006 and 2 September 2007). In 2006, PPFD was recorded inside the shadehouse of one block using eight quantum sensors (SD101QV-Cos, Macam Photometrics Ltd, Livingston, Scotland) positioned at plant level. PPFD in the open was measured simultaneously using one Macam quantum sensor. The sensors were connected to a CR10 datalogger (Campbell Scientific, Logan, UT, USA) with measurements taken every 10 seconds throughout the day, stored as 10-min averages.

In 2007, the light environment was assessed using a micrometeorological station. Light readings in open (HL) and shade (LL) conditions were obtained using two matched pairs of quantum sensors (two SD101QV-Cos sensors, Macam Photometrics Ltd, Livingston, Scotland; two SKP215 sensors, Skye Instruments, Llandindrod Wells, UK) placed at plant level and connected to a CR10 datalogger. Light measurements were taken every 30 seconds and stored as 30-min averages. During the 2007 growing season, air temperature and relative humidity measurements were also monitored continuously by placing a CS215 temperature and relative humidity probe (Campbell Scientific, Logan, UT, USA) at plant level within each light treatment in one block. The CS215 sensors were attached to the CR10 datalogger and readings were taken every 30 seconds and stored as 30-min averages.

All quantum sensors were calibrated against a reference quantum sensor (SKP215, Skye Instruments, Llandindrod Wells, UK). The relative average transmittance (the ratio between the PPFD in the open and that measured in the shade) in the LL shadehouses in 2006 and 2007 was $24 \pm 0.7\%$ (±SD) and $24 \pm 0.4\%$, respectively.

Mean daily variation in temperature and relative humidity from June to September 2007 in open and shade conditions are presented in Figure 2.2. During this period, differences in daily mean temperature and relative humidity between open and shade were less than 1 °C and 3.2 %, respectively (Fig. 2.2).

In 2007, spectral quality of the radiation in open and shade conditions were measured in one block with a GER1500 spectroradiometer, where red/far-red ratio (R/FR) was defined as the photon fluence rate between 655 and 665 nm divided by photon fluence rate between 725 and 735 nm (Leuchner et al., 2007). All the measurements were done on 19 September 2007. Under clear blue sky conditions and around solar noon, five consecutive measurements were taken, ~ 1 m above the ground. R/FR was $1.00 \pm 0.0006\%$ (±SD) in HL and $0.98 \pm$

0.003% in LL.

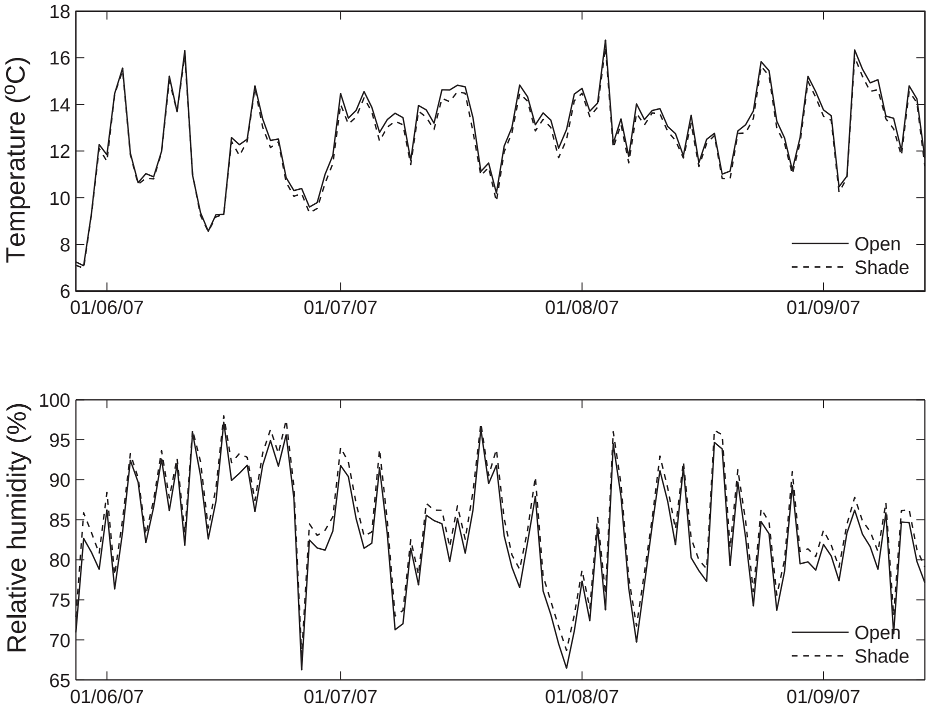

Fig. 2.2. Variation in daily mean temperature and daily mean relative humidity measured in open and shaded cages from 28 May 2007 to 14 September 2007.

2.2.5 Aphid and needle counting

Seedlings in the HL+ and LL+ were artificially infested with aphids on 28 April 2006 and 29 April 2007. *E. abietinum* populations characteristically peak in late spring, soon after bud-burst, when the nutritional quality of the phloem sap is at its highest (Williams et al., 2005). By infesting the seedlings at the end of April, aphid numbers on the trees build up rapidly to high densities and cause high rates of needle loss by the time of the usual population peak in May (Williams et al., 2005).

In 2006, the trees were infested by tying a 5 cm section of infested shoot onto the leader stem of each tree, according to the method used by Williams et al. (2005). In 2007, four 3 cm sections of infested shoot were tied at different positions on each tree, to ensure that all of the foliage was more evenly infested. All added material was removed after one week. Infested material was obtained from stock aphid cultures grown on fresh Sitka spruce branches in a growth chamber at 17 °C, 60% relative humidity and a 16 h light : 8 h dark photoperiod. The initial *E. abietinum* for the stock cultures were collected from Radnor Forest in Wales (autumn 2005), Royal Botanical Gardens, Edinburgh (spring 2006) and NRS (spring 2007).

During the first fortnight of August 2006 and August 2007, after aphid populations had reached their peak and numbers had collapsed, all of the trees were sprayed with pirimicarb (Aphox®) to prevent infestation of the current-year needles, which can be a problem on pot-grown trees in the autumn.

To carry out growth loss measurements, it was important to assess aphid and needle densities using a non-damaging method. Aphids and needles were counted directly at a number of set positions on each experimental tree in order to include all of the foliage where aphids were likely to be present (Fig. 2.3). Counts were made using a 3-cm window cut in a piece of plastic and held against the leader or the underside of a side shoot, as described by Straw et al. (1998a). The number of aphids visible through the 3-cm window and the number of needles originating along one side of the stem defined by the window, were counted. In 2006 and 2007, counts were made at each position on each tree on five occasions: 1) at the time of infestation (May), 2) at the aphid population peak (middle of June), 3) just after the peak (end of June), 4) once during the middle of the summer (end of July, beginning of August) and 5) at the end of the growing season (October). In 2006, aphids and needles were counted at three positions: one count on one-year old (C+1) needles (season 2005) on the leader shoot (L) and, two counts on C+1 needles on two separate side shoots (S) (Fig. 2.3a). In spring 2007, the C+1 needles of the previous year were renamed two-year old needles (C+2) and counts were made at several positions: two counts on the leader shoot on C+1 needles (season 2006) and C+2 needles (season 2005), three counts on C+1 needles on three separate side shoots (one on a shoot from the first whorl from the top of the tree and two on shoots from the second whorl from the top of the tree), and two counts on C+2 needles on two separate side shoots (Fig. 2.3b). Side branches were selected at random with the result that different shoots were chosen at different times. The measurements from the side shoots for the same needle age-class were averaged prior to analysis.

The percentage needle retention was a point estimate, calculated for each count on each tree for each position and foliage age-class by comparing the observed number of needles cm^{-1} at the time of the count with the initial observed number of needles cm^{-1} (i.e. at the beginning of the experiment in 2006 and in 2007).

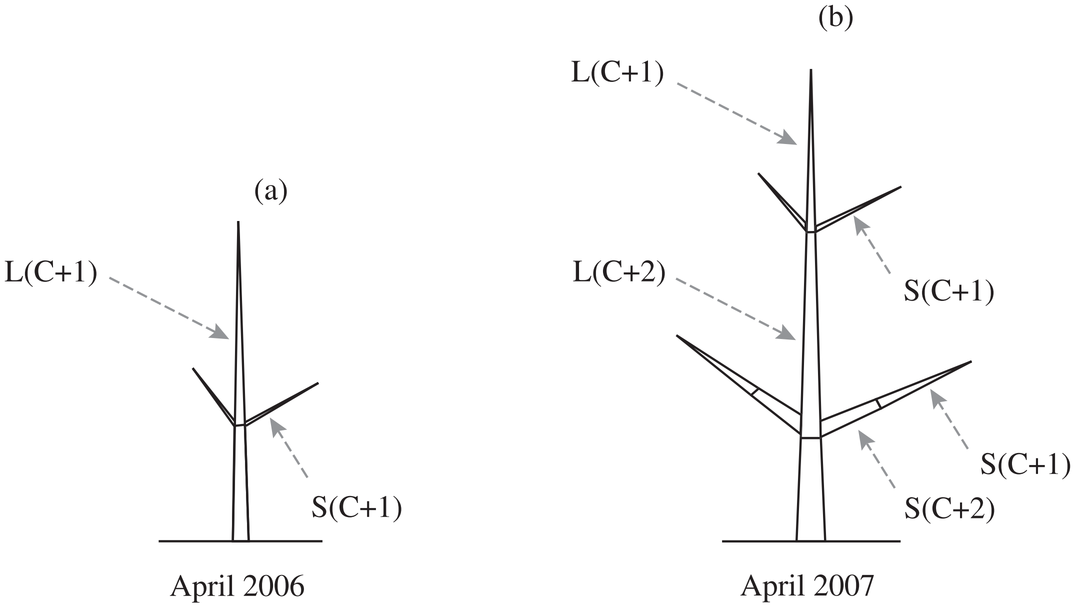

Fig. 2.3. Diagrammatic representation of the different positions and needle-age classes assessed to sample aphid and needle densities on the experimental trees during the growing season in a) 2006 and b) 2007. Sitka spruce seedlings were two-years old at the beginning of the 2006 growing season. L and S = leader and side shoots, respectively. C+1 and C+2 = one- and two-year old foliage age-classes, respectively.

2.2.6 Tree growth measurements

Analysis of stem growth was based on measurements of the trees carried through to the completion of the experiment in 2007 (n=60). Root collar diameter (RCD) on all of the experimental trees used in year 2007 was recorded in April 2006, January 2007, and October 2007 (as the mean of two measurements) and used to calculate stem diameter increment (i.e. annual diameter growth, mm yr^{-1}). Leader extension growth (cm yr^{-1}) in 2006 and 2007 was measured in October 2007.

Forty-eight of the experimental trees (i.e. three trees per subplot) were harvested in February 2007, and a further 48 trees (i.e. four trees per subplot) were harvested in February 2008. The stem was cut at the root-collar and the above-ground biomass was separated by position and age-class (Fig. 2.4). All of the shoot material was dried to constant weight at 80 °C for 48 hours. Afterwards, the shoots were separated into needles and stems. Total dry weight (DW) of needles and stems for each position and foliage age-class, and total root DW were determined.

At the end of the 2006 growing season, for each of the four treatments, one tree was randomly chosen from each block. Sub-samples of sixty current-year (C+0) needles were taken from two positions (leader and side shoots) from each tree and pooled together to determine projected needle area (A_N, mm^2) (Fig. 2.4a). A_N of C+1 needles from leader and side shoots were also determined but only from trees in the uninfested treatments (i.e. HL- and LL-). Pre-existing C+1 needles had fully developed prior to the start of the experiment. Consequently it was assumed that there would be no differences in needle morphological characteristics between infested and uninfested treatments. To obtain needle DW, needles were oven dried at 80 °C for 48 hours and then weighed. Projected leaf area of the needles was measured after oven-drying using ImageJ public domain software (U.S. National Institutes of Health, Bethesda, MD, USA).

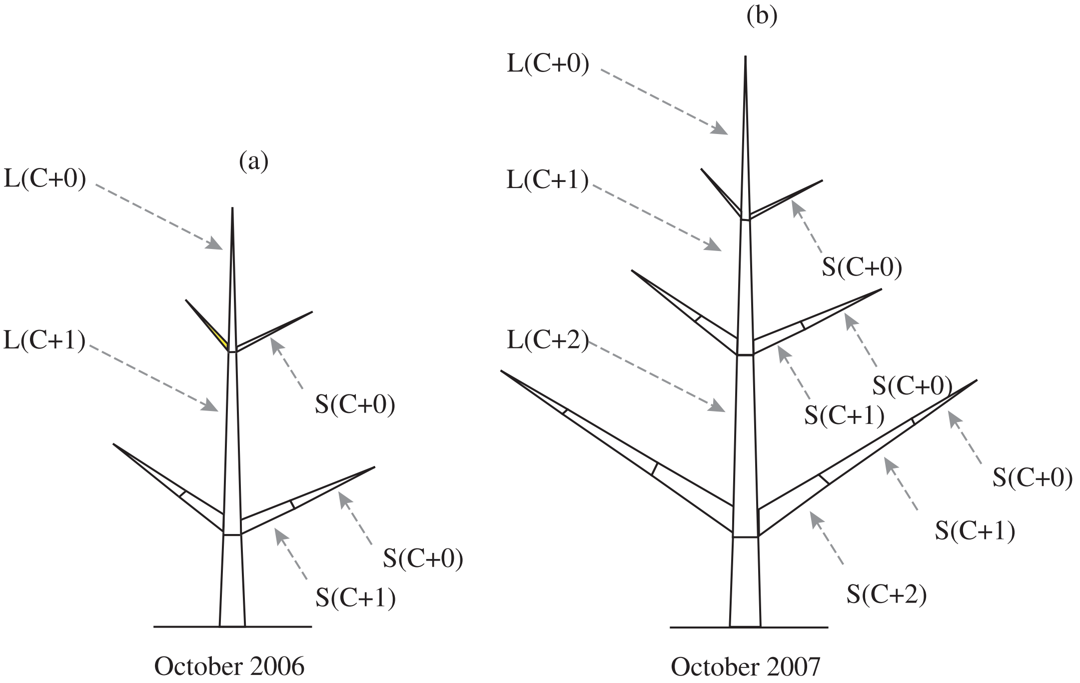

Fig. 2.4. Diagrammatic representation of the different above-ground positions and foliage age-classes harvested at the end of the growing season in a) 2006 and b) 2007. L and S = leader and side shoots, respectively. C+0, C+1 and C+2 = current-, one- and two-year old foliage age-classes, respectively.

2.2.7 Aphids per needle area

Aphid counts (E_S) were converted to estimates of total aphids·cm^{-2} (EA) to allow cross-comparison between the different light treatments. Needle count (N_S) was converted to total needle area per 3-cm shoot section (A_T) assuming the same number of needles along the two sides of the stem and using the following equation:

$$A_T = 2 \cdot N_S \cdot A_N \tag{2.1}$$

where A_N (mm^2) was the projected needle area. This relationship was used to convert aphid count (E_S) to EA, where:

$$EA = E_S / A_T \tag{2.2}$$

In addition, estimates of aphids·100 needles^{-1} (EN) were calculated to allow comparison with previous studies, where:

$$EN = 100 \cdot (E_S/(2 \cdot N_S)) \tag{2.3}$$

When either no aphids or no needles were observed, it was assumed that no aphids were actually present and that $EA = 0$ and $EN = 0$.

2.2.8 Statistical analysis

A mixed model approach (REML) was used to analyse the data with the statistical software Genstat version 8.01 (Genstat, 2005). Data were analysed with a split-plot analysis of variance model. Separate REML analyses of variance were performed for each position and foliage age-class and for each year, with light and aphid levels considered as fixed effects and plots within blocks, subplots within plots and trees within subplots considered as random factors. Tests of significance of main fixed effects (light, aphid) and interactions were based on the Wald statistics (W). In Genstat version 8.01, W is distributed approximately as χ^2. All continuous responses were assessed for normality and transformed where necessary. When data were transformed, back-transformed estimated means are presented. The significance level was set at $P = 0.05$. Means (predicted from the REML model) were compared using the estimated standard error of the differences (SED).

2.3 Results

2.3.1 Aphid densities and needle loss

E. abietinum **populations and percentage needle retention**

E. abietinum densities and percentage needle retention in the different treatments in 2006 and 2007 are presented in Figures 2.5 and 2.6. Artificial infestation of the trees in the infested treatments (LL+ and HL+) was successful and resulted in high aphid densities and appreciable needle loss in both years (Figs. 2.5 and 2.6). A few aphids were found in the uninfested treatments (LL- and HL-) in both years, but aphid densities in these treatments remained very low (Figs. 2.5 and 2.6). The final aphid-needle counts in October 2006 and October 2007 indicated that the majority of the trees had no aphids at this time in any of the four different treatments.

In both years, greater aphid populations occurred on the C+1 needles of the leader shoots than on the side shoots (Table 2.3). Higher aphid densities developed on C+1 needles in LL+ than HL+ in 2006 and 2007 (Table 2.3). However, the development of *E. abietinum* population on C+1 needles showed different patterns in 2006 and 2007 (Fig. 2.5). After inoculation with aphids in April 2006, aphid densities on C+1 needles in the two infested treatments followed the characteristic pattern of *E. abietinum*: a rapid population increase, peak densities in early June (week 24) and population collapse at the end of June (week 26) (Fig. 2.5a,c). In 2007, *E. abietinum* densities did not peak as distinctively on the C+1 needles of the infested seedlings (Fig. 2.5b,d). The aphid populations in LL+ never really collapsed and moderate aphid densities were maintained until insecticide was sprayed at the end of the season. In contrast, aphid densities in HL+ remained low through the whole of the 2007 experiment period on both the leader and side shoots (Fig. 2.5b,d). A similar trend was reported on C+2 needles in 2007 (Fig. 2.6a,b) but with in general higher aphid densities compared with populations found on C+1 needles in 2007 (Fig. 2.5b,d).

In 2006 and 2007, percentage needle retention in the two uninfested treatments followed a closely parallel pattern for both position and foliage age-classes, with needle retention in HL- being slightly lower than in LL- (Figs. 2.5 and 2.6). Percentage needle retention of C+1 needles was greater than 80% in HL- and LL- in 2006 and 2007, apart from needles on side shoots in 2006 which showed a bigger loss (Fig. 2.5). Conversely, percentage needle retention of C+1 needles in the infested treatments in 2006 and 2007 declined sharply after weeks 26–32, especially in the LL+ treatment (Fig. 2.5). A delay of a few weeks between the timing of the aphid populations peak and needle loss was observed. Percentage retention of C+2 needles in 2007 declined linearly and at a similar rate throughout the experiment period in all four treatments. Nevertheless the decline in percentage needle retention of C+2 needles was highest in LL+, closely followed by HL+. The decline in HL- and LL- was greater than in 2006 (Fig. 2.6).

Cumulative aphid densities and cumulative needle loss

Tables 2.3 and 2.4 summarise the total cumulative aphid densities over the growing season and percentage needle loss at the final count made in October (week 40 in 2006, week 42 in 2007) for the different position and foliage age-classes in 2006 and 2007. The percentage needle retention at the end of the growing season (i.e. October count) was used as an integrated measure of overall damage caused by the aphids over the whole of the growing season.

In 2006, cumulative aphid density was significantly different between both light and aphid treatments, together with a light x aphid interaction for C+1 needles on both leader and side shoots (Table 2.3). Aphid populations on leader and side shoots were 3.3 and 4.9 times higher, respectively, in LL+ than HL+ (Table 2.3). Within these treatments, cumulative aphid densities were 1.9 and 2.9 times greater, respectively, on leader than side shoots (Table 2.3).

In 2007, cumulative aphid densities on C+1 needles in the infested treatments were lower than in 2006, especially in HL+ (Table 2.3). Nevertheless, significant light, aphid and light x aphid effects were found in 2007 for C+1 needles on leader shoots, with again the highest populations present in LL+ (13.4-fold greater than in HL+) but smaller differences between HL+ and HL- populations. In C+1 needles on side shoots, only the aphid effect was significant (Table 2.3). However, a trend similar to that on leader shoots was observed. Cumulative aphid densities on C+1 needles in 2007 in LL+ was 1.7-fold greater on leader than side shoots. A similar trend was observed in 2007 on C+2 needles, with higher aphid densities in LL+ than in HL+. The population appeared to be greater than on C+1 needles on both leader and side shoots. C+2 needles in the infested treatments had already experienced one year of infestation by 2007, and needle densities were consequently lower at the start of 2007 season (Fig. 2.6).

Analysis of percentage needle loss by October indicated similar results to

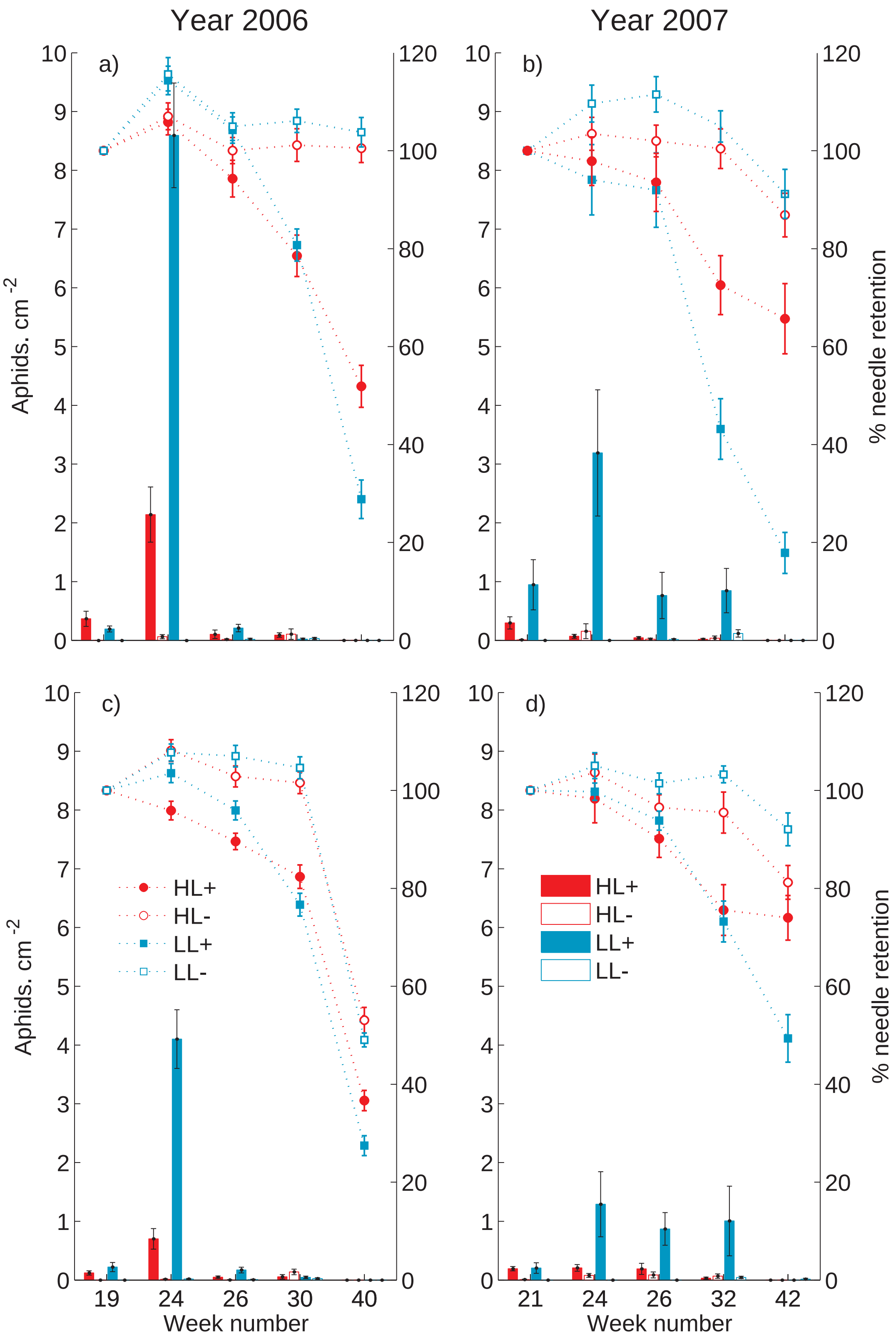

Fig. 2.5. Mean aphid densities (aphids·cm^{-2}; bars) and percentage needle retention (%; dashed lines) on one-year old (C+1) shoots in the four experimental treatments for a) leader shoots in 2006; b) leader shoots in 2007; c) side shoots in 2006; d) side shoots in 2007. Mean ±1 SEM shown for the whole population. See methods for light and aphid treatments.

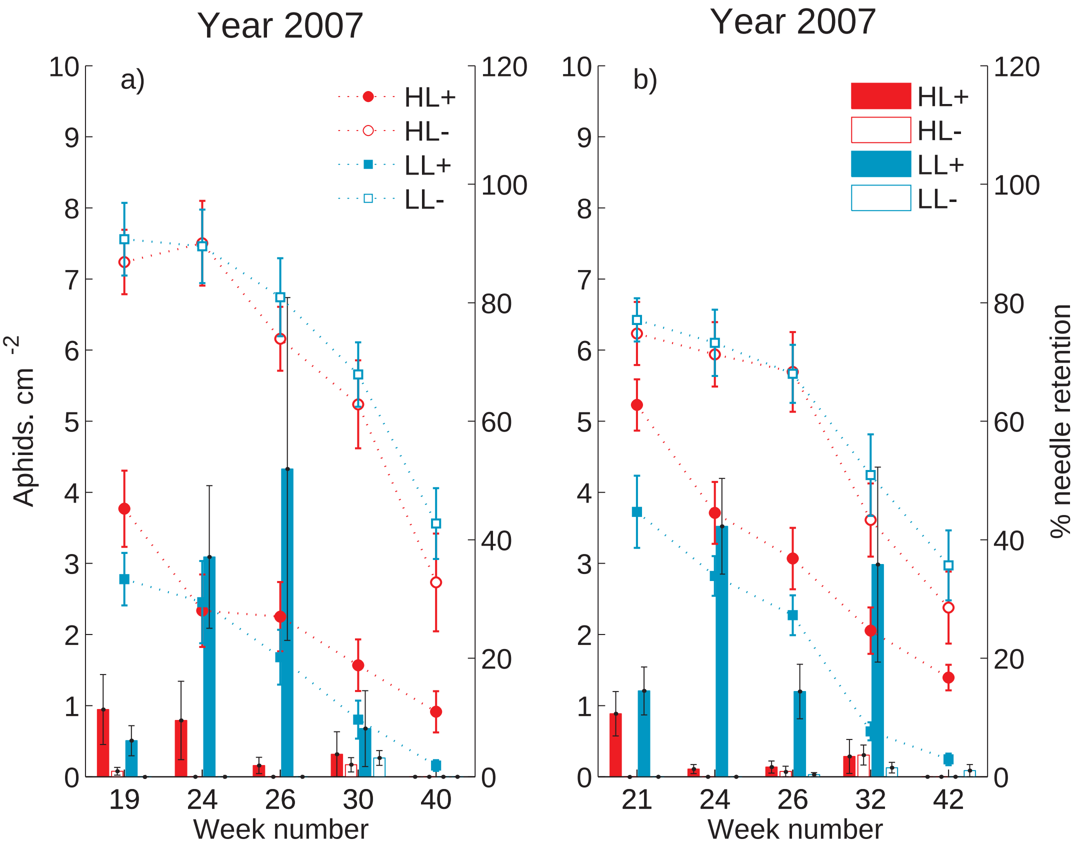

Fig. 2.6. Mean aphid densities (aphids·cm^{-2}; bars) and percentage needle retention (%; dashed lines) on two-year old (C+2) shoots in the four experimental treatments for a) leader shoots and b) side shoots in 2007. See Figure caption 2.5 for details.

those obtained for the cumulative aphid densities (Table 2.4). In 2006 and 2007, there were significant aphid and light x aphid effects at most positions and foliage age-classes. Only C+1 needles on side shoots in 2006 did not show an interaction between light and aphid. In 2006, trees in the high light treatments lost a significantly lower percentage of needles than those in the low light treatments. The highest loss of C+1 needles in 2006 occurred in the LL+ treatment, particularly on leader shoots. While C+1 needles on leader shoots in the uninfested treatments in 2006 did not seem to experience defoliation, uninfested C+1 needles on side shoots dropped by $47 - 51\,\%$ in 2006 (Table 2.4). In 2007, a significant light effect was found only on C+1 needles on leader shoots. The same trend as in 2006 was observed in terms of aphid damage on C+1 needles, with a greater aphid impact on leader than side shoots. The highest needle loss was also found in LL+, but the negative effect of aphid infestation was lower in HL+ compared with 2006. This result can be linked to the lower aphid population found in this treatment in 2007 (Table 2.3, Fig. 2.5). In 2007, percentage losses in C+2 needles were very high in all four treatments, on both leader and side shoots, due to the initially lower needle number at the time of infestation in 2007 compared with 2006. Almost all C+2 needles were lost in LL+, while trees in LL- retained slightly more needles than trees in HL-. Finally, in both years, when the percentage needle loss of infested seedlings was compared on a relative basis to that of undamaged seedlings for each light treatment, the greater needle loss in LL+ compared with HL+ was further emphasised due to the fact that the percent needle retention in HL- was initially lower as compared with LL- (Table 2.4).

Table 2.3. Summary of REML analysis of variance for the effects of light and aphid treatment on the cumulative aphid density at different shoot positions for different needle age-classes in 2006 and 2007. Bold numbers indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) and italic numbers indicate marginal significance (^{ms} $P < 0.1$).

Position (age-class)	**Estimated mean** (cumulative aphids·cm^{-2})				**SED**	***W***		
	HL+	HL-	LL+	LL-		light	aphid	light x aphid
2006								
L(C+1)	2.7	0.2	8.8	0.1	1.3	**11.3*****	**33.6*****	**10.3****
S(C+1)	0.9	0.2	4.6	0.1	0.5	**23.3*****	**69.1*****	**35.2*****
2007								
L(C+1)	0.4	0.2	5.8	0.1	0.4	**36.2*****	**137.3*****	**118.7*****
S(C+1)	0.6	0.2	3.4	0.1	1.2	2.3	**5.2***	*3.3*^{ms}
L(C+2)	2.2	0.2	8.6	0.3	2.6	2.3	**9.9***	*3.8*^{ms}
S(C+2)	1.5	0.4	8.9	0.2	1.5	**10.7****	**25.1*****	**14.5*****

Table 2.4. Summary of REML analysis of variance for the effects of light and aphid treatment on the point estimate percentage needle loss at the end of the growing season of 2006 and 2007 at different shoot positions for different needle age-classes. Bold numbers indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) and italic numbers indicate marginal significance (^{ms} $P < 0.1$).

Position (age-class)	**Estimated mean** (%)				**SED**	**Relative loss** [a] (%)		***W***		
	HL+	HL-	LL+	LL-		HL	LL	light	aphid	light x aphid
2006										
L(C+1)	48.1	-0.5	70.7	-4.4	0.1	48	72	**193.8*****	**197.9*****	**9.1***
S(C+1)	63.3	47.0	72.6	51.0	0.03	31	44	**5.1***	**80.0*****	1.6
2007										
L(C+1)	34.3	13.1	82.1	8.8	0.1	24	80	**6.1***	**41.0*****	**12.5*****
S(C+1)	26.0	18.8	50.6	7.9	0.1	9	46	1.4	**13.8*****	**7.0****
L(C+2)	94.6	78.8	99.5	60.0	0.1	75	99	0.0	**51.6*****	**9.2****
S(C+2)	84.5	77.6	98.7	67.3	0.1	31	96	0.6	**287.6*****	**110.0*****

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[a] Percentage loss of needles on infested seedlings relative to that on uninfested seedlings for the high light (HL) and low light (LL) treatments.

2.3.2 Tree biomass and allocation

Needle biomass

In 2006 and 2007, a significant light effect was found for C+1 and C+0 foliage, with higher needle biomass in HL (1.5- to 2.2-fold greater) compared with LL (Table 2.5). In 2006, a significant aphid effect was found for C+1 needles, corresponding to the needle age-class where aphids were present. Even though the analysis did not show any significant light x aphid interaction, the tendency was for greater relative needle loss due to aphid infestation under low light compared with high light (Table 2.5). The same trends were observed in 2007, i.e. no aphid effect on C+0 foliage but a significant aphid effect on C+1 foliage, with again larger relative losses caused by aphids in LL compared with HL. In 2007, a significant light x aphid interaction was recorded on leader shoots for C+1 needles, with a lower needle biomass in LL+ than in HL+. Patterns were less clear for C+2 needles in 2007, because needle biomasses were very low.

Stem biomass

Table 2.6 shows the stem biomass corresponding to the needle biomass presented in Table 2.5. A clearly significant light effect was found for all position and foliage age-classes in both 2006 and 2007, with higher stem biomass in HL than LL. The aphid effect on stem biomass was not as obvious as in needle biomass. In 2006, an aphid effect, though only marginally significant, was found on the current-year leader (L(C+0)) where the stem biomass tended to be lower in infested seedlings compared to those that were undamaged (Table 2.6). This effect was confirmed in 2007 where L(C+1) stem biomass was reduced significantly more in infested than uninfested treatments. On an absolute basis, stems in LL+ tended to show the lowest biomass among the four treatments for both shoot positions and needle age-classes (Table 2.6). In neither year was there a significant light x aphid interaction (Table 2.6).

Total root biomass

Trees that grew in HL had significantly higher root biomass in both years than trees grown in LL, with biomass in 2006 and 2007 being 4.8- and 3.9-fold greater, respectively, in HL than in LL (Table 2.7). Aphid infestation significantly reduced total root biomass in 2007. In neither year was there an interaction between the effects of aphid infestation and light on root biomass. However, in both years, trees in LL+ had the lowest root biomass.

Total seedling biomass and allocation

In 2006 and 2007, the total dry mass of the seedlings was significantly greater in HL than LL (Table 2.8). The aphid effect on total biomass, although difficult to visualise at the end of 2006 became marginally significant by the end of 2007, after two years of repeated infestation (Table 2.8). In neither year was there a light x aphid interaction on total biomass (Table 2.8). In 2006, total biomass in LL+ was reduced by 8% relative to LL-, while total biomass in HL+ was decreased by 11% compared with HL-. In 2007, total biomass in LL+ was 13% lower compared with LL-, whereas total biomass in HL+ was only 7% lesser than in HL-.

In both years, trees that grew in HL invested more biomass on a relative and absolute basis in below-ground (i.e. roots) biomass than trees in LL (Fig. 2.7). Relative biomass allocation to current foliage was greater in LL than HL in 2006 but similar in 2007 (Fig. 2.7c,d). In 2007, a higher percentage of biomass was distributed to old stems in LL than in HL (Fig. 2.7d).

Table 2.5. Summary of REML analysis of variance for the effects of light and aphid treatment on the total needle dry weight at the end of the growing season of 2006 and 2007 at different shoot positions for different needle age-classes. Bold numbers indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) and italic numbers indicate marginal significance (^{ms} $P < 0.1$).

Position (age-class)	**Estimated mean** (g DW)				**SED**	**Relative loss** [a] (%)		***W***		
	HL+	HL-	LL+	LL-		HL	LL	light	aphid	light x aphid
2006										
L(C+0)	1.9	2.1	1.2	1.2	0.2	9	3	**28.2*****	0.9	0.3
S(C+0)	18.5	20.5	10.0	10.7	2.0	10	7	**32.0*****	1.1	0.2
L(C+1)	0.4	0.7	0.2	0.5	0.1	44	64	**20.1*****	**26.8*****	0.0
S(C+1)	0.9	1.1	0.6	0.8	0.1	22	30	**17.4*****	**5.6***	0.0
2007										
L(C+0)	4.4	4.3	2.5	2.6	0.4	-1	2	**34.6*****	0.0	0.03
S(C+0)	95.8	98.5	41.8	46.1	6.2	3	9	**2240.5*****	0.4	0.02
L(C+1)	0.9	1.0	0.3	0.9	0.1	16	63	**94.5*****	**43.2*****	**13.6*****
S(C+1)	11.4	13.6	5.7	9.0	1.7	16	37	**19.8*****	**4.9***	0.2
L(C+2)	0.1	0.2	0.03	0.2	0.1	20	86	0.1	**10.7****	**5.1***
S(C+2)	0.2	0.3	0.1	0.3	0.1	40	64	0.1	**10.4****	1.0

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[a] Percentage loss of needle biomass on infested seedlings relative to that on uninfested seedlings for the high light (HL) and low light (LL) treatments.

Table 2.6. Summary of REML analysis of variance for the effects of light and aphid treatment on the total stem dry weight at the end of the growing season of 2006 and 2007 at different shoot positions for different needle age-classes. Bold numbers indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) and italic numbers indicate marginal significance (^{ms} $P < 0.1$).

Position (age-class)	**Estimated mean** (g DW)				**SED**	**Relative loss** [a] (%)		***W***		
	HL+	HL-	LL+	LL-		HL	LL	light	aphid	light x aphid
2006										
L(C+0)	2.9	3.7	2.1	2.6	0.5	21	18	**5.8****	*3.2*^{ms}	0.2
S(C+0)	10.3	10.5	5.1	6.2	1.8	2	17	**10.8*****	0.3	0.1
L(C+1)	9.7	10.6	5.7	5.6	0.2	8	-3	**20.2*****	0.1	0.2
S(C+1)	2.0	2.3	1.1	1.0	0.3	9	-3	**20.3*****	0.2	0.4
2007										
L(C+0)	11.9	12.9	6.1	7.4	1.3	8	17	**70.3*****	1.3	0.01
S(C+0)	55.1	54.6	26.4	29.7	4.5	-1	11	**114.9*****	0.2	0.3
L(C+1)	22.6	24.8	17.0	23.3	0.3	9	27	**53.5*****	**4.8***	1.3
S(C+1)	25.6	23.3	13.5	12.9	3.4	-10	-4	**15.0*****	0.5	0.2
L(C+2)	40.5	43.0	23.7	24.6	2.1	6	3	**112.0*****	1.5	0.4
S(C+2)	6.1	5.1	2.1	1.2	0.2	-20	-63	**169.9*****	*3.3*^{ms}	0.1

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[a] Percentage loss of stem biomass on infested seedlings relative to that on uninfested seedlings for the high light (HL) and low light (LL) treatments.

Table 2.7. Summary of REML analysis of variance for the effects of light and aphid treatment on the total root dry weight at the end of the growing season of 2006 and 2007. Bold numbers indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) and italic numbers indicate marginal significance (^{ms} $P < 0.1$).

Year	**Estimated mean** (g DW)				**SED**	**Relative loss** (%) [a]		***W***		
	HL+	HL-	LL+	LL-		HL	LL	light	aphid	light x aphid
2006	29.3	33.6	6.3	6.9	0.1	13	9	**444.6*****	0.9	0.04
2007	139.6	161.4	34.6	42.0	0.04	14	18	**243.3*****	**8.4*****	0.2

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[a] Percentage loss of root biomass on infested seedlings relative to that on uninfested seedlings for the high light (HL) and low light (LL) treatments.

Table 2.8. Summary of REML analysis of variance for the effects of light and aphid treatment on the total seedling biomass at the end of the growing season of 2006 and 2007. Bold numbers indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) and italic numbers indicate marginal significance (^{ms} $P < 0.1$).

Year	**Estimated mean** (g DW)				**SED**	**Relative loss** [a] (%)		***W***		
	HL+	HL-	LL+	LL-		HL	LL	light	aphid	light x aphid
2006	77.5	86.9	32.9	35.9	7.6	11	8	**62.8*****	1.5	0.4
2007	426.8	458.9	176.8	204.2	27.5	7	13	**99.7*****	*3.5*^{ms}	0.02

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[a] Percentage loss of total seedling biomass on infested seedlings relative to that on uninfested seedlings for the high light (HL) and low light (LL) treatments.

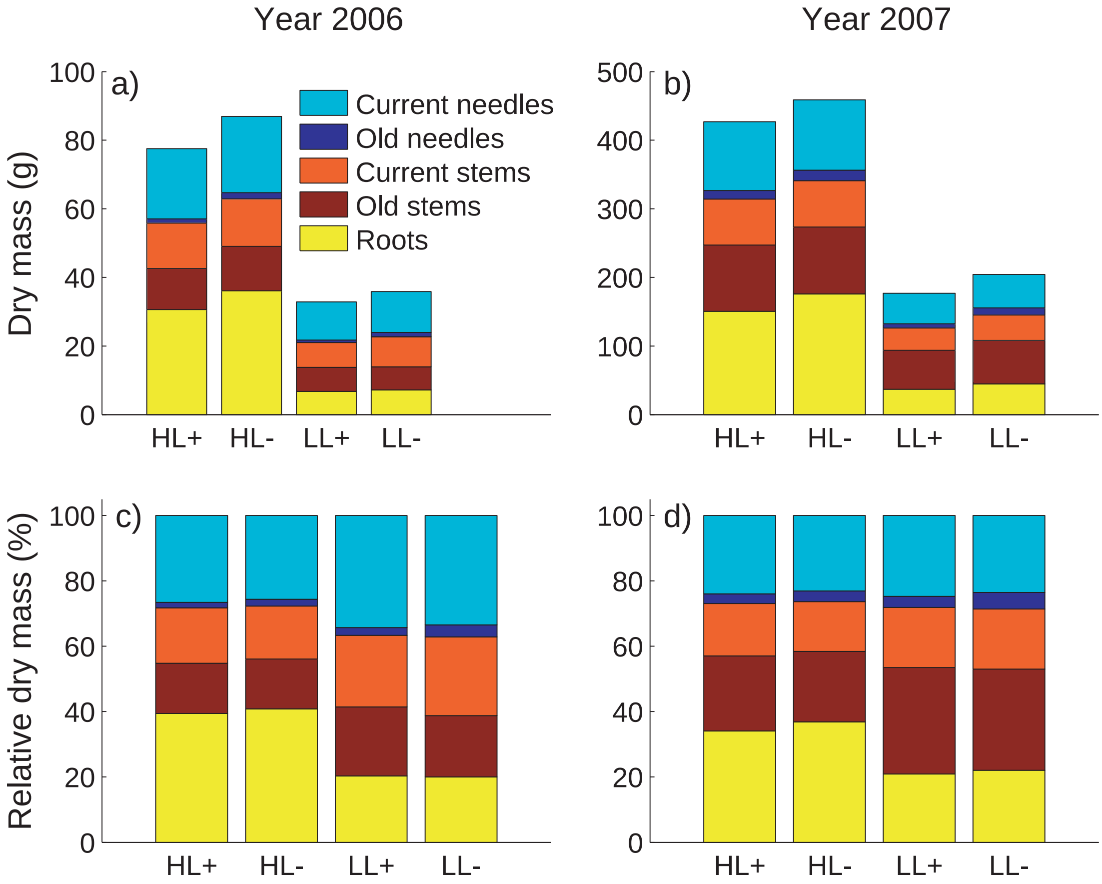

Fig. 2.7. Effect of light and aphid treatment on biomass allocation of the Sitka spruce seedlings on an absolute (a,b) and relative basis (c,d) in 2006 and 2007. Data are treatment means. Old needles and stems correspond to one-year old and older material.

2.3.3 Leader length extension and diameter growth

Prior to the start of the experiment, there were no significant differences between the treatments in total tree height (light: $P = 0.55$; aphid: $P = 0.12$; light x aphid: $P = 0.65$) or total diameter (light: $P = 0.69$; aphid: $P = 0.42$; light x aphid: $P = 0.09$) (Fig. 2.8). By the end of the first growing season, light was a significant main effect with respect to total tree height (light: $P < 0.001$), but not total diameter (light: $P = 0.12$). Total tree heights in LL were higher than in HL (Fig. 2.8a). By the end of the second growing season, total tree height was significantly reduced in HL compared with LL (light: $P < 0.001$), while total tree diameter was significantly greater in HL than in LL (light: $P < 0.001$). Infested trees had significantly smaller total heights than those uninfested (aphid: $P = 0.01$) (Fig. 2.8a). Total tree diameter tended to be smaller in infested trees compared with uninfested trees (aphid: $P = 0.08$) (Fig. 2.8b). Light x aphid interaction effect was marginally significant for total tree height (light x aphid: $P = 0.06$) but was not significant for total diameter ($P = 0.72$).

When looking at leader extension growth and stem diameter increment, the pattern was slightly different from total tree height and total diameter. By the end of the first season, leader extension in LL was significantly greater than in HL (Table 2.9). However in 2007, leader extension growth was similar in LL and HL, but the analysis showed a significant difference in leader length between infested and uninfested trees and a light x aphid interaction (Table 2.9). The reduction in leader extension caused by aphid infestation in 2007 was greater within LL than in HL: leader extension in LL+ was reduced by 17% relative to LL-, while leader extension in HL+ was only reduced by 2% compared with HL- (Table 2.9). A similar, although not significant, trend was observed in 2006 where infestation reduced leader extension by 15% and 3% in LL and HL, respectively (Table 2.9). In contrast, annual diameter increment was significantly affected by aphid infestation in 2006, but not by light, whereas in 2007 the light effect was large relative to the aphid effect (Table 2.9). There was no light x aphid interaction effect on annual diameter increment, but again the lowest increments were found in LL+.

Table 2.9. Summary of REML analysis of variance for the effects of light and aphid treatment on the leader extension growth and the diameter increment on the trees in experiment 2007 (n=60). Bold numbers indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$) and italic numbers indicate marginal significance (^{ms} $P < 0.1$).

Year	**Estimated mean**				**SED**	**Relative loss** [a] (%)		***W***		
	HL+	HL-	LL+	LL-		HL	LL	light	aphid	light x aphid
Leader extension growth ($\mathrm{cm\,yr^{-1}}$)										
2006	24.4	25.1	33.4	39.1	2.6	3	15	**41.0*****	*2.9*^{ms}	1.7
2007	59.5	60.6	51.5	62.1	3.0	2	17	2.5	**8.1****	**4.8***
Diameter increment ($\mathrm{mm\,yr^{-1}}$)										
2006	5.9	6.0	4.8	5.4	0.5	2	11	2.0	**4.5***	2.3
2007	12.5	13.3	8.1	8.7	0.6	6	7	**65.8*****	*3.7*^{ms}	0.1

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[a] Percentage loss of leader extension growth and diameter increment on infested seedlings relative to that on uninfested seedlings for the high light (HL) and low light (LL) treatments.

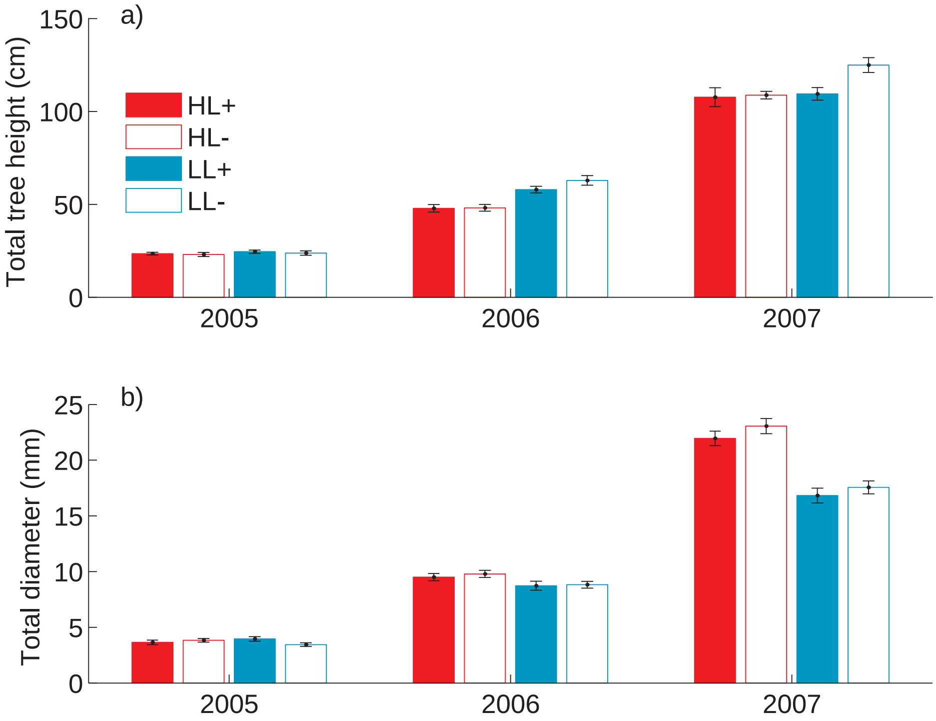

Fig. 2.8. Mean total tree height (a) and mean total diameter (b) in the four treatments in 2005, 2006 and 2007 (mean ±1 SEM, n=60).

2.4 Discussion

2.4.1 Aphid densities and needle loss

Aphid densities

Throughout the experiment, aphid population densities on the infested trees were significantly higher in the shade than in the open (Table 2.3). These results are in line with a recent field study looking at aphid population patterns under different forest environments (Bladon, 2009). Under field conditions, Bladon (2009) found that both the abundance and individual performance of *E. abietinum* was higher on young, naturally regenerated Sitka spruce seedlings growing below the tree canopy compared with seedlings planted in the open. A number of other studies have also shown increased performance of herbivorous insects on plants grown in shade rather than in open conditions (Jansen and Stamp, 1997; Henriksson et al., 2003; Moran and Showler, 2005; Roberts and Paul, 2006). A number of factors, operating separately or in combination, may contribute to differences in aphid density between open and shade conditions, such as plant defence mechanisms, plant nutritional quality (e.g. changes in nitrogen content, defensive terpene compounds or concentrations of particular amino-acids), predator abundance and microclimate (e.g. sensitivity to UV). Further investigation would be required to dissociate these factors. In contrast, the small differences in temperature, relative humidity and light quality in the current experiment in 2007 between open and shade were unlikely to have had a measurable direct effect on the variation in aphid densities between HL+ and LL+ (Fig. 2.2).

Beyond the higher aphid densities found in shade compared with open conditions, specific temporal and spatial variations in aphid numbers were observed that were similar in both light environments. Aphid densities on the artificially infested trees were generally lower in 2007 than in 2006 (Figs. 2.5 and 2.6). The population pattern on C+1 leader shoots during the first year following inoculation was similar to that of a typical moderate-to-severe natural outbreak (Straw et al., 1998a) in both infested treatments (28 ±5 and 94 ±9 aphids·100 needles^{-1} in HL+ and LL+, respectively), whereas densities in the second year were lower (5 ±1 and 69 ±15 aphids·100 needles^{-1} in HL+ and LL+, respectively) (Fig. 2.5a,b and Table 2.3). A characteristic feature of the year-to-year population dynamics of *E. abietinum* is that years in which high outbreaks occur are usually followed by a year with low aphid densities (Straw et al., 1998b). The factors causing low population numbers of *E. abietinum* in the year after an outbreak are still unclear (Williams et al., 2005). Williams et al. (2005) found no evidence for a change in host quality or an induced plant defence mechanism in previously infested Sitka spruce seedlings that could explain the low abundance of *E. abietinum* in the second year. On the other hand, the weather conditions in summer 2007 appeared to be less favourable for aphids compared with summer 2006, with lower temperatures and higher rainfall, both of which are known to limit aphid population growth (Harrington and Taylor, 1990; Harrington et al., 2007). In addition, a possible dilution effect of the greater amount of foliage may also partly explain the lower aphid densities in the second year of the experiment.

The distribution of aphids in the canopy of infested trees varied with aphids concentrated on leader shoots rather than on side shoots (Table 2.3). Similar differences in the distribution of aphids within the canopy of Sitka spruce seedlings have been observed in the field and have been attributed to a more suitable microclimate and better nutrient supply for aphids accessing needles at the apex of the tree (Straw et al., 1998a).

Needle loss

Aphids inject a toxin with their saliva during the feeding process which causes chlorosis, tissue death and premature needle abscission after a short period of time (Parry, 1974a). In this study, needle loss followed aphid densities with a $3-4$ week delay (Figs. 2.5 and 2.6). Defoliation was correlated with cumulative aphid densities and for both light treatments in both years, needle fall within the canopy was highest where the heaviest aphid densities were recorded (Tables 2.3 and 2.4). The infested trees in the shade lost, on average, 63 and 66 % of their older needles (one-year old and older) in 2006 and 2007, respectively, whereas trees in the open, which had lower aphid densities, suffered only 43 and 20 % defoliation in 2006 and 2007, respectively. The relationship between needle fall and the range of aphid densities observed in this experiment was similar to those reported in other studies carried out in full light conditions (Straw et al., 1998a, 2005; Williams et al., 2005). An asymptotic relationship exists between percentage needle loss and aphid density, with relatively high defoliation rates at low aphid densities (Straw et al., 1998a, 2005). For example, Straw et al. (1998a) found that in a Sitka spruce plantation, densities of 1 $\text{aphids}\cdot 100\,\text{needles}^{-1}$ caused 15 % defoliation. The same pattern was observed in this study, with relatively high needle loss in the infested trees in the open in 2007 even though aphid populations on these trees were very low (Table 2.3).

2.4.2 Tree biomass and allocation

The seedlings growing under shade conditions adapted quickly to the change of light environment. In terms of total seedling biomass, decreased light availability had a significant strong negative effect, with trees grown under shade conditions producing about 50 % less biomass than trees in full light in both years (Table 2.8). This reduction in biomass was observed in all the different organs of the seedlings over the two years (Tables 2.5 - 2.7). A large number of studies have shown that tree biomass is reduced with decreasing light availability (Shirley, 1929; Givnish, 1988; Wainhouse et al., 1998; Mason et al., 2004; Kennedy et al., 2007). Plants growing in a low light environment are also expected to allocate more biomass to above-ground parts (needles and stems) than below-ground parts (roots) to favour light interception (Givnish, 1988; Poorter and Nagel, 2000; Grassi and Minotta, 2000; Mori et al., 2008). This response to low light conditions was observed at the end of the first year of the current experiment and remained in the second year, with the seedlings in LL allocating more biomass to needles and stems than those in HL (Fig. 2.7). As trees grew bigger and older, they tended to allocate more biomass to branches, presumably to promote increased light capture through development of an expanding branch system.

In contrast to the effect of light, aphids had no overall effect on whole-seedling biomass, but just occasional effects on the biomass of particular components of the plant (Tables 2.5 - 2.8). Total DW of older needles at different shoot positions in infested trees were significantly lower than those in the uninfested trees and were correlated with defoliation rates (Tables 2.4 and 2.5). Total older needle biomass at harvest in LL+ was reduced by, on average, 41 and 43 % in 2006 and 2007, respectively, compared with LL-, while a decrease of 30 and 17 % occurred in HL+ in comparison with HL-, in 2006 and 2007, respectively. In a similar study, Straw et al. (2005) reported a significant decrease of 20 % in new foliage production in the year following heavy aphid infestation of Sitka spruce seedlings grown in the open. Although the range of aphid densities in LL+ was similar to those obtained in Straw et al. (2005), no significant aphid effect was found on total current-year needle biomass (Table 2.5). This result suggested that the impact of high aphid densities on the production of new needle biomass was modified by light availabilty.

Aphids had a significant effect on total root DW of infested trees in 2007 (Table 2.7). Interestingly, the relative reduction in total root biomass was similar ($14 - 18$ %) within high and low light environments, despite the large variation in aphid densities in the two light treatments. A decrease in total DW roots of young Sitka spruces grown in the open, and infested by aphids or artificially defoliated, have been reported in other studies (Day and Cameron, 1997; Straw et al., 1998a; Straw and Green, 2001). Whether this effect was directly due to aphids (e.g. drain on nutrients, injection of a toxin contained in the saliva) and/or to an effect of canopy reduction could not be ascertained.

Furthermore, rapid architectural changes that may improve light interception and growth, have been shown in some deciduous trees (e.g. *Eucalyptus blakely*, *Larix decidua*) exposed to sap-feeding insects or subject to artificial defoliation (Vranjic and Gullan, 1990; Krause and Raffa, 1996). However, in this study there were no differences (less than 3 %) in the relative proportions of dry matter allocated to needle, stem and root components between infested and uninfested trees within each light treatment (Fig. 2.7). These results show that the relative reduction in needle biomass component was accompanied by reductions of similar magnitude in the other organs of the seedlings, especially the roots. Straw and Green (2001) also observed that aphid infestation caused a decrease in total root biomass of Sitka spruce in proportion to reductions observed in above-ground growth, across high and low nutrient treatments. Krause and Raffa (1996) found the same pattern of response in another conifer species (*Pinus resinosa*) subjected to artificial defoliation, which was attributed to a static phenotypic character of some conifer species.

2.4.3 Leader length extension and diameter growth

In the field, leader length extension and stem diameter growth of natural or underplanted young Sitka spruce trees decline as light availability decreases (Page et al., 2001; Mason et al., 2004; Page and Cameron, 2006). In this study, diameter growth was lower in shade than open conditions, although a light effect was only significant in the second year (Table 2.9). In contrast, leader length extension was significantly higher in the shade than in the open in the first year, but the differences were not significant in the second year (Table 2.9). Similar results on leader extension growth were found by Kennedy et al. (2007) in a controlled study using shadehouses and potted Sitka spruce seedlings. In the current experiment, greater leader length extension in the first year might possibly be an artefact of the material used in the experiment. A large root system developed under full light conditions in the nursery prior to the experiment may have provided more resources for leader length extension than if the plants had been reared continuously under shade conditions.

In experiments on young Sitka spruce trees grown in the open and exposed to moderate-to-severe aphid populations, significant reductions in leader extension have been observed in the year of infestation and the subsequent year (Straw et al., 1998a; Straw and Green, 2001; Straw et al., 2005; Williams et al., 2005). In this experiment, leader growth of infested seedlings in shade conditions was 15 and 17 % shorter than uninfested seedlings in 2006 and 2007, respectively, but similar in the open with only 3 and 2 % differences in 2006 and 2007, respectively (Table 2.9). Although the trend was similar in 2006 and 2007, a light x aphid interaction effect was only significant in the second year (Table 2.9). An aphid effect was also observed on total tree height in 2007 (Fig. 2.8). Straw et al. (1998a) attributed the decline in leader growth to a direct aphid effect on shoot extension independent of needle loss. Williams et al. (2005) found that moderate aphid populations, such as in HL+ in 2006, reduced leader length of potted Sitka spruce seedling grown in full light conditions by 11 %. The absence of a relationship between leader length and aphid densities in the HL+ seedlings in 2006 may be due to a relative reduction in the effect of *E. abietinum* when trees have access to unlimited resources, as in a controlled environment (Straw and Green, 2001). Under low light conditions, an impact of high *E. abietinum* populations on leader extension was found. At similar aphid densities to those observed in LL+ seedlings, other studies carried out in full light conditions have found larger reductions (22 − 29 %) in leader growth (Straw et al., 1998a; Williams et al., 2005). When seedling growth is restricted by light, insect attack may have less effect on growth, because the seedling may lack the plasticity to respond to the additional constraints being imposed.

Stem diameter growth response across light treatments mirrored those observed for leader extension. Diameter growth often shows a different pattern of postponed response to aphid infestation compared with effects on leader extension (Straw et al., 1998a). In other experiments with young Sitka spruce trees, where plants developed in full light conditions, only a small effect on diameter growth was observed in the year of infestation, usually followed by a larger, delayed effect the subsequent year (Straw et al., 1998a, 2000; Halldórsson et al., 2003). In the current experiment, aphid infestation had a small effect on diameter increment of HL+ seedlings over the two years. However, there was a trend for a delayed effect with a relatively larger reduction in the second year (2 % in 2006 and 6 % in 2007), despite the very low aphid populations in HL+ in 2007. In contrast, diameter increment was reduced by 11 and 7 % in LL+ compared with LL- in 2006 and 2007, respectively. While a stronger impact was found on diameter increment of LL+ seedlings compared with HL+ seedlings in 2006, corresponding to the higher aphid densities in LL+, there was no difference in the effect in the second year. Again, the poorer growth in the shade may have muted the response to infestation.

2.5 Conclusion

The results reported here indicate that light was the main factor controlling Sitka spruce seedling growth and allocation. When seedling growth is restricted by light availability, severe natural *E. abietinum* outbreaks might have only a small effect on growth, due to a lack of plasticity in the seedlings to respond to an additional constraint. However, the defoliation rates and reduction in leader extension and total root dry weight associated with infestation in low light environments are likely to make the undertorey seedlings less able to compete with ground vegetation in field conditions. Therefore, a silvicultural system where the canopy is opened by creating scattered gaps (i.e. group selection system) with a minimum of 25 % incident light, rather than opening the canopy uniformly (i.e. shelterwood system) should be targeted in CCF management, with repeated thinning interventions every 3–4 years after seedling emergence to increase light levels and promote seedling growth, and minimise aphid impact.

2.6 Acknowledgements

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Chapter 3

Sun - shade acclimated Sitka spruce seedlings: aphid impacts on foliar morphology and photosynthetic performance

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Abstract

Under low light environments, larger populations of green spruce aphids (*Elatobium abietinum*) can develop on one-year old and older needles of Sitka spruce seedlings (*Picea sitchensis*) than under high light environments, causing greater defoliation. However, the effect of low light conditions and the presence of aphids on the morphological and physiological responses of Sitka spruce seedlings remains to be determined. This understanding is crucial in order to ensure successful seedling establishment below an existing canopy of Sitka spruce. A controlled experiment that combined two light levels (high light: 100 % and low light: 24 %) and two aphid infestation levels (absence/presence) was conducted in a split-plot design for two consecutive years to investigate the interactive influence of light availability and aphid infestation on photosynthesis, as measured by chlorophyll fluorescence, of one-year old needles with no apparent symptoms of aphid injury (e.g. chlorosis). Light was the main driving factor affecting the photosynthetic response. After two years, foliage produced on Sitka spruce seedlings had successfully acclimated, both morphologically and physiologically, to the prevailing light environment. In the second year, needles grown under high light conditions showed significantly lower total chlorophyll content and specific leaf area (SLA), and higher needle dry weight (DW), than needles grown under low light conditions. In comparison to the low light phenotype, the high light phenotype was characterised by reduced photosynthetic capacity (rETR_{max}) but an enhanced capacity for non-radiative dissipation of absorbed light energy of photosystem II (NPQ_{max}) over the whole second year period. In contrast, the presence of aphids had only a short-term and localised effect on photosynthesis. Following the first year of infestation, an increase in SLA of current-year needles on leader stems of seedlings grown under low light was found. The change in needle size was correlated with the highest maximum aphid densities and suggested a direct and local influence of aphids. An evidence of a photosynthetic response to aphid infestation on one-year old needles was only found in the second year of infestation in seedlings grown under low light conditions. Although only significant just after the time of aphid peak infestation, low light phenotype in the infested treatment consistently exhibited lower rETR_{max} than the uninfested low light phenotype, while no such trend was observed for high light phenotype. The decrease in rETR_{max} could be caused by a residual effect of the first year infestation and/or a direct aphid effect. We conclude that Sitka spruce seedlings should perform well under shaded environments even in the presence of aphids.

Keywords: *Elatobium abietinum*, light acclimation, non-photochemical quenching, photosynthetic electron transport, *Picea sitchensis*

3.1 Introduction

Continuous cover forestry (CCF), a forest management approach alternative to clearfelling, is increasingly being adopted in the UK. It aims to enhance stand structural diversity and favour natural regeneration and subsequent seedling growth below the existing canopy of plantation forests (Mason and Kerr, 2004; Mason et al., 2004). One area of limited knowledge is the critical level of below-canopy light for the growth of naturally regenerating seedlings (Malcolm et al., 2001; Hale et al., 2004). In addition, plant growth beneath canopies is influenced by other factors (e.g. herbivory) (Kotanen and Rosenthal, 2000; Löf, 2000). Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings under canopies have been observed to be more severely damaged by green spruce aphid (*Elatobium abietinum* (Walker)) than seedlings growing in the open. *E. abietinum*, a phloem-feeding insect, feeds on one-year old and older needles, and causes premature abscission of needles that have been attacked (Straw et al., 1998b). The combined effects of aphid attack and light availability on Sitka spruce seedling growth are not well understood, but this understanding is crucial in order to ensure successful management of regeneration within CCF systems.

Efforts to understand the effects of aphids on Sitka spruce seedlings have mainly focused on growth performance of plants that have developed under full light conditions (Straw et al., 1998a, 2000, 2002; Williams et al., 2005). To allow direct comparison with those studies, a controlled experiment, which mimicked different natural understorey light levels along with repeated artificial aphid infestation, was carried out over two years to look at seedling growth performance and changes in the allocation of resources (Bertin et al., 2009). Aphid population assessments showed significantly increased population densities under shade conditions (Bertin et al., 2009). At the whole-plant level, aphid impacts were observed on needle and root biomass, but effects on total plant biomass were small in comparison with the impact of light. Plant biomass allocation was affected strongly by light, while aphid presence did not result in significant changes. A significant interaction between light levels and presence/absence of aphid infestation was found for main leader extension of the seedlings during the second year of the experiment with lower leader extension at low light levels under infestation.

To comprehend changes in growth occurring at the whole-plant level, it is crucial to look at the physiological (e.g. photosynthetic) and morphological responses at the leaf-level. The effect of light on plant morphology, photosynthesis and growth have received considerable attention in the literature over a long period (Boardman, 1977; Givnish, 1988). It is now well established that as leaves form, they develop morphological and physiological characteristics that appear to be adaptive to the light conditions under which they develop (Boardman, 1977; Givnish, 1988). Plant responses induced by herbivore damage have also been studied extensively (see Crawley, 1999; Nykänen and Koricheva, 2004). However, no clear paradigm exists and the herbivory induced response in plants varies widely with often contradictory results between and within species, dependent upon resource availability among other factors (Nykänen and Koricheva, 2004). For example, photosynthetic efficiency in response to defoliation has been shown to increase (Lavigne et al., 2001), decrease (Macedo et al., 2003), not change (Aldea et al., 2005) or to be conditional (Vanderklein and Reich, 1999). Furthermore, the interactive effect of light availability and defoliation caused by insects on foliar morphology and photosynthetic performance of seedlings has received scant attention (McGraw et al., 1990). The effects of light, aphid and light x aphid at the leaf-level of Sitka spruce seedlings have never been investigated, yet it is needed to improve the overall understanding of Sitka spruce seedling responses and to identify the effect of these factors on light utilisation.

In this study, the morphological characteristics and the physiological status of one-year old undamaged needles of Sitka spruce seedlings, cultivated in the controlled experiment (i.e. seedlings grown under different light and aphid infestation levels), were investigated. The objective of the study was to assess the impact of aphid attack and light availability on the response of Sitka spruce seedlings at the leaf-physiology level.

3.2 Material and methods

3.2.1 Site and plant material

The design and methodology of the controlled experiment are described in detail in Bertin et al. (2009). The experiment was carried out over two years in 2006 and 2007 in an open field at the Forestry Commission's Northern Research Station (NRS), near Edinburgh, in Scotland (55°86′N, 03°20′W). The seedlings used in the shadehouse experiments were two-year old potted Sitka spruce seedlings (QSS 1U1) obtained from Delamere nursery in February 2006 before the establishment of the experiment.

3.2.2 Micrometeorological measurements

To characterise the light environment at the site, hourly global solar radiation ($\mathrm{W\,m^{-2}}$) measured by the Met Office in 2006 and 2007 in Edinburgh, UK (55°55′N, 03°20′W) was used as a proxy for photosynthetic photon flux density (PPFD, $\lambda = 400 - 700$ nm) fluctuations (Finch et al., 2004).

3.2.3 Experimental design

A split-plot design was used with two light levels as main plots, and two aphid infestation levels as subplots. Light was manipulated artificially by constructing shadehouses wrapped with shade clothes, except for a 30 cm strip at the bottom of the four sides to allow air circulation. Light levels of 100 % (frame only, no cloth; HL) and 24 % (LL) were used in the experiment. In one aphid subplot (+A), trees were artificially infested with aphids before the start of the measurements (end of April 2006 and 2007), while the other subplot served as a control (-A). In August 2006 and 2007, all of the trees were sprayed with pirimicarb (Aphox®) to prevent the current-year needles becoming infested by aphids, which can occur in the autumn.

In 2006, 12 shadehouses (1 x 4 x 6.5 m) were constructed on the site. The study was replicated across four blocks for a total of 144 trees (4 blocks x 2 plots (light treatments) x 2 subplots (aphid infestation levels) x 9 trees per aphid subplot). In 2007, six shadehouses were retained and raised to 2 m height. The study was reduced to three blocks and five trees per subplot, giving a total of 60 trees (3 blocks x 2 light treatments x 2 aphid infestation levels x 5 trees per aphid subplot). Four treatments were explored in the study: 1) full sunlight, with no aphids (HL-); 2) full sunlight, with aphids (HL+); 3) low light, with no aphids (LL-); 4) low light, with aphids (LL+).

3.2.4 Needle morphology and composition

Needle morphology

At the end of the growing season of 2006 and 2007, for each of the four treatments, one tree was chosen randomly from each block. Sub-samples of 60 needles were taken from the current-year age-class from two positions (leader and side shoots) from each tree to determine specific leaf area (SLA, leaf area per unit leaf biomass, $\mathrm{m^2\,g^{-1}}$). The needles from each position and age-class were pooled together for each tree, and were then oven dried at 80 °C for 48 hours and weighed. Projected needle area (A_N, $\mathrm{mm^2}$) was measured after oven-drying using ImageJ public domain software (U.S. National Institutes of Health, Bethesda, MD, USA).

Foliar analysis

Needles were collected in October 2006 and 2007 from all of the trees in the experiment for C:N analysis. One undamaged needle located on the leader stem and two undamaged needles located on the side shoots from current- and one-year old (C+1) age-classes were removed from each tree. The needles from the same position and age-class were pooled together for each block. The pooled needle samples were freeze dried for 24 hours, ground in a ball mill and stored in a desiccator until analysed for percentage dry weight of nitrogen ($\mathrm{N_{mass}}$) and carbon ($\mathrm{C_{mass}}$). In 2006, needles samples were first stored in a freezer at −20 °C until freeze dried. C:N analysis of the needle material was performed by the University of Edinburgh, Grant Institute of Earth Science, using a C/H/N analyzer (NA 2500 Elemental Analyser, Carlo Erba Strumentazione, Milan, Italy).

Chlorophyll

Samples of intact needles from each age-class were collected from each tree in October 2007 for measuring concentrations of chlorophyll *a*, chlorophyll *b* and total chlorophyll. The needles were removed from midway along side branches (eight needles for two-year old shoots, four needles for one-year old shoots and three needles for current-year shoots). Fully turgid fresh needle sample mass was measured immediately. The needles were then sectioned transversely and stored in a freezer at −80 °C until analysed. The concentration of chlorophylls was measured following the techniques described by Porra et al. (1989). Each sample was placed in $3\,\mathrm{cm^3}$ N,N-dimethylformamide (DMF) and kept in darkness at 4 °C for about seven days. The absorbance of the resulting extracts was then measured at 647, 664 and 750 nm with a spectrophotometer (model DR/2000, HACH Co., Loveland, Colorado, USA). Chlorophyll concentrations ($\mathrm{\mu g\,cm^{-3}}$) were calculated based on the following equations (Porra et al., 1989):

$$\begin{aligned}\text{Chl } a &= 12.00 \cdot (A^{664} - A^{750}) - 3.11 \cdot (A^{647} - A^{750}) \qquad (3.1)\\ \text{Chl } b &= 20.78 \cdot (A^{647} - A^{750}) - 4.88 \cdot (A^{664} - A^{750}) \qquad (3.2)\\ \text{Chl } a + b &= 17.67 \cdot (A^{647} - A^{750}) + 7.12 \cdot (A^{664} - A^{750}) \qquad (3.3)\end{aligned}$$

3.2.5 Chlorophyll *a* fluorescence

The chlorophyll *a* fluorescence methodology was adapted from Rascher (personal communication) and Rascher et al. (2000), avoiding common pitfalls described by Logan et al. (2007). Chlorophyll *a* fluorescence was measured with a portable pulse modulated fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany) using the leaf-clip holder (part no. 2030-B) or the dark leaf-clip (part no. DLC-8). Measurements of ambient PPFD were recorded with the MINI-PAM microquantum sensor fixed on the leaf-clip holder. Since aphids do not feed on current-year needles, fluorescence measurements were taken on previous year (C+1) undamaged needles (i.e. less than 20 % chlorosis) located midway along side shoots of the previous year whorl. In the 2006 experiment, three trees per subplot were chosen randomly at the beginning of the experiment and measurements repeated on three occasions on the selected trees through the growing season: 1) just after the aphid peak (week 26), 2) in the middle of the growing season (week 32), and 3) at the end of the growing season (week 42). In 2006, the C+1 sample needles corresponded to needles grown under the same light conditions in the nursery before being exposed to the different experimental treatments in April 2006. In the 2007 experiment, four trees per subplot were selected randomly before the start of the experiment and measurements taken four times: 1) before the aphid peak (week 21), 2) just after the aphid peak (week 26), 3) in the middle of the growing season (week 32), and 4) at the end of the growing season (week 42). In 2007, the C+1 needles had developed under the four different treatments applied during 2006.

In 2006, at each occasion, fluorescence measurements on different trees were completed within a week. In 2007, fluorescence measurements were made over two successive days. In both 2006 and 2007, for each tree, all sets of fluorescence measurements were recorded on the same day and at the same period of the day.

Steady state fluorescence (designated F_o when measured in dark-adapted tissue, F_{s} in light adapted tissue) was measured just before applying a 800 ms duration saturation pulse (intensity $> 5000\,\mathrm{\mu mol\,m^{-2}\,s^{-1}}$) after which the maximal fluorescence (designated F_{m} in dark-adapted tissue, F'_{m} in light adapted tissue) was recorded. The maximum effective quantum yield of photosystem II (PSII), $F_{\mathrm{v}}/F_{\mathrm{m}}$, was calculated as $(F_{\mathrm{m}} - F_{\mathrm{o}})/F_{\mathrm{m}}$, and was measured on plants that were dark-adapted using the dark leaf-clips attached at least 30 min prior to measurement, to minimise the effect of non-photochemical energy dissipation (Rascher et al., 2000). The effective quantum yield of PSII, $F'_{\mathrm{q}}/F'_{\mathrm{m}}$, was calculated as $(F'_{\mathrm{m}} - F_{\mathrm{s}})/F'_{\mathrm{m}}$ (Genty et al., 1989). Rapid light response curves (RLCs) of $F'_{\mathrm{q}}/F'_{\mathrm{m}}$ were obtained using the light curve program of the MINI-PAM (Rascher et al., 2000). Measurements of the RLCs were made in the dark so that all the actinic light received by the needles was delivered by the fibre optics of the MINI-PAM.

In 2006, for each tree, $F_{\mathrm{v}}/F_{\mathrm{m}}$ was an average of two measurements made on two spots that had been dark-adapted for at least 30 min with the dark leaf-clips. For each tree, the RLC was recorded on a different spot, using the 60 ° leaf clip holder. Each RLC took about 160 s to complete with 9 PPFD steps each of 20 s duration ranging in intensity from 0 to $\sim 2\,000\,\mathrm{\mu mol\,m^{-2}\,s^{-1}}$. The PPFD values for each step were recorded simultaneously with the MINI-PAM microquantum sensor mounted on the 60 ° leaf clip holder. The needles were not dark-adapted prior to the measurements of the RLCs.

In 2007, the design was adapted to the change in needle morphology between the treatments. To record the RLC, the dark leaf-clip was used instead of the leaf-clip holder for two reasons: 1) the fibreoptics could be adjusted to a smaller distance from the needles to increase the fluorescence amplitude, and 2) the measuring spot could be kept constant through the whole measurement sequence on a same tree. First, the needles were dark-adapted for at least 30 min with the dark leaf-clip. Subsequently, the fibre optic was mounted on the dark leaf-clip and a RLC was run. Each RLC took about 240 s to complete with 9 PPFD steps each of 30 s duration in intensity from 0 to $\sim 1\,300\,\mathrm{\mu mol\,m^{-2}\,s^{-1}}$. The first step of each RLC corresponded to $F_{\mathrm{v}}/F_{\mathrm{m}}$. For each plot, before and after running the RLCs of all the trees within a plot, the PPFD intensity emitted from the internal light source for each of the 9 PPFD steps was obtained by running the light curve program without any plant material but the dark leaf-clip placed above the MINI-PAM microquantum sensor in the same orientation as used for the needle sample. The two PPFD intensities for each PPFD step were then averaged and applied to the RLCs within the plot. This method was preferred to the automated light curve values because random variations in PPFD intensity through the course of the measurements over a day had been observed in a preliminary study (unpublished data). Since two subplots were nested within each plot, a RLC was alternatively run between each subplot to randomise the impact of a possible shift in PPFD intensities during the measurements within a plot.

Relative electron transport rate (rETR) was calculated at each step of the RLC and was obtained as rETR $= F'_{\mathrm{q}}/F'_{\mathrm{m}}$ x PPFD x 0.5 x 0.84 where the factor 0.5 assumes that only half of the absorbed photons go into PSII and 0.84 is the empirical mean absorption factor for green leaves (Ehleringer, 1981). Other studies have found similar absorptance factors for both Sitka spruce shoots grown under high and low light levels (Leverenz and Jarvis, 1979; Black et al., 2005). The calculated relative rates followed a curve which in principle is analogous to corresponding curves obtained from gas-exchange measurements for the light dependence of CO_2 fixation (Bilger et al., 1995).

Non-photochemical quenching (NPQ) was also calculated at each step of the RLC in experiment 2007 as NPQ $= (F_{\mathrm{m}} - F'_{\mathrm{m}})/F'_{\mathrm{m}}$ where F_{m} was measured during the first step of each RLC (Bilger and Björkman, 1990; Bilger et al., 1995). The different geometry used in the measurements of F_{m} and F'_{m} in experiment 2006 did not permit calculation of NPQ. The needles seldom covered the total surface of the clip introducing variation in fluorescence measures from one sample to another.

3.2.6 Statistical analysis

Linear mixed-effects (LME) and nonlinear mixed-effects (NLME) models approach (Pinheiro and Bates, 2000) was used to analyse the data in R (R Development Core Team, 2008). This method of variance component analysis was preferred because of its robustness to unbalanced designs and missing data, and its flexibility with complex multifactor designs (Quinn and Keough, 2003). In any built model, main fixed effects (light- and aphid- levels) and interaction were always considered. Different random effects were included in the models depending on the sampling procedure used (see below for a detailed description of each application). All continuous responses were assessed for normality and log transformed or weighted by least squares regression to account for heteroscedasticity in the residual variance where necessary. Tests for significance of main fixed effects and interaction were based on the Wald-test. The significance level was set at $P = 0.05$. Means (predicted from LME and NLME models) were compared using the estimated standard errors of differences (SED).

Needle morphology and composition

Data on needle morphology and composition were analysed with a split-plot analysis of variance using LME models.

Needle area, needle dry weight (DW), and SLA were analysed using separate LME models for each needle position and age-class, with plots within blocks and subplots within plots as random factors.

$\mathrm{N}_{\mathrm{mass}}$ and C/N ratio were analysed using separate LME models for each needle age-class, with plots within blocks, subplots within plots, and positions within subplots as random factors.

Total chlorophyll and chlorophyll *a/b* ratio were analysed using separate LME models for each needle age-class, with plots within blocks, subplots within plots, and trees within subplots as random factors.

Chlorophyll *a* fluorescence

Maximum effective quantum yield of PSII ($F_{\mathrm{v}}/F_{\mathrm{m}}$)

Analysis of $F_{\mathrm{v}}/F_{\mathrm{m}}$ was performed with a split-plot analysis of variance using separate LME models for each time occasion. Random factors were plots within blocks, subplots within plots and trees within subplots.

Rapid light response curves (RLCs)

A saturating exponential function constrained to pass through the origin was used to model the relationship between rETR and PPFD (Rascher, personal communication; Rascher et al., 2000), and NPQ and PPFD (Ritchie, 2008). A multilevel NLME model was used to fit these ecophysiological response curves, since this type of model provides biologically meaningful parameters to fit the nonlinear function (Peek et al., 2002). All parameters were estimated by maximum likelihood.

The rETR model is written:

$$\mathrm{rETR} = A \cdot (1 - \exp(-\exp(B) \cdot \mathrm{PPFD})) \tag{3.4}$$

The model parameters were A, the maximum rETR ($\mathrm{rETR_{max}}$), and $\mathrm{rPPFD_{sat}}$, defined as the PPDF level at which rETR was 90 % of $\mathrm{rETR_{max}}$ and calculated from B. The fitted model had mixed effects on both A and B. The fixed effects were light- and aphid- levels. The random effects included blocks and trees nested within blocks, but did not include plot- and subplot- levels in order not to overparameterise the model.

The NPQ model used in experiment 2007 is written:

$$\mathrm{NPQ} = C \cdot (1 - \exp(-\exp(D) \cdot \mathrm{PPFD})) \tag{3.5}$$

The model parameters were C, the maximum NPQ ($\mathrm{NPQ_{max}}$), and $\mathrm{PPFD_{sat}}$, defined as the PPDF level at which NPQ was 90 % of $\mathrm{NPQ_{max}}$, and calculated from D. The fitted model had mixed effects on both C and D. The fixed effects were light- and aphid- levels. The random effects were blocks and trees nested within blocks.

3.3 Results

3.3.1 Micrometeorological measurements

During the summer months of 2006 and 2007 in Eastern Scotland, maximum temperatures were 18.9 and 16.3 °C and minimum temperatures were 9.6 and 8.7 °C, total sunshine amounted to 537.5 and 369.3 hours, and total precipitation was 184.7 and 366.5 mm, respectively (Met Office, 2008).

The cumulative daily average global solar radiation at Edinburgh for the period 2006–2007 is illustrated by Figure 3.1. The global solar radiation values were slightly higher in April 2007 than in April 2006 (Fig. 3.1), whereas during the summer months (June to September) the solar energy received in 2007 was less than that received in 2006 (Fig. 3.1). Solar radiation intensities in autumn and winter months were similar in both years.

Ambient air temperatures and relative humidity within the shaded plots during summer months in 2007 were similar to those in the open, with less than 1 °C and 3.2 % differences, respectively (Bertin et al., 2009).

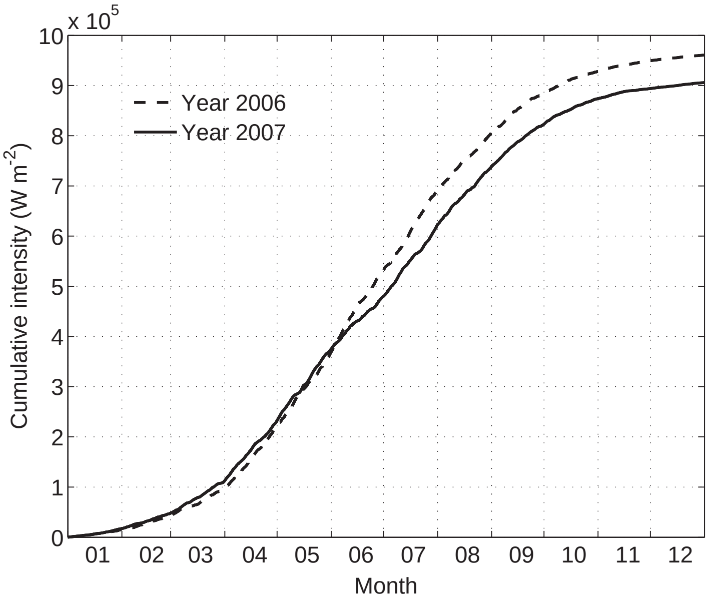

Fig. 3.1. Cumulative daily global solar radiation in 2006 and 2007.

3.3.2 Needle morphology and composition

Needle morphology

Projected area, DW and SLA of one-year old (C+1) needles on leader and side shoots were not significantly different in 2006, between the HL and LL treatments (data not shown). However, there was a significant difference in SLA of current-year needles between light treatments at both positions, and in both years with higher SLA in LL than HL (Fig. 3.2c,f and Table 3.1). The change in SLA was mainly attributed to a change in needle DW. DW of current-year needles was significantly lower in LL than in HL at any position, apart from on side shoots in 2006 (Fig. 3.2b,e and Table 3.1). In contrast, no differences were found in projected needle area at any position in either year (Fig. 3.2a,d and Table 3.1). A significant light x aphid effect was found for SLA of current-year needles situated on the 2006 leader, with higher SLA in LL+ than LL- but little difference between HL+ and HL- (Fig. 3.2c and Table 3.1). The higher SLA of LL+ relative to LL- seedlings appeared due to a non significant decrease in needle DW.

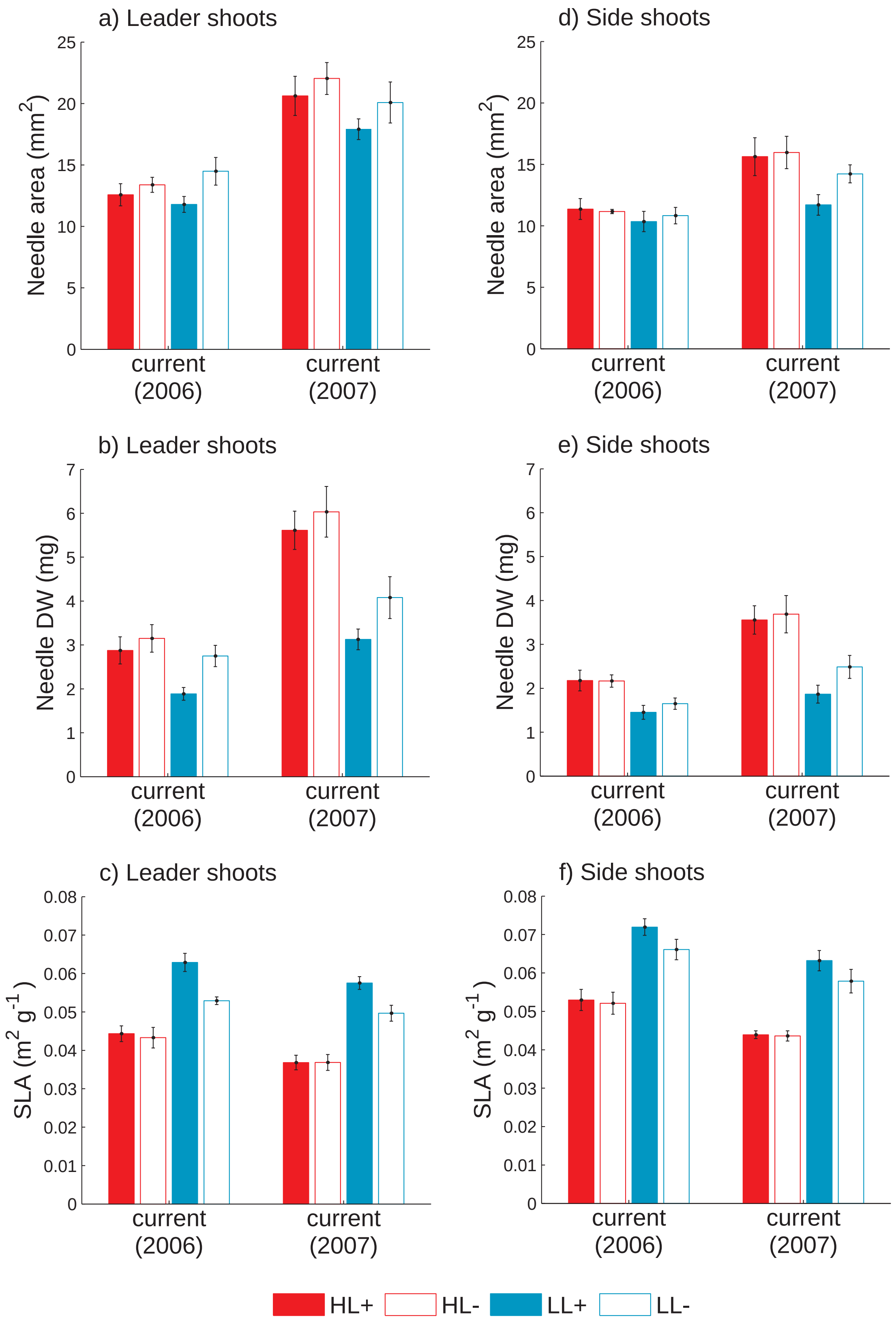

Fig. 3.2. Projected needle area, needle dry weight (DW) and specific leaf area (SLA) of current-year needles in 2006 and 2007 for (a-c) leader and (d-f) side shoots in each of the four treatments. Values are mean ±1 SEM (n = 4 in 2006, n = 3 in 2007). See methods for light and aphid treatments.

Table 3.1. Treatment effects on projected needle area, needle DW and SLA at different shoot positions in 2006 and 2007 (Wald tests for LME). Bold P-values indicate a significant effect at $P < 0.05$.

		Needle area		**Needle DW**		**SLA**	
	d.f.	F	P	F	P	F	P
Leader (2006)							
light	1,3	0.57	0.51	10.61	**0.05**	42.07	**0.007**
aphid	1,6	0.60	0.47	0.96	0.37	0.17	0.69
light x aphid	1,6	1.65	0.25	2.24	0.19	6.46	**0.04**
Leader (2007)							
light	1,2	2.02	0.29	19.94	**0.05**	53.24	**0.02**
aphid	1,4	1.28	0.32	0.29	0.62	0.00	1.00
light x aphid	1,4	0.22	0.66	1.10	0.35	2.91	0.16
Side (2006)							
light	1,3	1.10	0.37	8.93	0.06	26.14	**0.01**
aphid	1,6	0.06	0.81	0.00	0.97	0.05	0.83
light x aphid	1,6	0.35	0.58	0.37	0.57	0.91	0.38
Side (2007)							
light	1,2	6.45	0.13	19.36	**0.05**	38.86	**0.03**
aphid	1,4	0.11	0.75	0.07	0.81	0.01	0.93
light x aphid	1,4	2.58	0.18	2.06	0.23	1.33	0.31

Foliar analysis

In both years, there were no statistically significant differences in N_{mass} and C/N ratio between treatments for either of the needle age-classes (Fig. 3.3a,b), although in 2007 there was a tendency for higher N_{mass} in LL than in HL for both C+1 and current-year needles (Fig. 3.3c). C/N ratio in 2007 appeared to be higher in HL than LL in both age-classes (Fig. 3.3d). The lack of significance between HL and LL could be partly attributed to the sample design (i.e. lack of replicates at the tree level, see methods).

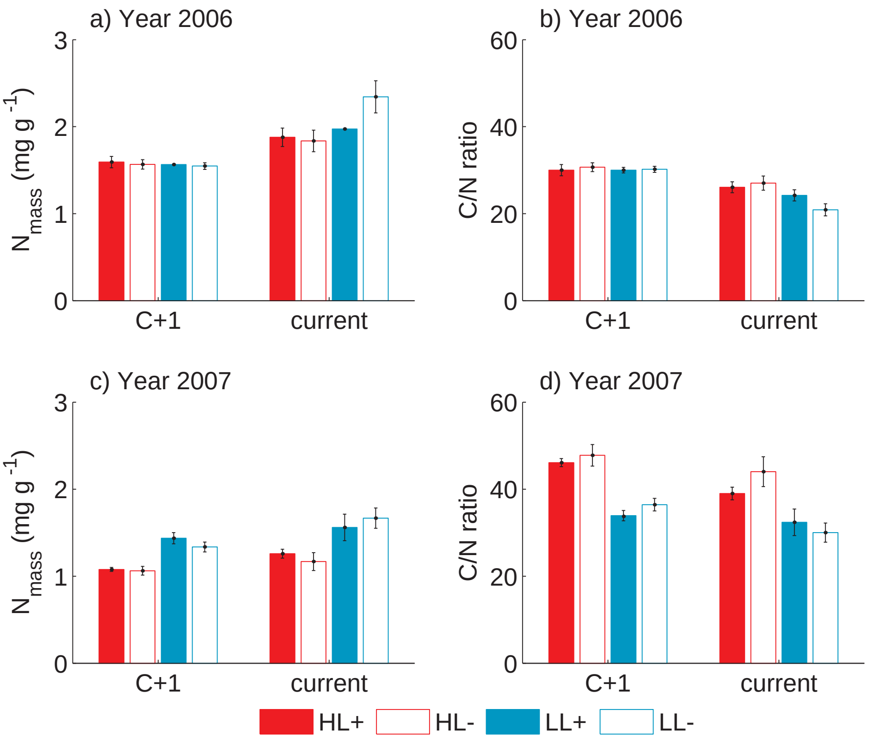

Fig. 3.3. Nitrogen content on a dry weight (DW) mass basis and C/N ratio of two needle age-classes in (a-b) October 2006 and (c-d) October 2007. Values are mean ±1 SEM (n = 4 in 2006, n = 3 in 2007).

Chlorophyll

Total chlorophyll content per unit mass of needles was significantly higher in LL compared with HL treatment in C+1 and current-year needles (Fig. 3.4a, light (C+1): $F_{1,2} = 46.69$, $P = 0.02$, light (current): $F_{1,2} = 94.08$, $P = 0.01$). The chlorophyll content of needles in the HL treatment gradually increased as they became older while it did not vary appreciably in LL (Fig. 3.4a). Although not significant, Chl a/b ratio was higher in HL than LL (Fig. 3.4b). The effect of aphid and the interaction between light and aphid were not significant for total chlorophyll or Chl a/b ratio (Fig. 3.4).

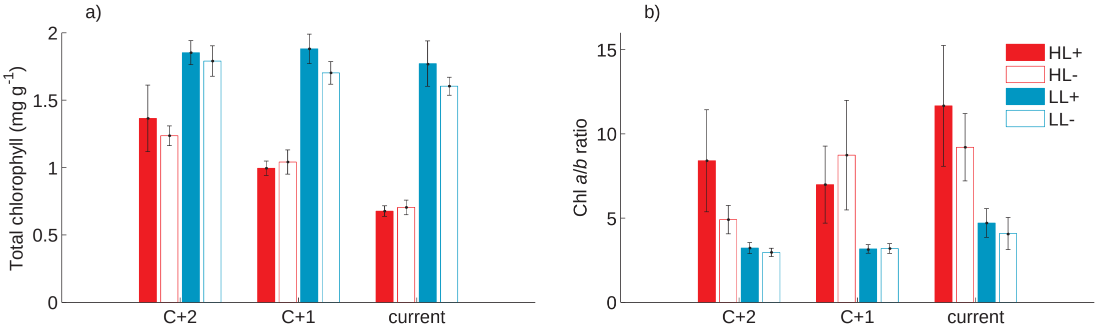

Fig. 3.4. Total chlorophyll content on (a) a mass basis and (b) Chl *a*/*b* ratio for three needle age-classes in October 2007. Values are mean ±1 SEM (n=15).

3.3.3 Chlorophyll *a* fluorescence

Maximum effective quantum yield of PSII ($F_\mathrm{v}/F_\mathrm{m}$)

No significant differences in maximum effective quantum yield of PSII ($F_\mathrm{v}/F_\mathrm{m}$) were found between treatments in 2006 and 2007 (Fig. 3.5 and Table 3.2). $F_\mathrm{v}/F_\mathrm{m}$ ratios were close to 0.8, i.e. characteristic of non-stressed needles. At the time of measurement, the LL treatment experienced less variation in PPFD than the HL treatment (Fig. 3.6). By the end of the growing season, incident PPFD in HL was much lower than had been the case at the time of the earlier PPFD measurements and, as a result, differences in PPFD between the LL and HL treatments were reduced (HL: 299 ±23 versus LL: 88 ±9 µmol m^{-2} s^{-1}, Fig. 3.6).

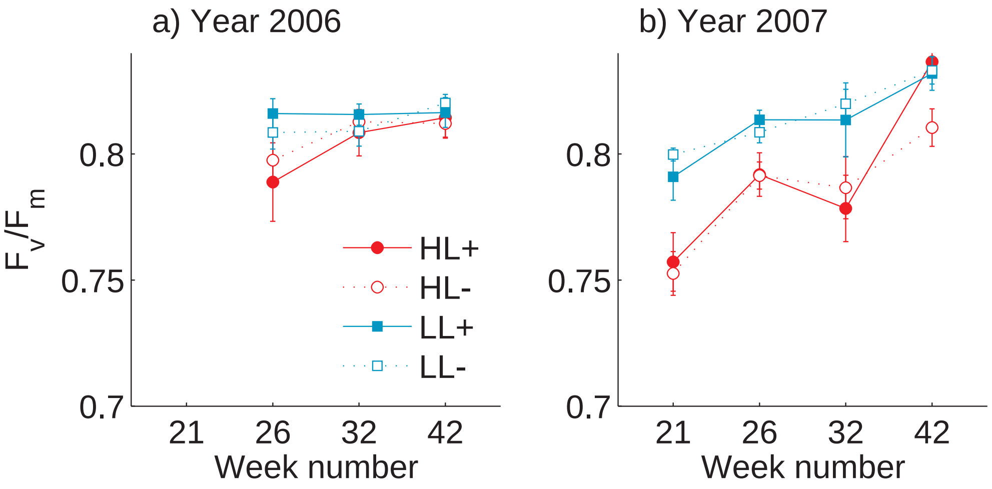

Fig. 3.5. Maximum effective quantum yield of PSII ($F_\mathrm{v}/F_\mathrm{m}$) in (a) 2006 and (b) 2007. Values are mean ±1 SEM (n=15).

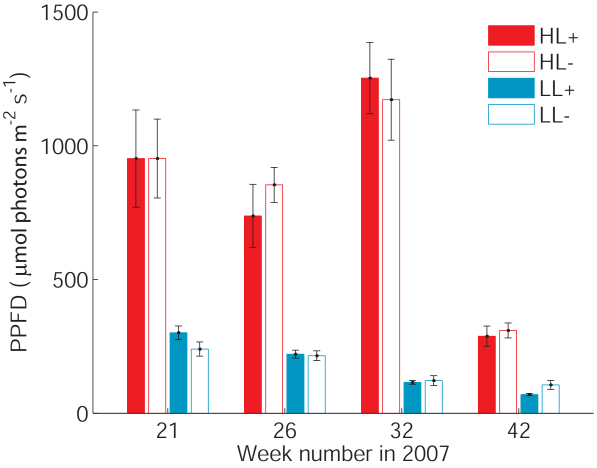

Fig. 3.6. Ambient PPFD experienced by the selected shoot before being dark-adapted in 2007. Values are mean ±1 SEM (n=12). Measurements were taken with the MINI-PAM microquantum sensor.

Table 3.2. Treatment effects on maximum effective quantum yield of PSII (F_v/F_m) in 2006 and 2007 (Wald tests for LME). Bold P-values indicate significant effect at $P < 0.05$.

	Year 2006			Year 2007		
	d.f.	F	P	d.f.	F	P
Week 21						
light	·	·	·	1,2	5.92	0.14
aphid	·	·	·	1,4	0.14	0.73
light x aphid	·	·	·	1,4	0.65	0.47
Week 26						
light	1,3	5.24	0.11	1,2	7.79	0.11
aphid	1,6	0.36	0.57	1,4	0.00	0.98
light x aphid	1,6	1.18	0.32	1,4	0.29	0.62
Week 32						
light	1,3	0.85	0.43	1,2	1.80	0.31
aphid	1,6	0.32	0.59	1,4	0.11	0.76
light x aphid	1,6	1.03	0.35	1,4	0.07	0.80
Week 42						
light	1,3	0.06	0.82	1,2	0.12	0.76
aphid	1,6	0.05	0.83	1,4	7.38	0.053
light x aphid	1,6	0.26	0.63	1,4	4.37	0.10

Rapid light response curves (RLCs)

The RLCs obtained in 2006 and 2007 are shown in the supplementary material section (Figs. 3.7 - 3.9). Fitted curves from the double exponential functions are also presented for information of $F'_\mathrm{q}/F'_\mathrm{m}$ versus PPFD (Fig. 3.7) .

rETR versus PPFD

In 2006, $\mathrm{rETR_{max}}$ and $\mathrm{rPPFD_{sat}}$ were significantly higher in HL than LL in all sampling periods (Fig. 3.8a-c, Tables 3.3 and 3.5). The lowest $\mathrm{rETR_{max}}$ and $\mathrm{rPPFD_{sat}}$ were measured in week 42. Aphid infestation and its interaction with light level were not found to cause any significant change in $\mathrm{rETR_{max}}$ and $\mathrm{rPPFD_{sat}}$ at any time during the first year of the experiment (Table 3.5). The differences in $\mathrm{rETR_{max}}$ between HL and LL remained relatively constant through the growing season (Table 3.3). In 2007, $\mathrm{rETR_{max}}$ and $\mathrm{rPPFD_{sat}}$ values occupied a different range compared with the values recorded in 2006 (Table 3.4), which was mainly attributed to the change of fluorescence methodology. From week 21 to week 32, $\mathrm{rETR_{max}}$ and $\mathrm{rPPFD_{sat}}$ were significantly lower in LL than HL, but the differences were not as marked as in 2006 (Fig. 3.8d-g). The only significant difference found in week 42 was a reduction in $\mathrm{rPPFD_{sat}}$ (Table 3.5). Throughout the whole experimental period in 2007, there was a trend for a lower $\mathrm{rETR_{max}}$ in LL+ compared to LL-, but no pattern was observed in HL (Fig. 3.8d-g, Table 3.4). A significant light x aphid interaction was found in week 21, with a lower $\mathrm{rETR_{max}}$ in LL+ compared to LL-, while at the same time there were no differences in $\mathrm{rETR_{max}}$ between HL+ and HL- (Table 3.5).

NPQ versus PPFD

In 2007, $\mathrm{NPQ_{max}}$ was significantly higher in HL than LL on all sampling dates (Fig. 3.9, Tables 3.6 and 3.7). In contrast, $\mathrm{PPFD_{sat}}$ did not differ significantly between the two light treatments, on any occasion (Table 3.7). Significant aphid and light x aphid effects were only found for $\mathrm{PPFD_{sat}}$ at week 21 (Table 3.7).

Table 3.3. Parameter estimates and SEs extracted from NLME models for the rETR versus PPFD relationship (equation 3.4) in 2006. 95 % confidence intervals calculated from B for $\mathrm{rPPFD_{sat}}$ ($\mu\mathrm{mol\,m^{-2}\,s^{-1}}$) are also given.

	HL+	HL-	LL+	LL-
$\boldsymbol{A} = \mathbf{rETR_{max}}$				
Week 26	165.2 ± 8.4	174.2 ± 8.5	107.5 ± 8.3	112.6 ± 8.3
Week 32	188.5 ± 7.6	180.7 ± 7.6	129.6 ± 7.5	131.7 ± 7.5
Week 42	135.4 ± 7.5	133.1 ± 7.5	78.9 ± 7.5	80.64 ± 7.5
$\boldsymbol{B} \sim \mathbf{rPPFD_{sat}}$				
Week 26	-6.41 ± 0.05	-6.48 ± 0.06	-5.87 ± 0.05	-5.90 ± 0.05
$\mathrm{rPPFD_{sat}}$ 95 % CI	(1262, 1567)	(1352, 1680)	(732, 907)	(754, 934)
Week 32	-6.39 ± 0.04	-6.39 ± 0.00	-6.05 ± 0.04	-6.09 ± 0.04
$\mathrm{rPPFD_{sat}}$ 95 % CI	(1262, 1499)	(1254, 1490)	(893, 1064)	(936, 1114)
Week 42	-6.09 ± 0.06	-6.06 ± 0.06	-5.61 ± 0.06	-5.66 ± 0.06
$\mathrm{rPPFD_{sat}}$ 95 % CI	(914, 1139)	(884, 1103)	(564, 707)	(591, 741)

Table 3.4. Parameter estimates and SEs extracted from NLME models for the rETR versus PPFD relationship (equation 3.4) in 2007. 95 % confidence intervals calculated from B for rPPFD$_{\text{sat}}$ (μmol m^{-2} s^{-1}) are also given.

	HL+	HL-	LL+	LL-
$\boldsymbol{A}$ **= rETR$_{\text{max}}$**				
Week 21	62.9 ± 4.7	61.9 ± 4.6	42.8 ± 4.5	49.2 ± 4.6
Week 26	58.1 ± 3.5	52.7 ± 3.3	48.1 ± 3.6	52.3 ± 3.3
Week 32	67.9 ± 6.4	66.0 ± 6.3	51.3 ± 6.1	60.5 ± 6.1
Week 42	52.2 ± 5.2	55.2 ± 5.0	42.8 ± 4.8	50.9 ± 4.8
$\boldsymbol{B}$ **∼ rPPFD$_{\text{sat}}$**				
Week 21	-5.74 ± 0.09	-5.78 ± 0.08	-5.09 ± 0.09	-5.25 ± 0.09
rPPFD$_{\text{sat}}$ 95 % CI	(602, 852)	(634, 885)	(315, 442)	(371, 518)
Week 26	-5.48 ± 0.06	-5.33 ± 0.06	-5.15 ± 0.07	-5.26 ± 0.06
rPPFD$_{\text{sat}}$ 95 % CI	(486, 628)	(419, 536)	(346, 452)	(391, 499)
Week 32	-5.67 ± 0.09	-5.62 ± 0.09	-5.21 ± 0.09	-5.41 ± 0.09
rPPFD$_{\text{sat}}$ 95 % CI	(555, 806)	(528, 759)	(355, 505)	(431, 613)
Week 42	-5.35 ± 0.11	-5.38 ± 0.10	-5.05 ± 0.10	-5.25 ± 0.10
rPPFD$_{\text{sat}}$ 95 % CI	(393, 599)	(409, 612)	(297, 438)	(364, 534)

Table 3.5. F- and P- values for treatment effects on A and B parameter estimates for rETR versus PPFD in 2006 and 2007 (Wald tests for NLME). Bold P-values indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$).

	Year 2006				**Year 2007**			
	$\boldsymbol{A} = \mathbf{rETR_{max}}$		$\boldsymbol{B} \sim \mathbf{rPPFD_{sat}}$		$\boldsymbol{A} = \mathbf{rETR_{max}}$		$\boldsymbol{B} \sim \mathbf{rPPFD_{sat}}$	
	F	P	F	P	F	P	F	P
Week 21								
light	·	·	·	·	12.94	***	31.56	***
aphid	·	·	·	·	0.03	0.86	0.15	0.70
light x aphid	·	·	·	·	0.88	0.35	0.50	0.48
Week 26								
light	23.83	***	57.93	***	5.11	**0.02**	13.26	***
aphid	0.56	0.45	0.92	0.34	1.58	0.21	3.05	0.08
light x aphid	0.05	0.82	0.15	0.70	2.47	0.12	4.41	**0.04**
Week 32								
light	30.49	***	30.42	***	7.38	**0.01**	22.66	***
aphid	0.54	0.46	0.01	0.92	0.10	0.76	0.31	0.58
light x aphid	0.44	0.51	0.35	0.56	1.71	0.19	3.44	0.06
Week 42								
light	67.91	***	57.08	***	1.79	0.18	4.12	**0.04**
aphid	0.11	0.74	0.28	0.60	0.17	0.68	0.04	0.84
light x aphid	0.18	0.67	0.79	0.38	0.27	0.60	0.70	0.40

Table 3.6. Parameter estimates and SEs extracted from NLME models for the NPQ versus PPFD relationship (equation 3.5) in 2007. 95 % confidence intervals calculated from D for PPFD$_{\text{sat}}$ (µmol m^{-2} s^{-1}) are also given.

	HL+	HL-	LL+	LL-
C = NPQ$_{\text{max}}$				
Week 21	2.94 ± 0.16	2.66 ± 0.16	2.04 ± 0.16	2.20 ± 0.16
Week 26	2.63 ± 0.21	2.76 ± 0.21	1.81 ± 0.22	1.87 ± 0.21
Week 32	3.28 ± 0.19	3.41 ± 0.17	2.68 ± 0.16	2.68 ± 0.16
Week 42	3.69 ± 0.25	3.46 ± 0.25	2.17 ± 0.22	2.10 ± 0.22
D = PPFD$_{\text{sat}}$				
Week 21	-5.75 ± 0.06	-5.53 ± 0.06	-5.69 ± 0.06	-5.73 ± 0.06
rPPFD$_{\text{sat}}$ 95 % CI	(636, 815)	(514, 650)	(601, 768)	(627, 799)
Week 26	-5.76 ± 0.09	-5.69 ± 0.09	-5.85 ± 0.09	-5.76 ± 0.09
rPPFD$_{\text{sat}}$ 95 % CI	(616, 871)	(575, 807)	(662, 958)	(611, 871)
Week 32	-5.68 ± 0.09	-5.70 ± 0.08	-5.59 ± 0.08	-5.76 ± 0.08
rPPFD$_{\text{sat}}$ 95 % CI	(567, 806)	(588, 813)	(527, 722)	(624, 858)
Week 42	-5.68 ± 0.10	-5.66 ± 0.10	-5.69 ± 0.10	-5.85 ± 0.10
rPPFD$_{\text{sat}}$ 95 % CI	(552, 821)	(544, 810)	(569, 816)	(661, 961)

Table 3.7. F- and P- values for treatment effects on C and D parameter estimates for NPQ versus PPFD in 2007 (Wald tests for NLME). Bold P-values indicate significant treatment effects at $P < 0.05$ (*** $P < 0.001$).

	$\boldsymbol{C = \mathrm{NPQ_{max}}}$		$\boldsymbol{D \sim \mathrm{PPFD_{sat}}}$	
	F	P	F	P
Week 21				
light	17.71	***	0.43	0.51
aphid	1.78	0.18	6.40	**0.01**
light x aphid	2.28	0.13	4.46	**0.04**
Week 26				
light	20.24	***	1.06	0.30
aphid	0.52	0.47	1.03	0.31
light x aphid	0.07	0.79	0.02	0.90
Week 32				
light	5.64	**0.02**	0.58	0.45
aphid	0.24	0.62	0.04	0.85
light x aphid	0.14	0.71	0.79	0.37
Week 42				
light	23.46	***	0.01	0.93
aphid	0.47	0.49	0.01	0.92
light x aphid	0.13	0.72	0.77	0.38

3.4 Discussion

3.4.1 Short-term and long-term structural and physiological acclimation to light

Short-term acclimation to light (2006)

In the first year of the experiment, the physiological measurements were conducted on pre-existing (C+1) needles that had developed under similar full light conditions in the nursery before being placed under the different light treatments. As a result the SLA values of C+1 needles in LL and HL were similar, confirming that the needles had kept their 'sun' morphological characteristics.

Throughout the experimental period in 2006, the pre-existing 'sun' needles, when exposed to the shade environment, showed significantly lower photosynthetic capacity (measured as $\mathrm{rETR_{max}}$) by $30-40\%$ and saturated at lower PPFD (measured as $\mathrm{rPPFD_{sat}}$) than those maintained in the open (Tables 3.3 and 3.5). This physiological response has been observed frequently when photosynthesis has been measured as CO_2 uptake (i.e. from gas exchange measurements) in 'sun' and 'shade' acclimated shoots on the same Sitka spruce tree (Leverenz and Jarvis, 1979, 1980; Meir et al., 2002). Sitka spruce foliage grown in the open was observed to rapidly acclimate physiologically to the reduced-light environment, even after the foliage has been morphologically established as 'sun' foliage. Similar quick responses to shading have been reported in other shade-tolerant evergreen conifers, and it has been suggested that the physiological acclimation involves reallocation of nitrogen among pools within the leaf to enhance light harvesting (Brooks et al., 1994; Grassi and Minotta, 2000; Niinemets, 2007).

Long-term acclimation to light (2007)

The new needles developed under the different light treatments in 2006 and 2007 adapted morphologically and physiologically to the change in light environment. Needles grown under HL had significantly lower total chlorophyll content and SLA, and higher needle DW, than needles grown in LL (Figs. 3.2 and 3.4). Although not significant, there was a trend for a lower Chl a/b ratio in LL which reflected a relative increase in the amount of chlorophyll b, a pigment forming a large part of the light-harvesting pigment of PSII. These changes are consistent with the typical pattern of 'sun' and 'shade' acclimated foliage of shade-tolerant species such as Sitka spruce (*c.f.* Norman and Jarvis, 1974; Lewandowska et al., 1976; Lewandowska and Jarvis, 1977; Leverenz and Jarvis, 1980). The lack of significant differences in N_{mass} with varying light conditions was in accord with another study on Sitka spruce when N was expressed on a mass basis (Meir et al., 2002).

In the second year of the experiment, the physiological measurements were conducted on C+1 needles that had acclimated to the differing light conditions the previous year. In contrast to the first year, differences in photosynthetic capacity (measured as $rETR_{max}$) and saturated PPFD (measured as $rPPFD_{sat}$) between sun-grown and shade-grown plants were much lower, although the differences were still mostly significant. Between week 26 and week 42, the foliage photosynthetic capacity in LL was lower by only $10-15\%$ of that in HL. Furthermore, the effective quantum yield of PSII (F'_q/F'_m) measured at different PPFD levels showed very similar patterns across the two light treatments (Fig. 3.7d-g). The relatively low capacity for photosynthetic electron transport of 'sun' phenotypes compared with 'shade' phenotypes is typical of shade-tolerant tree species. Similar results (based on chlorophyll *a* fluorescence and/or gas-exchange measurements estimated on a mass or area basis) have been found in Sitka spruce seedlings, where maximum photosynthetic capacity was similar or higher in 'shade' than 'sun' phenotypes (Denne and Smith, 1971; Smith, 1976; Leverenz and Jarvis, 1980; Black et al., 2005). The contrasting response in photosynthetic capacity between 2006 and 2007 emphasizes the importance of making a clear distinction between 'sun' and 'shade' acclimated foliage between and within trees of shade-tolerant species (*c.f.* Leverenz and Jarvis, 1980).

Sun-grown seedlings exhibited significantly higher asymptotic NPQ_{max} values compared with those grown in the shaded environment (an increase of $25-65\,\%$) and is most likely attributable to a greater non-radiative dissipation of absorbed light energy of PSII in sun-grown seedlings (Table 3.6, Fig. 3.9). It is now widely accepted that sun-acclimated leaves generally possess a higher capacity than shade-acclimated leaves for rapid increases in photoprotective xanthophyll cycle-dependent energy dissipation in order to avoid damage to foliage exposed to excess light (Demmig-Adams and Adams, 1992, 1996). The lower NPQ values of shade-grown seedlings could predispose them to photodamage if they experienced prolonged exposure to excess light.

Foliage produced by Sitka spruce trees grown in different light conditions exhibited both morphological and physiological long-term acclimation to their light environment. A combination of a similar capacity for photosynthetic electron transport alongside a higher capacity for thermal energy dissipation in sun-grown seedlings, suggests that Sitka spruce seedlings should perform well in shaded conditions. Black et al. (2005) supported the same conclusions in a study looking at the effect of irradiance and water availability on Sitka spruce seedlings, where 'sun' phenotypes suffered higher photodamage and slower recovery of photosynthesis compared with 'shade' phenotypes, particularly when grown under water stress. McKinnon and Mitchell (2003) attributed a lack of difference in photosynthetic capacity between sun- and shade-grown Engelmann spruce (*Picea engelmannii*) seedlings, another shade-tolerant conifer species, to a combination of downward adjustments in chlorophyll and upwards adjustments in photoprotective xanthophyll cycle carotenoids in the 'sun' phenotype, as a response to avoiding foliar damage from excess light.

3.4.2 Morphological and physiological responses to aphid impact

Localised changes in needle DW within the canopy have been found to develop in Sitka spruce trees during the year of aphid infestation, correlated with the canopy positions where aphid infestation was heaviest (Straw et al., 1998a). Therefore, the significant increase in SLA (mainly attributed to a decrease in needle DW) of current-year needles on leader stems of LL+ trees in the first year of this experiment was not surprising (Fig. 3.2, Table 3.1). Aphid population assessments showed a significant increase in population density under shade conditions in both years, although maximum densities were higher in the first year (Bertin et al., 2009). In addition, aphid densities on HL+ trees were higher on the leader shoots than on the side shoots (Bertin et al., 2009). The absence of changes in needle morphology in the infested treatments during the second year of the experiment, however, was in contrast to another study that reported a significant delayed effect on needle DW throughout the canopy of young Sitka spruce trees grown in the open (Straw et al., 2002). The results from the current study suggested that the mechanism causing changes in needle morphology in infested trees grown in shade conditions was likely to be directly linked to local aphid density. A direct and localised effect seems most likely to occur during the aphid peak period, which usually takes place in May-June (Day et al., 1998). It could be attributed, for example, to the toxin injected by aphids whilst they are feeding and/or to the diversion of photosynthates from new foliage (Parry, 1974;

Crawley, 1999). An indirect effect induced by defoliation caused by the aphid was improbable since needle loss occurred towards the end of the growing season, presumably after formation of the new foliage (Bertin et al., 2009).

Over the whole first year period, in both light treatments, there was no evidence of a photosynthetic response to aphid infestation on C+1 pre-existing healthy 'sun' needles (Table 3.5). Similar response (i.e. no effect after one year defoliation) has also been reported (Vanderklein and Reich, 1999). Since changes occurred in the morphology of needles formed during the year of infestation, it is possible that photosynthetic capacity was affected in the current foliage, but not in the pre-existing foliage. The second year of infestation showed different results. In terms of aphid and light x aphid effects, a consistent pattern on C+1 needles acclimated to their different light environment could be seen in photosynthetic capacity (measured as $\mathrm{rETR}_{\mathrm{max}}$). Although only significant at week 26 (i.e. just after the aphid peak), 'shade' shoots in the infested treatment consistently exhibited lower $\mathrm{rETR}_{\mathrm{max}}$ than the uninfested 'shade' shoots, while no such trend was observed for 'sun' shoots. The observed result was in agreement with the localised and short-term effect reported in SLA of needles formed during the first year of infestation in response to aphid attack. The lower $\mathrm{rETR}_{\mathrm{max}}$ in 'shade' shoots in the infested treatment during the second year could arise from a combination of factors such as a residual effect of the first year infestation (i.e. small, non significant, changes in needle morphology) linked to a direct aphid effect at the time of peak infestation. In addition, multi-year defoliation can lead to a cumulative response, which is not apparent after the first year of infestation (Vanderklein and Reich, 1999). In comparison, the absence of an impact of aphids on photosynthesis of C+1 needles on 'sun' shoots of infested trees is not surprising regarding the low abundance of aphid densities in HL+ in the second year of infestation (Bertin et al., 2009). Close and McArthur (2002) hypothesised that the levels of plant phenolics, a classic defence compound against herbivory, depends on the risk of photodamage to the plant. This hypothesis, in light with the upward adjustment in photoprotective mechanisms found in sun-grown trees, could partially explain the low aphid population densities assessed in HL+ in the second year of infestation. Further work on the primary role of phenolics in Sitka spruce seedlings may help to understand better the differences in aphid density between low and high light conditions.

3.5 Conclusion

Light was found to be the main driving factor affecting the photosynthetic response, whilst the presence of aphids had only a short-term, localised effect on photosynthesis, which was accentuated under shade conditions. Foliage developed on Sitka spruce trees grown in different light conditions exhibited both morphological and physiological long-term acclimation to their light environment, which was representative of a shade-tolerant tree species. Compared to 'shade' needles, 'sun' needles were characterised by reduced photosynthetic capacity but an enhanced capacity for non-radiative dissipation of absorbed light energy of PSII. The results of the study suggest that Sitka spruce seedlings should perform well below the existing canopy in CCF systems. The low magnitude of morphological and physiological responses to aphids in shade conditions suggested that two years repeated infestation should not be a threat to seedling establishment below the canopy. However, repeated high levels of aphid infestation may reduce the seedling's photosynthetic capacity and consequently the plant's ability to grow under low light environments.

3.6 Acknowledgement

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3.8 Supplementary material

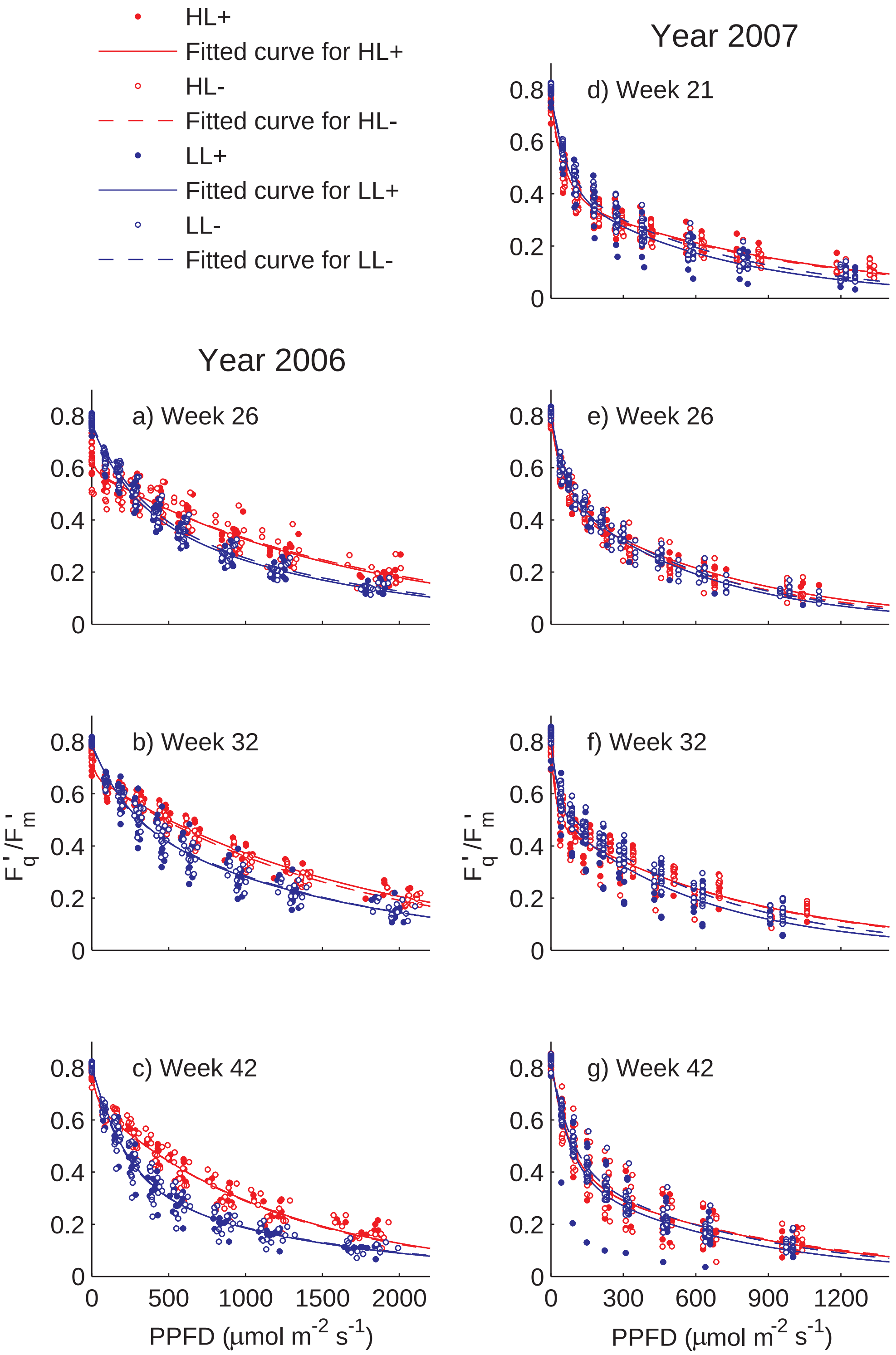

Fig. 3.7. F_q'/F_m' versus PPFD measured on selected trees (data points) in each of the four treatments (a-c) on three occasions in 2006 and (d-g) on four occasions in 2007. (Fitted curves from double exponential functions).

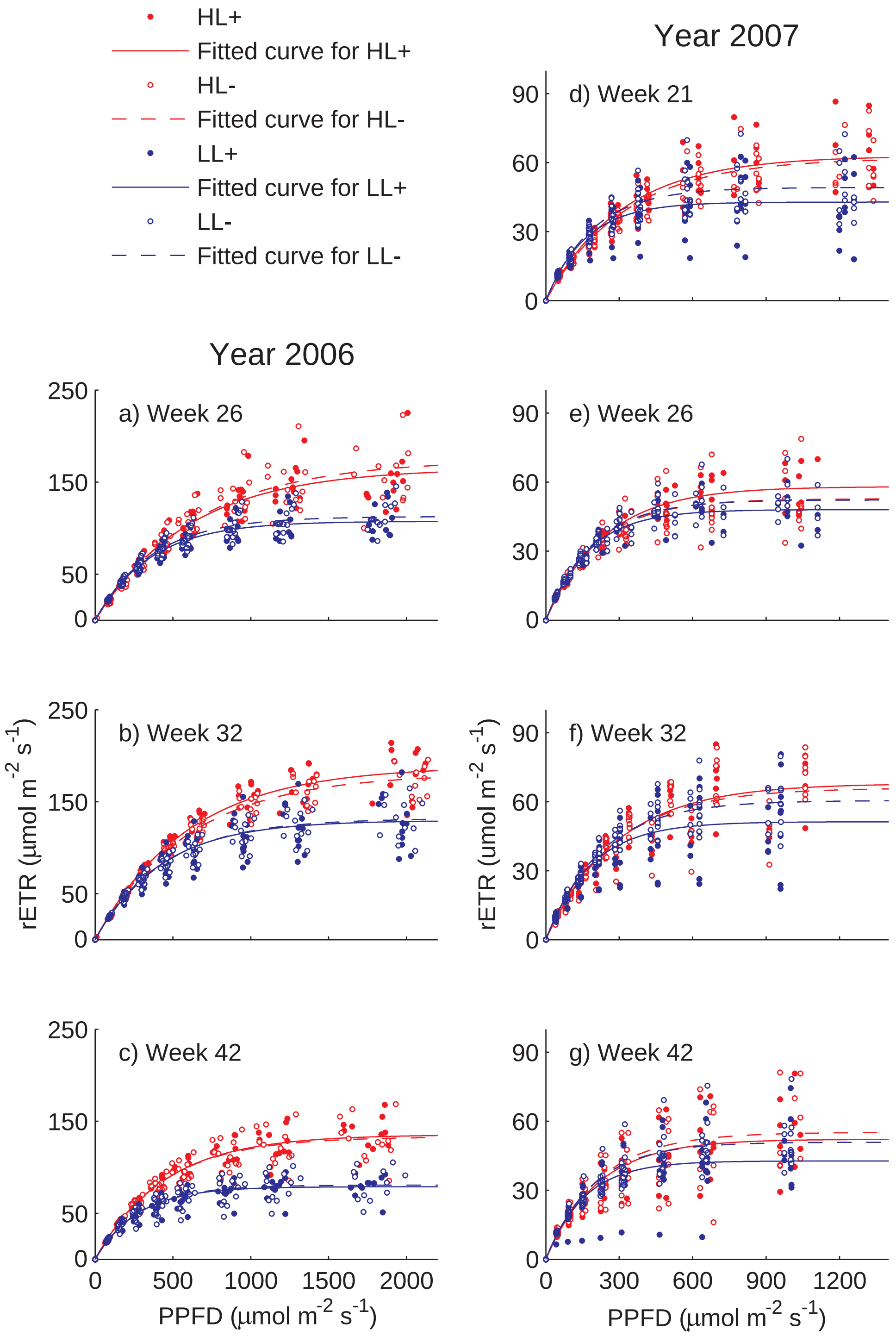

Fig. 3.8. rETR versus PPFD measured on selected trees (data points) in each of the four treatments (a-c) on three occasions in 2006 and (d-g) on four occasions in 2007. (Fitted curves from equation 3.4 and the extracted parameters given in Tables 3.3 and 3.4).

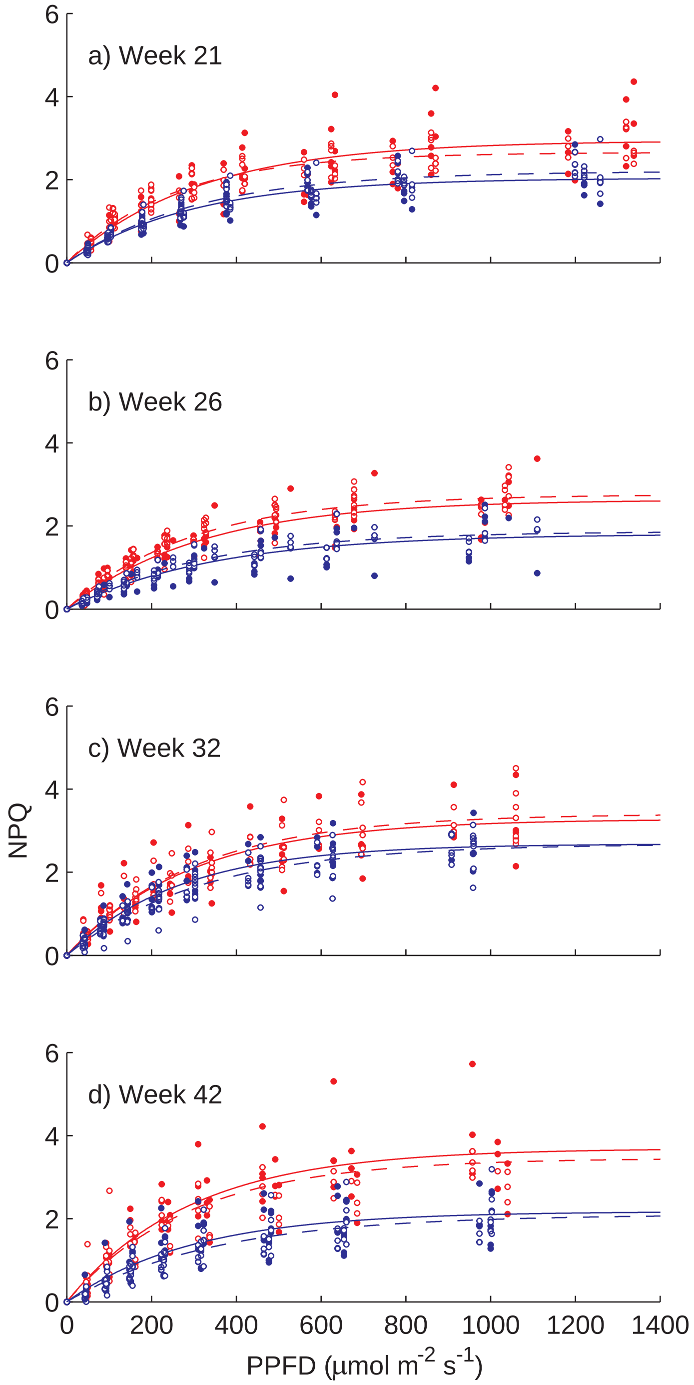

Fig. 3.9. NPQ versus PPFD measured on selected trees (data points) in each of the four treatments (a-d) on four occasions in 2007. (Fitted curves from equation 3.5 and the extracted parameters given in Tables 3.5 and 3.6; see Figure caption 3.8 for details).

Chapter 4

Physiological responses of Sitka spruce seedlings under variable light environments

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Abstract

A field experiment (F) was designed to look at the growth and the physiological response of naturally regenerated Sitka spruce seedlings across two light transmittance levels estimated at 23 % (intermediate light: F(IL)) and 17 % (low light: F(LL)), typical of the low impact silvicultural system named Continuous Cover Forestry (CCF). A comparison with the photosynthetic response of potted Sitka spruce seedlings grown in a controlled shadehouse experiment (C) that combined two levels of transmittance, 100 % (high light: C(HL)) and 24 % (low light: C(LL)), was also made. Chlorophyll fluorescence data indicated that field seedlings had acclimated to the shade environment. F(IL) field-grown seedlings exhibited a lower maximum photosynthetic capacity ($\mathrm{rETR_{max}}$) and a higher maximum heat dissipation energy ($\mathrm{NPQ_{max}}$) than C(LL) shadehouse-grown seedlings, despite the fact that both F(IL) and C(LL) seedlings had developed under similar 24% level of transmittance. At a similar transmittance level, the controlled C(LL) shadehouse light environment was more uniform with an absence of sunflecks whereas seedlings in the field experiment experienced a dynamic light regime with presence of sunflecks of moderate to high PPFD. We tentatively suggest that the requirement for photoprotective mechanisms was higher in F(IL) field-grown seedlings than in C(LL) shadehouse-grown seedlings. In the field experiment, the photosynthetic response of F(IL) and F(LL) seedlings was similar. This indicated that F(LL) seedlings may have acclimated to a dynamic light environment and developed a requirement for photoprotective mechanisms. However, F(IL) seedlings maintained current-year extension growth throughout the growing season, whereas F(LL) seedlings grew less than 2.5 cm $\mathrm{year^{-1}}$. The lack of correlation between extension growth and photosynthetic performance, yet a positive relationship between light transmittance and extension growth, suggests that integrated growing season percent PPFD is a primary factor controlling seedling growth in the field. Overall, the results of this study have important silvicultural implications regarding canopy interventions (i.e. thinning) to promote successful seedling growth during the transformation of Sitka spruce stands to CCF.

Keywords: natural regeneration, photoprotection, photosynthesis, *Picea sitchensis*, temporal heterogeneity

4.1 Introduction

Forests in the UK are dominated by even-aged stands of Sitka spruce (*Picea sitchensis* (Bong.) Carr.), mainly managed by clearfelling ($\sim$ 30% of total UK forest area; Anonymous, 2007). Recently, there has been much attention in the UK regarding adoption of low impact silvicultural management systems, often termed Continuous Cover Forestry (CCF), as an alternative to clearfelling. CCF aims at promoting structural diversity and natural regeneration (Mason et al., 2004; Mason and Kerr, 2004). A key factor in CCF systems is the successful establishment of natural regeneration. However, promoting natural regeneration in even-aged Sitka spruce stands faces several problems, which need to be taken into account, such as the light environment required and the damage that may be caused by the presence of green spruce aphid (*Elatobium abietinum* (Walker)), the main defoliator of Sitka spruce.

Much of our knowledge of the interactive effect of light environment and the presence of aphids on growth response and photosynthesis of Sitka spruce seedlings is based on large contrasting light levels (100 % versus 24 % transmittance level) for Sitka spruce seedlings grown in a controlled environment (i.e. artificial aphid infestation and light levels obtained with netting; Bertin et al., 2009b,c). Therefore, there is a need for experimental studies in the field to investigate if results obtained from a controlled experiment (Bertin et al., 2009b,c) are representative of the natural regeneration response. The objective of this study was to compare controlled and field conditions and to identify ways to improve Sitka spruce seedling regeneration success and rapidly evaluate plant performance below a Sitka spruce stand undergoing transformation to CCF.

In 2007, an experimental field site was established where plots were located across two light regimes typical of CCF conditions in upland UK coniferous forests. The experiment was initially designed to investigate the effect of natural outbreaks of green spruce aphid in combination with low light levels on the growth and the photosynthetic response of naturally regenerated Sitka spruce seedlings. However, no natural aphid outbreak occurred in the field in 2007 as very low aphid densities were encountered, and the results reflect the effect of light environment on Sitka spruce seedling growth and physiological characteristics.

4.2 Material and methods

4.2.1 Study site and sample plots

The design and methodology of the field experiment are described in detail in Bertin et al. (2009a). The study was carried out in Kielder forest, Northumberland, UK (55°13′N, 02°35′W, 190 m altitude), 120 km SW of Forestry Commission's Northern Research Station (NRS). Kielder forest is a 7-ha even-aged Sitka spruce stand planted in 1933 that had recently undergone transformation to CCF with thinning in scattered gaps carried out in 2000/2001 and 2005/2006 (Matthews, 1989). The site had an abundant natural regeneration of Sitka spruce seedlings ($> 5000\,\text{seedlings}\,\text{ha}^{-1} \leqslant 130\,\text{cm}$ height) occurring mainly in the $0 - 20\,\text{cm}$ height class. Understorey vegetation was scarce in the stand.

The experiment had four treatments with two light levels (intermediate light: IL and low light: LL) as main plots and two aphid infestation levels (presence: +A and absence: -A) as subplots. Each light level consisted of four plots of 2 m radius (Fig. 4.1; see method Bertin et al., 2009a). Mean transmittance level in IL and LL was $23.4 \pm 0.6\%$ (±SD) and $17.4 \pm 1.1\%$, respectively. The differences were narrow yet statistically significant ($P < 0.001$; Bertin et al., 2009a).

Each plot was manipulated to obtain two levels of aphid infestation (A+ and A-). Within the 2 m radius of each plot, two circular subplots of 1 m radius were set up opposite to each other. In the infested subplots, aphids were allowed to develop naturally (LL/A+ and IL/A+). Uninfested subplots were achieved by applying pirimicarb (Aphox®) insecticide to control green spruce aphid (LL/A- and IL/A-). Pirimicarb is non-systemic and has minimal phytotoxic properties (Straw and Fielding, 1998). Pirimicarb was sprayed to runoff on two occasions at the beginning of the study period (3 May 2007 (week 18) and 12 June 2007 (week 24)) on trees in the uninfested subplots, while the trees in subplots where aphids were maintained were sprayed with the equivalent volume of water. Because aphid densities were very low in the infested subplots, repeated applications of insecticide were not required during the study.

4.2.2 Seedling growth and aphid counting

Within each 1 m radius subplot, the initial number of seedlings was counted at the start of the 2007 growing season when no new recruits (newly germinated

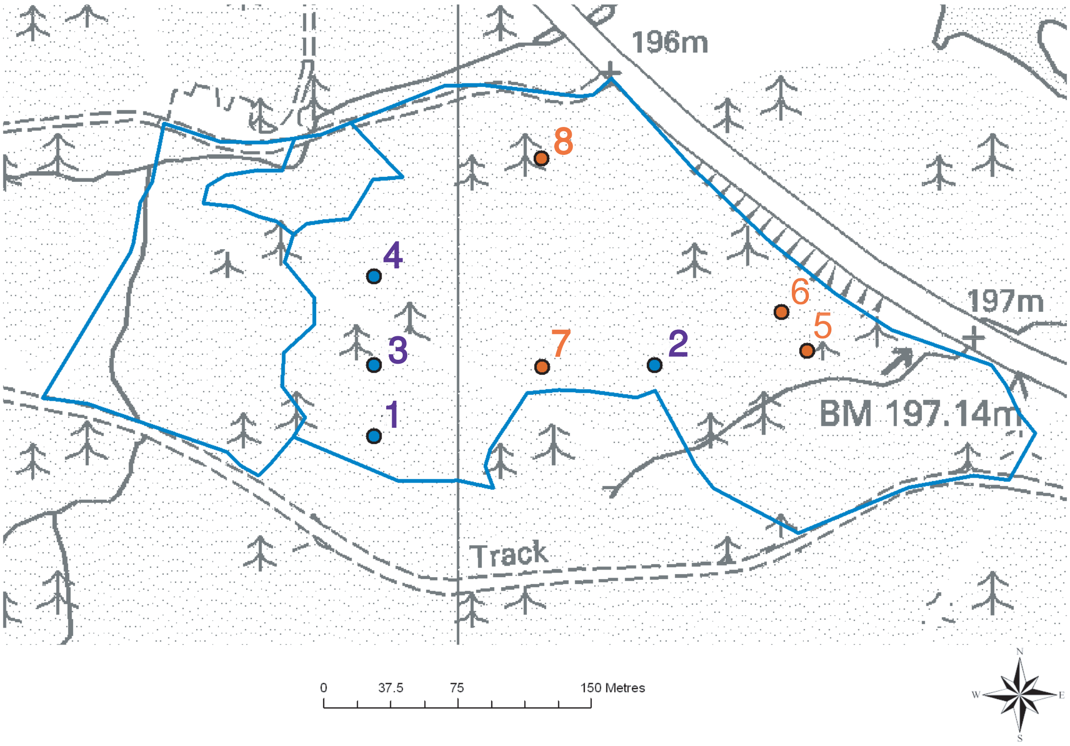

Fig. 4.1. Location of the eight selected plots in Kielder forest. The blue circles represent the low light level (plots 1 to 4; LL) and the red circles represent the intermediate light level (plots 5 to 8; IL). Plots 6, 7 and 8 correspond to plots where group selection was created during the last thinning operation (winter 2005/2006).

seedlings) were present. Seven seedlings were randomly collected four times throughout the 2007 growing season: at the beginning of the experiment (week 22); one month after budbreak (week 27), in August (week 33), and at the end of the growing season (week 15, 2008). New recruits were not sampled. Total aphid numbers per tree were counted in the laboratory on each sampling date. Total length height and current-year leader height of sampled seedlings were measured for the last three sampling periods (i.e. after budbreak had occurred).

4.2.3 Light environment

In the field experiment, mean transmittance level in the two light treatments was estimated from hemispherical photographs (see method in Bertin et al., 2009a). The transmittance calculated this way was equivalent to the method employed to measure the transmittance levels in the controlled experiment (Bertin et al., 2009b) where direct sensor measurements taken on an overcast day were used. Significant positive correlations have been reported between these two methods of light transmission estimation (Comeau et al., 1998; Gendron et al., 1998).

In addition, photosynthetic photon flux density (PPFD, $\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$) was measured at the centre of one IL plot (plot 6, Fig. 4.1) and one LL plot (plot 3, Fig. 4.1) during the growing season 2007 (June to November 2007). The two selected plots were considered to be representative of the two light classes explored in this study. Light readings in IL and LL plots were obtained using two matched pairs of quantum sensors (SD101QV-Cos, Macam Photometrics Ltd, Livingston, Scotland) placed at 1.3 m above the ground and connected to a CR1000 datalogger (Campbell Scientific, Logan, UT, USA) with measurements taken every 30 seconds and stored as 30-min averages. Light readings in the open were obtained from a matched pair of quantum sensors (SD101QV-Cos, Macam Photometrics Ltd, Livingston, Scotland) placed 1.3 m above the ground in a clearfelled area (open site) adjacent to the study area and connected to a CR10X datalogger (Campbell Scientific, Logan, UT, USA) with measurements every 30 seconds and stored as 30-min averages.

The spectral quality of the radiation in each plot was measured with a GER1500 spectroradiometer, where red/far-red ratio (R/FR) was defined as the photon fluence rate between 655 and 665 nm divided by photon fluence rate between 725 and 735 nm (Leuchner et al., 2007). All the spectroradiometer measurements were taken on 1 October 2007. Under clear blue sky conditions and around solar noon, six to ten consecutive measurements were taken at the centre of each plot, $\sim$ 1 m above the ground. An additional set of measurements was taken in an open site situated near the field experiment. R/FR was $1.00 \pm 0.03\%$ ($\pm$SD) in IL, $0.85 \pm 0.07\%$ in LL, and $1.06 \pm 0.02\%$ in the open site.

4.2.4 Chlorophyll *a* fluorescence

The fluorescence methodology employed at the field experiment is described in detail in Bertin et al. (2009c). To allow for comparison with the controlled experiment, the methodology applied in 2007 was used.

Chlorophyll *a* fluorescence was measured with a portable pulse modulated fluorometer (MINI-PAM, Heinz Walz GmbH, Effeltrich, Germany). Measurements of ambient PPFD were recorded with the MINI-PAM microquantum sensor fixed on the leaf-clip holder. The maximum effective quantum yield of photosystem

II (PSII), $F_{\mathrm{v}}/F_{\mathrm{m}}$, was calculated as $(F_{\mathrm{m}} - F_{\mathrm{o}})/F_{\mathrm{m}}$ (where F_{o} is the intrinsic fluorescence in dark-adapted tissue and F_{m} is the maximum fluorescence yield in dark-adapted tissue). The effective quantum yield of PSII, $F'_{\mathrm{q}}/F'_{\mathrm{m}}$, was calculated as $(F'_{\mathrm{m}} - F_{\mathrm{s}})/F'_{\mathrm{m}}$ (where F'_{m} is the maximum fluorescence yield in light adapted tissue and F_{s} is the steady state fluorescence in light adapted tissue) (Genty et al., 1989). Rapid light response curves (RLCs) of $F'_{\mathrm{q}}/F'_{\mathrm{m}}$ were obtained using the light curve program of the MINI-PAM (Rascher et al., 2000) and run on dark-adapted samples. Each RLC took about 240 s to complete with 9 PPFD steps each of 30 s duration in intensity from 0 to $\sim 1\,400\,\mu\mathrm{mol\,m^{-2}\,s^{-1}}$. The first step of each RLC corresponded to $F_{\mathrm{v}}/F_{\mathrm{m}}$. For each plot, before and after running the RLCs of all the trees within a plot, the PPFD emitted from the internal light source for each of the 9 PPFD steps was obtained by running the light curve program without any plant material but the dark leaf-clip placed above the MINI-PAM microquantum sensor in the same orientation as used for the needle sample. The two PPFD intensities for each PPFD step were then averaged and applied to the RLCs within the plot.

Relative electron transport rate (rETR) was calculated at each step of the RLC and was obtained as rETR $= F'_{\mathrm{q}}/F'_{\mathrm{m}}$ x PPFD x 0.5 x 0.84 where the factor 0.5 assumes that only half of the absorbed photons go into PSII and 0.84 is the empirical mean reflection factor for green leaves (Ehleringer, 1981). Non-photochemical quenching (NPQ) was also calculated at each step of the RLC as NPQ $= (F_{\mathrm{m}} - F'_{\mathrm{m}})/F'_{\mathrm{m}}$ where F_{m} was measured during the first step of each RLC (Bilger and Björkman, 1990; Bilger et al., 1995).

Five trees per subplot were chosen at the beginning of the experiment (one in each quarter of the subplot and one near the centre of the subplot). The measurements were repeated at three occasions on the selected trees through the growing season: 1) at the beginning of the experiment (week 22), 2) one month after budbreak (week 27), 3) in August (week 33). At each occasion, measurements were completed over one to two consecutive days. Each sample consisted of one-year old needles situated midway along side shoots of the previous year whorl.

4.2.5 Statistical analysis

Aphid densities were extremely low in the infested treatments through the whole study period. Consequently, for each light class, A+ and A- were combined. Mixed-effects models approach was used to analyse the data with light class considered a fixed effect. Different random effects were applied dependent upon the sampling procedure (see below for a detailed description of each method section). All continuous responses were assessed for normality and log transformed or weighted by least squares regression to account for heteroscedasticity in the residual variance where necessary. Tests for significance of main fixed effect were based on the Wald-test, with significance at $P = 0.05$.

Seedling growth

Growth data were analysed using a mixed effect model approach (REML) analysis of variance with the statistical software Genstat v8.01 (Genstat, 2005). Random factors were plots and subplots within plots.

Chlorophyll *a* fluorescence

Linear mixed-effects (LME) and nonlinear mixed-effects (NLME) models approach (Pinheiro and Bates, 2000) was used to analyse the fluorescence data in R (R Development Core Team, 2008).

Maximum effective quantum yield of PSII ($F_\mathrm{v}/F_\mathrm{m}$) and ambient PPFD

Analysis of $F_\mathrm{v}/F_\mathrm{m}$ and ambient PPFD were performed with an analysis of variance using separate LME models for each sampling date. Random factors were plots and subplots within plots.

Rapid light response curves (RLCs)

A saturating exponential function constrained to pass through the origin was used to model the relationship between rETR and PPFD (Rascher, personal communication; Rascher et al., 2000), and NPQ and PPFD (Ritchie, 2008). All parameters were estimated with maximum likelihood.

The rETR model is written:

$$\mathrm{rETR} = A \cdot (1 - \exp(-\exp(B) \cdot \mathrm{PPFD})) \tag{4.1}$$

The model parameters were A, the maximum rETR ($\mathrm{rETR}_\mathrm{max}$), and $\mathrm{rPPFD}_\mathrm{sat}$, defined as the PPDF level at which rETR is 90 % of $\mathrm{rETR}_\mathrm{max}$, calculated from

B. The fitted model had mixed effects on both A and B. The fixed effect was light class. The random effects included plots and trees nested within plots, but did not include subplot level in order not to overparameterise the model.

The NPQ model used is written:

$$\mathrm{NPQ} = C \cdot (1 - \exp(-\exp(D) \cdot \mathrm{PPFD})) \tag{4.2}$$

The model parameters were C, the maximum NPQ ($\mathrm{NPQ}_{\mathrm{max}}$), and $\mathrm{PPFD}_{\mathrm{sat}}$, defined as the PPDF level at which NPQ is 90 % of $\mathrm{NPQ}_{\mathrm{max}}$, and calculated from D. The fitted model had mixed effects on both C and D. The fixed effect was light class. The random effects were plots and trees nested within plots.

4.3 Results

Data from the field experiment (F) presented here and in the modelling paper in Bertin et al. (2009a) were compared with data from the second year (2007) of the controlled experiment (C) presented in Bertin et al. (2009b,c). In the controlled experiment only the data from the treatments called high light uninfested (HL-, 100 % transmittance level) and low light uninfested (LL-, 24 % transmittance level) were used. In the field experiment, we used the light treatment identifiers adopted in Bertin et al. (2009a), i.e. intermediate light (IL, 23 % transmittance level) and low light (LL, 17 % transmittance level). Low light (LL) treatment in the controlled experiment corresponded to similar 24% level of transmittance measured in the intermediate light (IL) treatment in the field experiment. Identifiers were kept to allow cross-comparison with the other studies. Therefore, for clarity, HL- and LL- from the controlled experiment will be named hereafter C(HL) and C(LL), respectively, while IL and LL from the field experiment will be called hereafter F(IL) and F(LL), respectively.

4.3.1 Aphid population

Natural green spruce aphid densities were low through the whole study period in the field experiment (Table 4.1). In comparison, aphid densities on infested seedlings in the controlled experiment reached 28 ±5 and 94 ±9 aphid·100 needles^{-1} in full light (100 %) and shade (24 %) conditions, respectively (Bertin et al., 2009b). Therefore, the two levels of aphid infestation (-A: uninfested and +A: infested) in the field experiment were combined together for each light class and only light effect was explored in the rest of the study.

Table 4.1. Total aphid number per tree in the four treatments in the field experiment in 2007. Values are mean ±1 SEM (n=28).

	F(IL)		**F(LL)**	
	-A	+A	-A	+A
Week 22	0.8 ± 0.3	3.9 ± 0.7	0.3 ± 0.1	0.6 ± 0.2
Week 27	0.1 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
Week 33	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1

4.3.2 Seedling growth

The initial number of seedlings per light treatment at the beginning of the field experiment was similar with 54 ± 6 (±SEM) seedlings m^{-2} in F(LL) and 45 ± 4 seedlings m^{-2} in F(IL). In both light treatments over 60 % of the seedlings were at least four-year old. The age of the seedlings was more widely distributed in F(IL) than in F(LL) (Table 4.2). Total length without current growth in F(IL) seedlings was not significantly different from that in F(LL) seedlings (light: $P = 0.52$, date: $P = 0.84$, light x date: $P = 0.74$), although there was a trend for F(IL) seedlings to be initially taller than F(LL) seedlings (Table 4.2).

The current-year leader height was used to represent seedling response to the different light classes in the field experiment. Current-year needles flushed in early June. In early July and at the end of the growing season, height of current-year leader was 3.4- and 4.9-fold greater, respectively, in F(IL) than in F(LL) seedlings (Table 4.2). The growth of the current-year leader continued during the whole growing season in F(IL) plots while in F(LL) plots the current-year elongation increased slowly until July and then ceased. Because the current-year leader growth might be affected by the initial height of the seedling, total height (without current year) was initially used as a covariate after log transformation. There was a significant difference between light ($P < 0.001$), date ($P < 0.001$) and light x date interaction ($P < 0.003$). A significant effect was also found for the covariate ($P = 0.015$) and for the interaction between the covariate and the light treatment ($P = 0.027$). The covariate had a positive effect and adjusted the log transformed mean of the current-year height by 0.22. However the covariate used here was probably a function of the treatment itself which in some respects invalidates it as a covariate (Quinn and Keough, 2003). The analysis without adjusting for seedling initial size (i.e. assuming that the plant response to treatment did not depend on the initial height) showed significant differences in current-year growth for light ($P < 0.001$) and date ($P < 0.001$) together with a light x date interaction ($P = 0.005$). The current leader height at the end of the growing season corresponded to the leader extension growth. Based on the standard errors of differences, both analyses showed that leader extension growth was significantly higher in F(IL) seedlings than in F(LL) seedlings at the end of the growing season.

Table 4.2. Descriptive statistics of seedling growth and age distribution in F(LL) and F(IL) classes in the field experiment during the growing season 2007. Values are mean ±1 SEM. For the age-class, the proportion of each age-class represents the relative amount of seedlings present in each age-class.

Light class	Sampling date	Leader 2007 height (cm)	Total length without current growth (cm)	Age-class (%)		
				2-yr	3-yr	$\geqslant$ 4-yr
F(LL)	Week 27 (2007)	1.4 ± 0.1	6.6 ± 0.4	5	13	82
	Week 33 (2007)	1.8 ± 0.2	6.8 ± 0.4	7	9	84
	Week 15 (2008)	1.7 ± 0.1	6.8 ± 0.5	9	13	79
F(IL)	Week 27 (2007)	4.7 ± 0.4	11.1 ± 1.3	16	24	60
	Week 33 (2007)	7.1 ± 0.6	10.0 ± 1.1	18	21	61
	Week 15 (2008)	8.4 ± 0.5	12.9 ± 1.6	21	21	58

4.3.3 Light environment

In order to compare the light environment in the controlled and field experiment, light measurements from the controlled experiment are presented (see method in Bertin et al., 2009b). The pattern of irradiance was different in the controlled and in the field experiment (Fig. 4.2). The light regime in the shadehouse was more uniformly distributed than in the forest. For example, on a representative sunny day, the C(LL) shadehouse never received PPFD $\geqslant 350\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$, whereas the selected F(IL) field plot experienced midday sunflecks of moderate to high PPFD ($> 500\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$; Fig. 4.2).

Over the study period in the field experiment, the frequency of PPFD $> 500\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ was above 35% in the open site, whereas it was less than 0.5% in the selected F(IL) and F(LL) field plots (Fig. 4.3). In the selected F(LL) field plot, PPFD was generally low with $\sim 85\%$ of PPFD $< 100\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$. PPFD frequency between $1\,00 - 300\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ was less than 10% in the selected F(LL) field plot while it was above 20% in the selected F(IL) field plot.

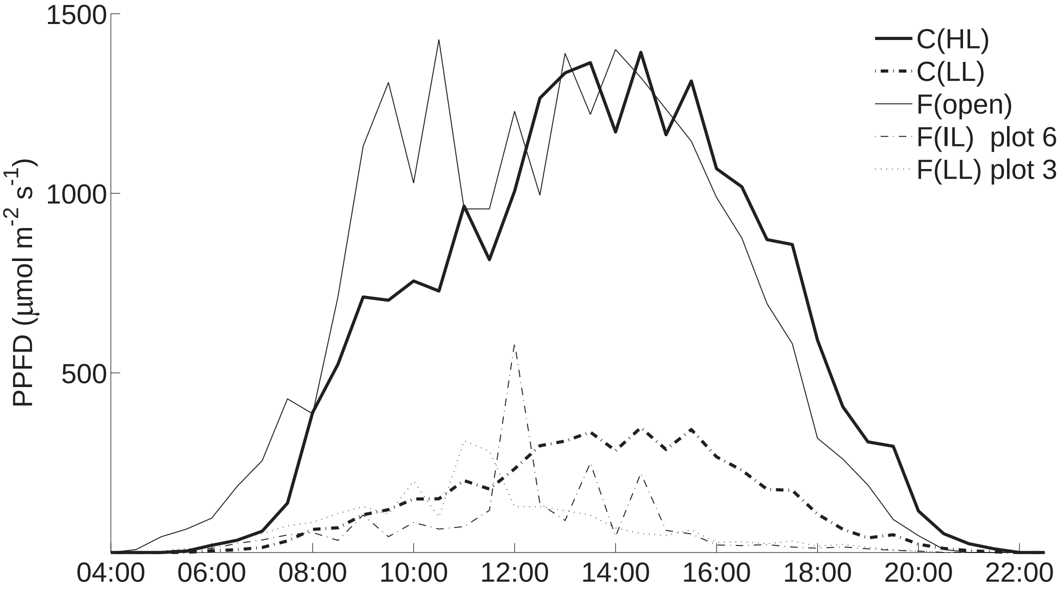

Fig. 4.2. Example of daily time course of PPFD in the different light treatments in controlled (C) conditions, in field (F) conditions in the selected IL and LL plots, and in an open clear-felled site in field (F) conditions on a representative sunny day (31 July 2007). Each point represents a 30-min average. Canopy transmittance determined by hemispherical photographs in F(IL) plot 6 and F(LL) plot 3 was 23.4 % and 18.7 %, respectively (see method in Bertin et al. (2009a)).

4.3.4 Chlorophyll *a* fluorescence

Maximum effective quantum yield of PSII (F_v/F_m) and ambient PPFD measurements

No significant differences in maximum effective quantum yield of PSII ($F_\mathrm{v}/F_\mathrm{m}$) were found between light treatments in the field experiment (Fig. 4.4 and Table 4.3). $F_\mathrm{v}/F_\mathrm{m}$ ratios were close to 0.8, a characteristic of non-stressed needles. At the time of measurement, ambient PPFD experienced by F(LL) and F(IL) seedlings were not significantly different (Fig. 4.5 and Table 4.3). Although not significant, ambient PPFD recorded in F(LL) class was always lower than in F(IL) class (Fig. 4.5).

Rapid light response curves (RLCs)

The RLCs obtained in the field experiment are shown in the supplementary material section (Figs. 4.6 - 4.8). Fitted curves from the double exponential

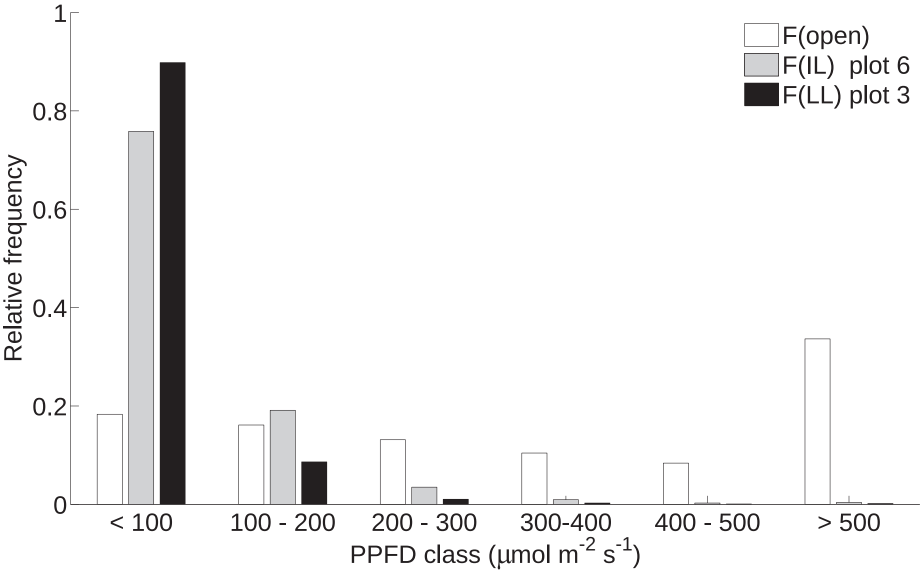

Fig. 4.3. Relative frequency of PPFD in the selected IL and LL plots, and in an open clear-felled site in field conditions over the growing season 2007 (01 June 2007 to 31 October 2007). The values were determined from the data averaged every 30 min during daylight hours.

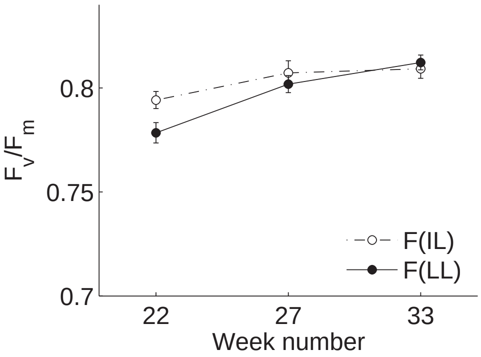

Fig. 4.4. Maximum effective quantum yield of PSII ($F_\mathrm{v}/F_\mathrm{m}$) in the field experiment in 2007. Values are mean ±1 SEM (n=40).

functions are also presented for information of $F'_\mathrm{q}/F'_\mathrm{m}$ versus PPFD (Fig. 4.6).

Throughout the whole field experimental period, there was a trend for lower $\mathrm{rETR_{max}}$ and $\mathrm{rPPFD_{sat}}$ in F(LL) compared with F(IL) (Fig. 4.7). The only significant differences found were a reduction in $\mathrm{rETR_{max}}$ in week 22 and a decrease in $\mathrm{rPPFD_{sat}}$ in week 33 (Table 4.4). $\mathrm{NPQ_{max}}$ and $\mathrm{PPFD_{sat}}$ did not differ significantly between the two light treatments, on any occasion (Fig. 4.8 and Table 4.5).

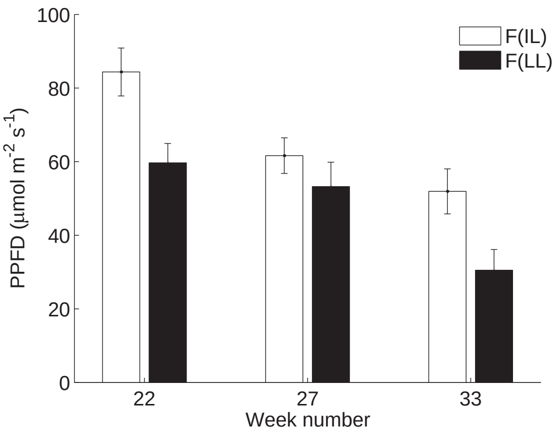

Fig. 4.5. Ambient PPFD experienced by the selected shoot before being dark-adapted in the field experiment in 2007. Values are mean ±1 SEM (n=40). Measurements were taken with the MINI-PAM microquantum sensor.

Table 4.3. Light treatment effect on maximum effective quantum yield of PSII (F_v/F_m) and ambient PPFD experienced by the selected shoot before being dark-adapted in the field experiment in 2007 (Wald tests for LME). Bold *P*-values indicate significant effect at $P < 0.05$.

		$\boldsymbol{F_v/F_m}$		**Ambient PPFD**	
	d.f	F	P	F	P
Light effect					
Week 22	1,6	1.59	0.25	0.97	0.36
Week 27	1,6	0.15	0.71	0.18	0.71
Week 33	1,6	0.19	0.68	0.63	0.46

Table 4.4. Parameter estimates and SEs extracted from NLME models for the rETR versus PPFD relationship (equation 4.1), and F- and P- values for light treatment effect on A and B parameter estimates in the field experiment in 2007 (Wald tests for NLME). 95 % confidence intervals calculated from B for rPPFD$_{\mathrm{sat}}$ ($\mu\mathrm{mol\,m^{-2}\,s^{-1}}$) are also given. Bold P-values indicate significant effect at $P < 0.05$.

	Parameter estimates		**Light effect**	
	F(IL)	F(LL)	F	P
$\boldsymbol{A} = \mathbf{rETR_{max}}$				
Week 22	39.9 ± 1.7	31.5 ± 1.7	11.85	**0.0006**
Week 27	33.4 ± 4.3	32.9 ± 4.3	0.01	0.94
Week 33	35.7 ± 3.6	26.4 ± 3.6	3.40	0.07
$\boldsymbol{B} \sim \mathbf{rPPFD_{sat}}$				
Week 22	-5.12 ± 0.06	-5.01 ± 0.06	1.48	0.22
Week 27	-4.83 ± 0.13	-4.88 ± 0.13	0.08	0.78
Week 33	-4.83 ± 0.12	-4.48 ± 0.12	3.94	**0.05**
$\mathbf{rPPFD_{sat} 95\%CI}$				
Week 22	(340, 433)	(305, 390)	·	·
Week 27	(222, 374)	(234, 395)	·	·
Week 33	(226, 367)	(159, 260)	·	·

Table 4.5. Parameter estimates and SEs extracted from NLME models for the NPQ versus PPFD relationship (equation 4.2), and *F*- and *P*- values for light treatment effect on *C* and *D* parameter estimates in the field experiment in 2007 (Wald tests for NLME). 95 % confidence intervals calculated from *D* for $\mathrm{PPFD}_{\mathrm{sat}}$ ($\mu\mathrm{mol\,m^{-2}\,s^{-1}}$) are also given. Bold *P*-values indicate significant effect at $P < 0.05$.

	Parameter estimates		**Light effect**	
	F(IL)	F(LL)	*F*	*P*
$C = \mathbf{NPQ_{max}}$				
Week 22	2.72 ± 0.25	3.07 ± 0.25	0.94	0.33
Week 27	2.29 ± 0.12	2.53 ± 0.12	2.07	0.15
Week 33	2.45 ± 0.15	2.42 ± 0.15	0.02	0.89
$D \sim \mathbf{PPFD_{sat}}$				
Week 22	-5.47 ± 0.07	-5.46 ± 0.07	0.01	0.93
Week 27	-5.43 ± 0.06	-5.43 ± 0.06	0.01	0.91
Week 33	-5.76 ± 0.07	-5.73 ± 0.07	0.12	0.73
$\mathbf{PPFD_{sat}}$ **95%CI**				
Week 22	(472, 628)	(468, 623)	·	·
Week 27	(467, 585)	(472, 591)	·	·
Week 33	(641, 837)	(619, 811)	·	·

4.4 Discussion

4.4.1 Comparison of photosynthetic response in field and controlled conditions

In order to compare fluorescence data across the four light regimes, two under field conditions (F(IL) and F(LL)) and two under controlled conditions (C(HL) and C(LL)), a summary of the data is compiled in Table 4.6. Since the design of the two experiments is different, the comparison of the observed results between the field and the controlled experiment is not based on statistical tests and only the observed trends are discussed here. The main interest was to compare the physiological response between F(IL) seedlings and 'shade' C(LL) phenotypes that have experienced similar 24% transmittance level but different experimental conditions.

The similar values of maximum effective efficiency of PSII ($F_{\mathrm{v}}/F_{\mathrm{m}}$) observed through the whole study period in F(IL) and F(LL) seedlings as compared to those in C(LL) (Table 4.6), which were grown with adequate nutrition, water and pot space conditions, indicated that field-grown seedlings were not exposed to any severe nutrient deficiency, temperature or water stress, or photodamaging conditions due to excess light (Huner et al., 1998; Grassi et al., 2001; Baker and Rosenqvist, 2004; Black et al., 2005).

Overall, photosynthetic capacity (measured as $\mathrm{rETR_{max}}$) and saturated PPFD (measured as $\mathrm{rPPFD_{sat}}$) of one-year old needles of F(IL) seedlings in the field experiment were closer to the values found in the 'shade' C(LL) phenotypes than in the 'sun' C(HL) phenotypes in the controlled experiment (Table 4.6). These results were consistent with the similar low transmittance level experienced by F(IL) and C(LL) seedlings, and indicated that F(IL) seedlings had acclimated to the shade environment. However, there was a shift toward lower $\mathrm{rETR_{max}}$ and $\mathrm{rPPFD_{sat}}$ values in F(IL) seedlings compared with C(LL) seedlings, whereas $\mathrm{NPQ_{max}}$ values in F(IL) seedlings were intermediate between the 'shade' C(LL) phenotypes and the 'sun' C(HL) phenotypes in the controlled experiment (Table 4.6). Interpretation of these results are given with the caveats required when comparing data from field and controlled experiments. Firstly, the history 'traits' of the seedlings were different. While shadehouse-grown seedlings were

Table 4.6. Ambient PPFD measured with the MINI-PAM microquantum sensor, maximum effective quantum yield of PSII ($F_\mathrm{v}/F_\mathrm{m}$) and fluorescence parameters obtained from the RLCs in the controlled and field experiments in 2007 (see Bertin et al. (2009c) for controlled experiment details).

	Trans-mittance level (%)	**Ambient PPFD**	$F_\mathrm{v}/F_\mathrm{m}$	**rETR parameter estimates**		**NPQ parameter estimates**	
				$\mathrm{rETR}_\mathrm{max}$	$\mathrm{rPPFD}_\mathrm{sat}$	$\mathrm{NPQ}_\mathrm{max}$	$\mathrm{PPFD}_\mathrm{sat}$
		Mean ± SEM	Mean ± SEM	Mean ± SE	(95 % CI)	Mean ± SE	(95 % CI)
Weeks 21-22							
C(HL)	100	952 ± 148	0.75 ± 0.009	61.9 ± 4.6	(634, 885)	2.66 ± 0.16	(514, 650)
C(LL)	24	240 ± 26	0.80 ± 0.003	49.2 ± 4.6	(371, 518)	2.20 ± 0.16	(627, 799)
F(IL)	23	84 ± 7	0.79 ± 0.004	39.9 ± 1.7	(340, 433)	2.72 ± 0.25	(472, 628)
F(LL)	17	60 ± 5	0.78 ± 0.005	31.5 ± 1.7	(305, 390)	3.07 ± 0.25	(468, 623)
Weeks 26-27							
C(HL)	100	854 ± 66	0.79 ± 0.005	52.7 ± 3.3	(419, 536)	2.76 ± 0.21	(575, 807)
C(LL)	24	215 ± 18	0.81 ± 0.004	52.3 ± 3.3	(391, 499)	1.87 ± 0.21	(611, 871)
F(IL)	23	62 ± 5	0.81 ± 0.006	33.4 ± 4.3	(222, 374)	2.29 ± 0.12	(467, 585)
F(LL)	17	53 ± 7	0.80 ± 0.004	32.9 ± 4.3	(234, 395)	2.53 ± 0.12	(472, 591)
Weeks 32-33							
C(HL)	100	1172 ± 151	0.79 ± 0.012	66.0 ± 6.3	(528, 759)	3.41 ± 0.17	(588, 813)
C(LL)	24	122 ± 19	0.82 ± 0.006	60.5 ± 6.1	(431, 613)	2.68 ± 0.16	(624, 858)
F(IL)	23	52 ± 6	0.81 ± 0.005	35.7 ± 3.6	(226, 367)	2.45 ± 0.15	(641, 837)
F(LL)	17	31 ± 6	0.81 ± 0.004	26.4 ± 3.6	(159, 260)	2.42 ± 0.15	(619, 811)

all of the same age and selected from improved genotypes, naturally regenerated seedlings would be expected to introduce greater structural (i.e. seedlings of different ages and sizes) and inherent genetic (i.e. seed quality) diversity (Nixon and Worrell, 1999). Photosynthetic capacity is also sensitive to provenance and plant age (Ludlow and Jarvis, 1971; Larcher, 1995). The rather higher photosynthetic capacity of C(LL) seedlings than that observed in F(IL) seedlings may be partly attributed to the improved genotypes of the seedlings measured in the controlled experiment. Secondly, the light environment experienced in field and shadehouse conditions differed. While the spectral quality (RFR) of the C(LL) shadehouse treatment mimicked RFR found in F(IL) field conditions (Bertin et al., 2009b), the distribution of the light regime provided by C(LL) shadehouse was relatively uniform with an absence of sunflecks (i.e. similar to filtered and diffuse sky radiations, Fig. 4.2). It differed from F(IL) field conditions below-canopy where light environment was highly dynamic due to the occurrence of sunflecks (i.e. short period of moderate to high PPDF alternating with long period of low PPDF, Fig. 4.2). A large body of literature has shown that temporal heterogeneity of natural dynamic light environment in the forest can have important implications for leaf physiology, light utilisation, photosynthesis and carbon gain (Ellsworth and Reich, 1992; Wayne and Bazzaz, 1993; Bloor, 2003; Leakey et al., 2005; Adams et al., 1999). Plants grown in low light or under a plant canopy have typically low photosynthetic capacity (Givnish, 1988). Consequently, when receiving sunflecks of moderate to high PPDF, only a fraction of the absorbed energy can be used in PSII photochemistry, while the remaining absorbed fraction can be harmful to the light-harvesting complexes if not dissipated adequately. Dissipation of heat energy in PSII by thermal mechanisms, as assessed by NPQ, represents an important photoprotection mechanism of plants under excess light and assists in the avoidance of photodamage of the photosynthetic apparatus (Müller et al., 2001). Over short-term or diurnal time-scales, the fraction of light that is dissipated thermally is usually mirrored by the total size of the xanthophyll cycle component pool and the engagement of the de-epoxidised components of the xanthophyll cycle (Demmig-Adams and Adams, 1992, 1996). It is now well established that the size and the conversion state of the xanthophyll cycle components acclimate to the prevailing light under which the plant has developed (Demmig-Adams, 1998; Demmig-Adams et al., 1996, 1995). Field studies have shown that shade leaves of plants acclimated to low light environments subjected to sunflecks show increased size and extent of de-epoxidised components of the xanthophyll cycle compared with shade leaves of low light acclimated plants without sunflecks. The presence of enhanced de-epoxidised xanthophyll components facilitates the harmless dissipation of excess light directly within PSII (Adams et al., 1999; Logan et al., 1997). Despite the fact that F(IL) and C(LL) seedlings had grown under similar light transmittance level (i.e. $\sim 24\%$), the observed higher maximum excess energy dissipation (as assessed by $\mathrm{NPQ_{max}}$) in F(IL) than in C(LL) seedlings suggested that the requirement for photoprotective mechanisms was higher in field- than in shadehouse-grown seedlings, due to the higher variability of solar irradiance in field conditions (Table 4.6, Fig. 4.2). The lower maximum photosynthetic capacity (as assessed by $\mathrm{rETR_{max}}$) of F(IL) seedlings than that of C(LL) seedlings under excess PPFD (Table 4.6) supports the finding of Demmig-Adams et al. (1995) who showed increased thermal energy dissipation to be accompanied by a decrease in the effective quantum yield of PSII (i.e. $F'_{\mathrm{q}}/F'_{\mathrm{m}}$) during exposure to excess light and associated it to a regulatory and photoprotective role. A decrease in $\mathrm{rETR_{max}}$ may constrain photosynthetic carbon gain in field conditions in comparison with controlled conditions. In addition, the lower $\mathrm{rPPFD_{sat}}$ (i.e. higher photochemical efficiency) by F(IL) seedlings than that of C(LL) seedlings may reflect the lower average ambient irradiance in the forest than in the shadehouses as evidenced by measures of ambient PPFD from the fluorometer microquantum sensor (Table 4.6). F(IL) field seedlings presented a tradeoff between shade adaptation and photoprotective mechanisms to avoid photodamage.

4.4.2 Linking photosynthetic response and extension growth in field conditions

Only small differences in photosynthetic parameters were observed between F(LL) and F(IL) seedlings in the field experiment (Tables 4.4 and 4.5). This result suggests that the one-year old foliage of F(LL) seedlings had developed under similar dynamic light environment than that of F(IL) seedlings (i.e. experiencing sunflecks). However, despite similar photosynthetic performance of the seedlings in the two light classes, the extension growth in F(LL) seedlings was lower than in F(IL) seedlings (Table 4.2). The fact that extension growth ceased in July in F(LL) seedlings while it was sustained through the whole growing season in F(IL) seedlings meant that differences in age structure and initial seedling size between the two light treatments were not enough to explain differences in leader growth (Table 4.2). Bertin et al. (2009a) found a positive relationship ($R^2 = 0.73$) between leader extension growth in 2007 and transmittance, measured with hemispherical photographs, in the eight Kielder plots in 2007. Transmittance measured by hemispherical photographs is a good proxy of integrated growing season percent PPFD in forests (Gendron et al., 1998). The results of Bertin et al. (2009a) suggests that the lower integrated growing season percent PPFD in F(LL) class than in F(IL) class resulted in a shorter assimilatory activity period in F(LL) seedlings, which may explain the observed lower leader extension growth in F(LL) seedlings despite similar photosynthetic parameters in F(LL) and F(IL) seedlings. In addition, the reduction in leader extension growth of F(LL) seedlings may also be attributed, in part, to other factors: 1) Changes in light quality may have affected extension growth. Light quality in conifer forest understorey shifts towards lower RFR as foliage increases (Leuchner et al., 2007). Ballaré et al. (1991) showed that higher RFR triggers stem elongation rate. Consequently, the lower RFR measured in F(LL) plots in comparison with F(IL) plots could partly explain the reduction in leader extension growth in F(LL) seedlings. 2) Seedlings may have adopted different strategies in the two light classes in the field experiment. The ability to temporarily slow growth in low light environments has been reported in shade-tolerant species and may improve the chance of survival by lowering costs associated with structural maintenance and by increasing allocation to attributes that benefit long-term survival such as root growth (Beaudet and Messier, 1998; Walters and Reich, 1999; Kobe, 1997). Thus, determinate extension growth at the beginning of the growing season in F(LL) seedlings suggests that preferential allocation to stems and roots took place for the rest of the growing season.

4.5 Conclusion

Light transmittance level and extension growth provide useful guidance to forest managers for evaluating if an increase in transmittance level is needed to promote seedling growth and recruitment. Having determined that an intervention is required, the manager must then decide which type of silvilcultural intervention to apply. However, transmittance level *per se* is not an adequate stand-alone indicator for deciding the type of intervention. The results of this study show that the ability to develop photoprotective mechanisms to avoid photodamage was most probably a function of the light temporal heterogeneity experienced by the seedlings rather than the transmittance level. Consequently, a uniform opening (i.e. thinning) of the stand may be required for seedlings that had grown under diffuse light conditions (i.e. seedlings with higher risk of suffering photodamage), while canopy interventions designed to develop scattered gaps may create a favourable environment for seedlings that had previously experienced dynamic light environments (i.e. seedlings with the ability to dissipate excess light energy when receiving sunflecks). A field portable fluorometer could be used as a rapid and non-invasive tool to evaluate the level of non-photochemical quenching of the seedlings, and consequently the type of thinning intervention required to promote successful seedling growth.

4.6 Acknowledgements

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4.8 Supplementary material

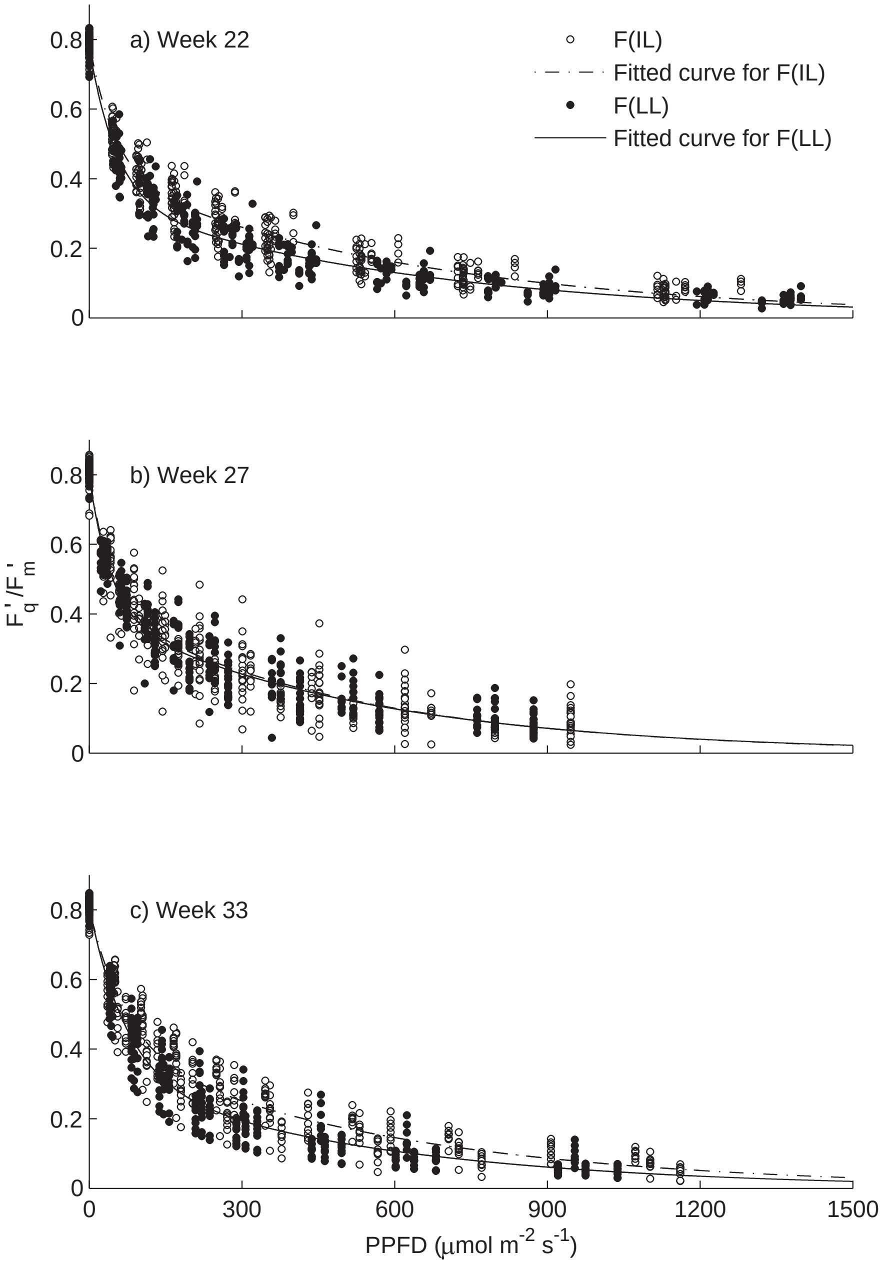

Fig. 4.6. $F'_\mathrm{q}/F'_\mathrm{m}$ versus PPFD measured on selected trees (data points) in each of the two light treatments (a-c) on three occasions in the field experiment in 2007. (Fitted curves from double exponential functions).

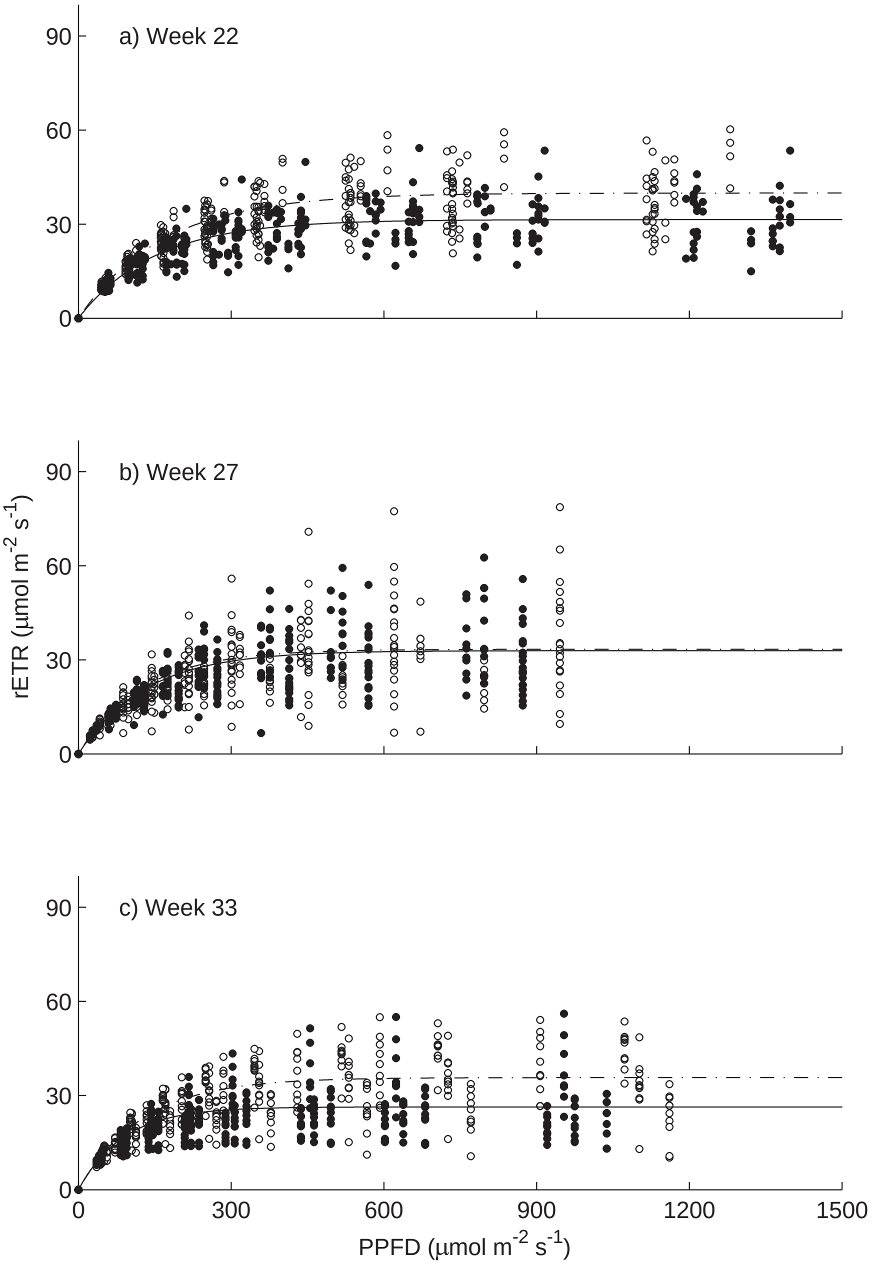

Fig. 4.7. rETR versus PPFD measured on selected trees (data points) in each of the two light treatments (a-c) on three occasions in the field experiment in 2007. (Fitted curves from equation 4.1 and the extracted parameters given in Table 4.4; see Figure caption 4.6 for details).

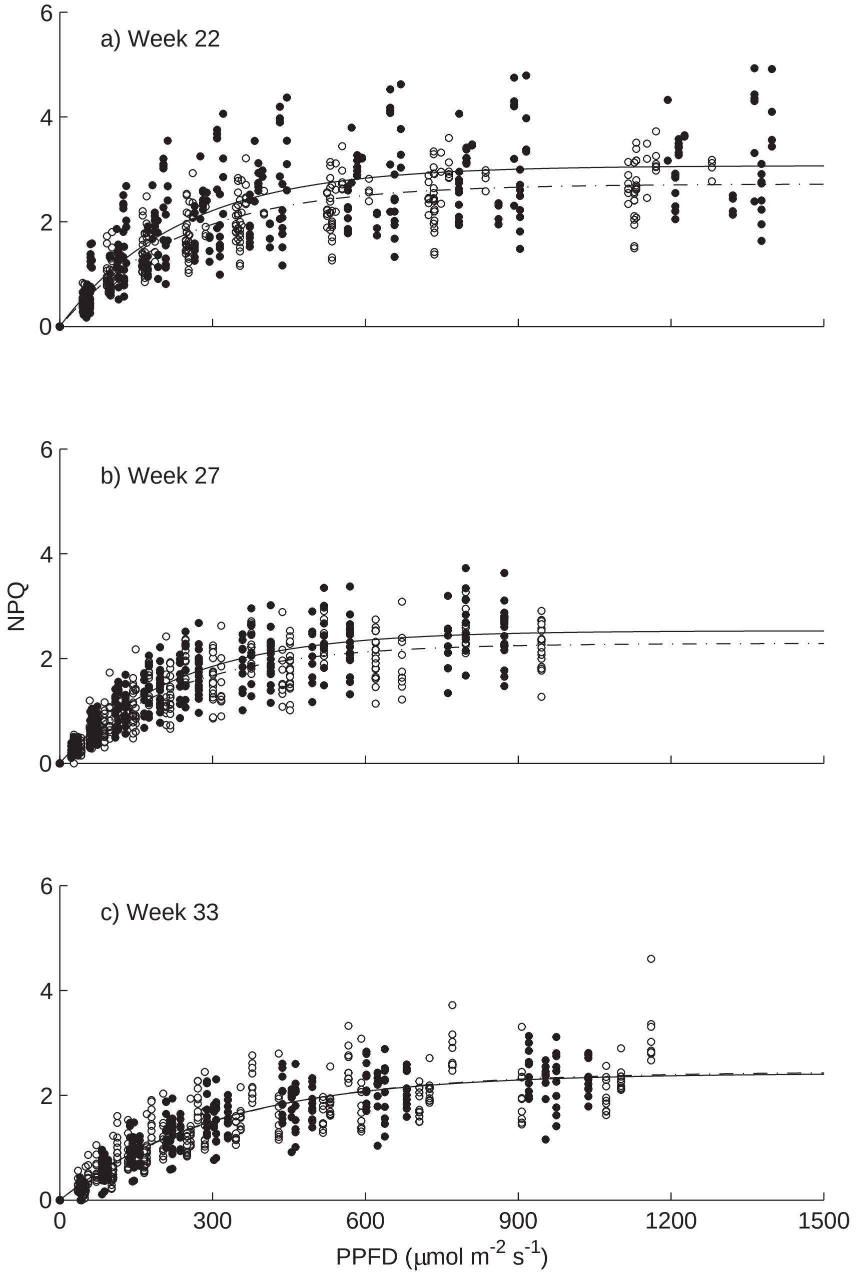

Fig. 4.8. NPQ versus PPFD measured on selected trees (data points) in each of the two light treatments (a-c) on three occasions in the field experiment in 2007. (Fitted curves from equation 4.2 and the extracted parameters given in Table 4.5; see Figure caption 4.6 for details).

Chapter 5

Modelling understorey light for seedling regeneration in Continuous Cover Forestry canopies

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Abstract

Continuous cover forestry (CCF), an alternative forest management approach to clearfelling, is increasingly being adopted in the UK. It aims at enhancing the diversity of the stand structure and favouring natural regeneration and subsequent seedling growth below the existing canopy. To give guidance on how to manage a CCF stand to achieve satisfactory seedling growth beneath an existing canopy an estimate of light transmitted through the canopy is required. So far, in the UK, only stand-level parameters such as basal area (BA) have been used to predict the understorey light environment in CCF stands. In this study, we assessed a UK Sitka spruce stand undergoing transformation to CCF and measured canopy transmittance in the field using hemispherical pictures. Stand-level characteristics were found to be highly stand-specific and not appropriate to predict seedling growth in CCF stands. We present parameterisation of a detailed light model, the 4C-A-RTM, for a UK stand and compared it with a simple Beer-Lambert law (BL). A sensitivity analysis was performed for both models to test the effect of key stand structural parameters on the estimation of transmitted light. We used transmittance assessment obtained from hemispherical photographs to test the models. The two light models tended to underestimate canopy transmittance. Despite the observed underestimation both approaches showed a very strong positive relationship with the current-year growth of the below canopy seedlings ($R^2 = 0.92$). Comparison of the two models showed that the 4C-A-RTM provided a better estimation of light transmittance across observed canopy structural differences. Furthermore the inclusion of stand characteristics in the 4C-A-RTM is likely to infer greater applicability across stands.

Keywords: Beer-Lambert law, Continuous Cover Forestry, 4C-A-RTM light model, seedling growth, Sitka spruce

5.1 Introduction

Alternative management systems to clearfelling are being increasingly adopted in Great Britain as a means of increasing species and structural diversity of plantation forests (Mason et al., 2004; Mason and Kerr, 2004). These regimes are often termed Continuous Cover Forestry (CCF). CCF aims to maintain canopy cover at one or more levels and is characterised by favouring natural regeneration for seedling recruitment. Nearly 30 % of total forest area in the UK is made up of plantations of even-aged Sitka spruce (*Picea sitchensis* (Bong.) Carr.), an exotic species representing the most important commercial species in the UK (Anonymous, 2007). Guidance in the identification of sites suitable for transformation to CCF in the UK to date has used information on wind risk, soils and stand management history (Mason and Kerr, 2004). The adoption of CCF systems requires changes in silvicultural practices, yet there is little experience in the UK in transforming even-aged stands of coniferous plantations to continuous cover management (Mason and Kerr, 2004).

Light is one of the most important factors controlling seedling survival and growth (Lieffers et al., 1999). The critical light level required for survival and growth of seedlings and young trees under an existing canopy of trees is not well quantified (Malcolm et al., 2001; Hale et al., 2004), whilst existing manual methods for estimating the quantity of light reaching the regeneration below the canopy are often not practical for routine use. Nevertheless estimates of light transmittance through canopies are required to underpin management guidance on creating a suitable light environment for natural regeneration.

So far, in the UK, stand-level characteristics have been used in management as indicators of understorey light levels (Hale, 2001; Page et al., 2001) and consequently the potential for regeneration success. UK field studies in Sitka spruce stands suggest that a minimum of $15 - 20\,\%$ canopy transmittance is needed to achieve seedling growth which can be obtained by thinning to reduce stand basal area (BA) below $30\,\mathrm{m}^2\,\mathrm{ha}^{-1}$ (Hale, 2001; Page et al., 2001). Current published guidance to assist UK forest managers in achieving successful natural regeneration utilises measurements of BA in conjunction with individual site assessment (Hale, 2004). However, because transformation to CCF is new to Britain, there is little data on the performance of natural regeneration in low BA stands, impeding the formulation of definitive guidance regarding the threshold light regime required for seedling growth (Hale, 2001). Hale (2001) recommended a focus upon measurements on stands with a $BA < 30\,\mathrm{m}^2\,\mathrm{ha}^{-1}$, thus generating more data to quantify better the relationships between BA, light under the canopy and the growth of naturally regenerated Sitka spruce seedlings.

The relationship between stand-level characteristics and light transmittance is likely to vary for a given species, depending on its stand structure and management history. Therefore, it is more adequate to measure below-canopy light than BA for instance (Sonohat et al., 2004). Kobe and Hogarth (2007) found that percent canopy openness provided the best indicator of growth for below canopy young trees. Transmittance of diffuse light, a proxy for canopy openness, can be assessed using (1) direct measurements of light intensity, (2) hemispherical photographs analysed for canopy gaps (Rich, 1990; Beaudet and Messier, 2002), and (3) radiative transfer models (RTMs). Quantifying light in forests through direct measurements is difficult due to high spatial and temporal variability (Gendron et al., 1998). Similarly hemispherical photographs can be useful if the sampling strategy accounts for site spatial variability. RTMs, or light models, provide an alternative method for estimating understorey light. RTMs are commonly categorised based on how they treat canopy architecture (i.e. the arrangement of the light intercepting elements). The simplest approach, often called a turbid medium model, and here referred to as BL (i.e. based on the Beer-Lambert law), is to apply a light extinction function through an assumed horizontally homogeneous, single-layer canopy (Hale, 2001). More detailed light models typically divide canopy into layers and account for the density profile of leaf area index, or divide the leaf area among individual tree crowns. Most of these models, if designed for coniferous stands, account for shoot characteristics, such as leaf angle distribution (mostly assumed spherical) and shoot clumping (the arrangement of needles around shoots), but for simplicity nearly all assume that these characteristics are constant with depth in the canopy (Wang et al., 1992). Some RTMs are capable of accounting for the effects of stand structure, explicitly (e.g. spatial models using tree coordinates; Brunner, 1998; Coates et al., 2003) or statistically (e.g. models using mean and distribution of tree and shoot clumping; Duursma and Makela, 2007; Chen et al., 2008; Giuliani and Brown, 2008).

In this study, light availability was considered to be the key factor for predicting seedling establishment and early seedling growth. Simple and complex RTMs were used to predict understorey light. The simple BL model was applied following the practical approach suggested by Hale (2001). The complex RTM model statistically accounted for variation in stand structure and represented a compromise between detailed 3-D models such as MAESTRA (Wang and Jarvis, 1990; Medlyn, 2004), and BL. It was originally constructed as a sub-model of the Canopy Conductance-Constrained Carbon Assimilation (4C-A) model, hereafter named 4C-A-RTM (Schäfer et al., 2003; Kim et al., 2008).

There were three objectives in this study: i) To explore the relationship between canopy transmittance assessed by hemispherical pictures and 1) stand-level characteristics (BA and stand density (SD)), and 2) seedling growth, in a

UK Sitka spruce stand undergoing transformation from even-aged silviculture to CCF management stand, assessed across a wide range of BA. ii) To parameterise and to test the sensitivity of BL and the 4C-A-RTM. 1) A sensitivity analysis of the 4C-A-RTM was conducted in order to test the effect of uncertainty in stand structural inputs on modelled transmittance. 2) Comparison between BL and the 4C-A-RTM was made over a range of selected inputs. iii) To validate the two models by 1) comparing modelled transmittance against measured transmittance from hemispherical photographs and, 2) evaluating modelled transmittance with respect to observed seedling growth.

5.2 Material and methods

5.2.1 Stand description

The study was carried out in an even-aged Sitka spruce stand located in Capon Burn, Kielder forest, Northumberland, UK (55°13′N, 02°35′W, 190 m altitude). The 7-ha stand, planted in 1933, has recently begun transformation to CCF using a ‘group selection’ silvicultural system (Matthews, 1989) and with thinning interventions carried out in 2000/2001 and 2004/2005. Thus the stand was at an early stage of transformation to CCF, ending its stem exclusion stage and entering the understorey reinitiation phase (Olivier and Larson, 1996, ; W.L. Mason, personal communication). The thinning operations were expected to provide fine-scale variation in below-canopy light environment. The stand had abundant natural regeneration of Sitka spruce ($\leqslant 130$ cm in height; $n > 5000\,\text{ha}^{-1}$) occurring mainly in the $0 - 20$ cm height class.

5.2.2 Sampling design and seedling growth measurements

The stand was assessed in January 2007 using the methodology for monitoring CCF stands described by Kerr et al. (2002). Thirty-seven 0.05 ha (12.6 m radius) circular plots were inventoried, recording diameter at breast height (d, cm) for trees $\geqslant 7$ cm. Of the 37 plots, 29 were located on a uniform grid and eight plots corresponded to the position of the group selections created during the last thinning operation. Seedling density was assessed in a 2 m radius regeneration circle at the centre of each 0.05 ha plot. Transmittance at a height of 1.3 m was recorded at the centre of each plot using hemispherical photographs (see below).

Following the site assessment in winter 2007, sampling points where either the seedling density was lower than $10\,\mathrm{seedling\,m^{-2}}$ or the understorey competition was influencing seedling growth were disregarded. The remaining sample plots were classified according to estimated transmittance. Four plots that experienced lowest light levels and four with highest light levels were retained for the purpose of the experiment and assigned to low (LL) and intermediate (IL) light classes, respectively (Table 5.1).

To assess accurately the abundant seedling regeneration within the eight selected plots, two circular subplots of 1 m radius were set up opposite to each other within the 2 m radius regeneration circle. Within each 1 m radius subplot, the initial number of seedlings was counted at the start of the growing season 2007 when no new recruits (newly germinated seedlings) were present. Within each 1 m radius subplot, seven seedlings were randomly collected at the end of the 2007 growing season and their current-year stem height (i.e. current-year leader extension growth) was measured. The extension growth was used to represent seedling response to the different light treatments. New recruits were not sampled.

Table 5.1. Range of observed values for the low light (LL) and intermediate light (IL) plots sampled in Kielder. For basal area (BA) and stand density (SD), only the mean value is given because they refer to the whole plot.

Light class	**Plot ID**	**Transmittance** (%)^a	$\boldsymbol{BA}$ ($\mathrm{m^2\,ha^{-1}}$)	$\boldsymbol{SD}$ ($\mathrm{trees\,ha^{-1}}$)	$\boldsymbol{d}$ (cm)	**Tree height** (m)	**Crown depth** (m)	**Crown width** (m)
LL	1	17.3–18.3	40.4	420	24.3–51.9	22.3–32.1	5.8–16.5	2.8–7.6
	2	15.8–16.7	40.6	320	24.0–57.2	21.6–31.7	5.1–15.5	3.2–9.7
	3	18.4–19.3	33.1	320	25.0–49.2	20.1–31.3	5.9–15.7	3.4–8.2
	4	16.4–16.8	42.0	400	20.8–58.2	23.3–35.8	4.7–17.9	2.2–9.7
IL	5	22.5–22.9	25.5	200	14.5–59.7	15.2–35.1	6.4–18.1	2.9–10.2
	6^b	22.7–24.1	26.5	200	30.0–52.1	24.2–33.8	7.6–16.9	5.4–9.0
	7^b	23.1–23.8	37.2	200	34.6–70.5	29.1–36.7	6.7–17.1	4.8–8.9
	8^b	24.0–24.3	40.6	180	42.2–68.8	31.4–35.7	9.6–17.0	6.2–12.1

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[a] % transmittance calculated from hemispherical photographs taken at 1.3 m height

[b] Group selection plot from 2004/2005 thinning

5.2.3 Canopy openness and transmittance

Canopy openness is defined as the fraction of open sky in a hemisphere that is visible from a point beneath the canopy (Jennings et al., 1999) and can be used as an index of light availability experienced by the seedlings. As a proxy for openness in this study, transmittance of diffuse light through the canopy gaps was used. Accordingly, the Indirect Site Factor (ISF) was estimated from the hemispherical photographs where ISF is defined as the proportion of diffuse solar radiation reaching a given location, relative to a location with no sky obstructions (Delta-T Devices, Cambridge, UK). A corresponding index was obtained from the two models used in this study, the 4C-A-RTM or BL, by calculating the transmittance of diffuse light averaged over the hemisphere. The transmittance calculated this way was equivalent to ISF and diffuse non-interceptance (DFIN) produced by the commonly used plant area analyser LAI-2000 (Li-Cor, Inc., Lincoln, Nebraska).

Three hemispherical photographs were used to characterise each of the eight study plots. One was taken at the centre of each plot and one at the centre point of each of the two 1 m radius regeneration subplots. A Nikon FC-E8 fish-eye lens with a field of view of 183 ° was used on a Nikon CoolPix 4500 digital camera. The camera was mounted on a tripod at a height of 1.3 m and the lens was levelled. The images were acquired under overcast sky conditions. To calculate the ISF, image analysis was undertaken using Hemiview (Delta-T Devices, Cambridge, UK), with manual thresholding to classify sky and canopy elements.

5.2.4 4C-A-RTM and BL models parameterisation for Sitka spruce

For the parameterisation of the 4C-A-RTM and BL, additional measurements were collected in the eight 0.05 ha selected plots during autumn 2007, recording total tree height, crown depth and crown width for trees $\geqslant 7$ cm d. Tree inventory data were summarised and SD and BA were calculated for each plot (Table 5.1). The base of the crown was defined as the lowest live branch, which was covered by foliage over 75 % of its length. Crown depth was calculated by subtracting the height to base of crown from the total tree height. Two crown widths were measured (N-S and E-W) and mean crown width was calculated for each tree.

5.2.4.1 Parameterisation of the 4C-A-RTM for the study plots

The 4C-A-RTM allows all shoot characteristics, as well as the average crown diameter to vary vertically. The 4C-A-RTM was first employed in an even-aged *Pinus taeda* L. plantation in Duke forest, North Carolina, USA (Schäfer et al., 2003). Kim et al. (2008) further developed the model by including tree distribution pattern and adapted it for application to broadleaved and mixed conifer-broadleaved stands.

To predict the transmittance for each of the eight plots, the 4C-A-RTM light model required inputs at three scales (Schäfer et al., 2003; Kim et al., 2008): i) site latitude, longitude and altitude, ii) mean values of the study plot characteristics (mean tree height, mean crown length, mean crown width, stand density) to account for grouping of tree distribution (i.e. tree clumping; Nilson, 1999), iii) vertical profiles of plant area index (PAI) comprised of leaf area index (LAI) and woody area index (WAI), and shoot characteristics (Campbell and Norman, 1998; Stenberg, 1998).

Estimation of PAI vertical profiles for each plot

The 4C-A-RTM uses PAI (L_t, $\mathrm{m}^2\,\mathrm{m}^{-2}$) defined as hemi-surface (i.e. half-total surface) area per unit ground surface area and takes into account hemi-surface LAI (L_l, $\mathrm{m}^2\,\mathrm{m}^{-2}$) and hemi-surface WAI ($L_w$, $\mathrm{m}^2\,\mathrm{m}^{-2}$) where $L_t = L_l + L_w$. This definition was used as opposed to the conventional definition where PAI is projected (i.e. one-sided) area per unit ground surface area (Chen and Black, 1992). For conifer needles which are shaped as circular cylinders, projected PAI is related to hemi-surface PAI by a factor of $2/\pi$.

For each plot, vertical profiles of PAI ($L_t(z)$, $\mathrm{m}^2\,\mathrm{m}^{-2}$) at 1-m canopy layer resolution were calculated from information collected at the stand level and from data available in the literature as described below. The variable z represented height in the canopy. Because the model was applied to a conifer stand composed of a species retaining needles for many years, it was assumed that L_t was invariant over the study period.

The hemi-surface leaf area for each tree (A_l, m^2) within a plot was estimated using the relationship between A_l and diameter at breast height (d, cm), developed by McIntosh (1984), in a 23-year-old Sitka spruce plantation in southern Scotland to calculate projected leaf area, adjusted by a factor of $\pi/2$

with:

$$A_l = (3.96 \cdot d - 26.92)(\pi/2), (d > 7 \text{ cm}) \tag{5.1}$$

The hemi-surface woody area (A_w, m^2) included branches (A_b, m^2) and stem (A_s, m^2) assuming $A_w = A_b + A_s$. The hemi-surface woody area was estimated using a woody-to-total area ratio (α). α was assumed to be a species-specific constant (Nilson, 1999). The ratio for individual trees was used to represent ratio per unit ground area based on the formula:

$$\alpha = A_w/(A_w + A_l) \tag{5.2}$$

hence:

$$A_w = (\alpha/(1 - \alpha)) \cdot A_l \tag{5.3}$$

Data on the woody-to-total area ratio have not been reported for Sitka spruce in the UK and α was therefore estimated from reports on other conifer species. Homolová et al. (2007) calculated a woody-to-total area ratio (hereafter named Homolová woody-to-total area ratio, α_H) of ~ 0.22 in two mature montane Norway spruce forest stands based on optical measurements (LAI-2000) of unaltered and then totally defoliated mature Norway spruce trees. However, indirect PAI measurements using LAI-2000 or similar instruments overestimate α by not taking into account the branch area masked by leaves or needles in intact canopies (Chen, 1996; Kucharik et al., 1998). In an 80-year-old Norway spruce plantation in Estonia the ratio of branches covered by needles within the canopy (c) was estimated at 0.35 ± 0.08 (E. Eensalu and M. Therezien, personal communication). α was therefore calculated by correcting α_H to account for c. Equations 5.2 and 5.3 were used to calculate the hemi-surface woody area estimated with α_H (A_{wH}):

$$A_{wH} = (A_l \cdot \alpha_H)/(1 - \alpha_H) \tag{5.4}$$

$$A_s = h_{cr} \cdot \pi \cdot (d/200) \tag{5.5}$$

where A_s was obtained using data collected from the stand: tree height at the base of the live crown (h_{cr}, m) and d (cm). h_{cr} and not total tree height was used as it was assumed that the bole within the crown was mostly covered by branches and leaves and therefore negligible in the model simulation. The surface area of branch participating in radiation attenuation was calculated as:

$$A_b = (A_{wh} - A_s) \cdot (1 - c) \tag{5.6}$$

applying the c obtained as described above.

The vertical distribution of A_l within the tree crown was described from the shape of the leaf area profile of a mature Norway spruce tree (H.S. Kim, personal communication; Ward et al., 2008) which was normalised over the canopy length and applied to the Sitka spruce data set. To generate the hemi-surface LAI profile ($L_l(z)$) of a plot, $A_l(z)$ of all individual trees within the plot were summed up for each zth layer and divided by the plot area. The same modelling approach was used to create the branch hemi-surface area index (BSAI) profile ($L_b(z)$) of a plot, using $A_b(z)$ and the shape of a branch surface area profile available for 25-year-old lobolly pine stand (H.S. Kim, personal communication), with more branches distributed at the bottom of the tree crown. A linear approach was used to create the stem hemi-surface area index (SSAI) profile ($L_s(z)$) of a plot by equally distributing A_s through each z^{th} layer of the bole. $A_s(z)$ was then divided by the plot area.

Naturally, the cumulative sum of $L_l(z)$, $L_b(z)$ and $L_s(z)$ along the vertical profile of a plot corresponded to its total L_l, total L_b, and total L_s, respectively. The total L_w was calculated by summing up total L_b and total L_s with $L_w = L_b + L_s$. The cumulative input values of the canopy components of PAI are summarised in Table 5.2.

Table 5.2. Characteristics of the eight Kielder plots where validation test was performed.

Light class	**Plot ID**	**No. of layers** z (m)	**Canopy length** (m)	**Total** L_l ($\mathrm{m^2\,m^{-2}}$)	**Total** L_b ($\mathrm{m^2\,m^{-2}}$)	**Total** L_s ($\mathrm{m^2\,m^{-2}}$)	**Total** L_t ($\mathrm{m^2\,m^{-2}}$)
LL	1	32	19	7.2	1.1	0.4	8.7
	2	32	19	6.4	0.9	0.4	7.7
	3	32	21	5.7	0.9	0.3	6.9
	4	36	21	7.1	1.0	0.4	8.5
IL	5	35	30	3.9	0.6	0.2	4.7
	6	34	22	4.2	0.6	0.2	5.0
	7	37	22	5.0	0.7	0.3	6.0
	8	35	17	5.2	0.7	0.3	6.2

Shoot characteristics

The 4C-A-RTM light model can account for vertical gradients in the characteristics of shoots by integrating shoot clumping factors (CF) and leaf angle distribution profiles. In this study, it was assumed that needles were spherically distributed (Campbell and Norman, 1998). CF profiles ($\beta(z)$) in this Sitka spruce stand were assumed to be similar to those calculated by Therezien et al. (2007) using input variables (shoot and needle dimensions) obtained from an 80-year-old Norway spruce plantation in Estonia (E. Eensalu, personal communication). CF profiles were normalized by the canopy height of each plot. The range of values used for CF was 0.43 – 0.47.

5.2.4.2 Parameterisation of BL model

In BL, the transmittance (τ) was calculated based on the formula:

$$\tau = \exp\left(-K \cdot L_e\right) \tag{5.7}$$

where L_e ($\mathrm{m}^2\,\mathrm{m}^{-2}$) was the hemi-surface area index of canopy elements cumulated from top of the canopy, and K is the extinction coefficient. The definition of L_e used in BL was equivalent to the one proposed by (Chen and Black, 1992) which was also used in the 4C-A-RTM. A constant value of 0.5 was assumed for K. It corresponded to the theoretical value of K when the radiation source (the sun) is directly above the canopy and with a leaf angle distribution assumed spherical (Campbell and Norman, 1998).

5.2.5 Sensitivity analysis and validation of the 4C-A-RTM and BL

First, a sensitivity analysis of the 4C-A-RTM was performed to test the effects of a number of selected inputs on transmittance predictions at 1 m above ground. The sensitivity analysis was run for each of the eight plots and results averaged by light class. The sensitivity of transmittance to the different canopy component of PAI (i.e. L_l, L_b and L_s) was tested. The values for each component of PAI in all layers were varied in turn by ±10% and ±30% without modifying the other base input values. For any input, the relative change in transmittance was calculated as (altered - base)/base.

Second, comparison between the 4C-A-RTM and BL was made over a range of L_l and a range of stand densities (SD). Input files from plot 5 were used as the base input values (Tables 5.1 and 5.2). Plot 5 was selected because it represented the plot which had the most variation along its vertical profile of LAI. The range of L_l used was 1.6, 3.9, 6.3 and $9.4\,\mathrm{m^2\,m^{-2}}$ and the range of SD used was 100, 200, 500, 1000, and $2000\,\mathrm{trees\,ha^{-1}}$, with no tree clumping. Two scenarios were run. First, woody material was not included in the models ($L_e = L_l$). In the second scenario, woody material was added to the models with $L_e = L_l + L_w$ with only L_l being varied. L_w base input value calculated for plot 5 was used and kept constant ($0.9\,\mathrm{m^2\,m^{-2}}$). Naturally the effect of the SD for a specific L_e could only be tested with the 4C-A-RTM where transmittance for each L_e was tested along the range of SD. The sensitivity of the two models predictions to variation in input values was assessed graphically.

Finally, validation of the two models was performed by two approaches: 1) by comparing the modelled transmittance versus the estimated transmittance outputs from the hemispherical pictures, 2) by evaluating the modelled transmittance against the current-year leader extension growth of seedlings measured for each of the eight studied plots. The base input values were used to run the 4C-A-RTM. In BL, transmittance was predicted using $L_e =$ total L_t (Table 5.2) and $K = 0.5$ for each plot.

5.2.6 Statistical analysis

Data were analysed using a mixed effect model approach (REML) with the statistical software Genstat v8.01 (Genstat, 2005). Light treatment was considered a fixed effect, with plots and subplots within plots as random factors. All continuous response data were assessed for normality and transformed where necessary: the typical transformation was logarithmic. The significance level was set at $P = 0.05$. R^2 was used as a measure of variation explained by the models.

5.3 Results

5.3.1 Stand structure and transmittance characteristics

The range of transmittance estimated from hemispherical photographs among the eight selected plots was narrow (Table 5.1), yet transmittance significantly discriminated the two light classes (LL = 17.4% versus IL = 23.4%; $P < 0.001$). SD was higher in the LL plots (range $3\,20 - 420\,\mathrm{trees\,ha^{-1}}$) than in the IL plots

($180 - 200\,\mathrm{trees\,ha^{-1}}$). The range covered by BA did not vary much between the two classes (range 33.1 – 42 $\mathrm{m^2\,ha^{-1}}$ for LL plots and 25.5 – 40.6 $\mathrm{m^2\,ha^{-1}}$ for IL plots). The diameter distribution of trees was that of an even-aged stand (Mason and Kerr, 2004) in the LL plots. In the IL plots, because of the recent thinning treatments, the distribution of diameters was more uneven.

5.3.2 Seedling growth in relation to light regime

The initial number of seedlings per light treatment at the beginning of the experiment was similar with 54 ± 6 (±SEM) $\mathrm{seedlings\,m^{-2}}$ in LL and 45 ± 4 $\mathrm{seedlings\,m^{-2}}$ in IL. By the end of the growing season 2007, the current-year leader extension growth was significantly higher in IL seedlings than in those of LL (IL $= 8.4 \pm 0.5$ (±SEM) $\mathrm{cm\,year^{-1}}$ versus LL $= 1.7 \pm 0.1$ $\mathrm{cm\,year^{-1}}$; Fig. 5.1).

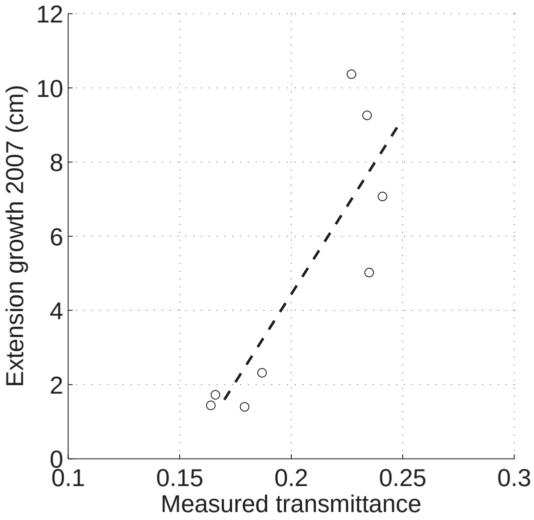

Fig. 5.1. Extension growth as a function of measured transmittance from hemispherical photographs at the eight Kielder plots ($y = 94.78x - 14.52$; $R^2 = 0.73$).

5.3.3 Parameterisation of the 4C-A-RTM for the study plots

Figure 5.2 illustrates the vertical profiles of LAI at each plot. On a relative basis, apart from plots 5 and 8, the shape of cumulative L_l did not vary much among the plots with L_l accumulation starting between range $30 - 40\,\%$ of the relative height (Fig. 5.2a). In all plots, L_l was concentrated at the upper canopy (Fig. 5.2a). In absolute terms, L_l was much higher for LL than IL plots (Fig. 5.2b). BSAI vertical profiles were forced to have a similar pattern to LAI vertical profiles.

The woody-to-total area proportion applied for this stand was 17 % with branches accounting for $73 \pm 2\%$ of the woody area. The higher SD, the higher was total L_t (Table 5.2).

Figure 5.3 shows the spatial distribution of the different canopy elements of PAI (i.e. L_l, L_b and L_s) along the height in the two different light classes. Mean total L_t for LL and IL reached 4.2 and $2.9\,\mathrm{m^2\,m^{-2}}$, respectively, with a peak of foliage and branches occurring at a greater height in LL than in IL (Fig. 5.3).

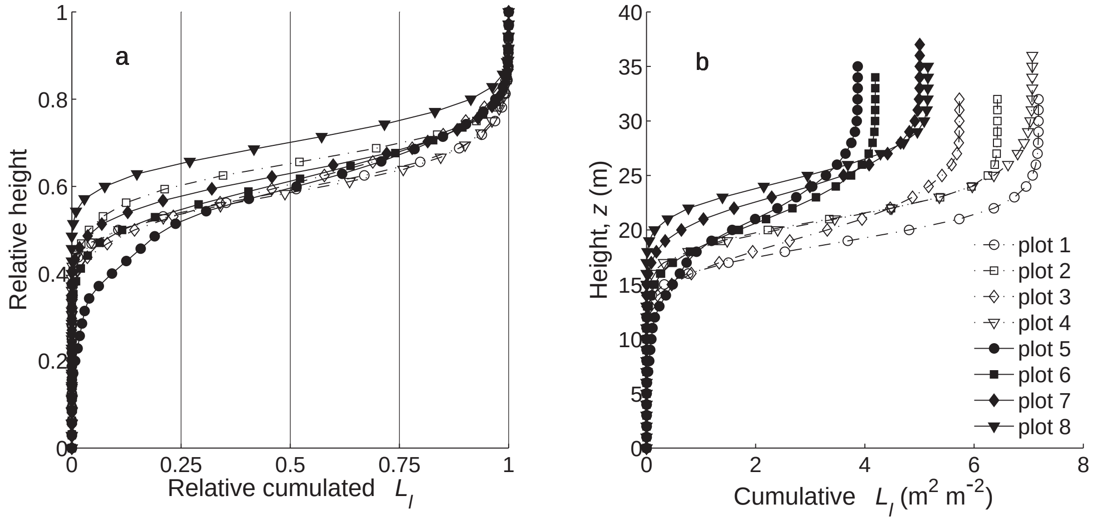

Fig. 5.2. a) Relative accumulation of L_l for each plot and, b) cumulated L_l for each plot. (Solid lines with filled symbols, and dashed lines with empty symbols correspond to LL and IL plots, respectively).

5.3.4 Sensitivity analysis of the 4C-A-RTM and BL

Figure 5.4 illustrates the sensitivity of the transmittance modelled with the 4C-A-RTM to the different canopy components of PAI tested in LL and IL. In both light classes, transmittance was highly sensitive to variation in L_l and, as it can be expected based on theoretical considerations, more influenced by a decrease than by an increase in L_l (Fig. 5.4a). The sensitivity of the transmittance to changes in L_l differed most between LL and IL when the base L_l value was reduced by 30 % (Fig. 5.4a). Since changes in transmittance were expressed on a relative basis, greater impact was found in LL where the base output transmittance was initially lower, as compared with IL. Change in transmittance was modest and almost linearly related to variations in L_b and L_s (Fig. 5.4b,c) with larger differences between LL and IL when the base input value was increased or decreased by 30 %. When running the model without woody material (i.e.

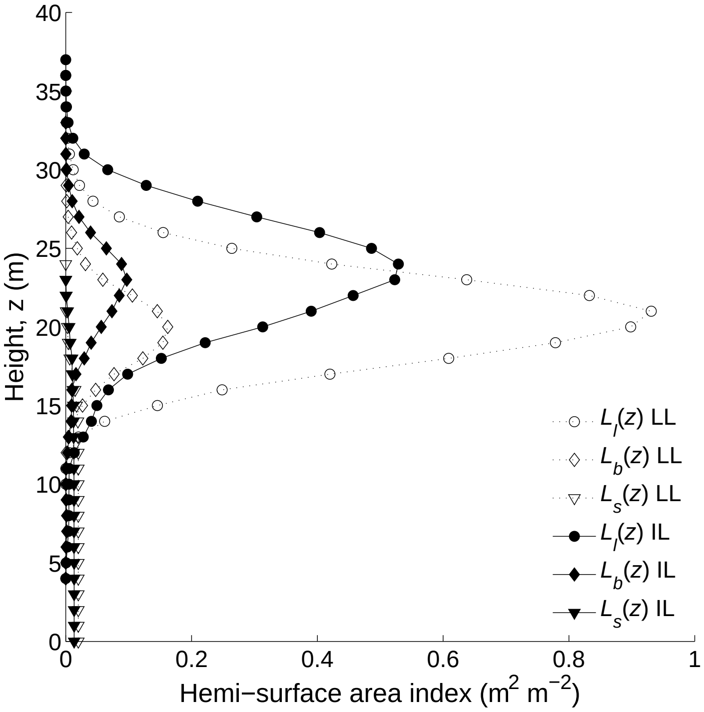

Fig. 5.3. Vertical profiles of the different canopy elements composing PAI for LL (dotted lines) and IL (continuous lines). Each light class represents the mean of 4 plots. Circles indicate L_l , diamonds L_b and inverted triangles L_s, where L_l, L_b and L_s are half-surface leaf area, branch area and stem area indices, respectively.

$L_t = L_l$), a substantial relative increase in transmittance estimates was observed (LL $= +138 \pm 17\%$ ($\pm$SD); IL $= +92 \pm 15\%$).

Estimation of transmittance of the 4C-A-RTM was sensitive to variations in shoot CF. When no shoot CF was applied in the model (i.e. $\beta(z) = 1$), a relative decrease in transmittance of $-84 \pm 2\%$ ($\pm$SD) and $-77 \pm 2\%$ was calculated in LL and IL, respectively. Not taking into account tree clumping in the model affected the relative decrease in transmittance less but with a stronger effect in LL ($-22 \pm 2\%$ ($\pm$SD)) than in IL ($-12 \pm 2\%$).

The comparison of the 4C-A-RTM with BL over a range of values of L_l and SD is presented in Figure 5.5. In the first scenario, when no woody material was included in L_e, and at all values of SD, BL always underestimated the transmittance compared with the 4C-A-RTM (Fig. 5.5a). In the second scenario where woody material was incorporated, the same trend was observed for a L_l of 3.9, 6.3 and 9.4 $\mathrm{m^2 m^{-2}}$ but lower in magnitude (Fig. 5.5b). In scenario 2, at the lowest L_e, BL and the 4C-A-RTM were in close agreement at all SD levels. In both scenarios, the lowest SD (100 trees $\mathrm{ha^{-1}}$) always corresponded to the highest transmittance compared with other values of SD for any given L_e (Fig. 5.5). Also, at high stand densities ($SD \geqslant 1000\,\mathrm{trees\,ha^{-1}}$) the estimated transmittance did not differ from that estimated when no tree clumping was applied in the 4C-A-RTM model (Fig. 5.5). Whether woody material was taken into account or not, most of the variation in transmittance was found at intermediate L_l (3.9 and $6.3\,\mathrm{m^2\,m^{-2}}$) where lower SD further increased the differences between the 4C-A-RTM and BL (Fig. 5.5).

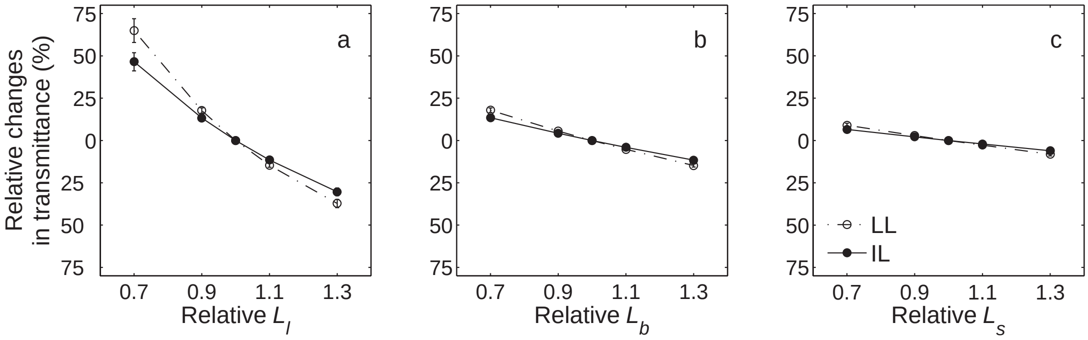

Fig. 5.4. Relative changes in transmittance predicted by the 4C-A-RTM as a result of variation in the different canopy elements composing PAI in LL and IL. Each light class represents the mean of 4 plots. Error bars are ±1 SD of the mean.

5.3.5 Validation of the 4C-A-RTM and BL

When looking at transmittance prediction from the two models compared with measured transmittance from photographs, Figure 5.6 shows that both the 4C-A-RTM and BL models systematically underestimated transmittance. Nevertheless both models differentiated between LL and IL plots (Fig. 5.6). Regression in each model showed increasing spread around the line for observed transmittance above 0.2, which corresponded to the plots in IL class (Table 5.1). BL predicted values were always much lower than the 4C-A-RTM predicted values. The quality of the fit was slightly higher in the 4C-A-RTM model ($R^2 = 0.63$) than in BL ($R^2 = 0.59$).

Despite underestimation of modelled transmittance values, results from both models predicted very well the seedling growth of the studied plots (Fig. 5.7). Modelled transmittances from both models were strongly and linearly related to measures of seedling growth (Fig. 5.7). The variation in transmittance values simulated by the two models explained most of the observed variation in seedling extension growth ($R^2 = 0.92$ for both the 4C-A-RTM and BL, Fig. 5.7).

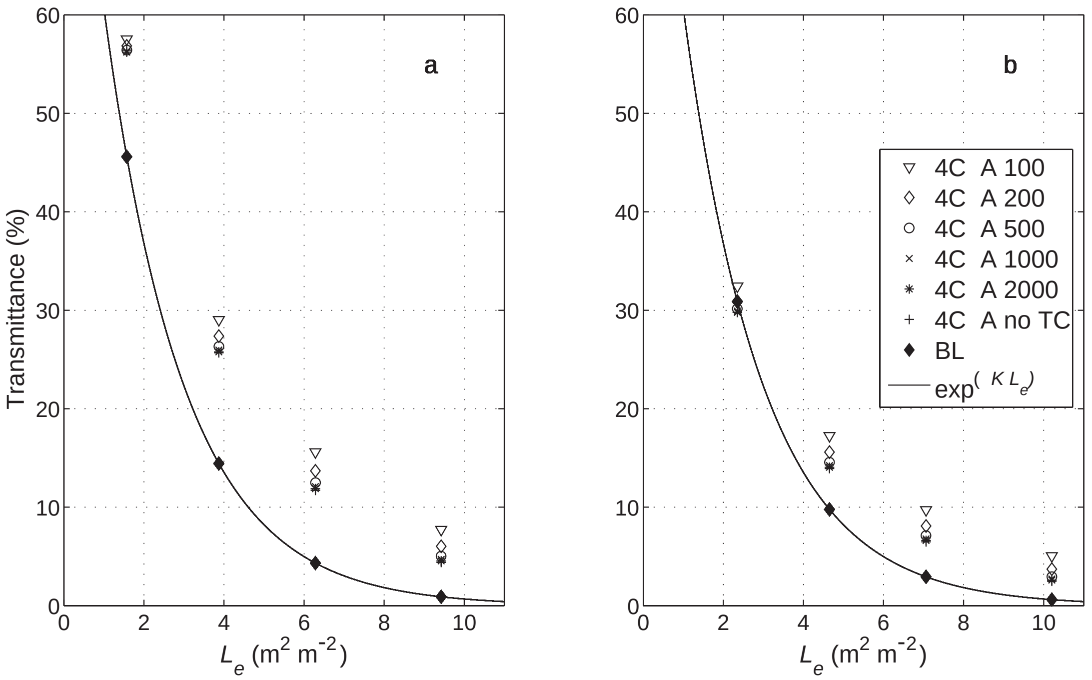

Fig. 5.5. Comparison of the transmittance output of the 4C-A-RTM and BL models over a range of L_l (1.6, 3.9, 6.3 and $9.4\,\mathrm{m^2\,m^{-2}}$) and SD (100, 200, 500, 1000, and $2000\,\mathrm{trees\,ha^{-1}}$, and no tree clumping (TC)). a) Scenario 1 with no woody material included ($L_e = L_l$). b) Scenario 2 with woody material incorporated ($L_e = L_l + L_w$ and $L_w = 0.9\,\mathrm{m^2\,m^{-2}}$). In the legend box, the number after the 4C-A-RTM corresponds to the SD examined. BL (♦) was calculated for each tested L_e from the equation 5.7.

5.4 Discussion

5.4.1 Relationship between stand structure and transmittance

Several studies have shown a relationship between below-canopy light levels and stand parameters (Kuusipalo, 1985; Vales and Bunnell, 1988; Hale, 2001, 2003; Korhonen et al., 2007) with BA being the most widely used stand parameter (Perry et al., 1969). An exponential relationship in the form underlying BL exists between BA and the percentage of transmitted light through the canopy, reflecting the allometric relationship between tree BA and its leaf area (Perry et al., 1969).

A theoretical curve of transmittance as proposed by Hale (2001) using BL with BA as an input and assuming a continuous canopy cover and a SD of 1000 identical trees per hectare is presented in Figure 5.8a. Hale (2001) used the equation developed by McIntosh (1984, see equation 5.1) to predict leaf area

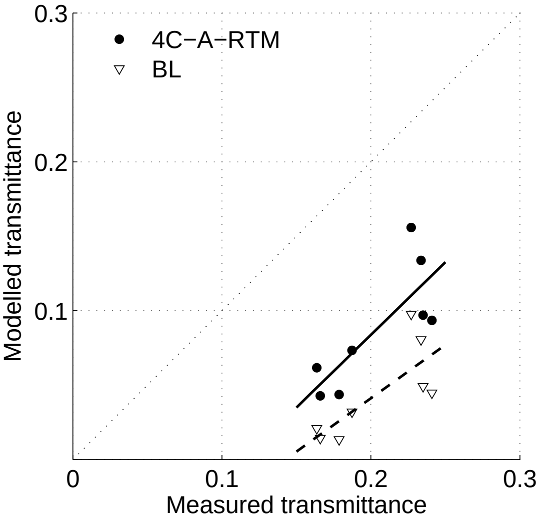

Fig. 5.6. Modelled transmittance calculated by the 4C-A-RTM and BL as a function of measured transmittance from hemispherical photographs at the eight Kielder plots. Regression lines are unbroken for the 4C-A-RTM ($R^2 = 0.63$), dashed for BL ($R^2 = 0.59$) and dotted for line 1:1.

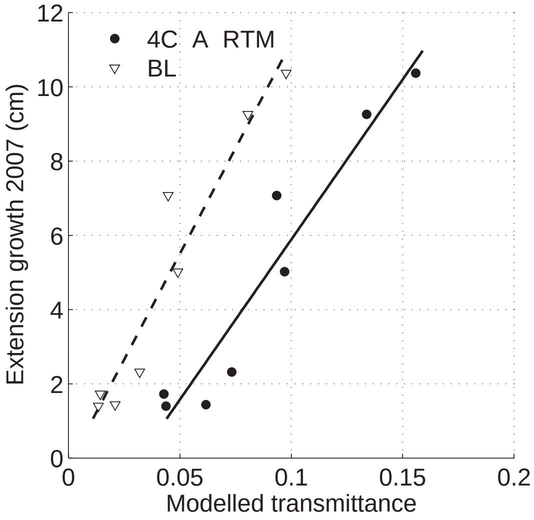

Fig. 5.7. Extension growth as a function of modelled transmittance calculated by the 4C-A-RTM and BL at the eight Kielder plots. Regression lines are unbroken for the 4C-A-RTM ($y = 86.23x - 2.74$; $R^2 = 0.92$) and dashed for BL ($y = 113.83x - 0.19$; $R^2 = 0.92$).

index from d and substituted d by the tree of average basal area ($\overline{BA}$) and SD. Figure 5.8a also shows the measured average transmittance from hemispherical pictures from this study and from Hale (2001) against corresponding BA for a range of Sitka spruce stands in Northern UK. Hale (2001) found good agreement between observations and the theoretical curve despite underlying assumptions that should lead to an overestimate of modelled transmittance: 1) projected area rather than hemi-surface area was used, which would tend to overestimate transmittance in conifer stands, and 2) openness from hemispherical pictures measures the gap fraction, or the proportion of sky obscured by vegetation (i.e. including leaf and woody material), yet woody material was not taken into account in BL ($L_e = L_l$) which would also lead to an increase in the predicted transmittance.

This study does not support the use of a threshold BA as a proxy measure of minimum light required for promoting the growth of Sitka spruce seedlings in UK CCF (*c.f.* Hale, 2001; Page et al., 2001; Mason et al., 2004). No general trend could be found between measured transmittance from this study and BA (Fig. 5.8 a, d and e symbols). In the study site BA was in the same range as the study of Hale (2001) for thinned and unthinned stands, yet transmittance was higher (Fig. 5.8a). These findings underline that the relationship between BA and transmittance is not universal but operates as a function of stand specificities and management history. The older trees, more heterogeneous stand structure, and a post-thinning response in IL plots were most likely the main reasons for the observed discrepancy.

Another determinant of canopy openness is SD (Nilson, 1999). Figure 5.8b shows transmittance versus SD for the same study plots as used in Fig. 5.8a. SD seemed to explain more of the variance in transmittance. Yet, just as in the case of BA, SD could not be considered as a useful predictor of openness in CCF, because a given SD can be comprised of trees of vastly different stature and crown dimensions with a concomitant impact on openness. Stand BA is a function of tree size and tree density, and best fits between transmittance from hemispherical photographs and BA occur in models that have included additional stand parameters such as SD and age (Kuusipalo, 1985; Korhonen et al., 2007). However, such empirical fits are stand-specific and should not be applied outside the range of stand conditions without testing their compatibility (Kuusipalo, 1985; Comeau, 2000).

5.4.2 Seedling growth in relation to light regime

The narrow $15 - 25\,\%$ range of transmittance measured from the hemispherical photographs investigated in this study corresponded with light levels critical for successful seedling growth in Sitka spruce stands in the UK. Hemispherical

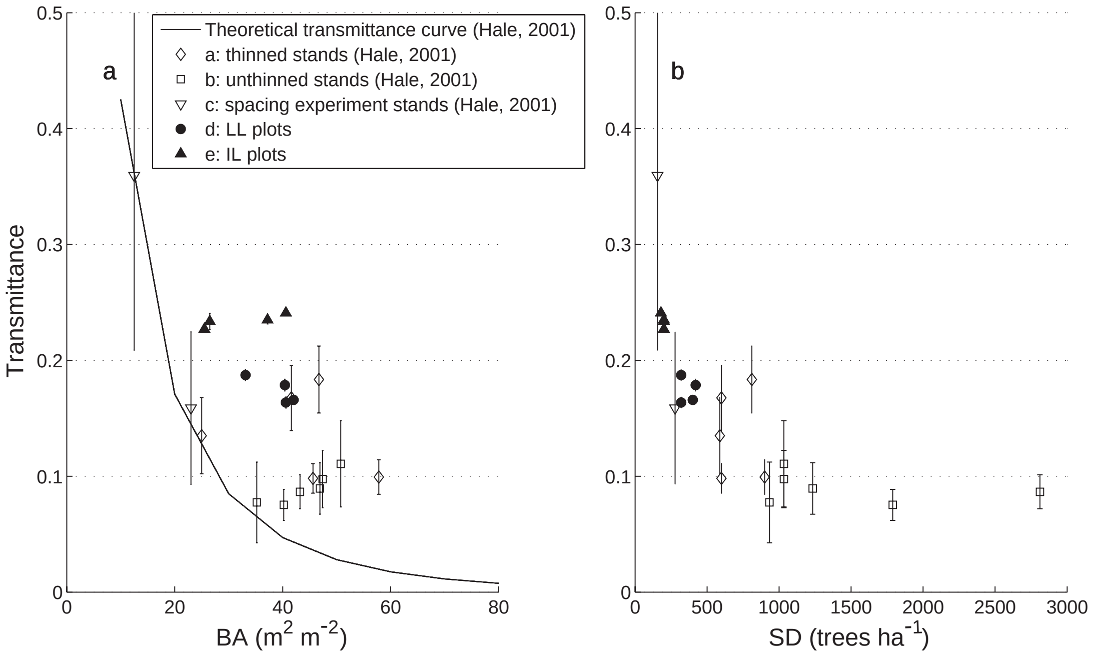

Fig. 5.8. Transmittance estimated from hemispherical photographs versus (a) plot basal area (BA) and (b) stand density (SD) for a range of Sitka spruce stands in northern Britain. The error bars are ±1 SD of the mean (for a, b, and c, n=7 pictures and plot of $900\,\mathrm{m}^2$ area; for d and e, n=3 pictures and plot of $500\,\mathrm{m}^2$ area). Panel a was adapted from Hale (2001). The transmittance values (ISF) from the hemispherical photographs of Hale (2001) were provided by S.E. Hale, so results were comparable with the current study.

photographs were able to predict well seedling growth ($R^2 = 0.73$, Fig. 5.1). This result supported Kobe and Hogarth (2007), finding that canopy openness was a reliable method to estimate seedling growth. In the current study, leader extension growth in the LL plots was less than $2.5\,\mathrm{cm\,year}^{-1}$, but more than $5\,\mathrm{cm\,year}^{-1}$ in the IL plots (Fig. 5.1). Other UK studies in Sitka spruce stands reported that $15 - 20\,\%$ light transmittance was the minimum to promote seedling growth (Hale, 2001; Hale et al., 2004; Mason et al., 2004; Page and Cameron, 2006). At such low light levels a small difference in light availability can correspond to the difference between regeneration success and failure.

5.4.3 Model parameterisation and sensitivity

In effect, a general approach to predict critical light levels for promoting seedling growth below the canopy should incorporate structural stand parameters via physically-based RTMs.

Sensitivity analysis found that the two models diverged to the greatest extent at low SD ($< 200\,\mathrm{trees\,ha^{-1}}$) and when only L_l was taken into account. Adding woody materials (L_w) to the models considerably reduced the differences. BL and the 4C-A-RTM were found to be in close agreement at both high SD and high L_l, the equivalent of a closed-canopy stand, which most closely corresponded to the assumption inherent in BL (i.e. assuming a horizontally homogeneous single-layer canopy). BL also yielded transmittance values close to the 4C-A-RTM in the range $4 - 9\,\mathrm{m^2\,m^{-2}}$ of L_e (with woody material), a range that was similar to the range of total L_t obtained from the parameterisation of the plots (with lower values corresponding to HL plots). The parameterisation results also showed that the vertical LAI profiles of the plots were similar on a relative base, with little vertical variability between plots.

The quantity most likely to produce error in both the 4C-A RTM and BL was L_l. The 4C-A-RTM was highly sensitive to reductions in L_l. No references for other L_l values in similar Sitka spruce stands were found in the literature. The fact that L_l was estimated from an allometric equation developed in a younger stand than this study site may have lead to an overestimation of L_l. With age, the ratio of sapwood to leaf area is expected to decrease (Tobin et al., 2006), and it tends to vary with density, thinning and site fertility (Whitehead et al., 1984; Mencuccini and Grace, 1995). Because transformation to CCF is a slow process, and because the first substantial seed crop is not expected before the age of $30 - 40$ years in Sitka spruce (Malcolm et al., 2001; Mason and Kerr, 2004) developing allometric equations in older Sitka spruce stands is essential, regardless of whether BL or the 4C-A RTM is employed to estimate canopy transmittance. Both models were sensitive to radiation interception by woody material. WAI is rarely estimated in studies of forest structural components even though it plays an important role in light interception (Weiskittel and Maguire, 2006). The impact of WAI is likely to be even greater in open CCF stands mainly because of the increase in branch area in response to thinning. Thus, there is a need to quantify the response of woody-to-total surface area ratio in Sitka spruce stands, and improve understanding of the response to silvicultural intervention, stand density, stand age and site quality.

5.4.4 Model validation

In absolute terms, the two models clearly underestimated measured transmittance, although the correct pattern was reproduced (Fig. 5.6). In part this can be explained by the high sensitivity of the two models to changes in L_l and L_w. The larger discrepancy found between BL estimated transmittance and measured transmittance, compared with the 4C-A-RTM estimated values, could be due to the fact that BL did not allow for stand parameters and shoot characteristics to be taken into account in the model.

Examination of the relationship between extension growth and the modelled canopy transmittance showed a high R^2 value for both models ($R^2 = 0.92$, Fig. 5.7). Growth of less than 5 cm year^{-1} was associated with modelled transmittance values below 9 % and 5 % in the 4-CA-RTM and BL, respectively. In relation to earlier work and in accordance with the hemispherical photograph measurements in this study, a higher transmittance threshold, between 15 − 20 %, would be expected to promote seedling growth (Hale et al., 2004; Mason et al., 2004; Page and Cameron, 2006). Those results further points toward the fact that BL, and to a lesser extent the 4C-A-RTM, underestimated transmittance.

The stand investigated in this study was at the beginning of its transformation to CCF (i.e. close to a simple structure), but evolving towards a more complex structure. A wider range of situations, in terms of both seedling height classes (above 20 cm) and variability in the stand structural parameters (i.e. more irregular structures) would most probably enhance the divergence between BL and the 4-CA-RTM with BL further underestimating transmittance.

A basic assumption in this study was that hemispherical photographs provided reliable measures of transmittance. Modelled and measured transmittance agreed in direction but not in quantity (Fig. 5.6), and the variability increased with higher transmittance. There are several possible reasons for these findings. Most of the IL plots were subject to group selection cutting two years prior to this study. A more variable transmittance measured from hemispherical photographs in the IL (thinned) plots compared to the LL (unthinned) plots was in agreement with Beaudet and Messier (2002). Differences in gap distribution and size is expected between the two light classes with larger gap fraction near the zenith angles in selection cuts (Frazer et al., 2000; Beaudet and Messier, 2002). Larger openings close to the zenith tend to be overestimated compared to smaller openings (Rich, 1990; Beaudet and Messier, 2002). Finally, errors may arise from picture analysis due to the subjective setting of a single threshold value to discriminate sky and non sky area over the entire picture when different thresholds could be applied to balance for the higher light scattering at smaller zenith angles (Rich, 1990; Chen and Black, 1991; Frazer et al., 2000).

5.5 Conclusion

Successful establishment of seedlings under tree canopy depends upon adequate beneath-canopy light to support regeneration and growth. In order to achieve this, management intervention (thinning) intensity can be varied. An understanding of the interaction between understorey regeneration and canopy transmittance is therefore key to the successful manipulation of forest stands. Stand parameters alone were not found to be useful predictors for CCF stand management. Although at this site the two model results correlated well with seedling growth, the models clearly underestimated light transmittance. The 4C-A-RTM represented the most widely applicable approach, but required more inputs than BL to produce accurate estimates across stands with large variability in structure. The 4C-A-RTM model has the potential to be integrated with stand management models to predict natural regeneration success in stands undergoing transformation in the UK across a wide range of structure.

5.6 Acknowledgements

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Chapter 6

Synthesis and Conclusions

The recent development of CCF in the UK and the concomitant lack of experience has resulted in a requirement for improved understanding of the ecophysiology of regenerating seedlings under CCF management, and appropriate data against which to draw conclusions. Specifically a lack of understanding of the effect of low light and aphid infestation on seedling performance is impeding the development of guidance for management practices to promote successful Sitka spruce regeneration (i.e. understorey reinitiation) in stands undergoing transformation to CCF in the UK.

The main contribution of this thesis has been to show how a combination of controlled and field experiments, but also modelling work, can lead to prediction of seedling growth success under different light and aphid infestation conditions beneath the canopy of CCF stands. In this chapter, the main conclusions drawn from the experimental manipulations are highlighted and then framed in the wider context of CCF management. Finally, recommendations for practical research methods on the delivery of guidance for forest managers are made.

6.1 Linking light availability and aphid invasion

Evidence from our results suggests that *Elatobium abietinum* population dynamics are influenced by the prevailing light environment. Our controlled experiment (C) showed that aphid densities increased with decreasing light availability. Aphid densities observed on Sitka spruce seedlings grown under 24 % transmitted light (hereafter named C(LL)) were 3–4 times higher than on seedlings grown in full light conditions (hereafter named C(HL)). Bladon's (2009) field studies also showed that the highest abundance of *E. abietinum* was observed on seedlings growing under shaded forest understorey conditions, suggesting that our experimental manipulations were representative of natural systems. In terms of *E. abietinum* population structure, Bladon's (2009) study found no consistent difference in aphid mean relative growth rate (MRGR) or aphid size between aphids reared on seedlings grown in shade or full light.

A direct aphid effect was observed in terms of needle loss, with defoliation rate of infested seedlings directly correlating with aphid density. After two years of repeated infestation, infested seedlings in C(LL) treatment, where larger *E. abietinum* populations were found, lost, on average, three times more older needles (one-year old and older) than infested seedlings in C(HL) treatment. Nevertheless, the potential impact of increased aphid exposure on seedling performance under the low light environment was mainly localised in extent. The impact of light was the major component that described seedling performance.

6.2 Effects of light and aphid on growth and photosynthesis in controlled conditions

The whole-plant biomass of C(LL) seedlings decreased by two orders of magnitude in comparison to C(HL) seedlings, regardless of whether seedlings were infested or not by aphids. Light was clearly the primary factor affecting total seedling biomass with C(LL) seedlings appearing mainly carbon-limited, because aphid presence or absence did not induce any observed changes to whole-plant biomass. In contrast to the overall effect of light, aphid had only localised effects on the total dry weight of older needles and roots, and on leader extension growth. Interestingly, after two repeated years of infestation, a similar relative reduction of $14-18\,\%$ of total root biomass of the infested seedlings was found within C(LL) and C(HL) treatments, despite the large variation in aphid densities in the two light treatments. Relative decrease in leader growth of infested seedlings was more pronounced in C(LL) treatment than in C(HL) treatment ($15\ -17\,\%$ versus $2-3\,\%$ reduction, respectively). But the impact of *E. abietinum* on C(LL) seedlings was of a lower magnitude to that which might be expected from the high aphid population assessed. Our results suggested that the magnitude of the impact of aphid densities on the seedlings were modified by light availability. When seedling growth is restricted by light, aphid attack may have less effect on growth, because the seedling may lack the plasticity to respond to the additional constraint being imposed. It is also possible that 1) the occurrence of compensatory photosynthesis at the whole-plant level and 2) a differing gene expression induced by the combination of low light and aphid infestation, might have partly mitigate the negative effect of aphid attack on the C(LL) seedlings, but it was not possible to investigate these aspects in the current study.

A shift in biomass allocation was also found in response to the light treatment, with C(LL) seedlings allocating relatively more dry weight to above-ground components than C(HL) seedlings, whereas presence or absence of aphids did not induce any changes in biomass allocation (i.e. seedling architecture). Since light resource was limited in C(LL) treatment, the seedlings allocated more biomass to above-ground parts (needles and stems) than below-ground parts (roots) in order to favour light interception. The absence of changes in biomass allocation of infested seedlings in comparison with uninfested seedlings suggested that the decrease in root biomass was accompanied by similar reductions in the above-ground parts of the seedlings.

The adjustments induced by light at the whole-plant level were accompanied by light-related physiological and morphological acclimation processes at the needle-level. No overall effect of aphid infestation on morphology and physiology were observed at the needle-level, supporting the lack of aphid effect on the whole-plant biomass. The higher total chlorophyll content and specific leaf area (SLA), but the lower needle dry weight in needles grown in C(LL) treatment than in needles grown in C(HL) treatment were identified as some of the typical responses that occur in 'sun' and 'shade' acclimated foliage of shade-tolerant species such as Sitka spruce (Leverenz and Jarvis, 1979, 1980; Meir et al., 2002). The analysis of the photosynthetic response of the one-year old needles of the 'sun' and 'shade' phenotypes showed that needle photosynthetic acclimation took also place in response to the level of transmitted light. A trend towards lower maximum photosynthetic capacity (rETR_{max}) and higher photosynthetic efficiency ($\text{rPPFD}_{\text{sat}}$) was observed in 'shade' phenotypes in comparison with 'sun' phenotypes. However, the magnitude of differences in rETR_{max} between 'sun' and 'shade' phenotypes was relatively small in regards to the large contrasting light levels experienced by the seedlings (i.e. 100 % versus 24 % transmitted light). The characteristics of the photosynthetic light response of seedlings were representative of a shade-tolerant species, where similar or higher maximum photosynthetic capacity can be found in 'shade' phenotypes in comparison with 'sun' phenotypes (*c.f.* Leverenz and Jarvis, 1980). The fact that growth was limited in C(LL) seedlings may be linked to a reduction in photosynthesis, while

C(HL) seedlings were expected to have an adequate level of photosynthates for growth. The relatively high maximum photosynthetic capacity and the increase in SLA and total chlorophyll content in ‘shade’ phenotypes in comparison with ‘sun’ phenotypes could be seen as a response to increase the effectiveness of carbon assimilation. The lack of an overall aphid effect on seedling growth was supported by similar photosynthetic light response characteristic between infested and uninfested seedlings.

In the controlled experiment, light was the main driving factor affecting growth and photosynthesis of the seedlings, whilst aphid infestation had only localised and short-term effects on the growth and photosynthetic responses of the seedlings. It seems that the strong constraint imposed by low light availability on the seedlings resulted in a reduced ability of the plant to respond to additional stress caused by aphid attack.

6.3 Estimation of understorey light levels in CCF stands

The importance of the transmittance light level on the growth response of Sitka spruce seedlings was confirmed in our field experiment (F) where a strong positive relationship ($R^2 = 0.72$) was found between extension growth and transmittance, measured with hemispherical photographs. Estimated transmittance from hemispherical photographs is a good proxy of integrated (i.e. total) growing season PPFD in forests (Gendron et al., 1998). In the field experiment, leader extension growth in the plots where transmittance measured from hemispherical photographs was 17 % (hereafter named F(LL)) was less than $2.5\,\mathrm{cm\,year^{-1}}$, but more than $5\,\mathrm{cm\,year^{-1}}$ in the plots where transmittance measured from hemispherical photographs was 24 % (hereafter named F(IL)). The fact that we found no differences in photosynthetic light response characteristics of F(LL) and F(IL) seedlings indicated that differences in integrated growing-season light transmission between the two light treatments was a primary cause of observed differences in leader extension growth. In our experiment, the narrow $15 - 25\,\%$ range of transmittance investigated corresponded to critical light level for Sitka spruce seedlings growth.

In order to develop guidance on how to manage a stand in order to promote successful seedling growth in CCF stands in the UK, a primary requirement is therefore the estimation of light transmittance through the canopy. CCF in the UK to date has used simple empirical stand-level relationships as indicators of understorey light levels. Our modelling study showed that stand-level characteristics are highly stand-specific and not adequate as predictors of transmitted light. As an alternative, we investigated the use of two radiative transfer models (RTMs): a simple model based on the Beer-Lambert law (BL) and a detailed pre-existing light model (4-CA-RTM) that statistically accounts for variation in stand structure. The sensitivity analysis indicated that leaf area index (LAI) was the input most likely to introduce errors in the two models. In addition, the two models were sensitive to radiation interception by woody material. Despite its importance in light interception, woody area index (WAI) is rarely estimated. Because the impact of WAI is likely to be greater in CCF stands due to the increase in branch area in response to thinning intervention, there is a need to better quantify it in Sitka spruce stands. The validation of the two models against measured transmittance from hemispherical photographs showed that, in absolute terms, they both underestimated measured transmittance, although the correct pattern was reproduced. The underestimation may be partly attributed to the high sensitivity of the two models to LAI. In addition, BL underestimated transmittance the most. This is most likely attributable to it not accounting for stand parameters. Despite the fact that the two models underestimated transmittance, they both evaluated correctly the observed extension growth of the seedlings ($R^2 = 0.92$). Finally, our modelling work showed that the complex 4-CA-RTM offered an improved estimate of light transmittance and adequately described seedling growth across structurally varied plots within stands at different stages of transformation. The 4-CA-RTM has the potential to be integrated with stand management models to predict seedling growth in stands undergoing CCF transformation.

6.4 Light environment and photoprotection

Light transmittance level and extension growth provide useful guidance to forest managers for evaluating if increased light availability is needed to promote seedling growth. If an intervention is required, the manager must then decide what type of silvicultural intervention to apply. The combination of our controlled and field experiments showed that transmittance level *per se* is not a adequate stand-alone indicator for deciding the type or level of intervention. Comparison of the photosynthetic response of Sitka spruce seedlings in both the controlled and field experiments highlighted the importance of considering the temporal heterogeneity of the light environment experienced by understorey seedlings to predict growth potential in CCF stands. In the controlled experiment, C(LL) seedling grown under 24 % transmitted light experienced low PPFD and absence of sunflecks (i.e. conditions of filtered and diffuse sky radiations). In contrast, under similar light transmittance level (i.e 23 %), F(IL) seedlings grown in the field experiment experienced a dynamic light environment with presence of sunflecks (i.e. fluctuations between long periods of low PPFD and short periods of moderate to high PPFD). Needle photosynthetic acclimation took place in response to the different patterns of irradiance in the field and under controlled conditions, with higher heat dissipation energy (as expressed by NPQ) in F(IL) seedlings in comparison with C(LL) seedlings. Acclimation of field needle photosynthetic apparatus provided photoprotection from excess light energy exposure under sunflecks. As a result, F(IL) seedlings presented a tradeoff between maximising photochemistry and providing photoprotection. The quenching of light as heat instead of photochemistry in PSII of F(IL) seedlings was linked to a downward adjustment of the photosynthetic efficiency (as expressed by rETR). Therefore a decrease in carbon gain in F(IL) seedlings in comparison with C(LL) seedlings may be expected. Our results suggested that, at a similar level of transmittance, Sitka spruce seedlings growing under diffuse light environment exhibit improved light capture than seedlings growing under a dynamic light environment. However, seedlings grown under diffuse conditions are likely to suffer higher risk of photodamage if they are exposed to sudden high irradiances. Such a situation may result from stand management through gap opening within the stand. Additional research examining the acclimation potential and photodamage susceptibility of Sitka spruce foliage grown under diffuse low PPFD in the field and transferred to a dynamic light environment with sunfleck presence are required to allow guidance on the risk posed by intervention through gap creation. Furthermore the results underline the importance of understanding the history of the irradiance pattern experienced by the seedlings before deciding the type of silviculutral intervention to apply. A uniform thinning of the stand may be more appropriate for seedlings that have grown under diffuse light in order to minimise the risk of photodamage. In contrast, canopy intervention designed to create small gaps would create a favourable environment for seedlings that have previously experienced a dynamic light environment and have therefore developed photoprotective mechanisms.

In parallel, the level of plant phenolics, classic defence compounds against herbivory, have been hypothesised to depend on the risk of photodamage to the plant (Close and McArthur, 2002). Further work is required on the primary role of plant phenolics in Sitka spruce seedlings to support this hypothesis. In this study higher aphid populations were found in C(LL) seedling grown under diffuse light conditions (i.e. with higher risk of photodamage) than in C(HL) seedlings grown in dynamic light environment (i.e. with higher levels of photoprotective mechanisms) which is in broad support of this hypothesis. If correct, the hypothesis would suggest that under similar low light levels but different patterns of irradiance seedlings grown under diffuse conditions may be exposed to higher aphid infestation. In comparison, under low transmittance level, Sitka spruce seedlings grown under a diffuse light environment may have higher carbon gain yet higher aphid infestation may be expected, whereas Sitka spruce seedlings grown under dynamic light environment may have lower carbon gain but may be less susceptible to aphid infestation. A study on the combined effect of temporal light variability and aphid infestation on Sitka spruce seedlings grown under low transmittance conditions would be required before definitive conclusions could be drawn.

6.5 Silvicultural management implications: Facts and Actions

Our results have important silvicultural management implications on how to promote successful seedling growth in stands undergoing transformation to CCF. Overall, light is the main driving factor affecting seedling growth below CCF canopies. The specific recommendations for management practices are:

Light transmittance level

Fact: 15 − 25 % range of transmittance correspond to critical light levels for Sitka spruce seedling growth.

Action point: Measures of seedling extension growth and light transmittance are useful indicators to evaluate if understorey transmittance needs to be increased.

Light environment history

Fact: History of the light environment that has been experienced by the seedling is needed before deciding how to open the canopy.

Action point: A field portable fluorometer could be used as a research service combined with assessments of stand basal area and spatial element (competition index).

Aphid invasion

Fact: Higher aphid population may be expected under low light conditions than under full light conditions. Following repeated moderate to large aphid outbreaks in low light conditions, cumulative reduction of two key attributes, leader extension growth and total root biomass, is likely to occur and reduce regeneration success.

Action point: Under the current climate, no action is required since the natural year-to-year variation observed in aphid population dynamics, with a high outbreak year usually followed by a year with low aphid densities, means that cumulative aphid effects on seedlings are unlikely to occur in nature.

6.6 References

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