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Morphological and physiological responses of *Fagus sylvatica* and *Quercus robur* seedlings to light availability

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The thesis is submitted to University College Dublin in fulfilment of the requirements for the degree of Doctor of Philosophy

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June 2016
I would like to thank a number of people who have helped me through this work. I would like to show gratitude to my Teagasc supervisor, Dr. Ian Short, and UCD supervisor, Dr. Conor O’Reilly, for their advice, guidance and support and also for giving me the chance to carry out my Ph.D. studies. I would like to express thanks to Jerry Campion, Teagasc, for his help and support and for sharing his expertise. I also wish to thank Dr. Jim Grant, Teagasc, for his help with statistical analysis during this project. I also want to express thanks to Dr. Olga Grant for her help and comments on the physiological part of the study. I would also wish to acknowledge Ricky Byrne for his assistance. I would also like to thank everyone in the Forestry Development Department, Teagasc, and UCD Forestry.

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Abstract

The need to develop forest management systems other than clearfelling has resulted in a requirement for improved understanding of the potential of continuous cover forestry (CCF). One suggested method for the conversion of forest stands into CCF systems and for bringing under-performing forests into productivity is thinning in conjunction with underplanting. This study was an attempt to provide information on species suitability for underplanting of two important trees in European forestry: pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.). To determine the morphological, physiological and growth responses of these two species to different light conditions, beech and oak seedlings previously grown at full light for two years were covered by shading nets that provide different shade levels (62%, 51% or 28% of full light) or continued to be exposed to full light. The different shade levels were intended to mimic a range of underplanted conditions and the process of acclimation to shade was studied to provide information on the ecology and adaptation of underplanted seedlings. In addition to the controlled-shade experiment another study to determine the physiological responses of beech natural regeneration to shade was conducted under natural light conditions (from open gaps to closed canopy).

Both oak and beech displayed similar acclimation in response to shade for most of the traits investigated. At the plant level, seedling acclimation to shade included higher biomass allocation to above than below-ground parts and greater energy investment on height than diameter growth. At the leaf level, seedlings grown under shade reduced their leaf thickness and photosynthetic rates per unit area and increased their specific leaf area. This increase in specific leaf area seems to be one mechanism that allows seedlings to perform well under shade conditions. Another acclimation to low light conditions was to increase the efficiency of the photosystem II under shade. Photosynthetic rates were higher and leaves were retained for longer in seedlings grown at full light than under shade. Hence, this probably led to a greater growth in the full light than under shade. Despite this greater growth at full light, the results of this study suggest that beech and oak seedlings would be able to acclimate and perform well if underplanted below overstories that reduce the available light to as low as 28% of
full light without having any significant adverse effect on the quality of the final crop.
Statement of Original Authorship

I hereby certify that the submitted work is my own work, was completed while registered as a candidate for the degree stated on the Title Page, and I have not obtained a degree elsewhere on the basis of the research presented in this submitted work.

Ignacio Sevillano
Publications associated with this work

Declaration of co-authorship

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Research Degree for which thesis is being submitted: Doctor of Philosophy (PhD)
Title of Research thesis: Morphological and physiological responses of Fagus sylvatica and Quercus robur seedlings to light availability

This co-authorship declaration applies to the following paper:
Effects of light availability on morphology, growth and biomass allocation of Fagus sylvatica and Quercus robur seedlings

The student’s contribution to the paper
(Outline briefly the student’s intellectual and practical contribution to the research presented in the paper. A percentage figure only will not suffice)

- Performed study conception and design, acquisition of data, statistical analysis and interpretation of data, drafting and critical revision of the manuscript.

Other Authors contribution to the paper
(Outline briefly the contribution of other authors to the paper)

<table>
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<tr>
<td>Ian Short</td>
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<td>Jim Grant</td>
<td>Performed study conception and design, helped with statistical analysis, critical revision of the manuscript</td>
</tr>
<tr>
<td>Conor O’Reilly</td>
<td>Performed study conception and design, supervised the work, critical revision of the manuscript</td>
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PhD student: [Signature]  Date: 22/06/16 (dd/mm/yy)
List of abbreviations and symbols

\( A \)  
Photosynthetic CO\(_2\) assimilation (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \))

\( A_{\text{max}} \)  
Maximum photosynthesis rate (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \))

\( A/g_s \)  
Intrinsic water use efficiency (\( \mu \text{mol CO}_2/\text{mol H}_2\text{O} \))

\( A/E \)  
Instantaneous water use efficiency (\( \mu \text{mol CO}_2/\text{mmol H}_2\text{O} \))

\( B/R \)  
Blue/Red ratio

\( C_3 \text{ plants} \)  
Those exhibiting only the \( C_3/\text{Calvin-Basham} \) pathway of photosynthetic carbon fixation

\( \text{CCF} \)  
Continuous Cover Forestry

\( \text{DBH} \)  
Diameter at breast height (cm)

\( \text{DLE} \)  
Duration of leaf emergence (days)

\( \text{DS} \)  
Duration of senescence (days)

\( E \)  
Transpiration (\( \text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \))

\( \text{ETR} \)  
Electron transport rate (\( \mu \text{mol photons m}^{-2} \text{s}^{-1} \))

\( F'_v/F'_m \)  
PSII maximum efficiency in the light

\( g_s \)  
Stomatal conductance (\( \text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \))

\( \text{H:D} \)  
Height:diameter ratios

\( \text{Ht / StemMass} \)  
Main stem length divided by stem dry weight

\( l_c \)  
Light compensation point (\( \mu \text{mol m}^{-2} \text{s}^{-1} \))

\( l_{\text{max}} \)  
Light saturation point beyond which there is no significant change in photosynthesis (\( \mu \text{mol photons m}^{-2} \text{s}^{-1} \))

\( l_{\text{sat}} \)  
Light saturation point (\( \mu \text{mol m}^{-2} \text{s}^{-1} \))

\( L_{\text{th}} \)  
Leaf thickness (\( \mu \text{m} \))

\( \text{LMA} \)  
Leaf mass per area (\( \text{g cm}^{-2} \))

\( \text{LWA} \)  
Leaf weight per area (\( \text{g cm}^{-2} \))

\( \text{LWC} \)  
Leaf water content (\%)

\( \text{PAR} \)  
Photosynthetically active radiation (\( \mu \text{mol m}^{-2} \text{s}^{-1} \))
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PPFD</td>
<td>Photosynthetic photon flux density ($\mu$mol m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>PSII</td>
<td>Photosystem II</td>
</tr>
<tr>
<td>$q$P</td>
<td>Photochemical quenching</td>
</tr>
<tr>
<td>$R_d$</td>
<td>Dark respiration ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity (%)</td>
</tr>
<tr>
<td>R/FR</td>
<td>Red/far-red ratio</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SFM</td>
<td>Sustainable forest management</td>
</tr>
<tr>
<td>SLA</td>
<td>Specific leaf area (cm$^2$ g$^{-1}$)</td>
</tr>
<tr>
<td>SLM</td>
<td>Specific leaf mass (g cm$^{-2}$)</td>
</tr>
<tr>
<td>SLW</td>
<td>Specific leaf weight (g cm$^{-2}$)</td>
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<tr>
<td>SWC</td>
<td>Soil water content (%)</td>
</tr>
<tr>
<td>T</td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>VPD</td>
<td>Vapour pressure deficit (kPa)</td>
</tr>
<tr>
<td>$\Delta$Dia</td>
<td>Diameter increment (mm)</td>
</tr>
<tr>
<td>$\Delta$Ht</td>
<td>Height increment (cm)</td>
</tr>
<tr>
<td>$\Phi_{CO2}$</td>
<td>Quantum yield of photosynthesis ($\mu$mol CO$_2$ (\mu$mol$ photons)$^{-1}$)</td>
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<tr>
<td>$\Phi_{PSII}$</td>
<td>PSII operating efficiency</td>
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Introduction

This Ph.D. study was part of the Broadleaf Silviculture Research and Development Project (a COFORD-funded project led by Teagasc in collaboration with UCD), focussing on the potential implications of light availability on the growth and physiological responses of underplanted broadleaf tree species. The project has six work packages and this study (“how trees in mixtures respond to environmental factors”) is part of Work Package 2: Establishment of Optimum Species Mixtures.

Foresters modify stand density and structure to address different challenges and meet different objectives. Many silvicultural systems have been used in Europe over the last centuries (Hart, 1995), mixing old forest practices with new techniques, to facilitate the establishment and growth of the remaining stand or regeneration. One of the main objectives of these silvicultural practices (from thinning to the application of different silvicultural systems) is to increase the amount of light that reaches the understory, which is a critical factor determining seedling establishment and survival.

More sustainable practices of forest management need to be developed. These demands can be achieved with the implementation of Continuous Cover Forestry (CCF), which makes use of the control of light to provide a wide range of benefits. Under CCF, forest canopy is maintained at one or more levels without clearfelling (Mason et al., 1999). Therefore, the understanding of how tree species utilise light under different scenarios plays an important role in the forest management under CCF systems.

The quality and quantity of solar radiation are a key factor for competition and growth within forest ecosystems. The establishment of gaps within a forest stand results in increased resources, such as light, nutrients and water. Although light is required for photosynthesis, both high and low sunlight can limit plant performance (Valladares and Niinemets, 2008). Therefore, efficient management of plant communities should be directed toward improving the amount and efficiency of photosynthesis (Kozlowski and Pallardy, 1997)\(^1\).

\(^1\) The specific point referred to is to be found on page 88 of the book referenced.
To date there has been little research on the functional basis of the tree responses to light, as most research has focussed on the growth responses. The main objective of the study was to investigate the effect of light on growth, morphology, phenology, tree architecture and physiology of underplanted pedunculate oak (*Quercus robur* L.) and European beech (*Fagus sylvatica* L.) trees. These species are an important component of forestry in Europe and are among the broadleaf species most suited to Irish forestry. The study focused on how these species respond to competition for light during the early establishment stage, which is critical for the sound management of tree species.

The specific objectives of this study were:

- To investigate the impact of light availability on survival, growth and biomass allocation in beech and oak seedlings planted under the same environmental conditions (Chapter 2).
- To determine the physiological responses (photosynthetic rates, chlorophyll fluorescence, water use efficiency) to different light levels of seedlings of beech and oak planted under the same environmental conditions (Chapter 3).
- To evaluate the phenology of growth of beech and oak seedlings planted under the same environmental conditions in response to light availability (Chapter 4).
- To determine the effect of light availability on stem form and quality of seedlings of beech and oak planted under the same environmental conditions (Chapter 5).
- To determine if similar physiological responses to those revealed in planted beech seedlings (Chapter 3) can be detected in natural regeneration of beech under an existing canopy (Chapter 6).

A controlled shade experiment provided most of the data used in the studies (Chapter 2, 3, 4 and 5). The two species were exposed to four different light levels (100%, 62%, 51% and 28% of full sunlight). Another study was carried out under forest conditions to determine if the responses were similar to those observed in the shadehouse trial (Chapter 6).
The understanding of the response of these tree species to different light levels might provide useful information to determine appropriate practices for the management of forests under alternative silvicultural systems to clear cutting.
1.1 Evidence from Irish forests

Ireland has one of the best climatic conditions for growing trees in Europe due to the high rainfall and long growing season. The potential natural vegetation of Ireland is temperate woodland and under present climatic conditions, and without human interventions, Ireland would be largely covered by deciduous forests (Cross, 1998, 2006). However, the current forest cover (11%) is substantially different from that expected to occur under natural conditions, being one of the lowest forest cover of all European countries (average around 32%) (Forest Europe et al., 2011). It is estimated that there were extensive forests in Ireland before 1600 but these forests had largely disappeared by 1800, probably due to centuries of logging as the population increased and the exploitation of the land was intensified (Forest Service, 2008). Since the foundation of the State in 1918 forest cover has grown from 1.4% to 10.5% in 2012 (Fig. 1.1). This increase in forest cover reflects an active State policy in favour of afforestation.

![Fig. 1.1. Changes in forest cover in Ireland, 1656-2012. All estimates prior to 1918 relate to the whole of the island of Ireland, thereafter estimates are just for the Republic of Ireland. Source: Department of Agriculture, Food and the Marine, 2014b.]
Farm forestry has increased in importance in recent years and plays a large role in economic, environmental and social development (Department of Agriculture, Food and the Marine, 2014a). A new policy has been recently published by the Department of Agriculture, Food and the Marine, which takes into account changes that occurred in Irish forestry and provides a guide to a sustainable and efficient expansion of the forest sector (Department of Agriculture, Food and the Marine, 2014a). The recommended policy and actions include:

- Expansion and management of the forest resource: to increase forest area and manage the forest resource according to sustainable forest management (SFM) principles.
- Environmental and public goods: to ensure that afforestation, forest management and development of the sector are undertaken according to environmental requirements and objectives.
- Supply chain and wood processing: to provide sustainable solutions for the development of the wood processing and the supply chain.
- Forest protection and health: to maintain a healthy forest environment through SFM, early detection and control of harmful invasive species, pests and diseases.
- Education training and research: to ensure the availability of suitable programmes of education, training and research.
- Quality, standards and certification: to focus on the quality of forest products and services, and the management of the forest resource.
- Policy and legislation: to be implemented and updated to meet changing needs supporting the principles of SFM.
- Funding: to support the development of the forest sector through EU and direct State funding, and facilitate private investment.
- Institutional arrangements: to support the development of the Forest Service to meet the needs of Government, national forest policy and forest sector.

The forests in Ireland amount to a total area of 758,761 ha, with approximately 360,834 ha (48%) being private forests. It is estimated that approximately 75% of the national forest state is predominantly conifer species,
while the remaining 25% is comprised mainly of broadleaf and mixed forest (Forest Service, 2008). Approximately half of this broadleaf and mixed forest is formed by native species such as oak, ash (*Fraxinus excelsior* L.), birch (*Betula* spp.) or alder (*Alnus* spp.).

In Ireland, a number of programmes provide financial support for forestry, including afforestation, native woodlands establishment, agro-forestry, forestry for fibre, forest reproductive materials, forest road and woodland improvement. Beech and oak, the species chosen for study, are eligible for the Grant and Premium Scheme under many of these programmes. The Thinning and Tending Scheme (woodland improvement) is associated with broadleaf forests to improve the health, quality and vitality of these forests by forest management (Forest Service, 2015a). Wood production managed through CCF silviculture is allowed under the Native Woodland Conservation Scheme, where it is compatible with the site’s ecological objectives (Forest Service, 2015a).

Broadleaf planting has become increasingly important in Ireland over recent years, with 30% of the area afforested each year planted with broadleaf species (Forest Service, 2008; Fig. 1.2). The increase in broadleaf planting is in agreement with a strategic plan for the development of Irish forest sector which put special emphasis on broadleaves and mixed species forests (Forest Service, 1996). This increase in broadleaf planting and the fact that some plantations have reached the stage where thinning interventions are required have heightened the demand for research to:

- Improve the quality and productivity of farm forests.
- Develop management and silvicultural intervention strategies to optimise the value of the forest.
- Identify different strategies for optimising financial returns from farm forests.
Fig. 1.2. Proportion of broadleaf planting in Ireland from 1998 to 2013 (Data collated from files posted on Teagasc website - https://www.teagasc.ie/crops/forestry/downloads/).

In Ireland, the majority of grant-aided forests (about 95%) are managed on a clearfell system (Department of Agriculture, Food and the Marine, 2014a). However, alternative silvicultural systems to clearfelling are being considered with the aim of delivering multipurpose objectives in forest management. This alternative silviculture generally involves partial harvesting, use of mixed species and natural regeneration. Thus, there is an increasing interest in CCF silvicultural systems, which are an alternative to clearfelling and can provide different economic and environmental benefits (Bosbeer et al., 2008). In spite of the interest and increase in broadleaf planting in Ireland over recent years, current observations indicate that many broadleaf plantations will have a high proportion of poor quality trees at the end of the first rotation (Hawe and Short, 2012). Therefore, there is growing demand for forestry research information in regard to the thinning of broadleaf forests in Ireland and the development of methods to manage under-performing broadleaf forests. One option for bringing these forests into productivity is thinning in conjunction with underplanting. However, little information of this kind has been produced in Ireland.

1.2 Planted forests

Planted forests are composed of trees established (either by afforestation or reforestation) through planting and/or seeding of native or introduced species. In its simplest form, plantation forestry describes the intensive management of a
forest crop for a limited range of products (i.e. more timber produced per ha), so may help offset declines in production elsewhere (e.g. forests set aside for parks and recreation). However, a more appropriate definition integrates other land uses and promotes an early and continuing production of different goods, services and values (Savill et al., 1999). Planted forests are established for different purposes, such as providing wood and non-woody products. Europe has the second greatest area of planted forests in the world, accounting for about 27% of global planted forest area (FAO, 2010). Although there was a significant increase in planted forests on a global and regional scale since 1990, there has been decline in the planted forest area in some countries. Planted forests now have the potential to provide wood and non-woody products, as well as environmental and social services (FAO, 2010).

Forests play a vital role in public recreation, biodiversity, water quality and the fight against climate change. Their heterogeneity and dynamic elements, together with all the interactions that occur in the forests, have increased the recognition of forests as complex systems (Puettmann et al., 2013). Similar to other types of forests, plantations could also be diversified and managed as complex adaptive systems, adapting them to natural, social and economic changes (Paquette and Messier, 2013). The understanding of forests as complex systems has raised the necessity to develop different approaches, tools and procedures to design (or redesign) new forest management methods (Puettmann et al., 2009). Silviculture is a discipline that studies the establishment, growth, composition, health and quality of forests to fulfil different needs and objectives. The discipline of forest ecology has essentially developed over the preceding century and its development and understanding now provide crucial information for forest management. Therefore, the disciplines of forest ecology and silviculture are strongly intertwined, as they offer complementary perspectives on how to apply sustainable management to forests (Puettmann et al., 2009).

1.3 Continuous Cover Forestry

Continuous Cover Forestry (CCF), sometimes known as Irregular Forest Management or Close to Nature Forestry, includes those silvicultural systems which involve continuous and uninterrupted maintenance of forest cover, and
avoid clearfelling (Pommerening and Murphy, 2004). Although many different terms have been used to refer to CCF, often some of them emphasise only a specific aspect of CCF (Pommerening and Murphy, 2004) or are too vague to be practical (Mason et al., 1999). CCF makes use of natural processes, such as the control of light, by contriving stand structures through thinning and selective felling to produce in a cost effective way, economic, environmental and quality benefits.

CCF describes one approach to forest management, which means that it is more than a silvicultural system. Silvicultural systems (e.g. shelterwood, selection systems, underplanting, coppice, free-growth) are means by which the chosen management objectives can be achieved. Mason et al. (1999) states that the guiding principles of CCF are the following:

- Managing the forest ecosystem rather than just the trees. Under continuous cover, the stands in the forests are considered as part of an ecosystem from which timber is harvested at intervals but aspects of the forest, such as conservation or protection, have the same importance.
- Avoidance of clearfelling. One of the distinctive elements of CCF is the avoidance of clearfelling of areas greater than 0.25 ha or more than two tree heights wide without the retention of some mature trees.
- Reliance on natural processes as the basis for stand management. CCF uses as much as possible natural processes for forest management. For example, wherever possible, natural regeneration is preferred. Although large-scale artificial regeneration is generally avoided, there are exceptions, such as conversion of stands that are not site-adapted or reforestation of sites where natural regeneration is accomplished with difficulty (Bauhus et al., 2013).
- Working within site limitations. Forest management makes use of site-adapted tree species that are compatible with site conditions.
- Creation of a diverse stand structure with a range of species. Increase the representation of stands with greater diversity (species and structure) since the recognition of the ecological value of mixed forests was one of the early motivations for CCF management (Bauhus et al., 2013).
CCF should not be seen in direct competition with plantation forestry, but rather is a management choice once forests have been established. The main advantages and disadvantages of CCF are summarised in Table 1.1.

In some parts of central Europe the destruction of forests from 1980 to 2000, the global climate change debate, and the interest in sustainable forest management and multi-purpose forestry have contributed to the promotion of the principles of CCF (Hasenauer and Sterba, 2000, Knoke and Plusczyk, 2001, Vítková et al., 2013). CCF systems are being introduced throughout Europe, focussing on the direct transformation of existing even-aged plantations to some form of mixed, uneven-aged woodland (Pommerening and Murphy, 2004).

In Ireland, although many forests are managed under the clearfell system, the commitment to sustainable forest management and interest in different forest management options has increased the concern for CCF (Kennedy et al., 2007). Among others, the shelterwood (shelterwood or shelterwood with reserves) and the selection systems (group selection or single tree selection) are silvicultural systems appropriate to Irish conditions that can deliver CCF (Ní Dhubháin, 2003). As the interest in CCF is increasing and CCF is implemented in different forests throughout Ireland, Irish foresters should become familiar with this kind of management.
Table 1.1. Advantages and disadvantages of CCF (adapted from Mason et al., 1999).

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low visual impact</td>
<td>More complex management (requires high-skilled staff)</td>
</tr>
<tr>
<td>Integration of multiple objectives</td>
<td>Focus on native species may restrict adaptation to future challenges</td>
</tr>
<tr>
<td>Greater structural and species diversity</td>
<td>Genetic diversity might be reduced when removing fittest trees</td>
</tr>
<tr>
<td>Less disturbance of forest ecosystem (promotes ecological stability and</td>
<td>Higher harvesting and infrastructure costs (dense road net needed)</td>
</tr>
<tr>
<td>natural processes)</td>
<td>Lack of disturbance may limit establishment of light-demanding species and</td>
</tr>
<tr>
<td>Protection of the environment by maintaining uniform forest conditions</td>
<td>reduce biodiversity</td>
</tr>
<tr>
<td>(e.g. carbon sequestration)</td>
<td>More site damage on heavy soils (less brash to provide brash-mats)</td>
</tr>
<tr>
<td>Protection of forest soil (erosion, nutrient cycling)</td>
<td>Less suited where there is heavy browsing pressure (damage to natural</td>
</tr>
<tr>
<td>Provide shelter for regeneration seedlings</td>
<td>regeneration)</td>
</tr>
<tr>
<td>Focus on development of individual trees</td>
<td>Wind damage risks when transforming regular stands</td>
</tr>
<tr>
<td>Flexibility to accommodate to local conditions</td>
<td>Time required to determine success</td>
</tr>
<tr>
<td>Minimizes establishment (if natural regeneration is possible and successful)</td>
<td>Yield prediction is more difficult</td>
</tr>
<tr>
<td>and tending costs (e.g. reducing need for weeding)</td>
<td></td>
</tr>
<tr>
<td>Increases the capital value of woodland</td>
<td></td>
</tr>
<tr>
<td>Greater productivities (large diameter and high quality sawlogs) and</td>
<td></td>
</tr>
<tr>
<td>continuous income (supply of wood of different dimensions, permanent jobs)</td>
<td></td>
</tr>
</tbody>
</table>
1.3.1 ProSilva

ProSilva was founded in 1989 and brings together foresters practising management which follows natural processes. ProSilva is a European federation of foresters who promote forest management strategies which optimise the maintenance, conservation and utilisation of forest ecosystems in such a way that the ecological and socioeconomic functions are sustainable and profitable, including market and non-market objectives and taking the whole forest ecosystem into consideration. ProSilva Ireland was founded in order to develop an alternative to clearfelling in Irish forestry and promote CCF throughout Ireland.

1.3.2 Transformation to CCF

As mentioned above, special emphasis has been put on the process of transformation to CCF since more sustainable management practices are pursued. In this section a few examples of transformation to CCF are described.

Kenk and Guehne (2001) described three different case studies of transformation in south-western Germany. The first case described the transformation of an even-aged stand to an uneven-aged stand structure. The other two cases outlined the transformation from coniferous to mixed stands. In one of them the authors reported the transformation of Norway spruce (*Picea abies* L. (Karst.)) stands on stable (by thinning and natural regeneration or underplanting of beech and/or silver birch) and unstable (by clearfelling at the end of the rotation, seeding and replanting with oak or underplanting with beech or silver birch, followed by single-tree harvesting) sites. In the other case, the authors discussed the transformation of pure Scots pine (*Pinus sylvestris* L.) stands into mixtures of different structures by clearfelling or partial reduction of the overstory and underplanting with beech, oak (*Quercus* spp.), lime (*Tilia* spp.) or hornbeam (*Carpinus betulus* L.).

In Scotland, a transformation trial was established in 1952 with the aim of creating and maintaining a continuous cover forest (Kerr *et al.*, 2010). The main species in the trial area were: 43% of Sitka spruce (*Picea sitchensis* (Bong.) Carr.), 25% of Norway spruce, 13% of Japanese larch (*Larix kaempferi* (Lamb.) Carr.) and 6% of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Although in the early phase of transformation the main objective was planting with Norway
spruce, European silver fir (*Abies alba* L.) and European beech, the main method of transformation was group felling followed by natural regeneration and planting. Kerr and Mackintosh (2012) found that beech saplings had the highest survival rates in this forest.

Another trial in Scotland focussed on transformation into an irregular stand (Cameron and Hands, 2010). This forest was originally a mixture of Norway spruce, Scots pine, European larch (*Larix decidua* Mill.), Douglas fir and European beech. Transformation involved planting with small groups of native and introduced conifer and broadleaf species within the existing mixed species stand (initial phase of the transformation), which were managed under the selection system (final phase of the transformation). The reduction in canopy cover increased the regeneration of seedlings in the understory, where the dominant species were shade-tolerant (western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and beech) followed by intermediate shade-tolerant species (Douglas-fir and Norway spruce) (Cameron and Hands, 2010).

In Ireland, different field experiments were established to assess the transformation of even-aged coniferous stands into continuous forest systems (Ní Dhubháin, 2010). The species used in this study included Sitka spruce, hybrid larch (*Larix x eurolepis* Henry), western red cedar (*Thuja plicata* Donn Ex D. Don), beech, downy birch (*Betula pubescens* Ehrh.) and sessile oak (*Quercus petraea* (Matt.) Liebl.). The results showed that the shelterwood system did not provide enough light for the survival and growth of the understory trees, even for the most shade-tolerant species. The study recommended that the process of transformation of a Sitka spruce stand 1) should start early (e.g. time of first thinning); 2) thinning should be heavy enough to increase light and facilitate establishment of the understory without risking stand stability (thinning can be carried out at short intervals as the overstory basal area quickly achieves the value before thinning); and 3) regeneration might be supplemented with underplanting.

### 1.4 Light

Light is one of the main environmental factors affecting stand development. Shade tolerance, considered as the minimum light required for survival, plays an
important role in forest dynamics. Light might affect, directly or indirectly, other environmental parameters, such as temperature, humidity, soil condition, and differences in light intensity and quality may result in changes in stand structure across forests (Larcher, 2003). Photosynthesis is the process by which light energy is captured by green plants and used to provide energy necessary for plant growth. Light provides the energy used in photosynthesis and the signals used in photoregulation of plant growth and development, and is, among the factors affecting plants, perhaps the most spatially and temporally heterogeneous (Pearcy, 1999). Understory light is the main driver of regeneration growth and its control is a key factor in regenerating mixed stands (Lieffers et al., 1999).

Incident radiation can reach the interior of a forest stand in different ways: as direct radiation through canopy gaps or margins, as scattered light after being reflected by leaves and/or soil surface, and as transmitted light through leaf blades (Larcher, 2003). In closed and dense canopies, understory plants can increase carbon gain by capturing more light (Pearcy and Sims, 1994). Plant species have a great capacity to modify leaf, shoot and canopy characteristics with changing light availability (Niinemets, 2010), resulting in different morphological structures to adapt to the light available within the canopy (Larcher, 2003). Therefore, the efficiency of light harvesting is of crucial importance for the growth and development of plants under shaded conditions.

Shade tolerance is an ecological concept referring to the capacity of a plant to tolerate low light levels. Shade tolerance is associated with different plant features, from leaf level to whole plant, affecting establishment, growth and survival under these low light conditions (Table 1.2). Tolerance is used to indicate the capacity of trees to develop and grow in the shade of other trees, and is one of the most important factors to affecting tree growth. Tree species that compete well under full shade are called shade-tolerant or tolerant species, while those that require high light are called shade-intolerant or light-demanding species. Between the extremes of shade-tolerant and light demanding species, intermediate shade-tolerant species can be found, depending on their ability to grow, develop and compete under shade. Some species with intermediate characteristics can survive and establish under shaded conditions at early stages but they need light to accelerate growth at some stage. Trees tolerance may vary
on the basis of age, region or site. Therefore, information on species shade tolerance is important for forest management.

**Table 1.2.** Expected value in low light conditions for different features of shade-tolerant species (adapted from Valladares and Niinemets, 2008).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Shade-tolerant plant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leaf level</strong></td>
<td></td>
</tr>
<tr>
<td>Light compensation point</td>
<td>Low</td>
</tr>
<tr>
<td>Quantum yield</td>
<td>High</td>
</tr>
<tr>
<td>Dark respiration rate</td>
<td>Low</td>
</tr>
<tr>
<td>Net photosynthesis rate</td>
<td>Low</td>
</tr>
<tr>
<td>Sunfleck utilization efficiency</td>
<td>High</td>
</tr>
<tr>
<td>Electron transport rate</td>
<td>Low</td>
</tr>
<tr>
<td>Sensitivity to photoinhibition</td>
<td>High</td>
</tr>
<tr>
<td>Stomatal conductance</td>
<td>Low</td>
</tr>
<tr>
<td>Carboxylation efficiency</td>
<td>Low</td>
</tr>
<tr>
<td>Rubisco content</td>
<td>Low</td>
</tr>
<tr>
<td>Nitrogen content per area</td>
<td>Low</td>
</tr>
<tr>
<td>Nitrogen content per dry mass</td>
<td>High</td>
</tr>
<tr>
<td>Chlorophyll content per area</td>
<td>High</td>
</tr>
<tr>
<td>Leaf mass per area</td>
<td>Low</td>
</tr>
<tr>
<td>Leaf thickness</td>
<td>Low</td>
</tr>
<tr>
<td>Stomatal density</td>
<td>Low</td>
</tr>
<tr>
<td>Leaf size</td>
<td>Large</td>
</tr>
<tr>
<td><strong>Crown level</strong></td>
<td></td>
</tr>
<tr>
<td>Leaf clumping</td>
<td>Low</td>
</tr>
<tr>
<td>Leaf turnover rate</td>
<td>Low</td>
</tr>
<tr>
<td>Apical dominance</td>
<td>Low</td>
</tr>
<tr>
<td>Canopy light transmittance</td>
<td>Low</td>
</tr>
<tr>
<td>Branching frequency</td>
<td>High</td>
</tr>
<tr>
<td>Elongation response to shade</td>
<td>Low</td>
</tr>
<tr>
<td>Number of foliage layers</td>
<td>Low</td>
</tr>
<tr>
<td>Leaf inclination angle</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Plant-level</strong></td>
<td></td>
</tr>
<tr>
<td>Root-shoot ratio</td>
<td>Low</td>
</tr>
<tr>
<td>Relative growth rate</td>
<td>High</td>
</tr>
<tr>
<td>Leaf area ratio</td>
<td>High</td>
</tr>
<tr>
<td>Carbohydrate storage</td>
<td>High</td>
</tr>
<tr>
<td>Fecundity</td>
<td>Low</td>
</tr>
<tr>
<td>Longevity</td>
<td>High</td>
</tr>
</tbody>
</table>
Niinemets and Valladares (2006) studied the shade tolerance of important temperate Northern Hemisphere woody species (conifers, deciduous and evergreen broadleaf species), considering it as the minimum light at which species are able to grow. They used a five-level scale for shade tolerance, from 0 (no tolerance) to 5 (maximal tolerance), which corresponds approximately to different light availabilities (Table 1.3).

**Table 1.3.** Shade tolerance scale and related light levels used by Niinemets and Valladares (2006).

<table>
<thead>
<tr>
<th>Shade tolerance scale</th>
<th>Available light (% of full sunlight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Very intolerant)</td>
<td>&gt;50</td>
</tr>
<tr>
<td>2 (Intolerant)</td>
<td>25-50</td>
</tr>
<tr>
<td>3 (Moderately tolerant)</td>
<td>10-25</td>
</tr>
<tr>
<td>4 (Tolerant)</td>
<td>5-10</td>
</tr>
<tr>
<td>5 (Very tolerant)</td>
<td>2-5</td>
</tr>
</tbody>
</table>

The quality and quantity of solar radiation are key factors affecting competition and growth within forest ecosystems. The establishment of gaps within a forest stand results in increased resources, such as light, nutrients and water. Although light is a required and crucial resource for photosynthesis, both high and low sunlight can limit plant performance (Valladares and Niinemets, 2008).

Photosynthetically active radiation (PAR) is the amount of light available for photosynthesis (normally considered between 400 and 700 nm) and it can be modified by different factors, such as sky conditions, shading by other trees and structures (e.g. terrain features) and air pollution. It is normally quantified as μmol m⁻² s⁻¹, which is a measure of the photosynthetic photon flux density (PPFD). PAR is needed for photosynthesis and is also implicated in plant morphogenesis (Christophe *et al.*, 2006). Light availability defines the regeneration niche of woody species (Gómez-Aparicio *et al.*, 2006; Poorter, 2007). In plant biology and ecology, PAR is probably one of the most relevant measures of light. The PAR region is where energy is more abundant (it represents on average 45% of the solar irradiance) and it is strong enough to drive electron transport in photosynthesis (Valladares, 2003).
Furthermore, in addition to light quantity, the spectral quality of light has effect on developmental processes (Kwesiga and Grace, 1986). By comparison with sunlight, shade light caused by a forest canopy shows a severe reduction of blue (400-500 nm) and red light (600-700 nm), a slightly weaker reduction of green light (500-600 nm) and a relatively poor reduction of far-red light (700-800 nm), due to the optical properties of green foliage (Casal, 2012). While both red and blue light have important roles in regulating photosynthesis, photoinhibition, stem elongation, pigment synthesis, flowering time and germination; blue light also influences stomatal opening, chloroplast movement and phototropism (Davis, 2015). Red/Far-Red ratio (R/FR) is the ratio between transmitted light in the red band and far-red band. Blue/Red ratio (B/R) is the ratio between transmitted light in the blue band and red band. Spectral ratios such as R/FR and B/R give important information about the light quality in a forest understory (Hertel et al., 2011).

On a clear, sunny day around noon, the R/FR in natural light is around 1, which means similar proportions of red and far-red light (Smith, 1982). The forest understory light is characterized by a low PAR and low R/FR (Holmes, 1981; St-Jacques et al., 1991). Usually, the R/FR ratio decreases under an intact canopy (decreases with increasing shade), which can have profound effects on plant growth and development (Leicht and Silander, 2006). The lower limit of the R/FR varies with sky conditions, species and time period, while the upper limit remains relatively constant in a mature mixed forest of *Picea abies* and *Fagus sylvatica* (Leuchner et al., 2007). Usually, the decrease in R:FR and B/R ratios is more pronounced in deciduous than conifer stands (Ross et al., 1986; Messier et al., 1989), with the B/L subject to higher variation (Hertel et al., 2011).

A non-linear relationship (which varies in function of phenological stage, species and meteorological conditions) between light quantity (expressed as the relative photosynthetic photon fluence rate) and light quality (represented by the R/FR) was found within a mixed forest stand in Germany (Leuchner et al., 2007). This relationship between the two parameters is described by a rectangular hyperbolic function. Lieffers et al. (1999) also reported a non-linear relationship between R/FR and PAR based on data from different studies (Fig. 1.3). A decrease in R/FR seems to be associated with a reduction in PAR at low levels of
PAR, but no great differences in R/FR have been found at high PAR levels (Lieffers et al., 1999; Capers and Chazdon, 2004; Leuchner et al., 2007). An easy method to determine R/FR with PAR measurements and vice versa under several conditions for a mature temperate mixed forest can be found in Leuchner et al. (2007).

![Fig. 1.3. Idealised relationships between R/FR and PAR under different forest stands (coniferous and deciduous) and conditions (sunny and cloudy). Source: Lieffers et al., 1999.](image)

Although in characterising light environments the light quality should always be determined as it might affect plant development (Larcher, 2003), light quantity appears to have more influence than light quality on tree growth and physiological acclimation to shade (Kitajima, 1994; Lee et al., 1996; Muth and Bazzaz, 2002b). Turnbull (1991) reported the influence of R/FR on photosynthetic characteristics and the effect appears to be dependent on the level of irradiance. However, only a few studies have investigated the effect of R/FR on tree seedlings development (Turnbull, 1991; Ammer, 2003), but far more research has been carried out on light availability. Since some studies suggest that R/FR changes little at high levels of PAR (as mentioned above), further research to consider the interactions between light availability and light quality should be considered, at least when carrying out experiments at low PAR. Besides, since R/FR and PAR can vary among different vegetation types (Muraoka et al. 2001; Leuchner et al., 2007), there is a need to assess this relationship locally to produce reliable estimations for a detailed characterisation of the light environment.
1.5 Managing light through silviculture

As mentioned previously, the amount of light transmitted to the understory is a crucial factor in determining regeneration dynamics and early growth. Foresters are aware of the benefits and possibilities offered when managing light levels under the forest canopy. Thinning is an important silvicultural practice which increases the amount of light between canopy positions by reducing the number of trees growing in a stand. Thinning reduces the number of trees competing for light, soil moisture and nutrients. A recent report of the 3rd International Congress on Planted Forests (2013) emphasized that stands that lack adequate management interventions, such as thinnings, are potentially more vulnerable to biotic and abiotic hazards.

Weed control is a fundamental step in establishing plantations and forests. The most likely period in which weeding is needed is during the regeneration, early establishment and any other stage that increases the amount of light below the canopy, such as first thinning. Although understory vegetation can have a positive effect on seedlings, by providing protection against browsing (Farris and Filigheddu, 2008) or extreme temperatures (Balandier et al., 2009), it competes for light, water and nutrients, and can be a serious threat to survival and early growth of new trees. It seems that the development of some herbaceous and shrubby competitive species could be controlled by decreasing light available in the understory (Gaudio et al., 2011). Therefore, managing light levels in the understory might help control weeds.

There is evidence that older and larger trees might respond differently from seedlings to environmental changes. As size usually increases with tree age, it is difficult to separate age response from size responses. In young plants with access to light and soil resources, productivity increases rapidly as a result of crown expansion and increased capture of light (Bond, 2000). Although there are some exceptions, the results of studies indicate that most physiological responses, such as leaf photosynthesis and stomatal conductance, decline with tree age (Bond, 2000).

Paquette et al. (2006) developed an overstory density index including available light, stocking values, canopy cover and the ratio of opening width to
canopy height (Table 1.4). This index might be useful for interpreting and comparing different thinning prescriptions.

Table 1.4. Overstory density index developed according to four descriptive variables used in the literature (adapted from Paquette et al., 2006).

<table>
<thead>
<tr>
<th>Overstory density</th>
<th>Available light (%)</th>
<th>Stocking (% of original basal area)</th>
<th>Canopy cover (%)</th>
<th>Width:height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncut</td>
<td>~0</td>
<td>100</td>
<td>~100</td>
<td>0</td>
</tr>
<tr>
<td>Light thinning</td>
<td>&lt;25</td>
<td>&gt;60</td>
<td>&gt;75</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Intermediate thinning</td>
<td>25-50</td>
<td>40-60</td>
<td>50-75</td>
<td>0.25-0.40</td>
</tr>
<tr>
<td>Heavy thinning</td>
<td>&gt;50</td>
<td>&lt;40</td>
<td>&lt;50</td>
<td>0.40-2</td>
</tr>
<tr>
<td>Clearfelling</td>
<td>~100</td>
<td>~0</td>
<td>~0</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

Hawe and Short (2012) reported that some silvicultural systems could be used to improve the production of poorly performed broadleaved stands, including the shelterwood (uniform or group system), underplanting, coppice and free-growth systems. In addition to the silvicultural systems mentioned above, selection systems can also be used to deliver CCF since these systems are tools to provide structural and species diversity while maintaining part of the existing canopy (Ní Dhubháin, 2010; Brang et al., 2014). These silvicultural systems can be combined and improved with elements from the others, as experienced in the Dinaric region (north-west Balkans) with freestyle silviculture (Boncina, 2011). Although some of the main aspects of all these systems are summarised below, this review pays special attention to underplanting as it is one of the methods recommended to manage under-performing broadleaf forests (Evans, 1984; Hawe and Short, 2012) and can be used in combination with other silvicultural methods.

In the shelterwood system, the old stand is felled in different stages leaving those trees needed to produce an appropriate microenvironment (e.g. sufficient protection) to encourage the establishment of a new age class under an overstory. The different ways in which the stand is felled (in time and space) will depend on circumstances and objectives of management. Cutting can be done uniformly throughout the stand (uniform shelterwood), in small gaps or groups (group shelterwood), in strips (strip shelterwood) and in different periods or cycles.
(irregular shelterwood). The shelterwood system, especially the uniform shelterwood, generally provides low structural diversity and tree species richness can be promoted (Brang et al., 2014).

In the coppice system the majority of the regeneration comes from vegetative sprouting of either shoots from stumps or suckers from roots. As the majority of broadleaf species respond well to coppice and most conifers do not, this system is largely limited to hardwood species. Different coppice methods are identified if all trees in the previous stand are cut (simple coppice) or reserve trees are retained (coppice with standards).

Free-growth is a silvicultural system in which the crown development of selected trees is stimulated to maximize radial stem increment (Jobling and Pearce, 1977). The system is focussed on a small number of potential crop trees (disease free, good stem form and dominant in the canopy) selected at an early stage. Free growth could be viewed as a heavy thinning regime (Short, 2013).

Selection systems maintain an uneven-aged structure by removing trees in all size classes. Tree removal can be done individually more or less uniformly throughout the stand (single tree selection) or in small groups (group selection). Brang et al. (2014) considered the group selection system one of the most flexible systems suitable for CCF since it creates a different range of light gradients that provide appropriate conditions for shade-tolerant and light-demanding species. While the single tree selection system is considered appropriate for increasing structural diversity, its disadvantage is that it does not increase species richness because small gaps are created that only favour a few shade-tolerant species.

Underplanting takes advantage of an existing canopy, which can be transformed by thinning, and seems to be a good way of regenerating a forest stand when natural regeneration is not possible or changes in species are prescribed. Besides, underplanting can be used to enrich of an existing stand or to supplement natural regeneration and introduce additional species, such as for example for the conversion of monocultures into more complex forests (e.g. CCF) and for the rehabilitation of poorly performed stands (Kenk and Guehne, 2001; Paquette et al., 2006; Brang et al., 2014). Underplanting also assists in natural pruning, vegetation control, stand productivity and flexible management.
As with all silvicultural practices, the tree species chosen should depend on management objectives and site conditions. Therefore, an understanding of species’ shade tolerances, and how the different species will survive, respond and grow during the early stages under different light availabilities is crucial when using underplanting systems.

1.6 Studied species

1.6.1 Pedunculate oak

The natural distribution encompasses the whole of central Europe, south to the northern Mediterranean coast, southern parts of Scandinavian countries, Russia and parts of central Asia. Provenances currently recommended for planting in Ireland are preferably Irish registered, although other sources, such as registered British (English and Welsh), northern French, Belgian, Dutch, Danish and northern German seed stands are also acceptable (Forest Service, 2015b). Oak is one of the most popular broadleaves planted in Ireland and is used for furniture, joinery, panelling and veneers.

Oak foliage is susceptible to late spring and early autumn frosts (COFORD, 2002a). In the early years there is usually a secondary midsummer flush called lammas growth. Oaks show rapid height growth in the early years and thereafter height growth gradually slows. Oak in Ireland is susceptible to grey squirrel damage and oak mildew, which reduces its growth. Young trees can be damaged by deer, hares, rabbits and domestic stock. Oaks may also suffer from oak decline or oak dieback, which weaken and deteriorate trees, sometimes resulting in premature death.

Pedunculate oak can tolerate a wide range of soil conditions and is best suited to well aerated but moist, deep and fertile soils. It is found on heavy, alkaline soils in the midlands. Pedunculate oak is a light demanding species and is relatively wind firm. According to the shade tolerance scale used by Niinemets and Valladares (2006) described above (Table 3), pedunculate oak has a shade tolerance score of 2.54 ± 0.28 (that would be between intolerant and moderately tolerant). According to that classification (Niinemets and Valladares, 2006), *Quercus robur* would not be able to grow under light environments lower than
20% of full sunlight. Oak is prone to develop epicormic branches and the timing and intensity of thinnings has an important influence on this development.

Once established, pedunculate oak generally exhibits a good ability to outcompete most competitors. *Quercus robur* exhibits a good coppicing capacity and some stands have been managed with that system, especially in the past. Several studies reported that oak seedlings are able to tolerate deep shade for the first one or two years of regeneration, but after which the canopy must be opened up to increase light availability (Ziegenhagen and Kausch, 1995; Lüpke, 1998; Welander and Ottosson, 1998). Incorporation of shade tolerant species as mixture species is crucial to avoid the development of epicormic shoots, which can result in small knots that can reduce the value of the wood. One of the most used species to avoid epicormic branches is *Fagus sylvatica*, but as indicated below, this species mixture might pose management problems due to the contrasting shade tolerance of oak and beech. Lüpke (1998) provided some guidelines for oak silviculture under permanent canopy cover: use of cleanings and thinnings to maintain a high of number of oaks in the overstory and minimise browsing by wild animals.

1.6.2 European beech

The natural distribution of beech extends from southern Norway to southern Italy, and from north-western Spain to the Black Sea. Although beech is not a native species in Ireland, it plays an important role in Irish forestry and has become naturalised after being introduced in the 16th century. Recommended beech provenances in Ireland are registered Irish, British, northern French, Belgian, Dutch and northern German seed stands (Forest Service, 2015b). Beech is one of the most flexible species of Irish forestry and is used in a wide range of products such as furniture and flooring.

Beech is susceptible to damage by late spring frost (COFORD, 2002a). Although less common in beech than oak, lammas growth is of silvicultural interest since it increases susceptibility to frost and insect damage, which could result in poor form (Joyce *et al.*, 1998). Beech is susceptible to grey squirrel damage and young trees can be affected by deer.
Beech grows under a wide range of soils but best development tends to occur where the soil has a neutral or slightly acid pH. For optimal growth it requires moist and free draining soils of moderate depth. It is a shade tolerant species and is frequently successful at establishing itself as an under-storey. According to the shade tolerance scale used by Niinemets and Valladares (2006) described above (Table 3), beech has a shade tolerance score of 4.56 ± 0.11 (that would be between tolerant and very tolerant). According to that classification (Niinemets and Valladares, 2006), beech would not be able to grow under light environments lower than 3% of full sunlight. Although tolerant of exposure, it grows best on sheltered sites. Beech is prone to coarse growth and forking with a medium ability for natural pruning.

Beech can be managed under a variety of silvicultural systems, from uniform shelterwoods to less regular systems that provides heavy shade in the understory. Beech is one of the most suitable species for underplanting (COFORD, 2002b; Savill, 2013). This kind of management approach, using beech underplanted in spruce shelterwoods, has been promoted around western Europe in recent years. When managing closed canopies through underplanting beech and other species with contrasting shade tolerance (e.g. Quercus robur), it should be taken into consideration that beech is highly competitive and very shade tolerant. Therefore, beech could suppress the development of other species and dominate the understory when light levels decrease. Nevertheless, Van Couwenberghe et al. (2013) found that controlling canopy cover is not an effective way of managing oak-beech mixtures, since natural regenerated beech seedlings were always more competitive than shade-intermediate sessile oak along different environmental gradients (light, density, mixture and seedling size). Emborg (2007) reported that beech has a “stop and go” competitive strategy, being able to survive periods of severe competition and then resume growth when conditions improve, and finally reach a dominant position in the canopy. For an overall consideration, beech shows the typical growth pattern of late successional, climax or shade-tolerant species.
1.7 Measurement practices

In this section, the most common practices to measure light, gas exchange and chlorophyll fluorescence are described. Gas exchange and fluorescence methods can be used to study the responses of photosynthesis to light.

1.7.1 Light

Light is one of the most important factors affecting plant growth and survival and foresters are aware of the benefits of managing understory light availability through silviculture. Although it is obvious that the amount of light available in the understory of a forest is strongly related to its stand density and structure, these processes are complex and not completely understood. Light is rarely measured by foresters, but indirect measures (e.g. height of the overstory and basal area) are sometimes strongly related to light levels (Jennings et al., 1999; Blizzard et al., 2013; Angelini et al., 2015).

Two common parameters used to measure forest canopies are canopy closure and canopy cover. Canopy closure is defined as the proportion of sky hemisphere obscured by the canopy when viewed from a single point on the ground (Jennings et al. 1999). Canopy cover is defined as the proportion of the ground covered by the vertical projection of the tree canopy (Jennings et al. 1999. Some of the most common direct and indirect methods used to measure light are described below:

- Quantum sensors. They measure the proportion of solar radiation that is available for photosynthesis (PAR). Quantum sensors are designed to measure PAR on a plane surface. Measurements can be made by attaching sensors to dataloggers. Sensors and dataloggers are generally expensive, and a considerable number of points should be measured for adequate data under a canopy. Some companies offer line quantum sensors (which average PAR over its length), also called ceptometers, that are specifically designed for measuring PAR under non-uniform radiation, such as forest canopies. One approach to measure PAR under canopies and relate it with PAR over the canopy is to simultaneously measure light levels in the open and under the canopy. Instantaneous
measurements of PAR on overcast days showed stronger correlations with mean daily PAR than measurements on cloudless days (Messier and Puttonen, 1995b; Parent and Messier, 1996). Consequently, instantaneous measurements of PAR in the understory and in the open under overcast conditions might provide an accurate and quick method of estimating light availability under a forest canopy (Messier and Puttonen, 1995b; Parent and Messier, 1996; Gendron et al., 1998; Lieffers et al., 1999).

- **Pyranometer.** A pyranometer is used to measure global solar radiation. Measurements can be made by attaching sensors to dataloggers. As PAR is not usually measured (despite being an important factor in determining plant growth) and global solar radiation is often observed in weather stations, it is possible to estimate PAR from global radiation data. The relationship between PAR and global solar radiation usually falls between 0.45 and 0.5 (Tsubo and Walker, 2005), and PAR is assumed to be 0.48 times the global incident radiation under European conditions (Gosse, 1995).

- **Hemispherical photography.** This approach is also known as fisheye or canopy photography and it is a common technique for measuring canopy transmittance and canopy closure of forests. A photograph is taken looking up into the canopy at the measurement point with a 180° fisheye lens. The resulting image can be analysed using specialised software to determine canopy closure, among others variables.

- **Spherical densiometer.** This instrument is used to measure canopy closure and consists of a spherical-shaped reflector mirror engraved with a cross-shaped grid. To take readings, four equally spaced dots in each square of the grid must be assumed and the number of these dots blocked by the canopy must be counted.

Engelbrecht and Herz (2001) studied the light conditions at 16 understory sites in a Panamanian forest. While they found high correlation between indirect measurements from hemispherical photographs and direct measurements
(quantum sensors), a weak relationship was found between measurements from the spherical densiometer and quantum sensors. However, Blizzard et al. (2013) reported that understory PAR was better predicted by canopy closure measured with a spherical densiometer than by hemispherical photographs, even though understory PAR was inversely proportional to both measures of canopy closure.

A large number of studies have focussed on the relationships between forest stand characteristics (DBH, basal area, number of trees, crown structure, forest composition) and understory light levels, which have largely confirmed the interest in predicting understory light conditions from attributes of stand overstory usually considered in forest management (Angelini et al., 2015). Understory light conditions depend on species characteristics, such as shade tolerance, since generally light demanding species transmit more light than shade tolerant species (Messier et al., 1999; Angelini et al., 2015). In general, light transmission is greater for species of boreal forests than for species of warmer and wetter temperate deciduous forests (Lieffers et al., 1999). There is also variation in understory light levels seasonally (different phenological stages) and by meteorological conditions (Gendron et al., 2001; Leuchner et al., 2007).

Blizzard et al. (2013) found a link between stand metrics and understory PAR, with the single-variable stand metrics (stocking, basal area and density) being closely correlated with the percentage of understory PAR. Comeau (2001) found that basal area is a useful predictor of understory light in trembling aspen (Populus tremuloides Michx.) stands, although results should not be extrapolated to different sites or stand types without careful evaluation. Similarly, Angelini et al. (2015) found that basal area is probably the preferable light predictor for managing young homogeneous stands, but considering parameters such as DBH, height and volume might increase the accuracy of light predictions. Therefore, further research is needed to provide relationships between understory PAR, stand metrics and forest composition for different sites, forest types and structures as they can provide useful information for forest management.

1.7.2 Gas exchange

Photosynthetic gas exchange involves the absorption of CO$_2$ and release of O$_2$ by plants. Along with photosynthesis, other parameters related with gas exchange
widely used in physiological studies are stomatal conductance, transpiration rate and water use efficiency. The equations for these variables, which are essentially those derived by Von Caemmerer and Farquhar (1981), are described below:

- **Net photosynthesis** ($A$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$). It is the net assimilation rate of CO$_2$ by the leaf and is calculated as follows:

  $$A = \frac{F \left( C_r - C_s \left( \frac{1000 - W_r}{1000 - W_s} \right) \right)}{1000S}$$

  where $F$ is air flow rate ($\mu$mol s$^{-1}$); $C_r$ and $C_s$ are reference and sample CO$_2$ concentrations respectfully ($\mu$mol CO$_2$ (mol air)$^{-1}$); $W_r$ and $W_s$ are reference and sample water mole fractions respectfully (mmol H$_2$O (mol air)$^{-1}$); and $S$ is leaf area (cm$^2$).

- **Stomatal conductance** ($g_s$, mol H$_2$O m$^{-2}$ s$^{-1}$). It is the measure of the rate of passage of water vapour exiting through the stomata of a leaf and is given by the following equation:

  $$g_s = \frac{1}{\frac{1}{g_{tw}} - \frac{k_l}{g_{bw}}}$$

  where $k_l$ is a factor based on the estimate of the stomatal ratio ($k$, which is the fraction of the stomatal conductances of one side of the leaf to the other) and calculated as $k_l=(k^2+1)/((k^2+1)^2$; $g_{tw}$ is the total conductance of the leaf (mol H$_2$O m$^{-2}$ s$^{-1}$); and $g_{bw}$ is the boundary layer conductance from one side of the leaf (mol H$_2$O m$^{-2}$ s$^{-1}$).

- **Transpiration** ($E$, mmol H$_2$O m$^{-2}$ s$^{-1}$). It is the process by which water is evaporated from plant leaves and is calculated as:

  $$E = \frac{F(W_s - W_r)}{100S(1000 - W_s)}$$

- **Water use efficiency.** As measured by gas exchange analysis and at leaf scale, it is the efficiency of the water consumed to produce net CO$_2$ assimilated by photosynthesis. Instantaneous water use efficiency ($A/E$, $\mu$mol CO$_2$/mmol H$_2$O) is the ratio of $A$ to $E$ and, on
the other hand, intrinsic water use efficiency \((A/g_s, \mu\text{mol CO}_2/\text{mol H}_2\text{O})\) is the ratio of \(A\) to \(g_s\). Since \(A/g_s\) is not influenced by vapour pressure deficit (VPD, kPa), which is the force that drives transpiration rate, it represents, therefore, a more consistent estimate of the relative water use efficiency than \(A/E\) (Meinzer et al., 1990) and is used in comparative studies with different evaporative demands (Tambussi et al., 2007).

Response curves of photosynthesis \((A)\) over a range of light intensities \((I)\) have been widely used to study leaf-level responses to light intensity. These curves describe the CO\(_2\) assimilation by a leaf as a function of light intensity from darkness to a high level of light. The vast majority of tree species are C\(_3\) plants, and for most of these the photosynthetic response to light intensity is roughly hyperbolic with 90\% of maximum net photosynthesis occurring between one-third and two-thirds of full sunlight (Kozlowski et al., 1991). Light-response curves provide useful information about the photosynthetic properties of leaves, such as dark respiration, light compensation point, photosynthetic quantum yield, light saturation point and maximum rate of photosynthesis (Taiz and Zeiger, 2002; Fig. 1.4):

- Dark respiration \((R_d, \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1})\). It is a form of respiration in plants in the absence of light when there is no photosynthetic carbon assimilation and CO\(_2\) is released by the plant. It is the gas exchange rate at zero PAR.

- Light compensation point \((I_c, \mu\text{mol m}^{-2} \text{ s}^{-1})\). It the PAR value at which net assimilation rate is equal to zero. At this point CO\(_2\) assimilated by photosynthesis is in balance with the CO\(_2\) produced by respiration.

- Quantum yield of photosynthesis \((\Phi_{\text{CO}_2})\). It is a measure of the photosynthetic efficiency expressed in moles of photons absorbed per mole of CO\(_2\) fixed or O\(_2\) evolved. The maximum quantum yield, with a theoretical value of 0.125, is measured when photosynthesis is light-limited and is typically calculated as the initial slope of the light response curve. This measure of maximum photosynthetic
efficiency should not be confused with instantaneous measures of photosynthetic efficiency. Instantaneous measures of photosynthetic efficiency vary with light availability, whereas the maximum quantum yield is an intrinsic characteristic of the photosynthetic tissue (Skillman, 2008).

- Light saturation point ($I_{sat}$, $\mu$mol m$^{-2}$ s$^{-1}$). It is the PAR value at which photosynthesis becomes saturated. Any increase in the amount of light beyond this point does not cause an increase in the photosynthesis rate.

- Maximum rate of photosynthesis ($A_{max}$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$). It is the maximum value of the photosynthesis rate.

The light-response curve generally presents several phases: the initial linear and rapid increase of photosynthesis as light levels increase; the curvature region, where photosynthesis does not increase proportionally to light and starts to saturate with light; and maximum value, where photosynthesis reaches the maximum value and an apparent plateau that sometimes is followed by a decrease in photosynthesis (Ye, 2007).

![Fig. 1.4. Idealised photosynthetic responses to PAR of plant leaves acclimated to high light (continuous line) and low light (dashed line). Some key parts of the curves are also shown. Source: Valladares et al., 2012.](image)
Different mathematical models have been used to describe the relationship between light levels and photosynthesis, such as the rectangular hyperbola based models (Baly, 1935; Smith, 1936; Thornley, 1998; Kaipiainen, 2009), the non-rectangular hyperbola based models (Prioul and Chartier, 1977; Leverenz and Jarvis, 1979; Farquhar et al., 1980; Marshall and Biscoe, 1980; Ögren, 1993; Marschall and Proctor, 2004; Chen et al., 2008), the hyperbolic tangent based models (Jassby and Platt, 1976; Abe et al., 2009), the exponential based models (Steele, 1962; Webb et al., 1974; Prado and de Moraes, 1997; Lootens et al., 2004; Devacht et al., 2009) and the Ye model (Ye, 2007). Lobo et al. (2013) developed an Excel routine to fit net photosynthetic light-response curves using the most common mathematical models found in the literature. This method is a useful tool for researchers to determine the best fit to the data from measured photosynthesis rates over a range of light intensities. Although many studies have used $I_{\text{sat}}$ and $\Phi_{\text{CO}_2}$ at low light to characterise light responses, Lobo et al. (2013) propose the use of $A_{\text{max}}$ (considered as the maximum photosynthesis rate beyond which no significant increment can be achieved with an additional increment in light level), $I_{\text{max}}$ (considered as the light point beyond which there is no significant change in photosynthesis rate), and $\Phi_{\text{CO}_2(l)}$ (considered as the apparent quantum yield at a particular value of light), as the last variables are more directly linked to plant ecophysiology.

### 1.7.3 Chlorophyll fluorescence

Chlorophyll fluorescence has become one of the most popular non-invasive techniques used in plant physiology. This technique provides information on the state of photosystem II (PSII), which is the first protein complex involved in the light-dependent reactions of photosynthesis, and has been used to study plant responses to environmental changes, among other applications. Some instruments allow researchers to assess simultaneously chlorophyll fluorescence and gas exchange analysis and both methods can be used to study the responses of photosynthesis to light.

Light energy absorbed by chlorophyll molecules in a leaf can be released in one of three pathways: to drive photosynthesis (photochemistry), excess energy dissipated as heat or re-emitted as light (fluorescence). Therefore, chlorophyll
fluorescence is light that has been re-emitted after being absorbed by chlorophyll molecules of plant leaves. This relationship can be expressed as $F + H + P = 1$; where $F$ is fluorescence, $H$ is heat and $P$ is photochemistry (also known as quantum yield or efficiency). These three processes occur in competition, and an increase in the efficiency of one will result in a decrease in the yield of the other two (Maxwell and Johnson, 2000). As the light incident on a leaf increases, $F$ and $H$ increase while $P$ decreases. At a saturating light intensity, $P$ will be zero and $F$ and $H$ will be at their maximum values.

The development of portable modulated fluorometers provides useful measurements that can be carried out easily under field conditions and without darkening the leaf (Murchie and Lawson, 2013). Some of the most useful and used parameters derived from fluorescence measurements in light-adapted leaves are described below:

- **PSII operating efficiency ($\Phi_{\text{PSII}}$)**. It is the quantum yield of PSII electron transport in the light and estimates the efficiency at which light absorbed by PSII antennae is used for photochemistry (Baker and Rosenqvist, 2004). It is one of the most useful and commonly-used light-adapted parameters that measure the yield of PSII photochemistry and can be used to calculate electron transport rate (Genty et al., 1989). This parameter has also been termed $F_{\text{q}}'/F_{\text{m}}'$ and $\Delta F/F_{\text{m}}'$ in the literature (Baker and Rosenqvist, 2004; Baker, 2008; Murchie and Lawson, 2013). It can be broken down into two products, PSII maximum efficiency and photochemical quenching (Baker and Oxborough, 2004), and is calculated as:

$$\Phi_{\text{PSII}} = \frac{(F_{\text{m}}' - F')}{F_{\text{m}}'}$$

where $F_{\text{m}}'$ is the maximum fluorescence from a light-adapted leaf; and $F'$ is the steady-state level of fluorescence in the light. Although Genty et al. (1989) found evidence of a linear relationship between $\Phi_{\text{PSII}}$ and $\Phi_{\text{CO2}}$ over a range of light intensities, a linear relationship between these two parameters is not found in many situations due to different stress factors and/or when photorespiration is operating (Baker and Oxborough, 2004; Baker, 2008; Valladares et al., 2012).
- **PSII maximum efficiency** ($F'_v/F'_m$): is the maximum quantum yield of PSII photochemistry under given light conditions, that is when all PSII centres are open (oxidized) at the point of measurement (Baker and Oxborough, 2004). It is generally determined by the level of quenching in PSII reaction centres and antenna (Baker and Rosenqvist, 2004). It is calculated as:

$$F'_v = \frac{F'_m - F'_0}{F'_m}$$

where $F'_v$ is the variable fluorescence of a light-adapted leaf defined as $(F'_m - F'_0)$, and $F'_0$ is the minimal fluorescence of a light-adapted leaf that has momentarily been darkened.

- **Photochemical quenching** ($q_P$). It is also known as PSII efficiency factor and provides an estimate of the fraction of the PSII maximum efficiency that is actually realized (Baker, 2008). It relates the PSII operating efficiency to the PSII maximum efficiency and can also be found as $F'_q/F'_v$ in the literature (Murchie and Lawson, 2013):

$$q_P = \frac{(F'_m - F'_v)}{(F'_m - F'_0)}$$

- **Electron transport rate** (ETR, $\mu$mol photons m$^{-2}$ s$^{-1}$). It is the actual flux of photons driving PSII and is given by the following equation:

$$ETR = \Phi_{PSII} \times f \times I \times \alpha_{leaf}$$

where $f$ is the fraction of absorbed photosynthetically active photon flux density (PPFD) that is received by PSII, $I$ is the incident PPFD on the leaf, and $\alpha_{leaf}$ is leaf absorptance or proportion of incident PPFD on the leaf that is absorbed by the leaf (Baker, 2008). There are generally a number of assumptions in the calculation of this parameter and, therefore, these assumptions should be considered when the results are being analysed (Maxwell and Johnson, 2000). Although $f$ is assumed to be 0.5 for C$_3$ plants (Ögren and Evans, 1993), it might be different in some situations and under environmental stresses (Baker 2008). Leaf absorptance can be calculated as (Bernacchi et al., 2002):
\[
\alpha_{\text{leaf}} = \frac{\alpha_{\text{blue}} B + \alpha_{\text{red}} (100 - B)}{100}
\]

The terms \(\alpha_{\text{blue}}\) and \(\alpha_{\text{red}}\) are absorptances at the blue and red light wavelengths, and \(B\) is an estimate of the percentage of incident light that is blue. Because it is not practical to measure \(\alpha_{\text{leaf}}\), it is assumed to be between 0.84 and 0.9, since overall mean absorptance for the entire PAR range is generally about 0.84-0.9 (Ritchie and Runci, 2014).

When measuring chlorophyll fluorescence in light adapted leaves, it is important to ensure that leaf material is at steady state (\(F^*\) signal is stable) and the light intensity at the leaf surface is known and stable during measurement (Murchie and Lawson, 2013).

1.8 Phenotypic plasticity

Plants are exposed to a great heterogeneity of conditions in the environment (i.e., climate change, invasiveness, natural disturbances, plant pests and pathogens) and, therefore, inter and intraspecific plant differences might result from the exposure to these factors. Schlichting (1986) defined phenotypic plasticity as the ability of an organism to modify its morphology/physiology in responses to changes in environmental conditions. In a wider sense, phenotypic plasticity means the expression of different phenotypes (organism’s observable characteristics or traits, such as growth, morphology, phenology and physiology) by the same genotype (genetic constitution of an organism) in response to different environmental conditions. Phenotypic plasticity is considered one of the most important ways by which plants can survive under different environmental conditions, and its study can play a key role for predicting crop productivity and species distribution under different conditions (Ackerly et al., 2000; Gratani, 2014). However, phenotypic plasticity is complex and further research is needed, as, despite all the studies about this topic, results are sometimes controversial. For example, sometimes it is hard to determine whether phenotypic changes are genetically based or they are just result of phenotypic plasticity (Merilä and Hendry, 2014).
Although most of the literature suggests that light-demanding and early successional species are more plastic than shade-tolerant and late successional species in response to different light conditions (Bazzaz and Carlson, 1982; Strauss-Debenedetti and Bazzaz, 1991, 1996; Kozloski and Pallardy, 1997; Muth and Bazzaz, 2002a; Portsmouth and Niinemets, 2007; Longuetaud et al., 2013), there is also evidence that indicates that changes are not related to the species’ successional status (Turnbull, 1991; Popma et al., 1992; Abrams and Mostoller, 1995; Beaudet et al., 2000). Phenotypic plasticity might also depend on the availability of other resources, such as soil nutrients and moisture (Sánchez-Gómez et al., 2006b; Portsmouth and Niinemets, 2007).

The quantitative estimation of phenotypic plasticity to environmental factors, such as irradiance, has been widely addressed in the literature. Indexes can be used to compare results across different studies (Weigelt and Jolliffe, 2003). However, many different indexes (at least 17 according to Gratani 2014) have been used to measure plasticity, which can complicate comparisons between studies. Valladares et al. (2006) analysed 17 different indexes to estimate phenotypic plasticity of tree seedlings in response to light and assessed their advantages and disadvantages. Some indexes should be used only when two different environments are considered (e.g., Larcher, 2003), or present statistical limitations for comparisons among species (e.g., Schlichting, 1986). Therefore, the development and use of a standard plasticity index would facilitate species comparisons and allow the results from different experiments to be compared.

A robust, simple and widely used index to assess phenotypic plasticity in response to light can be calculated as the difference between the maximum and minimum mean values among the different treatments divided by the maximum mean value (Valladares et al., 2006). This index, which ranges from 0 (no plasticity) to 1 (high plasticity), has been previously used in different studies (Valladares et al., 2000, 2002; Sánchez-Gómez et al., 2006a) and it might be useful for comparing changes in variables expressed in different units and across different environments. Valladares et al. (2002) reported that oak exhibited higher phenotypic plasticity in response to light than beech for variables related to anatomy and physiology while the opposite was found for chlorophyll content and leaf morphology. This suggests that shade tolerance is linked to plasticity in traits
associated with light harvesting (chlorophyll content and morphology) and tolerance to high irradiance is linked to physiological plasticity.

1.9 Plant response to shade

As light is one of the main environmental factors affecting plant development, information on the responses and acclimation of tree species to different light levels is crucial for forest management decision making. Shade tolerance may be reflected in differences in survival, growth and physiological performance (Niinemets and Valladares, 2006). When considering shade tolerance, characteristics of stand and site should be considered as responses to shade may vary with tree size and site (Beaudet and Messier, 1998). Similarly, co-occurring environmental factors (such as water stress, temperature, nutrients) should also be considered since shade tolerance is usually inversely related to drought and flooding tolerance (Niinemets and Valladares, 2006).

Two main hypotheses have been advanced to explain species’ shade tolerance: the carbon gain hypothesis (Givnish, 1988) and the stress tolerance hypothesis (Kitajima, 1994). While the carbon gain hypothesis suggests that shade tolerance is increased through improving carbon gain in low light; that is, improving light use efficiency when light is limited (Givnish, 1988); the stress tolerance hypothesis suggested that survival in shade is more related to the maximization of the resistance to biotic and abiotic stresses (Kitajima, 1994). Valladares and Niinemets (2008) proposed that shade tolerance depends on both the carbon gain efficiency in low light levels and the tolerance to environmental stresses.

The responses and adaptations of tree species to different light related to some of their characteristics, from growth to physiology, are described below.

1.9.1 Survival

Shade-tolerant species generally have greater survival rates than light demanding species when light is limited, based on the result from controlled environment studies (Walters and Reich, 1996) and those conducted under forest canopies (Pacala et al., 1994; Chen, 1997; Kaelke et al., 2001). On the other hand, excessive light can also reduce survival and under high light conditions plants
might have to cope with environmental stresses associated with high irradiance, such as high temperatures, desiccation and water stress.

While juveniles of shade-tolerant species may experience elevated survival rates under low light conditions and respond moderately to canopy openings, seedlings of shade intolerant species may suffer high mortality when light is limited and vigorous growth occurs in response to the formation of gaps or canopy openings (Canham, 1989). Nevertheless, Gravel et al. (2010) found that this interspecific low light survival/high light growth trade-off might not be the most important mechanism for tree species coexistence in variable environments. Therefore, this trade-off may be just one of the ways that trees can compete, among many others, to develop under different light gradients.

Paquette et al. (2006) studied the survival and growth of underplanted trees across different overstory densities and four biomes. They found that overall survival of underplanted seedlings was not affected significantly by the kind of overstory treatment in most of the biomes, with survival generally staying stable or improving slightly as overstory density (or shade levels) decreased. Although survival of planted trees under shelterwoods generally increases with reduction in overstory density (Johnson et al., 2009), stands managed through heavy thinning or clear-cutting experience less protection from climatic stresses, such as frost or wind (Langvall and Ottosson Lövenius, 2002; Agestam et al., 2003; Pomerening and Murphy, 2004; Paquette et al., 2006).

It has been reported that beech seedlings require a minimum light of about 3-5% of full sunlight to survive (Grosse, 1998; Niinemets and Valladares, 2006). While some studies reported high survival rates of beech seedlings across different light environments, even at very low light levels (Madsen, 1994, 1995; Petrițan et al., 2007; Lőf et al., 2010), the shade tolerance ranking for beech in all studies is consistent. Others found that seedling survival increased as light increased and higher mortality occurred at low light under natural conditions (Minotta and Pinzauti, 1996; Johnson et al., 1997). Results from a few studies under controlled conditions indicated high survival rates (≥ 90%) of oak seedlings under heavy shade, with the shade treatments ranging from 1% to 35% of full sunlight (Sánchez-Gómez et al., 2006a; Portsmouth and Niinemets, 2007). This would be in agreement with the finding that underplanted beech and oak
seedlings generally experienced high survival rates in all treatments under natural conditions, with beech exhibiting the lowest mortality and both species showing a good adaptation to shade (Löf et al., 2007). Similarly, Gemmel et al. (1996) found no significant effects of shelterwood density (providing 100%, 58% and 29% of PAR) on survival rates of beech and oak seedlings during a three-year study, although mortality was greater in the dense shelterwood and in oak than in beech. However, Löf (2000) reported higher survival of oak and beech seedlings three years after planting under the shelterwood (15-18% of PAR) than in the clearcut areas of stands (90% of PAR), although this trend was only true for beech seedlings after two years of planting. In contrast, beech and sessile oak seedlings growing under a 40-year old Sitka spruce stand had higher mortality rates by the end of the second season than after one growing season, with mortality increasing as light reaching the understory decreased (Ní Dhubháin, 2010). Several studies have suggested that it would be possible to regenerate oak as well as beech under dense shelterwood, as oak seedlings seem to be able to survive under shade during the first year, but, thereafter, light intensity would need to be increased for oak seedlings, as oak survival generally decreases after long periods under heavy shade (Ziegenhagen and Kausch, 1995; Welander and Ottosson, 1998). With time the shelterwood will grow denser leaving less light available in the understory, so thinning might be needed to promote survival and growth of the regeneration (Löf, 2000).

1.9.2 Growth

There is conflicting evidence in the literature in relation to the impact of shade on growth and morphology, with some studies reporting greater growth for shade-tolerant than light-demanding species in low light (Givnish, 1988; Walters and Reich, 1996) and others reporting the contrary (Kitajima, 1994; Pacala et al., 1994; Walters and Reich, 2000). Petrițan et al. (2009) found that light-demanding species exhibited greater growth rates than shade-tolerant species at both low and high light conditions.

1.9.2.1 Height growth

The height growth in response to light gaps is of particular interest since it determines how long an individual sapling can grow in high-light environments
before being overtopped by other competitors (King, 1994; Welander and Ottosson, 1998). Under low light conditions, gap specialist species generally invest much of their resources in height growth, which can cause mortality because of the lack of resources for other functions, while shade-tolerant species can survive long periods under deep shade, reducing their height growth (Messier et al., 1999). Several studies have reported that height growth increased with increasing light availability, regardless of shade tolerance ranking (King, 1994; Chen, 1997). However, increasing light availability does not always mean greater height growth since some species present optimal height growth under light levels below 100% of full sunlight, with no increment or even reduced growth above and below that optimal light level. For example, Gardiner and Hodges (1998) found that height growth of cherrybark oak (*Quercus pagoda* Raf.) increased as light increased from 8% to 53% of full sunlight but additional increases in light levels over 53% up to 100% of full sunlight did not result in a significant increase in height. This finding is in agreement with different studies in which little change in height growth was found as light levels increased above a certain (relatively high) light threshold (Farque et al., 2001; Petrițan et al., 2007, 2009; Ligot et al., 2013).

Height growth of light-demanding tree species tends to be greatest at low R:FR ratios, while shade tolerant species tend to grow slowly and display less pronounced responses to R:FR ratio (de la Rosa et al., 1998).

Similar to findings of Ĉater and Simončič (2010), a strong positive correlation between sapling height and light availability was found in beech saplings growing below a shelterwood canopy that reduced light conditions from 3% to 60% of full sunlight (Petrițan et al., 2009). Ligot et al. (2013) found that beech saplings reached optimum height growth rates at 10% of full sunlight while *Quercus petraea* (species with characteristics similar to *Quercus robur*) required more than 20% of full sunlight to achieve optimal growth rates, suggesting that sessile oak has greater light requirements than beech. In a controlled study of one-year-old seedlings, height growth of beech decreased as shade increased but no effect of shade was found for oak (Welander and Ottosson, 1998). Ammer (2003) also reported that height growth declined with increasing shade in beech, while height growth of oak seedlings increased with shade. This positive effect of increasing light availability on beech seedlings height was also found in other
studies carried out in natural conditions (Beaudet and Messier, 1998; Einhorn et al., 2004; Löf et al., 2007). Gemmel et al. (1996) reported a progressive decrease in height growth for oak seedlings from clearcuts to dense shelterwoods, while beech seedlings did not show differences in height when grown at different light intensities from clearcuts to shelterwoods of moderate density. Van Hees (1997) found similar responses to shading for beech and oak seedlings, with height growth increasing as light levels increased. On the other hand, Van Hees and Clerkx (2003) found that oak and beech seedlings had greater growth rates under shade (35% of full sunlight) than under full sunlight. Furthermore, Ziegenhagen and Kausch (1995) reported that oak seedling height increased with increasing shade up to 25% of full sunlight, although height growth was suppressed under heavier shade (10% of full sunlight). Other studies reported no effect of light availability on height growth of beech seedlings (Minotta and Pinzauti, 1996; Löf et al. 2010).

Some studies reported that height growth was found to be more affected by previous year than current year light conditions in oak (Welander and Ottosson, 1998) and beech (Welander and Ottosson, 1997) seedlings under controlled conditions. Therefore, both previous and current light conditions should be considered when planning silvicultural treatments such as underplanting.

1.9.2.2 Diameter growth

Besides increasing diameter growth of the remaining trees (Kerr, 1996; Yoshida and Kamitani, 1998; Savill and Evans, 2004), silvicultural practices that increase light availability and reduce competition, such as thinning, might have also a great impact on diameter growth of seedlings in the understory. Many studies have reported that stem diameter growth of oak and beech seedlings increases as light availability increases under natural conditions (Gemmel et al., 1996; Einhorn et al., 2004; Čater and Simončič, 2010; Ní Dhubhán, 2010) or controlled (Ammer, 2003) conditions. Similarly, Löf et al. (2007) reported that diameter growth of oak seedlings increased with a reduction in canopy density (diameter at ground level increased from 5% to 68% of full sunlight), although beech only showed increase in diameter growth as light levels increased from 5% to 19% of full sunlight and no additional diameter growth occurred with greater light availability. In contrast, Van
Hees and Clerkx (2003) found that shading levels of 30% of full sunlight had no
effect on root collar diameter in oak and beech seedlings.

1.9.2.3 Height to diameter ratio

Height to diameter ratio (H:D) has been used in forestry to assess tree quality,
vigour and strength and thus as a basis for thinning prescriptions. Opio et al.
(2000) proposed that H:D can be used as an alternative competition index in
young plantations. H:D is also considered a good indicator of vulnerability to
windthrow and snow break in Europe (Cremer et al., 1982) and America (Wonn
and O’Hara, 2001). H:D can be considered as a slenderness or sturdiness
coefficient, and is calculated for each tree by dividing tree height by either the
diameter at the root collar or diameter at breast height.

Height and diameter growth are influenced by environmental factors, and
thus H:D will also be affected. Light levels might affect the allocation of resources,
such as carbon, which can produce morphological changes such as modifications
in H:D. Mustard and Harper (1998) concluded that light is the most important
factor affecting H:D, and therefore this ratio can be used to choose appropriate
silvicultural treatments when removing overstory canopy to promote the
development of regeneration. Reduction of light levels can cause long and weak
stems since under shade height growth might be prioritised over diameter and
crown growth, resulting in larger H:D. The elongation and weakening of stems are
some of the characteristics of a process called etiolation, which occurs in partial
or complete absence of light (Drew and Ferrell, 1977). It is generally accepted
that as H:D increases tree vigour and growth rates decrease, and thus trees with
high H:D may benefit if available light is increased (Mustard and Harper, 1998).

It is apparent that H:D is affected by thinning, resulting in higher values
when no thinning is carried out and lower values progressively from moderate to
heavy thinning (Cremer et al., 1982). Similarly, many studies have reported a
decrease of H:D with increasing light (Harrington and Chan, 1992; Lieffers and
Stadt, 1994; Wang et al., 1994; Messier and Puttonen, 1995a; Mailly and
Kimmins, 1997; Chen and Klinka, 1998).

Beech exhibited higher H:D as shade increased (Petrițan et al., 2009).
Similarly, Prévosto and Balandier (2007) reported an increase in H:D under
strong competition from neighbours, which was explained by a reduction in light availability. Ní Dhubháin (2010) also reported that after two growing seasons, H:D of beech seedlings in the understory was lower when thinning was heavier (higher transmittance through the canopy into the understory) than if thinning was not carried out, but no differences across overstory treatments were found for sessile oak seedlings during the two growing seasons or beech after one growing season.

1.9.2.4 Biomass

Total biomass (or partial biomass of different parts of the plant) and biomass allocation or distribution within the plant should be considered when assessing tree responses to different light environments.

It is generally accepted that biomass accumulation increases with light availability, as found in many studies. The aboveground, belowground and/or total biomass increased in oak and beech with increasing irradiance (Madsen, 1994; Gemmel et al., 1996; Minotta and Pinzauti, 1996; Welander and Ottosson, 1998; Ammer, 2003; Van Hees and Clerkx, 2003; Einhorn et al., 2004; Curt et al., 2005; Čater and Simončič, 2010). It has been found that the normal reduction in biomass production that occurs as shade levels increase is related to the shade tolerance ranking of the species, with shade-tolerant species having a greater reduction in biomass than the less shade-tolerant ones (Walters et al., 1993b; Kitajima, 1994; Chen, 1997). Less shade-tolerant oak should experience less reduction in biomass than beech (Ammer, 2003; Van Hees and Clerkx, 2003). However, Gemmel et al. (1996) reported that oak seedling biomass decreased with increased shade while no differences across overstory treatments were found in beech.

It is generally assumed that plants optimise their resource use by partitioning more biomass to the part of the plant that acquires the most limiting resource, such as light. One expression of that optimisation is the biomass allocated to roots and shoots, which can be expressed as the root:shoot ratio (root dry mass divided by the shoot dry mass). Another way to assess the distribution of biomass along the plant is the root-mass fraction (also known as root-mass ratio), which is calculated as the proportion of plant dry mass in roots (Pérez-Harguindeguy et al.,
When light is the limiting resource and plants are growing in deep shade, plants generally display high biomass allocation to leaves (Givnish, 1988), suggesting that more biomass is allocated to the shoot than to the root as a morphological adjustment to shade. As a consequence, the root:shoot ratio is expected to decrease under shade in comparison to higher light conditions or full sunlight, as found for oak, beech or both species in several studies (Minotta and Pinzauti, 1996; Niinemets, 1998; Valladares et al., 2002; Ammer, 2003; Van Hees and Clerkx, 2003; Čater and Simončič, 2010). Welander and Ottosson (1998) reported similar results (root:shoot ratio increased with light) for beech seedlings that had been grown under different light conditions, but this trend was found in oak only up to 21% of PAR. However, some exceptions to the general trend have been reported. Light availability had little or no effect on root:shoot ratio of beech and/or oak seedlings, suggesting a low impact of light regime on biomass allocation (Gemmel et al., 1996; Einhorn et al., 2004; Curt et al., 2005). On the other hand, Madsen (1994) found that the root:shoot ratio of beech seedlings decreased or increased with increasing light depending on the soil water content, suggesting that the interaction between light and soil water content should be taken into consideration when studying responses to light intensity. Kitajima (1994) reported that seedlings of shade-intolerant species had lower root:shoot ratios than shade-tolerant ones.

1.9.3 Tree quality

The shape and branching habit of a tree can affect its commercial value and marketability. Different methods to assess tree/seedling quality can be found in the literature. Some of these methods are based on a subjective visual assessment of the tree (e.g., standard quality grades) while others are more objective (e.g., number of first-order and second-order branches, diameter and length in the major branch, branch relative height). Different indexes have been used to classify tree quality from very good to very poor based on tree characteristics, such as straightness, single leader, number of forked stems and branchiness (Rock et al., 2004; Teagasc, 2005; Saha et al., 2012).

Light availability in the understory does not only influence survival and growth, but also might affect the quality of seedlings found in the understory. The
branches of trees, especially in the juvenile stage, adapt architecturally to light availability (Aussenac, 2000). Thinning of forest stands generally improves wood quality since stem diameter increases, producing wood with fewer knots and warps (Kozlowski et al., 1991). Similarly, Cameron (2002) reported that early reduction of tree density through selective thinning improved individual tree quality without compromising stand stability. The lower branches of intermediate shade-tolerant yellow birch (Betula alleghaniensis Britt.) died in response to reduction in light, so shade affected their crown morphology, especially in smaller trees (Delagrange et al., 2004). Huuskonen et al. (2014) found that moderate thinning was a useful method to reduce the average branchiness of remaining young Scots pine trees.

1.9.4 Leaf characteristics

1.9.4.1 Single leaf area

The area of a leaf (also referred to as leaf area or leaf size) is defined as the one-sided or projected area of an individual leaf. Variation in leaf area has been related to external factors, such as climatic conditions, soil, elevation and latitude, and internal factors of the stand, such as plant size, tree architecture or leaf number (Pérez-Harguindeguy et al., 2013).

Plants adapted to low light tend to produce thin leaves with a larger surface area (Larcher, 2003). These patterns have also been observed within individual trees, with sun leaves growing on the exposed parts of the tree’s crown usually being smaller and thicker than those growing in shade inside the canopy (Pérez-Harguindeguy et al., 2013).

Van Hees (1997) found that shading increased leaf area of beech and oak seedlings under controlled conditions. Similar to Valladares et al. (2002), Ziegenhagen and Kausch (1995) reported that oak seedlings exhibited an increase in total leaf area with reduction of light intensity up to 25% of full sunlight in a controlled environment study, but heavier shade (10% of full sunlight) suppressed total leaf area. However, the opposite response has been found in other studies, with leaf area increasing as light increased in beech (Minotta and Pinzauti, 1996; Valladares et al., 2002; Einhorn et al., 2004; Petriţan et al., 2009).
and oak (Welander and Ottosson, 1998) seedlings. Welander and Ottosson (1998) found that beech seedlings increased leaf area with increasing PAR at very low light levels (from 2% to 9% of PAR at full sunlight), but leaf area remained the same at higher light levels (from 9% to 43% of full sunlight). On the other hand, Beaudet and Messier (1998) did not find any variation in leaf area of beech seedlings with increasing light. The contrasting findings regarding leaf area under different light conditions may be due to the use of different light intensities across studies, as well as to the adjustments of different species to increase light interception.

Parallel to the increase of leaf area with increasing light mentioned above, some studies have reported a similar increase in leaf area from the bottom to the top layer of different saplings (Paquette et al., 2007; Petrițan et al., 2009).

1.9.4.2 Leaf thickness

Leaf thickness ($L_{th}$) plays a key role in plant functioning. $L_{th}$ determines the physical strength of leaves and is one of the main components of the specific leaf area (Vile et al., 2005; Pérez-Harguindeguy et al., 2013).

Among other leaf characteristics, the $L_{th}$ influences the amount of light absorbed by leaves. Vendramini et al. (2002) suggested that $L_{th}$ can be used as an indicator of plant resource-use strategy, although the suitability of $L_{th}$ to understand the resource-use strategy in plants might vary across sites and growth environments. Regardless of leaf size, shade leaves that develop under low light levels generally have less mesophyll tissue and larger intercellular spaces, which results in thinner leaves than sun leaves growing at high light (Taylor and Davies, 1988). $L_{th}$ increases as irradiance levels increase in many tree species (St-Jacques et al., 1991; Ashton and Berlyn, 1994), including European beech and pedunculate oak (Aranda et al., 2001; Valladares et al., 2002). The greater leaf thickness exhibited under high levels of light might promote higher water use efficiency and lower evapotranspiration demands (Ashton and Berlyn, 1994) and can be a consequence of optimising photosynthetic balance against costs of respiration and transpiration (Pérez-Harguindeguy et al., 2013).
Valladares et al. (2002) found that $L_{th}$ was higher in less shade-tolerant oak than in beech. However, other studies found that thickness of leaves was approximately the same for species with different shade tolerance (Taylor and Davies, 1988) or no clear link was found between $L_{th}$ and shade tolerance ranking of different *Quercus* species (Ashton and Berlyn, 1994).

1.9.4.3 *Specific leaf area*

Specific leaf area (SLA) is defined as the leaf area divided by its oven-dry mass. Different terms such as leaf mass per area (LMA), leaf weight per area (LWA), specific leaf mass (SLM) and specific leaf weight (SLW) can also be found in the literature, and are simply $1/\text{SLA}$.

SLA describes the efficiency of light interception, expressed as leaf area, relative to the biomass invested in leaf tissue. SLA is a function of $L_{th}$ and leaf water content (LWC), but the contribution of each component depends on the conditions and the plant group and, therefore, they are not always related to each other (Pérez-Harguindeguy et al., 2013). Although sometimes it might not always be the best descriptor, SLA has been suggested as a good indicator of plant resource-use strategy (more widely applicable than LWC or $L_{th}$) since it has been found to be strongly related to resource use (Vendramini et al., 2002). SLA is often positively related to relative growth rate and mass-based light-saturated photosynthesis rate, and generally exhibits a negative relationship with leaf longevity and C investment in secondary compounds such as tannins or lignin (Pérez-Harguindeguy et al., 2013).

Usually, species grown in heavy shade conditions (resource-limited environment) have high SLA and low $L_{th}$ (Pérez-Harguindeguy et al., 2013). This increase in SLA with increasing shade has been reported for beech and oak, grown under natural (Minotta and Pinzauti, 1996; Beaudet and Messier, 1998; Niinemets, 1998; Aranda et al., 2001; Einhorn et al., 2004; Curt et al., 2005; Kunstler et al., 2005; Gardiner et al., 2009; Petrițan et al., 2009; Čater and Simončič, 2010) and controlled (Van Hees, 1997; Welander and Ottosson, 1997, 1998; Valladares et al., 2002; Van Hees and Clercx, 2003) conditions. Greater SLA under shading indicates that morphological adjustments occur in response to shade (Curt et al., 2005; Čater and Simončič, 2010) and increases the efficiency...
of light interception by maximising leaf area per unit of leaf biomass (Wang et al., 1994; Beaudet and Messier, 1998; Petrițan et al., 2009).

However, the results regarding the relationship between SLA and shade tolerance ranking of species has been controversial. While some studies have reported a tendency for shade-tolerant species to have greater SLA than less shade-tolerant ones (Niinemets and Kull, 1994; King, 2003; Kunstler et al., 2005; Klooster et al., 2007; Petrițan et al., 2009), others did not observe this trend (Beaudet and Messier, 1998; DeLucia et al., 1998; Stancioiu and O'Hara, 2006). While Van Hees and Clerkx (2003) and Valladares et al. (2002) found higher SLA values in beech than oak, Van Hees (1997) did not find differences between both species for SLA. This greater SLA values with increasing shade tolerance suggest that some species might produce greater leaf area under shade conditions, thereby increasing light utilisation at low cost and contributing to their higher survival under heavy shade conditions.

1.9.4.4 Leaf phenology

Phenology is the study of the timing of the recurring biological events such as seed emergence, leaf unfolding, flowering, leaf senescence and leaf fall. Changes in the timing of phases of the plant life cycle (phenophases) are affected by temperature, rainfall and day length. The evaluation of phenological records provides valuable information about the duration of these phenophases in different species. Bud flushing advances as spring temperature increases and as day length gets longer (Ray et al., 2010). Leaf phenology is also an important aspect of light harvesting (Kikuzawa, 1995). Three main characteristics must be considered in the study of leaf phenology: leaf emergence, leaf senescence and leaf longevity (Harada and Takada, 1988; Kikuzawa, 1990). Plant phenology can also be used as a useful tool to predict responses to different threats, such as climate change or tree diseases. For example, European ash (Fraxinus excelsior L.) trees that display bud flushing early in the spring (Bakys et al., 2013) and early leaf senescence in the autumn (Pautasso et al., 2013) are less susceptible to infection by ash dieback (Hymenoscyphus fraxineus (T. Kowalski) Baral et al.), an emerging invasive fungal disease, and, therefore, ash trees that flush and senesce early are preferred by forest managers. Furthermore, early flushing
species may be better able to exploit the growing season than late-flushing species so may accumulate more carbon, which might favour survival and growth (Augspurger, 2008; Lopez et al., 2008). Lopez et al. (2008) also suggested that differences in leaf emergence times might vary in relation to shade tolerance levels. However, early flushing may also be disadvantageous, such as by increasing susceptibility to late spring frosts (McGee, 1975) and herbivore damage (Wesolowski and Rowinski, 2008). Fu et al. (2014) described that oak and beech seedlings that had early leafing experienced earlier senescence too. Although different studies have addressed how phenology is affected by different factors (Vitasse et al., 2011; Fu et al., 2014; Laube et al., 2014; Way and Montgomery, 2015) few have focussed on phenological responses to light availability.

Two types of leaf-emergence patterns have been recognized in temperate deciduous broadleaf forests: flushing or synchronous leafing in which all the leaves burst in a short period and successive leafing in which leaves burst one by one over a longer period (Kikuzawa, 1983). In early successional conditions, where resources are usually available, plants expand leaves successively; while in shaded environments plants can attain higher production by simultaneous leaf emergence (Kikuzawa, 1995). In temperate deciduous forests, improvement in light conditions not only occurs by increasing tree height but also by earlier leaf development (Jones et al., 1997; Seiwa, 1998). Leaf senescence constitutes the final stage of leaf development, and it is basically governed by the developmental age, but it is also influenced by internal and environmental factors, such as drought, nutrient availability, day length and shade (Lim et al., 2007). Lammas growth is the secondary midsummer flush of height growth from the newly formed terminal bud. It can adversely affect productivity (higher probability to suffer autumn frosts and decrease growth) and quality (higher number of branches, increased probability of double leader, spike knots) in trees.

Although different studies have reported differences in leaf phenology in different tree species (Lopez et al., 2008; Chuine, 2010; Caffarra and Donnelly, 2011), few studies have compared the effect of different light availabilities on phenological responses of different species. For example, northern red oak (Quercus rubra L.) broke bud an average of 7 days before late-leafing American
beech (*Fagus grandifolia* Ehrh.) but the latter required less time to achieve full leaf expansion (Lopez *et al.*, 2008), suggesting that species leafing out earlier require more time to expand their leaves. McGee (1975) reported that the presence or absence of a tree canopy and the time of the year when changes in canopy cover occurs can affect the phenology of *Quercus rubra* and scarlet oak (*Quercus coccinea* Muenchh.) seedlings, with those grown under shade breaking dormancy earlier than those grown in full light. Differences in budburst were also affected by previous year light conditions in that study. Augspurger (2008) did not find differences in spring phenology (budburst and full expansion) of Ohio buckeye (*Aesculus glabra* Willd.) saplings grown under shade (7-8% of full sunlight) or ambient light (83% of full sunlight), while she found that leaves of sugar maple (*Acer saccharum* Marsh.) broke bud and expanded leaves slightly earlier under shade than under ambient light conditions. Caffarra and Donnelly (2011) found that high light intensity noticeably advanced budburst in beech and small-leaved lime (*Tilia cordata* Mill.), but budburst of broad-leaved osier (*Salix x smithiana* Willd.) and downy birch (*Betula pubescens* Ehrh.) was not affected by light intensity.

1.9.4.5 *Leaf physiology*

Physiological responses to light are of great importance since the light microclimate within the forest or plantation can be modified by silvicultural practices, such as thinning or pruning. In photosynthesis, light is harvested by chlorophyll-protein complexes associated with photosystem reaction centres (Kozlowski *et al.*, 1991). Stand density, thinning intensity, site quality and tree species, age and vigour are characteristics apparently related to photosynthetic acclimation (Han, 2012). Photosynthesis rates are highly sensitive to different environmental conditions: shaded plants in the understory generally have lower photosynthetic capacity than sun-adapted plants (Boardman, 1977). The leaves within the canopy of a single tree may also differ; e.g. sun leaves compared with shade leaves (Legner *et al.*, 2013).

Light stresses due to low light (shade) and high light (full sunlight) should both be considered when the effects of light conditions on physiological responses are studied. Among the physiological processes and modifications
generally quantified for plants under light stress are photosynthesis rate, stomatal conductance, transpiration, water status, electron transport rate, PSII efficiency and other parameters related to photosynthetic capacity.

Although the photosynthetic apparatus is capable of using light efficiently, sometimes leaves are exposed to more light than they can use for photosynthesis, which results in low quantum utilization and low assimilation yield (Larcher, 2003). This process is called photoinhibition or photoinactivation, and is defined as the slow reversible decline of photosynthetic efficiency that occurs when absorbed light is in excess of that required for carbon assimilation (Kitao et al. 2000). Chlorophyll fluorescence can be used to measure photoinhibition (Kitao et al., 2000; Gardiner et al., 2001). Although plants may show photoinhibition regardless of their shade tolerance, shade-tolerant species generally have a greater tendency for photoinhibition (Kozlowski et al., 1991). Photoinhibition might have important ecological implications in the understory of forests stands after changes in forest canopy cover, such as thinning, since leaves grown in shade are more vulnerable to photoinhibition after being suddenly exposed to high light (Larcher, 2003). Photoinhibition is also accentuated by stresses other than excess of light, such as by high temperature, drought and salinity (Kozlowski and Pallardy, 1997). The effect of photoinhibition declines after a period of acclimation to high light levels (Naidu and DeLucia, 1997) or the tree produces leaves acclimated to the new environment (Mulkey and Pearcy, 1992). Therefore, tree seedlings that may be able to minimise photoinhibition and reduce acclimation time might have advantage in survival and growth under forest gaps (Kitao et al., 2000).

In addition to the physiological responses to low and high light levels, sunflecks also play an important role in light utilisation. Efficient utilisation of sunflecks might depend on the establishment of a balance among the different processes occurring in chloroplasts (Kokzowski et al., 1991). Sunflecks are an important component of the carbon gain for shaded leaves (Way and Pearcy, 2012). There is evidence that beech seedlings can exploit sunflecks, suggesting that forest gaps (in which sunflecks may play an important role) are favorable for photosynthesis in beech seedlings (Johnson et al., 1997; Tognetti et al., 1997).
Chlorophylls are the most important pigments in photosynthesis and may play an important role in the balance between light absorption and light use. The chlorophyll content of plants might be influenced by shade, perhaps limiting the photochemical process, especially under intense light conditions (Larcher, 2003). Other pigments present in the leaves of plants are carotenoids and anthocyanins. Carotenoids contribute to light-harvesting and protect the photosynthetic systems (Gitelson et al., 2003). Anthocyanins protect leaves from excess light (Gitelson et al., 2002). The carbon gain hypothesis suggests that shade-tolerant species have higher chlorophyll content in their leaves than light-demanding ones under low light environments (Valladares and Niinemets, 2008). Givnish (1988) reported that leaves of seedlings grown in shaded conditions have greater chlorophyll contents than those grown under full light. This is in agreement with results found for oak and beech seedlings, where chlorophyll content increased with decreasing light and was significantly higher under low light conditions (Johnson et al., 1997; Tognetti et al., 1997; Valladares et al., 2002). Chlorophyll content might vary depending on how it is expressed, since shaded leaves generally have higher chlorophyll content on a weight basis but less on an area basis (Kozlowski et al., 1991). The greater chlorophyll content of shaded leaves in conjunction with a reduction in leaf thickness might provide greater light-use efficiency (Boardman, 1977). Chlorophyll deficiency in leaves might occur under excess of light but also when there is too little light (when the mineral balance is disturbed), and may reduce photosynthesis rates (Larcher, 2003).

1.9.4.5.1 Gas exchange

Many studies have revealed that photosynthetic light-response curves vary across light environments for a great number of different species, from shade-tolerant to light-demanding species (Loach, 1967; Bazzaz and Carlson, 1982; Kubiske and Pregitzer, 1996; Beaudet et al., 2000; Gardiner and Krauss, 2001; Gardiner, 2002; Rodríguez-Calcerrada et al., 2007; Wong et al., 2012). However, Gardiner et al. (2001) found that light-response curves of photosynthesis of leaves of nuttall oak (Quercus nuttallii Palm.) seedlings in the understory were not substantially altered by a cotton-wood (Populus deltoides Bartr. Ex Marsh.) canopy. The lack of physiological differences in response to different light availabilities could be due to 1) photoinhibition of leaves under full light, which is
characterised by reduction in maximum capacity for photosynthesis and quantum efficiency (Kozlowski and Pallardy, 1997); or 2) light in the understory was enough to let the photosynthetic mechanism develop properly, as reported by Dean et al. (1982) for black walnut (Juglans nigra L.) under shade.

Similar to findings mentioned above, considerable photosynthetic plasticity in beech and oak in response to different light environments has been reported (Johnson et al., 1997; Gardiner et al., 2009; Kuehne et al., 2014). Reynolds and Frochot (2003) found that photosynthetic saturation curves for beech seedlings that had been released (after a windstorm) or were in full sunlight differed from that observed for shaded seedlings, but the response did not differ between the released and full sunlight seedlings.

Generally maximum photosynthetic rates are greater for plants grown under high light intensities than the same plants grown at low light levels (Kozlowski and Pallardy, 1997). Additionally, leaves acclimated to the understory of closed forest canopies usually have lower $A_{\text{max}}$, $R_d$, $l_c$ and $I_{\text{sat}}$ than those acclimated to open sites (Bazzaz and Carlson, 1982; Teskey and Shrestha, 1985; Naidu and DeLucia, 1997; Balandier et al., 2007). Similar results have also been found in studies for beech and oak under controlled and natural conditions where $A_{\text{max}}$, net photosynthesis, $l_c$, $I_{\text{sat}}$ and/or $R_d$ increased with increasing light (Gross et al., 1996; Johnson et al., 1997; Tognetti et al., 1997; Valladares et al., 2002; Reynolds and Frochot, 2003; Aranda et al., 2004; Einhorn, 2007; Gardiner et al., 2009; Kuehne et al., 2014). Although Kuehne et al. (2014) found that $R_d$ of oak seedlings increased in large canopy openings (>40% of full sunlight) the differences from seedlings grown in canopy gaps (11-40% of full sunlight) and closed canopy (<11% of full sunlight) were not significant. It has also been reported that stomatal conductance, an important limiting factor for photosynthesis, was greater at full sunlight than at 50% full light for 4 years-old oak seedlings, although the values depended both on PAR and water supply (Gross et al., 1996). Intrinsic water use efficiency increased with increasing irradiance for pedunculate and sessile oak growing in a common garden experiment (Ponton et al., 2002). These results highlighted the importance of taking into consideration water supply when analysing physiological responses to
light intensity, since drought stress can reduce net photosynthesis and stomatal conductance (Raftoyannis and Radoglou, 2002).

The improvement of physiological responses, such as net photosynthesis rate, in response to increasing light have been observed in stands where different thinning treatments were applied (Wang et al., 1995), although in some cases the response was manifested one year after thinning (Gauthier and Jacobs, 2009; Gauthier and Jacobs, 2010), suggesting that some species might require a full growing season before responding to thinning treatments.

At low light, leaves grown in shade frequently have higher quantum yield of photosynthesis, $\Phi_{\text{CO}_2}$, than leaves at full sunlight. This type of response to light intensity has been found in a variety of species (Loach, 1967; Dean et al., 1982). Similar to these findings Johnson et al. (1997) found that $\Phi_{\text{CO}_2}$ of beech seedlings decreased with increasing PAR from understory to clearing, whereas Aranda et al. (2004) found no differences among one year-old beech established in thinned and non-thinned stands. In contrast, some studies have reported that $\Phi_{\text{CO}_2}$ increased with increased light for *Q. robur* seedlings (Gardiner et al., 2009; Kuehne et al., 2014).

In a study on photosynthetic characteristics of beech and oak established under a Norway spruce canopy (Gardiner et al., 2009) physiological responses relative to leaf mass were in contrast to those found relative to leaf area. While seedlings established in open stands showed greater area-based $A_{\text{max}}$, $R_d$ and $I_{\text{sat}}$ than regeneration established in closed stands, $A_{\text{max}}$ relative to dry mass of beech seedlings established in the shelterwoods (closed canopy) were significantly higher than beech established in patches. This reduction in the photosynthesis rates on leaf area basis compared with those on leaf mass basis of beech seedlings in the shelterwoods (which was not observed in oak seedlings since photosynthesis increased with increasing light regardless of if it was measured relative to leaf mass or area) suggests that beech and oak differ in their mechanisms of photosynthetic acclimation to light availability (Valladares et al., 2002).

Although beech seedlings seem to be able to acclimate, through morphological and physiological changes, to new light conditions created after
opening the overstory canopy (Aranda et al., 2001), higher physiological plasticity (maximum photosynthesis, stomatal conductance and rubisco activity) in response to light was found in oak than in beech (Valladares et al., 2002).

Under high light environments, light-demanding species are expected to exhibit greater area-based photosynthesis rates than the shade-tolerant ones (Bazzaz, 1979; Bazzaz and Carlson, 1982; Walters et al., 1993a; Kubiske et al., 1996; Kubiske and Pregitzer, 1996; Niinemets et al., 1998; Morecroft and Roberts, 1999; Kitao et al., 2000; Valladares et al., 2002; Gardiner et al., 2009). In contrast, lower photosynthesis rates in shade-tolerant species were not found between Acer species of different shade tolerance (Hanba et al., 2002) or between beech and light-demanding ash (Einhorn et al., 2007).

1.9.4.5.2 Chlorophyll fluorescence

Under high irradiance, plant leaves may absorb more photons than they can use, and this excess of energy can lead to reduced PSII efficiency (Kato et al., 2003). It has also been found that PSII efficiency might decrease with decreased photosynthesis when photosynthesis is inhibited by environmental or physiological factors (Weng, 2009). PSII quantum yields ($\Phi_{PSII}$) are usually greater under low light conditions than under high light, considering that in low light a large proportion of the absorbed light is used in photochemistry while in high light a greater proportion of the absorbed energy is dissipated through non-photochemical processes (LI-COR, 2011). For example, it has been reported that $\Phi_{PSII}$ decreased as the incident light increased in beech leaves under field conditions (Bilger et al., 1995; Einhorn et al., 2004). The same trend with PAR was found for $\Phi_{PSII}$, and its two components, PSII maximum efficiency and photochemical quenching, in a controlled study of oak and beech seedlings grown in different light environments (Valladares et al., 2002). Values decreased with increasing light availability (Valladares et al., 2002).

Previous studies have shown that photoinhibition occurs in plants when photochemical quenching ($qP$) falls below 0.6 (Ögren, 1991; Öquist et al., 1992; Einhorn et al., 2004). Measurements of $qP$ suggested that beech seedlings under open and gap conditions experienced photoinhibition (Einhorn et al., 2004).
Electron transport rate (ETR) can be used to study the dynamic of photosynthetic induction since ETR induction is generally quicker than that of CO₂ assimilation (Han et al., 1999). It was found that beech seedlings exhibited greater ETR under high light (Einhorn et al., 2004; Balandier et al., 2007).

1.10 Controlled light environment studies

Controlled-shade studies (shadehouses) have been carried out in an attempt to simulate the effect of tree canopy on the growth of underplanted seedlings. Controlled studies allow the effects of light to be separated from other effects and reduce confounding effects due to climatic, edaphic factors and competition. However, light availability and other factors (e.g., temperatures and water availability) will often vary unpredictably in a natural environment, making it difficult to separate the effects of light from other environmental factors. A wide range of nets (shadecloth) have been used to provide shadow and protection, which simulate different shade levels artificially (Walters and Reich, 1996; Oren-Shamir et al., 2001; Ammer, 2003; Valladares et al., 2005; Gómez-Aparicio et al., 2006; Leicht and Silander, 2006; Kennedy et al., 2007; Cummings et al., 2008; Gaudio et al., 2011; Mugnozza et al., 2011). These nets, widely used in horticulture, can be utilised outdoors as well as in glasshouses. Some concerns of most artificial field experiments in which light is controlled is the possibility that light could be affected by the frames or any other part of the shading construction (not only by the nets), and the modification of the microenvironment under the shadehouses, as factors different to light might be altered (such as temperature, relative humidity, soil moisture).

Greenhouse construction and shading material can be classified into non-selective (transmits wavelengths uniformly) and selective (transmits wavelengths disproportionately). Shade nets may differ in their efficiency in transmitting diffused or scattered light, and in their ability to scatter direct light passing through them. Plastic nets are generally constructed from polypropylene or polythene. Nets available differ in colours (e.g., green, red and black) and may range from neutral (do not modify the R/FR but can reduce PAR levels in a similar way to a forest canopy) to those that can modify R/FR ratio and other characteristics of the incident light (Oren-Shamir et al., 2001). Therefore, using and combining different
type of nets can modify light quantity (PAR) and light quality (R/FR) in order to get the desired effect.
1.11 References


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Chapter 2
Effects of light availability on morphology, growth and biomass allocation of *Fagus sylvatica* and *Quercus robur* seedlings

**Abstract**
The survival, morphological, and growth responses of European beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.) seedlings to different light intensities, from full sunlight to heavy shade, were studied over two growing seasons in a shadehouse experiment. Although shade treatments significantly affected seedling growth, they did not influence seedling survival. Both growth and biomass increased as light intensity increased. Diameter growth of oak seedlings was higher than that of beech. Beech and oak seedlings showed typical acclimation to shade, including greater specific leaf area and height to diameter ratios, and lower leaf thickness and root:shoot ratios with increasing shade. Beech seedlings exhibited greater specific leaf area, and lower leaf thickness and root:shoot ratios than oak seedlings. In spite of the greater growth at full sunlight, the results from this study suggest that beech and oak seedlings would have high survival rates and would acclimate well if underplanted below overstories that reduce the available light to as low as 28% of full light.

2.1 Introduction
Silviculture is an old discipline which must be adapted to address different forest management challenges, such as sustainability and multi-purpose objectives. Although natural regeneration is preferred and is the most common method of replacing forests on a worldwide scale (Savill *et al.* 1997), it is not always successful or practical. In these cases, underplanting may be a feasible alternative regeneration method. In Ireland, planting is the most common method of establishment (Woodlands of Ireland, n.d.). Underplanting in an existing stand is a common practice in Central Europe (Hawe and Short, 2012) and is carried out in shelterwoods and thinned stands (Lüpke *et al.*, 2004). Underplanting has been applied for the enrichment of an existing stand, for the conversion of even-aged monocultures into more complex systems and for the rehabilitation of non-productive stands (Kenk and Guehne, 2001; Paquette *et al.*, 2006). Therefore, one suggested method for improving under-performing broadleaf forests is
thinning in conjunction with underplanting (Evans, 1984; Hawe and Short, 2012). An understory of trees will help control weed growth and give some flexibility in management.

In Europe many different silvicultural systems have been used for centuries, but in recent years there is increasing interest in Continuous Cover Forestry (CCF), which has gained in recognition worldwide as an alternative to clearfelling to promote tree species and structural diversity, and multi-objective forests (Hart, 1995; Department of Agriculture, Food and the Marine, 2014). CCF uses the control of light through thinning and includes those silvicultural systems in which there is a continuous maintenance of forest cover (Pommerening and Murphy, 2004). The shelterwood and selection systems are preferred in CCF since these systems are considered to meet some principles of close-to-nature silviculture (COFORD, 2003; Brang et al., 2014). The interest in broadleaf species and alternatives to clearfelling has now heightened the demand for research on how tree species develop under different light environments as a result of forest management intervention. The response of species to light conditions is a complex function (Valladares et al., 2002) and understanding how light influences seedling survival and growth in the early years after planting may reveal important information for the management of broadleaf species.

Pedunculate oak (Quercus robur L.) and European beech (Fagus sylvatica L.) are two important trees in Europe and play a key role in European forestry. These species vary in their shade tolerance, with oak being considered less shade-tolerant than beech at the seedling stage (Brzeziecki and Kienast, 1994). However, Welander and Ottosson (1998) suggested that one-year-old seedlings of oak and beech adapt similarly to low light conditions. Seedlings from nurseries, adapted to higher light before underplanting, may experience planting shock, but there is little information on this aspect for underplanted stock compared with stock planted on open forest sites. Therefore, responses to change in light intensity may be different from that of naturally regenerated plants. The performance of oak (Ziegenhagen and Kausch, 1995; Welander and Ottosson, 1998) and beech (Welander and Ottosson, 1997; Tognetti et al., 1998) seedlings can be influenced by previous and current light conditions. Beech responds well to thinning, but, if thinning or clearfelling is carried out suddenly in a previously
shaded stand, the cambium may die as a result (Savill, 2013). Beech is one of the most suitable species for underplanting and the prescription involves underplanting after the first thinning of the overstorey (COFORD, 2002b).

While various studies have addressed the response of beech or oak to light availability (Madsen, 1994; Tognetti et al., 1994, 1998; Gross et al., 1996; Aranda et al., 2001), little research has been done with these two species under similar environmental conditions (Welander and Ottosson, 1998; Valladares et al., 2002). The responses of different species to light availability under a forest canopy are difficult to investigate since other factors may also vary and it can be difficult to find sites where the same species are present in the understory. Therefore, studies performed under artificial shade may be alternative approaches to investigate the response of various species to light intensity (Madsen, 1994).

The aim of this study was to investigate the impact on survival, growth and biomass allocation in beech and oak seedlings grown under different shade conditions. The different shade conditions were intended to mimic a range of underplanting conditions. The results were expected to provide information on the acclimation of underplanted oak and beech seedlings to light levels, particularly in relation to CCF.

2.2 Material and methods

2.2.1 Study site and tree species
The study was conducted in a controlled-shade experiment located at Teagasc Ashtown Food Research Centre, Dublin 15, Ireland (53°22′45″ N, 6°20′13″ W, 40 m above sea level). Two year-old seedlings (1u1) of pedunculate oak (*Quercus robur* L.) and three year-old (1u1u1) European beech (*Fagus sylvatica* L.) were purchased from a Coillte Nursery, Ardattin, Co. Carlow, Ireland (52°43′47″ N, 6°41′13″ W, 104 m above sea level) and planted at Teagasc Food Research Centre in March 2011. Because 1u1 beech seedlings of similar size to the oak seedlings (50-80 cm) were not available, 1u1u1 beech seedlings were used instead. The provenances used were according to recommendations in Ireland (COFORD, 2002a): beech provenance was Cirencester Region 404, United Kingdom, origin unknown (51°43′0″ N, 2°0′0″ W, 140 m above sea level), and oak provenance was NL.S. Nuenen 03, Netherlands, origin unknown.
(51°29'9" N, 5°32'9" E, 20 m above sea level). The experimental area was fenced to exclude rabbits and hares. Weeding was carried out when required. The mean annual total rainfall in the region is 774 mm and the mean annual air temperature is 9.8 °C (all means are from the period 1981-2010). The weather conditions from 2011 to 2014, the period when this study was conducted, were similar with respect to temperature but differed in rainfall during the growing season (Table 2.1). Climate data were collected by an Automatic Weather Station (Met Éireann, Phoenix Park station) located 1.93 km away at an open site.

Table 2.1. Temperature (°C) and rainfall (mm) during the years of the study. Growing season was calculated considering the period from April to October.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year 2011</th>
<th>Year 2012</th>
<th>Year 2013</th>
<th>Year 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.4</td>
<td>9.8</td>
<td>9.9</td>
<td>10.4</td>
</tr>
<tr>
<td>Growing season</td>
<td>13.2</td>
<td>12.4</td>
<td>13.2</td>
<td>13.6</td>
</tr>
<tr>
<td>Rainfall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual</td>
<td>675</td>
<td>869</td>
<td>711</td>
<td>885</td>
</tr>
<tr>
<td>Growing season</td>
<td>287</td>
<td>564</td>
<td>282</td>
<td>336</td>
</tr>
</tbody>
</table>

The mean seedling heights at planting were 61.1 ± 0.5 cm for *F. sylvatica* and 75 ± 0.6 cm for *Q. robur*. The mean stem diameters were 8.7 ± 0.1 mm for *F. sylvatica* and 7.3 ± 0.1 mm for *Q. robur*.

2.2.2 Experimental design and shade treatments

The experimental design was a randomised block design with split-plots: light as the whole plot factor and species as subplot factors, replicated across five blocks. This resulted in twenty plots (11 m long, 4.3 m wide and 2.9 m high, including the shading nets), each containing two subplots and corresponding to the two broadleaf species. Plots were spaced apart from each other to minimise any interaction effects. Forty-two seedlings were planted in each subplot at 0.5 × 0.5 m spacing to encourage the early onset of interplant competition. The subplot measurement area entailed 16 seedlings per species. Each subplot was surrounded by a buffer zone and included an additional line of plants.

Green polythene shade nets (Colm Warren Polyhouses Ltd., Kilmurray, Trim, Co. Meath, Ireland) were erected on frames to simulate different light environments (representing a spectrum of thinning intensities) in September
2012, about one and a half years after the seedlings were planted. Four different light treatments were established in each block (one treatment per plot): full sunlight, light shade, medium shade and heavy shade. The proportion of photosynthetically active radiation (PAR) below the nets was calculated as the difference between readings taken simultaneously with a data logger, LI-1400 (LI-COR Inc., Lincoln, Nebraska), using a LI-190SA Quantum Sensor (LI-COR Inc., Lincoln, Nebraska) outside the plot and a LI-191SA Line Quantum Sensor (LI-COR Inc., Lincoln, Nebraska) inside the plot in October 2013. LI-COR quantum sensors monitor PAR in the 400 to 700 nm waveband. Soil water content (SWC, %) was measured in January 2014 in each plot to determine the amount of rainfall interception. Measurements were carried out in the corners and centre of the plot with a WET sensor and a moisture meter that allowed SWC measurement at a depth of 68 mm (Delta-T Devices Ltd, Cambridge, UK). Red/far-red ratio (R/FR) was measured in March 2014 with a Skye SKR 110 sensor connected to a display meter (Skye Instruments, Powys, UK) that reports quantum flux at 660 and 730 nm. In each light treatment of the first block, air temperature and relative humidity were recorded every 10 min from 26 May to 8 October during 2015 using dataloggers (SF-LOG-M, Solfranc Tecnologias SL, Tarragona, Spain) with shelter to prevent direct solar radiation and rainfall. Temperature and humidity loggers were located in the middle of each oak subplot (after checking there were no differences between oak and beech subplots), ≈70 cm above-ground. The different light treatments averaged 100%, 62%, 51% and 28% of PAR. A description of the conditions in the different treatments is shown in Table 2.2. The shadehouses had little effect on R/FR, as this ratio inside and outside the shadehouses was similar in the two intermediate treatments, and slightly lowered in the heavy shade treatment (Table 2.2). The rainfall interception in the soil decreased with increasing shade (Table 2.2). Air temperature and relative humidity did not differ among the different light environments.
Table 2.2. Light properties, soil water content (SWC), air temperature (T) and relative humidity (RH) in the different shade environments. Data are the means ± standard errors.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAR (%)</th>
<th>R/FR</th>
<th>SWC (%)</th>
<th>T (°C)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (full sunlight)</td>
<td>100</td>
<td>1.00</td>
<td>47.7 ± 0.8</td>
<td>13.6</td>
<td>82.2 ± 0.1</td>
</tr>
<tr>
<td>Light shade</td>
<td>62</td>
<td>0.98</td>
<td>46.0 ± 0.9</td>
<td>13.6</td>
<td>82.4 ± 0.1</td>
</tr>
<tr>
<td>Medium shade</td>
<td>51</td>
<td>0.98</td>
<td>44.3 ± 0.9</td>
<td>13.7</td>
<td>81.1 ± 0.1</td>
</tr>
<tr>
<td>Heavy shade</td>
<td>28</td>
<td>0.92</td>
<td>39.8 ± 1.0</td>
<td>13.5</td>
<td>81.3 ± 0.1</td>
</tr>
</tbody>
</table>

2.2.3 Morphological measurements

Survival, seedling height and stem diameter at 3 cm above the ground were assessed during the dormant season before and after erecting the shadehouses (2011, 2012, 2013, 2014 and 2015). Height was measured from the ground to the highest point of the live crown (and drooping leaders were extended to full length for measurement). The height and diameter increments for each growing season were the differences in the two consecutive sets of values. Height:diameter ratios (H:D ratios) were calculated from recordings before starting the growing season as: height (mm) / stem diameter (mm). During the summer of 2014 the elongation of the main stem was measured during June and August.

In 2013, dead or missing seedlings in the measurement area of each plot were replaced with randomly selected seedlings from the "spare area" of the same plot before the beginning of the growing season. If there were not enough plants in the spare line for each plot (light treatment) to be replaced, seedlings from a nearby plot with the same treatment were chosen.

2.2.4 Destructive sampling

Three plants of each species and treatment were randomly selected to carry out destructive measurements at the end of the study. Five leaves of each selected plant were harvested to analyse leaf area using a LI-3000 area meter (LI-COR Inc., Lincoln, Nebraska, USA). All leaf material was healthy and collected from the same position between 8:00am and 11:00am on 8th of October 2014. Leaves were placed in sealed plastic bags and stored in a cool box in the dark until further processing in the laboratory. Fresh weights of the selected leaves were measured and they were dried at 80 °C until constant weight was reached, after which the samples weights were recorded. From these data, leaf size, leaf dry
mass, specific leaf area (SLA) and leaf thickness ($L_{th}$) were calculated. SLA was determined as the leaf area divided by its oven-dry mass. $L_{th}$ was estimated by dividing leaf fresh mass by leaf area, which allows for the estimation of leaf thickness from easily measured leaf traits and works well as an approximation (Vile et al., 2005; Pérez-Harguindeguy et al., 2013).

At the end of the experiment (February 2015) the selected seedlings were harvested and separated into stems, branches and roots. Seedlings were lifted by hand maintaining a soil core of 50 cm of diameter. Any remaining soil was removed by washing the roots. The stem was separated from the roots at the root collar, and the remaining dead leaves were removed from the branches. Samples were stored in bags and placed in a cold store until further processing. Samples were dried in an oven at 105 °C until constant dry weight was obtained. Root mass, branch mass, stem mass, aerial biomass (branches plus stems, no leaves included), total biomass (above-ground plus below-ground mass, no leaves included) and root:shoot ratios were determined from these data.

2.2.5 Data analysis

All statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Growth responses were analysed using the MIXED procedure of SAS. Dead seedlings were excluded. Fixed effects were light, species and their interaction. Random effects were block and block x light interaction; the latter to account for the split plot structure. For those parameters measured for the two years of the study (height, diameter and H:D ratios) and at different dates (elongation 2014), repeated measures models were used. Following a significant effect or interaction, pairwise comparisons of least square means (Tukey’s test) were used to detect treatment differences. All tests for significance were conducted at $p \leq 0.05$. Normal distribution of errors and homogeneity of variance were assessed graphically. Data with residuals that did not conform to assumptions of normality and/or homogeneity of variances were transformed using Box-Cox transformations (Box and Cox, 1964).

Additionally, Pearson correlation analyses were used to identify relationships between some morphological variables (height and growth) with biomass and
SLA. Correlations between SLA and $L_{th}$; and aboveground biomass and root biomass were also carried out.

As survival was a binary response (alive or dead), maximum likelihood estimation was used to study relationships between survival and species, light conditions and species x light interaction, employing the LOGISTIC procedure of SAS.

A plasticity index (from 0 to 1) was determined for the parameters studied in during the growing season of 2014 (growth, biomass and foliage characteristics). It was calculated as the difference between the maximum and the minimum mean values between the different light treatments divided by the maximum mean value (Valladares et al., 2002). This index allowed the comparison of changes in variables expressed in different units.

2.3 Results

2.3.1 Tree survival
The study conducted during two growing seasons (2013 and 2014) showed that there were no significant differences in tree survival between species ($p = 0.489$ and 0.677 for the first and second growing season, respectively) or light treatments ($p = 0.779$ and 0.637 for the first and second growing season, respectively). Over the two growing seasons survival rates were greater than 90% for the different species and light treatments.

2.3.2 Seedling growth
Seedling height increment ($\Delta H_t$) was significantly influenced by year, species x light, species x year and light x year interactions (Table 2.3). Beech seedlings had higher $\Delta H_t$ (averaged over years) than oak seedlings at full sunlight but no significant differences were found between species and light treatment combinations for $\Delta H_t$ (Table 2.4). While $\Delta H_t$ (averaged over treatments) decreased in beech seedlings from the first to the second growing season, the opposite was found for oak seedlings. In 2013 the higher $\Delta H_t$ was found in beech seedlings, but in 2014 $\Delta H_t$ was higher in oak than in beech. With increasing shade, $\Delta H_t$ (averaged over species) increased during 2013 but the opposite
occurred in 2014, with significant differences between the heavy shade and full sunlight during both years of the study (Table 2.4).

Table 2.3. Repeated-measures analysis of variance testing 1) the effects of species (df = 1), light (df = 3), year (df = 1) and their interactions on height increment (ΔHt), diameter increment (ΔDia) and height to diameter ratio (H:D); 2) the effects of species, light, month (df = 1) and their interactions on main stem elongation during the growing season of 2014. Significant effects are in bold (p < 0.05).

<table>
<thead>
<tr>
<th>Traits 1)</th>
<th>Species (S)</th>
<th>Light (L)</th>
<th>S x L</th>
<th>Year (Y)</th>
<th>S x Y</th>
<th>L x Y</th>
<th>S x L x Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔHeight</td>
<td>0.290</td>
<td>0.307</td>
<td>0.029</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.305</td>
</tr>
<tr>
<td>ΔDiameter</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.115</td>
<td>0.092</td>
<td>0.055</td>
<td>0.011</td>
<td>0.145</td>
</tr>
<tr>
<td>H:D</td>
<td>0.068</td>
<td>&lt;0.001</td>
<td>0.105</td>
<td>0.002</td>
<td>0.778</td>
<td>0.719</td>
<td>0.386</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Traits 2)</th>
<th>Species</th>
<th>Light</th>
<th>S x L</th>
<th>Month (M)</th>
<th>S x M</th>
<th>L x M</th>
<th>S x L x M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongation 2014</td>
<td>0.030</td>
<td>0.023</td>
<td>0.002</td>
<td>0.007</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.456</td>
</tr>
</tbody>
</table>

Table 2.4. Height increment (on a year basis) and elongation in 2014 (on a monthly basis) of beech and oak seedlings in the different light treatments. Data are the means. Where species x treatment interaction is significant, combination means followed by the same upper case letter are not significantly different (n = 5 reps). Where treatment x year (or treatment x month) interaction is significant, combination means followed by the same lower case letter are not significantly different (n = 5 reps).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species</th>
<th>Height increment (cm)</th>
<th>Elongation2014 (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Beech</td>
<td>42.75 41.8 42.27A</td>
<td>33.87 7.37 20.61A</td>
</tr>
<tr>
<td></td>
<td>Oak</td>
<td>23.76 37.4 30.53B</td>
<td>14 17.18 15.53ABC</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>33.26bc 39.55ab</td>
<td>23.90a 12.25cd</td>
</tr>
<tr>
<td>51</td>
<td>Beech</td>
<td>43.45 28.24 35.81AB</td>
<td>20.12 7.24 13.67BC</td>
</tr>
<tr>
<td></td>
<td>Oak</td>
<td>36.86 36.12 36.48AB</td>
<td>10.42 23.68 17.06ABC</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>40.15ab 32.14bc</td>
<td>15.27bcd 15.46bcd</td>
</tr>
<tr>
<td>28</td>
<td>Beech</td>
<td>48.43 21.11 34.67AB</td>
<td>14.47 6.57 10.41G</td>
</tr>
<tr>
<td></td>
<td>Oak</td>
<td>38.82 36.53 37.61AB</td>
<td>7.66 28.11 17.89AB</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>43.62a 28.66c</td>
<td>11.03d 17.27bc</td>
</tr>
</tbody>
</table>

Stem elongation in 2014 was significantly affected by species, light, month, species x light, species x month and light x month interactions (Table 2.3). While stem elongation (averaged over months) decreased with increasing shade for beech seedlings, no significant differences between treatments were found for oak seedlings (Table 2.4). Elongation decreased in beech seedlings from June to August, but the opposite was found for oak seedlings, resulting in greater elongation in June for beech and greater elongation in August for oak regardless
of light level (Table 2.4). While elongation (averaged over species) in June decreased with increasing shade, no differences between light treatments were found in August (Table 2.4).

Diameter increment (ΔDia) was significantly affected by species, light and light x year interaction (Table 2.3). ΔDia (averaged over treatments and years) was significantly greater in oak (4.91 ± 0.09 mm) than beech (3.80 ± 0.09 mm). ΔDia decreased with increasing shade but significant differences were not found between the intermediate treatments (Fig. 2.1A).

The H:D ratio was significantly affected by light and year (Table 2.3). The H:D ratio was significantly greater in 2014 (73.57 ± 1.17) than in 2013 (71.32 ± 1.16). Seedlings at full sunlight exhibited lower H:D ratios than seedlings in the other light treatments (Fig. 2.1B).

2.3.3 Biomass and foliage characteristics
Leaf size was more affected by decreasing light levels in oak than in beech, as indicated by the significant species x light interaction (Table 2.5). Leaf size of beech was not significantly different between the different light treatments while oak leaf size increased with decreasing light availability (Table 2.6). Oak leaves in the full sunlight were 27% smaller in area than those in the heavy shade. Although beech seedlings had generally smaller leaves in all the light treatments, significant differences between species were only found in the heavy shade treatment (Table 2.6). Unlike leaf size, the significant species x light interaction (Table 2.5) indicated that leaf mass in beech was more affected by decreasing
light than in oak (Table 2.6). Leaf mass of beech decreased with decreasing light, with leaves in the heavy shade being 43% lower in mass than those in the full sunlight (Table 2.6). Leaf mass was significantly greater in oak than in beech in the 51% and 28% of PAR (Table 2.6). The greatest difference in leaf characteristics between species was found in the heavy shade treatment, where oak leaves were 63% greater in area and 113% heavier in mass than beech leaves (Table 2.6).

Table 2.5. Summary of analysis of variance for the main effects of species (df = 1), light (df = 3) and their interaction (df = 3) on growth, biomass and foliage characteristics. Significant effects are in bold ($p < 0.05$).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Species</th>
<th>Light</th>
<th>Species x Light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Leaf size</td>
<td>39.84</td>
<td>&lt;0.001</td>
<td>1.19</td>
</tr>
<tr>
<td>Leaf mass</td>
<td>72.18</td>
<td>&lt;0.001</td>
<td>2.32</td>
</tr>
<tr>
<td>Root mass</td>
<td>0.25</td>
<td>0.621</td>
<td>3.99</td>
</tr>
<tr>
<td>Branch mass</td>
<td>8.53</td>
<td>0.010</td>
<td>2.29</td>
</tr>
<tr>
<td>Stem mass</td>
<td>0.49</td>
<td>0.487</td>
<td>2.53</td>
</tr>
<tr>
<td>Aerial biomass</td>
<td>2.62</td>
<td>0.109</td>
<td>2.36</td>
</tr>
<tr>
<td>Total biomass</td>
<td>1.54</td>
<td>0.218</td>
<td>3.06</td>
</tr>
<tr>
<td>Root:shoot ratio</td>
<td>26.03</td>
<td>&lt;0.001</td>
<td>6.20</td>
</tr>
<tr>
<td>SLA</td>
<td>50.51</td>
<td>&lt;0.001</td>
<td>18.53</td>
</tr>
<tr>
<td>Leaf thickness</td>
<td>155.61</td>
<td>&lt;0.001</td>
<td>34.48</td>
</tr>
<tr>
<td>Ht / StemMass</td>
<td>3.08</td>
<td>0.082</td>
<td>4.35</td>
</tr>
</tbody>
</table>

SLA and $L_{th}$ were strongly influenced by species and light with no significant interaction between the two effects (Table 2.5). As expected, SLA increased with increasing shade (Table 2.6). $L_{th}$ was significantly greater in oak than beech while the opposite was true for SLA (Table 2.6). $L_{th}$ was significantly and negatively correlated with SLA for both species, showing a clear pattern of association between SLA and $L_{th}$ (Fig. 2.2A).
Table 2.6. Foliage characteristics of beech and oak seedlings grown under four percentages of PAR. Data are the means ± standard errors. Where species x treatment interaction is significant, combination means followed by the same letter are not significantly different (n = 5 reps). Where no interaction, species (n = 10 reps) or treatment (n = 5 reps) means followed by the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Light (PAR)</th>
<th>Beech</th>
<th>Oak</th>
<th>Treatment mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>22.01 ± 1.49 bc</td>
<td>22.82 ± 1.49 bc</td>
<td>22.41 ± 1.26</td>
</tr>
<tr>
<td>Leaf size (cm²)</td>
<td>62%</td>
<td>20.74 ± 1.49 bc</td>
<td>22.89 ± 1.49 bc</td>
<td>21.82 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>21.27 ± 1.49 bc</td>
<td>26.48 ± 1.49 ab</td>
<td>23.88 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>19.02 ± 1.49 c</td>
<td>31.06 ± 1.49 a</td>
<td>25.04 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>20.76 ± 0.63</td>
<td>25.81 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>Leaf mass (g)</td>
<td>100%</td>
<td>0.14 ± 0.01 ab</td>
<td>0.18 ± 0.01 a</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>0.12 ± 0.01 bc</td>
<td>0.15 ± 0.01 ab</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>0.11 ± 0.01 bc</td>
<td>0.17 ± 0.01 a</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>0.08 ± 0.01 c</td>
<td>0.17 ± 0.01 a</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>0.11 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>SLA (cm² g⁻¹)</td>
<td>100%</td>
<td>161.48 ± 8.76</td>
<td>132.89 ± 8.76</td>
<td>147.18 ± 6.61</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>191.14 ± 8.76</td>
<td>151.32 ± 8.76</td>
<td>171.23 ± 6.61 b</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>208.01 ± 8.76</td>
<td>158.17 ± 8.76</td>
<td>183.09 ± 6.61 b</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>231.26 ± 8.76</td>
<td>186.02 ± 8.76</td>
<td>208.64 ± 6.61 a</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>197.97 ± 5.05</td>
<td>157.10 ± 5.05</td>
<td></td>
</tr>
<tr>
<td>Leaf thickness (µm)</td>
<td>100%</td>
<td>148.8 ± 5.6</td>
<td>189.0 ± 5.6</td>
<td>168.9 ± 4.5 a</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>125.6 ± 5.6</td>
<td>165.7 ± 5.6</td>
<td>145.7 ± 4.5 b</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>112.0 ± 5.6</td>
<td>160.8 ± 5.6</td>
<td>136.4 ± 4.5 b</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>102.3 ± 5.6</td>
<td>139.2 ± 5.6</td>
<td>120.8 ± 4.5 c</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>122.2 ± 3.8 a</td>
<td>163.7 ± 3.8 b</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2.2. Correlations between specific leaf area (SLA) and leaf thickness (Lth) (A); aerial biomass and root biomass (B); total biomass and final diameter (C), and final height (D). Solid triangles and continuous lines indicate beech seedlings; open circles and dotted lines indicate oak seedlings.
Root mass was significantly influenced by light treatments, with seedlings at full sunlight having longer mean values than seedlings in the heavy shade (Table 2.5; Table 2.7). Beech seedlings allocated significantly greater biomass to branches than did oak seedlings (Table 2.5; Table 2.7). There was a significant interaction of species and light for stem, aerial and total biomass, indicating different responses to light treatments between species (Table 2.5). Stem, aerial and total biomass was significantly greater at full sunlight than heavy shade in beech seedlings, while no significant differences between treatments were found in oak seedlings (Table 2.7). The root:shoot ratio was significantly greater for seedlings grown in full sunlight and was higher in oak than in beech (Table 2.7). The length gained per unit of mass invested (calculated as the main stem length divided by stem dry weight, Ht/StemMass) increased with increasing shade (except in 51% of PAR), although the differences were only significant between the two extreme treatments, and was greater, but not significantly different, in oak than in beech (Table 2.5; Table 2.7). Shoot growth correlated strongly with root growth (Fig. 2.2B). Diameter and height at the end of the study were significantly and positively correlated with total biomass, but diameter was more strongly correlated with total biomass (Fig. 2.2C and 2.2D).

2.3.4 Morphological plasticity

Morphological plasticity in response to light diverged among species and variables studied (Table 2.8). The mean plasticity index for all variables was 31% higher in beech than oak.
Table 2.7. Biomass characteristics of beech and oak seedlings grown under four percentages of PAR. Data are the means ± standard errors. Where species x treatment interaction is significant, combination means followed by the same letter are not significantly different (n = 5 reps). Where no interaction, species (n = 10 reps) or treatment (n = 5 reps) means followed by the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Light (PAR)</th>
<th>Beech</th>
<th>Oak</th>
<th>Treatment mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root mass (g)</td>
<td>100%</td>
<td>322.95 ± 33.58</td>
<td>197.59 ± 33.58</td>
<td>260.27 ± 25.16 a</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>176.17 ± 33.58</td>
<td>167.1 ± 33.58</td>
<td>171.64 ± 25.16 ab</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>144.42 ± 33.58</td>
<td>225.12 ± 33.58</td>
<td>184.77 ± 25.16 ab</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>99.96 ± 33.58</td>
<td>117.22 ± 33.58</td>
<td>108.59 ± 25.16 b</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>185.87 ± 12.42 a</td>
<td>176.76 ± 12.42 a</td>
<td>200.30 ± 27.93 a</td>
</tr>
<tr>
<td>Branch mass (g)</td>
<td>100%</td>
<td>293.31 ± 36.99</td>
<td>107.29 ± 36.99</td>
<td>203.93 ± 13.6</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>215.91 ± 36.99</td>
<td>124.99 ± 36.99</td>
<td>203.93 ± 13.6</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>197.19 ± 36.99</td>
<td>183.82 ± 36.99</td>
<td>203.93 ± 13.6</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>194.90 ± 36.99</td>
<td>218.11 ± 36.99</td>
<td>203.93 ± 13.6</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>187.83 ± 13.6</td>
<td>185.24 ± 13.6</td>
<td>190.50 ± 27.93 a</td>
</tr>
<tr>
<td>Stem mass (g)</td>
<td>100%</td>
<td>475.69 ± 54.94 a</td>
<td>294.99 ± 54.94 ab</td>
<td>312.05 ± 18.97</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>306.72 ± 56.14 ab</td>
<td>254.58 ± 54.94 ab</td>
<td>312.05 ± 18.97</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>270.89 ± 54.94 ab</td>
<td>379.16 ± 54.94 ab</td>
<td>312.05 ± 18.97</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>194.90 ± 54.94 b</td>
<td>218.11 ± 54.94 ab</td>
<td>312.05 ± 18.97</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>310.87 ± 31.39</td>
<td>286.71 ± 31.39</td>
<td>325.02 ± 41.32</td>
</tr>
<tr>
<td>Aerial biomass (g)</td>
<td>100%</td>
<td>768.99 ± 88.39 a</td>
<td>402.29 ± 80.48 ab</td>
<td>510.87 ± 31.39</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>502.21 ± 90.67 ab</td>
<td>379.56 ± 80.48 ab</td>
<td>510.87 ± 31.39</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>468.08 ± 80.48 ab</td>
<td>562.98 ± 80.48 ab</td>
<td>510.87 ± 31.39</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>304.19 ± 80.48 b</td>
<td>321.38 ± 80.48 b</td>
<td>510.87 ± 31.39</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>510.87 ± 31.39</td>
<td>416.55 ± 31.39</td>
<td>510.87 ± 31.39</td>
</tr>
<tr>
<td>Total biomass (g)</td>
<td>100%</td>
<td>1091.94 ± 119.24 a</td>
<td>599.88 ± 119.24 b</td>
<td>691.41 ± 42.68</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>657.05 ± 122.37 ab</td>
<td>546.66 ± 119.24 ab</td>
<td>691.41 ± 42.68</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>612.49 ± 119.24 ab</td>
<td>788.10 ± 119.24 ab</td>
<td>691.41 ± 42.68</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>404.15 ± 119.24 b</td>
<td>438.60 ± 119.24 b</td>
<td>691.41 ± 42.68</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>691.41 ± 42.68</td>
<td>593.31 ± 42.68</td>
<td>691.41 ± 42.68</td>
</tr>
<tr>
<td>Ht / StemMass (cm g⁻¹)</td>
<td>100%</td>
<td>0.67 ± 0.22</td>
<td>0.91 ± 0.22</td>
<td>0.79 ± 0.16 a</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>0.79 ± 0.23</td>
<td>1.41 ± 0.22</td>
<td>1.10 ± 0.16 ab</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>0.89 ± 0.22</td>
<td>1.10 ± 0.22</td>
<td>0.99 ± 0.16 ab</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>1.13 ± 0.22</td>
<td>1.43 ± 0.22</td>
<td>1.28 ± 0.16 b</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>0.87 ± 0.10 a</td>
<td>1.21 ± 0.10 a</td>
<td>0.87 ± 0.10 a</td>
</tr>
<tr>
<td>Root:shoot ratio</td>
<td>100%</td>
<td>0.43 ± 0.03</td>
<td>0.55 ± 0.03</td>
<td>0.49 ± 0.02 a</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>0.32 ± 0.03</td>
<td>0.48 ± 0.03</td>
<td>0.40 ± 0.02 b</td>
</tr>
<tr>
<td></td>
<td>51%</td>
<td>0.35 ± 0.03</td>
<td>0.45 ± 0.03</td>
<td>0.40 ± 0.02 b</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>0.34 ± 0.03</td>
<td>0.44 ± 0.03</td>
<td>0.39 ± 0.02 b</td>
</tr>
<tr>
<td></td>
<td>Sp mean</td>
<td>0.36 ± 0.02 a</td>
<td>0.48 ± 0.02 b</td>
<td>0.36 ± 0.02 a</td>
</tr>
</tbody>
</table>
Table 2.8. Plasticity index in response to different light levels of beech and oak seedlings for the variable studied during the growing season of 2014.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasticity index</th>
<th>∆Beech-oak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beech</td>
<td>Oak</td>
</tr>
<tr>
<td>Leaf size</td>
<td>0.14</td>
<td>0.27</td>
</tr>
<tr>
<td>Leaf mass</td>
<td>0.43</td>
<td>0.17</td>
</tr>
<tr>
<td>Root mass</td>
<td>0.69</td>
<td>0.48</td>
</tr>
<tr>
<td>Branch mass</td>
<td>0.63</td>
<td>0.44</td>
</tr>
<tr>
<td>Stem mass</td>
<td>0.59</td>
<td>0.42</td>
</tr>
<tr>
<td>Root:shoot ratio</td>
<td>0.26</td>
<td>0.20</td>
</tr>
<tr>
<td>SLA</td>
<td>0.30</td>
<td>0.29</td>
</tr>
<tr>
<td>L_{th}</td>
<td>0.31</td>
<td>0.26</td>
</tr>
<tr>
<td>∆Ht</td>
<td>0.49</td>
<td>0.20</td>
</tr>
<tr>
<td>∆Dia</td>
<td>0.60</td>
<td>0.49</td>
</tr>
<tr>
<td>H:D</td>
<td>0.17</td>
<td>0.28</td>
</tr>
<tr>
<td>Ht / Stem Mass</td>
<td>0.41</td>
<td>0.36</td>
</tr>
<tr>
<td>Mean</td>
<td>0.42</td>
<td>0.32</td>
</tr>
</tbody>
</table>

2.4 Discussion

In this study survival rates of oak and beech were not affected by shade and both species had low mortality over the two growing seasons. Greater mortality in the less shade-tolerant species (oak) was expected in the heavy shade as lower survival rates than those of shade-tolerant species have been reported for these species in previous studies in controlled (Walters and Reich, 1996) and natural conditions (Pacala et al., 1994; Gemmel et al., 1996; Chen, 1997; Kaelke et al., 2001). However, Paquette et al. (2006) reported that survival of underplanted temperate deciduous species was not affected by overstory density.

Annual height growth increased with increasing shade level during the first growing season, but it decreased during the second growing season. Similar to the trend found during the second growing season, several studies have reported a reduction in height growth with increasing shade (King, 1994; Chen, 1997). Čater et al. (2012) also reported greater height increment with increasing light availability for beech seedlings underplanted below Norway spruce (Picea abies L. (Karst.)) canopy. The greater height growth under shade found during the first growing season in this study may suggest that the expected decline in height increment with shade may be time dependent (Kennedy et al., 2007). The fact that the seedlings were grown at full sunlight for a number of growing seasons before being exposed to shade may have delayed the response to treatment, as
height growth might be more affected by previous than by current light conditions (Welander and Ottosson, 1997, 1998). Height increment was significantly different between species only at full sunlight, with beech having greater height increment than oak over the two seasons. Diameter growth decreased with increasing shade and was greater in oak than beech. A decline in diameter increment with increasing shade has been widely reported for beech and oak seedlings growing in natural (Gemmel et al., 1996; Löf, 2000; Einhorn et al., 2004; Balandier et al., 2007; Ni Dhubháin, 2010) and controlled conditions (Ammer, 2003). Löf et al. (2007) found the same trend in diameter growth for oak but they did not find an additional growth response at higher light levels for beech. In contrast to the results from this study, Van Hees and Clercx (2003) found that shading levels of 30% of full sunlight had no effect on root collar diameter in oak and beech seedlings.

The H:D ratios of beech and oak during both years of the study were higher in all shade levels than at full sunlight, with no differences between species. The fact that H:D ratios were greater with increasing shade suggests that seedlings under shade prioritised the allocation of biomass to leader height growth at the expense of diameter growth. Prévosto and Balandier (2007) reported similar results for beech seedlings growing under strong competition and low light availability. This trend is also confirmed by the greater height growth per unit of stem biomass under the heavy shade observed in the current study, as found by Einhorn et al. (2004).

As expected, shoot elongation was greater in June and lower in August in beech than oak seedlings. In the early years, oak seedlings usually experience two periods of shoot growth (the initial elongation in May and June, and the lammas growth in July and August), while lammas growth in beech is much less common, with elongation taking place mainly in May and June (Evans, 1984).

The reduced seedling dry mass under decreasing light quantity found in the current study is consistent with previous studies on the effect of shading on biomass production on beech, oak and other species (Loach, 1970; Welander and Ottosson, 1998; Ammer, 2003; Einhorn et al., 2004; Kennedy et al., 2007; Gardiner et al., 2009; Čater and Simončič, 2010; Brown et al., 2014). The only exception to that in the present study was for oak under 51% of PAR (medium
shade), where seedling biomass was greater than in all the other treatments. As found by Ammer (2003), branch dry mass was greater in beech than oak, which might suggest a better ability of oak to prune naturally. In contrast, root mass or total biomass did not differ significantly between species in this study. Plants with higher root:shoot ratios can compete more effectively for soil nutrients, while those with lower root:shoot ratios can collect more light energy (Allaby, 1998). Kitajima (1994) reported that shade-intolerant species had lower root:shoot ratios. Although the root:shoot ratios in this study did not follow that pattern, they were consistent with findings on beech and oak in some previous studies (Welander and Ottosson, 1998; Valladares et al., 2002; Ammer, 2003). Shading generally reduced root biomass more than aerial biomass, resulting in lower root:shoot ratios under shade than at full sunlight in this study. Many studies have found a reduction in root:shoot ratios with increasing shade (Welander and Ottosson, 1998; Valladares et al., 2002; Ammer, 2003; Van Hees and Clercx, 2003; Kennedy et al., 2007; Čater and Simončič, 2010).

SLA increased as shade levels increased, which is a common response of plants to shade that has been well documented in beech and oak (Van Hees, 1997; Aranda et al., 2001; Valladares et al., 2002; Curt et al., 2005; Kunstler et al., 2005; Gardiner et al., 2009; Goisser et al., 2013). Similarly, Lth decreased with increasing shade, which has also been reported in other studies (St-Jacques et al., 1991; Ashton and Berlyn, 1994; Valladares et al., 2002). The acclimation of plants to shade results in larger and/or thinner leaves, as shown by the frequently reported higher SLA in shaded leaves (Abrams and Kubiske, 1990; Abrams and Mostoller, 1995). Thinner leaves typically capture more light per unit area than thicker leaves and distribute nitrogen, which plays an important role in healthy growth, over a larger leaf area optimising the light harvesting (Niinemets, 1997). The low SLA values at full sunlight was associated with greater growth increment, which is in agreement with a previous study on beech seedlings (Curt et al., 2005). Rebbeck et al. (2012) also found greater SLA in foliage of northern red oak (Quercus rubra L.) and white oak (Quercus alba L.) when grown in low light but they found the opposite response in chestnut oak (Quercus prinus L.). Similar to our findings, Špulák (2011) found that beech seedlings planted under a young spruce (Picea sp.) stand had significantly greater SLA than seedlings found in a
nearby gap. Shade-tolerant beech seedlings had greater SLA than less shade-tolerant oak seedlings (Table 2.6), a similar trend to that reported in previous studies of beech and oak seedlings (Valladares et al., 2002; Gardiner et al., 2009) and in other species differing in shade tolerance (Kitajima, 1994; Niinemets and Kull, 1994). The results of this study showed that the increase of SLA with increasing shade was associated with lighter leaves in beech (leaf mass decreased as shade levels increased) and larger leaves in oak (leaf size increased as shade levels increased). Similarly, oak species maximised their light interception by increasing their leaf area in response to increasing shade levels (Callaway, 1992; Gardiner and Krauss, 2001). The strong correlation between $L_{th}$ and SLA might suggest that $L_{th}$ could be as useful as SLA as an indicator of plant light-use strategy.

Beech seedlings showed a greater morphological plasticity than oak, although it was not as great as that reported by Valladares et al. (2002). Kunstler et al. (2005) also observed that beech exhibited higher morphological plasticity than less shade-tolerant downy oak (Quercus pubescens Willd.) as a function of light. In contrast, Van Hees (1997) found a similar morphological plasticity between oak and beech. The seedlings in the study of Van Hees (1997) were younger than those used in this study, so differences in the effect of shading on growth might increase as the plants age. The results reported herein are in agreement with Canham (1988), who suggested that shade-tolerant species generally show greater morphological plasticity than less tolerant ones.

In addition to light availability, other environmental factors affect tree growth; such as water availability, light quality, nutrient levels and temperature. While water stress may not seem to be a major issue in Ireland due to the high and frequent rainfall, exposure can increase moisture stress despite the availability of water in the soil and is believed to be the main cause of poor field performance of broadleaved species newly planted in open fields. Frost damage is also an impediment in establishing some broadleaf species in open fields. Therefore, underplanting in an existing stand may be a good practice as the existing canopy will provide shelter for underplanted seedlings (Paquette et al., 2006; Dey et al., 2012; Hawe and Short, 2012). The nets used in this study did not modify light
quality in the same way than a forest canopy might do, and the possible effects of other environmental factors should also be taken into consideration.

Light availability affected the growth of beech and oak seedlings in this study, with growth decreasing as shade increased, but it did not affect seedling survival. Therefore, low growth rates might be expected for seedlings underplanted into shade conditions, such as stands where silvicultural treatments different from clearfelling or heavy thinning are applied. The above findings suggest that oak seedlings would perform well under light conditions as low as 28% of PAR, acclimating to shade as well as beech seedlings. Differences in the responses in the intermediate treatments were small for most parameters in this study, probably because PAR did not differ sufficiently to elicit strong responses. Although the best growth and biomass accumulation in both species were found at full sunlight, beech and oak seedlings would be able to acclimate both morphologically and physiologically to allow them to survive and grow well under alternative systems to clearfelling, such as shelterwood systems, or a wide range of thinning intensities. Although both species can tolerate shade levels as low as 28% of full sunlight, these species might also respond favorably to canopy openings, as suggested by others (Lüpke, 1998; Collet et al., 2001; Coll et al., 2003; Curt et al., 2005).

2.5 Conclusions
The results from this study confirm that light levels strongly affect seedling growth and morphology. The survival rates of beech and oak seedlings were not influenced by light availability and were greater than 90% during two growing seasons, regardless of the shade level applied. A decrease in light availability reduced diameter increment in beech and oak seedlings during both years of the study. Height increment increased as the level of shade was increased during the first growing season, but the opposite was found for the second growing season. Both species exhibited morphological acclimation to increasing levels of shade, such as by increasing SLA and H:D ratios and decreasing leaf thickness and root:shoot ratio. The acclimation of leaves to shade would increase the seedling ability to intercept light, while the changes in H:D and root:shoot ratios suggest that plants allocate more biomass to the above-ground than below-ground parts in response to shade. Based on these findings, both species may be suitable for
underplanting under a wide range of shade levels (from light to heavy shade), although they showed a reduction in growth and biomass as the shade level increased.
2.6 References


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different light and vegetation composition conditions. *Annals of Forest Science* 60, 593–600.


Chapter 3
The impact of shade on photosynthetic characteristics in *Fagus sylvatica* and *Quercus robur* seedlings

Abstract

Efforts to use alternative silvicultural systems to clearfelling should be underpinned by a better understanding of how tree species develop and acclimate when planted under forest canopies. To this end, the physiological responses of European beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.) to different light intensities, from full light to heavy shade, were investigated over two growing seasons in a shadehouse experiment. The different shade conditions were intended to mimic a range of underplanting conditions (62%, 51% and 28% of full light). The shade treatments resulted in lower photosynthetic capacity, with beech seedlings exhibiting the greatest shade-induced reduction in photosynthetic rates at saturating light in the first growing season. There were no differences between species in their physiological response to shade in the second growing season. Efficiency of PSII generally increased with increasing shade. Despite the greater photosynthetic performance at full light, results suggest that canopy conditions that reduce available light to 28% of sunlight might still provide enough light for the introduction of beech and oak seedlings through underplanting.

3.1 Introduction

Continuous cover forestry (CCF) is increasing as an alternative to clearfelling systems and uses the control of light through thinning, which can have a large impact on the development of broadleaf forests, to produce stand benefits (Han, 2012). CCF includes those silvicultural systems which involve continuous and uninterrupted maintenance of forest cover and avoid clearcutting (Pommerening and Murphy, 2004). To date there has been little research on the functional basis of the broadleaf tree responses to light, as most research has focussed on the growth responses. The increase in broadleaf planting, the fact that some plantations have reached the stage where thinning interventions are required, and the increased interest in CCF, have heightened the demand for research information on the response of tree seedlings to the light environment. Such
information may lead to the development of better management and silvicultural intervention strategies to optimise the value of forests. Although natural regeneration is generally preferred for several reasons, planting under existing stands (underplanting) can be a useful practice when 1) species are failing to regenerate naturally (Dey et al., 2012), 2) the objective is to improve the productive capacity and rehabilitate poorly performing stands (Hawe and Short, 2012), 3) diversity in species, age and canopy structure is required (Hart, 1995). Underplanting can also be used in conjunction with different thinning intensities to encourage early growth or when species need open sites/gaps for establishment.

Light is one of the main environmental factors affecting stand development, so shade tolerance has an important influence on tree physiology and plays an important role in plant competition. Tree species have different capacities for light use. Shade tolerance is an ecological concept referring to the capacity of a plant to tolerate low light levels. The establishment of gaps within a forest stand results in increased availability of resources, such as light, nutrients and water (Canham et al., 1990; Aussenac, 2000; Han, 2012). Although light is required for photosynthesis, both high and low sunlight can limit plant performance (Valladares and Niinemets, 2008). Acclimation to different light environments has been associated with changes in the anatomy, morphology and physiology of plant leaves (Kamaluddin and Grace, 1993; Kloeppe, 1993). Photosynthetically active radiation (PAR) is the spectrum of light available for photosynthesis (between 380nm and 710nm), and is used as a measure of light quantity. Red/Far-Red ratio (R/FR) is the ratio between transmitted light in the red band (660 nm) and far-red band (730 nm), and gives information about the aspect of light quality that can directly induce changes in plant development (Hertel et al., 2011). Changes in PAR can have important effects on plant growth processes mediated by variations in photosynthesis, while changes in R/FR can trigger low-energy switches that alter development and morphogenesis (Ballaré et al., 1990; Christophe et al., 2006).

Stand density, thinning intensity, site quality, tree species age and vigour affect the duration, magnitude and occurrence of photosynthetic acclimation to increasing light intensity (Han, 2012). Possible differences in the relative shade tolerance of species can be approached by comparing the photosynthetic light
responses between species. Response curves of photosynthesis over a range of light intensities (light-response curve) have been widely used to study differences between shade and sun-grown plants to light intensity (Givnish, 1988; Kozlowski and Pallardy, 1997). Light-response curves provide useful information about the photosynthetic properties of leaves, such as dark respiration, light compensation point and light saturation point (Taiz and Zeiger, 2002).

Phenotypic plasticity is the ability of an organism to alter its physiology/morphology in response to changes in environmental conditions (Schlichting, 1986). The understanding of phenotypic plasticity can be crucial for predicting changes in species distribution, community composition and crop productivity under different environmental conditions (Ackerly et al., 2000; Gratani, 2014). However, phenotypic plasticity is a complex phenomenon and somewhat controversial. While light-demanding species may exhibit greater photosynthetic plasticity than shade tolerant species (Strauss-DeBenedetti and Bazzaz, 1991, 1996; Muth and Bazzaz, 2002; Valladares et al., 2002; Longuetaud et al., 2013), there is also evidence that phenotypic plasticity does not necessarily favour light-demanding early successional species (Turnbull, 1991).

European beech (Fagus sylvatica L.) and pedunculate oak (Quercus robur L.) are two important broadleaf trees in Europe, that are not only of great ecological importance, but also of considerable economic value for the wood processing industry. The two species differ in shade tolerance: oak is considered to be less shade-tolerant than beech (Hill et al., 1999, Valladares et al., 2002). The objective of this study was to gain a better knowledge about the physiological acclimation and plasticity of these two broadleaf species when planted under different shade conditions. The different shade conditions were intended to mimic a range of underplanting conditions. The results were expected to provide information on the ecology and physiological adaptation of underplanted oak and beech seedlings.

3.2 Materials and methods

3.2.1 Study site and tree species
The study was conducted in a controlled-shade experiment located at Teagasc Ashtown Food Research Centre, D15 DYO5, Ireland (53°22'45" N,
6°20'13" W, 40 m ASL). Two year-old (1u1) pedunculate oak and three year-old (1u1u1) European beech seedlings supplied by Coillte Nursery, Ardattin, Co. Carlow (Ireland) were planted at Teagasc Food Research Centre in March 2011. Because 1u1 beech seedlings of similar size to the oak seedlings (50-80 cm) were not available, 1u1u1 beech seedlings were used instead. The mean (± SE) seedling heights at time of planting were 61.1 ± 0.5 cm for F. sylvatica and 75 ± 0.6 cm for Q. robur. The mean (± SE) stem diameters at time of planting were 8.7 ± 0.1 mm for F. sylvatica and 7.3 ± 0.1 mm for Q. robur.

Seedling provenances used were according to provenance recommendations in Ireland (COFORD, 2002): beech provenance was Cirencester Region 404, United Kingdom, origin unknown (51°43'0" N, 2°0'0" W, 140 m above sea level), and oak provenance was NL.S. Nuenen 03, Netherlands, origin unknown (51°29'9" N, 5°32'9" E, 20 m above sea level). The experimental area was fenced to exclude rabbits and hares. Weeding was carried out when required. The mean annual total rainfall in the region is 774 mm and the mean annual air temperature is 9.8 °C (all means are from the period 1981-2010). The weather conditions from 2011 to 2014, the period when this study was conducted, were similar with respect to temperature but differed in rainfall during the growing season (Table 3.1). The climatic data were collected by an Automatic Weather Station (Met Éireann, Phoenix Park station) located 1.93 km away at an open site.

Table 3.1. Temperature (°C) and rainfall (mm) during the years of the study. Growing season was calculated considering the period from April to October.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2011</td>
</tr>
<tr>
<td>Temperature</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Growing season</td>
</tr>
<tr>
<td>Rainfall</td>
<td>Annual</td>
</tr>
<tr>
<td></td>
<td>Growing season</td>
</tr>
</tbody>
</table>

3.2.2 Experimental design and shade treatments

The area was divided into five blocks, each with four randomly assigned plots, each randomly split into two subplots (split plot design). Light was the whole plot
factor and species was the subplot factor, replicated across five blocks. This resulted in twenty plots (11 m long, 4.3 m wide and 2.9 m high, including the shading nets), each containing two subplots. Each subplot contained 42 seedlings per species planted at 0.5 m spacing. The subplots (species) were planted 1 m apart. This arrangement encouraged interplant competition within rows, but minimised competition between rows. The subplot measurement area, located in the centre of each subplot, entailed 16 seedlings per species and was surrounded by a buffer line, with an additional line of plants towards the front.

Green polythene shade nets (Colm Warren Polyhouses Ltd., Kilmurray, Trim, Co. Meath, Ireland) were erected on frames to simulate different light environments (representing a spectrum of thinning intensities) in September 2012, about one and a half years after the seedlings were planted. Four different light treatments were established in each block (one treatment per plot): full sunlight, light shade, medium shade and heavy shade. The proportion of PAR below the nets was calculated as the difference between readings taken simultaneously with a data logger, LI-1400 (LI-COR Inc., Lincoln, Nebraska), using a LI-190SA Quantum Sensor (LI-COR Inc., Lincoln, Nebraska) outside the plot and a LI-191SA Line Quantum Sensor (LI-COR Inc., Lincoln, Nebraska) inside the plot in October 2013. LI-COR quantum sensors monitored PAR in the 400 to 700 nm waveband. Soil water content (SWC, %) was measured in each plot to determine the amount of rainfall interception, which was carried out in January 2014. Measurements were carried out in the corners and centre of the plot with a WET sensor and a moisture meter that allowed SWC measurement at a depth of 68 mm (Delta-T Devices Ltd, Cambridge, UK). Red/far-red ratio (R/FR) was measured in March 2014 with a Skye SKR 110 sensor connected to a display meter (Skye Instruments, Powys, UK) that reports quantum flux at 660 and 730 nm. In each light treatment of the first block, air temperature and relative humidity were recorded every 10 min from 26 May to 8 October during 2015 using dataloggers (SF-LOG-M, Solfranc Tecnologias SL, Tarragona, Spain) with shelter to prevent direct solar radiation and rainfall. Temperature and humidity loggers were located in the middle of each oak subplot (after checking there were no differences between oak and beech subplots), ≈70cm above-ground. The different light treatments averaged 100%, 62%, 51% and 28% of PAR,
respectively. A description of the conditions in the different treatments is shown in Table 3.2. The shadehouses had little effect on R/FR ratio, as this ratio inside and outside the shadehouses was similar in the two intermediate treatments, and slightly lowered in the heavy shade treatment (Table 3.2). The rainfall interception in the soil decreased with increasing shade (Table 3.2). Air temperature and relative humidity did not differ between the different light environments (Table 3.2).

Table 3.2. Light properties, soil water content (SWC), air temperature (T) and relative humidity (RH) in the different shade environments. Data are the means ± standard errors.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAR (%)</th>
<th>R/FR</th>
<th>SWC (%)</th>
<th>T (°C)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (full sunlight)</td>
<td>100</td>
<td>1.00</td>
<td>47.7 ± 0.8</td>
<td>13.6</td>
<td>82.2 ± 0.1</td>
</tr>
<tr>
<td>Light shade</td>
<td>62</td>
<td>0.98</td>
<td>46.0 ± 0.9</td>
<td>13.6</td>
<td>82.4 ± 0.1</td>
</tr>
<tr>
<td>Medium shade</td>
<td>51</td>
<td>0.98</td>
<td>44.3 ± 0.9</td>
<td>13.7</td>
<td>81.1 ± 0.1</td>
</tr>
<tr>
<td>Heavy shade</td>
<td>28</td>
<td>0.92</td>
<td>39.8 ± 1.0</td>
<td>13.5</td>
<td>81.3 ± 0.1</td>
</tr>
</tbody>
</table>

3.2.3 Physiological measurements

Physiological measurements were carried out on oak and beech seedlings from the measurement area during the summer in 2013 and 2014. Gas exchange and chlorophyll fluorescence measurements, expressed on a leaf area basis, were conducted with a portable photosynthesis system LI-6400XT in conjunction with an integrated LI6400-40 fluorescence chamber head (LI-COR Inc., Lincoln, Nebraska, USA). Three seedlings per species and treatment were randomly selected and used for physiological measurements. All measurements were carried out on fully expanded, non-senescent and healthy leaves from the upper terminal shoot of each seedling. Physiological responses were recorded in all treatments in 2014, while only the full sunlight and heavy shade treatments were assessed in 2013.

3.2.3.1 Photosynthetic light-response curves

Photosynthetic light-response curves were generated on a randomly selected subset of seedlings to determine the impact of the light treatments on the photosynthetic response. Because light-response curves are time intensive, light-response curves with irradiance expressed as PAR (μmol m$^{-2}$ s$^{-1}$) were only
measured on three seedlings per species chosen at random from the measurement area in the extreme treatments without replications (100 % and 28 % of PAR, Block 1). Light-response curves were measured in the first week of August 2013 according to protocol recommended by LI-COR (LI-COR, Inc. 2011) and other sources (Gauthier and Jacobs, 2010, Kuptz et al., 2010, Legner et al., 2013). Light-response curves at 25 °C (block temperature), 400 μmol mol⁻¹ (reference CO₂) and around 50 % humidity were established in nine steps: 2000, 1500, 1000, 500, 200, 100, 50, 20 and 0 μmol m⁻² s⁻¹ PAR, with each light level provided by the light source of the chamber head and maintained for 3 minutes. Light-response curves were fitted using the Solver function of Microsoft Excel 2010. Following Lobo et al. (2013), the most common mathematical models employed to describe photosynthetic light-response curves were used. The model which presented the lowest value of the sum of the squares of the errors was chosen. Parameters obtained from the light curves were dark respiration (Rd, μmol CO₂ m⁻² s⁻¹), light compensation point (Ic, μmol photons m⁻² s⁻¹), quantum yield (ΦCO₂, μmol CO₂ (μmol photons)⁻¹), light saturation point beyond which there is no significant change in photosynthetic rate (Imax, μmol photons m⁻² s⁻¹) and maximum photosynthesis obtained at Imax (Amax, μmol CO₂ m⁻² s⁻¹). Rd was derived from the light curve at a PAR of 0 μmol m⁻² s⁻¹, light level at which there is no photosynthetic carbon assimilation and therefore net CO₂ flux is negative because of mitochondrial respiration. Ic is the value of PAR at which the CO₂ assimilated by photosynthesis is in balance with the CO₂ produced by respiration, determined at the intersection of the fitted light curve with the light axis (x-axis). ΦCO₂ is a measure of photosynthetic efficiency and was calculated as the slope of the near-linear portion of the response curve between PAR levels of Ic and 200 μmol m⁻² s⁻¹. The PAR value at which differences in photosynthesis among species and treatments became significant was also determined from the light curves.

3.2.3.2 Leaf gas exchange

Leaves of selected seedlings were exposed to 1500 μmol m⁻² s⁻¹ (Imax), 500 μmol m⁻² s⁻¹ (light value at which differences in photosynthesis became apparent) and 0 μmol m⁻² s⁻¹ from the light source of the chamber head, and the values of photosynthetic CO₂ assimilation (A, μmol CO₂ m⁻² s⁻¹) were recorded to test
whether the responses observed in the light curves were consistent over all blocks. Stomatal conductance \( (g_s, \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}) \) and transpiration rate \( (E, \text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}) \) were also measured. Measurements were done in the last week of August \( (\text{PAR} = 1500 \mu\text{mol m}^{-2}\text{s}^{-1}) \) and first week of September \( (\text{PAR} = 0 \text{ and } 500 \mu\text{mol m}^{-2}\text{s}^{-1}) \). While conducting these measurements during the two years of the study, the reference CO\(_2\) concentration, temperature and humidity were kept at the same values as for the light curve measurements. Preliminary measurements determined that 150 seconds was an adequate equilibration time.

Photosynthetic rate, stomatal conductance and transpiration rate were also measured under ambient conditions \( (\text{amb}) \) of air temperature, humidity and radiation, with the reference CO\(_2\) concentration maintained at 400 \( \mu\text{mol mol}^{-1} \). Measurements were made during the second week of August.

The ratio of \( A \) to \( E \) and \( A \) to \( g_s \) were calculated to determine instantaneous \( (A/E, \mu\text{mol CO}_2/\text{mmol H}_2\text{O}) \) and intrinsic \( (A/g_s, \mu\text{mol CO}_2/\text{mol H}_2\text{O}) \) water use efficiency, respectively. \( A/g_s \) is not influenced by vapour pressure deficit (VPD, the force which drives transpiration rate) and therefore represents a more consistent estimate of the relative water use efficiency than \( A/E \) (Meinzer et al., 1990), and is used in comparative studies with different evaporative demands (Tambussi et al., 2007).

### 3.2.3.3 Chlorophyll fluorescence

Chlorophyll fluorescence was simultaneously assessed over the same leaf area when measuring leaf gas exchange under ambient conditions. Fluorescence parameters were estimated according to common protocols for fluorescence analysis at a known light intensity (Murchie and Lawson, 2013). A number of fluorescence parameters were derived from the measurements:

1) PSII (photosystem II) operating efficiency \( (\Phi_{\text{PSII}} = F'_q/F'_m = [F'_m - F']/F'_m; \) where \( F' \) is the steady-state level of fluorescence in the light, and \( F'_m \) is the maximal fluorescence under ambient light conditions). It is the quantum efficiency of PSII electron transport in the light, and estimates the efficiency at which light absorbed by PSII antennae is used for photochemistry.
2) PSII maximum efficiency \( (F'_v/F'_m = (F'_m - F'_o)/F'_m) \); where \( F'_v \) is the variable fluorescence of a light-adapted leaf defined as \( (F'_m - F'_o) \), and \( F'_o \) is the minimal fluorescence of a light-adapted leaf that has momentarily been darkened). It provides an estimate of the maximum efficiency of PSII photochemistry at a given light intensity, which is the PSII operating efficiency if all the PSII centres were open.

3) Photochemical quenching \( (F'_v/F'_v = qP = (F'_m - F)/(F'_m - F'_o)) \). It is also known as PSII efficiency factor, and relates the PSII operating efficiency to the PSII maximum efficiency.

4) Electron transport rate (ETR, \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \)) was calculated as \( [(F'_m - F)/F'_m] \times f \times l \times \alpha_{\text{leaf}} \); where \( f \) is the fraction of absorbed quanta that is used by PSII, which was assumed to be 0.5 (Laish and Loreto, 1996); \( l \) is the incident photon flux density; and \( \alpha_{\text{leaf}} \) is leaf absorptance (Maxwell and Johnson, 2000). A standard leaf absorptance value of 0.84 was used (Björkman and Demmig, 1987), since the true leaf absorptance could not be measured.

Chlorophyll content was determined on the same seedlings used for the gas exchange and chlorophyll fluorescence measurements during the first week of September 2014 with a chlorophyll content meter (CCM-300, Opti-sciences, Inc., Hudson, USA).

3.2.4 Plasticity index

A plasticity index ranging from 0 to 1 was calculated for each physiology variable and species as the difference between the minimum and the maximum mean values between the different shade treatments divided by the maximum mean value (Valladares et al., 2000a; Valladares et al., 2000b; Valladares et al., 2002). This index allows changes in variables expressed in different units to be compared. Mean physiological plasticity was calculated for each species by averaging the plasticity indices obtained for all physiological traits.
3.2.5 Data analysis

All statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Physiological responses were analysed using the MIXED procedure of SAS. Fixed effects were shading treatment, species and their interaction. Random effects were block and block x treatment interaction, the latter one to account for the split plot structure. For those parameters measured for the two years of the study, repeated measures models were used to account for correlation within plots. When the treatment x species x year or treatment x year interaction was significant, analysis was conducted separately for each year to facilitate the interpretation of treatment and species effects. Physiological traits recorded in 2014 were also analysed separately to compare all shading treatments. Following a significant effect or interaction, pairwise comparisons of least square means (Tukey’s test) were used to detect treatment differences. Means are reported as least square means ± their standard errors. All tests for significance were conducted at $p \leq 0.05$. Normal distribution of errors and homogeneity of variance were assessed graphically. Data with residuals that did not conform to assumptions of normality and/or homogeneity of variances were transformed using Box–Cox transformations (Box and Cox, 1964).

Additionally, Pearson correlation analyses were carried out to identify linear relationships among physiological variables.

3.3 Results

3.3.1 Photosynthetic light-response curves

The light-response curves showed different photosynthetic responses between beech seedlings grown in the open and heavy shade conditions, but differences could not be treated statistically. There were small differences for oak between both treatments (Fig. 3.1; Table 3.3). Beech seedlings at full light exhibited $A_{\text{max}}$ and $\Phi_{\text{CO}_2}$ rates 257% and 193% higher, respectively, than in the heavy shade (Table 3.3). $I_c$ and $R_d$ of beech in the heavy shade were 130% and 165% higher, respectively, than values obtained in the full light (Table 3.3). There was no difference in $A_{\text{max}}$ or $\Phi_{\text{CO}_2}$ between the tree species when grown at full light (Table 3.3). Fitted light-response curves revealed that $A_{\text{max}}$ for both species in both treatments was obtained at around 1500 $\mu$mol m$^{-2}$ s$^{-1}$ (Table 3.3), a value
that was used to determine saturating gas exchange parameters in the remainder of the study (see below). Since differences between treatments emerged at 500 $\mu$mol m$^{-2}$ s$^{-1}$ ($p < 0.001$), this PAR value was also used to test gas exchange responses during the study.

**Fig. 3.1.** Light-response curves of photosynthesis for beech and oak seedlings grown under full sunlight (100% of PAR) and heavy shade (28% of PAR). Regression lines represent fitted curves and symbols are observed field data (each observed point represents the average for the observations taken).

**Table 3.3.** The parameters obtained from the fitted curves for beech and oak seedlings under full sunlight (100%) and heavy shade (28%). $I_{\text{max}}$ (μmol photons m$^{-2}$ s$^{-1}$): light saturation point beyond which there is no significant change in photosynthesis; $A_{\text{max}}$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$): photosynthesis at $I_{\text{max}}$; $I_c$ (μmol photons m$^{-2}$ s$^{-1}$): light compensation point; $R_d$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$): dark respiration; $\Phi_{\text{CO}_2}$ (μmol CO$_2$ (μmol photons)$^{-1}$): quantum yield.

<table>
<thead>
<tr>
<th>Species</th>
<th>Light (PAR)</th>
<th>$I_{\text{max}}$</th>
<th>$A_{\text{max}}$</th>
<th>$I_c$</th>
<th>$R_d$</th>
<th>$\Phi_{\text{CO}_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech</td>
<td>100%</td>
<td>1526.29</td>
<td>14.53</td>
<td>11.81</td>
<td>-0.63</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>1422.56</td>
<td>4.07</td>
<td>27.13</td>
<td>-1.67</td>
<td>0.014</td>
</tr>
<tr>
<td>Oak</td>
<td>100%</td>
<td>1482.37</td>
<td>14.67</td>
<td>34.07</td>
<td>-1.82</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>28%</td>
<td>1490.00</td>
<td>14.80</td>
<td>31.19</td>
<td>-2.43</td>
<td>0.041</td>
</tr>
</tbody>
</table>

### 3.3.2 Leaf physiological responses to shade

Different physiological responses at saturating light of beech and oak seedlings grown under different shade conditions were found between the two years of the study, as indicated by the species x treatment x year or treatment x year interaction (Table 3.4). Although photosynthetic rate and stomatal conductance at saturating light declined in both species with increasing shade in 2013 ($A_{1500}$ and $g_{S1500}$, respectively), greater reductions in $A_{1500}$ and $g_{S1500}$ were found for beech
than oak (Fig. 3.2A and 3.2B). However, $A_{1500}$ and $g_{s1500}$ of beech and oak seedlings were equally affected by shade in 2014 (Table 3.5; Table 3.6). There was a significant interaction of treatment and species for intrinsic and instantaneous leaf water use efficiency at saturating light, $(A/g_s)_{1500}$ and $(A/E)_{1500}$, in 2013 ($p = 0.033$ and $p = 0.002$, respectively), indicating different responses to shade between species in water use efficiency at saturating light. While $(A/g_s)_{1500}$ and $(A/E)_{1500}$ decreased with shade in beech seedlings, no significant differences were found for oak seedlings between shading treatments (Fig. 3.2C and 3.2D). However, $(A/g_s)_{1500}$ and $(A/E)_{1500}$ were not affected by species, treatment and treatment x species interaction in 2014 (Table 3.5).

Table 3.4. Repeated-measures analysis of variance testing the effects of species (df = 1), shading treatment (df = 1, only extreme treatments), year (df = 1) and their interactions on some physiological variables under 1) PAR = 1500 μmol m$^{-2}$ s$^{-1}$; 2) PAR = 500 μmol m$^{-2}$ s$^{-1}$; 3) PAR = 0 μmol m$^{-2}$ s$^{-1}$; 4) ambient PAR. $A$: photosynthetic rate; $g_s$: stomatal conductance; $A/E$: instantaneous water use efficiency; $(A/g_s)$: intrinsic water use efficiency; $R_d$: dark respiration; $\Phi_{psii}$: PSII operating efficiency; $F'/F_m$: PSII maximum efficiency; $qP$: photochemical quenching; ETR: electron transport rate. Significant effects are in bold ($p < 0.05$).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Species (S)</th>
<th>Treatment (T)</th>
<th>S x T</th>
<th>Year (Y)</th>
<th>S x Y</th>
<th>T x Y</th>
<th>S x T x Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) PAR = 1500</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{1500}$</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.444</td>
<td>0.268</td>
<td>0.025</td>
<td>0.007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$g_{s1500}$</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.340</td>
<td>0.502</td>
<td>0.046</td>
<td>0.054</td>
<td>0.211</td>
</tr>
<tr>
<td>$(A/E)_{1500}$</td>
<td>0.081</td>
<td>0.048</td>
<td>0.217</td>
<td>0.028</td>
<td>0.230</td>
<td>&lt;0.001</td>
<td>0.012</td>
</tr>
<tr>
<td>$(A/g_s)_{1500}$</td>
<td>0.612</td>
<td>0.759</td>
<td>0.378</td>
<td>0.723</td>
<td>0.660</td>
<td>0.015</td>
<td>0.167</td>
</tr>
<tr>
<td>2) PAR = 500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{500}$</td>
<td>&lt;0.001</td>
<td>0.194</td>
<td>0.068</td>
<td>0.001</td>
<td>0.072</td>
<td>0.648</td>
<td>0.031</td>
</tr>
<tr>
<td>$g_{s500}$</td>
<td>&lt;0.001</td>
<td>0.700</td>
<td>0.094</td>
<td>&lt;0.001</td>
<td>0.154</td>
<td>0.085</td>
<td>0.069</td>
</tr>
<tr>
<td>$(A/E)_{500}$</td>
<td>0.095</td>
<td>0.042</td>
<td>0.213</td>
<td>0.512</td>
<td>0.289</td>
<td>0.057</td>
<td>0.366</td>
</tr>
<tr>
<td>$(A/g_s)_{500}$</td>
<td>0.379</td>
<td>0.083</td>
<td>0.281</td>
<td>0.248</td>
<td>0.273</td>
<td>0.018</td>
<td>0.314</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_d$</td>
<td>0.159</td>
<td>0.677</td>
<td>0.122</td>
<td>0.753</td>
<td>0.507</td>
<td>0.285</td>
<td>0.147</td>
</tr>
<tr>
<td>4) Ambient PAR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{amb}$</td>
<td>0.425</td>
<td>&lt;0.001</td>
<td>0.555</td>
<td>0.060</td>
<td>0.195</td>
<td>0.620</td>
<td>0.763</td>
</tr>
<tr>
<td>$g_{samb}$</td>
<td>0.078</td>
<td>0.212</td>
<td>0.195</td>
<td>0.007</td>
<td>0.023</td>
<td>0.880</td>
<td>0.176</td>
</tr>
<tr>
<td>$(A/E)_{amb}$</td>
<td>0.910</td>
<td>&lt;0.001</td>
<td>0.571</td>
<td>&lt;0.001</td>
<td>0.993</td>
<td>0.002</td>
<td>0.063</td>
</tr>
<tr>
<td>$(A/g_s)_{amb}$</td>
<td>0.727</td>
<td>0.005</td>
<td>0.988</td>
<td>0.215</td>
<td>0.847</td>
<td>0.407</td>
<td>0.196</td>
</tr>
<tr>
<td>$\Phi_{psii}$</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.141</td>
<td>0.002</td>
<td>0.381</td>
<td>0.275</td>
<td>0.045</td>
</tr>
<tr>
<td>$F'/F_m$</td>
<td>&lt;0.001</td>
<td>0.053</td>
<td>0.059</td>
<td>&lt;0.001</td>
<td>0.312</td>
<td>0.244</td>
<td>0.075</td>
</tr>
<tr>
<td>$qP$</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.412</td>
<td>0.005</td>
<td>0.482</td>
<td>0.261</td>
<td>0.056</td>
</tr>
<tr>
<td>ETR</td>
<td>0.328</td>
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<td>0.977</td>
<td>0.546</td>
<td>0.920</td>
<td>0.189</td>
<td>0.961</td>
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</table>
**Fig. 3.2.** Parameters of leaf gas exchange and chlorophyll fluorescence of beech and oak seedlings grown at 100% and 28% of PAR in 2013. $A_{1500}$: photosynthetic rate at saturating light (A); $g_{1500}$: stomatal conductance at saturating light (B); $(A/g_s)_{1500}$: intrinsic water use efficiency at saturating light (C); $(A/E)_{1500}$: instantaneous water use efficiency at saturating light (D); $A_{500}$: photosynthetic rate at 500 μmol m$^{-2}$ s$^{-1}$ (E); $(A/g_s)_{500}$: intrinsic water use efficiency at 500 μmol m$^{-2}$ s$^{-1}$ (F); $(A/E)_{amb}$: instantaneous water use efficiency at ambient PAR (G); $\Phi_{PSII}$: PSII operating efficiency (H). Bars indicate means and standard errors ($n = 5$ reps). Within each graph, bars with different letters are significantly different to each other.
Table 3.5. Summary of analysis of variance for the main effects of species (df = 1), shading treatment (df = 3, all treatments) and their interaction (df = 3) on some physiological variables measured in 2014 under 1) PAR = 1500 μmol m$^{-2}$ s$^{-1}$; 2) PAR = 500 μmol m$^{-2}$ s$^{-1}$; 3) PAR = 0 μmol m$^{-2}$ s$^{-1}$; 4) ambient PAR. A: photosynthetic rate; $g_s$: stomatal conductance; $A/E$: instantaneous water use efficiency; $A/g_s$: intrinsic water use efficiency; $R$: dark respiration; $\Phi_{PSII}$: PSII operating efficiency; $F'/F'_m$: PSII maximum efficiency; qP: photochemical quenching; ETR: electron transport rate. Significant effects are in bold ($p < 0.05$).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Species</th>
<th>Shading treatment</th>
<th>Species x Shade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>F $p$</td>
<td>F $p$</td>
</tr>
<tr>
<td>1) PAR = 1500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{1500}$</td>
<td>14.71</td>
<td>0.002</td>
<td>4.10</td>
</tr>
<tr>
<td>$g_{s1500}$</td>
<td>17.34</td>
<td>0.001</td>
<td>17.34</td>
</tr>
<tr>
<td>$(A/E)_{1500}$</td>
<td>1.04</td>
<td>0.317</td>
<td>1.23</td>
</tr>
<tr>
<td>$(A/g_s)_{1500}$</td>
<td>3.54</td>
<td>0.070</td>
<td>1.58</td>
</tr>
<tr>
<td>2) PAR = 500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{500}$</td>
<td>10.11</td>
<td>0.004</td>
<td>3.61</td>
</tr>
<tr>
<td>$g_{s500}$</td>
<td>10.02</td>
<td>0.006</td>
<td>2.73</td>
</tr>
<tr>
<td>$(A/E)_{500}$</td>
<td>0.00</td>
<td>0.959</td>
<td>0.48</td>
</tr>
<tr>
<td>$(A/g_s)_{500}$</td>
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<td>0.472</td>
<td>0.48</td>
</tr>
<tr>
<td>3) PAR = 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_d$</td>
<td>0.26</td>
<td>0.616</td>
<td>0.18</td>
</tr>
<tr>
<td>4) Ambient PAR</td>
<td></td>
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</tr>
<tr>
<td>$A_{amb}$</td>
<td>0.54</td>
<td>0.467</td>
<td>7.44</td>
</tr>
<tr>
<td>$g_{samb}$</td>
<td>1.50</td>
<td>0.231</td>
<td>1.60</td>
</tr>
<tr>
<td>$(A/E)_{amb}$</td>
<td>0.49</td>
<td>0.496</td>
<td>9.99</td>
</tr>
<tr>
<td>$(A/g_s)_{amb}$</td>
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<td>0.786</td>
<td>9.35</td>
</tr>
<tr>
<td>$\Phi_{PSII}$</td>
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<td>0.019</td>
<td>2.71</td>
</tr>
<tr>
<td>$F'/F'_m$</td>
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<td>0.006</td>
<td>2.48</td>
</tr>
<tr>
<td>qP</td>
<td>3.81</td>
<td>0.061</td>
<td>2.53</td>
</tr>
<tr>
<td>ETR</td>
<td>3.31</td>
<td>0.089</td>
<td>3.43</td>
</tr>
</tbody>
</table>
Table 3.6. Physiological characteristics of beech and oak seedlings grown under different shading treatments (% of PAR) in the summer of 2014. Because there were no significant interactions, means and standard errors (SE) are presented for each treatment (averaged over species, n = 5 reps) and for each species (averaged over treatments, n = 10 reps). Within either the treatment or species, means followed by different letters are significantly different. A (μmol CO_{2} m^{-2} s^{-1}): photosynthetic rate; g_{s} (mmol H_{2}O m^{-2} s^{-1}): stomatal conductance; (A/g_{s}) (μmol CO_{2}/mol H_{2}O): instantaneous water use efficiency; (A/E) (μmol CO_{2}/mmol H_{2}O): instantaneous water use efficiency; Φ_{PSII}: PSII operating efficiency; F_{v}/F_{m}: PSII maximum efficiency; qP: photochemical quenching; ETR (μmol photons m^{-2} s^{-1}): electron transport rate; ChlCont (mg m^{-2}): chlorophyll content.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Light treatment (PAR)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
<td>62%</td>
</tr>
<tr>
<td>A_{500}</td>
<td>13.92a</td>
<td>12.41ab</td>
</tr>
<tr>
<td>g_{s1500}</td>
<td>194.9a</td>
<td>161.6ab</td>
</tr>
<tr>
<td>A_{500}</td>
<td>10.50a</td>
<td>8.48ab</td>
</tr>
<tr>
<td>g_{s500}</td>
<td>173.8a</td>
<td>142.9a</td>
</tr>
<tr>
<td>A_{amb}</td>
<td>11.43a</td>
<td>8.13b</td>
</tr>
<tr>
<td>(A/E)_{amb}</td>
<td>4.51a</td>
<td>3.90a</td>
</tr>
<tr>
<td>(A/g_{s})_{amb}</td>
<td>64.95a</td>
<td>52.97a</td>
</tr>
<tr>
<td>Φ_{PSII}</td>
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<td>0.58a</td>
</tr>
<tr>
<td>F_{v}/F_{m}</td>
<td>0.69a</td>
<td>0.71a</td>
</tr>
<tr>
<td>ETR</td>
<td>62.56a</td>
<td>33.72b</td>
</tr>
<tr>
<td>ChlCont</td>
<td>544.8a</td>
<td>554.1a</td>
</tr>
</tbody>
</table>

The species x treatment x year interaction indicated different responses to shade between the both years for photosynthetic rate at 500 μmol m^{-2} s^{-1} (A_{500}) (Table 3.4). In 2013, A_{500} in beech leaves was significantly higher at full light than in the heavy shade treatment but was not affected by shade in oak leaves (Fig. 3.2E). A_{500} was equally affected by shade in both species in 2014 (Table 3.5), decreasing from 100% to 51% of PAR but increased thereafter with increasing shade levels (Table 3.6). In contrast to A_{500}, shade conditions did affect stomatal conductance at 500 μmol m^{-2} s^{-1} (g_{s1500}) in both years of the study (Table 3.4: Table 3.5). Similar to A_{500}, intrinsic water use efficiency at 500 μmol m^{-2} s^{-1} ((A/g_{s})_{500}) was higher in beech leaves grown at full light than in the heavy shade treatment in 2013 (Fig. 3.2F). However, (A/g_{s})_{500} was not significantly affected by either treatment or species in 2014 (Table 3.5) and neither was instantaneous water use efficiency ((A/E)_{500}) in both years of the study (Table 3.4).

Repeated measures analysis of R_{S} showed no significant effect of treatment, species, year or their interactions (Table 3.4). Similarly, no significant effect of treatment or species was found in 2014 (Table 3.5).
All environmental conditions except PAR remained steady and did not differ significantly between treatments during gas exchange and chlorophyll fluorescence measurements under ambient conditions in the two years (Table 3.7). PAR under ambient conditions decreased with increasing shade in both years of the study and were 25% and 61% higher in 2014 than in 2013 at full light and under heavy shade, respectively (Table 3.6). Lower Tair and VPD, and higher RH were recorded in 2013 than in 2014 (Table 3.7). Repeated measures analysis of photosynthetic rate and intrinsic and instantaneous water use efficiency at ambient PAR ($A_{amb}$, ($A/g_s$)$_{amb}$ and ($A/E$)$_{amb}$, respectively) showed a significant effect of treatment in both years of the study, with no differences between species (Table 3.4). Averaging $A_{amb}$ over species and year for each treatment, showed 114% higher rates at full light ($10.90 \pm 0.64 \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$) than in the heavy shade ($5.10 \pm 0.64 \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$). Similar to $A_{amb}$, ($A/g_s$)$_{amb}$ was 81% higher at full light ($67.47 \pm 4.10 \mu\text{mol CO}_2$/mol H$_2$O) than in the heavy shade ($37.19 \pm 4.10 \mu\text{mol CO}_2$/mol H$_2$O). This decline in $A_{amb}$, ($A/g_s$)$_{amb}$ and ($A/E$)$_{amb}$ with increasing shade levels was also noted in 2014 (Table 3.5; Table 3.6). The significant treatment x year interaction for ($A/E$)$_{amb}$ (Table 3.4) indicated greater values at full light in 2013 than in 2014 for both species (Fig. 3.2G; Table 3.6).

**Table 3.7.** Mean environmental conditions during gas exchange and chlorophyll fluorescence measurements under ambient conditions during the first (2013) and second (2014) growing season after erecting shadehouses. Values are means ± standard errors ($n$ = 5 reps). Means within rows followed by the same letter are not significantly different. PAR: photosynthetically active radiation; Tair: air temperature; RH: relative humidity; VPD: vapour pressure deficit.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year</th>
<th>Treatment (% PAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)</td>
<td>2013</td>
<td>560.4 ± 51.6 a</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>703.1 ± 104.2 a</td>
</tr>
<tr>
<td>Tair (°C)</td>
<td>2013</td>
<td>18.57 ± 0.24 a</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>20.28 ± 0.32 a</td>
</tr>
<tr>
<td>RH (%)</td>
<td>2013</td>
<td>45.65 ± 0.38 a</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>38.20 ± 0.42 a</td>
</tr>
<tr>
<td>VPD (kPa)</td>
<td>2013</td>
<td>1.08 ± 0.02 a</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>1.36 ± 0.04 a</td>
</tr>
</tbody>
</table>
For both species, shading significantly affected ETR in 2013 and 2014 (Table 3.4). ETR values of seedlings grown at full light (72.24 ± 5.26 μmol photons m⁻² s⁻¹) were 118% higher than those for seedlings grown under heavy shade (33.12 ± 5.26 μmol photons m⁻² s⁻¹) (averaged over species and year for each treatment). This reduction in ETR with increasing shade was also observed in 2014 (Table 3.5; Table 3.6). The species x treatment x year interaction for Φ_PsII indicated different responses to shade between the two years (Table 3.4). In 2013, Φ_PsII was significantly higher for oak seedlings grown under heavy shade than at full sunlight, but no significant differences were found for beech seedlings (Fig. 3.2H). In 2014, Φ_PsII increased with increasing shade in both species, although differences were not significant (Table 3.5; Table 3.6). Although repeated measures analysis of qP indicated significantly higher values under heavy shade (0.82 ± 0.02) in both species than at full light (0.71 ± 0.02) (Table 3.4), differences were smaller and statistically insignificant in 2014 (Table 3.5). F'/Fm was not significantly affected by shading treatment (Table 3.4; Table 3.5).

Chlorophyll content was strongly influenced by species (p < 0.001) with no significant effect of shading treatment (p = 0.791) or species x treatment interaction (p = 0.490). Chlorophyll content was significantly greater in oak than beech seedlings (Table 3.6). Similar to chlorophyll content, many physiological traits studied (A_1500, g_s1500, A_500, g_s500, Φ_PsII, F'/Fm, qP) were significantly greater in oak than in beech (Table 3.5; Table 3.6).

3.3.3 Physiological plasticity

Physiological plasticity in response to light diverged between years, species and variables studied (Table 3.8). In 2013, the physiological plasticity to shade was higher in beech than oak for almost all gas exchange traits at saturating PAR, 500 μmol m⁻² s⁻¹ and ambient PAR but was greater in oak than beech for most of the chlorophyll fluorescence traits. Differences in plasticity between species were small in 2014. Plasticity index values were 84% and 19% higher in beech than oak for 2013 and 2014, respectively.
3.3.4 Relationships between physiological variables

At saturating light and 500 μmol m$^{-2}$ s$^{-1}$, $A$ was positively correlated with $g_s$ ($p < 0.001$) for both species in 2013 (Fig. 3.3A and 3.3B) and 2014 ($r^2 = 0.317$, 0.622 for beech; $r^2 = 0.595$, 0.666 for oak). $A_{amb}$ was positively correlated with $g_{samb}$ for beech seedlings but no correlation was found for oak in 2013 (Fig. 3.3C). However, the opposite was found in 2014: $A_{amb}$ was positively correlated with $g_{samb}$ for oak ($r^2 = 0.411$) but only a weak correlation was found for beech.

$(A/g_s)$ and $(A/E)$ were always positively correlated for both species (at saturating light, 500 μmol m$^{-2}$ s$^{-1}$ and ambient PAR) in 2013 (Fig. 3.3D, 3.3E and 3.3F) and 2014 ($r^2 = 0.963$, 0.962, 0.842 for beech; $r^2 = 0.896$, 0.927, 0.901 for oak).

$A_{amb}$ was strongly and positively correlated with ETR in 2013 for both species (Fig. 3.3G) but no correlation between these two variables was found in 2014 (data not shown). A positive and strong relationship was found between $qP$ and

### Table 3.3.8. Plasticity index, calculated as [(max-min)/max] (Valladares et al., 2000a), along a light gradient of beech and oak seedlings for the physiological variables studied during two growing seasons. Variables are arranged by PAR conditions. ΔBeech-oak is the difference in the plasticity index between beech and oak.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Variable</th>
<th>PI-2013</th>
<th></th>
<th>PI-2014</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Beech</td>
<td>Oak</td>
<td>ΔBeech-oak</td>
<td>Beech</td>
</tr>
<tr>
<td>PAR = 1500</td>
<td>$A_{1500}$</td>
<td>0.74</td>
<td>0.28</td>
<td>0.46</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>$g_s_{1500}$</td>
<td>0.69</td>
<td>0.22</td>
<td>0.47</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>$(A/g_s)_{1500}$</td>
<td>0.30</td>
<td>0.06</td>
<td>0.24</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>$(A/E)_{1500}$</td>
<td>0.43</td>
<td>0.16</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.54</td>
<td>0.18</td>
<td>0.36</td>
<td>0.28</td>
</tr>
<tr>
<td>PAR = 500</td>
<td>$A_{500}$</td>
<td>0.52</td>
<td>0.19</td>
<td>0.33</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>$g_s_{500}$</td>
<td>0.30</td>
<td>0.29</td>
<td>0.01</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>$(A/g_s)_{500}$</td>
<td>0.47</td>
<td>0.22</td>
<td>0.25</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>$(A/E)_{500}$</td>
<td>0.49</td>
<td>0.20</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.45</td>
<td>0.22</td>
<td>0.23</td>
<td>0.17</td>
</tr>
<tr>
<td>Ambient PAR</td>
<td>$A_{amb}$</td>
<td>0.70</td>
<td>0.50</td>
<td>0.20</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>$g_s_{amb}$</td>
<td>0.43</td>
<td>0.05</td>
<td>0.38</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>$(A/g_s)_{amb}$</td>
<td>0.48</td>
<td>0.50</td>
<td>-0.02</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>$(A/E)_{amb}$</td>
<td>0.45</td>
<td>0.53</td>
<td>-0.08</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>$\Phi_{psii}$</td>
<td>0.15</td>
<td>0.32</td>
<td>-0.17</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>$F'/F'_m$</td>
<td>0.05</td>
<td>0.15</td>
<td>-0.10</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>$qP$</td>
<td>0.11</td>
<td>0.24</td>
<td>-0.13</td>
<td>0.14</td>
</tr>
<tr>
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<td>ETR</td>
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<td>0.60</td>
<td>0.04</td>
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</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.38</td>
<td>0.36</td>
<td>0.02</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Total mean</td>
<td>0.46</td>
<td>0.25</td>
<td>0.21</td>
<td>0.25</td>
</tr>
</tbody>
</table>
ΦPSII during the two growing seasons (2013 shown in Fig. 3.3H; r² = 0.925, 0.960 for beech and oak in 2014).

Fig. 3.3. Relationships between photosynthetic rate (A) and stomatal conductance (gₛ) (A, B and C); instantaneous (A/E) and intrinsic water use efficiency (A/gₛ) (D, E and F); photosynthetic rate at ambient PAR (Aₐmb) and electron transport rate (ETR) (G); photochemical quenching (qP) and PSII operating efficiency (ΦPSII) (H). Solid triangles and continuous lines indicate beech seedlings; open circles and dotted lines indicate oak seedlings. Measurements were made at saturating light (A and B), 500 μmol m⁻² s⁻¹ (C and D) and ambient PAR (E, F, G and H) during the summer of 2013.
3.4 Discussion

The results of this study showed that seedlings of European beech and pedunculate oak exhibited different physiological responses to shade in the first growing season after being established under shade conditions (2013). However, there were no differences between the species in their physiological responses to shade in the second growing season (2014). Although both species showed a greater photosynthetic capacity following a sudden increase in light intensity ($A_{1500}$) when grown in the open than under shade over the two growing seasons, $A_{1500}$ of beech seedlings were much more affected by shade than oak seedlings in the first growing season. Thus, in 2013 oak seedlings grown under 28% of full light were better able to respond, as measured by CO$_2$ assimilation, to increased incident light (applied artificially) than beech seedlings grown under the same shade levels. In contrast, both species grown in the open responded similarly to increased light intensity applied artificially. Although $A_{amb}$ and ETR decreased with increasing shade during both years of the study, both species were able to photosynthesise efficiently (higher $\Phi_{PSII}$ under shade), not only in the intermediate treatments (62% and 51% of full light) but also under 28% of full light.

The shade-induced reductions in photosynthetic capacity found for beech and oak seedlings in this study are consistent with the results obtained in previous studies in natural and controlled conditions (Gross et al., 1996; Johnson et al., 1997; Tognetti, et al., 1997; Reynolds and Frochot, 2003; Aranda et al., 2004; Einhorn, 2007; Kuehne et al., 2014). However, at 28% of full light, beech seedlings reduced their photosynthetic capacity ($A_{1500}$ and $A_{500}$) more than oak during the first growing season of being exposed to shade, which is not consistent with previous studies on beech and oak seedlings grown at similar conditions (Valladares et al., 2002; Gardiner et al., 2009). One possible explanation for the low rates of $A_{1500}$ and $A_{500}$ in shaded beech seedlings is that photosynthesis was limited by photoinhibition. Strong irradiance can lead to photoinhibition, especially in shade tolerant species and plants acclimated to shade (Anderson and Osmond, 1987; Valladares and Pearcy, 1997; Kitao et al., 2000). Wyka et al. (2007) reported a high degree of photoinhibition in shade acclimated beech (5% of full light) exposed to high light. Therefore, it is possible that beech seedlings in the shade were limited by an inability to acclimate to a sudden increase in light.
conditions (applied artificially). The fact that shaded beech responded in a similar way than shaded oak to sudden increase in light intensity ($A_{1500}$ and $A_{500}$) during 2014 suggest that beech seedlings could reduce the likelihood of occurring photoinhibition, after a period of acclimation (Naidu and DeLucia, 1997) or by producing leaves adapted to the environment (Mulkey and Pearcy, 1992). Another explanation for the greater impact of shade on $A_{1500}$ and $A_{500}$ could be the combination of high light with another stress, such as high temperatures (Gamon and Pearcy, 1990; Mulkey and Pearcy, 1992), water stress (Gamon and Pearcy, 1990) and/or nutrient content (Johnson et al., 1997). Nevertheless, these factors did not seem to be limiting in this study.

Under high light environments, light-demanding species typically have higher maximum photosynthetic rates relative to leaf area than shade tolerant species (Bazzaz, 1979; Bazzaz and Carlson, 1982; Walters et al., 1993; Kubiske et al., 1996; Kubiske and Pregitzer, 1996; Niinemets et al., 1998; Morecroft and Roberts, 1999; Kitao et al., 2000; Valladares et al., 2002; Gardiner et al., 2009). Therefore, greater photosynthetic rates at light saturation were expected for light-demanding oak than for beech seedlings grown at full light. However, this trend was not found in this study since there were no differences in photosynthetic capacity between species at full light, similar to findings reported for light-demanding ash (Fraxinus excelsior L.) and beech (Einhorn, 2007), and Acer species with contrasting shade tolerance (Hanba et al., 2002).

Similar to $A_{1500}$, $g_{S1500}$ also decreased with shade and differences among treatments were more pronounced for beech seedlings in 2013. However, shade had a greater impact on $A_{1500}$ than $g_{S1500}$ in beech seedlings during the first growing season, resulting in reduced ($A/g_s)_{1500}$ under heavy shade. The strong correlation found between instantaneous and intrinsic water use efficiency suggests that shade has a similar effect on stomatal conductance and transpiration. The strong positive relationship between $A_{1500}$ and $g_{S1500}$ found for both species, suggests that stomatal limitation of photosynthesis might occur at saturating light. However, $A_{amb}$ and $g_{samb}$ were not always correlated for both species during the two growing seasons of the study, suggesting that light availability may alter the linear relationship between CO$_2$ assimilation and stomatal conductance and factors other than stomatal closure, such as
biochemical limitations, caused the reduction of $A_{amb}$ under low light (Peri et al., 2009). Since $A_{amb}$ decreased with shade and $g_{samb}$ was not affected by shade, it resulted in seedlings exhibiting greater water use efficiencies at full light than under heavy shade. Similar to this finding, Reynolds and Frochot (2003) reported greater water use efficiencies of beech seedlings grown at full light than those grown under shade.

Increasing the efficiency of PSII as shade increased is a common acclimation of leaves to the light environment, by using light most efficiently under low light conditions (Valladares et al., 2002; Einhorn et al., 2004; Baker, 2008; Špulák, 2011). In this study, $\Phi_{PSII}$ and $q_P$ were greater under shade than full light conditions, although differences were not always significant. The smaller variation between light environments showed by $F'_v/F'_m$ compared to that found for $q_P$ and the strong correlation between $\Phi_{PSII}$ and $q_P$ suggest that changes in $\Phi_{PSII}$ are determined by changes in $q_P$.

Leaves grown under low light may maximize photosynthesis by increasing total chlorophyll content (Johnson et al., 1997). In this study total chlorophyll content was significantly higher in oak than beech seedlings but it did not change with increasing shade. This does not agree with the results reported in several studies, wherein chlorophyll content increased with decreasing light availability (Johnson et al., 1997; Tognetti et al., 1997; Valladares et al., 2002) and was found to be strongly correlated with photosynthetic rates (Tognetti et al., 1995; Koike at al., 2004). However, the opposite trend of sun leaves exhibiting larger chlorophyll content than that of shade leaves has also been found (Demarez, 1999). One possible explanation for this discrepancy is that chlorophyll content in this study was measured on different dates to photosynthetic rates and at the end of the summer, and chlorophyll content may decline at the end of the growing season (Demarez, 1999; Gond et al., 1999).

Similar to $A_{amb}$, lower values of ETR were found in seedlings growing under shade, which had been reported in previous studies (Wyka et al., 2007; Špulák, 2011). The same trend in the response of $A_{amb}$ and ETR to shade in both years and the strong relationship found between these two physiological traits in 2013 suggest that changes in ETR are apparently connected with changes in $A_{amb}$ (Balandier et al., 2007; Jurásek et al., 2010). Several studies have focussed on
leaf-level photosynthetic responses as an indicator of growth (Harrington et al., 2004; Kruger and Volin, 2006; Long et al., 2006; Malhi et al., 2015). As reported earlier (Chapter 2), oak and beech seedlings grown under shade had lower growth rates than at full sunlight. Hence, the reduction in $A_{\text{amb}}$ and ETR of seedlings grown under shade coincided with a reduction in growth, suggesting that the lower photosynthetic capacity under shade was apparently connected with a decline in growth. However, other factors such as total leaf area and leaf architecture/leaf display, which were not investigated in this study, should be considered if photosynthesis at the plant level is used to predict growth.

While beech exhibited higher physiological plasticity in response to shade than oak in 2013, mainly determined by the low ability of shaded seedlings to respond to a sudden increase in light intensity, the response to shade was similar between species in 2014. This finding does not agree with the results from several studies that suggest that light-demanding species have a more plastic photosynthetic response than shade tolerant species (Bazzaz and Carlson, 1982; Ellsworth and Reich, 1996; Kozlowski and Pallardy, 1997; Valladares et al., 2000b; Sánchez-Gómez et al., 2006; Portsmouth and Niinemets, 2007). Valladares et al. (2002) reported a greater physiological plasticity for oak than beech, which was linked to a greater photosynthetic performance of oak seedlings at full light. However, no differences in the photosynthetic capacity of oak and beech seedlings at full light were found in this study.

An understanding of physiological and growth responses to light availability of beech and oak seedlings can be used to inform decision-making on the development of silvicultural systems for forest regeneration and rehabilitation of these species. This study provides evidence that light conditions affected the physiological response of beech and oak seedlings. Although best photosynthetic and growth performance was observed at full light, findings from this study suggest that both species can photosynthesise efficiently after being exposed to shade levels of 62%, 51% and 28% of full light. This suggests that beech and oak seedlings can be underplanted below forest canopies that reduce light conditions as low as 28% of full light. Another implication is the period that seedlings can remain in an understory before being released to full sunlight. The increase in $A_{1500}$ (photosynthetic rates at PAR values much higher than experienced during
the growing seasons) for beech grown in the heavy shade from the first to the second growing season may suggest that beech would be able to benefit from increase in light availability after one growing season of being exposed to heavy shade. Reynolds and Frochot (2003) reported that beech seedlings released from shading had begun to acclimate to high light conditions by the first growing season, but the process remained incomplete and acclimation continued in the successive growing season. On the other hand, Valladares et al. (2002) reported a lack of acclimation of photosynthetic capacity of beech and oak seedlings to an increase in light; maximum photosynthetic rates remained constant between shaded plants and plants transferred from the shade to full light over one growing season. In contrast to beech, shaded oak exhibited a great photosynthetic capacity ($A_{1500}$) throughout the two years of the study, suggesting that shaded oak seedlings may benefit from an increase in light availability during the first year after being exposed to shade. However, caution is advised as the increase in irradiance ($1500 \, \mu\text{mol m}^{-2} \text{s}^{-1}$) was applied artificially and only for a short period of time (until photosynthetic rates were stable) instead of the full growing season. Therefore, there is no evidence that photosynthetic responses will not change after longer exposures to these PAR levels. Since the increase in light intensity was only applied for a short period of time, the results could also be seen as similar responses to those induced by sunflecks (brief and intermittent periods of high light that can significantly affect photosynthetic responses in shaded forest understories). Therefore, shaded beech may be capable of exploiting sunflecks, as suggested by Tognetti et al. (1997), just one year after being exposed to shade.

### 3.5 Conclusions

The results from this study confirm that light strongly affected photosynthetic performance of beech and oak seedlings. Although both species displayed greater photosynthetic capacity ($A_{1500}$, $A_{amb}$ and ETR) at full light than under shade conditions, beech and oak seedlings were able to photosynthesize efficiently at the intermediate treatments (62% and 51% of full light) and also under 28% of full light. The ability of shaded seedlings to photosynthesize at saturating light and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was more limited in beech than oak during the first growing season. However, there were no differences between the
species in their photosynthetic responses to shade in the second growing season. The efficiency of the PSII generally increased with shade levels for both species. Based on these findings, both species might survive and grow well under a wide range of shade levels and, therefore, may be suitable for underplanting under these light conditions.
3.6 References


Chapter 4
The influence of light availability on leaf phenology in $Fagus$ $sylvatica$ and $Quercus$ $robur$ seedlings

Abstract
Different growth strategies have evolved in trees to cope with varying environmental factors, so different phenological responses among species can be expected. Leaf phenology of shade tolerant European beech ($Fagus$ $sylvatica$ L.) and more light-demanding pedunculate oak ($Quercus$ $robur$ L.) seedlings grown under different light availability were studied in a shadehouse experiment over a two-year period. The buds of oak seedlings broke dormancy earlier than those of beech and had a longer period of leaf development each year from budburst to full leaf expansion. Oak leaves had a longer duration of leaf senescence which, along with early spring leaf out, translated into greater leaf longevity. The buds of seedlings in full sunlight flushed earlier and had a longer duration of leaf senescence than those grown under shade, with greater differences between the heavy shade and control (open grown) treatments. This information should be considered when establishing or managing beech and oak forests.

4.1 Introduction
Phenology is the study of the timing of recurring biological events such as leaf unfolding, flowering, leaf senescence and leaf fall. Changes in the timing of phases of the plant life cycle are typically controlled by environmental conditions, such as temperature, rainfall and day length. Phenological observations have been used for centuries by farmers to maximize crop production and determine the beginning, end and length of the growing season; and have gained scientific recognition in recent years to show the responsiveness of species to climate change. Besides its sensitivity to climate change, leaf phenology plays an important role in the forest ecosystem, water and carbon balances, and species distribution (Chuine, 2010). Phenological differences result from the capacity of a plant to optimize the period of activity under different environmental conditions, such as temperature, light and water availability (Chuine, 2010). Some of the main points which should be considered in the study of leaf phenology are leaf emergence and leaf senescence. While most previous phenological studies have
focused on responses to changes in temperature affected by climate change (Chmielewski and Rotzer, 2001; Carroll et al., 2009; Morin et al., 2010; Vitasse et al., 2011; Fu et al., 2014) or photoperiod (Falusi and Calamassi, 1990; Fracheboud et al., 2009; Basler and Könder, 2014; Laube et al., 2014; Way and Montgomery, 2015), there has been little research on how light intensity affects phenology.

Two types of leaf emergence patterns have been found in tree species (Kikuzawa, 1983; Kikuzawa, 1984; Kikuzawa, 1988). One is the simultaneous emergence, where many leaves appear simultaneously within a short period. The other is the successive type, where leaves appear one by one successively over a longer period.

Leaf phenology is also an important aspect of the light-harvesting strategy of plants (Kikuzawa, 1995). Plants harvest light by expanding leaves to gain energy, therefore the appearance of leaves in time is critical in any strategy of plant carbon gain. In high light conditions, plants produce leaves that can utilize full sunlight and attain high photosynthetic rates (Kikuzawa, 1995). This capacity to utilize high irradiance declines quickly with leaf age or height of trees (Koike, 1988; Kikuzawa, 1995). In shade environments, plants produce leaves that can utilize low light efficiently and are retained longer (Kikuzawa, 1995). The buds of seedlings growing under forest canopies tend to flush earlier than in those growing in the open (McGee, 1975). Early leafing in the presence of forest canopies has been attributed to changes in temperature and quicker spring warming near the forest soil (Augspurger, 2004). The early bud burst and development of seedlings under canopies can be an important adaptation to maximize plant carbon gain before the buds of shoots in the overstory start to flush (Harrington et al., 1989; Gill et al., 1998). However, if early flushing occurs in the open, seedlings can be more susceptible to late spring frost (McGee, 1975). Augspurger (2008) suggested that the role of the early spring leaf out should be taken into account when considering species shade tolerance.

Leaf senescence constitutes the final stage of leaf development, and is an integral part of plant growth and development (Kim et al., 2011). Besides the developmental age of the plant, leaf senescence is also influenced by internal and environmental factors, such as drought, temperature, nutrients and shading by
other plants (Lim et al., 2003, 2007). If senescence occurs too early, the growing season will be shortened and the photosynthetic carbon gain will be reduced; while if senescence occurs too late, green leaves could be killed by frost.

Light availability is one of the main factors that can be used in forest management to favour continuous and uninterrupted maintenance of forest cover (continuous cover forestry, CCF) and sustainable management. Thinning increases the amount of light under the forest canopy and can be used to favour regeneration as part of continuous cover forestry objectives. Depending on the main objectives and species used, different thinning intensities should be carried out, from heavy (for light demanding species) to light thinning (for shade-tolerant species). Underplanting is also used to transform forests managed under clearcutting into CCF, so the phenological responses to light availability of species involved should be considered.

To date there has been little research on the phenology of shoot growth and leaf senescence of different species under different light environments. In this study, the impact of light availability on leaf phenology in two common tree species was assessed, European beech (Fagus sylvatica L.) and pedunculate oak (Quercus robur L.). Beech and oak are two important temperate forest species with a wide distribution across Europe (Savill, 2013). While oak saplings are light-demanding, beech saplings are more shade tolerant (Hill et al., 1999). Oak foliage is more susceptible than most broadleaved species to late spring frosts (Evans, 1984). Beech can also suffer from late spring and early autumn frost (Savill, 2013).

The aim of this study was to investigate the impact of shade conditions on leaf phenology, from leaf emergence to leaf senescence, in beech and oak seedlings.

4.2 Material and methods

4.2.1 Study site and tree species
The study was conducted in a controlled-shade experiment located at Teagasc Ashtown Food Research Centre, D15 DYO5, Ireland (53 °22 ’45 ” N, 6 °20 ’13 ” W, 40 m ASL). Two-year old seedlings (1u1) of pedunculate oak and three-year old (1u1u1) European beech were purchased from a Coillte Nursery,
Ardattin, Co. Carlow, Ireland (52 °43'47" N, 6 °41'13" W, 104 m ASL) and planted at Teagasc Food Research Centre in March 2011. Seedling provenances used were according to provenance recommendations in Ireland (COFORD, 2002): beech provenance was Cirencester Region 404, United Kingdom, origin unknown (51 °43'0" N, 2 °0'0" W, 140 m ASL) and the oak provenance was NL.S. Nuenen 03, Netherlands, origin unknown (51 °29'9" N, 5 °32'9" E, 20 m ASL). The experimental area was fenced to exclude rabbits and hares. Weeding was carried out when required. The mean annual total rainfall in the region is 774 mm and the mean annual air temperature is 9.8 °C (all means are from the period 1981-2010). The weather conditions from 2011 to 2014, the period when this study was conducted, were similar with respect to temperature but differed in rainfall during the growing season (Table 4.1). Climate data were collected by an Automatic Weather Station (Met Éireann, Phoenix Park station) located 1.93 km away at an open site.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Mean</td>
<td>10.4</td>
<td>9.8</td>
<td>9.9</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td>Growing season</td>
<td>13.2</td>
<td>12.4</td>
<td>13.2</td>
<td>13.6</td>
</tr>
<tr>
<td>Rainfall</td>
<td>Annual</td>
<td>675</td>
<td>869</td>
<td>711</td>
<td>885</td>
</tr>
<tr>
<td></td>
<td>Growing season</td>
<td>287</td>
<td>564</td>
<td>282</td>
<td>336</td>
</tr>
</tbody>
</table>

Seeding heights and stem diameters (3 cm above root collar) were recorded at time of planting. The mean (± SE) seedling heights were 61.1 ± 0.5 cm for beech and 75 ± 0.6 cm for oak. The mean (± SE) stem diameters were 8.7 ± 0.1 mm for beech and 7.3 ± 0.1 mm for oak.

4.2.2 Experimental design and shade treatments

The area was divided into five blocks, each with four randomly assigned plots, each randomly split into two subplots. This resulted in twenty plots (11 m long, 4.3 m wide and 2.9 m high when covered by shading nets), each containing two subplots separated by 1 m and corresponding to the two broadleaf species. Plots were spaced apart from each other to minimise any interaction. Forty-two
seedlings were planted in each subplot at 0.5 × 0.5 m spacing to encourage the onset of interplant competition. The subplot measurement area entailed 16 seedlings per species and was surrounded by a buffer line to help mitigate any potential edge effects.

Green polythene shade nets (Colm Warren Polyhouses Ltd., Kilmurray, Trim, Co. Meath, Ireland) were erected on frames to simulate different light environments (representing a spectrum of thinning intensities) in September 2012, about 18 months after the seedlings were planted. Four different light treatments were established in each block (one treatment per plot): full sunlight, light shade, medium shade and heavy shade.

The proportion of photosynthetically active radiation (PAR) below the nets was calculated as the difference between readings taken simultaneously with a data logger, LI-1400 (LI-COR Inc., Lincoln, Nebraska), using a LI-190SA Quantum Sensor (LI-COR Inc., Lincoln, Nebraska) outside the plot and a LI-191SA Line Quantum Sensor (LI-COR Inc., Lincoln, Nebraska) inside the plot in October 2013. LI-COR quantum sensors monitored PAR in the 400 to 700 nm waveband. Soil water content (SWC, %) was measured in each plot to get some indications about rainfall interception. Measurements were carried in January 2014 in the corners and centre of the plot with a WET sensor and a moisture meter that allowed SWC measurement in 68 mm of depth (Delta-T Devices Ltd, Cambridge, UK). Red/far-red ratio (R/FR) was measured in March 2014 with a Skye SKR 110 sensor connected to a display meter (Skye Instruments, Powys, UK) that reports quantum flux at 660 and 730 nm. In each light treatment of the first block, air temperature and relative humidity were recorded every 10 min from 26 May to 8 October during 2015 using dataloggers (SF-LOG-M, Solfranc Tecnologias SL, Tarragona, Spain) with radiation and rain shelter to prevent direct solar radiation. Temperature and humidity loggers were located in the middle of each oak subplot (after checking there were no differences between oak and beech subplots), ~70cm above-ground. The different light treatments averaged 100%, 62%, 51% and 28% of PAR, respectively. A description of the conditions in the different treatments is shown in Table 4.2. The shadehouses had little effect on the light quality, as the R/FR ratio inside and outside the shadehouses was similar in the two intermediate treatments, and slightly reduced
in the heavy shade treatment (Table 4.2). Air temperature and relative humidity did not differ among the different light environments. Air temperature and relative humidity outside the treatments averaged 15.03 ± 0.05°C (mean ± SE) and 72.59 ± 0.18% (mean ± SE).

**Table 4.2.** Light properties, soil water content (SWC), air temperature (T) and relative humidity (RH) in the different shade environments. Data are the means ± standard errors.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAR (%)</th>
<th>R/FR</th>
<th>SWC (%)</th>
<th>T (°C)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (full sunlight)</td>
<td>100</td>
<td>1.00</td>
<td>47.72 ± 0.83</td>
<td>13.63 ± 0.03</td>
<td>82.16 ± 0.10</td>
</tr>
<tr>
<td>Light shade</td>
<td>62</td>
<td>0.98</td>
<td>45.98 ± 0.91</td>
<td>13.57 ± 0.03</td>
<td>82.42 ± 0.10</td>
</tr>
<tr>
<td>Medium shade</td>
<td>51</td>
<td>0.98</td>
<td>44.33 ± 0.85</td>
<td>13.66 ± 0.03</td>
<td>81.06 ± 0.10</td>
</tr>
<tr>
<td>Heavy shade</td>
<td>28</td>
<td>0.92</td>
<td>39.81 ± 1.03</td>
<td>13.50 ± 0.03</td>
<td>81.33 ± 0.10</td>
</tr>
</tbody>
</table>

### 4.2.3 Leaf phenology

Leaf phenology of oak and beech seedlings from the measurement area was monitored in all treatments. Since the time of flushing and leaf senescence may vary within the tree, the bottom half and top half part of each tree was assessed separately. In 2013, the bottom part was considered to be from the ground to about 50 cm, and the upper part from 50 cm to the seedling top at about 1m (data from the height means). In 2014, the boundary between bottom and top was at about 80 cm.

The timing of leaf development was monitored three times weekly in 2013 and 2014 using a categorical index adapted from Liang and Schwartz (2009) (Table 4.3). Bud flushing was considered complete when more than half of the tree’s buds or leaves had flushed.

In autumn 2013, symptoms of leaf senescence on the different species were visually scored on a scale of 0-4 (Table 4.3) used by Zhao *et al.* (2012). Leaf senescence on seedlings was rated at 7-day intervals (once per week) from October.
Table 4.3. Scores used for the description of flushing and leaf senescence.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description of flushing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Initiation of flushing: green leaf material visible</td>
</tr>
<tr>
<td>2</td>
<td>Leaf form visible</td>
</tr>
<tr>
<td>3</td>
<td>Leaf fully expanded</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Leaf senescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms, whole plant leaves are green and healthy</td>
</tr>
<tr>
<td>1</td>
<td>Senescence leaves with yellowing symptoms accounting for no more than 25% of the whole plant leaves</td>
</tr>
<tr>
<td>2</td>
<td>Senescence leaves with yellowing symptoms accounting for 25-50% of the whole plant leaves</td>
</tr>
<tr>
<td>3</td>
<td>Senescence leaves with yellowing symptoms accounting for 50-75% of the whole plant leaves</td>
</tr>
<tr>
<td>4</td>
<td>More than 75% senescent leaves shown to have extensive yellowing, desiccation and had abscissed</td>
</tr>
</tbody>
</table>

The Leaf Senescence Index (LSI) was calculated according to the following formula (Zhao *et al.*, 2012):

$$LSI = \left( \frac{\sum \text{leaf senescence scores} \times \text{number of senescence plants for each scores}}{\text{total number of investigated plants} \times \text{the highest grade senescence score}} \right) \times 100$$

In this study, the initiation of flushing (score 1) was used to determine leaf flushing date. Duration of leaf emergence (DLE) was defined as the period from the initiation of flushing to the date when the leaves where fully expanded (score 3). The start of autumn senescence was defined as the date when yellow colour appeared (score 1), and senescence was considered complete when more than 75% of the leaves had fallen or there was extensive yellowing and desiccation (score 4). The duration of senescence (DS) was determined as the number of days between these two dates. Leaf longevity (LL) was defined as the period from the date when leaves where fully expanded (score 3 of flushing) to the date when more than 75% senescence symptoms were found (score 4 of senescence).

4.2.4 Data analysis

All statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Data analysis was performed using PROC MIXED statement of SAS. Light
treatment, species and their interaction were included as fixed effects. Block and block x light interaction were included as random effects. Repeated measures were used to take account of year effect and light, species, year and their interaction were included in the model as fixed effects. Where significant effects (interactions, or main effects if no interaction) were found, means were separated using Tukey’s adjustment. Means are reported as least square means and their standard errors. All tests for significance were conducted at $p \leq 0.05$. Normal distribution of errors and homogeneity of variance were assessed visually. Data with residuals that did not conform to assumptions of normality and/or homogeneity of variances were transformed using Box-Cox transformations (Box and Cox, 1964).

Pearson correlation analyses were carried out to identify the relationships between phenology (leaf longevity, flushing and senescence) and growth (diameter and height increment). Correlations between flushing date between the two years and senescence values in the first year were also carried out.

4.3 Results

4.3.1 Spring phenology
The timing of leaf development (all phases) was significantly influenced by species, light, year and the species x year interaction (Table 4.4). Although the light x year interaction was significant for the first phase of leaf development in the bottom part of the seedlings (Table 4.4), this interaction was marginal compared with the main effects and no differences between years was found in the response to light treatments. Seedlings at full sunlight reached the different leaf stages earlier than seedlings under medium and heavy shade and no significant differences were found between the other treatments during the two years of the study (Fig. 4.1). This trend was found in both parts of the seedlings, bottom half (Fig. 4.1) and top half (data not shown). All leaf development phases occurred earlier in oak than beech seedlings, but differences were generally greater in the bottom part and became less evident with the progress of leaf development (Table 4.5). Buds flushed earlier in 2014 than in 2013 in both species (Table 4.5). A similar pattern of development was found in both parts of the seedlings.
Table 4.4. Repeated-measures analysis of variance testing the effects of species (S, df = 1), light (L, df = 3), year (Y, df = 1) and their interactions on the different phases of leaf development (Phase1, Phase2 and Phase3) and the duration of leaf emergence (DLE) during the two years of the study. Significant effects are in bold ($p \leq 0.05$).

<table>
<thead>
<tr>
<th>Traits</th>
<th>S</th>
<th>L</th>
<th>S x L</th>
<th>Y</th>
<th>S x Y</th>
<th>L x Y</th>
<th>S x L x Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.353</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.049</td>
<td>0.279</td>
</tr>
<tr>
<td>Top</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.432</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.225</td>
<td>0.152</td>
</tr>
<tr>
<td>Phase2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.245</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.054</td>
<td>0.661</td>
</tr>
<tr>
<td>Top</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.374</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.224</td>
<td>0.258</td>
</tr>
<tr>
<td>Phase3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.227</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.057</td>
<td>0.395</td>
</tr>
<tr>
<td>Top</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.168</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.302</td>
<td>0.142</td>
</tr>
<tr>
<td>DLE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;0.001</td>
<td>0.173</td>
<td>0.140</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.007</td>
<td>0.848</td>
</tr>
<tr>
<td>Top</td>
<td>&lt;0.001</td>
<td>0.367</td>
<td>0.040</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td>0.003</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Fig. 4.1. Timing of initiation of flushing (Phase 1), leaf form visible (Phase 2) and leaf fully expanded (Phase 3) during two growing seasons for each percentage of full PAR in the bottom part of the seedlings. Bars indicate means and standard errors (n = 5 reps). Within each phase, means with the same letter are not significantly different.
Table 4.5. Timing of initiation of flushing (Phase1, day of year), leaf form visible (Phase2, day of year), leaf fully expanded (Phase3, day of the year) and duration of leaf emergence (DLE, days) in different parts of the seedlings averaged for species and year. Data are the means ± standard errors (n = 10 reps). Species means over time followed by the same letter are not significantly different.

<table>
<thead>
<tr>
<th></th>
<th>Bottom</th>
<th>Top</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beech</td>
<td>Oak</td>
</tr>
<tr>
<td>Phase1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>141.17 ± 0.48 a</td>
<td>130.51 ± 0.49 c</td>
</tr>
<tr>
<td>2014</td>
<td>132.39 ± 0.49 b</td>
<td>117.69 ± 0.49 d</td>
</tr>
<tr>
<td>Phase2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>144.67 ± 0.40 a</td>
<td>139.33 ± 0.41 c</td>
</tr>
<tr>
<td>2014</td>
<td>137.28 ± 0.41 b</td>
<td>125.37 ± 0.41 d</td>
</tr>
<tr>
<td>Phase3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>153.73 ± 0.38 a</td>
<td>151.16 ± 0.39 c</td>
</tr>
<tr>
<td>2014</td>
<td>141.98 ± 0.39 b</td>
<td>133.74 ± 0.39 d</td>
</tr>
<tr>
<td>DLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>12.56 ± 0.25 a</td>
<td>20.67 ± 0.26 c</td>
</tr>
<tr>
<td>2014</td>
<td>9.63 ± 0.26 b</td>
<td>16.06 ± 0.26 d</td>
</tr>
</tbody>
</table>

The duration of leaf emergence was significantly affected by species, year and species x year interaction (Table 4.4). Although the species x light interaction was significant in the top part of the seedlings (Table 4.4), this interaction was small and no differences were found in the response of species between treatments. The duration of leaf emergence was longer in 2013 than 2014 for both species and in oak than beech each year, regardless of the light treatments applied (Table 4.5). The same trend was found in the bottom and top part of the seedlings.

4.3.2 Autumn senescence

The leaf senescence index was always lower in the open than in the other treatments for beech seedlings until the 19th of November (day 323 of the year) and 26th of November (day 330 of the year) in the bottom (Fig. 4.2A) and the top part (Fig. 4.2C) of the tree, respectively. Shade treatments had little effect on the leaf senescence index in oak seedlings (Fig. 4.2B and 4.2D). After the 29th of October (day 302 of the year) the percentage of senescence started to increase, regardless of species or treatment (Fig. 4.2). Leaf senescence index followed a similar trend in the bottom and top parts of the seedlings (Fig. 4.2).
Fig. 4.2. Leaf senescence index of the bottom (A and B) and top part (C and D) of the species studied in response to different percentages of PAR versus day of the year (2013).

The duration of leaf senescence was significantly influenced by species, light and their interaction (Table 4.6). The duration of leaf senescence decreased with increasing shade level, and was always longer in seedlings growing in the open than in the other treatments (Fig. 4.3). Leaf senescence occurred over a longer duration in oak than in beech (Fig. 4.3), with 2.3 days longer in the bottom and 3.2 days longer in the top of the tree. Only the bottom part of beech under full sunlight had a longer senescence period than oak (Fig. 4.3A). Except at full sunlight, the bottom and top parts of the seedlings had a similar trend in response to treatment for the duration of leaf senescence (Fig. 4.3).

Table 4.6. Summary of analysis of variance for the main effects of species (S), light (L) and their interaction (S × L) on duration of senescence (DS) and leaf longevity (LL) during 2013. Significant effects are in bold (p ≤ 0.05).

| Effects | df | DS Bottom | | DS Top | | LL Bottom | | LL Top |
|---------|----|-----------| |       | |          | |       |
|         |    | F P       | |       | |          | |       |
| S       | 1  | 11.78 0.001 | | 51.21 <0.001 | | 35.64 <0.001 | | 59.77 <0.001 |
| L       | 3  | 9.81 0.002 | | 5.03 0.018 | | 18.49 <0.001 | | 20.27 <0.001 |
| S × L   | 3  | 12.66 <0.001 | | 3.46 0.016 | | 12.15 <0.001 | | 2.36 0.070 |
4.3.3 Leaf longevity

Leaf longevity was strongly affected by species and light conditions in the bottom and top part of the seedlings (Table 4.6). While there was significant interaction between species and light for leaf longevity in the bottom part of the seedlings, it was not the case for the top part (Table 4.6). Leaf longevity was greater in oak than beech seedlings (Fig. 4.4), with leaves living 5.2 and 5 days longer in the bottom and top part, respectively. As occurred with the duration of leaf senescence, only the bottom part of beech seedlings under full sunlight experienced longer senescence period than in oak (Fig. 4.4A). Leaf longevity usually decreased with increasing shade, but not always significantly, and was significantly longer in the full sunlight than in the heavy shade (Fig. 4.4). Leaf longevity showed a similar trend in the different parts of the seedlings except in the full sunlight treatment (Fig. 4.4).
Fig. 4.4. Leaf longevity in the bottom (A) and top part (B) of beech and oak seedlings exposed to different percentages of PAR during 2013. Bars indicate means and standard errors (n = 5 reps). Within a graph, different letters indicate significant differences between light treatments where there was no species*light interaction (B), or between species and light treatments where there was an interaction (A).

4.3.4 Relationship between phenology and growth

Leaf longevity and stem diameter growth decreased with increasing shade for both species but the differences were greater in beech than in oak seedlings (Fig. 4.5A and 4.5B). In addition, leaf longevity was significantly and positively correlated with stem diameter growth (Fig. 4.6A). In contrast, the opposite pattern was observed for height growth in response to shade: height growth generally increased with increasing shade and differences were much greater in oak than in beech seedlings (Fig. 4.5C and 4.5D). Moreover, no correlation was found between leaf longevity and tree height growth (data not shown).

A significant positive linear relationship was found between leaf flushing dates (initiation of flushing) observed in 2013 and 2014 for both species (fig. 4.6B). No correlation was found between leaf flushing dates and leaf senescence in 2013, or between leaf senescence date in 2013 and leaf flushing date in 2014 (data not shown).
Fig. 4.5. Stem diameter growth (dotted bars) and leaf longevity (solid bars) of beech (A) and oak (B) seedlings; height growth (dotted bars) and leaf longevity (solid bars) of beech (C) and oak (D) seedlings. Bars indicate means for each light treatment (% of PAR) (n = 5 reps).

Fig. 4.6. Correlations between stem diameter growth and leaf longevity (A); initiation of flushing in 2013 and 2014 (B).

4.4 Discussion
Most previous research has focused on phenological responses to temperature (climate warming) and photoperiod (Basler and Körner, 2014; Fu et al., 2014; Kuster et al., 2014; Way and Montgomery, 2015), but the effect of light intensity on phenology has been rarely investigated. Some studies have documented how
changes in canopy closure of deciduous forests affect leaf phenology (McGee, 1975; Augspurger, 2008; Lopez et al., 2008). Since phenological development might vary according to the presence/absence of canopy cover and the time when canopy changes (McGee, 1975; Augspurger, 2008), data derived from phenological studies may also depend on the forest type. While in temperate deciduous forests (mainly broadleaves) there is greater seasonal variation in light availability in the understorey, evergreen forests (mainly conifers) maintain quite steady-state light environments (Dreiss and Volin, 2014).

Leaf flushing occurred earlier in seedlings growing in the open than in those growing in the heavy shade in beech and oak seedlings in this study. The greater longevity of beech and oak leaves was due to the earlier leaf development in the full sunlight than in the other treatments. In this study, the stage of leaf senescence was more advanced in beech grown in shade than in full sunlight, but similar percentages of senescence between the different treatments were found for oak. Temperature and relative humidity did not differ between the light environments (Tables 4.1 and 4.2), so light levels probably account for most of the phenological response differences. Lebourgeois et al. (2010) concluded that global solar radiation, which is linked to light intensity, plays an important role in determining phenological events. Partanen et al. (2001) suggest that besides temperature, light conditions might have an effect on spring phenology of Norway spruce (Picea abies L. (Karst.)). According to McGee (1975), oak seedlings (northern red and scarlet oak; Quercus rubra and Q. coccinea respectively) growing in the shade in a deciduous forest broke dormancy significantly earlier than those growing in the open. However, Augspurger (2008) found that spring phenology did not differ between light treatments for Ohio buckeye (Aesculus glabra Willd.) while there were slight differences in bud break and leaf expansion between treatments for sugar maple (Acer saccharum Marsh.). In this study, the buds of seedlings of both species grown in the open and light shade treatments flushed earlier than those grown in the medium or heavy shade during the two years of the study. This finding is consistent with previous research conducted on beech, where high light intensity advanced budburst by about 4 days on average (Caffarra and Donnelly, 2011).
Leaf phenology differed markedly between beech and oak seedlings in this study. Several studies suggest that leaf phenology is species dependent (Lopez et al., 2008; Chuine, 2010; Caffarra and Donnelly, 2011). Oak seedlings reached equivalent flushing stages earlier than beech seedlings (from budburst to full leaf expansion), but differences were less evident during the last stage of leaf expansion. In contrast, the period of leaf emergence was rapid in all of the beech treatments compared with oak. This suggests that while beech buds burst much later than oak buds, leaf development of beech was much quicker after the initiation of flushing. Bud flushing is strongly affected by temperature, with warming temperatures advancing the date of flushing (Morin et al., 2010; Fu et al., 2014). The fact that the buds of beech broke dormancy later in the season might affect the duration of leaf emergence since temperatures are expected to be higher on average than earlier in the season. Spring phases, from bud-burst to full expansion of leaves, started later in the first than in the second year of the study. The earlier leaf flushing in 2014 might have been the result of the higher temperatures, as the mean temperatures were higher in April and May (main period when trees flush) in 2014 (10.0 and 12.1°C respectively) than in 2013 (7.6 and 10.6°C respectively). Moreover, the earlier flushing in 2014 was not likely an artefact of plant ageing since the increasing age of trees would be expected to delay the start of leaf emergence (Menzel and Fabian, 1999; Seiwa, 1999). The duration of leaf senescence was longer for oak seedlings which, linked to the early budburst, resulted in greater leaf longevity (except for the bottom part of seedlings growing in full sunlight). This difference in the bottom half part for seedlings in the open might be due to self-shading. In contrast to a recent study which suggests that early leaf flushing translated into earlier leaf senescence (Fu et al., 2014), this trend in leaf phenology did not emerge in this study, suggesting that date of leaf senescence does not relate to chronological leaf age and might be triggered when environmental conditions starting to be less favourable in the autumn season.

A longer growing season is likely to contribute to increase biomass and growth (Menzel and Fabian, 1999). In this study, shading reduced leaf longevity as well as diameter growth in both species, with a greater reduction in beech than in oak. In addition to this, leaf longevity was correlated with stem diameter growth,
similar to the findings of Augspurger (2008). On the other hand, height growth did not mirror leaf longevity in response to light availability. This might be due to the fact that height growth does not provide reliable information about seedling performance, as found by previous research (O'Reilly et al., 2001).

Early leaf development might increase plant vulnerability to frost (McGee, 1975) and herbivore damage (Wesolowski and Rowinski, 2008). On the other hand, early spring leaf-out might result in greater carbon gain, which might favour greater survival and growth (Augspurger, 2008). Therefore, differences in phenology between species and light environments might have important forest management implications. Although further investigations are required, differences in phenology in response to light levels, which were also associated with differences in diameter growth, were evident in this study of beech and oak seedlings. Phenological information should be considered when planting beech and oak seedlings at open sites or under existing canopies. Since oak seedlings flushed earlier than beech, special attention should be paid to spring frost risk when planting oak in the open because of the absence of shelter. While both species had similar duration of leaf longevity and diameter growth at full light, oak seedlings responded better than beech, having a longer growing season and greater growth, as shade levels increased. This suggests that despite the higher shade tolerance of beech widely reported in the literature (e.g. Joyce et al., 1998; Welander and Ottosson, 1998), oak seedlings would have greater growth than beech as a result of greater leaf longevity under an existing canopy.

The results showed a similar trend in the bottom and top part of beech and oak seedlings for all the variables studied in response to light availability. Only two of the variables studied (duration of leaf senescence and leaf longevity) showed a different trend in the bottom half and top half parts, which occurred in the full sunlight treatment only. This suggests that the phenological assessments could have been simplified, relying on observations from one part of the seedling. Although treatment trends were similar in the bottom and top part of a tree, differences were more pronounced for some variables in the bottom part (timing of leaf flushing and leaf longevity) and for others in the top part (leaf senescence index).
4.5 Conclusions
Seedlings of both species in the open flushed earlier, had longer duration of leaf senescence and greater leaf longevity than those grown under lower light environments, although all differences were not significant. The greatest differences were found between seedlings grown under full sunlight and those under heavy shade. The buds of seedlings flushed earlier and leaf senescence occurred over a longer period in oak than in beech, resulting in greater leaf longevity in oak than in beech. This meant that seedlings growing in the open could exploit the growing season for longer than those growing in the shade, thus probably leading to greater growth rates in the open. The results suggest that both species, especially oak, are suitable for underplanting as these species might perform well under different light conditions similar to those used in this study. However, further work over longer periods of time are required to gain a better understanding of phenological adaptations of tree species to light availability and their implications on silvicultural management.
4.6 References


Chapter 5

The stem quality in seedlings of *Fagus sylvatica* and *Quercus robur* seedlings under different light availabilities

Abstract
The production of high quality timber is necessary for a multipurpose management of broadleaf forests. The quality of the trees is influenced by competitive interactions, where light plays an important role and can be modified by silvicultural practices. The effects of light conditions, from full light (100 % of photosynthetically active radiation, PAR) to heavy shade (28 % of PAR), on seedling quality and form of 4-year-old oak (*Quercus robur* L.) and 5-year-old beech (*Fagus sylvatica* L.) seedlings were assessed during two growing seasons in this study. Seedling form, shoot dieback and branchiness (branch number and branch weight) were evaluated. Light condition had no effect on the quality and architecture of beech and oak seedlings. Beech seedlings showed better quality in all treatments during the second growing season, but oak seedlings performed similarly over the two growing seasons. The results of this study indicate that both species had a good growth form when grown under different light regimes, and therefore, are suitable for underplanting under a wide range of silvicultural options.

5.1 Introduction
In addition to environmental and social services, the production of timber is one of the most important forest management objectives. The good growth and form of forest stands is important to maintain timber supply to the forest industry. Despite the increasing interest in broadleaves in European forestry, in comparison to some conifers, there has been little research on tree growth and quality of broadleaf stands, from environmental factors to silvicultural interventions. While conifers are relatively easy to grow and manage (they tend to have good apical dominance and are less prone to forking), it is more difficult to grow high quality broadleaves (Joyce *et al.*, 1998; Savill, 2003). From a commercial point of view the lower section of the stem is the most valuable part of any tree as this is the part which returns the greatest final income (Bulfin, 2003).
The quality of a forest stand is influenced by silvicultural management, such as choice of species and provenances (COFORD, 2002a), spacing (Kerr and Morgan, 2006), weed control (Evans, 1984), mammal protection measures (Kerr and Evans, 1993), shaping (Bulfin and Radford, 1998) and thinning (Savill and Evans, 2004). Assuming that site preparation and planting methods are optimal, post-planting maintenance is the next important step to maximize future timber revenue and other benefits. Therefore, the quality of broadleaf seedlings following planting is a factor to consider because it is difficult to convert poor quality young stands into good quality mature stands. Some of the major determinants of wood quality potential and timber value are size dimensions, presence of knots and forks, straightness of stem, branchiness, vigour, regular growth and colour (Evans, 1984; Joyce et al., 1998; Struck and Dohrenbusch, 2000; Rock et al., 2004; Short and Radford, 2008; Saha et al., 2012). Shoot dieback, which has an effect on flowering and crown density, may lead to the development of poor quality stems (O’Reilly and Cabral, 2008).

It has been demonstrated that branchiness of young broadleaf species depends on a number of factors such as site conditions (Struck and Dohrenbusch, 2000; Kint et al., 2010), genotype (Jensen, 1993; Kleinschmit, 1993; Harmer, 2000), tree density (COFORD, 2002a; Kint et al., 2010; Sagheb-Talebi and Schütz, 2012), stand development (Nicolini, 1998; Kint et al., 2010) and light availability (Wilson, 1990; Nicolini and Caraglio, 1994; Rozenbergar and Diaci, 2014). In addition to the effect on branchiness, variation in understory light may also affect other factors involved in tree architecture and, therefore, cause differences in the quality of trees (Oliver and Larson, 1996; Rozenbergar and Diaci, 2014). Thinning, which reduces the density of trees per unit area and hence increases the amount of light available to the remaining trees, can be used to improve stem quality in both conifers and broadleaf stands (Stirling et al., 2000; Cameron, 2002; Savill, 2003). It is well known that broadleaf tree species are capable of altering their architecture and growth in low light environments (Beaudet and Messier, 1998; Takyu, 1998; Cho et al., 2005; Rozenbergar and Diaci, 2014). While these modifications, such as plagiotropic growth (Sagheb-Talebi and Schütz, 2002), may have a positive impact on seedling survival, they can negatively affect the potential future quality of the trees.
To fulfil the increasing interest in continuous cover forestry (CCF), especially for broadleaves, it is essential to find suitable species to grow under different CCF management scenarios. In addition to a range of environmental (conservation of ecosystems, protection of soil and environment) and social (recreation, amenity and cultural aspects) benefits, management under CCF silvicultural systems might provide higher productivity and a continuous income of wood products (Mason et al., 1999; Vítková and Ní Dhubháin, 2013; Puettmann et al., 2015). Although natural regeneration is preferred, planting is appropriate when natural regeneration fails (lacking seed trees or having ones not adapted to a site), a change in tree species/genotypes is required or managing poor quality woodlands (Evans, 1984; Wagner et al., 2010; Dey et al., 2012). In order to identify what level of shading is appropriate when underplanting, it is necessary to have a good knowledge of the effects of light availability on form and quality of the seedlings.

The species chosen for study, European beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.), are among the most important broadleaf species of the European Continent. Beech is described as shade-tolerant, especially suitable for underplanting procedures and it is generally accepted that beech shows better form when developing under moderate light levels (COFORD, 2002b; Mountford et al., 2006). On the other hand, oak is considered a light-demanding species and underplanting is advisable, in many cases, after overstory density has been reduced (Dey et al., 2012).

The main purpose of this study was to determine the impact of shade on stem form and quality of planted oak and beech seedlings. Since the aim was to focus on the response of the study species to different shade levels, beech and oak seedlings were exposed to different light conditions in a simulated semi-controlled experiment in a green-field site.

### 5.2 Material and methods

#### 5.2.1 Study site and tree species

The study was conducted in a controlled-shade experiment located at Teagasc Ashtown Food Research Centre, Dublin, D15 DYO5, Ireland (53°22′45″ N, 6°20′13″ W, 40 m ASL). Two-year-old seedlings (1u1, 50-80 cm height) of pedunculate oak and three-year old (1u1u1, 50-80 cm height) European beech
were purchased from a Coillte nursery, Ardattin, Co. Carlow, Ireland (52°43'47" N, 6°41'13" W, 104 m ASL) and planted at the Teagasc Food Research Centre in March 2011. The provenances used were according to provenance recommendations in Ireland (COFORD, 2002a): beech provenance was Cirencester Region 404, United Kingdom, origin unknown (51°43'0" N, 2°0'0" W, 140 m ASL) and the oak provenance was NL.S. Nuenen 03, Netherlands, origin unknown (51°29'9" N, 5°32'9" E, 20 m ASL). The experimental area was fenced to exclude rabbits and hares. Weeding was carried out when required. The mean annual total rainfall in the region is 774 mm and the mean annual air temperature is 9.8 °C (all means are from the period 1981-2010). The weather conditions from 2011 to 2014, the period when this study was conducted, were similar with respect to temperature but rainfall during the growing season was much higher in 2012 (564 mm) than in the other years of the study (287, 282 and 336 mm for 2011, 2013 and 2014, respectively. Climatic data were collected by a weather station (Met Éireann, Phoenix Park) located 1.93 km away at an open site.

Seedling heights and stem diameters (3 cm above root collar) were recorded at time of planting. The mean (± SE) seedling heights were 61.1 ± 0.5 cm for beech and 75 ± 0.6 cm for oak. The mean (± SE) stem diameters were 8.7 ± 0.1 mm for beech and 7.3 ± 0.1 mm for oak.

5.2.2 Experimental design and shade treatments
The experiment had four light treatments (full sunlight, light shade, medium shade and heavy shade; Table 1) and two species (beech and oak). Each species and light treatment combination was replicated five times using a split plot design, with the light treatment corresponding to the whole plot and the tree species to the subplot. This resulted in twenty plots (11 m × 4.3 m × 2.9 m high), each containing two subplots separated by 1 m. Plots were spaced apart from each other to minimise any interaction effects. The subplot measurement area entailed 16 seedlings (planted at 0.5 m spacing to encourage interplant competition) and was surrounded by a buffer line to help mitigate any potential edge effects. Green polythene shade nets (Colm Warren Polyhouses Ltd., Kilmurray, Trim, Co. Meath, Ireland) were erected on frames to simulate the different shade environments
(light, medium and heavy shade) in September 2012, about 18 months after the
seedlings were planted. Plots without shade nets served as controls (full sunlight).

On October 2013, photosynthetically active radiation (PAR, \( \lambda = 400\text{–}700 \text{ nm} \)) was measured in each plot. A LI-190SA quantum sensor (LI-
COR Inc., Lincoln, Nebraska) placed in the open and a LI-191SA line quantum
sensor (LI-COR Inc., Lincoln, Nebraska) placed in each plot were used to record
PAR in each environment. These sensors were linked to a datalogger (LI-1400
(LI-COR Inc., Lincoln, Nebraska)) which recorded instantaneous readings of both
sensors simultaneously. The %PAR in each treatment was determined as the
ratio of PAR below the nets to the PAR in the open (using PAR values recorded
at the same time). On January 2014, soil water content was measured in each
plot using a WET sensor (68 mm of depth, Delta-T Devices Ltd, Cambridge, UK)
to determine the level of rainfall interception by the covers. Red/far red ratio
(R/FR, \( \lambda = 660\text{–}700 \text{ nm} \)) was measured in March 2014 with a Skye SKR 110
sensor (Skye Instruments, Powys, UK). Air temperature and humidity were
recorded in each treatment plot of the first block during the growing season of
A description of the environmental conditions in the different treatments is shown
in Table 5.1.

### Table 5.1. Light properties, soil water content (SWC), air temperature (T) and relative humidity (RH) in the different shade environments. Data are the means ± standard errors.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PAR (%)</th>
<th>R/FR</th>
<th>SWC (%)</th>
<th>T (°C)</th>
<th>RH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (full sunlight)</td>
<td>100</td>
<td>1.00</td>
<td>47.72 ± 0.83</td>
<td>13.63 ± 0.03</td>
<td>82.16 ± 0.10</td>
</tr>
<tr>
<td>Light shade</td>
<td>62</td>
<td>0.98</td>
<td>45.98 ± 0.91</td>
<td>13.57 ± 0.03</td>
<td>82.42 ± 0.10</td>
</tr>
<tr>
<td>Medium shade</td>
<td>51</td>
<td>0.98</td>
<td>44.33 ± 0.85</td>
<td>13.66 ± 0.03</td>
<td>81.06 ± 0.10</td>
</tr>
<tr>
<td>Heavy shade</td>
<td>28</td>
<td>0.92</td>
<td>39.81 ± 1.03</td>
<td>13.50 ± 0.03</td>
<td>81.33 ± 0.10</td>
</tr>
</tbody>
</table>

5.2.3 Field measurements

Seedlings from the measurement area were used for quality assessment using a
four-grade quality categorisation (Teagasc, 2005). This grading system was
based on assessment of stem straightness, leader dominance, the presence or
absence of form defects along the main stem (heavy branches, forks) and the
need of shaping (Fig. 5.1). In this standard grading system, Grade 1 is a very
good quality, well-formed seedling and a Grade 4 seedling has such a poor form
that formative shaping is not worthwhile. Seedlings of intermediate categories (Grade 2 and Grade 3) may be remedied by shaping or other silvicultural treatments or may improve gradually over a period of time in response to natural processes (Bulfin, 2003). Quality assessments were carried out after leaf fall and before the start of the growing season during the two years of the study (2013 and 2014). All seedlings were also measured for height (from the ground to the tip of the highest living branch and extending leaders to full length for measurement) and stem diameter at 3 cm above the ground.

![Fig. 5.1](image.png)

Fig. 5.1. Standard quality grades for young broadleaved trees (Teagasc, 2005).

For all seedlings from the measurement area in each treatment plot, the total number of first-order living branches (without branch diameter limit) was counted before the start of the growing season.

At the end of the two growing seasons (2013 and 2014) in September (before the beginning of leaf senescence), the seedlings were assessed using the following categorical index to determine dieback symptoms: 0, dead shoot; 1, < 25% of the shoot system had healthy leaves; 25%–75% of the shoot system had healthy leaves; 3, > 75% of the shoot system had healthy leaves (Cabral and O’Reilly, 2005).

At the end of the experiment in February 2015, three seedlings per species and treatment were randomly selected and removed from the ground. The
branches of each of the seedlings were separated from the stem and dried in an oven at 105 °C until a constant dry weight was obtained.

5.2.4 Data analyses
Data were analysed using the MIXED procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Fixed effects were light, species and their interaction. Random effects were block and block x light interaction; the latter to account of the split plot structure. Repeated measures models (including year in the model) were used where the same assessment was repeated on several years (quality and dieback index). Where significant effects (interactions, or main effects if no interaction) were found, means were separated using Tukey’s adjustment at a significance level of 0.05. Means are reported as least square means and their standard errors. All data were checked for normality and homoscedasticity.

The proportion of seedlings categorised as Grade 1 (very good form) and Grade 2 (good form) are presented, but treatment effects were analysed for all categories using MIXED procedure of SAS.

Since there was no change in the number of first-order living branches over 2013 and 2014, only the data for 2013 are presented.

The degree of association between the growth parameters (stem diameter and height) and quality parameters (stem quality and dieback index) was evaluated using the simple linear correlation procedure in SAS.

5.3 Results
In both years most of beech and oak seedlings were classified in the intermediate quality categories (Grade 2 and Grade 3) regardless of the light treatment (Table 5.2). The highest percentage of very poor quality stems (18.7 %) were found in 2013 (first growing season with seedlings growing at different light conditions) for beech grown at full sunlight (Table 5.2). The highest percentage of very good quality stems (14.1 %) were found in 2013 for oak seedlings grown at full sunlight (Table 5.2). The effect of light availability on stem quality was not significant (Table 5.3; Fig. 5.2). The mixed model indicated that the species x year interaction had a significant effect on the quality index of seedlings regardless of
light treatment (Table 5.3; Fig. 5.2). The percentage of beech seedlings that had good quality stems increased significantly from 2013 (39.4%) to 2014 (54.8%), indicating an increase in the quality of beech form through the years of the study (Fig. 5.2). In contrast, the percentage of good quality stems was not significantly different between years for oak seedlings (Fig. 5.2). During both growing seasons, oak had a higher percentage of seedlings with good quality than beech (Fig. 5.2).

Table 5.2. Percentage of trees of each species in each quality category for the different light treatments over the period 2013-2014. The quality categories range from 1 (highest quality) to 4 (lowest quality) (Fig. 5.1).

<table>
<thead>
<tr>
<th>Year</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality category</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Species</td>
<td>Light (%)</td>
<td></td>
</tr>
<tr>
<td>Beech</td>
<td>100</td>
<td>1.3</td>
</tr>
<tr>
<td>62</td>
<td>2.7</td>
<td>38.4</td>
</tr>
<tr>
<td>51</td>
<td>1.3</td>
<td>29.5</td>
</tr>
<tr>
<td>28</td>
<td>3.9</td>
<td>40.3</td>
</tr>
<tr>
<td>Oak</td>
<td>100</td>
<td>14.1</td>
</tr>
<tr>
<td>62</td>
<td>3.0</td>
<td>61.2</td>
</tr>
<tr>
<td>51</td>
<td>10.6</td>
<td>62.1</td>
</tr>
<tr>
<td>28</td>
<td>4.1</td>
<td>67.1</td>
</tr>
</tbody>
</table>

Table 5.3. Repeated-measures ANOVA summary for the main effects of species (S, df = 1), light (L, df = 3), year (Y, df = 1) and their interactions on the quality and dieback index. Significant effects are in bold (p ≤ 0.05).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Species (S)</th>
<th>Light (L)</th>
<th>Year (Y)</th>
<th>S x L</th>
<th>S x Y</th>
<th>L x Y</th>
<th>S x L x Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality index</td>
<td>&lt;0.001</td>
<td>0.900</td>
<td>&lt;0.001</td>
<td>0.699</td>
<td><strong>0.005</strong></td>
<td>0.458</td>
<td>0.453</td>
</tr>
<tr>
<td>Dieback index</td>
<td>0.273</td>
<td>0.318</td>
<td>&lt;0.001</td>
<td>0.551</td>
<td>0.864</td>
<td>0.904</td>
<td>0.845</td>
</tr>
</tbody>
</table>
Fig. 5.2. Percentage of beech and oak seedlings with good stem quality (Grade 1 and Grade 2 categories combined) in each PAR treatment during two growing seasons. Data are means and standard errors (n = 5 reps). Black circles are the means (averaged over light treatments) by species and year (means with the same letter are not significantly different).

Species or light treatment did not significantly influence the dieback index but it was significantly affected by year (Table 5.3; Fig. 5.3). Although the percentage of the shoot system with healthy leaves was generally high (over 60%), it was lower in the second growing season regardless of species or light treatments (Fig. 5.3).

Fig. 5.3. Dieback for beech and oak seedlings grown at different percentage of PAR during two growing seasons. Data are means and standard errors (n = 5 reps). Black circles are the means (averaged over light treatments) by species and year (means with the same letter are not significantly different).
The results of mixed model analysis indicated that the light treatment had no significant influence on branch number or branch weight at the end of the second growing season (Table 5.4). While there was no significant effect of species with respect to number of branches this was not true for branch weight (Table 5.4). Branch weight averaged over light treatments was significantly greater in beech (203.93 ± 18.86 g) than in oak seedlings (129.84 ± 18.86 g).

Table 5.4. Summary of ANOVA for the main effects of species (df = 1), light (df = 3) and the interaction (df = 3) on the number of first-order living branches (BranchNumber) and branch weight. Significant effects are in bold (p ≤ 0.05).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Species</th>
<th>Light</th>
<th>Species x Light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>BranchNumber</td>
<td>2.29</td>
<td>0.141</td>
<td>0.11</td>
</tr>
<tr>
<td>BranchWeight</td>
<td>8.53</td>
<td><strong>0.010</strong></td>
<td>2.29</td>
</tr>
</tbody>
</table>

The linear correlations between growth and seedlings quality were low. There was only a moderate negative relationship between height and quality index for beech seedlings during the first growing season, which indicated that the quality of the seedlings increased (grade scores declined) as height increased (Fig. 5.4).

Fig. 5.4. Correlation between height and stem quality in beech seedlings during the first growing season of the study (2013). The quality index range from 1 (highest) to 4 (lowest quality) (Fig. 5.1).

5.4 Discussion
In the present study, light availability did not have a significant effect on the quality and architecture of beech and oak seedlings: quality grade, dieback index, branch number or branch weight. A similar response on architectural quality of beech saplings was reported by Diaci and Kozjek (2005) in a light interval from
30-80%. This result is in contrast with previous studies that found light availability significantly affected the quality of broadleaf seedlings (Nicolini and Caraglio, 1994; Stancioiu and O’Hara, 2006; Petriţan et al., 2009; Rozenbergar and Diaci, 2014), although Rozenbergar and Diaci (2014) reported that the influence of light on the architecture of young beech decreased substantially with time (5-year period). However, these authors suggested that PAR below 30% should be avoided to reduce the risk of poor stem quality in naturally regenerated beech and PAR values lower than 28% were not used in this study. Furthermore, in this study seedlings were grown at full sunlight before being exposed to shade, unlike the published findings compared above. Therefore, responses to light levels may be different in underplanted seedlings than in naturally regenerated plants. Nevertheless, light quantity was one of the main factors that affected the quality of underplanted beech seedlings, with seedlings exhibiting better form as light intensity increased (Kazda, 2005).

The stem quality (based on an assessment of straightness, apical dominance and existence of forks) of beech seedlings increased from 2013 to 2014, but no differences between years were found for oak seedlings. Similarly, Rozenbergar and Diaci (2014) found that the probability of plagiotropic (horizontally oriented) growth and stem forking of beech decreased over time (5-year period). The better stem quality of beech seedlings in the second growing season was probably due to an increase in the number categorised as grade 2 seedlings. This is in agreement with Bulfin (2003), who reported that trees of intermediate quality categories may be remedied by natural processes over a period of time. Collet et al. (1998) found that in sessile oak (Quercus petraea (Matt.) Liebl.) the reduction in the number of forked stems was related to a reduction in the persistence of the existing forks, rather than to a reduction of the appearance of new forks. The percentage of healthy leaves of the shoot system (which gives an indication of the degree of shoot dieback) decreased from the first to the second growing season of the study for both species, but this did not affect stem quality.

Although a reduction of the intensity of branch development in response to shading was expected (Steingraeber et al., 1979; Holbrook and Putz, 1989; Cornelissen, 1993), this did not occur in this study. Kint et al. (2010) reported that
dense tree spacing, which results in reduction in light availability for the whole tree, led to greater self-pruning (increased the mortality of shaded branches) of oak and beech trees. Beech and oak seedlings in this study did not differ significantly in the number of branches, but beech seedlings had heavier branches than oak seedlings.

Several studies have reported poor performance of Quercus robur plantations (Bulfin and Radford, 1998; O’Reilly, 2006). However, in this study oak seedlings exhibited greater quality than beech seedlings regardless of light level. It is in contrast with findings of Bulfin and Radford (1998), who found that beech trees tend to have better quality stems than oak trees. The results from this study suggest that oak seedlings may have an acceptable stem quality when grown under light conditions that favour growth and survival (see Chapter 2). Although the quality of beech seedlings was not as good as the oak seedlings, their quality improved from the first to the second growing season.

Although this study provides some information on the short-term effects of light conditions on the stem quality of two important broadleaf species, it also has some shortcomings. The study would have probably benefited from including a more extreme shade level, considering that several studies have reported a threshold for risk of poor stem quality below 30% of full light (Stancioiu and O’Hara, 2006; Petrițan et al., 2009; Rozenbergar and Diaci, 2014). Since the rotation lengths of oak and beech are long, more time is required to provide reliable information on the stem form of these species growing under different light levels.

5.5 Conclusions
The results of the study showed that stem quality, shoot dieback, branch number and branch weight of beech and oak seedlings did not change in response to different light conditions over a short period of time. Stem quality of beech improved with time (from the first to the second growing season) regardless of the light treatment, while the stem quality of oak seedlings was similar during both growing seasons. Overall, both species had an acceptable stem form, although oak exhibited better form than beech, which indicates that both species are suitable for underplanting under a wide range of light conditions and there is no
evidence that this will have a long-term negative effect on the future quality of the crop.
5.6 References


Kleinschmit, J. 1993. Intraspecific variation of growth and adaptive traits in European oak species. *Annals of Forest Sciences* 50 (Supplement), 166s–185s.


Chapter 6
Photosynthetic performance of *Fagus sylvatica* seedling regeneration in response to a natural light gradient

Abstract
Commitment to sustainable forest management (alternatives to clearfelling) has led to a renewed interest in continuous cover forestry systems, which promote the control of light to produce stand benefits. To examine the photosynthetic performance of shade-tolerant European beech (*Fagus sylvatica* L.) seedlings in response to light availability under natural conditions (in contrast to the core experimental results under artificial-shade conditions), the physiological responses of naturally regenerated beech seedlings were studied under five canopy types, from open gaps to closed canopy, during the summer of 2014 in a forest located in the Mid-East Region of Ireland. Although beech seedlings had lower photosynthetic capacity under a closed canopy than in the gaps, they were able to maintain positive assimilation rates under low light levels. Leaves of seedlings under closed canopy had the ability to use light more efficiently (higher PSII efficiency) than those in the gaps. The current photosynthetic observations offer some physiological explanation for the ability of beech seedlings to regenerate naturally under very low light conditions, although they might grow more rapidly in open gaps.

6.1 Introduction
European beech (*Fagus sylvatica* L.) is a dominant late successional species covering a large geographic area of Europe. Beech is an important tree in Europe, in terms of ecology and also commercial value. Although beech is not a native broadleaf species in some parts of Europe, such as Ireland and north of England, it has become widely naturalised there (Joyce *et al*., 1998). For trees growing in a forest understory shade has been considered an important factor that limits growth and survival (Chen, 1997). Besides light availability, other resources such as water and nutrients may be also important for seedling performance in the understory (Kloeppel *et al*., 1993; Abrams and Mostoller, 1995; Walters and Reich, 1997). However, it is possible to artificially manipulate light levels in a forest. For example, canopy gaps can result from silvicultural
practices (e.g., thinning), as well as from natural causes (e.g., windblow, storm damage). Tree seedlings can respond to changes of light conditions by modifying several traits to increase light utilisation, including leaf physiology (Bazzaz, 1979). European beech is considered to be a shade-tolerant species, being able to grow under shade levels as low as 5% of full sunlight (Niinemets and Valladares, 2006).

Silvicultural methods can be successfully used to manipulate the growth of beech stands, with natural regeneration commonly being used (provided seed source is adequate) to restock the stand (Wagner et al., 2010). Forest management objectives in Europe currently include sustaining multiple services and values from forests (FAO, 2010), often by using continuous cover forestry (CCF) silvicultural systems (Vítková and Ní Dhubháin, 2013), which promote the full use of natural dynamic forest processes (e.g. natural regeneration). There is also interest in reducing regeneration and management costs, while also maintaining structural and species diversity and producing high quality timber (Diaci and Kozjek, 2005). CCF promotes forest management which optimises the maintenance, conservation and use of forest ecosystems in such a way that the ecological and socio-economic functions are sustainable and profitable (Pro Silva, 2012). Therefore, where practical and appropriate, natural regeneration should be the preferred method of regenerating broadleaf stands, since it offers many benefits in terms of costs, genetics, silviculture and the environment (Joyce et al., 1998; Brang et al., 2014).

Photosynthesis is a physiological process of primary importance for plants and the photosynthetic response of leaves and physiological plasticity to light availability are of great interest. Previous studies have considered the influence of light availability on the physiological responses of beech seedlings (Tognetti et al. 1997; Valladares et al. 2002; Parelle et al., 2006; Balandier et al., 2007; Čater and Simončič, 2009; Gardiner et al., 2009; Čater, 2010). These authors reported that beech seedlings acclimate to shade, such as by decreasing maximum photosynthetic rates (Valladares et al., 2002; Čater and Simončič, 2009; Gardiner et al., 2009) and electron transport rates (Parelle et al., 2006; Balandier et al., 2007) with increasing shade levels. While most of these studies were carried out exclusively under artificial shade (Tognetti et al. 1997; Valladares et al. 2002) or
natural conditions (Balandier et al., 2007; Čater and Simončič, 2009; Gardiner et al., 2009; Čater, 2010), only Parelle et al. (2006) examined beech acclimation to shade under both natural and controlled conditions. Although studies conducted under artificial shade may provide useful information on the physiological responses to light availability, such experiments may also have some drawbacks, especially if the results are to be extrapolated to field conditions. Therefore, more research is required to determine whether the physiological responses to light availability in seedlings growing under artificial shade differ from that of naturally regenerated seedlings growing under natural shade conditions in the field.

In the main experimental study, changes in physiological responses of beech and oak seedlings grown under different shade conditions were investigated in a simulated semi-controlled experiment (Chapter 3). It had not been possible to identify a natural-shade control site for *Q. robur*, so the following experiments were restricted to *F. sylvatica*. In this study, the physiological responses of naturally regenerated beech seedlings under different light regimes in a forest were compared to determine which light levels optimise the photosynthetic performance of beech seedlings. The main objectives were: (i) determine if beech seedlings exhibited physiological acclimation in response to light availability (e.g., decrease in CO₂ assimilation rate with increasing shade); (ii) determine if the seedlings responded similarly under natural-shade as compared with artificial-shade conditions.

6.2 Material and methods

6.2.1 Study area

The study was conducted in Knockrath Forest, which is located in the Vale of Clara between Laragh and Ruthdrum, Co. Wicklow, Ireland (52°57′13″ N, 6°14′32″ W, 115 m above sea level). There is a long history of forest management at Knockrath using a wide range of conifer and broadleaved species, both as pure stands and in mixtures. Since it is located adjacent to the Wicklow Mountains National Park, the Vale of Clara nature reserve, the Avonmore River (a recovering salmonid habitat) and is in an important scenic and recreational area, Knockrath Forest is in the process of conversion to CCF
management which aims to achieve the multipurpose objectives, including recreation, amenity, timber production, carbon sequestration and biodiversity.

The soil type is an acid brown earth. Mean temperatures range from 5.7 °C in January to 15.8 °C in July, with a mean annual temperature of 10.2 °C, based on data obtained for the nearby Glenealy weather station (Met Éireann, Glenealy), located 13 km from the site (all means are from the period 1999-2014). The region receives 1213 mm in average annual precipitation. In 2014, the year this study was conducted, about 486 mm of precipitation was recorded between April and October.

The stand is composed of beech, Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Scots pine (*Pinus sylvestris* L.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Norway spruce (*Picea abies* (L.) Karst.). The site has abundant natural regeneration of beech seedlings. The plantation age is mixed and the mature beech from which the regeneration arises is estimated to be approximately 110 years old, but the trees vary in age. Beech regeneration is of mixed age but generally from 1 to 15 years old.

6.2.2 Experimental design and canopy treatments

This experiment was conducted as a larger research effort to examine the physiological responses of beech seedlings in different light environments. Five small research plots of 3 m radius were laid down to cover a range of different light regimes, from closed canopy to open gaps.

Light availability in each plot was evaluated using the method described in Parent and Messier (1996) and verified for mixed-species stands with irregular canopies (Messier and Parent, 1997; Gendron *et al*., 1998). These authors showed that an instantaneous measurement of the percentage of above-canopy photosynthetically active radiation (PAR, 400–700 nm) taken under overcast sky conditions provides an accurate estimate of the mean daily percentage of PAR reaching a location in the understory (%PAR). Instantaneous light measurements were taken on 12th of September 2014. Above-canopy PAR (PARa) was measured using a point quantum sensor (LI-190SA, LI-COR Inc., Lincoln, Nebraska) installed in the open gap. A second line quantum sensor (LI-191SA, LI-
COR Inc., Lincoln, Nebraska) was used to measure PAR above understory seedlings (PAR$_a$) in each plot. Both sensors were linked to a datalogger (LI-1400, LI-COR Inc., Lincoln, Nebraska). The time of each measurement was recorded and %PAR was calculated as (PAR$_u$/PAR$_a$)×100, where PAR values were recorded at the same time. Percentages of above-canopy light reaching the understory in each plot are presented in Table 6.1.

Table 6.1. Mean values and ranges of the PAR measurements from the five plots in this study.

<table>
<thead>
<tr>
<th>Plot number</th>
<th>Canopy type</th>
<th>Mean PAR</th>
<th>Range PAR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Open gap (OP)</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Moderate shade (MS)</td>
<td>65</td>
<td>63.1–65.2</td>
</tr>
<tr>
<td>3</td>
<td>Medium shade (MES)</td>
<td>37</td>
<td>36.3–38.3</td>
</tr>
<tr>
<td>4</td>
<td>Heavy shade (HS)</td>
<td>25</td>
<td>24.9–25.4</td>
</tr>
<tr>
<td>5</td>
<td>Very heavy shade (VHS)</td>
<td>14</td>
<td>12.9–14.5</td>
</tr>
</tbody>
</table>

Five beech seedlings 1–2 m in height were randomly selected in each plot. The selected seedlings were approximately the same size as those used in the shadehouse experiment in 2014 (Chapter 3).

6.2.3 Physiological measurements
CO$_2$ assimilation rate (A, μmol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance ($g_s$, mmol H$_2$O m$^{-2}$ s$^{-1}$) and transpiration rate ($E$, mmol H$_2$O m$^{-2}$ s$^{-1}$), expressed on a leaf area basis, were measured between 15 and 19 September 2014 with a portable photosynthesis system LI-6400XT (LI-COR Inc., Lincoln, Nebraska, USA) on previously tagged beech seedlings located in the study plots. Measurements were carried out on fully expanded, non-senescent and healthy leaves from the upper terminal shoot of each seedling and leaves were kept as close to their natural position as possible during measurements. In each plot, leaf gas exchange measurements were recorded under ambient conditions of air temperature, humidity and PAR, with the reference CO$_2$ concentration maintained at 400 μmol mol$^{-1}$. Gas exchange measurements were also carried out at common light levels in all plots: 1500 and 500 μmol m$^{-2}$ s$^{-1}$. These PAR values were used because data collected during gas exchange measurements in a shadehouse experiment revealed that photosynthesis of beech seedlings was saturated at around 1500 μmol m$^{-2}$ s$^{-1}$ and differences in photosynthesis became
apparent at 500 μmol m^{-2} s^{-1} (see Chapter 3). While conducting these measurements, CO₂ concentration was kept at 400 μmol mol^{-1}, block temperature was set to 25°C and relative humidity was around 40%. Values were recorded after short adaptation when CO₂ exchange had remained stable. The ratio of A to E and A to gₛ were calculated to determine instantaneous (A/E, μmol CO₂/mmol H₂O) and intrinsic (A/gₛ, μmol CO₂/mol H₂O) water use efficiency, respectively.

Chlorophyll fluorescence was measured simultaneously with gas exchange under ambient conditions using the portable LI-6400XT equipped with a leaf chamber fluorometer LI-6400-40 (LI-COR Inc., Lincoln, Nebraska, USA). Under known light conditions, the steady-state level of fluorescence (F'), the maximum fluorescence (F’ₘ) and the minimal fluorescence (F’₀) were estimated according to common protocols for fluorescence analysis at a known light intensity (Murchie and Lawson, 2013). F’₀ and F’ₘ were determined by applying a dark and a saturating pulse to a light-adapted leaf, respectively. The operating efficiency of photosystem II (ΦₚₛₛᵢⅡ), PSII maximum efficiency (F’ₙ/F’ₘ) and photochemical quenching (qP) were calculated as $(F’ₘ – F’)/F’ₘ$, $(F’ₘ – F’₀)/F’ₘ$ and $(F’ₘ – F’)/(F’ₘ – F’₀)$, respectively. The photosynthetic electron transport rate (ETR, μmol (e⁻) m^{-2} s^{-1}) was calculated as $ΦₚₛₛᵢⅡ × f × I × α_{leaf}$, where $f$ is the fraction of absorbed quanta that is used by PSII, $I$ is the incident PAR and $α_{leaf}$ is the leaf absorptance (LI-COR, 2011). $f$ was assumed to be 0.5 (Laisk and Loreto, 1996) and the average value of $α_{leaf}$ for green leaves of 0.84 was used (Björkman and Demmig, 1987).

6.2.4 Phenotypic plasticity
Plasticity of physiological traits for beech seedlings were calculated based on the phenotypic plasticity index, PIᵥ (Valladares et al., 2006). This index, ranging from zero to one, is the difference between the minimum and the maximum mean values of a trait divided by the maximum mean value (Valladares et al., 2000). This index allows changes in traits expressed in different units to be compared. Mean physiological plasticity was the average plasticity value for all physiological traits.
6.2.5 Data analysis
All statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Physiological responses were analysed using the MIXED procedure of SAS with light availability below each canopy type considered a fixed effect. Because of the large variability within plots and the small sample sizes used, regression analysis was used to determine if nominal light availability below each canopy type explained most of the variation in the responses. The light availability was treated as a quantitative variable that explained the photosynthetic response. A linear regression as a function of available light was fitted for each physiological trait. All tests for significance were conducted at $p \leq 0.05$. Normal distribution of errors and homogeneity of variance were assessed graphically.

Additionally, Pearson correlations were carried out to identify linear relationships between physiological variables.

6.3 Results
6.3.1 Leaf gas exchange and chlorophyll fluorescence
The photosynthetic capacity and stomatal conductance of beech seedlings at saturating light ($A_{1500}$ and $g_{s,1500}$, respectively) were significantly influenced by shade (Table 6.2). In contrast, water use efficiency at saturating light was not affected by shade (Table 6.2). $A_{1500}$ and $g_{s,1500}$ did not change from gap (100% PAR) to moderate shade (65% PAR), but decreased thereafter with increasing shade (Fig. 6.1A and 6.1B). Beech seedlings exhibited the lowest $A_{1500}$ and $g_{s,1500}$ under very heavy shade (Fig. 6.1A and 6.1B). A positive relationship between $A_{1500}$ and $g_{s,1500}$ was found (Fig. 6.2A).

Shade did not influence photosynthetic rate, stomatal conductance or water use efficiency at 500 μmol m$^{-2}$ s$^{-1}$ PAR (Table 6.2).

Mean PAR values were 106, 60, 41, 34 and 19 μmol m$^{-2}$ s$^{-1}$ in the OP (100% PAR), MS (65% PAR), MES (37% PAR), HS (25% PAR) and VHS (14% PAR) plots, respectively. At ambient PAR, beech seedlings exhibited similar trends for photosynthetic rate ($A_{amb}$) and ETR, with values for both variables decreasing as shade levels increased (Table 6.2; Fig. 6.1C and 6.1G). $A_{amb}$ was significantly and positively correlated with ETR (Fig. 6.2E). In contrast, $\Phi_{PSII}$,
$F'_{v}/F'_{m}$ and $qP$ increased with increasing shade (Table 6.2; Fig. 6.1D, 6.1E and 6.1F). A strong, positive relationship was found between $qP$ and $\Phi_{PSII}$ (Fig. 6.2F).

Instantaneous and intrinsic water use efficiencies were always positively correlated for beech seedlings at 1500 μmol m$^{-2}$ s$^{-1}$, 500 μmol m$^{-2}$ s$^{-1}$ and ambient PAR in 2013 (Fig. 6.2B, 6.2C and 6.2D).

Table 6.2. Regression equations used to model the dynamics of different physiological traits as function of percentage of light found below each canopy type for beech seedlings ($y = a + b \times$PAR). Given are the estimates ($a$ and $b$), standard errors (SEa and SEb) and p-values ($p_a$ and $p_b$) for the coefficients and the correlation coefficient ($r^2$) of the regression equations for some physiological traits at 1500 μmol m$^{-2}$ s$^{-1}$ (1), 500 μmol m$^{-2}$ s$^{-1}$ (2) and ambient PAR (3). Traits: $A$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$): photosynthetic rate; $g_s$ (mmol H$_2$O m$^{-2}$ s$^{-1}$): stomatal conductance; ($A/g_s$) (μmol CO$_2$/mol H$_2$O): intrinsic water use efficiency; ($A/E$) (μmol CO$_2$/mmol H$_2$O): instantaneous water use efficiency; $\Phi_{PSII}$: PSII operating efficiency; $F'_{v}/F'_{m}$: PSII maximum efficiency; $qP$: photochemical quenching; ETR (μmol photons m$^{-2}$ s$^{-1}$): electron transport rate. Significant effects are in bold ($p < 0.05$).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Regression model coefficients</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a$</td>
<td>SEa</td>
</tr>
<tr>
<td>1)PAR=1500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{1500}$</td>
<td>4.4485</td>
<td>0.3358</td>
</tr>
<tr>
<td>$g_s_{1500}$</td>
<td>0.0589</td>
<td>0.0091</td>
</tr>
<tr>
<td>($A/g_s$)$_{1500}$</td>
<td>72.1950</td>
<td>5.8651</td>
</tr>
<tr>
<td>($A/E$)$_{1500}$</td>
<td>3.6771</td>
<td>0.2891</td>
</tr>
<tr>
<td>2)PAR=500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{500}$</td>
<td>4.1500</td>
<td>0.3504</td>
</tr>
<tr>
<td>$g_s_{500}$</td>
<td>0.0954</td>
<td>0.0104</td>
</tr>
<tr>
<td>($A/g_s$)$_{500}$</td>
<td>45.1668</td>
<td>4.8633</td>
</tr>
<tr>
<td>($A/E$)$_{500}$</td>
<td>3.3920</td>
<td>0.3406</td>
</tr>
<tr>
<td>3)Ambient PAR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_{amb}$</td>
<td>0.2973</td>
<td>0.2796</td>
</tr>
<tr>
<td>$g_s_{amb}$</td>
<td>0.0939</td>
<td>0.0105</td>
</tr>
<tr>
<td>($A/g_s$)$_{amb}$</td>
<td>11.715</td>
<td>3.379</td>
</tr>
<tr>
<td>($A/E$)$_{amb}$</td>
<td>1.4846</td>
<td>0.4023</td>
</tr>
<tr>
<td>$\Phi_{PSII}$</td>
<td>0.7193</td>
<td>0.0080</td>
</tr>
<tr>
<td>$F'<em>{v}/F'</em>{m}$</td>
<td>0.7553</td>
<td>0.0030</td>
</tr>
<tr>
<td>$qP$</td>
<td>0.9492</td>
<td>0.0096</td>
</tr>
<tr>
<td>ETR</td>
<td>4.7756</td>
<td>1.1392</td>
</tr>
</tbody>
</table>
Fig. 6.1. Photosynthetic rate at 1500 μmol m⁻² s⁻¹ (A), stomatal conductance at 1500 μmol m⁻² s⁻¹ (B), photosynthetic rate at ambient PAR (C), PSII operating efficiency (D), PSII maximum efficiency (E), photochemical quenching (F) and electron transport rate (G) as a function of light found below each canopy type. Regression lines represent fitted equations and symbols are the mean of the observed data for each canopy type.
Fig. 6.2. Relationships between photosynthetic rate and stomatal conductance (A), between instantaneous and intrinsic water use efficiency (B, C and D), between photosynthetic rate and electron transport rate (E), and between photochemical quenching and PSII operating efficiency (F). Measurements were made at 1500 μmol m$^{-2}$ s$^{-1}$ (A and B), 500 μmol m$^{-2}$ s$^{-1}$ (C) and ambient PAR (D, E and F).
6.3.2 Phenotypic plasticity

Physiological plasticity of beech seedlings in response to light availability was greater under ambient PAR than under controlled conditions (500 and 1500 μmol m$^{-2}$ s$^{-1}$ PAR) (Table 6.3). In response to the controlled light conditions applied in the field, beech seedlings had greater phenotypic plasticity at 1500 μmol m$^{-2}$ s$^{-1}$ (saturating light) than at 500 μmol m$^{-2}$ s$^{-1}$ (Table 6.3). The responses ranged from highly plastic (e.g., photosynthetic rate, stomatal conductance and electron transport rate) to less plastic (e.g., most chlorophyll fluorescence variables) (Table 6.3). Water use efficiency had variable plasticity depending on the PAR conditions used during measurements, i.e. highly plastic under ambient PAR but far less plastic under 500 and 1500 μmol m$^{-2}$ s$^{-1}$ PAR (Table 6.3).

Table 6.3. Plasticity index in response to different light availabilities of naturally regenerated beech seedlings for the leaf gas exchange and chlorophyll fluorescence variables studied during 2014. Variables are arranged by PAR conditions used during measurements.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Variable</th>
<th>Plasticity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR = 1500</td>
<td>$A_{1500}$</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>$g_s_{1500}$</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>$(A/g_s)_{1500}$</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>$(A/E)_{1500}$</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.31</td>
</tr>
<tr>
<td>PAR = 500</td>
<td>$A_{500}$</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>$g_s_{500}$</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>$(A/g_s)_{500}$</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>$(A/E)_{500}$</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.20</td>
</tr>
<tr>
<td>Ambient PAR</td>
<td>$A_{amb}$</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>$g_s_{amb}$</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>$(A/g_s)_{amb}$</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>$(A/E)_{amb}$</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>$\Phi_{PSII}$</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>$F'/F'_m$</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>$qP$</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>ETR</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Total mean</td>
<td>0.33</td>
</tr>
</tbody>
</table>
6.4 Discussion

Photosynthetic capacity ($A_{1500}$, $A_{amb}$ and ETR) decreased with increasing shade. Similarly to $A_{1500}$, $g_{s1500}$ also decreased with increasing shade, indicating that changes in CO$_2$ assimilation might result in changes in stomatal conductance at saturating conditions. The fluorescence responses ($\Phi_{PSII}$, $F'_v/F'_m$ and $qP$) increased as shade increased, indicating that beech leaves have the ability to use light more efficiently under low light conditions than in gaps. Water use efficiency of beech seedlings was not affected by the light regime. Overall, the trend in the physiological responses to light availability observed in this study under field conditions was similar to that observed under more controlled conditions in a separate study (Chapter 3).

The reduction of photosynthetic capacity with increasing shade agrees with the results from previous studies carried out under field conditions in planted (Balandier et al., 2007; Gardiner et al., 2009) and naturally regenerated (Parelle et al., 2006; Čater and Simončič, 2009; Čater, 2010) beech seedlings. Stomatal conductance may also be an important limiting factor for photosynthesis. Seedlings with high $A_{1500}$ also showed high $g_{s1500}$, similar to previous findings on different plant species (Wong et al., 2012b). Furthermore, a positive correlation between these $A_{1500}$ and $g_{s1500}$ was found, suggesting that stomatal limitation of photosynthesis was substantial at saturating light. However, no correlation between CO$_2$ uptake and stomatal conductance was found under ambient conditions. This suggests that, under some conditions, the relationship between CO$_2$ exchange and stomatal conductance may not hold. During damp morning conditions for example, stomatal conductance under shade was generally high enough and it was not a limiting factor for photosynthesis for four species of tropical shrubs in the family Rubiaceae (Allen and Pearcy, 2000). CO$_2$ uptake ($A$) was previously found to be related to ETR in woody plants (Han et al., 1999; Bai et al., 2008; Wong et al., 2012a, 2012b). In this study, $A_{amb}$ was positively correlated with ETR. Similarly, Jurásek et al. (2010) found that the higher ETR in beech seedlings grown in the sun (compared to seedlings grown in shade) was apparently connected with higher $A$.

The increase of $\Phi_{PSII}$, as well as $F'_v/F'_m$ and $qP$, as shade increased is a common acclimation feature of beech seedlings, which has been reported in
previous studies (Valladares et al., 2002; Einhorn et al., 2004; Špulák, 2011). Leaves acclimate to the light environment by adjusting the photochemical processes, using light most efficiently under shade conditions (Einhorn et al., 2004; Baker, 2008). The strong correlation between $\Phi_{\text{PSII}}$ and $qP$ indicated that the increase in $\Phi_{\text{PSII}}$ is associated with an increase in $qP$, which is in agreement with the current research’s study of beech seedlings under similar (artificial) shade levels (Chapter 3). Since $qP$ gives an indication of the proportion of PSII reaction centres that are open, this suggests that the reason for changes in $\Phi_{\text{PSII}}$ is the proportion of open reaction centres and not the maximum efficiency capacity of the photosystem. Exposure of leaves to high light levels can result in photoinhibition, especially in shade-tolerant species and shade-acclimated individuals (Valladares and Pearcy, 1997; Kitao et al., 2000). In this study, beech seedlings maintained $qP$ above 0.6, indicating that photoinhibition had not occurred (Ögren, 1991; Öquist et al., 1992; Einhorn et al., 2004).

Physiological plasticity (i.e. plasticity in traits related with gas exchange, chlorophyll fluorescence and water use efficiency) has been linked to an enhanced capacity to grow in high light, whereas morphological plasticity has been linked to an enhanced capacity to grow in the understory (Valladares et al., 2002; Niinemets and Valladares, 2004). The phenotypic plasticity of beech seedlings to light varied with the trait studied. While it was lower for certain physiological variables (including those related with PSII efficiency), it was higher for traits related with photosynthetic capacity. The physiological plasticity found in this study was similar to that obtained for beech seedlings under controlled light conditions in a previous study (Chapter 3).

Fig. 6.3 represents the main results of this study, compared with the findings from a previous one conducted under artificial shade conditions (Chapter 3). Although a statistical comparison was not possible, a similar overall trend was found in the physiological responses to shade of beech seedlings grown under controlled and field conditions (Fig. 4). In both experiments, $\text{CO}_2$ assimilation rates, stomatal conductance and ETR decreased with increasing shade, while PSII efficiencies ($\Phi_{\text{PSII}}$ and $F'_v/F'_m$) and $qP$ increased with shade. Similarly, Parelle et al. (2006) reported that maximum carboxylation rates and ETR decreased with shade in natural regeneration (field conditions) and potted
saplings (controlled) of beech. These findings suggest that studies conducted under controlled conditions could be used to investigate the physiological response to light availability in beech. Higher values for CO2 assimilation rate, ETR and water efficiency (A/E and A/gs) and lower values for ΦPSII and Fv'/Fm' and qP were generally found in the controlled than in the field experiment. One possible reason for this is that seedlings growing in the gaps were considered to have received 100% PAR, given that it was not possible to find an area close to the stand that received full sunlight, whereas the control seedlings in the shadehouse experiment received full sunlight. Therefore, PAR values were always much lower in the field than in the controlled experiment at similar %PAR.

![Fig 6.3](image-url)

**Fig 6.3.** Photosynthetic rates at saturating light (A1500) and ambient PAR (Aamb), PSII operating efficiency (ΦPSII) and electron transport rates (ETR) in response to different light availabilities obtained in the field and a previous controlled experiment in 2014. Data are means and standard errors. See Chapter 3 for controlled experiment details.

From a silvicultural perspective, light is the main factor that can be controlled and manipulated in forest stands. The greater Aamb in the open gaps than in the other plots suggest that beech seedlings are expected to have higher growth rates in the open gaps than in the heavy shade, as found in a previous study.
under controlled conditions (Chapter 3). Beech seedlings can maintain positive carbon assimilation rates and therefore can survive and grow under heavy shade (14% of PAR of that found in the gap) conditions. Similarly, previous studies have reported that *F. sylvatica* can survive and perform well in deep shade, but grows much better under higher light availability (Ammer, 2003; Coll *et al.*, 2003; Chapter 2). According to this, *F. sylvatica* could be managed under CCF systems.

### 6.5 Conclusions

The results from this study confirm that shade strongly affected physiological responses of beech seedlings. The increase in shade reduced photosynthetic capacity ($A_{1500}$, $g_{s1500}$, $A_{amb}$ and ETR) and increased the efficiency of PSII ($\Phi_{PSII}$, $F'_v/F'_m$ and $qP$). It is likely that this ability to increase PSII efficiency with increasing shade (i.e. use light more efficiently) contributes to the success of beech regeneration under low and moderate light levels. Compared with the traits measured in a previous controlled study, beech seedlings in the field exhibited a similar trend in physiology with increasing shade levels.
6.6 References


Han, Q., Yamaguchi, E., Odaka, N. and Kakubari, Y. 1999. Photosynthetic induction responses to variable light under field conditions in three species
grown in the gap and understory of a *Fagus crenata* forest. *Tree Physiology* 19, 625–634.


Chapter 7
General Discussion

The need to develop forest management systems other than clearfelling has resulted in a requirement for improved understanding of the potential of continuous cover forestry (CCF). CCF promotes tree species and structural diversity, multi-purpose objectives (social, economic and environmental functions), and the continuous maintenance of forest cover. When natural regeneration cannot be used for the transformation to CCF, underplanting is regarded as one method to achieve this conversion (Lüpke et al., 2004; Löf and Oleskog, 2005). Therefore, understanding the differences in the ecology and adaptation of seedlings to different light conditions may be critical in determining and promoting the successful establishment of tree species under CCF systems. In this study the morphological, physiological and growth responses of two important European tree species were assessed. Another objective of CCF systems is to produce good quality stems (less branching, greater straightness, etc.). In this study seedlings of light demanding pedunculate oak and shade-tolerant European beech were grown in the open (100% of full light) or in shadehouses simulating a range of underplanted conditions (62%, 51% and 28% of full light), over two growing seasons to evaluate the performance and acclimation of seedlings under different light conditions. The physiological performance of beech seedlings that had regenerated naturally under different levels of shade under a forest canopy (Chapter 6) was compared to the responses of planted seedlings grown under a range of different artificial shade conditions (Chapter 3) to determine if the response to shade was similar in both studies.

7.1 Effects of light availability on survival, growth, leaf morphology and physiology, phenology and tree quality in underplanted beech and oak seedlings

Although greater mortality with increasing shade might be expected for light demanding than for shade-tolerant species (Walters and Reich, 1996; Chen, 1997), in this study survival rates of light demanding oak and shade-tolerant beech were not affected by shade and both species had high survival rates
(> 90%) over the two growing seasons (Chapter 2). According to Niinemets and Valladares (2006) about 20% of full light is the minimum required for oak to grow, while Löf et al. (2007) reported high survival rates of oak seedlings underplanted below a Norway spruce canopy at lower light levels than 20%. The densest shade level used in this study was 28% of PAR, perhaps not sufficiently dense enough to cause significant mortality in either species.

Root collar diameter growth decreased with increasing shade for both species over the two years of the study (Chapter 2). The greater H:D ratios of shaded seedlings in comparison to seedlings at full sunlight suggest that seedlings under shade prioritised the allocation of biomass to leader height growth at the expense of diameter growth. This trend is also confirmed by the greater height growth per unit of stem biomass under shade than at full sunlight. Compared to those grown in full sunlight, oak and beech seedlings invested more resources into height than diameter growth, presumably as a means of exploiting as much light energy as possible when light availability is limited, a growth strategy that has been suggested also for other species (Canham, 1988; Lei and Lechowicz, 1990; Wang et al., 1994; Chen and Klinka, 1998). Whole-plant biomass and both components of this measure, above-ground (aerial) and below-ground (roots) biomass, generally declined for both species as shade increased (Chapter 2). This decline in growth with increasing shade has been widely reported for beech and oak seedlings (Gemmel et al., 1996; Welander and Ottosson, 1998; Löf, 2000; Ammer, 2003; Einhorn et al., 2004; Balandier et al., 2007; Gardiner et al., 2009; Čater and Simončič, 2010). However, shading generally reduced below-ground biomass more than above-ground biomass, resulting in lower root:shoot ratios with increasing shade (Chapter 2). Therefore, when light resources were limited (under shade conditions) seedlings allocated more biomass to above than below-ground parts, perhaps allowing the plants to produce more leaves and therefore increase light interception (Allaby, 1998; Welander and Ottosson, 1998). For example, several studies have reported that leaf area ratio (the ratio of the total leaf area to above-ground biomass) increased with increasing shade (Valladares et al., 2002; Gardiner et al., 2009). High leaf area ratios in the shade allow seedlings to capture more light, as it optimises leaf display for the amount of carbon invested in foliage (Delagrange et al., 2004).
Unfortunately, the leaf area ratio was not analysed in this study and, therefore, it was not possible to confirm this morphological acclimation to shade.

Oak and beech seedlings also exhibited developmental acclimation to shade at the leaf-level (Chapter 2). For example, shade acclimated beech and oak seedlings had higher specific leaf area (SLA) and lower leaf thickness ($L_{th}$) than seedlings grown at full sunlight, which is a common response of plants to shade that has been well documented for oak and beech (Van Hees, 1997; Aranda et al., 2001; Valladares et al., 2002; Curt et al., 2005; Kunstler et al., 2005; Gardiner et al., 2009; Goisser et al., 2013). This increase in SLA and reduction in $L_{th}$ is believed to optimise light interception under shade by maximising the leaf area displayed per unit of leaf biomass (Wang et al., 1994; Beaudet and Messier, 1998; Petrițan et al., 2009). However, the mechanisms to achieve these changes in SLA in response to increasing shade differed between beech and oak. While the increase of SLA with increasing shade was associated with lighter leaves in beech, it was associated with larger leaves in oak. These findings suggest that beech seedlings grown in the shade produce lighter leaves in comparison to seedlings grown at full sunlight as a way to achieve greater SLA and, therefore, optimise light interception. In contrast, oak seedlings respond to shade by increasing their leaf area, a finding that is consistent with the results of earlier studies on the acclimation of oak species to low light availability (Van Hees, 1997; Jensen et al., 2012).

At the physiological level, both species displayed greater photosynthetic capacity (including CO$_2$ assimilation at ambient and saturating light, and electron transport rate) in full light than under shade conditions (Chapter 3), which is consistent with the results of previous studies (Wyka et al., 2007; Jurásek et al., 2010; Špulák, 2011). However, the ability of shaded seedlings to photosynthesise under (applied artificially) high light conditions was more limited in beech than oak during the first growing season after the shadehouses had been erected (greater reduction in $A_{1500}$ and $A_{500}$ under heavy shade in beech than oak - Chapter 3). These results suggest that beech seedlings grown in 28% of full light were most susceptible to photoinhibition (e.g. if opened up to full sunlight, as might happen in canopy gaps following a heavy thinning operation) during the first growing season, which is in agreement with the view that photoinhibition is more
commonly found in shade tolerant than light-demanding species and in plants grown under shade than those grown at full light (Anderson and Osmond, 1987; Valladares and Pearcy, 1997; Kitao et al., 2000). However, in the second growing season beech and oak seedlings grown in the shade exhibited similar photosynthetic responses to artificially applied high light ($A_{1500}$ and $A_{500}$) levels, suggesting that beech seedlings could reduce the likelihood of incurring photoinhibition.

This change in the photosynthetic response of shaded beech to sudden light increase could be attributed to the combination of high light with another stress (e.g. high temperature, drought or lack of nutrients). For example, Robson et al. (2009) reported that drought stress adversely affected photosynthetic performance of beech seedlings in the understory. Therefore, the fact that the growing season in 2013 was drier than in 2014 could have had a negative effect on the photosynthetic response of shaded beech to high light. Mulkey and Pearcy (1992) reported that the degree of photoinhibition and the potential for recovery are dependent on leaf temperatures and high light. Since leaf temperatures during measurements at saturating light were slightly higher in the first than in the second growing season, high light in combination with high temperature could have caused photoinhibition in the first growing season and, as consequence, lower values of $A_{500}$ and $A_{1500}$. The fact that shadehouses retained leaf litter could have altered the nutrient contents in the soil, with more leaf litter in the second than in the first growing season. According to Johnson et al. (1997) beech growing in soils with high N availability experienced less photoinhibition. Furthermore, it could be that a mix of all the above mentioned stress in combination with high light led to the low $A_{500}$ and $A_{1500}$ in 2013. Another hypothesis for the change of shaded beech to high light over the two growing seasons is that taller seedlings responded better to sudden increase in light intensity.

The efficiency of the PSII generally increased with shade levels for both species, indicating that leaves increase the efficiency of light utilisation at low light. Despite the expected negative impact of shade on photosynthetic capacity, both species photosynthesised efficiently (exhibiting high photosynthetic capacity) under 62%, 51% and 28% of full light. The physiological performance of beech
seedlings in response to light availability in the field (Chapter 6) were consistent with the physiological observations from the shadehouse study, even though lower photosynthetic values and higher values related with PSII efficiency were found in the field (probably due to lower absolute PAR values in the field than in the shadehouses). Likewise, Parelle et al. (2006) reported an increase in the photosynthetic capacity of beech seedlings with increasing irradiance in natural and controlled conditions.

The buds of seedlings flushed earlier and leaf senescence occurred over a longer period under full sunlight than under shade conditions in both species (Chapter 4), so leaf photosynthesis occurred over a longer period of the growing season under full sunlight conditions. This finding agrees with a previous study where high light intensity advanced leaf flushing in *F.sylvatica* seedlings (Caffarra and Donnelly, 2011). The greater leaf longevity, along with the greater photosynthetic rates, of beech and oak leaves grown at full sunlight was likely one of the main mechanisms that led to an increase in growth at full sunlight, as suggested by the positive relationship between leaf longevity and stem diameter growth. However, early leaf development might result in an increased risk of frost (McGee, 1975; Lopez et al., 2008) and herbivore damage (Wesolowski and Rowinski, 2008). Although beech and oak seedlings responded similarly to increasing shade, delaying bud flushing and advancing leaf senescence, leaf longevity was greater in oak than beech regardless of the light conditions.

The densest level of shade (28% of full sunlight) used did not affect stem form and tree architecture in beech and oak seedlings in this study (Chapter 5). This finding is in agreement with a previous study of naturally regenerated beech saplings (Diaci and Kozjek, 2005), but contrasts with the main findings from the literature for naturally regenerated broadleaf seedlings where shade negatively affected stem form (Nicolini and Caraglio, 1994; Stancioiu and O'Harra, 2006; Petrițan et al., 2009; Rozenbergar and Diaci, 2014). However, the great majority of these authors recommended that light conditions in excess of 30% of full light are required to reduce the risk of adverse effects on stem quality. Reassuringly, the densest experimental 28% shade regime used in this study was very little different from that recommended densest shade tolerance level. Although there has been little research on how light intensity or overstory density affects the
growth and form of underplanted young trees, there is some evidence that overstory density might influence growth and form of seedlings growing in the understory (Lüpke, 2005). For example, Lüpke et al. (2004) reported that underplanted beech seedlings were straighter when grown below moderate than dense shelter, suggesting that the number of crooked stems increases with increasing shade. Perhaps the densest shade level used in this study (28% of PAR) was not sufficiently dense enough to produce changes in stem form.

Beech seedlings showed greater morphological plasticity in response to changes in light availability than oak (Chapter 2), similar to the findings of others (Valladares et al., 2002; Niinemets and Valladares, 2004; Kunstler et al., 2005). This high shade-induced morphological plasticity indicated that beech seedlings modified their morphology (including leaf, root, branch and stem mass, height and diameter growth) to a greater extent than oak seedlings, which does not necessarily imply greatest fitness across the different light conditions (Portsmuth and Niinemets, 2007), just a greater ability to alter their morphology in response to shade. Beech seedlings also exhibited greater physiological plasticity than oak seedlings in 2013 (mainly linked to a lower ability of shaded beech to respond to a sudden increase in light intensity), while small differences were found in 2014 (Chapter 3). This is in contrast to the findings of Valladares et al. (2002), who found that oak displayed greater physiological plasticity than beech (linked to a greater photosynthetic capacity of oak at full light). The SLA, $L_{\text{th}}$, root:shoot ratio, $A_{\text{lamb}}$ and ETR treatment responses were similar in both species in this study, suggesting that the species acclimation responses to different light levels are similar.

Considering all the morphological and physiological traits investigated, the main findings of this study suggest that oak seedlings planted under shade tend more towards being as shade tolerant as beech seedlings rather than being less shade tolerant, as has been generally reported in the literature (Brzeziecki and Kienast, 1994; Hill et al., 1999; Valladares et al., 2002; Niinemets and Valladares, 2006). Table 7.1 summarises the responses of beech and oak seedlings to increasing shade in this study and is compared with the responses reported previously in the literature. For example, variables such as survival rate and diameter increment, that were previously reported in the literature as being more
reduced by shade in light demanding than shade-tolerant species, did not differ between oak and beech seedlings in response to the shade levels studied (Table 7.1). Similarly, oak and beech seedlings in this study exhibited a similar trend in root:shoot ratio and SLA in response to shade, while previous studies reported greater reduction in root:shoot ratio and greater increase in SLA for shade-tolerant than light demanding species (Table 7.1). Physiological traits, such as photosynthetic rates at saturating light, decreased to a greater extent in beech than oak during the first growing season, which contrasts with findings in the literature. Other characteristics, such as stem quality or branch development, were not affected by shade, while the opposite was found in the literature. Therefore, the results of the morphological and physiological traits of oak seedlings in response to the shade levels used in this study (ranging from 62% to 28% of full light) indicated a similar, or even better, performance to those obtained for beech grown under the same conditions. The findings mentioned above suggest that at the seedling stage oak is not as shade-intolerant as previous works suggest.
Table 7.1. Summary of the responses to increasing shade levels compared with those reported previously in the literature. The most relevant characteristics in response to shade found in this study are in bold.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>General responses (findings in the literature)</th>
<th>Response per shade tolerance (findings in the literature)</th>
<th>General response (findings in the present study)</th>
<th>Response per shade tolerance (findings in the present study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>Decreases with shade (Ni Dhubháin, 2010)</td>
<td>Decreases to a greater extent in light demanding than in shade-tolerant (Kaelke et al., 2001)</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Height increment</td>
<td>Decreases with shade (Chen, 1997)</td>
<td>Decreases to a greater extent in shade-tolerant than in light demanding (Ammer, 2003)</td>
<td>First growing season increased with shade but it decreased with shade during the second growing season</td>
<td>Greater increment in beech seedlings at full light over the two growing seasons</td>
</tr>
<tr>
<td>Diameter increment</td>
<td>Decreases with shade (Ammer, 2003)</td>
<td>Decreases to a greater extent in light demanding than in shade-tolerant (Pacala et al., 1994)</td>
<td>Decreased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Height:Diameter ratio</td>
<td>Increases with shade (Petrițan et al., 2009)</td>
<td>-</td>
<td>Increased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Total biomass</td>
<td>Decreases with shade (Curt et al., 2005)</td>
<td>Decreases to a greater extent in shade-tolerant than in light demanding (Chen, 1997)</td>
<td>Decreased with shade</td>
<td>Greater reduction with increasing shade in beech than in oak</td>
</tr>
<tr>
<td>Above-ground biomass</td>
<td>Decreases with shade (Curt et al., 2005)</td>
<td>Decreases to a greater extent in shade-tolerant than in light demanding (Chen, 1997)</td>
<td>Decreased with shade</td>
<td>Greater reduction with increasing shade in beech than in oak</td>
</tr>
<tr>
<td>Below-ground biomass</td>
<td>Decreases with shade (Curt et al., 2005)</td>
<td>Decreases to a greater extent in shade-tolerant than light demanding (Chen, 1997)</td>
<td>Decreased with shade</td>
<td>Greater reduction with increasing shade in beech than in oak</td>
</tr>
<tr>
<td>Root:shoot ratio</td>
<td>Decreases with shade (Valladares et al., 2002)</td>
<td>Decreases to a greater extent in shade-tolerant than in light demanding (Valladares et al., 2002)</td>
<td>Decreased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Characteristic</td>
<td>General responses (findings in the literature)</td>
<td>Response per shade tolerance (findings in the literature)</td>
<td>General response (findings in the present study)</td>
<td>Response per shade tolerance (findings in the present study)</td>
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</tr>
<tr>
<td>Leaf mass</td>
<td>Decreases with shade (Takahashi and Goto, 2012)</td>
<td>No difference in response to shade between shade-tolerant and light demanding (Takahashi and Goto, 2012)</td>
<td>Response differed between species</td>
<td>Beech: decreased with shade</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No difference in response to shade between shade-tolerant and light demanding (Takahashi and Goto, 2012)</td>
<td>Beech: not affected by shade</td>
<td>Oak: not affected by shade</td>
</tr>
<tr>
<td>Leaf area</td>
<td>Controversial</td>
<td>-</td>
<td>Response differed between species</td>
<td></td>
</tr>
<tr>
<td>Specific leaf area (SLA)</td>
<td>Increases with shade (Valladares et al., 2002)</td>
<td>Increases to a greater extent in shade-tolerant than in light demanding (Valladares et al., 2002)</td>
<td>Increased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Leaf thickness ($L_{th}$)</td>
<td>Decreases with shade (Valladares et al., 2002)</td>
<td>No difference in response to shade between shade-tolerant and light demanding (Valladares et al., 2002)</td>
<td>Decreased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Height/stem mass</td>
<td>Increases with shade (Einhorn et al., 2004)</td>
<td>No difference in response to shade between shade-tolerant and light demanding (Einhorn et al., 2004)</td>
<td>Increased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Photosynthetic CO$<em>2$ assimilation at saturating light ($A</em>{1500}$)</td>
<td>Decreases with shade (Gardiner et al., 2009)</td>
<td>Decreases to a greater extent in light demanding than in shade-tolerant (Gardiner et al., 2009)</td>
<td>Decreased with shade</td>
<td>Greater reduction in beech in the first growing season</td>
</tr>
<tr>
<td>Stomatal conductance at saturating light ($g_{s1500}$)</td>
<td>Decreases with shade (Gross et al., 1996)</td>
<td>-</td>
<td>Decreased with shade</td>
<td>Greater reduction in beech in the first growing season</td>
</tr>
<tr>
<td>Instantaneous water use efficiency (saturating light)</td>
<td>-</td>
<td>-</td>
<td>Response differed between species and year</td>
<td>2013: decreased with shade in beech</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>2014: not affected by shade</td>
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<tr>
<td>Intrinsic water use efficiency (saturating light)</td>
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<td>Response differed between species and year</td>
<td>2013: decreased with shade in beech</td>
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<td>2014: not affected by shade</td>
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<td>Response per shade tolerance (findings in the literature)</td>
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<td>Response per shade tolerance (findings in the present study)</td>
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<tr>
<td>Photosynthetic CO$<em>2$ assimilation at PAR=500 ($A</em>{500}$)</td>
<td>Decreases with shade (Gardiner et al., 2009)</td>
<td>Decreases to a greater extent in light demanding than in shade-tolerant (Gardiner et al., 2009)</td>
<td>Response differed between species and year</td>
<td>2013: decreased with shade in beech 2014: not affected by shade</td>
</tr>
<tr>
<td>Stomatal conductance at PAR=500</td>
<td>-</td>
<td>-</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Instantaneous water use efficiency at PAR=500</td>
<td>-</td>
<td>-</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Intrinsic water use efficiency at PAR=500</td>
<td>-</td>
<td>-</td>
<td>Response differed between species and year</td>
<td>2013: decreased with shade in beech 2014: not affected by shade</td>
</tr>
<tr>
<td>Photosynthetic CO$<em>2$ assimilation at ambient PAR ($A</em>{amb}$)</td>
<td>Decreases with shade (Reynolds and Frochot, 2003)</td>
<td>-</td>
<td>Decreased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Stomatal conductance at ambient PAR</td>
<td>Decreases with shade (Reynolds and Frochot, 2003)</td>
<td>-</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
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<td>Decreases with shade (Reynolds and Frochot, 2003)</td>
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<td>Decreased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
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<td>PSII operating efficiency</td>
<td>Increases with shade (Valladares et al., 2002)</td>
<td>Increases to a greater extent in shade-tolerant than in light demanding (Valladares et al., 2002)</td>
<td>Increased with shade (not significant differences)</td>
<td>Greater increase with shade in oak and beech during the first growing season</td>
</tr>
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<td>PSII maximum efficiency</td>
<td>Increases with shade (Valladares et al., 2002)</td>
<td>Increases to a greater extent in shade-tolerant than in light demanding (Valladares et al., 2002)</td>
<td>Increased with shade (not significant differences)</td>
<td>Greater increase with shade in oak and beech during the first growing season</td>
</tr>
<tr>
<td>Photochemical quenching</td>
<td>Increases with shade (Valladares et al., 2002)</td>
<td>Increases to a greater extent in shade-tolerant than in light demanding (Valladares et al., 2002)</td>
<td>Increased with shade (not significant differences)</td>
<td>Greater increase with shade in oak and beech during the first growing season</td>
</tr>
<tr>
<td>Characteristic</td>
<td>General responses (findings in the literature)</td>
<td>Response per shade tolerance (findings in the literature)</td>
<td>General response (findings in the present study)</td>
<td>Response per shade tolerance (findings in the present study)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>--------------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td>---------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Electron transport rate (ETR)</td>
<td>Decreases with shade (Wyka et al., 2007)</td>
<td>No difference in response to shade between shade-tolerant and light demanding (Wyka et al., 2007)</td>
<td>Decreased with shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Dark respiration</td>
<td>Decreases with shade (Gardiner et al., 2009)</td>
<td>Decreases to a greater extent in light demanding than in shade-tolerant (Gardiner et al., 2009)</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Chlorophyll Content</td>
<td>Increases with shade (Valladares et al., 2002)</td>
<td>Increases to a greater extent in shade-tolerant than in light demanding (Valladares et al., 2002)</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Leaf flushing</td>
<td>Occurs later in shade (Caffarra and Donnelly, 2011)</td>
<td>-</td>
<td>Occurred later in shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Duration of leaf senescence</td>
<td>-</td>
<td>-</td>
<td>Decreased with shade</td>
<td>Greater reduction with shade in beech than oak</td>
</tr>
<tr>
<td>Leaf longevity</td>
<td>-</td>
<td>-</td>
<td>Decreased with shade</td>
<td>Greater reduction with shade in beech than oak</td>
</tr>
<tr>
<td>Stem quality</td>
<td>Decreases with shade (Rozenbergar and Diaci, 2014)</td>
<td>-</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Dieback</td>
<td>-</td>
<td>-</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Branch number</td>
<td>Decreases with shade (Kint et al., 2010)</td>
<td>-</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
<tr>
<td>Branch weight</td>
<td>Decreases with shade (Ammer, 2003)</td>
<td>-</td>
<td>Not affected by shade</td>
<td>No difference in response to shade between species</td>
</tr>
</tbody>
</table>
7.2 Implications of the methodology

The approach used to investigate the impact of different shade levels on growth, morphological, physiological and phenological responses and tree architecture of oak and beech seedlings may have some shortcomings. It may be difficult to extrapolate results from studies performed under artificial shade (shadehouses) to field conditions.

For example, the light regime provided by the nets is more uniformly distributed than that found in the forest understory. Furthermore, sunflecks can locally increase PAR in shaded forest understories for short periods of time and, therefore, improve carbon gain under relatively heavy shade conditions. Thus, it means that seedlings grown in the shadehouses may receive less PAR over the period of study than if they were grown below a forest canopy experiencing similar shade levels, perhaps mainly as a result of the additional energy received during sunflecks, assuming that the occasional bursts of extra light (sunflecks) in the gaps do not cause photoinhibition. Greater growth rates were found in two birch species grown in shadehouses (uniform light) than in forest gaps, despite the fact that PAR averaged same values in both situations (Wayne and Bazzaz, 1993). This suggests that studies carried out under relatively homogeneous light regimes, such as shadehouses, may provide more favourable growing conditions than may be expected in natural gaps. Although green polythene (neutral shade) nets were used to mimic the colour of a forest canopy, these nets are assumed to not modify the spectral light distribution (Cummings et al., 2008). Therefore, the downside of the nets used in this study is that while they may reduce overall irradiance in a similar way to a forest canopy, they might not have an equivalent effect on the quality of transmitted light. Since both light quantity and quality are crucial for growth and competition (Hertel et al., 2011) and forest canopies alter both aspects of irradiance, these two aspects should be considered when studying responses of tree seedlings to shade.

The responses of different species to light availability under a forest canopy are difficult to investigate. First, the light conditions in the forest understory are heterogeneous and, therefore, difficult to measure. Secondly, there are confounding factors associated with measuring responses to variable light availability, such as variation in water and nutrient availability, temperatures,
competition effects and other factors under a forest canopy that may also limit growth. Hence, the use of shadehouses may be an alternative way of assessing the potential response of underplanted seedlings to light availability, since environmental conditions can be controlled better in shadehouses than in the field.

The different shade conditions used in this study were intended to mimic a range of underplanted conditions. According to Paquette et al. (2006), the light levels used in this study would correspond approximately with those found in forest stands managed through clearfelling (100% of full light), heavy thinning (62% of full light), intermediate thinning (51% of full light) and light thinning (28% of full light). Kennedy et al. (2007) used shadehouses that provided 75%, 50% and 25% of full light to reflect the range in shade that might be found in stands considered for CCF in Ireland, similar to this study. Nevertheless, it would have been preferable to have included a more extreme shade level in the artificial shadehouse experiment to reflect the densest shade found under closed canopies. Discrepancies between the results from this study and other studies with the same species may be related to the different environmental conditions used (light levels, temperature, rainfall, etc.).

A similar overall trend was found in the physiological responses to shade of beech seedlings grown under controlled and field conditions, with lower photosynthetic rates and higher PSII efficiency found in the field than in the shadehouses. Although these changes could be attributed to lower PAR absolute values in the field than in the shadehouses, the fact that shadehouses did not alter light quality should also be considered. The fact that forest canopies alter the R/FR and B/R ratios suggests that these different photosynthetic values between the field and controlled experiment could have also been due to changes in light quality.

Although this study provides information on the responses of oak and beech seedlings to different shade levels, which may inform decision making in relation to underplanting in CCF scenarios, further research should be conducted to confirm the practical evidence regarding the suitability of these species for underplanting in the field in Ireland.
7.3 Silvicultural management implications

The results of this study showed that light availability strongly affected the performance of beech and oak seedlings. Although, as expected, the best growth was observed at full sunlight, both species were able to tolerate shade (as low as 28% of full sunlight), through morphological and physiological acclimation. This suggests that beech and oak seedlings are suitable for underplanting under permanent canopy cover that reduces light to about this level and can be managed under CCF systems, such as shelterwood and selection systems.

Previous studies have reported the better performance of beech than oak under low light conditions, which is not surprising since beech is considered to be more shade tolerant than oak (Gemmell et al., 1996; Löf et al., 2007; Gardiner et al., 2009). Similarly, Dey et al. (2008) suggested that oaks perform better than more shade tolerant species as stocking decrease and understory light intensity increases. This reported greater shade tolerance of beech seedlings has resulted in beech being one of the most popular species used for underplanting (Oleskog and Löf, 2005). However, the results from this study showed that oak seedlings have the ability to perform well, even better than beech seedlings, to a reduction in light availability (from 100% to 28% of full light), which would be similar to the effect of underplanting below an existing canopy. Therefore, oak seedlings would be compatible with forest practice alternatives to clearfelling and should be considered as a suitable for underplanting in Ireland, as this species might offer a wide range of planting scenarios under existing canopies. Although seedlings from nurseries, adapted to higher light before used for underplanting, may experience planting shock, the results of this study suggest that beech and oak seedlings will acclimate well to a reduction in light levels as low as 28% of full sunlight.

Moreover, beech and oak seedlings had good stem form, regardless of the shade treatment applied, which suggests that underplanting will not have a negative effect on the future quality of the crop. However, this result in relation to stem quality is premature due to the early stage of the seedlings used for the study and further long-term research should be conducted. One stage when it would be important to assess stem quality is before carrying out the first thinning.
Despite the suitability of beech and oak seedlings for underplanting beneath a canopy of trees, the ability of these species to respond favourably to canopy openings should be considered also, as suggested by previous studies (Lüpke, 1998; Collet et al., 2001; Coll et al., 2003; Curt et al., 2005). Although oak seedlings may tolerate low light conditions during the first one or two years of establishment, increasing light availability is essential to promote oak growth thereafter (Ziegenhagen and Kausch, 1995; Lüpke, 1998; Welander and Ottosson, 1998). The results of this study suggest that beech seedlings planted under heavy shade might benefit from an increase in light availability after one growing season, since photosynthetic capacity ($A_{1500}$ and $A_{500}$) increased from the first to the second growing season and shaded seedlings seem to be more susceptible to photoinhibition during the first growing season. In contrast, shaded oak exhibited a good ability to use high light (high $A_{1500}$ and $A_{500}$) over the two years of the study, suggesting that oak seedlings grown under shade (ranging from 62% to 28% of full sunlight) would benefit from an increase in light availability in the first two growing seasons.

The greater photosynthetic rates on exposure to saturating light as compared with those at ambient light observed over the two years of the study (except for shaded beech in the first growing season) also suggest that oak and beech seedlings may be capable of exploiting sunflecks. Therefore, sunflecks could be an important contribution to carbon gain under forest canopies. Tognetti et al. (1997) reported that beech seedlings showed a clear capability to exploit sunflecks. Photosynthesis during sunflecks provides 10-90% of daily carbon gain (Leakey et al., 2005). Thus, the capability to exploit sunflecks of the species growing in the understory would suppose an advantage to increase carbon gain and, therefore, growth.

A forest can be thinned to increase light levels in the understory. Therefore, thinning (to create appropriate light levels for establishing beech and oak seedlings) in conjunction with underplanting can be used to enhance regeneration in under-performing forest stands, to increase tree species richness and structural diversity and/or to transform into CCF systems (Evans, 1984; Kenk and Guehne, 2001; Paquette et al., 2006; Hawe and Short, 2012). This method will give some flexibility in management, since if needed, further thinning can be used to reduce
overstory density according to species and management objectives. For example, Ní Dhubháin (2010) reported that using a shelterwood approach to transform a mature Sitka spruce (Picea Sitchensis (Bong.) Carr.) stand into a CCF one in Ireland resulted in inadequate light levels (<11% of full light) in the understory to allow the successful establishment of beech and sessile oak (Quercus petraea (Matt.) Liebl.). In this case, an intervention, such as thinning, would be required to achieve higher light levels that promote survival and growth of the understory species.

This study focussed on how oak and beech seedlings previously grown at full light conditions performed under different shade levels that simulate a range of underplanted conditions, since understory light is a key parameter in seedling survival, establishment and growth. However, other factors that affect species performance should also be considered when underplanting. Among these, water and nutrient availability (Valladares and Niinemets, 2008) and competition with the regeneration layer (Annighöfer et al., 2015) play a key role.

Frost damage, along with exposure, might be one impediment to the establishment of broadleaf species in open fields, so it is suggested (Hawe and Short, 2012) that frost-tender species, such as beech and oak, should not be planted on sites subject to frost although Gemmel et al. (1996) reported that shelterwood systems could be used when planting oak and beech on frost-prone sites. It was observed that oak and beech seedlings under shade flushed later than seedlings at full sunlight, suggesting that underplanting with these two species would reduce the risk of frost damage in comparison with seedlings planted in open sites. Underplanting could have other advantages since conditions created after heavy thinnings or clearfelling could substantially increase the risk of windthrow, which is a major constraint to profitable forestry in Ireland (Ní Dhubháin et al. 1996). Taking advantage of an existing canopy will also help control weed competition and might be a good practice if overstory density is managed in a way that balances the resource needs of the introduced species with that of its competitors (Dey et al., 2012). This is of particular importance in Ireland, where weed competition represents one cause of plant loss and poor growth (Bulfin, 1992). Therefore, the underplanting in an existing stand may be good practice as the existing canopy will provide shelter for underplanting
seedlings and may lessen transplant shock by moderating environmental stress (Dey et al., 2012). However, forest managers should be aware of the risk of wind damage when carrying out thinning in conjunction with underplanting.

In relation to Irish conditions, where observations indicated that many first rotation broadleaf plantations have a high proportion of poor quality stems (Hawe and Short, 2012), thinning in conjunction with underplanting could be a practice to rehabilitate these stands. This method may be also carried out to enrich plantations, to bring about change in species or to manage CCF systems. The type of thinning should be dependent on the light requirements of the underplanted species. This study suggests that light availability about 28% of full sunlight would suffice for the success of underplanted oak and beech over two years. However, intermediate levels such as 62% and 51% of full sunlight would increase growth rates of the underplanted beech and oak. After establishment of the underplanted species, subsequent thinning could manipulate the amount of light reaching the understory.

Provenance choice, as well as species selection, is also a crucial element of successful plantation establishment. Therefore, to match a suitable and well-adapted provenance to a site is of great importance. It is known that beech populations can be differentiated on the basis of growth parameters (Borghetti et al. 1993), morphology and phenology (Borghetti and Giannini, 1982), and susceptibility to drought stress (Tognetti et al., 1995). Likewise, differences among oak provenances in relation to growth (Harmer, 2000), sensitivity to drought and warming (Arend et al., 2011) have been found. Although seedling provenances used in this study were according to provenance recommendation in Ireland (Dutch and British for oak and beech, respectively), home-collected material (i.e. material from registered Irish stands) should be a first choice (COFORF 2002, 2007). This suggests that material from Irish registered stands might have had better performance than the provenances used in the study. Nevertheless, sometimes home-collected material is unavailable and material must come from another sources.
7.4 Conclusions

The results of this study suggest that both beech and oak seedlings can be underplanted in forests in Ireland without greatly limiting the silvicultural options for these sites. One suggested option is underplanting following a thinning carried out to achieve appropriate light levels (≥ 28% of full light) for the survival and growth of these species. Although oak is generally considered less shade-tolerant than beech at the seedling stage, the results of this study suggest that oak seedlings have a similar response to beech seedlings in the first two years of being exposed to shade levels as low as 28% thereafter. This information should be considered when establishing or managing forest stands through underplanting.

Both oak and beech displayed similar acclimation in response to shade for a great number of the traits investigated, even if the mechanisms resulting in these changes were different, from leaf to plant level. At the plant level, seedlings acclimation to shade included higher biomass allocation to above-ground than below-ground parts and greater energy investment on height than diameter growth. At the leaf level, seedlings grown under shade increased their SLA and reduced leaf thickness. Since leaves of seedlings grown under shade had lower CO₂ assimilation rates, expressed per unit leaf area, than the same species grown at full sunlight, changes in SLA seem to be one of the main mechanisms that allow seedlings to perform well under shade conditions. Shaded seedlings also increased the efficiency of the PSII, compared to seedlings at full light, which is another typical acclimation to low light conditions. Photosynthetic rates were higher and leaves were retained for longer (buds flushed earlier and senescence was delayed) in beech and oak seedlings grown at full light, leading to a greater growth and biomass production than in those grown under shade conditions. Despite this greater growth at full sunlight, the results of this study suggest that beech and oak seedlings would be able to acclimate and perform well if underplanted below overstories that reduce the available light to as low as 28% of full light without having any significant adverse effect on the quality of the final crop.
7.5 References


different light and vegetation composition conditions. *Annals of Forest Science* 60, 593–600.


Appendix A: Flushing assessment

Flushing date was recorded in all the oak (right) and beech (left) seedlings in each plot. We recorded flushing (break-bud or budburst) in the bottom half and top half of the plants, and three separate stages/phases were identified:

*Phase 1: initiation of flushing (green leaf material visible: the colour of the new leaves is visible through openings in the swollen bud). Date when the protective scale coating is shed from the bud exposing tender new growth tissues of one or more leaves.

*Phase 2: leaf form visible. Leaves open, and recognisably the shape (not the full size of the adult leaves).

*Phase 3: leaves fully expanded. Date the leaves are completely unfolded from the bud. Leaves need to be opened completely (flat) and the leaf stem or base must be visible.
Appendix B: Senescence scoring

Senescence scoring for oak (top) and beech (bottom) seedlings: 1) leaves with yellowing symptoms < 25% of the whole plant leaves; 2) leaves with yellowing symptoms from 25-50% of the whole plant leaves; 3) leaves with yellowing symptoms from 50-75% of the whole plant leaves; 4) more than 75% of the leaves of the whole plant with extensive yellowing, desiccation and abscission.