

PLANT WATERLOGGING:
CAUSES, RESPONSES, ADAPTATIONS AND
CROP MODELS

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ABSTRACT

Waterlogging has been reported to reduce crop yields by up to 80 %, although the lack of a consistent definition of waterlogging or specific effects on plants makes it hard to accurately ascribe crop yield losses to waterlogging relative to other abiotic stresses. After reviewing the available literature I suggest that recording soil profile information, topographic data, meteorological information, plant morphological appearance and areas with visible surface water are the most important factors for describing waterlogging in the field.

An above ground plant response to waterlogging that is easily identifiable in some species is leaf wilting. Reduced root hydraulic conductance was investigated as the possible cause of leaf wilting by waterlogging *Glycine max* L. and *Nicotiana glutinosa* L. under greenhouse conditions. During these experiments a defined sequence of plant responses and adaptations to waterlogging was established. Waterlogged soybean showed very little change in plant physiology or morphology implying a low sensitivity to reduced root zone soil oxygen concentration [O₂]. At the other end of the waterlogging sensitivity scale before [O₂] reached 10 % there was a 50 % reduction in root dry weight of *N. glutinosa* on day 2 of waterlogging. On day 3 of waterlogging there was decreased stomatal conductance and leaf water potential, both measures indicating water deficit stress. However, apparent root hydraulic conductance measured with a hydraulic conductance flow meter (HCFM) increased, as did petiole and leaf hydraulic conductance. There was no evidence of aerenchyma formation in roots although there was extensive breakdown of endodermal cells in the waterlogged roots. It is suggested that root water uptake was severely impaired by this loss of cellular integrity. An implication from this is that water uptake is primarily in response to osmotic gradients and active water transfer across root cell membranes rather than a response to the hydrostatic potential gradient from the free water surrounding the roots into the root xylem. The breakdown of root anatomical integrity seems likely to be associated with the apparent increase in measured root

hydraulic conductance. Care should be taken in applying the HCFM measurement technique to root systems that are anatomically damaged.

Evidence from the literature and observations from the current experiments highlight the multiple and varied responses of different species to waterlogging. This apparent variation makes the development of general plant waterlogging response models very challenging. To address this, a framework was developed that identifies three stages of response by plants to the onset of waterlogging; an initial increase in plant growth and function, followed by decreased growth and function as $[O_2]$ decreases, and finally, a species specific adaptation phase that places the species in a range from highly sensitive to highly tolerant.

Using this response framework, the generic crop growth and yield simulation model SWAGMAN Destiny was modified to improve the representation of waterlogging response in common crop species with a particular focus on wheat. An empirical representation of decreased gas filled pore space by soil layer, the depth of the layer, the root length and the duration of saturated conditions were used to derive a waterlogging stress factor. This stress factor was then used to change the distribution of roots in the soil profile and aggregated to provide a plant stress factor that modified carbohydrate production from the plant leaf area. In essence, the waterlogging stress factor is used as a collective representation of the above empirical processes, and changing root hydraulic conductivity that we observed in response to low $[O_2]$. The simulated output yields were consistent with experimental results and published field trial results.

In compiling information on specific species sensitivity to waterlogging in field conditions it became obvious that rigorous comparison was extremely difficult since there is a lack of consistency around the duration and timing of waterlogging, the soil profile, topography and climate. This reality means that simulation modelling that represents the physiological processes of waterlogging and the response processes of plants has an important role in

assisting understanding of a waterlogged soil plant system. I recommend any crop model that explicitly includes waterlogging as an abiotic stress should demonstrate the three stage response as supported by outputs from SWAGMAN Destiny.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Ruth Shaw

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Final thanks goes to my friends and family who have supported me throughout my PhD. Love you. Xo.

LIST OF PUBLICATIONS

Shaw RE, Meyer WS, Mcneill A, Tyerman SD (2013) Waterlogging in Australian agricultural landscapes: a review of plant responses and crop models. *Crop & Pasture Science*, **64**: 549-562.

Shaw RE, Meyer WS Empirically representing plant responses to waterlogging for simulating crop yield. *Agronomy Journal*. Submitted, manuscript ID AJ-14-0625-A.

CHAPTER 1
INTRODUCTION

1 CHAPTER 1

1.1 INTRODUCTION

Approximately 12 million ha of irrigated land in the developing world suffers productivity loss due to waterlogging and salinity (Mancuso and Shabala, 2010). Worldwide it is estimated that one-tenth of irrigated cropland is waterlogged (Mancuso and Shabala, 2010) either permanently or transiently. In 2006 to 2007 Australian farmers spent \$649 million in the combined prevention and management of soil erosion, compaction, soil acidity and surface waterlogging (Statistics, 2010). It is not clear what proportion of this total was directed at reducing the effects of waterlogging. Part of the difficulty in obtaining aggregate statistics about the extent of waterlogging and its effects is that it is poorly defined. It is most commonly described as excess soil water in the plant root zone that results in a decrease in soil oxygen flux and concentration and hence oxygen levels that limit optimal root and plant function. While this description can be understood it is not necessarily readily observed and this, along with many different above ground plant responses makes reports of waterlogging effects difficult to interpret.

This thesis systematically reviews the literature of plant and root zone responses to waterlogging, investigates the curious leaf wilting response of waterlogged *Nicotiana glutinosa* L. plants in the light of new knowledge about aquaporins in root cell membranes and the effect on water uptake. Finally this thesis proposes and implements changes to how waterlogging is represented in a plant growth water use and yield model.

There is an inconsistency within the literature regarding waterlogged crops in the field (Shaw *et al.*, 2013). Very few papers record a comprehensive description of the soil, the plants and the climatic conditions (Table 1 in Shaw *et al.* (2013)). This lack of data makes it hard to understand plant mechanisms, to compare data and hence prevent or avoid waterlogging. There are a variety of plant adaptations that occur during waterlogging, dependent upon plant

species, plant development stage, climatic conditions, soil profile and the length of the waterlogging event. Plant adaptations during waterlogging range from observed responses such as wilting (Kramer and Jackson, 1954; Jackson, 1956), leaf yellowing (McDonald, 1995), root blackening and root rotting to physiological adaptations such as aerenchyma (Armstrong, 1979; Colmer, 2003b), adventitious roots (Belford, 1981), within cell barriers to radial oxygen loss (Colmer *et al.*, 1998) and a reduction in root hydraulic conductance (Bramley *et al.*, 2007). Curiously, leaf wilting occurs in some plants during waterlogging. Wilting is most commonly seen in plants that are subject to water deficit. The relationship between leaf wilting (an easily observed aboveground response) and the change in aquaporin activity, represented by the reduction in root hydraulic conductance (a physiological adaptation) has been investigated to better understand the mechanisms of plant adaptations during waterlogging.

One relatively recent method of improving the diagnosis of yield reducing stresses, including waterlogging, is with crop growth, water use and yield simulation models. With representations of the major physiological processes and environmental drivers it is possible to both diagnose the contributing effect of various stresses retrospectively and importantly to identify areas and conditions that will likely lead to yield reduction. However, the success of these models is highly dependent on the adequacy of the process representations in the simulation model. As better understanding of plant physiological processes develops, the representation of these processes in models should be modified. One such process that requires implementation into models is plant response to waterlogging. Current crop models that incorporate waterlogging stress (APSIM (Asseng *et al.*, 1998), SWAGMAN Destiny (Meyer *et al.*, 1996) and DRAINMOD (Skaggs, 2008)) do not include plant physiological adaptations that occur during waterlogging. With my increased understanding, by reviewing literature and performing my own experiments I have incorporated plant adaptations,

including the change in root hydraulic conductance during waterlogging in the crop growth, water use and yield simulation model SWAGMAN Destiny. Including biological processes into crop models should lead to improved accuracy and better representations of estimated final crop yields due to the stresses of waterlogging.

To summarise the aim of my thesis, the above research can be divided into three sections, comprising seven chapters:

1. An investigation into reported waterlogging in the field within Australia and the identification of a minimum data set to benchmark potential waterlogging areas (Chapter 2);
2. Greenhouse experiments to understand plant physiological mechanisms and adaptations during waterlogging (Chapters 3 and 4);
3. Including plant adaptations during waterlogging into the crop growth and yield simulation model SWAGMAN Destiny (Chapters 5 and 6).

Details of the thesis Chapters are as follows. Chapter 2 was published as a critical review in *Crop & Pasture Science*. It examines past literature and reported observations of waterlogging on field crops and the inconsistencies found in those reports. It suggests a possible minimum data set for predicting and monitoring waterlogging. It then goes on to discuss crop growth and yield simulation models that incorporate waterlogging stresses and the recommendation of including plant adaptations when waterlogged into crop growth and yield simulation models, which forms the basis for study in the following chapters. Chapter 3 describes experiments designed to understand plant physiological mechanisms and adaptations during a waterlogging event, specifically looking at the relationship between the observation of leaf wilting, the physiological changes in root hydraulic conductance and the changes in aquaporin activity. Chapter 4 examines the relationship between the observed response and physiological adaptation of *N. glutinosa* during waterlogging. Chapter 5 has been submitted to *Agronomy Journal* as a concept for improvement of crop growth and yield simulation models. It proposes an empirical representation of plant adaptations during

waterlogging to incorporate into the crop model SWAGMAN Destiny, by way of example, thereby including plant physiological processes and improving model accuracy. Chapter 6 details the changes made to SWAGMAN Destiny and the improvement resulting from better representation of soil and plant processes in the waterlogging module. Finally, Chapter 7 provides an overall conclusion of my findings and recommendations for future work.

CHAPTER 2
LITERATURE REVIEW

2 CHAPTER 2

The work contained in this chapter has been published as a critical review paper in *Crop & Pasture Science*.

2.1 STATEMENT OF AUTHORSHIP

Shaw RE, Meyer WS, McNeill A, Tyerman SD (2013) Waterlogging in Australian agricultural landscapes: a review of plant responses and crop models. *Crop & Pasture Science*, **64**: 549-562.

Author contributions: By signing the statement of authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Shaw RE (Candidate)

Collected, analysed and interpreted literature, wrote manuscript. I hereby certify that the statement of the contribution is accurate.

Signed

Date 20.10.2014.

Meyer WS

Supervised writing, reviewed and edited manuscript. I hereby certify that the statement of the contribution is accurate.

Signed

Date 23/10/2014

McNeill A

Supervised writing, reviewed and edited manuscript. I hereby certify that the statement of the contribution is accurate.

Signed

Date 21/10/2014.

Tyerman SD

Supervised writing, reviewed and edited manuscript. I hereby certify that the statement of the contribution is accurate.

Signed

Date 21/10/2014.

Shaw, R.E., Meyer, W.S., Mcneill, A. & Tyerman, S.D. (2013) Waterlogging in Australian agricultural landscapes: a review of plant responses and crop models. *Crop & Pasture Science*, v. 64(6), pp. 549-562.

NOTE:

This publication is included on pages 8-21 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.1071/CP13080>

CHAPTER 3
MATERIALS, METHODS
& PILOT EXPERIMENTS

3 CHAPTER 3

This chapter is set out with the following sections:

- (a) it describes the materials and methods common to the experiments reported in this thesis that aren't described elsewhere,
- (b) it reports results from several pilot experiments undertaken to standardise measurements and enable familiarisation with the techniques, and
- (c) it provides comparative data for *Glycine max* L. (soybean) and *Nicotiana glutinosa* L. under similar edaphic and environmental conditions to compare waterlogging tolerances between species.

A brief summary of all experiments undertaken for this thesis is given in Table 3.1. Experiments were conducted at staggered planting dates with measurements of different parameters at the same growth stage.

Table 3.1: Summary of all experiments reported in this thesis.

Experiment number	Number of waterlogged plants	Number of freely drained plants	Plant species used	Aim of experiment
1	6	6	<i>N. glutinosa</i>	Emulating experiments of Kramer and Jackson (1954) to observe wilting and measure plant physiological responses to waterlogging.
2	6	6	<i>N. glutinosa</i>	Increased pot sizes, familiarisation with techniques and equipment.
3	6	6	<i>N. glutinosa</i>	Familiarisation with techniques.
4	2	2	<i>Glycine max</i>	Observing soybean wilting response and adaptations during waterlogging. Familiarisation of techniques with a different species.
5	10	10	<i>N. glutinosa</i>	Changed soil mix. Increased plant replicates (Chapter 4).
6	10	10	<i>Glycine max</i>	Repeat of experiment 5 with soybean. Results in Section 3.11 of this chapter.
7	4	4	<i>N. glutinosa</i>	Investigating leaf wilting response, measuring leaf ion content.
8	12	11	<i>N. glutinosa</i>	Investigating the apparent increase in root hydraulic conductivity (L_o), measuring leaf hydraulic conductivity (L_{leaf}).
9	4	4	<i>N. glutinosa</i>	Investigating the apparent increase in root hydraulic conductivity (L_o), examining root cross sections for aerenchyma formation (Chapter 4).

3.1 PLANT CHOICE AND PREPARATION

Two tobacco species were considered for use in this work. *Nicotiana tabacum* L. is widely used as a host plant in plant pathology (Creager *et al.*, 1999; Fluhr, 2001) and biotechnology research (Fiedler and Conrad, 1995; McCormick *et al.*, 1999; Scholthof, 2004). *N. tabacum* has been observed to wilt when waterlogged under both field (Hunt *et al.*, 1981; Kramer and Boyer, 1995) and greenhouse conditions (Kramer and Jackson, 1954; Willey, 1970). A pilot experiment with *Nicotiana glutinosa* L. demonstrated a similar response to waterlogging as

N. tabacum, consequently ease of access and availability lead me to use *N. glutinosa* instead of *N. tabacum*. *N. glutinosa* seeds were planted in soil mix, seedlings were pricked out into 1000 mm² plastic pots 14 days after sowing (DAS), and transferred into 200 mm diameter pots approximately 40 DAS. All experiments were performed on *N. glutinosa* 63 – 80 DAS when plants were flowering.

Glycine max L. (soybean) experiments were performed as a direct comparison to *N. glutinosa* to compare waterlogging tolerances between species using the same greenhouse conditions. Soybean physiological measurements during waterlogging conditions have been recorded in the greenhouse (Bacanamwo and Purcell, 1999) and in the field (Evans *et al.*, 1990; Heatherly and Pringle, 1991).

In experiments 4 and 6 (Table 3.1) soybean seeds were washed in commercial bleach, rinsed in deionised water and then germinated in a Petri-dish containing filter paper that was kept continuously moist. After germination (at 7 days) two seeds were planted in each 1000 mm² plastic pot (Figure 3.1) and seedlings were transplanted into 200 mm diameter pots 19 DAS. Experiment 6 (Table 3.1) began 54 DAS with 20 plants (results in Section 3.11 of this Chapter).



Figure 3.1: Soybean seedlings growing in 1000 mm² plastic pots in University of California soil mix.

3.2 SOIL MIX

Coco mix was initially used as the potting medium in *N. glutinosa* experiments 1, 2 and 3, totalling 36 plants (Table 3.1). This mix contained coco peat, Waikerie sand, dolomite lime, agricultural lime, hydrated lime, gypsum, superphosphate, iron sulphate, iron chelate, MicroMax[®] (a nutrient blend; <http://www.scottsaustralia.com.au/miracle-gro.aspx>), calcium nitrate and Osmocote[®] (a controlled release plant fertiliser; <http://www.scottsaustralia.com.au/osmocote.aspx>). Large fibres within the coco mix caused root cleaning to be extremely time-consuming and impractical for studies with multiple plants. University of California soil mix (UC mix) was used in all subsequent experiments. The high sand content in UC mix made it much easier to clean from plant roots. UC mix contained Waikerie sand, peat moss, hydrated lime, agricultural lime and Osmocote[®].

3.3 PLANT MAINTENANCE

Prior to the onset of waterlogging in each experiment all plants were watered daily. During the waterlogging period only freely drained (control) plants were watered daily. Waterlogged plants were not watered since applying fresh water could re-oxygenate the root zone and reduce any physiological effects of waterlogging.

For the initial pilot experiment (experiment 1, Table 3.1) 12 plants were grown in 1000 mm² plastic pots, however the soil water holding capacity was less than evapotranspiration, quickly resulting in water deficit of all plants. Subsequently, 200 mm diameter plastic pots, providing extra soil volume and holding more plant available water, were used for all subsequent experiments.

During experiment 6 (Table 3.1) soybean was treated for a thrip infestation 30 DAS. To control this infestation the greenhouse was fumigated three times in 1 week with dichlorvos.

3.4 GREENHOUSE TEMPERATURES

Plants were grown in greenhouses at the South Australian Research and Development Institute (SARDI) at the Waite Campus, Urrbrae, South Australia; 34.9670° S, 138.6360° E. Temperatures over a period of 5 days were recorded within the greenhouse and compared to outside temperatures during experiment 6 (Table 3.1). Greenhouse temperatures were recorded using a Gemini Data Loggers Tinytag Transit 2 sensor. The Bureau of Meteorology Kent Town station (station number 023090) was used for outside temperatures (Meteorology). Although the day and night maximum and minimum temperatures within the greenhouse varied between 20 to 25 °C they were moderated relative to the fluctuations observed in outside temperatures (Figure 3.2). Temperature sensors weren't continuously available and temperatures were only recorded during experiment 6.

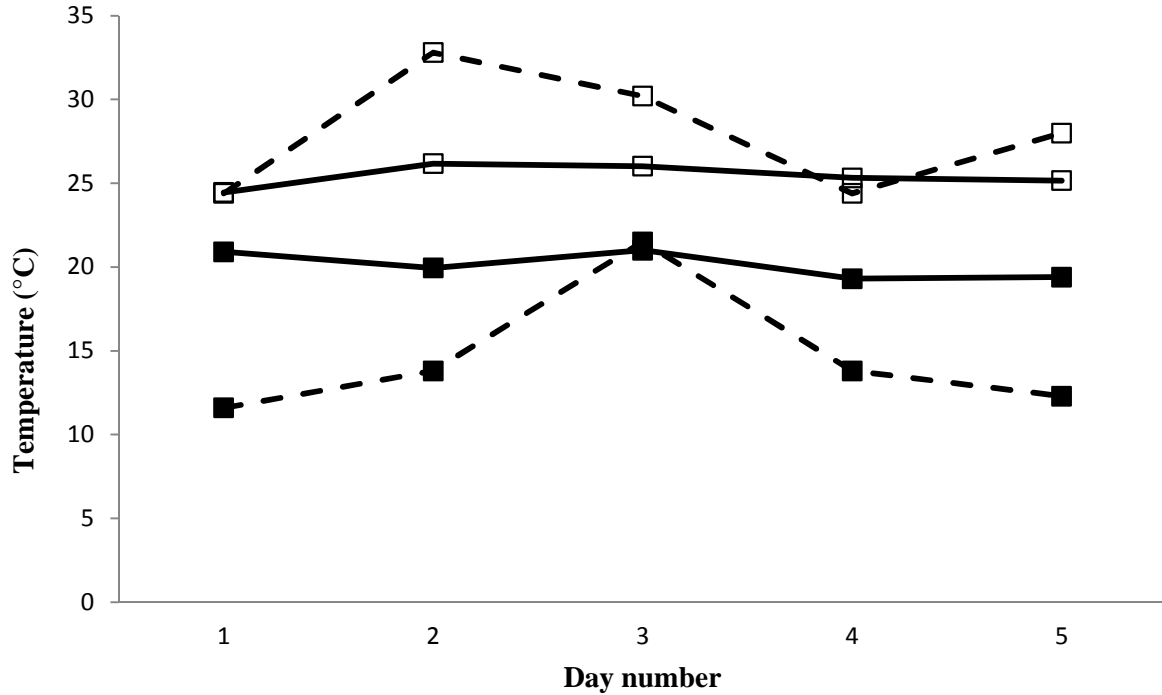


Figure 3.2: Daily minimum (filled markers) and maximum (unfilled markers) greenhouse temperatures (continuous lines) compared to outside temperatures (dashed lines) recorded over 5 days in December 2012 during experiment 6 (Table 3.1).

3.5 SOIL OXYGEN CONCENTRATION MEASUREMENTS

Measuring soil oxygen concentration is important when simulating waterlogging. Often waterlogging is defined as the soil having less than 10 % air filled pore space (Grable, 1966; Moore and McFarlane, 2004) (see Chapter 4 and 5 for more details). The soil oxygen concentration for the experiments reported in this thesis was measured in the soil mix, and the oxygen concentration logged every hour using an ICT International soil oxygen sensor and data logger. The sensor measured oxygen density within the soil in millivolts, which was converted to a percentage of atmospheric oxygen concentration. Before beginning experiments the sensors were calibrated. Calibration was done using the sensors linear voltage readings with oxygen concentrations of zero, atmospheric oxygen (20.9 %) and a

calculated midpoint. The measured soil oxygen concentrations were downloaded from the data logger using ICT International's software. Before beginning experiments with plants (Table 3.1) oxygen concentration measurements were successfully performed in saturated soil conditions (without plants) to check the sensitivity of the sensor for measuring oxygen concentrations in waterlogged conditions (Figure 3.3). This was the first time ICT International's soil oxygen sensors were used for measuring oxygen concentrations in hypoxic conditions, consequently a case study was written summarising my initial experiments and circulated to their client base (Forster, 2012).

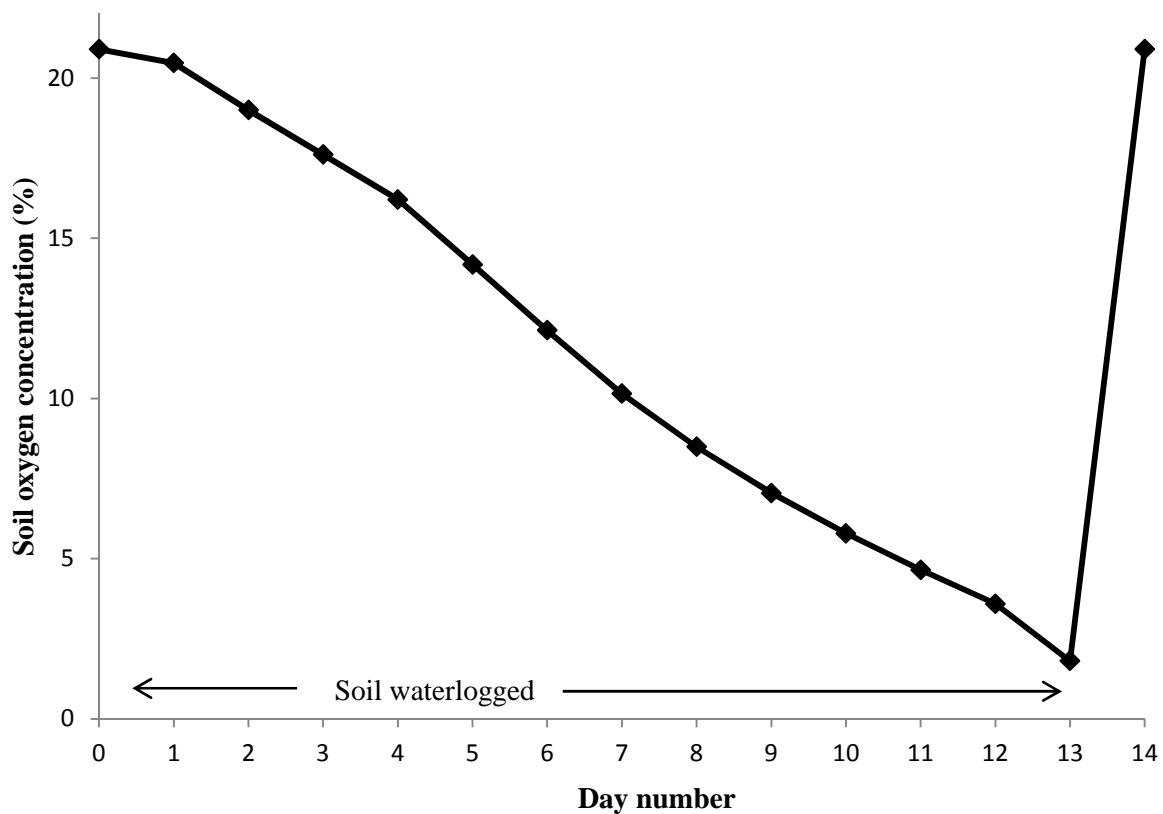


Figure 3.3: Soil oxygen concentration (%) measured in coco peat in pots (no plants) in the greenhouse. Soil was waterlogged on day 0 and drained on day 13. Soil oxygen concentration was 1.81 % on day 13.

3.6 STOMATAL CONDUCTANCE MEASUREMENTS

For all experiments (Table 3.1) stomatal conductance (g_s) was measured around 1200 h each day for consistency, using cycling porometry (Monteith *et al.*, 1988) with a Delta T Devices AP4 Porometer. A calibration plate was constructed more than 1 h before calibrating, as per the calibration instructions in the porometer manual (Bragg *et al.*, 2004). Calibration plates lasted up to 3 days before a fresh plate was required. Calibration was performed daily. The Bureau of Meteorology Kent Town station (station number 023090) daily barometric pressures were used as part of the calibration process (Meteorology, 2014).

Light intensity (measured in $\mu\text{mol m}^{-2} \text{s}^{-1}$) was recorded for each g_s measurement. The porometer head was equipped with a gallium arsenide phosphide (GaAsP) photodiode designed to estimate leaf irradiance between each measurement. It was important that light intensity remained reasonably constant for each leaf measurement to ensure differences in g_s weren't due to variations in light intensity (Figure 3.4).

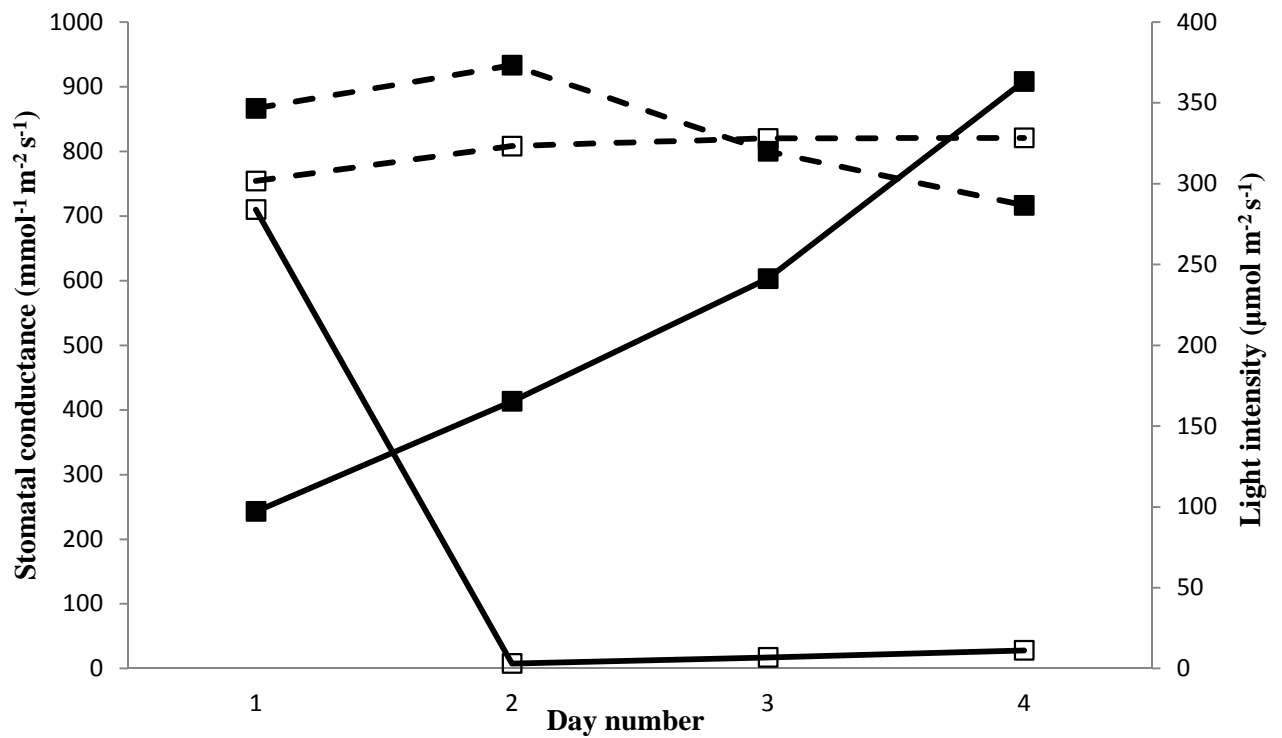


Figure 3.4: An example of stomatal conductance (continuous line) and light intensity (dashed line) measured coincidentally for freely drained (filled markers) and waterlogged (unfilled markers) plants measured over 4 days ($n = 3$ performed on one plant) during experiment 7 (Table 3.1).

3.7 ROOT AND LEAF HYDRAULIC CONDUCTANCE MEASUREMENTS

A Dynamax Incorporated Hydraulic Conductance Flow Meter (HCFM) was used to measure root (L_o) and leaf (L_{leaf}) hydraulic conductance (see Chapter 4 Materials and Methods for details) (Tyree *et al.*, 1995). Root hydraulic conductance (L_o) was measured in experiments 2, 3, 4, 5, 6, and 7, for a total of 76 plants. Leaf hydraulic conductance (L_{leaf}) was measured in experiment 8, for 23 plants (Table 3.1). The HCFM pumped water into the root or leaf at varying pressures and measured the rate of water flow (measured in $\text{kg s}^{-1} \times 10^{-6}$) through the root (Figure 3.5) or leaf. The slope of flow against pressure was normalised relative to dry

matter or leaf area to give hydraulic conductance (L_o measured in $\text{kg s}^{-1} \text{MPa}^{-1} \text{g}^{-1}$, L_{leaf} measured in $\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-2}$).

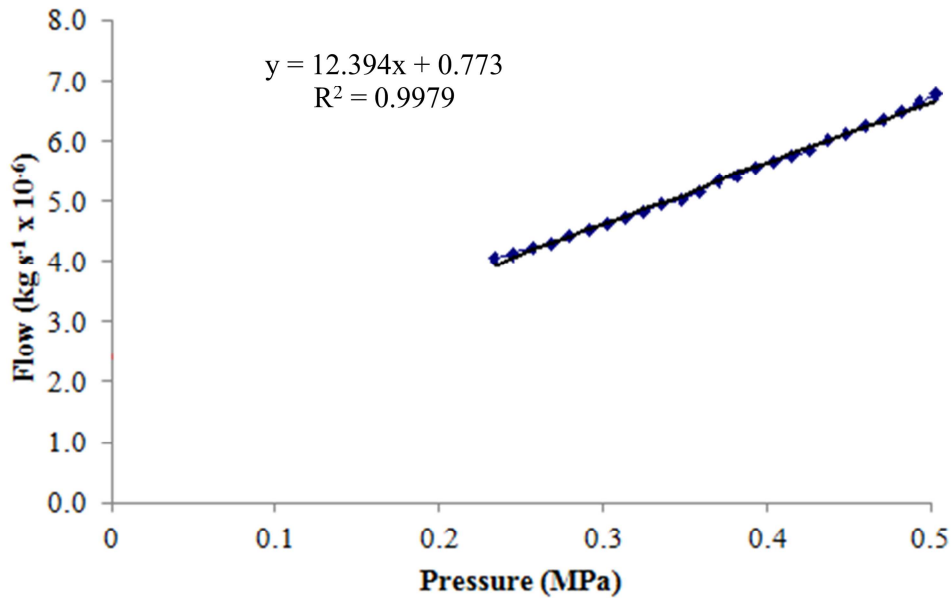


Figure 3.5: Example HCFM output for roots.

Root hydraulic conductance can be normalised by root surface area (m^2) or root dry weight (g). Root surface area (cm^2), average root diameter (mm), total root length (cm) and number of root tips were measured using a high resolution scanner (600 dpi) and Regent Instruments WinRhizo software for experiments 1 and 2 (Table 3.1), for a total of 24 plants. Roots were stained with 1 part methylene blue to 1000 parts water then mounted in a thin film of water in a tray on the scanner (Figure 3.6). Whilst accurate for plants with small root length this method proved impractical, from both a time and technical perspective as agreed by Bauhus and Messier (1999). Both soybean and *N. glutinosa* plants developed substantive root length by the time measurements were made. Consequently L_o was normalised using root dry weight. The roots were dried in an oven for 24 h at 85°C and weighed with a Sartorius

BP4105 balance. Leaf hydraulic conductance (L_{leaf}) was normalised by leaf area (Section 3.9).

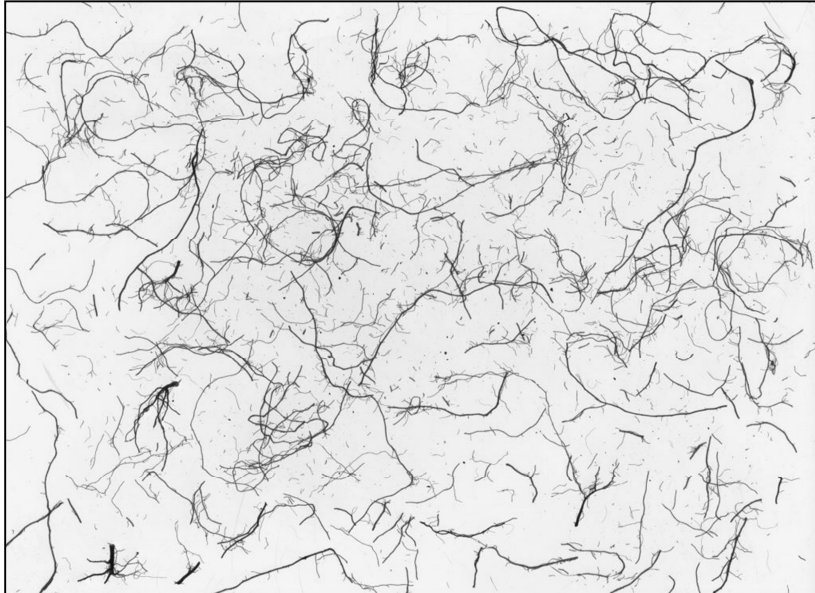


Figure 3.6: One of three trays of *N. glutinosa* roots for a single plant scanned with a high resolution scanner and evaluated using WinRhizo software.

3.8 DETAILED CALCULATIONS OF HYDRAULIC CONDUCTANCE FOR WHOLE PLANTS

Hydraulic conductance is calculated for *N. glutinosa* plants in experiment 5 (Table 3.1) using measured stomatal conductance and leaf water potential values (the method for measuring leaf water potential is described in Chapter 4). Porometer theory (Bragg *et al.*, 2004) uses the relationship:

$$E = \frac{\delta\phi}{r} \quad (3.1)$$

where E ($\text{mol m}^{-2} \text{s}^{-1}$) is the flux density of water vapour, $\delta\phi$ (mol m^{-3}) is the concentration difference across the resistance r (s m^{-1}).

The concentration difference across the resistance can be expressed as:

$$\delta\phi = \left[\frac{\text{mol}_{\text{H}_2\text{O}}}{\text{mol}_{\text{air (saturated)}}} - \frac{\text{mol}_{\text{H}_2\text{O}}}{\text{mol}_{\text{air (unsaturated)}}} \right] \quad (3.2)$$

By expressing the concentration in dimensionless units of mole of water vapour per mole of air (mol mol^{-1}) the unit of resistance becomes $\text{m}^2 \text{s mol}^{-1}$.

Conductance to water vapour pressure loss is derived from Fick's Law of diffusion (Percy *et al.*, 1989):

$$g_w = \frac{E}{\Delta W} \quad (3.3)$$

where g_w ($\text{mmol m}^{-2} \text{s}^{-1}$) is the water vapour loss (stomatal conductance) measured, E ($\text{mmol m}^{-2} \text{s}^{-1}$) is the evapotranspiration and ΔW (Pa Pa^{-1}) is the water concentration gradient.

Rearranging Equation 3.3:

$$E = g_w \times \Delta W \quad (3.4)$$

Past experiments suggest the assumption of water vapour saturation in the intercellular spaces near the cell walls for well watered plants is valid (Sharkey *et al.*, 1982) therefore:

$$\Delta W = w_i - w_o \quad (3.5)$$

where w_i (Pa Pa^{-1}) is calculated from the saturated vapour pressure at the leaf temperature and w_o (Pa Pa^{-1}) is found using the relative humidity and the saturated vapour pressure at air temperature. Saturated vapour pressure values (used to calculate w_i and w_o) are found using the Goff-Gratch formulation at specific temperatures (Percy *et al.*, 1989).

Using Dalton's law of partial pressures to calculate both w_i and w_o :

$$w_i = \frac{v_{w \text{ sat leaf}}}{P} \quad (3.6)$$

where $v_{w \text{ sat leaf}}$ (kPa) is the saturated vapour pressure at leaf temperature and P (hPa) is the atmospheric pressure. Leaf temperature (in $^{\circ}\text{C}$) is calculated using measurements obtained

from the porometer. The porometer measures the cup temperature (T_{cup}) and the cup temperature minus leaf temperature (T_{leaf}) as ($T_{\text{cup}} - T_{\text{leaf}}$). From this, actual leaf temperature is derived as:

$$\text{Leaf Temperature} = - (T_{\text{cup}} - T_{\text{leaf}}) + T_{\text{cup}} \quad (3.7)$$

w_o is calculated as:

$$w_o = RH \left(\frac{V_{\text{w sat air}}}{P \times 100} \right) \quad (3.8)$$

where RH (%) is the relative humidity, assumed to be 40 %, and $V_{\text{w sat air}}$ (kPa) is the saturated vapour pressure at air temperature. Air temperature is taken to be the mean leaf temperature for each day of measurement.

Plant conductivity ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) is calculated by:

$$\text{Plant conductivity} = \frac{E}{\Delta\psi} \quad (3.9)$$

where:

$$\Delta\psi = \psi_{\text{soil}} - \psi_{\text{leaf}} \quad (3.10)$$

$\Delta\psi$ (MPa) is the difference in water potential between the saturated soil ($\psi_{\text{soil}} = 0$) and the leaf water potential (ψ_{leaf}). The leaf water potential (in bar) was measured using a PMS Instrument Company model 1000 pressure chamber (Albany, OR, USA).

Plant conductivity is normalised by total plant leaf area (m^2) to derive plant conductance ($\text{mmol s}^{-1} \text{MPa}^{-1}$):

$$\text{Plant conductance} = \text{Plant conductivity} \times \text{Total plant leaf area} \quad (3.11)$$

Root hydraulic conductivity ($\text{mmol s}^{-1} \text{MPa}^{-1} \text{g}^{-1}$) is found using:

$$\text{Root conductivity} = \frac{\text{Plant conductance}}{\text{Root dry weight}} \quad (3.12)$$

where *Root dry weight* (g) is measured.

To convert into a root hydraulic conductance value that directly compares to measured values:

$$\begin{aligned} & \text{Root conductivity (kg s}^{-1} \text{ MPa}^{-1} \text{ g}^{-1}) \\ &= \frac{\text{Root conductivity (mmol s}^{-1} \text{ MPa}^{-1} \text{ g}^{-1}) \times \text{Molecular weight of water (kg mol}^{-1})}{1000} \end{aligned} \quad (3.13)$$

Results comparing this method to measured root hydraulic conductance values from experiment 5 (Table 3.1) are given in Chapter 4 (Figure 4.8).

3.9 MEASURING LEAF AREA

A LI-COR leaf area meter (LI-3000C) with conveyer belt (LI-3050C) was used during experiments 1 and 2 (Table 3.1) to measure leaf area of twenty four *N. glutinosa* plants. Unfortunately the scanning head of the meter when mounted in the conveyer proved too narrow for the bulky *N. glutinosa* leaves to fit through. Additionally *N. glutinosa* leaves deposited a sticky residue on the conveyer belt. Hence in subsequent experiments leaves were mounted between two transparent sheets, scanned at 300 dpi and analysed in Adobe Photoshop CS6. The histogram function within Photoshop was used to highlight leaf areas. The number of pixels were counted within the highlighted areas and converted to calculate leaf area (m²).

3.10 STATISTICAL ANALYSIS

For all experiments measured values were assessed using standard error of the mean (SEM) in Microsoft Excel, n representing sample size. Two tailed, two sample t-tests were performed assuming either equal or unequal variance (depending on sample size), also in Microsoft Excel. Treated and untreated plants were compared within the same experimental batch at the same number of days after sowing for comparable plant sizes within a greenhouse.

3.11 SOYBEAN WATERLOGGING EXPERIMENT

This section provides results of experiment 6 (Table 3.1). Experiment 6 measured the physiological responses and adaptations to waterlogging of *Glycine max* L. (soybean). Experiment 6 repeated the methods used with *Nicotiana glutinosa* L. in experiment 5. Results for experiment 5 are in Chapter 4. Experiment 5 and 6 were performed under similar edaphic and environmental conditions to compare waterlogging tolerances between species.

Although plant responses and adaptations to waterlogging have been observed in the field and in greenhouses for a variety of species there is a lack of consistently measured data for direct comparisons of plant tolerances to waterlogging (see Chapter 2 for details). Bennett and Albrecht (1984) found no changes to leaf water potential or signs of visible plant stress when waterlogging soybean for 14 days in a greenhouse. Board *et al.* (1998) found a reduction in yield when waterlogging soybean in a greenhouse and in the field. Several investigations report reduced soybean yield due to waterlogged conditions in the field, thus soybean has been labelled as susceptible to waterlogging (Evans *et al.*, 1990; Oosterhuis *et al.*, 1990; Heatherly and Pringle, 1991; Bacanamwo and Purcell, 1999).

Since there was wide variation in the literature on waterlogging experiments with regard to different measured variables and hence variation in the interpretation of results (Chapter 2), a more unifying approach was adopted for this thesis. Experiments were performed in the same size pots, same soil mix and under the same waterlogging conditions to directly compare the waterlogging tolerance of soybean (experiment 6, Table 3.1) to *N. glutinosa* (experiment 5, Table 3.1). Soil oxygen concentrations, leaf water potential (ψ_{leaf}), leaf area, stomatal conductance (g_s), root hydraulic conductance (L_o) and shoot and root dry weights were measured for waterlogged and freely drained soybean over a 5 day period with a total of 20 plants.

Leaf water potential (ψ_{leaf}) (Figure 3.7) measurements of waterlogged soybean indicated waterlogged plants had generally higher (less negative) values than freely drained plants. During 5 days of waterlogging, plants were more hydrated with excess water in the root zone, showing significant differences on days 1, 2 and 5 (using a two tailed, two sample t-test assuming equal variance in Microsoft Excel).

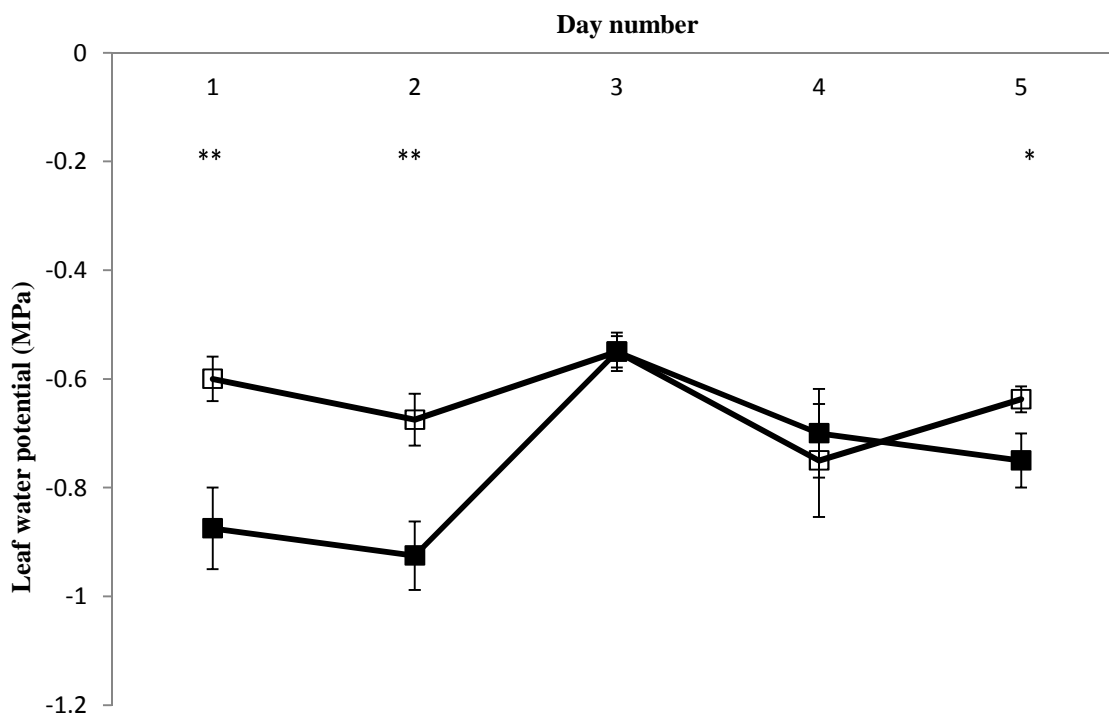


Figure 3.7: Leaf water potential (ψ_{leaf}) for waterlogged (unfilled markers) and freely drained (filled markers) soybean. Bars represent SEM (n = 4 from two plants). ** represents $p < 0.01$, * represents $p < 0.05$ significant differences between waterlogged and freely drained plants.

Leaf area (Figure 3.8) over 5 days of waterlogging showed no significant differences (using a two tailed, two sample t-test assuming equal variance in Microsoft Excel) for waterlogged compared to freely drained plants.

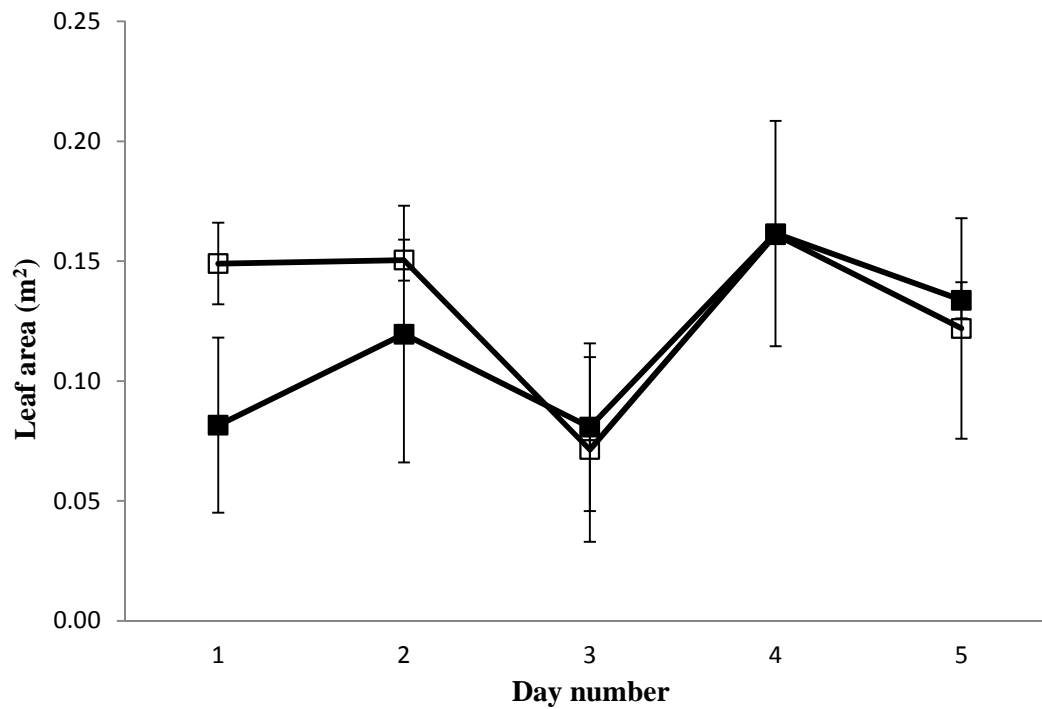


Figure 3.8: Leaf area for waterlogged (unfilled markers) and freely drained (filled markers) soybean. Bars represent SEM (n = 2 from two plants).

Stomatal conductance (g_s) trended higher for waterlogged plants (Figure 3.9) indicating waterlogged plants had higher evapotranspiration rates (Else *et al.*, 1995). The trend was significantly different on day 3 and day 5 (using a two tailed, two sample t-test assuming equal variance in Microsoft Excel). Measured root hydraulic conductance (L_o) showed no significant differences between waterlogged and freely drained soybean until day 5 (Figure 3.10) (using a two tailed, two sample t-test assuming unequal variance in Microsoft Excel) when L_o for waterlogged plants was lower than L_o for freely drained plants.

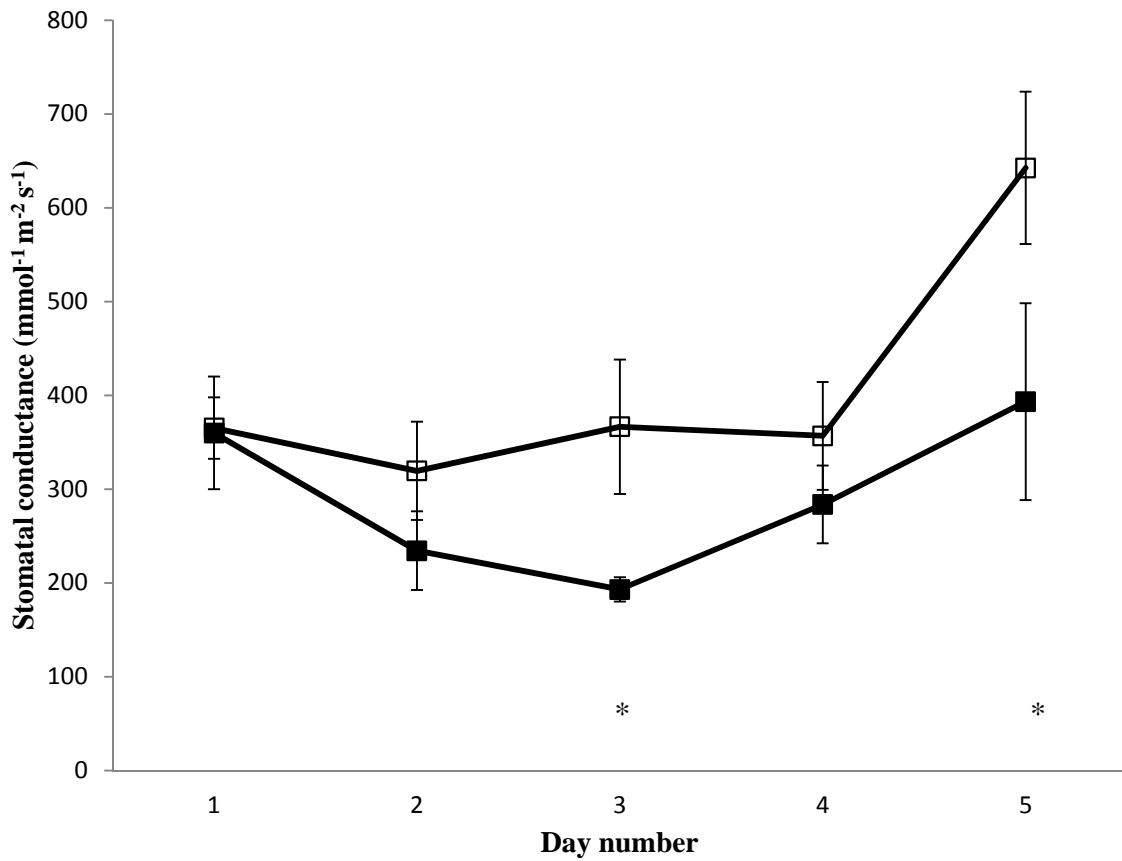


Figure 3.9: Stomatal conductance (g_s) for waterlogged (unfilled markers) and freely drained (filled markers) soybean. Bars represent SEM ($n = 6$ from two plants). * represents $p < 0.05$ significant differences between waterlogged and freely drained plants.

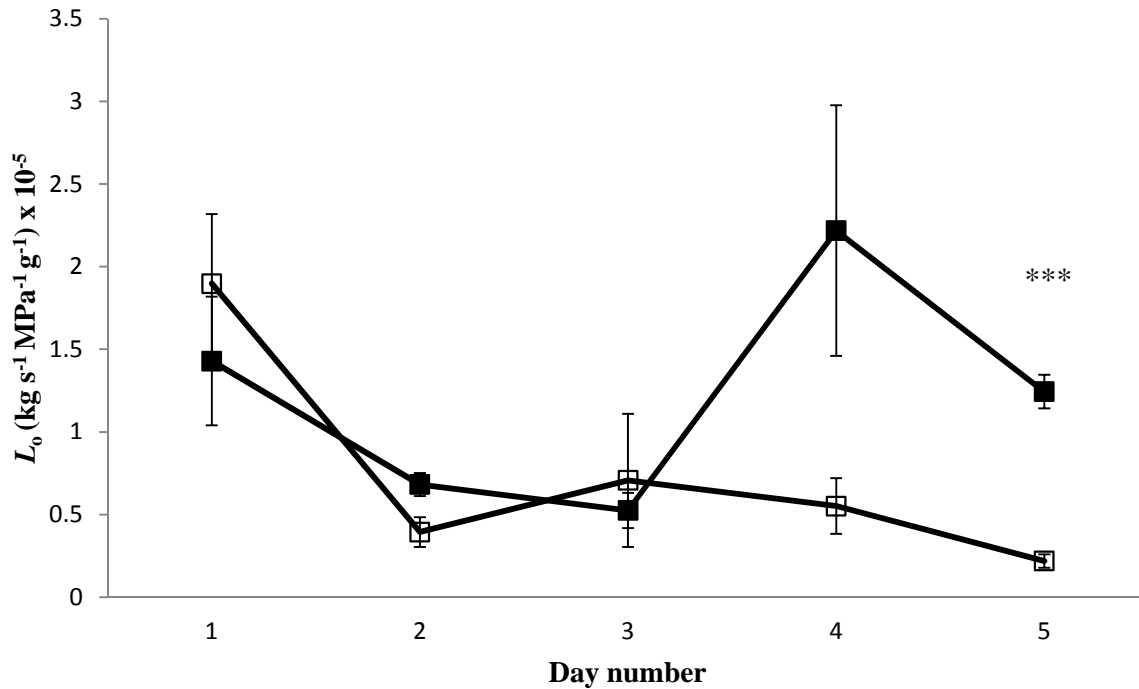


Figure 3.10: Measured root hydraulic conductance (L_0) for waterlogged (unfilled markers) and freely drained (filled markers) soybean. Bars represent SEM (n = 3 to 6 from two plants). *** represents $p < 0.001$ on day 5 showing significant differences between waterlogged and freely drained plants.

Shoot dry weights (Figure 3.11) of waterlogged compared to freely drained plants were not significantly different over 5 days of waterlogging (using a two tailed, two sample t-test assuming equal variance in Microsoft Excel). Root dry weights (Figure 3.12) were also not significantly different until day 5 (using a two tailed, two sample t-test assuming equal variance in Microsoft Excel) when the root dry weight of waterlogged plants was less than freely drained plants. Adventitious roots were visible on soybean stems on day 4 of waterlogging (Figure 3.13). No wilting (loss of turgor) was seen in soybean leaves, even up to 17 days of waterlogging (Figure 3.14).

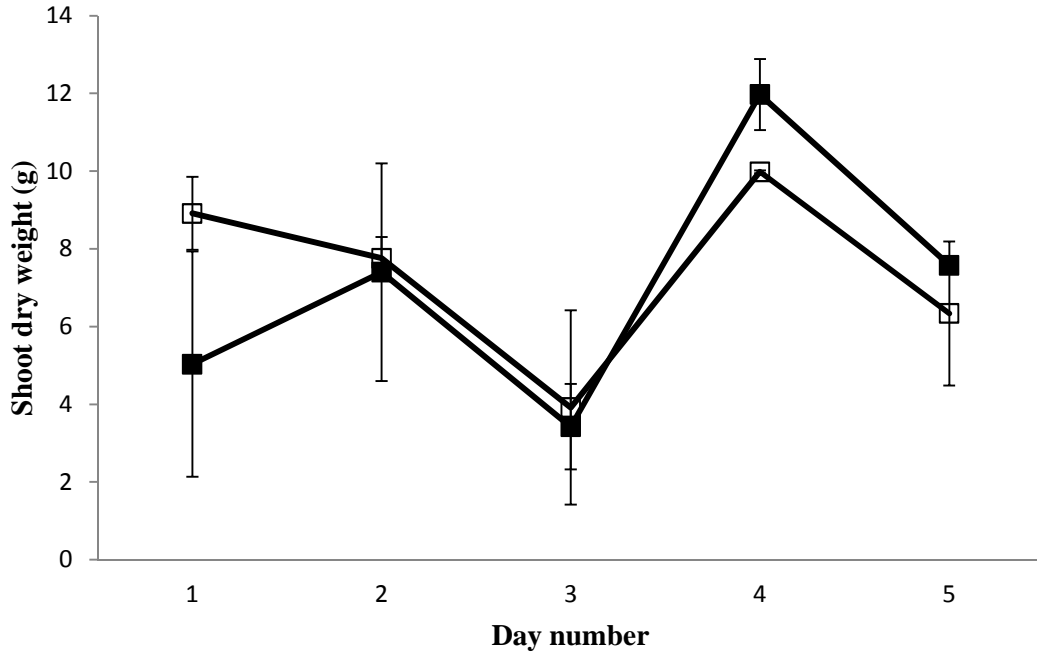


Figure 3.11: Shoot dry weight for waterlogged (unfilled markers) and freely drained (filled markers) soybean. Bars represent SEM (n = 2 from two plants).

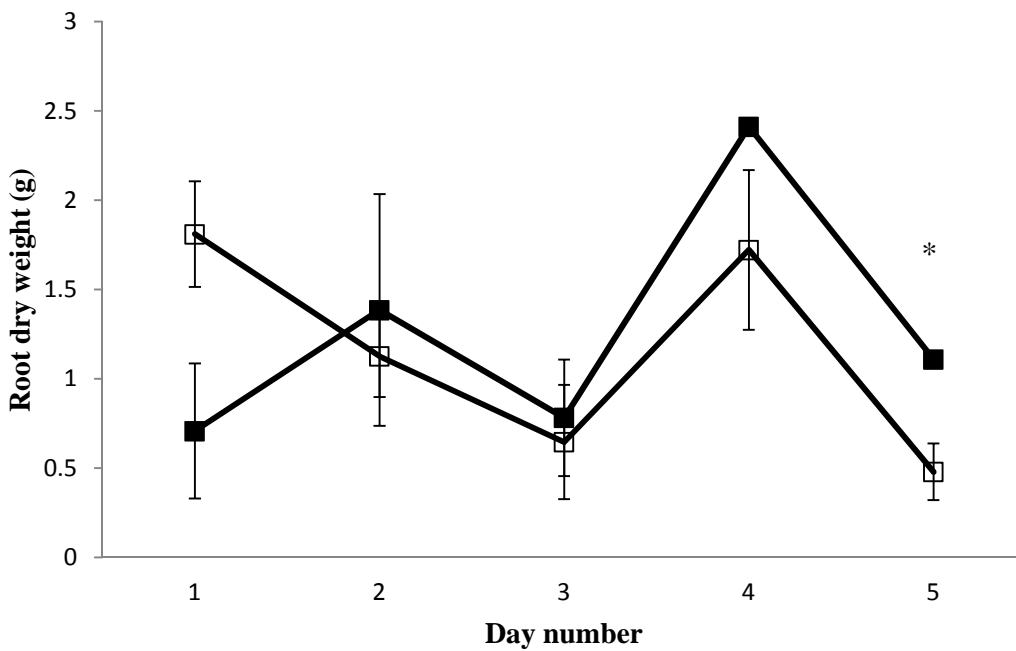


Figure 3.12: Root dry weight for waterlogged (unfilled markers) and freely drained (filled markers) soybean. Bars represent SEM (n = 2 from two plants). * represents $p < 0.05$ on day 5.



Figure 3.13: Adventitious roots developing on the stem of soybean just above the water level on day 4 of waterlogging.

Leaf water potential (Figure 3.7) and g_s (Figure 3.9) results indicate increased water transport to leaves of soybean under waterlogged conditions compared to freely drained soybean and waterlogged *N. glutinosa* (Chapter 4). Above ground plant growth of waterlogged compared to freely drained soybean exhibited no differences in leaf area (Figure 3.8) or shoot dry weights (Figure 3.11). Below ground plant function showed changes between waterlogged and freely drained soybean on day 5. On day 5 of waterlogging, root dry weight (Figure 3.12) and L_o (Figure 3.10) were both reduced compared to freely drained soybean. Repeat experiments that waterlog soybean for more than 5 days would be required to verify a significant reduction in root dry weight and L_o at 5 days of waterlogging. The formation of adventitious roots (Figure 3.13) indicates the plants are adapting to waterlogged conditions, forming roots that have access to atmospheric oxygen to maintain plant function (Chapter 2).

A single plant was waterlogged for 17 days showing no signs of leaf wilting, but the soil did show signs of hypoxic conditions (Chapter 2) with the smell of hydrogen sulfide (Ponnamperuma, 1972; Bennett and Albrecht, 1984) and a visible film on the water surface (Figure 3.14).

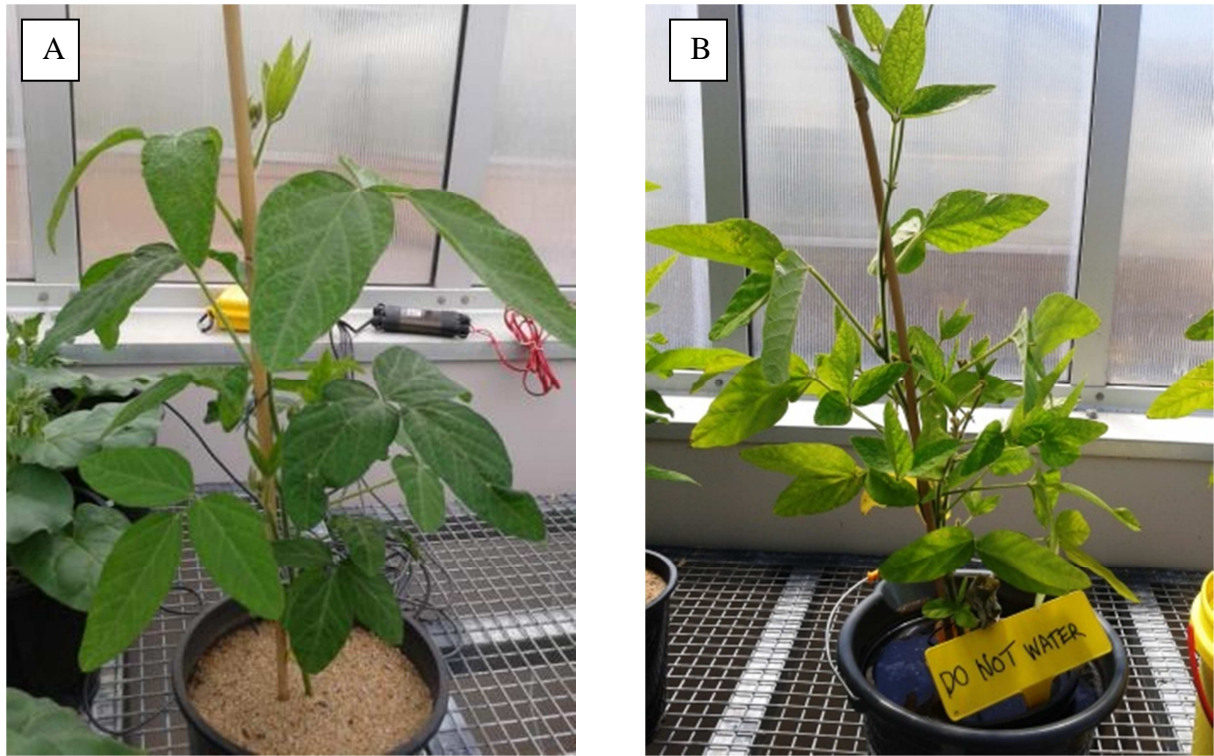


Figure 3.14: (A) Soybean before waterlogging and (B) after 17 days of waterlogging, showing no signs of leaf wilting, but signs of hypoxic conditions in the soil.

3.12 CONCLUSION

Directly comparing waterlogged soybean to *N. glutinosa* (results in Chapter 4) indicates that soybean is more tolerant to waterlogging than *N. glutinosa* and the response of leaf wilting to waterlogging is not consistent across all plant species. My results confirm that waterlogging causes or results in a variety of plant responses and adaptations, dependent upon plant species. This understanding has been used to determine an empirical representation of plant adaptations to waterlogging (Chapter 5) and to incorporate the effects of waterlogging on

individual plant species into the crop growth and yield simulation model SWAGMAN
Destiny (Chapter 6).

CHAPTER 4

BEHAVIOUR OF PLANT AND ROOT
HYDRAULIC CONDUCTANCE DURING
WATERLOGGING IN
NICOTIANA GLUTINOSA L.

4 CHAPTER 4

Chapter 4 details greenhouse experiments using *Nicotiana glutinosa* L. to understand the sequence of plant responses and adaptations during waterlogging. This chapter examines plant function during waterlogging and is used to construct an empirical representation of waterlogging in Chapter 5 for use in a crop growth and yield simulation model in Chapter 6.

Additionally the results of waterlogging *N. glutinosa* in this Chapter can be directly compared to the results from waterlogging soybean (Section 3.11) in Chapter 3 as similar conditions were used.

4.1 INTRODUCTION

There has been a reported increase in extreme precipitation events due to climate change (Rosenzweig *et al.*, 2002; Tubiello *et al.*, 2007; Hartmann *et al.*, 2013). This is likely to result in increased flooding and waterlogging, negatively affecting productivity of farmland (Bailey-Serres and Voesenek, 2008; Shaw *et al.*, 2013). In the United States estimated agricultural production loss from excessive soil water (waterlogging) associated with climate change could be up to US\$3 billion per year by 2030 (Rosenzweig *et al.*, 2002).

Soil waterlogging occurs when the rate of incoming water exceeds the outgoing drainage rate, most commonly due to poor flow within the soil profile (Cannell, 1977). This condition is widespread with approximately 10 % of the global land area, and up to 20 % of some particular areas, affected by waterlogging due to poor soil drainage (Setter and Waters, 2003).

Water displaces air in the pore spaces of the soil matrix, the amount of oxygen present decreases, the soil and plant gas exchange changes and plant growth and development is affected (Ponnamperuma, 1972). As the amount of oxygen decreases due to gas displacement by water and by respiratory consumption, the oxygen status is variously described as hypoxic (low soil oxygen concentration) and eventually anoxic (zero soil oxygen concentration).

Different plant species respond variably to reduced soil oxygen and experiments have attempted to determine the critical oxygen point, the minimum oxygen concentration required before plants begin to show signs of stress (Girton, 1979; Saglio *et al.*, 1984). Soil oxygen status has been measured and reported in a variety of ways; as oxygen partial pressures below which oxygen consumption rates are inhibited (Drew, 1997), as oxygen concentrations in soil (in $\text{mg}_{\text{oxygen}} \text{L}^{-1}_{\text{soil}}$) (Meyer *et al.*, 1985), as an oxygen diffusion rate (in $\text{g}_{\text{oxygen}} \text{cm}^{-2} \text{min}^{-1}$) (Bertrand and Kohnke, 1957) or as a volume of air filled pore space within the soil matrix (Grable, 1966; Moore and McFarlane, 2004) and is generally reported to be between 10 % to 15 % by volume of normal oxygen concentration of atmospheric air at 25 °C.

When root systems are oxygen deprived from waterlogging in soil, one curious response is the observation that some species exhibit wilted leaves (Kramer and Jackson, 1954; Else *et al.*, 2001). This suggests that hydraulic conductance through the plant and most likely in the roots has decreased substantially, perhaps as the result of disruption in the normal signalling that adjusts shoot evapotranspiration to the capacity of the root system to transport water (Chaumont and Tyerman, 2014). Thus far there has been little research reported that examines the relationship between root hydraulic conductance and leaf wilting during waterlogging (Else *et al.*, 2001).

The appearance of wilting in waterlogged plants has been ascribed to effects of ethylene production by roots and an insufficiency of water to maintain leaf turgor (through a reduction of root water uptake) (Cannell and Jackson, 1981; Bramley and Tyerman, 2010). Recent research has shown that root hydraulic conductance is substantially moderated by water conducting proteins called aquaporins (Maurel, 1997). Aquaporins can rapidly change the water permeability of cell membranes by mechanisms including gating (opening and closing) and insertion or withdrawal from the membrane (Chaumont and Tyerman, 2014). A reduction in root hydraulic conductance may suggest a decrease in aquaporin activity (Aroca *et al.*,

2012), but anatomical changes can also have a large effect on hydraulic conductance (Bramley *et al.*, 2009). It has also been shown that ethylene can have an effect on the activity of aquaporins, increasing cell permeability (Kamaluddin and Zwiazek, 2002; Chervin *et al.*, 2008; Tungngoen *et al.*, 2009).

The observation by Kramer and Jackson (1954) of leaf wilting by *Nicotiana tabacum* L. when subjected to waterlogging has not been satisfactorily explained. The response of leaf wilting, yellowing and death during waterlogging has been observed in both field (Kramer and Jackson, 1954; Hunt *et al.*, 1981; Kramer and Boyer, 1995) and greenhouse conditions (Kramer and Jackson, 1954; Willey, 1970). A preliminary experiment with *Nicotiana glutinosa* L. showed that this species responded similarly to *N. tabacum* and so *N. glutinosa* was subsequently used in this study. The purpose was to develop a better understanding of the relationship between hypoxic root zone conditions, leaf wilting, and plant hydraulic conductance during waterlogging. The kinetics of responses to waterlogging were examined using several greenhouse experiments with potted *N. glutinosa*. Observations of soil oxygen, plant growth, stomatal conductance, leaf water potential, hydraulic conductances, and element and ion concentrations of leaf xylem were used to propose a sequence of responses that may explain plant water (deficit) stress with waterlogged conditions.

4.2 METHODS

4.2.1 Experimental design

Experiments were conducted with a total of seventy eight *N. glutinosa* plants grown in a glasshouse and using staggered planting dates in order to measure different parameters at the same growth stage. Plants were grown at day lengths ranging from 10.5 to 13 h at temperatures from 15 to 25 °C. All plants were grown in University of California soil mix (Waikerie sand, peat moss, hydrated lime, agricultural lime and mini Osmocote) in 200 mm

diameter pots. Over a series of experiments, a total of 40 plants were waterlogged for up to 5 days by placing pots in a bucket of water with the water level 10 mm above the soil. No additional water was added to the waterlogged plants to prevent aerated water replenishing the root zone oxygen concentration. Thirty eight control plants were watered daily and allowed to freely drain. Waterlogging started 63 – 80 days after sowing (DAS) and measurements of both the control and waterlogged plants occurred between 63 – 80 DAS when the plants were flowering. All measurements were performed around 1200 h for consistency.

4.2.2 Experimental Measurements

Leaf growth, stomatal conductance (g_s), leaf temperature and soil oxygen concentration ($[O_2]$) were measured throughout the life of the plants. Stomatal conductance and leaf temperature were measured for the fourth, fifth and sixth youngest leaves on each plant using a Delta T Devices AP4 Porometer (Cambridge, UK).

Soil oxygen concentration was measured within random pots and logged every hour using ICT International's (Armidale, NSW, Australia) soil oxygen sensor (ICT02) and data logger (SOM). The soil oxygen sensor was placed in the pot when planting so as not to disturb or damage roots at a later time.

Leaf growth, leaf area, shoot dry weight, root dry weight, leaf stomatal conductance (g_s), leaf temperature, leaf water potential (ψ_{leaf}), root hydraulic conductance (L_o), leaf hydraulic conductance (L_{leaf}) and $[O_2]$ were measured for two or three replicate pots for both freely drained (control) and waterlogged (treated) plants sequentially over 5 days over a number of experiments. Images were taken of root cross sections to identify if aerenchyma development occurred during the waterlogged days. On the last day of waterlogging, leaf area, shoot dry weight and root dry weight were measured. Soil was washed from the roots using water at

low pressure over a fine sieve. Scanned images of leaves were used to determine leaf area (m^2) and processed in Adobe Photoshop CS6 V13.0 x64 (Sydney, NSW, Australia). Shoot and roots were dried in an oven for 24 h at 85 °C and weighed with a Sartorius BP4105 balance (Germany).

Leaf water potential was measured using a PMS Instrument Company (Albany, OR, USA) model 1000 pressure chamber instrument and mean values for the fourth and fifth oldest leaves on each plant calculated. The leaf petiole was cut close to the stem and placed immediately into the pressure chamber. The chamber was pressurised with nitrogen until sap appeared at the cut surface of the petiole and the ψ_{leaf} measured.

Leaf xylem sap samples were collected for 8 plants (4 control, 4 waterlogged) over 4 sequential days while the leaves were under pressure within the pressure chamber. Enough pressure was applied to the leaves to exude 5 μL of sap in about 60 s. Xylem sap was tested for nitrate concentration using an RQ flex 10 Merck Nitrate Meter (Darmstadt, Germany). 5 μL of sap was diluted in 2 mL of distilled water and calcium, magnesium, sodium, potassium, phosphorus and sulphur concentrations were measured using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES) (performed at Waite Analytical Services, <http://www.adelaide.edu.au/was/>).

Root hydraulic conductance (L_o) and leaf hydraulic conductance (L_{leaf}) were measured using a Dynamax Hydraulic Conductance Flow Meter (HCFM) (Houston, TX, USA) (Tyree *et al.*, 1995). The HCFM forces water into the root system or leaf at varying pressures and measures the rate of water flow against pressure. The rate of flow was plotted against pressure and the slope normalised by the root dry weight (g) giving L_o (measured in $\text{kg}_{\text{water}} \text{s}^{-1} \text{MPa}^{-1} \text{g}^{-1}_{\text{root}}$). The mean of L_o was calculated from three measurements on each root system.

To measure L_{leaf} , leaves were cut under degassed deionised water leaving at least 30 mm of petiole for connection to the HCFM. A perfusion solution of filtered degassed 10 mM KCl

was used to minimise ionic effects on pit membrane conductance (Sack *et al.*, 2002; Pou *et al.*, 2013). Leaves were illuminated by a 400 W Sylvania MEYTALARC lamp (Sylvania Lighting Australasia Pty Ltd, Cavan, South Australia) providing approximately $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR). Leaves were immersed in a water bath during the measurements to prevent evapotranspiration and maintain temperature (Sack *et al.*, 2002). The water bath temperature was regulated to $21 \text{ }^{\circ}\text{C}$ (ambient temperature) to normalise for the temperature effects of the viscosity of water (Nardini *et al.*, 2005; Pou *et al.*, 2013). The slope of the rate of flow against pressure was normalised against leaf area (m^2) giving L_{leaf} (measured in $\text{kg}_{\text{water}} \text{s}^{-1} \text{MPa}^{-1} \text{m}^{-2}_{\text{leaf}}$) (Nardini *et al.*, 2005; Pou *et al.*, 2013). Leaf hydraulic conductance was measured three times for each leaf to get the mean. Root hydraulic conductance (L_o) and L_{leaf} were measured within 2 min of plant decapitation (for L_o) and leaf removal (for L_{leaf}) to minimise changes in L_o or L_{leaf} due to time (Vandeleur *et al.*, 2014).

For days 1 to 4 of waterlogging and for freely drained plants, root cross sections were processed and visually analysed for aerenchyma formation and changes in root structure. The cross sections of primary and first order lateral roots were imaged at the basal (10 – 20 mm from root/shoot junction), mid (centre of root length) and apical (within 30 mm of the root tip) regions. Sections of roots were prepared and embedded in an acrylic resin based on hydroxyethyl methacrylate at Adelaide Microscopy (<http://www.adelaide.edu.au/microscopy/>). $5 \mu\text{m}$ cross sections of roots within polymerised blocks were cut using a Leica RM2265 Rotary Microtome (Wetzlar, Germany). Sections were stained with 0.1 % toluidine blue and mounted with DPX Mountant. Cross sections were viewed and captured using an Olympus C-3030 Camedia camera and Olympus BH-2 BHT microscope, using Olympus Camedia Master and VideoPro 32 software (Center Valley, PA, USA).

4.2.3 Calculation of hydraulic conductance for whole plants

Described in detail in Section 3.8 and briefly below, hydraulic conductance for intact whole plants was obtained from measured leaf water potentials (ψ_{leaf} (MPa)) and a calculated evapotranspiration rate (E (mmol m⁻² s⁻¹)) from measured stomatal conductances (g_w (mmol m⁻² s⁻¹)) where:

$$E = g_w \times \Delta W \quad (4.1)$$

and the water concentration gradient (ΔW (Pa Pa⁻¹):

$$\Delta W = w_i - w_o \quad (4.2)$$

w_i was calculated from the saturated vapour pressure at the leaf temperature and w_o was found using the relative humidity and the saturated vapour pressure at air temperature. Saturated vapour pressure values (used to calculate w_i and w_o) were found using the Goff-Gratch formulation at specific temperatures (Percy *et al.*, 1989). Using Dalton's law of partial pressures to calculate both w_i and w_o :

$$w_i = \frac{v_{w \text{ sat leaf}}}{P} \quad (4.3)$$

where $v_{w \text{ sat leaf}}$ (kPa) was the saturated vapour pressure at leaf temperature and P (hPa) was the atmospheric pressure.

Plant conductivity (mmol m⁻² s⁻¹ MPa⁻¹) was calculated as:

$$\text{Plant conductivity} = \frac{E}{\Delta\psi} \quad (4.4)$$

where:

$$\Delta\psi = \psi_{\text{soil}} - \psi_{\text{leaf}} \quad (4.5)$$

$\Delta\psi$ (MPa) was the difference in water potential between the saturated soil ($\psi_{\text{soil}} = 0$) and the leaf water potential (ψ_{leaf}). The leaf water potential (in MPa) was measured using the pressure chamber.

Plant conductivity was normalised by multiplying by total plant leaf area (m²) to get plant conductance (mmol s⁻¹ MPa⁻¹):

$$\text{Plant conductance} = \text{Plant conductivity} \times \text{Total plant leaf area} \quad (4.6)$$

Plant conductance was normalised to root dry weight for comparison with HCFM measurements.

4.2.4 Statistical analysis

Measured values were assessed using standard error of the mean (SEM) in Microsoft Excel and n represents sample size. Two tailed, two sample t-tests were performed assuming either equal or unequal variance depending on sample size, also in Microsoft Excel. Leaf hydraulic conductance results were analysed using a 2 way ANOVA. ICPAES results were analysed using linear regression and comparison of slopes (treated versus untreated) with time. The 2 way ANOVA and linear regression were performed in GraphPad Prism (V6.0 GraphPad Software Inc., CA, USA). Treated and untreated plants were compared within the same experimental batch at the same number of days after sowing for comparable plant sizes within a greenhouse.

4.3 RESULTS

4.3.1 Soil effects

There was a linear reduction in soil oxygen concentration within waterlogged pots. It took approximately 4 days for the soil oxygen concentration to reach 10 % (Figure 4.1) for waterlogged plants.

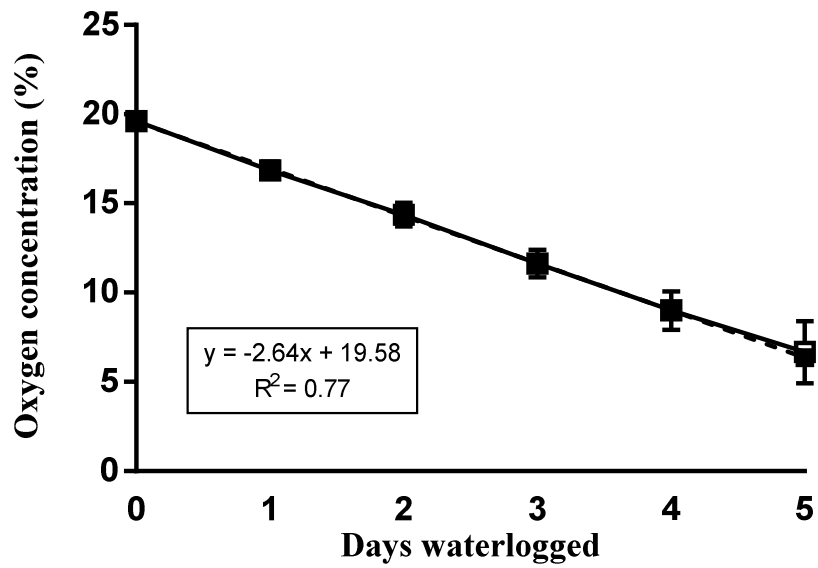


Figure 4.1: Soil oxygen concentrations for waterlogged soil (continuous line) with regression line (dashed line). Bars represent SEM (n = 17 on days 0 and 1, n = 15 on day 2, n = 12 on day 3, n = 9 on day 4, n = 5 on day 5).

4.3.2 Observed morphological responses

N. glutinosa plants showed signs of leaf wilting (Figure 4.2A) 3 days after waterlogging. Silver spots and yellowing occurred on the leaves from day 3 onwards (Figure 4.2B), but there was no apparent sign of apoplast flooding in leaves. Plants waterlogged for 5 days showed no signs of adventitious root formation.



Figure 4.2: A) Plant waterlogged for 4 days showing severe leaf wilting. B) Plant waterlogged for 3 days showing signs of leaf yellowing.

4.3.3 Plant growth

There was negligible reduction in shoot dry weight (Figure 4.3) over the first 4 days of waterlogging (mean from 31 waterlogged plants and 32 freely drained plants). A reduction in shoot dry weight in waterlogged plants was evident in plants waterlogged for 5 and 6 days. The dry weight of roots from waterlogged plants was less than untreated plants on day 2 and beyond (Figure 4.3). Waterlogging for 2 days resulted in a reduction of 50 % on average of root dry weight with no further decline up to 4 days (mean from 32 waterlogged plants and 30 freely drained plants).

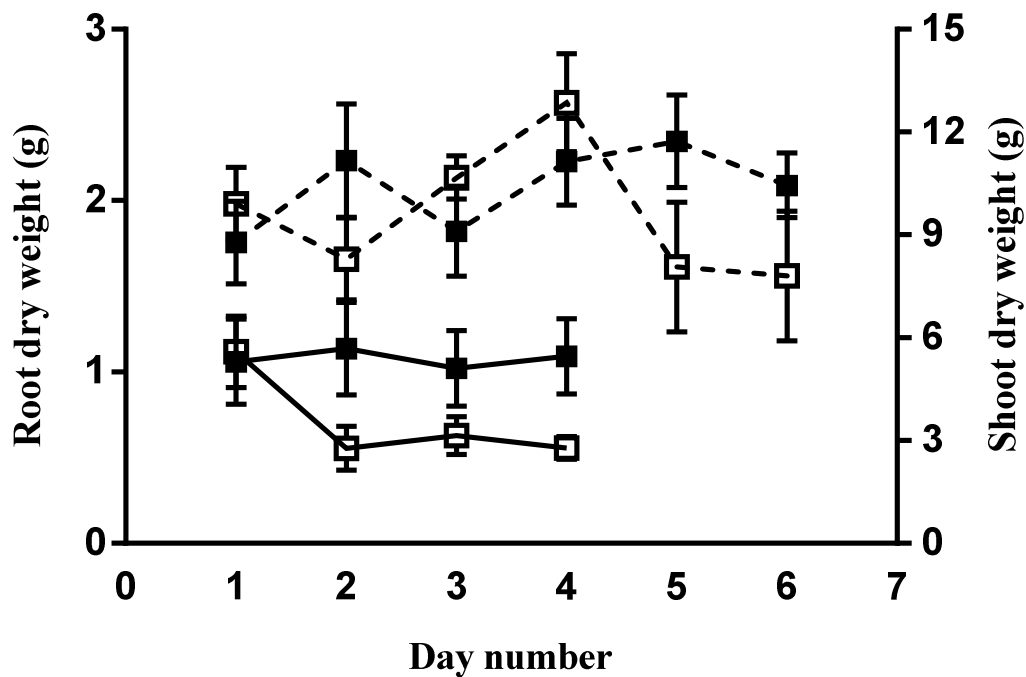


Figure 4.3: Root dry weight (solid line) and shoot dry weight (dashed line) measured for waterlogged (unfilled markers) and freely drained (filled markers) plants. Bars represent SEM (For root dry weight $n = 6$ for day 1, 2 and 3, $n = 7$ for day 4. For shoot dry weight $n = 6$ for day 1, 2, 3 and 4, $n = 4$ for day 5, $n = 2$ for day 6).

Steady leaf growth occurred for 2-3 days after waterlogging began. Treated plants had less growth than control plants. Waterlogging resulted in a 15 % reduction in leaf area (mean from 28 waterlogged plants and 27 freely drained plants) from freely drained plants over a 5 day waterlogging period. The onset of reduced leaf growth and therefore leaf area was evident from day 4 onwards (Figure 4.4).

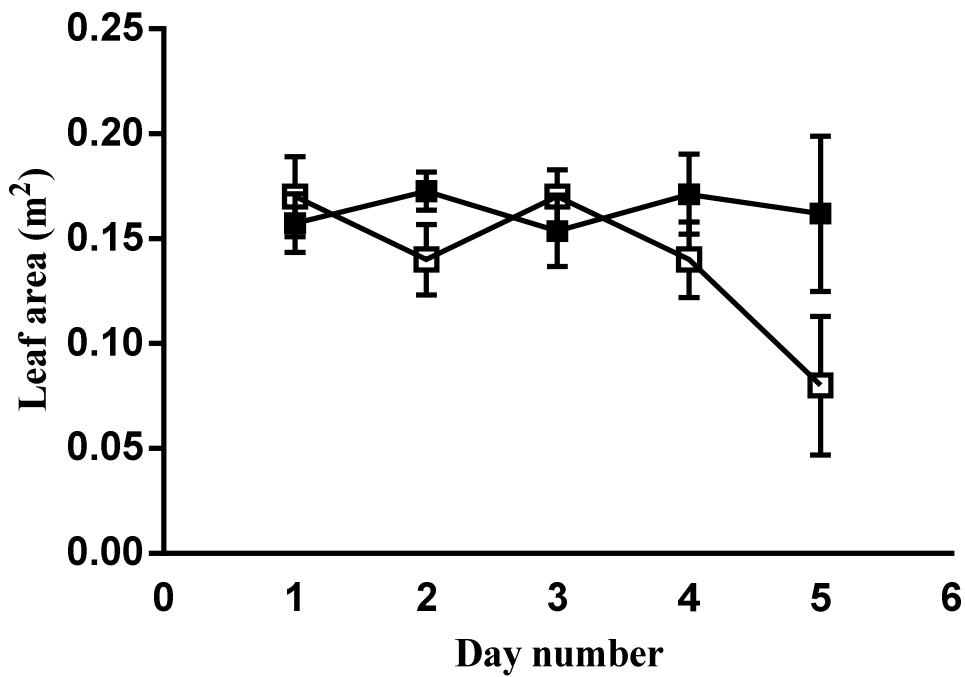


Figure 4.4: Leaf area measured for waterlogged (unfilled markers) and freely drained (filled markers) plants. Bars represent SEM (n = 6 for day 1, 2, 3 and 4, n = 3 for day 5).

4.3.4 Leaf water relations

Waterlogging *N. glutinosa* for more than 2 days caused a reduction in g_s (Figure 4.5). The increase in g_s of control plants followed an increase in ambient temperature during the experiment. Waterlogging caused a reduction in ψ_{leaf} on day 4 onwards (Figure 4.6). Leaf hydraulic conductance (L_{leaf}) of treated plants was the same as control plants until day 4 and then it became significantly greater (Table 4.1).

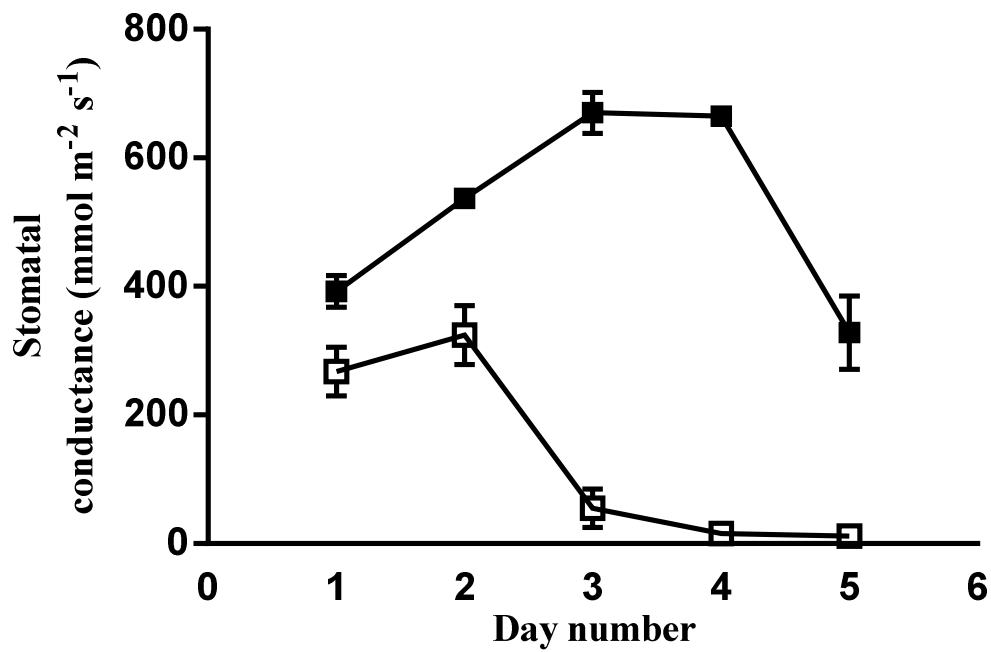


Figure 4.5: Leaf stomatal conductance (g_s) measured for days waterlogged (unfilled markers) and freely drained (filled markers) plants. Bars represent SEM ($n = 6$).

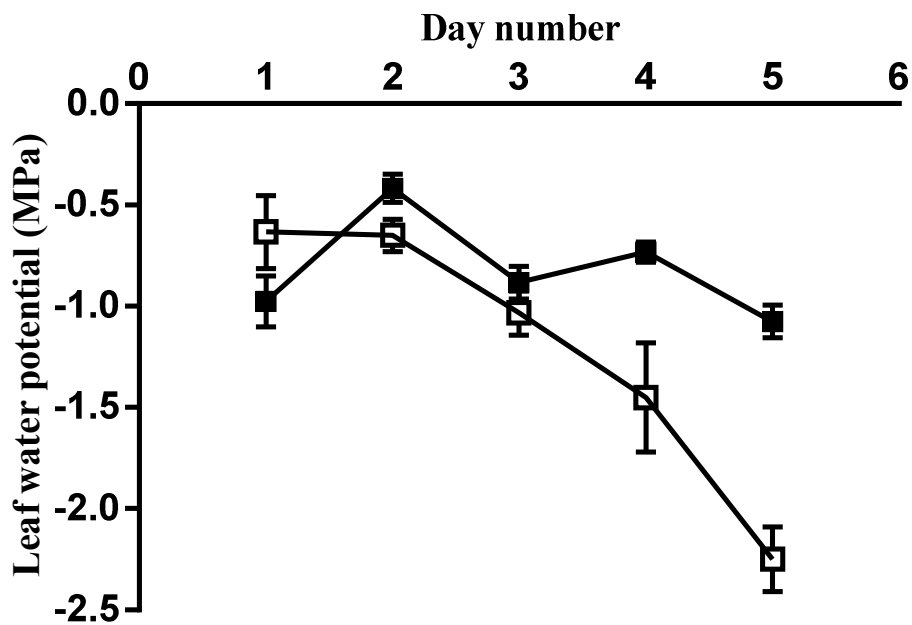


Figure 4.6: Leaf water potential (ψ_{leaf}) measured for days waterlogged (unfilled markers) and freely drained (filled markers) plants. Bars represent SEM ($n = 5$).

Table 4.1: Leaf hydraulic conductance (L_{leaf}) measured for waterlogged and freely drained plants. Values are means \pm SEM. A 2 way ANOVA showed that day was not significant but treatment was ($P = 0.0031$), with no significant interaction. Day 4 waterlogging was significantly different from day 4 freely drained compared to day 3.

Day number	L_{leaf} ($\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-2}$) $\times 10^{-5}$	
	Freely drained	Waterlogged
Day 3 (P = not significant)	19.64 \pm 2.77	28.50 \pm 4.52
Day 4 (**P < 0.01)	14.99 \pm 1.22	32.67 \pm 5.05

4.3.5 Leaf xylem ion concentrations

Linear regression analysis of the xylem ion concentrations over time showed no significant changes for control or waterlogged plants ($P > 0.05$) except for magnesium, which significantly increased in the waterlogged plants ($P < 0.0064$). Further analysis was precluded by large variations over time between individual plants (Table 4.2).

Table 4.2: Mean (SEM) xylem sap concentrations of elements analysed by ICPAES, nitrate concentration and sap volume taken from leaves over 4 days of waterlogging or freely drained (control). Only magnesium showed a significant increase in concentration over time with waterlogging.

	Xylem sap elements (mg kg ⁻¹)							Sap volume
	Calcium	Magnesium	Sodium	Potassium	Phosphorus	Sulphur	Nitrate	(mL)
Control	440.10	145.40	338.60	1890	74.54	99.52	11200	0.030
SEM	211.40	49.59	137.40	1134	26.74	28.13	5203	0.014
Waterlogged	320.30	163.80	537.50	1305	122.40	128.30	6300	0.018
SEM	85.31	37.34	161.60	276	19.76	27.56	1340	0.009

4.3.6 Root and plant hydraulic conductances

There was a significant positive linear correlation between detached root L_o from HCFM measurements and calculated evapotranspiration in freely drained control plants (Figure 4.7), but this was not observed for waterlogged plants where measured root L_o increased substantially at day 3 and calculated evapotranspiration declined. Calculated whole plant conductance and measured root L_o were compared for both waterlogged and freely drained plants over time (Figure 4.8). Calculated whole plant conductance normalised to root dry weight was similar to that of the root conductance measured using the HCFM for control plants and for day 1 of waterlogging. However, for waterlogged plants the two conductances diverged by orders of magnitude, with the calculated whole plant conductance decreasing by over an order of magnitude, while measured root L_o increased by an order of magnitude. There were no significant differences over time between calculated plant hydraulic conductance and measured root L_o for freely drained plants but there was a large and significant difference between measured root L_o and calculated plant hydraulic conductance after day 2 of waterlogging ($P < 0.05$).

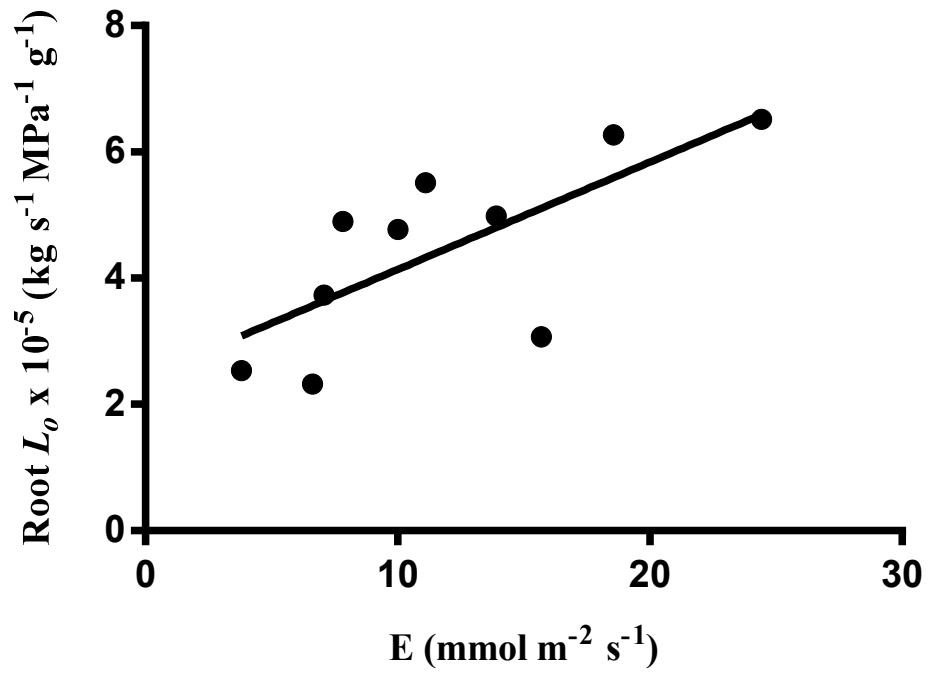


Figure 4.7: Root hydraulic conductances (L_o) measured from HCFM on detached roots plotted against calculated evapotranspiration (E) for freely drained plants. Regression shows a linear correlation with a significant slope ($P = 0.0186$), $y = 0.1701x + 2.437$.

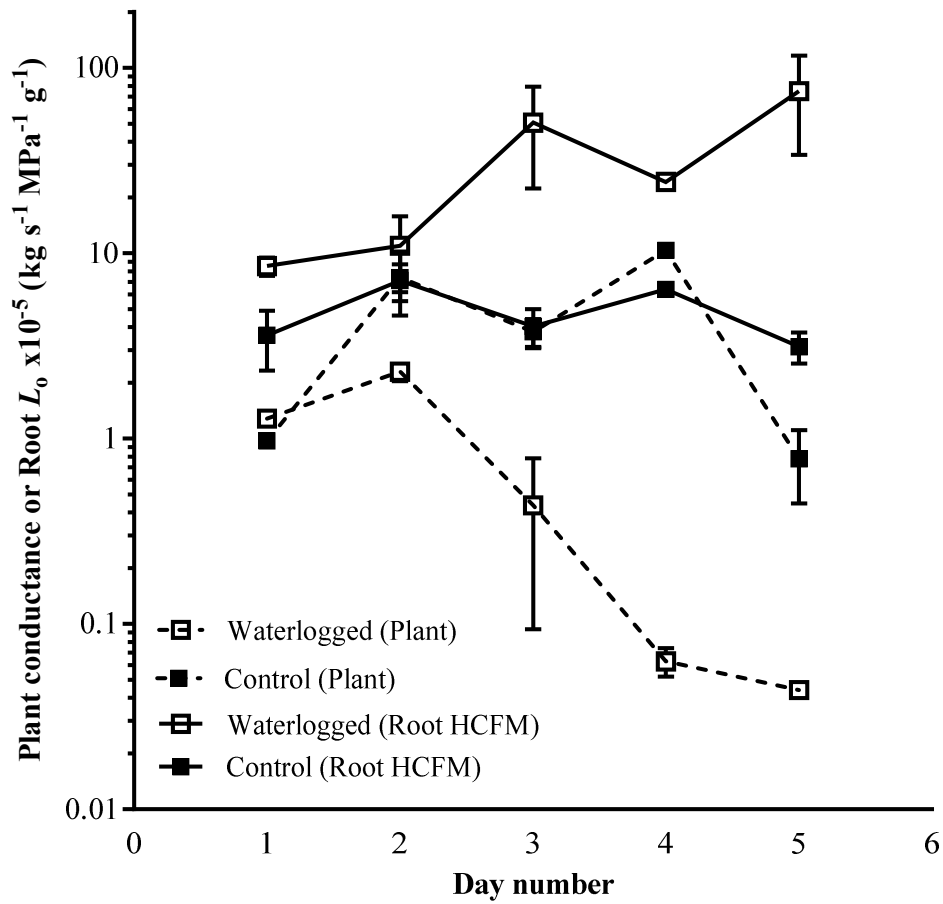


Figure 4.8: Hydraulic conductances measured from HCFM on detached roots (solid lines) and from whole plants using calculated evapotranspiration and measured leaf water potentials (dashed lines) under waterlogging (open squares) and freely drained (filled squares) conditions. Data is plotted on a log scale to better observe the large divergence in measurements using the two techniques after day 2 of waterlogging. Bars represent SEM (n = 6).

4.3.7 Root anatomy

Cross sections of the primary roots in the basal, mid and apical regions showed that the cellular structure began to break down on day 4 of waterlogging (Figure 4.9). The cytoplasm within some cell walls (Figure 4.9B) appeared shrunken and presumably non-functional on day 4 and beyond. Endodermal cells and associated cells appeared to break down (Figure 4.9F) which resulted in separation of the stele and cortex.

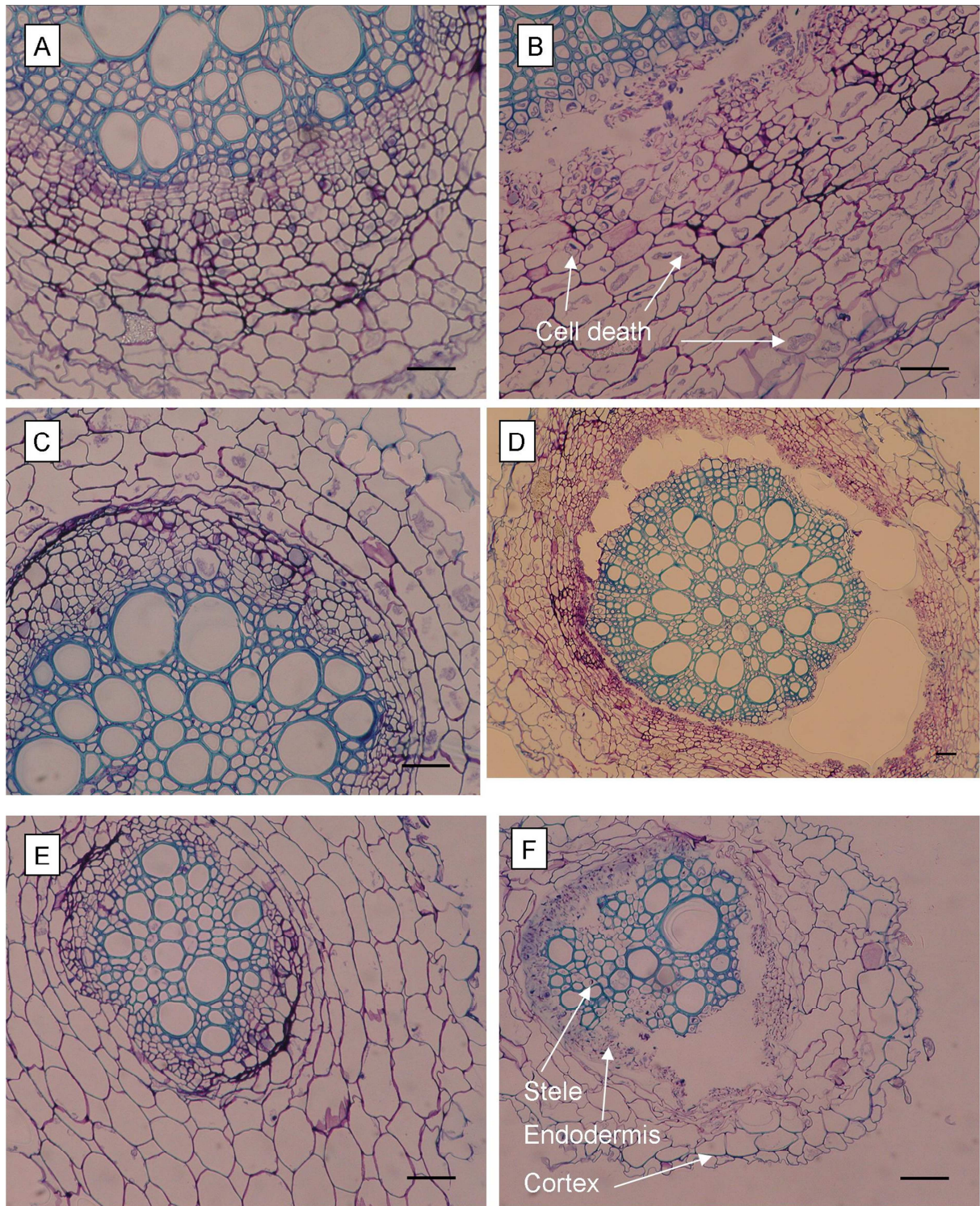


Figure 4.9: Primary root cross sections of *N. glutinosa*. A), C), and E) are freely drained plants. B), D) and F) are plants after 4 days of waterlogging. A) and B) are from the basal root zone. C) and D) are from the mid root zone. E) and F) are the apical zone. B) shows cell death. F) identifies relevant aspects of the root structure. Scale bars represent 100 μm .

4.4 DISCUSSION

Leaf wilting is a common response to waterlogged soil. I investigated a sequence of physiological and morphological responses of *N. glutinosa* to waterlogging (summarised in Figure 4.10), and I shall discuss them in sequence, in order to better understand the causes of leaf wilting and more specifically the purported reduction in root hydraulic conductance during waterlogging that could induce a shoot water (deficit) stress (Kramer and Jackson, 1954).

It is possible that increased transport of toxins to the xylem associated with root cell damage could cause leaf wilting. Hiatt and Lowe (1967) showed that oxygen deficient roots leaked solutes following the breakdown of the root plasma membrane. Jackson *et al.* (1996) found that the concentrations of protein amino acids, ions (apart from nitrate) and sucrose increase in the xylem sap of tomato plants during waterlogging due to the loss of membrane integrity in root cells. With root cell breakdown, ions and compounds that can be potentially toxic can increasingly enter the xylem stream. The regression analysis of the leaf xylem element concentrations over time showed a significant increase in magnesium with no apparent changes in the concentrations of calcium, phosphorus, sulphur, sodium or nitrate. Therefore, it is unlikely that toxic effects from excessive ion transport are the cause of the leaf wilting symptoms.

One of the earliest responses I observed in roots and before a significant decrease in stomatal conductance was a large reduction in root dry weight (Figure 4.3). This occurred before changes in root and plant hydraulic conductance and before soil oxygen concentrations reached 10 %. The decline in root dry weight has been seen previously in waterlogged plants. Hurng and Kao (1993) saw a decrease of more than 60 % root dry weight in *N. tabacum* after 4 days of waterlogging, but they did not report earlier measurements.

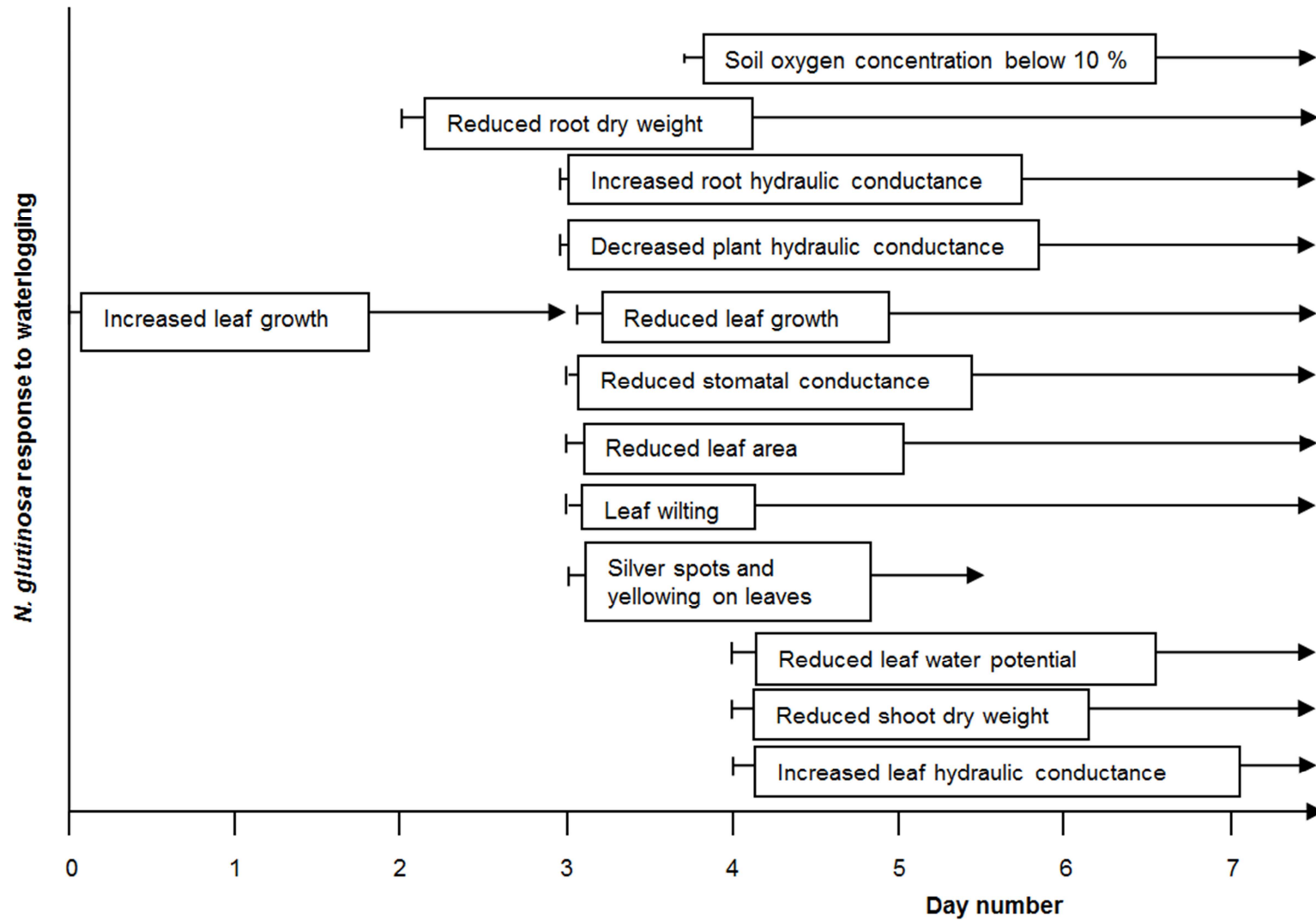


Figure 4.10: Summary of *N. glutinosa* response to waterlogging.

Trought and Drew (1980) saw a 43 % reduction in root dry weight on day 4 of waterlogging wheat relative to controls, explained by a cessation of root growth and a breakdown of root tissue. Smith *et al.* (1990) saw a reduction in root dry weight when waterlogging kiwifruit vines and a separation of the cortex from the stele with affected roots. They reported carbohydrate (starch) in the cortical cells immediately surrounding the endodermis of control roots, but noted an absence of starch in the waterlogged roots. It is possible that reduced starch in waterlogged *N. glutinosa* roots could account for the observed reduction in root dry weight and this requires further investigation.

For waterlogged plants, measured root L_o increased at day 3 (Figure 4.8) which is contrary to other reports (Everard and Drew, 1989; Gibbs *et al.*, 1998; Tournaire-Roux *et al.*, 2003). This increase in measured root L_o under waterlogging cannot be solely attributed to the decline in root dry weight (root conductance is normalized by root dry weight), since the increase in measured root L_o was much larger than can be accounted for by the reduced dry weight, additionally the same trend was observed when root conductance was normalised to shoot dry weight. At the same time I observed a large reduction in calculated plant hydraulic conductance corresponding to leaf wilting (Figure 4.2A), a concurrent reduction in g_s (Figure 4.5) and somewhat later a reduction in ψ_{leaf} (Figure 4.6).

For freely drained control plants detached root L_o measured by HCFM showed a positive correlation with calculated evapotranspiration (Figure 4.7) similar to that observed by Vandeleur *et al.* (2014) for soybean and grapevine. *N. glutinosa* fitted to the same trend line as soybean (Vandeleur *et al.*, 2014), but had higher overall measured L_o and calculated leaf evapotranspiration rates. This association between measured L_o and calculated evapotranspiration can explain the relatively large variation that is often observed in root L_o measurements if evapotranspiration is varying due to changed environmental conditions.

The increase in detached root L_o would usually be interpreted to indicate that waterlogged plants could potentially increase water uptake through the roots, hence increase water availability to the stem and leaves. However leaf wilting and reduction in both g_s and ψ_{leaf} indicate a water deficit in the leaves. Hunt *et al.* (1981) also saw leaf wilting and a reduction in ψ_{leaf} in field trials of waterlogged *Nicotiana tabacum* L. These responses correspond with the calculated reduction in plant hydraulic conductance. Interestingly, for well drained plants detached measured root L_o and calculated plant hydraulic conductances were similar indicating that the roots constituted the main resistance to water flow.

The divergence between the measured root L_o and calculated plant hydraulic conductance due to waterlogging (Figure 4.8) may be explained by the following possibilities:

1) It is possible that another component of the water flow pathway in the plant had a large decrease in hydraulic conductance. Hence I measured leaf hydraulic conductance to test this possibility (Table 4.1). Leaf hydraulic conductance (L_{leaf}) has previously been shown to be plant, light (Nardini *et al.*, 2005), temperature (Yang and Tyree, 1993; Sack *et al.*, 2002) and stress dependant (Pou *et al.*, 2013). The comparison of L_{leaf} of waterlogged and freely drained plants over 4 days found there were no significant differences until day 4 when L_{leaf} of the waterlogged plants increased. The fact that L_{leaf} was higher for the waterlogged plants cannot explain the reduced ψ_{leaf} (Figure 4.6) since others have consistently found that decreased L_{leaf} is associated with decreased g_s (Tsuda and Tyree, 2000; Pou *et al.*, 2013). My values of L_{leaf} are similar to those observed for other species (Sack and Holbrook, 2006). There is therefore no evidence that the observed leaf wilting in the waterlogged plants is caused by a restriction in the flow path of the leaf petiole and lamina. Hence the reduced calculated whole plant conductance must be elsewhere in the flow pathway, almost certainly within the roots.

2) Measurements of root L_o using the HCFM are in response to changing the hydrostatic pressure gradient across the root, while calculated whole plant hydraulic conductance is

likely to be in response to both hydrostatic and osmotic gradients. Gambetta *et al.* (2013) found for grapevine roots that root L_o from hydrostatic gradients (as with HCFM) gave values more than 100-fold higher than those measured with osmotic gradients. During waterlogging, plant water transport in *N. glutinosa* may be more dependent on osmotic gradients than hydrostatic gradients so that calculated whole plant conductance may largely reflect measured root L_o in response to osmotic gradients, indicating a large difference between root L_o under hydrostatic and osmotic gradients.

3) The HCFM measurement forces water out through roots in the opposite direction to normal flow and may result in an overestimation of conductance when barriers in the root are altered. Consequently I must caution the use of the HCFM to measure L_o when there is cell breakdown in roots. My observations show cell breakdown around the stele, outside the vascular tissue, on day 4 of waterlogging (Figure 4.9). This breakdown of the inner cortex near the endodermis has been associated with secondary root growth or thickening and is thought to be an adaptation to waterlogging as described for *Rumex acetosella* by Justin and Armstrong (1987). The thickening results from phellogen activity and vascular expansion of the stele which in turn causes breakdown of cortex cells (Justin and Armstrong, 1987). With cell breakdown there is no longer cell to cell protoplast membrane resistance and it would be expected that this would result in increased water conductance (Else *et al.*, 1995). Aerenchyma formation may also result in an apparent increase in L_o using the HCFM technique. *N. glutinosa* root has radially packed cortical cells, which, along with cubic packing, is generally associated with a greater likelihood of aerenchyma development compared to a hexagonally packed root cortex (Justin and Armstrong, 1987). However, there was no visible evidence of aerenchyma formation and there is no current literature that reports aerenchyma in *N. glutinosa* roots. McDonald and Visser (2003) reported the absence of aerenchyma in two tobacco genotypes, while Willey (1970) chose *N. tabacum* to work

with because aerenchyma were not known to form under hypoxic conditions. It has been shown that aerenchyma develop in the mid to outer cortex, but never near the endodermis (Fagerstedt, 2010) where I observed cell breakdown.

Fiscus (1975) and others since (e.g. Passioura and Munns (1984)) have observed changes in hydraulic conductance with changes in hydrostatic gradients or rooting medium, indicating that there were components in the root that acted as a variable conductance (Fiscus, 1977). We now know that this variable conductance is accounted for by aquaporins in the short term combined with anatomical changes in the longer term (Chaumont and Tyerman, 2014). Aquaporins as proteins need a constant and probably high proportion of energy supplied by root respiration to maintain normal function. Hypoxia and anoxia under waterlogging conditions will considerably diminish this supply of energy and this will disrupt the normal operation of aquaporins or the cell membranes in which they are imbedded. Consequently physical models of plant responses to stresses, such as waterlogging, where it is assumed that water flow depends on a fixed hydraulic conductance (Sieben, 1964; Hiler, 1969; Meyer *et al.*, 1996; Asseng *et al.*, 1998; Skaggs, 2008) are physiologically flawed. This has been suspected for a long time (Fiscus and Kramer, 1975; Passioura, 1988; Else *et al.*, 1995; Steudle, 2000) but there has been little evidence provided to support the design of models in which the uptake of water becomes a plant energy dependant process. The work reported in this paper goes some way towards addressing this knowledge gap. I have shown water stress in leaves can be explained by large reductions in root conductance. While there is a measured increase in root L_o when water is pushed down the root xylem vessels, the whole plant conductance using g_s and ψ_{leaf} measurements indicates that root L_o most likely decreased under waterlogged conditions. This suggests that the water flow from saturated soil through the cell protoplasmic and apoplasmic pathways is impeded. My results suggest that root cellular integrity and function is very important to maintain adequate water flow to the leaves and that

osmotic gradients and membrane aquaporin activity within root cells is the primary determinant of water flow from the rhizosphere into xylem vessels of the roots.

4.5 CONCLUSION

I examined a sequence of plant water relation responses to waterlogging that has revealed complexities in water transport during waterlogging of *N. glutinosa*. Stomata responded concurrently with changes in root water transport and before soil oxygen concentrations were below 10 %. This occurred before there were measurable differences between control and waterlogged plants in leaf water potential. Water (deficit) stress (observed as leaf wilting) could be explained by large reductions in root hydraulic conductance in intact transpiring plants, but it remains difficult to explain why detached root hydraulic conductance measured with hydrostatic gradients showed the opposite response. Further research is required to test the contribution of hydrostatic and osmotic gradients to water flow in *N. glutinosa* roots under waterlogged conditions. The sequence and time (days) for observed responses, plant growth, physiological effects and soil effects for *N. glutinosa* to respond to waterlogging was consistent between experiments and used to construct an empirical representation of waterlogging in Chapter 5.

CHAPTER 5

EMPIRICAL REPRESENTATION OF PLANT
ADAPTATIONS TO WATERLOGGING

5 CHAPTER 5

The work contained in this chapter has been submitted as a research article to *Agronomy Journal*, manuscript ID AJ-14-0625-A.

5.1 STATEMENT OF AUTHORSHIP

Shaw RE, Meyer WS (2014) Empirically representing plant responses to waterlogging for simulating crop yield. *Agronomy Journal*. Submitted.

Author contributions: By signing the statement of authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Shaw RE (Candidate)

Experimental development, data collection, analysis, critical interpretation and manuscript writing. I hereby certify that the statement of the contribution is accurate.

Signed

Date 23.10.2014

Meyer WS

Supervised development of work, data analysis and interpretation, reviewed and edited manuscript. I hereby certify that the statement of the contribution is accurate.

Signed

Date 23/10/2014

Shaw, R.E. & Meyer, W.S., (2015) Improved Empirical Representation of Plant Responses to Waterlogging for Simulating Crop Yield.
Agronomy Journal, v. 107 (5), pp. 1711-1723

NOTE:

This publication is included on pages 76-99 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

<http://dx.doi.org/10.2134/agronj14.0625>

CHAPTER 6

MODELLING CROP GROWTH AND YIELD
AFFECTED BY WATERLOGGING

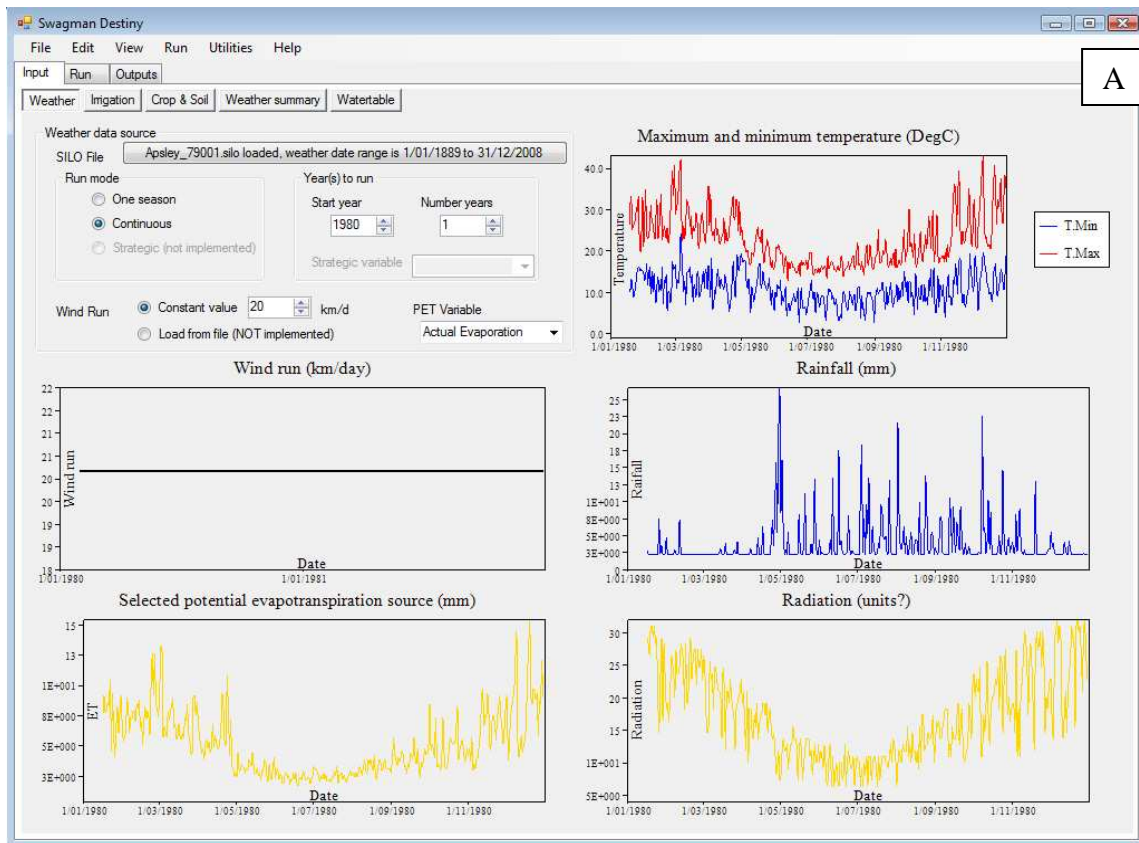
6 CHAPTER 6

As described in Chapters 2 and 5 SWAGMAN Destiny is a crop growth and yield model that considers the effects of abiotic stresses to estimate crop yields. Chapters 3 and 4 focused on understanding the physiological effects and morphological responses that waterlogging has on plants. Chapter 6 incorporates the new understanding of crop physiology gained from Chapters 3 and 4 into SWAGMAN Destiny to improve crop yield estimation due to waterlogging. This Chapter explains and justifies the changes made to SWAGMAN Destiny and the outcomes.

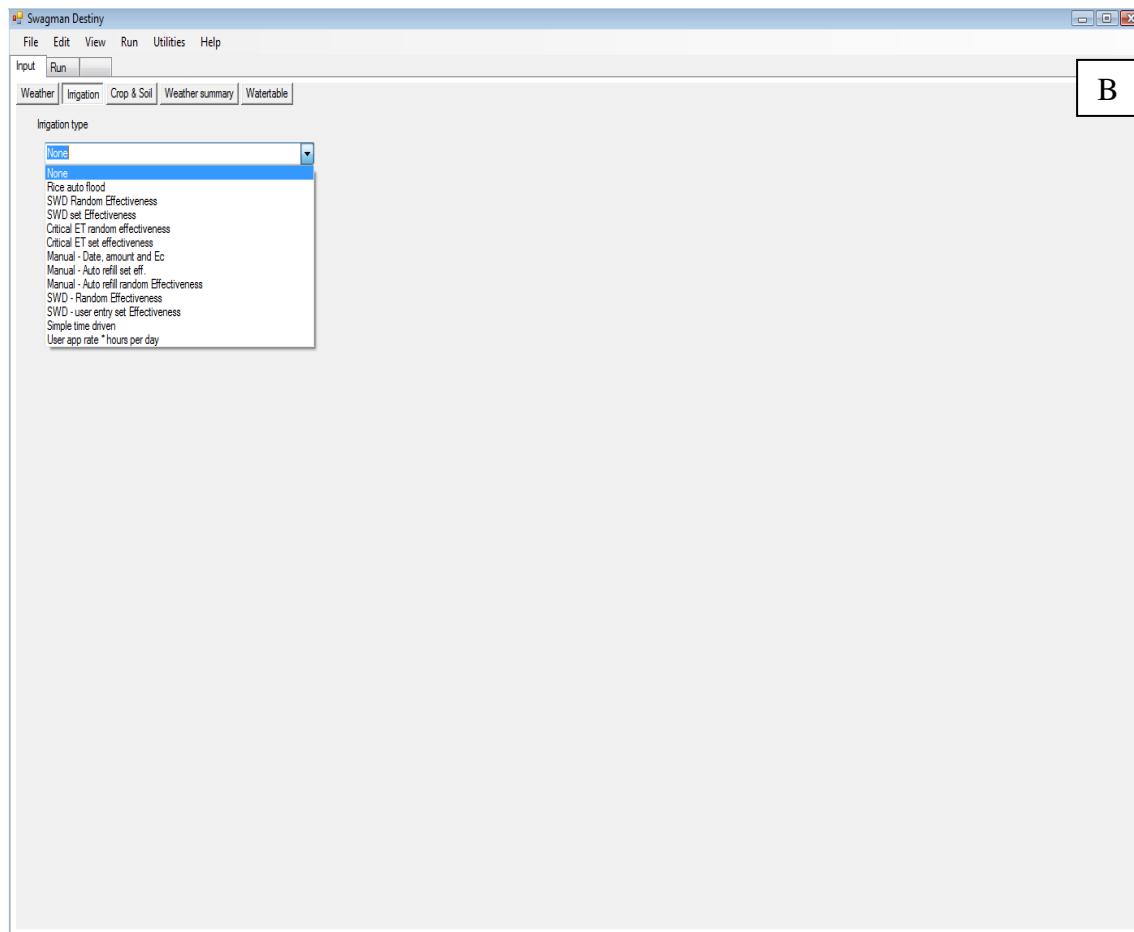
6.1 A BRIEF OVERVIEW OF SWAGMAN DESTINY

Details of the theory behind SWAGMAN Destiny (Destiny) can be found in Chapters 2 and 5. Destiny was originally written in Fortran and more recently converted to Microsoft Visual Basic.

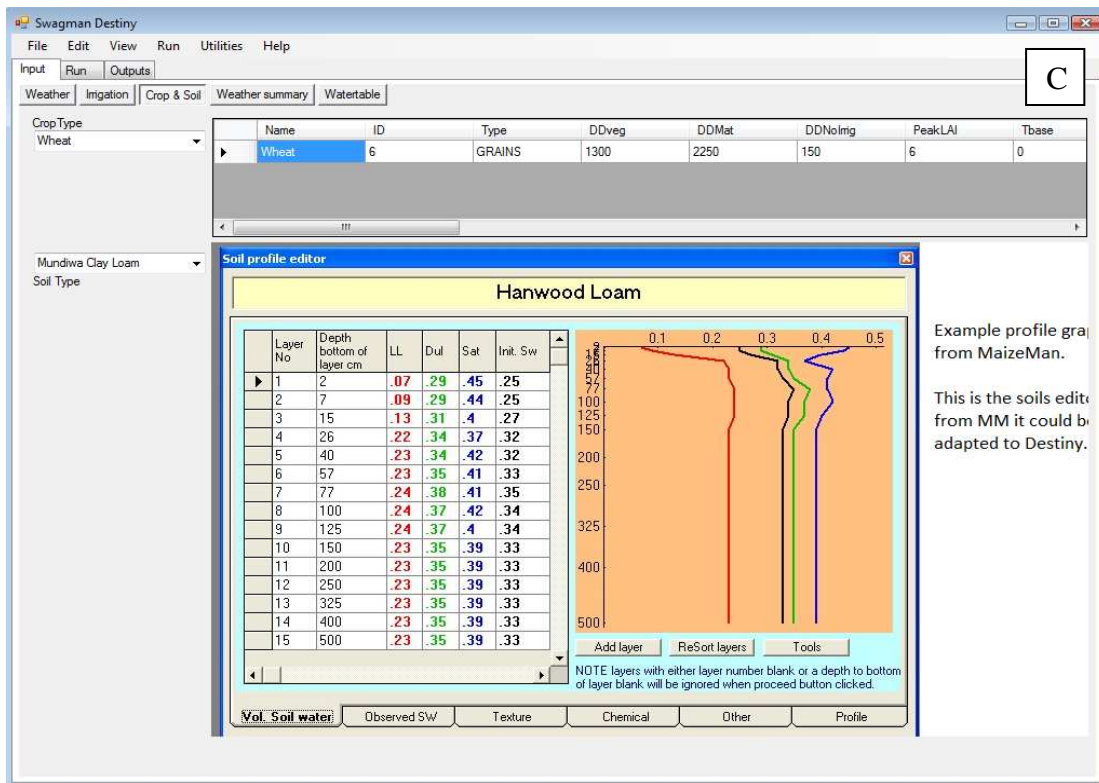
Through the graphical user interface (GUI) the user selects a simulation year (Figure 6.1A), inputs weather (Figure 6.1A), irrigation (Figure 6.1B), crop and soil information (Figure 6.1C) along with watertable information (Figure 6.1D). The program runs and produces an output summary (Figure 6.2A) along with graphed (Figure 6.2B) and tabulated outputs (Figure 6.2C).



A



B



Example profile gra from MaizeMan.

This is the soils edito from MM it could be adapted to Destiny.

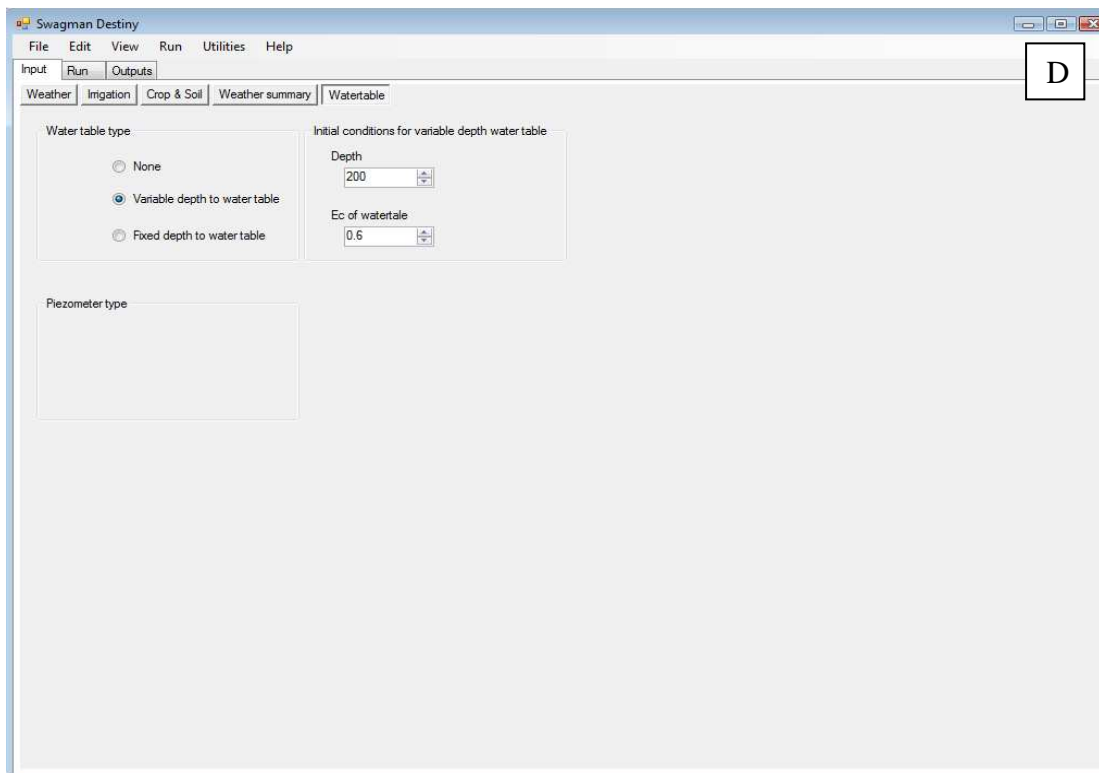
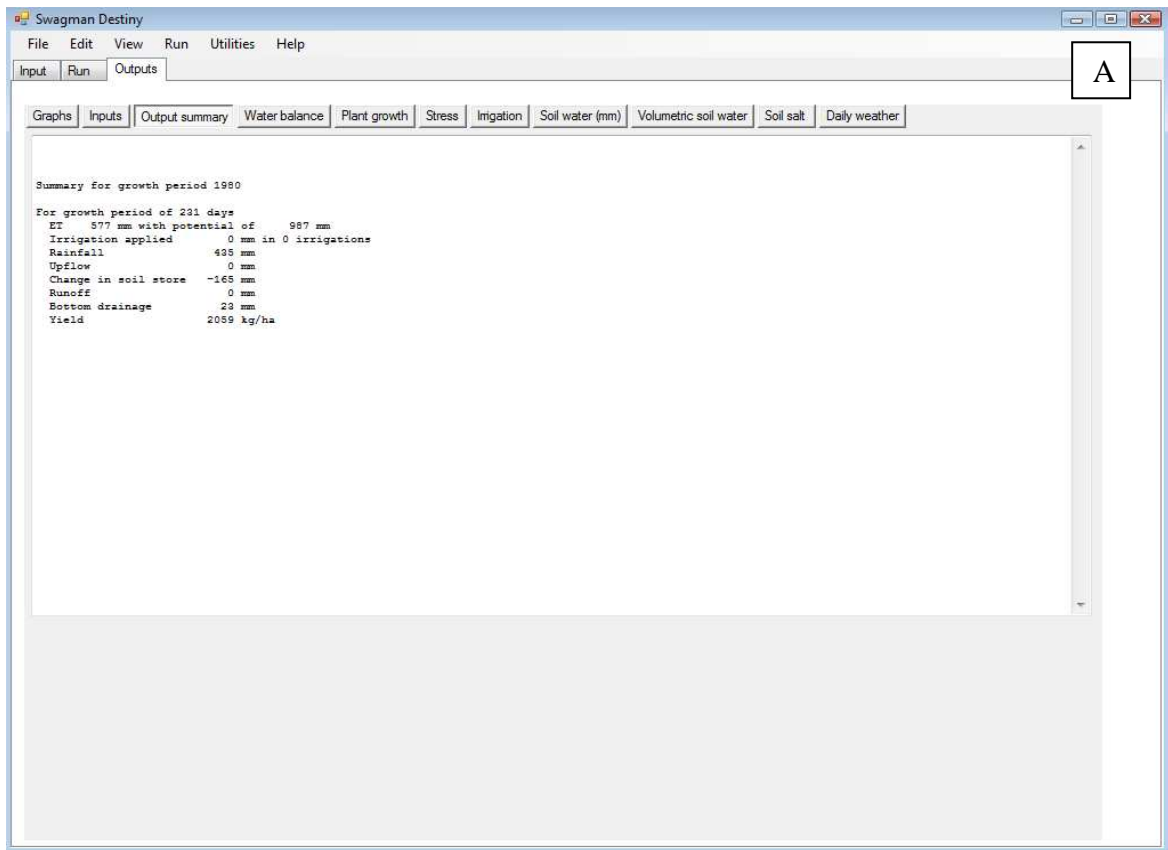
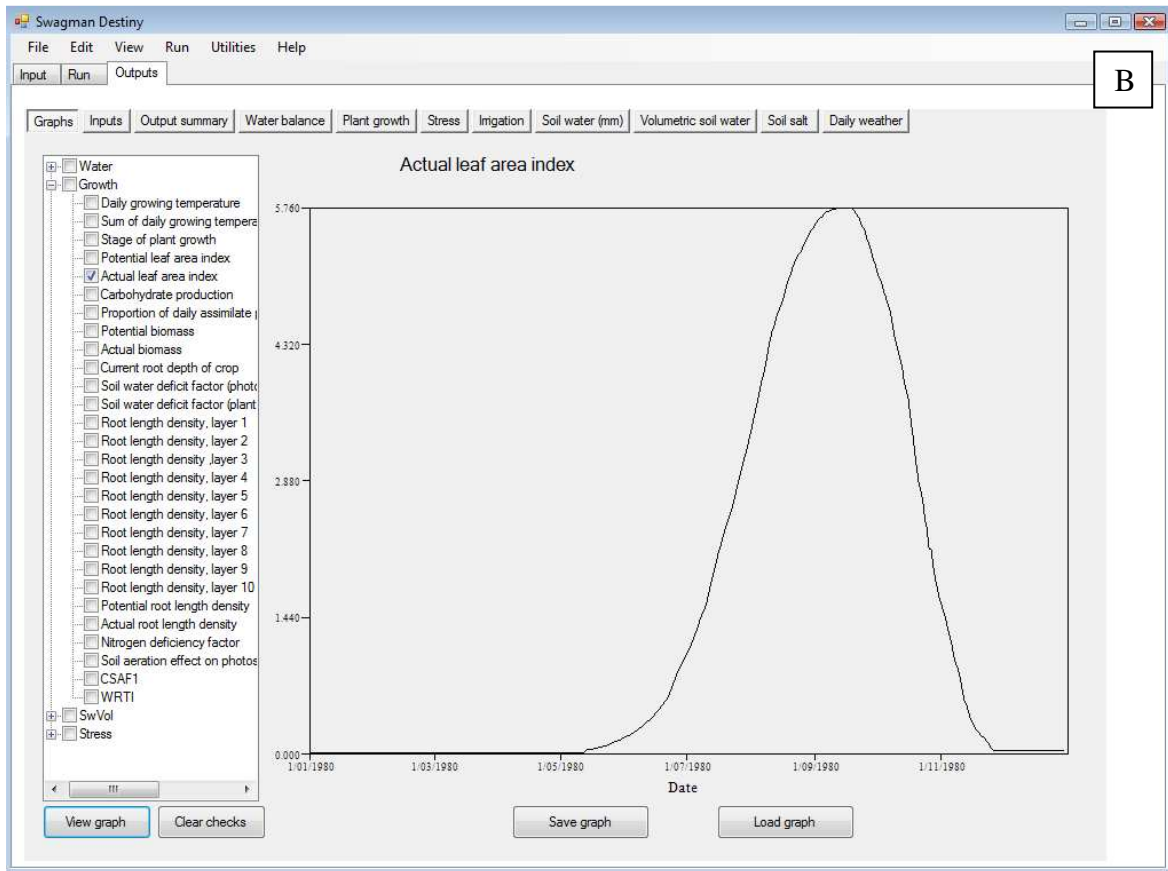


Figure 6.1: SWAGMAN Destiny graphical user interfaces showing input options. A. Year and weather inputs, B. Irrigation options, C. Crop and soil information, D. Watertable options.



A



B

6.2.2 Irrigation inputs

SWAGMAN Destiny was originally developed to simulate the water and salt balance effects associated with irrigated crops. Hence there are many options available to trigger irrigation. These range from a simple time driven system which specifies a day and amount of water added, to irrigation events that are automatically triggered once a cumulative evapotranspiration (ET) or cumulative soil water deficit is reached. The time driven method was used to simulate waterlogging periods at particular times by specifying a date and a large volume of water to be added. For prolonged or more frequent waterlogging simulations a trigger ET value was specified along with an irrigation amount that had effectiveness greater than 100 %. This ensured that an amount of water in excess of that which had been evaporated was added and would potentially fill the soil profile to saturation.

6.2.3 Crop and soil inputs

Crop and soil inputs for Destiny are called from tables composed and stored in Microsoft Access.

The choice of crops are cotton, established summer pasture (*Dactylis* L., *Phalaris aquatica*), winter pasture (*Trifolium* L., *Lolium* L.), established woodlot (*Eucalyptus*), young woodlot (*Eucalyptus*), maize, rice, soybean, sunflower, vines and wheat. The relevant crop input parameters defined by the crop input table are summarised in Table 6.1.

Each soil type (Table 6.2) is separated into 15 soil layers (L), each of fixed thickness. For each layer, volumetric water content at lower limit, drained upper limit, saturation and the initial water content of the layer, salinity, bulk density, ammonium and nitrate content, organic carbon, a root growth factor and macropore hydraulic conductivity are defined as inputs that are specific for each soil type.

6.2.4 Watertable inputs

The presence or absence of a watertable can be selected. If a watertable is present it can be assigned as a variable or fixed depth from the ground surface with the initial depth and its salinity specified by the user (Figure 6.1D).

Table 6.1: Crop input parameters used in SWAGMAN Destiny that are relevant to this chapter.

Name	DDveg	DDmat	Ddnoirrig	Peak LAI	Tbase	ParvConv Fac	PotYield	SppAer Fact	InitRoot Depth	Crop factor	RootDrate	Isow	Summer	RtVol Mass	Root Diam	Aero stress threshold
	Degree days from sowing until the end of vegetative growth stage	Degree days from sowing until the end of crop maturity	Elapsed degree days before commencement of irrigation	Peak leaf area index	Temp of zero growth for computing degree days (°C)	Photosynthetically active radiation factor used to convert radiation (MJ m ⁻²) to kilograms of dry matter (Kg MJ ⁻¹)	Maximum potential crop yield under optimum conditions (kg ha ⁻¹)	Aeration factor (0 - 1 factor)	Initial rooting depth at sowing (cm)	Crop coefficient used for scaling reference potential evapotranspiration to crop potential evapotranspiration at full cover	The root development rate (cm °C ⁻¹ day ⁻¹). Used to calculate the rate of increase in depth of roots as constrained by root growth rate and salt factors	Sowing date specified as day number of the year	A boolean field indicating whether it is a summer crop or otherwise	Volume of root mass indicating the total volume (cm ³) occupied by one gram of roots (cm ³ g ⁻¹)	Average diameter of first order lateral root (cm)	0-1 factor
Cotton	1300	1700	100	9	12	4	2000	1	8	1	0.2	300	TRUE	10	0.032	0.8
Established summer pasture	3500	4500	50	9	0	2	0	1	8	0.85	0.08	183	TRUE	10	0.02	0.8
Established Woodlot	900	2100	100	5	5	2	70000	1	325	0.6	0.05	1	TRUE	10	0.03	0.8
Maize	1100	2000	100	8	8	3.5	15000	1	8	0.85	0.2	300	TRUE	7.5	0.035	0.8
Rice	1295	1895	100	10	8	3.05	15000	1	15	1	0.1	290	TRUE	10	0.01	0.8
Soybean	1000	1895	100	5	8	1.1	5000	1	8	1.1	0.1	322	TRUE	10	0.028	0.8
Sunflower	1300	2000	100	7.5	4	2.5	5000	1	8	0.95	0.1	320	TRUE	10	0.025	0.8
Vines	2000	3500	50	5	5	3	40000	1	8	0.75	0.05	245	TRUE	10	0.03	0.8
Wheat	1300	2250	150	6	0	2.5	7000	1	8	1.05	0.15	135	FALSE	10	0.025	0.8
Winter pasture	1500	2100	50	9	0	2.5	0	1	8	0.95	0.08	90	FALSE	10	0.018	0.8
Young wood lot	900	2100	100	9	5	2	70000	1	20	0.6	0.05	100	TRUE	10	0.03	0.8

Table 6.2: Soil types available in SWAGMAN Destiny.

Soil type	Soil type continued
Black earth, low water holding capacity	Non-restrictive duplex soil with thin well structured topsoil
Brown clay loam (15 - 18 cm) over reddish brown heavy clay subsoil	Red Brown Earth
Brown clay loam (shallow < 10 cm) over dark red-brown heavy clay	Red brown loam (~25 cm) over light brown clay (~20 cm). Clay loam to depth
Brown heavy clay to depth, self-mulching	Red Earth
Carrathool ripped	Restrictive duplex soil with thick hard topsoil
Carrathool unripped	Restrictive duplex soil with thick well structured topsoil
Clayey calcareous soil	Restrictive duplex soil with thin hard topsoil
Deep clayey calcareous unigrad	Restrictive duplex soil with thin well structured topsoil
Deep hard clayey	Rubbly calcareous soil
Deep hard loamy unigrad	Sandy calcareous soil
Deep loamy calcareous unigrad	Shallow calcareous non-restrictive soil
Deep sandy gradational soil	Shallow calcareous restrictive soil
Deep sandy uniform soil	Shallow clay over calc rock
Deep stony soil	Shallow cracking clay over calc-rock
Deep well structured clayey unigrad	Shallow loamy soil over calc-rock
Deep well structured loamy unigrad	Shallow loamy soil over rock/pan
Fine sandy loam	Shallow sandy soil
Grey heavy clay to depth, self-mulching (Northern NSW)	Soil compiled from Greenwood's data (Kyabram area)
Grey heavy clay to depth, self-mulching (Southern NSW)	Very shallow loamy/clayey soil over calc-rock
Lithosol	Very shallow non-rippable soil
Loamy calcareous soil	Very shallow sandy soil over calc-rock
Modified Wunnamurra (found in Hay)	Very shallow soil over rock
Non-restrictive duplex soil with thick hard topsoil	Well structured over restrictive cracking clay
Non-restrictive duplex soil with thick well structured topsoil	Well structured throughout cracking clay
Non-restrictive duplex soil with thin hard topsoil	Whitton soil
	Yellow podzolic

6.3 THE WATERLOGGING COMPONENT OF SWAGMAN DESTINY

As discussed in Chapters 2 and 5, Destiny assigns a potential crop yield then calculates the effects of daily abiotic stresses on that crop yield. Stresses considered are water deficit (drought), nitrogen deficiency, salinity and aeration (waterlogging). The effects of stresses are determined from the combination of the daily values of weather, soil, crop, watertable and irrigation inputs within the year or continuous years selected. The final part of my research has been to include process informed representations of my understanding of plant physiological responses and adaptations to waterlogging (Chapters 3 and 4) into the waterlogging component of Destiny.

Up to this point I have discussed crop yield as the main output of Destiny and the effect that waterlogging has on crop yield. There are several steps, inputs and calculations that must first be satisfied within the waterlogging component of Destiny to determine final crop yield (summarised in Figure 6.3). These steps, inputs and calculations are discussed in the sections below.

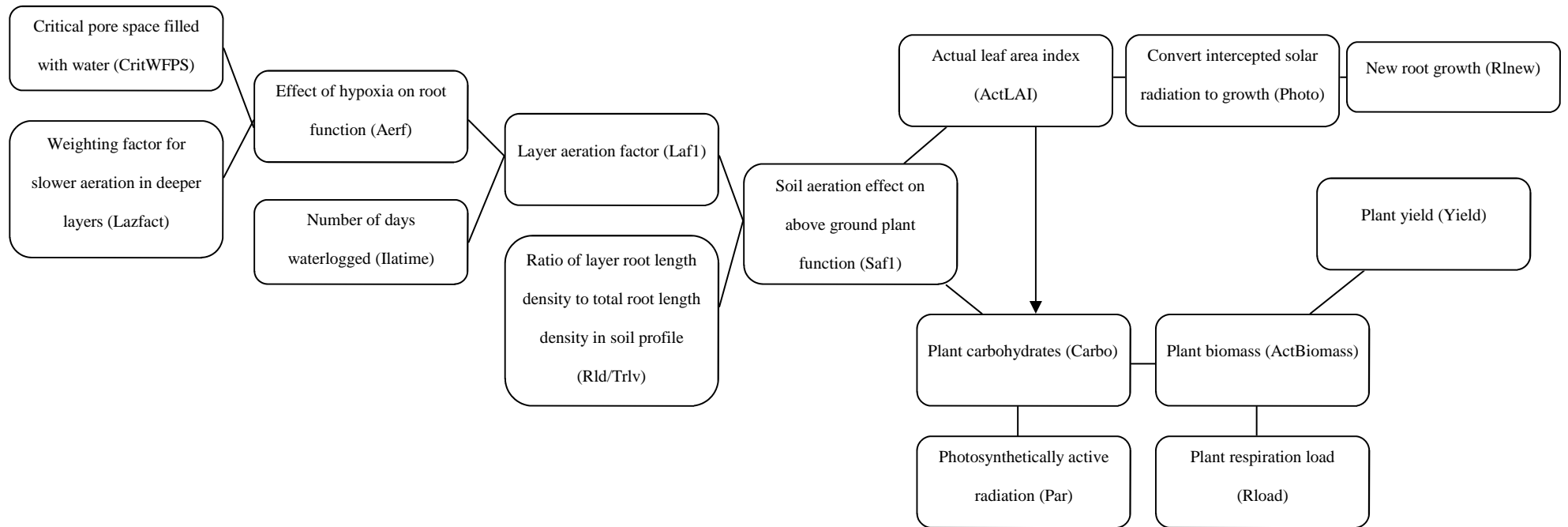


Figure 6.3: Sequence diagram of components required to calculate the effect of waterlogging on crop yield within SWAGMAN Destiny.

6.3.1 SWAGMAN Destiny glossary of terms

Destiny uses many abbreviations and a list of the terms used within this chapter can be found in Table 6.3.

Table 6.3: Abbreviations discussed in this chapter, used in SWAGMAN Destiny.

Abbreviation	Meaning	How they are determined
ActBiomass	Actual biomass (kg ha ⁻¹)	Calculated (Section 6.3.12)
ActLAI	Actual leaf area index	Calculated (Section 6.3.9)
AerDelay	Delay before hypoxia begins to affect plant processes (days)	Set at 3 days
Aerf	Aeration factor derived from water filled pore space and soil layer depth (0 – 1 factor)	Calculated (Section 6.3.4)
BD	Bulk density of each soil layer (g cm ⁻³)	Input from soil file
Carbo	Carbohydrate generated from PARConvFac and Par (kg ha ⁻¹)	Calculated (Section 6.3.12)
CritWFPS	Critical water filled pore space for root growth	Set as 0.65
DAS	Days after sowing	Counted
DeltaDep	Change in root depth on a daily basis	Calculated (Section 6.3.11)
DDmat	Day degrees from the end of the vegetative phase until maturity	Input from the crop file
DDveg	Day degrees for the vegetative phase	Input from the crop file
DL2	Depth to the bottom of the soil layer (cm)	Input from soil file
Dlayr	Thickness of the soil layer (cm)	Input from soil file
DTT	Daily growing degrees (°C)	Calculated (Section 6.3.9)
DUL	Drained upper limit of soil water content in a layer (cm ³ cm ⁻³)	Calculated (Section 6.3.11)
Grort	Daily increment of root growth (g cm ⁻²)	Calculated (Section 6.3.11)
Ilatime	Counter for number of days of waterlogging (days)	Calculated (Section 6.3.5)
Isow	Date of sowing for each crop (day of year)	Input from crop file
L	Layer number (layer 1 at top of profile, 15 in total)	Input from soil file
Laf1	Layer aeration factor (0 – 1 factor)	Calculated (Section 6.3.6)
Lafact	Relative amount of total pore space filled with water (0 – 1 factor)	Calculated (Section 6.3.2)

Table 6.3 continued

Lazfact	Weighting factor for depth of layer effect on aeration of the layers layer (0 – 1 factor)	Calculated (Section 6.3.3)
LL	Lower limit of plant available soil water in a layer ($\text{cm}^3 \text{cm}^{-3}$)	Calculated (Section 6.3.11)
Par	Photosynthetically active radiation (MJ m^{-2})	Calculated (Section 6.3.10)
ParConvFac	Efficiency of conversion from intercepted photosynthetically active radiation into biomass carbohydrate (g MJ^{-1})	Input from crop file
PCarbo	Potential carbohydrate (kg ha^{-1})	Calculated (Section 6.3.12)
PeakLAI	Peak leaf area index	Input from crop file
Photo	Converts the intercepted radiation to carbohydrate (kg ha^{-1})	Calculated (Section 6.3.10)
PotBiomass	Potential biomass (kg ha^{-1})	Calculated (Section 6.3.12)
PotLAI	Potential leaf area index	Calculated (Section 6.3.9)
PotYield	Potential yield (kg ha^{-1})	Calculated (Section 6.3.13)
Prft	Temperature index for photosynthesis and respiration	Calculated (Section 6.3.10)
Ptf	Partitioning factor (assigns carbohydrates to the root system)	Assigned
Rcoef	Rate of leaf appearance	Assigned
Rdtt	Thermal time used to drive rooting depth increase (set as DTT)	Calculated (Section 6.3.11)
Rld	Root length density of the soil layer (cm cm^{-3})	Calculated (Section 6.3.8)
Rldf	Root length density factor for soil layer used to calculate new root growth distribution between layers	Calculated (Section 6.3.11)
Rlnew	New root length (cm cm^{-2})	Calculated (Section 6.3.11)
Rload	Respiration load (kg ha^{-1})	Calculated (Section 6.3.12)
Rnlf	Temporary variable used for root length distribution (cm cm^{-2})	Section 6.3.11
RootDrate	Root development rate ($\text{cm } ^\circ\text{C}^{-1} \text{day}^{-1}$)	Input from crop file
RootLWRatio	Root length to weight ratio (cm g^{-1})	Input from crop file
RtVolMass	Amount of mass per root volume ($\text{cm}^3 \text{g}^{-1}$)	Input from crop file
Saf1	Soil profile aeration factor (0 – 1 factor)	Calculated (Section 6.3.7)
SDTT	Sum of daily growing degrees ($^\circ\text{C}$)	Calculated (Section 6.3.9)
Solrad	Amount of daily solar irradiance (MJ m^{-2})	Input from weather file
SppAerFact	Species sensitivity factor (0 – 1 factor)	Input from crop file
Sw	Volumetric soil water content ($\text{cm}^3_{\text{water}} \text{cm}^{-3}_{\text{soil}}$)	Input from soil file

Table 6.3 continued

SWWFPS	Water filled pore space at current soil water content	Calculated (Section 6.3.2)
Tbase	Base temperature at which species starts to grow (°C)	Input from crop file
TEMPMN	Minimum daily temperature (°C)	Input from weather file
TEMPMX	Maximum daily temperature (°C)	Input from weather file
Tpore	Total porosity (%)	Calculated (Section 6.3.2)
Trlv	Total root length density over the whole profile (cm cm ⁻³)	Calculated (Section 6.3.8)
WR	Weighting factor for soil depth to determine new root growth distribution	Input from soil file
Xstage	Plant development stage (0, 1 or 2)	Calculated (Section 6.3.9)
Yield	Yield, grain or vegetative dry matter (kg ha ⁻¹)	Calculated (Section 6.3.13)

6.3.2 Calculating the relative amount of critical pore space filled by water

The first step towards calculating yield reductions due to waterlogging is to calculate the relative amount of pore space filled with water (Lafact) within the soil. This is a zero to unity factor where zero indicates the soil pores are completely full of water (the soil is saturated) and one indicates there is no water in the soil pores (the soil is oven dry) (Figure 6.4). The critical water filled pore space (CritWFPS) is set to 0.65, as the threshold when new root growth within the affected layer will slow relative to root growth in less affected (better aerated) layers (Meyer and Barrs, 1991). Doran *et al.* (1990) found that respiratory activity of microbial function in soils decreased when the water filled pore space increased to a value above 0.65. If there are roots in the layer and the water filled pore space (SWWFPS) at a particular soil water (Sw) content is more than the critical water filled pore space (SWWFPS > CritWFPS) then Lafact is calculated as:

$$\text{Lafact} = 1 - \frac{(\text{SWWFPS} - \text{CritWFPS})}{(1 - \text{CritWFPS})} \quad (6.1)$$

where

$$SWWFPS = \frac{Sw}{Tpore} \quad (6.2)$$

and

$$Tpore = 1 - \frac{BD}{\text{Soil particle density}} \quad (6.3)$$

T_{pore} is the total porosity of the soil ($\text{cm}^3_{\text{pore space}} \text{cm}^{-3}_{\text{soil}}$) and soil particle density (2.68 g cm^{-3}) is the same for all layers. Volumetric water content (Sw) ($\text{cm}^3_{\text{water}} \text{cm}^{-3}_{\text{soil}}$) for each soil layer is calculated for each day of the simulation starting with an initial input value from the soil file. BD is the dry soil bulk density given as an input for each layer. If $SWWFPS$ is less than $CritWFPS$ then $Lafact$ is set equal to one.

In the current configuration, low aeration in any particular layer does not affect the rate of growth of roots but will affect the distribution of roots by having roots preferentially grow in those areas that are better aerated. There is no direct effect of low aeration conditions on either water uptake or nitrogen supply to the above ground portion of the plant. An aeration factor is calculated for each layer ($Lafact$) and a final overall factor ($Saf1$, Section 6.3.8) is calculated.

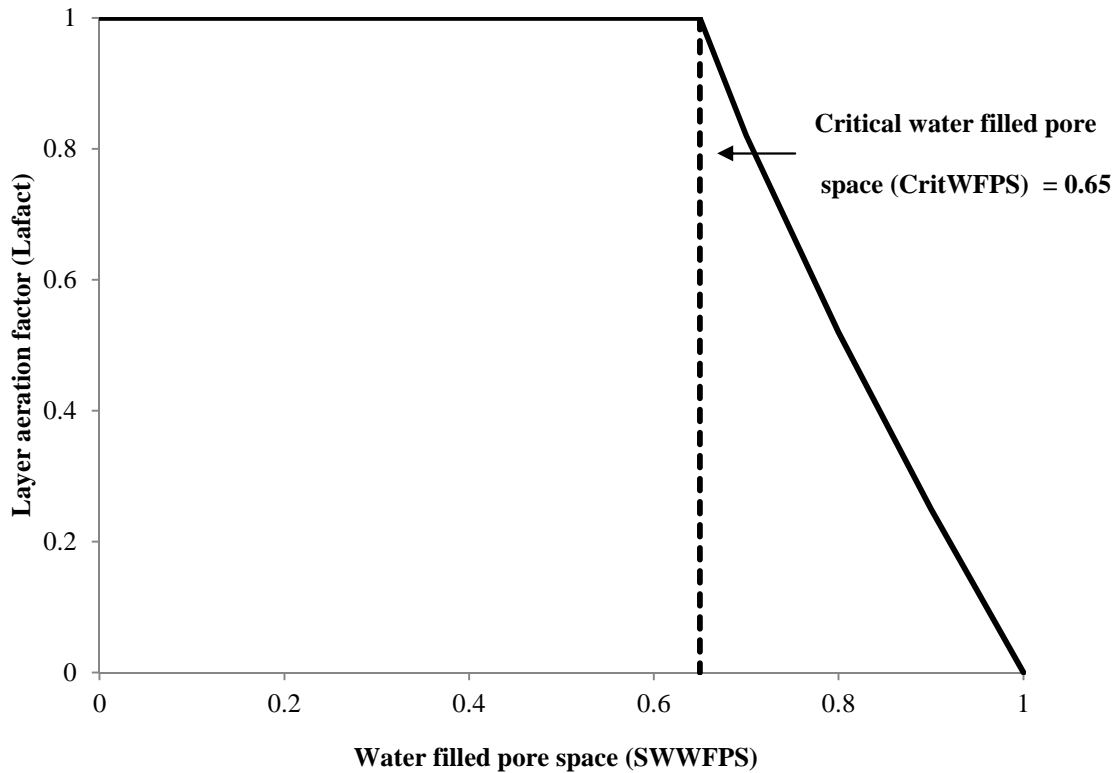


Figure 6.4: Aeration factor (Lafact) as a function of water filled pore space (SWWFPS).

A coding error was found in the Microsoft Visual Basic version of Destiny where Equation 6.1 was given as:

$$\text{Lafact} = 1 - \frac{(\text{SWWFPS} - \text{CritWFPS})}{(\text{Tpore} - \text{CritWFPS})} \quad (6.4)$$

This function gives similar values at the lower end of the Lafact range but incorrect values at the upper end. This error had been counteracted in the previous Microsoft Visual Basic version of the code by rearranging the equation used to calculate the final layer aeration factor (Laf1), which includes the time of exposure to low aeration (Ilatime) and the species sensitivity to waterlogging (SppAerFact). The correct equation for Laf1 (which had been present in the original Fortran code) is discussed below (Equation 6.10, Section 6.3.6).

6.3.3 Calculating the depth weighting factor for the aeration effect on soil layers

The depth weighting factor for aeration effect on soil layers (Lazfact) is a zero to unity factor describing the effects of soil depth on soil aeration (Figure 6.5). If the depth to the bottom of the soil layer (DL2) is more than 50 cm then:

$$\text{Lazfact} = \frac{50}{\text{DL2} - \text{Dlayr} \times 0.5} \quad (6.5)$$

where Dlayr is the thickness of the soil layer (in cm). If DL2 is less than 50 cm then Lazfact is made equal to one.

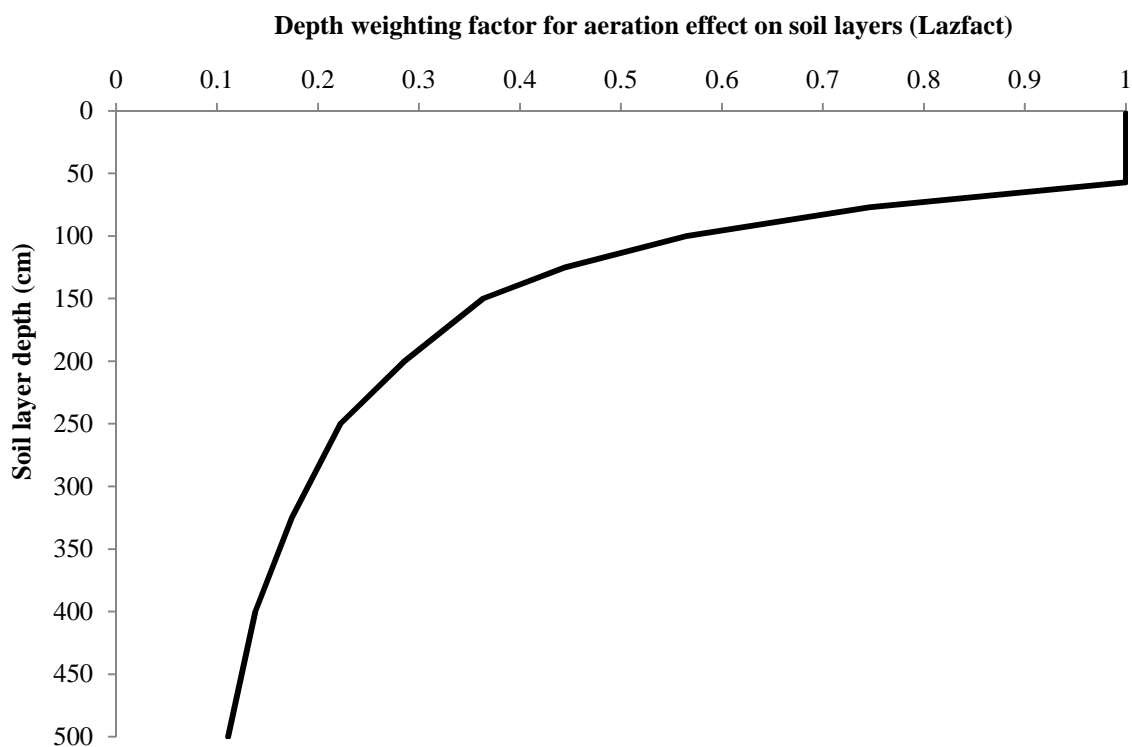


Figure 6.5: An example of the depth weighting factor for aeration effect (Lazfact) as soil layer depth increases.

6.3.4 Calculating the effects of hypoxia on root water uptake

The effects of waterlogging (low aeration) on the soil have been described by Lafact (Equation 6.1, Section 6.3.2) and Lazfact (Equation 6.5, Section 6.3.3). Using Lafact and

Lazfact for each layer the effects of hypoxia on plant root function (Aerf) are calculated as a zero to unity factor:

$$\text{Aerf} = \text{Lazfact} \times \text{Lafact} \quad (6.6)$$

However, during low aeration Lazfact and Lafact are very small values, resulting in an even smaller value when calculating Aerf using Equation 6.6. On testing it became apparent that the resultant product value of the two factors was excessively small. To address this problem Aerf was changed to:

$$\text{Aerf} = \frac{(\text{Lazfact} + \text{Lafact})}{2} \quad (6.7)$$

to give a better representation of the effects in magnitude of waterlogging stress compared to other stresses such as soil water deficit stress. For a deep wet soil layer (Aerf equals zero) there will be less aeration than a shallow dry (less than the critical water filled pore space (CritWFPS)) soil layer (Aerf equals one). This is because the shallow soil layers proximity to atmospheric oxygen means it returns to aerated conditions more rapidly than deep wet soil layers (Donohue *et al.*, 1984; Meyer and Barrs, 1988; Maher, 1997; Maher, 1999).

6.3.5 Calculating the number of waterlogged days

Chapters 2, 3 (Section 3.5), 4 (Section 4.3.1) and 5 (Section 5.6.3) discuss the effects that the duration of waterlogging has on soil oxygen concentration (soil aeration) and consequently plant growth. Within Destiny, plant response delay to reducing aeration, hence increasing hypoxia (AerDelay) can be a variable but is set at 3 days (Hunt *et al.*, 1981; Meyer and Barrs, 1988). This is the commonly observed time it takes for plants to respond to a waterlogging event and for the water filled pore space to increase above the critical value of 0.65 (Section 6.3.2). The number of waterlogged days (Ilatime) (in days) is calculated as:

$$\text{Ilatime} = 0 - \text{AerDelay} \quad (6.8)$$

$$\text{llatime} = \text{llatime} + 1 \quad (6.9)$$

With continuous waterlogging llatime will accumulate to a maximum of 60 days after which llatime is fixed at 60.

6.3.6 Calculating the layer aeration factor

The layer aeration factor (Laf1) is a zero to unity factor that brings together the effects of soil water content (and hence water filled pore space), depth of the layer in the profile, duration of the waterlogging event (llatime) and species sensitivity to waterlogging (SppAerFact). The function is specified as a power decay function:

$$\text{Laf1} = [(\text{Aerf})^{\text{llatime}^{0.167}}] \times [\text{SppAerFact}] \quad (6.10)$$

where SppAerFact (a zero to unity factor) is the species sensitivity factor with the default value set at one. The form of the function with various values of SppAerFact is shown in Figure 6.6.

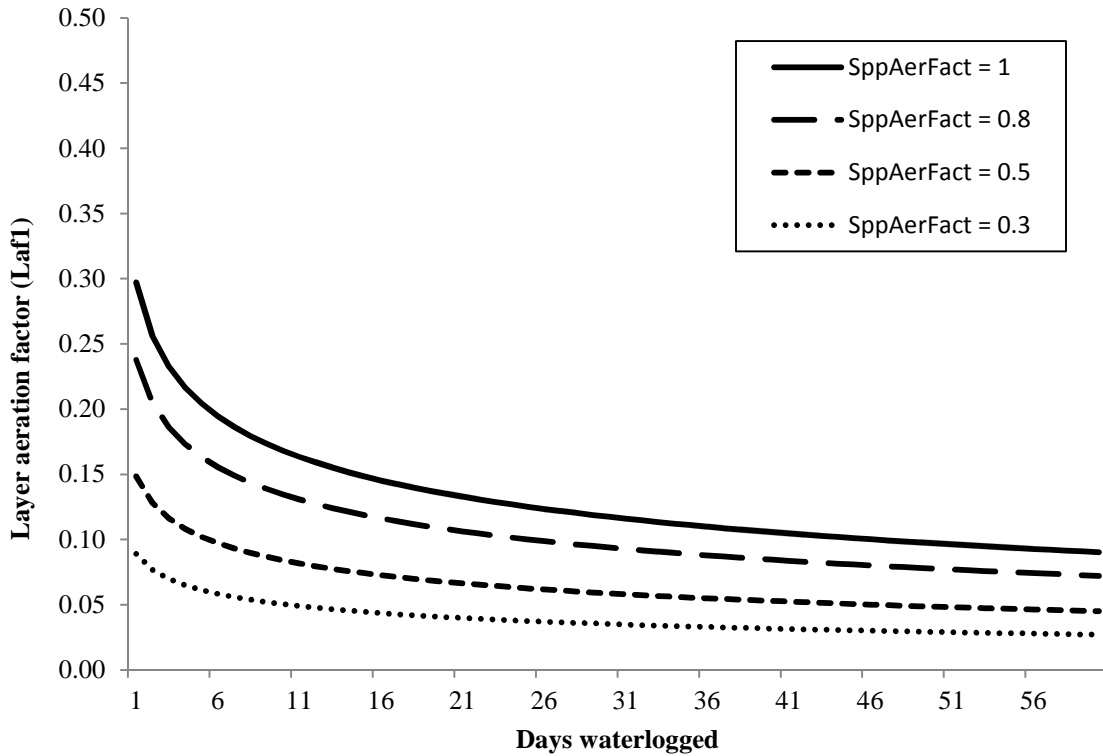


Figure 6.6: An example of the layer aeration factor (Laf1) at varying species sensitivity factors (SppAerFact). For this example the effect of hypoxia on plant root function (Aerf) is set to 0.3 to represent partially saturated soil (Section 6.3.4).

As indicated above (Section 6.3.2) the coding error in Equation 6.4 was compensated for by applying the following equation for Laf1:

$$\text{Laf1} = 1 - [(1 - \text{Aerf})^{\text{llatime}^{0.167}}] \times [\text{SppAerFact}] \quad (6.11)$$

A plot of Laf1 using the coding errors (Equations 6.4 and 6.11) shows with increasing days of waterlogging Laf1 increases towards one (Figure 6.7), resulting in less stress (in all Destiny zero to unity factor cases, zero is the maximum stress and one represents no stress). Stress from waterlogging increases with time (Chapters 2, 3, 4 and 5) concluding that this representation of Laf1 is incorrect. By the same principal (zero is the maximum stress and one represents no stress) when SppAerFact is closer to zero the stress should be worse, but

Laf1 is closer to one resulting in less stress. Equation 6.11 has been replaced with Equation 6.10.

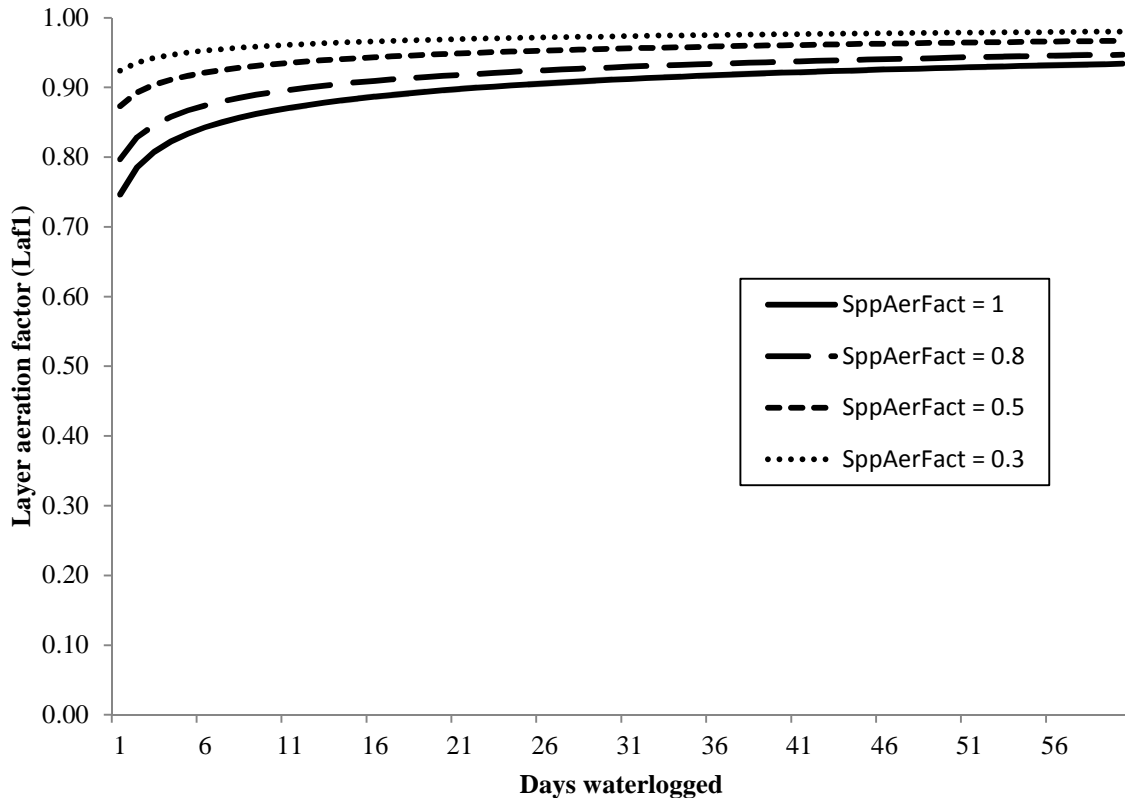


Figure 6.7: The incorrect representation of the layer aeration factor (Laf1) previously used in the Microsoft Visual Basic version of SWAGMAN Destiny at varying species sensitivity factors (SppAerFact). For this example the effect of hypoxia on plant root function (Aerf) is set to 0.3 to represent partially saturated soil (Section 6.3.4).

6.3.7 Assigning the effect of soil aeration on plant functions – effect on roots

One of the most unequivocal responses of plants to low soil oxygen concentrations is observed changes in root distribution (Meyer and Barrs, 1991; Maher, 1999). This effect is represented in Destiny by considering the calculated layer aeration factor (Laf1) through the whole profile and assigning the daily inferred carbohydrate for root growth from the leaves to root growth in layers with the highest Laf1 values. The effect of this is that soil layers that

have an inferred low oxygen concentration (Laf1 values closer to zero) will have less root growth assigned compared with layers with higher inferred oxygen concentrations (Laf1 values closer to one). This effect, combined with a uniformly applied root death rate of nominally 1 % will result in a gradual decline in root length density within layers that are waterlogged.

6.3.8 Assigning the effect of soil aeration on plant functions – effect on above ground plant functions

While the effect of Laf1 on roots is assigned on a layer basis (Equation 6.10, Section 6.3.6), the effect of aeration status on above ground plant functions is assigned using a whole soil profile factor (Saf1). This soil profile aeration factor (Saf1) is a zero to unity factor calculated as:

$$\text{Saf1} = \text{Saf1} + \sum_{L=1}^{L=15} \left(\left[\frac{\text{Rld}(L)}{\text{Trlv}} \right] \times \text{Laf1}(L) \right) \quad (6.12)$$

where

$$\text{Trlv} = \sum_{L=1}^{L=15} \text{Rld}(L) \quad (6.13)$$

Rld(L) is the root length density (cm cm⁻³) for layer L calculated daily and Trlv is the total root length density (cm cm⁻³) over the whole profile. Rld(L) is discussed in Section 6.3.11. The effect of Saf1 is that inferred low soil aeration in layers with more roots (larger root length density) will have a greater effect on the final value of Saf1 than layers with fewer roots. The root profile aeration factor (Saf1) is then used, along with other stress factors, to influence both leaf growth and photosynthesis.

6.3.9 Calculating canopy growth

Potential leaf area index (PotLAI) is estimated using the input values of peak leaf area index (PeakLAI) for a given crop, day degrees for the vegetative phase (DDveg), the date of sowing (Isow) (all assigned from the crop input file) and the sum of the degree days (SDDT). An actual leaf area index (ActLAI) is calculated from incrementing the daily change in leaf area which is the product of daily potential leaf growth and the worst (smallest index value) daily stress, for example:

$$\text{ActLAI} = \text{PotLAI} \times \text{Saf1} \quad (6.14)$$

where in the case of waterlogging, soil profile aeration (Saf1) is the worst stress on that day. The daily actual and potential leaf areas are accumulated and presented as leaf area indices (Figure 6.8).

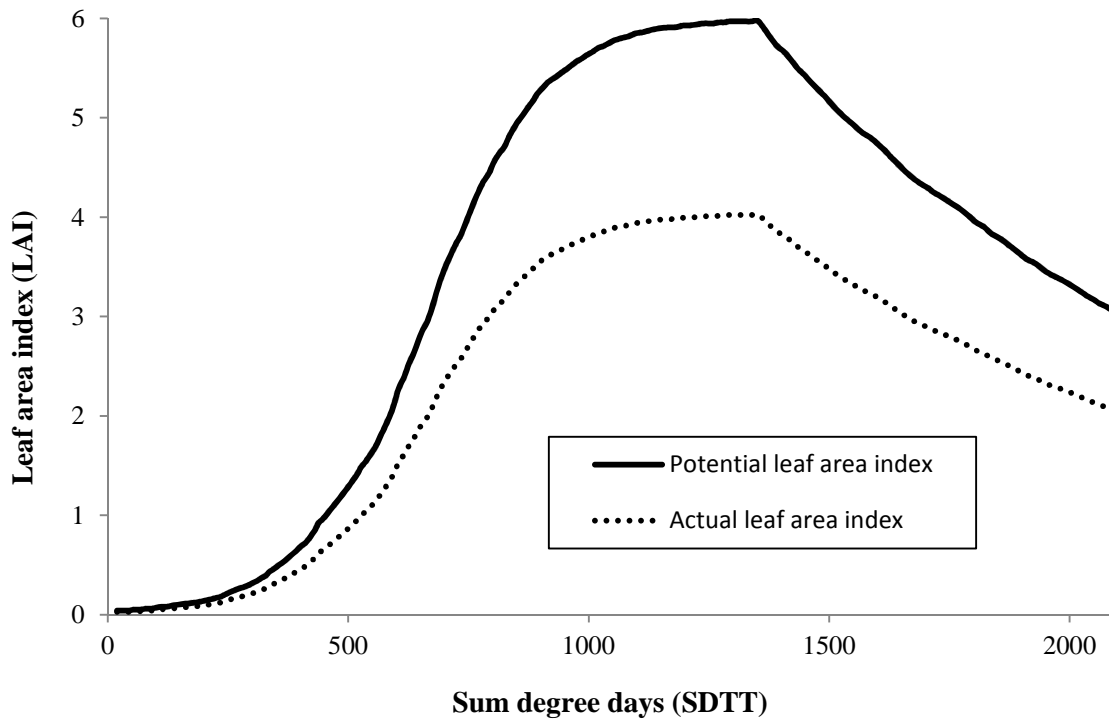


Figure 6.8: Example of potential leaf area index (PotLAI) and actual leaf area index (ActLAI) for a persistently waterlogged wheat crop in Griffith, Australia (34°17'S, 146°03'E, 130 m above mean sea level), 1993.

The sum of growing degree days (SDTT) is accumulated from the daily growing degree days (DTT):

$$DTT = \left(\frac{TEMPMX + TEMPMN}{2} \right) - T_{base} \quad (6.15)$$

where T_{base} (°C) is assigned for each crop as the minimum (base) temperature for plant growth (Table 6.1) and TEMPMN and TEMPMX are the minimum and maximum daily temperatures (in °C) respectively from the input SILO weather file.

The plants development stage (Xstage) is calculated as:

$$Xstage = \frac{SDTT}{DDveg} \quad (6.16)$$

If X_{stage} is less than or equal to one then the plant is in the vegetative stage of growth (Equation 6.16), if X_{stage} is more than one then the plant is deemed to be in the reproductive phase when new leaf growth will slow, eventually cease growth, mature and lose area:

$$X_{stage} = 1 + \left(\frac{SDTT - DD_{veg}}{DD_{mat} - DD_{veg}} \right) \quad (6.17)$$

where DD_{mat} is the degree days until plant maturity and is a crop input.

6.3.10 Converting incoming solar radiation to growth

Photosynthetically active radiation (Par) (in $MJ\ m^{-2}$) is calculated as:

$$Par = Solrad \times 0.5 \quad (6.18)$$

where $Solrad$ (in $MJ\ m^{-2}$) is the daily total solar radiant energy and is an input from the SILO weather file (Section 6.2.1).

The temperature index for photosynthesis and respiration ($Prft$) is calculated as:

$$Prft = 1 - 0.0025 \times [(0.25 \times TEMP_{MN}) \times (0.75 \times TEMP_{MX}) - 26]^2 \quad (6.19)$$

Converting Par to an inferred amount of carbohydrate ($Photo$) is calculated as:

$$Photo = Par \times ParConvFac \times [1 - e^{-0.65 \times ActLAI}] \times Saf1 \times 10 \quad (6.20)$$

where $ParConvFac$ is the efficiency of converting intercepted Par into biomass and is defined as a crop input (Table 6.1). Actual leaf area index ($ActLAI$) is calculated from the potential leaf area index ($PotLAI$) multiplied by the worst daily stress (assumed to be $Saf1$ in the case of waterlogging) as described above (Equation 6.14, Section 6.3.9).

6.3.11 Calculating root growth

There are multiple calculations and inputs required for representing crop root growth. A root weighting factor (WR) for soil depth is used to distribute new root growth in soil layers

where root growth has been initiated because root depth has extended to these layers. The root weighting factor by layer is an input from the soil file (Figure 6.9).

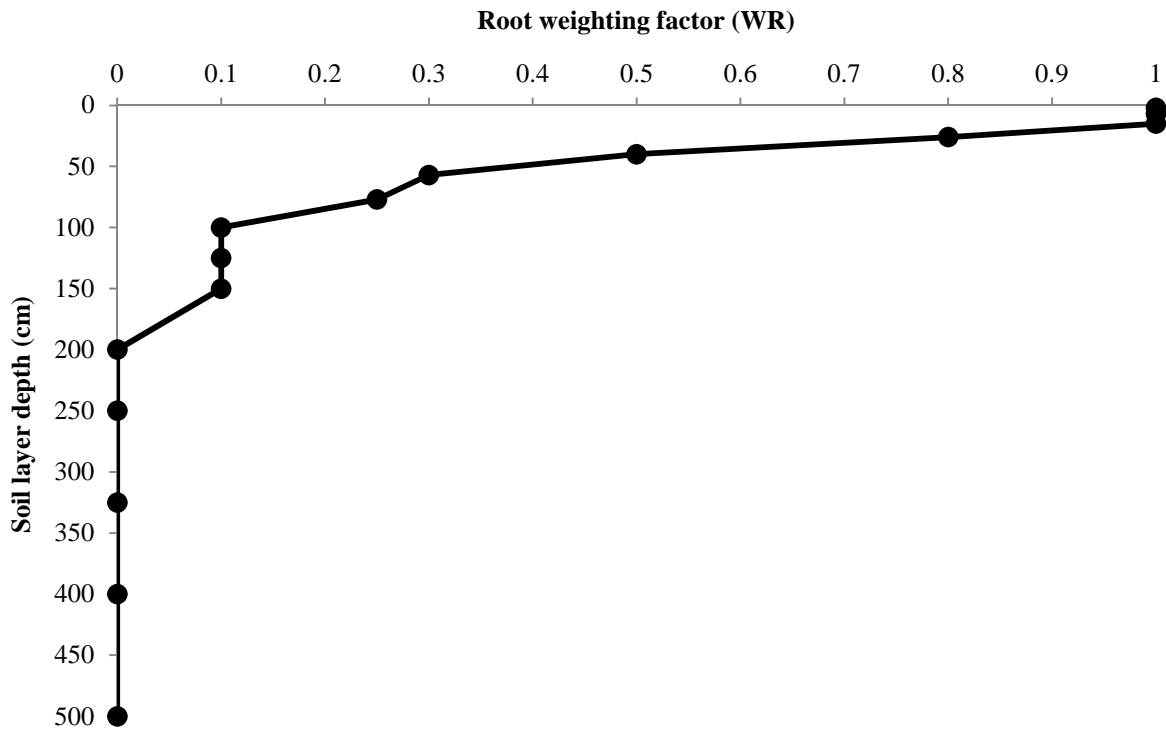


Figure 6.9: Example of the root weighting factor (WR) over the soil profile for Hanwood loam.

The amount of carbohydrate available for daily root growth (G_{root}) (in g cm^{-2}) is calculated as a proportion of the amount of intercepted radiation that has been converted to growth (Photo; Equation 6.20, Section 6.3.10) using a partitioning factor (Ptf) that assigns the amount of carbohydrates going to the roots:

$$G_{\text{root}} = \text{Photo} \times (1 - \text{Ptf}) \times 0.1 \quad (6.21)$$

A root length to weight ratio (RootLWRatio) (in cm g^{-1}) is calculated as:

$$\text{RootLWRatio} = \frac{\text{RtVolMass}}{\pi \left(\frac{\text{Root diameter}}{2} \right)^2} \quad (6.22)$$

where root diameter (cm) and root volume mass (RtVolMass) (in $\text{cm}^3 \text{g}^{-1}$) are crop input values. New root growth (R_{new}) (in cm cm^{-2}) in each soil layer (L) is calculated as:

$$R_{\text{new}} = G_{\text{ort}} \times \text{RootLWRatio} \quad (6.23)$$

A root length density factor (Rldf) for each soil layer (L) is used to calculate the distribution of root length density between layers over the soil profile:

$$R_{\text{ldf}} = R_{\text{ldf}} \times WR \times D_{\text{layr}} \quad (6.24)$$

The new root length density (Rld) for the soil profile is calculated by:

$$R_{\text{ld}} = R_{\text{ld}} + R_{\text{ldf}} \times \frac{R_{\text{nlf}}}{D_{\text{layr}}} - \left(0.01 + 0.01 \times \left[1 - \left\langle \frac{S_w - LL}{DUL - LL} \right\rangle \right] \right) \times R_{\text{ld}} \quad (6.25)$$

where Rnlf (in cm cm^{-2}) is a temporary variable used for root length distribution, Sw (in $\text{cm}^3_{\text{water}} \text{cm}^{-3}_{\text{soil}}$) is the volumetric soil water content, DUL (in $\text{cm}^3 \text{cm}^{-3}$) is the drained upper limit and LL (in $\text{cm}^3 \text{cm}^{-3}$) is the lower limit of the plant extractable soil water in a layer.

The change in root depth on a daily basis (DeltaDep) is calculated from the product of thermal time since sowing or the start of growth (Rdtt) and the root development rate (RootDrate) which is a crop input (in units of $\text{cm } ^\circ\text{C}^{-1} \text{day}^{-1}$). This rate of depth increase will be slowed if the layer in which root depth growth is active is subject to poor aeration. This is done by applying the layer aeration factor (Laf1; Equation 6.10, Section 6.3.6) when it is less than 0.25 in the equation:

$$\text{DeltaDep} = R_{\text{dtt}} \times \text{RootDrate} \times (\text{Laf1} \times 4) \quad (6.26)$$

6.3.12 Calculating plant biomass

Calculating plant biomass is the final step towards calculating final yield. Firstly potential carbohydrate (PCarbo) is found by:

$$\text{PCarbo} = \text{Par} \times \text{ParConvFac} \times \left(1 - e^{(-0.65 \times \text{PotLAI})} \right) \times \text{Saf1} \times 10 \times \text{Prft} \quad (6.27)$$

assuming, in this case, the limiting stress is aeration stress (Saf1).

The actual carbohydrates are found using Equation 6.27, but with the actual leaf area index (ActLAI) rather than the potential leaf area (PotLAI) index:

$$\text{Carbo} = \text{Par} \times \text{ParConvFac} \times (1 - e^{(-0.65 \times \text{ActLAI})}) \times \text{Saf1} \times 10 \times \text{Prft} \quad (6.28)$$

again assuming Saf1 is the limiting stress.

A respiration load (Rload) is calculated as a function of plant development stage (Xstage; Equations 6.16 and 6.17, Section 6.3.9), temperature (DTT and SDTT; Equation 6.15, Section 6.3.9) and potential (PotBiomass) or actual (ActBiomass) biomass:

$$\text{Rload} = \frac{\text{Xstage}}{\text{Rcoef}} \times \frac{\text{DTT}}{\text{SDTT}} \times \text{PotBiomass} \quad (6.29)$$

$$\text{Rload} = \frac{\text{Xstage}}{\text{Rcoef}} \times \frac{\text{DTT}}{\text{SDTT}} \times \text{ActBiomass} \quad (6.30)$$

where Rcoef is the rate of leaf appearance and is set to 1.8.

Actual biomass (ActBiomass) (in kg ha⁻¹) is accumulated each day after Rload is subtracted and the amount of PCarbo remaining in the plant tops is added:

$$\text{ActBiomass} = (\text{Accumulated ActBiomass} - \text{Rload}) + \text{PCarbo} \times \text{Ptf} \quad (6.31)$$

Similarly potential biomass (PotBiomass) (in kg ha⁻¹):

$$\text{PotBiomass} = (\text{Accumulated PotBiomass} - \text{Rload}) + \text{PCarbo} \times \text{Ptf} \quad (6.32)$$

6.3.13 Calculating final yield

Final yield (Yield, kg ha⁻¹) is calculated using the potential yield (PotYield, kg ha⁻¹), which is an input from the crop file (Table 6.1) reduced by the ratio of the actual biomass (ActBiomass) relative to the potential biomass (PotBiomass):

$$\text{Yield} = \text{PotYield} \times \frac{\text{ActBiomass}}{\text{PotBiomass}} \quad (6.33)$$

6.4 IMPROVING WATERLOGGING REPRESENTATIONS IN SWAGMAN DESTINY

By analysing waterlogging field trials (Chapter 2), performing greenhouse experiments (Chapters 3 and 4) and proposing an empirical representation of waterlogging (Chapter 5) it

became apparent that the current version of Destiny overestimated reductions in crop yield due to waterlogging. Consequently the equations for the relative amount of pore space filled with water (Lafact; Equation 6.1, Section 6.3.2), the effect of hypoxia on plant root function (Aerf; Equation 6.6, Section 6.3.4) and the layer aeration factor (Laf1; Equation 6.10, Section 6.3.6) were changed to better represent physical conditions occurring when soil is waterlogged. Scenarios (Table 6.4) were run in Destiny to compare outputs before (Scenario 1) and after (Scenario 2) changes to Lafact, Aerf and Laf1, and are discussed in this section.

Table 6.4: The definition of 2 simulation scenarios to compare equation changes in SWAGMAN Destiny.

	Relative amount of pore space filled with water (Lafact)	Effect of hypoxia on plant root function (Aerf)	Layer aeration factor (Laf1)
Scenario 1	$\text{Lafact} = 1 - \frac{(\text{SWWFPS} - \text{CritWFPS})}{(\text{Tpore} - \text{CritWFPS})}$	$\text{Aerf} = \text{Lazfact} \times \text{Lafact}$	$\text{Laf1} = 1 - [(1 - \text{Aerf})^{\text{llatime}^{0.167}}] \times [\text{SppAerFact}]$
Scenario 2	$\text{Lafact} = 1 - \frac{(\text{SWWFPS} - \text{CritWFPS})}{(1 - \text{CritWFPS})}$	$\text{Aerf} = \frac{(\text{Lazfact} + \text{Lafact})}{2}$	$\text{Laf1} = [(\text{Aerf})^{\text{llatime}^{0.167}}] \times [\text{SppAerFact}]$

The same inputs used to mimic transient waterlogging in the field in Chapter 5 (Section 5.8.1) are used in this section. The inputs are wheat (cv. Egret) at Griffith, NSW, Australia (34°17'S, 146°03'E, 130 m above mean sea level), on Hanwood Loam, using SILO climate data (<http://www.longpaddock.qld.gov.au/silo/>) for 1994 with total rainfall of 93 mm during the 181 day growing period, with 500 mm of irrigation applied over 6 irrigation events (Table 5.4, Chapter 5).

Figure 6.10 shows the effect of irrigation and rainfall (used to induce waterlogging) on Saf1 in Destiny. In Scenario 1 Saf1 approaches zero, however, in Scenario 2 Saf1 reaches a minimum of 0.5 (zero being the worst stress, one being no stress). The effect of Saf1 on plant biomass surrounding the waterlogging event (irrigation) on 105 days after sowing (DAS) can

be seen in Figure 6.11 and Figure 6.12 for Scenarios 1 and 2 respectively. Scenario 1 (Figure 6.11) shows a decrease in the rate of growth of plant biomass (shown by a negative slope) as soon as Saf1 is in effect 107 DAS (3 days after irrigation) until 119 DAS. Scenario 2 (Figure 6.12) takes 7 days after the waterlogging event (112 DAS) to show a decrease in the rate of growth of plant biomass, with the decrease lasting only 1 day.

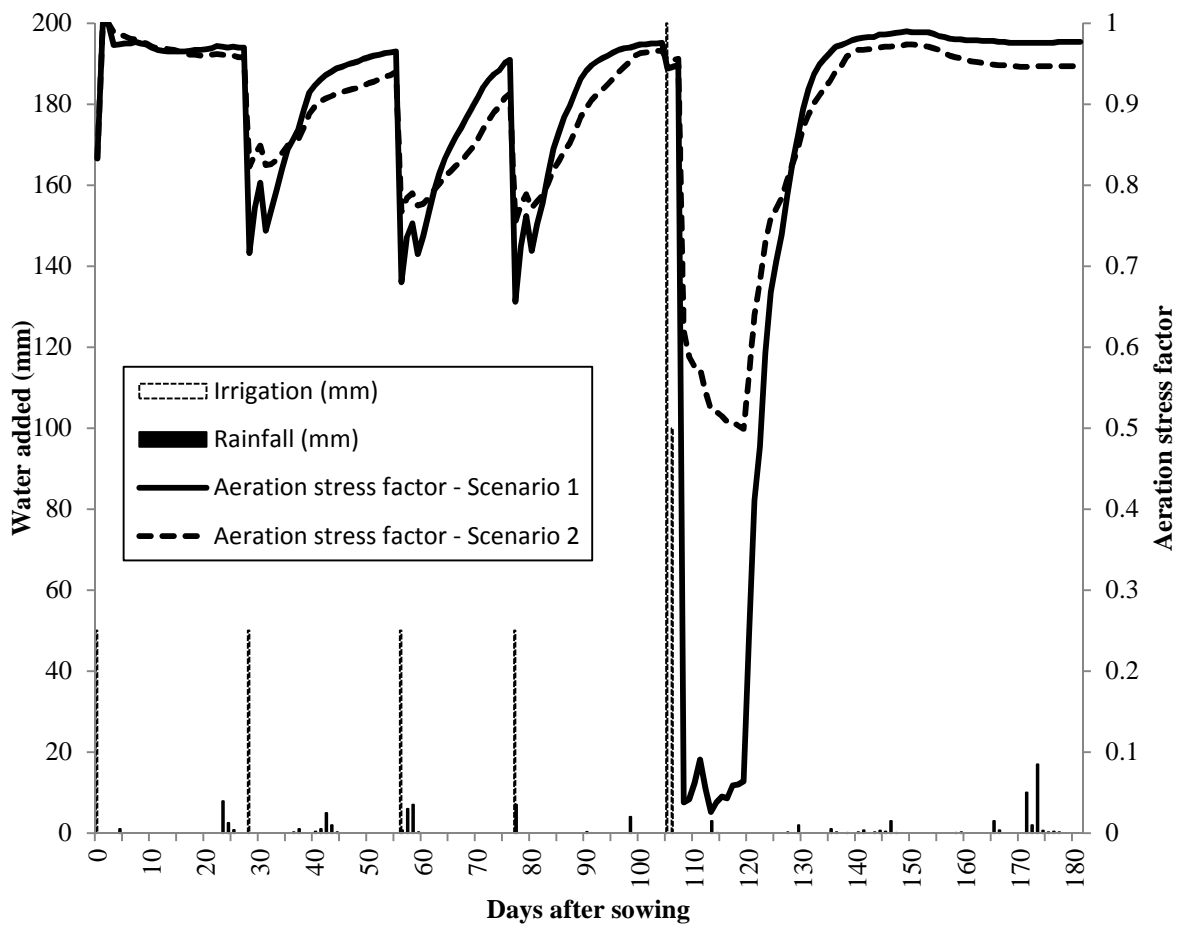


Figure 6.10: The effect of irrigation and rainfall on the aeration stress factor (Saf1) throughout the soil profile during transient waterlogging for Scenarios 1 and 2 in SWAGMAN Destiny.

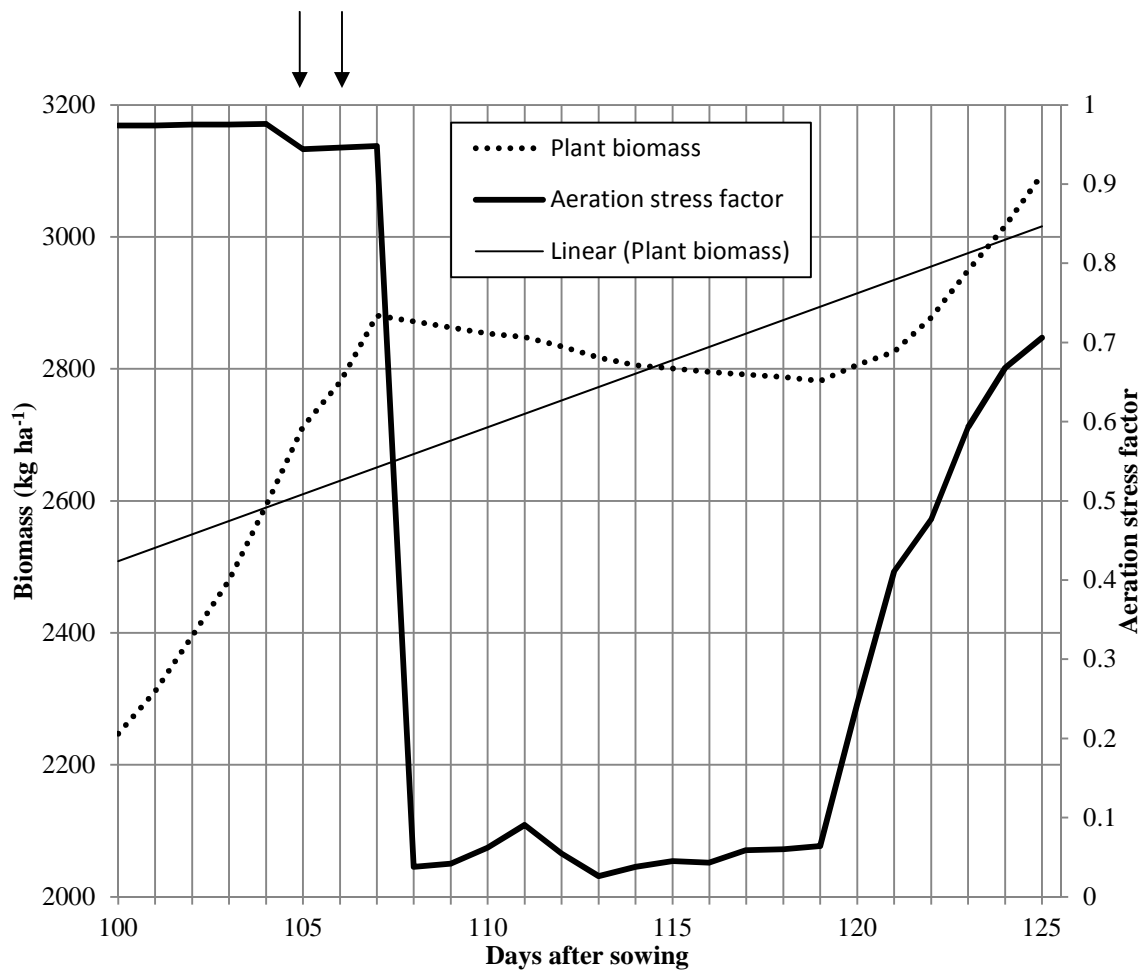


Figure 6.11: The effect of the aeration stress factor (solid line) on plant biomass (dotted line) during transient waterlogging for Scenario 1 in SWAGMAN Destiny. Arrows indicate an irrigation event. Equation of the plant biomass trend line is $y = 20.29x + 2488.2$, $R^2 = 0.59$.

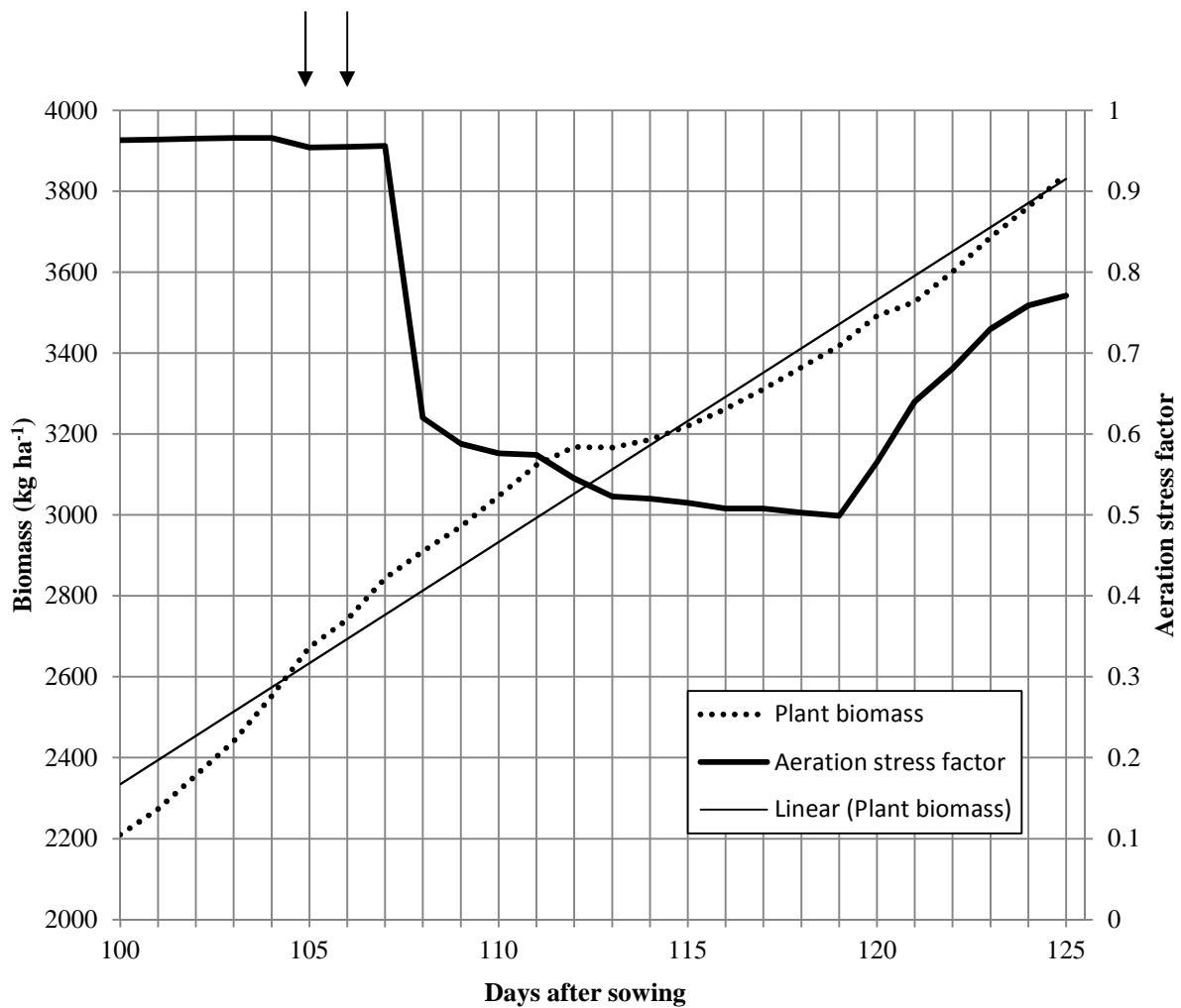


Figure 6.12: The effect of the aeration stress factor (solid line) on plant biomass (dotted line) during transient waterlogging for Scenario 2 in SWAGMAN Destiny. Arrows indicate an irrigation event. Equation of the plant biomass trend line is $y = 59.85x + 2247.5$, $R^2 = 0.97$.

Scenario 1 produces a final yield of 3429 kg ha^{-1} , Scenario 2 produces a yield of 3936 kg ha^{-1} . As discussed in Chapter 5, Section 5.8.1 the changes made to Lafact, Aerf and Laf1 in Destiny (Scenario 2) better represent plant adaptations during waterlogging that occur in the field (Meyer *et al.*, 1985; Meyer and Barrs, 1988). Although transient waterlogging has been shown to reduce some crop yields in the field (Cannell *et al.*, 1980; Belford, 1981), transient waterlogging rarely reduces yield to the extent shown by Scenario 1. Consequently equation changes to Lafact, Aerf and Laf1 from Scenario 2 have been used for all subsequent tests.

6.5 THE EFFECT OF THE SPECIES SENSITIVITY FACTOR IN SWAGMAN DESTINY

The species sensitivity factor (SppAerFact) is an input from the crop file of Destiny that represents a plant species sensitivity to low aeration conditions (waterlogging). It is a zero to unity factor; zero representing high sensitivity to waterlogging and one representing no effect to the plant from waterlogging. Currently the SppAerFact is set to 1 for all crops in Destiny (Section 6.3.6). The effects of changing the SppAerFact of wheat on the aeration stress factor (Saf1), plant biomass (Actbiomass) and yield (Yield) were tested in Destiny. The same inputs were used from Section 6.4 and Chapter 5, Section 5.8.1 to represent waterlogging in the field.

The changes in Saf1 and plant biomass for a wheat SppAerFact of 1, 0.5 and 0 can be seen in Figure 6.13, Figure 6.14 and Figure 6.15 respectively. As the SppAerFact decreases Saf1 approaches zero for longer periods of time, consequently the rate of plant growth decreases, represented by a reduction in Actbiomass (Figure 6.13, 6.14 and 6.15). A reduction in Actbiomass results in reduced final yield (Table 6.5). Table 6.5 highlights the sensitivity of the SppAerFact and its effect on crop yield.

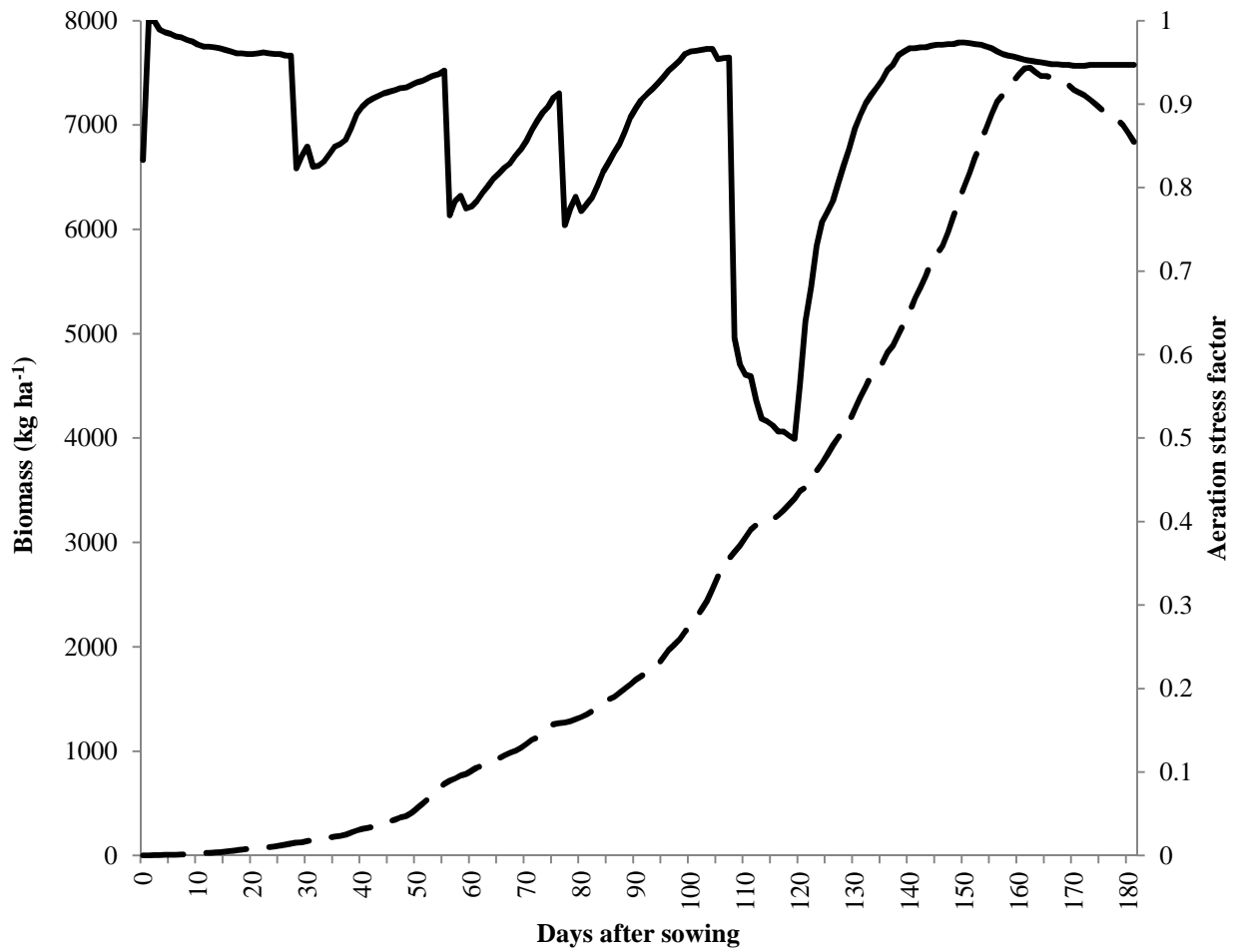


Figure 6.13: The effect of the aeration stress factor (Saf1) (solid line) on plant biomass (Actbiomass) (dashed line) with the species sensitivity factor (SppAerFact) set to 1 during transient waterlogging in SWAGMAN Destiny.

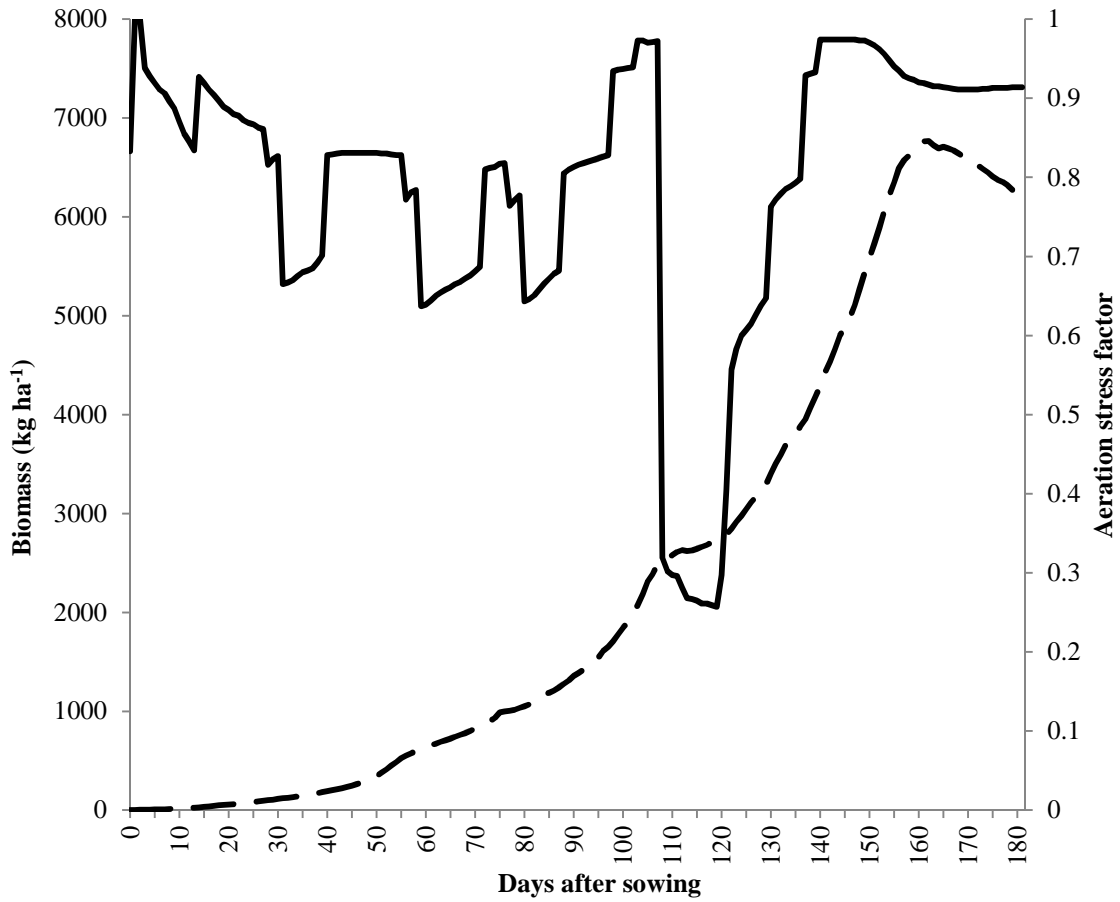


Figure 6.14: The effect of the aeration stress factor (Saf1) (solid line) on plant biomass (Actbiomass) (dashed line) with the species sensitivity factor (SppAerFact) set to 0.5 during transient waterlogging in SWAGMAN Destiny.

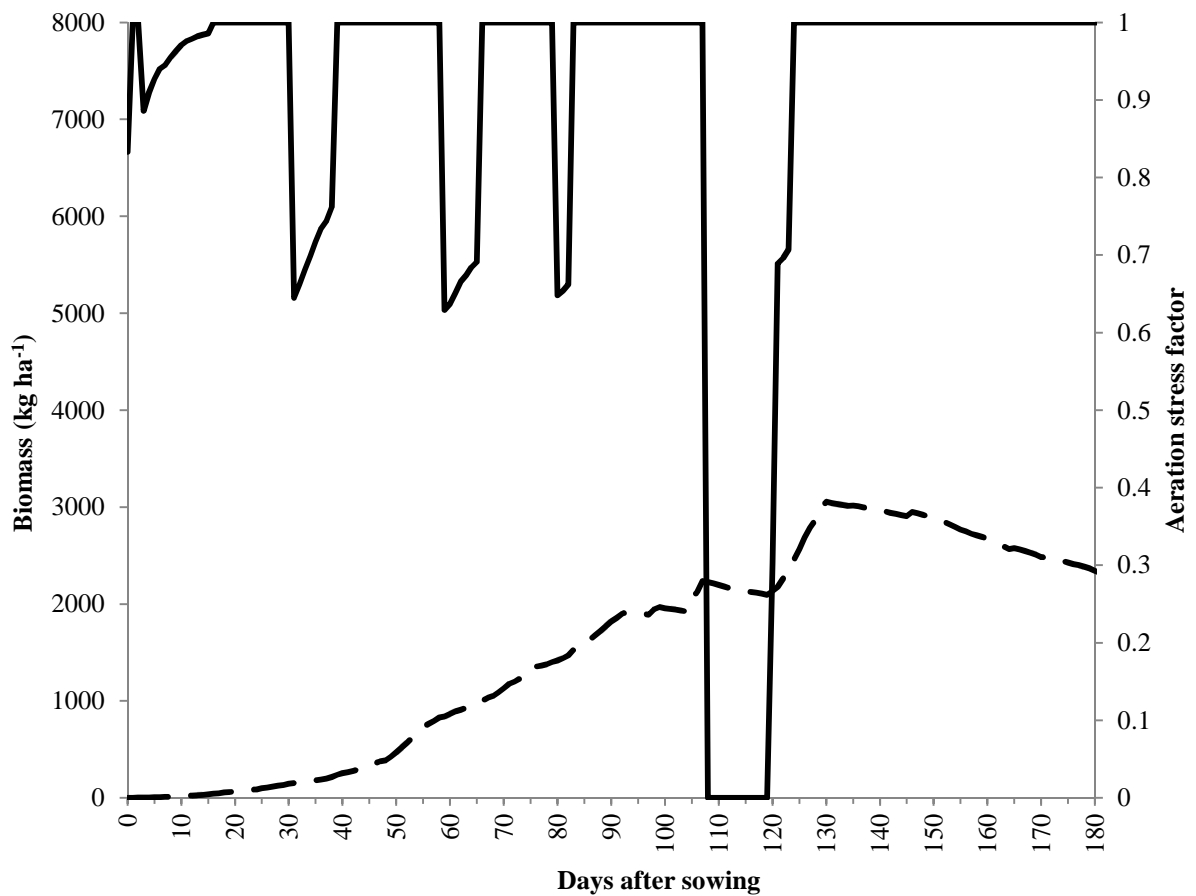


Figure 6.15: The effect of the aeration stress factor (Saf1) (solid line) on plant biomass (Actbiomass) (dashed line) with the species sensitivity factor (SppAerFact) set to 0 during transient waterlogging in SWAGMAN Destiny.

Table 6.5: The effects of changing the species sensitivity factor (SppAerFact) on final yield (Yield) in SWAGMAN Destiny.

Species sensitivity factor (SppAerFact)	Grain Yield (kg ha ⁻¹)
1	3936
0.8	3795
0.5	3527
0.1	1829
0	1334

The sensitivity of the SppAerFact means that for the same waterlogging duration, soil profile and climate the SppAerFact could be used to estimate reductions in crop yields according to

the plant species tolerance or sensitivity to waterlogging. There have been numerous comparisons of waterlogging tolerances between plant species (Jones and Marshall, 1992; Crawford and Braendle, 1996; Bell, 1999; Colmer, 2003b; Moore and McFarlane, 2004; Bramley *et al.*, 2007; Aroca *et al.*, 2012). However, the waterlogging conditions between and within the comparisons are often very different. These plant species tolerance comparisons are currently the best available, but given the often very large differences in waterlogging conditions the ranking of species tolerance should be interpreted as indicative only. Consequently I suggest using them with great caution to compare simulated yield outputs during waterlogging for different species. Based on the tolerances between plant species a SppAerFact could be assigned to each species, hence effecting estimated final yield. For example, a SppAerFact could be based around the waterlogging loss factor defined by Jones and Marshall (1992). Their waterlogging loss factor is based on yield reductions during waterlogged conditions, rice being the most tolerant species ranging to perennial pastures as the least tolerant. Similarly the SppAerFact could be based on a reduction in root hydraulic conductivity summarised by Bramley *et al.* (2007); *Agave deserti* being the most tolerant, wheat being the least tolerant to waterlogging. Root porosity (a measure of aerenchyma formation in roots (Chapter 2; Plant responses to waterlogging)) might represent the SppAerFact. Colmer (2003b) summarises root porosity during waterlogging for a variety of species; rice being the most tolerant to waterlogging, *Festuca rubra* being the least tolerant. Crawford and Braendle (1996) monitored new root growth (SppAerFact representing new root growth) for a variety of species during waterlogging ranging from *Acorus calamus* as the most tolerant to potato as the least tolerant to waterlogging. From these few examples of a plant species tolerance to waterlogging it is obvious there are variations between reports, hence care must be taken in understanding what crop inputs are being used and what final yield estimates truly represent.

6.6 MAKING THE CONNECTION BETWEEN THE OBSERVED (AND MEASURED) PHYSIOLOGICAL RESPONSES TO WATERLOGGING AND THEIR REPRESENTATION IN A CROP GROWTH, WATER USE AND YIELD MODEL

It has been extensively established that there are many and varied responses observed between species. With this reality, application of detailed physiological modelling would need to be constructed species by species. This is not practical for more generally applied crop growth, water use and yield models. To partially account for species response differences the unifying response concept in Chapter 5 was developed. It follows then that outputs from a model that includes waterlogging effects should show the expected sequence and form of day-to-day responses, i.e. initial improved function and growth, then decreased function and growth followed by an adaptation response dependant on species aeration sensitivity. Outputs from the modified Destiny model show this sequence.

The physiological effect of waterlogging on *N. glutinosa* explained in this study has identified that impairment of the water transport pathway from rhizosphere into the root xylem is the likely cause of the observed leaf wilting. Since leaf wilting is observed only in a few species subject to waterlogging the inclusion of this representation of this specific physiological effect into general crop growth and yield models is not warranted. Nonetheless, the representation of decreasing [O₂] on root growth and distribution and then an effect of this abiotic stress on the critical above ground process, namely photosynthesis and hence carbohydrate production is warranted. In essence, the effect of water deficit stressed leaves on a plant species that has this response to waterlogging is approximated by this representation. Similarly in plant species that show leaf yellowing and necrosis on

waterlogging, the inference of impaired carbohydrate production can be calibrated to produce modelled growth and yield responses consistent with observed values.

6.7 CONCLUSION

With a better understanding of plant responses and adaptations during waterlogging gained in Chapters 2 to 5, I found that the modelled reduction in crop yield due to a waterlogging event in Destiny was too great compared to waterlogging field and greenhouse trials. I was able to modify the equations for the relative amount of pore space filled with water, the effect hypoxia has on plant root function and the layer aeration factor in Destiny to better represent waterlogging in the field. Additionally, I have shown the sensitivity of the species sensitivity factor for comparing crop yields for tolerances of plant species to waterlogging. However, care must be taken to only compare plant species waterlogging tolerances for the same edaphic and environmental conditions.

CHAPTER 7
CONCLUSIONS AND FUTURE RESEARCH

7 CHAPTER 7

7.1 CONCLUSIONS AND FUTURE RESEARCH

Waterlogging has been said to reduce crop yields by up to 80 % (MacEwan *et al.*, 1992), although my literature review (Chapter 2: Shaw *et al.* (2013)) revealed there are many discrepancies between what is reported around yield and economic loss due to the differences in the way that areas effected by waterlogging are defined. There are also limited measurements of plant physiological responses and adaptations reported following waterlogging in the field. The lack of information available to farmers makes it hard to estimate crop yield losses due to waterlogging. In Chapter 2 I have suggested a minimum data set that will be useful to estimate the potential effect of waterlogging. This data set should include:

- Soil profile information (areas with duplex soils);
- Topographic data (slope and the proportion of area on the downside of slopes);
- Meteorological information (seasonal weather data sufficient to estimate a daily water balance and identify when periods of soil saturation occurred and the likely duration);
- Plant morphological appearance (careful observations of plant growth and development prior to, during and following any suspected waterlogging);
- The areas with visible surface water, its extent, depth and duration.

Careful compilation of these data and observations can assist in identifying the likelihood of plant effects associated with waterlogging events. This would also assist in separating likely waterlogging effects from other abiotic stresses that can occur during a crop season. These data and observations would also provide case studies to assist validate and refine crop growth and yield models that explicitly incorporate waterlogging effects.

An above ground plant response to waterlogging that is easily identifiable in some species is leaf wilting (Chapters 2 and 4). A possible reason for leaf wilting is a reduction in root hydraulic conductance. I investigated the relationship between wilting and root water uptake in waterlogged *Glycine max* L. (soybean) and *Nicotiana glutinosa* L. and was able to establish a sequence of plant responses to waterlogging (soybean; Chapter 3, *N. glutinosa*; Chapter 4).

Waterlogged soybean showed very little change in plant physiology or morphology, with no observed leaf wilting or significant top growth impairment, implying a high tolerance to low aeration in the root zone. Both leaf water potential and stomatal conductances indicated well-functioning plants with no indication of stress from waterlogging. Root hydraulic conductance and root dry weight showed no significant differences until a reduction on day 5 of waterlogging. Waterlogging for longer periods would be required to identify if the development of adventitious roots formed on day 4 of waterlogging overcame the apparent reduction in root growth and function.

At the other end of the waterlogging sensitivity scale I found that before root zone soil oxygen concentrations reached 10 % there was a 50 % reduction in root dry weight of *N. glutinosa* on day 2 of waterlogging. This large reduction in root dry weight, so soon after waterlogging, could be due to the absence of carbohydrate (starch) in waterlogged roots and requires further investigation. A decrease in measured stomatal conductance and from this a decrease in estimated plant hydraulic conductance were accompanied by leaf wilting and leaf yellowing. However, measurements of root hydraulic conductance with the Dynamax Hydraulic Conductance Flow Meter (HCFM) indicated increased root conductance. Measurements of hydraulic conductance in petioles and leaf laminae were similar for well drained and waterlogged plants until day 4 when waterlogged plants had higher conductance values. Leaves showing and having measurements consistent with water deficit stress while

measures of increased root and upper plant apparent hydraulic conductivity is anomalous. This result casts doubt on the suitability of the HCFM for measuring root conductivity when root integrity is compromised by anatomical breakdown. It can also suggest that plant water flow during waterlogging is more dependent on osmotic rather than hydrostatic gradients. This hypothesis requires further research to investigate the different results obtained using different measuring techniques. Additionally, stomatal conductance was used to calculate evapotranspiration, in turn estimating plant hydraulic conductance. Confirmation of plant hydraulic conductance using measured evapotranspiration should be performed.

After the soil oxygen concentration dropped below 10 % on day 4, there was a reduction of leaf water potential and shoot dry weight and an increase in leaf hydraulic conductance. Other researchers have found a decrease in measured (Tsuda and Tyree, 2000) and calculated (Pou *et al.*, 2013) leaf hydraulic conductance together with a decrease in stomatal conductance, this anomaly requires further research. During my trials *N. glutinosa* showed no signs of morphological adaptations to waterlogging such as development of aerenchyma and/or adventitious roots.

The results from my greenhouse experiments suggest that current physical models (Hiler, 1969; Meyer *et al.*, 1996; Skaggs, 2008) representing waterlogged plants having fixed hydraulic conductance are physiologically flawed. I have shown that observed leaf wilting is associated with reduced stomatal conductance and leaf water potential. It is therefore consistent with water deficit even though roots are surrounded by water. The breakdown of root anatomical integrity seems likely to have been associated with the impedance of root water uptake. If this is the case then it further suggests that water uptake in roots is more influenced by cellular and membrane integrity and hence osmotic and active uptake influence than by hydrostatic gradients exerted through liquid continuity in the plant. Using this

knowledge and the sequence of responses of waterlogged plants during my greenhouse experiments (Chapters 3 and 4) I was able to make headway into updating current models by proposing an empirical representation of waterlogging (Chapter 5). I proposed basing waterlogging severity around the length of time a crop is waterlogged and the crops tolerance to waterlogging. The crop tolerance to waterlogging was based on previous field trial results measuring plant adaptations such as leaf and stem growth, leaf area or evapotranspiration.

Using the knowledge I gained proposing an empirical representation of waterlogging (Chapter 5) I was able to improve the crop growth and yield simulation model, SWAGMAN Destiny (Destiny), to better represent the estimates of crop yield changes during waterlogging (Chapters 5 and 6). Modifications to the relative amount of pore space filled with water, the effect hypoxia has on plant root function and the layer aeration factor were implemented and simulated output yields reflected published field trial results. Further improvements and future work to the waterlogging module of simulation models could be made by including plant species tolerances to waterlogging. However, care must be taken when comparing species tolerances during a waterlogging event. For direct comparisons between species there must be consistency around the amount of time waterlogged, the soil profile, topography and climate. Given growing season differences between species and the lack of control on root zone variability in field waterlogging trials it is highly unlikely that definite field comparison of species sensitivity to waterlogging is possible. This reality means that simulation modelling that represents the critical processes causing waterlogging and the response processes of plants has an important role in assisting understanding a waterlogged soil plant system. Comparison of the Destiny model outputs with field waterlogging trials has shown general agreement. However additional validation is needed as is further experimentation to improve the representation of the numerous species specific responses to waterlogging.

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