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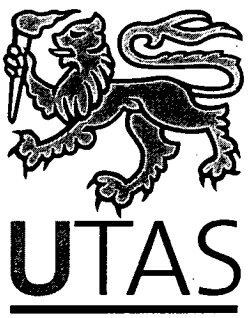

An Investigation into the Influence of Variation in Controlled Environment Plant Research Facilities on Growth Responses.

Submitted in fulfilment of the requirements for the Degree of Master of Science

By

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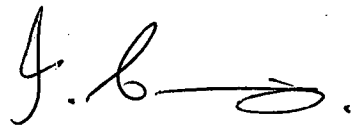

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Abstract

In this study, controlled environment plant growth facilities were examined through both physical measurement and plant growth response studies in order to characterise the degree of variation between environments and to identify those variations that may influence experimental results. Plant growth facilities consist of greenhouses, where temperature and light is influenced by seasonal variations, and growth chambers, where temperature and light quantity is considered to be accurately controlled, but where all light is artificial.

Natural light spectral properties were found to be quite consistent temporally and seasonally, but quantity was highly variable and influenced by greenhouse design and covering material. In winter, light quantity was found to influence plant morphology, particularly in greenhouse areas with heavy structural components. Plants showed increased shoot elongation relative to higher light areas under such conditions. Growth chamber experiments that varied irradiance but not temperature confirmed shoot length was closely associated with light quantity, with longest shoot lengths under lowest irradiance and shortest shoot lengths under highest irradiance. Covering material also had an influence. In a study of the spectral properties and growth responses under glass and polycarbonate clad greenhouses with the same design, orientation and temperature profiles, light quantity was always lower under polycarbonate relative to glass. In spring, with longer day-length and higher irradiance relative to winter, this had little influence on plant morphology or development. In winter, however, plants under polycarbonate showed significant shoot elongation relative to plants grown under glass. The minor differences in spectral properties between glass and polycarbonate (polycarbonate had lower UV and blue, and higher far-red proportions relative to glass and natural light) did not appear to be a significant influence on results, as flowering node was not significantly different between treatments. The UV reduction under polycarbonate and laminated glass relative to natural light and horticultural glass also did not appear to be a significant influence on plant morphology, as supplementing UV back to natural levels did not produce significant differences between treatments.

Light quantity reductions in winter can be somewhat compensated for by supplementary lighting. A range of high pressure sodium lamps were tested, and most would be suitable for this purpose, including some non-plant specific brands.

Irradiances of 50- 100 μmol $m^{-2}s^{-1}$ over an 18h photoperiod produced dramatic growth improvements in pea, with significantly increased leaf size, dry weight and yield. Although high pressure sodium lamps have a high red to far-red ratio (R:FR), which could be expected to delay flowering, there was no delay in flowering node relative to 18h extension lighting with a low R:FR. Diffusing covers over the lamps improved light distribution, and there was no significant benefit from using a moving light system relative to a fixed system.

Photoperiod control systems were examined, and the importance of total light exclusion for day-length studies was confirmed. Inductive light levels for pea were less than 0.1 μmol $m^{-2}s^{-1}$. While traditional photoperiod extension is with incandescent lamps because of their low R:FR, white, blue, red and far-red light were all inductive to flowering for pea. The low R:FR of incandescent and far-red light induced typical shade avoidance responses of increased shoot length and reduced leaf size, which the other wavelengths did not.

Seasonally, both light quantity and temperature varied widely in the glasshouse environments. Various shade methods are commonly employed in summer to reduce radiant load, and a range of these were examined. All of the methods were found to be spectrally neutral compared to unshaded conditions, and did not influence plant morphology. Plants grown in summer had significantly reduced shoot length, leaf size, flowering time and yield compared to plants grown in other seasons. Both growth chamber and natural light experiments indicated these were primarily responses to elevated temperature, particularly the reductions in yield.

For more accurate control over environmental parameters, plant growth chambers are commonly used in plant research. However, all of the light sources used were found to have very different spectral properties to natural light, even when mixed to broaden the spectrum. Thermal load was found to be significant with high intensity discharge lamps even with a separately ventilated light loft, although the use of double glass barriers and water filters reduced the impact. The addition of incandescent lamps to the light mix in an attempt to mimic more natural R:FR ratios was found to be ineffective and significantly increased thermal load. Plants showed clear signs of temperature influence, with reduced shoot length, leaf size and yield, and did not flower at a lower node as expected from reduced R:FR. However, far-red light emitting diodes added to the light mix produced natural R:FR ratios without thermal

load influences, and plants responded as expected with increased shoot length and reduced flowering node.

Spectral distribution and growth responses under fluorescent and mixed metal halide/high pressure sodium lamps were quite similar at equal temperature and irradiance. However, plants grown under metal halide flowered at a significantly earlier node than the other sources, while under high pressure sodium lamps, shoot length was significantly longer. Metal halide has high blue, and high pressure sodium has low blue irradiance. Supplementation of high pressure sodium with blue light induced reduced shoot length and flowering node. However, R:FR also varied between light sources and natural light. The role of blue light was further investigated using photo-selective shade screens, which were found to alter blue proportion but not R:FR relative to natural light. Under red shade cloth (low blue, high red proportions) shoot length was significantly increased and under blue shade cloth (high blue, low red proportions) shoot length was significantly reduced relative to spectrally neutral shade cloth. Blue light receptor *cry1* mutant plants did not respond to shade cloth treatment, as shoot elongation was not significantly different in *cry1* mutant plants grown under neutral, red or blue shade. This indicates a clear role of blue light quantity in pea shoot length responses, and specifically, the *cry1* photoreceptor in these changes.

This study has identified that light and temperature are the most important factors that vary between controlled environments, and are a potential influence on results. Taken together, the results from this study will allow future plant researchers, and facility managers, to identify the equipment variations that may influence plant responses.

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Abbreviations

B	Blue light (400-500 nm)
B:R	Quantum ratio of blue to red light
B:FR	Quantum ratio of blue to far-red light
cry1	A cryptochrome 1 defective mutant selection from L107 pea
CWF	Cool white fluorescent lamps
DLI	Daily light integral, the total PPF quantity of light received in a 24h period (mol $m^{-2}d^{-1}$)
DW	Shoot dry weight
FR	Far-red light (700-800 nm)
FT	Flowering time from planting date
HPS	High pressure sodium lamps
Inc	Incandescent lamps
L107	Hobart line 107 pea, a selection from *Pisum sativum* L. 'Torsdag'
L218	Hobart line 218 pea, a day neutral selection from *Pisum sativum* L. 'Torsdag'
L1-9	Length between nodes 1 to 9
LED	Light emitting diodes
LL	Leaflet length at node 9
LW	Leaflet width at node 9
MH	Metal halide lamps
NFI	Node of flower initiation
PAR	Photosynthetic active radiation (400-700 nm, W m^{-2})
PPF	Photosynthetic photon flux (400-700 nm, μmol $m^{-2}s^{-1}$)
R	Red light (600-700 nm)
R:FR(b)	Broad band quantum ratio or red (600-700 nm) to far-red (700-800 nm)
R:FR(n)	Narrow band quantum ratio of red (655-666 nm) to far-red (725-735 nm) light
TL	Total shoot length
TN	Total number of nodes
UV	Ultra-violet light (< 400 nm)

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Chapter 1 Introduction

To survive, plants need only minerals, water, air and light (Spalding and Folta 2005). Plant development is influenced by environmental factors, such as light, temperature, CO_2, humidity and nutrients (Moe and Heins 1990), as well as water status (Hanan 1998). Water status and nutrients are largely controlled in protected horticulture, and air movement is less variable than outside conditions (Downs and Krizek 1997). Light and temperature are considered the major environmental determinants (Cathey and Campbell 1977). In plant science research, it is important to optimise these parameters, or at least minimise their possible influence, on the environmental factor or factors being examined. Moe and Heins (1990) examined the effects of light and temperature and found they had a similar influence for many morphological characteristics in a wide range of plants. Red light (R) or high R to far-red (FR) ratios suppressed shoot elongation and promoted lateral branching. Plants grown with a lower day temperature than night temperature exhibited the same morphology. FR light or low R:FR strongly enhanced shoot elongation and inhibited lateral branching. Plants grown with a higher day temperature than night temperature exhibited the same morphology. Thus, it is important to optimise control parameters so that known variables are being examined.

Plants respond to their environment in a variety of ways. For example, photoperiodic responses of species can be classified into 25 interactions of light and temperature (Hanan 1998). These responses are generally examined in isolation; it is rare for multiple influences to be examined together (Moe and Heins 1990). Even for molecular and genetic studies, if there are multiple environmental influences, it would be difficult to assign a cause to an observed effect. This is the basis for controlled environment facilities- environmental variables can be controlled so that cause and effect can be examined. For example, plant growth chambers with accurate temperature and light control are used to examine temperature gradient effects under the same light conditions or light effects under the same temperature conditions.

There are three classes of plant growth structures: greenhouses, where radiation is supplied by the sun; phytotrons, which use solar radiation supplemented by artificial

sources; and growth chambers, where all radiation is artificial (Aldrich and White 1969).

Greenhouses are largely concerned with modification of temperature by trapping long wave radiation. Modification of the light environment is a consequence of the choice of covering material (Hanan 1998). Structure, geometry and orientation all influence the light environment inside the greenhouse (Mermier and Baille 1988).

Photoperiod studies in greenhouses involve the natural photoperiod being modified by extension lighting and/or screening to exclude light. Screening in greenhouses can modify the difference in temperature between short day (SD) and long day (LD) or control plants and can lead to incorrect conclusions (Heins and Faust 1994).

Growth chambers provide accurate control of light and temperature, allowing (theoretically) for uniform, reproducible conditions (Carlson and Giger 1978; Hammer 1978). However, the artificial light sources used have very different spectral properties to sunlight (Runkle 2004), while thermal load from the lamps can be a significant and often unmeasured component (Bubenheim *et al.* 1988).

Thus, the facilities used to control the environment may in themselves influence plant responses. Characterisation of the experimental environment is the first step required before quantifying plant responses (Sager *et al.* 1988). That is the intention of this thesis. Through physical measurement of the variation in representative controlled environment growth facilities, and by relating these to measured plant responses, valuable insights can be gained into the influence of equipment variations that may inadvertently influence experimental results.

1.1 Temperature

Temperature influences plants at all stages of their development (Ormrod 1978b). Rate of development is temperature dependent, rates increase up to a maximum, which is the optimum temperature for that species, and above this optimum, growth rapidly declines (Heins *et al.* 2000).

Average day temperature influences rate of leaf and flower development in a wide range of species in a near linear fashion over a set temperature range (usually 10 - 30^{0}C). At lower and higher temperatures rates are reduced (Moe and Heins 1990). The rate of flower development in particular is strongly influenced by temperature (Kaczperski *et al.* 1991; Pramuk and Runkle 2005).

Temperature is also an important conditioning factor in germination with optimum germination temperature varying with species. For example, for *Sinapsis arvensis* the optimum is 15°C while in *Plantago major* it is between 25-30°C (Frankland 1981). Some species, such as *Rumex obtusifolia,* are stimulated by fluctuating temperature (Frankland 1981). Regular temperature changes can also entrain the endogenous clock in the absence of light signals (Fankhauser and Staiger 2002). Many species show a thermo periodic response, with improved growth when there is a daily temperature fluctuation (Ormrod 1978b). Lower day than night temperature can reduce shoot length (Moe and Heins 1990; Vogelezang 2000) through reduced gibberellin levels (Grindal *et al.* 2000).

Optimum temperature also varies with developmental stage. Germination and seedling optimum temperatures are often higher than later growth stages (Ormrod 1978b). Cooler temperatures during maturation increase yield in many crop species (Heins *et al.* 2000) and yield and seed weight is negatively correlated with temperature in pea (Chetia and Kumar 2005; Poggio *et al.* 2005).

Temperature extremes can be very damaging to plants. High temperatures increase moisture stress within the plant and can damage cells, destroy proteins, and interfere with enzyme activity (Ormrod 1978b). Low temperatures reduce growth rates, while freezing temperatures physically damage cells from ice crystal formation and desiccation (Ormrod 1978b). However, within the normal temperature range it is the average temperature that influences plant development, not short term fluctuations (Adams 2006; Cockshull *et al.* 2002).

Soil temperature also influences plant growth and development. Water and nutrient availability is influenced by soil temperature (Ormrod 1978b), which can be significantly increased from radiant heat in growth chambers, particularly in individual pots (Hamasaki and Okada 2000). High root temperature impairs plant growth by increasing respiration and reducing water and nutrient uptake, while root cooling can partially offset growth reductions from high air temperature (Incrocci *et al.* 2000).

Temperature also interacts with other factors, notably air velocity and light. High air velocity and low humidity can have a cooling effect on leaves by increasing transpiration rates, so that leaves can be cooler than ambient temperature (Ormrod 1978b). Absorbed radiation increases plant temperature, particularly at the shoot tip, unless removed by transpiration, emission or convection (Faust and Heins 1997).

Flowering in many species is controlled by modulation of photoperiod and thermo period, allowing for onset of flowering and seed set in favourable conditions (Fankhauser and Staiger 2002). Plant phenology can be predicted by interactions of light and temperature (Yan and Wallace 1998). Flowering delays from low light quantity can be somewhat offset by higher temperature (Pramuk and Runkle 2005), while low temperature exposure of imbibed seed (vernalization) can counteract short day photoperiod flowering delays in sensitive species (Beveridge and Murfet 1996; Yan and Wallace 1998; Inoue 2002). Photosynthesis continues over a wide temperature range in most species (Salisbury and Ross 1992), but low temperature can severely limit photosynthesis in cold sensitive species, such as soybean (Tambussi *et al.* 2004).

Temperature in greenhouses is generally controlled by heating and cooling systems. Ventilation rates, active cooling systems, and use of thermal screens all contribute to temperature control but wide temperature ranges are common (Hanan 1998). Growth chambers generally provide accurate temperature control, but thermal exchange needs to be considered (Hicklenton and Heins 1997). A major source of thermal load is heat from lamps (Hicklenton and Heins 1997). To reduce the influence of lamp heat, many growth chambers have a separately ventilated light loft with a glass or plexiglass barrier (Cathey and Campbell 1977). However, radiant heat load can still be significant even with a barrier (Bubenheim *et al.* 1988; Hamasaki and Okada 2000). McCree (1984) examined radiation from high intensity discharge lamps and found high irradiance can be accompanied by an abnormally high thermal radiation load on plants. Near infra red radiation is largely transmitted or reflected from leaves, but incandescent and high intensity discharge lamps in particular emit large quantities of far infrared radiation (Bubenheim *et al.* 1988; Faust and Heins 1997; Hicklenton and Heins 1997; McCree 1984). Thus plants in growth chambers can be subject to far greater thermal loads than in the natural environment (Hicklenton and Heins 1997), 5-10 times larger than on a sunny outdoor day (Hamasaki and Okada 2000).

Some light source influences on growth may also be related to temperature. Increased growth of lettuce under high pressure sodium lamps compared to cool white fluorescent tubes at equal irradiance was attributed to wavelength and temperature contribution from wavelengths above 700nm (Koontz *et al.* 1987). Most studies that demonstrate the value of photoperiod extension with incandescent lamps have not established if the benefits are from a phytochrome ratio effect, the additional

photosynthetic contribution of 700-750 nm radiation, or a temperature effect on the plants (Tibbitts *et al.* 1983). The radiant thermal effects need to be separated from other environmental temperature effects (Sager *et al.* 1982).

1.2 Light

For plants, light is the energy source and therefore of primary importance in plant development, physiology and metabolism. Most aspects of plant life are influenced by the qualities and quantities of light (Spalding and Folta 2005). Plant responses to light can be categorised as photosynthetic or photomorphogenic (Sager *et al.* 1982).

Photosynthesis determines vegetative growth (Sager *et al.* 1982). Intact leaves absorb more green than the isolated pigments used in studies as the carotenoids act in a light harvesting capacity for photo systems 1 and 2, contributing to the high quantum efficiency of photosynthesis over a wide spectral range (Barber *et al.* 1981).

Photosynthetic rates amongst species vary by nearly two orders of magnitude, even under optimum conditions (ideal temperature, saturating light, normal oxygen and CO_2 levels, high humidity). C_4 species, such as corn, have the highest rates, fixing CO_2 at up to twice the rate of C_3 crop plants, such as pea (Salisbury and Ross 1992). Interactions between exogenous and endogenous factors determine photosynthetic rates and patterns, corresponding primarily with diurnal changes in air vapour-pressure deficit and light quantity (Singsaas *et al.* 2000). Natural light quantity can be highly variable, particularly when cloudy (Smith and Morgan 1981), but transferring plants from high to low irradiance show acclimation responses are rapid, with rapid changes in photosynthetic rate and chlorophyll a/b ratio (Chow and Anderson 1987a; Walters and Horton 1994). Lower irradiance over longer periods stimulates an increase in light harvesting complexes (Chow and Anderson 1987b). Chloroplasts accumulate towards light under low fluence to maximise photosynthetic efficiency, and relocate away from high fluence light to minimise photo-damage (Wada 2005). Thus, plants adapt to the light environment through physiological responses (Walters and Horton 1994).

Photomorphogenesis refers to the responses of plants to their light environment (Kendrick and Weller 2003a). Light acting as information rather than an energy source affects a wide range of photomorphogenic responses. These include germination, seedling development, photosynthetic and photo-protective pigment

synthesis, and morphological development including shoot elongation and leaf expansion, leaf movement, and flower initiation (Sager *et al.* 1982).

The ability of a plant to respond to the radiation environment depends on its capacity to detect and respond to those changes. Photoreceptors detect the changes and are involved in the translation of the environmental signal to a biological signal (Smith 1981). Photomorphogenesis involves multiple photoreceptors and multiple interacting signalling pathways, and depends on the environmental conditions and developmental stage of the plant (Iino and Haga 2005). Many light controlled processes, based on modulation of gene activity, occur in response to changes in light (Fankhauser and Staiger 2002). Light mediated responses range from within minutes, such as de-etiolation, to hours or days, such as entrainment of circadian rhythms (vonArnim and Deng 1996). Inductive responses can be induced by a pulse of light, while other responses require long periods of light and can show increased response with increased irradiance. Thus photoreceptors can act as a switch or as photon counters (Kendrick and Weller 2003b).

Photoreceptors work through proteins, ions and hormones that form interacting and branching signalling pathways so that a relatively simple input (light) creates a complex output (Fankhauser and Staiger 2002). Phytochromes and cryptochromes mediate many of the same physiological responses, and interactions may result in relatively stable responses over a wide range of fluence rates and photoperiods (Platten 2003). Examples include interaction between phyA and phyB in near neighbour detection (shade avoidance); and cry2, phyB and phyA interactions in flower initiation (Casal 2005).

Many responses are not all or none, but quantitative (vonArnim and Deng 1996). Responses in the natural environment involve complex interactions of light and hormones in response to environmental cues, as many light regulated responses also respond to hormone application, notably auxin, ethylene, cytokinins and gibberellins (vonArnim and Deng 1996). Light and gibberellin (GA) interactions occur in germination, de-etiolation, stem growth, tuber formation, and flowering (Garcia-Martinez and Gil 2001). Low R:FR and/or end of day FR treatments that increase shoot elongation affect GA metabolism and/or responsiveness in many species. Application experiments and phytochrome mutant studies suggest light quality alters plant responses to GA (Garcia-Martinez and Gil 2001).

In controlled environments, and in horticulture, the problem of supply and measurement of light for plant growth has long been known. Visible light to the human eye (generally measured in lumens) is between 400-750 nm, with peak luminosity at 555 nm (Canham 1966). Plants respond to wavelengths beyond these limits, 300-800 nm is generally used to define the morphogenetic active range (Sager and McFarlane 1997). Light between 500-600 nm, although bright to us, is of relatively less importance to the plant (Canham 1966). Lamp manufacturers are primarily concerned with human visibility (Ryer 1997), and thus with luminous flux (a weighted measure of the overall stimulation to the average human eye from both intensity and wavelength between 400 and 750 nm). Lamps are thus quoted in terms of luminous flux (Ryer 1997). All of these measures are meaningless for plant growth. Different lamp sources with the same luminous intensity produce quite different plant relative dry weights (Canham 1966).

Most plant physiology literature quote the quantity of light in the 400-700nm range, i.e. photosynthetically active radiation (PAR). McCree (1972a) examined definitions of PAR by measuring the action spectrum, absorptance and spectral quantum yield of 22 crop plants in both growth chambers and the field. CO_2 uptake was measured over 350-750 nm in a wide range of conditions including leaf age, orientation, temperature and CO_2 concentration (McCree 1972a). Regardless of the condition, all species showed a quantum yield curve with 3 maxima: 2 broad maxima at 440 nm and 620 nm, as well as a shoulder at 670 nm. Average height of the blue peak was 70% of the red peak, although data by Sager *et al.* (1982) suggest it is higher than this.

Photosynthetic activity occurs between 360-760 nm, but the tails below 400nm and above 700nm are minimal (McCree 1972b). McCree (1972b) concludes that although none of the definitions of the PAR range are strictly accurate, and that leaves do not have a constant response rate between this range, it is still an acceptable definition if measured in μmoles $m^{-2}s^{-1}$ (i.e. PPF- photosynthetic photon flux). However, there is considerable variation between PPF and the biological responses when using narrow spectrum lamps; PPF is only accurate for broad spectrum sources (Sager *et al.* 1982). In addition, single number measurements, such as PAR or PPF, often ignore the wavelength/energy per photon nature of light, as well as direction. Light may also be polarized by reflecting surfaces in many experimental set ups. The time factor is also often ignored- i.e. whether an instantaneous (e.g. fluence rate) or time integrated (e.g. fluence) measurement is being given (Bjorn and Vogelmann 1994). Plants respond to

light quality as well as light quantity, so ideally a full description of light would include information on spectral distribution and wavelength ratios using spectroradiometer data, but this is rarely done (Bjorn and Vogelmann 1999).

At the very least, light descriptions should include information on the light sources used for the study (Krizek and McFarlane 1983). This thesis includes comprehensive spectroradiometer analysis of the light sources commonly used in plant research, including relative spectral distribution and wavelength ratios. As such, it can be used as a guide to the spectral properties of artificial lights, the influence of covering materials and shade methods on the properties of natural light, and the influence these may have on plant growth responses.

1.2.1 Light sources

1.2.1.1 Natural light

Solar radiation is absorbed (by ozone, oxygen and water) and scattered (Raleigh and Mie scattering) by the atmosphere (Smith and Morgan 1981). Daylight is the total global radiation received at the earth's surface and is therefore the sum of the resulting incident and diffuse light. Total irradiance (400-800nm) on clear days is above 1600 μmoles $m^{-2}s^{-1}$. Fluence rate between 450-850 nm is quite uniform, but cloud cover and dust haze produce some variations. Clouds reflect a portion of blue (400-500 nm) wavelengths, but cause little alteration to longer wavelengths (600-800 nm); only altering R:FR ratios by 5%. Dust reduces blue and increases the proportion of red wavelengths. Aspect affects the daylight spectrum received, where plants may not be irradiated by direct light for some time and are primarily lit by diffuse light, which is higher in blue wavelengths (Smith and Morgan 1981).

At sunrise and sunset, when the solar elevation is less than 10 degrees, the spectrum is relatively rich in blue and far red. The normal daylight R:FR ratio is quite constant (655-665/725-735) at 1.1, rising from or dropping to about 0.7 at sunrise and sunset. The duration of this change depends on latitude and solar declination, as well as being affected by weather and dust haze (Smith and Morgan 1981).

1.2.1.1.1 Shade

Radiation under canopies consists of unfiltered daylight that has passed through the canopy; and filtered daylight modified by absorption, reflection and transmission. Shade spectrums thus typically have troughs in the blue and red regions due to

absorption, a minor peak in green, and a major peak in far red from reflection. The degree of shading corresponds to the R:FR ratio- the lower the ratio the greater the degree of shading (Kendrick and Weller 2003a; Smith and Morgan 1981). Under overcast skies the R:FR ratio within canopies is less markedly reduced due to the diffusing nature of cloud cover and a larger proportion of diffuse radiation penetrates canopy gaps. Shade spectrums are also affected by solar elevation and seasonal change, as well as weather patterns, particularly wind gaps in vegetation cover and its effect on sun flecks (Holmes 1981).

Shade responses are highly varied according to species and maturity, as well as canopy architecture and soil fertility. Different parts of the plant experience different degrees of shade, and many species have different strategies during juvenile and adult phases (Grime 1981). Many shade tolerant species tend to be slower growing with comparatively low respiratory rates even at higher irradiance. Responses to shade in such species may be more physiological than morphogenic (Grime 1981). Shade tolerant species have a lower response to spectral changes than shade intolerant species, particularly for stem extension rates (Morgan 1981).

In competitive situations there is an adaptive advantage in rapid elongation responses to reduced irradiance or low R:FR (Ballare *et al.* 1997). Phytochrome perceives the spectral changes in FR. The decreased blue quantity in shade light can affect plant growth independently of the R:FR ratio (Morgan 1981). As a canopy grows, mutual shading triggers movement towards better lit areas. FR reflection from neighbouring plants provides an early warning proximity detection mechanism triggering anticipatory shade avoidance responses termed foraging for light. Projection of shoots towards canopy gaps is mediated by blue light and negative phototropism to reflected FR (Ballare *et al.* 1997).

Thus although sunlight is relatively constant, many plant species respond to the reduced R:FR and blue quantity in shade light by increasing shoot elongation, reducing leaf area, and earlier flowering, collectively called shade avoidance (Kendrick and Weller 2003a). In contrast, high R:FR can signal non-competitive conditions to the plant, resulting in reduced plant height, and later flowering in many species (Runkle and Heins 2001). As such, any alteration in the spectral properties of light by controlled environment equipment, such as covering material or artificial light source, could have a significant impact on plant growth and development.

1.2.1.2 Artificial sources

Spectrally, sunlight is quite constant seasonally and temporally (Smith and Morgan 1981), but the artificial lights used in plant research and horticulture vary markedly. There are many types and brands of lamps used in plant research, and lamp types are often mixed to produce broader spectra. As plant responses can vary according to wavelength, intensity and duration (Sager *et al.* 1982), it is important to understand the properties of the light sources being used and to report those properties in correct radiometric terms (Salisbury and Ross 1992).

The commonly used artificial light sources are incandescent globes, fluorescent tubes, and, where higher irradiance is required, high intensity discharge lamps: metal halide or high pressure sodium (Bubenheim *et al.* 1988). Although many different kinds of fluorescent lamps have been developed, cool white fluorescent has been the standard used for most horticultural and research applications, including in growth chambers (Cathey and Campbell 1977). Metal halide and high pressure sodium lamps have a higher luminous efficiency, but the greater heat radiation requires increased ventilation, and the point source nature of globes rather than tubes must be managed to avoid variation in uniformity at the plant level (Bubenheim *et al.* 1988; Cathey and Campbell 1977).

Artificial light sources all have very different spectral properties to natural light. Cool white fluorescent tubes, for example, have a much higher R:FR ratio (up to 8.8) compared to sunlight (around 1.1). This can affect growth and development responses, particularly of LD plants (Runkle 2004). Small changes in R:FR ratios between 0-2 can have significant effects, thus canopy shading, twilight, and light sources with different light spectra will influence phytochrome photo equilibrium and thus shoot elongation, lateral branching and flowering in many species. This has important implications for plant research studies, particularly those involving artificial light sources, either as the sole source or as a supplement to natural light (Moe and Heins 1990). Incandescent globes, unlike the other lamp types, are rich in far red wavelengths, hence are often added to other sources to broaden the spectral mix or to reduce R:FR. However, this is usually in insufficient quantities to reduce R:FR meaningfully in growth chambers without significant thermal effects (Smith 1994), as most of the output of incandescent globes is heat (McFarlane 1978).

Blue wavelengths may be involved in the perception of light quantity (Smith and Morgan 1981). High pressure sodium lamps are relatively deficient in blue, while

metal halide lamps are rich in blue. Excess shoot elongation under high pressure sodium has been attributed to low blue quantity (Tibbitts *et al.* 1983). In contrast, more compact growth can occur under metal halide than natural light and high pressure sodium (Yorio *et al.* 1995; Zheng *et al.* 2005). High UV levels are possible from depleted ozone levels and can be found in some artificial light sources, such as metal halide lamps, particularly on start up. High UV is associated with tissue damage, shortened internodes, smaller and thicker leaves, increased branching, and decreased biomass. However, the effects are modified by other wavelengths in the PAR range, and plants can acclimate (Kakani *et al.* 2003; Nogues *et al.* 1999; Teramura 1983).

Light source can influence flowering and shoot length even as a supplement or extension to natural light. Flowering of many species is delayed by day length extension with high R:FR sources such as fluorescent, compared to low R:FR sources, such as incandescent (Lane *et al.* 1965). A combination of cool white fluorescent and incandescent improves the flowering response in many LD species (Vince-Prue 1994). R:FR close to the natural level of 1.1 is the most effective at flower induction for LD plants without increasing elongation, which lower ratios (such as under incandescent lighting) tend to induce (Runkle 2004). Irradiance can also affect flowering time of many plants, increasing irradiance can reduce flowering time in some species (Mattson and Erwin 2005).

Traditional photoperiod day length extension lighting is with incandescent, as it is effective due to its low R:FR ratio, and inexpensive to install and run (Cathey and Campbell 1977). However, incandescent lamps are inefficient for photosynthetic (supplementary) lighting (McCree 1972b) and the low R:FR can increase shoot elongation relative to other sources (Runkle and Heins 2001). Cool white fluorescent, high pressure sodium, metal halide and incandescent lamps were compared for flower induction through weak day extension in *Campanula* and *Coreopsis*. Irradiance above 1 $\mu mol\ m^{-2}s^{-1}$ from any of the lamp sources was sufficient to induce flowering, and shoot elongation was reduced compared to incandescent lighting (Whitman *et al.* 1998).

Light leakage is an important aspect of photoperiod control. Fluence rates as low as 0.2 $\mu moles\ m^{-2}s^{-1}$ induced flowering in *Campanula* (Whitman *et al.* 1998). Light leakage of high pressure sodium light was tested on neighbouring crops from a greenhouse with a photoperiod extended morning and evening to provide 18 h.

Flowering was delayed in the short day plants (*Chrysanthemum, Poinsettia*) from measured leakage levels of 0.05-0.2 μmol $m^{-2}s^{-1}$ PPF, and promoted in the long day plants (*Fuchsia* and *Callistephus chinensis*) and cucumber elongation was reduced and fruiting delayed (Bakker and Blacquiere 1992). Hence when studying photoperiod sensitive species, any light from any source is a potential influence on results, and the growth chambers or greenhouse areas used should exclude all external light.

Thus choice of light source can be a strong influence on results, even as a low level extension to natural light. Low R:FR sources, such as incandescent, can accelerate flowering but increase shoot length, that is, induce a shade avoidance response. High R:FR sources, such as fluorescent, metal halide and high pressure sodium, can signal non-competitive conditions and may relatively delay flowering compared to natural light. Blue quantity may also influence morphology, with low blue sources such as high pressure sodium associated with increased shoot length. However, at higher irradiance wavelength differences between lamps may be less important, total blue quantity increases and plants have more energy for growth, masking wavelength effects (Cathey and Campbell 1977, Tibbitts *et al.* 1983; Walters and Horton 1994). In addition, as a supplement to natural light, wavelength differences between lamps may be less important (Moe 1997). What is needed when choosing light sources for plant growth and experiments is assessment of the likely impacts, if any, of those choices on plant development and morphology.

Natural light quantity is highly variable, but in growth chambers is usually constant. It is common for plants in growth chambers to be given high irradiance to simulate more 'natural' conditions (Bubenheim *et al.* 1988). Total photosynthesis depends on irradiance and duration (Hanan 1998), and plants exposed to lower PPF for longer periods generally accumulate more dry matter than those exposed to high PPF for shorter periods (Warrington and Norton 1991). Many growth chambers are capable of up to 1000 μmol $m^{-2}s^{-1}$ of constant irradiance, which over a 16h photoperiod equates to almost 58 mol m^{-2} dy^{-1}, well above peak summer daily light integrals. Such abnormally high irradiance can produce abnormal growth (Warrington and Norton 1991). High PPF can produce photo bleaching while the radiant heat load can produce desiccation (McFarlane 1978). Growth chamber guidelines (Sager and McFarlane 1997) point out that irradiances based on peak summer PPF values are unnecessary, 3-400 μmol $m^{-2}s^{-1}$ are sufficient for 16 h photoperiods- around 17-23 mol $m^{-2}d^{-1}$.

It is clear that light is a major influence on plant growth and development, and thus it is important to understand the variation in the qualities and quantities of light in controlled environment facilities for plant growth. PPF alone is not adequate for determining the growth effects of various lamp types and filters (Bubenheim *et al.* 1988), as PPF gives information on light quantity but no information on light quality. Thus data in non-photon units and that does not go beyond 700nm is limiting (Smith and Morgan 1981). Quantum sensors weighted in the PPF range for plant physiology give a reasonable approximation, but their limitations should be noted (Bjorn and Vogelmann 1994). For these reasons, this thesis uses spectroradiometer analysis of the commonly used light sources, and can be used as a reference to their relative spectral properties. Growth responses under these sources are also presented, along with analysis of the influence of spectral, quantity, and thermal differences.

1.3 Context

Plants respond to their environment through a wide range of environmental cues, and plant research frequently involves measurement of responses to one particular environmental cue while attempting to keep other parameters constant. This is the reason for using controlled environment plant growth facilities. Greenhouses modify temperature, but do not completely control it. Glass enriches the PAR component relative to sunlight, while many other materials enrich the far-red component relative to sunlight, and UV is generally reduced. Shading and filtering of such structures can also influence wavelength ratios. All of these factors can affect photosynthesis and photomorphogenesis (Kittas *et al.* 1999). Thus it is important to characterise these parameters and the effects they may have.

Growth chambers provide accurate control over temperature and light quantity, but the light sources used all vary markedly from sunlight. Photomorphogenic effects can be induced by a wide range of wavelengths, well beyond the normal PAR range, which is often the only light parameter measured. Some light quality influences may be reduced at higher irradiance, but responses vary between and within species. There may also be unmeasured radiant temperature effects from lamps. When using artificial light sources, which will have different spectral properties to sunlight even when mixed, it is important to characterise the full spectral distribution of the light sources in use and to understand the effects they may have on the species being examined.

In addition to temperature and light, CO_2, air velocity and exchange, and humidity also vary within and between controlled environments. CO_2 can become depleted in close cultivation, or elevated from human activity without adequate ventilation (Peet and Krizek 1997), and air velocity is much less variable than outside conditions (Downs and Krizek 1997).

By measuring physical differences between controlled environments and examining plant responses to these variations, insights into which parameters are important under which circumstances can be gained. The controlled environment facilities at the School of Plant Science, University of Tasmania, have evolved since the 1960s with construction and/or purchase of phytotrons, glasshouses, and controlled environment plant growth chambers as need and funds allowed. The facilities now consist of 2 phytotron glasshouses, several glasshouses and shade houses, a multi chambered controlled environment glasshouse, and 24 growth chambers of various age, manufacture, design and lighting source. An overview of the facilities can be found at www.utas.edu.au/glasshouse. Thus, there are a diverse range of glasshouse and growth chamber environments. This allowed for study of the influence of physical variation on growth responses. To study the influence of polycarbonate or glass, the usual choices in greenhouse covering material, measurements and growth studies were conducted at the Department of Primary Industry facility at Knoxfield, Victoria.

A number of other institutions' facilities were surveyed and examined, both to determine the general relevance of this study and to identify common issues. From this, it was found that most such facilities also have a diverse range of greenhouses and growth chambers of various design, age and level of control. Many different light sources were in use, and there was general uncertainty about the potential influence of variations in equipment design and set-up on experimental results. Reports of observed differences in plant responses in different growth chambers and greenhouses were common, but the reasons for these differences were generally not known. While most facilities routinely measured light quantity, none were measuring light quality.

The intention of this thesis is to analyse and characterise a wide range of controlled environments and levels of control available for plant research in terms of light, temperature, humidity, CO_2 levels, air exchange rates and air velocity. These variables will be related to their effects on plant morphogenesis, through analysis and experimentation, with emphasis on the implications this may have for research outcomes. How interchangeable controlled environments are will be examined,

particularly by experiments on pea parental lines, the major source of morphogenic research mutants used at this facility. Pea was chosen as it is sensitive to light quality, quantity, and temperature changes, thus a good indicator of the influence of variation in these factors in controlled environment research.

Generally, controlled environment plant growth equipment is used to minimise environmental variables to examine specific plant responses. This study takes a different approach by using the plant responses to study variation in the controlled environment equipment. To identify the variables of importance, initial chapters are by necessity largely concerned with the physical variations and general plant responses to these variations. In later chapters, a more focused approach could be taken to examine specific responses in more detail.

It is hoped the information in this thesis will assist plant researchers with decisions over experimental design, and controlled environment facility managers with decisions on equipment design, set-up, and use. Thus, inadvertent influences on experimental results from equipment differences can be avoided.

Chapter 2 Glasshouse environments

2.1 Introduction

In this chapter, physical variation between glasshouses is measured. Plant responses in the different environments are examined, and specific experiments are reported on that examined the influence of the physical factors identified as important, notably light quantity and temperature.

Under natural conditions, plants encounter considerable variation in light intensity, quality and duration (Walters and Horton 1994), as well as in temperature, air movement, nutrients, and water status (Hanan 1998; Khaoua *et al.* 2006; Moe and Heins 1990). Greenhouses are largely concerned with modification of temperature by trapping long wave radiation. Modification of the light environment is a consequence of the choice of covering material (Hanan 1998). Structure, geometry and orientation all influence the light environment inside the greenhouse (Mermier and Baille 1988). For example, east-west orientation of the long axis improves light transmission in autumn and winter compared to north-south, but reduces the uniformity of light distribution within the greenhouse (Soriano *et al.* 2004).

Water status and nutrients are largely controlled in protected horticulture, and air movement is less variable than outside conditions (Downs and Krizek 1997). Air movement influences include heat transfer, transpiration, and CO_2 uptake, and thus can influence leaf size, crop yield and shoot length (Downs and Krizek 1997). Greenhouse ventilation is required to assist temperature and humidity control and distribution, and for air exchange to maintain CO_2 levels in particular (Khaoua *et al.* 2006). Mechanical stress from high air velocity can reduce shoot elongation (Morrow and Wheeler 1997), while low air velocity has been blamed for elongated shoots (Downs and Krizek 1997). Generally in controlled environments air velocities between 0.3-0.7 m s^{-1} are recommended (Downs and Krizek 1997). Plants and infrastructure within the controlled environment can strongly influence air velocity distribution and thus microclimate (Khaoua *et al.* 2006).

In regions with short day-length in winter, light is often the limiting factor for plant growth in greenhouses, thus the covering material and greenhouse design become important (Aldrich and White 1969). Typical responses to low light levels are smaller, longer leaves, increased internode length, reduced chlorophyll concentration, and lower dry weight at maturity (McFarlane 1978). The structural frame can reduce light

by up to 70% (Aldrich and White 1969); lightweight frames, larger panel size and lower roof pitches can improve transmission (Aldrich and White 1969). Greenhouse covering material also becomes more important at moderate to high latitudes with glass providing the highest transmission (Aldrich and White 1969; Hanan 1998; Kittas *et al.* 1999).

While in winter light can be limiting, in summer the radiation load needs to be managed in greenhouses to reduce thermal load (Hanan 1998). Various methods are employed, including whitewash, shade cloths and thermal screens. However, excess shading can induce elongation (Potter *et al.* 1999) independently of wavelength (Christophe *et al.* 2006; Gawronska *et al.* 1995). Spectral properties of shade methods have been examined in sunny conditions and found to slightly alter wavelength distribution (Kittas *et al.* 1999), but an examination in overcast conditions or comparative growth responses have not been described.

Study of greenhouse transmission is complex. Transmission varies with material, superstructure orientation, design, shape, slope and height as well as time of day, season, latitude and climatic conditions (Hanan 1998). Greenhouse energy balance involves measurement of global (300-3000nm) transmission. For plant physiology, *in situ* measurements of PAR (400-700nm) and morphogenetically active radiation (300-800nm) is more relevant, with information on the cryptochrome and phytochrome related parameters included (Kittas *et al.* 1999). This study takes that approach. However, such studies generally compare sunny summer conditions. Included here are comparisons during light limiting conditions when transmission and wavelength differences are likely to be of most importance. Hence sunny and overcast measurements were made in summer and winter, as well as examining growth responses in all seasons using garden pea as an indicator of temperature and light variations.

Most aspects of plant life are influenced by the qualities and quantities of light (Spalding and Folta 2005), while temperature is considered to be the major influence on horticultural crops (Faust and Heins 1997; Hanan 1998). Greenhouses are generally used to modify natural temperature variation for more even growth, but temperature can still vary markedly. The rate of development is temperature dependent, with rates increasing up to a maximum, which is the optimum temperature for that species (Heins *et al.* 2000). Cooler temperatures during maturation increase yield in many crop species (Heins *et al.* 2000). Peas are particularly sensitive to water and

temperature stress during maturation (Roche *et al.* 1999). Net photosynthesis decreases with increasing leaf temperature in pea (Haldimann and Feller 2005), and yield and seed weight is negatively correlated with temperature in pea (Chetia and Kumar 2005; Poggio *et al.* 2005). Peas are a cool season crop, hence well suited to the study of the influence of even moderate temperature increases over summer.

The controlled environment facilities at the School of Plant Science, University of Tasmania consist of a range of glasshouses of varying age, structure and orientation allowing examination of glass type, structure and orientation influences on spectral properties and light distribution, both seasonally and temporally. Both laminated glass and horticultural glass are used. Winter light levels can be limiting at this location (42^0 S), while in summer, various shading methods are employed ranging from whitewash through shade cloths and thermal shade screens. This allowed for study of the physical variation between typical research glasshouses, and the influence these variations may have on plant development and morphology.

Other sources of variation between greenhouses include heating and cooling methods which will influence temperature distribution and air velocity. These factors were also examined along with CO_2 levels, which could be depleted with crowded conditions in cool weather (vents closed). To further examine some observed plant responses to measured environmental variations, a number of specific experiments were conducted using garden pea.

2.2 Materials and methods

2.2.1 Glasshouse environments

All the glasshouses examined are aluminium framed with similar orientation, but vary in the type of glass covering and in summer shading method (Table 2.1). The main phytotron is covered in laminated glass (Pilkington, Australia, 6.4mm), the top phytotron by horticultural glass (Pilkington, Australia, 3mm). Summer shading methods are also listed. Various shading methods were examined: whitewash (Parosoline glasshouse paint, Plantecnic, Belgium), 70% shade cloth (Sarlon, Australia) and internal and external thermal shade screens (XLS aluminium/polyester 60% thermal shade screen, Ludvig Svensson Ltd., Kinna, Sweden). Shade cloth measurements were conducted in a small adjacent horticultural glasshouse. The two phytotron glasshouses include a series of automatic plant trolleys on rails that can be moved into climate controlled dark bays. The bays are used for photoperiod control

and can be dark or have extension lighting. The trolley system adds to the shading influence of the structure and for this reason the main phytotron is divided into apron area (the growing area without the trolleys) and bay area (with the trolleys) for much of the analysis.

Heating of the main phytotron glasshouses is provided by electric tubular heaters activated below 15^0C; cooling by vents opening at 23^0C, gable extraction fans at 25^0C, and evaporative cooling at 30^0C. The top phytotron is also heated by electric tubular heaters activated below 15^0C and cooled by air conditioners activated at 25^0C, hence giving a smaller degree of temperature variation. The controlled environment glasshouse is divided into 6 individually controllable cells with heating and cooling provided by fan coil units and chilled water; temperature control is generally within 1-2^0C of set point. Temperature in all the glasshouses is continuously recorded and controlled via Honeywell (Australia) TC205F17 sensors and EBI software.

This variation in glasshouse environments allowed for the examination of a number of factors including the influence of glass type, degree of infrastructure shading, shade method, and level of temperature control, in different seasons.

Table 2.1. Greenhouse environments examined at the Hobart site

Greenhouse	Abbreviation	Cover	Orientation	Shading method
Main phytotron	Main apron	Laminated glass	E-W	External Al screen
Main phytotron	Main bays	Laminated glass	E-W	External Al screen
Top phytotron	Top	Hort. Glass	E-W	Whitewash
Eucalypt house	Euc	Hort. Glass	E-W	Whitewash
Controlled env.	Cell	Laminated glass	NE-SW	Internal Al screen

2.2.2 Light measurements and analysis

Light measurements were taken with a LI-1800 spectroradiometer (LI-COR, Lincoln, NB, USA) with a cosine corrected sensor. All natural light measurements were taken unless otherwise stated at midday in stable conditions: clear sky for sunny measurements, fully overcast for cloudy conditions. Comparative measurements, including transmission percentages, were taken on the same day in the same conditions in immediate succession. Growth chamber light measurements were at an air temperature of 20^0C with external light excluded.

Spectral irradiance was downloaded in W m^{-2} nm^{-1} and as quantum intergrade (μmol m^{-2} s^{-1}) averaged over 3 scans in the range 300-800nm, following measurement and reporting guidelines (Bjorn and Vogelmann 1994; Sager *et al.* 1982). Selected measurements were also taken with an Apogee UV-PAR spectroradiometer (Apogee Instuments Inc., Logan, UT, USA) to check for accuracy. Instrument agreement was generally within 1% in all wavebands. For comparisons of waveband proportions at different irradiances the percentage of quantum intergrade (300-800) was calculated for PPF (photosynthetic photon flux, 400-700 nm) and for each 100nm band.

Wavelength ratios follow published methods (Kittas *et al.* 1999) and were calculated from the quantum data as: R:FR narrow band (R:FRn) 655-665 nm/725-735 nm; R:FR broad band (R:FRb) 600-700 nm/700-800 nm; blue to red (B:R) 400-500 nm/600-700 nm; B:FR 400-500 nm/700-800 nm. Figures quoted for R:FR are broad band unless otherwise stated.

Light distribution measurements were taken using a LI-185B Quantum radiometer with quantum sensor (LI-COR, Lincoln, NB., USA). Daily light integrals (DLI) are given as mol m^{-2} d^{-1} and follow standard calculation methods (Faust 2003) from the light measurement data.

2.2.3 Temperature, air velocity and CO_2 measurements

Air temperature in all the controlled environments is continuously recorded via Honeywell (Australia) TC205F17 sensors and EBI software. This data was used for temperature analysis. Temperature and air velocity distribution within the environments were taken with a Kane-May Ltd (Welwyn, Herts, Great Britain) KM-4000 thermo-anemometer in a range of external weather conditions. Soil temperature was measured 1 cm below the pot surface at weekly intervals with a CPS Inc (Hialeah, Florida, USA) Tempseeker T200 digital thermometer with silicon temperature sensors. Three sensors were used per measurement with 10 pots per location. Surface and leaf temperature measurements were taken with a CPS Inc (Florida, USA) infra red thermometer. CO_2 measurements were taken at weekly intervals with a LI-COR LI-6400 portable photosynthesis system.

2.2.4 Plant growth and measurements

To compare growth responses under the different environments peas (a selection of *Pisum sativum* L. 'Torsdag') were grown in the various environments. This line (Hobart line 107, wild type to a range of photoperiod and shoot length mutants), is a

quantitative long day plant (Reid *et al.* 1996). Plants were sown 2 per pot using even sized seed in 14 cm slimline pots in a 1:1 mixture of grade 3 vermiculite (Australian Vermiculite and Perlite Co., Fairfield, Victoria, Australia) and 10 mm dolerite aggregate (HBMI, Kingston, Tasmania, Australia) topped with 2-3 cm of a pasteurised 1:1 mix of peat moss (Te - Em, New Brunswick, Canada) and coarse river sand (Island Resources, Scottsdale, Tasmania, Australia) with added macronutrients (Osmocote 18N-2.6P-9.9K , Scotts-Sierra, Marysville, OH, USA) at 1 kg m^{-3}, pH was adjusted to 7 with dolomite lime and limestone. All plants were watered as needed and fertilised with nutrient solution weekly consisting of Aquasol (Hortico, Australia), N:P:K 23:4:18 at a rate of 1g l^{-1} plus Iron Chelate (Kendon Chemicals, Sydney, NSW, Australia) at 0.05g l^{-1}.

Plants were sown in the respective treatments and germination recorded. Stem diameter (mid point between nodes 9 and 10), leaf width (LW) and leaf length (LL) of 1 leaflet per plant was measured at node 9 at the commencement of flowering for selected treatments. During growth, node of flower initiation (NFI) and days from planting to first open flower (FT) was recorded. At maturity (senescence) length of internodes 1-9 (L1-9), total shoot length (TL), number of nodes (TN); number of seed (Seed) and number of pods (Pods) were measured. Dry weight was measured after air drying of the harvested plants for at least 72 h.

Statistical analysis using JMP software (SAS Institute, Cary, NC, USA) included ANOVA, Students t-tests, Dunnetts method, and Tukeys test.

2.3 Results

2.3.1 Spectral properties

Spectral properties can alter between summer and winter, mainly due to sun angle, and with cloud cover, mainly due to Rayleigh scattering (Kittas *et al.* 1999). Thus measurements were taken in winter (July) and summer (December) under sunny (clear skies) and cloudy (fully overcast) conditions to quantify the spectral differences that would be experienced by plants in the different glasshouses in different seasons.

2.3.1.1 Winter

Figure 2.1 shows spectral distribution as a function of wavelength of the glasshouses and outside under winter overcast conditions, when light parameters are likely to be most important due to short days and low irradiance. The horticultural glass covered

top phytotron and the laminated glass main phytotron (apron and bays) are shown as the measurements for the other glasshouses covered with the same materials were similar (eucalypt glasshouse, horticultural glass; controlled environment glasshouse cells, laminated glass). Hence this figure is representative of material and structural influences.

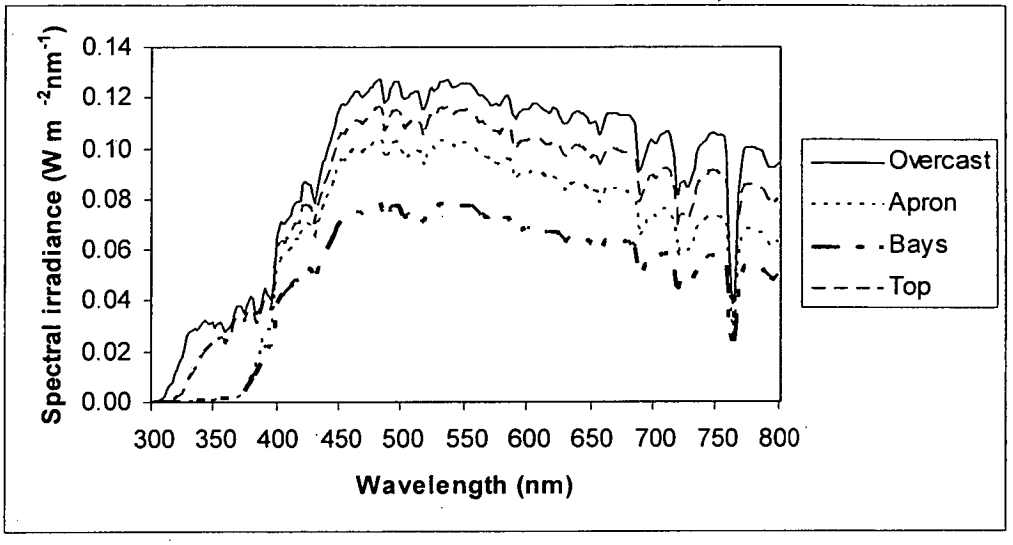


Figure 2.1. Spectral distribution of the glasshouse environments under winter overcast conditions

Lower transmission in the phyotoron bay area relative to the other glasshouse areas can also be seen (Bays, Fig. 2.1), showing the influence of heavier infrastructure shading in this area. Spectral distribution was largely unaltered in any of the glasshouses relative to natural light, apart from the reduced UV-B component through the laminated glass covered apron and bays compared to the horticultural glass covered top glasshouse (Fig. 2.1).

Spectral distribution on a proportional basis also shows this reduced UV-B transmission through laminated glass compared to horticultural glass and natural light under both sunny and overcast conditions (Table 2.2). Otherwise there was little variation between the glasshouses under winter sunny or overcast conditions and natural light (Table 2.2). However, the heavily shaded phytotron bays do show some variation in sunny conditions, with slightly more UV (300-400 nm) and blue (400-500 nm), less red (600-700 nm) and less far red (700-800 nm) proportionally, similar proportions to natural overcast conditions (Table 2.2). Wavelength ratios (Table 2.2) also indicate the slightly higher blue proportion under overcast conditions, and in the

shaded phytotron bays under sunny conditions. Diffuse light is higher in blue wavelengths proportionally (Smith and Morgan 1981), thus heavy infrastructure produced some shade like light qualities even under sunny conditions. However, R:FR was largely unaltered by sun conditions, glass type, or infrastructure shading.

2.3.1.2 Summer

Spectral characteristics of the glasshouse environments were also examined in summer and compared to winter (Fig. 2.2). The slightly higher blue (400-500 nm) proportion in overcast conditions occurs during both summer and winter. In winter sun, red (600-700 nm) and far red (700-800 nm) proportions are slightly higher than summer, but red to far red ratio is unaltered (1.1 in winter and summer).

Spectral distribution and wavelength ratios are largely unaltered winter to summer, outside or in the glasshouse environments, apart from the reduction in UV component under laminated glass (Tables 2.2, 2.3).

In summer, various shade methods are employed to reduce thermal load (Table 2.1). Shade cloth (70% green, Sarlon Australia) is used on some smaller glasshouses, or within glasshouses for specific experiments, hence was included in the analysis. The influence of these common shade methods on spectral properties was examined (Table 2.3).

The apron (main phytotron apron) is covered on the eastern side by aluminium thermal screen. Measurements were taken on this side with the screen up, then rolled down in immediate succession. Top (top phytotron glasshouse) is whitewashed with strips of Parasoline glasshouse paint, applied with a roller. Measurements were taken on adjacent whitewashed and un-whitewashed areas. The euc house (eucalypt glasshouse) is also shaded by this method and spectral properties were no different to the top phytotron. Shade cloth was measured before and after applying the screen to a small glasshouse on site clad in 3mm horticultural glass, the same material as the top phytotron and the euc house. Spectral properties of these 3 glasshouses uncovered was the same, hence only the figures for the top phytotron are included in Table 2.3.

Table 2.2. Waveband proportions (% quantum intergrade 300-800nm) and wavelength ratios of the glasshouse environments under winter sunny and overcast conditions. Main and cell glasshouses are laminated glass, *top and euc glasshouses are horticultural glass.

Environment		Wavelength proportion (% total irradiance)					Wavelength ratios			
	PPF	300-400 UV	400-500 Blue	500-600 Green	600-700 Red	700-800 Far-red	R:FR (n)	R:FR (b)	B:R	B:FR
Sunny										
Outside	69.4	2.4	15.5	25.0	29.0	28.0	1.1	1	0.5	0.6
Main apron	72.9	0.9	16.9	26.9	29	26.1	1.1	1.1	0.6	0.7
Main bay	73.3	1.9	20.8	26.8	25.7	24.5	1	1	0.8	0.9
Cell	73.9	0.9	17.0	27.5	29.3	25.2	1.2	1.2	0.6	0.7
Top*	71.9	2.3	16.7	26.4	28.7	25.8	1.1	1.1	0.6	0.7
Euc*	69	2.5	15.9	24.7	28.3	28.5	1	1	0.6	0.7
Overcast										
Outside	69.8	3.8	17.9	24.9	27	26.4	1.1	1	0.7	0.7
Main apron	74.1	1.2	19.6	27.2	27.3	24.7	1.1	1.1	0.7	0.8
Main bay	73.5	1.4	19.3	27.0	27.2	25.2	1.1	1.1	0.7	0.8
Cell	74.5	1.2	19.6	27.4	27.5	24.3	1.1	1.1	0.7	0.8
Top*	71.6	3.1	18.4	26.2	27.1	25.3	1.1	1.1	0.7	0.7
Euc*	71	3.2	18.3	25.8	26.9	25.7	1.1	1.1	0.7	0.7

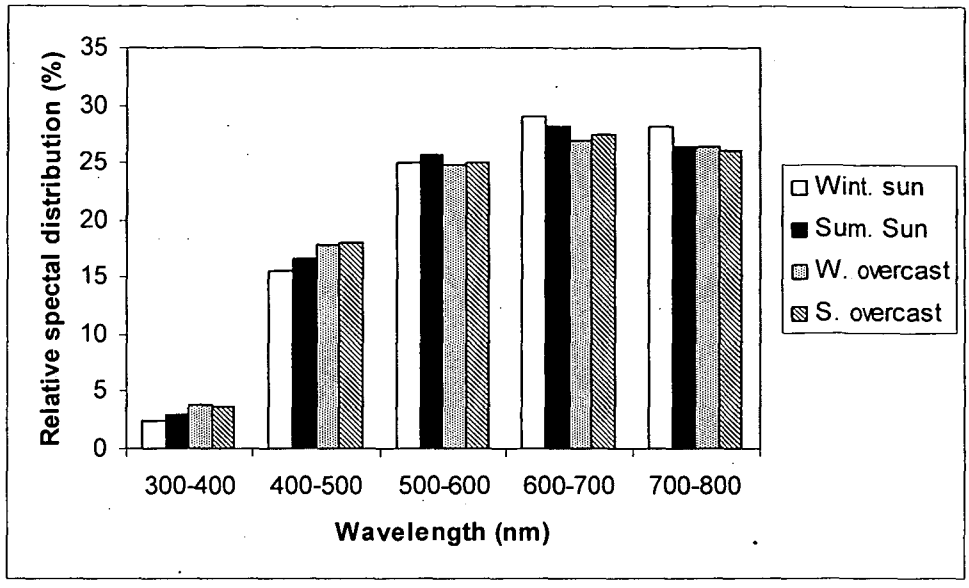


Figure 2.2. Waveband proportions (% 300-800nm) of sunny and overcast conditions, winter and summer, outside.

As for winter, there was little difference in spectral properties between the glasshouses, apart from the UV reduction under laminated glass (main apron). None of the shade methods employed significantly altered the spectral properties of sunlight (Table 2.3), or of overcast conditions (data not shown). However, shading with whitewash and thermal screens did slightly increase blue proportions and wavelength ratios, although R:FR was largely unaltered by any of the shade methods (Table 2.3). The shade cloth examined was wavelength and ratio neutral (Table 2.3), i.e. spectral properties were not altered by shade cloth, only light quantity.

Table 2.3. Spectral distribution (% 300-800 nm) and wavelength ratios for glasshouse environments with and without shading. Abbreviations Apron- main phytotron apron area (laminated glass), Top- top phytotron apron area (horticultural glass), SS- aluminium thermal shade screen, WW- Parosoline glasshouse paint, SC- 70% green shade cloth

Environment		Wavelength proportion (% total irradiance)					Wavelength ratios			
	PPF	300-400	400-500	500-600	600-700	700-800	R:FR	R:FR		
		UV	Blue	Green	Red	Far-red	(n)	(b)	B:R	B:FR
Outside	71.0	2.9	16.7	25.7	28.2	26.5	1.1	1.1	0.6	0.6
Apron sun	74.0	1.2	18.1	27.4	28.5	24.9	1.1	1.1	0.6	0.7
Apron SS	75.0	1.3	19.4	27.7	27.7	23.9	1.1	1.2	0.7	0.8
Top sun	71.0	2.6	16.8	26.1	28.2	26.3	1.1	1.1	0.6	0.6
Top WW	72.0	3.1	18.3	26.6	27.0	25.1	1.1	1.1	0.7	0.7
SC	70.0	2.7	16.5	25.7	27.8	27.3	1	1	0.6	0.6

2.3.2 Light quantity

Natural light quantity is affected by weather, season, cloud cover and day-length. Under intermittent cloud cover, natural light quantity variation can be large (Fig. 2.3). Sun angle variations, time of day and seasonal, also have an influence (Smith and Morgan 1981). In greenhouses, covering material and superstructure also influence light quantity and distribution (Hanan 1998).

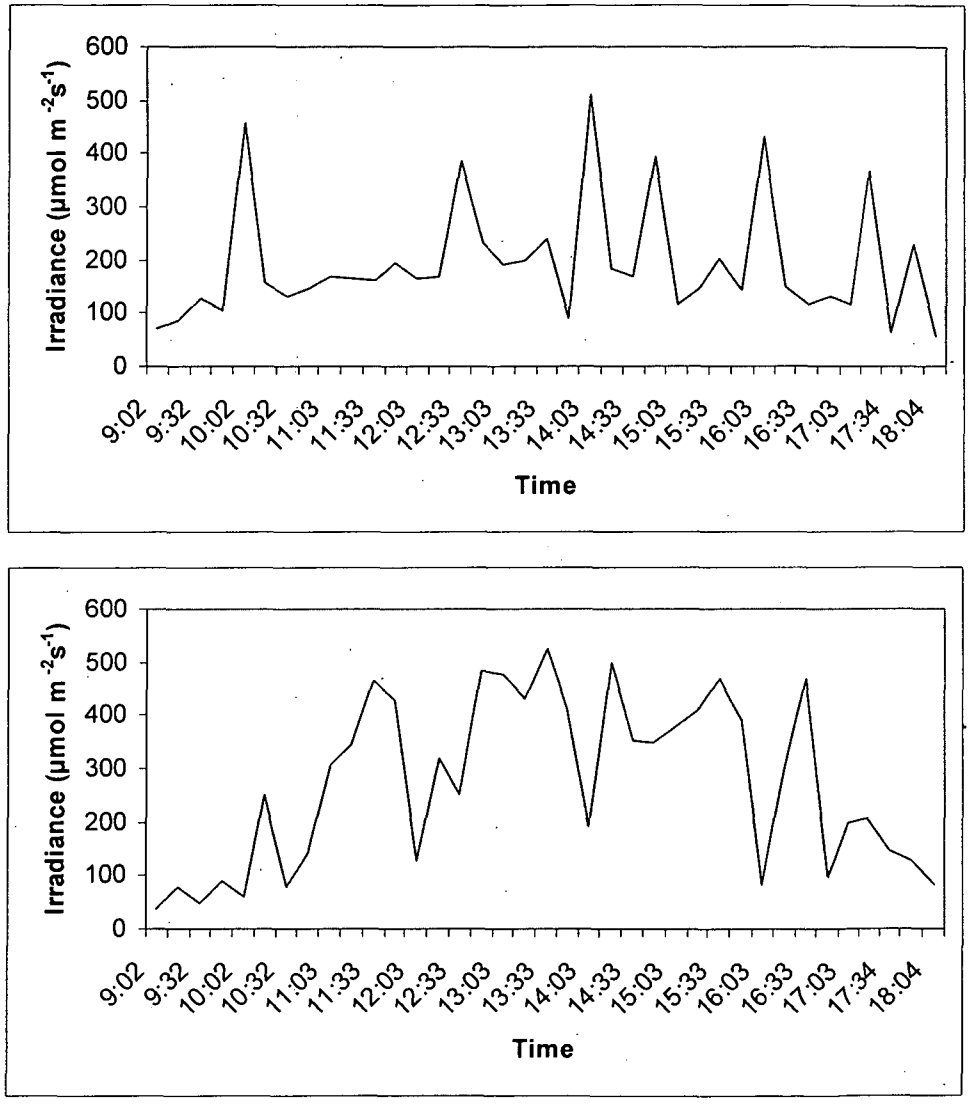


Figure 2.3. Irradiance measurements taken at 15 minute intervals inside a glasshouse on days (11 and 13 March 2007) with intermittent cloud cover.

2.3.2.1 Season

Seasonal light quantity figures for the main phyotoron apron were similar, mean DLI was approximately 14, 11, 14 & 16 mol $m^{-2}d^{-1}$ for autumn, winter, spring and summer (under a shade screen in summer) respectively. Photoperiod was maintained

at 18 h in all seasons, consisting of natural daylight extended morning and evening with weak (10-20 μmol $m^{-2}s^{-1}$, 0.5 mol $m^{-2}d^{-1}$) mixed fluorescent/incandescent lighting with a R:FR of 0.8. Variation within and between glasshouses was examined in more detail for winter and summer.

2.3.2.2 Winter

In situ transmission percentages (Table 2.4) show the strong influence of superstructure shading under sunny conditions in particular, as noted by a number of authors (eg Hanan, 1998; Aldritch and White, 1969). Testing of glass materials *ex situ* gave PPF transmission of 90% in sunlight for laminated glass and 82% for horticultural glass, but inside glasshouse measurements show transmission of only around 50%. The heavy infrastructure in the phytotron bays reduced transmission to less than 14% at plant level in sunny conditions (Table 2.4). The controlled environment glasshouse (Cell) has larger glass panels, smaller extension lighting systems and the highest *in situ* transmission in sunlight, over 80% (Table 2.4). Under overcast conditions, transmission percentages are much higher, over 90% of available light is transmitted in the top phytotron, although in the phytotron bays transmission percentages were still much lower (50-60%, Table 2.4). Under overcast conditions, transmission was higher through horticultural glass (euc house, top phytotron) compared to laminated glass (main glasshouse, controlled environment glasshouse), the reverse of the sunny situation (Table 2.4).

Table 2.4. Transmission percentages (PPF) of the glasshouse environments under winter sunny and overcast conditions

	Laminated glass			Hort glass	
	Main apron	Main bay	Cell	Top	Euc
Sunny	53.6	14.4	81.5	47.2	47.6
Overcast	78.4	59.5	80.3	95.8	89.4

Light distribution measurements were taken at midday in winter in sunny and overcast conditions. Measurements within the greenhouses under overcast conditions were very even, although low, ranging from 100-160 μmol m^{-2} s^{-1}. Under sunny conditions there were stark contrasts from structural shading, ranging from 140-820 μmol m^{-2} s^{-1}. Under heavy cloud, light levels were very low, and there was large variation in winter light levels (Table 2.5). Natural photoperiod averaged 9.6 h in

winter, and mean daily light integrals (DLI) were low, particularly in the phytotron bays (less than 9 mol $m^{-2}d^{-1}$, Table 2.5). Photoperiod in the glasshouses was maintained at 18 h, extending morning and evening by weak (20 µmol $m^{-2}s^{-1}$) fluorescent/incandescent lighting, which contributed minimally to DLI.

Table 2.5. PPF (µmol $m^{-2}s^{-1}$) and daily light integrals (mol $m^{-2}d^{-1}$) for the glasshouse environments during winter 2005

Environment	Range	Mean PPF	Extension PPF	DLI
Outside	43-940	415	0	14.3
Main Apron	32-810	319	20	10.7
Main Bay	24-620	228	20	8.5
Top phytotron	34-820	324	20	11.8

2.3.2.3 Summer

Fluence rates were much higher in summer, averaging almost 3 times higher than winter outside (Table 2.6 and 2.7), and natural photoperiod was up to 15.5 hours. Even on overcast days, close to 600 µmol $m^{-2}s^{-1}$ was measured outside at midday.

The major effect of the shade methods was a dramatic reduction in fluence (Table 2.6). Fluence rate was reduced to less than 30% under the shade screen and to less than 15% under white wash in all wavebands.

Table 2.6. Fluence rates (µmol $m^{-2}s^{-1}$) with and without shading and % transmission under aluminium thermal shade screen (SS), and Parosoline glasshouse paint (WW).

	Apron sun	Apron SS	% trans	Top sun	Top WW	% trans
300-800	1553	438	28.2	1853	276	14.9
PPF	1148	328	28.6	1317	199	15.1
300-400	18	6	30.4	48.5	9	17.5
400-500	282	85	30.2	312	51	16.3
500-600	425	121	28.5	483	74	15.2
600-700	442	121	27.4	522	75	14.3
700-800	386	105	27.1	468	69	14.8

On overcast days, the reduction in transmission was much less- transmission percentages were 58% under the shade screen and 54% under whitewash. Hanan (1998) warns that shading can be overdone in an attempt to control temperature, particularly for dense plantings, but natural photoperiod in summer is long and light quantities high. To quantify the available light, mean midday measurements of PPF in

the glasshouses through summer 2005/6 were recorded and daily light integrals (DLI) calculated (Table 2.7).

Table 2.7. Calculated PPF (μmol $m^{-2}s^{-1}$) and daily light integrals (mol $m^{-2}d^{-1}$) for the glasshouse environments during summer 2005/6

Environment	Range	Mean PPF	Extension PPF	DLI
Outside	580-1710	1184	0	65.8
Apron	310-1260	850	20	47.7
Apron SS	180-360	286	20	16.3
Main Bay SS	130-280	217	20	12.3
Top phytotron	250-1110	576	20	30.6

The significant influence on light quantity of the shade screens can be seen, reducing mean PPF by almost 70% on the apron. Added to the infrastructure shading in the bays, DLI is reduced to12.3 mol $^{-2}d^{-1}$ compared to almost 48 mol $^{-2}d^{-1}$ on the unshaded portion of the same glasshouse apron area (Table 2.7). However, all measurements are within the 12-14 mol $^{-2}d^{-1}$ recommended for peak flowering by Mattson and Erwin (2005). The top phytotron was painted with whitewash, which had the lowest transmission % of around 15% compared to shade screen transmission of 30% (Table 2.6). The whitewash was applied in strips, however, rather than a blanket application, and this method created a higher transmission level than shade screen of around 36% of the uncovered glass. The striped nature also created a moving pattern of light and shade rather than a blanket reduction, with no area in permanent sun or shade. This affected distribution- under the shade screens light distribution was very even, under the striped whitewash, stark contrasts were measured, as indicated by the high range figures (Table 2.7). The net result was significantly higher light levels in the whitewashed glasshouses than the shade screen areas (Table 2.7). Thus in summer, there were large light quantity differences between the glasshouses according to shade method or absence of shade.

2.3.3 Temperature variation

Greenhouses are generally used to modify natural temperature variation, and the climate modifying influence of the glasshouses examined compared to outside conditions can be seen (Table 2.8). However, temperature ranges were still large, and

seasonal temperature variations even within the same glasshouse were significant, with mean temperature ranges of 9-31^{0}C in the main phytotron glasshouse (Table 2.8). These figures also demonstrate that the degree of temperature moderation depends on the level of control equipment, as the evaporatively cooled main glasshouse was appreciably more variable than the air conditioned top phytotron (Table 2.8), while the chilled water fan coil units in the cell glasshouse produce mean temperature variations of less than 2^{0}C in all seasons (data not shown).

Thus effective temperature control in glasshouses is possible, but the control equipment required is significant and costly, both to purchase and to operate. Most research institutions examined had a similar range of greenhouses with varying levels of control, with the bulk of plant material grown in areas with moderate temperature control and smaller areas with high level temperature control for specific experiments.

As for light quantity, then, temperature variation seasonally is large for the bulk of plant material in plant research facilities and a closer examination of the influence of such variation is warranted. Further detail of the temperature variations experienced by the plants seasonally are included in the growth response studies (Section 2.4).

Table 2.8. Mean day and night air temperatures and temperature ranges (^{0}C) by season in selected glasshouses compared to outside conditions.

	Outside			Main phytotron			Top phytotron		
Season	Day	Night	Range	Day	Night	Range	Day	Night	Range
Autumn	15.6	10.9	3-29	20.3	14.5	11-28	19.3	13.8	10-24
Winter	12.5	8.3	2-19	18.2	12.8	9-23	18.1	13.6	10-22
Spring	16.8	11.0	4-31	21.4	14.6	10-31	21.9	14.7	10-27
Summer	21.5	16.2	9-30	24.9	18.5	15-31	22.8	17.4	13-26

2.3.4 CO_2, Air velocity and Humidity variation

CO_2 levels can become limiting in dense canopies and in controlled environments without adequate ventilation (Hanan 1998; Peet and Krizek 1997). Levels can also be enhanced from human activity in closed or poorly ventilated environments (Peet and Krizek 1997). However, measurements taken in all the glasshouse environments in all seasons and weather conditions did not show any significant variation from ambient levels.

Air velocity can vary with greenhouse ventilation type and location, internal structures and plant position, as well as being influenced by external weather conditions (Khaoua *et al.* 2006). External measurements showed wide variation in air velocity, but this was not reflected in the internal measurements. In winter, with vents generally closed, air velocity ranged from 0.1-0.4 m s^{-1} and was generally evenly distributed, although lowest readings were in the phytotron bays. In summer, with vents generally open and active cooling on (evaporative coolers, air conditioning), air velocity ranged from 0.1-0.6 m s^{-1}. Active cooling vents are all shielded with perforated aluminium to reduce air velocity gradients. Distribution within the greenhouses was again relatively even, although velocities as high as 1.4 m s^{-1} could be measured at vent level during active cooling. In the top phytotron, the first plant row had measurements above 0.7 m s^{-1} under such conditions.

Generally in controlled environments air velocities between 0.3-0.7 m s^{-1} are recommended (Downs and Krizek 1997). While often below this, the results suggest ventilation rates within the greenhouses were sufficient to maintain ambient CO_2 levels, and that air velocity distribution was relatively even. Some plant rows adjacent to active vents were above the recommended range.

Humidity influences plant transpiration rates through vapour pressure deficit. Transpiration rate is influenced by temperature, air movement, irradiance, and soil moisture (Spomer and Tibbitts 1997). In well watered plants, low humidity is unlikely to cause desiccation. However, high humidity is associated with plant diseases and can be an issue in controlled environments (Spomer and Tibbitts 1997). Humidity monitoring by glasshouse sensors showed levels vary widely, ranging from 33-81%. In the controlled environment glasshouse humidity was maintained at 60% or above, values ranged from 62-83%. However, no evidence of plant desiccation from low humidity or increased disease incidence at high humidity was observed.

2.3.5 Growth responses

The glasshouse environments showed physical variations in light quality, quantity and temperature, even in the same season. To examine the influences of these variations, if any, a standard pea line known to be sensitive to temperature and light variations (a selection from *Pisum sativum* L. 'Torsdag', Hobart line 107) was grown concurrently in representative glasshouses in the extreme seasons, winter and summer. As well, seasonal variation influences were examined by comparing growth responses

of the same pea line in different seasons in the same glasshouse. Glasshouses used were the laminated glass main phytotron apron and bay areas, which varied in light quantity but not air temperature; and the top phytotron, which is clad in horticultural glass.

2.3.5.1 Winter

In winter, measured light levels were potentially limiting, particularly in the phytotron bays, with a mean daily light integral (DLI) of less than 9 mol m^{-2} d^{-1} (Table 2.5). Light wavelength differences are likely to be more important at low irradiance (Cathey and Campbell 1977), allowing for examination of any influence of the slight spectral variations between the environments, such as the UV reduction under laminated glass (main glasshouse) compared to under horticultural glass (top glasshouse). Mean temperature parameters for the study period were similar in the different environments (Table 2.9). As well as continuous monitoring results (mean day/night temps), weekly midday measurements of air and soil temperature were taken at each growing site (mid. air/soil temp, Table 2.9).

Table 2.9. Mean winter temperature parameters (^{O}C) for the glasshouse environments during the study period (winter 2005)

Environment	Midday air temperature	Midday soil temperature	Mean day temperature	Mean night temperature
Outside	15.2	-	13.3	8.8
Main apron	21.6	19.8	18.5	13.3
Main bay	21.6	19.4	18.5	13.3
Top phytotron	22.4	19.8	18.6	13.4

Photoperiod in all the areas was 18 h, consisting of natural daylight (average 9.6 h) extended morning and evening by weak (20 μmoles m^{-2} s^{-1}) mixed fluorescent incandescent lighting with a red to far-red ratio (R:FR) of 0.8. PPF and DLI measurements over the study period (Table 2.5) show lowest light measurements were in the main phytotron bays, averaging only 8.5 mol m^{-2} d^{-1}, followed by the main apron and top phytotron (10.7 and 11.8 mol m^{-2} d^{-1}). Thus the environments allow comparison of growth parameters in a standard pea line under different light quantity conditions. Plants were grown and analysed as described in Materials and Methods.

In the lowest light environment of the phytotron bays total shoot length (TL, Table 2.10) was significantly increased relative to the other environments. Flowering was also delayed as node of flower initiation (NFI) and flowering time (FT) were significantly ($P<0.05$) delayed compared to the phytotron apron and the top glasshouse (Table 2.10), and stem diameter and leaf size (LW, LL) were significantly ($P<0.05$) reduced. Yield (seed, pods) was also reduced in the bays relative to the other environments, but not significantly so (Table 2.10). There were no significant differences between the apron and top glasshouse (Table 2.10), apart from TL and FT, which decreased with increased light quantity (r^2 0.74 and 0.89 respectively). Dry weight was not significantly different between environments (Table 2.10) in spite of the growth differences. For example, plants in the bays were taller but had smaller stems and leaves, perhaps explaining the lack of significant difference in dry weight relative to the other environments.

In summary, the results show there were significant differences in growth and development parameters between the glasshouse environments during winter, and there was a strong association with light quantity. Even within the same glasshouse (i.e. the main phytotron apron and bays) large differences were observed. Plants grown in the bays, with low light levels, showed increased shoot length and number of nodes, delayed flowering, reduced stem diameter and leaf size.

Table 2.10. Winter and summer mean growth results (± SE) for L107 pea, n = 20. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Daily light integrals: Winter- Bay, 8.5; Apron, 10.7; Top, 11.8; Summer- Bay, 12.3, Apron shaded, 16.3, Apron unshaded 47.7, Top, 30.6 mol m^{-2} d^{-1}. Abbreviations: Bay- main phytotron bay area, Apron- main phytotron apron area, Top- top phytotron apron area, Apron shaded- main phytotron apron area under thermal shade screen, L1-9- length between nodes 1-9, TL- total shoot length, TN- total no. of nodes, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaf let length at node 9, Dry W- shoot dry weight.

Winter											
	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	DryW (g)
Bay	57.1 ± 1.85 a	191.5 ± 8.08 a	20.8 ± 0.39 a*	16.9 ± 0.23 a*	55.3 ± 0.69 a*	17.4 ± 2.58 a	4.9 ± 0.64 a	2.6 ± 0.08 a*	29.0 ± 0.72 a	35.5 ± 0.08 a*	6.9 ± 0.70 a
Apron	62.1 ± 0.83 b	169.9 ± 2.62 b*	19.4 ± 0.16 b	16.1 ± 0.05 b	50.3 ± 0.37 b*	21.0 ± 1.00 a	5.4 ± 0.28 a	3.2 ± 0.07 b	30.8 ± 0.50 b	40.4 ± 0.67 b	6.4 ± 0.20 a
Top	55.0 ± 0.67 a	148.2 ± 3.16 c*	19.5 ± 0.17 b	16.1 ± 0.06 b	47.0 ± 0.40 c*	18.4 ± 1.61 a	5.1 ± 0.36 a	3.2 ± 0.06 b	31.6 0.58 b	42.5 ± 0.80 b	6.9 ± 0.39 a
Summer											
Bay	37.7 ± 0.44 a	100.4 ± 2.20 a	17.9 ± 0.11 a	15.6 ± 0.18 a	33.4 ± 0.44 a	3.7 ± 0.83 a	1.7 ± 0.24 a	2.2 ± 0.06 a	26.5 ± 0.87 a	39.3 ± 1.25 a	3.5 ± 0.15 a
Apron Shaded	34.7 ± 0.83 b	92.4 ± 2.68 a	17.7 ± 0.29 a	15.8 ± 0.24 a	34.9 ± 0.45 a	3.4 ± 0.63 a	1.7 ± 0.19 a	2.3 ± 0.06 a	26.7 ± 0.95 a	39 ± 1.04 a	3.2 ± 0.20 a
Apron	35.1 ± 0.48 b	93.9 ± 2.67 a	17.1 ± 0.31 a	15.1 ± 0.20 a	31.7 ± 0.60 b*	3.0 ± 0.41 a	1.7 ± 0.17 a	2.3 ± 0.05 a	28.3 ± 0.81 a	41 ± 0.92 a	3.6 ± 0.16 a
Top	31.4 ± 0.76 c*	91.6 ± 2.13 a	17.9 ± 0.26 a	15.2 ± 0.25 a	39.1 ± 0.90 c*	7.3 ± 0.71 b*	2.5 ± 0.22 b	2.5 ± 0.10 b	28.0 ± 1.06 a	38.5 ± 1.32 a	3.5 ± 0.08 a

2.3.5.2 Summer

As for winter, to compare growth responses under the different environments described above, peas were grown concurrently in the same glasshouses. In summer, shading methods were employed- whitewash in the top phytotron; shade screens in the main phytotron (eastern side only). This also allowed for the examination of the influence of shade method on growth responses, as plants were grown concurrently under the non-shaded portion of the apron. Mean PPF and DLI for each environment were very variable in the different environments, ranging from 12-48 mol $m^{-2}d^{-1}$ in shaded and unshaded conditions respectively (Table 2.7). Photoperiod was maintained at 18 h, consisting of natural daylight (averaging 15h over summer) extended morning and evening by weak (20 μmoles $m^{-2}s^{-1}$) mixed fluorescent and incandescent lighting. Plants were grown and analysed as described in Materials and Methods.

Temperature parameters were quite variable between the environments in summer (Table 2.11). The main phytotron environments (apron unshaded, apron shaded and bays) were comparable in mean and midday air temperature, but midday soil temperature was significantly higher in non-shaded plants in this environment (Table 2.11). Temperatures were cooler on average in the air conditioned top phytotron than the evaporatively cooled main phytotron, with midday air and soil temperatures up to 5^0C cooler. This demonstrates the influence of cooling method on growing conditions. Thus, unlike winter, there was significant temperature variation between the environments.

Table 2.11. Mean summer temperature parameters (^{o}C) for the glasshouse environments during the study period (summer 2005/6)

Environment	Midday air temperature	Midday soil temperature	Mean day temperature	Mean night temperature
Outside	26.6	-	21.5	16.2
Main apron SS	29.5	24.8	24.9	18.5
Apron no SS	29.5	28.9	24.9	18.5
Main bay	29.5	23.8	24.9	18.5
Top phyt.	25.9	23.8	22.8	17.4
Cell 3	20.4	19.6	20.5	19.8

PPF and DLI measurements (Table 2.7) were also variable. Lowest light measurements were in the main phytotron bays, averaging 12.3 mol $m^{-2} d^{-1}$, while highest measurements were in the same glasshouse on the unshaded side, averaging almost 48 mol $m^{-2}d^{-1}$. Shading dramatically reduced light levels (and soil temperature), on the apron area to 16 mol $m^{-2} d^{-1}$. Light levels in the whitewashed top phytotron were relatively high, over 30 mol $m^{-2} d^{-1}$ (Table 2.7). Thus the glasshouses examined for the growth response studies varied markedly in light quantity and temperature.

Results (Table 2.10) suggest the major influence in summer was temperature, not light quantity. Shoot length (L1-9, TL) was not significantly different on the unshaded and shaded sides of the apron even though daily light integral was almost 3 times higher, nor was stem diameter, leaf size and dry weight. While L1-9 was higher in the relatively low light of the bays, all other parameters were not significantly different to the shaded and unshaded portions of the apron. The exception was flowering time (FT), which was not significantly different between the bays and shaded apron, but significantly earlier ($P<0.01$) on the unshaded side, and significantly later ($P<0.01$) in the top phytotron (Table 2.10). Seed number was also significantly higher ($P<0.01$) in the top phytotron, but not significantly different in the other environments (Table 2.10). Dry weight was not significantly different between any of the environments.

Shade methods dramatically reduced light transmission (Table 2.6) and irradiance (Table 2.7). In the main phytotron, the shade screen reduced DLI from around 48 to 16 mol $^{-2}d^{-1}$. The reduction in thermal load is reflected in a significant reduction in mean midday soil temperature from 29 to 25°C (Table 2.11). However, there were no significant differences for any of the growth and development parameters measured between the shaded and unshaded sides of the apron, apart from earlier flowering time on the unshaded side (Table 2.10).

Overall, the results show there was much less variation in growth responses between the environments in summer compared to winter in spite of the large differences in light quantity. This would suggest that light quantity in the lower light areas was still sufficient for normal growth. Variation appears to be more related to temperature than light in summer. There are large reductions in all growth parameters in the higher temperatures of summer compared to winter, particularly in shoot length, flowering time, and yield (Table 2.10), even in the phytotron bays where light levels were still relatively low. Node of flower initiation (NFI) was relatively stable between the

environments and winter to summer, reducing by around 1 node in summer. Reduction of DLI by shading had little influence on growth parameters, but in the cooler top phytotron, plants had longer internodes, later flowering, and higher yields and larger leaves (Table 2.10). However, this house also varied in covering material (horticultural rather than laminated glass), and shade method (whitewash rather than shade screen).

2.3.5.3 Season

The influence of season was examined by comparing the growth of L107 pea on the main glasshouse apron area in different seasons. In the same glasshouse environment under the same photoperiod (18h) growth responses were variable between seasons, particularly shoot length, flowering time and yield (Table 2.12). Summer figures are from plants grown under the shade screen. As can be seen from Table 2.12, DLI figures were relatively even between the seasons in this area, although lower in winter. The major variations were in mean temperature (Table 2.12). Shoot length decreased with increasing temperature between the seasons (TL r^2=0.76), and yield figures reduce (seed r^2=0.65) (Table 2.12). Flowering node (NFI) was earlier in spring and summer, but was relatively stable between seasons. Days to first open flower (FT), i.e. the vegetative phase, was also influenced by temperature (r^2=0.89). The vegetative phase was longest in the coolest seasons (50 days in winter) and reduced in warmer periods (35 days in summer) (Table 2.12). Dry weight also decreased with increasing temperature (Table 2.12). Late summer figures were included to examine the influence of decreasing temperature- plants were maturing in autumn as temperature was moderating. Shoot length figures were significantly increased relative to spring (when temperatures were increasing) and summer, as were yield figures (Table 2.12).

Late summer plants were also grown in the main glasshouse and outside in a shade house to examine the influence of the lower average temperature outside, and of decreasing temperature after flowering. Compared to glasshouse grown plants, plants outside in the shade house had significantly (at $P < 0.01$) increased total shoot length, flowering time (vegetative phase) and yield (Table 2.12). Plants were similar to winter and autumn grown plants, when temperatures were cooler but DLI was less.

Table 2.12. Mean environmental and growth parameters (± SE, n = 20) for L107 pea grown on the main phytotron apron in different seasons. Late summer plants were also grown concurrently in a shade house (Late summer shade house). Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Abbreviations: L1-9- length between nodes 1-9, TL- total shoot length, FT- flowering time from planting date, NFI- node of flower initiation, LW- leaflet width at node 9, LL- leaflet length at node 9, DW-shoot dry weight.

Season	Mean day temp (^{0}C)	Mean DLI ($molm^{-2}d^{-1}$)	L1-9 (cm)	TL (cm)	FT (days)	NFI	Seed	Pods	Stem (mm)	LW (mm)	LL (mm)	DW. (g)
Winter	18.2	10.7	62.1 ± 0.83 a*	169.9 ± 2.62 a*	50.3 ± 0.37 a*	16.1 ± 0.05 a	21.0 ± 1.00 a*	5.4 ± 0.28 a*	3.2 ± 0.07 a*	30.8 ± 0.50 a	40.4 ± 0.67 a	6.4 ± 0.20 a*
Autumn	20.3	14.2	37.7 ± 0.66 b	151 ± 2.20 b*	45.3 ± 0.35 b*	16.0 ± 0.13 ab	18.2 ± 0.77 a*	4.6 ± 0.18 a*	2.6 ± 0.08 b	28.3 ± 0.84 a	39.4 ± 1.24 a	5.7 ± 0.18 a*
Spring	21.4	14.4	39.4 ± 0.92 b	118.9 ± 4.00 c*	36.4 ± 0.46 c*	15.3 ± 0.14 b*	10.2 ± 1.42 b*	3.5 ± 0.28 b*	2.7 ± 0.06 b*	31.3 ± 0.68 a	44.9 ± 0.86 b*	4.4 ± 0.19 b*
Summer	24.9	16.3	34.7 ± 0.83 b	92.4 ± 2.68 d*	34.9 ± 0.45 c*	15.8 ± 0.24 ab	3.4 ± 0.63 c*	1.7 ± 0.19 c*	2.3 ± 0.06 c*	26.7 ± 0.95 b*	39.0 ± 1.04 a	3.6 ± 0.16 c*
Late Summer	22.2	15.6	42.6 ± 0.56 c	127.3 ± 2.18 c*	36.1 ± 0.67 c*	16.0 ± 0.31 a	11.9 ± 0.69 b*	3.5 ± 0.20 b	2.7 ± 0.06 b*	32.2 ± 0.65 a	42.5 ± 3.72 a	5.3 ± 0.21 ab*
Late summer-shade house	21.1	15.6	39.6 ± 0.78 b	156.8 ± 6.63 a*b*	43.6 ± 0.55 b*	15.9 ± 0.17 a	14.1 ± 1.09 b*	5.2 ± 0.32 a*	3.3 ± 0.07 a*	32.6 ± 0.63 a	43.8 ± 0.92 b	5.9 ± 0.20 a*

2.3.6 Examining the reasons for growth response variations

2.3.6.1 Light quality (spectral properties)

Spectral differences between the glasshouse environments were minor. The shading methods examined did not significantly alter the spectral properties of sunlight (Table 2.3), and there were no significant differences between plants grown on the shaded and unshaded sides of the apron apart from flowering time (Table 2.10). The largest spectral difference was in the reduced UV-B component through laminated glass compared to horticultural glass (Fig. 2.1). In winter, plants grown in the main glasshouse (laminated glass) were significantly longer and days to first flower was significantly later (L1-9, TL, FT, Table 2.10) than those grown concurrently in the top glasshouse (horticultural glass), even though light and temperature profiles were similar and photoperiod extension type was the same. In summer, plants had longer L1-9 under horticultural glass, and flowering time and yield was increased (Table 2.10), but there were significant temperature differences (Table 2.11).

To examine the influence of UV, independently of temperature, L107 pea was grown in the controlled environment glasshouse at 20^0C day, 18^0C night, ± 1^0C under laminated glass. Supplementary UV was supplied to half the plants over the full 18 h photoperiod by fluorescent black lights (Fig. 2.4) at a fluence rate of 6 μmol m^{-2} s^{-1}. This was to provide 0.4 mol m^{-2} d^{-1} UV, the calculated deficit from natural light UV levels during the study period (autumn). Also grown was pea L218, a day neutral selection from L107, to examine if there was any difference in response between a photoperiodic and non photoperiodic line.

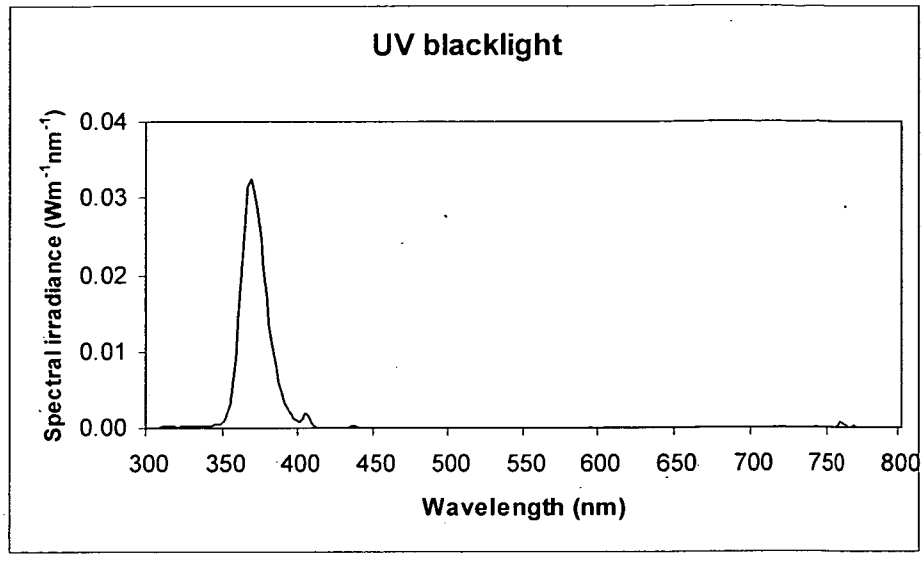


Figure 2.4. Spectral distribution of Sylvania (Tokyo, Japan) F36W/BLB-T8 Black light

Results (Table 2.13) show that for L107, there were no significant differences between UV depleted and UV added plants, apart from a slight increase in number of nodes (TN) without UV. For L218, total shoot length, number of seed and stem diameter was significantly increased ($P< 0.05$, Table 2.13) with UV added. Other parameters were not significantly different. There was no significant difference in flowering parameters for either the photoperiodic (L107) or day neutral lines (L218).

2.3.6.2 Light quantity

2.3.6.2.1 Duration

L107 pea is a quantitative long day photoperiodic line and wild type to a range of photomorphogenic mutants (Weller *et al.* 2001) grown at the Hobart site. The phytotron bay system (automated trolleys) allows for photoperiod control, and is examined in Chapter 5. As a quantitative plant, L107 flowers later under short days, (node 23 under 8h, Chapter 5, Table 5.4), and around node 16 under 18h (Table 2.12). Standard photoperiod in the glasshouses is 18h. It was identified that in winter plants were influenced by low light levels (Table 2.12), and one way of increasing light quantity is to extend the photoperiod. Some species show improved growth under a 24h photoperiod, while others do not. For example, increasing photoperiod from 16 to 24 h with the same total daily light integral produced a 50% yield increase in lettuce (Koontz *et al.* 1987). However, some species (e.g. radish and chrysanthemum) show reduced leaf development and dry weight, and some growth abnormalities under continuous light (Warrington and Norton 1991). The response of pea appears to have

not been described in these terms; hence the specific response of increasing photoperiod from 18h to 24h was examined under controlled conditions.

To examine the growth response of pea to a 24h photoperiod, L107 was grown in growth chambers under fluorescent/incandescent lighting at 20^0C at 18h and 24h. Irradiance was 200 μmoles $m^{-2}s^{-1}$ under 18h and 150 μmoles $m^{-2}s^{-1}$ under 24h to give equal total daily light integral of 13 mol $m^{-2}d^{-1}$, allowing for examination of the difference in photoperiod without temperature or total light quantity differences.

Compared to 18h, plants under 24h at equal DLI showed significantly ($P < 0.01$) reduced total shoot length, number of nodes, earlier flowering node and time, reduced yield and dry weight (Table 2.14). Leaf size was not significantly different (Table 2.14).

Table 2.13. Mean growth parameters (± SE, n = 20) for lines 107 and 218 pea grown concurrently in a controlled environment laminated glass glasshouse (UV depleted) with and without 0.4 mol m^{-2} d^{-1} UV-B supplement provided by Sylvania (Japan) F36W/BLB-T8 Black light. Photoperiod 18h consisting of natural light extended by high pressure sodium globes. Temperature 22^0 day, 18^0 night ±1^0C. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Abbreviations: L1-9- length between nodes 1-9, TL- total shoot length, TN- total no. of nodes, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaflet length at node 9, Dry W- shoot dry weight.

L107											
	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
- UV	46.0 ± 0.66 a	144.0 ± 2.54 a	20.8 ± 0.16 a	17.5 ± 0.12 a	36.3 ± 0.32 a	11.8 ± 0.79 a	4.1 ± 0.29 a	2.6 ± 0.07 a	31.6 ± 0.59 a	43.4 ± 0.73 a	4.7 ± 0.12 a
+ UV	47.0 ± 0.59 a	140.2 ± 1.41 a	20.3 ± 0.14 b	17.3 ± 0.11 a	36.0 ± 0.26 a	11.0 ± 0.51 a	3.9 ± 0.21 a	2.7 ± 0.07 a	32.6 ± 0.58 a	45.5 ± 0.94 a	4.6 ± 0.06 a
L218											
	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
- UV	40.0 ± 0.56 a	94.8 ± 2.00 a	16.4 ± 0.23 a	13.4 ± 0.17 a	29.9 ± 0.39 a	7.4 ± 0.65 a	4.4 ± 0.31 a	2.8 ± 0.04 a	32.9 ± 0.39 a	44.9 ± 0.67 a	3.7 ± 0.07 a
+ UV	40.9 ± 0.38 a	100.7 ± 1.24 b	16.8 ± 0.17 a	13.4 ± 0.22 a	29.7 ± 0.54 a	10.0 ± 0.69 b*	4.9 ± 0.32 a	3.0 ± 0.04 b*	32.5 ± 0.41 a	45.9 ± 0.46 a	4.0 ± 0.17 a

Table 2.14. Mean environmental and growth parameters (± SE, n = 20) for L107 pea grown concurrently in controlled environment chambers under fluorescent/incandescent lighting at 20^0C at 18 and 24h. Irradiance was 200 µmoles $m^{-2}s^{-1}$ under 18h and 150 µmoles $m^{-2}s^{-1}$ under 24h to give equal total daily light integral of 13 mol $m^{-2}d^{-1}$. * signifies significant differences at $P<0.05$, ** significant at $P<0.01$. Abbreviations: L1-9- length between nodes 1-9, TL- total shoot length, TN- total no. of nodes, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaflet length at node 9, Dry W- shoot dry weight.

	L1-9 (cm)	TL (cm)	TN	NFI	FT (days)	Seed	Pods	Stem (mm)	LW (mm)	LL (mm)	Dry W (g)
18h	31.4 ± 0.58	131.5 ± 3.23	23.3 ± 0.33	18.1 ± 0.18	35.4 ± 0.44	19.9 ± 1.71	7.8 ± 0.70	2.8 ± 0.08	35.0 ± 1.02	46.3 ± 1.30	5.7 ± 0.16
24h	33.2 ± 0.54*	107.9 ± 2.0**	21.5 ± 0.24**	16.7 ± 0.15**	31.2 ± 0.57**	12.8 ± 0.93**	6.2 ± 0.50 ns	2.9 ± 0.07 ns	33.0 ± 0.57 ns	45.6 ± 0.98 ns	4.7 ± 0.16**

2.3.6.2.2 Irradiance

Most species have an optimum irradiance range for maximum growth rates. Dry weight accumulation increases with irradiance for many species up to a threshold (Mattson and Erwin 2005), but responses vary widely by species (Warrington and Norton 1991). Maximum flowering for many crop species is between 12 and 14 mol $m^{-2}d^{-1}$ (Mattson and Erwin 2005). Photosynthetic rates also vary with species, but most C_3 species reach light saturation by 600 μmol $m^{-2}s^{-1}$ (McCree 1972a). For peas, maximum rates are at less than 500 μmol $m^{-2}s^{-1}$, photosynthetic rates reduce above 500 μmol $m^{-2}s^{-1}$ (Chow and Anderson 1987a).

Low light levels can be a stress to plants. Peas typically respond to low light with shoot elongation (Gawronska *et al.* 1995) and this appeared to be the case for winter grown plants in the glasshouse bays (Table 2.10). Light levels in the glasshouse environments varied widely between each other (e.g. 8.5-10.7 mol $m^{-2}d^{-1}$ in winter in the main phytotron bays and apron, Table 2.6) and between seasons (eg 12.3-16.3 mol $m^{-2}d^{-1}$ for the same areas in summer, up to 47.7 mol $m^{-2}d^{-1}$ on the unshaded apron, Table 2.7). However, temperature also varied (Table 2.12); hence the specific response to irradiance was examined under controlled conditions.

To examine the growth response of pea to irradiance, L107 was grown in growth chambers under fluorescent/incandescent lighting at 20^0C under an 18h photoperiod. Irradiance was 80, 150, 220 and 300 μmoles $m^{-2}s^{-1}$ PPF, giving total daily light integrals of 5.2, 9.7, 14.3, and 19.4 mol $m^{-2}d^{-1}$ respectively.

Results show that at equal temperature, increased irradiance significantly ($P < 0.01$) reduced shoot length (Table 2.15). Length of nodes 1-9 was strongly correlated with irradiance (r^2=0.88), decreasing with increasing irradiance. Dry weights, however, were not significantly different between treatments (Table 2.15). Optimum irradiance appeared to be at 14 moles $m^{-2}d^{-1}$, with earlier flowering time, highest yield and stem diameter, and dramatically increased leaf size (Table 2.15).

Table 2.15. Mean environmental and growth parameters (± SE, n = 20) for L107 pea grown concurrently in controlled environment chambers under fluorescent/incandescent lighting at 20^{0}C at 80, 150, 220 and 300 µmoles $m^{-2}s^{-1}$, photoperiod 18h. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Abbreviations: L1-9- length between nodes 1-9, TL- total shoot length, TN- total no. of nodes, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaflet length at node 9, Dry W- shoot dry weight.

PPF/DLI	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
80/5.2	46.1 ± 0.27 a*	150.3 ± 1.64 a	22.6 ± 0.21 a	18.7 ± 0.18 a*	39.8 ± 0.20 a	15.2 ± 1.33 a	4.4 ± 0.23 a*	2.1 ± 0.05 a*	28.3 ± 0.50 a	37.0 ± 0.71 a	5.4 ± 0.16 a
150/9.7	43.5 ± 0.48 b*	141.5 ± 2.99 b	22.1 ± 0.22 a	17.5 ± 0.11 b*	39.5 ± 0.29 a	8.5 ± 0.60 b*	7.2 ± 0.33 b*	2.9 ± 0.13 b	29.4 ± 0.74 a	39.3 ± 1.31 a	5.2 ± 0.42 a
220/14.3	33.4 ± 0.42 c*	122.2 ± 1.46 c*	22.9 ± 0.26 a	17.5 ± 0.16 b*	33.4 ± 0.34 b*	16.1 ± 1.09 a	5.8 ± 0.42 c	3.4 ± 0.05 c	37.6 ± 0.44 b*	50.3 ± 0.53 b*	5.2 ± 0.16 a
300/19.4	29.6 ± 0.85 d*	117.4 ± 2.09 d*	22.9 ± 0.21 a	18.6 ± 0.17 a*	38.7 ± 0.38 a	13.3 ± 1.11 a	5.7 ± 0.37 c	3.1 ± 0.09 b	29.9 ± 0.81 a	39.0 ± 0.92 a	5.3 ± 0.15 a

2.3.6.3 Temperature

The major differences in the glasshouse environments in summer appeared to be temperature related, with reductions in shoot length, yield and flowering time in particular associated with increased temperature (Table 2.12). To examine the influence of temperature, independently of irradiance, L107 was grown in growth chambers with fluorescent/incandescent lighting under an 18h photoperiod at 15, 20, and 25^{0}C at optimum irradiance (shown above to be 220 μmoles $m^{-2}s^{-1}$ PPF, 14.3, mol $m^{-2}d^{-1}$) Results (Table 2.16) show that at 15 ^{0}C, total shoot length, number of nodes, flowering node, flowering time, yield and dry weight were significantly increased relative to 20^{0}C. At 25^{0}C, shoot length, flowering time, yield, stem diameter, leaf size and dry weight were significantly reduced relative to plants grown at 20^{0}C. Development time was slower at lower and earlier at higher temperature, reflected in flowering time (Table 2.16), but also in days to senescence (90 days at 15^0, 65 days at 20^0, 60 days at 25^{0}C). Thus, relative to 20^{0}C, lowering temperature increased yield and biomass, but strongly delayed development time. Higher temperature reduced yield and biomass significantly and slightly accelerated development time. At 20^{0}C, leaf size was significantly ($P < 0.01$) larger than at 15 and 25^{0}C, smallest leaf size was at the higher temperature (Table 2.16). Total shoot length, flowering time, yield, and dry weight appear to be closely associated with temperature in these results (Table 2.16), with r^2 values of 0.93, 0.98, 0.79 and 0.77 respectively. Node of flower initiation (NFI, Table 2.16) was least influenced by temperature (r^2=0.28).

To confirm these responses under natural light conditions, L107 plants were also grown concurrently in adjacent controlled environment glasshouse cells. Temperature was 20^{0}C day 15^{0}C night and 25^{0}C day, 20^{0}C night, both ± 2^{0}C. Results (Table 2.16) also showed significantly ($P < 0.05$) reduced total shoot length, flowering time, yield, stem diameter, leaf size and dry weight at 25^{0}C compared to 20^{0}C. Again NFI was not significantly different (Table 2.16).

Table 2.16. Mean growth parameters (± SE, n = 20) for L107 pea grown concurrently in controlled environment chambers under fluorescent/incandescent lighting at 15, 20 and 25°C at 220 μmoles $m^{-2}s^{-1}$, photoperiod 18h; and in adjacent controlled environment glasshouse cells under natural light with photoperiod extended to 18 h with high pressure sodium lamps at 20 and 25° ±2°C. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Abbreviations: L1-9- length between nodes 1-9, TL- total shoot length, TN- total no. of nodes, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaflet length at node 9, Dry W- shoot dry weight.

Growth chambers											
°C	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
15	31.8 ± 0.40 a	156.3 ± 1.49 a*	25.8 ± 0.22 a*	18.4 ± 0.13 a	55.0 ± 0.46 a*	30.0 ± 1.51 a*	10.0 ± 0.51 a*	3.4 ± 0.06 a	32.0 ± 0.47 a*	40.8 ± 0.48 a	6.1 ±0.12 a*
20	33.4 ± 0.42 b	122.2 ± 1.46 b*	22.9 ± 0.26 b	17.5 ± 0.16 b*	33.4 ± 0.34 b*	16.1 ± 1.09 b*	5.8 ± 0.42 b*	3.4 ± 0.05 a	37.6 ± 0.44 b*	50.3 ± 0.53 b*	5.2 ± 0.16 b*
25	27.9 ± 0.41 c*	93.7 ± 2.01 c*	22.3 ± 0.34 b	18.2 ± 0.12 a	27.7 ± 0.21 c*	6.6 ± 0.66 c*	3.8 ± 0.34 c*	2.5 ± 0.04 b*	26.0 ± 0.64 c*	38.9 ± 0.79 c	3.7 ± 0.08 c*
Controlled temperature glasshouse											
°C	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
20	32.5 ± 0.84 a	121.4 ± 4.11 a	20.7 ± 0.41 a	17.0 ± 0.24 a	40.0 ± 0.29 a	15.2 ± 1.32 a	5.3 ± 0.67 a	3.5 ± 0.19 a	37.4 ± 1.15 a	48.8 ± 1.66 a	5.7 ± 0.32 a
25	33.1 ± 0.82 a	97.3 ± 4.74 b*	20.0 ± 0.47 a	17.4 ± 0.31 a	30.8 ± 0.42 b*	11.1 ± 1.04 b	3.7 ± 0.54 b	2.7 ± 0.11 b*	28.4 1.64 b*	40.6 ± 1.62 b*	3.3 ± 0.35 b*

2.3.6.4 Air velocity, CO_2

In common with most research greenhouses, the glasshouses examined have staged cooling. Initially vents open at 20^{0}C (passive cooling), and extraction fans or air conditioning operating at 23-25^{0}C (active cooling). Air velocity measured in the glasshouses was generally within the recommended range for controlled environments of 0.3-0.7 m s^{-1} (Downs and Krizek 1997), even with active cooling. However, up to 1.4 m s^{-1} was measured close to vents during active cooling. To examine the influence of air velocity, L107 was grown concurrently under glasshouse conditions at 0.1-0.3 m s^{-1} (i.e. the normal air velocity range measured in the ventilated glasshouse with passive ventilation), and with air velocity increased with fans to 0.7 and 1.4 m s^{-1} (the ranges measured with active cooling).

There were no significant differences between treatments for number of nodes, flowering node, flowering time, yield, stem diameter, leaf size or dry weight. However, length of nodes 1-4, 1-9 and total shoot length all showed significant ($P < 0.01$) reductions at 0.7 m s^{-1} compared to 0.3 m s^{-1}. Although shoot length was further reduced at 1.4 m s^{-1}, the reductions were not significantly different (e.g. Fig. 2.5 for L1-9). Thus increased air velocity reduced shoot length but not other growth parameters.

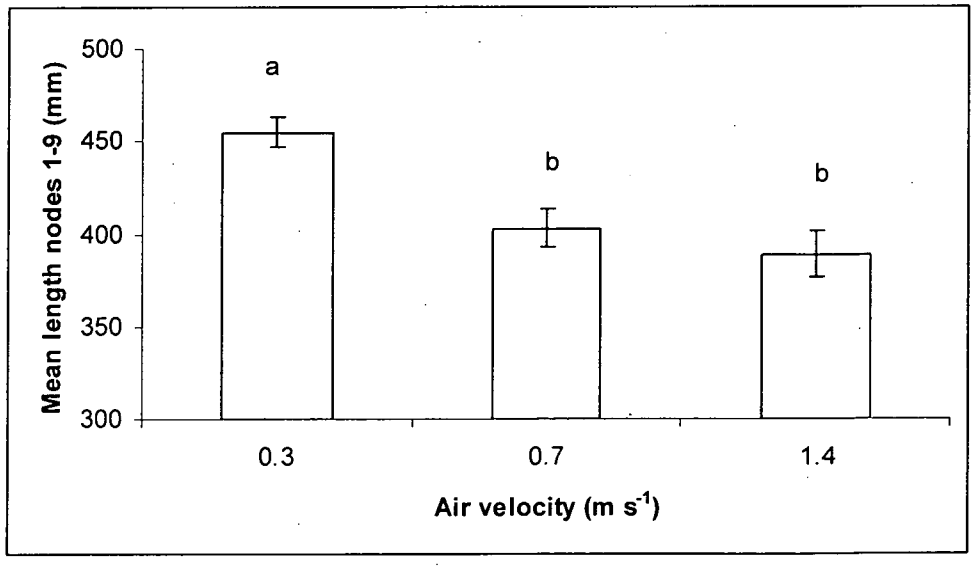


Figure 2.5. Mean length of nodes 1-9 for L107 pea grown concurrently in glasshouse conditions at air velocities of 0.3, 0.7 and 1.4 m s^{-1}. Different letters signify significant differences at $P<0.01$, n = 20.

2.4 Discussion

2.4.1 Spectral properties

There was little difference in natural light spectral properties between winter and summer either in sunny or overcast conditions (Fig. 2.2). In overcast conditions, blue proportions are increased, but R:FR ratios are unaltered (Table 2.2). Increased blue has been attributed to light scatter from cloud cover (Smith and Morgan 1981), and also appears to be a property of scattered light in the glasshouses from structural shading, as the phytotron bays showed similar increases in blue proportions, as did areas under thermal shade screens. The light scattering properties of the heavy infrastructure in the bays, and of the reflective aluminium shade screens may be responsible. However, differences were minor with around a 1% increase in blue in shaded conditions.

Apart from this difference in the bays, there was also little difference in spectral properties in the glasshouses in any season, apart from the UV reduction under laminated glass (main phytotron) compared to horticultural glass (top phytotron), (Fig. 2.1, Table 2.2). In winter, when these areas had similar temperature and light quantity profiles, shoot length and flowering time was reduced in the top phytotron, but all other growth parameters were the same (Table 2.10). In summer, shoot length was also reduced but flowering time was later, and yield was increased in the top phytotron (Table 2.10). However, it was markedly cooler in the top phytotron compared to the apron over this period. To test if UV reduction had an influence, both L107 and a day neutral pea line (L218) were grown at controlled temperature in a laminated glass glasshouse with and without supplementary UV radiation. Results (Table 2.13) showed no significant differences for L107, but slight increases in shoot length and yield of L218 with UV added to restore ambient levels. There have been numerous studies on the influence of above ambient UV levels: high UV can induce tissue damage, stunting, reduced leaf area and increased thickness, and increased branching. Stomata may close, photo protective pigments increase and chlorophyll content reduces, thus photosynthesis is reduced (Beggs and Wellman 1994; Teramura 1983), including in pea (Nogues *et al.* 1999). However, these effects may be modified by other wavelengths in the PAR range, and plants can acclimate (Kakani *et al.* 2003). Although UV can be damaging, a lack of UV can reduce anthocyanin synthesis and

produces "weaker" plants (Hashimoto 1994). For pea, restoring UV to ambient levels produced slight but not significant growth improvement, and the UV reduction under laminated compared to horticultural glass is unlikely to be a significant influence on growth responses.

None of the shade methods employed significantly altered the spectral properties of sunlight (Table 2.3), unlike canopy shade where far red proportions in particular are significantly enhanced (Morgan 1981; Smith and Morgan 1981). Both whitewash and shade cloth can be considered wavelength neutral with respect to sunlight (Table 2.4). Thermal shade screens are commonly used in research greenhouses, as they provide insulation at night and shade during summer days. The thermal shade screen used in the main phytotron over summer did induce a slight increase in R:FR, from 1.1 to 1.2, and slightly higher blue proportion (Table 2.4). This was in agreement with previous reports (Kittas *et al.* 1999), but growth responses were not examined. In this study, there were no significant differences (apart from flowering time, which appeared to be temperature related, Table 2.12) in growth parameters between plants grown under shade screen and those with no shading (Table 2.10). This suggests the slight spectral alterations under shade screens can also be considered spectrally neutral for plant responses, and that the common methods of artificial shade do not mimic the spectral properties of the filtered light in plant canopies where R:FR ratio is reduced in proportion to the degree of shading (Morgan 1981).

Spectral properties, then, showed little variation seasonally or between glasshouses, were little influenced by shading methods, and the slight changes recorded did not appear to influence the growth responses of a pea line known to be sensitive to such changes.

2.4.2 Light quantity

In contrast to spectral properties, light quantity was highly variable, both seasonally and daily. Ranges in winter and summer (Tables 2.5, 2.7) showed large variation and large quantity variations can also be found on an hourly basis with cloud cover (Fig. 2.3). *In situ* measurements showed the strong influence of greenhouse structures on light quantity (Table 2.4). While glass transmitted up to 90% of sunlight, within the glasshouse transmission was reduced to around 50%, and was under 15% amongst the heavy infrastructure of the phytotron bays. In the controlled environment glasshouse, with little structural shading, transmission was over 80%. Thus mean daily light

integrals (DLI) was very low in the phytotron bays in winter (Table 2.5), and plants grown in this period showed significantly increased shoot length and flowering time, with reduced stem diameter and leaf size (Table 2.10), characteristics of low light responses (McFarlane 1978). The effect of infrastructure shading could also be observed from light distribution measurements, particularly under sunny conditions where midday measurements on the same day ranged from, for example, 140-820 $\mu mol\ m^{-2}\ s^{-1}$. Lower figures were under extension lights and stands on the phytotron apron. However, with the earth's rotation, the sun position is not fixed relative to the glasshouse, and there was no area of the glasshouses in permanent shade. Rather, there was a moving pattern of light and shade within the glasshouses throughout the day, and photosynthetic adaptation to light levels is rapid (Walters and Horton 1994). While higher total crop yields are reported under diffused light, attributed to more even light distribution (Hanan 1998; Aldritch and White 1969), these glasshouses are not used for whole crops but for a series of small scale independent experiments. Nevertheless, there was no evidence of improved growth under the diffusing nature of the shade screens compared to unshaded conditions (Table 2.10).

Winter light levels can be limiting under diffusing materials and maximum transmission needs to be considered (Aldritch and White 1969). Under overcast conditions, winter light levels measured were at times very low, 5 times lower than sunny conditions outside, even though transmission was relatively high under overcast conditions (Table 2.4), and in winter plants were relatively elongated compared to other seasons (Table 2.12). One way to increase daily light integral is to increase the photoperiod, for example from 18 to 24h. Some species such as lettuce, show improved growth under a 24h photoperiod (Koontz *et al.* 1987), while others do not (Warrington and Norton 1991). To examine the response of pea to a 24h photoperiod, L107 was grown in growth chambers at 18 and 24h but at equal total daily light integral of 13 $mol\ m^{-2}d^{-1}$. Results (Table 2.14) showed the longer photoperiod was detrimental to growth for pea, with reduced shoot length, yield and dry weight compared to 18h, but earlier flowering.

In contrast to winter, summer light levels were very high, 3 times higher than winter outside (Tables 2.5, 2.7), and was highly variable between environments, ranging from means of 12 $mol\ m^{-2}\ d^{-1}$ in the phytotron bays to almost 48 $mol\ m^{-2}\ d^{-1}$ on the unshaded main phytotron apron (Table 2.7). A common method for reducing thermal load in greenhouses in summer is to use shading to reduce solar gain. The primary aim

of shading is not the creation of shade but to limit unacceptable temperature increases from high solar irradiance (Hanan 1998). Whitewash is simple and cheap, but can produce heavy shading and is considered inflexible (Connellan 1996). Retractable shade screens offer the flexibility to be opened on overcast days, and closed when needed. Shade screens with insulating properties (thermal screens) can be opened during low light, closed during high light and at night to reduce heat loss (Connellan 1996; Hanan 1998). While measurements under whitewash confirmed the heavy shading properties of this material (transmission of 15%, Table 2.6), there is flexibility in how the material is applied. With striped application, summer light levels in the top phytotron were relatively high, over 30 mol m^{-2} d^{-1}, compared to 16 mol m^{-2} d^{-1} under shade screen (Table 2.7), even though shade screen transmission was close to 30% (Table 2.6). The striped application method created a higher transmission level of around 36% of the uncovered glass. The striped nature also created a moving pattern of light and shade rather than a blanket reduction. This affected distribution- under the shade screens light distribution was very even, under the striped whitewash, stark contrasts could be found. However, as for uncovered glass, no area was in permanent shade. The net result was significantly higher light levels in the whitewashed glasshouses than the shade screen areas (Table 2.7). Photosynthetic adaptation to light is rapid, as plants transferred from high to low light intensity show rapid changes in photosynthetic rate and chlorophyll a/b ratio and an increase in light harvesting complexes (Walters and Horton 1994). Peas also adapt rapidly, photosynthetic capacity increased on transfer from low light (60 μmoles $m^{-2}s^{-1}$) to high (390 μmoles $m^{-2}s^{-1}$) with no obvious lag phase, and both high and low light adapted plants showed similar utilisation efficiency of low light (Chow and Anderson 1987a).

Use of shading maintained relative even light levels between seasons, yet growth responses were highly variable (Table 2.12). In winter, with relatively even temperature profiles in the glasshouses, shoot length and flowering time showed a close association with light quantity (Table 2.10). In summer shoot length was not significantly different on the unshaded and shaded sides of the apron (Table 2.10) even though daily light integral was almost 3 times higher, although flowering time was earlier under higher light. To further examine the role of light quantity, plants were grown in growth chambers at equal temperature (20^0C) but different irradiances (80, 150, 220 and 300 μmol m^{-2} s^{-1}). There were clear reductions in shoot length with increasing irradiance (Table 2.15). Flowering time was not significantly different at

80, 150 and 300 μmol m^{-2} s^{-1}, but was significantly earlier at 220 μmol m^{-2} s^{-1}. This irradiance (around 14 mol m^{-2} d^{-1}) appeared to be the optimum irradiance for pea growth, with highest yield, stem diameter and leaf size (Table 2.15).

Overall, the results show that light quantities are highly variable and structural shading can dramatically reduce light levels. In winter, plant responses suggested light was limiting in the phytotron bays, with increased shoot length, known to be associated with irradiance in pea (Gawronska *et al.* 1995). This was confirmed by growth chamber experiments (Table 2.15). In summer, the much higher irradiance can be managed with shading, and the commonly employed methods examined can be considered as spectrally neutral, both physically and in terms of pea responses. Although light quantity was substantially reduced by shading, light quantities were still adequate for growth, and its use resulted in relatively even DLI between seasons (Table 2.12). Peak irradiance for pea growth was around 14 mol m^{-2} d^{-1}.

2.4.3 Temperature

Mean day temperature ranges were moderate between seasons, ranging from 18-25^{0}C in the main phytotron (Table 2.8), but pea is a cool season crop strongly influenced by temperature (Chetia and Kumar 2005). Temperature appeared to exert a major influence, independently of irradiance. The seasonal results at relatively even DLI (Table 2.12) suggest a strong influence of temperature on shoot length and yield in pea, and the growth chamber experiments at equal irradiance confirmed this. Seasonally, total shoot length, flowering time, seed number, and stem diameter appeared to be closely associated with temperature, decreasing with increasing temperature. Leaf size reduced, but was less variable between seasons. Node of flower initiation was relatively stable between seasons. Plants grown in the cooler shade house over summer were similar to winter and autumn grown plants, when temperatures were cooler but DLI was less, suggesting the shoot length, yield and flowering time reductions in spring and summer were related more to temperature than light quantity.

Growth variation also appeared to be more related to temperature than irradiance in summer. There were large reductions in all growth parameters in the higher temperatures of summer compared to winter, particularly in shoot length, flowering time, and yield (Table 2.10), even in the phytotron bays where light levels were still relatively low. Node of flower initiation (NFI) was relatively stable between the

environments and winter to summer, reducing by around 1 node in summer. In the main phytotron, the shade screen reduced DLI from around 48-16 mol $^{-2}d^{-1}$ (Table 2.7). The reduction in thermal load was reflected in a significant reduction in mean soil temperature from 29 to 25^{0}C, but air temperature was not altered (Table 2.11). However, there were no significant differences for any of the growth and development parameters measured between the shaded and unshaded sides of the apron apart from earlier flowering time on the unshaded side (Table 2.14). The air conditioned top phytotron was on average 2^{0}C cooler in summer (Table 2.11), and plants had longer internodes, later flowering, higher yields and larger leaves (Table 2.10). Most species have an optimum temperature, above which growth and yield declines rapidly (Heins *et al.* 2000), and under field conditions, yield has been negatively correlated with increasing temperature for pea (Chetia and Kumar 2005; Poggio *et al.* 2005).

Under the same light source at equal irradiance in growth chambers, and under natural light in a controlled environment glasshouse, the strong influence of temperature on pea could be seen. Total shoot length, flowering time, yield, and dry weight were closely related to temperature in these results (Table 2.16), while node of flower initiation (NFI) was least influenced by temperature, (r^2 of 0.28). At 15^{0}C, total shoot length, number of nodes, flowering node, flowering time, yield and dry weight were significantly increased relative to 20^{0}C. At 25^{0}C, shoot length, flowering time, yield, stem diameter, leaf size and dry weight were significantly reduced relative to plants grown at 20^{0}C. Development time was slower at lower and earlier at higher temperature. Higher temperature reduced yield and biomass significantly and reduced leaf size (Table 2.16).

2.4.4 Air velocity and CO_2

Light quantity and temperature varied between environments and seasons, and had significant influences on growth. However, the influence of air velocity, CO_2 and humidity was minor. While humidity was often variable, ranging from 40% to over 80%, there was no evidence of growth impacts on well watered plants. CO_2 levels showed little variation from ambient readings in any of the glasshouse environments. Air velocity and distribution, while highly variable outside, was generally within the 0.3 to 0.7 m s^{-1} range recommended for controlled environments (Downs and Krizek 1997). Increasing air velocity above this level did reduce shoot length (Fig. 2.5), but all other growth measurements were not significantly different.

2.4.5 Indicative plant responses

In L107 pea, shoot length was the most variable factor in response to environmental variation. Shoot length was increased by low irradiance, and reduced by increased temperature and high air velocity. Node of flower initiation was relatively stable at different irradiances and temperature, but was reduced by a 24h photoperiod. Flowering time was increased by low irradiance, but shows close association with temperature, reducing as temperature increases, and a reflection of development time. Yield (seed and pods) showed close correlation with temperature, reducing as temperature increased, as did dry weight. However, dry weight, commonly used as a biomass indicator, was not always representative of growth differences. For example, in winter plants in the phytotron bays had significantly smaller leaves and stems relative to the other environments, but dry weight was not significantly different, as these plants also had increased shoot length. Stem diameter and leaf size was relatively stable between environmental conditions, but was reduced at low irradiance and by higher temperature. Optimum growing conditions for this pea line would appear to be at 20^0C under an 18h photoperiod at around 200 μmol $m^{-2}s^{-1}$, or 14 mol m^{-2} d^{-1}, at air velocities below 0.7 m s^{-1}. Least favorable growing conditions would be above 23^0C under a 24h photoperiod at less than 100 μmol $m^{-2}s^{-1}$, or 9 mol m^{-2} d^{-1}, at air velocities above 0.7 m s^{-1}.

Chapter 3 Glass or Polycarbonate: influence of covering material

3.1 Introduction

A common decision in greenhouse design is the choice of covering material. Many plastic films are available, but for long term structures, the choice is usually between glass and polycarbonate (Lexan). In Australia in recent years, the choice has tended to lean towards polycarbonate for research greenhouses, as its impact resistance more easily meets containment regulations. In the commercial sector, the trend has been towards glass because of its high light transmission and longevity. Surprisingly, given the costs involved, there have been few studies on comparative growth responses under these materials. In this section, spectral properties and growth responses of a standard research pea line under glass and polycarbonate is examined.

In Chapter 2, the strong influence of glasshouse infrastructure on light quantity was shown in sunny conditions (Fig. 2.1); the influence was less under overcast conditions (Table 2.4). Shoot length of pea was influenced by light quantity, with reduced leaf size and relatively increased shoot length at lower irradiance under both natural (Table 2.10) and controlled conditions (Table 2.15).

The spectral properties of natural light varied according to cloud cover, with increased blue proportion relative to sunny conditions (Table 2.2), but little difference by season (Fig. 2.2). Commonly used summer shading methods also did not influence spectral properties appreciably (Table 2.3). Both laminated and horticultural glass did not alter the spectral properties of natural light significantly, apart from the UV reduction under laminated glass (Table 2.2). However, UV supplementation to natural levels under laminated glass did not influence growth responses (Table 2.13).

At moderate to high latitudes, light is often the limiting factor for plant growth in greenhouses during short winter days, thus type of covering material and greenhouse design become more important (Aldrich and White 1969). Most studies confirm glass provides the highest transmission of greenhouse covering materials; several types of reinforced plastic materials had visible light transmission values 15-37% lower with up to 20% further losses in transmission when aged for 5 years (Aldrich and White 1969). Double layer covers have higher absorption and, although energy efficient, can limit growth in low light climates (Hanan 1998). Polycarbonate is 10 times lighter

than glass, thus requiring less supporting structure. Transmission is 7-14% less than glass but even with less infrastructure, light transmission inside the greenhouse is usually less than inside a glasshouse (Mermier and Baille 1988). Plastic films are available in a wide range of chemical compositions and additives, mainly UV stabilisers for durability and infrared absorbers to reduce thermal heat loss. Transmission levels range from 75 to 90%, and the light weight requires less supporting structure (Connellan 1998; Mermier and Baille 1988). Transmission deteriorates with age, with longevity for plastic films estimated at 2-4 years, 7-12 years for polycarbonate, and 30-50 years for glass (Connellan 1998).

Transparency of covering material also has an influence. Transparent materials provide sharp contrasts, while translucent materials provide more light scatter and thus more even distribution (Aldrich and White 1969). Some studies report up to 25% increase in dry weight under translucent coverings, even though light quantity was lower. However, for many species examined, lower winter light levels under fibreglass and reinforced plastics resulted in elongated stems and abnormal development compared to glass grown plants (Aldrich and White 1969). Tomato plant height was significantly higher under acrylic and polyethylene compared to glass (Erhioui *et al.* 2002). There are also temperature interactions. Growth and flowering of snapdragon and stock was better under glass than double layer polyethylene film, although dry weight was similar, attributed to the improved thermal properties under the double layer film (Dansereau *et al.* 1998). Carnation and cucumbers had lower yields and flowering was delayed by 1-4 days under double acrylic than under single glass greenhouses with identical design and orientation (Reiersen and Sebesta 1981). Heat loss was 50% less and light transmission 15% lower in the acrylic compared to the glasshouse in this study (Sebesta and Reiersen 1981). While many of these papers relate the observed growth differences to temperature and/or light quantity, rarely is consideration given to alterations in light quality under the different covering materials. Kittas *et al.* (1999) measured spectral differences between greenhouse covering materials under clear sky conditions but did not examine plant responses. While supplementary lighting tended to correct any growth anomalies under non-glass materials, suggesting a light quantity response (Erhioui *et al.* 2002; Hao and Papadopoulos 1999), the influence of spectral properties is rarely described. Height reduction by filtering far red light is well described (Cerny *et al.* 2003), as is growth

improvements by supplementing natural light with high pressure sodium lamps, which can offset light quantity reductions (Moe 1997).

Kittas *et al.* (1999) examined the spectral properties of glass, plastic film, polyethylene and fibreglass greenhouses with and without various shading methods. They found for similar structures and dimensions, glass transmits the highest PAR, although the lighter structure of the plastic film greenhouse allowed for higher total transmission. There was no influence of covering material or shading method on phytochrome related parameters, but blue wavelengths were reduced significantly under all covers except glass. Glass also contributes to a relative enrichment of PAR relative to total radiation, and is recommended for areas where light is limiting in winter (Kittas *et al.* 1999). Transmission, spectral properties and growth responses were examined at this location (Hobart, Tasmania, Australia, latitude 42^0 S) under laminated and horticultural glass. Glass and polycarbonate properties and growth responses were examined in more detail at the Department of Primary Industries at Knoxfield, Victoria, Australia, which has parallel climate controlled glass and polycarbonate greenhouses, allowing for a clear comparison of the influence of these materials on light properties and plant responses.

3.2 Materials and methods

3.2.1 Greenhouse environments

Glasshouses examined at the Hobart site are listed in Table 2.1. All glasshouses are aluminium framed and clad in either laminated glass (Pilkington Australia, 6.38 mm) or horticultural glass (Pilkington Australia, 3mm). Also on the Hobart site is a CSIRO greenhouse framed with galvanised steel and clad in polycarbonate 8mm glazing sheet (Allplastics Engineering, Sydney, Australia). Glass and polycarbonate properties and growth responses were examined in more detail at the Department of Primary Industries at Knoxfield, Victoria, which has parallel climate controlled horticultural glass and polycarbonate greenhouses. This greenhouse complex was opened in 1994, thus the covering materials were 12-13 years old at the time of the study. Each greenhouse has the same design (galvanised steel frame clad in either horticultural glass or polycarbonate), size (7 x 4 metres), control equipment (fan coil units and evaporative cooling) and orientation (long axis north-south). Environments are continuously monitored and controlled via a Nelan Industries (Melbourne, Victoria, Australia) greenhouse control system.

3.2.2 Light measurements and analysis

Light measurements were taken with a LI-1800 spectroradiometer (LI-COR, Lincoln, NB, USA) and/or with an Apogee UV-PAR spectroradiometer (Apogee Instruments Inc., Logan, Utah, USA), both with cosine corrected sensors. Measurements at the Hobart site were taken of the isolated materials (horticultural glass, laminated glass and polycarbonate sheeting) in sunny conditions by placing the spectroradiometer in a box with the top cut at an angle of 60^0 (to simulate roof pitch) and placing a sheet of the material over the top. Measurements were also taken in the greenhouses in immediate succession under clear sky conditions. Greenhouses measured were the main phytotron (laminated glass), top phytotron (horticultural glass) and the CSIRO greenhouse (polycarbonate). This allowed for *in situ* measurements of the materials under the same conditions.

Spectroradiometer measurements were taken under both sunny and overcast conditions under horticultural glass and polycarbonate during the growth response studies at DPI, Knoxfield, Victoria. Adjacent greenhouses with the same design and orientation were used. This allowed for *in situ* comparisons under a range of light conditions and at various times of the day. In addition, light quantity (in kilo lux) is monitored and recorded at 15 min intervals in the DPI greenhouses. This data was downloaded and analysed for the growth response study periods.

Comparative measurements, including transmission percentages, were taken on the same day in the same conditions in immediate succession. Growth chamber light measurements were at an air temperature of 20^0 C with external light excluded. Spectral irradiance was downloaded in W m^{-2} nm^{-1} and as quantum intergrade ($\mu mol \cdot m^{-2} \cdot s^{-1}$) averaged over 3 scans in the range 300-800nm, following the measurement and reporting recommendations of Sager *et al.* (1982) and Bjorn and Vogelmann (1994). For comparisons of waveband proportions at different irradiances the percentage of quantum intergrade (300-800) was calculated for PPF (photosynthetic photon flux, 400-700 nm) and for each 100nm band.

Red to far-red ratios (R:FR) were calculated as narrow band (R:FR n): 655-665 / 725-735 nm; broad band (R:FR b) 600-700 / 700-800 nm. Blue to red ratios were calculated as 400-500 / 600-700 nm (B: R); blue to far red as 400-500 / 700-800 nm (B:FR). Ratio calculations follow the recommendations outlined in Kittas *et al.* (1999).

Light distribution measurements were taken using a LI-185B Quantum radiometer with quantum sensor (LI-COR, Lincoln, NB, USA). Daily light integrals (DLI) are given as mol m^{-2} d^{-1} and follow standard calculation methods (Faust 2003) from the light measurement data.

3.2.3 Plant material and culture

To compare growth responses under the different environments peas (a selection of *Pisum sativum* L. 'Torsdag') were grown concurrently in adjacent greenhouses clad with either polycarbonate or horticultural glass in both spring (17/10-29/11/2006) and winter (4/6-6/8/2007). This pea line (Hobart line 107) is a quantitative long day plant. As no supplementary lighting was used for the study periods (so that only the influence of covering material was being examined) a day neutral isoline of L107 was also grown (Hobart line 218). This allowed for collection of flowering data under short day conditions (winter), and also allowed for examination of any growth differences between a photoperiodic and non-photoperiodic line under the different covering materials. Plants were sown 2 per pot using a commercial pine bark based potting mix with added macro and micro nutrients (Bio-Gro, Bayswater, Victoria, Australia). Plant spacing was sufficient to avoid self shading.

Plants were watered with timed sprinklers for 10 min twice daily. Mean temperatures for the study periods were spring- maximum 24.5^{0}C, minimum 12.6^{0}C (glass), 24.4^{0}C and 13.0^{0}C (poly); winter- maximum 21.7^{0}C, minimum 10.2^{0}C (glass) 21.6^{0}C and 10.5^{0}C (poly). Thus temperatures were very even between the environments and the major difference was the covering material.

3.2.4 Data collection and analysis

At harvest, length of each internode from nodes 1 to 12 was recorded. Stem diameter (mid point between nodes 9 and 10), leaf width (LW) and leaf length (LL) of 1 leaflet per plant was measured at node 9. Node of flower initiation (NFI) and days from planting to first open flower (FT) was recorded during growth. Fresh weight of harvested shoots was also recorded.

Statistical analysis using JMP software (SAS Institute, Cary, NC, USA) included ANOVA, Students t-tests, Dunnetts method, and Tukeys test.

3.3 Results

3.3.1 Transmission and spectral properties

Transmission percentages of the isolated materials were 68, 82 and 90% for polycarbonate, horticultural glass and laminated glass, respectively, in the PPF (400-700 nm) range. In the polycarbonate, horticultural glass and laminated glass greenhouses at the Hobart site, however, there was little difference in transmission, all were around 58% when measured in immediate succession. Figure 3.1 shows *in situ* spectral distribution measurements in the greenhouses at the Hobart site, and it is clear from this figure that the transmission losses are relatively even. This highlights that differences in greenhouse design and orientation can influence light transmission.

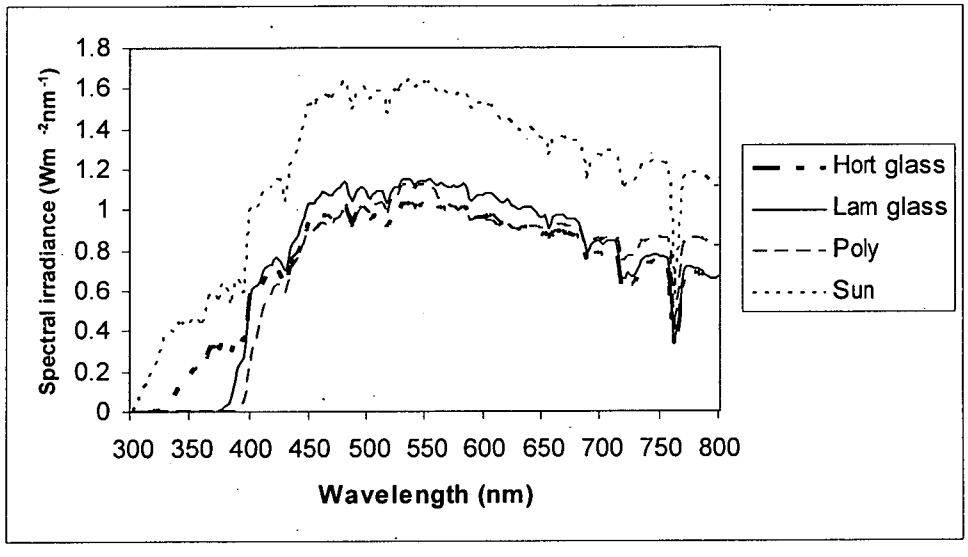


Figure 3.1. Spectral distributions of sunlight, and *in situ* measurements of laminated glass, horticultural glass, and polycarbonate. Measurements were conducted in immediate succession, as described in Materials and Methods.

There is little difference in spectral distribution through any of the tested covering materials and sunlight, apart from the elimination of UV-B radiation below 390 nm by polycarbonate and laminated glass (Fig. 3.1). Wavelength ratios were largely unaltered from sunlight ratios under any of the materials, although blue wavelengths were slightly reduced (by 2%) and far-red proportions slightly increased (by 2-3%) under polycarbonate (Fig. 3.2). The slight enhancement of PAR wavelengths reported for glass (Kittas *et al.* 1999) can also be seen, particularly for laminated glass (Fig. 3.2). This conforms with previous reports (Young *et al.* 1994) for polycarbonate (Lexan) and for polyethylene and fibreglass greenhouses (Kittas *et al.* 1999).

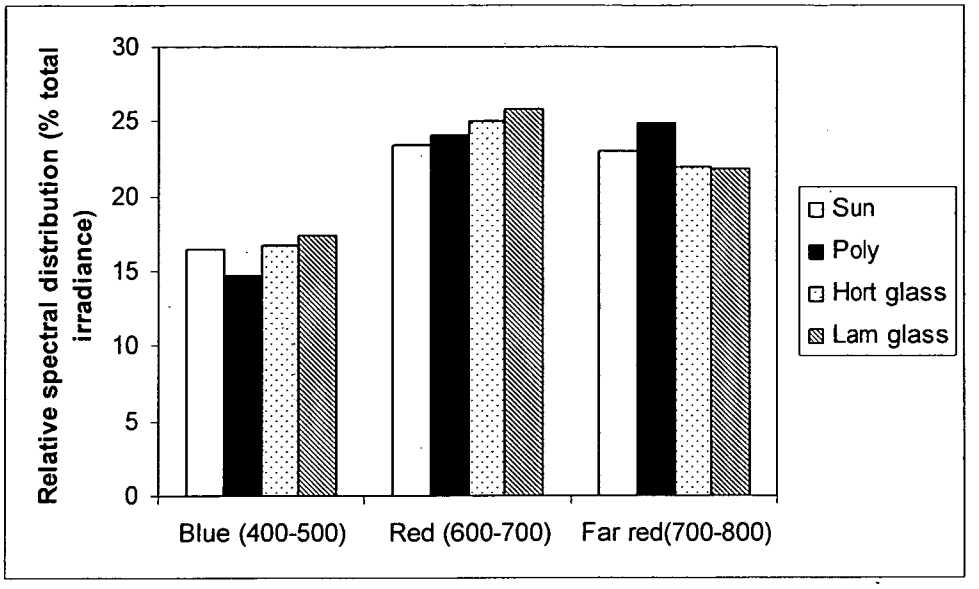


Figure 3.2. Relative spectral distribution as a percentage of total irradiance (300-800 nm) for sunlight, polycarbonate, horticultural glass and laminated glass.

It is interesting to note that *in situ* measurements in the glasshouses were comparable to those in the polycarbonate greenhouse at the Hobart site (Fig. 3.1). However, design, orientation and internal structures of this house differed from the glasshouses, which have more structural components than the polycarbonate house. By comparison, transmission measurements in the controlled environment glasshouse, which has larger panel size and less structural components than the other glasshouses, were over 80% (Table 2.6), again highlighting the influence of design on light properties. Thus further analysis was conducted at the DPI Knoxfield site, which has adjacent greenhouses with the same design and orientation. This allowed for *in situ* comparisons under a range of light conditions and at various times of the day. Measurements were conducted in both spring and winter during the growth response studies.

Analysis of spectral distribution seasonally and by time of day showed the same relative spectral properties of the covering materials. The slightly reduced blue and slightly enhanced far-red proportions of polycarbonate relative to glass and natural light was observed at all times of the day and under both sunny and overcast conditions (Table 3.1). Representative spectral distributions at different times of the day and under sunny and overcast conditions are shown in Figure 3.3. Clear from these figures, and confirmed by the continuous monitoring data, was that at all times under all conditions, transmission was lower through polycarbonate compared to glass. With lower sun angle in the morning and afternoon, and in overcast conditions,

transmission differences were less (Table 3.2). However, midday figures showed large differences in transmission, particularly under sunny conditions (Fig. 3.3 A), in spring and winter.

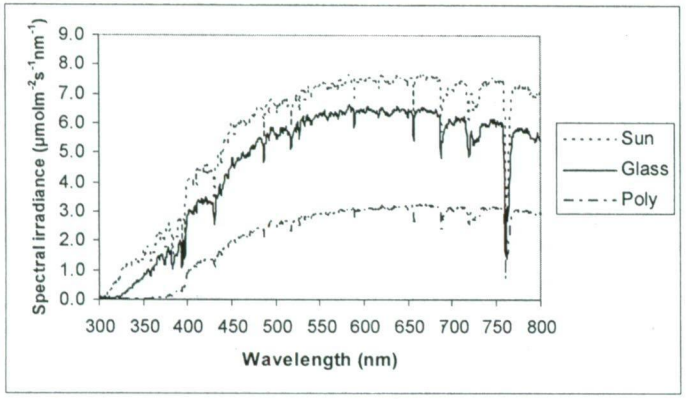
3.3.2 Growth responses

Growth response studies were conducted in adjacent greenhouse cells in both spring and winter, as described in Materials and Methods. In spring, growth differences between plants grown under glass or polycarbonate were slight (Table 3.3). Shoot length was slightly reduced under polycarbonate in both pea lines even though light quantity was reduced under this material, suggesting light quantity was not a limiting factor. Flowering measurements were not significantly different under glass and polycarbonate for either line, suggesting light quality was not a significant factor as NFI in particular is most influenced by light quality. Leaf size was reduced under polycarbonate relative to glass in L218, and fresh weights were reduced. Overall, while differences were small, plants under glass were taller, healthier plants in appearance with higher fresh weights.

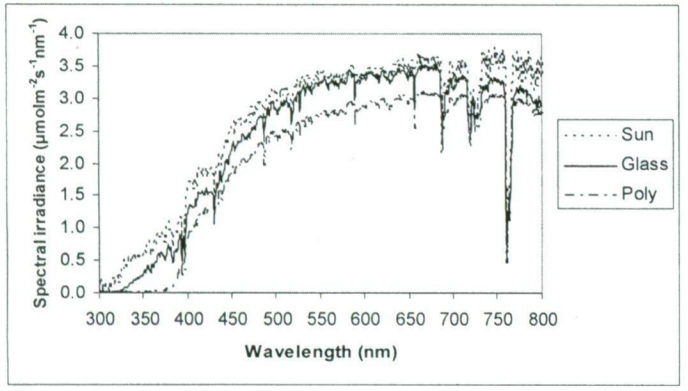
In winter, plants under polycarbonate showed clear responses to low light levels. Compared to plants grown under glass, both L107 and L218 showed significant ($P \leq 0.01$) shoot elongation at all growth stages. L218 also had significantly reduced leaf size and fresh weight compared to plants grown under glass (Table 3.3). Flowering node was not significantly different between treatments for either line, suggesting light quality differences were not a significant influence, but flowering time was delayed under polycarbonate relative to glass.

Table 3.1. Waveband proportions (% quantum intergrade 300-800nm) and wavelength ratios of natural light and adjacent greenhouse environments under winter sunny and overcast conditions.

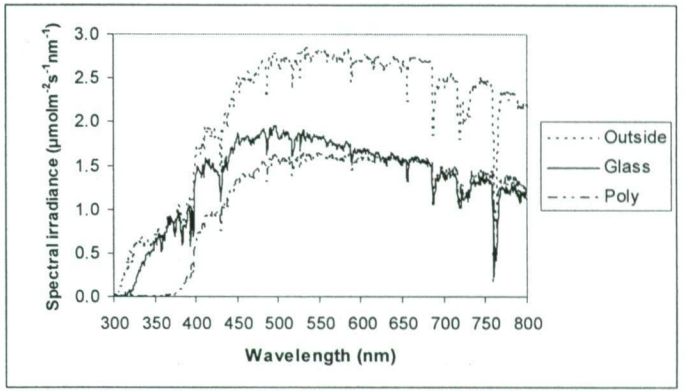
Environment	Wavelength proportion (% total irradiance)						Wavelength ratios			
	PPF	300-400 UV	400-500 Blue	500-600 Green	600-700 Red	700-800 Far-red	R:FR (n)	R:FR (b)	B:R	B:FR
Sunny										
Outside	70.5	5.1	19.1	25.1	26.2	24.6	1.1	1.1	0.7	0.8
Glass	72.4	3.5	18.6	26.4	27.2	24.2	1.1	1.1	0.7	0.8
Poly	72.4	0.8	17.0	26.5	28.8	27.0	1.1	1.1	0.6	0.6
Overcast										
Outside	69.2	5.4	19.9	24.7	24.8	25.6	1.0	1.1	0.8	0.8
Glass	71.9	4.8	21.3	26.0	24.8	23.5	1.1	1.2	0.9	0.9
Poly	73.4	1.0	21.4	26.7	25.8	25.8	1.0	1.2	0.8	0.8



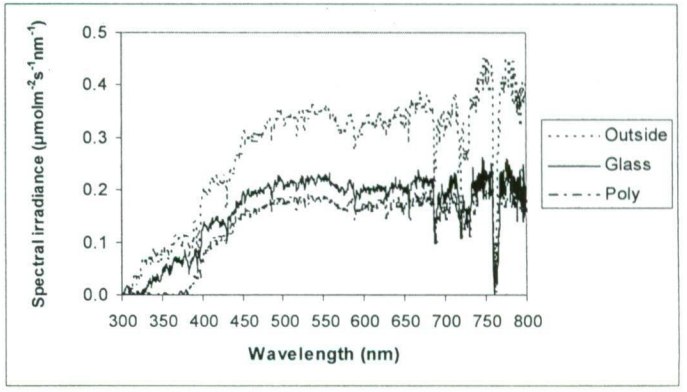
A. 12 noon sunny, October 17 (spring)



B. 4 pm sunny, October 17 (spring)



C. 12 noon overcast, June 4 (winter)



D. 4 pm overcast, June 4 (winter)

Figure 3.3. Spectral distributions of sunlight, and *in situ* measurements of horticultural glass, and polycarbonate in adjacent greenhouses. Measurements were conducted in immediate succession, as described in Materials and Methods.

Table 3.2 Calculated transmission percentages of glass and polycarbonate greenhouses examined at selected times on a sunny spring day (17 October 2006) and a winter overcast day (4 June 2007).

Sunny, spring		Transmission % by Wavelength (nm)						
Time	Material	300-800	PPF	300-400	400-500	500-600	600-700	700-800
10 am	Glass	73.1	75.3	62.5	76.3	76.0	73.6	69.3
10 am	Poly	59.3	61.6	7.1	56.2	62.8	65.2	65.7
Noon	Glass	82.2	84.4	57.5	80.2	86.5	85.5	81.0
Noon	Poly	38.3	39.3	5.9	33.0	40.3	42.1	42.1
4 pm	Glass	91.8	94.6	56.4	88.3	96.6	97.2	90.5
4 pm	Poly	78.8	81.2	11.1	73.1	82.3	85.6	84.5
Cloudy, winter		Transmission % by Wavelength (nm)						
Time	Material	300-800	PPF	300-400	400-500	500-600	600-700	700-800
10 am	Glass	59.1	51.9	43.7	60.9	65.2	66.9	59.1
10 am	Poly	48.1	50.8	6.8	47.8	45.2	43.2	48.1
Noon	Glass	64.3	65.8	79.3	74.6	65.3	58.8	54.6
Noon	Poly	53.8	56.7	8.6	54.0	57.9	57.7	57.5
4 pm	Glass	60.1	62.5	53.1	64.4	63.3	60.2	55.2
4 pm	Poly	48.6	51.5	9.1	51.6	52.4	50.5	48.9

It is claimed light diffusing materials such as polycarbonate result in more even plant growth, but plants under polycarbonate were not less variable than those under glass, either in appearance or as indicated by standard error calculations (Table 3.3).

Overall, the results suggest that the observed light quantity reductions under polycarbonate relative to glass were not significant during spring but resulted in shoot elongation, reduced leaf size and lower fresh weight in winter.

Table 3.3. Mean growth parameters (± SE, n = 20) for lines 107 and 218 pea grown concurrently in controlled environment glass and polycarbonate greenhouses under natural light in spring and winter. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Abbreviations: L1-x- shoot length between nodes 1-x, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaflet length at node 9, FW- shoot fresh weight.

L107 Spring									
	L1-4(mm)	L1-9(mm)	L1-12(mm)	NFI	FT(days)	Stem(mm)	LW(mm)	LL(mm)	FW (g)
Glass	60.4 ± 1.34 a	271.2 ± 7.61 a	427.3 ± 16.01 a	16.2 ± 0.12 a	34.2 ± 0.42 a	2.4 ± 0.09 a	25.4 ± 0.51 a	34.5 ± 0.74 a	5.3 ± 0.58 a
Poly	57.3 ± 1.34 a	235.8 ± 9.92 b*	387.2 ± 21.24 a	16.3 ± 0.16 a	33.9 ± 0.46 a	2.4 ± 0.05 a	26.5 ± 0.51 b	35.1 ± 0.69 a	3.4 ± 0.51 a
L218 Spring									
	L1-4(mm)	L1-9(mm)	L1-12(mm)	NFI	FT(days)	Stem(mm)	LW(mm)	LL(mm)	FW (g)
Glass	42.9 ± 1.16 a	300.7 ± 7.24 a	517.7 ± 12.64 a	12.7 ± 0.14 a	28.9 ± 0.59 a	2.9 ± 0.14 a	28.2 ± 0.65 a	38.2 ± 0.74 a	6.7 ± 1.16 a
Poly	46.8 ± 1.76 a	286.6 ± 6.85 a	445.7 ± 15.90 b*	13.1 ± 0.18 a	29.3 ± 0.56 a	2.7 ± 0.08 a	24.5 ± 0.72 b*	33.7 ± 0.95 b*	4.5 ± 0.47 b
L107 Winter									
	L1-4(mm)	L1-9(mm)	L1-12(mm)	NFI	FT(days)	Stem(mm)	LW(mm)	LL(mm)	FW (g)
Glass	58.9 ± 1.88 a	277.2 ± 5.27 a	435.6 ± 7.17 a	16.4 ± 0.20 a	53.0 ± 0.24 a	2.6 ± 0.07 a	24.6 ± 0.64 a	31.8 ± 0.86 a	7.2 ± 0.44 a
Poly	77.3 ± 1.98 b*	316.9 ± 7.03 b*	503.1 ± 10.00 b*	16.7 ± 0.20 a	55.0 ± 0.44 b*	2.6 ± 0.07 a	24.3 ± 1.02 a	32.2 ± 0.94 a	6.7 ± 0.41 a
L218 Winter									
	L1-4(mm)	L1-9(mm)	L1-12(mm)	NFI	FT(days)	Stem(mm)	LW(mm)	LL(mm)	FW (g)
Glass	61.9 ± 2.79 a	311.9 ± 5.00 a	515.0 ± 8.53 a	12.4 ± 0.12 a	42.4 ± 0.22 a	2.9 ± 0.06 a	27.6 ± 0.47 a	36.1 ± 0.56 a	9.6 ± 0.41 a
Poly	81.3 ± 1.07 b*	338.8 ± 4.50 b*	541.3 ± 7.01 b	12.6 ± 0.11 a	46.7 ± 0.25 b*	2.8 ± 0.10 a	25.3 ± 0.45 b*	33.2 ± 0.65 b*	7.4 ± 0.25 b*

3.4 Discussion

When considering greenhouse design, covering material options need to be examined. Although glass has the highest transmittance (Aldrich and White 1969), the lower superstructure requirements, better diffusing properties and better insulating properties of other materials such as polycarbonate (Connellan 1998; Mermier and Baille 1988) should be considered. In Australia, new construction standards (Australian Standard 1288) requires sloping roofed glass structures, when being built or modified, to be constructed of laminated or toughened glass. Containment regulations (both quarantine and gene technology regulations) also require impact resistant materials to be used. Thus re-development of existing glasshouses and construction of new greenhouses requires a change from standard 3mm horticultural glass to another material: acrylic, polycarbonate, laminated glass, or toughened glass. Acrylics tested had relatively low light transmission (under 50%, data not shown). Toughened glass required by regulation is thicker (8 mm) and heavier than laminated glass (6mm), requiring heavier superstructure. Thus the choice, realistically, is between laminated glass and polycarbonate, although greenhouses are still being constructed using 4 mm horticultural glass.

Spectral properties of laminated and horticultural glass and polycarbonate were examined in detail using both the isolated materials and *in situ*, as suggested by Kittas *et al.* (1999), for possible photomorphogenic effects from altered wavelength ratios (Fig. 3.1, 3.2). There was little difference in spectral distribution through any of the tested covering materials and sunlight, apart from the elimination of UV radiation below 390 nm by both polycarbonate and laminated glass. The influence of reduced UV levels is unclear. High UV can be damaging to plants, increasing leaf thickness, and reducing chlorophyll content and photosynthesis (Kakani *et al.* 2003; Teramura 1983). Filtering UV improved growth and yield of eggplant, and with UV eliminated plants were 20% taller (Kittas *et al.* 2006), but responses may be species dependent (Kittas *et al.* 2006). Cucumber and lettuce growth was inhibited by ambient UV levels (Krizek *et al.* 1998; Krizek *et al.* 1994), but tomato and radish growth was improved by ambient UV levels (Tezuka *et al.* 1993). It has also been suggested that a lack of UV can reduce anthocyanin synthesis and produces "weaker" plants (Hashimoto 1994). For pea, supplementation with UV to ambient levels under laminated glass

(which filters UV, unlike horticultural glass) did not alter growth and there were no shoot length alterations (Table 2.13). Thus, the observed shoot length increases in peas under polycarbonate (UV depleted) compared to horticultural glass in winter (non- depleted) (Table 3.3), were unlikely to be an influence of UV levels. In addition, shoot length differences were small in spring; plants grown under polycarbonate were actually slightly reduced in shoot length relative to glass grown plants.

Another possible influence on shoot length is the slightly reduced blue and increased far-red proportions of polycarbonate compared to glass and natural light (Fig. 3.2). Both increased far-red and reduced blue were shown to increase shoot length in pea (Chapters 7, 8: Figs. 7.4, 8.3). However, the alterations are slight, around 2%, wavelength ratios were largely unaltered (Table 3.1), and again while shoot length was increased in winter under polycarbonate, plants were slightly shorter in spring under this material relative to glass. Flowering node for L107 was found to be relatively stable across irradiance and temperature differences, but is influenced by light quality (Table 2.16, Fig 7.4). For plants grown under glass and polycarbonate in both seasons, flowering node was not significantly different. This suggests the measured light quality differences between glass and polycarbonate were not a significant influence on results. Interestingly, both a non-photoperiodic and a photoperiodic line were grown as the later was not expected to flower under short day conditions during the growth period. While no photoperiod extension lighting was used in the greenhouses during the study, as only the influence of covering material was to be examined, security lighting around the complex appeared to be sufficient to induce a long day response in L107 pea. This highlights the importance of total light exclusion for photoperiod studies, as previous studies have suggested light leakage even at very low levels can be sufficient to induce a photoperiodic response (Bakker and Blacquiere 1992), and the presence of flowering in L107 in winter during the study period confirm this.

In greenhouses with different design and orientation, light quantity differences between glass and polycarbonate were small (Fig. 3.1), highlighting the importance of these factors in greenhouse design. However, transmission measurements through the isolated materials, and in greenhouses with the same design and orientation, light quantity was reduced by polycarbonate relative to glass (Fig. 3.3, Table 3.2). In spring, with relatively longer days and higher overall irradiance, this reduction in light quantity was not a significant influence on results (Table 3.3). In winter, however,

plants showed clear responses to low irradiance, with significantly increased shoot length, smaller leaves, and reduced fresh weight under polycarbonate relative to glass (Table 3.3). In controlled environment experiments, increased shoot length was clearly associated with low irradiance (Table 2.15). Previous studies with pea have also demonstrated elongation in response to reduced irradiance (Gawronska *et al.* 1995).

In summary, spectral differences between materials was not a significant influence on results. Reduced light quantity by lower transmission through polycarbonate relative to glass was not a significant influence on results except under light limiting conditions (i.e. during short days in winter). It should be noted, however, that supplementary lighting offers significant growth improvements under such conditions (Table 4.3) and would be likely to correct any light quantity deficit responses under polycarbonate.

Chapter 4 Supplementary lighting

4.1 Introduction

Natural radiation is highly variable with cloud cover, dust and shading; varying from as little as 70 μmoles $m^{-2}s^{-1}$ under heavy cloud to over 2000 μmoles $m^{-2}s^{-1}$ under clear skies. Greenhouses typically only transmit 60% of the available light, and under short winter days radiation is often deficient. Supplemental irradiation can provide significant growth improvements where natural light is limiting, such as under short winter days and overcast conditions (Hanan 1998; Hao and Papadopoulos 1999).

Despite the spectral variation, any of the artificial light sources can be used to grow plants or to supplement natural radiation (Hanan 1998). Incandescent lamps have low photosynthetic efficiency and are little used to supplement radiation. Fluorescent are more efficient, but their size with reflectors produce considerable shading. High pressure sodium (HPS) are the most efficient- fewer, smaller lamps are needed for the same irradiance, thus reducing interference with natural light through shading (Hanan 1998). HPS are long life and have sufficiently high red levels to promote both growth and flowering in many long day plants (Fisher *et al.* 2001). Metal halide (MH) are also suitable for supplementary lighting, but compared to HPS have lower energy efficiency and lower photosynthetic efficiency from the lower red component (Sager *et al.* 1988) thus are little used in commercial greenhouses (Hanan 1998). For example, HPS lighting required 25% less fixtures than MH to achieve the same PPF, and produced more flowers per plant in roses at equal PPF (Menard and Dansereau 1995). Most crops show increased yield with HPS supplemental lighting of 40-80 μmoles $m^{-2}s^{-1}$ to increase daily light integrals (Fisher *et al.* 2001). For example, 65 μmoles $m^{-2}s^{-1}$ of HPS supplementing natural light for 16 h daily increased cucumber yield 28% (Hao and Papadopoulos 1999). Sweet pepper growth rates, yield and dry weight was substantially increased by HPS supplements of 75-125 μmoles $m^{-2}s^{-1}$ for 16 h (Demers *et al.* 1991). While unnecessary during sunny and/or long days, during winter or overcast conditions, there is a near linear positive relationship between daily light integral (DLI) increases up to 12 moles $m^{-2}d^{-1}$ and yield (Fisher *et al.* 2001).

Light distribution may be further enhanced by using a moving light system. Stationary lights must be evenly spaced for even light distribution. While reflector type or diffusing covers can even out distribution, there will still be patterns of light and shade from other structures and the crop itself. There is some evidence that

pulsing supplementary lights can further improve net assimilation rates by avoiding light saturation and allowing time for photosynthetic reactions (Hanan 1998). Under HPS pulse lighting, geraniums had superior quality and more flowers in less time than under non pulsed HPS supplements (Tardif and Dansereau 1993). The moving system can be used in this manner to produce a slow increase and decrease in light intensity at the plant level as well as, in theory, allowing greater penetration of light into the canopy by reducing self shading (Zheng *et al.* 2006). However, under fixed and moving HPS supplementary lighting, growth of gerbera was reduced relative to the fixed system (Zheng *et al.* 2006).

As described in Chapter 2, winter light levels can be limiting at the Hobart study site, particularly in the phytotron bays, where DLI averaged less than 9 mol m^{-2} d^{-1} (Table 2.5), and plants were relatively elongated in response (Table 2.10). Supplemental irradiation with HPS is reported to provide significant growth improvements under such conditions (Hanan 1998; Hao and Papadopoulos 1999), but the primary lighting systems in use is traditional photoperiodic lighting using mixed fluorescent/incandescent lamps. While such a mix promotes flowering in many LD species (Vince-Prue 1994), such as pea, and only very low fluence rates are required (Kendrick and Weller 2003a) photosynthetic efficiency is low. These have been chosen for their low R:FR ratio to accelerate flowering (0.7-0.8), but have low photosynthetic efficiency, and such low R:FR can increase shoot extension (Moe and Heins 1990). However, high R:FR lighting such as HPS can signal non-competitive conditions and relatively delay flowering in some species. While only low irradiance extension lighting is required for photoperiod induction (Kendrick and Weller 2003a), there would be some advantage to a higher irradiance photoperiodic lighting system which could double as photosynthetic lighting when natural light levels are limiting, and to provide more even seasonal daily light integrals. Spectral differences between lamps, however, may be less important when used as a supplement to natural radiation (Moe 1997), and photoperiod extension with weak HPS can produce an equivalent flowering response with less shoot extension than incandescent lamps in some species (Whitman *et al.* 1998).

There are a range of HPS lamps and reflectors available, many marketed as plant specific. In this section, spectral properties and growth responses to supplementary HPS lighting are examined, with particular emphasis on growth improvements, shoot extension and flowering response relative to traditional fluorescent/incandescent

photoperiodic lighting. Also examined were light delivery systems, and specifically whether a moving light system could deliver the claimed further growth improvements relative to a fixed system.

4.2 Materials and Methods

Photoperiodic lighting in the glasshouses examined is provided by mixed cool white fluorescent (CWF) and 100W incandescent lamps. A range of plant specific HPS lamps are available commercially, generally marketed as having higher blue, red and far-red proportions than regular HPS globes. Several of these, with different housings, were tested. Light measurements, plant growth and analysis were as previously described in Materials and Methods (Sections 2.2.2 and 2.2.4). Measurements were taken at night to exclude other light sources, except for daylight overcast supplemental light measurements. All measurements were taken at 1.5 m from the light source.

The spectral properties of a range of HPS lamps were compared relative to sunlight, traditional photoperiodic lighting (CWF, INC), and to each other. Plant specific HPS tested were Lucagrow LU400/HO (GE Lighting, Budapest, Hungary); Vialox Planta-T 400 (Osram, Munich, Germany); SON-T AGRO 400 (Philips, Brussels, Belgium); and SL600W.U15.VRD Super HPS Deluxe (Sunmaster, Solon, OH, USA). Non plant specific HPS tested were Vialox NAV-E (SON-E) 400W (Osram, Munich, Germany) and SON-E GES Elliptical 400W (Thorn, Bucharest, Romania). Relative spectral distribution as a percentage of total irradiance (300-800nm, quantum data) and wavelength ratios of these HPS was also compared to photoperiod extension lighting and to sunlight. Photoperiod extension lighting used was 40W L40 W/ 20S cool white, (Osram, Munich, Germany) and 100W pearl incandescent (Thorn, Smithfield, NSW, Australia), housed in aluminium reflectors that hold 3 x CWF and 4 x incandescent lamps (spectral properties, Section 5.3.1). In addition, the influence of increasing the far-red component by adding incandescent to the supplementary light mix in a 1:1 ratio (1 x 400W HPS, 1 x 100W incandescent) was examined.

Also tested were the spectral properties of supplementary light in daylight: i.e. does supplementary lighting alter the spectral properties of sunlight, and thus potentially influence growth responses. Spectroradiometer measurements were taken under both sunny and overcast conditions at midday beneath Osram Vialox NAV-E (SON-E) 400W HPS at a distance of 1.5 m from the light source.

To examine the influence of supplementary light on growth responses, L107 pea plants were grown and analysed as previously described (Section 2.2.4) concurrently under glasshouse conditions in both winter and summer. Photoperiod was 18 h consisting of natural daylight (average 9.6 h winter, 15.2 h summer) supplemented and extended with either HPS or mixed fluorescent/incandescent lighting. HPS was Vialox NAV-E (SON-E) High Pressure Sodium 400 W globes (Osram, Munich, Germany) delivering approximately 100 μmol m^{-2} s^{-1} at plant level; mixed fluorescent/ incandescent was 3 x 40 W L40 W/ 20S cool white to 4 x 100 W pearl incandescent delivering approximately 20 μmol m^{-2} s^{-1}. R:FR ratios measured were 4 and 0.8 respectively. Mean temperatures were winter, 18.6^{0}C day, 13.4^{0}C night; summer 22.8^{0}C day 17.4^{0}C night.

To aid light distribution, different housings and reflectors are available for supplementary lighting. Some have diffusing covers or larger reflectors for more even light distribution (manufacturers' data). Light distribution measurements were taken beneath various housings and reflectors fitted with GE Lucagrow lamps using a LI-185B quantum sensor (LI-COR, Lincoln, NB, USA). Tested were housings with acrylic diffusing covers from Ruud Lighting (Sydney, NSW, Australia); and Pierlite (Padstow, NSW, Australia) HL400HPSCG pendant mount fittings. Open reflectors tested were LL 400 Lowbay fittings (Philips, Sydney, NSW, Australia), and Adjust-A-Wings Avenger reflectors (Accent Hydroponics, NSW, Australia).

The influence of a moving light system on growth responses was examined by growing L107 concurrently in growth chambers at 20^0 ± 0.5^{0}C with an 18 h photoperiod. An automated light mover system (Nicoponics, Sydney, NSW, Australia) was installed in one of the chambers. Lights are attached to small motors that run along aluminium rails, length of travel is determined by length of rail and adjustable stoppers. When the motor hits a stopper, it reverses direction. The claim is that shaded spots are avoided, and combined with the pulsing of light intensity, growth is improved (manufacturer's literature). SL600W.U15.VRD Super HPS Deluxe (Sunmaster, Hungary) with Adjust-A-Wings Avenger reflector (Accent Hydroponics, NSW, Australia) were attached to the light mover in one cabinet, the same light source was fixed in position in the other cabinet. Irradiance (PPF) was 220 μmol m^{-2} s^{-1} at pot level in each case directly beneath the lights, reducing to 150 μmol m^{-2} s^{-1} at the edges of the plant area. Twenty plants were grown per treatment with the aim of determining if the moving system improved and provided more even growth.

4.3 Results

4.3.1 Relative spectral properties of HPS light sources

HPS lamps are generally used for supplementary lighting because of the relatively high red component and high light output (Fisher *et al.* 2001). There are many brands and types of HPS available, some marketed as plant specific, with higher blue and red proportions. However, of those tested, spectral distribution (Fig. 4.1) is very similar between plant specific products (Lucagrow, Agro, Sunmaster) and regular HPS (SON-E). The low UV and blue, and high red components of HPS lighting relative to sunlight is clear from these figures and Table 4.1. Relative spectral distribution and wavelength ratios (Table 4.1), however, reveal that most of the spectral output is in the 500-600 nm (green/yellow) range. Far-red proportions are also low in HPS lamps relative to sunlight and photoperiod extension lighting, and hence R:FR ratios are high (Table 4.1).

For plant growth, blue, red and far-red light are needed. Plant specific HPS are marketed as having higher blue and far-red proportions to regular HPS, but of the lamps tested, only the Lucagrow lamps met these claims (Table 4.1). UV and blue proportions, however, are still very low compared to sunlight in all the lamps tested, and R:FR remains high. In fact the next best lamp tested in terms of these wavelengths was the non-plant specific Osram SON-E. Blue proportion was higher and R:FR lower than the Lucagrow lamps, but red proportion was lower. The Planta lamps had very high green/yellow, and low blue, red and far-red proportions (Table 4.1) and on this basis would be the least suitable for plant growth, even though they are marketed as such.

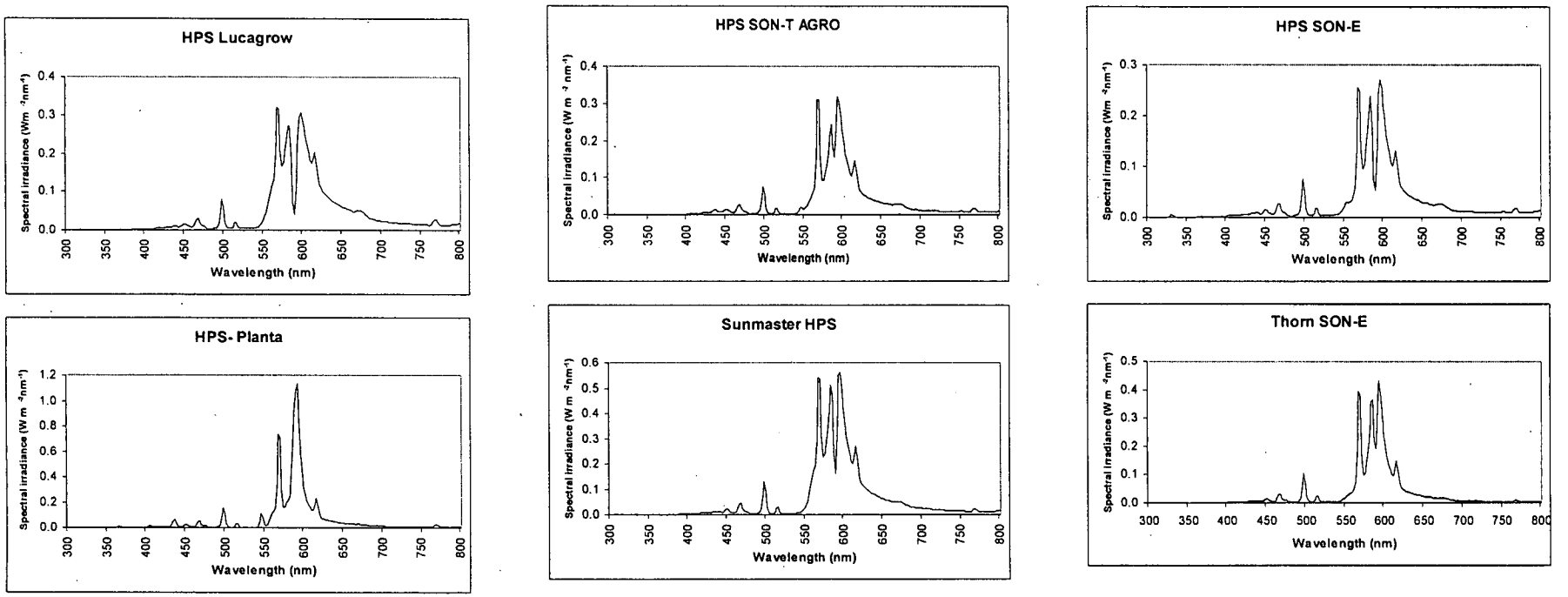


Figure 4.1. Spectral distribution of tested HPS light sources. Abbreviations: Lucagrow , GE Lucagrow; Planta, Osram Vialox Planta HPS; SON-T-Agro- Osram SON-T AGRO HPS; Sunmaster, Sunmaster 600W HPS Deluxe; SON-E, Osram Vialox NAV-E (SON-E) 400W; Thorn SON-E, SON-E GES Elliptical 400W.

Table 4.1. Relative spectral distribution as the percentage of total irradiance (300-800 nm) and wavelength ratios of high pressure sodium (HPS) light sources, photoperiod extension lighting and natural light with and without HPS supplement. Fluorescent/ incandescent extension lighting and sunlight proportions and ratios are included for comparative purposes. Both plant specific (Lucagrow, Planta, Son-T Agro, Sunmaster) and non- plant specific (Osram, Thorn SON-E) HPS globes were compared. Sun+HPS and Overcast+HPS measurements used Osram SON-E. Full light details and measurement protocols are in Materials and Methods. Abbreviations: Lucagrow , GE Lucagrow; HPS+INC, 1 x GE Lucagrow (400W) and 1 x 100W Incandescent; Planta, Osram Vialox Planta HPS; Agro- Osram SON-T AGRO HPS; Sunmaster, Sunmaster 600W HPS Deluxe; Osram SON-E, Vialox NAV-E (SON-E) 400W; Thorn SON-E, SON-E GES Elliptical 400W; Fl/Inc, 3x cool white fluorescent + 4x 100W incandescent; INC, 100W incandescent.

Light source	Wavelength proportion (% total irradiance)						Wavelength ratios		
	PPF	300-400 UV	400-500 Blue	500-600 Green	600-700 Red	700-800 Far-red	R:FR (n)	R:FR (b)	B:R	B:FR
HPS										
Lucagrow	89.6	0.2	4.2	41.6	43.9	10.1	2.8	4.3	0.1	0.4
HPS+INC	88.8	0.1	4.1	40.3	44.5	11.0	2.6	4.0	0.1	0.4
Planta	96.5	0.2	5.3	68.1	23.1	3.3	3.5	7.1	0.2	1.6
Agro	92.2	0.1	5.1	51.0	36.2	7.8	2.8	4.7	0.1	0.7
Sunmaster	93.0	0.1	4.2	52.4	36.5	6.7	2.9	5.4	0.1	0.6
Osram SON-E	90.3	0.5	5.6	47.8	36.8	9.3	2.5	4.0	0.2	0.6
Thorn SON-E	96.2	0	5.5	61.6	29.1	3.7	3.7	7.8	0.2	1.5
Extensions										
Fl/Inc	64.5	0	8.1	26.6	29.8	35.5	0.8	0.8	0.3	0.2
Inc	45.7	0	2.0	12.0	31.0	55.0	0.7	0.6	0.1	0.04
Natural light										
Sunlight	69.4	2.4	15.5	25.0	29.0	28.0	1.1	1	0.5	0.6
Sun+HPS	72.0	2.6	15.5	28.4	28.4	25.2	1.1	1.1	0.5	0.6
Overcast	71	3.2	18.3	25.8	26.9	25.7	1.1	1.1	0.7	0.7
Overcast+HPS	75	3.0	16.7	29.1	28.9	22.4	1.2	1.3	0.6	0.8

The addition of incandescent to the HPS light mix did little to alter spectral properties. Far-red proportion was only slightly increased, and hence R:FR only slightly reduced, remaining very high relative to sunlight at a ratio of 4 (Table 4.1).

Traditional photoperiod extension lighting is with incandescent (Inc) or mixed fluorescent/incandescent (FL/Inc), which have lower R:FR than natural light, and considerably lower than HPS (Table 4.1). However, irradiance was comparatively low, PPF measurements were 5 and 10 μmol m^{-2} s^{-1} for Inc and FL/Inc at 1.5 m; under HPS PPF was 100 μmol m^{-2} s^{-1} at the same distance. Thus while the photoperiodic lighting has sufficient irradiance for day length extension, it would be insufficient for use as a photosynthetic supplement.

On spectral properties, the high red proportion of HPS should aid photosynthesis, but the high R:FR and low blue component could delay flowering relative to sunlight. However, spectral properties of lamps may be less important when used as a supplement to natural radiation (Moe 1997). Hence the spectral properties of supplemented natural light were examined under HPS lamps. The significant contribution to irradiance can be seen (Fig. 4.2), particularly in the 500-650 nm range. In sunny conditions, the HPS supplement had little influence on fluence rate, but under overcast conditions, the HPS supplement increased PPF by almost 1/3. However, there is little change in the relative wavelength proportions or ratios of natural light with the HPS supplement added (Table 4.1) in spite of the increased yellow/red irradiance, and R:FR is largely unaltered from natural proportions.

HPS supplementation increased average DLI by 4 mol m^{-2} d^{-1} in winter (Table 4.2). In comparison, the fluorescent/incandescent extensions only contribute 0.6 mol m^{-2} d^{-1} to DLI. Winter light levels were significantly boosted by supplementing natural radiation with HPS lighting, particularly during overcast conditions. In summer, similar increases in DLI occurred under HPS supplementation (Table 4.2), but DLI was already high at over 30 mol m^{-2} d^{-1}. This was well above the 14 mol m^{-2} d^{-1} used for optimum growth in growth chamber experiments (Table 2.15).

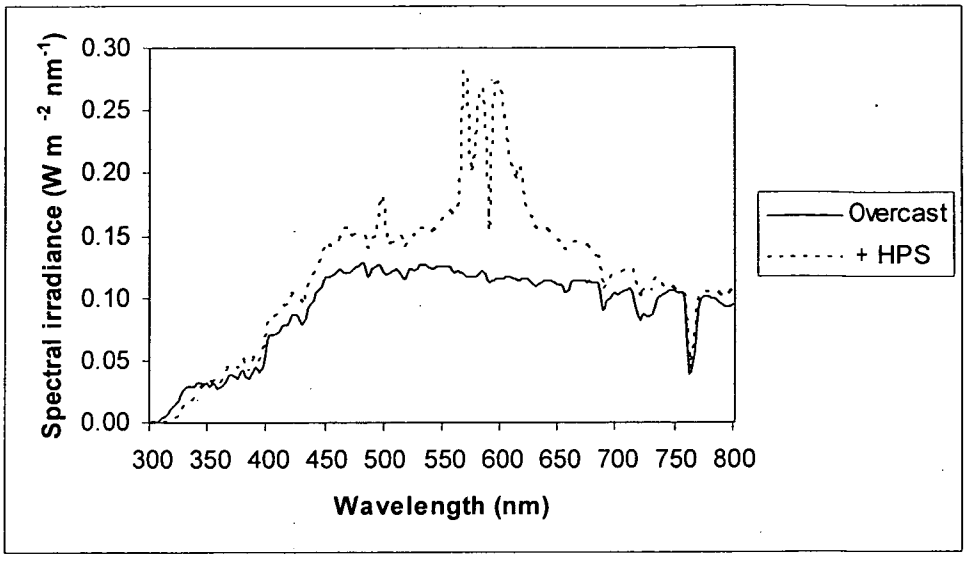


Figure 4.2. Natural light within the glasshouse during winter overcast conditions and with HPS supplement (Osram Vialox NAV-E (SON-E) High Pressure Sodium 400W in Philips (Australia) LL 400 Lowbay fitting, no cover

Table 4.2. Winter and summer light quantities (PPF in μmol $m^{-2}s^{-1}$, DLI in mol $m^{-2}d^{-1}$) under 18h HPS or mixed fluorescent/incandescent (Fl/Inc)supplementation of natural light.

Winter				
Supplement	Range PPF	Mean PPF	Ext. PPF	DLI
HPS	80-910	371	100	15.8
Fl/Inc	34-820	324	20	11.8
Summer				
HPS	360-1110	631	100	35.2
Fl/Inc	250-1080	576	20	30.6

4.3.2 Growth responses with supplementary lighting

While HPS supplementation did not greatly alter the spectral properties of natural light (Table 4.1), plants would be subjected to high R:FR after dark. In particular this could delay flowering relative to low R:FR extension lighting. HPS also significantly increased light quantity in winter, which can increase growth responses when light may be limiting. To examine the influence of supplementary light on growth responses, L107 pea plants were grown and analysed concurrently under glasshouse conditions in both winter and summer as described in Materials and Methods. Photoperiod was 18 h consisting of natural daylight (average 9.6 h winter, 15.2 h summer) supplemented and extended with either 100 μmol m^{-2} s^{-1} HPS or 20 μmol m^{-2} s^{-1} mixed fluorescent/incandescent lighting. R:FR ratios measured were 4 for HPS and 0.8 for Fl/Inc. Light quantity was also increased. HPS supplementation increased average DLI by 4-5 mol m^{-2} d^{-1} (Table 4.2).

In winter, plants grown with HPS supplementation showed dramatic increases in yield, almost double the number of pods and a 75% increase in seed compared to plants grown under the Fl/Inc supplement (Table 4.3). Dry weight, stem diameter and leaf size (LW and LL, Table 4.3) were also dramatically increased under HPS. Internode length (L1-9) was reduced under HPS, but not total shoot length (TL, Table 4.3). Flowering time was earlier under HPS, but not flowering node (Table 4.3). Thus in winter, HPS supplementation dramatically increased growth parameters relative to Fl/Inc supplementation. Internode length was reduced as expected from high R:FR, but increased irradiance also reduces shoot length in pea (Table 2.15). Flowering node, however, was not delayed as could be expected from high R:FR (Table 4.3).

In summer, stem diameter, leaf size and dry weight were also increased under HPS supplementation compared to Fl/Inc supplementation (Table 4.3), but the increases were not so dramatic. Again flowering node was not delayed by the high R:FR of HPS (Table 4.3). Shoot length was reduced, including total shoot length, and yield was reduced relative to Fl/Inc supplementation. These factors (shoot length and yield) were shown to be associated with increased temperature (Table 2.16) and radiant heat from the higher wattage HPS lamps may be an influence in these results. Flowering time was earlier under HPS in both winter and summer, which may also be associated with temperature (Table 2.12, 2.16).

Table 4.3. Winter and summer mean growth parameters (± SE, n = 20) for L107 peas grown concurrently in glasshouse conditions under 18h photoperiod consisting of natural light supplemented with 100 µmoles $m^{-2}s^{-1}$ high pressure sodium lamps (HPS) or 20 µmoles $m^{-2}s^{-1}$ fluorescent/incandescent (Fl/Inc) lighting. Full details are in Materials and Methods. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$.

Winter											
	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
Fl/Inc	55.0 ± 0.67 a	148.2 ± 3.16 a	19.5 ± 0.17 a	16.1 ± 0.06 a	47.0 ± 0.40 a	18.4 ± 1.61 a	5.1 ± 0.36 a	3.2 ± 0.06 a	31.6 ± 0.58 a	42.5 ± 0.80 a	6.9 ± 0.39 a
HPS	46.4 ± 0.70 b*	146.8 ± 2.27 a	21.0 ± 0.21 b*	15.8 ± 0.13 a	45.6 ± 0.16 b*	34.1 ± 2.49 b*	9.9 ± 0.46 b*	4.3 ± 0.07 b*	38.6 ± 0.79 b*	52.3 ± 0.93 b*	7.7 ± 0.52 a
Summer											
Fl/Inc	31.4 ± 0.76 a	91.6 ± 2.13 a	17.9 ± 0.26 a	15.2 ± 0.25 a	39.1 ± 0.90 a	7.3 ± 0.71 a	2.5 ± 0.22 a	2.5 ± 0.10 a	28.0 ± 1.06 a	38.5 ± 1.32 a	3.5 ± 0.08 a
HPS	29.4 ± 0.84 a	76.0 ± 3.05 b*	17.6 ± 0.26 a	15.3 ± 0.18 a	34.1 ± 0.61 b*	5.9 ± 1.0 a	2.5 ± 0.34 a	3.1 ± 0.15 b*	29.1 ± 1.26 a	43.8 ± 2.18 b	4.0 ± 0.20 b

4.3.3 Light distribution systems

For even growth, even light distribution is important. Thus supplementary lighting needs to be evenly distributed to avoid growth differences. To aid light distribution, different housings and reflectors are available for supplementary lighting, usually with diffusing covers or larger reflectors. Light distribution measurements were taken beneath various housings and reflectors fitted with GE Lucagrow lamps using a LI-185B quantum sensor. Tested were housings with acrylic covers, open lowbay reflectors, and open winged reflectors as described in Materials and Methods.

Spectral distribution measurements were taken on the Lucagrow globes with and without the acrylic diffusing cover (data not shown).There was little difference in spectral distribution between the covered and uncovered Lucagrow globes. The major effect of the acrylic covers was on light distribution. The diffusing nature of the covers resulted in very even light distribution. With the lights placed 1.5m apart there was little alteration in fluence rate measured between lights at 1.5m from the lights. With the uncovered lights in the lowbay reflectors at the same spacing measured at the same distance, fluence rate reduced by 35% between lights. While higher irradiance was measured directly beneath the uncovered lights at a distance of 1 m, the diffusing nature of the acrylic covers actually results in higher PPF measured at a distance of 1.5m from the lights, directly underneath (120 and 80 μmoles $m^{-2}s^{-1}$, respectively). The winged reflectors also produced very even light distribution with little alteration in fluence rate between lights, and a much broader light distribution area from single lights compared to the open lowbay reflectors. Thus, both diffusing covers and larger reflectors aid light distribution, but the increased shading influence of larger reflectors during daylight was noticeable.

Light distribution can also be improved by moving light systems. Light distribution measurements undertaken on this system confirmed the slow increase/decrease in fluence, and higher canopy penetration. Growth responses were compared under a fixed or moving system using the same lamps and reflector type as described in Materials and Methods. Results (Table 4.4) showed the moving system did improve some growth responses relative to the fixed system, but development was delayed. Seed number was increased, but flowering node and flowering time were delayed relative to the same lights in a fixed system (Table 4.4). In fact the plants were slower to develop under the moving system, as reflected in the later flowering time and

increased total shoot length (Table 4.4). Hence dry weight was significantly higher under the moving system (Table 4.4), but this was a result of the increased shoot length from slower development times. Harvest time was two weeks later under the moving system. The moving system also did not result in more even growth between the plants.

Table 4.4. Mean growth parameters (± SE, n = 20) for L107 peas grown concurrently in growth chambers at $20^0 \pm 0.5^0$C under HPS lamps fixed in position or moving backwards and forwards across the plants. Photoperiod 18h, irradiance 220 µmoles $m^{-2}s^{-1}$ at the pot surface. Full details are in Materials and Methods. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$.

	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
Stationary	54.1 ± 0.49 a	138.5 ± 2.62 a	22.2 ± 0.25 a	18 ± 0.13 a	31.6 ± 0.19 a	16.9 ± 1.05 a	6.1 ± 0.53 a	3.2 ± 0.05 a	37.4 ± 0.42 a	48.1 ± 0.61 a	5.6 ± 0.22 a
Moving	53.7 ± 0.41 a	150.6 ± 2.76 b*	22.5 ± 0.26 a	18.8 ± 0.10 b*	37.7 ± 0.18 b*	21.1 ± 1.17 b	6.2 ± 0.42 a	3.1 ± 0.06 a	37 ± 0.63 a	47.8 ± 0.71 a	6.6 ± 0.13 b*

4.4 Discussion

All of the HPS sources tested have low blue and high red components compared to sunlight and other sources (Fig. 4.1, Table 4.1). However, the highest proportional output is in the 500-600 nm (green/yellow) range. This is in the peak range of the human eye photopic response (Ryer 1997) and hence the lights appear very bright to humans. Photosynthesis, however, has peak responses to blue and red, with red wavelengths the most efficient for photosynthesis (Sager *et al.* 1982).

The FR proportion from HPS sources is very low compared to sunlight and traditional photoperiod extension lighting (Table 4.1), and hence R:FR is very high. Addition of far red rich incandescent globes to HPS, however, had little effect, only reducing R:FR by 0.2 (Table 4.1). HPS globes do contain some FR; in the plant specific Lucagrow lamps it is relatively high at 9.3% compared to Osram Planta lamps tested (3.3%, Table 4.1). These latter globes are also marketed as plant specific, with higher blue and red proportions than regular HPS (manufacturers' data). While blue levels are similar (both only 4-6%), the Lucagrow globes have 20% less green, 14% more red and 6% more far red than the Planta globes, thus come closer to meeting the plant specific claim. In fact, the non-plant specific Osram SON-E globes tested in this section have less green, more red and more far red than Planta globes and thus come closer to meeting the plant specific claim, even though they are not marketed as such and are considerably less expensive. Overall, given the aim of supplementary lighting to maximise photosynthesis (Hanan 1998) and red wavelengths having the highest photosynthetic efficiency (Sager *et al.* 1988), the Lucagrow globes appear to be the best choice of the globes tested for this purpose, followed by Osram SON-E, which out-performed more expensive plant specific lamps, including Sunmaster and the widely used SON-T-Agro lamps.

Far-red proportions are low in HPS lamps relative to sunlight, and hence R:FR ratios are high (Table 4.1). High R:FR is known to reduce internode extension and to delay flowering in photoperiodic sensitive species (Runkle and Heins 2001). Low blue proportion is also associated with later flowering and increased internode extension (Runkle and Heins 2001, Eskins 1992, Wheeler *et al.* 1991). Hence on spectral properties, the high red proportion of HPS should aid photosynthesis, but the high R:FR and low blue component could delay flowering relative to sunlight. Shoot length responses are less clear, as high R:FR can reduce shoot length but the low blue

proportion of HPS has been associated with increased internode length (Wheeler *et al.*, 1991, Tibbitts *et al.* 1983), as is low irradiance (Gawronska *et al.* 1995). In addition, in a natural light background, HPS provided significant contribution to total irradiance (Table 4.2), but did not significantly alter wavelength ratios of natural light (Table 4.1). There are numerous studies that report growth improvements under HPS supplementation of natural light during winter (Bredmose 1995; Demers *et al.* 1991; Dorais *et al.* 1991; Vezina *et al.* 1991), but the influence of HPS supplementation on flowering node or internode extension has received less attention.

Thus to examine the influence of altered wavelength properties and increased irradiance, growth responses under HPS and fluorescent/incandescent supplementary lighting were examined using a photoperiodic pea line (L107). In winter, compared to Fl/Inc supplement, supplementary lighting with HPS significantly reduced L1-9 and increased TN, but not TL significantly (i.e. internode length was reduced, Table 4.3). Flowering node was not significantly altered in spite of the high R:FR ratio of the HPS lighting (R:FR 4), which could be expected to delay flowering (Whitman *et al.* 1998). Twilight R:FR ratios (such as in the Fl/Inc mix used in this study, R:FR 0.8) can increase shoot extension (Moe and Heins 1990; Runkle and Heins 2001), but so can low irradiance in pea (Gawronska *et al.* 1995) and the observed internode length reductions in this study could be from high R:FR during the after dark extension period or from the increased irradiance. While low R:FR promotes flowering in many LD species (Vince-Prue 1994), such as pea, and only very low fluence rates are required (Kendrick and Weller 2003a) photosynthetic efficiency is low. This was reflected in the large increase in DLI with HPS supplement compared to Fl/Inc supplementation (Table 4.2). Supplemental irradiation can provide significant growth improvements where natural light is limiting, such as under short winter days and overcast conditions (Hanan 1998; Hao and Papadopoulos 1999). In garden pea, yield was dramatically increased, as was stem diameter and leaf size under HPS supplementary lighting during winter (Table 4.3). Thus the DLI increase from HPS supplementary lighting dramatically improved growth and yield under winter conditions, internode length was reduced, and flowering unaltered.

In summer, supplementary light had less influence (Table 4.3). Light use efficiency is highest at low PPF, thus supplementing light has a greater effect at low light levels than high levels (de Koning 1997). Summer results also showed a temperature influence, with reduced shoot length, yield and flowering time. Thermal radiation

from lamps can influence growth (Faust and Heins 1997; Graper and Healy 1991), and L107 pea showed reduced shoot length, yield and flowering time with increased temperature, both under natural light and in growth chamber experiments (Table 2.16).

Flowering node was again not delayed under HPS compared to Fl/Inc lighting in summer (Table 4.3), in spite of the large difference in R:FR. Spectral differences between lamps may be less important when used as a supplement to natural radiation (Moe 1997), and ratio measurements (Table 4.1) confirm little influence of HPS on natural ratios as a supplement. As well, flowering can be promoted by an increase in photosynthesis supply and/or radiant heat from the lamps (Moe 1997), which may counteract a ratio induced delay. It is interesting that when HPS lighting was applied as an extension to photoperiod rather than over the full lighting period, NFI was delayed relative to Fl/Inc extension. Under these conditions, L107 NFI was around node 17 (Table 2.16) compared to node 15-16 under both continuous light period supplementation with HPS or Fl/inc (Table 4.3) or with Fl/inc extension (Table 2.12). In the growth chamber studies, plants under HPS lighting alone showed delayed flowering node relative to natural light supplemented with HPS (around nodes 18 and 16 respectively, Tables 4.4, 4.3). Thus HPS lighting can delay flowering, but when used as a supplement to natural light, these delays were not observed.

When ambient DLI is low, supplemental lighting at photosynthetic levels improves quality and flowering (Pramuk and Runkle 2005). Results obtained for a standard line pea plant support this, and if yield or biomass (as indicated by dry weight) increase is the primary aim, significant growth improvements can be obtained in winter by supplementing natural radiation with HPS lighting.

For even growth, even light distribution is important, thus the spacing and reflector type is important with supplementary lighting. The high output and small size of HPS allows for fewer lamps and less shading for a given light level compared to other light sources, but light distribution must be managed- generally less than 30% variation should be the aim (Fisher *et al.* 2001). Higher wattage lamps with broad reflectors can be mounted high in the greenhouse, or if head height does not allow this, a closer spacing of lower wattage lamps is required (Fisher *et al.* 2001). Results with various reflector types showed that acrylic covers improved light distribution and did not alter the spectral properties of uncovered lamps. Larger reflectors also improved light distribution, but increased infrastructure shading during natural light. The covered

housings also protect the lamps from moisture and sprays, and provide safety protection from lamp breakage.

Moving light systems are claimed to improve and provide more even growth by aiding light distribution and penetration into the canopy. While such a system did produce some growth improvements (Table 4.4), development time was delayed relative to a fixed system, and growth was not less variable. Under fixed and moving HPS supplementary lighting, growth of gerbera was reduced relative to the fixed system, even though photosynthetic capacity was increased, perhaps because of the slower response time of photosynthesis compared to the rate of change in irradiance (Zheng *et al.* 2006). Even light distribution under HPS can be achieved by adequate spacing of lamps, particularly if diffusing covers are used. Most lighting manufacturers provide a service to calculate the required lamp spacing for a given irradiance over an area.

In summary, HPS supplements are an established method of improving growth rates under light limiting conditions. A range of plant specific HPS lamps are available and of those tested GE Lucagrow globes best meet the manufacturers claims, although some non-plant specific (and less expensive) HPS globes performed well. Winter growth of pea was dramatically improved by the use of 80-100 μmol $m^{-2}s^{-1}$ HPS supplementation of natural light, and flowering was not delayed relative to low R:FR lighting. Diffusing acrylic covers or adjustable reflectors improve light distribution, but the reflectors have a larger footprint and thus produce more infrastructure shading. Light moving systems reduced self shading and increased canopy penetration, but delayed development times relative to a fixed system. HPS lighting could also be used as photoperiod lighting for more even seasonal daily light integrals, and did not delay flowering when used as a continuous supplement over the full LD photoperiod.

Chapter 5 Photoperiod control

5.1 Introduction

Photoperiod studies in greenhouses involve the natural photoperiod being modified by extension lighting and/or screening to exclude light (Heins and Faust 1994). Growth chambers are also useful for photoperiod studies, as the duration of the light period can be controlled. Issues identified of concern in photoperiod control were what types of lights can be used for photoperiod extension, the quantity of light required and what level of light leakage can be inductive in sensitive species. This Chapter examines those issues using the facilities at the School of Plant Science, which include growth chambers in total light exclusion zones and phytotron glasshouses with a series of automated plant trolleys and light proofed bays. This allowed for examination of the influence of light extension type to natural daylight (eg fluorescent or incandescent), examination of inductive light levels and the quantification of light leakage in controlled environment equipment used for photoperiod studies.

Photoperiod responses vary between species and even within cultivars of the same species (Mattson and Erwin 2005). Garner and Allard (1920, cited in Cathey and Campbell, 1977) characterised plant responses to photoperiod and classified plants into four types- short day (SD), long day (LD), day length intermediate and day neutral. Continuous light inhibits flowering and promotes vegetative growth in short day plants, promotes flowering and vegetative growth in long day plants, reduces flowering in intermediate plants, and has no effect on flowering but may increase shoot length in day neutral plants. Long night photoperiodic responses (SD) depend on the period of darkness exceeding some critical value, following a period of light. In LD plants, long photoperiods of continuous light are required for flowering (Vince-Prue 1981). In SD plants the duration of darkness is the primary determinant, as night break experiments prevent flowering. Also important is when the night break occurs- the night break response is a transient period of sensitivity related in time to the beginning of the dark period. However, a preceding period of light is needed for the dark period to be effective. Thus both light and dark are necessary components of the photoperiodic mechanism in SD plants. Light quantity is also important, SD plants will not flower if the photoperiod is very short or irradiance is very low. Although the

light requirement for induction in SD plants is not of photosynthetic levels, there may be an interaction with photosynthesis in that photosynthate is required (Vince-Prue 1994).

Plants measure the length of darkness. In a number of LD plants, a night break of light under short day conditions has the same effect as long day conditions. R is the most effective wavelength and FR can reverse the effects. Since this is a phytochrome mediated response, only low energy levels are needed to artificially extend photoperiodic day length (Kendrick and Weller 2003a).

Traditional photoperiod extension lighting is with incandescent lamps, which have a low R:FR ratio and low photosynthetic efficiency. Only very low fluence rates are required for photoperiodic responses- less than 1 μmole $m^{-2}s^{-1}$ can be inductive (Whitman *et al.* 1998). Threshold illuminance values for responsive species vary by two to three orders of magnitude, but between 1 and 50 lux of incandescent is generally sufficient (Summerfield and Roberts 1987). Using the formulas and conversion tables of Thimijan and Heins (1983), 50 lux of incandescent equates to 1 μmol $m^{-2}s^{-1}$. Saturation irradiance (above which there is no increase in response) also varies widely, from less than 10 lux for chickpea to over 1000 lux (20 μmol $m^{-2}s^{-1}$) in lentil (Summerfield and Roberts 1987). Hence, accurate light exclusion is necessary to effectively examine photoperiod responses.

Comparative photoperiod studies generally use a photosynthetic lighting level followed by either dark or weak photoperiodic extension at a non photosynthetic level. Incandescent for this purpose is simple to install, effective, and inexpensive (Cathey and Campbell 1977; McCree 1972b; Whitman *et al.* 1998).

Many studies confuse photoperiod with daily light integral (DLI) when comparing light duration effects (Adams and Langton 2005). LD photoperiod treatments include high irradiance SD preceded and/or followed by low irradiance lighting (day extension lighting); photosynthetic lighting of different photoperiods and intensity to give the same DLI (equal integral lighting); and low irradiance lighting applied during the dark period (night break lighting) (Adams and Langton 2005). LD treatments frequently improve growth of plants compared to SD, even with equal DLI. LD commonly promotes leaf area and photosynthetic efficiency. Typically thinner, larger shade type leaves are produced under such conditions, as plants appear to respond to the average irradiance over the lit period. Such leaves are more photosynthetically efficient, and due to the hyperbolic relationship between PAR and photosynthesis, it is

5 times more efficient to give weak additional light (13 μmol $m^{-2}s^{-1}$ in this study) during the night than during the day (Adams and Langton 2005). Thus it is important that photoperiod studies use non- photosynthetic levels of extension lighting, or are at equal DLI.

Spectral qualities of the extension lighting can be important. Incandescent extensions to natural short days stimulated earlier flowering in henbane, sugar beet, barley, dill, *Lolium* and petunia than fluorescent extensions (Lane *et al.* 1965). However, incandescent can produce excessive elongation growth and suppression of branching in many species due to their low R:FR ratio (i.e. stimulates the shade avoidance response) (Whitman *et al.* 1998). Maximum flowering responses in many LD species is seen at R:FR ratios close to sunlight (about 1.1), such as under a mix of fluorescent and incandescent (Runkle 2004; Vince-Prue 1994). Lowering R:FR ratios below 1.1 can accelerate flowering but also increases shoot extension (Runkle and Heins 2001). A number of studies show inhibition of flowering under CWF for many species due to the high R:FR ratio, while others show no difference (reviewed in Whitman *et al.* 1998). For example, cool white fluorescent, high pressure sodium and metal halide (which all have high R:FR ratios) day length extensions all produced equal flowering responses and less shoot elongation than incandescent at PPF above 1 μmol $m^{-2}s^{-1}$ in campanula and coreopsis (Whitman *et al.* 1998). Thus in some species the end of day R:FR ratio may be important, in others it may not.

The end of day signal is also unclear. The light to dark transition at dusk may be marked in plants by the lowered R:FR ratio (from about 1.1 in daylight to 0.7-0.8), or by the lowering of irradiance (Vince-Prue 1994). Most photoperiodic species examined show a threshold of irradiance required for a response, the demarcation between day and night appears to be related to this value rather than a change in spectral quality (Summerfield and Roberts 1987). No clear effect from removal of twilight by blackout curtains before sunset was found (Mortensen and Moe 1992).

A major research species grown at the School of Plant Science is the garden pea, which includes many LD lines, including L107. Thus normal growing conditions involve photoperiod extensions to create LD conditions even in winter, when day length is naturally short in Hobart (mean winter day length 9.6 h). To study the influence of photoperiod, a range of day length control options are in use, from automated phytotron bays to growth chambers in light exclusion areas. A range of light sources are used for photoperiod extensions. Spectral properties of these sources

are examined, particularly R:FR ratios, and the likely implications for morphological growth parameters in the garden pea. To examine the influence of photoperiod control methods, L107 peas were grown under a range of conditions described below. Specifically examined were inductive light levels, light leakage and effectiveness of control methods, and the influence of photoperiod extension type.

5.2 Materials and methods

Light measurements, plant growth and analysis were as previously described in Materials and Methods (Sections 2.2.2 and 2.2.4).

Normal glasshouse conditions were an 18 h photoperiod consisting of natural light extended morning and evening by mixed fluorescent/incandescent lighting. This is a mix of 3 x 40 watt L40 W/ 20S cool white fluorescent tubes (Osram, Munich, Germany) to 4 x 100w pearl incandescent (Thorn, Smithfield, NSW, Australia) with R:FR measured of 0.8.

Inductive light level was examined by growing L107 under natural light for 8 h followed by 16 h low fluence incandescent extension or no light extension in light proof dark bays. The extension was provided by a single 25W incandescent bulb (Thorn Australia). Ten plants per treatment were placed at intervals from this light to create a total irradiance gradient of 0.6, 0.3, 0.2 and 0.1 μmol $m^{-2}s^{-1}$, as measured with the Li-Cor spectroradiometer. Mean day temperature for the study period was day, 23.2^0C, night 16.0^0C.

Light leakage levels were measured in closed dark bays and growth chambers during daylight, and in growth cabinets grouped in a dark area with the lights on in an adjacent chamber.

End of day light measurements were taken with both the Li-Cor and Apogee spectroradiometers at 15 min intervals until after sunset under both overcast and clear sky conditions.

To examine the effects of photoperiod extension type, plants were grown concurrently in phytotron bays for 8 h photoperiods (natural daylight) extended by either incandescent (25W pearl incandescent Thorn, Australia) or cool white fluorescent (40 watt L40 W/ 20S cool white, Osram, Germany) for 16 h. Extension fluences used were 5 μmol $m^{-2}s^{-1}$ (300-800 nm) in both cases. R:FR ratios were 0.7 and 5.6, respectively. Mean daytime PPF was 228 μmoles $m^{-2}s^{-1}$, DLI around 8 mol m^{-2} d^{-1}. The phytotron bays consist of a series of moveable trolleys and light proofed

bays. Plants can be grown under natural light and programmed to move into the light proofed bays at the end of the desired photoperiod automatically. The light proof bays can be dark or be fitted with photoperiod extension lighting. In this way a series of photoperiods or light extension types can be examined. Mean day temperature was 18.5°C, night temperature in the bays was maintained at 16°C.

To examine the influence of specific wavelengths in photoperiod responses, plants were also grown under an 8 h photoperiod with no extension, and 8 h natural light with 5 μmoles $m^{-2}s^{-1}$ 16 h extensions provided by monochromatic blue, red and far-red light emitting diodes (LED). These were locally constructed strips of blue- NSPB510S WF3 Super Blue (Nichia Corporation, Singapore), peak output at 460nm; red- KL450-660GDDH (Shinkoh Electronics, Tokyo, Japan), peak output at 660 nm; and far-red- KL450-730GDDH (Shinkoh Electronics), peak output at 730 nm (spectral distributions, Fig. 5.1).

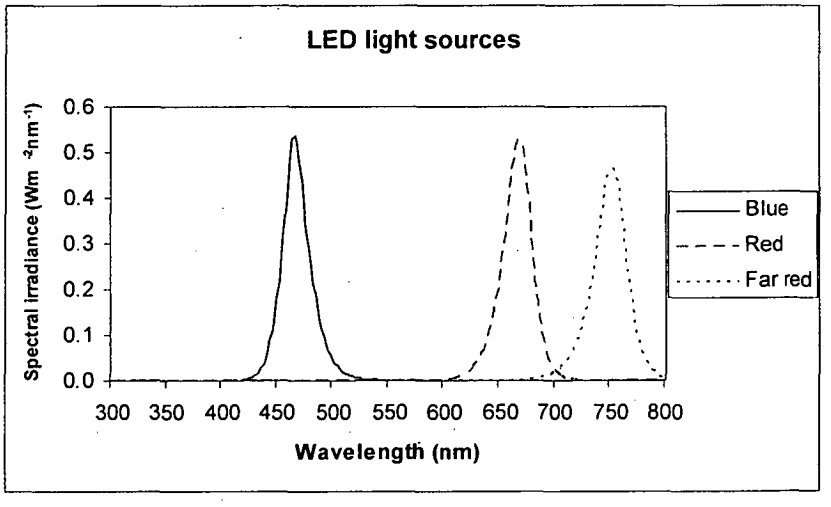


Figure 5.1. Spectral distribution of described blue, red and far-red LEDs

5.3 Results

5.3.1 Spectral properties of light sources

Photoperiod extension methods examined were pearl incandescent, cool white fluorescent, and mixed fluorescent incandescent. Spectral distribution of fluorescent and incandescent lamps used are shown in Chapter 6 (Fig. 6.1 & 6.2). Mixed fluorescent/incandescent (Fig. 5.2) is similar to the fluorescent distribution, apart from the enhanced far-red component from the incandescent lamps. In this proportion R:FR

is reduced from 5.6 (for fluorescent) to 0.8, which is equivalent to the natural light twilight ratio (Smith and Morgan 1981).

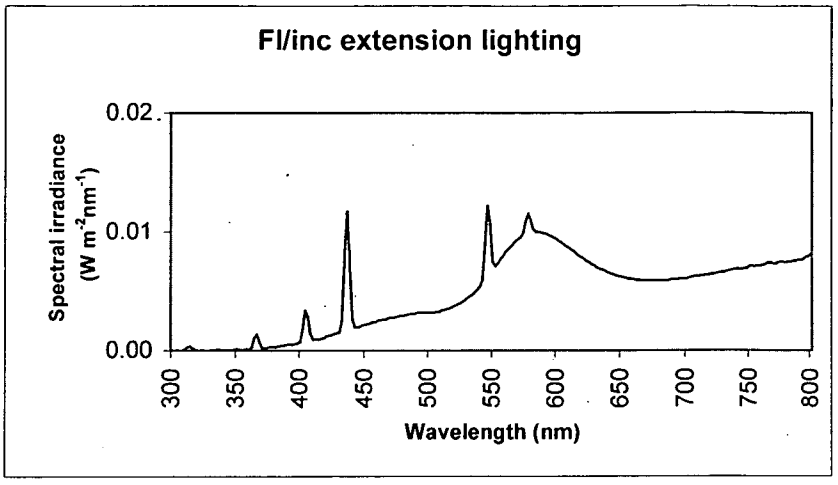


Figure 5.2. Spectral distribution of standard photoperiod extension using 3 x 40 watt L40 W/ 20S cool white fluorescent, (Osram) to 4 x 100w pearl incandescent (Thorn).

Arrangement of this lighting in the phytotrons at the School of Plant Science consists of one light bank over every second row of plants, set 2 m above ground level. This gave measured light distribution ranges of 8-20 μmol $m^{-2}s^{-1}$ at pot level. This is above the range of threshold to saturation irradiance given for photoperiodic responses by Summerfield and Roberts (1987), who also note that even distribution is not as important for photoperiodic lighting, as long as levels are above thresholds.

5.3.2 Inductive light level

To examine inductive light level, plants were grown under 8 h natural light with a very weak light extension gradient (0.1 to 0.6 μmol $m^{-2}s^{-1}$), and with no extension, as described in Materials and Methods. Results show that even an irradiance of 0.1 μmol $m^{-2}s^{-1}$ can be considered inductive for pea, as flowering node (NFI, Table 5.1) was significantly earlier ($P < 0.01$) than no extension. At 0.2 and 0.3 μmol $m^{-2}s^{-1}$ NFI was not significantly different to 0.1 μmol $m^{-2}s^{-1}$. At 0.6 μmoles $m^{-2}s^{-1}$ NFI was significantly earlier ($P < 0.01$) than 0.1 μmol $m^{-2}s^{-1}$ and was not significantly different to an extension irradiance of 5 μmoles $m^{-2}s^{-1}$ used to compare extension light source (Table 5.2). Thus, for L107 pea inductive irradiance was less than 0.1 μmoles $m^{-2}s^{-1}$ while saturating irradiance was above 0.6 μmoles $m^{-2}s^{-1}$.

Table 5.1. Node of flower initiation (NFI) ± SE, n = 10 for L107 pea grown under 8 h natural light with no extension, and with 16 h extensions of 0.1 to 0.6 μmoles $m^{-2}s^{-1}$ as described in Materials and Methods. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$.

Extension irradiance (μmoles $m^{-2}s^{-1}$)	0	0.1	0.2	0.3	0.6
NFI	22.1 ± 0.21 a*	17.2 ± 0.18 b	16.8 ± 0.16 b	16.7 ± 0.16 b	15.9 ± 0.16 c*

5.3.3 Light leakage measurements

Given the very low fluence required for inductive responses, growth chambers and dark bays used for photoperiod studies were tested for light leakage levels, as plants growing in SD conditions may be influenced by light leakage from natural light or from adjacent growth chambers. In closed dark bays during daylight, light levels were under 0.001 μmol $m^{-2}s^{-1}$.

In the phytotron bays and in growth chambers in a darkened area, light leakage was below detectable levels. With lights on in an adjacent chamber, light levels in the dark chamber were under 0.001 μmol $m^{-2}s^{-1}$. In dark cabinets not in a light proof area, light levels measured were between 0.01 and 0.03 μmol $m^{-2}s^{-1}$. L107 plants grown in this chamber under SD conditions (8 h) flowered at node 25.2 ± 0.28, suggesting light leakage at this level (0.01-0.03 μmol $m^{-2}s^{-1}$) was not inductive.

5.3.4 End of day R:FR ratio

R:FR ratio of the mixed fluorescent/incandescent photoperiod extension lighting examined mimic twilight levels of 0.8. However, it is uncertain whether end of day time measurement is a response to the lowering of R:FR ratio at twilight or to lowered irradiance (Vince-Prue 1994), but appears to be marked by irradiance below threshold levels (Moe 1997; Summerfield and Roberts 1987, Tibbitts *et al.* 1983).

Twilight ratios were measured under both clear sky and overcast conditions, both in a glasshouse (through horticultural glass) and outside. To avoid light contamination, all supplementary light sources were turned off for these measurements. It has previously been reported (Smith and Morgan 1981) that daylight is remarkably constant over the course of the day, but that R:FR drops to 0.8 during twilight. This was confirmed for broad band R:FR (Fig. 5.3), with a lowering of R:FR to 0.8 at

twilight due to an increase in far-red proportion. However, there was little alteration in narrow band calculation. Also measured were blue ratios (Fig. 5.3). Blue proportion increases dramatically at twilight, from 0.6 to 1.2 at sunset. Thus although far-red does increase at twilight, the large change in spectral properties is in blue proportion. There was no alteration in ratios through glass compared to open sky, and results were similar under both clear and overcast conditions.

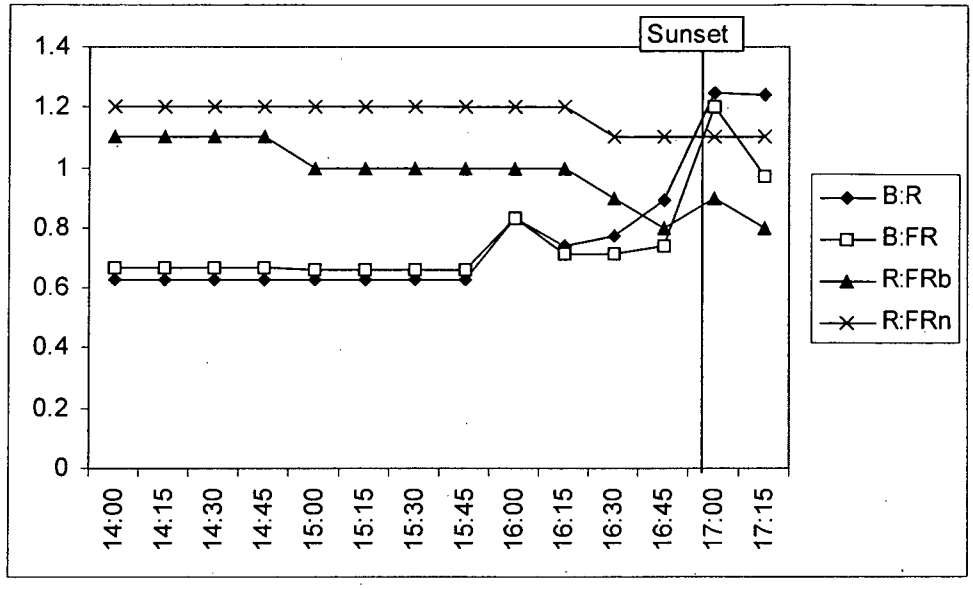


Figure 5.3. Measured wavelength ratios from 2 PM to after sunset (twilight), overcast conditions

The specific role of blue light at twilight appears to have not been investigated, but screening to exclude twilight had no influence in previous studies (Mortensen and Moe 1992). In addition, plants in the SD system at the School of Plant Science usually enter the dark bays before twilight and do not receive the extension lighting. Such plants therefore do not receive a twilight signal, but respond to the SD treatment, suggesting lowered irradiance acts as an end of day signal, as well as or instead of a twilight ratio.

5.3.5 Responses to photoperiod extension R:FR

In Chapter 4, plants grown under high pressure sodium (high R:FR) did not flower at a later node than plants grown under fluorescent/incandescent lighting (low R:FR), as could be expected. However, since this comparison was not at equal DLI, there may have been no delay in flowering due to the extra photosynthate from increased irradiance. A number of species show inhibition of flowering under fluorescent due to

the high R:FR, while others show little difference (Whitman *et al.* 1998). Lowering R:FR below daylight levels of around 1.1 can accelerate flowering, but also increases shoot extension as part of the shade avoidance response (Runkle and Heins 2001). However, spectral differences between lamps may be less important when used as a supplement to natural radiation (Moe 1997). To examine the influence of photoperiod extension R:FR at equal DLI, L107 plants were grown concurrently under 8 h, and 8 h + 16 h weak photoperiod extension with either incandescent or fluorescent lamps. R:FR ratios of the extensions were 0.7 and 5.6, respectively, allowing for examination of the influence of low or high R:FR.

Results (Table 5.2) confirm the facultative LD response of L107 pea (Reid *et al.* 1996): plants will flower under SD, but flowering is delayed by 6-7 nodes and 15-20 days. Thus total shoot length (TL) and total nodes (TN) is significantly greater than under inductive (long day) photoperiods. In spite of the very large difference in R:FR between the incandescent and fluorescent extensions, however, NFI was not significantly different, although slightly earlier under incandescent. Flowering time, however, was significantly earlier under incandescent compared to fluorescent (Table 5.2).

Table 5.2. Photoperiod extension type mean growth results (± SE) for L107 pea, n = 20. Plants grown concurrently under 8 h natural light with no extension, or 16 h weak fluorescent or incandescent extension as described in Materials and Methods. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Abbreviations: inc- incandescent, fl- fluorescent, L1-9- length between nodes 1-9, TL- total shoot length, TN- total no. of nodes, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaflet length at node 9, Dry W- shoot dry weight.

	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
8 h	31.5 ± 1.09 a*	189.8 ± 5.15 a*	30.8 ± 0.60 a*	22.7 ± 0.29 a*	69.3 ± 1.29 a*	15.5 ± 2.58 a*	10.3 ± 0.99 a*	2.5 ± 0.08 a	25.3 ± 0.50 a*	34.5 ± 0.58 a	6.1 ± 0.28 a*
8 h + inc	58.2 ± 0.64 b*	147.5 ± 2.64 b*	18.0 ± 0.10 b	16.0 ± 0.05 b	48.7 ± 0.33 b*	8.3 ± 0.51 b	2.6 ± 0.20 b	2.5 ± 0.05 a	26.3 ± 0.28 b	33.8 ± 0.43 b*	4.6 ± 0.24 b
8 h + fl	44.4 ± 1.09 c*	129.9 ± 2.78 c*	19.1 ± 0.14 c	16.4 ± 0.06 b	53.6 ± 0.44 c*	11.1 ± 1.13 b	3.9 ± 0.36 b	2.5 ± 0.07 a	28.0 ± 0.59 c	36.7 ± 0.75 c*	4.5 ± 0.24 b

Shoot length was significantly increased relative to fluorescent extension under incandescent. Internode length (L1-9) was significantly increased (by almost 14 cm) as was total shoot length (TL). Total nodes (TN) was significantly decreased, and leaflet size (LW and LL) was significantly decreased under incandescent. These are typical shade avoidance responses to low R:FR ratio (Ballare *et al.* 1997). Yield (seed and pods) was also reduced under incandescent extension. Thus extension type (fluorescent or incandescent) significantly influenced morphology of a standard line garden pea.

5.3.6 Response to monochromatic photoperiod extensions

Flowering node (NFI, Table 5.2) was not significantly different under fluorescent or incandescent extensions in spite of the large difference in R:FR. Other species have also shown this lack of response, for example high R:FR day length extensions all produced equal flowering responses and less shoot elongation than low R:FR incandescent extensions in campanula and coreopsis (Whitman *et al.* 1998). To test the response of pea, the experiment was repeated with the day length extension provided by weak monochromatic blue, red and far-red LED lighting as described in Materials and Methods. This allowed for examination of specific wavelengths on the LD response, including blue light. While light sources such as fluorescent and high pressure sodium have high R:FR, they still contain some far-red light. By using monochromatic wavelengths as the photoperiod extension, the specific roles of red, far-red and blue can be examined. The flowering response under the extension wavelengths would also indicate if a far-red component is required for flowering, or whether other wavelengths are also inductive.

Results show that any of the light extension wavelengths induce significantly earlier flowering than SD conditions (Table 5.3), all were around the natural LD flowering node for this pea line of node 16. Thus any of the wavelengths tested can be considered inductive. However, plants flowered at a significantly later node under red with significantly reduced internode lengths compared to blue and far-red extensions (Table 5.3). The far-red extension induced significantly earlier flowering, increased internode length and smaller leaves, typical shade avoidance responses to low R:FR. Under blue, shoot length and leaf size was not significantly different to SD conditions, but flowering was significantly earlier. The results suggest light of any wavelength is

effective at flower induction in pea as an extension to natural light, but high R:FR will delay flowering and reduce shoot length, while low R:FR has the opposite effect. Blue wavelengths were inductive for flowering without altering shoot length.

Table 5.3. Photoperiod extension type mean growth results (± SE) for L107 pea, n = 20. Plants grown concurrently under 8 h natural light with no extension, or 16 h weak blue, red or far-red light emitting diodes extension as described in Materials and Methods. Different letters signify significant differences at $P<0.05$, letter plus asterisk (e.g. b*) significant at $P<0.01$. Abbreviations:, L1-X- length between nodes 1-X, NFI- node of flower initiation, FT- flowering time from planting date, LW- leaflet width at node 9, LL- leaflet length at node 9.

	L1-4 (cm)	L1-9 (cm)	L1-12 (cm)	NFI	FT (days)	LW (mm)	LL (mm)
8 h	10.9 ± 0.26 a	44.8 ± 0.36 a*	73.2 ± 0.71 a*	23.6 ± 0.28 a*	66.3 ± 2.01 a*	20.4 ± 0.56 a	28.1 ± 0.63 a
8h + Blue	11.5 ± 0.17 a*	46.0 ± 0.50 a*	72.4 ± 0.66 a*	15.9 ± 0.06 b*	46.1 ± 0.23 b	19.7 ± 0.33 a	27.3 ± 0.41 a
8h + Red	9.3 ± 0.23 b*	40.2 ± 1.05 b*	61.9 ± 1.60 b*	16.8 ± 0.18 c*	45.5 ± 0.31 b	21.4 ± 0.69 a	29.5 ± 1.13 a
8h + Far-red	12.7 ± 0.36 c*	55.6 ± 0.97 c*	83.2 ± 1.43 c*	15.3 ± 0.13 d*	51.0 ± 0.34 c	17.7 ± 0.65 b	23.6 ± 0.50 b*

5.4 Discussion

Only very low fluence was required for an inductive response in L107 pea. Inductive irradiance was less than 0.1 μmol $m^{-2}s^{-1}$ while saturating irradiance was above 0.6 μmol $m^{-2}s^{-1}$ (Table 5.1). This is in line with other results for photoperiod sensitive species (Summerfield and Roberts 1987). Fluence rates as low as 0.2 μmol $m^{-2}s^{-1}$ induced flowering in *Campanula* (Whitman *et al.* 1998). This highlights that for SD experiments to be effective, external light must be excluded, including from adjacent areas. For example, light leakage of HPS light was tested on neighbouring crops from a greenhouse with a photoperiod extended morning and evening to provide 18 h by Bakker and Blacquiere (1992). Flowering was delayed in the SD plants (*Chrysanthemum, Poinsettia*) from measured leakage levels of 0.05 to 0.2 μmol $m^{-2}s^{-1}$ PPF, and promoted in the LD plants (*Fuchsia* and *Callistephus chinensis*). Cucumber elongation was reduced and fruiting delayed (Bakker and Blacquiere 1992). As part of the study of glass and polycarbonate (Chapter 3), L107 was grown under non-inductive conditions in winter: short days without any photoperiod extension. However, security lighting around the complex was sufficient to induce a LD

flowering response (Table 3.3). This again highlights the importance of light exclusion for SD conditions.

For different photoperiods incorporating natural light, a system of phytotron bays is in use at the School of Plant Science, Hobart. These consist of automatic trolleys that run into a series of dark bays, which can be dark or contain photoperiod extensions. The phytotron bays tested were effectively light proof, but other systems in common use, such as photoperiod curtains, may not exclude all light.

Photoperiod control is also commonly conducted in growth chambers. As for natural light systems, care must be taken to exclude all other light sources (such as light leakage from other chambers through vents) if the photoperiod being examined is to be relied upon. Testing of light leakage levels in chambers and the dark bays showed that levels were very low, less than 0.03 μmol m^{-2} s^{-1}. Plants grown under SD conditions with this level of light leakage did not show an inductive response, suggesting light leakage was not an issue in the growth chambers examined. Nevertheless, for added safety, light exclusion zones (including light proof vents) are in place at the School of Plant Science around selected growth chambers, and the phytotron bays can be considered light proof.

It is uncertain if the end of day signal is marked in plants by lowered R:FR (from about 1.1 in daylight to 0.7-0.8), or by the lowering of irradiance (Vince-Prue 1994). It has previously been reported (Smith and Morgan 1981) that daylight is remarkably constant over the course of the day, but that R:FR drops to 0.8 during twilight. Twilight ratios were measured (Fig. 5.3) and it was found that although broad band R:FR does decrease, narrow band calculation did not. Blue proportion increased dramatically at twilight, from 0.6 to 1.2 at sunset (Fig. 5.3). That light spectral properties are relatively constant through the day was confirmed and there was no alteration in ratios through glass compared to open sky, under both clear and overcast conditions. The role of blue at twilight has not been investigated specifically, but screening to exclude twilight had no influence in previous studies (Mortensen and Moe 1992). The demarcation between day and night appears to be related to irradiance dropping below threshold values rather than a change in spectral quality (Summerfield and Roberts 1987). In addition to these studies, plants in the SD system at the School of Plant Science usually enter the dark bays before twilight and do not receive any extension lighting. Such plants therefore do not receive a twilight signal, but respond

to the SD treatment, suggesting lowered irradiance acts as an end of day signal, as well as or instead of a twilight ratio.

The influence of extension lighting type on flowering is unclear. R:FR has a clear influence on plant responses, sensitive species show increased elongation, earlier flowering and small leaf size in response to low R:FR (e.g. Ballare *et al.* 1997), and relatively delayed flowering and reduced shoot length at high R:FR (e.g. Runkle and Heins 2001). However, as an extension to natural light, plant responses vary and wavelength differences may be less important under such circumstances (Moe 1997). Flowering in a range of species was relatively delayed under high R:FR fluorescent compared to incandescent photoperiod extensions (Lane *et al.* 1965), and maximum flowering response tends to be at natural R:FR (Vince-Prue 1981), thus traditional photoperiod extensions use low R:FR lighting such as described in Figure 5.2. However, other studies do not show inhibition of flowering at high R:FR. For example, CWF, HPS and MH (which all have high R:FR) day length extensions all produced equal flowering responses and less shoot elongation than incandescent at PPF above 1 $\mu mol\ m^{-2}s^{-1}$ in campanula and coreopsis (Whitman *et al.* 1998). R:FR may be less important in the LD flowering response than the mere presence of light. Any light from any wavelength can stimulate de-etiolation, for example (Kendrick and Weller 2003b). Thus in some species the end of day R:FR ratio may be important, in others it may not.

The response of pea was examined by comparing L107 growth with incandescent (R:FR 0.7) and fluorescent (R:FR 5.6) at equal irradiance. Results (Table 5.2) showed that for L107 pea, shoot length was significantly increased relative to fluorescent extension under incandescent, with reduced leaf size and yield. These are typical shade avoidance responses to low R:FR (Ballare *et al.* 1997). However, flowering node was not significantly different. Further analysis using 8 h natural light plus weak blue, red and far-red monochromatic extensions (Table 5.3) suggested light of any of the tested wavelength is effective at flower induction in pea relative to SD conditions. However, high R:FR will slightly delay flowering and reduce shoot length, while low R:FR has the opposite effect. Blue wavelengths were inductive for flowering without altering shoot length.

Many studies that demonstrate the value of photoperiod extension with incandescent have not established if the benefits are from a phytochrome ratio effect, the additional photosynthetic contribution of 700-750 nm radiation, or a temperature effect on the

plants (Tibbitts *et al.* 1983). Incandescent lamps have a high thermal component (McFarlane 1978), and the radiant thermal effects need to be separated from other environmental temperature effects (Sager *et al.* 1982). The low fluence rates, and high distance to lights in the climate controlled phytotron bays helps to minimise these effects, but the radiant influence of incandescent lamps in closer environments should be considered. There was no evidence of thermal load influence on the L107 results under either light source. Increased temperature reduced shoot length in pea (Table 2.16), as did the influence of thermal load from incandescent globes in growth chambers (Chapter 7). L107 grown under the incandescent extension showed shoot length increases relative to the fluorescent extension, as expected from lowered R:FR, and under far-red LED, which provide far-red light without the thermal implications of incandescent, the same typical shade avoidance responses of increased shoot length, smaller leaves and earlier flowering were observed. Thus this response can be considered to be a wavelength effect, not a thermal influence.

In summary, inductive light levels for photoperiodic sensitive species are very low, and care should be taken to exclude all external light for SD studies. Any light from any wavelength is likely to be inductive. Growth cabinets tested, however, did not show light leakage above threshold levels. The end of day signal is likely to be irradiance below threshold levels rather than a change in spectral properties at twilight. Photoperiod extension R:FR can influence results- low R:FR can induce shade avoidance responses, but high R:FR lighting and even monochromatic blue lighting extension still produced a LD response of earlier flowering.

Chapter 6 Growth Chambers

6.1 Introduction

A range of growth chamber types are used at the School of Plant Science Controlled Environment Facility, as in most such facilities. As research needs develop, growth chambers are added, thus all facilities surveyed had a range of brands and types with different light sources. Growth chambers are used for their ability to accurately control environmental variables, notably temperature and light quantity. However, there are many design and control method variations, and hence differences in air velocity, direction and exchange rates, temperature distribution, and light. What influence do these parameters have on plant development and morphology? How comparable are results using different growth chambers? What is the influence of light source on results? And even where the same light type is used (eg cool white fluorescent), are there differences between the various brands and wattages used?

This chapter looks in detail at growth chamber issues by both physical measurement and by examining growth responses of a standard pea line. The major difference between growth chambers (and natural light) is light source. Spectral characteristics of all the light sources used at the School of Plant Science facility are examined, which are representative of the commonly used artificial lights used in plant research. The interchangeability of sources, possible photomorphogenic effects of these sources, and comparative growth responses are examined.

Under natural conditions, plants encounter considerable variation in light quantity, quality and duration (Walters and Horton 1994). Temperature and other environmental parameters also vary spatially and temporally (Carlson and Giger 1978). The variability of natural light environments was examined in Chapter 2, and both descriptive (eg light quantity and temperature) and experimental (growth responses of the garden pea in these environments) measurements showed significant variation between environments and seasons. This makes comparisons of results difficult unless plants are grown concurrently. Growth chambers can provide uniform reproducible conditions for assessment of physiological and morphological development (Carlson and Giger 1978). However, in growth chambers all radiation is

artificial (Aldrich and White 1969) and all artificial light sources have very different spectral characteristics to sunlight (Deitzer 1994; Runkle 2004).

Plant responses vary according to wavelength, intensity and duration (Sager *et al.* 1988). Light source becomes particularly important in growth chambers where all radiation is artificial (Aldrich and White 1969), and all artificial light sources have very different spectral characteristics to sunlight (Runkle 2004). There may also be some growth effects from the radiant component of artificial light sources above 700nm, either from the additional photosynthetic contribution, or a temperature effect (Koontz *et al.* 1987; Tibbitts *et al.* 1983). Cool white fluorescent (CWF) are the traditional light source in growth chambers, with or without incandescent (Cathey and Campbell 1977). R: FR ratios as high as 8.8, with virtually no far-red (FR) component, can be found in CWF, and this can inhibit flowering and shoot extension in many species (Runkle 2004). R:FR ratios of natural light are generally not replicated in growth chambers, even with the addition of incandescent lamps to the light mix (Downs 1994).

Where high PPF levels are required, usually with the aim of simulating "natural" conditions, fluorescent sources are considered inadequate, and high intensity discharge (HID) lamps tend to be used (Bubenheim *et al.* 1988). Of the HID sources, metal halide and high pressure sodium are the most common, due to their radiant efficiency, long life and high output (Bubenheim *et al.* 1988). HID lamps can provide higher PPF levels, but also increase short and long wave radiation, often to levels well above those found in the natural environment (McCree 1984). This can affect leaf temperature, increasing transpiration and reducing water use efficiency (Bubenheim *et al.* 1988). Leaf temperatures 16^{0}C higher than air temperature have been measured under HID lamps without filtering (McCree 1984). Hence growth chambers often have separately ventilated, temperature controlled light lofts to reduce thermal load and reduce output variation from temperature influences (Sager and McFarlane 1997).

Even with a barrier, management of heat load in the chamber is difficult, particularly with lamps with a high thermal component such as HID and incandescent. Radiation not reflected is absorbed and radiated as heat (Ormrod 1978a). Plexiglass (polycarbonate) reduces the UV component more than glass; and both reduce transmission of long wave radiation. Water barriers are the most effective long wave radiation filters, reducing levels by a maximum of 51% at a depth of 40mm yet reducing PPF by less than 2% (Bubenheim *et al.* 1988). Unfortunately, water filters

increase design costs and maintenance so are often not practical (Downs 1994). Differences in spectral output and light filtering methods (e.g. plexiglass, glass or water barrier filters) may affect growth and development by causing differences in photosynthesis, photomorphogenesis, and leaf and soil temperature (Tibbitts *et al.* 1983). At the School of Plant Science, growth chambers with no barrier, single and double glass, and with water filters are in use. This allowed for study of the influence of barrier type on both thermal load and growth responses.

There are many brands and types of lamps used in plant research, and they are often mixed to produce broader spectra or specific light ratios (Sager *et al.* 1988). The lack of conformity in lamp types and mixes makes comparison of results difficult (Tibbitts *et al.* 1983). Spectral distribution, particularly in the far red region, has rarely been considered in selection and use of growth chamber lighting systems (Downs 1994). Phytochrome parameters have largely been ignored, with the addition of "token" amounts of incandescent to provide limited far red proportions, well below sunlight levels (Smith 1994). Equal wattage of incandescent has been recommended to provide a far red component equivalent to sunlight proportions (Warrington 1978). However, incandescent sources have peak output at 900nm, producing significant long wave radiation. Consequently radiant heat load must be managed, and barrier type becomes more important (Smith 1994). Although early recommendations for addition to fluorescent lighting were 30% of the installed wattage, output of fluorescents has improved (Downs 1994) and this figure appears to be inadequate, particularly for higher output high intensity discharge lighting (Downs 1994; Warrington 1978).

In addition to photosynthesis, light also acts as environmental information for the plant, affecting a wide range of photomorphogenic responses, including germination, de-etiolation, elongation, leaf expansion, and flowering (Spalding and Folta 2005; Weller 2004). The wavelengths most important for plant development are UV-blue (350-500 nm), red (600-700 nm), and far-red (700-800 nm). The usual practice of reporting PAR or PPF alone (i.e. 400-700 nm) are limiting (Downs 1979; Smith and Morgan 1981). A full description of light should give the amount of light in each waveband active in plant responses, 300-800nm, using spectroradiometer data expressed in photometric terms in $\mu mol\ m^{-2}s^{-1}$ (Bjorn and Vogelmann 1994). Ratios of blue to red (B:R), blue to far-red (B:FR), and R:FR should also be included for possible photomorphogenic effects (Kittas *et al.* 1999).

Specific ratios can have specific effects. High R:FR generally signal a favourable light environment, encouraging germination, de-etiolation, and flowering in many species. Low R:FR signal competitive conditions and can encourage shade avoidance responses- increased elongation, reduced leaf area, reduced branching and accelerated flowering (Kendrick and Weller 2003a). Even very small changes in R:FR can induce such responses (Casal 2005).

The importance of a far red component is well documented, particularly as an incandescent addition to fluorescent sources, often increasing plant fresh weight, shoot length and rate of flowering (Downs 1979). Less clear is the addition of incandescent to HID sources. Addition of incandescent had little effect on mustard and wheat growth, but increased soybean growth and accelerated flowering in Lolium (Downs 1994). Addition of incandescent in equal wattage to HID increased dry weight, plant height and leaf area in tomato and sorghum when heat load was managed (Warrington 1978). The system described use water barrier filters and distance to reduce thermal load effects, but most growth chambers do not have these options. Light mixes that most closely resemble the natural spectrum produced the most "normal" plant growth and development (Warrington *et al.* 1976), but growth chamber light sources have very different spectral properties to natural light..

Blue light has been associated with light quantity/quality perception, as increasing the blue component of white light is associated with shorter internodes (Thomas 1981), and the reduction of the blue component in canopy shade can induce elongation (Ballare *et al.* 1997). Excess hypocotyl elongation in some species under high pressure sodium (HPS) lamps has been attributed to the low blue component of this light source (Tibbitts *et al.* 1983). HPS can produce excess elongation, while metal halide (MH, relatively high blue component) induced less elongation than control plants under high irradiance (Sager *et al.* 1982), and tomatoes grown under HPS were taller than those grown under MH (Zheng *et al.* 2005). Soybean internode length under HPS became progressively shorter with increasing blue supplementation or increased irradiance, up to a blue level of 30 $\mu mol\ m^{-2}s^{-1}$ after which increasing blue had no effect (Wheeler *et al.* 1991). Hypocotyl and petiole extension may be regulated by light irradiance through blue light perception (Christophe *et al.* 2006; Walters and Horton 1994), but inhibition of extension is also under phytochrome control (Walters and Horton 1994). Large differences in shoot elongation may be seen under low irradiance due to phytochrome responses, under high irradiance (above 300 $\mu mol\ m^{-}$

$^{2}s^{-1}$) with the same light source only small differences may be seen due to the influence of blue receptors (Tibbitts *et al.* 1983). However, light quantity is also a strong influence on shoot length (Gawronska *et al.* 1995), both blue quantity and irradiance can influence plant morphology (Christophe *et al.* 2006). Plants grown under higher irradiance have more energy available for growth, masking wavelength effects (Walters and Horton 1994).

Other possible sources of variation between growth chambers include humidity, air velocity, CO_2 levels and temperature distribution. Generally in growth chambers air velocities between 0.3-0.7 m s^{-1} are recommended for adequate air exchange and to avoid CO_2 depletion (Downs and Krizek 1997). A downward airflow creates more even temperature gradients than upward and horizontal flow, but horizontal flow better mimics natural conditions (Downs and Krizek 1997). Air velocity was examined as an influence on growth in Chapter 2 and found to reduce shoot length at velocities above this level (Fig. 2.5). Temperature profiles, air velocity and CO_2 levels were measured in the growth chambers during the growth response studies to determine the range of variation and to identify any issues with these factors. Plants were also grown in different chambers concurrently under the same light source, irradiance, photoperiod and temperature to determine if there was any significant growth chamber influence on results. Position of plants in the chambers was also analysed for any such influence, such as edge effects.

However, the major difference in growth chambers is the light source, which can exert an influence on photosynthetic, photomorphogenic and temperature responses. The same model of growth chamber was used to study the influence of light source on temperature and growth responses within the chamber. These chambers can be fitted with fluorescent or HID lamps, with and without incandescent, allowing for a comprehensive analysis of the influence of light source.

6.2 Materials and methods

6.2.1 Growth chambers examined

Growth chambers used were Thermoline (Sydney, NSW, Australia) models 3540 and PG11.12.6.TD with externally ventilated light lofts separated from the growing area by Pilkington (Dandenong, Victoria, Australia) 6TF 6 mm toughened glass barriers with horizontal air flow; Conviron (Winipeg, Manatoba, Canada) model EF7H with plexiglass barriers and upward airflow, locally constructed chambers (Tri-Tec, Hobart, Tasmania, Australia) with double 6mm toughened glass light loft barriers with and without 40 mm water filters beneath the light loft; and locally constructed growth chambers with no light loft barrier and downward airflow.

6.2.2 Light sources and treatments

Cool white fluorescent sources were L40 W/ 20S (Osram, Munich Germany), 36 W/W 41 (Thorn, Smithfield, NSW, Australia), 40SSCW/37-EXP (NEC, Tokyo, Japan); F36W/840 Luxline Plus (Sylvania, Munich, Germany); 115W F48T12/CW/VHO (Sylvania, Danvers, MA, USA); and 37W HGX Quadphosphor (NEC, Tokyo, Japan). Gro-Lux lamps were F36W GRO-T8 (Sylvania, Munich, Germany).

Blue and red fluorescents were TLD 36W/15 Blue and TLD 36W/15 Red (Philips, Eindhoven, Holland).

Fluorescent light source growth response studies were conducted in growth chambers at an air temperature of $20^0 \pm 0.2^0$C, under an 18 h photoperiod at 220 μmol m^{-2} s^{-1} PPF at the pot surface. Light sources used were L40W/20S, 36W/W 41, and 36W/840 CWF, and Gro-Lux lamps.

High intensity discharge lamps used were metal halide: Kolorarc MBID 400/T/H (GE Lighting, Budapest, Hungary), and MH 400W/C/U (Thorn, Mumbai, India); high pressure sodium lamps were Vialox Planta T400W (Osram, Munich, Germany), 400W SON-E GES Elliptical (Thorn, Bucharest, Romania), and SL600W.U15.VRD Super HPS Deluxe (Sunmaster, Solon, OH, USA).

Incandescent source used were 100W pearl incandescent lamps (Thorn, Smithfield, NSW, Australia), MR16 12V 50W halogen, and 150W PAR38 (Mirabella, Tullamarine, Victoria, Australia).

Comparative plant growth responses under growth chamber artificial light source studies were conducted in the same model chambers (model 3540, Thermoline, Sydney, Australia,) at equal irradiance (photosynthetic photon flux 220 μmol m^{-2} s^{-1} at the pot surface), photoperiod (18 h) and temperature ($20^0 \pm 0.2^0$C). These reach-in chambers can accommodate high intensity discharge (HID) lamps (6 x 400W) and incandescent (5 x 100W) or fluorescent lamps (16 x 36W) and incandescent (4 x 100W). This allowed for study of all the light sources in the same growth chamber type and model. Light sources used were cool white fluorescent- F36W/840 Luxline Plus (Sylvania, Munich, Germany); incandescent globes- 100W pearl (Thorn, Smithfield, NSW, Australia); metal halide lamps- 400W Kolorarc MBID 400/T/H (GE Lighting, Budapest, Hungary); and high pressure sodium lamps- Vialox Planta T400W (Osram, Munich, Germany).

All other experiments and measurements were conducted in the growth chambers under an 18 h photoperiod at 220 or 425 μmol m^{-2} s^{-1} PPF, at an air temperature of $20^0 \pm 0.2^0$C.

6.2.3 CO_2 and humidity measurements

CO_2 and humidity were measured in a range of growth cabinets with a LI-6400 portable photosynthesis system (LI-COR, Inc., Lincoln, NB, USA) during selected growth response studies.

6.2.4 Light measurements

All light measurements were taken in controlled environment chambers with external light excluded at an air temperature of 20^0 C using a LI1800 spectroradiometer (LI-COR, Inc., Lincoln, NB, USA) with a cosine corrected sensor. Spectral irradiance was downloaded in W m^{-2} nm^{-1} and as quantum intergrade (μmol m^{-2} s^{-1}) averaged over 3 scans in the range 300-800nm, following measurement and reporting guidelines (Bjorn and Vogelmann 1994; Sager *et al.* 1982). Selected measurements were also taken with an Apogee UV-PAR spectroradiometer (Apogee Instuments Inc., Logan, UT, USA) to check for accuracy. Agreement between instruments was generally within 1%. Figures and spectral distributions were checked against data from the manufacturers, lists of spectral data (Deitzer 1994; Deitzer 2005) and other published data using light source comparisons (Bubenheim *et al.* 1988; Koontz *et al.* 1987; Sager *et al.* 1982; Tibbitts *et al.* 1983).

For comparisons of waveband proportions of a light source and/or between light sources at different irradiances, the percentage of quantum intergrade (300-800 nm) was calculated for PPF (photosynthetic photon flux, 400-700 nm) and for each 100nm band. Wavelength ratios follow published methods (Kittas *et al.* 1999) and were calculated from the quantum data as: R:FR narrow band (R:FRn) 655-665 nm/725-735 nm; R:FR broad band (R:FRb) 600-700 nm/700-800 nm; blue to red (B:R) 400-500 nm/600-700 nm; B:FR 400-500 nm/700-800 nm.

Light source details are as given by the manufacturer. As fluence rate can decrease with lamp age, particularly in the first 100 h, all measurements were taken on sources that had burned for 100 h or more. Light sources were measured a minimum of 1 h after start up. Light distribution measurements in the growth chambers were taken using a LI-COR LI-185B quantum radiometer with quantum sensor. This instrument and an Apogee model QMSW quantum meter were also used to compare measurement ranges and sensors.

6.2.5 Air and temperature measurements

Air temperature measurements in the growth chambers were continuously monitored and controlled by BrainChild (Taipei, Taiwan) BTC 9090 sensors (Thermoline models) and by Honeywell (Sydney, NSW, Australia) customised software and sensors (model TE205F17, Mamac Systems, Holden Hill, South Australia). Air velocity was measured with a Kane-May Ltd (Welwyn, Herts, UK) KM-4000 thermo-anemometer. Radiant temperature in chambers was measured with a CPS Inc (Hialeah, Florida, USA) Tempseeker T200 digital thermometer with silicon temperature sensors, 3 sensors were used per measurement, at 10 cm intervals from the light loft barrier. Soil temperature was measured 1 cm below the surface using the probe sensor of this instrument. Results given are means of hourly measurements over three days. Surface and leaf temperature measurements were taken with a CPS Inc (Hialeah, FL, USA) infra-red thermometer.

6.2.6 Plant growth and measurements:

To compare growth responses under the light sources and in the growth chambers, peas (a selection of *Pisum sativum* L. 'Torsdag') were grown in the various environments. This line (Hobart line 107) is a quantitative long day plant (Reid *et al.* 1996). Plants were grown in adjacent pots, 2 per 14 cm slimline pot in a 1:1 (v/v) mix of vermiculite and dolerite chips topped with 2-3 cm of peat-sand potting mixture. All

plants were watered as needed and fertilised with nutrient solution weekly consisting of Aquasol (Hortico, Sydney, NSW, Australia), N:P:K 23:4:18 at a rate of 1g l^{-1} plus Iron Chelate (Kendon Chemicals, Sydney, NSW, Australia) at 0.05g l^{-1}. Relative humidity, while not controlled, ranged from 40-65% in all experiments. Twenty plants were sown per treatment. During growth, stem diameter (mid point between nodes 9 and 10), leaf width (LW) and leaf length (LL) of 1 leaflet per plant was measured at node 9 at the commencement of flowering, node of flower initiation (NFI) and days from planting to first open flower (FT) were recorded. At maturity (senescence) length of internodes 1-9 (L1-9), total shoot length (TL), number of nodes (TN); number of seed (Seed) and number of pods (Pods) were measured. Shoot dry weight was measured after air drying of the senesced plants for at least 72 h.

Statistical analysis using JMP software (SAS Institute, Cary, NC, USA) included ANOVA, Students t-tests, Dunnetts method, and/or Tukeys test.

6.3 Results

6.3.1 Air velocity, CO_2 and humidity

A range of growth chamber types were used in these studies, with a range of air flow directions. Generally in growth chambers air velocities between 0.3-0.7 m s^{-1} are recommended for adequate air exchange and to avoid CO_2 depletion (Downs and Krizek 1997). Downward airflow creates more even temperature gradients than upward and horizontal flow, but horizontal flow better mimics natural conditions (Downs and Krizek 1997).

Results of measurements within the growth chambers tested showed that air velocities ranged from 0.3 to 1.1 m s^{-1}. Largest gradients were in the horizontal flow chambers (Thermoline models), which ranged from 1.1 m s^{-1} at the fan inlet side to 0.5 m s^{-1} at the opposite wall. However, decrease in velocity was rapid, measuring around 0.7 m s^{-1} within 10 cm of the inlet. Velocity between plants was reduced to around 0.3 m s^{-1}. Most even were the downward airflow models, with velocities ranging from 0.5 to 0.6 m s^{-1}. In the growth response studies (Fig. 2.5), air velocity above 0.7 m s^{-1} was shown to reduce shoot length. However, during growth response studies in the growth chambers, there was no significant variation in shoot length from plant position in the chamber (data not shown). In addition, fluorescent growth response studies were conducted in different chamber types, but there were no

significant differences in growth parameters. This would suggest air velocity within the ranges measured was not a significant factor in growth responses.

CO_2 levels can become limiting in dense canopies and in controlled environments without adequate ventilation (Hanan 1998; Peet and Krizek 1997). Levels can be also be enhanced from human activity in closed or poorly ventilated environments (Peet and Krizek 1997). However, measurements taken in various growth cabinets during growth did not show any significant variation from ambient levels, suggesting air exchange rates were adequate and CO_2 was not a limiting factor.

Humidity influences plant transpiration rates through vapour pressure deficit. Transpiration rate is influenced by temperature, air movement, irradiance, and soil moisture (Spomer and Tibbitts 1997). In well watered plants, low humidity is unlikely to cause desiccation. However, high humidity is associated with plant diseases and can be an issue in controlled environments (Spomer and Tibbitts 1997). Humidity was not controlled in the growth cabinets used, but monitoring showed levels varied much less than ambient conditions, ranging from 55-70%. No evidence of plant desiccation from low humidity or increased disease incidence at high humidity was observed.

6.3.2 Light measurement ranges and sensors

As a guide, Table 6.1 lists fluence rates at the same distance from the light sources in the growth chambers using both PPF meters (Li-Cor and Apogee) and spectroradiometers (Li-Cor LI 1800 and Apogee) in the 400-700 nm PPF range. Also included is fluence over 300-800 nm, the morphologically active radiation range, measured with the spectroradiometers. All measurements were 80 cm from the light source at 20° C. Table 6.1 illustrates a number of points. It shows how important it is to use a calibrated, or at least the same, instrument to measure light parameters, as the PPF meters give very different readings to the LI1800 spectroradiometer over the same range. The PPF meters under register the PPF of CWF by 14%, and of HID sources by 25%. Errors are larger with incandescent added, and the wider range measurements illustrate the significant unmeasured contribution to irradiance of longer wavelength sources- incandescent and HID. PPF meter readings vary according to light source, calibration and weighting function, a more accurate measure of quantum flux is a spectroradiometer, which totals the number of photons in each waveband (Bjorn and Vogelmann 1994). Although the accepted PPF range is 400-700nm, some photosynthetic activity occurs between 360-760 nm (McCree 1972a),

and the unmeasured photosynthetic contribution of longer wavelength light sources can be significant (Tibbitts *et al.* 1983). Both PPF meters used in this study gave very similar readings, as did the two spectroradiometers, hence this was not a calibration issue, but an indication that the weighting function used in such meters under estimate the actual fluence rate when calculated on an energy flux per nanometre basis. However, PPF meters can be used as a guide to setting up equal PPF of the same light source (e.g. CWF in several growth cabinets). Because of wavelength differences between lamps, caution should be used when setting equal irradiance of different light sources unless a spectroradiometer is used.

Wattage is not a guide to PPF, as the monochromatic sources illustrate (blue and red tubes). Blue wavelengths have almost twice the energy of red (Salisbury and Ross 1992) and in this study red fluorescent tubes have only 1/3 of the output of blue tubes of the same wattage. Similarly, the 115W high output (VHO) tubes (tested in the Conviron chamber) did not produce higher irradiance than 40W CWF. A number of authors point out the different photosynthetic efficiency of various lamp types, with red wavelengths being the most efficient (e.g. Sager *et al.* 1982, Cathey and Campbell, 1977). Thus the actual spectral distribution of a lamp type is important, particularly at lower fluence (Cathey and Campbell 1977; Tibbitts *et al.* 1983; Walters and Horton 1994). PPF meters give an indication of irradiance, but, unlike spectroradiometers, no information on spectral distribution.

Table 6.1 also illustrates the high PPF levels achievable with HID lamps, over six times the levels achievable with a full bank of CWF tubes in the same cabinets at the same distance.

Table 6.1. Fluence rate measurements of various light sources in μmol m^{-2} s^{-1} at 80 cm.

Light Source	No. lamps	PPF meter 400-700 nm	Spectro-radiometer 400-70 nm	Spectro-radiometer 300-800 nm
Blue fluorescent	6 x 36 W	23	27.9	30.1
Red fluorescent	6 x 36 W	7	9.3	12.1
CWF.	16 x 40W	150	184.0	193.0
CWF + inc	16, 4 x 100W	175	215.6	275.3
Inc	4 x 100W	25	27.1	78.4
CWF	8 x VHO	75	98.0	102.8
Thermolines				
CWF	14 x 36W	75	87.3	101.7
CWF + inc	14, 4 x 60W	85	112.8	145.4
HID	6 x 400W	390	521.4	606.8
HID + inc	6, 5 x 100W	410	552.9	671.2

6.3.3 Light distribution

Light distribution measurements were taken in the growth cabinets under both fluorescent and high intensity lighting. Growth cabinets are generally designed for even light distribution, with white reflective walls and the entire growing area covered by lighting. Fluorescent tubes run the full length of the growing area, and HID lighting generally consists of more lower wattage lamps rather than fewer high wattage lamps to produce more even distribution. Nevertheless there was around 10-15% reduction in irradiance towards the edges under both lighting types. Thus edge effects should be considered in experimental design, or the very edges avoided. Light distribution otherwise was very even.

6.3.4 Comparison of cool white fluorescent tubes

The commonly used light source for plant research in both growth chambers and greenhouses is CWF (Heo *et al.* 2002, Runkle 2004). Various CWF tubes are available, many with claimed higher output.

Fluorescent tubes pass a current through mercury vapour to emit radiation. The inner walls of the tubes are coated with phosphors that fluoresce when activated by the radiation, broadening the spectral output. Thus spectral distribution depends on the particular phosphors used (Sager and McFarlane 1997). Although broader spectrum fluorescents are available (eg warm white) and specific plant fluorescents (Gro-Lux) the additional phosphor coatings reduce lamp output, making them a less efficient (and more expensive) option (Sager and McFarlane 1997). Comparative studies generally show higher dry matter accumulation under CWF than warm whites and Gro-Lux (Sager and McFarlane 1997). CWF emit light primarily in the PAR range, and little far red radiation is produced contributing to the characteristically high R:FR ratios (Sager and McFarlane 1997). This is confirmed by Fig. 6.1 and Table 6.2 below.

Fluorescent tubes are available in Australia mainly as 36W and 40W tubes, the latter being the standard tube used at the School of Plant Science. However, 40W fluorescent tubes are to be discontinued in Australia, and some of the more recent growth chambers do not take these larger tubes. Higher output tubes are also available, such as triphosphor 36W840 tubes. A comparison was made between CWF light sources- spectral properties and fluence- to examine how interchangeable such tubes are (what are the spectral differences and are there any differences in growth responses as a consequence), and whether higher irradiances are achievable from the fixed tube capacity of the growth chambers. In addition, spectral properties and growth responses under plant specific Gro-Lux tubes were compared with standard CWF. For comparative purposes, spectral distributions of representative fluorescent lamps are shown in Fig. 6.1. Also examined were the various brands of 36W and 40W cool white tubes. There were no significant differences between any of the brands examined (Sylvania, Philips, Thorn and Osram, data not shown) of the same wattage.

A more detailed analysis of the quantum data using relative wavelength proportions and quantum ratios (Table 6.2) allows for a better comparison of the CWF sources to each other, and to natural light.

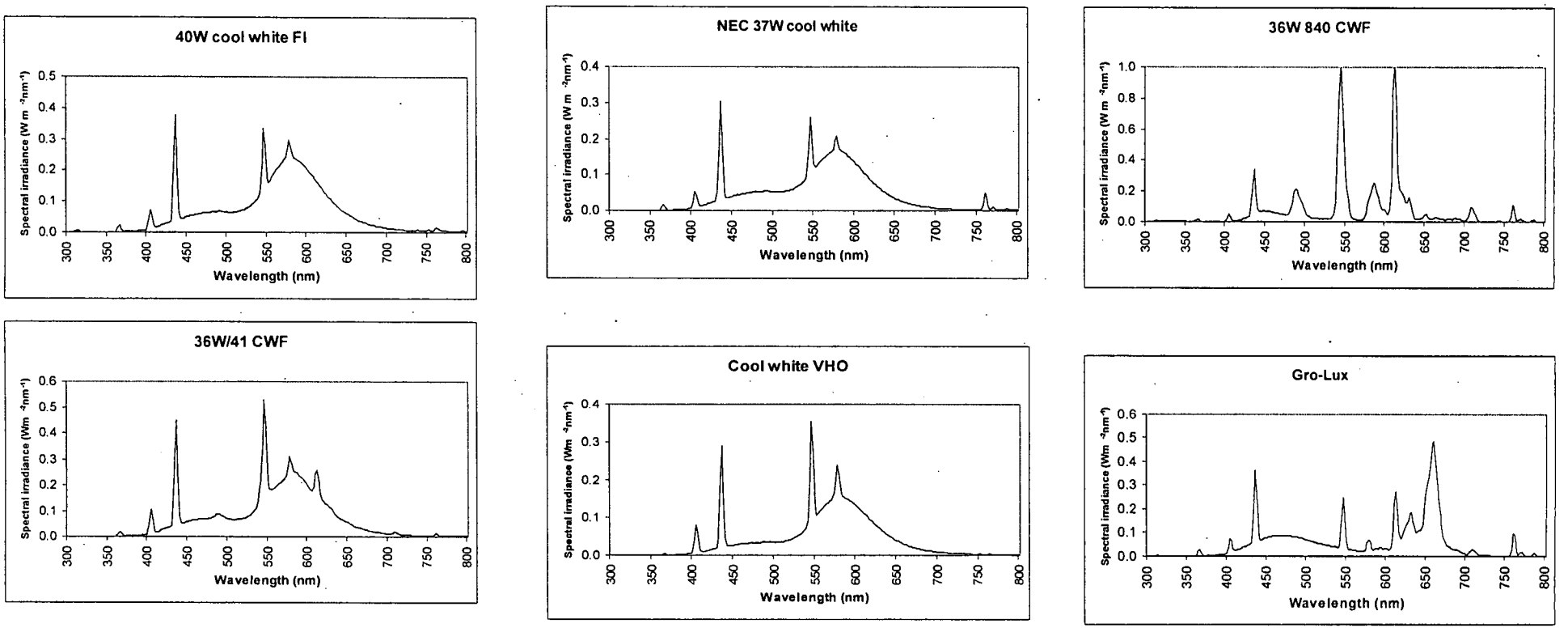


Figure 6.1. Spectral distribution of measured fluorescent light sources. Full light details and measurement protocols are in Materials and Methods.

Table 6.2. Relative spectral distribution as the percentage of total irradiance (300-800 nm) and wavelength ratios of cool white fluorescent (CWF), incandescent (INC) and natural light. Full light details and measurement protocols are in Materials and Methods.

Light source		Wavelength proportion (% total irradiance)					Wavelength ratios			
	PPF	300-400 UV	400-500 Blue	500-600 Green	600-700 Red	700-800 Far-red	R:FR (n)	R:FR (b)	B:R	B:FR
CWF										
40W	96.6	0.6	16.5	52.3	27.8	3.0	5.6	9.3	0.6	5.5
36W/41	97.3	0.5	19.8	53.1	24.3	2.1	5.8	11.4	0.8	9.3
37W	96.6	0.5	19.4	53.8	23.7	2.1	6.5	11.2	0.8	9.2
VHO	97.3	0.3	15.5	54.3	27.4	2.5	6.1	11.2	0.6	6.3
36W/840	95.3	0.6	19.4	41.0	35.0	4.0	2.2	8.7	0.6	4.8
Quadphos.	95.8	0.5	20.0	41.2	34.6	3.7	2.2	9.5	0.6	5.5
Gro-Lux	94.7	0.7	22.5	16.7	55.5	4.6	4.4	12.2	0.4	4.9
INC										
Inc	45.7	0	2.0	12.0	31.0	55.0	0.7	0.6	0.1	0.04
PAR38	49.1	0.6	3.7	14.0	31.5	50.3	0.7	0.6	0.1	0.06
QH	58.2	1	5.2	19.2	33.7	40.9	0.8	0.8	0.2	0.1
Natural light										
Sunlight	69.4	2.4	15.5	25.0	29.0	28.0	1.1	1	0.5	0.6
Overcast	69.8	3.8	17.9	24.9	27	26.4	1.1	1	0.7	0.7

Results (Fig 6.1, Table 6.2) show the very low UV and far red output of CWF relative to sunlight. Blue and red proportions are comparable to sunlight, but green/yellow proportions (500-600 nm) are much higher. This is in the peak range of the human eye photopic response (Ryer 1997) hence the lights appear bright to people, but for photosynthesis blue and red wavelengths are the most efficient, green the least efficient (McCree 1972a). Of the CWF sources tested, the triphosphor 36W/840 tubes had highest red proportions and lowest R:FR, and on this basis are likely to be the most suitable for plant growth. These tubes also produced approximately 15% more light in the PPF range than the other sources, including VHO tubes. Recently released quadphosphor tubes had a near identical spectral profile and a further 15% increase in fluence. Otherwise, spectral differences between CWF tubes were minor. Plant specific Gro-Lux lamps have high blue, low green and high red (Table 6.2), hence should be more photosynthetically efficient at equal irradiance, but far-red proportions are also very low. This is reflected in the wavelength ratios, all the fluorescent sources have very high R:FR, which has been associated with delayed flowering and reduced shoot extension (Runkle and Heins 2001).

6.3.5 Growth responses under fluorescent sources

Fluorescent light source growth response studies were conducted using L107 peas grown in growth chambers at an air temperature of $20^0 \pm 0.2^0$C, under an 18 h photoperiod at 220 μmol m^{-2} s^{-1} PPF at the pot surface. Light sources used were L40W/20S, 36W/W 41, 36W/840 CWF, and Gro-Lux lamps.

There were no significant growth differences between any of the sources used at equal irradiance. Representative figures for growth under CWF are included in Table 6.4. Shoot length, flowering time, yield, leaf size and dry weight all showed no significant differences from these means, nor did flowering node. However, flowering node (means around node 18) show significant delays to natural light grown plants (around node 16), reflecting the high R:FR of the fluorescent sources. The higher red proportion of 36W840 tubes did not improve growth relative to the other CWF sources, and the lower R:FR did not result in a lower flowering node. R:FR was still very high relative to sunlight at over 2 (Table 6.2). Conversely, the higher R:FR of the other sources (all around 6) did not result in further delays in flowering node. Gro-Lux tubes at equal irradiance did not improve growth of peas relative to CWF (data not shown).

6.3.6 Incandescent sources

Incandescence is the radiation created by a heated body, for incandescent bulbs this is created by passing a current across a tungsten filament (Sager and McFarlane 1997). Incandescent bulbs are used largely for photoperiod extensions as their high far red component makes them highly suitable for this purpose (Hanan 1998). They are commonly used either as the only light source for relatively weak photoperiod extensions or mixed with other light sources in growth chambers to reduce the high R:FR of these sources. The low R:FR of incandescent is clear (Table 6.2). Most of the spectral output is in the far-red range, hence R:FR is lower than sunlight at 0.7, and much lower than fluorescent sources (Table 3.2).

Incandescent sources have a continuous spectra (Fig. 6.2) rather than lined spectra as found in most other light sources. Bulb wattage did not influence results- 25W, 40W and 60W incandescent bulbs all had the same spectral properties. A large proportion of incandescent output is heat, and photosynthetic output is low. Other incandescent sources, such as higher output PAR 38 floodlights and low voltage quartz halogen, also have the same spectral properties (Fig. 6.2, Table 6.2). While higher irradiance can be achieved with floodlights, most of the output is still in the far-red and above (thermal) range.

The very low blue proportion of incandescent is reflected in the B:R and B:FR ratios (Table 6.2). Most of the photosynthetic output is in the red portion of the spectrum, and PPF is less than half of the output. The high proportion of far-red output and low photosynthetic efficiency make incandescent globes suitable for photoperiod extension but unsuitable for photosynthetic lighting. Incandescent globes are also a useful source of additional far-red light for more efficient light sources such as CWF and HID, but the thermal component is significant. The influence of adding incandescent to the light mix in growth chambers is examined in detail in Chapter 7.

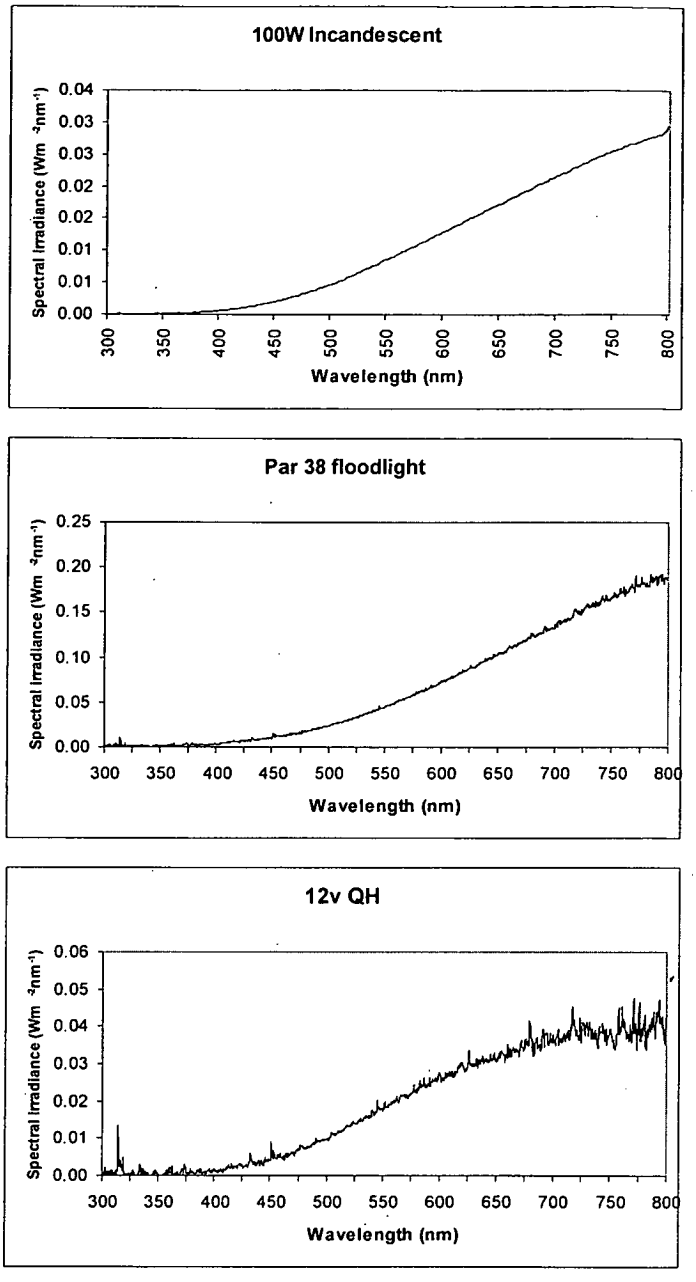


Figure 6.2. Spectral distribution of incandescent sources- 100W pearl incandescent globes, PAR38 floodlamp, and 12 volt quartz halogen. Full details are in Materials and Methods.

6.3.7 HID Lamps

High intensity discharge (HID) lamps are used in growth chambers when higher irradiances are required. In the same growth chamber type (Thermoline model 3540), irradiance with the HID lamps were up to 6 x higher than the fluorescent bank of lamps (Table 6.1). Fluence rates up to 600 μmol m^{-2} s^{-1} (PPF) are achievable in these chambers with the HID lamps. The lights are mounted in a separately ventilated light loft to reduce thermal heat load, with a Pilkington (Australia) 6TF 6mm toughened glass barrier. Light banks can be switched separately to control light source (eg HID

with or without INC) and intensity (eg half on, all on). Irradiance can also be altered by adjustable height growing racks to move plants closer or further from the light sources. These growth chambers also have a removable fluorescent/ incandescent rack. This allowed for the same growth cabinet type to be used for the growth response studies, thus minimising the possible influence of other sources of variation, such as air exchange and velocity.

The commonly used HID sources are metal halide (MH) and high pressure sodium (HPS). Spectral properties of HPS sources were described in Chapter 4 (supplementary light), but are also included here for comparative purposes (Fig. 6.3).

Relative spectral distribution and wavelength ratios of the light sources as a percentage of total fluence (300-800 nm) are shown in Table 6.3. Fluorescent lights and sunlight figures are included for comparative purposes. It can be seen from these tables and figures that metal halide is a relatively broad spectrum light source, with high blue, green and red proportions (Table 6.3). Unlike all the other light sources, UV component (300-400 nm) is similar to sunlight. Blue component is higher than sunlight, hence blue ratios are well above natural light levels (Table 6.3). Far-red proportion is still low relative to sunlight, hence R:FR is high at over 2 (Table 6.3). However, far-red component is higher than all of the artificial light sources tested, apart from incandescent (Table 6.2).

HPS lamps have a low UV with high green/yellow and red components. Blue proportion is very low, only 5.3% compared to 23.6% for MH and 16-20% for sunlight and CWF (Table 6.3). Far-red proportion is very low, hence R:FR is very high. Mixed MH/HPS (2:1 ratio) produces blue proportions, and B:R ratio, similar to sunlight, but green/yellow proportions remain high, and far-red very low compared to sunlight (Table 6.3).

All of these HID sources tested were plant specific products, but far-red content is still very low, and most of the output is in the human photopic peak of 500-600 nm. As all MH lamps provide high blue proportion and even plant specific HPS have low blue proportion, in a mix of the two lamp types it may be unnecessary to use plant specific lamps. Thus the spectral properties of non-plant specific lamps in the same ratio (2 x 400W MH to 1 x 400W HPS) were tested. Lamps used were Thorn (India) MH 400W/C/U Metal Halide and Thorn (Romania) HPS 400W SON-E GES Elliptical, both non-plant specific. However, analysis of wavelength proportions

(Table 6.3) shows the non plant specific mix does have slightly lower blue and far-red proportions compared to the plant specific MH/HPS mix.

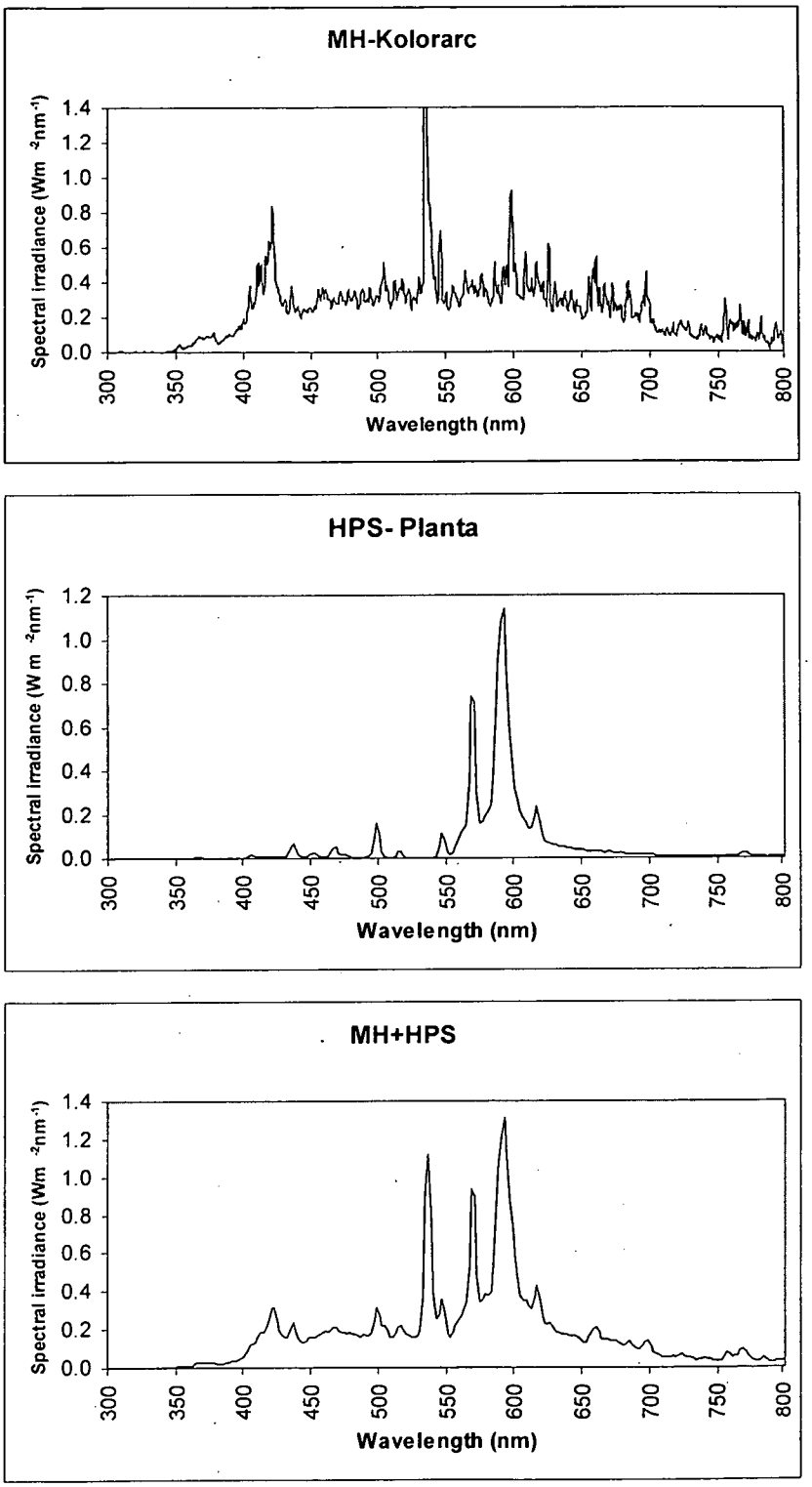


Figure 6.3. Spectral distribution of GE Kolorarc MBID 400/T/H metal halide lamps, Vialox Planta T400W HPS lamps, and a mix of these two sources in a 2:1 ratio (MH/HPS). Full details are in Materials and Methods.

Table 6.3. Relative spectral distribution as the percentage of total irradiance (300-800 nm) and wavelength ratios of high intensity discharge (HID) lamps compared to sunlight and cool white fluorescent (CWF). Light sources were: metal halide (MH)- GE Kolorarc; high pressure sodium (HPS)- Osram Planta; mixed (MH+HPS) - 2x Kolorarc to 1 x Planta; mixed non-plant specific (MH+HPS*) Thorn MH 400W/C/U Metal Halide and Thorn HPS 400W SON-E GES Elliptical: CWF- Sylvania 36W/840. All lights were measured in Thermoline model 3540 growth chambers through 6mm toughened glass barrier with external light excluded. The same light sources and chambers were used in the growth response studies.

Light source		Wavelength proportion (% total irradiance)					Wavelength ratios			
	PPF	300-400 UV	400-500 Blue	500-600 Green	600-700 Red	700-800 Far-red	R:FR (n)	R:FR (b)	B:R	B:FR
HID										
MH	84.2	2.0	23.6	36.1	24.5	10.2	2.4	2.4	1.0	2.3
HPS	96.5	0.2	5.3	68.1	23.1	3.3	3.5	7.1	0.2	1.6
MH+HPS	91.4	0.9	16.5	49.6	25.3	7.7	3.3	3.3	0.7	2.2
MH+HPS*	95.4	1	12.0	58.0	25.3	3.6	3.4	7	0.5	3.3
CWF										
36W/840	95.3	0.6	19.4	41.0	35.0	4.0	2.2	8.7	0.55	4.8
Natural light										
Sunlight	69.4	2.4	15.5	25.0	29.0	28.0	1.1	1	0.5	0.6

6.3.8 Growth responses under HID lamps

The high R:FR of HID and fluorescent lamps could result in delayed flowering and reduced internode extension (Runkle 2004). Previous studies have also suggested the low blue levels in HPS can induce shoot elongation, while the relatively high blue levels in MH can inhibit elongation and produce more compact growth (Sager *et al.* 1982; Tibbitts *et al.* 1983; Zheng *et al.* 2005). To test the response to light source, a photoperiodic pea line was grown under both HID and CWF at equal irradiance (220 $\mu mol\ m^{-2}\ s^{-1}$), photoperiod (18 h) and temperature ($20^0 \pm 0.2^0C$) in the same model growth chambers (Thermoline model 3540). Thus the major difference was the light source.

Results did vary with light source (Table 6.4), but growth responses under CWF, MH, and mixed MH/HPS were quite consistent. Shoot length measurements were similar under CWF, MH and mixed MH/HPS (Table 6.4). However, internode length was significantly longer under HPS compared to the other sources, flowering node and flowering time was delayed, and dry weight was significantly higher (Table 6.4). Under MH, plants flowered significantly earlier (FT) at a lower node (NFI, Table 6.4) than the other light sources. Leaflets were significantly smaller (LW, Table 6.4) and dry weight was significantly reduced relative to the other sources (Table 6.4). Yield (seed and pods, Table 6.4) was similar for all the sources; although pod number was higher under MH, total seed number was not (i.e. under MH plants produced more pods but with fewer seeds per pod). Thus under CWF and mixed MH/HPS, growth responses were similar at equal temperature and irradiance. However, plants under MH showed earlier flowering, reduced leaf size and dry weight. Under HPS, plants were significantly longer with delayed flowering relative to the other sources.

Table 6.4. Mean growth parameters (± SE, n = 20) for L107 peas grown in Thermoline model 3540 growth chambers under cool white fluorescent (CWF) and high intensity discharge (HID) lamps at equal irradiance (220 µmoles $m^{-2}s^{-1}$), photoperiod (18h) and temperature (20^{0}C) Light sources were: CWF- Sylvania 36W/840; metal halide (MH)- GE Kolorarc; high pressure sodium (HPS)- Osram Planta; mixed (MH+HPS) - 2x Kolorarc to 1 x Planta. Full details are in Materials and Methods. Different letters signify differences significant at $P<0.05$, * significant at $P<0.01$.

	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
CWF	36.4 ± 0.58 a	131.5 ± 3.23 a	23.3 ± 0.33 a	18.1 ± 0.18 a	35.4 ± 0.44 a	19.9 ± 1.71 a	7.8 ± 0.70 a	3.0 ± 0.05 a	35.0 ± 1.02 a	46.3 ± 1.02 a	5.7 ± 0.16 a
MH	36.6 ± 0.37 a	133.9 ± 2.36 a	23.0 ± 0.23 a	16.8 ± 0.12 b*	34.3 ± 0.25 b	20.0 ± 0.94 a	9.5 ± 0.46 b*	3.0 ± 0.07 a	33.1 ± 0.44 b*	46.1 ± 0.67 a	5.4 ± 0.19 b
HPS	53.7 ± 0.41 b*	150.6 ± 2.76 b*	22.5 ± 0.26 a	18.8 ± 0.10 c	37.7 ± 0.18 c*	21.1 ± 1.17 a	6.2 ± 0.42 c	3.1 ± 0.06 a	37.0 ± 0.63 a	47.8 ± 0.78 a	6.6 ± 0.13 c*
MH/HPS	36.2 ± 0.41 a	129.1 ± 2.27 a	23.1 ± 0.23 a	18.5 ± 0.16 a	35.4 ± 0.38 a	21.1 ± 1.09 a	6.6 ± 0.36 c	3.1 ± 0.07 a	36.2 ± 0.62 a	50.7 ± 0.86 b	5.7 ± 0.13 a

6.3.9 High irradiance influences, barrier types and water filters

Significantly higher irradiance is achievable with HID lamps compared to fluorescent sources in the Thermoline model chambers (Table 6.1). However, HID sources increase thermal load on the plants, particularly at high irradiance (McCree 1984) unless managed with the use of effective light loft barriers (Bubenheim *et al.* 1988). A 4 cm water barrier is reported to be an effective filter of up to 50% radiant heat – i.e. the infra- red and above range, but not reduce the PAR range (Bubenheim *et al.* 1988). Shallower depths are less effective, increasing depth a further 20cm only provides an additional 2% radiant reduction (Bubenheim *et al.* 1988).

Thermal load and growth responses of L107 pea were measured under mixed MH/HPS in the Thermoline chambers, with a single glass barrier, and in locally constructed chambers (Tri-Tec Ltd) specifically designed to operate at high irradiance. These latter chambers have a double glass barrier and 4cm removable water barrier filters to further reduce lamp thermal load. This allowed for examination of barrier type on thermal load, spectral properties and irradiance, and on growth responses. L107 was shown to be sensitive to moderate temperature increases (Chapter 2), with reduced shoot length, yield and flowering time (Table 2.17).

Plants were grown at an air temperature of 20^0C under an 18 h photoperiod at 425 μmol m^{-2} s^{-1} PPF.

6.3.9.1 Light measurements

Lamps used were Thorn (India) MH 400W/C/U Metal Halide and Thorn (Romania) HPS 400W SON-E GES Elliptical in a ratio of 2 MH lamps to 1 HPS lamp. Measurements with and without the water filter were taken 1 metre from the light barriers at 20^0 C.

Spectral distribution of the light mix with and without the water barrier filter is shown in Fig. 6.4. There is a reduction in UV wavelengths below 380 nm, and a slight reduction in the far-red waveband above 720 nm with the water filter in place, otherwise there is little difference in the spectral distribution with or without the water filter. The LI-1800 spectroradiometer does not measure beyond 850 nm, so it is difficult to assess the impact on infra red and beyond wavelengths (i.e. the thermal load); however, low temperature performance of the cabinets is significantly improved by addition of the water filter. A comprehensive analysis of global radiation

characteristics of water barrier filters using similar light sources is provided by Bubenheim *et al.* (1988).

Relative spectral distribution of the light mix with and without the water filter confirms little alteration in spectral properties from the addition of a water filter (Fig. 6.5). Irradiance was not reduced in the PPF range by the water filter. For example, measurements at 1 m from the lights were 485 μmol $m^{-2}s^{-1}$ without and 484 μmol $m^{-2}s^{-1}$ with the 4 cm filter.

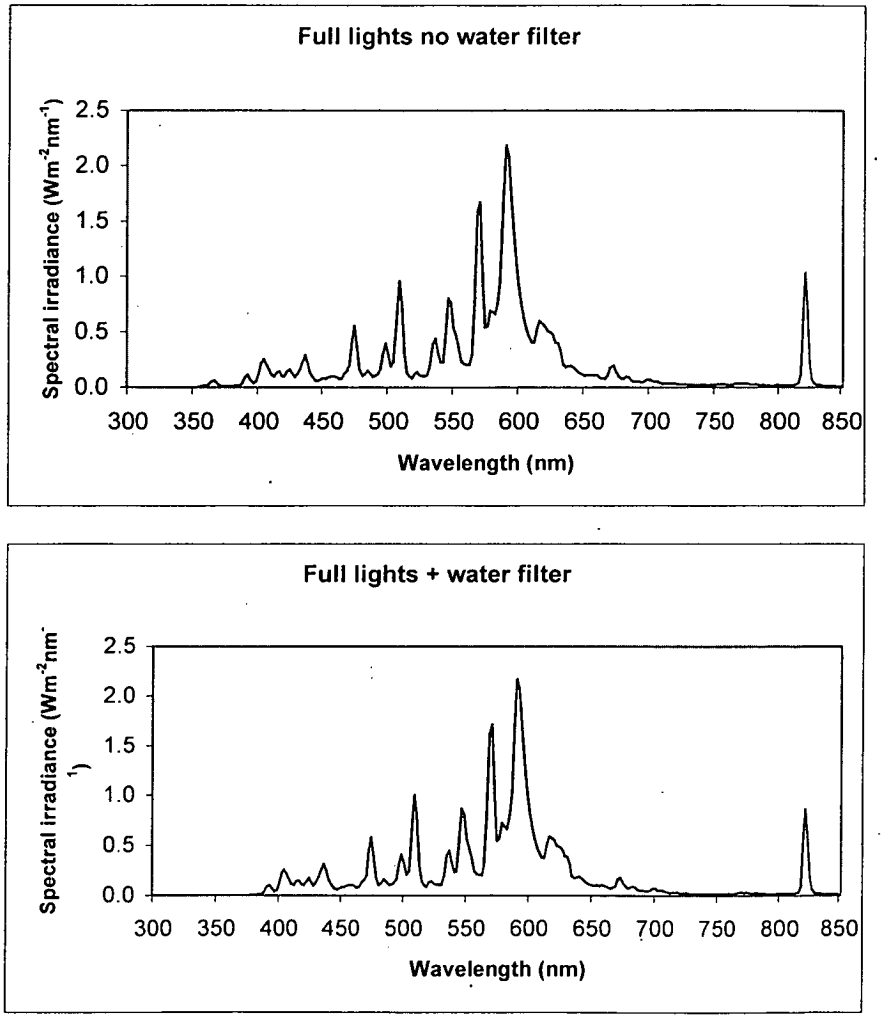


Figure 6.4. Spectral distribution of Thorn (India) MH 400W/C/U Metal Halide and Thorn (Romania) HPS 400W SON-E GES Elliptical in a 2:1 ratio measured through double layer toughened glass (top), and as above with a 4cm depth water barrier filter (bottom).

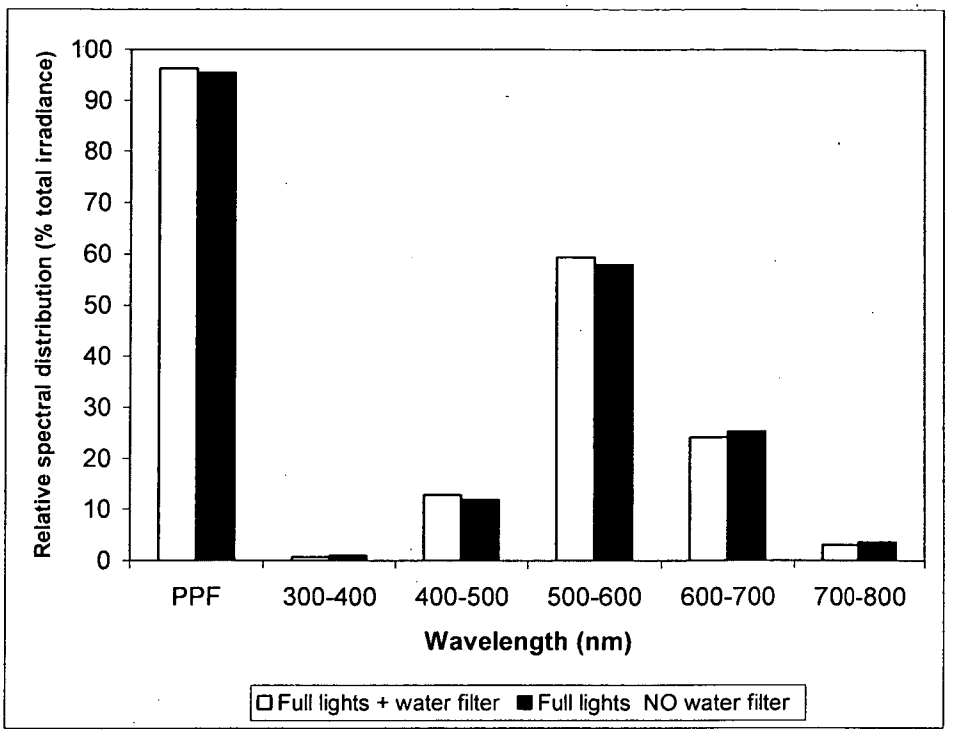


Figure 6.5. Relative spectral distribution of Thorn (India) MH 400W/C/U Metal Halide and Thorn (Romania) HPS 400W SON-E GES Elliptical through double layer toughened glass, with and without 4cm water barrier filter, as a percentage of total irradiance (300-800nm).

6.3.9.2 Radiant temperature measurements

Radiant temperature was measured in the chambers with and without the 4 cm depth water filter during the growth response studies. Irradiance at plant level was 425 μmol $m^{-2}s^{-1}$; air temperature in both chambers was $20^0 \pm 0.5^0$C. With lights off, radiant temperature was close to air temperature (Fig. 6.6). With lights on, radiant temperature in both chambers was above air temperature in both cases (Fig. 6.6). With the water barrier in place, radiant temperature was 1-2.5^{0}C above air temperature; through the double glass barrier alone, temperatures were 2-4^{0}C above air temperature (Fig. 6.6). In the Thermoline chambers, with a single glass barrier, radiant temperatures were consistently 4-5^{0}C above air temperature at this irradiance (Figure 6.6).

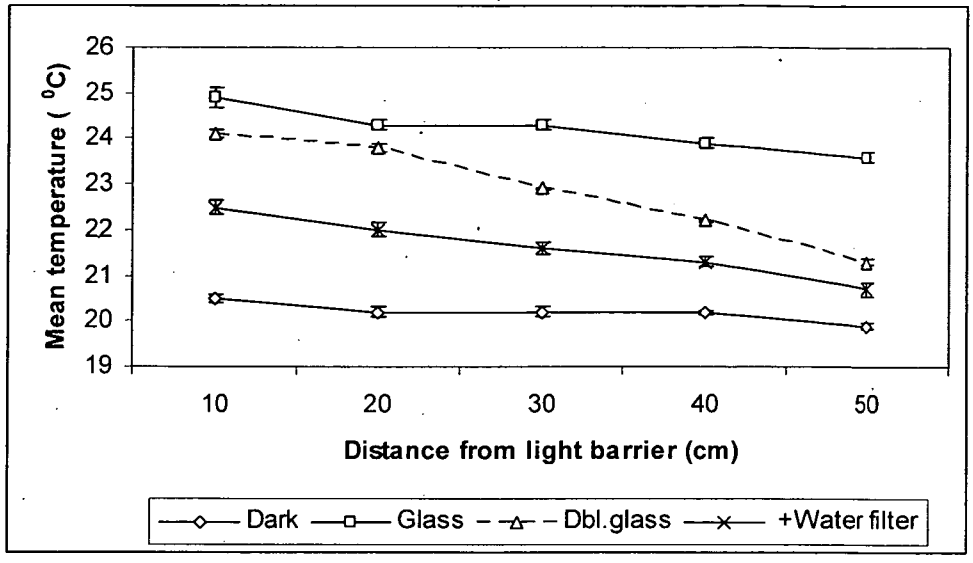


Figure 6.6. Mean (± SE) radiant temperature measurements inside growth chambers with separately ventilated light lofts with single glass (Glass), double glass (Dbl. glass) and double glass plus a 4 cm depth water filter (+ Water filter). Air temperature in all cases was 20^0 ± 0.2^0C; distance is cm from the barrier. Measurements were taken with lights off (dark) and with lights on, PPF 425 µmol m^{-2} s^{-1}.

6.3.9.3 Growth responses

Compared to plants grown at 220 µmol $m^{-2}s^{-1}$ (Table 6.5), plants grown at higher irradiance under single glass (425 µmol $m^{-2}s^{-1}$) had significantly reduced shoot length, yield, leaf size and dry weight. Plants were more compact with smaller leaves and significantly thicker stems at higher irradiance in all categories (Table 6.5). Both increased irradiance and temperature was shown to have these influences on pea (Tables 2.16, 2.17). Under double glass and double glass with a 4 cm water filter, radiant temperature was progressively reduced (Fig. 6.6), and total shoot length was significantly increased relative to single glass at the higher irradiance. However, although seed number was increased under the water filter, other growth parameters were not significantly increased relative to double glass alone. Overall, the results suggest higher irradiance produces more compact plants with smaller leaves, which is slightly offset by reducing thermal load. Higher radiant temperature reduced seed number, but the growth improvements from further radiant filtering by the use of a 4 cm water filter were slight compared to a double glass barrier alone.

Table 6.5. Mean growth parameters (± SE, n = 20) for L107 peas grown in growth chambers under mixed metal halide and high pressure sodium lighting at equal irradiance (425 µmoles $m^{-2}s^{-1}$), photoperiod (18h) and temperature (20^0C) Full details are in Materials and Methods. Different letters signify differences significant at $P<0.05$, * significant at $P<0.01$.

	L1-9(cm)	TL(cm)	TN	NFI	FT(days)	Seed	Pods	Stem(mm)	LW(mm)	LL(mm)	Dry W (g)
Single glass	22.5 ± 0.36 a	93.7 ± 1.62 a*	22.8 ± 0.40 a	18.2 ± 0.18 a	35.7 ± 0.40 a	17.2 ± 2.13 a	6.2 ± 0.53 a	3.6 ± 0.08 a	27.8 ± 0.59 a	42.3 ± 0.97 a	4.7 ± 0.36 a
Dbl glass	22.7 ± 0.22 a	99.7 ± 1.67 b*	22.6 ± 0.27 a	17.9 ± 0.15 a	34.8 ± 0.46 a	16.9 ± 1.10 a	6.2 ± 0.51 a	3.5 ± 0.07 a	27.7 ± 0.39 a	42.7 ± 1.00 a	5.3 ± 0.32 b
Dbl glass + water filter	23.0 ± 0.29 a	103.0 ± 2.03 b*	23.6 ± 0.41 a	17.9 ± 0.15 a	36.5 ± 0.16 a	21.0 ± 2.01 b	7.1 ± 0.64 a	4.2 ± 0.10 b*	29.1 ± 0.74 b	43.2 ± 0.73 a	5.4 ± 0.30 b
Single glass 220 µmol $m^{-2}s^{-1}$	36.2 ± 0.41 b*	129.1 ± 2.27 c*	23.1 ± 0.23 a	18.5 ± 0.16 a	35.4 ± 0.38 a	21.1 ± 1.09 b	6.6 ± 0.36 a	3.1 ± 0.07 c*	36.2 ± 0.62 c*	50.7 ± 0.86 c*	5.7 ± 0.13 c

6.4 Discussion

Growth chambers allow for accurate environmental control over light and temperature regardless of season. Hence they are favoured to allow for reproducible experimental conditions. A range of growth chamber types were tested and all had relatively low and even air velocity, and humidity, with no evidence of CO_2 limitation. However, the light sources used have very different spectral properties to sunlight, and to each other.

The commonly used light source in growth chambers is cool white fluorescent (CWF), which offer relatively broad spectral output and low heat output. Many brands and types are available, including tri and quadphosphor tubes with claimed higher output. Of the CWF tubes tested, spectral distribution was similar (Fig. 6.1, Table 6.2); altered growth effects from substituting either the NEC 37W or triphosphor tubes for the standard 40W or 36W cool white tubes on this basis would be unlikely. On a proportional basis, CWF have similar blue and red levels to natural light, but high output in the 500-600nm range. R:FR ratios are well above sunlight levels, reflecting the very low far-red component of CWF. Ratios between tubes were similar. Output in the 600-700 nm band was higher for the tri and quadphosphor tubes (36W840) and lower in the green 500-600nm band. The higher red component of these tubes (Table 6.2) should also be advantageous for plant growth, as should the high blue and red components of Gro-Lux tubes. However, growth response studies did not show any significant differences for L107 peas under any of these sources at equal irradiance and temperature, including Gro-Lux tubes. Previous studies have shown no growth advantage from these plant specific tubes due to the lower output (Sager and McFarlane 1997, Sager *et al.* 1988). In this study, even at equal irradiance, growth of pea was not improved by Gro-Lux tubes relative to the considerably less expensive CWF tubes tested.

Both the NEC and tri phosphor tubes (36W840) had a higher total fluence (15% and 17.5% respectively) than the other CWF tubes, which can be significant where light may be limiting such as in a growth cabinet under short day conditions. Recently released quadphosphor CWF produced a further 15% increase in irradiance (i.e. more than 30% higher output in the PPF range than regular CWF of equal wattage). Thus while wavelength differences between CWF tubes were relatively unimportant in terms of growth responses, the higher irradiances achievable with tri and quad phosphor tubes are

an advantage when higher fluence rates are required. Using standard CWF it was difficult to maintain the identified peak irradiance of 14 mol m^{-2} d^{-1} for peas (plants had to be very close to the canopy), while with the tri and quad phosphor tubes, this irradiance was easily achieved. Under short day conditions, the higher irradiance achievable becomes particularly important if adequate daily light integrals for normal growth are to be achieved.

When higher irradiances are required, high intensity discharge (HID) lamps are often used in growth cabinets. For example, in the same growth chamber type (Thermoline model 3540), irradiance with the HID lamps was up to 6 x higher than the fluorescent bank of lamps (Table 6.1). Such lamps have a high thermal component, however, and need to be separated from the growing area by separately ventilated light lofts to manage thermal load. However, high irradiance fluorescent growth cabinets are available, using electronic ballasts and VHO and/or T5 fluorescents irradiances up to 1000 µmol m^{-2} s^{-1} are claimed at 15cm from the lights. Tests on a Conviron model E15 were conducted; irradiance averaged 875 µmol m^{-2} s^{-1} at this distance.

Of the HID lamps in common use, metal halide (MH) provides the closest approximation to sunlight where high PPF levels are required, but the spectral properties remain very different to sunlight. R: FR and blue proportions are very high relative to natural light. High pressure sodium (HPS) have a high green and red component, and red wavelengths are the most effective for photosynthesis (Sager *et al.* 1982), but blue content is very low. The MH+HPS mix (2:1 ratio) reduces the blue proportion to close to sunlight levels, but R: FR remains very high relative to natural light (Table 6.3).

When grown at equal temperature and irradiance, plants under CWF and mixed MH/HPS, had similar growth responses. However, plants under MH showed earlier flowering, reduced leaf size and dry weight. Under HPS, plants were significantly longer with delayed flowering relative to the other sources (Table 6.4). Thus at equal irradiance and temperature in the same growth chamber type, growth results for L107 pea varied with light source. Under MH, which has the highest blue proportion, flowering was significantly earlier than under the other light sources, while under HPS, which has a low blue proportion, flowering was delayed and shoot length increased relative to the other sources. All of the light sources have low far-red content, hence high R: FR ratios, and

flowering was delayed relative to natural long day conditions (Table 2.13, around node 16 for L107). Flowering node was found to be relatively stable across light quantity and temperature variations (Chapter 2), and a good indicator of light quality. Thus delayed flowering node under the high R:FR of artificial light sources was expected. However, flowering node under MH (node 16.8) was significantly earlier than the other sources even though R:FR was high, and less than one node later than under natural light. These results suggest that light quality influences shoot length and flowering node, but overall growth (number of nodes, yield, stem diameter, leaf size and dry weight) were relatively uninfluenced by light quality.

It could be expected that sources rich in blue and red would produce improved growth, as these wavelengths are the most efficient for photosynthesis, while light in the 500-600nm band is less efficient (Sager *et al.* 1982). Artificial light sources are primarily developed for luminous intensity (i.e. for human visibility), which is maximal in the green waveband (Canham 1966). Hence most artificial light sources have peak output in this 500-600nm band, as this corresponds with the human eye peak photopic response (Ryer 1997), and even plant specific HID products tested here retain peak output in this band (Table 3.3). However, under Gro-Lux tubes, which would appear to have the ideal profile for plant growth with high blue and red and low yellow-green proportions, growth was not improved relative to CWF. This confirms previous studies that did not find growth improvements under these lights. Under MH, which is rich in blue, growth was reduced relative to CWF, and under HPS, which are high in red wavelengths, shoot length was increased (possibly from the low blue component) but yield and leaf size were not significantly increased. While light in the vicinity of the absorption peaks of chlorophylls a and b (662 and 642 nm) is the most efficient (Tamulaitis *et al.* 2005), the carotenoids act in a light harvesting capacity for photo systems 1 and 2 contributing to the high quantum efficiency of photosynthesis over a wide spectral range (Barber *et al.* 1981). Even under near monochromatic yellow low pressure sodium lamps, tested plants grew adequately (Cathey and Campbell 1977). There has been little study on the influence of green wavelengths on growth, but addition of 24% green to red and blue LED improved lettuce growth and colour (Kim *et al.* 2004). Thus it would appear that for general growth

what is required is sufficient light in the PPF range of 400-700nm, but sources low in blue can result in increased shoot length and further delays to flowering.

Under mixed MH/HPS at higher irradiance (425 μmol m^{-2} s^{-1}) plants were more compact with thicker stems and smaller leaves. Radiant temperature was appreciably increased relative to air temperature by HID lighting, even with the lights separated from the growth area with ventilated light lofts. With a single glass barrier, radiant temperature was 4-5^{0}C higher than air temperature, with double glass 2-4^{0}C higher, and with the addition of a 4 cm depth water filter, 1-2.5^{0}C higher. Thus further separation of the light loft by double glass and/or water filters reduced thermal load in the growth chambers. The observed shoot length reduction in the plants was slightly offset by reducing thermal load by the use of a double glass barrier and/or a 4 cm depth water filter. Higher radiant temperature reduced seed number, but the growth improvements from further radiant filtering by the use of a water filter were slight compared to a double glass barrier alone. Thus as for the irradiance and temperature studies under CWF (Table 2.17), high irradiance and temperature reduces shoot length and leaf size while high temperature reduces yield.

Chapter 7 Red to far-red ratio correction in growth chambers

Note: The contents of this chapter have largely been published (Cummings I, Reid JB, Koutoulis A (2007) Red: Far-Red Ratio Correction in Plant Growth Chambers - Growth Responses and Influence of Thermal Load on Garden Pea. Physiologia Plantarum 131, 171-179).

7.1 Introduction

For long day (LD) plants a red to far red ratio (R:FR) close to the natural level of around 1 is considered the most effective at flower induction (Vince-Prue 1981). Most growth chamber light sources have high R:FR (greater than 2), which can produce responses different from those observed in the natural environment, including delayed flowering in photoperiodic sensitive species, and inhibited internode extension (Runkle 2004; Runkle and Heins 2001; Whitman *et al.* 1998). For example, as R:FR increased (i.e. relatively more R) shoot extension progressively decreased and flowering was inhibited in five LD species tested (Runkle 2004). Hence far red rich incandescent lamps are frequently added to the light mix in growth chambers to reduce R:FR, but usually in insufficient quantities to correct the far-red deficit (Smith 1994). The high thermal component of incandescent sources can lead to heat load problems from adding sufficient FR (Smith 1994). To reduce the influence of heat from lamps, many growth chambers have a separately ventilated light loft with a glass or plexiglass barrier (Cathey and Campbell 1977). However, radiant heat load can still be significant even with a barrier (Bubenheim *et al.* 1988; Hamasaki and Okada 2000). McCree (1984) examined radiation from high intensity discharge (HID) lamps and found high irradiance can be accompanied by an abnormally high thermal radiation load on plants. Near infra red radiation is largely transmitted or reflected from leaves, but incandescent and HID lamps in particular emit large quantities of far infrared radiation (Bubenheim *et al.* 1988; Faust and Heins 1997; Hicklenton and Heins 1997; McCree 1984). Thus plants in growth chambers can be subject to far greater thermal loads than in the natural environment (Hicklenton and Heins 1997), 5-10 times larger than on a sunny outdoor day (Hamasaki and Okada 2000). Absorbed radiation increases plant temperature, particularly at the shoot tip, unless

removed by transpiration, emission or convection (Faust and Heins 1997). While air temperature in growth chambers is generally closely monitored and controlled, giving the user confidence in their accuracy, the thermal load from lamps may be an un-monitored influence on results. The common practice of adding incandescent lamps to the light mix to increase the far-red component may not reduce R:FR meaningfully in the proportions provided, but may well add to thermal load.

This section examines the spectral properties of common growth chamber light sources, with and without incandescent lamps, and examines the growth responses of a photoperiodic garden pea line (*Pisum sativum* L. cv. Torsdag) under these sources. The shoot length and flowering response of this pea line to a R:FR gradient was examined. Commonly used growth chamber light sources (fluorescent, metal halide and high pressure sodium) were measured with and without incandescent in the same brand and model of growth chambers, allowing a comparative analysis of the spectral properties. An analysis of radiant temperature under these light sources in the cabinets is also presented. Growth responses of pea were examined under the light sources, focusing on the influence of adding incandescent lamps. This suggested thermal load to be a major influence with HID lamps and supplementary incandescent sources. Hence the influence of increased temperature on pea was examined. An alternative low heat method of correcting for far red deficiency using light emitting diodes (LED) was examined.

7.2 Materials and methods

7.2.1 Light measurements

To ensure the light source comparisons were at equal irradiance, all light measurements were taken in controlled environment chambers with external light excluded at an air temperature of 20^0 C using a LI1800 spectroradiometer (LI-COR, Inc., Lincoln, NB, USA) with a cosine corrected sensor. Spectral irradiance was downloaded in W m^{-2} nm^{-1} and as quantum intergrade (μmol m^{-2} s^{-1}) averaged over 3 scans in the range 300-800nm, following measurement and reporting guidelines (Bjorn and Vogelmann 1994; Sager *et al.* 1982). Selected measurements were also taken with an Apogee UV-PAR spectroradiometer (Apogee Instuments Inc., Logan, Utah, USA) to check for accuracy. Agreement between instruments was generally within 1%. Figures and spectral

distributions were checked against data from the manufacturers, lists of spectral data (Deitzer 1994; Deitzer 2005) and other published data for light source comparisons. For comparisons of waveband proportions of a light source and/or between light sources at different irradiances, the percentage of quantum intergrade (300-800 nm) was calculated for PPF (photosynthetic photon flux, 400-700 nm) and for each 100nm band. Ratio calculations follow published methods (Kittas *et al.* 1999): R:FR as narrow band (R:FR n) 655-665 / 725-735 nm; broad band (R:FR b) 600-700 / 700-800 nm. Figures quoted for R:FR are broad band unless otherwise stated.

Light source details are as given by the manufacturer. As fluence rate can decrease with tube or globe age, particularly in the first 100 h, all measurements were taken on sources that had burned for 100 h or more. Light sources were measured a minimum of 1 h after start up.

7.2.2 Growth chambers and light sources

Growth chambers used were Thermoline (Sydney, NSW, Australia) model 3540 with externally ventilated light lofts separated from the growing area by Pilkington (Dandenong, Victoria, Australia) 6TF 6 mm toughened glass barriers. These reach-in chambers can accommodate high intensity discharge (HID) lamps (6 x 400W) and incandescent (5 x 100W) or fluorescent lamps (16 x 36W) and incandescent (4 x 100W). This allowed for study of all the light sources in the same growth chamber type and model.

7.2.3 Temperature measurements

Air temperature measurements in the growth chambers were continuously monitored and controlled by BrainChild (Taipei, Taiwan) BTC 9090 sensors. Air temperature and velocity were checked with a Kane-May Ltd (Welwyn, Herts, UK) KM-4000 thermo-anemometer. Radiant temperature in chambers was measured with a CPS Inc (Hialeah, FL, USA) Tempseeker T200 digital thermometer with silicon temperature sensors, 3 sensors were used per measurement, at 10cm intervals from the light loft barrier. Soil temperature was measured 1 cm below the surface using the probe sensor of this instrument. Results given are means of hourly measurements over three days. Surface and

leaf temperature measurements were taken with a CPS Inc (Hialeah, Florida, USA) infra-red thermometer.

7.2.4 Plant growth and measurements

To compare growth responses under the light sources, peas (a selection of *Pisum sativum* L. cv. Torsdag) were grown in the various environments. This line (Hobart line 107) is a quantitative long day plant (Reid *et al.* 1996). Plants were grown in adjacent pots, 2 per 14 cm slimline pot in a 1:1 (v/v) mix of vermiculite and dolerite chips topped with 2-3 cm of peat-sand potting mixture. All plants were watered as needed and fertilised with nutrient solution weekly consisting of Aquasol (Hortico, Sydney, NSW, Australia), N:P:K 23:4:18 at a rate of 1g l^{-1} plus Iron Chelate (Kendon Chemicals, Sydney, NSW, Australia) at 0.05g l^{-1}. Relative humidity, while not controlled, ranged from 40-65% in all experiments. Twenty plants were sown per treatment. During growth, stem diameter (mid point between nodes 9 and 10), leaf width (LW) and leaf length (LL) of 1 leaflet per plant was measured at node 9 at the commencement of flowering, node of flower initiation (NFI) and days from planting to first open flower (FT) were recorded. At maturity (senescence) length of internodes 1-9 (L1-9), total shoot length (TL), number of nodes (TN); number of seed (Seed) and number of pods (Pods) were measured. Shoot dry weight was measured after air drying of the senesced plants for at least 72 h.

Statistical analysis using JMP software (SAS Institute, Cary, NC, USA) included ANOVA , Students t-tests, Dunnetts method, and/or Tukeys test.

7.2.5 Experimental design

For consistency, all measurements and growth response studies were conducted in the same model growth chambers- Thermoline Pty. Ltd., (Sydney, NSW, Australia) model 3540, except for one experiment on temperature responses under natural light conditions. This was conducted in adjacent controlled environment glasshouse cells under an 18 h photoperiod consisting of natural light extended morning and evening by approximately 100 µmol m^{-2} s^{-1} high pressure sodium lamps (GE Lighting, Budapest, Hungary). Mean daily light integral (DLI) was approximately 16 mol m^{-2} d^{-1}. Air temperatures were $20^0 \pm 2^0$ and $25^0 \pm 2^0$C respectively. Response to R:FR was examined in the growth chambers at a constant $20^0 \pm 0.2^0$C under a 24 h photoperiod at a PPF of 150 µmol m^{-2} s^{-1}. Light

sources were: R:FR 8.7- F36W/840 Luxline Plus cool white fluorescent (Sylvania, Munich, Germany); R:FR 6.7- 40SSCW/37-EXP cool white fluorescent (NEC, Tokyo, Japan); R:FR 1.9- 40SSCW/37-EXP cool white fluorescent tubes + 100W pearl incandescent lamps (Thorn, Smithfield, NSW, Australia). All other experiments and measurements were conducted in the growth chambers under an 18 h photoperiod at 220 or 425 μmol m^{-2} s^{-1} PPF, at an air temperature of $20^0 \pm 0.2^0$C. Light sources used were 40SSCW/37-EXP cool white fluorescent; 100W pearl incandescent; Kolorarc MBID 400/T/H metal halide lamps (GE Lighting; Budapest, Hungary) and Vialox Planta T400W high pressure sodium lamps (Osram, Munich, Germany). LED sources were locally constructed 40 cm strips of 120 far red KL450-730GDDH (Shinkoh Electronics, Tokyo, Japan) mounted on velo board 10 mm apart in 3 rows and encased in polycarbonate tubes.

7.3 Results

7.3.1 Spectral distribution

Spectral distributions of cool white fluorescent (CWF), metal halide (MH), high pressure sodium (HPS) and incandescent globes (INC) relative to sunlight are illustrated in Fig. 7.1A. Because the light sources were at different irradiances to sunlight, percentage distribution of each waveband is shown to allow for direct comparison. The very different spectral properties of these common growth chamber light sources can be seen, both in relation to each other and in particular to sunlight. While cool white fluorescent and metal halide are relatively broad spectrum, both have a higher green (500-600 nm) and lower red (600-700 nm) fraction than sunlight. Metal halide also has a higher blue (400-500 nm) proportion. High pressure sodium has very little blue, but a very high proportion of green. All of these sources are deficient in far red (700-800 nm), with broad band R:FR ratios of 6.7, 2.4 and 7.1, respectively, compared to the sunlight ratio measured of 1.1. Incandescent is blue and green deficient with high red and particularly far red proportions, with a R:FR of 0.6. Thus, based on R:FR, cool white fluorescent, metal halide and high pressure sodium should delay flowering in long day (LD) plants and inhibit internode extension, while incandescent would have the opposite effects (Runkle 2004; Runkle and Heins 2001; Whitman *et al.* 1998).

It is clear why incandescent lamps are often mixed with other sources in growth chambers, and most chambers have provision for this. However, the quantities are usually insufficient to correct the far-red deficit (Smith 1994). This is demonstrated in this study (Fig. 7.1B). For cool white fluorescent, R:FR is reduced from 6.7 to 1.6, with metal halide from 2.4 to 2.1 and with mixed metal halide/high pressure sodium from 3.3 to 2.4 with the addition of the full bank of 500W of incandescent in these chambers.

All these R:FR ratios are still above the natural light ratio of 1.1. To achieve a R:FR close to natural light levels, it was necessary to add higher than equal proportions of incandescent (as suggested by Warrington, 1978). For example, 1100W of incandescent to 800W of metal halide produced a R:FR of 1.2 at 220 μmol m^{-2} s^{-1} PPF. Using far- red light emitting diodes (LED) it was possible to achieve the same ratio with a single LED strip (30W) added to 800W of metal halide. The monochromatic nature of this light source compared to incandescent is also clear (Fig. 7.2). Using far-red LED R:FR ratios as low as 0.1 has been measured with cool white fluorescent at 150 μmol $m^{-2}s^{-1}$. By varying the number of LED strips and the power input, R:FR can be manipulated to the desired level with an adequate photosynthetic level of light and without significant thermal load influences.

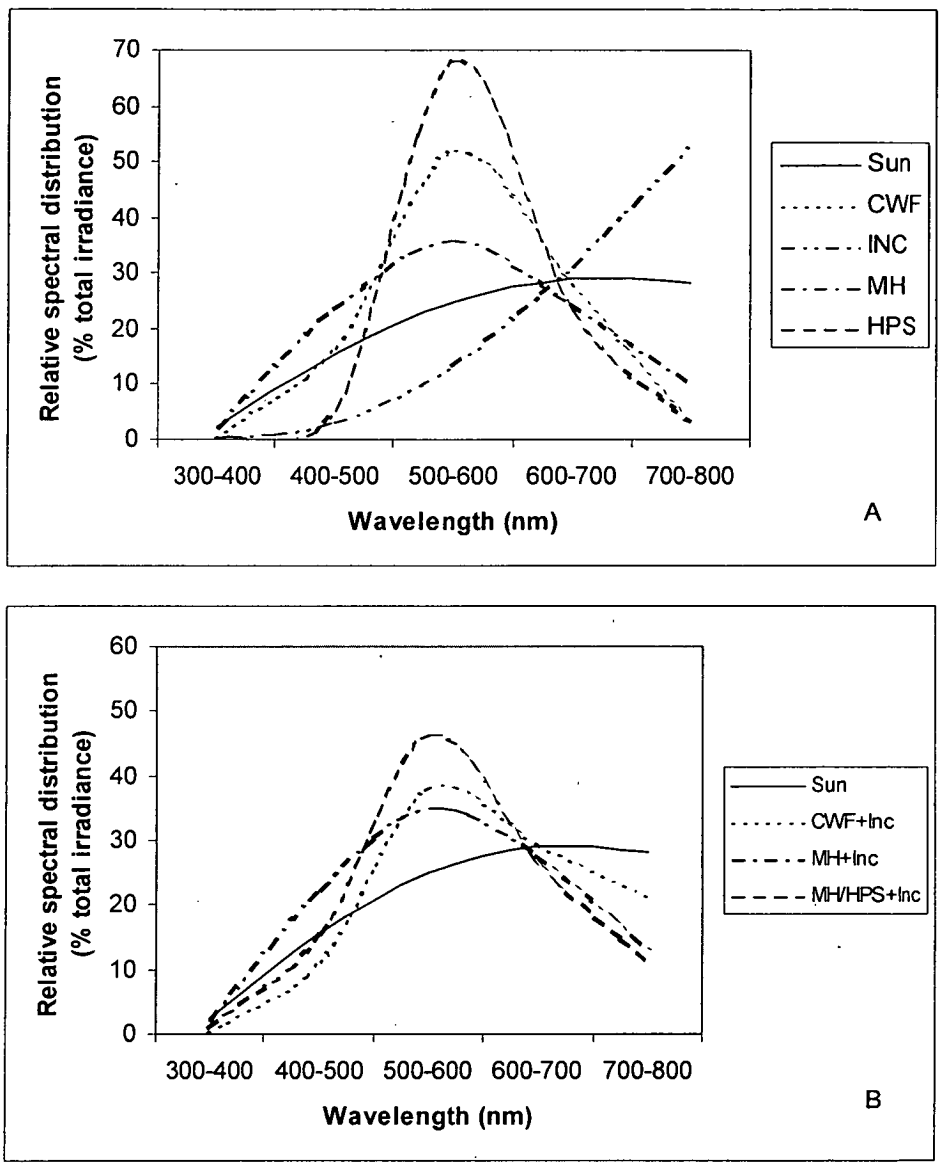


Figure. 7.1. Relative spectral distribution as a percentage of total irradiance over 300-800nm for common growth chamber light sources compared to sunlight. Abbreviations- CWF – cool white fluorescent; INC- incandescent; MH- metal halide; HPS- high pressure sodium; full details in materials and methods. A) measured through the glass barrier in Thermoline growth chambers, R:FR ratios- CWF-6.7, INC- 0.6, MH- 2.4 and HPS-7.1. B) As above, measured in the same chambers with the full bank of incandescent added- 16x 37W CWF + 4 100W Inc, R:FR 1.6; 6x 400W MH + 5 100W Inc, R:FR 2.1; 4 x400W MH + 2 x 400W HPS + 5 100W Inc, R:FR 2.4

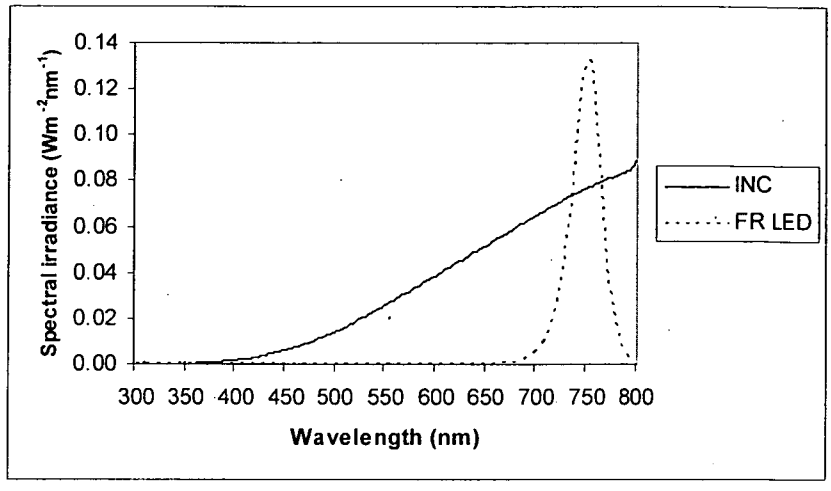


Figure 7.2. Spectral distribution (300-800nm) of 3 x 100W incandescent and approximately 30W (120) far red KL450-730GDDH LED (Shinkoh Electronics, Tokyo, Japan).

The spectral variation between the plants growing in the chambers was also examined, as radiation under canopies consists of unfiltered light that has passed through gaps in the canopy and filtered light modified by absorption, reflection and transmission (Holmes 1981). Natural shade spectrums thus typically have troughs in the blue and red regions due to absorption, a minor peak in green, and a major peak in far red from reflection (Smith and Morgan 1981). The degree of shading corresponds to R:FR; the lower the ratio the greater the degree of shading (Kendrick and Weller 2003a; Smith and Morgan 1981). As growing conditions are often crowded in growth chambers, the possibility of corresponding variations in spectral properties was investigated. Slight reductions were found in blue (400-500nm) and red (600-700nm) regions and increases in green (500-600 nm) and far red (700-800 nm) regions. However, with the white reflective walls of the growth chambers, the within canopy variations were minor, in the order of 1-2%, with R:FR reductions of 0.1-0.5. For example, the highest R:FR reduction was under mixed metal halide, high pressure sodium and incandescent, with a reduction from 2.4 above canopy to 1.9 below. Under cool white fluorescent and incandescent, the reduction in R:FR under the canopy was 0.1.

7.3.2 Thermal load

Radiant temperature measurements were taken in the chambers at 10cm intervals from the light loft barrier at 220 (Fig. 7.3A) and 425 μmol m^{-2} s^{-1} PPF (Fig. 7.3B) during the growth studies. Soil temperature at 1cm depth was also measured. Under cool white fluorescent, temperature variation was $+1^0$C or less. However, adding 400W of incandescent increased the radiant temperature at 10cm from the barrier by 4^0C. By 50cm, the variation was less than $+1^0$C (Fig. 7.3A).

At the same irradiance using high intensity discharge (HID) lamps (metal halide and high pressure sodium) temperature was up to 3^0C higher than cool white fluorescent (Fig. 7.3A). Adding incandescent to the HID lamps did not appreciably increase temperature, but did elevate mean soil temperature by close to 3^0C (Fig. 7.3A). Under HID at 425 μmol $m^{-2}s^{-1}$ (Fig. 7.3B) radiant temperature was consistently 3-4^0C higher than air temperature. With the incandescent bank turned on (500W) and at the same irradiance, temperature variation was 4-5 ^{0}C higher than air temperature, with soil temperature more than 4^0C above air temperature. With incandescent added, R:FR was reduced from 3.3 to 2.4, still well above the natural ratio of 1.1.

Under metal halide, with a R:FR of 1.2 achieved by the addition of sufficient incandescent or far red LED, radiant temperature measurements (Fig. 7.3C) show both chambers were above the air temperature of 20^0C. However, under incandescent radiant temperature was 2-3^0C higher than under far red LED.

Leaf temperatures in the various environments were not significantly different from the air temperature (data not shown), as expected for well watered plants, as transpiration has a cooling effect. However, leaves within 10cm of the barrier under high irradiance and with incandescent added were 1-2^0C above the ambient air temperature. Growth chamber internal surface temperatures were also similarly elevated when incandescent light was added. At the higher irradiance, the under surface of the light loft barrier was well over 40^0C, 7-8^0C higher than without incandescent at the same irradiance.

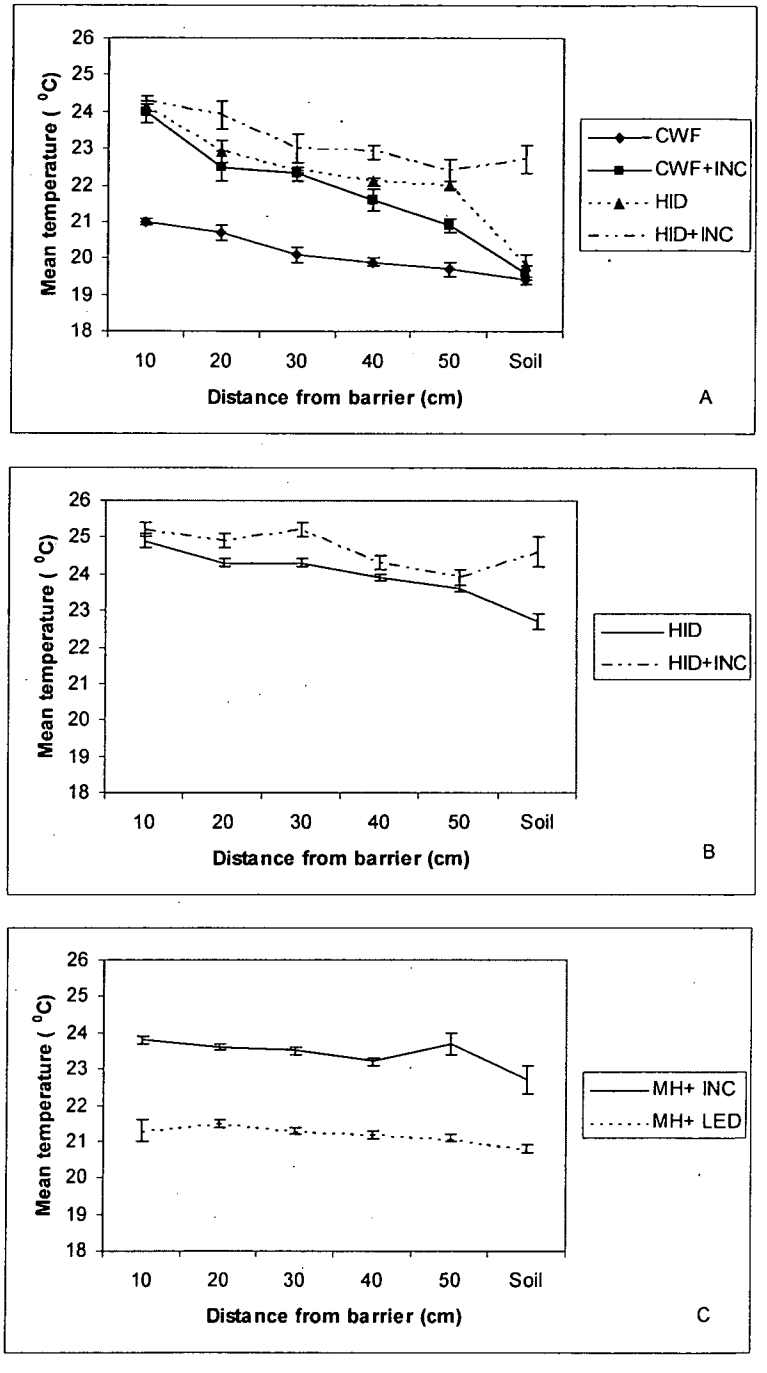


Figure 7.3. Mean (± SE) radiant temperature measurements inside growth chambers with separately ventilated light lofts. Air temperature in all cases was 20± 0.2°C, distance is cm from the barrier, soil temperature was at 1 cm depth. Irradiance (PPF at 50 cm) : (A)- 220, (B)- 425, (C)- 220 μmol $m^{-2}s^{-1}$. (C) was under metal halide with a R:FR of 1.2 achieved by adding incandescent lamps or far red LED. Abbreviations: CWF, cool white fluorescent; INC, incandescent; MH, metal halide; HID, mixed MH and high pressure sodium lamps; LED, far red light emitting diodes; full details in Materials and Methods.

7.3.3 Growth responses

To examine the influence of increasing R:FR, the photoperiodic pea line (L107) was grown under a R:FR gradient generated using cool white fluorescent and incandescent lamps (R:FR 1.9) and different brands of cool white fluorescent (R:FR 6.7 and 8.7). Internode length was significantly ($P \leq 0.01$) inhibited and flowering significantly ($P \leq 0.01$) delayed with increasing R:FR (Fig. 7.4).

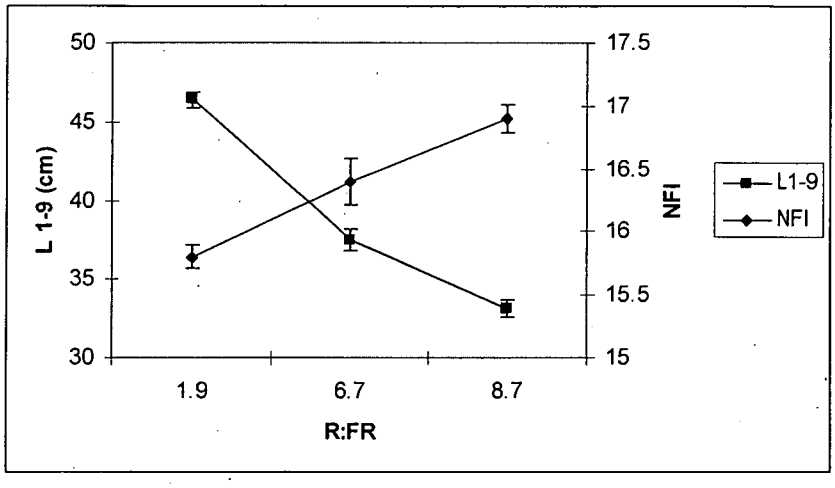


Figure 7.4. Mean node of flower initiation (NFI) ± SE, and shoot length between nodes 1-9 (L1-9) ± SE at different R:FR ratios. Light sources for the ratios: 8.7- F36W/840 Luxline Plus cool white fluorescent (Sylvania, Munich, Germany); 6.7- FL 40SSCW/37-T8 cool white, (NEC, Tokyo, Japan) 1.9- FL 40SSCW/37-T8 cool white + 4x 100Wpearl incandescent bulbs (Thorn, Australia). All plants were grown under a 24h photoperiod, PPF was 150 µmol m^{-2} s^{-1}, temperature was 20 ± 0.2°C, n = 20.

Growth responses under high intensity discharge lamps (mixed metal halide, high pressure sodium) with and without incandescent lamps are shown in Table 7.1. At the same irradiance in the same growth chamber type the plants show significant ($P \leq 0.01$) reductions in shoot length (L1-9, TL), yield (seed, pods), stem diameter and leaflet size (LW, LL) with incandescent added in spite of the R:FR reduction. At 220 µmol m^{-2} s^{-1}, flowering node (node of flower initiation, NFI) was not significantly different with the R:FR reduction from 3.3 to 2.1. At higher irradiance (425 µmol m^{-2} s^{-1}) flowering node was earlier (from 18.2 to 17.5), as was flowering time (FT) with incandescent added.

However, under natural LD light in the glasshouse this pea line flowers at node 16 (mean 16.1 ± 0.12, n=100), so flowering node is still delayed compared to natural conditions.

Table 7.1. Mean (± SE) growth measurements of pea L107 grown in growth chambers with separately ventilated light lofts. Air temperature in all cases was 20 ± 0.2°C, photoperiod 18h. Light source metal halide/high pressure sodium (MH/HPS) in a 2:1 ratio. Irradiance was PPF at 50 cm, 220 µmol m^{-2} s^{-1} and 425 µmol m^{-2} s^{-1} both ± 500W incandescent, R:FR ratios 3.3 (no inc), 2.1 (+inc). Abbreviations- L1-9- length between nodes 1-9; TL- total shoot length; FT- flowering time; NFI- node of flower initiation; LW- leaflet width; LL- leaflet length, full details in materials and methods. Significance **$P \leq 0.01$, *$P \leq 0.05$, ns- not significant, n=20.

	L1-9 (cm)	TL (cm)	FT (days)	NFI	Seed	Pods	Stem (mm)	LW (mm)	LL (mm)
220 -INC	36.2 ± 0.41	129.1 ± 2.27	35.4 ± 0.38	18.5 ± 0.20	21.1 ± 1.09	6.6 ± 0.36	3.1 ± 0.07	36.2 ± 0.62	50.7 ± 0.86
220 + INC	32.9 ± 0.53**	112.4 ± 3.03**	36.6 ± 0.43*	18.3 ± 0.3 ns	15.6 ± 1.37**	4.9 ± 0.47**	2.5 ± 0.08**	30.2 ± 0.93**	40.7 ± 1.34**
	L1-9 (cm)	TL (cm)	FT (days)	NFI	Seed	Pods	Stem (mm)	LW (mm)	LL (mm)
425 - INC	22.5 ± 0.36	93.7 ± 1.62	35.7 ± 0.4	18.2 ± 0.18	17.2 ± 2.13	6.2 ± 0.53	3.6 ± 0.08	27.8 ± 0.59	42.3 ± 0.97
425 + INC	18.6 ± 0.37**	64.8 ± 1.57**	34.0 ± 0.30**	17.5 ± 0.17*	8.0 ± 0.8**	2.9 ± 0.3**	2.5 ± 0.07**	20.3 ± 0.55**	28 ± 0.69**

These responses mimic the results for temperature increase both in the glasshouse and growth chamber environments (Table 2.16). In growth chambers, increasing air temperature from 20 to 25°C at the same irradiance (18 h photoperiod, 220 µmol m^{-2} s^{-1}- 14.4 mol m^{-2} d^{-1}) produced similar significant reductions in shoot length, yield, stem diameter and leaflet size (Table 2.16). Plants also showed significant ($P \leq 0.01$) reductions in shoot dry weight and flowering time at higher temperature, although the flowering node was delayed at the higher temperature. L107 plants grown in adjacent controlled environment glasshouse cells show significantly reduced ($P \leq 0.01$) total length, yield, stem diameter and leaflet size at 25°C compared to 20°C (Table 2.16). Shoot dry weight and flowering time were also significantly ($P \leq 0.01$) reduced, from means of 5.7 to 3.3 g and 40 to 30.8 days respectively. However the flowering node was only slightly delayed. At the higher temperature plants showed faster development, hence

flowering time was significantly earlier, as was harvest time (data not shown). Flowering node, however, appears to be a better physiological indicator of flowering as it is less influenced by temperature, and does reflect R:FR alterations.

To further examine if the influence on growth was from temperature variations under the different light sources or R:FR, plants were grown concurrently in adjacent growth chambers under metal halide with the far-red correction provided by either incandescent or far-red LED to give a R:FR ratio of 1.2. Thus the major difference was radiant temperature (Fig. 7.3C). The results show dramatic differences in shoot length, weight and leaflet size of the plants (Table 7.2).

Table 7.2. Mean (± SE) growth measurements of pea L107 grown in growth chambers with separately ventilated light lofts. Air temperature was 20^0 C ± 0.2^0C, irradiance was 220 µmol m^{-2} s^{-1} PPF at 50 cm, photoperiod 18h. Light sources were metal halide with 1100W incandescent (MH + INC) or 30W far red light emitting diodes (MH + LED), R:FR ratio of both was 1.2. Abbreviations- L1-x- length between nodes 1-x; NFI- node of flower initiation; FW- shoot fresh weight; DW- shoot dry weight; LW- leaflet width; LL- leaflet length, full details in materials and methods. Significance **$P \leq 0.01$, *$P \leq 0.05$, n=20.

R:FR 1.2	L1-4 (cm)	L1-9 (cm)	L1-15 (cm)	NFI	FW (g)	DW (g)	Stem (mm)	LW (mm)	LL (mm)
MH + INC	5.7 ± 0.13	28.9 ± 0.29	68.5 ± 0.59	16.7 ± 0.11	13.3 ± 0.56	3.3 ± 0.08	2.5 ± 0.04	28.2 ± 0.49	41.5 ± 0.63
MH + LED	7.6 ± 0.18**	39.3 ± 0.67**	82.5 ± 1.0**	17.1 ± 0.15*	20.6 ± 1.10**	4.0 ± 0.16**	3.1 ± 0.07**	36.2 ± 0.76**	48.2 ± 0.92**

Under far-red LED, plants were 14 cm longer between nodes 1-15, mean fresh weight was 7.3 g higher, and leaflets were approximately 7 mm longer and wider. The flowering node was only slightly later (by 0.4 nodes). This demonstrates that when the thermal load associated with the incandescent bulbs is removed, the reduced R:FR has the expected impact on shoot length.

7.4 Discussion

The high R:FR ratio of the light sources used in this study (cool white fluorescent, high intensity discharge lamps) delayed flowering and inhibited internode extension in the photoperiodic pea L107. However, adding incandescent to increase the far-red component and to reduce R:FR produced results similar to increasing temperature (Tables 7.1 and 2.16). Shoot length was reduced (Table 7.1), not increased as expected by the lower R:FR

(Runkle 2004), and yield, leaf size and shoot dry weight were significantly reduced. These responses are normally seen in pea as temperature is increased, both in growth chamber and natural light conditions (Table 2.16). Correcting the far-red deficit with far-red LED, a low heat source of far-red light, reduced the radiant load (Fig. 7.3C) and dramatically increased shoot length and leaflet size compared to plants grown with the ratio correction provided by incandescent lighting (Table 7.2). Yield in particular appeared to be closely related to temperature in these results. Cooler temperatures during maturation increase yield in many crop species (Heins *et al.* 2000) and yield and seed weight is negatively correlated with temperature in pea (Chetia and Kumar 2005; Poggio *et al.* 2005).

This pea line does respond to R:FR, although the response is not large (Weller *et al.* 2001). Under cool white fluorescent, reducing R:FR from 6.7 (no incandescent) to 1.9 (with incandescent) significantly ($P \leq 0.01$) increased internode length and reduced the node of flower initiation (NFI) in L107 (Fig. 7.4). Temperature differences were not large under this source (Fig. 7.3A), and adding incandescent was relatively effective at reducing R:FR, although irradiance achievable was relatively low. Under high intensity discharge light sources (metal halide, high pressure sodium), reducing R:FR by adding incandescent reduced internode length and had little influence on NFI (Table 7.1). Thus, if the aim of adding incandescent is to modify shoot length reduction and the delayed flowering resulting from high R:FR, it was not achieved. Increasing light quantity had similar effects under high intensity lamps- reduced shoot length, yield, leaflet size and dry weight (Table 7.1). Again these responses are similar to those observed as temperature is increased (Table 2.16).

While temperature differences were only in the order of 2-3^{0}C in most cases, the differences were sustained over the entire photoperiod of 18h. Within the normal temperature range it is average temperature that influences plant development, not short term fluctuations (Adams 2006; Cockshull *et al.* 2002) and peas are particularly sensitive to water and temperature stress during maturation (Roche *et al.* 1999). Once temperature rises above a species optimum, growth responses can reduce rapidly (Heins *et al.* 2000). While light loft barriers can reduce thermal load by up to 75% they do not completely remove it. The relatively low air speed (0.1-0.4 m s^{-1}) of most chambers can exacerbate

this residual thermal load on plants (McCree 1984). Air velocities measured in the chambers in our study ranged from 0.3-0.6 m s^{-1}. Increasing air velocity decreases the difference between leaf and air temperature, but high velocities can reduce growth from mechanical stress (Salisbury 1979). Higher temperatures can also increase moisture stress within the plant (Ormrod 1978b). Pallas and Michel (1971) examined infrared radiation on leaf temperature and growth on warm and cool weather species, including pea. While leaf temperature was similar due to transpiration, growth of the cool weather species was significantly better in the low radiation chamber, and "strikingly better" for pea (Pallas and Michel 1971). We measured leaf temperature in the various environments and generally did not find significant differences, as expected in well watered plants. However, growth chamber surface temperatures were significantly higher with incandescent added, and at high irradiance the under surface of the light loft barrier was well over 40^0C. Leaves within 20cm of the barrier were 1-2^0C above ambient under the high irradiance sources with or without incandescent added.

Soil temperature can be significantly increased from the radiant load in growth chambers, particularly in individual pots (Hamasaki and Okada 2000). In this study, while air temperatures were often similarly elevated with or without incandescent, soil temperature was always higher (Fig. 7.3). For example, under high intensity discharge lamps at 425 μmol m^{-2} s^{-1}, air temperature was similarly elevated but soil temperature was 2^0C higher with incandescent added (Fig. 7.3B) and significantly reduced growth parameters (Table 7.1). Growth parameters were also significantly reduced with the increase in irradiance from 220 to 425 μmol m^{-2} s^{-1}.

R:FR correction in growth chambers without additional thermal load influences is achievable by using far-red LEDs. Under metal halide, a R:FR of 1.2 was achieved with 1100W of incandescent or approximately 30W of far red LED. Radiant temperature was 2-3^0C less under LED (Fig. 7.3C) and plants were significantly longer with larger leaflets and higher weights (Table 7.3), similar to autumn and spring grown glasshouse plants (Table 2.12). With incandescent added, the plants were more like plants grown in the glasshouse over summer (Table 2.12). Most of the output of incandescent lamps is infrared radiation (McFarlane 1978). The narrow band specific wavelength of LED and low heat output make them useful for plant research and growth (Bula *et al.* 1991). R:FR

ratios as low as 0.1 can be achieved using far-red LED supplementation of photosynthetic levels of fluorescent lighting, a useful feature for studies on canopy shading.

The results suggest that caution should be used when using incandescent lamps in growth chambers and/or high intensity discharge lamps at relatively high irradiance. Although air temperatures were maintained throughout these experiments at 20^0C, radiant temperature was significantly higher up to 50cm from the light loft barriers and strongly influenced results. Addition of incandescent in the normal proportions provided in growth chambers did not correct R:FR sufficiently to mimic natural light (flowering node was still delayed) and increased radiant heat problems (reduced shoot length, yield, leaflet size, shoot dry weight). Higher than equal wattage was required to reproduce sunlight R:FR, which produced greater heat load and hence growth effects. However, supplementary far-red LED can produce sunlight or even deep canopy shade R:FR ratios without the significant growth inhibiting thermal influences.

Chapter 8 The role of blue light

8.1 Introduction

The ratio of red to far-red light (R:FR), which is perceived by the phytochrome photoreceptors, is the most intensively studied of the light quality changes that influence plant growth (Franklin and Whitelam 2005; Rajapakse *et al.* 1999). Due to the absorption of red light by photosynthetic tissue, canopy shade has a higher far-red proportion, and this can stimulate increased internode length, reduced leaf area, increased apical dominance, and accelerated flowering, collectively called shade avoidance (Kendrick and Weller 2003a). In contrast, high R:FR can signal non-competitive conditions and reduce plant height, as well as delay flowering in many species (Runkle and Heins 2001). In addition to low R:FR, canopy shade also has reduced levels of blue light and an overall reduction in light quantity, which can play a role in shade avoidance (Pierik *et al.* 2004), although the role of these factors has received considerably less attention (Christophe *et al.* 2006; Franklin and Whitelam 2005).

In growth chambers the commonly used light sources (fluorescent, metal halide, high pressure sodium) all have a R:FR above 2, well above the natural R:FR of approximately 1, which can reduce shoot length and delay flowering (Cummings *et al.* 2007; Moe and Heins 1990; Runkle 2004). Far-red rich incandescent globes are often added to the light mix in growth chambers, but this is usually in insufficient quantities to reduce R:FR significantly (Cummings *et al.* 2007; Smith 1994). All of these light sources vary not only in R:FR (from sunlight and each other), but also in blue irradiance. Artificial light sources deficient in blue, such as high pressure sodium (HPS) can induce elongation (Tibbitts *et al.* 1983), while sources high in blue, such as metal halide, can reduce height (Zheng *et al.* 2005) even though both sources have high R:FR. Increasing the blue component of HPS by supplementation or increasing irradiance reduced internode length in soybean (Wheeler *et al.* 1991), and blue supplementation of HPS reduced shoot length in cucumber and tomato (Menard *et al.* 2006). However, the role of blue light may be species dependent (Dougher and Bugbee 2001; Hirai *et al.* 2006), and the role of blue

light needs to be examined independently of both R:FR and irradiance alterations, which also influence shoot length (Christophe *et al.* 2006; Gawronska *et al.* 1995).

For horticultural crops, shoot height control through manipulation of wavelength ratios is receiving increasing interest, as it allows manipulation of plant height without long-term breeding programs or the use of chemical growth regulators (Rajapakse *et al.* 1999). Most research has focused on manipulating R:FR, particularly shoot height reduction by filtering far-red light (thus increasing R:FR) with plastic greenhouse films (Cerny *et al.* 2003; Fletcher *et al.* 2005; Li *et al.* 2003; van Haeringen *et al.* 1998). Shoot height reduction through copper sulphate filters has also been attributed to reduced far red (Bachman and McMahon 2006; Rajapakse and Kelly 1992; Rajapakse *et al.* 1999). However, height reduction by increasing R:FR can also have the potentially negative effect of delaying flowering (Runkle and Heins 2002; Runkle and Heins 2003). In contrast, manipulation of blue light to alter plant height has not been widely explored, even though filtering blue light can induce elongation (Maas 1992; Mortensen and Moe 1992) independently of R:FR (Runkle and Heins 2001), and increasing the blue component of white light may be associated with shorter internodes (Thomas 1981).

Photo selective shade cloths have recently been developed with the aim of providing crop protection and manipulation of plant growth (Shahak *et al.* 2004). However, reduced irradiance due to spectrally neutral shading can induce elongation and reduced leaf area (Christophe *et al.* 2006; Dougher and Bugbee 2001; Gawronska *et al.* 1995). Red and blue photo selective shade cloths reportedly enhance or reduce specific wavelengths, particularly in the blue and red regions; while R:FR is largely unaltered (Shahak *et al.* 2004). Blue shade cloth reduced and red shade cloth increased shoot length in *Pittosporum variegatum* (Oren-Shamir *et al.* 2001), chrysanthemum height was reduced under blue shade cloth (Kobayashi 2005) and *Dracaena* height increased under red shade cloth (Kobayashi *et al.* 2006). However, the mechanism of action of these shade cloths has not been fully explored and their potential use to examine photomorphogenic responses has not been described. It is unclear whether the growth responses are to altered red or blue wavelengths, or both.

One way to distinguish this is by examining the role of specific photoreceptors. Phytochromes mediate red and far red and some blue responses, while cryptochromes are

exclusively involved in blue responses (Neff and Chory 1998{Platten, 2005 #184; Platten *et al.* 2005; Weller *et al.* 2001). In pea a range of photoreceptor mutants are available, including a cryptochrome 1 (*cry1*) mutant with specifically altered blue light response (Platten *et al.* 2005). For example, *cry1* mutant seedlings grown under blue light are longer than wild type plants as the CRY1 protein is required to suppress shoot elongation in response to blue light (Platten *et al.*, 2005). As the CRY1 photoreceptor only absorbs light in the blue region of the spectrum (Ahmad *et al.* 2002), a change in the response of *cry1* mutants to red and/or blue shade cloths would indicate that the shade cloths modify plant growth due to changes in the proportion of blue rather than red light.

In this section, the role of blue light is explored using pea growth responses through to maturity. A range of methods were used to specifically examine the role of blue light. Shoot length and flowering in particular were examined under artificial light sources which varied in both R:FR and blue irradiance. The role of blue light was further examined by supplementation experiments under high pressure sodium lamps, which are blue deficient. To examine the role of blue light under natural light conditions, the spectral properties and growth responses under photo selective shade cloths were analysed. This allowed for the examination of the role of blue independently of R:FR, as well as the influence of light quantity. In addition to using a wild-type selection of pea, a mutant deficient in *cryptochrome 1* (*cry1*) was used to determine if changes in blue light were responsible for the differences observed under the shade cloth treatments.

8.2 Materials and methods

8.2.1 Plant material and growth conditions

Pea (*Pisum sativum* L.) lines used were a quantitative long day wild type selection of cv. Torsdag, L107 (Reid *et al.* 1996), and the *cry1* mutant on this genetic background (Platten *et al.* 2005). Plants were sown two per pot using even sized seed in 14 cm slimline pots in a 1:1 mixture of grade three vermiculite (Australian Vermiculite and Perlite Co., Fairfield, Victoria, Australia) and 10 mm dolerite aggregate (HBMI, Kingston, Tasmania, Australia) topped with two cm of a pasteurised 1:1 mix of peat moss (Te - Em, New Brunswick, Canada) and coarse river sand (Island Resources, Scottsdale, Tasmania, Australia) with added macronutrients (Osmocote 18N-2.6P-9.9K , Scotts-Sierra, Marysville, OH, USA) at 1 kg m^{-3} ; pH was adjusted to 7 with dolomite lime and limestone. All plants were watered as needed and fertilised with nutrient solution weekly consisting of Aquasol (Hortico, Sydney, NSW, Australia), N:P:K 23:4:18 at a rate of 1g l^{-1} plus Iron Chelate (Kendon Chemicals, Sydney, NSW, Australia) at 0.05g l^{-1}.

Growth chamber artificial light source studies were conducted in the same model chambers (model 3540, Thermoline, Sydney, Australia,) at equal irradiance (photosynthetic photon flux 220 µmol m^{-2} s^{-1} at the pot surface), photoperiod (18 h) and temperature ($20^0 \pm 0.2^0$C). Light sources used were cool white fluorescent- F36W/840 Luxline Plus (Sylvania, Munich, Germany); incandescent globes- 100W pearl (Thorn, Smithfield, NSW, Australia); metal halide lamps- 400W Kolorarc MBID 400/T/H (GE Lighting, Budapest, Hungary); and high pressure sodium lamps- Vialox Planta T400W (Osram, Munich, Germany).

Blue and white light supplementation of high pressure sodium studies were conducted in locally constructed chambers (Tri-Tec, Hobart, Tasmania, Australia) under an 18h photoperiod and air temperature of $20^0 \pm 0.2^0$C. Plants were grown under 400W SON-E GES Elliptical (Thorn, Bucharest, Romania) high pressure sodium lamps at an irradiance of 100 µmol m^{-2} s^{-1}, which provided a blue proportion of 5 µmol m^{-2} s^{-1} and a R:FR of 7.8. To examine the influence of blue quantity, monochromatic blue was added to this level of HPS by suspending blue fluorescent tubes (TLD 36W/15 Blue, Philips, Eindhoven,

Holland) in the chamber, which created a gradient from 5-70 μmol m^{-2} s^{-1} additional blue. To examine if any observed growth responses were due to blue wavelength or simply increased irradiance, the experiment was repeated with a white fluorescent gradient (F36W/840 Luxline Plus, Sylvania, Munich, Germany) in the range of interest: 20-45 μmol m^{-2} s^{-1}. The additional blue irradiance of the white fluorescent treatment was 4-9 μmol m^{-2} s^{-1}.

Shade treatment plants were grown in a glasshouse, average temperatures for the study period (late spring) were day 23.9°C, night 16.6°C. Photoperiod was 18 h, consisting of natural daylight (average 14 h) extended morning and evening by weak (5 μmol m^{-2} s^{-1}) incandescent lighting. Repeats were conducted in autumn and early spring in the same glasshouse, average temperatures were day 20.1 and 22.4°C, night 13.5 and 13.8°C, respectively.

8.2.2 Light measurements and treatments

Light measurements were taken with a LI-1800 spectroradiometer (LI-COR, Lincoln, NB, USA) with a cosine corrected sensor following measurement and reporting guidelines (Bjorn and Vogelmann 1994; Sager *et al.* 1982). For comparisons of waveband proportions the percentage of total irradiance (quantum intergrade 300-800 nm) was calculated for PPF (photosynthetic photon flux, 400-700 nm) and for each 100nm band. Quantum ratios were calculated using described methods (Kittas *et al.* 1999): Red to far red narrow band (R:FR n) 655-665 / 725-735 nm; red to far red broad band (R:FR b) 600-700 / 700-800 nm; blue to red (B: R) 400-500 / 600-700 nm; blue to far red (B:FR) 400-500 / 700-800 nm. Light distribution measurements were taken using a LI-185B quantum radiometer with quantum sensor (LI-COR, Lincoln, NB, USA). All natural light measurements were taken on the same day in the same conditions in immediate succession. Growth chamber measurements were at an air temperature of 20^0 C with external light excluded.

8.2.2.1 Shade treatments

Shade treatment plants were grown in a climate modified glasshouse clad with 3mm horticultural glass (Pilkington, Dandenong, Victoria, Australia), which the spectral measurements showed to be effectively visible light wavelength neutral with respect to

sunlight (Table 3.3.1). Plants were grown unshaded and under 50% shade cloths: green (Sarlon, Moonee Ponds, Victoria, Australia); red and blue (ChromatiNet, Polysack Industries, Negev, Israel). Green shade cloth (neutral shade) was also wavelength neutral with respect to sunlight (Table 8.1). In contrast, the red and blue photo selective shade cloths dramatically altered spectral distribution. Blue shade cloth enhanced blue (400-500 nm) and reduced red (600-700nm) proportions; while red shade cloth reduced blue and green/yellow (500-600 nm) proportions and enhanced red and far-red (Table 8.1, Fig. 8.1). Thus green shade cloth was used to examine the effects of light quantity (neutral shade), while red and blue shade cloths were used to examine the effects of blue and red light proportions largely independently of R:FR. Although broad band R:FR was reduced under blue shade cloth (0.8, Table 8.1), narrow band R:FR was not and neither calculation range is altered under green or red shade cloth. Photosynthetically active radiation (PAR, 400-700nm) measurements taken with a quantum sensor under the various treatments during the growth response studies were consistently equal. Typical values were 950 μmol m^{-2} s^{-1} on sunny days in un-shaded conditions, 450 μmol m^{-2} s^{-1} under each shade treatment. Thus although PAR was the same for each shade treatment, spectral distribution varied markedly (Fig. 8.1).

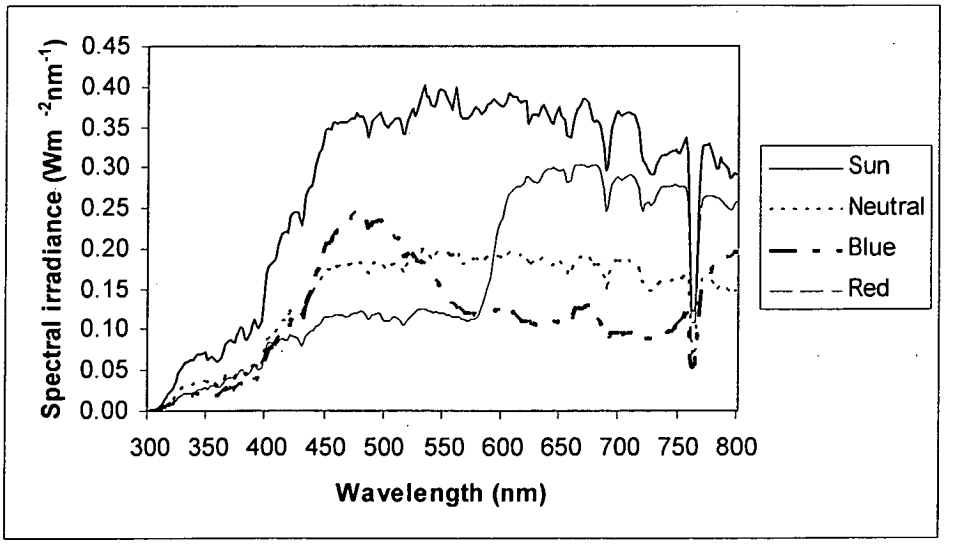


Figure 8.1. Spectral distribution as a function of wavelength of sunlight, neutral and photo selective shade cloths. Red, Blue- ChromatiNet® 50% red & blue shade cloth, neutral- 50% green shade cloth.

Table 8.1. Relative spectral distribution (% total irradiance 300-800 nm) and wavelength ratios for sun, glass and shade methods. Abbreviations SC- 50% green shade cloth; Red, Blue- ChromatiNet® 50% red & blue photo selective shade cloth.

	Sun	Glass	SC	Red	Blue
Wavelength					
PAR	71	72	70	70	71
300-400	2.9	2.6	2.7	2.1	2.3
400-500	16.7	16.8	16.5	9.4	23.8
500-600	25.7	26.1	25.7	13.9	25.3
600-700	28.2	28.2	27.8	36.8	21.5
700-800	26.5	26.3	27.3	37.8	27.1
Ratios					
R:FR (N)	1.1	1.1	1	1	1.1
R:FR (B)	1.1	1.1	1	1	0.8
B:R	0.6	0.6	0.6	0.3	1.1
B:FR	0.6	0.6	0.6	0.3	0.9

8.2.3 Plant growth measurement and analysis

Under each treatment, stem diameter, and leaflet width and length were measured at node 9 at the commencement of flowering. Node of flower initiation (NFI) and days from planting to first open flower (FT) were recorded. At harvest, length of internodes, total shoot length, number of nodes, and seed and pod number were measured. Shoot dry weight was measured after air drying of senesced plants for 72 h. Statistical analysis using JMP software (SAS Institute, Cary, NC, USA) included ANOVA, Students t-tests, Dunnetts method, and/or Tukeys test.

8.3 Results

8.3.1 Shoot length and flowering node varied with light source

To examine the influence of light source, L107 pea was grown under mixtures of fluorescent, metal halide, high pressure sodium and incandescent lamps. The same model growth chambers were used at equal irradiance (220 μmol m^{-2} s^{-1}), photoperiod (18 h) and temperature (20 ± 0.2^{0}C).

Both internode length and flowering node varied with light source (Fig. 8.2). L107 does responds to reduced R:FR with delayed flowering and reduced shoot length (Cummings *et al.* 2007; Weller *et al.* 2001). However, under the light sources used, a response based on R:FR was not clear (Fig. 8.2A, C). Shoot length did not decrease with increasing R:FR as expected (Fig. 8.2A). However, ranking the light sources by blue irradiance suggested a correlation of shoot length with blue quantity (Fig. 8.2B). Plants displayed the longest internodes under the lowest blue irradiance (9 μmol $m^{-2}s^{-1}$, HPS). Light sources with higher blue irradiance had significantly shorter internodes and above 30 μmol $m^{-2}s^{-1}$ of blue no further reduction in shoot length was observed. Node of flower initiation (NFI) was significantly earlier ($P < 0.01$) under metal halide than the other light sources even though R:FR was high (Fig. 8.2C). Ranking the light sources by total blue (400-500 nm) irradiance (Fig. 8.2D) suggested earlier flowering at a blue irradiance above 40 μmol m^{-2} s^{-1}.

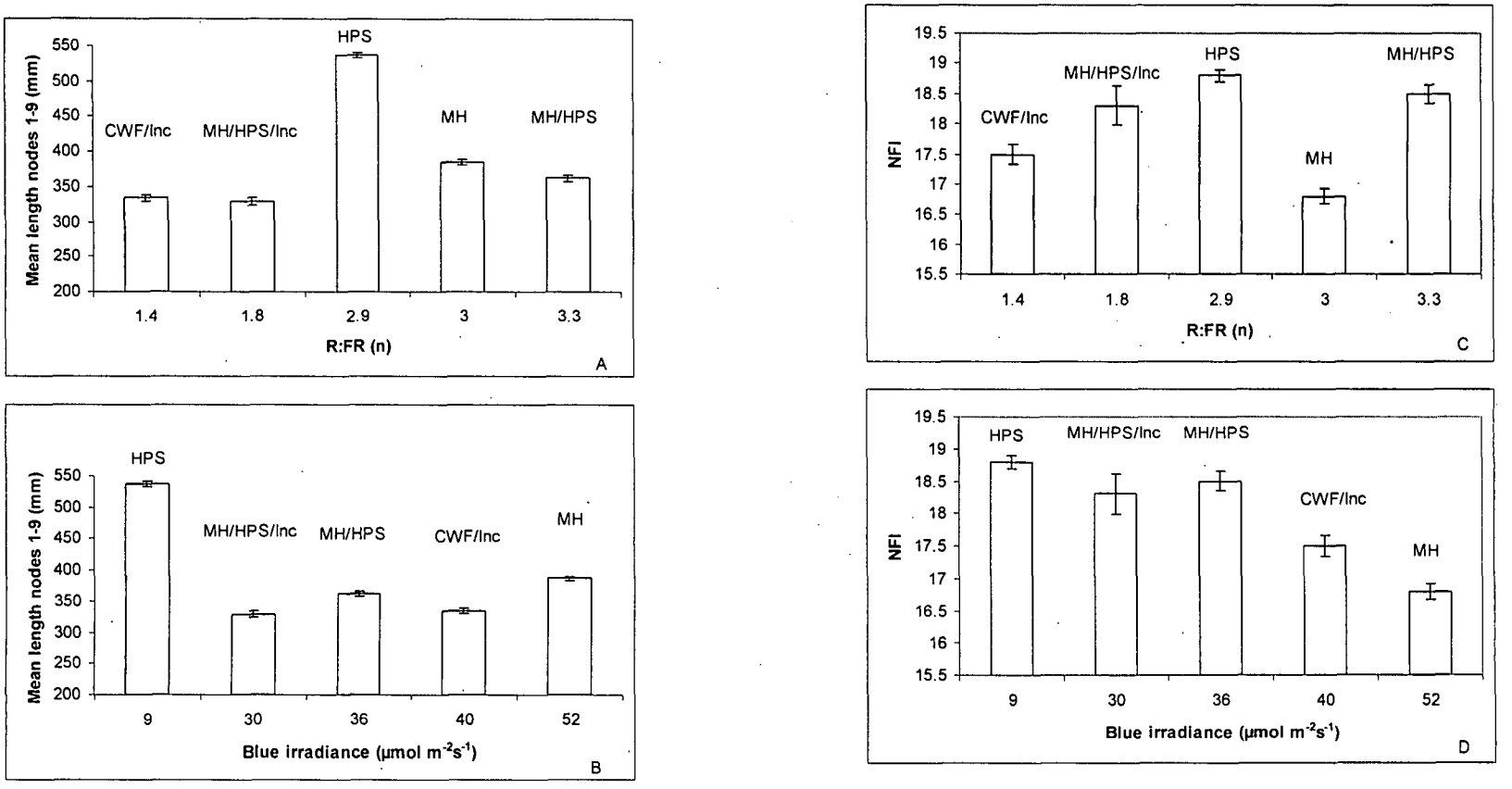


Figure 8.2. Mean shoot length ± SE between nodes 1 and 9, and node of flower initiation (NFI) for pea L107 by R:FR (A, C) and blue irradiance (B, D), n = 20. Plants were grown at 220 µmol m^{-2} s^{-1} PPF, photoperiod 18 h, temperature 20 ± 0.2^{0}C in Thermoline (Sydney, Australia) model 3540 growth chambers under combinations of cool white fluorescent tubes (CWF), incandescent globes (Inc), metal halide lamps (MH), and high pressure sodium lamps (HPS) to give the R:FR and blue irradiances specified.

8.3.2 Blue supplementation reduced shoot length and flowering

To examine this blue response in more detail L107 was grown under high pressure sodium lamps, the light source with lowest blue fraction but high R:FR. To this background was added a monochromatic blue light gradient from 5-70 μmol m^{-2} s^{-1}.

Significant shoot length reductions ($P < 0.05$ at 20 and $P < 0.01$ at 30 μmol m^{-2} s^{-1}, n=10) occurred at additional irradiance of 20-30 μmol m^{-2} s^{-1} blue compared to additional irradiance of 5 - 15 μmol m^{-2} s^{-1} blue. As for the light source experiments (Fig. 8.2), no further significant reduction in shoot length was observed above 30 μmol m^{-2} s^{-1} of blue.

To examine if this was simply an effect of the additional irradiance, the experiment was repeated with a white fluorescent gradient in the range of interest (20-45 μmol m^{-2} s^{-1}). The additional blue irradiance of the white fluorescents was 4-9 μmol m^{-2} s^{-1}. Under high pressure sodium with white supplementation, shoot length did not significantly differ. In contrast, the equivalent blue irradiance significantly reduced shoot length (Fig. 8.3, $P < 0.01$).

Node of flower initiation (NFI) was progressively lower under increasing blue irradiance, from means of 17.6 ± 0.24 with 5 μmol m^{-2} s^{-1} additional blue to 16.3 ± 0.21 with 45 μmol m^{-2} s^{-1} additional blue (significant at $P < 0.01$, n=10). The results suggest a blue light specific effect on flowering, as increasing irradiance with the white fluorescent gradient had no effect on NFI; and NFI was reduced under light sources with a higher blue proportion (Fig. 8.2D). In addition, when grown under monochromatic blue L107 NFI was node 15.9 (± 0.10, n=20); under monochromatic red NFI was node 18.3 (± 0.39, n=20).

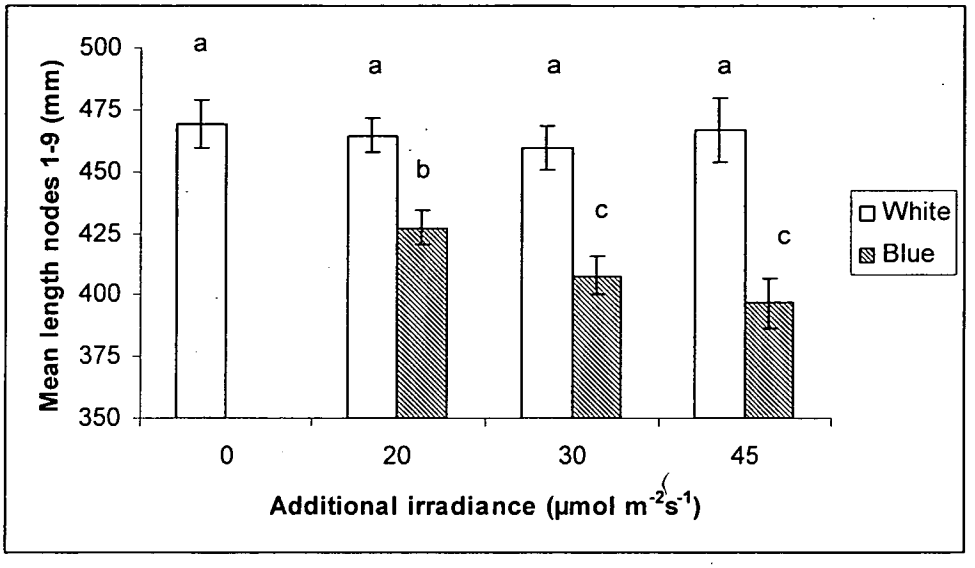


Figure 8.3. Mean shoot length ± SE between nodes 1 and 9 for L107 pea grown under 100 µmol m^{-2} s^{-1} high pressure sodium lamps, and with additional white or blue fluorescent irradiance of 20-45 µmol m^{-2} s^{-1}. Photoperiod 18 h, temperature 20 ± 0.2 ^{0}C. Different letters signify significant differences at $P < 0.01$, n=10.

8.3.3 Reduced irradiance increased shoot length and delayed flowering

To further examine the influence of light quantity, L107 pea was grown under spectrally neutral shade cloth and unshaded in the glasshouse. PAR was reduced by approximately 50% under neutral shade but light quality relative to sunlight was not affected (Table 8.1). Compared to neutral shade plants, unshaded plants had significantly reduced shoot length throughout the experiments (Fig. 8.4, $P < 0.01$). Leaf size was not affected by light quantity, but stem diameter was significantly larger in unshaded plants (data not shown, $P < 0.01$). Shoot dry weight was significantly higher for unshaded plants (Table 8.2, $P < 0.01$). Unshaded plants also flowered significantly earlier and at a lower node (FT, NFI, Table 8.2, $P < 0.01$), with significantly higher yield (seed, and pods, Table 8.2, $P < 0.01$) compared to plants under neutral shade. Thus reducing PAR by 50% without altering light quality produced taller, thinner plants with longer internodes, reduced dry weight and reduced yield.

Table 8.2. Mean flowering node and time, yield (seed, pods) and shoot dry weight ± SE for L107 peas grown under neutral, photo selective shade cloths and unshaded treatments as described in Materials and Methods. Significance comparisons used Dunnetts method with neutral shade as the control group. Different letters signify differences significant at $P<0.05$, * significant at $P<0.01$, n=20. Abbreviations: NFI, node of flower initiation; FT, flowering time.

Treatment	NFI	FT(days)	Seed	Pods	Dry w (g)
Neutral	16.5	42.5	9.4	3.2	4.2
	±0.11 a	±0.18 a	±0.87 a	±0.20 a	±0.11 a
Blue	16.1	42.3	10.3	3.9	5.9
	±0.10 b	±0.22 a	±0.52 a	±0.18 b	±0.07 b*
Red	16.1	41.1	10.0	3.2	5.3
	±0.13 a	±0.09 b*	±0.92 a	±0.25 a	±0.10 c*
Unshaded	15.5	39.3	17.6	4.8	5.4
	±0.14 c*	±0.18 c*	±1.36 b*	±0.33 c*	±0.32 c*

8.3.4 Blue irradiance influenced pea growth independently of R:FR

The artificial light studies have both altered blue quantity and R:FR. In contrast, the photo selective shade cloths had altered blue irradiance but largely unaltered R:FR (Table 8.1, Fig. 8.1), and are thus a useful tool to further examine the role of blue light, independently of R:FR. Each shade cloth treatment reduced PAR by approximately 50%.

Compared to neutral shade, L107 peas under blue shade cloth were significantly shorter and under red shade cloth significantly taller, respectively, at all growth stages. For example, length between internodes 1-17 was significantly longer under red and significantly shorter under blue shade cloth (Fig. 8.4, $P < 0.01$). Under red shade, R:FR was the same as neutral shade and unshaded treatments but blue quantity was reduced and red quantity enhanced (Table 8.1). Under blue shade broad band R:FR was actually reduced relative to other treatments, which would be expected to increase internode length, but length was actually reduced, presumably due to the enhanced blue quantity (Table 8.1). Repeats of the experiments in different seasons (spring and autumn) consistently produced significantly shorter plants under blue and significantly taller plants under red shade cloths (data not shown, $P < 0.01$).

Node of flower initiation (NFI) and flowering time (FT) were slightly earlier under both red and blue coloured shade cloths compared to neutral shade (Table 8.2).

Leaflets and stems were slightly larger under both red and blue shade compared to neutral shade, and leaflets were significantly longer under blue shade (data not shown, $P < 0.05$). Yield was also increased under blue shade compared to neutral shade (Pod number, Table 8.2). More importantly, both blue and red shaded plants had significantly higher dry weights than neutral grown plants (Table 8.2, $P < 0.01$).

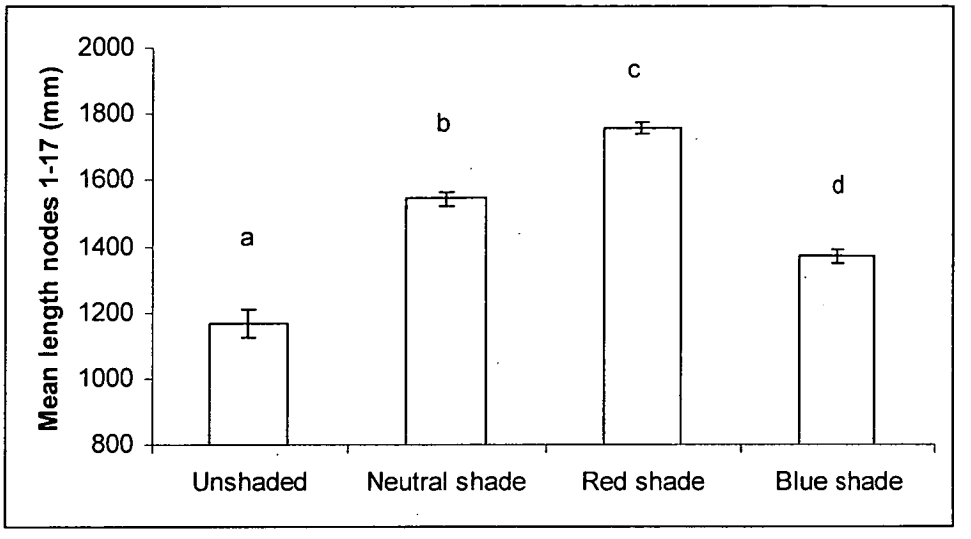


Figure 8.4. Mean shoot length ± SE between nodes 1 and 17 for L107 pea grown under shaded and unshaded conditions. Neutral shade- 50% green shade cloth; Red, Blue shade- ChromatiNet® 50% red & blue shade cloth. Different letters signify significant differences at $P < 0.01$, n=20.

8.3.5 Examining the role of the cryptochrome photoreceptor in the shade cloth response

While R:FR was largely unaltered by all three shade cloth treatments examined, the red and blue shade cloths result in substantial changes in the proportions of both red and blue light when compared to sun and neutral shade (Table 8.1, Fig. 8.1). However, it was still unclear if the observed shoot elongation under red shade and shoot length reductions under blue shade relative to neutral shade (Fig. 8.4) were due to changes in the proportion of red light or blue light or both. Thus the response of the blue light receptor mutant *cry1* of pea to neutral, blue and red shade cloths was examined to determine if it was changes in blue light under these treatments that modify growth.

As observed in previous experiments (Fig. 8.4), compared to neutral shade wild type (L107) pea plants exhibited significantly increased elongation under red shade and significantly reduced shoot elongation under blue shade (Fig. 8.5, $P < 0.05$). In contrast, *cry1* mutant plants did not respond to either shade cloth treatment, as shoot

elongation was not significantly different in *cry1* mutant plants grown under neutral, red or blue shade (Fig. 8.5). This indicates a clear role of blue light quantity in pea shoot length responses, and specifically, the CRY1 photoreceptor in these changes.

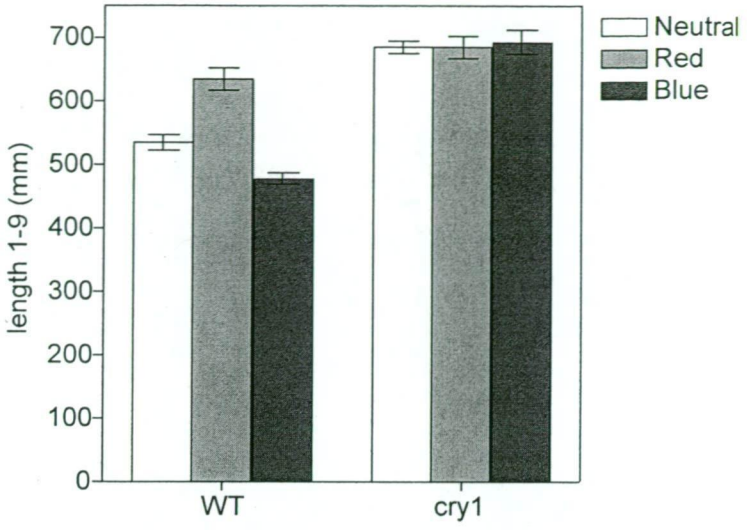


Figure 8.5. Mean shoot length ± SE between nodes 1 and 9 for L107 (WT) and *cry1* peas grown under the shade treatments as. Neutral- 50% green shade cloth; Red, Blue- ChromatiNet® 50% red & blue shade cloth.

8.4 Discussion

In this study, blue irradiance had clear effects on shoot length and flowering in pea independently of R:FR, in a range of conditions. Under different artificial light sources, shoot length and flowering was associated with blue quantity as well as R:FR, and blue supplementation reduced shoot length and flowering node independently of the increased irradiance. Using a novel method for varying blue quantity under natural light, it has been shown that blue light mediated alteration in shoot length was at least partially mediated by the CRY1 photoreceptor in pea.

Like many photoperiodic species, L107 pea has reduced shoot length and delayed flowering with increased R:FR (Weller *et al.* 2001). The commonly used artificial light sources used in growth chambers (fluorescent, metal halide, high pressure sodium) all have high R:FR. However, when grown under these sources at equal irradiance, wild type peas did not show clear correlations between increasing R:FR and shoot length or flowering, but were influenced by the blue irradiance (Fig. 8.2). Low blue irradiance was associated with increased shoot length even at high R:FR. This highlights the impact light source can have on plant growth and indicates that

care should be taken with which light sources are selected for growth chamber experiments.

Previous studies in soybean have associated shoot length with the low blue irradiance of high pressure sodium lamps, as increasing irradiance to provide a minimum blue light threshold of 30 μmol m^{-2} s^{-1} reduced shoot length (Wheeler *et al.* 1991). This single study has been used as a guideline to avoid increased elongation in growth chamber studies under artificial light (Sager and McFarlane 1997). However, increasing irradiance in itself influences shoot length (Christophe *et al.* 2006), particularly of pea (Gawronska *et al.* 1995). It is clear examination of such a threshold in other species is required, and for pea it would appear this threshold of 30 μmol m^{-2} s^{-1} also applies. Under HPS, shoot length was significantly longer than the other light sources at equal irradiance, all of which had a blue proportion above 30 μmol m^{-2} s^{-1} (Fig. 8.2). Furthermore, blue light supplementation of HPS above 30 μmol m^{-2} s^{-1} significantly reduced pea shoot length, whereas supplementation with white fluorescent light of equal irradiance did not, presumably because the blue fraction did not meet this apparent threshold (Fig. 8.3), and providing a clear indication that blue quantity can influence shoot length, not just increased irradiance.

High blue irradiance also influenced flowering. In WT plants, flowering node was reduced when blue irradiance was above 45 μmol m^{-2} s^{-1}, but was not significantly different when the additional irradiance was supplied by white fluorescent. Under monochromatic blue, flowering was at a similar node to light grown glasshouse plants (node 16), and under blue shade cloth, flowering was earlier than neutral shade (Table 8.2). In *Arabidopsis*, blue irradiance can influence flowering even when R:FR is high through CRY2 (Casal 2005; Mockler *et al.* 2003), and in mixtures of red, blue and far red light, flowering was in direct relation to blue irradiance (Eskins 1992). However, the role of blue light in regulating flowering in pea is less clear. The promotion of flowering in pea is mediated by phyA under high R:FR; under low R:FR promotion is via a reduction in phyB mediated inhibition, but blue may also promote flowering (Weller *et al.* 2001). Pea *cry1* (blue light receptor) mutants still retain a blue induced promotion of flowering, suggesting the role of additional blue receptors in this response in pea, possibly cry2 (Platten *et al.* 2005).

The artificial light sources that were studied not only have altered blue irradiance, but different R:FR, and are well above the sunlight R:FR of 1.1. In studies under natural light, height reduction by filtering far red light is well documented (e.g.

Rajapakse *et al.* 1999) but the role of blue light has received less attention. Thus photo selective shade cloths with altered blue irradiance (Oren-Shamir *et al.* 2001) were examined as a potential research tool. Blue shade cloth increased and red shade cloth decreased the blue proportion respectively relative to natural light and neutral shade, but R:FR was largely unaltered (Table 8.1, Fig. 8.1). This allowed for a novel method of examining the influence of blue light, and of light quantity, independently from R:FR, and on large adult plants.

Under blue shade cloth, shoot length was significantly reduced relative to neutral shade (Fig. 8.4). In fact, blue shade cloth had slightly reduced broad band R:FR (0.8), which may be expected to induce elongation, yet plants were significantly reduced in height. Plants grown under red shade cloth showed significant elongation relative to neutral shade (Fig. 8.4) although R:FR was unaltered. Thus it would appear that the shoot length alterations under red and blue shade cloths were due to alterations in blue or red irradiance, or both. To resolve this problem, L107 (WT) and a mutant blue light receptor line (*cry1*) were used to clarify the influence of blue light in the shade cloth response. In contrast to WT plants, there were no significant shoot length differences in the *cry1* mutant plants between the shade treatments (Fig. 8.5). CRY1 only absorbs in the blue spectrum (Ahmad *et al.* 2002), confirming the shoot length modifications under blue and red shade cloths are due to the alterations in blue proportion, not red proportion, and that CRY1 is the photoreceptor mediating this response.

Photosynthetically active radiation (PAR) level is also implicated in plant morphogenesis (Christophe *et al.* 2006; Dougher and Bugbee 2001) and in this study increased irradiance reduced shoot length in pea with no change in wavelength proportions compared to neutral shade (Fig. 8.4). In *Trifolium*, both blue and PAR reductions contributed to petiole elongation (Christophe *et al.* 2006). In the shade treatments, PAR was similar yet there were shoot length reductions under blue and shoot length increases under red shade cloths (Fig. 8.4), clearly regulated by blue light. While blue shade grown plants were taller than un-shaded plants, they were significantly shorter than plants under neutral shade. This indicates that photo selective shade cloths could have potential horticultural applications. Protection of horticultural crops by shading is common to reduce radiant heat load, but reducing PAR by shading can induce elongation and reduce yield (Hanan 1998).

Chemical growth regulators are also commonly used to manipulate height of horticultural crops, and there is considerable interest in photo selective filters to

reduce chemical use (Rajapakse *et al.* 1999). Height reduction by filtering far red light (i.e. increasing R:FR) can have the potentially negative effect of delaying flowering (Runkle and Heins 2001; Runkle and Heins 2002). Blue shade cloth may be a potentially beneficial form of shading compared to neutral shaded plants. Pea plants grown under blue shade cloth were significantly shorter, had higher shoot dry weight, higher yield and earlier flowering, all potentially beneficial growth responses (Table 8.2).

Thus the photo selective shade cloths examined have potential for examining the influence of blue irradiance on photomorphogenic responses in adult plants and, in species with a strong response to blue irradiance, have horticultural potential for manipulating height and flowering whilst offering crop protection.

In conclusion, a clear role for blue light in shoot elongation and flowering in mature pea plants has been defined. The significant impact of blue irradiance on pea development appears to act independently of R:FR, and indicates blue may be an important component of the shade avoidance response of this species. Under the artificial light sources commonly used in growth chambers, both R:FR and blue irradiance can influence shoot length and flowering, indicating that the choice of light source should be carefully considered. Photo selective shade cloths have both horticultural and photomorphogenic research potential. Using this novel method, it has been shown that under sunlight R:FR, both irradiance and blue light fraction influences shoot length and flowering in garden pea, with shoot length at least partially mediated by the CRY1 photoreceptor.

Appendix: Indicative plant responses

The following table has been compiled from indicative responses of peas in the different experimental environments. By using the plants' responses as indicators of their environment, management decisions can be made based on such information and the growing conditions of the plants. Thus if, for example, the plant response is long internodes, it could be from low light, low blue or low R:FR. If the plants are in relatively short days and/or if the PPF is low, increasing irradiance and/or the DLI should correct the problem. If the plants are under HPS, it is probably from low blue irradiance, so increasing blue (eg add some MH to the light mix) should correct this. The third option is unlikely in growth chambers as R:FR is usually high, but possible from incandescent photoperiod extensions, in which case one could add/switch to Fluorescent for the extension. In this way the plant responses can be used as a management tool.

Symptom	Causes	Solutions
Long internodes	Low light	↑ irradiance
	Low blue	↑ blue
	Low R:FR	↑ red, ↓ far red
Short internodes	High temp.	↓ temp.
	High light	↓ irradiance
	High blue	↑ red, ↓ blue
	High R:FR	↑ far red, ↓ red
Small leaves	High light	↓ irradiance
	High temp.	↓ temp.
	Low R:FR	↑ red, ↓ far red
Late flowering	High R:FR	↑ far red, ↓ red, ↑ blue
Early flowering	Low R:FR	↑ red, ↓ far red
Low yield	High temp.	↓ temp.
	High light	↓ irradiance
	Low R:FR	↑ red, ↓ far red

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