Gene expression variation under dynamic light in *Arabidopsis thaliana*

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of

Bachelor of Philosophy (Science) (Honours)

Word Counts:

Introduction:	x words
Results:	y words
Discussion:	z words

Abstract

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Contents

Todo list

Acknoweledgements

Thanks everyone! write this

Chapter 1

Design and Implementation of Dynamic Growth Conditions

1.1 Background, Aims and Hypotheses

In this chapter I am to create artificial growth conditions which vary on diurnal and circannual cycles in a similar manner to the regional climates cultivated or naturally growing plants experience. I hypothesise that plants grown under such conditions will exhibit phenotypes more similar to those grown under natural environments, given their growth conditions are more similar to natural environments. The Borevitz lab and I are terming this class of laboratory growth condition “dynamic growth conditions”. The term dynamic is in contrast with the unnaturally benign, highly static growth conditions typically used in the propagation, growth and experimentation of plants in laboratory settings. Such static conditions are throughout this thesis referred in the broad sense as “static growth conditions”.

As a key driver behind the development of dynamic growth conditions is the ability to simulate regional climates in the reliable and reproducible manner required for scientific study, a secondary aim for this chapter of my thesis is to create software to allow dynamic growth conditions to be implemented with hardware available to the Borevitz lab. This software should be able to use the model outputs of software previously written to create models underlying dynamic growth conditions to control multi-spectral LED lamps and computerised reach-in growth chambers. Successful completion of this sub-aim will allow the implementation of dynamic growth conditions, and their use in research.

1.2 Materials and Methods

1.2.1 The SpectralPhenoClimatron

The SpectralPhenoClimatron is a new facility within the Research School of Biology, consisting of computer controllable plant growth cabinets featuring multi-spectral LED lamps, and real-time imaging hardware. Conviron PGC20 reach-in growth chambers (Conviron, Winnipeg, Canada) have been retro-fitted with Heliospectra Model L4A Series 10 multi-spectral LED growth lamps (Heliospectra AB, Sweden) and image-based phenomics systems (Canon EOS DSLR cameras and other consumer hardware). The Conviron PGC20 cabinets have a capacity of 320 5cm by 5cm plant growth containers, or 16 <+dimension+> standard nursery seed trays (e.g. <+gcp part number+>). The temperature and humidity within the growth cabinets is tightly controlled, with operating range of 5-35 °C and 50-80 % RH. The Heliospectra L4A Series 10 LED lamps contain 7 LED wavelength channels: 400nm (sub-blue), 420nm (blue), 450nm (blue), 530nm (green), 630nm (red), 660nm (red) and 735nm (far red); the intensity of these channels is not equal (see subsection 1.2.2). needs concluding sentence

Hardware components of the SpectralPhenoClimatron are both manually and automatically programmable. Components are manually programmable either directly or over a web interface, however this control is limited to approximately 15-20 updates per day, with a single schedule (no day-to-day changes). In addition, both Conviron PGC20 cabinets and Heliospectra L4A lamps can interface with a computer using commands communicated over the telnet protocol. This enables a programmer to create software which automates the process of controlling each component together. The functionality of the SpectralPhenoClimatron would then only be limited by the operating range of its components and the software used to control it.

1.2.2 Design of dynamic growth conditions

Novel growth conditions were designed to emulate some characteristics of growth conditions experienced by plants in natural environments. Using SolarCalc (version AusFebC) (Spokas and Forcella 2006), I have created simulated climates derived from historical observations and models of the climate of the region surrounding Temora, New South Wales. This is a trial location for various agricultural crops, and is in the same climate zone as much of the native range of *Arabidopsis thaliana* (Peel, Finlayson, and McMahon 2007). SolarCalc has a very extensive list of parameters which can be set. The settings I used in the creation of the dynamic conditions are described in Table 1.1. SolarCalc was run with these model parameters, to produce a model of growth conditions which simulate elements of a natural environment.

SolarCalc by default simulates the climate of a location without any weather, however I aimed to create condition which mimic cloudy and intermittently cloudy days. Thus, to create such con-

Parameter	Temora Setting
Simulation End Date	31/12/12
Simulation Start Date	01/09/12
Chamber Max RH	85
Chamber Min Temp	5C
Chamber Date	01/03/13
Date Format	US
Site Elevation	300m
Site Latitude	-34.446556
Site Longitude	147.53334
Timezone	Sydney
Update Frequency	1 min
Weather Year	2010
Lighting	LED
Set to Threshold	Yes
LED1 Power Threshold	5.00%
LED1 Wavelength	400nm
LED1 Weight Multiplier	5.2
LED2 Power Threshold	5.00%
LED2 Wavelength	420nm
LED2 WeightMultiplier	4.12
LED3 Power Threshold	5.00%
LED3 Wavelength	450nm
LED3 Weight Multiplier	4
LED4 Power Threshold	5.00%
LED4 Wavelength	530nm
LED4 Weight Multiplier	3.92
LED5 Power Threshold	5.00%
LED5 Wavelength	630nm
LED5 Weight Multiplier	4.88
LED6 Power Threshold	5.00%
LED6 Wavelength	660nm
LED6 Weight Multiplier	0.68
LED7 Power Threshold	5.00%
LED7 Wavelength	740nm
LED7 Weight Multiplier	3.64
AOD	0.27
Azimuth	180
Slope	10
Soil Albedo	0.5
Solar Tilt	3.5
Solar Constant	1360
Track Sun	FALSE

Table 1.1: Parameter settings of SolarCalc used in the creation of dynamic conditions.

ditions, post-processing work is required. Firstly, SolarCalc was re-run with a neutral density shade parameter of 45%, meaning that the model sunlight intensity was 45% that of the original model, forming the shaded model, which aimed to emulate a cloudy day. Then, the result of both the original model, and this shaded model were spliced together to form a condition whose light intensity changed between shaded and original models on a two hour shaded, one-hour original rotation, using the `spliceSolarCalc.py` script described in **??**. This created the fluctuating condition designed to emulate an intermittently cloudy day. The temperature, humidity and light quality of these conditions remained unchanged. These additional conditions allow us to compare the effect of different light regimes within the framework of such pseudo-natural dynamic growth conditions which SolarCalc creates.

This sounds too much like what got destroyed in the initial examiners meeting. Also it's to wordy.

1.2.3 Measurement of Spectral Power Density

Raw spectral power density data was obtained from Dr. Pip Wilson of the Borevitz lab. The <+model+> radio spectrophotometer was used to record spectral power density across the spectrum between light of wavelengths 400nm to 800nm, with 2nm wavelength resolution. Sun and shade spectra were obtained on <+date+> at the Acton campus of the ANU, in a clear, open space and under heavy shade from mature trees of various species, in the courtyard between buildings 46 and 48. Spectra of laboratory growth conditions were obtained from a Conviron PGC20 by placing the spectrophotometer on the lowest shelf level, while fluorescent lamps or Heliospectra L4A series 10 lamps were illuminated at their highest intensities. In the case of the Heliospectra L4A series 10 LED lamps, measurement from directly under a single unit were recorded. Intensity-normalised spectral power density was calculated by normalising the intensity between spectra to an intensity of 1 μmol photons $m^{-2}s^{-1}nm^{-1}$ per total μmol photons $m^{-2}s^{-1}$.

1.3 Results

1.3.1 Computer Control of the SpectralPhenoClimatron

To create dynamic growth conditions which change on diurnal and circannual cycles, high temporal resolution is required. Whilst SpectralPhenoClimatron hardware has such capabilities, external software is required to make such control feasible. Thus, I have created software which is able to control these cabinets, enabling the creation of dynamic growth conditions. The software I have created, `spcControl`, gives control commands to both growth cabinets and retrofitted LED light lamps. This software is implemented in the Python programming language, and allows conditions generated in a defined format by external software to be parsed, converted to control commands, and sent in concert to all components of the SpectralPhenoClimatron. It runs on a computer situated next to the chambers, and can control many chambers at once. python mentioned twice

This software has many capabilities required for the implementation of dynamic growth conditions. Given a climate model generated by SolarCalc, or a derived model in the same format, `spcControl` will, at times specified in the input model, send control commands to both the master LED array, and plant growth cabinet, updating lighting and temperature and humidity respectively. This process takes around 30-45 seconds, and thus can occur up to every minute, giving extreme temporal resolution in growth condition control. Additionally, as the commands are sent out synchronously, lighting, temperature and humidity will never go “out of sync” if a power outage or device failure occurs. Furthermore, to ensure reliable operation and detection of faults, at every time-point specified in the SolarCalc model, the success or failure is communicated to an off site database, and any error message is emailed to an administrator. An additional module, `spcControl.monitor`, polls this database and guards against failure of hardware, control computers and software, informing an administrator upon any failure. Together, these features allow highly reliable control of growth cabinets with temporal resolution suitable for the implementation of dynamic growth conditions.

The `spcControl` module and sub-modules run with python version 3.2 or later. It is modular in design, with a main program loop which sends a control line to each sub-module per the schedule given by the SolarCalc model. Sub-modules then parse this line, and a configuration file, to formulate commands sent to the relevant device(s); sub-modules for Heliospectra L4S LED lamps and Conviron PCG20 chambers have been implemented. This modular design means that, given hardware specifications, creating new sub modules to control other hardware configurations would be relatively trivial. Status reports are reported to an external PostgreSQL database, and email error messages are generated using the Google mail API from within python. In all, this consists of over 740 lines of code and configuration, and 134 minor versions (git commits) 16 releases.

concluding sentence

1.3.2 Sufficient, Excess and Fluctuating Dynamic Growth Conditions

To investigate the effect of altered light intensity on the *Arabidopsis* transcriptome, three novel growth conditions have been created. These conditions have been specifically designed to emulate a difference in light intensity which may be encountered naturally, that of neutral density shading by clouds. The Sufficient Light dynamic growth condition (hereafter "Sufficient") corresponds to approximately the same daily integral of light as "standard growth conditions" of 120-150 μ mol photons $m^{-2}\,s^{-1}$on a 12-hour photoperiod. The Excess light condition is an approximate 250 % excess over the sufficient condition. The Fluctuating light condition is designed to simulate the pattern of light intensity variation caused by partial cloud or patchy sun. These conditions simulate the spring season, and display circannual variation in temperature, light and humidity. As time progresses, daily minimal and maximal temperature and peak light intensity increase, while minimal relative humidity decreases.

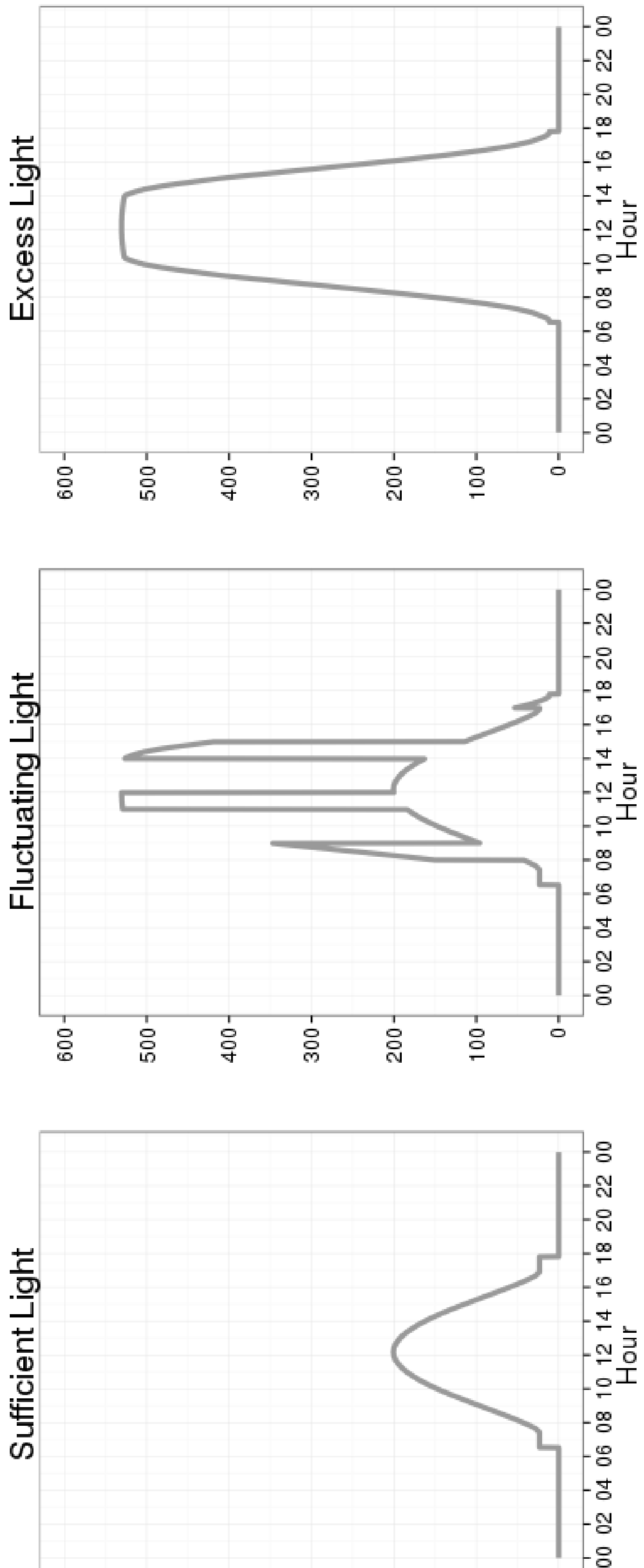

Figure 1.1: Diurnal variation in approximate light intensity of sufficient, fluctuating and excess light dynamic growth conditions (for model date 1 March).

finish captions describing the trends in my conditions

The light quality of all dynamic growth conditions created using this system are markedly different to other light sources. Compared to the fluorescent lamps typically used in laboratory growth chambers the spectral power density of LED arrays is less variable across the visible and adjacent spectrum, with intensity-normalised spectral power density of fluorescent and LED array light sources of 1.00 ± 1.74 and 1.00 ± 0.92 μ mol photons $m^{-2}\,s^{-1}nm^{-1}$ per total μ mol photons $m^{-2}\,s^{-1}$respectively (means ± SD; see Figure 1.7). The intensity-normalised spectral power density of sunlight on a clear day is remarkably even (1.00 ± 0.19 μ mol photons $m^{-2}\,s^{-1}nm^{-1}$ per total μ mol photons $m^{-2}\,s^{-1}$; Figure 1.7). The intensity normalised spectral power density of shaded light is similarly even at wavelengths lower than approx 700nm, above which sunlight is not filtered by vegetation and thus is over-represented. Overall, the spectral power density of LED lamps is more even than that of fluorescent lamps, however is nowhere near as even as that of sunlight.

The overall light intensity of these light sources varies drastically. Open sunlight is very bright, with a total intensity of over 2480 μ mol photons $m^{-2}\,s^{-1}$in the visible and adjacent spectrum on the day of measurement. The intensity of naturally shaded sunlight is much lower, at approximately 88 μ mol photons $m^{-2}\,s^{-1}$on the day of measurement. The light intensity under a single Heliospectra L4A series 10 LED lamp was 370 μ mol photons $m^{-2}\,s^{-1}$, while the intensity of light from fluorescent lamps was 140 μ mol photons $m^{-2}\,s^{-1}$(data shown graphically in Figure 1.7).

1.3.3 Phenomic dataset generation

does this go here or ch4?

Tim: images from Col, Cvi, Ler from all time-points

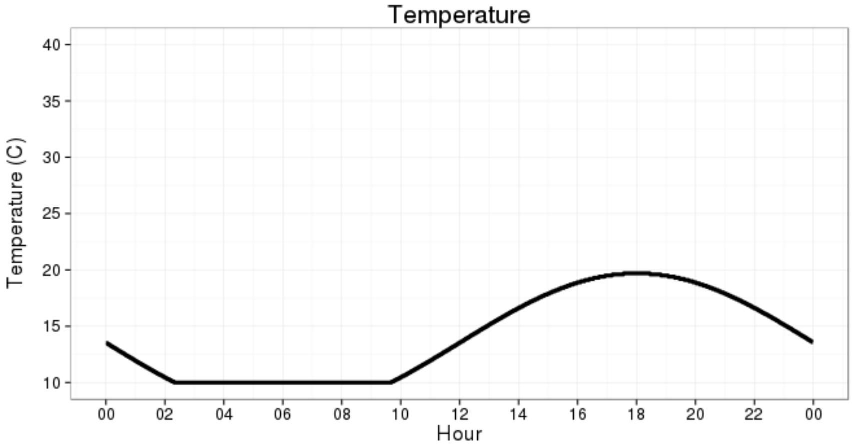

Figure 1.2: Diurnal variation in dynamic growth condition model temperature (for model date 1 March). Temperatures follow an approximation of those observed in temperate climates, reaching a minimum before sunrise (06:00), steadily increasing after sunrise to a peak immediately prior to sunset (18:00). Growth chamber hardware limitations prevent temperatures falling below 10 °C for extended periods, thus the model "bottoms out" where temperatures below 10 °C would have occurred (02:00 - 10:00).

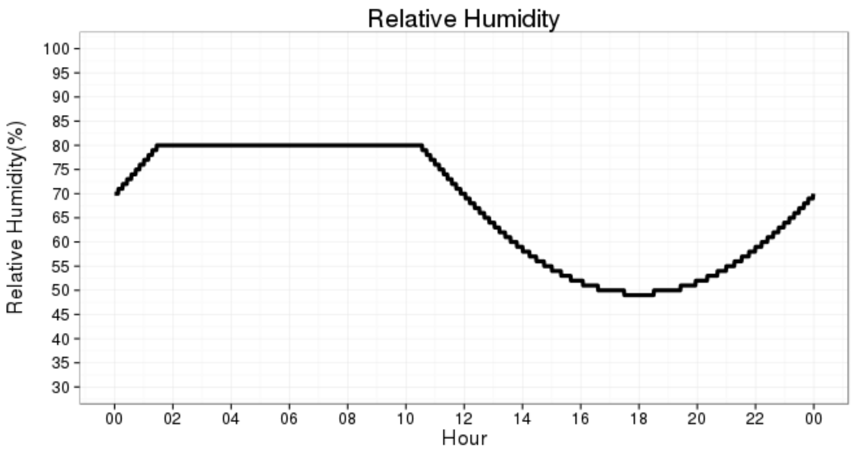

Figure 1.3: Diurnal variation in Dynamic Growth Condition model relative humidity (for model date 1 March). Humidity follows an inverse trend to temperature, peaking before sunrise (06:00) and reaching its minimum at approximately sunset (18:00). Similarly to temperature, growth chamber hardware limitations prevent relative humidities greater than 80% for long periods, and therefore humidity is capped at 80% between the hours of 02:00 and 10:00.

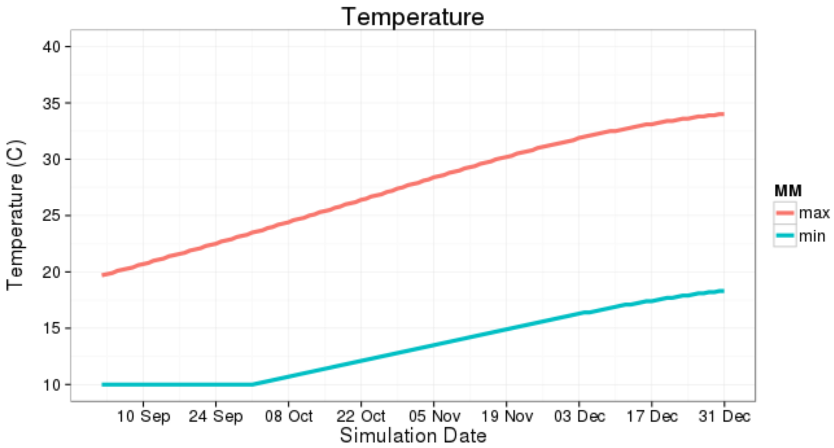

Figure 1.4: Circannual variation in daily minimal and maximal Dynamic Growth Condition model temperature

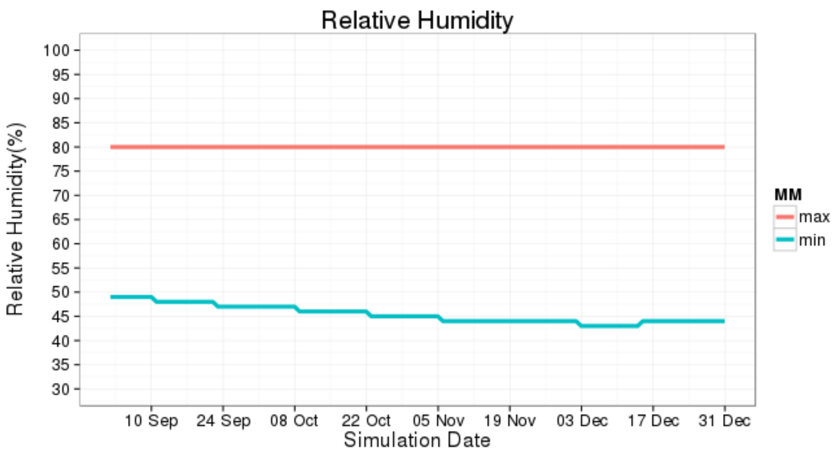

Figure 1.5: Diurnal variation in daily minimal and maximal Dynamic Growth Condition model relative humidity

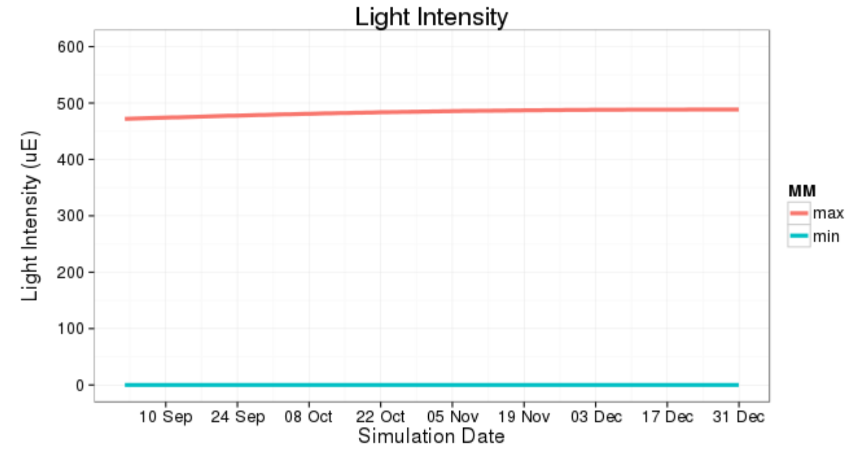

Figure 1.6: Diurnal variation in daily minimal and maximal Dynamic Growth Condition model light intensity

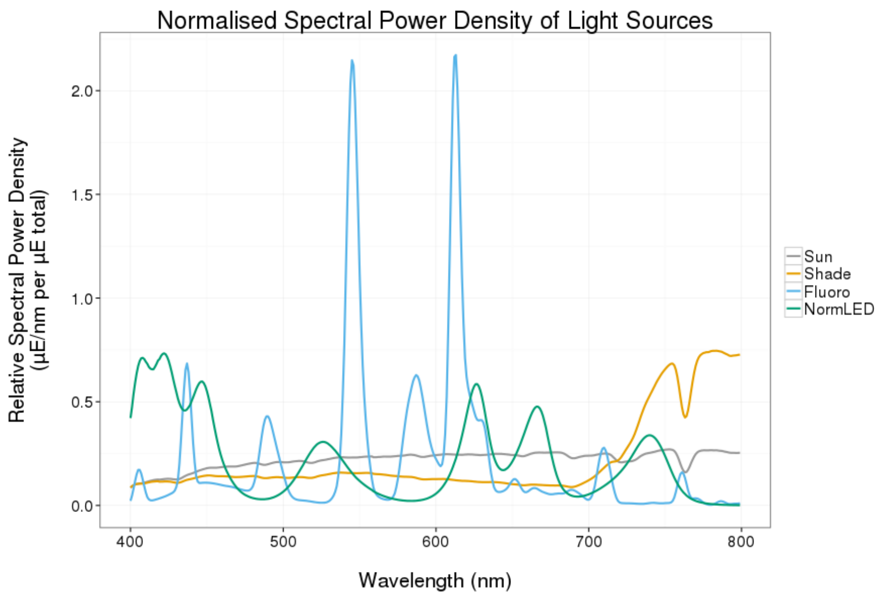

Figure 1.7: Intensity-normalised spectral power density of sunlight, shaded sunlight, fluorescent lamps and Heliospectra L4A series 10 LED lamps.

1.3.4 Novel Dynamic Growth Conditions That Simulate a Continental Gradient

In addition to conditions created to test hypotheses around altered light intensity, I have created conditions to answer others' hypotheses. Two conditions have been created to test the overall effect of environments with higher light and more diurnal variation in temperature, such as may be experienced in inland regional climates, compared to conditions with lower light intensity and lower diurnal variation in temperature, such as those often encountered in coastal climates. These conditions, named "NSW inland" and "NSW coastal" respectively, generally are more harsh than the sufficient, fluctuating and excess light dynamic growth conditions created for my experiments. Plots describing their conditions follow.

should this be included in my thesis?

polish this if it is to be included, and input plots. also needs a subsection in the methods section of this chapter if included.

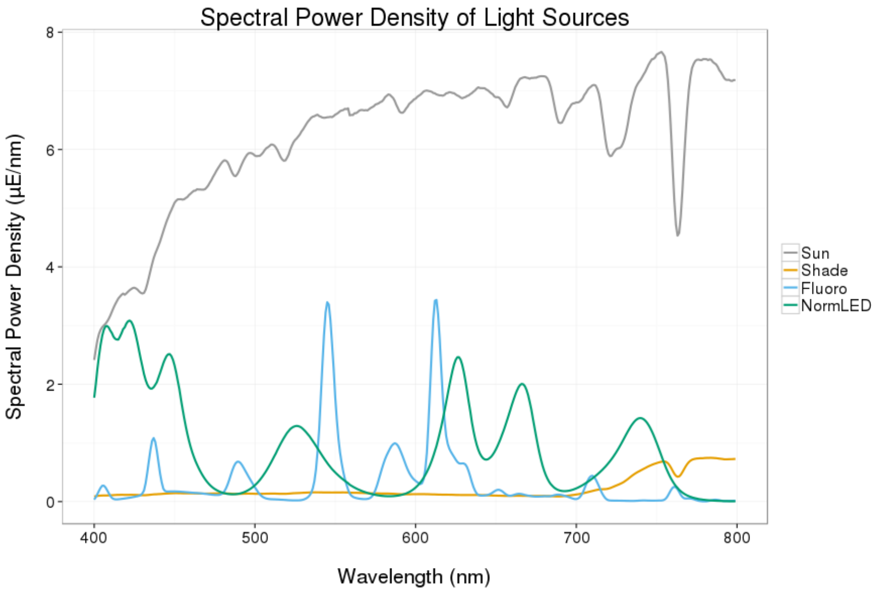

Figure 1.8: Spectral power density of sunlight, shaded sunlight, fluorescent lamps and Heliospectra L4A series 10 LED lamps.

Bibliography

Peel, MC, Finlayson, BL, and McMahon, TA (Oct. 2007). Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci.* 11.5, pp. 1633–1644. DOI: 10.5194/hess-11-1633-2007 (cit. on p. 6).

Spokas, K and Forcella, F (Jan. 2006). Estimating hourly incoming solar radiation from limited meteorological data. *Weed Science* 54.1, pp. 182–189. DOI: 10.1614/WS-05-098R.1 (cit. on p. 6).

Chapter 2

Appendix

Notes:

- Code listings, where included, are illustrative. Full source code of all software developed is large (over 5000 lines of code), and will be distrubuted as a gzipped tar archive. The latest code for all pipleines, scripts, is available online. See Appendix **??** and **??**