

CHAPTER 5

Growth, allocation and morphological responses of four broadleaved species to different developmental stages of *Pinus sylvestris* woodland

5.1 Introduction

The aim of this study was to develop an understanding about the establishment and shade tolerance of four broadleaved species in Caledonian Forest. This involved planting one-year-old tree seedlings under different developmental stages of *Pinus sylvestris* woodland (Highland region, Inverness-shire) and monitoring their growth, allocation, morphology and survival over a two year trial period.

The negative impact of browsing from large herbivores such as sheep and deer on tree establishment is well established (Cummins, 1982; Miller *et al.*, 1982, 1998; Stains, 1994; Miller & Cummins, 1998; Beaumont *et al.*, 1995; Harmer & Gill, 2000). However, in the absence of browsing, the successful establishment of broadleaves on pinewood sites still depends on an ability to tolerate an array of limiting environmental resources. Many of the pinewoods are in a degenerative state (Malcolm, 1957). Degeneration has been triggered by the opening up of the canopy by deforestation increasing and permitting the invasion of undesirable shrubs. There follows rather a rapid decline in site quality due to a rise in the water table and a deterioration in the chemico-physical qualities of the soil, particularly of the humus layers. Thus, the establishment of broadleaves is not just subjected to limitations of light under the shade of invasive ericaceous shrubs and tree canopy but also subjected to poor nutrient availability exacerbated by poor soil moisture regimes. Increased water-logging of sites restricts the availability of oxygen which influences growth of plants directly by impairing root function (Drew, 1997) and thus nutrient acquisition (Ohlson, 1995). In dry years, seedlings can also be subjected to drought especially on well-drained sites (McVean, 1964b; Miles & Kinnaird, 1979).

In the Caledonian forest, the ability of tree seedlings to tolerate shade will thus depend on an ability to tolerate limiting below ground conditions. The importance of below ground resources in modifying shade tolerance in the field is slowly receiving more attention. Kimmins (1987) and Carter & Klinka (1992) define shade tolerance not just as an ability to tolerate low light conditions but also high root competition. Coomes and Grubb (2000) state how “it is widely recognised that shade tolerance is not an immutable property of a species but varies from site to site, depending on the availability of below ground resources”. The theory of multiple resource limitation is introduced which predicts that the performance of plants will depend on the

availability of water and nutrients as well as the depth of shade cast by the established canopy (Chapin *et al.*, 1987).

Many studies have investigated how nutrients and light interact to modify the shade tolerance of temperate woody species (Corre, 1983b; Latham, 1992; Ellsworth & Reich, 1992; Crabtree & Bazzaz, 1993; Grubb *et al.*, 1996; Walters & Reich, 1996; 1997; 2000; Reich *et al.*, 1998b). Few temperate studies have investigated interactions between water availability and light (Osonubi & Davies, 1981); even less tackle the effects of high water tables (Nisbet *et al.*, 1989; Battaglia *et al.*, 2000). Most studies that focus on water availability and light are based in the tropics where seasonal drought under shade is an important factor influencing survival (Turner, 1990; Veenendaal *et al.*, 1995). A number of studies have adopted the multiple resource limitation approach investigating interactions of light, nutrient and water availability on seedling growth (Kolb *et al.*, 1990; Knox *et al.*, 1995; Canham *et al.*, 1996; Walters & Reich, 1997; Welander & Ottosson, 2000).

A worldwide review of trenching experiments showed that light alone limited seedling growth in forests on moist, nutrient-rich soils, but competition for below ground resources became important on infertile soils and in drier conditions (Coomes & Grubb, 2000). There was some evidence to suggest that trenching had the greatest impact under trees which cast little shade, reflective perhaps of a positive correlation found between shade and fertility. It was thus suggested that root competition plays an important role in Northern temperate forests which are found on highly acidic peats and sandy soils and are often dominated by early-successional, open-canopied conifers (generally pines).

Shade tolerance studies have focused on strategies by which plants obtain limiting resources, e.g.: adjustments in leaf morphology, enzyme activity, branching patterns and root:shoot allocation which tends to maximise photosynthetic efficiency. Chapin *et al.* (1987) believe that the maintenance of a favourable carbon:nutrient balance is the key to survival in habitats of fluctuating resource availability. They explain how plants adjust to resource imbalance by allocating more biomass to the organs that acquire the most strongly limiting resource. When plants become short of carbon (by shading or herbivory), they compensate by partitioning more biomass to the shoot and less to the root. This decreases the carbon stress and returns carbon and nitrogen reserves to a balance more favourable for growth. When nutrient limitations lead to low nutrient concentrations and to accumulation of carbohydrates, plants respond by increasing their energy allocation to root growth. The shade induced shift in partitioning differs between species and is considered indicative of difference in

shade tolerance between tree species (Grime, 1979; Walters *et al.*, 1993b; in van Hees & Clercx, 2003).

It is argued that a reduction in the root:shoot ratio, reduces the capacity of plants to capture and retain water and nutrients and thus limits the extent to which plants can tolerate drought or infertility in the shade (Smith & Huston, 1989). Indeed some species (less able to balance their C:N efficiencies) decline in such conditions with shade-tolerance improving with the enhancement of nutrient and /or water availability (Coomes & Grubb, 2000). In Scottish *P. sylvestris* woodland, the removal of root competition led to large increases in the growth of *Oxalis acetosella* and *Deschampsia flexuosa* (Watt & Fraser, 1933). Furthermore, Fricke (1904) discovered that after trenching in pinewood, trees that normally occur on moister soils invaded spontaneously (in Coomes & Grubb, 2000). Other species have naturally evolved specialised traits which allow them to tolerate limited below ground resources in the shade. For example, many shade tolerant woody plants in tropical and mediterranean forests have thick, tough, long-lived leaves with large root systems which confers drought tolerance (Coomes & Grubb, 2000).

The main objective of this study was to determine how well broadleaved seedlings could grow and survive within pinewood sites of varying shade and resource availability. This was achieved by i/ assessing the growth, allocation and morphological responses of broadleaved seedlings to different development stages of Caledonian pinewood and ii/ assessing their degree of morphological and physiological plasticity. As well as irradiance, other environmental variables (such as root competition and soil water content) that characterised the pinewood stands were assessed to analyse their potential impact on seedling growth and survival.

The following questions were asked:

- i/ Do species show classical responses to shade in the field?
- ii/ What physiological or environmental factors play an important role in driving growth rates across different irradiances / pinewood stands?
- iii/ How can differences in growth, allocation and morphology distinguish degrees of shade tolerance between species?
- iv/ How do physiological responses match traditional notions of shade tolerance and Ellenberg light indicator values?

5.2 Methods

5.2.1 Study site

Seven woodland sites were located around *Loch Beinn a Mheadhoin*, in Glen Affric, Highland Region, Inverness-shire, each containing three *Pinus sylvestris* stand development stages: stand initiation (open woodland), stem exclusion (pole plantation), and canopy break-up (natural old growth). All stands were selected based on similarities in stand density, understorey vegetation and slope. The research was carried out over a two year period from April 2001 to September 2002. This allowed time for adequate seedling establishment and measurement of below-ground competition.

5.2.2 Experimental design

A hierarchical factorial design was used. This involved seven blocks with the three stand development treatments within each block ($7 \times 3 = 21$ plots). Each treatment plot was divided up into two sub-plots representing two randomised Latin squares (see Appendix 1). Each Latin square contained 16 individuals (4 species \times 4 replications) with each row and column containing one of each of the four species in random order. A hexagonal pattern was used for planting to ensure that individuals were equi-distance to each other by 0.3 m.

On the edge of each treatment plot, an enclosure (2.72 m by 1.95 m) made from four wooden stakes (1.7 m) and chicken wire was erected to deter browsing from deer, rabbits and hares. A 0.2 m high nylon skirting was attached from the ground to the surrounding chicken wire to deter voles and mice. A buffer zone of 0.45 m was created between fence and plot. Generally each enclosure was placed on moderately flat ground, orientated with the long axis aligned north-south.

5.2.3 Species and establishment

One year old cell-grown seedlings were obtained from Christie Elite Nurseries Ltd (Forres, Moray). The four species were *Betula pubescens*, *Sorbus aucuparia*, *Alnus glutinosa* and *Ilex aquifolium*. These species were chosen as they represent a shade tolerance range according to the original Ellenberg light indicator values (Ellenberg, 1988). All species were from provenance 201 except for *S. aucuparia*, which was from 102 (Herbert *et al.*, 1999).

A month before field transplantation, each seedling was potted up in a peat / perlite medium (Irish peat pH 5.5) within a 0.75 litre plastic net pot. The net pot had a mesh size (1 cm^2), adequate to allow drainage or horizontal and vertical root growth. All pots were buried below the litter layer and approximately 20 mm into the main humus layer. On planting, care was taken to reduce any air gaps between pot and surrounding soil and any over-shading vegetation (mostly *Calluna vulgaris*) was cut back to aid initial establishment. This was not repeated in the second season so that the effect of above-ground competition on seedling growth could be assessed.

5.2.4 Climate data

Daily total rainfall (mm) data for Glen Affric (Affric Lodge 2 meteorological station) was downloaded from the UK Land Surface Station database (courtesy of the British Atmospheric Data Centre (BADC)). Measures of accumulated temperature, moisture deficit, windiness and continentality for each site were obtained from the Ecological Site Classification Decision Support System (Ray, 2001) (see Appendix 4 for details).

5.2.5 Light

Hemispherical photography

Hemispherical photography was used to obtain seasonal estimates of total photosynthetic photon flux density (PPFD / PAR) which are difficult and expensive to obtain from direct measurements (Rich, 1990; Brown *et al.*, 2000). The approach provides a consistent estimate of transmittance under a range of conditions over an annual period (Comeau, 2000). Full details of the approach have been outlined in Appendix 3.

PAR measurements

Direct measurements of PAR were taken in the field for comparison with hemispherical photographic estimates and shade house readings (Chapter 3).

PAR logging was carried out over both growing seasons due to the limitation of four sensors and loggers. Between July and August, one data logger with its quantum sensor was placed in each of the three stand treatments of one block, mounted on a central wooden platform at seedling top height. Another quantum sensor was placed in a very open site, void of trees, on a peninsula overlooking *Loch Beinn a Mheadhoin*. Under each treatment, light measurements were recorded

continuously in each block over 4 consecutive days. The data were recorded on a Skye Instrument datalogger system (version DH / MM 63, Skye Instruments Ltd.) which was set to record measures of PAR at 30 second intervals and record values as an integrated mean over each 30 minute period in $\mu\text{mol m}^{-2} \text{s}^{-1}$. The percentage of light transmittance in each of the three different stand treatments was calculated as a percentage of total PAR in the open area. An estimate of total daily PAR for each light treatment was calculated by summing the PAR readings for each light treatment pooled over 28 days. Readings were taken from 11:00 to 15:00 hrs so comparisons in mean daily PAR could be made with the shade house trial.

R:Fr measurements

The Red:Far-red ratio (R:Fr) within each stand treatment was measured using a sensor type 660-730 nm (SKR 110 0797 15447, SK3, Skye Instruments Ltd., Ddole Industrial Estate, Llandrindod Wells, Powys, UK). The sensor and its leveller were placed on top of the same platforms used for the PAR data loggers. Measurements were carried out every month between June and August between 11:00-14:00 hrs when there was continuous cloud cover to minimise variability. The mean R:Fr ratio for each treatment was calculated as a mean of the recordings throughout the experimental period.

5.2.6 Stand assessments

The co-ordinates of each site were recorded using a GPS and elevation estimated from the 1:25000 Ordnance Survey Map of Glen Affric. Number and diameter at breast height (dbh) (1.3 m) of all trees and height of the tallest tree were measured within a radius of 5.6 m (0.01 ha) central to each plot. Unfortunately forest patches were generally too small to obtain a reliable estimate of basal area and stem density per hectare. Tree species including natural regeneration present within each radial plot were also recorded. Management notes for each stand were obtained from the sub-compartment database for Glen Affric and the Ancient Woodland Site Inventory.

5.2.7 Other environmental variables

Indirect and direct measurements of soil nutrients and soil moisture were obtained for each treatment plot. Once data on soil (type, stoniness, root depth) and vascular plants (including *Calluna vulgaris*) were collected from each plot, a broad classification of soil nutrient regime (SNR) and soil moisture regime (SMR) could be

generated from the Ecological Site Classification Decision Support System (ESC DSS) (Ray, 2001). pH measurements (x four replicates per plot) and vascular plant indicator species were used to obtain indirect quantitative measures of soil nutrients (Wilson, 2001; 2002). Volumetric soil moisture content was recorded using a thetaprobe sensor (Type ML2x, Delta-T Devices Ltd, Cambridge). Four measurements were taken in each plot in August of each year, after 2-3 days of no rain. An estimate of aerobic soil conditions was also obtained by incubating mild steel welding rods (0.5 m in length) for 68 days in the ground adjacent to each treatment plot in the Autumn of 2002 (Armstrong *et al.*, 1976; Carnell & Anderson, 1986; Bridgham *et al.*, 1991). Above ground competition was assessed (July 2002) as local frequency by placing a grided quadrat (0.25 m x 0.25 m) (grids: 0.05 m x 0.05 m) over each seedling, and counting the squares in which over-topping vegetation was found. A full description of all the above techniques can be found in Appendix 5.

5.2.8 Growth, performance and survival measurements

Measurements were carried out over a two year trial period running from April 2001 to September 2002. The first year of the trial was predominately for establishment purposes only with growth analysis and above and below ground competition assessed in the second year. This was to ensure sufficient magnitude of measured effect for both seedling growth and ingrowth of competing roots in peat (Finer & Laine, 2000). Before onset of growth in April and towards the end of the growing season in September, the stem height (± 5 mm) (soil level to tip of the apical stem) and basal diameter (± 0.01 mm) was measured for each seedling using a metre rule and digital callipers. A basal diameter:height ratio could then be calculated to obtain a measure of etiolation for each seedling. In September 2002, the number and branch length (± 5 mm) of all branches per seedling were also measured. This enabled the calculation of a leader:lateral ratio (total stem height / mean branch length) for the assessment of seedling architecture (modified from Page *et al.*, 2001).

In April, July and September of each year, the status of the seedlings was recorded by assessing their performance and amount of damage.

Each seedling was assigned a performance score (I-IV) for each of the three performance categories: leaf colour, leaf loss and stem necrosis (Table 5.1). The highest score of any of the three categories determined an overall performance class (1-4) for each seedling. Seedling mortality was confirmed by growth cessation in the second year with the assignment of class 5.

Table 5.1 Performance class, categories and scores

Class	Leaf colour	Leaf loss	Stem necrosis
1. Healthy	I green	I no loss	I < no necrosis
2. Weakened	II green-yellow	II < 50% loss	II < 5% necrosis
3. Suffering	III yellow-brown	III > 50% loss	III > 5 - 20% necrosis
4. Dying	IV brown	IV total loss	IV > 20% necrosis

The percentage of damage was recorded for each damage class below with a damage score on a four point scale: I: 0-5%; II: > 5; III > 20% and IV > 50%.

Damage class:

- 1 = No damage
- 2 = Mechanical Damage
- 3 = Mammal damage
- 4 = Fungal damage
- 5 = Insect damage
- 6 = Frost damage

5.2.9 Harvesting

Seedlings

After 18 weeks of growth (t_2) (May-September 2002), seedlings within treatments were harvested to obtain total dry mass and dry mass of leaves, stems and roots. Plants were divided up into separate components and oven-dried until constant dry mass was obtained (48 hrs @ 80°C). Methodology from Evans (1972) was followed (as in Chapter 3). Samples were then weighed using a digital top pan balance (± 0.001 g). Before fresh leaves were placed in the oven, total leaf area for half of all seedling samples (individuals from one Latin Square of each treatment plot) were calculated with a leaf area meter (Delta T Devices Ltd). All roots were washed free of soil by hand with the aid of sieves and a lightly powered water spray.

From the primary data, a number of growth variables were calculated (Hunt, 1990). These were: leaf area ratio (LAR: leaf area/dry plant mass ($\text{m}^2 \text{g}^{-1}$)); specific leaf area (SLA: leaf area/dry leaf mass ($\text{m}^2 \text{g}^{-1}$)); leaf mass ratio (LMR: dry leaf mass/dry plant mass); stem mass ratio (SMR, dry stem mass/dry plant mass) and root mass ratio (RMR, dry root mass/dry plant mass). Calculations have been modified slightly from descriptions in Hunt (1990) as "lamina" was used instead of leaf, and "stem" also included petioles or rachis. Henceforth, the term "leaf" is used to mean leaf lamina and "stem mass" is taken to include petiole and rachis mass.

Relative growth rate (RGR) and net assimilation rate (NAR) were determined using the equations 3.1 and 3.2 (Chapter 3). Relative height growth and relative basal diameter growth were calculated by adopting the RGR equation for biomass.

In the calculation of RGR, plant mass was log-transformed before averaging; adopting the estimator 2 approach outlined by Hoffmann & Poorter (2002) (as in Chapter 3). Total dry mass (W_1) was obtained by an initial harvest of 10 extra seedlings per species at the beginning of the experiment. Seedlings were very different in size and form after the first year, so another harvest was done in April 2002. This involved randomly removing one seedling per species from each stand treatment from six randomly chosen blocks. This equated to the removal of less than 10% of the total number of plants from the experimental design which is statistically acceptable (Tom Connolly, personal communication). Regressions using seedlings from this harvest were used to obtain estimates of initial total biomass of seedlings in the field. Best subsets regression was carried out to find the best predictors of initial plant mass using non-destructive measures. A nominal value (0.0001 m^2) was assigned for initial leaf area (A_1) for the calculation of NAR for all deciduous species. NAR and RGR were not calculated for *I. aquifolium* due to complications in the estimation of W_1 and A_1 .

Foreign roots

During the root washing process, care was taken to separate out tree seedlings roots from other foreign roots which had invaded the net pots. Foreign roots within pots of half of the samples (one Latin square) were kept and weighted separately to obtain an estimate of dry mass. Root identification involved a prior study of heathland plant root systems with aids from the literature (Heath *et al.*, 1938; Persson, 1980) and specimens from the field.

5.2.10 Statistical analysis

ANOVA (GenStat / Windows Ed. 6, Rel. 6.1) was used to analyse differences in growth and morphological responses of species and other abiotic factors between stand development treatments. Within Genstat, the treatment terms that were analysed by ANOVA included species, stand type and species-stand interactions. The main experimental design involved a nested and crossed structure which was

incorporated into the BLOCKSTRUCTURE model (Block/Strip/Rep/(Row*Column)¹. This model was used to analyse the majority of seedling growth variables. Total leaf area and ingrowth of foreign roots into pots, however, was only measured for one Latin square so "Rep" was removed from the above model for these variables. As environmental variables were not measured at the individual seedling level but at a higher stratum level, "Row*Column" and or "rep" terms were removed from the model and if necessary replaced with an appropriate term. The ANOVA tables (Appendix 2) clearly illustrate how the design structure was incorporated into the analysis with sources of variation explained at each stratum level¹.

Before ANOVAs were interpreted, residual plots were drawn up for each parameter to check for normality and homogeneity of variance. Data were transformed where necessary using natural logs for plant mass and for plant ratios, arcsine square-roots for proportions and square roots for counts. Missing values in the orthogonal design were dealt with by substituting estimated values generated by Genstat's missing value estimation function¹.

S. aucuparia individuals within some of the plots were subjected to preferential grazing but the extent was such that browsed seedlings could be treated as missing data along with dead and snapped individuals. Least significant difference error bars (LSD) were attached to ANOVA plots as in Chapter 3. Tukey multiple comparison tests were only carried out to clarify differences in environmental variable measures between stand types.

Environmental variables could not be treated as co-variants in ANOVA due to a lack of independence between co-variate and treatment groups and or a lack of linearity between co-variate and RGR (Quinn & Keough, 2002). Instead best subsets regression was used in exploratory analysis to determine which environment variables (PAR%, soil moisture, Wilson value, percentage cover of *C. vulgaris*, ingrowth of foreign roots and over topping vegetation) explained most variation in the growth of individual species across stands. Exclosures were treated as independent sites for this analysis, regardless of stand type and block and statistical significance values have not been attributed for this reason. However regressions were run with both linear and quadratic models to explore relationships with the more important variables across stands. Separate regressions were also run between growth rates and environmental variables within individual stand treatments.

¹ See Appendix 2 for ANOVA tables (including degrees of freedom and F ratio calculations), explanation of directive terms, sources of variation at each stratum level and calculation of missing values.

5.3 Results

5.3.1 Climatic data

ESC classifies the Glen Affric climate as cool and wet with a moisture deficit ranging from 56-78 mm and accumulated temperature ranging from 987–1071°C across sites (Pyatt *et al.*, 2001) (Table 5.3). Windiness is in the lower ranges with DAMS (Detailed Aspect Method of Scoring) from 11-14. Over the past 10 years, the driest months of the year in Glen Affric have been from May to August with February being the wettest month (Figure 5.1). Precipitation was normal in the first growing season (2001) but the second season was wetter than average. Seedlings growing over the autumn and winter period from 2001 to 2002 were also subjected to wetter than average conditions.

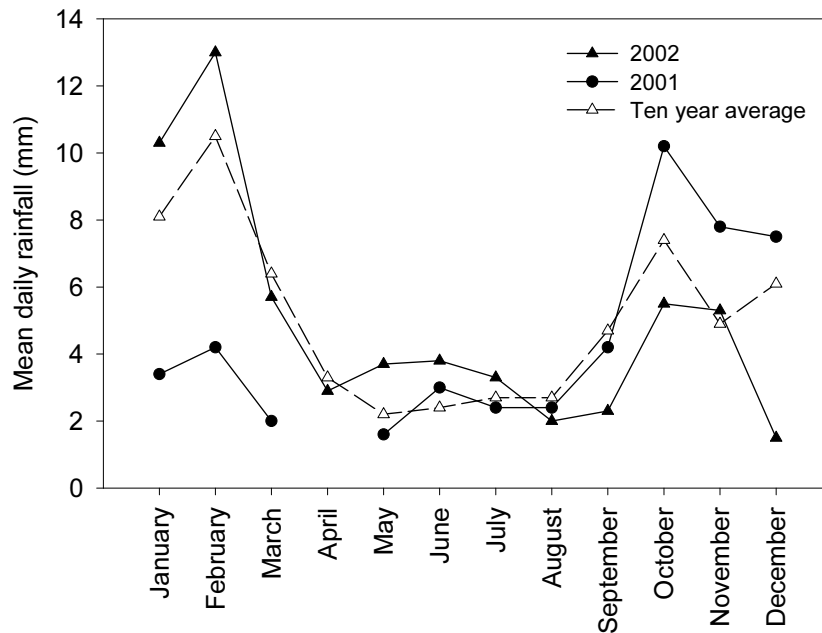


Figure 5.1 Trends in mean daily rainfall (mm) for 2001 and 2002 in Glen Affric (Affric Lodge 2 meteorological station, UK Land Surface Stations Data, BADC).

5.3.2 Light

Mean total seasonal estimates of PPFD from hemispherical photographs were lowest under pole stage (15% PAR), followed by old-growth (44% PAR) and then the open (71% PAR) (Table 5.2). The mean seasonal R:Fr ratio was 0.86 under pole, followed by 0.98 under old-growth and 1.05 in the open.

Table 5.2 Light data including estimates of mean total seasonal PAR (PPFD) and PAR% from hemispherical photographs (indirect) as well as PAR% calculated from data logged in the field in July and August (direct). A seasonal estimate of R:Fr is also provided. Means pooled across blocks (n = 7) (\pm standard deviation).

Light variables	Open	Pole	Old-growth
Total seasonal PAR (PPFD) (indirect)	2081 (\pm 503)	424 (\pm 136)	1254 (\pm 325)
Total seasonal PAR% (indirect)	71 (\pm 12)	15 (\pm 5)	44 (\pm 14)
July / August PAR% (direct)	65 (\pm 16)	12 (\pm 4)	36 (\pm 14)
Mean seasonal R:Fr	1.05 (\pm 0.03)	0.85 (\pm 0.04)	0.98 (\pm 0.06)

5.3.3 Stand structure and management

Natural pole stage stands were extremely limited in Glen Affric and so Forestry Commission *P. sylvestris* plantations provided the crucial low light treatment. Pole sites were generally about 40 years old with a mean height of 13 m and mean diameter of 15 cm (Table 5.3). Old-growth stands had a mean height of 17 m and a mean diameter of 66 cm. Natural regeneration was present in nearly all plots including *P. sylvestris*, *B. pubescens* and *S. aucuparia*. Approximately half of all sites were on ancient woodland sites. The only native broadleaf commercial forestry species which was found suitable for growth according to site quality classifications was *B. pubescens* (ESC DSS, Ray, 2001).

Table 5.3 Stand structure data and climatic and edaphic factors from the ESC decision support system (Ray, 2001) for open, pole and old-growth stands. Means have been pooled across blocks ($n = 7$) (\pm standard deviations) and ranges provided for categorical data. Stand structural means have been calculated from measurements taken within a 0.01 ha area around each plot. Natural regeneration present within plots include *Pinus sylvestris* (SP), *Betula pubescens* (DBI) and *S. aucuparia* (RWN). Climatic factors include a measure of summer warmth (accumulated temperature above 5°C), dryness of the growing season (monthly rainfall – monthly evaporation = moisture deficit mm), windiness (Detailed Aspect Method of Scoring (DAMS) range: 3 - 36), and seasonal variability (continentality range: 1-13). Soil nutrient regime (SNR) ranges from very poor (VP); poor (P); medium (M); rich (R) to very rich (VR). Soil moisture regime (SMR) ranges from wet (W); very moist (VM), fresh (F); slightly dry (SD); moderately dry (MD) to very dry (VD).

Variables	Open	Pole	Old-growth
dbh (cm)	-	15.1 (\pm 1.8)	66.4 (\pm 14.5)
Height (m)	-	12.8 (\pm 2.3)	16.7 (\pm 2.3)
Stem density	-	21.3 (\pm 5.7)	2.4 (\pm 1.1)
Natural regeneration	SP, DBI, RWN	SP, DBI, RWN	SP, RWN
Elevation (m)	235 (\pm 26)	236 (\pm 22)	236 (\pm 24)
Acc. Temperature (°C)	1033 (\pm 31)	1032 (\pm 27)	1032 (\pm 29)
Moisture deficit (mm)	64.4 (\pm 6.9)	64.3 (\pm 6.1)	64.3 (\pm 6.6)
Windiness (DAMS)	12.6 (\pm 1.0)	12.7 (\pm 0.8)	13.6 (\pm 2.6)
Continentality	5.0	5.0	4.9
Soil Nutrient Regime	VP	P-VP	VP
Soil Moisture Regime	VM-W	MD-W	F-W

5.3.4 Competition

A difference in ingrowth of foreign roots was found between stand types though not quite significant at $P = 0.05$ ($P = 0.09$). No significant species effects were found but there was a significant species-stand interaction ($P < 0.05$) (ANOVA tables, Appendix 2). Generally a lower level of ingrowth of foreign roots was found in the open stand and a higher but similar level found in both pole and old-growth stands where *P. sylvestris* and *Vaccinium* roots dominated samples (Figure 5.2).

Significant species, stand ($P < 0.001$) and species-stand interaction ($P < 0.01$) effects were found for local frequency of over-topping vegetation. Higher levels were found in the open stand with lower but similar levels found in the pole and old-growth stands for most species (Figure 5.2). Generally *I. aquifolium* was subjected to the most over-topping vegetation and *S. aucuparia* the least in all stands.

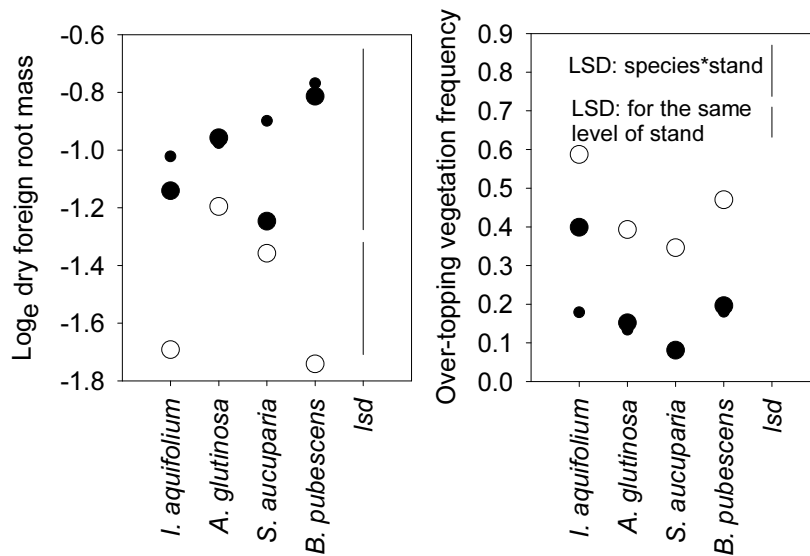


Figure 5.2 ANOVA plots displaying species-stand interactions for ingrowth of foreign roots and local frequency of overtopping vegetation (arcsine SQRT) for 2002. LSD error bars have been appended to each plot; the upper for the interaction between species and stand and the lower for the comparison of means with the same level of stand. Open (71% PAR) (o); old-growth (44% PAR) (o); and pole (15% PAR) (•). Species have been put along the x axis in order of increasing original Ellenberg light indicator value or shade intolerance.

5.3.5 Other environmental variables

Open sites were dominated by wet to very moist soil moisture regimes (SMR) (Table 5.3). Pole and old-growth stands were more variable encompassing drier regimes with a range from moderately dry to wet. These broad Ecological Site Classifications match direct measurements of soil moisture to some extent. A significant difference in volumetric soil moisture content was found between stands in August 2001 (ANOVA: $F = 5.36$; d.f. = 2, 12; $P < 0.05$). The open stand was much wetter than old-growth (Tukey $P < 0.05$) but pole remained intermediate and similar to both (Table 5.4). In August 2002, no significant difference in volumetric soil moisture content was found between stand ($P > 0.05$) perhaps reflective of the high summer water table that year (c.f. Figure 5.1). A difference in the mean depth of rusting on steel rods was found between stand treatments, though not quite significant at 0.05 (ANOVA: $F = 3.30$; d.f. = 2, 11; $P = 0.08$) suggesting that aerobic soil conditions were deeper in old-growth, followed by pole then the open stands. A

soil moisture gradient is apparent with soils becoming increasingly drier and more aerated with stand development².

Sites were dominated by the ESC soil nutrient regime (SNR) class of “very poor”, the lowest class on a 5 point scale (Table 5.3). Exceptions included two pole stands with a slightly higher class of “poor”. Differences in new Wilson mean site values were found between stands (ANOVA: $F = 14.22$; d.f. = 2, 12; $P < 0.001$) with a higher nutrient level indicated in the pole (Tukey, $P < 0.05$) compared with old-growth and open stands which had lower but similar levels (Table 5.4). However, no significant difference in pH was found between stand treatments with a mean pH of 3.9 ($P > 0.05$). Accumulated results suggest that the nutrient status of soils across stands are all low with some evidence of improved fertility in the pole stand.

There was a significant difference in percentage cover of *Calluna vulgaris* between stand types (ANOVA: $F = 39.92$; d.f. = 2, 12; $P < 0.001$) with a much lower cover found in the pole stand (Tukey, $P < 0.05$) compared with higher but similar levels found in open and old-growth stands (Table 5.4).

Table 5.4 Means of environmental parameters recorded in the three stand treatments of open pole and old-growth pooled across blocks ($n = 7$) (\pm standard deviations).

Environmental variables	Open	Pole	Old-growth
Volumetric soil moisture content (m m^{-1}) 2001	0.61 _a (± 0.09)	0.42 _{ab} (± 0.16)	0.39 _b (± 0.12)
Volumetric soil moisture content (m m^{-1}) 2002	0.60 _a (± 0.05)	0.49 _a (± 0.17)	0.48 _a (± 0.09)
Aerobic soil depth (mm)	122 _a (± 154)	211 _a (± 136)	302 _a (± 139)
pH	3.82 _a (± 0.22)	3.82 _a (± 0.19)	3.95 _a (± 0.13)
Wilson mean site plant indicator value (new)	2.13 _a (± 0.15)	2.41 _b (± 0.11)	2.16 _a (± 0.04)
% of <i>Calluna vulgaris</i> (arcsine SQRT)	42.9 _b (± 16.3)	2.9 _a (± 3.7)	43.6 _b (± 19.7)

Means followed by the same letter code do not differ significantly (Tukey test, $P > 0.05$).

5.3.6 Plant size (Primary data)

For all primary and secondary data (derived variables), the main effects of species and stand treatments have been reported in the ANOVA tables in Appendix 2. However, the more interesting effects of species-stand interaction have been reported here along with descriptions of the main trends.

ANOVA results revealed that there were highly significant species-stand interactions ($P < 0.001$) for leaf number, leaf dry mass, stem, root and total dry mass of seedlings at final harvest (2002) (Appendix 2). The species-stand interaction was not significant for branch number nor leaf area ($P > 0.05$). Generally, deciduous tree

² It is a possibility that these patterns have also been influenced by forest management with trees planted / retained on drier sites.

species were found to increase all plant mass components with irradiance up to 44% PAR (old-growth) with no further production found in the open (71% PAR) (Table 5.5). The single evergreen, *I. aquifolium* showed an opposite pattern with higher dry mass in the pole stand. Generally tree seedlings had a greater number of leaves and branches in open and old-growth stands but larger total leaf areas in the pole stand.

In the first season (2001), ANOVA revealed a highly significant species-stand interaction for relative diameter growth (RDG) ($P < 0.001$) but not for relative height growth (RHG) or diameter:height ratio (d/h ratio) ($P > 0.05$). In the second season (2002), highly significant species-stand interactions were found for RHG, RDG and the d/h ratio ($P < 0.001$).

In 2001, RHG was highest in the pole stand followed by old-growth with lowest rates found in the open for all species (Figure 5.3). The opposite pattern was found for RDG with higher rates found in open and old-growth stands rather than in the pole stand. Differences in growth rates were not so distinct in 2002. In 2001, *S. aucuparia* had the highest RHG of all species across all stands but in 2002, *A. glutinosa* outgrew *S. aucuparia* in height. *A. glutinosa* also maintained the highest RDG of all species in open and old-growth for both years. Generally *I. aquifolium* had the lowest RHG of all species and was the only species which maintained a higher RHG in the pole stand in both 2001 and 2002. It also maintained a higher RDG in the pole stand in 2002.

In 2001 and 2002, d/h ratio was lowest in the pole stand with higher but similar ratios found in open and old-growth stands (Figure 5.3). This suggests tree seedlings had longer thinner stems in the deeper shade treatment (14% PAR) than at higher irradiance: an indication of etiolation as well as a lower level of stem robustness. Generally, *S. aucuparia* and *B. pubescens* had longer thinner stems than *I. aquifolium* and *A. glutinosa* across stands.

Table 5.5 Mean seedling performance of four native broadleaves growing in Caledonian pine-woodland sites at final harvest (September 2002). Dry mass and leaf area was \log_e (ln) transformed, and counts square rooted prior to ANOVA. Calculated means include estimated values for missing values. LSD = least significant difference of means at $P < 0.05$.

Stand	Pole	Old-growth	Open	
PAR %	15	44	71	
R/Fr	0.85	0.98	1.05	LSD
	<i>I. aquifolium</i>			
SQRT mean number of leaves	6.538	5.796	5.844	0.828
SQRT mean number of branches	1.992	1.898	1.918	0.214
ln mean leaf area	0.026	0.020	0.015	0.453
ln mean leaf mass	1.479	1.088	0.696	0.422
ln mean stem mass	1.364	1.199	1.221	0.235
ln mean root mass	1.288	1.341	1.215	0.231
ln mean total plant mass	2.506	2.367	2.234	0.226
	<i>A. glutinosa</i>			
SQRT mean number of leaves	4.685	4.990	4.723	0.828
SQRT mean number of branches	2.378	2.519	2.677	0.214
ln mean leaf area	0.043	0.037	0.038	0.453
ln mean leaf mass	0.156	0.431	0.326	0.422
ln mean stem mass	1.109	1.624	1.586	0.235
ln mean root mass	0.870	1.698	1.643	0.231
ln mean total plant mass	1.926	2.507	2.473	0.226
	<i>S. aucuparia</i>			
SQRT mean number of leaves	9.051	8.016	9.757	0.828
SQRT mean number of branches	1.242	1.331	1.427	0.214
ln mean leaf area	0.012	0.016	0.013	0.453
ln mean leaf mass	-0.876	-0.661	-0.731	0.422
ln mean stem mass	1.373	1.449	1.433	0.235
ln mean root mass	0.755	1.175	0.990	0.231
ln mean total plant mass	1.909	2.108	2.001	0.226
	<i>B. pubescens</i>			
SQRT mean number of leaves	5.616	6.938	5.311	0.828
SQRT mean number of branches	2.550	2.661	2.523	0.214
ln mean leaf area	0.010	0.009	0.003	0.453
ln mean leaf mass	-1.484	-1.071	-1.765	0.422
ln mean stem mass	0.674	1.089	0.804	0.235
ln mean root mass	0.241	0.935	0.610	0.231
ln mean total plant mass	1.253	1.792	1.461	0.226

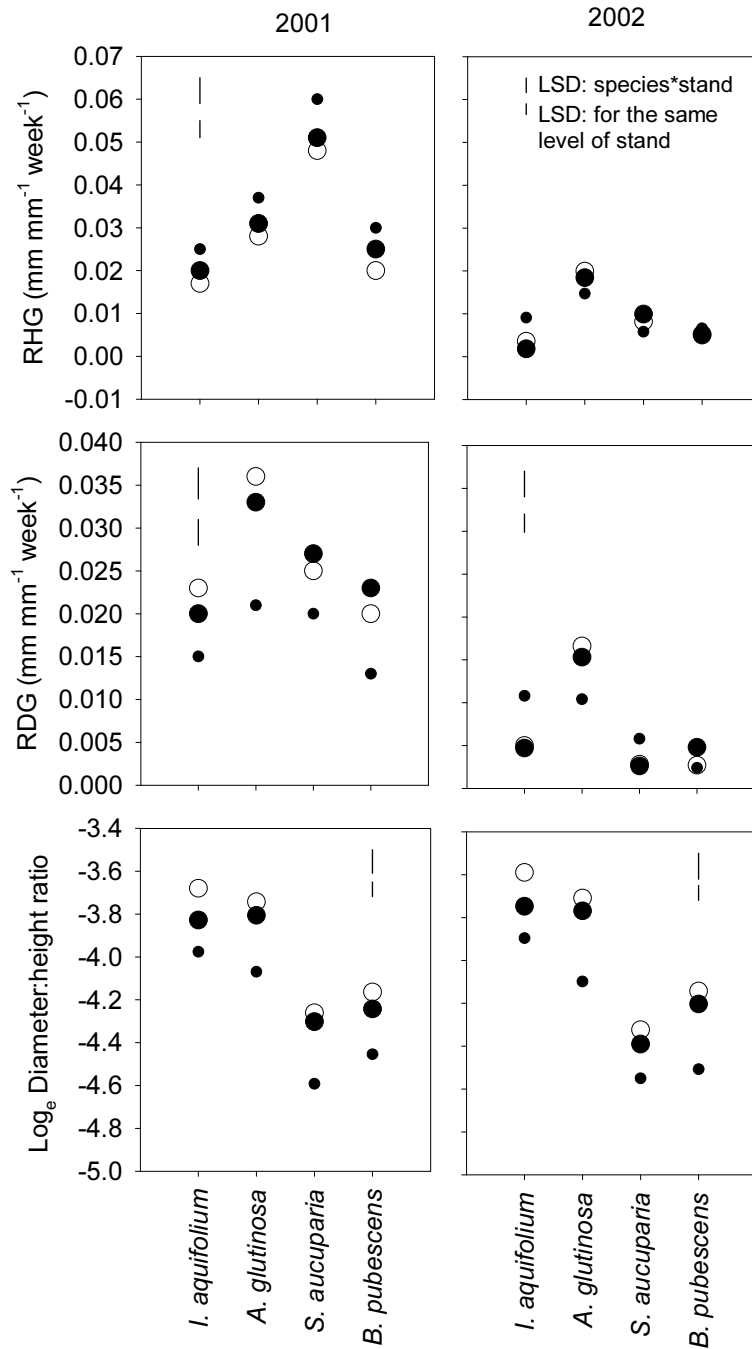


Figure 5.3 ANOVA plots displaying species-stand interactions for relative height growth (RHG), relative diameter growth (RDG) and \log_e diameter:height ratio for 2001 and 2002. LSD error bars have been appended to each plot; the upper for the interaction between species and stand and the lower for the comparison of means with the same level of stand. Open (71% PAR) (o); old-growth (44% PAR) (◐); and pole (15% PAR) (●).

5.3.7 Seedling architecture

ANOVA revealed a highly significant species-stand interaction for the leader:lateral ratio (LL ratio) (stem length / mean branch length) ($P < 0.001$). The LL ratio remained similar between stands for all species except *S. aucuparia* which had a higher LL ratio in the old-growth stand with lower but similar ratios found in pole and open stands (Figure 5.4).

5.3.8 Relative growth rate, morphology and physiology

ANOVA revealed a highly significant species-stand interaction for relative growth rate (RGR) for 2002 ($P < 0.001$) (ANOVA tables, Appendix 2).

B. pubescens grew most successfully in the old-growth stand with lower but similar RGRs found in open and pole stands (Figure 5.4). *A. glutinosa* had similar higher RGR in both old-growth and the open stands. The RGR of *S. aucuparia* did not differ between stand types. Generally *A. glutinosa* had the highest RGR of all deciduous species across stands except in the pole stand, where *S. aucuparia* and *A. glutinosa* shared similarly high rates of growth.

Highly significant species-stand interactions ($P < 0.001$) were found for net assimilation rate (NAR). Means display a positive response of NAR to irradiance (Figure 5.4). However NAR between stands for *S. aucuparia* and *B. pubescens* were similar and *A. glutinosa* was the only deciduous species that displayed a marked difference in NAR between the pole stand (15% PAR) and stands with higher irradiance.

Significant stand-species interactions ($P < 0.05$) were found for specific leaf area (SLA) and leaf area ratio (LAR). Generally species had higher SLA and LAR in the pole stand with lower ratios found at higher irradiance (Figure 5.4). *S. aucuparia* was an exception where there was little difference in LAR between stands. *A. glutinosa* had the highest LAR in the pole stand of all species, followed by *I. aquifolium* with lower but similar ratios found for *B. pubescens* and *S. aucuparia* (Figure 5.4). *I. aquifolium* had a much lowest SLA than all deciduous species with the latter not differing much in SLA within stands.

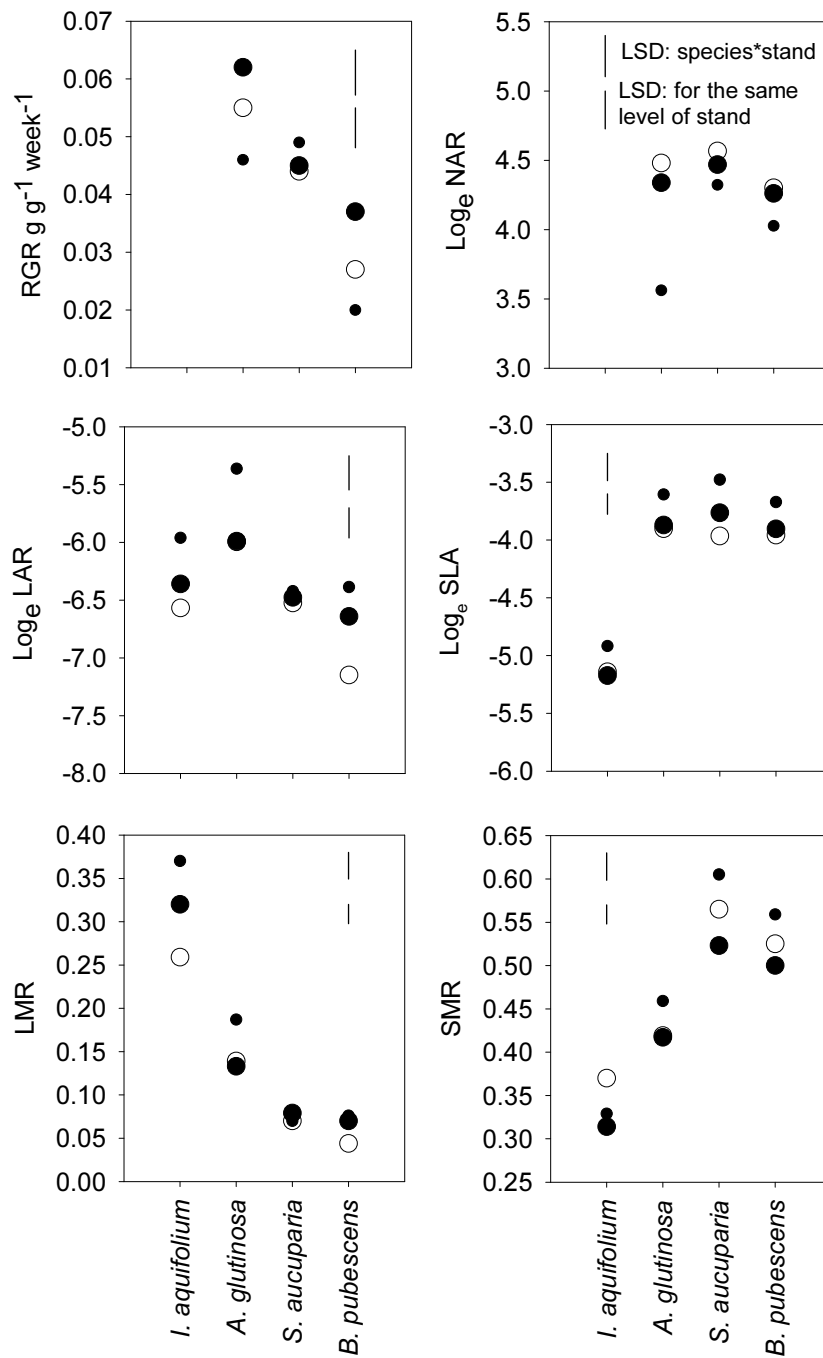


Figure 5.4 ANOVA plots displaying species-stand interactions for growth, morphological and biomass allocation variables: relative growth rate (RGR), leaf area ratio (LAR) and specific leaf area (SLA), net assimilation rate (NAR), leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR) and leader: lateral ratio (stem length:mean branch length). LSD error bars have been appended to each plot; the upper for the interaction between species and stand and the lower for the comparison of means with the same level of stand. Open (71% PAR) (o); old-growth (44% PAR) (o); and pole (15% PAR) (•).

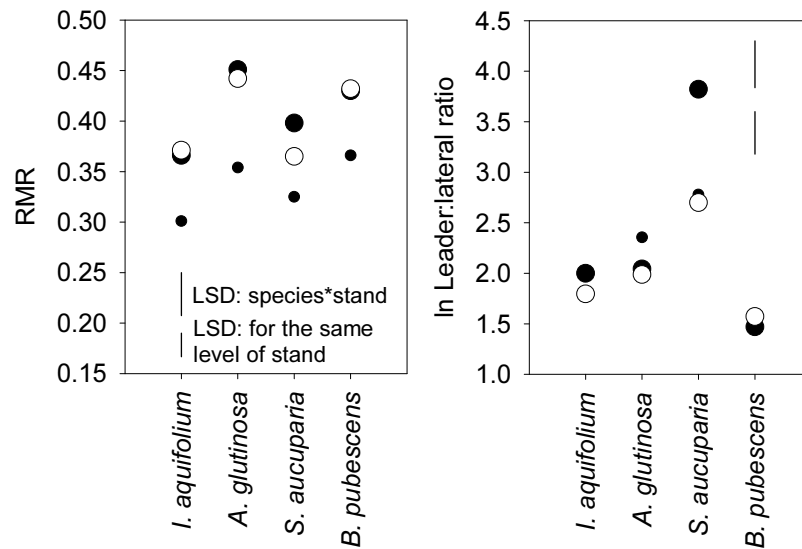


Figure 5.4 continued

5.3.9 Biomass allocation and partitioning patterns

ANOVA results revealed significant species-stand interactions ($P < 0.05$) for leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) (ANOVA tables, Appendix 2). LMR was higher in pole (15% PAR) compared with stands in the open (71% PAR) for all species except *S. aucuparia* where no differences in LMR were found between stands (Figure 5.4). Within stand comparisons revealed that *I. aquifolium* maintained the highest LMR and *S. aucuparia* and *B. pubescens* the lowest. SMR was higher in the pole stand with lower but similar ratios found in the open and old-growth stands for all species except *I. aquifolium* which showed an opposite pattern with a higher SMR in the open. Within stands, *S. aucuparia* had the highest SMR and *I. aquifolium* the lowest. RMR was reduced in the pole stand with higher but similar ratios found in old-growth and the open. Within stands, *A. glutinosa* and *B. pubescens* had the highest RMR and *S. aucuparia* and *I. aquifolium* the lowest.

S. aucuparia and *B. pubescens* shared similar biomass partitioning patterns where a higher proportion of carbon was allocated to the stem followed by root then leaves (Figure 5.5). The shift in biomass partitioning between stands was not dramatic with most change occurring in the pole stand where there was a reduction in root mass with most carbon being re-allocated to the stem. *A. glutinosa* also showed a decline

in root mass in the pole stand but with re-allocation of carbon to the leaves as well as the stem. *I. aquifolium* stands out from the deciduous species with a more equal balance of carbon allocation between plant components. Similarities with deciduous species include a decline in root mass in the pole stand accompanied with an increase in leaf mass.

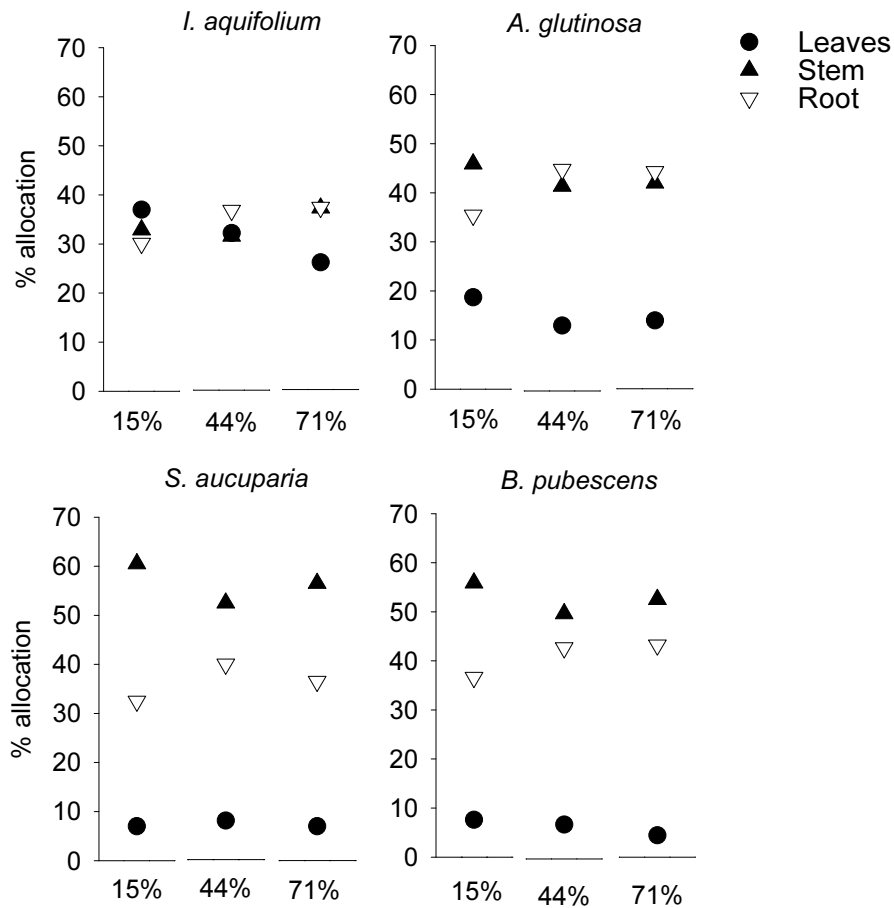


Figure 5.5 Biomass partitioning patterns at three different light regimes of 15% PAR (pole), 44% PAR (old-growth) and 71% PAR (open) for one evergreen (*I. aquifolium*) and three deciduous broadleaves (*S. aucuparia*, *B. pubescens* and *A. glutinosa*).

5.3.10 Survival and vigour

In July 2002, a greater percentage of healthy seedlings were found in the pole stand for *I. aquifolium* and *S. aucuparia* and in old-growth for *A. glutinosa* and *B. pubescens* (Figure 5.6). The greatest percentage of unhealthy seedlings were found amongst *I. aquifolium* in the open followed by *B. pubescens* in the pole stand.

Mortality was not recorded at all for *I. aquifolium* but this could just be reflective of difficulty in assessing death in the second season. There was certainly a larger proportion of dying stems in the open. Mortality amongst deciduous species was low (< 3%) occurring randomly across plots in the field. Relationships between vigour and damage caused primarily by insects and disease will be analysed elsewhere but are discussed at the end of this chapter.

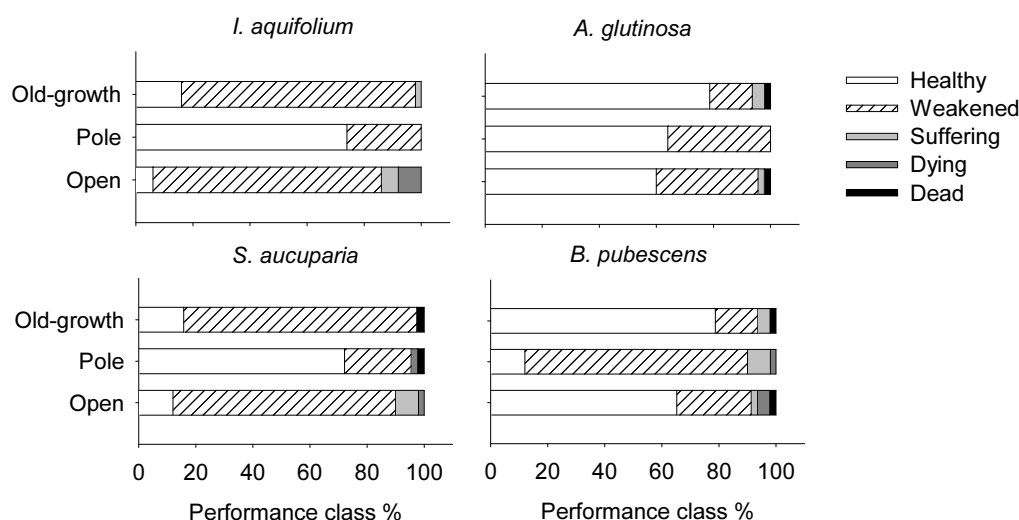


Figure 5.6 Percentage of seedlings within each performance class for all four broadleaved species (*I. aquifolium*, *A. glutinosa*, *S. aucuparia* and *B. pubescens*) under open, pole and old-growth pinewood stands in July 2002. Seedlings browsed by mice and accidentally snapped have not been included.

5.3.11 Interrelationships

RGR was found to be positively correlated with NAR across stand types for the deciduous species (Table 5.6). The RGR of *A. glutinosa* was negatively correlated with LAR across stands with other species displaying weaker negative trends. Correlations were found between LAR and SLA and LMR across stands for most species. *B. pubescens* was an exception, with a correlation only found between LAR and LMR. Generally only weak correlations were found between the RGR of species and other growth parameters (SMR, RMR, leader:lateral ratio) except for *A. glutinosa*.

Table 5.6 Person's correlations between RGR and LAR and other growth variables across all stand levels for individual species. Correlation co-efficients (r). Exclosures have been treated as independent sites (n = 21) hence statistical significance of tests are not shown.

	lnLAR	lnNAR	lnSLA	LMR	SMR	RMR	ln Leader:lateral ratio
				<i>I. aquifolium</i>			
LAR	-	-	0.72	0.78	-0.40	-0.66	0.16
				<i>A. glutinosa</i>			
RGR	-0.50	0.72	-0.48	-0.21	-0.52	0.47	-0.29
LAR		-0.84	0.83	0.74	0.53	-0.83	0.50
				<i>S. aucuparia</i>			
RGR	-0.31	0.52	-0.04	-0.03	0.21	-0.19	-0.38
LAR		-0.63	0.60	0.48	0.16	-0.28	0.44
				<i>B. pubescens</i>			
RGR	-0.06	0.63	-0.41	0.09	-0.28	0.21	0.09
LAR		-0.55	0.27	0.65	-0.20	-0.18	0.01

An exploratory analysis using best subsets regression revealed that PAR% and percentage cover of *C. vulgaris* (*C. vulgaris*%) were most commonly found to be the most important single predictors of growth rate (RGR, RHG, RDG) yielding high R² values. Other factors that were important included Wilson value (representing soil fertility) for *I. aquifolium* and *A. glutinosa* and soil moisture and frequency of overtopping vegetation for *S. aucuparia* and *B. pubescens*.

Regressions between growth rates and 1-3 of these environmental variables were found for each species using either a linear or quadratic model. Some interesting patterns emerge from these regressions of growth rate against environmental variables across all sites (Figure 5.7 & 5.8). Both PAR% and *C. vulgaris*% were clearly negatively associated with growth rates of *I. aquifolium*. Wilson value was also clearly positively associated with *I. aquifolium* growth. PAR%, *C. vulgaris*% and Wilson value were also clearly associated with *A. glutinosa* growth but with positive slopes for PAR% and *C. vulgaris*% in contrast to *I. aquifolium*. Both PAR% and soil moisture were most clearly associated with growth rates of *B. pubescens*. A curvilinear response is revealed with growth rates declining at extreme ends of both these environmental gradients. No one variable was clearly associated with the growth of *S. aucuparia* with similar contributions from PAR%, *C. vulgaris*%, Wilson value and soil moisture.

The regression plots (with the different stand types highlighted), however, also clearly show how growth responses to environmental variables are confounded with treatment effects. As irradiance levels (PAR%) increase from pole to old-growth to open, so does the percentage cover of *C. vulgaris*. Furthermore Wilson values (soil nutrients) are generally higher in the pole stand and the soil moisture content higher

in the open compared with all other stands. Thus, cautious interpretation of growth responses to environmental variables across all sites is required. Particular relationships may just be a consequence of higher or lower levels of one environmental variable in a particular stand type.

The regressions plots (Figure 5.7 & 5.8) also display the general lack of strong relationships between growth rates and environmental variables within individual stand types. When within-stand correlations between growth rates and environmental variables (including ingrowth of foreign roots and overtopping vegetation) were analysed, very few significant and/or strong correlations were found. Exceptions included strong positive correlations between RGR and RDG of *B. pubescens* and PAR% ($r = 0.76$, $r = 0.74$ respectively) and RDG of *B. pubescens* and *C. vulgaris*% ($r = 0.78$) across all pole stands. No strong correlations were found between the ingrowth of foreign roots and growth rates for individual species within individual stand types or across stand types. Correlations were performed using mean values calculated per plot as well as values from individual seedlings.

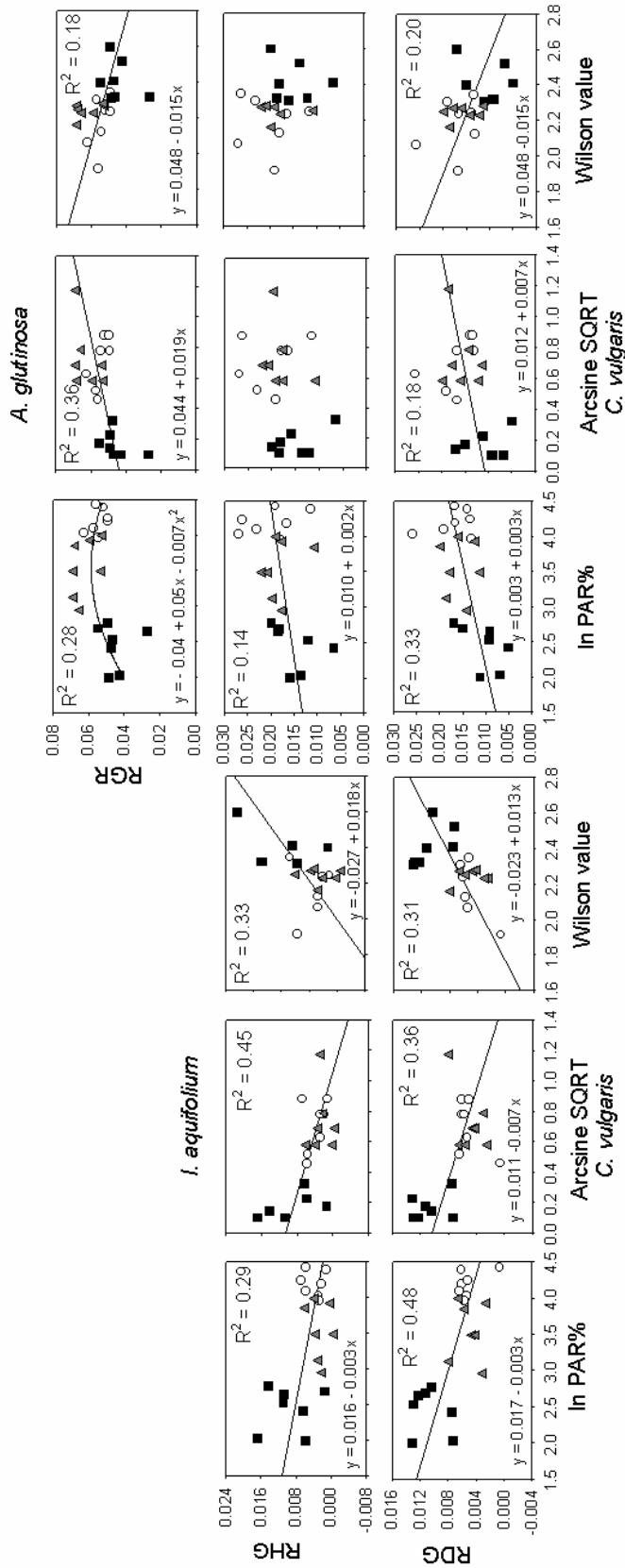


Figure 5.7 Relative growth rate (RGR $\text{g g}^{-1} \text{ week}^{-1}$), relative height growth (RHG $\text{mm mm}^{-1} \text{ week}^{-1}$) and relative diameter growth (RDG $\text{mm mm}^{-1} \text{ week}^{-1}$) regressed against environmental variables, \log_e (ln) PAR %, arcsine square-root (SQRT *C. vulgaris*), and Wilson value for *I. aquifolium* and *A. glutinosa*. The quadratic model was only used when both terms in the model were significant and thus justified its use over a linear model. Significance levels have not been attached to R² values due to a lack of independent between sites (n = 21). : pole; Δ : old-growth; o: open.

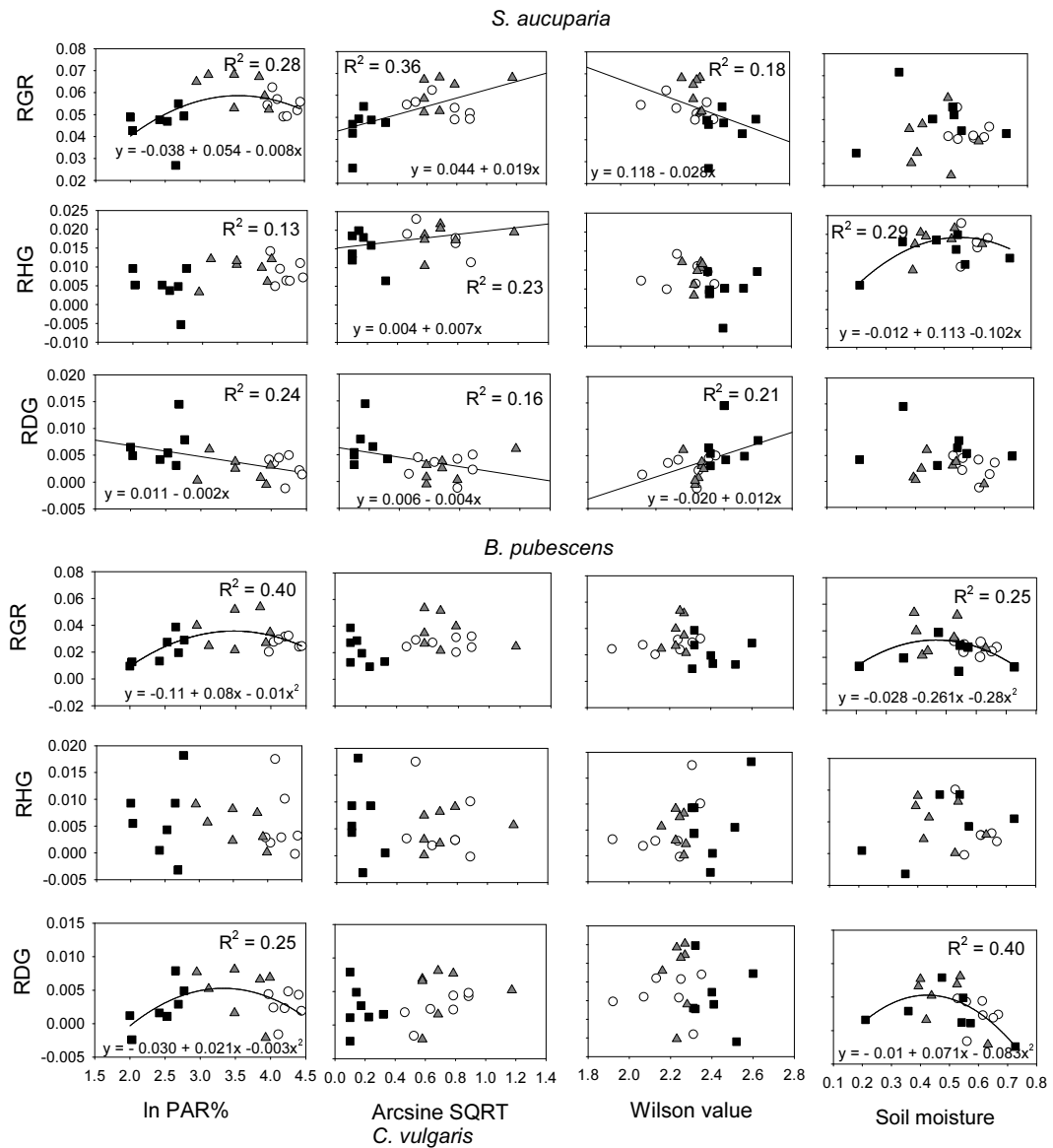


Figure 5.8 Relative growth rate (RGR $\text{g g}^{-1} \text{week}^{-1}$), relative height growth (RHG $\text{mm mm}^{-1} \text{week}^{-1}$) and relative diameter growth (RDG $\text{mm mm}^{-1} \text{week}^{-1}$) regressed against environmental variables, \log_e (ln) PAR%, arcsine square-root (SQRT) *C. vulgaris*%, Wilson value and soil moisture for *S. aucuparia* and *B. pubescens*. The quadratic model was only used when both terms in the model were significant and thus justifying its use over a linear model. Significance levels have not been attached to R^2 values due to a lack of independent between sites ($n = 21$). \square : pole; Δ : old-growth; \circ : open.

5.4 Discussion

Do species show classical responses to shade in the field?

The broadleaved tree seedlings displayed some of the typical responses that plants make to shade when light is the most limiting resource. These responses were displayed at 15% PAR in the pole stand rather than under lighter shade cast by old-growth canopies (44% PAR). Morphological adjustments to low light (15% PAR) for most species included an increase in SLA and LAR where the production of thinner leaves (higher SLA) and subsequent increases in LAR enhance light interception (Figure 5.4) (see Chapter 3). This conformed with responses of *B. pubescens* grown under varying structures of *P. sylvestris* forest in Finland (Messier & Puttonen, 1995) as well as with other temperate tree seedling studies (Loach, 1970; Latham, 1992; Grubb *et al.*, 1996; Reich *et al.*, 1998a). Typical reduction in growth rates with corresponding reductions in photosynthetic rate expressed in NAR due to light limitation was not so clear. *B. pubescens* and *S. aucuparia* displayed reductions in RGR to low light (15% PAR). However *S. aucuparia* showed little variation in RGR across stand irradiances and *I. aquifolium* actually had a higher rate of RGR in low light. *A. glutinosa* was the only deciduous species to reduce NAR significantly in low light where NAR remained similar across stands for *S. aucuparia* and *B. pubescens*.

All species displayed reductions in diameter growth (RDG) in low light (15% PAR) initially in 2001 but not in the following year. Height growth was actually enhanced in the pole stand in 2001, rather than reduced, due to etiolation of the stems. Height growth may have been enhanced by the potting compost which probably aided initial root establishment. *S. aucuparia* seedlings that were planted next to plots without the additional potting compost (unfertilised but well-aerated peat) did not grow so well as trial *S. aucuparia* seedlings (mean diameter and height increments were approximately 40% lower). Substantial reductions in the growth rates of seedlings were noticeable in the second year, perhaps a reflection of the compost effect wearing off. It is also well known that as woody seedlings increase in size, growth rates decline as a consequence of greater allocation of carbon to stems for support at the expense of the photosynthetic apparatus (ontogenetic drift phenomenon) (Givnish, 1988; Walters *et al.*, 1993).

When below-ground resources are not limiting, most herbaceous species and woody species grown in low light invest proportionally more to shoots than roots improving the capture of photon energy (Chapin *et al.*, 1987; Lambers & Poorter, 1992). All species displayed reductions in root in low light (15% PAR) but re-

allocation of carbon to other plant components was not consistent across species (as in Grubb *et al.*, 1996; Veneklaas & Poorter, 1998). *A. glutinosa* and *I. aquifolium* re-allocated carbon to both stems and leaves (Figure 5.5) (as in Atkinson, 1984; Walters *et al.*, 1993b; Kitajima, 1994; Messier & Puttonen, 1995; Walters & Reich, 1996). *B. pubescens* and *S. aucuparia* however re-allocated more carbon to just the stem with the percentage allocation to leaf mass staying fairly constant across difference irradiances (as in Reich *et al.*, 1998a).

According to Horn (1971) and Whitney (1976), plants well acclimated to shaded habitats should have a crown architecture that optimises light interception. The development of long lateral branch systems that bear leaves growing in shaded habitats can be seen as a way of avoiding the overlapping of leaves within a seedling crown (Kohyama, 1987). Generally broadleaved seedlings did not change their architecture in this way in response to shade (Figure 5.4). Messier and Puttonen (1995) also found no apparent architectural differences in *B. pubescens* between *P. sylvestris* stands of varying structure which they attributed to its lack of morphological plasticity. However, it is possible that seedling architecture in this trial remained similar between stands because of the influence of understorey plants.

What physiological and environmental factors play an important role in driving growth rates across different pinewood stands?

In theories of growth analysis, RGR is a product of a physiological component, NAR (net increment of plant mass expressed per unit leaf area) and a morphological component, LAR (leaf area relative to plant dry mass) (Evans, 1972). Positive relationships between RGR and NAR and negative relationships between RGR and LAR (except *B. pubescens*) across stands were found (Table 5.6). This result suggests that NAR was important in driving RGR in stands with higher irradiances but LAR was more important in lower light (15% PAR). A positive response of NAR to irradiance was apparent in the ANOVA plot (Figure 5.4) but *S. aucuparia* and *B. pubescens* actually retained similar NARs between stands. *A. glutinosa* was the only deciduous species that displayed a marked decline in NAR in low light (15% PAR). *I. aquifolium*, *A. glutinosa*, and *B. pubescens* responded to low light by increasing LAR but *S. aucuparia* retained a constant LAR across all irradiances. Furthermore, species differences in RGR in high light could not be clearly explained by differences in NAR as rates remained similar between species. In low light, *S. aucuparia* and *B. pubescens* have similar LARs, but their RGRs were quite different. Other factors are obviously contributing to variation in RGR in the field.

Both LMR and SLA were found to have similar positive relationships with LAR across stand types for all species which may suggest that both play an equal role in driving LAR ($LAR = SLA \times LMR$, Evans, 1972). *B. pubescens* was an exception, however, where only LMR was found to be positively related to LAR. The lack of relationships between RGR and other plant allocation / structural variables (SMR, RMR, leader:lateral ratio) across stand types suggests that leaf morphology (LAR, SLA, LMR) and physiology (NAR) were more important in driving RGR.

The measurement of several environmental variables at the stand level indicated that differences in irradiance alone would not be the sole determinant of differences in tree seedling growth between pinewood stands. Both pole and old-growth stands were characterised by lower light regimes, higher root competition, lower above ground competition from understorey vegetation, better aerated soils and lower soil moisture content than open stands (Table 5.4). The pole stands were distinguished with a much lower percentage cover of *C. vulgaris* in its understorey.

PAR% and soil moisture were most clearly associated with RGR and RDG of *B. pubescens* across stand types (Figure 5.8). The growth response to both variables was curvilinear, increasing at first but then decreasing at the top end of the resource gradient with a noticeable sharp decline in RDG. This may indicate particular sensitivity to an excess of soil moisture as well as sensitivity to very low light conditions. The latter is supported by the strong positive relationship found between growth rates and irradiance within pole stage stands alone.

B. pubescens is known to tolerate badly drained heathlands (even more so than *S. aucuparia* and *I. aquifolium*; Ellenberg, 1998) (Aas & Riedmiller, 1996; Savill, 1998) but it obviously has its limits. The poor establishment of *B. pubescens* in high water table conditions has been linked to root sensitivity to low oxygen tensions and mechanical damage of flooding (McVean, 1956b). It is also possible that *I. aquifolium* and *S. aucuparia* suffered from these factors. Strong correlations were not found between RGRs and soil moisture within the open treatment for these two species but that can easily be explained by uniform suppression.

Positive correlations were found between RGR ($r = 0.61$) and RDG ($r = 0.60$) of *A. glutinosa* and soil moisture across open stands. This positive response is not surprising as it conforms with the ecology of *A. glutinosa* which favours very wet conditions (Ellenberg, 1988; Aas & Riedmiller, 1996; Savill, 1998). It is able to tolerate water-logged conditions due to its deep penetrating root system and hypertrophied lenticels which are believed to increase the efficiency of the aeration system (McVean, 1956a, b).

Light (PAR%) and percentage cover of *C. vulgaris* were most commonly associated with growth rates of *I. aquifolium* and *A. glutinosa* across stands (Figure 5.7 & 5.8). It is well known that ericaceous shrubs like *C. vulgaris* can prevent root expansion (Mallik, 1995), chemically inhibit root growth and mycorrhization (Jalal & Read, 1983a, b) and competitively reduce the available supply of mineral nutrients (Leake, 1992). This explains the negative growth response of *I. aquifolium* to *C. vulgaris* cover. *I. aquifolium* also showed a strong positive response to Wilson value (nutrient availability) across sites which perhaps also emphasises a particular sensitivity to nutrient deficiency.

Most tree seedlings have been reported to be suppressed by "heather check" but this was not reflected in the growth rates of *A. glutinosa* or *S. aucuparia* where responses to *C. vulgaris* were (weakly) positive rather than negative (Figure 5.8). The differing age and structure of *C. vulgaris* across stands may have influenced results where older shrubs were found in the open. *B. pubescens* and *S. aucuparia* saplings have frequently been found on open ground in association with heather (McNeill, 1955; Kinnaird, 1974), particularly old heather (Miles & Kinnaird, 1979) which is less competitive in its degenerative state.

It should be stressed that the above growth responses may just be a consequence of higher or lower levels of one environmental variable in a particular stand type. For example, the percentage cover of *C. vulgaris* naturally increases with irradiance, a reflection of its light demanding nature (Gimingham, 1960; Ellenberg, 1988). Low levels of *C. vulgaris* in pole stands are in fact a characteristic of the low light treatment just as high levels would be a characteristic of open stands. This may explain in part the negative growth responses of *I. aquifolium* to an increase in *C. vulgaris*% but also the positive growth responses of *A. glutinosa*. The effects of *C. vulgaris*% on seedling growth is thus confounded with light. Grime (1965) pointed out that many environmental factors vary in concert and it is often difficult to recognise which of many interrelated variables are controlling plant distribution. However a prior knowledge of the physiology of species does aid interpretation.

Ingrowth of foreign roots and frequency of overtopping vegetation were not found to be closely associated with growth rates of the broadleaved species after two years of establishment. No significant negative correlations were found between the ingrowth of foreign roots and growth rates of individual seedlings. This conforms with Jones *et al.* (1989) and Jones & Sharitz (1990) who also found a great deal of scatter in their results for individual species and few significant relationships. This may lead one to conclude that root competition is not important in this infertile open Caledonian pinewood ecosystem. McVean (1956a) proposed that root competition is

perhaps more prevalent in woodland communities of drier soils but absent in woods (e.g.: alderwoods) where the water table is constantly high. It is possible that the potentially deleterious effect of foreign root ingrowth on seedling growth becomes more pronounced in later years with further root growth. If root competition was to have an effect, seedlings growing under pole and old-growth stands would probably be more susceptible where *P. sylvestris* roots were found to be particularly invasive.

The majority of unhealthy seedlings were allocated a weakened performance class (Figure 5.6), primarily due to the presence of yellow-green leaves or chlorosis. Usually chlorotic leaves are an indication of nutrient deficiency especially nitrogen which is important for the maintenance of photosynthesis and growth. Although relationships between growth and foliar nutrients were not analysed, it is highly likely that overall growth in all seedlings were suppressed by nutrient deficiency (nitrogen and phosphorus particularly) across all stands (see Chapter 7). All soils were poor to very poor according to ESC classification (Ray, 2001) and very acidic (pH 3.8).

Although all of the species in this trial do tolerate peaty acidic conditions (Peterken, 1967; Ellenberg, 1988; Savill, 1998) (even *A. glutinosa* has been shown to freely colonize peat of pH 3.5; McVean, 1956a), additional nutrients would most definitely have improved growth. The Forestry Commission have shown that neither birch, alder or rowan grow well when planted on heath or moorland peat in Scotland due to phosphate deficiency (Evans, 1984) and manuring with alkaline phosphate at the time of planting can improve growth (Zehetmayr, 1954). Gillham (1980) and Findlay (1999) have shown how *S. aucuparia* will grow on nutrient poor soils but growth rate is higher on more fertile soils. Furthermore Peterken (1967) found that *I. aquifolium* seedlings growing in the New Forest, rooted in acidic humus and mineral horizons possessed a markedly slower rate of weight increase than frequently watered, garden-grown seedlings rooted in John Innes No.1 compost. The positive effects of enhanced nutrients on tree seedling growth is well established (e.g: Crabtree & Bazzaz, 1993; Wang *et al.*, 1998; Keski-Saari & Julkunen-Tiitto, 2003) especially in more than 5% daylight (Coomes & Grubb, 2000) and with light demanding species (Grubb *et al.*, 1996). The lack of mycorrhizal associations on poor soil in the Highlands is commonly reported to retard the early growth of seedlings (McVean, 1963a, Miles & Kinnaird, 1979; Crowell, 1998). Mycorrhizal work was not carried out in this trial but its impact on growth is acknowledged.

Performance recordings of damage on individual tree seedlings indicated that pests and disease may have an important role in the growth of broadleaved seedlings in the field (analysed in a separate paper). In July 2002, generally 50-80% of all

seedlings suffered from some form of damage (Table 5.7). Insect leaf herbivory was the most common form of damage caused primarily by caterpillars and leaf miners followed by fungal damage. *A. glutinosa* leaves were infected with *Taphrina torquinettii* and a rust (*Melampsora* sp) was found on the leaves of *B. pubescens*. In 2001, a frost in June caused widespread damage to *I. aquifolium* seedlings particularly in the open. However most seedlings recovered by re-sprouting in the second year except for severely frost damaged individuals in the open stand. The holly leaf miner (*Phytomyza ilicis* Curtis) proved to be its biggest pest. The amount of damage on leaves (fungi/insects) and stems (frost) of individual seedlings, however was not substantial with mean damage scores indicating less than 20% damage. It is difficult to ascertain how much this damage affects overall growth of seedlings but any leaf surface damage would restrict photosynthesis to some degree introducing additional carbon stress especially in the shade. Consequences of pests and diseases on shade tolerance will be discussed later.

How can differences in growth, allocation and morphology in the field distinguish different degrees of shade tolerance?

Growth, allocation and morphological responses of *I. aquifolium* to shade were reflective of a species with much shade tolerance. *I. aquifolium* had the lowest growth rate of all the species reflecting its evergreen physiology. Low growth rates and tissue turnover rates are presumed to be adaptations to habitats characterised by chronically low resource availability such as shaded or nutrient poor environments (Grime & Hunt, 1975; Chapin, 1980; Reich *et al.* 1992). A slow, steady growth and conservation of reserves is believed to be of prime importance for shade tolerance (Loach, 1970).

Plant biomass, RHG and RDG of *I. aquifolium* in 2002 was found to be higher in the pole stand rather than in old-growth or open stands. Maintenance of these growth rates in the shade can be closely linked to adjustments in morphology where increases in LAR (explained by increases in both LMR and SLA) aid photon capture. *I. aquifolium* had a much higher LMR in the pole stand compared with the other species which in part is a consequence of its evergreen nature but also aids photosynthesis in low light. A reduction in roots with re-allocation to shoots (leaves and stems) also aids the maintenance of a positive carbon budget. In addition to these mechanisms, Del Hierro *et al.* (2000) has shown that *I. aquifolium* in low light can move its chlorophylls in the leaf mesophyll to a position where light absorption can be increased.

	Damaged%	Mechanical%	Mammal%	Fungi%	Insect%	Insect/Fungus%	Frost%	Other%
				<i>I. aquifolium</i>				
Open	71	0	0	0	49 (1.2 ± 0.4)	0	18 (1.9, ± 0.5)	4 (2.5 ± 0.7)
Pole	58	0	0	0	58 (1.3 ± 0.6)	0	0	0
Old-growth	82	0	0	0	78 (1.4 ± 0.6)	0	0	4 (1.5 ± 0.7)
				<i>A. glutinosa</i>				
Open	77	2 (4)	0	23 (1.7 ± 0.6)	15 (1.3 ± 0.5)	6 (1 ± 0.0)	0	30 (1.9 ± 0.5)
Pole	86	4 (1 ± 0.0)	2 (1)	12 (0.5 ± 0.5)	44 (1.9 ± 0.8)	18	0	6 (1.7 ± 0.6)
Old-growth	78	2 (1)	0	12 (1.2 ± 0.4)	34 (1.4 ± 0.5)	6 (1 ± 0.0)	0	24 (1.6 ± 0.7)
				<i>S. aucuparia</i>				
Open	78	0	2 (1)	0	76 (1.6 ± 0.8)	0	0	0
Pole	77	5 (3, ± 0.7)	2 (1)	0	68 (1.5 ± 0.6)	2 (1)	0	0
Old-growth	74	0	0	0	67 (1.6 ± 0.6)	5 (3 ± 0.0)	0	3 (2)
				<i>B. pubescens</i>				
Open	67	2 (4)	0	0	63 (1.8, ± 0.9)	0	0	2 (1)
Pole	83	4 (4 ± 0.0)	0	0	67 (1.6, ± 0.7)	9 (1.8 ± 0.9)	0	2 (4)
Old-growth	51	0	0	4 (1 ± 0.0)	43 (1.2, ± 0.6)	2 (1)	0	2 (1)

Table 5.7 Total percentage of damaged seedlings of four broadleaved species (*B. pubescens*, *S. aucuparia*, *A. glutinosa*, *I. aquifolium*) under open, pole and old-growth pinewood stands in July 2002 including percentage damage for each damage type (mean amount of damage and standard deviation included). Dead seedlings, seedlings damaged by mice and snapped artificially have been excluded from the data set). Mean percentage damage has been calculated from damage scores ranging from 1 – 4 (I: 0 – 5%; II: > 5 – 20%; III > 20 – 50%; IV: > 50%).

The majority of the leaves of *I. aquifolium* under the pole stand were very green and healthy suffering from less chlorosis and damage caused by holly leaf miner (*Phytomyza ilicis*) than in stands of higher irradiance (Table 5.7). *I. aquifolium* also suffered from the least amount of damage in the pole stand compared to all other species. Faster growth rates under shade (15% PAR) probably helped combat insect attack with thick evergreen leaves with spines providing additional defence (Grubb, 1992). Calcium oxalate crystals which are abundant in the abaxial cell layer of leaves may also provide additional mechanical constraints on larvae feeding within shaded leaves (in Potter, 1992). If defence against insect/ fungal attack plays an important role in determining shade tolerance in pinewood, *I. aquifolium* is shown to have a clear advantage over its deciduous counterparts.

The ability of *I. aquifolium* to tolerate higher irradiances in the field seems to be limited by *C. vulgaris* and frost. Seedlings were most susceptible to frost damage in the open and recovery was limited due to the additional stress imposed by water-logged soils. The negative association between *C. vulgaris* and growth of *I. aquifolium* conforms with observations by Peterken (1966) where exclusion on *C. vulgaris* heaths was caused by competition from the surrounding vegetation. Another factor which may have contributed to the reduction of growth at higher irradiances is photoinhibition where *I. aquifolium* in the UK has been found to be particularly sensitive to this under cold, high light conditions (Groom *et al.*, 1991).

Veneklaas and Poorter (1998) distinguish shade intolerant species from shade tolerants by their much higher RGRs in both low and high light driven by much higher values of LAR and NAR respectively. This can be linked closely to the ability of shade intolerants to be generally more flexible in form than shade tolerants (Grime, 1979; Grubb *et al.*, 1996; Reich *et al.*, 1998a). *A. glutinosa* was the only species that displayed some of these shade intolerant traits with a relatively high degree of morphological plasticity. It had the highest growth rates (RHG, RDG and RGR) in 2002 of all species, growing best in the old-growth and open stands. Growth rate in the pole stand was higher than most species except *S. aucuparia* which had a similar rate. A high RGR in low light was explained by a high LAR (driven by both SLA & LMR), which was much higher than all other species.

A higher RGR of *A. glutinosa* in high light (old-growth & open stands) could not be explained by a higher NAR as it remained similar to all other species. However, successful growth in these sites can be explained by *A. glutinosa*'s roots and their plasticity. It grew an extensive root system in the open sites enabling it to maintain relatively high growth rates in water-logged open conditions but was also able to reduce root mass in response to shade. (Results do not clearly indicate that

A. glutinosa had a substantially larger root system in the open compared to all other species but coarse lateral roots were lost accidentally in the harvesting process). Furthermore, a unique ability to fix atmospheric nitrogen in association with bacteria (*Frankia* spp.) in very large root nodules (Savill, 1998) probably also contributed to *A. glutinosa*'s successful growth rates across sites.

Other characteristics which suggest that *A. glutinosa* is shade intolerant include a slightly higher susceptibility to insect herbivory and disease in pole than in other stands (Table 5.7). The production of thinner leaves in the shade can be seen as detrimental as leaves are more susceptible to pests and disease. Its high SLA is a more beneficial trait in grassy swards in the open, where it can allow a plant to outgrow its competitors quickly and emerge from the shade stratum (Loach, 1970). Low etiolation and an ability to reduce roots with re-allocation of carbon to the leaves (as well as the stem), however suggests some degree of shade tolerance.

Responses of *B. pubescens* and *S. aucuparia* to different pinewood stands were similar in some respects suggesting they belong to the same functional group. Both displayed the highest rates of etiolation in low light (15% PAR) amongst all the species. They also shared very similar biomass partitioning where carbon was re-allocated from the roots to the stems rather than the leaves in low light. Growth rates (RHG, RDG, RGR) were also similar, particularly in the second season across stand types. The main difference in RGR was found in the open stands, where *S. aucuparia* grew much faster than *B. pubescens*. It is very likely that the growth rate of *B. pubescens* would have increased further in higher light if it had not been restricted by water-logged conditions. *B. pubescens* possessed a few traits that suggest a slightly higher degree of intolerance compared with *S. aucuparia*. It clearly maximised RGR in old-growth stands whereas *S. aucuparia* maintained similar growth rates across all stand types. *B. pubescens* also suffered from a greater proportion of weakened forms (Figure 5.6) with much higher susceptibility to insect damage in the pole stand (Table 5.7) compared with *S. aucuparia* (as well as the other species).

How do physiological responses match traditional notions of shade tolerance and Ellenberg light indicator values?

I. aquifolium has been classified as one of the most shade tolerant of all British trees (Savill, 1998). It has been given an Ellenberg light indicator value of 4 which indicates a shade to semi-shade plant, found between 5% and 30% PAR (Ellenberg, 1988) (Table 5.8). This value fits physiological responses in the field well, where the performance of *I. aquifolium* was most successful under 15% PAR.

A. glutinosa grew best at higher irradiances (44-71% PAR) with much morphological plasticity to different irradiances. These responses conform well to its common classification as a light demander (Evans, 1988; Aas & Riedmiller, 1996) which is very sensitive to shading (McVean, 1953a; Savill, 1998). Its ability to withstand semi-shade when young is acknowledged, however (Hart, 1988), and this fits well with successful growth under shade cast by old-growth pine and an ability to re-allocate carbon to leaves under shade. However long term survival is questionable where large thin leaves under shade are susceptible to insect herbivory and fungal attack. An Ellenberg light indicator value of 6 or 7 seems most appropriate. An intermediate value of 6 was also assigned to *S. aucuparia* which showed both light-demanding and shade tolerant traits.

B. pubescens is commonly classified as a light demanding pioneer (Miles & Kinnaird, 1979; Evans, 1988; Atkinson, 1992; Aas & Riedmiller, 1996; Hart, 1998; Savill, 1998). *Betula* saplings in Scotland are commonly reported to be fewer in number under dense canopy of trees or ferns with higher densities found on adjacent unwooded ground (Kinnaird, 1974; Miles & Kinnaird, 1979). However in Glen Affric, adverse soil conditions restricted fast growth rates in higher light and responses were more similar to *S. aucuparia*. It has thus been assigned a light indicator value of 6.

Table 5.8 Original Ellenberg values (Ellenberg, 1988) and Ellenberg light indicator values for British saplings (Hill *et al.*, 1999) with revised indicator values based on physiological responses in the field.

Species	Original Ellenberg light indicator values	Ellenberg light indicator values for British saplings	Physiological light indicator values
<i>I. aquifolium</i>	4	5	4
<i>A. glutinosa</i>	5	5	6-7
<i>S. aucuparia</i>	6	6	6
<i>B. pubescens</i>	7	7	6

5.5 Summary

A. glutinosa grew most successfully at higher irradiance in old growth and open stands but also did well across all stands due to its high plasticity in both root and leaf morphology. *B. pubescens* grew most successfully in old-growth and less well on sites with very high soil moisture content. *I. aquifolium* performed best in the pole stand with a negative response to increasing light and the percentage cover of *C. vulgaris*. The growth of all seedlings was most probably limited by nutrient deficiency and insect herbivory across stands. *I. aquifolium*, however showed the

lowest susceptibility of all species to these variables in the pole stand. *I. aquifolium* was most shade tolerant followed by *S. aucuparia* and *B. pubescens* and then *A. glutinosa*.