Impact of episodes of high temperature on physiology, growth and yield performance of potato (*Solanum tuberosum* L.)

This thesis is presented by **Charles Otieno Obiero** for the degree of Doctor of Philosophy of Murdoch University

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Declaration

I declare that this thesis is my original work and its content has not been presented at any University for the award of any diploma/degree.

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Abstract

Potato is the most important non-cereal crop for world food security. It is adapted to mild conditions, but its production is rapidly shifting to warmer regions where more and longer episodes of high temperatures are likely to reduce its yield. This will exacerbate the effects of global warming on potato production. Nevertheless, little is understood about how episodes of high temperatures influence potato physiology, growth and tuber yields because most of the research has been conducted under persistent high temperature treatments. This study assessed the potato-growing regions of Western Australia for the actual temperatures experienced by potato plants during the critical period of tuber growth (Chapter 3). It then investigated how the identified episodes of high temperatures influenced leaf growth, photosynthetic rate and dry matter partitioning to the tubers (Chapter 5, 6, 7 and 8). Lastly, it explored whether an agronomic intervention through application of plant growth regulators could overcome the negative impacts of the high temperatures (Chapter 9).

Climate analysis showed the number of hot days above 25 °C increased from 16 to 22 in the various regions in 1985 to 36 to 54 days in 2014. More than seven consecutive days above 25 °C occurred at least once per year in all regions except in Albany. The glasshouse study showed that tuber dry matter was reduced to a similar extent when a 9-day episode of 26 or 30 °C was applied before or after tuber initiation. Even one-day exposure of potato plants to high temperatures subsequently reduced tuber growth. Plants in the high temperature treatments had the same or slightly more tubers per plant than those in the control. More small-sized tubers (tubers with the diameter at the widest part of less than 2.5 cm) were produced when the episode of 30 °C exceeded three consecutive days. There was less starch and sucrose content in the tubers of the high temperature treatments relative to the control, but more sucrose accumulated in the leaves of plants that had been exposed to the high temperature episodes before tuber initiation. The shoot grew at the same rate regardless of the impact of the episode of the high temperatures. After the end of the high temperature period, the high temperature treatments only had 60 % of the leaf area on the main shoot as in the control. The net photosynthetic rate per unit leaf area measured between 12.00 and 2.00 pm during the high temperature period was also reduced more in the older than in the younger leaves. The high temperatures increased the morning (7.00 am to 10.00 am) and the
night-time (7.00 pm to 1.00 am) dark respiration rates per unit leaf area regardless of the leaf age. Exogenously applied auxin inhibitor (TIBA) caused similar effects to the high temperature but applying auxin (IAA) did not overcome the negative impact of high temperature on the potato plants.

The reduction in tuber growth in this study is inconsistent with the proposed inactivation of starch synthase (which reduces the conversion of sucrose into starch in the tubers hence reduces tuber growth) or increased production of gibberellic acid (which not only stimulates shoot growth which diverts carbon away from the tubers but also reduces starch synthase activity in potato plants grown at high temperatures). This is because, in the present study, the reduction in tuber growth occurred mostly after the end of the high temperature period when plants were grown back at cooler conditions. Further, the reduction occurred even in plants that had no tubers present at the end of the high temperature period. Moreover, the tubers in the high temperature treatments had less sucrose and starch content rather than the expected increase in sucrose content with inhibition of starch synthase activity. Lastly, the shoot grew at the same rate while the laterals grew more after the end of the high temperature period.

In this study, the reduction in tuber growth was more consistent with lower whole plant carbon supply due to reduced leaf area, lower net photosynthesis in older leaves, higher respiration rates regardless of the leaf age and the continued shoot growth. These findings provide new insights into how potato responds to high temperatures.
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<tbody>
<tr>
<td>®</td>
<td>Trade mark symbol</td>
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<tr>
<td>ACS</td>
<td>American Chemical Society</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AR5</td>
<td>Fifth assessment report</td>
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<tr>
<td>BAP</td>
<td>6-Benzyl Amino Purine</td>
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<tr>
<td>CCC</td>
<td>Chlorocholine chloride</td>
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<tr>
<td>CCI</td>
<td>Chlorophyll concentration index</td>
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<tr>
<td>CLIMARC</td>
<td>Computerizing the Australian Climate Archives</td>
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<tr>
<td>CORR</td>
<td>Correlation</td>
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<td>D</td>
<td>Diameter</td>
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<td>D/N</td>
<td>Day/Night</td>
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<tr>
<td>DAA</td>
<td>Days after anthesis</td>
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<td>DAP</td>
<td>Days after planting</td>
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<td>DAS</td>
<td>Days after sowing</td>
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<tr>
<td>DD</td>
<td>Data drill</td>
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<tr>
<td>DDI</td>
<td>Double deionized water</td>
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<td>DD-water</td>
<td>Double distilled water</td>
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<tr>
<td>DF</td>
<td>Dilution factor</td>
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<tr>
<td>DSITI</td>
<td>Department of Science, Information Technology, and Innovation (Queensland Government)</td>
</tr>
<tr>
<td>DTR</td>
<td>Diurnal temperature range</td>
</tr>
<tr>
<td>EAL</td>
<td>Environmental Analysis Laboratory</td>
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<tr>
<td>EPA</td>
<td>The United States Environmental Protection Agency</td>
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<tr>
<td>Expt.</td>
<td>Experiment</td>
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<tr>
<td>FAOSTAT</td>
<td>Food and Agriculture Organization Statistics</td>
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<tr>
<td>Fm</td>
<td>Maximum fluorescence</td>
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<tr>
<td>Fo</td>
<td>Minimum fluorescence</td>
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<td>Fv</td>
<td>Variable fluorescence</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>Fv/Fm</td>
<td>Maximum quantum efficiency of photosystem II</td>
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<tr>
<td>GA</td>
<td>Gibberellic acid</td>
</tr>
<tr>
<td>GHG</td>
<td>Greenhouse gas</td>
</tr>
<tr>
<td>GLM</td>
<td>General Linear Model</td>
</tr>
<tr>
<td>GS</td>
<td>Starch or sugar concentration (Absorbance divided by the slope of the curve) (mg ml⁻¹)</td>
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<tr>
<td>GSS</td>
<td>Starch or sucrose concentration of the plant part (g g⁻¹ dry matter)</td>
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<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
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<tr>
<td>IAA</td>
<td>Indole-3-acetic acid</td>
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<tr>
<td>IPCC</td>
<td>Intergovernmental Panel on Climate Change</td>
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<tr>
<td>LAR</td>
<td>Leaf area ratio</td>
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<td>Lateral shoot leaves</td>
<td>Leaves on the lateral shoots</td>
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<tr>
<td>Lateral shoot</td>
<td>Branches of the main stem with the leaves and petioles</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>m.a.s.l</td>
<td>Meters above sea level</td>
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<tr>
<td>Main shoot leaves</td>
<td>Leaves on the main shoot</td>
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<tr>
<td>Main shoot petioles</td>
<td>Leaf petioles on the main shoot</td>
</tr>
<tr>
<td>Main shoot</td>
<td>Main stem of the potato plant together with its leaves and petioles excluding the lateral shoots</td>
</tr>
<tr>
<td>Main stem</td>
<td>Stem of the main shoot of the potato plant without petioles, leaves and the lateral branches</td>
</tr>
<tr>
<td>NAA</td>
<td>Naphthalene Acetic Acid</td>
</tr>
<tr>
<td>NAR</td>
<td>Net assimilation rate</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically active radiation</td>
</tr>
<tr>
<td>PEA</td>
<td>Plant efficiency analyser</td>
</tr>
<tr>
<td>PGRs</td>
<td>Plant growth regulators</td>
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<tr>
<td>Pn</td>
<td>Net photosynthesis</td>
</tr>
<tr>
<td>PPD</td>
<td>Patched point data</td>
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<tr>
<td>ppm</td>
<td>Parts per million</td>
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<tr>
<td>PROC</td>
<td>Procedure</td>
</tr>
<tr>
<td>PRWA</td>
<td>Potato Research Western Australia</td>
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</table>
psi  Pounds per square inch (pressure)
PSII  Photosystem II
QC  Quality control
RCPS  Representative Concentration Pathways
REG  Regression
RGR  Relative growth rates
rpm  Revolution per minute
RTP  Research Training Program (Australian Government)
RUBISCO  Ribulose-1,5-bisphosphate carboxylase/oxygenase
s  Seconds
SABC  State Agricultural and Biotechnology Centre
SAS  Statistical Analysis Software
SD  Standard deviation
Shoot  All the above ground plant parts of the potato plant including the petioles, leaves on the main shoot and the lateral shoots
SLA  Specific leaf area
SPS  Sucrose phosphate synthase
TDM  Total dry matter
TGA  Total glycoalkaloid
TIBA  2,3,5-triiodobenzoic acid
TM  Trade mark symbol
UHPLC  Ultra-High-Performance Liquid Chromatography
UNFCCC  The United Nations Framework Convention on Climate Change
UV-VIS  Ultraviolet-visible spectroscopy
V  Volume
W  Width
WS  Weight of sample
Conference and seminars

A list of presentations made at conferences, meetings and seminars attended during my PhD candidature.

Oral presentations

Charles Obiero, Stephen Milroy and Richard Bell (2018). New insights into how potato responds to high temperatures. A five-minute talk given during the State Agricultural and Biotechnology Centre (SABC), Western Australia, 25th Anniversary at Kim Beazley Lecture Theatre, Murdoch University, 8th June 2018.


Seminars

Charles Obiero, Stephen Milroy and Richard Bell (2017). Climate and production changes threaten a key crop for global food security: Has potato had its chips? A seminar given at the State Agricultural and Biotechnology Centre (SABC), Western Australia, 2nd June 2017.

Posters


Charles Obiero, Stephen Milroy and Richard Bell (2016). Leaf position alters photosynthetic and respiratory response of potato leaves exposed to an episode of high temperature. A poster presented during the School of Veterinary and Life Science Poster Days held at Murdoch University Library, 7th November 2016.

Charles Obiero, Stephen Milroy and Richard Bell (2016). Increasing frequency of hot days: Implications for yield of potatoes and food security. A poster presented during the Combined Biological Sciences Meeting held at the University Club, University of Western Australia, 26th August 2016.
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Lastly, I am also very grateful to colleagues, friends and those who assisted in anyway and whose names may not have been mentioned here.

God bless you all.
Dedication

I dedicate this thesis to my mum, my late dad, brother and fiancée whose inspiration has always been my strength.
CHAPTER ONE

General introduction
General introduction

1.1 Background information

The atmospheric concentration of carbon dioxide (CO₂) has risen by over 40% since 1750 and continues to rise at the rate of 2.0 ± 1.0 ppm yr⁻¹ (IPCC, 2014). Currently, atmospheric CO₂ is between 380–400 ppm and could reach 550 ppm by the end of this century (IPCC, 2007, 2014). The rising concentration of CO₂ and other greenhouse gases has caused a significant increase in global mean air temperatures (Allen et al., 2004). Since 1880, global air temperatures have risen by 1.7 °C and is predicted to rise to more than 5 °C above the global annual average by the end of this century (IPCC, 1996, 2014). On a global scale, the rising air temperatures have led to decreased frost days, changed the length of growing seasons, increased frequency of heat waves and increased the frequency of warm nights (Easterling et al., 2000; Meehl and Tebaldi, 2004; IPCC, 2007, 2014; Nastos and Kapsomenakis, 2015). At regional levels, evapotranspiration rates have increased, and heat waves have become more frequent (Tebaldi et al., 2006; Kharin et al., 2007; Diffenbaugh et al., 2007; Lhotka et al., 2017). In places such as Australia and New Zealand, the frequency of extremely warm days and nights has increased while the number of cold days and nights has become less since 1961 (Plummer et al., 1999; Collins et al., 2000; Manton et al., 2001; Griffiths et al., 2005). For Southwest Australia, annual mean temperatures are predicted to increase by 2.5 to 3.75 °C by 2050 (Turner et al., 2011). These climatic changes present challenges for future crop production.

Potato is the most important food crop after wheat (Triticum aestivum L.), rice (Oryza sativa L.) and maize (Zea mays L.) (Liu et al., 2006; Ahmadi et al., 2014). It is produced on over 19 million hectares worldwide yielding over 19 tonnes fresh tubers per hectare per year (FAOSTAT, 2018). Potato is an important component of world food security (Birch et al., 2012). Asia, with a population of 4.4 billion, produces over 190 million tonnes fresh tubers per year (FAOSTAT, 2018). Potato originated in cooler temperate regions of South America (Smith, 1968; Hawkes, 1978) and has been bred mostly within cooler environments of Europe (Horton, 1987). Thus, it is adapted to cooler environments with optimum temperatures between 18 to 20 °C where it produces maximum tuber yields (Smith, 1968; Hawkes, 1978; Haerkort, 1990; Van Dam et al., 1996).
The low optimum temperature required for tuber growth means that potato is highly vulnerable to high temperatures. However, due to climate change, traditional production and breeding centres for potatoes are warming (FAOSTAT, 2018). Further, potato production is shifting to hotter environments (Haverkort, 1990; Bustan et al., 2004; Muthoni and Kabira, 2015). In the Americas, where potato originated, its area of production has remained below 5 million hectares per year although yields up to 5 tonnes above the global annual average are obtained. Europe, with its suitable climate and where intensive potato breeding has been conducted, is experiencing a sharp decline in production with only 60 % of the 17.7 million hectares in 1961 currently under production. On the other hand, more potato is being produced in Asia and Africa. China, for instance, is currently the world’s largest potato producer with an annual production of 70-80 million tonnes fresh tubers per year from over 5 million hectares (FAOSTAT, 2018).

 Seriously concerning is the fact that none of the potato growing or breeding regions is spared from climate change. In the Northern region of the United States of America (USA), which is responsible for most potato production in the USA, the temperature has increased by 2-4 °C; more than four times the 20th century global average of 0.6 °C (Backlund et al., 2008). The temperatures are projected to rise again by 1-4 °C. Further, duration and frequencies of heatwaves have also increased (Backlund et al., 2008). In Europe, climate projections indicate doubled frequency of heatwaves by 2020-2049 compared to 1970-1996 (Lhotka et al., 2017). Northern China, where close to 50 % of potato is produced in China, has also experienced a 2.7 °C increment in annual temperatures for the period between 1987-2000 compared to 1961-1986. Again, these temperatures are projected to continue rising (Ma et al., 2003; Lin and Qian, 2003; Shi et al., 2007). The number of hot days within the same region has also increased since 1990 (Zhai and Pan, 2003). Thus, it is likely that potato is experiencing increased frequency of high temperature episodes which might limit its tuber growth hence yield. However, how much is known about influence of episodes of high temperature on plant and tuber growth in potato plants?

High temperatures impair shoot and tuber growth in potato plants but tuber growth is impacted more (Hawkes, 1978; Thornton et al., 1996). This is a conclusion from several empirical and simulation studies. From empirical studies, it is shown that that high soil and/or air temperatures above 25 °C significantly impair tuber growth (Epstein, 1966; Benoit et al.,
On the other hand, simulation studies indicate that potato will incur 18-32 % tuber dry matter losses should global temperatures increase by 1-1.4 °C (Hijmans (2003). Gobin (2010) also estimated 23-44 % loss in tuber yields through climate change simulation of potato production in Belgium. However, it is still unclear how episodes of high temperature might affect both plant and tuber growth in potatoes. This is because empirical studies were mostly concerned with the response of tuber growth at high temperatures (Menzel, 1980; Van Dam et al., 1996) and potato plants were exposed to high temperatures for either the whole growing period or from when the treatments were initially imposed (Lafta and Lorenzen, 1995; Timlin et al., 2006). No investigations have been made on plant performance after the end of high temperature episodes representative of events predicted to occur in the future. Secondly, potato simulation models are unreliable in accounting for in-season climatic variability. For instance, Fleisher et al. (2017) found that as the temperature rose the amount of uncertainty increased in a potato crop multi-model assessment aimed at quantifying variation among models and evaluating response of potato plants to climate change. In other crops, such as maize and soybean (Glycine max L.), Riha et al. (1996) found that the in-season temperature and precipitation variability could not be accounted for after assessing four crop models. With more frequent episodes of high temperatures, the in-season climatic variability will be increasingly important.

A number of experiments and simulation studies have been conducted to understand how other species might respond to these episodes of high temperatures. But still little can be learnt in terms of performance of potato plants in the same conditions. This is because most of the these studies used crops where the anthesis period was highly susceptible to high temperatures (Haynes and Haynes, 1988; Pressman et al., 2002; Alva, 2008; Zinn et al., 2010; Snider and Oosterhuis, 2011). For instance, Savin et al. (1996); Savin and Nicolas (1996) and Savin et al. (1997) found that even a few days of 35 °C at anthesis reduced grain weight in barley cultivars. Devasirvatham et al. (2015) showed up to 39 % yield loss in chikpea exposed to 35 °C. Asseng et al. (2011), through simulation, found that wheat lost close to 20 % grain yield after only 5 days of 34 °C temperatures after the anthesis period. Innes et al. (2015) also simulated 5.3 % grain loss for every 1 °C rise in average daily temperatures during wheat growing season in New South Wales, Australia. However, potato tubers are asexually
reproduced and the anthesis period is irrelevant to tuber production (Simmonds, 1997; Fernie and Willmitzer, 2001). Further, tubers grow below the soil surface where they are not directly influenced by high air temperatures. This means that high temperature might influence plant and tuber growth through other mechanisms such as alteration in whole plant carbon production through interference with leaf development, reduced photosynthesis, altered partitioning of plant dry matter or changes in tuber number. However, even if the leaf is paramount in plant growth as the main source of carbon (Sowokinos, 1990; Ghosh and Biswas, 1991; Sowokinos and Varns, 1992; Milroy and Bange, 2003; Zhao et al., 2007) and studies show that high temperatures negatively influence vegetative growth including leaf expansion (Reynolds and Ewing, 1989; Reynolds et al., 1990; Asseng et al., 2011), only a few studies have linked yield response in plants exposed to high temperature to whole plant leaf growth and expansion. For a vegetatively reproduced plant such as potato, this could be worth investigating.

This study explored a number of knowledge gaps. Firstly, cropping environments are becoming hotter due to climate change. However, little is known about the actual frequencies and duration of episodes of high temperatures experienced by potato plants during critical period of tuber bulking. Secondly, little understanding exists about whether potato plants recover after the end of the high temperature period. Lastly, reduction in tuber growth in potato plants exposed to high temperatures is typically linked to either reduced activity of starch synthase at temperatures above 25 °C or increased production of gibberellic acid. This is because starch synthesis is reported to be severely impaired at temperatures of above 25 °C (Krauss and Marschner, 1984; Keeling et al., 1993; Savin and Nicolas, 1996); while gibberellic acid, which is thought to stimulate shoot growth that diverts carbon away from the tubers, is produced in potato plants exposed to high temperatures (Menzel, 1980, 1983, 1985). However, in these experiments plants were not exposed to cooler conditions after the high temperature period. Given that the structure of the potato plant changes during high temperatures, is growth also altered after the end of an episode of high temperature or do the plants recover?

The diurnal cycle of temperatures influence plant growth (Benoit et al., 1986). However, while some studies suggest that high nighttime temperatures reduce tuber growth more than the high daytime temperatures (Driver and Hawkes, 1943; Gregory, 1965; Slater, 1968),
others show that there are no significant differences between the low nighttime and high daytime temperatures (Menzel, 1980). In this study, the potato plants were exposed to constant day and night temperatures except in experiment 5 (Chapter 9) in which plants were exposed to low night temperatures due to a malfunctioning of an air conditioner.

1.2 Research question and objectives

1.2.1 Main research questions

The main research questions were: How did episodes of high temperature affect potato physiology, and plant and tuber growth? Moreover, did growth stage and agronomic interventions such as exogenous application of plant growth regulators (PGRs) alter such responses?

1.2.2 Broad objectives

a. To determine the frequency and duration of episodes of high temperatures experienced during the critical period of tuber bulking in the main potato growing regions of Western Australia.

b. To investigate the impact of an episode of high temperatures applied shortly before or after tuber initiation on: canopy development, photosynthetic gain and dry matter, starch and sucrose partitioning, especially to the tubers.

c. To determine the influence of varying duration of high temperatures shortly after tuber initiation on canopy development, growth and dry matter partitioning, especially to the tubers.

d. To determine whether exogenous application of PGRs can be used to help correct the changes in canopy development and tuber growth in potato plants exposed to an episode of high temperature.
CHAPTER TWO

General literature review
General literature review

Summary
Cropping environments all over the world are getting hotter and experiencing more frequent episodes of high temperature; due to climate change. Potato production is also shifting to warmer regions of the world where the plant is likely to experience more frequent episodes of high temperatures. Soil and or air temperatures above 25 °C reduce tuber growth and this is mostly explained based on the inactivation of starch synthase, which limits the conversion of sucrose to starch in the tuber, and/or increased production of gibberellins which not only stimulate shoot growth which diverts carbon away from the tubers but also reduce starch synthase activity. High temperatures might also limit tuber growth through other mechanisms such as a reduction in sink capacity, photosynthesis or sucrose transport but these have been less explored. The current understanding is based on experiments in which the tubers or the potato plants were exposed to continuous high temperatures from planting through to final sampling or else plants were grown at cooler temperatures before being exposed to high temperatures until the final sampling. However, plants do not experience constant high temperature in the field, rather they experience episodes. Very few experiments have considered the responses of potato plants to episodes of high temperatures.

2.1 Introduction
Cropping environments all over the world are experiencing increasing temperatures, increased number of warm nights and more frequent episodes of high maximum temperatures (IPCC, 2014). These climatic changes are likely to impair plant growth and crop yield (Wheeler et al., 2000; Lobell and Field, 2007), particularly in crops such as potatoes that are adapted to cool temperate conditions (Hawkes, 1978; Haverkort, 1990). For instance, Hijmans (2003) assessed the impact of climate change on global potato production using the LINTUL-Potato model. He found that a 1 to 4 °C change in the global mean temperatures conditions from the 1961 to 1990 records will lead to a global potential tuber yield loss of 18 to 32 % (without improvement on the existing potato production technologies) or 9 to 18 % (with improvements) between 2010 to 2039 and 2040 to 2049. Raymundo et al. (2017) also simulated the responses of the potato crop to atmospheric CO₂ and high temperatures over
all potato-growing regions in the world using the SUBSTOR-Potato crop model. They found 6 to 26% loss in the global tuber yields by 2085 depending on the Representative Concentration Pathways (RCPS). At regional scales, they showed that high reductions will occur in higher latitudes such as in Eastern Europe and Northern America and in the lowlands of Africa and less reductions in the mid-latitudes and the tropical highlands. Thus, there is need to safeguard potato yields against the negative impacts of the high temperatures. However, this requires first the understanding of the actual growth conditions experienced by field crops and how these conditions influence their growth and yields. This chapter will: (1) demonstrate the shift in the global and regional temperatures; (2) show the global shift in potato production and what it means for future food security; (3) outline the current understanding of the impact of high temperatures on potato plants and highlight the limitations in that understanding.

2.2 Climate change

2.2.1 The definition of climate change

Climate change has been defined in various ways. The Intergovernmental Panel on Climate Change (IPCC) defines climate change as the change in climate over time, whether due to natural variability or as a result of human activity (IPCC, 2007). The United States Environmental Protection Agency (EPA) defines it as, “any significant change in measures of climate (such as temperature, precipitation, or wind) lasting for an extended period (decades or longer) which may result from: natural factors, such as changes in the sun’s intensity or slow changes in the earth’s orbit around the sun; natural processes within the climate system (e.g. changes in ocean circulation); human activities that change the atmosphere’s composition (e.g., through burning fossil fuels) and the land surface (e.g., deforestation, reforestation, urbanization, desertification, etc.)” (EPA, 2018). The United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as, “a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over

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*Representative Concentration Pathways (RCPS) are four time-dependent projections of atmospheric greenhouse gas (GHG) concentrations: RCP 8.5, 6.0, 4.5 and 2.6 (3.0) adopted by the Intergovernmental Panel on Climate Change (IPCC) for its Fifth Assessment Report (AR5) in 2014 (IPCC, 2014).
comparable time periods”, (Philippe, 1992). The IPCC and EPA definitions recognizes the contributions of both natural systems and humans to the long-term alteration of the earth’s atmosphere. The UNFCCC definition majorly attributes the climate change to human activity. However, all the definitions recognize the fact that the earth’s climate is changing. Thus, the need to understand the influence of climate change on plant growth and yields.

2.2.2 The global and regional surface temperatures

The global and regional temperatures have shifted, continue to rise and are more unpredictable. The global atmospheric carbon dioxide (CO$_2$) concentration has risen by over 40% since 1750 and continues to rise at the rate of 2.0 ± 1.0 ppm year$^{-1}$ (IPCC, 2014). The current atmospheric CO$_2$ concentration ranges from 380 to 400 ppm and may reach up to 550 ppm by the end of this century (IPCC, 2007, 2014).

The rising CO$_2$ has had a significant impact on the global mean air temperatures (Allen et al., 2004; IPCC, 2014). The Global Climate Models or the General Circulation Models show that global mean air temperatures have risen by 1.7 °C since 1880 and this rise could reach more than 5 °C by the end of this century (IPCC, 2014). Further, there is evidence of increased frequency of episodes of high temperatures at regional scales. Griffiths et al. (2005) analysed trends in the maximum and minimum temperatures and the number of hot days (frequency of days with maximum temperatures above the 1961-2003 mean 99th percentile) and warm nights (frequency of days with minimum temperatures above the 1961-2003 mean 99th percentile) in 89 weather stations across the Asia-Pacific region (46 °N to 47 °S). They showed that most of the stations had increased annual maximum (64 % of the stations) and minimum (79 %) temperatures over the 1961-2003 period. Forty percent of the stations recorded increased number hot days per year while 55 % showed increased number of warm nights per year.

In Australia, hot days with maximum temperatures of above 25 °C are common in summer (Wardlaw and Moncur, 1995; Collins et al., 2000). Climatic records also show that regions north of Perth such as Dandaragan, experience mean monthly maximum temperatures of over 30 °C that can last for seven months between October and April. South of Perth, regions such as Pemberton and Manjimup experience mean monthly maximum temperatures of 28 °C and above for almost five months between December and April (Australia Bureau of
Meteorology, 2018a, 2018b). This is not the only problem. The hot days have also become more. The Australian area-average mean daily temperature indicate that the number of hot days (days with maximum temperatures of more than 35 °C) per year is likely to increase from an average of about 28 in 2013 to 50 days annually by 2070 (Australia Bureau of Meteorology, 2016).

The information about the global, regional, yearly and monthly mean temperatures is important. However, it is limited in its application in understanding the actual temperatures experienced by plants during the critical period of crop growth. Firstly, crops are usually grown only during specific seasons of the year around the globe. There are those that are grown only during the warm season such as sweet potato ([Ipomoea batatas] L. (Lam.)), watermelon ([Citrullus lanatus] L. (Thunb.) Matsum and Nakai) and Okra ([Abelmoschus esculentus] L. (Moench)) and can tolerate the daily maximum temperatures of up to 35 °C. There are also those cool season crops like potato ([Solanum tuberosum] L.) and cabbage ([Brassica oleracea] L.) that require the daily maximum temperatures of less than 25 °C (Hatfield et al., 2008). Given that the global mean temperature is still only 14.7 °C, there might be little to worry about. Secondly, 20 to 50 hot days in a year demonstrate little information in terms of the distribution of the hot days in a year or during the critical period of crop growth. For, instance hotter days in summer, means little to crops that might be grown during cooler seasons such as potato. Further, under field conditions, crops are not usually exposed to high temperatures lasting from planting to harvesting rather to episodes of high temperatures which can occur at any stage during their growing season.

2.3 Potato and the geographical relocation in its production

2.3.1 Importance of the potato production

Potato ([Solanum tuberosum] L.) is the most important food crop after wheat ([Triticum aestivum] L.), rice ([Oryza sativa] L.) and maize ([Zea mays] L.) (Fabeiro et al., 2001; Liu et al., 2006; Ahmadi et al., 2014). With an annual production from over 19 million hectares worldwide yielding close to 19 tonnes fresh tubers per hectare annually (FAOSTAT, 2018), potato is one of the most important components of world food security (Birch et al., 2012). Potato is also the most water efficient in terms of the amount of energy produced per cubic metre (m³) of water. It provides up to 23430 kilojoules (kJ) per cubic metre of water (kJ/m³)
compared to wheat (9623 kJ/m$^3$) or maize (16318 kJ/m$^3$). Thus, is it able to provide the per capita daily energy needs of about 11297 kJ/day with just a half cubic metre of water (Birch et al., 2012).

2.3.2 The geographical relocation in potato production

The potato plant originated from the Andean region of South America (Hawkes, 1978; Cribb and Hawkes, 1986; Hosaka and Hanneman, 1988); and has been bred mostly within cooler temperate regions of the world such as North America and Europe (Horton, 1987; Haverkort and Harris, 1987). Thus, current commercial cultivars are best adapted to cooler environments with maximum temperatures ranging from 14 °C to 22 °C where they produce high tuber yields (Bodlaender, 1963; Kooman, 1995). Temperature above 25 °C adversely affects potato growth and tuber yields (Bodlaender, 1963; Van Dam et al., 1996). In spite of this, potato production is shifting geographically from the cooler environments of America and Europe to warmer climates of Africa, the Middle East and Asia (Haverkort, 1990; Birch et al., 2012; Muthoni and Kabira, 2015).

Since 1961 the potato production in America has stagnated below 2.5 million hectares per year (Fig. 2.1) (FAOSTAT, 2018). Europe has experienced 40 % drop in the land area allocated to potatoes compared to the 17 million hectares that were under production in 1961. In contrast, production has increased strongly in Africa and in Asia. The area under potato production in Asia has increased by over 40 % from 2.5 million in 1961 to over 10 million hectares per year in 2016.

This movement to warmer production environments together with global warming (Haverkort, 1990; IPCC, 2014; FAOSTAT, 2018), means that potato crops are likely to be more frequently exposed to episodes of high temperatures which might limit its tuber growth.
Figure 2.1: World potato production trend in million hectares 1961-2016 (FAOSTAT, 2018)

2.4 Impacts of high temperature on potato tuber growth

This section focusses on what is known about the influence of high temperatures on tuber growth and the theories that have been used to explain the responses of tuber growth at high temperature.

Tuber growth in potato plants is influenced by both air and soil temperatures (Bodlaender, 1963; Van Dam et al., 1996). The influence is also cultivar dependent (Struik et al., 1989b; Reynolds and Ewing, 1989). The maximum tuber growth, and hence yield, is favoured by maximum temperatures of between 14 and 24 °C (Borah et al., 1960; Bodlaender, 1963; Sale, 1979; Kooman, 1995; Kim and Lee, 2016).

Air and or soil temperatures above 25 °C impair tuber growth leading to less tuber dry matter, and hence lower yields. Epstein (1966) found 44 % loss in tuber dry matter in potato cv. Katahdin grown at soil temperatures of 29 °C compared to 22 °C for 30 days. The soil temperatures were increased shortly after tuber initiation. Menzel (1985) observed 62.3 % loss in tuber dry matter in potato cv. Sebago grown for 70 days from emergence at 30/26 °C rather than at 22/18 °C day/night (D/N) temperatures. Lafta and Lorenzen (1995) found 98.4 % (potato cv. Norchip) and 99.8 % (potato cv. Up-to-Date) less tuber dry matter when the plants were exposed to temperatures of 31/29 °C for 28 days compared to 19/17 °C D/N. The high temperatures were applied from the tuber initiation stage and the plants were sampled
at the end of the high temperatures. They also found 44.7 % (cv. Norchip) and 36 % (Up-to-Date) less tuber matter when the plants were exposed to 29/27 °C compared to 19/17 °C D/N temperatures. In this experiment, the high temperature was applied 10 days after the tuber initiation stage and lasted for 14 days. The plants were sampled at the end of the high temperatures period. The differences in tuber dry matter in these two studies could have been due to either the number of tubers per plant or the average weight per tuber. However, the information on tuber numbers per plant was not provided in any of these studies. Timlin et al. (2006) also observed 94.7 % loss in tuber dry matter when the potato cv. Atlantic was grown at 32 °C than at 24 °C constant temperatures from emergence to the final sampling (on day 52 after emergence), but again the number of tubers per plant or weight per tuber were not presented.

2.5 Mechanism by which tuber growth is reduced at high temperatures

2.5.1 Inhibition of starch synthase
The conversion of sucrose into starch, and hence starch deposition in storage organs such as grains and potato tubers, is controlled by starch synthase and ADP-glucose pyrophosphorylase (Hawker et al., 1979; Mares and Marschner, 1980; Jenner, 1991; Savin and Nicolas, 1996; Edwards et al., 1999; Geigenberger et al., 2004). At temperatures above 25 °C, the activity of both starch synthase and ADP-glucose pyrophosphorylase is reduced, leading to less sucrose conversion into starch in potato tubers or grains (Mohabir and John, 1988; Keeling et al., 1993; Jenner, 1994; Denyer et al., 1994). Krauss and Marschner (1984) found depressed starch synthase activity, lower starch content and reduced tuber growth rates when tubers of three potato cultivars, Ostara, DTO 28 and Mariva, were exposed for 6 days to 30 °C compared to 20 °C constant temperatures. Lafta and Lorenzen (1995) observed a reduction in the activity of sucrose synthase, ADP-glucose pyrophosphorylase and tuber growth rate, in potato plants that were grown at 31/29 °C compared to 19/17 °C D/N. Geigenberger et al. (1998) also observed reduced starch and in the activity of ADP-glucose pyrophosphorylase in developing potato tubers incubated at 30 °C compared to 25 °C. In these studies, the plants or tubers were under the direct influence of high temperatures. How does tuber growth relate to starch synthase when plants are exposed to high temperatures before or after tuber initiation and grown back at cooler conditions?
2.5.2 Increased production of gibberellins

Increased production of gibberellins is also proposed as one of the mechanisms influencing tuber growth in potato plants exposed to high temperatures. Gibberellins are linked to three responses of the potato plant to temperature. Gibberellic acid (GA) inhibits tuber initiation hence reduce the number of tubers produced per plant (Lovell and Booth, 1967; Menzel, 1983). It also stimulates both shoot growth and stem elongation leading to increased shoot dry matter and taller plants (Lovell and Booth, 1967; Kumar and Wareing, 1974; Fernie and Willmitzer, 2001). Further, GA is known to reduce the activity of starch synthase at the tuber leading to repartitioning of the sucrose that would otherwise go to tubers, into the shoots and stolons (Lovell and Booth, 1967; Booth and Lovell, 1972; Mares et al., 1981).

Gregory (1956) suggested hormonal-like responses in potato plants grown at high temperatures or exposed to different photoperiods. He found that tuber formation in potato resulted from a stimulus formed or activated by specific conditions of temperature and photoperiod. Further, he found that this stimulus remained active in the plants for some time even when plants were moved to conditions unfavourable for tuber formation. Menzel (1980) found 29 % higher shoot (stems plus leaves) dry matter in potato cv. Sebago grown at 32/18 °C or 32/28 °C compared to 22/18 °C D/N. He also found that the shoot dry matter in plants treated with GA in the control (22/18 °C) increased in the same manner as in the high temperature treatments. Moreover, he found that the negative impact of the GA was reversed by an application of gibberellic acid inhibitor chlorocholine chloride (CCC). Menzel (1983) found increased gibberellins in the crude extracts from the buds of potato plants (cv. Sebago) grown at 35/30 °C compared to 20/15 °C D/N. He therefore concluded that tuber growth in potato plants grown at high temperatures is reduced due to the production of gibberellins that stimulate shoot growth which diverts carbon away from the tubers (Menzel, 1985).

The proposed influence of GA in potato plants grown at high temperatures is inconsistent with several studies. Menzel (1985) proposed that the reduced tuber growth was because of the greater shoot using the available carbon. However, there are studies that indicate that tuber dry matter is still reduced even when shoot growth is reduced or unaffected in plants grown at high temperatures. Lafta and Lorenzen (1995) reported 25 to 40 % less shoot dry matter and 36 to 99.8 % less tuber dry matter in potato cv. Up-to-Date exposed to 29/27 °C
or 31/29 °C compared to 19/17 °C D/N. Similarly, Timlin et al. (2006) found 15.2 % less shoot dry matter and 94.7 % less tuber dry matter in potato cv. Atlantic grown at a constant temperatures of 32 °C compared to 24 °C.

Although changes in shoot growth have been reported, there is little data demonstrating the influence of GA on leaf area, and so not much is known about the effect on the whole plant carbon production. Further, with episodes of high temperatures, the changes in shoot and leaf growth, and potentially the tuber number, may alter how the plant grows after the end of the high temperature period. However, potato plants in the studies mentioned above were exposed to a persistent influence of high temperatures without being grown in cooler conditions after the end of the high temperatures. If GA changes the plant structure, it will be important to investigate how this affects growth during and then after an episode of high temperature.

2.5.3 Sink capacity in terms of number of tubers per plant and the number of cells in each tuber

High temperatures impair tuber initiation hence the number of tubers produced per plant (Ewing, 1981). Menzel (1980) found that the number of tubers per plant was reduced by 77 % in potato cv. Sebago plants grown at 32/18 °C or 32/28 °C compared to 22/18 °C D/N. The temperatures were applied before tuber initiation and lasted for 30 days. However, the total tuber dry matter per plant is the product of the total number of tubers per plant and their individual dry matter (Kim and Lee, 2016). Thus, the high temperatures can reduce the total tuber dry matter per plant by only inhibiting the production of more tubers per plant.

The number of tubers per plant, and hence the available sink capacity for the deposition of photosynthates, also effects whole plant carbon production. Higher tuber demand increases whole plant growth rate (Kolbe and Stephan-Beckmann, 1997; Chen and Setter, 2003). Burt (1964) found reduced net assimilation in potato cv. Sebago in which the tubers were removed 21 days after tuber initiation compared to the intact plants. Nösberger and Humphries (1965) also observed a reduction in the net assimilation rate and in the total dry matter in plants of potato (cv. Epicure) in which tubers had been removed compared to those whose tubers remained intact.
The reduction in the available sinks in terms of the number of tubers for starch deposition can lead to repartitioning of the photosynthates meant for the tubers elsewhere on the plant. Burt (1964) and, Nösberger and Humphries (1965) showed that carbon that would otherwise have gone to the tubers was partitioned into the stems and leaves. Further, Nösberger and Humphries (1965) found that more than double of the numbers of the tubers per plant was produced in plant in which tubers were removed a soon as they formed. Moreover, there was increased production of leaves and lateral stems in the same plants in which the tubers had been removed. This might mean that the available carbon occasioned by the lack of tubers was used for the initiation of more tubers and the growth of the leaves and the lateral stems. Consistent with this, Lorenzen (1989) found that the rapid growth in tubers coincided with higher starch accumulation and export from the leaves. However, the number of tubers, the photosynthetic gain, or the possible interaction are not always considered when relating the reduction in tuber growth per plant to high temperatures (Menzel, 1980; Lafta and Lorenzen, 1995; Timlin et al., 2006). To gain a whole plant understanding of potato’s response to high temperature these aspects need to be studied.

The number of cells in each tuber modulates the individual growth rate of the tuber. Tubers with fewer cells are usually slow growers due to insufficient storage space for starch (Plaisted, 1957; Reeve et al., 1973; Xu et al., 1998b). The total number of cells in each tuber can be influenced by the available photosynthate and tuber growth stage. Chen and Setter (2003, 2012) found that when plants were exposed to elevated CO$_2$ (700 compared to 350 µmol CO$_2$ mol$^{-1}$) applied from the tuber initiation stage, not only did the whole plant dry matter increase as a result of increased supply of photosynthates, the number of cells in each tuber was 67% higher at elevated CO$_2$ condition compared to the control. The tubers in plants exposed to elevated CO$_2$ from the tuber bulking stage only had 18% more cells relative to the control. Further, when the plants were shaded (150 µmol m$^{-2}$ s$^{-1}$ cf. 600 µmol m$^{-2}$ s$^{-1}$) they showed that the total number of cells in the tubers was reduced by 40% while their average cell volume reduced by 30%. The high number of cells per tuber in plants exposed to elevated CO$_2$ from tuber initiation compared to tuber bulking could be because of higher cell division in young tubers (Xu et al., 1998b). In older tubers, there is usually relatively less cell division and growth usually proceed with the available cells (Reeve et al., 1973; Xu et al., 1998b). Although Chen and Setter (2003, 2012) experiments demonstrate the significance of the whole plant supply
of photosynthates on the initial and subsequent tuber growth, the experiments were conducted under a light limiting environment compared to what the field grown potato plants would normally experience. The 600 μmol m$^{-2}$ s$^{-1}$ used as the control in these experiments, for instance, is three times as low as the light in which potato plants might be exposed to in Western Australia of about 1500 to 2000 μmol m$^{-2}$ s$^{-1}$ (Thomas and Turner, 1998).

Little information is available on the influence of high temperatures on cell number in tubers especially for plants exposed during tuber initiation. Nicolas et al. (1984) found increased rate but shorter duration of cell division in the endosperm of wheat grain in plants exposed to 28/20 °C compared to 23/18 °C D/N. Further, they found that the number of cells in the endosperm was reduced whether a 10-day temperature of 28/20 °C was applied at an early stage during the cell division: between day 3 to 13 after anthesis (DAA) or later between 10 to 20 DAA. In maize (cv Mo17), Commuri and Jones (2001) found reduced number of cells in the endosperm of kernels exposed to 35 °C for 4 or 6 days starting five days after pollination, both in field and in vitro, compared to plants maintained at 25 °C. In contrast, Morita et al. (2005) found higher number of cells in the endosperm of the grain of rice plants at heading stage exposed to 34/22 °C or 34/22 °C compared to 22/22 °C D/N. Thus, it is not clear whether cell number could be one of the factors in the reduced tuber growth in potato plants especially for plants exposed to high temperatures during tuber initiation. If this is the case, it will also be important to know whether the reduced cell number will continue to limit tuber growth after the end of a high temperature episode.

### 2.5.4 The net photosynthetic gain

Studies demonstrate that photosynthesis per unit leaf area of the potato plant is highly sensitive to high temperatures (Burton, 1981; Hammes and De Jager, 1990; Prange et al., 1990). This loss in photosynthesis has been related to negative impacts of the high temperature on the plants’ photosystem II (PSII), enzymatic capacity, leaf chlorophyll florescence and on the stomatal conductance (Makino et al., 1994; Chaitanya et al., 2001; Morita et al., 2004; Cen and Sage, 2005; Way and Sage, 2008; Ashraf and Harris, 2013; Bita and Gerats, 2013). In particular, high temperatures disrupts the PSII process, leading to inhibition of the electron transport chain and inactivation of the reaction centers (Prange et al., 1990; Havaux et al., 1996; Cornic and Fresneau, 2002; Hu et al., 2006; Slabbert and Krueger, 2011). Similar findings have been demonstrated in many other species such as

In addition to the effect on photosynthesis per unit of leaf area, Fleisher et al. (2006) observed 64 % less canopy photosynthesis in potato cv. Kennebec grown at 34/29 °C than at 23/18 °C. Timlin et al. (2006) also showed 84 % less canopy photosynthesis in potato cv. Atlantic grown at 32 °C compared to 24 °C constant temperatures. Although Fleisher et al. (2006) and Timlin et al. (2006) showed negative impacts of high temperatures on canopy photosynthesis, their data did not indicate the degree to which high temperatures might have influenced the whole plant leaf area. Thus, the relative contribution of a reduction in the photosynthetic rate per unit leaf area and of a reduction in the leaf area remains unclear.

Results of experiments on the photosynthetic rate per unit area of the potato plants at high temperatures are, however, inconsistent. Hammes and De Jager (1990) observed 37 % lower photosynthetic rates in potato cv. Up-to-Date grown at 40 °C compared to 20 °C. Wolf et al. (1990b) found 40 % lower photosynthetic rates of the same potato cv., Up-to-Date, and C1-884 when plants grown at 22 °C were exposed to 32 °C for 2 hours, although no reduction was found in the cultivar Desiree. In dramatic contrast, Hancock et al. (2014) showed a 70 % increase in photosynthesis in potato cv. Desiree plants that were grown at 30/20 °C compared to 22/16 °C; which were similar to the conditions used by Wolf et al. (1990b).

A difficulty in collating data on leaf photosynthetic rates is that leaves at different positions on the plant have been used. It has been shown that leaf age influences the photosynthetic capacity of the potato plants (Vos and Oyarzun, 1987; Suzuki et al., 1987; Katny et al., 2005). Frier (1977) conducted photosynthetic measurements on the first two subterminal leaflets of the six and of every third fully expanded leaf as numbered from the soil level. Ku et al. (1977) determined the rates from the terminal leaflet of the third leaf from the top of the plant. Reynolds et al. (1990) used the terminal leaflet of the leaf on the sixth node as numbered from the top of the plant. Dwelle et al. (1983) and Wolf et al. (1990) used the fourth and the fifth leaf interchangeably; while Midmore and Prange (1992) only described the leaves as “the uppermost fully expanded”. Hammes and De Jager (1990) and Katny et al. (2005) did not specify the leaves used in their measurements.
The net photosynthesis is gross photosynthesis less respiration (Dutton et al., 1988). Considerations on plants photosynthetic responses based only on net photosynthetic rates at a leaf level do not demonstrate how the whole plant responses might be. This is because the diurnal responses in the net photosynthesis and respiration rates are not the same (Dwelle et al., 1983; Manhas and Sukumaran, 1988; Timlin et al., 2006; Fleisher et al., 2006). Little is known of how this pattern could be altered under different environments. Investigating the diurnal response of the gross photosynthesis and respiration rates to temperature will add to our understanding of how changes in net photosynthesis measured at one time of the day can relate to the change in net photosynthetic gain over the whole day.

Importantly, the photosynthetic data in previous studies were collected when plants were under persistent influence of high temperatures. However, potato plants in the field experience episodes of high temperatures. That is, high temperature events followed by cooler conditions. It is not known whether photosynthesis in potato leaves recovers after exposure to an episode of high temperatures. Further, because the plant continues to develop during an episode of high temperatures, there are leaves that will emerge during this period. Little is known of whether these new leaves are better acclimated to higher temperatures than those that had emerged before the high temperatures started. Likewise, there is little information about the performance of leaves that expanded during the high temperatures when the plant returns to cooler conditions after the end of the high temperature period.

### 2.5.5 Sucrose transport and unloading into the tubers

Sucrose is the main transport sugar in plants (Zrenner et al., 1995; Winter and Huber, 2000; Koch, 2004; Lemoine et al., 2013). The rate of sucrose transport from the source (leaves) to the sink (tubers, grains, stems and other leaves) is controlled by sucrose transporter (SUT) genes (Aoki et al., 2003). In tomatoes (*Solanum lycopersicum* L.), it has been shown that there was earlier flowering, increased net photosynthesis and improved concentration of sucrose in mature fruits with the overexpression of the sucrose transporter gene PbSUT2 from *Pyrus bretschneideri* (Wang et al., 2016). Significantly, sucrose transporter genes are highly susceptible to high temperatures. Phan et al. (2013) found that in rice grown at 30 °C relative to 25 °C, the expression of the sucrose transporter gene OsSUT1 was suppressed along with that of the starch synthase genes SuSyz, AGPS26, BEIIb, and this coincided with a reduced grain weight. Thus, less tuber growth is reported with reduced expression of SUT1 in...
transgenic potato plants (Kühn et al., 2003). High temperature has a direct influence on the availability of photosynthates at a whole plant level and the expansion of individual tubers. Little is known of how high temperatures might regulate the expression of sucrose transporter genes hence the sucrose transport and uploading into the tubers.

2.6 Factors that might alter the response of plants to high temperatures

This section focusses on factors that might alter the response of potato plants to high temperature. Some of these factors may need to be taken into account when assessing the likely impact of a high temperature episode and some might be useful tools for the development of agronomic methods to moderate the impact of the high temperatures on crop yield.

2.6.1 Duration of the episode of high temperatures

In the field, potato crops are expected to be exposed to episodes of high temperature of varying duration. Thus, understanding how duration of high temperatures alter plant growth and yields becomes important. Working with wheat, Wardlaw and Moncur (1995) showed a reduction in kernel weight as early as 2 days after exposure to temperatures of 36/31 °C D/N. Prasad et al. (2000) grew groundnuts (Arachis hypogaea L.) cv. ICGV 86015 plants at 28/22 °C D/N until 9 days after flowering then exposed them to day temperatures of 28, 34, 42, 48 °C for 2, 4 and 6 days. They found a significantly reduced proportion of flowers forming fruit and reduced number of pegs and pods per plant with longer duration of high temperatures. Wardlaw et al. (2002) also observed that only 4 days of 36/31 °C applied in wheat from 20 days after anthesis was sufficient to reduce grain growth compared to 30/25 °C that were applied 6 days after anthesis. Talukder et al. (2014) exposed wheat plants at the near flowering or green anther stage, or early grain set to a single heat event in which high temperatures was increased by 3-4 °C per hour from about 25 °C at 10.00 am to a maximum of 35 °C at midday and maintained for three hours before being allowed to steadily decrease to ambient temperatures. They found that this single heat event was able to significantly reduce grain number, individual grain weight and the grain yield.

In potato, the only variable for which there is information demonstrating a response of high temperature is photosynthesis. Wolf et al. (1990b) found that the leaf level photosynthesis of
two cultivars (Up-to-Date and C1-884) was not altered when plants were grown at 38 °C for more than 42 days but all plants experienced a reduction in photosynthesis when they were exposed to 40-42 °C or within 2 hours after plants grown at 22 °C were exposed to 32 °C.

2.6.2 Pre-conditioning of plants at warmer temperatures
In the field, the transition from cool to hot conditions does not typically happen abruptly. Pre-exposure of plants to warm temperatures prior to high temperatures might moderate the impact on plant growth and hence yields. Thus, Bhullar and Jenner (1986) found higher starch accumulation rate in wheat plants that were first acclimatized at 25 °C then exposed to 33 °C than in those plants that were transferred directly from 21 °C to 33 °C. Corbellini et al. (1997) found that wheat plants subjected to a progressive increase of temperature from 30 to 40 °C or exposed to a previous heat shock, were better able to tolerate high temperatures than plants that were subjected immediately to the high temperatures. Little is known about the influence of pre-exposure of potato plants to warmer conditions on their performance during an episode of high temperatures. Likewise, if a plant experiences a second episode of high temperature is it affected more or less than during the first episode?

2.6.3 The day and night temperatures
It has been suggested that high night-time temperatures are more deleterious to tuber growth than the high daytime temperatures (Driver and Hawkes, 1943). Gregory (1956) found that tuber growth was greater at night temperatures of 20 °C than at 26 °C. However, Menzel (1980) compared three high temperature treatments: the control (22/18 °C) and two high temperatures treatments in which the night time temperatures were different, 32/18 and 32/28 °C. Significantly, the two high temperature treatments reduced tuber dry matter to a similar extent whether the night-time temperature was the same as the control or 10 °C higher. In other species, Morita et al. (2005) also showed appreciable grain loss of rice grown at 22/34 °C compared to 34/22 °C. Prasad et al. (2008) found that grain yields decreased linearly with increasing night-time temperatures in wheat plants grown under 14, 17, 20, 23 °C night-time temperatures while the daytime temperatures was maintained at 24 °C. Thus, both the day and night-time temperatures are important for carbon deposition in storage organs, but it is not particularly clear whether one is more important than the other. As both day and night temperatures are increasing globally (IPCC, 2014), further information on their relative importance are needed.
2.6.4 Moisture stress
The impacts of high temperature can be enhanced by too much or less water (Levy et al., 1990; Hatfield and Prueger, 2015). The air temperature is exponentially related to the saturation vapor pressure: the maximum pressure possible by water vapor at a given temperature (Hatfield and Prueger, 2015). A combined impact of high temperatures and drought impair whole plant carbon production hence plant growth and yield through reduced leaf area, lower photosynthetic rates and altered partitioning (Levy et al., 1990; Rizhsky et al., 2002; Awasthi et al., 2014; Zhou et al., 2017). Thus, plants exposed to extreme temperatures might experience moisture stress than those under cooler environments.

2.7 Conclusions
Due to climate change, cropping environments all over the world are getting hotter and experiencing more frequent episodes of high temperatures. The effect of this on potato productivity is made worse because production is shifting to warmer regions of the world where the potato plant is likely to experience more frequent episodes of high temperatures which could limit its yields. However, there is limited information about the actual growth temperatures experienced by field grown potato plants at the critical period of tuber bulking.

Soil and or air temperatures above 25 °C reduce tuber growth. The reduction in tuber growth is usually explained based on two mechanisms: the inactivation of starch synthase at temperatures above 25 °C which limits the conversion of sucrose to starch in the tuber, and/or the increased production of gibberellins which stimulate shoot growth and thus diverts carbon away from the tubers. It also reduces the activity of starch synthase. The significance of other mechanism such as the reduction in sink capacity, photosynthesis or sucrose transport at high temperatures have been less explored.

It is particularly important that the current understanding provides little information about the impact of episodes of high temperatures on potato physiology, growth and tuber yields. In the field, plants are not exposed to constant high temperatures, rather they experience episodes of high temperatures. However, our current understanding is based on experiments in which the tubers or the potato plants were exposed to continuous high temperatures from planting through to the final sampling or else, plants were initially grown at cooler
temperatures before being exposed to high temperatures that then continued until the final sampling. Only a few experiments have considered the responses of potato plants to episodes of high temperature and the researchers have not tried to separate plant responses during the high temperature period from those after the high temperature period. Thus, the need for further investigations.

The review raises a number of important questions:

a. Climate analysis of the potato growing regions is proposed to understand the actual temperatures experienced by potato plants during the critical period of tuber bulking. What are these temperatures? Are they higher than the 25 °C critical thresholds for tuber growth? What is their duration in terms of the number of consecutive days of high temperatures and how frequently does episode of high temperature occur?

b. How does an episode of high temperature influence canopy growth, net photosynthesis and tuber growth? In particular, how do the changes in growth caused by high temperature alter crop growth after the end of the high temperature episode? How do these results fit with the proposed effects of deactivation of starch synthase and the higher production of gibberellic acid at high temperatures?

c. How do high temperature episodes of different duration influence plant and tuber growth in potato plants and the ability of the crop to recover?

d. How does a repeat of an episode of high temperature influence plant and tuber growth in potatoes; that is, do they acclimate?
CHAPTER THREE

Occurrence of hot days during tuber bulking in potato growing regions of Western Australia
Occurrence of hot days during tuber bulking in potato growing regions of Western Australia

Abstract
Temperatures above 25 °C impair potato yields. In Western Australia, days with maximum temperatures above 25 °C are common but potatoes are grown throughout the year. Thus, it was important to known about the actual temperatures experienced by potatoes during the critical period of tuber bulking and whether these temperatures threaten tuber yields. It was hypothesized that potato grown in Western Australia frequently experience episodes of temperatures above the 25 °C critical thresholds during tuber bulking. Thirty-year (1985-2014) temperature data from nine potato growing regions of Western Australia were assessed for: (1). Trends in annual maximum, mean and minimum temperatures; (2). Trends in monthly maximum temperatures during tuber bulking; (3). The number of hot days of above 25, 30 or 35 °C and, (4). The occurrence of different durations of 25, 30 and 35 °C hot spells. Results showed that most regions had significant trend of increased annual maximum and mean temperatures. The annual minimum temperatures showed strong trends of declining temperatures in some regions. All regions except Albany, Manjimup, Pinjarra and Medina had increased monthly maximum temperatures during tuber bulking and were already at or above 25 °C in all regions except Albany (all months) and Jindong (October-November). The number of hot days of above 25 °C increased by between 10 and 18 days from 22 to 36 days in 1985 to 26 to 54 days in 2014 in more than half of the regions. The number of hot days of 30 °C increased by 9 to 16 days from 7 to 15 days in 1985 to between 16 and 25 days in 2014 in five regions. Three consecutive hot days above 25 °C were common in all regions. Five consecutive hot days above 30 °C occurred at least once a year in most regions except Albany, Pinjarra and Medina. Potatoes grown in Western Australia are frequently exposed to episodes of high temperatures which might be limiting their yields.

3.1 Introduction
Cropping environments all over the world are becoming hotter due to climate change (Alexander et al., 2006; IPCC, 2014). Both global and regional mean surface temperatures are expected to continue to rise (IPCC, 1996; Tebaldi et al., 2006; IPCC, 2014; Lhotka et al., 2017).
In the cropping zones of south-western Australia temperatures have risen by 0.2 °C per decade since 1950 (Turner et al., 2011). Importantly, the rise in mean temperatures has led to an increased frequency of extremely hot days and nights and a reduction in the number of cold days and nights in places such as Australia (Plummer et al., 1999; Collins et al., 2000; Manton et al., 2001; Griffiths et al., 2005). These conditions pose new challenges for crop production. However, there is very little published information on the occurrence and the duration of hot spells experienced by potato plants during the critical period of tuber growth.

Potato is very sensitive to high temperatures. Temperatures above 25 °C impair its tuber growth and hence yields (Gregory, 1956; Smith, 1968). In Australia, hot days with maximum temperatures of above 25 °C are common in summer (Collins et al., 2000). In Western Australia, regions to the north of Perth such as Dandaragan, experience mean monthly maximum temperatures of over 30 °C that can last for seven months between October and April. Regions to the south of Perth, such as Pemberton and Manjimup experience mean monthly maximum temperatures of 28 °C and above for almost five months from December to April (Australia Bureau of Meteorology, 2018a). The mean annual temperatures for the cropping zones of southwestern Australia is still projected to increase by 1.25 to 1.75 °C by 2050 (Hennessy et al., 2008; Turner et al., 2011). Not only are these mean monthly maximum temperatures hot for potato production, climate projections of the city of Perth and the surrounding areas also indicate an increase in the number of hot days with the maximum temperatures of more than 35 °C for an average of about 28 to 50 days annually for 2070 (Australia Bureau of Meteorology, 2016). These conditions threaten the productivity of potatoes grown in Western Australia.

In Western Australia, potatoes are grown throughout the year. In winter potatoes are grown mostly to the north of Perth in regions such as Dandaragan, Gingin, Pinjarra and Myalup. The peak tuber growth (i.e. the tuber bulking phase of the crop) in these regions occurs in October and November. In summer the production shifts to the south-west corner of Western Australia where peak tuber growth happens during the months of January and February in Pemberton and Manjimup, or November and December in Albany (McPharlin et al., 2013; Australian Venture Consultants Pty Ltd, 2014). Therefore, it is likely that potatoes in Western Australia are already experiencing temperatures above the 25 °C during their growth period but there has been no analysis of the historical trends in the annual maximum, mean and
minimum temperatures within the different potato growing regions of Western Australia especially of the temperatures experienced during tuber bulking in the various production regions. Importantly, little is known about the trend in the number of hot days during the period of tuber bulking in these production regions and there is no information on the frequency and the duration of hot spells during these times.

It was hypothesized that potato grown in Western Australia frequently experiences episodes of temperatures above the 25 °C critical thresholds during tuber bulking. In this chapter, thirty years (1985-2014) of temperature data from nine potato growing regions in Western Australia were analysed for: (1) Trends in annual maximum, mean and minimum temperatures; (2) Trends in the monthly maximum temperatures during tuber bulking in the nine potato regions; (3) The number of hot days of over 25, 30 and 35 °C and, (4) The occurrence of different durations of hot spells during tuber bulking.

3.2 Methods
3.2.1 Study regions
Nine potato growing regions were selected. Each location either currently produces ware or seed potato or is being considered for future production (Fig. 3.1). The regions, from the south of Western Australia to the North of Perth were: one seed potato region (Albany), seven commercial ware potato regions (Manjimup, Pemberton, Marybrook, Myalup, Medina, Gingin, Dandaragan) and one region being explored for future potato production (Forrest Grove).
Figure 3.1: Potato growing regions of Western Australia considered in this analysis. Climate data for Jindong, Pinjarra, Medina and RAAF Pearce were used for Marybrook, Myalup, Baldivis, and Gingin respectively.

3.2.2 The temperature data

Thirty years’ (1985-2014) of temperature data were used for assessing long-term trends in maximum temperatures, and duration and occurrence of high temperature episodes during tuber bulking. The temperature data were from weather stations based at each region. In cases where data were not available for any region, the nearest weather station was considered. This was the case for Marybrook, Myalup, Baldivis and Gingin for which data from Jindong, Pinjarra, Medina and RAAF Pearce respectively were used.

All the data were obtained from the Australian Government Bureau of Meteorology (BOM) in November 2015. The data were either patched point data (PPD) or data drill (DD) (Table 3.1). Patched point data contained the actual observed data from the weather station and any missing data have been supplemented by interpolated estimates. The estimates are obtained by using an anomaly-interpolation spline algorithm that computes for the daily maximum and minimum temperature (Queensland Government, 2018). The interpolation gives data that
are proper representation of the actual weather of the region. The data obtained using the data drill approach, on the other hand, consist of data that are derived by interpolating between nearby weather stations. In this study, the temperature data were limited to the 30-years (1985-2014) to avoid too much of reliance on the patched points. There were about 1-14 % estimates in the patched point data used in this study (Table 3.2).

Table 3.1: Geographical and data characteristics of the potato growing regions of Western Australia

<table>
<thead>
<tr>
<th>Region</th>
<th>Data characteristic</th>
<th>Location (Lat/Long)</th>
<th>Elevation (m.a.s.l)</th>
<th>Tuber bulking period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany</td>
<td>PPD</td>
<td>-34.94/117.80</td>
<td>68</td>
<td>Nov-Dec</td>
</tr>
<tr>
<td>Pemberton</td>
<td>PPD</td>
<td>-34.45/116.04</td>
<td>174</td>
<td>Jan-Feb</td>
</tr>
<tr>
<td>Manjimup</td>
<td>PPD</td>
<td>-34.25/116.14</td>
<td>286</td>
<td>Jan-Feb</td>
</tr>
<tr>
<td>Forrest Grove</td>
<td>DD</td>
<td>-34.15/115.15</td>
<td>73</td>
<td>Jan-Feb</td>
</tr>
<tr>
<td>Jindong</td>
<td>DD</td>
<td>-33.75/115.25</td>
<td>76</td>
<td>Jan-Feb, Oct-Nov</td>
</tr>
<tr>
<td>Pinjarra</td>
<td>DD</td>
<td>-32.60/115.85</td>
<td>58</td>
<td>Oct-Nov</td>
</tr>
<tr>
<td>Medina</td>
<td>PPD</td>
<td>-32.22/115.81</td>
<td>14</td>
<td>Oct-Nov</td>
</tr>
<tr>
<td>RAAF Pearce</td>
<td>PPD</td>
<td>-31.67/116.02</td>
<td>40</td>
<td>Oct-Nov</td>
</tr>
<tr>
<td>Dandaragan</td>
<td>DD</td>
<td>-30.65/115.70</td>
<td>240</td>
<td>Oct-Nov</td>
</tr>
</tbody>
</table>

Lat/Long = Latitude/Longitude; m.a.s.l = meters above sea level; PPD = Patched point data; DD = Data Drill

Table 3.2: Percentage of the observed data in the whole 30-year temperature data sets for the potato production locations of Western Australia that had the Patched Point Data (PPD).

<table>
<thead>
<tr>
<th>Region</th>
<th>Maximum temperature records</th>
<th>Minimum temperature records</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>PPD</td>
</tr>
<tr>
<td>Albany</td>
<td>10597</td>
<td>359</td>
</tr>
<tr>
<td>Pemberton</td>
<td>10812</td>
<td>145</td>
</tr>
<tr>
<td>Manjimup</td>
<td>10526</td>
<td>431</td>
</tr>
<tr>
<td>Medina</td>
<td>9702</td>
<td>1255</td>
</tr>
<tr>
<td>RAAF Pearce</td>
<td>9431</td>
<td>1526</td>
</tr>
</tbody>
</table>

Source: Queensland Government Department of Science, Information Technology, and Innovation (DSITI) and Bureau of Meteorology (BoM). Computerizing the Australian Climate Archives (CLIMARC) Project data contain computerized daily maximum and minimum temperature using an anomaly-interpolation spline algorithm.
3.3 Data analysis

The data were analysed for: (1) The long-term trends in maximum, mean and minimum annual temperatures; (2) Trends in maximum monthly temperatures during tuber bulking; (3) Trends in the number of hot days (above 25, 30 or 35 °C) during tuber bulking and, (4) The occurrence of different durations of hot spells above 25, 30 or 35 °C during tuber bulking. A hot spell is defined as a sequence of consecutive days when the maximum temperature is above the given threshold.

The long-term trends in maximum, mean and minimum annual temperatures were analysed from all the months in the thirty-year data sets. Trends in the maximum monthly temperatures and the number of hot days during tuber bulking were analysed only for the two months in each region when the tubers were bulking. The frequency and duration of hot spells during tuber bulking were analysed from the last ten years of data (2005-2014).

The maximum, mean and minimum annual temperatures were the averages of daily maximum, mean or the minimum temperatures in each year for every region. The diurnal temperature range was the difference between the annual maximum and the annual minimum temperature in each region. The maximum monthly temperatures during tuber bulking were the average of the daily maximum temperature in each month during tuber bulking for each region. Hot days were taken as days with maximum temperatures of above 25, 30 or 35 °C. A hot spell was when two or more consecutive days had maximum temperatures of above 25, 30 or 35 °C.

Trends were calculated using Linear Regression Procedure (Proc Reg) of the Statistical Analysis Software (SAS), University Edition by the SAS Institute, North Carolina, USA. Correlations between the diurnal temperature range and the annual maximum and the minimum temperatures were derived using the Correlation Procedure (Proc Corr) of the SAS software. Where applicable, the data were subjected to Analysis of Variance (ANOVA). Unless otherwise stated, significant differences or trends are reported at 5 % level of probability.
3.4 Results

3.4.1 Trends in annual maximum, mean and minimum temperatures

All the potato regions have warmed. Regions closer to the city of Perth were warmer than those to the southern part of Western Australia. The annual maximum temperatures increased more strongly than either the annual mean or minimum temperatures (Table 3.3; Fig. 3.2; Fig. 3.3). In all regions, the annual maximum temperature increased by about 0.8 to 1.3 °C over the period, representing about 0.04 to 0.06 °C per year (Table 3.3). Pemberton, Manjimup, Jindong, RAAF Pearce and Dandaragan had the strongest increase in the annual maximum temperatures. In some regions such as Dandaragan, Medina and RAAF Pearce the annual maximum temperatures were already more than the 25 °C threshold for optimum tuber growth. By 2014, the range in the annual maximum temperature was between 21.0 °C in Albany and 26.3 °C in Dandaragan.

In all regions, the annual mean temperature in each year increased by about 1.5 to 2.0 °C since 1985 except for Medina, Jindong, and Forrest Grove, representing an increase of about 0.03 to 0.05 °C per year. However, the annual mean temperature in all locations is still below the 25 °C thresholds. By 2014, the range in the annual mean temperature was from 16.2 °C in Albany to 19.5 °C in Dandaragan; cooler in the south and warmer closer to Perth.

The annual minimum temperature had different trends for different potato regions. In Albany, Pemberton, Pinjarra and Median, there was a significant trend of increasing minimum temperatures in each year. The minimum temperatures had increased by about 0.5 to 1.4 °C since 1985 representing an increase of 0.02 to 0.05 °C yr⁻¹. In Forrest Grove, Jindong and RAAF Pearce, the minimum temperatures in each year were decreasing. Jindong and Forrest Grove had significant trends of decreasing annual minimum temperatures.

All regions had significant trends of increasing diurnal temperature range of between 0.02 to 0.09 °C per year except for Albany, Pemberton, Medina and Pinjarra (Table 3.3). There were strong increases in the diurnal temperature range in Forrest Grove, RAAF Pearce and Jindong corresponding to the locations with significant trends of the decreasing annual minimum temperatures.

In all regions there was strong positive relationship between the annual maximum temperatures and the diurnal temperature range (Table 3.4). The annual minimum
temperatures were negatively related to the diurnal temperature range and strongly only in a few regions.

Figure 3.2: Trends in annual maximum, mean and minimum temperatures in Albany and Pemberton (left) and Manjimup (right).
Figure 3.3: Trends in annual maximum, mean and minimum temperatures in Forrest Grove, Pinjarra and Medina (Left) and Jindong, RAAF Pearce and Dandaragan (right).
Table 3.3: Trends in annual maximum, mean and minimum temperatures and diurnal temperature range (DTR) in potato growing regions of Western Australia

<table>
<thead>
<tr>
<th>Region</th>
<th>Trends (°C yr⁻¹)</th>
<th>Maximum temperature</th>
<th>Mean temperature</th>
<th>Minimum temperature</th>
<th>Diurnal temperature range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany</td>
<td>0.02*</td>
<td>0.02*</td>
<td>0.02*</td>
<td>0.004NS</td>
<td></td>
</tr>
<tr>
<td>Pemberton</td>
<td>0.04**</td>
<td>0.04**</td>
<td>0.03*</td>
<td>0.010NS</td>
<td></td>
</tr>
<tr>
<td>Manjimup</td>
<td>0.05**</td>
<td>0.03*</td>
<td>0.01NS</td>
<td>0.04***</td>
<td></td>
</tr>
<tr>
<td>Jindong</td>
<td>0.05**</td>
<td>0.01NS</td>
<td>-0.04**</td>
<td>0.06***</td>
<td></td>
</tr>
<tr>
<td>Forrest Grove</td>
<td>0.04**</td>
<td>0.01NS</td>
<td>-0.02*</td>
<td>0.09***</td>
<td></td>
</tr>
<tr>
<td>Pinjarra</td>
<td>0.03*</td>
<td>0.03*</td>
<td>0.05*</td>
<td>-0.00NS</td>
<td></td>
</tr>
<tr>
<td>Medina</td>
<td>0.04*</td>
<td>0.05*</td>
<td>0.05*</td>
<td>-0.01NS</td>
<td></td>
</tr>
<tr>
<td>RAAF Pearce</td>
<td>0.06**</td>
<td>0.03*</td>
<td>-0.01NS</td>
<td>0.07***</td>
<td></td>
</tr>
<tr>
<td>Dandaragan</td>
<td>0.05**</td>
<td>0.03*</td>
<td>0.01NS</td>
<td>0.02***</td>
<td></td>
</tr>
</tbody>
</table>

*, **, and *** means significant at 5, 1 and 0.1% level of probability, respectively; NS = Not significant

Table 3.4: Correlations (as percentage) between the diurnal temperature range (DTR) and the annual maximum and minimum temperatures in potato growing regions of Western Australia.

<table>
<thead>
<tr>
<th>Region</th>
<th>Pearson Correlation Coefficients (as percentage), N = 30; P Value (in brackets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany</td>
<td>Maximum temperature: 66.3 % (&lt;.0001) Minimum temperature: -10.8 % (0.56)</td>
</tr>
<tr>
<td>Pemberton</td>
<td>Maximum temperature: 50.7 % (0.004) Minimum temperature: -8.7 % (0.64)</td>
</tr>
<tr>
<td>Manjimup</td>
<td>Maximum temperature: 82.8 % (&lt;.0001) Minimum temperature: -0.30 % (0.98)</td>
</tr>
<tr>
<td>Jindong</td>
<td>Maximum temperature: 76.1 % (&lt;.0001) Minimum temperature: -76.5 % (&lt;.0001)</td>
</tr>
<tr>
<td>Forrest Grove</td>
<td>Maximum temperature: 67.9 % (&lt;.0001) Minimum temperature: -60.8 % (0.0004)</td>
</tr>
<tr>
<td>Pinjarra</td>
<td>Maximum temperature: 56.3 % (0.001) Minimum temperature: -53.6 % (0.002)</td>
</tr>
<tr>
<td>Medina</td>
<td>Maximum temperature: 37.4 % (0.041) Minimum temperature: -61.3 % (0.0003)</td>
</tr>
<tr>
<td>RAAF Pearce</td>
<td>Maximum temperature: 78.2 % (&lt;.0001) Minimum temperature: -47.6 % (0.007)</td>
</tr>
<tr>
<td>Dandaragan</td>
<td>Maximum temperature: 75.9 % (&lt;.0001) Minimum temperature: -16.8 % (0.37)</td>
</tr>
</tbody>
</table>
3.4.2 Trends in monthly maximum temperatures during tuber bulking

The monthly maximum temperatures during tuber bulking increased rapidly in all regions since 2005 and are above the 25 °C threshold for maximum tuber growth in most potato growing regions of Western Australia (Fig. 3.4; Fig. 3.5; Fig. 3.6. **Note the differences in the months of tuber bulking**). Except the month of October in Jindong, there was a significant trend of increased monthly maximum temperatures during the first month of tuber bulking in all regions (Table 3.5); the rate of increase being 0.06 °C yr$^{-1}$ or above. The second month of tuber bulking also had increased monthly maximum temperatures with trends being significant in more than half of the regions.

November, January and February were the hottest months during tuber bulking. In 2014, monthly maximum temperatures of up to 32 °C were recorded for the month of November in Dandaragan. In Pemberton, Manjimup and Jindong, the temperatures were 30 °C and above in January of 2014.

![Figure 3.4: Trends in monthly maximum temperatures during tuber bulking in the months of November and December in Albany.](image-url)
Figure 3.5: Trends in monthly maximum temperatures during tuber bulking in the months of January and February in Pemberton and Manjimup (left) and Jindong and Forrest Grove (right).
Figure 3.6: Trends in monthly maximum temperatures during tuber bulking in the months of October and November in Jindong, Pinjarra and Medina (left) and RAAF Pearce and Dandaragan (right).
Table 3.5: Trends in monthly maximum temperatures during tuber bulking in potato growing regions of Western Australia.

<table>
<thead>
<tr>
<th>Region</th>
<th>Trends in monthly maximum temperature during tuber bulking (°C)</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>October</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany</td>
<td></td>
<td>0.06*</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pemberton</td>
<td></td>
<td>0.06*</td>
<td>0.03*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manjimup</td>
<td></td>
<td>0.07*</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jindong</td>
<td></td>
<td>0.09**</td>
<td>0.01*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forrest Grove</td>
<td></td>
<td>0.07*</td>
<td>0.06*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jindong</td>
<td></td>
<td>0.08</td>
<td>0.07*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinjarra</td>
<td></td>
<td>0.06*</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medina</td>
<td></td>
<td>0.08*</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAAF Pearce</td>
<td></td>
<td>0.09*</td>
<td>0.08*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dandaragan</td>
<td></td>
<td>0.09*</td>
<td>0.06*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, **, and *** means significant at 5, 1 and 0.1 % level of probability, respectively

3.4.3 Trends in the number of hot days during tuber bulking

Hot days with maximum temperatures of equal to or over 25, 30 and 35 °C during tuber bulking have been experienced in potato growing regions in Western Australia (Fig. 3.7; Fig. 3.8; Fig. 3.9). All regions showed significant trends of an increased number of days with the maximum temperatures of 25 °C and above during tuber bulking (Table 3.6). The number of hot days with maximum temperatures above 30 °C also increased and showed a significant trend for over half of the regions. All regions recorded hot days with maximum temperatures of above 35 °C during tuber bulking but the trend was not significant and sometimes negative; for instance, in Pemberton.

Manjimup, Jindong, Forrest Grove, RAAF Pearce and Dandaragan had the strongest trend of increased number of days with maximum temperatures of above 25 °C and 30 °C. Albany had the fewest days above 25 °C and experienced even fewer days of over 30 °C maximum temperatures during tuber bulking. Jindong, Forrest Grove, RAAF Pearce and Dandaragan had
the highest rate of increase in the number of days of over 30 °C maximum temperatures of between 0.24 and 0.51 hot days per year during tuber bulking period. Over the thirty years from 1985, the number of days during the January-February window that were 30 °C and above increased from around 10 to 28 per year at Jindong. In 2014, there were about 10 to 18 more days with maximum temperatures of above 25 °C in Jindong, Forrest Grove, RAAF Pearce and Dandaragan.

Figure 3.7: Trends in the number of hot days during tuber bulking in Albany.
Figure 3.8: Trends in the number of hot days during tuber bulking in Pemberton and Manjimup (left) and Jindong and Forrest Grove (right).
Figure 3.9: Trends in the number of hot days during tuber bulking in Pinjarra and Medina (left) and RAAF Pearce and Dandaragan (right).
Table 3.6: Trends in the number of hot days during tuber bulking in potato growing regions of Western Australia.

<table>
<thead>
<tr>
<th>Region/Months</th>
<th>Trends in the number of hot days during tuber bulking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 25 °C</td>
</tr>
<tr>
<td>November-December</td>
<td></td>
</tr>
<tr>
<td>Albany</td>
<td>0.12*</td>
</tr>
<tr>
<td>January-February</td>
<td></td>
</tr>
<tr>
<td>Pemberton</td>
<td>0.30**</td>
</tr>
<tr>
<td>Manjimup</td>
<td>0.34**</td>
</tr>
<tr>
<td>Jindong</td>
<td>0.61**</td>
</tr>
<tr>
<td>Forrest Grove</td>
<td>0.63**</td>
</tr>
<tr>
<td>October-November</td>
<td></td>
</tr>
<tr>
<td>Pinjarra</td>
<td>0.15*</td>
</tr>
<tr>
<td>Medina</td>
<td>0.32**</td>
</tr>
<tr>
<td>RAAF Pearce</td>
<td>0.49**</td>
</tr>
<tr>
<td>Dandaragan</td>
<td>0.45**</td>
</tr>
</tbody>
</table>

*, **, and *** means significant at 5, 1 and 0.1 % level of probability, respectively

3.4.4 Occurrence of different durations of hot spell during tuber bulking

Analysing the data from the last ten years (2005-2014) showed that the potato regions experienced frequent hot spells that lasted for up to seven consecutive days during the tuber bulking. Jindong had the most frequent occurrence of hot spells. (Fig. 3.10; Fig. 3.11; Fig. 3.12). Forrest Grove, Pinjarra and Medina had the lowest frequency of the hot spells above 30 °C and no hot spell of above 35 °C. Albany had the lowest frequency of any hot spells.

Jindong, Manjimup, RAAF Pearce and Dandaragan experienced hot spells of up to seven consecutive days above 25 and 30 °C. Two-day hot spells of above 25 °C were common in all regions and occurred five to eight times per year in all regions except in Albany. Hot spells of five consecutive days above 25 °C occurred up to four times a year in Jindong and thrice in Manjimup, Pemberton, Forrest Grove and Dandaragan. Hot spells of three consecutive days above 30 °C occurred up to four times per year in Jindong. But only three times in Manjimup and twice in RAAF Peace and Dandaragan. Hot spells of three consecutive days above 35 °C occurred at least once a year in most regions except Albany or Pinjarra.
Figure 3.10: The number of occurrence of different durations of hot spell during tuber bulking in Albany. Data from 2005 to 2014.
Figure 3.11: The number of occurrence of different durations of hot spell during tuber bulking in Manjimup and Pemberton (left) and Jindong and Forrest Grove (right). Data from 2005 to 2014.
Figure 3.12: The number of occurrence of different durations of hot spell during tuber bulking in Pinjarra and Medina (left) and RAAF Pearce and Dandaragan (right). Data from 2005 to 2014.

3.5 Discussion

This study investigated how frequently potatoes grown in Western Australia are exposed to episodes of temperatures of 25 °C or above during the critical period of tuber bulking. The following were the key results: (1) All potato regions had increased annual maximum and mean temperatures; (2) Monthly maximum temperatures during tuber bulking in most regions were above 25 °C since 2005; (3) Most regions had increased number of hot days with maximum temperature above 25 or 30 °C and, (4) Hot spells of up to five days above 25 °C occurred in all regions, with durations up to seven days in most regions.

The warming in the annual mean and maximum temperatures and the changes in the annual minimum temperatures in this study is consistent with global and regional records (Plummer et al., 1999; Manton et al., 2001; IPCC, 2007, 2014; Australia Bureau of Meteorology, 2016). It is argued that the warming in the global mean air temperatures is driven by the strong increases in the minimum temperatures (Karl et al., 1991; Easterling et al., 1997; Easterling et al., 2000). In this analysis, however, only the annual maximum temperatures consistently
showed significant trends of increasing temperature. The annual minimum temperatures even declined in some of the regions, including some that showed strongly increasing annual maximum temperatures such as Jindong, Forrest Grove and RAAF Pearce (Table 3.3). Further, the diurnal temperature range was only strongly correlated to maximum temperature and not the minimum temperatures (Table 3.3; Table 3.4). This is unlike previous reports (Karl et al., 1991; Easterling et al., 1997; Easterling et al., 2000; Alexander et al., 2006). This means that the changes in the maximum temperatures are not always associated with the declining diurnal temperature range occasioned by increases in the minimum temperatures. The maximum and minimum temperatures and hence the diurnal temperature range can be influenced by other factors such as proximity to the coastline or elevation (Lima and Wethey, 2012). In this study, however, the changes in the maximum and minimum temperatures seemed not to have been influenced by the proximity to the coastline. This can be seen in the increase in the annual minimum temperatures in Albany, Medina and Pinjarra compared to the decreases in Jindong and Forrest Grove even though all these locations were proximate to the coast.

The information about the warming in the annual mean, maximum and minimum temperatures say little about the actual growth temperatures experienced by potato plants in the different regions. Firstly, the annual mean temperatures in all regions were still below the 25 °C thresholds for optimum tuber growth and may not warm to this level anytime soon. Secondly, although the annual maximum temperatures in some regions were already at or above the 25 °C thresholds, potatoes are produced only during a specific period of the year which is also regionally dependent. Further, the critical period of tuber bulking is only confined to specific months during growth of the potato plant. Therefore, knowledge on the prevailing maximum temperatures, the spread the maximum temperatures, the duration and frequency of hot spells during tuber bulking is crucial.

In this analysis, the monthly maximum temperatures during the two-month period of tuber bulking in all regions were already above the 25 °C threshold for optimum tuber growth except for both the months in Albany and the first months in Jindong, Pinjarra and Medina (Fig. 3.4; Fig. 3.5; Fig. 3.6). Further, there were significant increases in the number of hot days with maximum temperatures of above 25 °C in all regions and in hot days above 30 °C in more than half of the regions. In Pemberton, Manjimup, Jindong, RAAF Pearce and Dandaragan, the
number of hot days with maximum temperatures of over 25 °C increased by 10 to 18 days from 22 to 36 days in 1985 to about 36 to 54 days in 2014. Hot days above 30 °C increased from 10 in 1985 to between 16 and 25 days in 2014 in Manjimup, Jindong, RAAF Pearce and Dandaragan. Closely related results have been demonstrated elsewhere. Hennessy et al. (2008) and Australia Bureau of Meteorology (2016) showed increased number of hot days with maximum temperatures above 35 °C in the broad acre cropping zones of southwestern Australia. In this study, however, the increased number of hot days not only means over half the period of tuber bulking happens at maximum temperatures of above 25 °C in all the regions, the tuber is also exposed to hot days of over 30 °C for almost a third of the bulking period in most of the regions. Thus, potatoes in Western Australia are already being produced in environments that could be limiting their tuber growth. At least two hot spells per year of five days or more over 25 °C were experienced during tuber bulking in all regions except in Albany. These results demonstrate that potatoes grown in Western Australia are not only exposed to hot spells that could impair tuber growth and yield, they also experience repeated exposure to hot spells of varying durations during tuber bulking. In other studies, Collins et al. (2000) showed that the frequency of hot spells of three to five days of 35 °C had increased between 1957 to 1996 in Australia and it is still projected that the number of hot days with over 35 °C are likely to increase from annual average of 28 to 50 days in by 2070 for regions around Perth (Australia Bureau of Meteorology, 2016).

These temperature conditions can be expected to negatively impact the productivity of field grown potato. It is known that tuber growth in potato plants is highly sensitive to high temperatures and is reduced at temperatures above 25 °C (Gregory, 1956; Smith, 1968). However, previous studies have concentrated on investigating the long-term impacts of high temperatures (Menzel, 1985; Lafta and Lorenzen, 1995; Timlin et al., 2006). In the few experiments in which potato plants were exposed to episodes of high temperatures, the data were only collected at the end of the experiment (Rykaczewska, 2015). Therefore, the changes that occurred during the high temperature period and after the end of the high temperatures could not be studied. Further, these experiments only dealt with the impact of a single event of the high temperatures on the potato plants. With these episodes of high temperatures questions related to the performance of potato plants after the end of the high temperatures become important. Further, it is imperative to understand the responses of
both the potato plant and tuber growth to high temperatures applied before tuber initiation. In addition, it is worth finding out how pre-exposure to warmer conditions before an episode of high temperatures or how a repeat of the episodes of high temperatures can influence plant and tuber growth in potatoes.

Crop yields were not correlated with temperature in this study. While this approach has been used in previous studies of major crops (Lobell and Field, 2007; Gobin, 2010; Peltonen-Sainio et al., 2010), correlating commercial tuber yield and temperature data over time would not have given a measure of the impact of episodes of high temperatures on production of potato. Commercial yield is not only influenced by weather conditions but also by varieties and management. Overtime, new varieties, and new management practices (fertilizers, irrigation, cropping patterns, pest control) have been introduced. Thus, it would have been a complex analysis to attempt to separate the effect of temperature from other variables.

The analysis of monthly and annual means in average, minimum and maximum temperature trends in this study were similar to those used in previous studies (Manton et al., 2001; Griffiths et al., 2005; Lobell and Field, 2007; Gobin, 2010; Peltonen-Sainio et al., 2010; Perkins and Alexander, 2012). The additional analyses which focus on the critical stage for yield development and incorporate the known threshold temperatures at which crop growth is impaired or yield is reduced provides a more accurate reflection on the problem in terms of crop performance. Analysing the number and duration of hot spells during the critical period of plant growth in different crop production regions provides a more detailed description of the temperature problem at the crop level. This is important because field grown crops are not always exposed to hot temperatures throughout their growth period. Rather they experience high temperature episodes at various growth stages during their development. Thus, the analysis of the number and duration of high temperature episodes describes the real concern for crop production.

### 3.6 Conclusions

a. Potatoes grown in Western Australia are frequently exposed to episodes of high temperatures which might be limiting their yields. All potato regions have experienced increases in the monthly maximum temperatures during tuber bulking and these
temperatures are already above the 25 °C threshold for maximum tuber growth in most regions.
b. The impact of these episodes of high temperatures on plant and tuber growth of potato has not been studied. The increasing temperatures in potato growing areas, means there is a need for urgent studies.
CHAPTER FOUR

General experimental materials and methods
General experimental materials and methods

4.1 Introduction
In Chapter 3, it was shown that potatoes grown in Western Australia are already experiencing episodes of high temperature above the 25 °C critical threshold for maximum tuber growth during the bulking stage. These episodes of high temperature can last for more than seven consecutive days. However, there is little information in the literature about how an episode of high temperatures before or after tuber initiation influenced potato physiology, canopy development and tuber yield; in particular, the performance after the end of the high temperature period. Further, little was known about how varying the duration of the high temperature event alters whole plant and tuber growth. This study was aimed to address these gaps. It also investigated how agronomic interventions such as exogenous application of PGRs altered responses of potato plants to an episode of high temperature.

Five glasshouse experiments were conducted. Experiment (Expt.) 1 and 2 investigated the impact of a 9-day episode of high temperatures applied before (Expt. 1) or shortly after (Expt. 2) tuber initiation on shoot development, carbon gain and tuber growth. Expt. 3 determined how varying durations (1, 3, 6 and 9 days) of high temperature imposed after tuber initiation affected shoot development and tuber growth. Expt. 4 and 5 explored the impact of either auxin or an auxin inhibitor on shoot development, carbon gain and tuber growth of potato plants exposed to an episode of high temperature shortly after tuber initiation.

This chapter presents materials and methods that were common in all experiments. Materials and methods specific to each experiment are described in the appropriate experimental chapter.

4.2 Site description
All experiments were conducted in glasshouses located at Murdoch University, Perth (32 ° 04' S; 115 ° 50' E) Western Australia.
4.2.1 Glasshouses

In all experiments, a glasshouse set at 22 °C was used to grow potatoes before high temperatures were applied and after the end of the high temperatures. During the high temperature period, two to three separate glasshouses were used depending on number of temperature treatments in each experiment. All glasshouses were heated and cooled by reverse cycle air conditioners (Hitachi Australia Pty Ltd, Model No. RAS-4HVRN) but the glasshouse which was used for 30 °C treatments had an additional reverse cycle air conditioner (Temperzone Australia Pty Ltd, Model No. ISD 86KY-D/OSA 86RKSH.zx) to allow maintenance of constant temperatures of 30 ± 1 °C.

Air temperature within the glasshouses was monitored at about 1.2 meters above the glasshouse floor (i.e. at about mid-plant height) for all treatments in all experiments. This was done with the aid of Extech Temperature and Humidity Data Logger Model No. 42270 in solar radiation shields (6-Plate Solar Radiation Shield Model. 41303) sourced from Campbell Scientific, Australia. The data from the loggers were collected at the final sampling of each experiment and the loggers were reset for the next experiment. Air temperatures are described in each experimental chapter.

Photoperiod and light intensity affect tuber growth (Demagante and Vander Zaag, 1988; Levy and Veilleux, 2007). In this study, all glasshouses were naturally lit. The photoperiod and relative humidity for the individual experiments are described in each experimental chapter.

4.2.2 Planting and plant management

Potato (Solanum tuberosum L.) cv. Royal Blue was used in these experiments. The variety is a high value potato cultivar in Western Australia (McPharlin et al., 2013; Australian Venture Consultants Pty Ltd, 2014). It is also considered to be relatively more sensitive to high temperatures than many other varieties although there is no data to confirm this. In all experiments, uniformly sprouted seed potatoes were planted, one tuber per planting bag, at a depth of 10 cm in the centre of bags filled with growth medium. The bags containing the growth medium were already in a glasshouse at 22 °C. The growth medium used was based on a potting mix sourced from Richgro, Western Australia. The potting mix contained about 2-parts composted pine bark, 2-parts coarse river sand, and 1-part coco peat. Chemical and physical properties of the potting mix are presented in Table 4.1.
Table 4.1: Chemical and physical properties of the potting mix used to make the growth medium. Data are mean of nine samples analysed by the Environmental Analysis Laboratory (EAL), Southern Cross University, New South Wales.

<table>
<thead>
<tr>
<th>Chemical/Physical Property</th>
<th>Method of Analysis</th>
<th>Unit</th>
<th>Amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nitrogen (N)</td>
<td>LECO IR Analyzer</td>
<td>%</td>
<td>0.12</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>Colwell</td>
<td>mg/kg</td>
<td>164</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>Morgan 1</td>
<td>mg/kg</td>
<td>753</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>Morgan 1</td>
<td>mg/kg</td>
<td>738</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>Morgan 1</td>
<td>mg/kg</td>
<td>306</td>
</tr>
<tr>
<td>Carbon/Nitrogen (C/N)</td>
<td>Calculated</td>
<td>ratio</td>
<td>89</td>
</tr>
<tr>
<td>Organic matter (OM)</td>
<td>Calculated</td>
<td>%</td>
<td>39</td>
</tr>
<tr>
<td>pH</td>
<td>1:5 water</td>
<td>-</td>
<td>5.78</td>
</tr>
<tr>
<td>Texture</td>
<td>Basic Texture</td>
<td>-</td>
<td>Sandy soil</td>
</tr>
<tr>
<td>Colour</td>
<td>Basic Colour</td>
<td>-</td>
<td>Brownish</td>
</tr>
</tbody>
</table>

About 30 kg of the potting mix was mixed thoroughly with 40 g of Osmocote®, 40 g of Grower’s Blue®, 20 g dolomite (CaMgCO₃), 12 g lime (CaCO₃) and five litres of tap water using an electric mixer. Osmocote® contained 14 % N, 3 % P, 18 % K and 3 % Mg while Grower’s Blue® had 12 % N, 5 % P, 14 % K, 9.8 % S. Then 7.5 kg of this mixture was filled into 20-Litre (28 x 33 cm) woven green planting bags. Additional Osmocote® was applied on 35 days after planting (DAP) (Expt. 1, 3, 4 and 5) or 43 DAP (Expt. 2) at the rate of 16 g per plant. The growth medium was kept moist at all times throughout the experiment. Plants were watered twice a day: morning (8.00 am) and evening (3.00 pm). During the high temperature period, the high temperature treatments received an additional watering at 11.00 am to keep them moist.

A preliminary study was conducted to investigate how solar radiation in the glasshouse influenced temperature of the growth medium and how to overcome the problem. Three planting bags filled with growth medium were wrapped either with aluminium foil, bubble wrap or nothing (NoWrap). Then iButton Temperature Loggers DS1904L from ALPHAOmega Electronics, Spain, were buried at a depth of 10 cm in the centres of the planting bags already filled with the growth medium to monitor the temperature. The buttons were first placed in
zip-lock polythene bags to prevent them from moisture damage. The data were collected after nine days.

Results of this test showed that solar radiation within the glasshouse increased the temperature of the growth medium excessively. During the day, the temperature of the growth medium increased to 25 °C when the air temperature was set at 22 °C (Fig. 4.1). Aluminium foil was found to significantly reduce soil heating. Therefore, to avoid the confounding effect of different air and root temperatures, planting bags in all experiments were wrapped with aluminium foil. The surface of the growth medium was also covered with a white shade cloth to minimise chances of warming from the surface of the growth mix while still allowing air and water movement. The temperature of the growth medium was also monitored in all treatments of Expt. 1 and 2. The temperature buttons were still buried at a depth of 10 cm in the growth medium at planting and left until the end of the experiment. Data from the buttons were collected and described later in Chapter 5.

![Figure 4.1: Temperatures of growth medium in planting bags wrapped with different materials as influenced by solar radiation within the glasshouse. Data are means of three potting bags in each treatment averaged over nine days. Bars are Standard Errors.](image)
The number of the shoots growing from a single tuber can be highly variable. The number can range from just a few to more than five shoots per tuber (Oliveira, 2000; Vreugdenhil et al., 2011; Shayanowako et al., 2014). Therefore, to obtain and maintain uniform plants, one vigorously growing shoot per tuber was selected and the rest removed at 14 DAP in all experiments (Fig. 4.2). Thereafter, shoots were removed as soon as they emerged. The remaining shoot was supported by a fibreglass stake to grow in an upright position. Plants were randomised within and between the benches in the glasshouse on 14 DAP to minimise positional effects. Thereafter, plants were re-randomized weekly. In all experiments, an additional 4 (Expt. 1) or 6 (Expt. 2 to 5) plants were maintained to check tuber initiation (Expt. 1) or early tuber growth (Expt. 2 to 5) to allow appropriate timing of treatments.

Figure 4.2: Single-stemmed potato plants (cv. Royal Blue) supported by white fibreglass stakes. Planting bags are covered with aluminium foil and the top of growth medium covered with white shade cloth. Plants were in 22 °C glasshouse.

4.2.3 Experimental design and treatment structure

The diurnal cycle of temperature has an influence of on crop growth and yields. Literature suggests that high night-time temperatures could cause more harmful effect on tuber growth than the high daytime temperatures (Driver and Hawkes, 1943; Gregory, 1956). The same impact is also shown in other plant species such as rice and wheat (Morita et al., 2005; Prasad
et al., 2008). In these experiments, however, the daytime and nighttime temperatures were maintained at constant, except in Expt. 5 (Chapter 9 Section 9.5.1) in which the daytime temperatures were higher than the nighttime temperatures due to a breakdown of one air conditioner.

The experiments had different number of treatments. There were three treatments in Expt. 1 and 2, five in Expt. 3, six in Expt. 4 and eight in Expt. 5. Specific details about design and treatment structure are given in each experimental chapter.

4.3 Data collection

There were two types of data: that collected by non-destructive measurements relating to gas exchange, and that from destructive measurements for leaf area development, dry matter accumulation, and starch and water-soluble carbohydrate content in different parts of the potato plant. Non-destructive data were net photosynthetic rates, respiration rates, leaf chlorophyll content and chlorophyll fluorescence. Methods relating to the measurement of photosynthesis, respiration leaf chlorophyll fluorescence are described in Chapter 7 and 9 and those relating to the determination of starch and water-soluble carbohydrate content are in Chapter 8.

Three destructive samplings were conducted in all experiments except for Expt. 4 and 5 where only one sampling was conducted at the end of the experiment. The first sampling was conducted on the day before commencement of the high temperature treatments. The second sampling was on the same day the high temperatures were stopped. The third (final) sampling was at the end of each experiment after plants had been grown for 28 (Expt. 1, 2, 4 and 5) or more (Expt. 3) days after the end of the high temperature episode. The first sampling was conducted only on the control (22 °C) treatment. The second and third samplings were conducted on all treatments.

All plants were cut at the surface of the growth medium, put in polythene bags and immediately transferred to a cool room at 4 °C where they were held until all plants from the sampling were processed. The planting bags with tubers still intact were also transferred and held in the cool room until all processing was completed. During data collection, the tubers, stolons and roots were washed using tap water to remove sand and debris. The total number
of tubers per plant and the number of different size categories of tuber (based on the diameter at the widest part): small ($D \leq 2.5$ cm) and large ($D \geq 2.5$ cm) tubers was then recorded. All tubers were chopped into smaller pieces to enhance drying. The above ground plant parts were partitioned into leaves on the main shoot, leaves on lateral shoots, senesced leaves on the main shoot, senesced leaves on lateral shoots, petioles on the main shoot, main stem, lateral shoots (including their petioles), stolons, tubers, and roots. All leaflets were plucked from the petiolules. Then all senesced sections of the leaf laminae were removed (Fig. 4.3). The area of the leaflets was then determined using a portable laser leaf area meter (C1-202, CID Bio-Sciences Inc. USA) (Fig. 4.4). The length of the stripped main shoot was measured from the level of the growth medium to the growing tip. All plants parts were put in separate labelled paper bags and dried in a forced draught oven at 60 °C to a constant weight. The dry weights were used to derive biomass accumulation and partitioning ratios.

![Figure 4.3: Scanned leaflets (left) and the senesced sections of the leaflets (right) that were removed before scanning was conducted.](image)
Figure 4.4: Leaflets ready for area scans using a portable laser leaf area meter (C1-202, CID Bio-Sciences Inc. USA). Leaves were made flat before scanning.

Dried plant samples from Expt. 1 and 2 were ground into fine powder using an electric blender (Rocket Blender XJ-11401BO). This was found to give a more complete and more uniform particle size than a hammer mill. Each plant part in each experiment was ground separately, and the blender thoroughly cleaned using compressed air before another sample was processed. Ground samples were kept in 50-ml air tight containers in the dark until they were analysed for starch and water-soluble carbohydrates. Detailed description of the analysis and results are described in Chapter 8.

4.4 Calculations

Two levels of calculations were conducted: those relating to the content of starch and water-soluble carbohydrate are described in Chapter 8. Below are the calculations relating to derived classical growth parameters.

4.4.1 Biomass partitioning

Shoot growth is usually increased while tuber weight is reduced in potatoes grown at high temperatures (Lorenzen and Lafta, 1996; Gunn and Farrar, 1999). To investigate whether episodes of high temperatures had the same influence on shoot and tuber growth, shoot and tuber partitioning ratio was calculated as follows.
**Shoot or tuber to whole plant ratio**

\[
\text{Shoot or tuber to whole plant ratio} = \frac{\text{Total shoot or tuber dry matter (g)}}{\text{Total plant dry matter (g)}}
\]

### 4.4.2 Specific leaf area

Specific leaf area is the ratio between leaf area and leaf weight (Poorter and Remkes, 1990). It is a measure of how leaf dry matter is spread over the leaf surface (Evans and Poorter, 2001) and can be used to make inferences about plants photosynthesis performance (Basu and Minhas, 1991), radiation interception (Knops and Reinhart, 2000), transpiration (Sims and Pearcy, 1994) and biomass partitioning (Sims and Pearcy, 1994; Poorter and Nagel, 2000):

\[
\text{Specific Leaf Area (SLA)} = \frac{\text{Area of green leaf per plant}}{\text{Weight of green leaf per plant}} \text{ (cm}^2 \text{g}^{-1})
\]

### 4.4.3 Leaf area ratio

Leaf area ratio is the ratio between total leaf area and total plant weight (Poorter and Remkes, 1990) and is thus a measure of photosynthetic surface relative to respiratory load:

\[
\text{Leaf Area Ratio (LAR)} = \frac{\text{Area of green leaf per plant}}{\text{Whole plant dry matter per plant}} \text{ (cm}^2 \text{g}^{-1})
\]

### 4.4.4 Net assimilation rate

Net assimilation rate is the rate of increase in plant dry weight per unit leaf area over time (Poorter and Remkes, 1990). It is a measure of the efficiency of the leaves in generating biomass:

\[
\text{Net Assimilation Rate (NAR)} = \frac{1}{A} \times \frac{\partial w}{\partial t} \text{ (g cm}^{-2} \text{ d}^{-1})
\]

Where: \( A = \text{Area of green leaf (cm}^2) \)

\( \partial w = \text{Change in whole plant biomass (g)} \)

\( \partial t = \text{Change in time (days)} \)
4.4.5 Relative growth rate

Relative growth rate is rate of mass increase per unit mass present (efficiency of growth with respect to mass) (Hunt, 1982; Hoffmann and Poorter, 2002):

$$Relative \text{ Growth Rate (RGR)} = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1} \left( \text{g g}^{-1} \text{ d}^{-1} \right)$$

Where: $\ln(W_1)$ and $\ln(W_2)$ are the means of the natural logarithm-transformed plant weights, $W_1$ and $W_2$ are plant dry weights at times $t_1$ and $t_2$, respectively.

4.5 Data analysis

In all experiments, the data were subjected either to a one-way or a two-way analysis of variance (ANOVA) using Statistical Analysis Software (SAS) University Edition by the SAS Institute, North Carolina, USA and the General Linear Model (GLM) procedure of the SAS software. Where a significant treatment effect was found, the means were compared using Least Significant Difference (LSD). Unless otherwise specified, significant differences are reported at the 5 % level of probability. In some cases, such as in Expt. 3 and 5, a regression approach was used. The SAS REG procedure was used to calculate the coefficients of the regression. Regression lines were fit for the response of each variable to duration or concentration of the PGRs and the regression coefficients were tested for significance. Unless otherwise stated, they are only presented when significant at 5 % level of probability. Correlation analysis was also conducted in Expt. 3 using the Correlation procedure (Proc CORR) of the SAS Software. The resultant Pearson Correlation Coefficients are presented in percentages against their P Values.
CHAPTER FIVE

Impact of an episode of high temperatures before or after tuber initiation on plant and tuber growth of potatoes
Impact of an episode of high temperatures before or after tuber initiation on plant and tuber growth of potatoes

Abstract

Potato is adapted to mild temperatures, but its production is shifting to warmer regions of the globe, where episodes of high temperatures may limit its yield. This is compounded by the problem of climate change leading to global warming. It was hypothesized that an episode of high temperatures after tuber initiation would reduce tuber growth but not an episode that occurred before tuber initiation. This study investigated the physiology and growth response of the potato plant to an episode of high temperature before (Expt. 1) or after tuber initiation (Expt. 2). In both glasshouse experiments, potato (cv. Royal Blue) was grown at 22 °C. Then, at 35 days after planting (DAP) (Expt. 1) or 43 DAP (Expt. 2), the plants were transferred to high temperature treatments of either 26 °C or 30 °C and grown for nine days before being returned to 22 °C for 26 days. The final tuber dry matter was depressed to a similar extent whether the high temperatures were applied before or after tuber initiation. At the final sampling, the 30 °C treatment had 33-34 % less tuber dry matter (both experiments) than the 22 °C treatment. However, the number of tubers in both experiments was the same in all treatments. The main shoot leaf area was 34-41 % lower in the 30 °C treatment than in the control at the end of the high temperature period and at the final sampling in both experiments. By contrast, leaf area on the lateral shoots in the 30 °C treatment was 56-60 % higher than in the main shoot (both experiments) and slightly more dry matter (Expt. 2) than the 22 °C treatment. The reduction in tuber dry matter when potato plants were exposed to the 9-day episode of high temperatures before or after tuber initiation is inconsistent with the previously proposed mechanisms for high temperature impairment of tuber growth, involving starch synthase or gibberellic acid mechanisms. In this study, the reduction in tuber growth is consistent with reduced whole plant carbon supply from a reduced leaf area and exacerbated by the continued growth of the shoot, mainly in the lateral shoots.
5.1 Introduction

Potato is increasingly exposed to frequent episodes of high temperature due to climate change which can be expected to reduce its yields (Haverkort, 1990; Hijmans, 2003; Pereira et al., 2008; Jovanovic et al., 2012; Muthoni and Kabira, 2015). In some regions, such as Western Australia and Northwest China, potato already experience episodes that can last for more than seven days of temperatures above the 25 °C critical thresholds for tuber growth (Hou et al., 2010); see also Chapter 3 Section 3.4.4. While some studies have examined long term exposure of potato to increased temperatures (Lafta and Lorenzen, 1995; Timlin et al., 2006), limited understanding exists on the influence of episodes of high temperatures on the physiology, plant and tuber growth of the potato plants.

Episodes of high temperatures can occur at any stage of development during the growth of plants but thereafter plants often return to a lower temperature (see Chapter 3). Thus, the responses of plants during the high temperature period and after the end of the high temperature period becomes important. It is known that prolonged soil and/or air temperatures above 25 °C impair tuber growth (Hawkes, 1978; Wheeler et al., 1986; Reynolds and Ewing, 1989; Haverkort, 1990; Prange et al., 1990) and can cause up to 100 % loss in tuber dry matter (Bushnell, 1927; Menzel, 1980; Lafta and Lorenzen, 1995). However, most studies only consider the influence of the high temperatures at the tuber growth stage (Lafta and Lorenzen, 1995; Hancock et al., 2014; Rykaczewska, 2015). Further, in most studies the potato plants were exposed to long-term high temperatures sometimes lasting from planting until harvesting and the data collected at the end of that period (Bushnell, 1927; Prange et al., 1990; Timlin et al., 2006). Hence there are questions about the relevance of these studies to the actual temperature conditions experienced by field grown potato plants. There is limited information about how potato plants behave under conditions of episodic high temperature. Does the return of plants to cooler conditions after the end of an episode of high temperatures allow plant and tuber growth to recover or are there persistent effects of the high temperature episode?

In potatoes, the growth of other plants parts is reduced after tuber initiation (Moorby, 1978). However, it is reported that continuous high temperatures reduce dry matter partitioning to the tubers but promote shoot growth (Borah et al., 1960; Struik et al., 1989b, 1989a). There is also evidence that continuous high temperatures alter the shoot structure in potatoes.
plants (Fleisher et al., 2006) which may contribute to the change in supply of photosynthates to the tubers (Monteith and Moss, 1977; Ingram and McCloud, 1984; Boyd et al., 2002). However, in studies of whole plant responses to continuous high temperatures, the responses in leaf expansion are inconsistent (Prange et al., 1990; Lafta and Lorenzen, 1995) although tuber dry matter is always reduced (Lafta and Lorenzen, 1995; Timlin et al., 2006). Thus, it remains unclear whether impacts of high temperatures on whole plant carbon production and distribution could partly explain the reduction in tuber dry matter.

Depressed starch synthase activity has been proposed as a mechanism to explain the reduction in tuber growth at high temperatures. Starch synthase is responsible for starch deposition in storage organs including tubers (Savin and Nicolas, 1996; Edwards et al., 1999). At temperatures above 25 °C, starch synthase is inactivated leading to less starch accumulation in the organ and hence less growth (Keeling et al., 1993). Potatoes grown at temperatures above 25 °C also experience reduced starch synthase activity and hence less tuber growth (Krauss, 1981). A second proposed mechanism involves increased gibberellic acid because it was stimulated in potato plants grown at high temperatures (Menzel, 1983). This stimulation of gibberellic acid is thought to promote shoot growth that diverts carbon away from the tubers leading to less tuber growth (Menzel, 1980, 1985). Nonetheless, these are conclusions that were derived from studies in which potato plants were exposed to long-term effects of high temperatures and plants were never exposed to cooler conditions after the end of the high temperature period. Further, plants were never exposed to high temperatures shortly before tuber initiation and the subsequent impacts on tuber growth investigated. Thus, the contribution of these mechanism is unclear in situations when the plants are exposed to episodes of high temperatures shortly before or even after tuber initiation and then grown back at cooler conditions afterwards.

It was hypothesized that an episode of high temperatures after tuber initiation would reduce tuber growth but not an episode that occurred before tuber initiation. This chapter explored whole plant and tuber growth of potato plants exposed to nine days of high temperatures applied either shortly before or after tuber initiation.
5.2 Materials and methods

Expt. 1 involved the application of high temperatures shortly before tuber initiation while in Expt. 2, the same high temperatures were applied after tuber initiation. Expt. 1 was conducted from December 2015 to March 2016 and Expt. 2 from March to June 2016.

5.2.1 Experimental design and treatment structure

Each experiment had three treatments: the control, in which plants were grown and maintained at a constant temperature of 22 °C until final sampling and two high temperature treatments in which plants were exposed to either 26 °C or 30 °C temperatures for nine days. The high temperatures in both experiments were applied from 10.00 am (Western Australian Time) on the first day of the high temperature period. In Expt. 1, the high temperatures were applied from day 35 after planting (DAP); shortly before tuber initiation. In Expt. 2, the same temperatures were applied from 43 DAP; after tuber initiation was confirmed. After nine days, the high temperature treatment groups were transferred back to 22 °C and grown until final sampling: 70 (Expt. 1) and 77 (Expt. 2) DAP.

5.2.2 Planting and management

Glasshouses operation is outlined in Chapter 4. Solar duration in Expt. 1 ranged from 14 hours in December (at planting) to 12 hours in March (at the final sampling). In Expt. 2, the solar radiation ranged from 12 hours in March (at planting) to 10 hours in June (at the final sampling). The mean relative humidity during the high temperature period was 83.7 % in the 26 °C treatment and 47.6 % in 30 °C treatment. In 22 °C, the mean relative humidity was 68.1 %. The growth medium, planting bags, planting and subsequent plant management were as described in Chapter 4. Four (Expt. 1) or six extra potted plants (Expt. 2) were used to check for tuber initiation before the high temperatures were applied.

5.3 Data collection

Three destructive samplings were conducted in both experiments. The first sampling was conducted on the day before commencement of the high temperature treatment. The second sampling was at the end of the 9 days of the high temperatures. The third (final) sampling was conducted at the end of each experiment after plants had been grown back at 22 °C for 26 days in both experiments.
The first sampling was conducted before the high temperatures were applied, thus only values for 22 °C are reported. During the data collection, four (Expt. 1) and six (Expt. 2) plants were sampled for leaf area, plant height, dry weights and the total tuber number and the size categories of tubers following the methods described in Chapter 4.

5.4 Data analysis

Calculations involving classical growth parameters in both experiments were conducted as described in Chapter 4. The high temperature response of the leaf area, the number of tubers and their size distribution per plant and the dry matter parameters (tuber, below ground, leaf, stem, shoot, and whole plant) per plant were subjected to one-way analysis of variance (ANOVA) as described in Chapter 4. The analysis on the number and size distribution of tubers per plant was conducted on the square root-transformed data but only the back-transformed data are reported. Analysis of leaf senescence was performed as a percent of the senesced leaf dry matter in relation to the whole plant leaf dry matter per plant.

5.5 Results

5.5.1 Air and root temperatures during the high temperature period

During the high temperature period, the air temperature in each treatment was the same in both experiments (Fig. 5.1). The root temperature in each treatment was also the same in both experiments. The air temperature in the 30 °C treatment was between 29.9 and 31.6 °C with a mean of 30.8 °C. In the 26 °C treatment, the air temperature ranged from 25.6 to 26.5 °C with a mean of 26.1 °C. In the 22 °C treatment, the same air temperature ranged from 21 to 22.2 °C with an average of 21.5 °C. The soil temperature in the 30 °C treatment ranged from 29 to 30.7 °C with a mean of 29.7 °C. In the 26 °C treatment, the soil temperature was between 24.3 and 25.9 °C with a mean of 25.1 °C while in the 22 °C treatment, the soil temperature ranged from 21.3 to 23.8 °C and had a mean of 22.5 °C.
5.5.2 Tuber dry matter

The 9-day episode of high temperatures applied shortly before or after tuber initiation impaired tuber growth and the reduction in tuber growth per plant continued even after the end of the high temperature period in Expt. 2 (Fig. 5.2). In Expt. 1, the tubers had not formed by the end of the high temperature period (on day 44 after planting). In Expt. 2, plants in the 30 °C treatment had 85 % less tuber dry matter accumulation than the control at the end of the high temperature period. In both experiments, the reduction in tuber dry matter in the high temperature treatments continued after the end of the high temperature period. At the final sampling of Expt. 1, there was 34 % less tuber dry matter per plant in the 30 °C treatment than the control. In Expt. 2, the 30 °C treatment had 33 % less tuber dry matter per plant than
the control. The 26 °C treatment also had 16 % less tuber dry matter per plant than the control in Expt. 2.

![Figure 5.2: Tuber dry matter per plant as influenced by a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. Error bars are LSD at 5 % probability.](image)

5.5.3 Whole plant, shoot and below ground dry matter

The 9-day episode of high temperatures applied shortly before or after tuber initiation impaired the whole plant and the below ground dry matter per plant and the reduction continued even after the end of the high temperature period. In Expt. 1, there was 19 % less whole plant dry matter per plant in the 30 °C treatment than the control both at the end of high temperature period and at the final sampling (Fig. 5.3). The below ground dry matter at the end of the high temperature period was the same in all treatments: the tubers had not been formed in all treatments. However, at the final sampling, plants in the 30 °C treatment had 32 % less below ground dry matter than the control. In Expt. 2, plants in all treatments had the same whole plant dry matter at the end of the high temperature period. The below ground dry matter per plant in the 30 °C treatment was 75 % less than the control. At the final sampling, the 30 °C treatment had 22 % less whole plant and 32 % below ground dry matter than the control. The change in below ground dry matter per plant mirrored the loss in tuber dry matter per plant both at the end of high temperature period and at the final sampling in both experiments. The shoot continued to accumulate dry matter during and after the end of
the high temperature period in both experiments and the shoot dry matter per plant was not significantly influenced by the high temperature episode in either case.

Figure 5.3: Whole plant, shoot and below ground dry matter per plant as influenced by a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. Error bars are LSD at 5 % level of probability.
5.5.4 Shoot biomass partitioning

The dry matter per plant on the main shoot, the lateral shoot and the main shoot petioles was influenced differently by the 9-day episode of high temperatures when applied either shortly before or after tuber initiation. There was 20-22 % less main shoot dry matter per plant in the high temperature treatments than the control at the end of high temperature period in Expt. 1 (Fig. 5.4). At the final sampling of Expt.1, plants in all treatments had the same dry matter on the main shoot. In Expt. 2, plants in all treatments had the same dry matter on the main shoot both at the end of the high temperature period and at the final sampling. Plants in all treatments had the same dry matter on the lateral shoots at end of the high temperature period (both experiments) and at the final sampling (Expt. 1). At the final sampling in Expt. 2, there was 39 % more dry matter per plant on the lateral shoots in the 30 °C treatment than the control. Plants in all treatments had the same amount of dry matter on the main shoot petioles at the end of the high temperature period (Expt. 1 and 2) and at the final sampling (Expt. 2). At the final sampling of Expt. 1, there was 36 % less dry matter per plant on the main shoot petioles in the 30 °C treatment than the control.
Figure 5.4: Shoot biomass partitioning per plant as influenced by an episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. Error bars are LSD at 5 % level of probability.

5.5.5 Main shoot elongation and growth

In Expt. 2, plants in the 30 °C treatment were 22 % shorter than the control at the end of the high temperature period (Fig. 5.5). At the final sampling of both experiments, plants in all treatments were of the same height. The dry matter per plant on the main stem in all treatments was the same at the end of the high temperature period and at the final sampling in both experiments.
Figure 5.5: Main stem length and dry matter per plant as influenced by an episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. Error bars are LSD at 5 % level of probability.

5.5.6 Dry matter partitioning ratios

The 9-day episode of high temperatures applied shortly before or after tuber initiation changed the dry matter partitioning of potato. In Expt. 1, the biomass partitioning between the shoot and the whole plant or the tuber and the whole plant did not change either at the end of the high temperature or at the final sampling. The only change in dry matter partitioning was between the lateral shoots and the main shoots in which the lateral shoots accumulated 36 % more biomass in the 30 °C treatment compared to the control at the final sampling (Table 5.1). In Expt. 2, there was a 26 % increase in partitioning to the main shoots relative to the whole plant dry matter in the 30 °C treatment than in the control at both the end of the high temperature period and at the final sampling. The partitioning to the tubers decreased by 76 % (at the end of the high temperature period) and 16 % (at the final sampling) less dry matter in the 30 °C treatment than in the control. The lateral shoots accumulated the
same amount of dry matter compared to the main shoot in all treatments at the end of the high temperature period. At the final sampling, however, partitioning to the lateral shoots relative to main shoot was 39 % higher in the 30 °C treatment than in the control.

Table 5.1: Dry matter partitioning per plant (as a percentage) as influenced by a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. DAP = Days after planting.

<table>
<thead>
<tr>
<th>DAP</th>
<th>Partitioning ratios</th>
<th>22 °C</th>
<th>26 °C</th>
<th>30 °C</th>
<th>LSD 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt.1 (Before tuber initiation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44 DAP</td>
<td>Shoot/Whole plant</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Lateral/Main shoot</td>
<td>2.8ab</td>
<td>1.6b</td>
<td>4.9a</td>
<td>3.2</td>
</tr>
<tr>
<td>70 DAP</td>
<td>Shoot/Whole plant</td>
<td>54</td>
<td>58</td>
<td>61</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Tuber/Whole plant</td>
<td>39</td>
<td>33</td>
<td>32</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Lateral/Main shoot</td>
<td>59b</td>
<td>62b</td>
<td>92a</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Expt. 2 (After tuber initiation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52 DAP</td>
<td>Shoot/Whole plant</td>
<td>63b**</td>
<td>68b*</td>
<td>85a</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Tuber/Whole plant</td>
<td>34a</td>
<td>29a</td>
<td>8b**</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Lateral/Main shoot</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>77 DAP</td>
<td>Shoot/Whole plant</td>
<td>29bc t</td>
<td>31b</td>
<td>39a***</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Tuber/Whole plant</td>
<td>70a</td>
<td>67a†</td>
<td>59b***</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Lateral/Main shoot</td>
<td>19b</td>
<td>24ab</td>
<td>31a</td>
<td>10</td>
</tr>
</tbody>
</table>

*, **, and *** means significant at p < 0.05, 0.001 and, 0.0001 level of probability respectively, means marked with the same letter within a row are not significantly different, † and †† significant at 7 and 8 %, respectively, NS = Not significant.

5.5.7 Number of tubers per plant

The 9-day episode of high temperatures applied shortly before or after tuber initiation did not impair the production of tubers per plant (Table 5.2). However, more small-sized tubers were produced at the final sampling in plants that had been exposed to the 9-day episode of high temperatures applied after tuber initiation. In both experiments, plants in all treatments had the same total number of tubers and the tubers with diameter of more than 2.5 cm.
However, there were 45% more tubers with the diameter of less than 2.5 cm in the 30 °C treatment than in the control at the final sampling of Expt. 2.

Table 5.2: Total number and size distribution of tubers per plant as affected by a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. Analysis was conducted on square root-transformed data. Only back-transformed means are presented in the table. D = tuber diameter at the widest part.

<table>
<thead>
<tr>
<th>DAP</th>
<th>22 °C</th>
<th>26 °C</th>
<th>30 °C</th>
<th>LSD 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22 °C</td>
<td>26 °C</td>
<td>30 °C</td>
<td>LSD 0.05</td>
</tr>
<tr>
<td>Expt. 1 (Before tuber initiation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70 DAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>35</td>
<td>28</td>
<td>NS</td>
</tr>
<tr>
<td>D &gt; 2.5 cm</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>D &lt; 2.5 cm</td>
<td>17</td>
<td>24</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>Expt. 2 (After tuber initiation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52 DAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18.6</td>
<td>16.5</td>
<td>18.1</td>
<td>NS</td>
</tr>
<tr>
<td>D &gt; 2.5 cm</td>
<td>1.3</td>
<td>1.5</td>
<td>0.0</td>
<td>NS</td>
</tr>
<tr>
<td>D &lt; 2.5 cm</td>
<td>17.3</td>
<td>15.0</td>
<td>18.1</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>21.2</td>
<td>20.6</td>
<td>30.7</td>
<td>NS</td>
</tr>
<tr>
<td>77 DAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D &gt; 2.5 cm</td>
<td>8.7</td>
<td>6.7</td>
<td>8.0</td>
<td>NS</td>
</tr>
<tr>
<td>D &lt; 2.5 cm</td>
<td>12.5b</td>
<td>14.0ab</td>
<td>22.7a</td>
<td>9.19</td>
</tr>
</tbody>
</table>

Means marked with the same letter within a row are not significantly different, NS = Not significant.

5.5.8 Leaf growth and expansion

5.5.8.1 Leaf growth

The 9-day episode of high temperatures applied shortly before or after tuber initiation impacted leaf growth differently. In Expt. 1, plants in the high treatment had 24% less dry matter on the whole plant leaves than in the control at the end of the high temperature period (Fig. 5.6). At the final sampling, plants in all treatments had the similar amount of whole plant leaf dry matter. In Expt. 2, plants in all treatments had the same whole plant leaf dry matter at the end of the high temperature period. At the final sampling, the 30 °C treatment had 22% less whole plant leaf dry matter per plant than the control.
In Expt. 1, plants in the 30 °C treatment had 24 and 40 % less dry matter on main shoot leaves at the end of the high temperature period and at the final sampling, respectively than in the control. In Expt. 2, plants in all treatments had the same dry matter per plant on the main shoot leaves at the end of the high temperature period. At the final sampling, the 30 °C treatment had 35 % less dry matter per plant on the main shoot leaves than the control. There was a tendency of increased dry matter in the lateral shoots in the 30 °C treatment compared to the control at the final sampling of both experiments, but it was not significant.

The lateral shoots had not been formed in all treatments at the end of the high temperature period in both experiments. Therefore, there was no enough data for comparable analysis. At the final sampling, however, dry matter partitioning to the leaves of the lateral shoots relative to main shoot was 57 % (Expt. 1) and 36 % (Expt. 2) higher in the 30 °C treatment than in the control.
Figure 5.6: Leaf dry matter per plant of whole plant, main shoot and lateral shoots as influenced by a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. Error bars are LSD at 5 % level of probability.

5.5.8.2 Leaf expansion

The whole plant leaf area was influenced differently by a 9-day episode of high temperature applied shortly before initiation compared to after tuber initiation (Fig. 5.7). However, the impact on the main and the lateral shoot leaf area had a similar pattern whether the high temperatures were applied before or after tuber initiation. At the end of the high temperature period in both experiments, there was 35 % (Expt. 1) and 36 % (Expt. 2) less whole plant leaf area per plant in the 30 °C treatment than in the control (Fig. 5.7). At the final sampling, plants in all treatments in Expt. 1 had the same amount of whole leaf area. In Expt.
there was no recovery. Plants in the 30 °C treatment had 23 % less whole plant leaf area than the control.

The main shoot leaf area per plant in the 30 °C treatment was 35 % (Expt. 1) and 34 % (Expt. 2) less than the control at the end of the high temperature period (Fig. 5.7; Fig. 5.8). At the final sampling, the main shoot leaf area per plant was 41 % (Expt. 1) and 37 % (Expt. 2) less in the 30 °C treatment than in the control. The main shoot leaf area per plant in the 26 °C treatment in Expt. 2 was also 25 % less than the control. Lateral shoots had not been formed in all treatments at the end of the high temperature period in both experiments. Therefore, there was not enough data for comparable analysis. However, at the final sampling, leaf area lateral shoots relative to main shoot was 60 % (Expt. 1) and 56 % (Expt. 2) higher in the 30 °C treatment than in the control.

The high temperature period strongly increased leaf senescence in Expt. 2, particularly on the main shoot (Table 5.3). The leaf senescence on the main shoot at the final sampling in Expt. 2 was 37 % (26 °C treatment) and 58 % (30 °C treatment) more than in the control. Leaf senescence on the lateral shoots was not significantly different in plants in any treatments at the final sampling of Expt. 2.
Figure 5.7: Whole plant, main shoot and lateral shoot leaf area per plant as influenced by a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. Error bars are LSD at 5 % level of probability.
Figure 5.8: Potato plant showing the main and the lateral shoots. The plant was growing at the control condition (22 °C) after an exposure to a 9-day episode of 30 °C in Expt. 1.
Table 5.3: Whole plant, main shoot and lateral shoots senesced leaf dry matter per plant as affected by a 9-day episode of high temperatures applied after (Expt. 2) tuber initiation. Percent leaf senescence is based on respective leaf dry matter.

<table>
<thead>
<tr>
<th>Growth parameter</th>
<th>Senesced leaves as % of the whole plant leaf dry matter in Expt. 2</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAP</td>
<td>22 °C</td>
<td>26 °C</td>
<td>30 °C</td>
<td>LSD 0.05</td>
</tr>
<tr>
<td>Whole plant leaves</td>
<td>52 DAP</td>
<td>2.1b</td>
<td>2.1b</td>
<td>7.2a**</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>77 DAP</td>
<td>15.9c**</td>
<td>24.4b*</td>
<td>34.2a</td>
<td>8.44</td>
</tr>
<tr>
<td>Main shoot leaves</td>
<td>77 DAP</td>
<td>16.9c***</td>
<td>26.7b*</td>
<td>40.0a</td>
<td>9.19</td>
</tr>
<tr>
<td>Lateral shoot leaves</td>
<td>77 DAP</td>
<td>11.2</td>
<td>14.8</td>
<td>14.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*, **, and *** means significant at p < .05, .001 and .0001 respectively, means with same letters in the same row are not significantly different, NS = Not significant.

5.5.9 Classical growth parameters

All growth parameters showed no significant differences among treatments either at the end of the high temperature period or at the final sampling in both experiments. There was a slight decrease (p = 0.07) in the net assimilation rate in the high temperature treatments relative to the control at the final sampling of Expt. 1 (Table 5.4).
Table 5.4: Specific Leaf Area (SLA), Leaf Area Ratio (LAR), Net Assimilation Rate (NAR) and Relative Growth Rate (RGR) per plant as influenced by a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation. The high temperatures were applied from 35-44 (Expt. 1) and 43-52 (Expt. 2) DAP, respectively. DAP = Days after planting.

<table>
<thead>
<tr>
<th>DAP</th>
<th>Growth parameter</th>
<th>22 °C</th>
<th>26 °C</th>
<th>30 °C</th>
<th>LSD 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. 1 (Before tuber initiation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44 DAP</td>
<td>SLA (cm² g⁻¹ plant⁻¹)</td>
<td>428</td>
<td>500</td>
<td>370</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>LAR (cm² g⁻¹ plant⁻¹)</td>
<td>192</td>
<td>211</td>
<td>160</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NAR (mg cm⁻² day⁻¹ plant⁻¹)</td>
<td>0.33</td>
<td>0.28</td>
<td>0.32</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>RGR (mg g⁻¹ day⁻¹ plant⁻¹)</td>
<td>60</td>
<td>50</td>
<td>50</td>
<td>NS</td>
</tr>
<tr>
<td>70 DAP</td>
<td>SLA (cm² g⁻¹ plant⁻¹)</td>
<td>314</td>
<td>341</td>
<td>351</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>LAR (cm² g⁻¹ plant⁻¹)</td>
<td>66.4b†</td>
<td>82.6a</td>
<td>80.2ab</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>NAR (mg cm⁻² day⁻¹ plant⁻¹)</td>
<td>0.5a</td>
<td>0.42b†</td>
<td>0.42b†</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>RGR (mg g⁻¹ day⁻¹ plant⁻¹)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Expt. 2 (After tuber initiation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52 DAP</td>
<td>SLA (cm² g⁻¹ plant⁻¹)</td>
<td>549</td>
<td>571</td>
<td>666</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>LAR (cm² g⁻¹ plant⁻¹)</td>
<td>202</td>
<td>232</td>
<td>313</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NAR (mg cm⁻² day⁻¹ plant⁻¹)</td>
<td>0.49</td>
<td>0.33</td>
<td>0.14</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>RGR (mg g⁻¹ day⁻¹ plant⁻¹)</td>
<td>50</td>
<td>50</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>77 DAP</td>
<td>SLA (cm² g⁻¹ plant⁻¹)</td>
<td>475</td>
<td>470</td>
<td>452</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>LAR (cm² g⁻¹ plant⁻¹)</td>
<td>67</td>
<td>61</td>
<td>68</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>NAR (mg cm⁻² day⁻¹ plant⁻¹)</td>
<td>0.49</td>
<td>0.46</td>
<td>0.43</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>RGR (mg g⁻¹ day⁻¹ plant⁻¹)</td>
<td>29</td>
<td>28</td>
<td>31</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means with same letters in the same row are not significantly different, † significant at 7% level of probability. NS = Not significant.
5.6 Discussion

Based on the growth and yield responses of potato plants exposed to nine days of high temperature applied shortly before or after tuber initiation, there were five key findings: (1) Tuber dry matter were reduced to a similar extent whether an episode of high temperatures was applied shortly before or after tuber initiation; (2) The whole plant and the tuber dry matter per plant were suppressed even after the end of the 9-day high temperature period; (3) The shoot dry matter did not change both at the end of the high temperature period and at the final sampling and neither was the relative growth rates of plants after the high temperature episode affected by the antecedent high temperatures; (4) The reduction in whole plant leaf area at the end of the high temperature period recovered after plants were grown back at cooler base temperatures in plants that were exposed to the 9-day episode of high temperatures before tuber initiation; (5) In contrast, there was a pronounced change in shoot dry matter partitioning with more lateral shoots growth in the period following the high temperature episode.

Reduced tuber dry matter at the end of the high temperature period relative to the control plants is consistent with previous studies (Marinus and Bodlaender, 1975; Kooman, 1995; Lafta and Lorenzen, 1995; Kim et al., 2017). However, there are two surprising aspects. Firstly, the reduction in tuber dry matter in Expt. 1 was very similar to that in Expt. 2 even though there were no tubers present when the high temperature was applied in Expt. 1. Secondly, in Expt. 2, tuber dry matter accumulation continued to be suppressed in the high temperature treatments (26 and 30 °C) compared to the control (22 °C) between the end of the episode and the final sampling, even though the plants were growing at 22 °C. In both cases, the suppression of tuber growth happened in cooler temperatures (that should have been favourable for tuber growth) following the high temperature episode.

In this study, the reduction in the tuber dry matter per plant at the final sampling was not likely to have been caused by a reduction in starch synthase activity, or the stimulation of gibberellic acid production, or a limitation due to insufficient tuber sinks for the deposition of starch. Starch synthase is responsible for the incorporation of starch in to potato tubers and hence tuber growth (Hawker et al., 1979; Mares and Marschner, 1980; Geigenberger et al., 1997). However, starch synthase is highly susceptible to high temperatures with activity reducing quickly at temperatures above 25 °C which therefore hinders the conversion of
sucrose to starch (Jenner, 1991; Keeling et al., 1993; Jenner, 1994). The inactivation of starch synthase has been linked to the reduction in tuber growth (Krauss, 1981; Krauss and Marschner, 1984). However, previous studies on the impact of starch synthase on tuber growth used experiments in which plants were either under long-term persistent influence of high temperatures from planting to harvesting or in which individual tubers were exposed to high temperatures without plants being grown to cooler temperatures after the end of the high temperature period (Krauss, 1981; Krauss and Marschner, 1984; Geigenberger et al., 1998). By contrast, in the current study, tuber dry matter were not only reduced in plants that had no tubers present during the exposure to high temperatures, there was also less tuber dry matter accumulation after all the plants that had been exposed to high temperatures were grown back at the lower temperature of 22 °C; a temperature that would be optimal for starch synthase activity hence tuber growth. This suggests that some other mechanism might have influenced tuber growth following an episode of the high temperatures.

Increased gibberellic acid (GA) is also proposed as a mechanism responsible for the reduction in tuber dry matter in potato plants grown at high temperatures (Menzel, 1983). The application of GA on potato plants has been shown to impair tuber initiation and reduced the number of tubers per plant but stimulated the shoot growth and stem elongation (Lovell and Booth, 1967; Kumar and Wareing, 1974; Fernie and Willmitzer, 2001). It has also been demonstrated that GA application to potato plants reduces starch synthase activity at the tuber level while more starch was deposited in the shoots and stolons (Lovell and Booth, 1967; Mares and Marschner, 1980). Gibberellic acid-like responses have been observed in potato plants grown at high temperatures (Menzel, 1980). Thus, the proposed GA mechanism for a reduction in tuber dry matter is attributed to inhibited conversion of sucrose to starch at the tuber level and the production of fewer tubers per plant leading to the reduction in tuber sinks for the deposition of starch and the stimulation of the shoot growth that diverts the dry matter from tubers (Gregory, 1956; Menzel, 1980, 1983, 1985). However, there are questions about the involvement of the gibberellins in reduced tuber growth of potato plants exposed to the high temperatures because some reports show that tuber dry matter is still reduced even when the shoot dry matter is also reduced (Lafta and Lorenzen, 1995).

The results of the current study are not consistent with an increase of GA at high temperatures. Firstly, there was no indication of tuber initiation being inhibited. Plants in all
treatments had the same number or more tubers per plant than the control (Table 5.2). In Expt. 2, the number of tubers per plant in the 30 °C treatment increased by 41 % from 18.1 tubers per plant at the end of the high temperature period to 30.7 tubers per plant at the final sampling. In the control, the increase in the number of tubers per plant was only by 13 % during the same period. Secondly, growth and height of the main shoot was impaired at the end of the high temperature period and continued to be less even when plants were grown back at cooler conditions. However, the lateral shoots expanded more after the end of the high temperature period (Table 5.1; Fig. 5.4, Fig. 5.6, Fig. 5.7). This is contrary to a GA response which would have been expected to cause the main shoot to elongate and laterals to be suppressed. Fleisher et al. (2006) also reported that it is the lateral shoots that grow under high temperature, not the main stem. Plants in all treatments had the same shoot dry matter both at the end of the high temperature period and at the final sampling in both experiments (Fig. 5.5). Collectively these responses suggest a different mechanism to increased gibberellins. In fact, the shorter plants after the end of the high temperature period in Expt. 2 and the rapid lateral shoot growth after the end of the high temperature period while main shoot growth was continually impaired are more consistent with an auxin response (Sachs and Thimann, 1967; Kumlay, 2014).

Insufficient sinks for the deposition of starch in terms of the number of tubers per plant was also not responsible for the lower tuber dry matter per plant. As discussed above, the high temperature treatments either had the same number or more tubers per plant than the control in both experiments (Table 5.2) even though the high temperature treatments had less tuber dry matter per plant (Fig. 5.2). This means that the tubers were smaller in the high temperature treatments. Therefore, what might have led to the loss in tuber dry matter in the high temperature treatments? Was this due to less carbon production in terms of leaf area and the net photosynthetic efficiency? What about the dry matter partitioning and the intraplant competition? Or are there any other plausible explanations?

In this study, the reduction in both plant and tuber growth was likely caused by the reduction in the leaf area. The main shoot leaf area per plant was reduced by 35 % (Expt. 1) and 34 % (Expt. 2) in the 30 °C treatments relative to the control at the end of the high temperature period. It continued to be suppressed even when plants were grown back at 22 °C for 26 days in both experiments. At the final sampling, the main shoot leaf area was 41 % (Expt. 1) and 37
% (Expt. 2) less in the 30 °C treatment compared to the control. The reduction in the main shoot leaf area both at the end of the high temperature period and at the final sampling means that the whole plant carbon production would have been negatively influenced even if the high temperatures did not significantly alter the photosynthetic efficiency of the potato plants.

The continued shoot growth in the high temperature treatments with a reduced ability of the plant to supply carbon because of the lower leaf area, was consistent with the reduction in the tuber dry matter. It is expected that once tuber initiation starts, growth of other plant organs is diminished (Moorby, 1978). The continued accumulation of biomass by the shoot was mainly through the growth of the lateral shoots when plants were grown back at cooler temperatures. At the final sampling, the lateral shoots had accumulated 36 % (Expt. 1) and 39 % (Expt. 2) more biomass in the 30 °C treatment than in the control. Studies indicate that not only are young rapidly developing leaves photosynthetically inefficient (Smillie, 1962; Geronimo and Beevers, 1964; Weerakoon et al., 2000), but also the initiation of more lateral shoots, leaves and tubers is sucrose demanding (Xu et al., 1998a). This would have exacerbated the competition for sucrose caused by the unchecked growth of the shoot as a whole, meaning less sucrose for partitioning to the tubers and hence conversion into starch. But further question arises. Why were the lateral shoots stimulated to grow? Was this purely a hormonal response resulting from the impaired growth on the main shoot? Or was it a compensatory response by the potato plant due to the reduction in the leaf area on the main shoot?

None of the classical growth parameters (specific leaf area, leaf area ratio, net assimilation rate and relative growth rates) showed any significant difference among treatments either at the end of the high temperature period or at the final sampling in both experiments (Table 5.4). The 9-day episode of the high temperature reduced both leaf area and total dry weight. The classical growth parameters suggest the change in total dry matter and the rate of dry matter accumulation were consistent with the change in leaf area. While the high temperature episode altered dry matter partitioning between the shoot and the tubers and growth on the main shoot was reduced in favour of the lateral shoots, because the classical growth parameters were calculated for the whole plant, these changes could not be detected.
There are other plausible reasons that might have contributed to the lower tuber dry matter in the high temperature treatments. The growth of an individual tuber can be limited by the number of the dividing cells in each tuber (Bradbury, 1953; Plaisted, 1957; Reeve et al., 1971; Xu et al., 1998b). Tubers in which the mitotic process is arrested at the early stages of growth expand less and the growth proceeds with the fewer number of cells but more slowly (Reeve et al., 1973; Xu et al., 1998b). In Expt. 1, the tuber dry matter per plant and the average weight of individual tubers were reduced even though potato plants had no tubers during the episode of the high temperatures. However, it is possible that the initiation of the tubers began during the high temperature period. Therefore, could the high temperatures have impaired the cell division during tuber initiation or, in Expt. 2, during early tuber development, and resulted in fewer cells for the deposition of starch when plants were grown back at cooler temperatures? There is little information about how high temperatures influence cell division during early tuber growth hence this is worth further investigations.

Suppression of the sucrose transporter genes (Riesmeier et al., 1993; Geigenberger et al., 1998; Kühn et al., 1999) and increased concentration of auxins at the tuber level (Zhongqi et al., 1992; Purgatto et al., 2001) are also some of the possible mechanisms which can lead to the less sucrose available for conversion into starch at the tuber level. A shift between apoplastic and symplastic uploading of sucrose into the tubers has also been reported to change the balance between the number and size of tubers but little is known about how high temperature affects the mechanism of sucrose uploading in the tubers and hence is worth investigation. Some of these factors, such as impaired sucrose transport or a restriction in the deposition of starch, might be expected to lead to an accumulation of sucrose (Nösberger and Humphries, 1965; Lafta and Lorenzen, 1995) or starch (Basu and Minhas, 1991) in stems and leaves and that can negatively influence photosynthesis (Basu and Minhas, 1991; Paul and Pellny, 2003). These effects can be measured. Measurements concerning the photosynthetic responses, the content of sucrose and starch of the various potato plant parts and the hormonal influence on tuber growth are dealt with in later chapters.
5.7 Conclusions

a. A 9-day episode of high temperatures either before or after tuber initiation impaired whole plant and tuber growth.

b. The episode of high temperature impaired leaf area substantially and suppressed whole plant carbon production. As the growth of the whole shoot was unaffected by temperature, dry matter partitioning to the tubers, and hence tuber growth, were reduced.

c. The loss in tuber dry matter might have been exacerbated by competition for photo-assimilates from the rapidly expanding lateral shoots and the initiation of slightly more tubers after the end of the high temperature period.

d. A high temperature episode had both an immediate and a long-lasting impact on both plant and tuber growth.

e. Suppressed tuber growth cannot be attributed solely to the inactivation of starch synthase because the reduction in tuber growth in the current study continued after the end of the high temperature period when plants were growing back at cooler temperatures (22 °C). In Expt. 1, the reduction in tuber dry matter occurred even though there were no tubers present when the plants were exposed to the high temperature.

f. Short plants at the end of the high temperature period, the production of more tubers after the end of the high temperature period and reduced growth on main shoot both at the end of the high temperature period and at final sampling, together with the stimulation of the lateral shoots after the end of the high temperature period were inconsistent with increased gibberellic acid. They were more of consistent with an effect of auxins, which is explored further in Chapter 7.
CHAPTER SIX

Impact of varying duration of high temperature shortly after tuber initiation on plant and tuber growth of potatoes
Impact of varying duration of high temperature shortly after tuber initiation on plant and tuber growth of potatoes

Abstract
Potatoes grown in Western Australia are exposed to frequent episodes of 25 and 30 °C temperatures lasting from one to more than seven consecutive days during the tuber bulking period. However, there is little understanding of how the duration of a high temperature episode influences whole plant and tuber growth. It was hypothesized that a longer episode of high temperature shortly after tuber initiation would have a proportionally larger effect on plant and tuber growth of potatoes. This chapter investigated the impact of four durations of high temperature applied shortly after tuber initiation on plant and tuber growth of potato plants. Potato plants cv. Royal Blue were first grown in 22 °C. On day 35 after planting (DAP), plants assigned to four high temperature treatments were moved to 30 °C and grown for 1, 3, 6 or 9 days. Plants were moved back to 22 °C at the end of each duration of the high temperature and grown until final sampling (79 DAP). Plants exposed to 9 days of 30 °C had 74 % fewer tubers per plant than the control at the end of the high temperature period. With shorter duration at 30 °C, more tubers with diameter greater than 2.5 cm were produced. Plants that had been exposed to 3 days of 30 °C had 70 % of the tubers with diameter greater than 2.5 cm. In contrast, the longer durations of 30 °C encouraged the production of tubers with diameter of less than 2.5 cm; in plants that had been exposed to 9 days of 30 °C, only 40 % tubers had diameter greater than 2.5 cm. There was less whole plant, main shoot and tuber dry matter and main shoot leaf area in plants that were exposed to the longer duration of 30 °C. In conclusion, even one-day exposure of potato plants to high temperature subsequently reduced tuber growth and the impact was proportional to the duration of the episode. In addition, the duration of high temperature altered the size distribution of tubers towards small-sized tubers.

6.1 Introduction
Potatoes grown in Western Australia experience frequent episodes of above 25 or 30 °C temperatures that can last from one to more than seven consecutive days during the critical period of tuber bulking (Chapter 3 Section 3.4.4). In Chapter 5, potato plants cv. Royal Blue
exposed to 9 days of constant 26 and 30 °C temperatures shortly before and after tuber initiation experienced a similar reduction in tuber dry matter even when the plants were grown back at 22 °C for another 26 days. There was less leaf area on the main shoots in the high temperature treatments than in the control at the end of the high temperature period and at the final sampling. Growth was reduced during the high temperature period and the reduction in plant growth and tuber dry matter continued when plants were returned to 22 °C. Therefore, it was concluded that the 9-day episode of the 26 and 30 °C temperatures had both immediate and a long-lasting influence on plant and tuber growth. However, some important questions that also relate to the climate analysis arose from this study. What is the relationship between the duration of high temperatures and plant growth, tuber dry matter and, the total number of tubers per plant and size distribution of tubers in potato plants? Would potato plants recover more effectively if exposed to shorter durations of high temperatures?

The literature conveys very little understanding on how different durations of high temperature influence shoot and tuber growth in potato plants. The only available reports are in the area of the influence of the duration of high temperature on leaf photosynthesis, the activity of a number of carbohydrate enzymes and the concentration of sugars and starch in mature leaves in potato plants. Wolf et al. (1990b) studied the leaf level photosynthetic responses of two potato cultivars cv. Up-to-Date and C1-884 to short and long periods of high temperatures. They showed that the photosynthetic rates per unit area of the fourth and fifth youngest fully expanded leaves were not altered when plants were grown at 38 °C for more than 42 days. But the same leaf photosynthetic rates declined when plants were exposed to 40-42 °C or within 2 hours after plants were transferred from 22 °C to 32 °C. Further, they found higher leaf photosynthetic rates in the potato plants cv. C1-884 after 24 hours than during the first 3 hours of the 32 °C. Thus, the leaf photosynthetic rates increased with longer exposure time at that temperature.

Lafta and Lorenzen (1995) also studied the leaf photosynthetic response of two potato cultivars Norchip and Up-to-Date during the high temperature period. The potato plants were exposed to high temperatures at tuber initiation and the photosynthetic measurements were conducted on day 3 and day 8 during the high temperature period. The leaf photosynthetic rates increased with the longer duration of the high temperatures in both cultivars. In
Norchip, the leaf photosynthesis was 3.5 % higher in the high temperature treatment (31/29 °C) than in the control (19/17 °C) D/N temperatures on day 3. On day 8, the rates in the high temperature treatment were 34 % higher than in the control. In Up-to-Date, although the rates were 13 % less in the high temperature treatment than in the control on day 3, the high temperature treatment was 27 % higher than the control on day 8. Lafta and Lorenzen (1995) also found higher concentration of both glucose and sucrose but less starch in the mature potato leaves with longer duration of high temperatures.

Lorenzen and Lafta (1996) investigated the activity of sucrose phosphate synthase on three different days under high temperatures (29/27 °C) in mature potato leaves and after three days when plants had been grown back at cooler base temperatures (21/19 °C). The activity of sucrose phosphate synthase (SPS) increased with longer duration of high temperatures and was higher than in the control on all the sampling days. Further, the high temperatures did not impair the activity of the SPS. Leaves exposed to 6 days of the high temperatures and grown back to cooler temperatures for 3 days had the same level of SPS activity as in the control plants.

Lack of the negative impacts of the long-term exposure to high temperature on the leaf photosynthetic rates and the sucrose phosphate synthase activity combined, and the higher leaf photosynthetic performance with longer duration of high temperature are possible indicators that the potato plants might be acclimating to high temperatures. The increased sucrose and lower starch accumulation in mature leaves is also important. It suggests the possibility that problems related to sucrose transport and/or its conversion into starch and the deposition of starch into the potato tubers might be exacerbated at longer duration of high temperatures. However, the photosynthetic response per unit leaf area, the enzymatic activity and the accumulation of sucrose on the potato leaves alone are insufficient to fully illustrate how duration of high temperature is likely to alter whole plant growth, dry matter partitioning and hence the tuber yields. Further, except for sucrose phosphate synthase activity, these studies did not demonstrate how the duration of the high temperatures is likely to alter the performance of the potato when grown back to cooler base temperatures as expected with episodes of the high temperatures. But the whole plant dry matter production is dependent on the total leaf surface area for radiation interception and enough storage sinks (tubers) for the deposition of photosynthates (Moorby, 1968; Monteith and Moss, 1977;
Khedher and Ewing, 1985; Wilson et al., 1999; Boyd et al., 2002). Leaf development and individual tuber growth are also independently influenced by high temperature (Monteith and Moss, 1977; Midmore and Prange, 1992). For instance, Benoit et al. (1983) found maximum leaf expansion at 25 °C, the same temperature considered as the maximum threshold for tuber growth. Thus, there is need for (a) whole plant considerations of the response to the duration of high temperature and (b) an understanding on how the duration of the high temperatures influence the responses when plants are grown back at cooler temperatures.

It was hypothesized that a longer episode of high temperature shortly after tuber initiation would have a proportionally larger effect on plant and tuber growth of potatoes. This chapter investigated the impact of four durations of high temperature applied shortly after tuber initiation on canopy development and dry matter partitioning in potato plants. The data were recorded before high temperature treatment was applied, at the end of the high-temperature period and at the final sampling when plants had been returned to cooler conditions for 26-34 days depending on the duration of high temperatures during the high temperature period.

6.2 Materials and methods

This experiment (Expt. 3) was conducted from August to November 2016 in the glasshouse at Murdoch University, Perth as described in Chapter 4.

6.2.1 Experimental design and treatment structure

The climate analysis revealed that that potato grown in Western Australia experience up to 5 consecutive hot spells of 30 °C during tuber bulking in all regions (Chapter 3 Section 3.4.4). These temperatures could last from two to more than seven consecutive days in some regions. Therefore, in this experiment, there were five treatments: the control, in which plants were grown at constant temperature of 22 °C until final sampling (79 DAP) and four treatments in which plants were exposed to 30 °C for either 1, 3, 6 or 9 days shortly after tuber initiation. The 30 °C treatment was initiated at 10.00 am (Western Australian Time), 35 days after planting (DAP); after tubers were confirmed.
6.2.2 Planting and management

Potato plants were grown in a glasshouse at 22 °C before and after the end of the high temperature period. During the high temperature period, plants assigned to four high temperature treatments were moved to 30 °C and grown for 1, 3, 6 or 9 days. After the end of each duration (days), the plants in the relevant treatment were moved back to 22 °C conditions and together with the control (22 °C) treatment, grown until the final sampling. Both glasshouses were heated and cooled as described in Chapter 4. The glasshouses were naturally lit. Solar duration ranged from 10.5 hours in August (at planting) to 14 hours in November (at the final sampling). The mean relative humidity during the high temperature period was 41 % in 30 °C treatment. At 22 °C, the mean relative humidity was 61 %. Growth medium, planting bags, planting and subsequent plant management were conducted as described is Chapter 4. Six extra plants were used to check for the presence of tuber before the high temperatures were applied.

6.3 Data collection

Three destructive samplings were also conducted in Expt. 3. The first sampling was conducted on the day before commencement of the high temperature treatment. The second sampling was at the end of the high temperature episode for each treatment. The third (final) sampling was conducted at the end the experiment (79 DAP) after plants had been grown back at 22 °C for more than 28 days depending on the number of days spent in the 30 °C treatment. This is because final sampling was conducted on the same day, but plants such as the 1-day temperature treatment were returned to 22 °C eight days earlier than the 9-day temperature treatment.

As no differential treatments had yet been applied, the first sampling was conducted on only one set of plants at 22 °C. The second and third samplings were conducted on all treatments. Plants were sampled for the total number and size category of tubers. The data on leaf area, plant height, and dry weights were also collected following the methods described in Chapter 4. Six plants per treatment were sampled during the data collection.
6.4 Calculations and data analysis

Calculation of classical growth parameters and overall data analysis were conducted as described in Chapter 4. The dry matter and leaf area of the lateral shoots were calculated as a percent of the dry matter or leaf area of the main shoot. The size distribution of tubers was calculated as the number of tubers of a given size expressed as a percent of the total number of tubers per plant.

The effect of each the high temperature treatment on the leaf area, the number of tubers and their size distribution per plant and the dry matter components (tuber, below ground, leaf, stem, shoot, and whole plant) per plant was determined using a one-way analysis of variance (ANOVA) at the end of each of the high temperature treatments using procedures as described in Chapter 4 Section 4.5. The significant effects are reported at 5 % level of probability unless otherwise specified.

A regression model was used to test the effect of duration of high temperature on the leaf area, the number of tubers and their size distribution and the dry matter variables (tuber, below ground, leaf, stem, shoot, and whole plant) at the final sampling (79 DAP). The regression equations and coefficients were calculated using the Regression procedure (Proc REG) of the SAS Software.

A correlation analysis was conducted between the tuber dry matter and the whole plant, shoot, main shoot, lateral shoot, main shoot leaf, lateral shoot leaf, below ground and the tuber dry matter per plant and the leaf area per plant on the whole plant, main shoot and lateral shoots. The correlation coefficients were calculated using the Correlation procedure (Proc CORR) of the SAS Software. The regression and correlation coefficients were tested for significance at 5 % unless otherwise specified. The analysis of the calculated classical growth parameters gave no significant results hence are omitted from the results and discussion of this chapter.

6.5 Results

6.5.1 Day and night-time air temperature during the high temperature period

The mean air temperature during the high temperature period was 29.5 °C in the 30 °C treatment (Fig. 6.1). In the same 30 °C treatment, the night-time (7.00 pm to 7.00 am) air temperature was 28.5 °C on average while the daytime (8.00 am to 6.00 pm) temperature
was 30.5 °C on average. In the 22 °C treatment, the mean air temperature was 23.7 °C while the day and night-time temperatures were lower than 24 °C except from 7.00 am to 2.00 pm when the temperatures were somewhat higher than 24 °C.

![Graph showing mean air temperatures of different treatments averaged over the nine days of the high temperature episode of Expt. 3.](image)

**Figure 6.1:** Mean air temperatures of the different treatments averaged over the nine days of the high temperature episode of Expt. 3.

### 6.5.2 Tuber dry matter

The tuber dry matter of plants in all treatments did not differ significantly from the control at the end of the high temperature period (Fig. 6.2). At the final sampling, there was a significant linear relationship between duration of high temperature and the accumulated tuber dry matter per plant. Significantly less tuber dry matter per plant accumulated in treatments with longer duration of high temperature ($p = 0.0019$). There was 3% reduction in tuber dry matter for each day the potato plants were exposed to 30 °C.
Figure 6.2: Tuber dry matter per plant as influenced by duration of high temperature. Data at the end of each of the duration of the 30 °C and at the final sampling (79 DAP) of Expt. 3. NS = Not significant at 5 % level of probability.

6.5.3 Total number of tubers per plant

Plants exposed to 9 days of 30 °C had 74 % fewer number of tubers than the control at the end of the high temperature period (Fig. 6.3). However, at the final sampling, there was the same number of tubers per plant in all treatments; that is, there was no significant response to duration.

Figure 6.3: Total number of tubers per plant as influenced by duration of high temperature. Data at the end of each of the duration of the 30 °C and at the final sampling (79 DAP) of Expt. 3. Error bars are LSD at 5 % level of probability. NS = Not significant.
6.5.4 Size distribution of tubers per plant

In contrast to the number of tubers per plant, there was a significant cubic polynomial effect in the response of the percentage of tubers in the different size classes at the final sampling to the duration of high temperature (Fig. 6.4). In both the control and 6-day temperature treatments, the number of tubers with diameter of greater than 2.5 cm was equal to those with diameter less than 2.5 cm. However, for the 3-day high temperature treatment, 70 % of the total number of tubers had a diameter of greater than 2.5 cm and for the 9-day treatment only 40 % of the tubers had a diameter greater than 2.5 cm.

\[
\geq 2.5 \text{ cm} = 0.21x^3 - 3.5x^2 + 13x + 51 \\
 \quad p = .03 \text{ (x}^3\text{), } p = .01 \text{ (x}^2\text{ and x) }
\]

Figure 6.4: Percent size distribution of tubers per plant as influenced by varying duration of high temperatures. Tubers were categorised based on diameter \((D) \leq 2.5 \text{ or } \geq 2.5 \text{ cm}\). The classification of tubers was based on their diameter \((D)\) at the widest part. Data at the final sampling (79 DAP) of Expt. 3.

6.5.5 Whole plant, shoot and below ground dry matter

Plants in all treatments had the same whole plant dry matter as the control at the end of the high temperature period. Shoot and below ground dry matter were also the same as the control for all treatments (Fig. 6.5). At the final sampling, longer durations of high temperature resulted in significant less whole plant \((p = 0.012)\) and below ground \((p = 0.002)\) dry matter per plant. There was 3.5 % (whole plant) and 3 % (below ground) reduction in dry matter for each day the potato plants were exposed to 30 °C.
Figure 6.5: Whole plant, shoot and below ground dry matter per plant as affected by duration of high temperature. Data at the end of each of the duration of the 30 °C and at the final sampling (79 DAP) of Expt. 3. NS = Not significant at 5 % level of probability.

### 6.5.6 Shoot biomass partitioning

Plants in all treatments had the same main shoot dry matter per plant as the control at the end of the high temperature period. Lateral shoot and main shoot petioles dry matter were also the same in all treatments (Fig. 6.6). At the final sampling, significantly less dry matter had been accumulated by the main shoot and main shoot petioles in treatments with longer duration of high temperature. There was 1.2 % (main shoot) and 0.2 % (main shoot petioles) reduction in dry matter for each day the potato plants were exposed to 30 °C. In contrast, the lateral shoots accumulated 4 % more dry matter per plant than the main shoots for each day the potato plants were exposed to 30 °C.
Figure 6.6: Influence of duration of high temperature on dry matter deposition per plant on shoot components. Data at the end of each of the duration of the 30 °C and at the final sampling (79 DAP) of Expt. 3. NS = Not significant at 5 % level of probability.

6.5.7 Main shoot length and main stem dry matter

At the final sampling, plants in all treatments were of similar height (i.e. no significant response to duration), but significantly less dry matter accumulated in the main stem with longer durations of high temperature (Fig. 6.7). There was 0.4 % reduction in the main stem dry matter per plant with each day the potato plants were exposed to 30 °C.
Figure 6.7: Main shoot length and dry matter as influenced by duration of high temperature. Data at the final sampling (79 DAP) of Expt. 3.

6.5.8 Leaf growth and expansion

6.5.8.1 Leaf growth

At the end of the high temperature period, plants in all treatments had the same whole plant leaf dry matter as the controls (Fig. 6.8). Leaf dry matter accumulated on main or lateral shoots was also the same in all treatments. At the final sampling, significantly less dry matter accumulated in main shoot leaves with longer duration of high temperature. There was 1 % reduction in the dry matter accumulated on the main shoot leaf with each day of exposure of the potato plants to 30 °C. On the other hand, there was 4 % more dry matter per plant in the
leaves of the lateral shoots than in the main shoot with longer duration of the high temperatures. Nonetheless, same dry matter accumulated in the whole plant leaves with longer duration of the high temperatures.

![Graphs showing leaf dry matter of whole plants, main shoots and lateral shoots as influenced by duration of high temperature. Data at the end of each of the duration of the 30 °C and at the final sampling (79 DAP) of Expt. 3.](image)

**Figure 6.8:** Leaf dry matter of whole plants, main shoots and lateral shoots as influenced by duration of high temperature. Data at the end of each of the duration of the 30 °C and at the final sampling (79 DAP) of Expt. 3.

### 6.5.8.2 Leaf expansion

Plants in all treatments had similar whole plant leaf area at the end of the high temperature period. Main and lateral shoot leaf expansion also did not change (Fig. 6.9). At the final sampling, all treatments also had the same whole plant leaf area per plant. The main shoot leaf area was significantly less with the longer duration of high temperature. The 6 and 9-day
temperature treatments had 31-34 % less main shoot leaf area than the control. On the other hand, more leaves expanded more on the lateral shoots than on the main shoots with longer duration of the high temperatures. With each duration of the 30 °C, the leaf area on the lateral shoots increased by 8 % than in the main shoots.

Figure 6.9: Leaf area of the whole plant, main shoot and lateral shoots as affected by duration of high temperature. Data at the end of each of the duration of the 30 °C and at the final sampling (79 DAP) of Expt. 3.
6.5.8.3 Leaf senescence

There was greater senesced leaf dry matter on the main shoot in treatments with greater duration of high temperature (Fig. 6.10). The 9-day temperature treatment had 40 % more senesced leaf dry matter on the main shoot than the control.

At final sampling

<table>
<thead>
<tr>
<th>Duration (Days) of 30°C</th>
<th>Dry matter of senesced leaves on main shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
</tr>
<tr>
<td>6</td>
<td>8.0</td>
</tr>
<tr>
<td>7</td>
<td>9.0</td>
</tr>
<tr>
<td>8</td>
<td>10.0</td>
</tr>
<tr>
<td>9</td>
<td>11.0</td>
</tr>
</tbody>
</table>

\[ y = 0.28x + 3.3 \]

\[ R^2 = 0.39, \ p = 0.03 \]

Figure 6.10: Senesced main shoot leaf in grams per plant as influenced by duration of high temperature. Data at the final sampling (79 DAP) of Expt. 3.

6.5.9 Correlation between growth parameters and tuber dry matter

There was a significant positive relationship between the accumulated dry matter per plant in the whole plant, main shoot, main shoot leaf dry matter, and the main shoot leaf area and the tuber dry matter per plant at the final sampling of Expt. 3. (Table 6.1) Both the dry matter and the leaf area on the lateral shoot showed insignificant but a negative relationship with the accumulated dry matter per plant in the tubers.
Table 6.1: Correlation (as percentage) between growth parameters and the tuber dry matter at the final sampling (79 DAP) of Expt. 3.

<table>
<thead>
<tr>
<th>Plant parameter</th>
<th>Pearson Correlation Coefficients (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole plant dry matter</td>
<td>90</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Shoot dry matter</td>
<td>35</td>
<td>0.12</td>
</tr>
<tr>
<td>Main shoot dry matter</td>
<td>76</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Lateral shoot dry matter</td>
<td>-21</td>
<td>0.36</td>
</tr>
<tr>
<td>Whole plant leaf dry matter</td>
<td>40</td>
<td>0.07</td>
</tr>
<tr>
<td>Main shoot leaf dry matter</td>
<td>69</td>
<td>0.0007</td>
</tr>
<tr>
<td>Lateral shoot dry matter</td>
<td>-21</td>
<td>0.35</td>
</tr>
<tr>
<td>Whole plant leaf area</td>
<td>35</td>
<td>0.12</td>
</tr>
<tr>
<td>Main shoot leaf area</td>
<td>69</td>
<td>0.0007</td>
</tr>
<tr>
<td>Lateral shoot leaf</td>
<td>-29</td>
<td>0.20</td>
</tr>
</tbody>
</table>

6.6 Discussion

This experiment investigated the response of potato plants at early tuber growth to four durations (1, 3, 6 and 9 days) of 30 °C. The potato plants were grown at 22 °C before and after the end of high temperature period.

Results of this experiment demonstrated that each additional day of 30 °C applied on potato plants during early tuber development caused the same impact on canopy development dry matter accumulation and dry matter partitioning. However, the size distribution of tubers showed a different response to the duration of high temperature.

Short durations of the high temperature reduced potato yield and the reduction occurred even when plants were exposed to only 1 day of high temperature and grown back to cooler base temperatures for 35 days. This was surprising. Not only did plants in the 1-day high temperature treatment spend the shortest time at 30 °C, they were also grown at the more favourable, cooler temperature (22 °C) for a longer period than the other high temperature treatments.

The results of this experiment support the findings of Chapter 5, that the reduction in tuber growth in potato plants exposed to episodes of high temperatures is not predominantly
controlled by reduced starch synthase activity or increased gibberellic acid concentration. At the end of the high temperature period, the tuber mass was very small and did not differ significantly from the control. Most of the tuber growth occurred after the end of the high temperature period at temperatures favourable for tuber growth. Secondly, these results demonstrate the potential threats of even the short duration of hot spells identified in the climate analysis for plant and tuber growth (Chapter 3). The responses observed in this experiment might be altered by factors such as cooler night temperatures, nutrition, plant density, the multi-stemmed plants and irrigation. These require more understanding.

The results of this experiment showed a significant negative linear relationship between the duration of high temperature and the accumulated whole plant, main shoot, main stem, below ground and tuber dry matter and main shoot leaf area per plant. The tuber dry matter per plant was less by 3 g plant\(^{-1}\) for each additional day of 30 °C (Fig. 6.2). The whole plant dry matter was less by 3.5 g plant\(^{-1}\) day\(^{-1}\) while main shoot leaf area was also less by 318 cm\(^2\) plant\(^{-1}\) for each additional day of the 30 °C (Fig. 6.5; Fig. 6.9). Each additional day of the high temperatures caused a similar loss in both the dry matter and leaf area per plant. This suggests that a repeat of the high temperature episode is expected to cause a consistent loss in whole plant and tuber growth. This is very important given that potatoes grown in Western Australia are not only exposed to hot spells of varying durations during the tuber bulking period, the plants are also exposed to repeated exposures of the high temperatures (Chapter 3). However, there is no data available about the response of potato canopy development and tuber growth to a repeated episode of high temperatures. In particular, it is not known whether potato plants would acclimate to high temperature and whether this depends on the duration of high temperatures. The only available information is on the thermotolerance of the photosystem II (PSII). Havaux (1993) found increased thermotolerance of PSII in the leaves of the potato plant cv. Haig grown at 25 °C and first exposed to 35 °C before being exposed to 40 °C. Leaves that transitioned directly from 25 °C to 40 °C experienced complete and irreversible inhibition of the PSII but not the leaves that were first exposed to 35 °C before the 40 °C temperatures.

Other plant species have been shown to acclimate well at high temperatures and might provide useful insights into whether potato plants could respond similarly. Li et al. (1991) found better plant dry weight, pod set, pod weight and yield in common beans that had been
grown at 25/18 °C D/N and first acclimated at 37 °C D/N for 24 hours before being exposed to 50 °C. Improved photosynthetic performance, PSII, Rubisco activity, Rubisco activation state, chlorophyll content and carotenoid content has also been demonstrated in maize that were first exposed to 50 °C then grown at 41 °C than those that grew at 25 °C then exposed to temperatures of over 35 °C (Sinsawat et al., 2004). However, there is also information that not all plants or even plants from the same species acclimate at high temperatures. Larigauderie and Körner (1995) showed that only 3 out of 19 alpine species showed similar respiration rates when the plants were grown at 10 and 20 °C. They also found that only the prostrate alpine species had the lowest acclimation potential. Acclimation to high temperatures may have a major influence on the impact of high temperature in the field where plants are typically exposed to repeated episodes. Thus, more studies are needed with the potato plants.

The duration of high temperature influenced the size distribution of tubers per plant. At the final sampling, there was a significant curvilinear relationship between the duration of high temperature and the size distribution of tubers (Fig. 6.4). More tubers with diameter greater than 2.5 cm were produced in plants that had been exposed to shorter durations of high temperature. With 70 % of these tubers being produced in plants that had been exposed to 3 days of 30 °C. On the other hand, fewer tubers with diameter of more than 2.5 cm were produced in plants that had been exposed to longer durations of high temperatures. From the point of view of seed production, this is important because the size of seed tubers can influence germination (stand count), shoot growth (plant vigour) and hence yields of potato plants (Masarirambi et al., 2012). Seed potatoes smaller than 2.5 cm in diameter tend to give poor plant set, less plant vigour and lower yields compared to those more than 2.5 cm in diameter (Struik and Wiersema, 1999). When potato is grown for processing, sizes larger than 224 g give premium fries (Iritani et al., 1983). Thus, the complex impact of duration of high temperature on tuber size distribution is important for management of crops intended for either seed or processing potato.
6.7 Conclusions

a. High temperature caused a long-lasting influence on tuber growth. Even the impacts of the short durations of 30 °C continued to negatively influence tuber growth of potato plants after the end of the high temperature period.

b. The duration of high temperature influenced the size distribution of tubers. Shorter durations of less than 3 consecutive days of the 30 °C increased the production of tubers with diameter greater than 2.5 cm. In contrast, longer durations of more than 6 consecutive days of the same temperature increased the production of tubers with diameter of less than 2.5 cm.

c. Potatoes grown in Western Australia are exposed to frequent episodes of varying durations of hot spells. Therefore, further investigation is needed with a repeat of the high temperatures after plants have been grown back at cooler conditions.
CHAPTER SEVEN

Photosynthetic and respiratory response of potato plants grown under an episode of high temperatures
**Photosynthetic and respiratory response of potato plants grown under an episode of high temperatures**

**Abstract**

There is inconsistent information about the leaf level photosynthetic responses of the potato plant at high temperatures. Moreover, little is known about the photosynthetic performance of the potato plants when plants are exposed to cooler conditions after the end an episode of high temperature. It was hypothesized that an episode of high temperatures shortly after tuber initiation would reduce photosynthesis and increase respiration in potato leaves. This study investigated the photosynthetic and respiratory response of potato plants exposed to a 9-day episode of high temperatures applied shortly after tuber initiation in two glasshouse experiments: Expt. 2 (Chapter 5) and 4 (Chapter 9). Expt. 2 had three treatments: the control (22 °C) and two high-temperature treatments (26 °C and 30 °C). Expt. 4, had only two treatments: the control (22 °C) and 30 °C. In all experiments, potato plants were grown at 22 °C before and after the end of the high-temperature period. High temperatures increased photosynthetic rates measured between 12.00 and 2.00 pm in the terminal leaflet of the seventh leaf both during the high temperatures (both experiments) and when plants were grown back at cooler conditions except in Expt. 4 in which treatments were not significantly different on day 64 after planting. The high temperature increased photosynthetic rates at cooler conditions in the terminal leaflet of a leaf that emerged during the high temperature period. The high temperatures impaired photosynthesis in older leaves even though there was brief recovery for a few days during the high temperature period. The high temperature treatments increased morning (7.00 am to 10.00 am) and night-time (7.00 pm to 1.00 am) respiration rates relative to the control. In conclusion, the 9-day episode of the high temperatures impaired the whole plant carbon production in the potato plants through a reduction in the photosynthetic efficiency of the older leaves, increased morning and night-time respiration rates and a reduction in leaf area on the main shoots as reported in Chapter 5 and 6.
7.1 Introduction

The reduction in tuber dry matter at the end of the high temperature period and at the final sampling in Chapter 5 and 6 was related to the loss in leaf area and the continued shoot growth (Chapter 5 Section 5.5.8 and 5.5.3; Chapter 6 Section 6.5.9). The whole plant carbon production is dependent not only on the available leaf surface for radiation interception but also on photosynthetic rates and the available sinks for the deposition of the photosynthates (Moorby, 1968; Spence and Humphries, 1972; Monteith and Moss, 1977; Khedher and Ewing, 1985; Wilson et al., 1999; Boyd et al., 2002). Thus, a reduction in total available carbon can be due to a reduced leaf area, a reduction in photosynthetic rates per unit leaf area, or a combination of the two. It is unclear to what extent altered photosynthetic rates contributed to the reduced tuber growth in Chapter 5 and 6.

High temperatures impair canopy photosynthesis in potato plants. Fleisher et al. (2006) found 64 % less whole canopy photosynthesis in potato cv. Kennebec when grown at 34/29 °C compared to 23/18 °C D/N. Similarly, Timlin et al. (2006) showed 84 % less whole canopy photosynthesis in potato cv. Atlantic grown at 32 °C compared to 24 °C constant temperature. However, the canopy photosynthesis is influenced by both the photosynthetic rates of the individual leaves and the available whole plant leaf area (Peng and Krieg, 1991; Fleisher et al., 2006). Information with regards the influence of high temperatures on the photosynthetic rates of individual potato leaves is inconsistent. Hammes and De Jager (1990) found 37 % reduction in leaf-level photosynthesis of potato cv. Up-to-Date when growth temperature was raised from 15 °C to 40 °C at the rate of 5 °C per hour. Wolf et al. (1990b) showed 40 % reduction in the photosynthesis of the same potato cv. Up-to-Date and on C1-884 when these plants were transferred from 22 °C to 32 °C and grown for 2 hours. However, they found no influence on cultivar Desiree. Aien et al. (2011) found 16-22 % less photosynthesis in potato cv. Kufri Chipsona-3 and Kufri Surya grown at 29-39 °C compared to 25 °C.

Importantly, in studies of the influence of temperature on potato photosynthesis, leaves at different positions on the plant have been used. In some case, the leaves used in the photosynthetic measurements were not indicated. Frier (1977) conducted photosynthetic measurements on the first two sub-terminal leaflets of the six and of every third subsequent fully expanded leaf as numbered from the soil level. Reynolds et al. (1990) used the terminal leaflet of the leaf on the sixth node as numbered from the top of the plant. Dwelle et al. (1983)
and Wolf et al. (1990) used the fourth and the fifth leaf interchangeably; while Midmore and Prange (1992) described the leaves as “the uppermost fully expanded”. Hammes and De Jager (1990) and Katny et al. (2005) did not specify the leaves used in their measurements. This inconsistency is important because photosynthesis has been showed to be related to leaf age. Lower photosynthetic rates have been demonstrated in older than in younger leaves in plant species such as cotton (*Gossypium* spp.) cv. Sri Sumrong60, wheat (*Triticum* spp.), Asian pear (*Pyrus serotine*) Rehd cv. Cultura and Rubbertree (*Hevea brasiliensis* (Willd.) Müll.-Arg] (Constable and Rawson, 1980; Kasemsap et al., 1997; Xie and Luo, 2003; Kositsup et al., 2010).

As little is known about how potato leaves at different positions respond differently to temperature, the importance of the different leaves used in the published studies cannot be determined.

There is also little information on the diurnal respiratory responses of potato leaves and how such responses might vary among leaf positions or change with high temperatures. This information is important for understanding the changes in whole plant carbon dynamics. Wolf et al. (1990b) reported a doubling in the rates of dark respiration in the potato plant cv. Up-to-Date grown at 40-42 °C relative to those at 30-32 °C. However, the time of day or the night when these data were collected was not indicated. Fleisher et al. (2006) reported a tripling in rates of dark respiration in potato plants grown at 28/23 °C and 34/29 °C compared to plants gown at lower temperatures (i.e. 23/18 °C, 20/15 °C, 17/12 °C or 14/10 °C D/N temperatures. These data showed the means of the dark respiration data collected between 2000 to 2200 hours (considered as the daytime when the PAR was zero) and between 0100 and 0400 hours (night-time). Timlin et al. (2006) also observed tripling effect in the dark respiration of potato plants grown at 28 °C and 32 °C relative to those between 20 to 24 °C. Nonetheless, these reports also showed only the means of the dark respiration collected between 0100 to 0400 hours (night-time). From other sources, it is documented that respiration is usually high during the first few hours of darkness (Byrd et al., 1992; Bunce, 2007). It is also known that the newly unfolded leaves have higher respiration and lower net photosynthesis (Smillie, 1962; Geronimo and Beevers, 1964; Hardwick et al., 1968; Sesták, 2012). On the other hand, photosynthetic rates usually reach a peak at around midday (Dwelle et al., 1983; Timlin et al., 2006). Therefore, it is important to understand how high temperatures and leaf age might alter respiration in potato plants and how this relates to the whole plant carbon balance.
The maximum quantum efficiency of photosystem II (Fv/Fm) is a major determinant of the rate of CO₂ assimilation (Harbinson et al., 1990; Krall and Edwards, 1991). Light energy absorbed by the leaf chlorophylls is used to drive photosynthesis (photochemistry) (Krause and Weis, 1991; Maxwell and Johnson, 2000). The maximum quantum efficiency of a leaf is linearly related to its CO₂ assimilation rate (Harbinson et al., 1990). Thus, changes in chlorophyll fluorescence directly relate to the photosynthetic performance of plants (McAlister and Myers, 1940; Genty et al., 1989). In potato plants, the reduction in the Fv/Fm has been showed to correlated with reduction in net photosynthetic rates even at temperatures as low as 25 °C and above (Prange et al., 1990). These measurements could provide an additional reflection on how episodes of high temperatures influence photosynthetic rates in potato plants.

It was hypothesized that an episode of high temperatures shortly after tuber initiation would reduce photosynthesis and increase respiration in potato leaves. This chapter investigated: (1) the photosynthetic and chlorophyll fluorescence response of potato plants before high temperatures were applied, during the high temperature period and at the end of an episode of high temperatures when plants were grown at cooler conditions; (2) the photosynthetic performance at cooler conditions of a leaf that emerged during the high temperature period; (3) the influence of leaf position on the photosynthetic performance of potato leaves and how their performance was altered by high temperature; and (4) the diurnal respiratory response of leaves at different leaf position on the main shoot of potato plants and how they were influence by an episode of high temperatures. A series of preliminary experiments were also conducted to ensure an appropriate method was used (Appendix A).

7.2 Materials and methods

Photosynthetic measurements were conducted in two experiments: Expt. 2 and 4. Expt. 2 investigated the influence of a 9-day episode of 26 or 30 °C temperature on the photosynthetic response of potato plants (Chapter 5 Section 5.2). Expt. 4 explored the impact of exogenously applied PGRs on performance of potatoes during a 9-day episode of 30 °C (Chapter 9 Section 9.2). The data on the photosynthetic and the diurnal respiratory response of the plants in the treatments which had received no hormonal application were used in this
chapter. The high temperatures in both experiments were applied shortly after tuber initiation. Expt. 2 was conducted from March to June 2016 and Expt. 4, from March to June 2017.

7.2.1 Experimental design and treatment structure
Expt. 2 had three treatments: the control (22 °C) and two temperature treatments (26 and 30 °C, Chapter 5 Section 5.2.1). Only two treatments were used from Expt. 4: the control (22 °C) and one high-temperature treatment (30 °C, Chapter 9 Section 9.2.1). In all experiments, plants in the control were grown and maintained at a constant temperature of 22 °C until final sampling while plants in high temperature treatments were grown at 22°C until the time of the heat stress, then exposed to the high temperatures for nine days before being returned to 22°C.

In both experiments, high temperatures were initiated shortly after tuber initiation was confirmed; on 43 DAP (Expt. 2) or 37 DAP (Expt. 4).

7.2.2 Glasshouse conditions and plant management
The glasshouses were operated as described in Chapter 4. In Expt. 2 and 4, the solar radiation ranged from 12 hours in March (at planting) to 10 hours in June (at the final sampling). The growth medium, planting bags, planting and subsequent plant management in all experiments were as described in Chapter 4. Six plants were used to check for tuber initiation before the high temperatures were applied.

7.3 Measurements
Two types of measurements were conducted. The first measurements related to gas exchange while the second targeted the efficiency of photosystem II (PSII). Measurements related to gas exchange were the net photosynthetic and respiration rates per unit leaf area. The net photosynthesis and respiration were recorded using a LCpro + System (Serial No. 32125, ADC BioScientific Ltd. UK) and an open leaf chamber. The influx of CO₂ into the leaf (µmol CO₂ m⁻² s⁻¹) represented net photosynthetic rate whereas CO₂ efflux from the leaf was taken as the respiration rate as documented by Dutton et al. (1988); Wolf et al. (1990b); Raja et al. (2001); Fleisher et al. (2006).
Measurements related to the photosynthetic efficiency per unit leaf area were the chlorophyll fluorescence parameters. These were recorded using the Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd. UK). All measurements were conducted *in-situ*.

**7.3.1 Photosynthetic measurements**

The photosynthetic performance of potato leaves exposed to the 9-day episode of the high temperatures was measured on the terminal leaflet of the seventh leaf as determined before the high temperatures were applied. This leaf was then tagged for repeated measurement during the high temperature period and after the end of the episode of high temperature (Fig. 7.1). The measurements before high temperatures were applied were made only on control plants on the day before the application of the high-temperature treatments. Measurements during the high-temperature period were made daily on all treatments. Measurements after the end of the high-temperature treatment were continued in both experiments until the final sampling.

To determine the influence of a 9-day episode of high temperatures on the photosynthetic response of potato leaves of different leaf age, two sets of photosynthetic measurements were conducted. The first set of measurements were conducted only on the control plants before the high temperatures were applied. These measurements were recorded from the terminal leaflets of the third, fifth, seventh and eleventh leaf numbered from the growing tip on the main shoot of single-stemmed potato plants at 22 °C in Expt. 2 (Fig. 7.1). The leaf positions were selected to reflect the developmental stages of the leaves: the third and fifth leaves were rapidly expanding young leaves, the seventh leaf was young fully expanded leaf and the eleventh leaf was a fully mature leaf. The measurements were conducted on six randomly selected potato plants on day 18, 19 and 21 after planting.

The second set of the photosynthetic measurements was conducted during the high temperature period in Expt. 2 and 4. Measurements in Expt. 2 were conducted on the third, fifth, seventh, eleventh and fifteenth leaf representing the different leaf age as described above. The fifteenth leaf was included to represent older leaves. These measurements in Expt. 2 were conducted 45, 47 and 49 DAP in all treatments: the control (22 °C) and the two high-temperature treatment (26 and 30 °C). However, results are presented only for the control and the 30 °C treatment. In Expt. 4, the measurements were made only from the
terminal leaflet of the seventh, eleventh and fifteenth leaves and only for the two treatments: control (22 °C) and 30 °C. These measurements in Expt. 4 were conducted 41, 44 and 47 DAP.

To investigate the photosynthetic performance at cooler growth conditions of leaves that emerged during the high temperatures, photosynthetic measurements were conducted on the terminal leaflet of the third leaf that emerged during the high temperature period. These leaves were tagged after plants had been transferred back to cooler conditions on day 56 after planting. The photosynthetic measurements were conducted weekly from 56 to 70 DAP on six randomly selected potato plants per treatment; only in Expt. 2.
Figure 7.1: Potato plants with leaf discs (white) attached on the terminal leaflet of a tagged seventh leaf. The pink ribbons show tagged leaves at different leaf position on the main shoot of the single-stemmed potato plants. Leaves were tagged a day before the high temperatures were applied in Expt. 2. The plants were in the 30 °C temperature treatment.

7.3.2 Chlorophyll fluorescence measurements

Measurements of chlorophyll fluorescence were conducted on the same terminal leaflets of the seventh leaf used to determine the photosynthetic responses of potato plants (Fig. 7.1 above). These measurements were also conducted on six randomly selected potato plants per treatment at intervals of two days from 42 to 52 DAP during the high-temperature period in Expt. 2. Before chlorophyll fluorescence measurements were made, the terminal leaflets
were dark adapted for 30 minutes. Leaf discs with the shutter closed were placed on the terminal leaflets of the seventh leaf and left for 30 minutes. Then, the leaf chamber of the Fluorometer was fitted into the leaf discs, the shutter opened to illuminate the leaf with blue-red light at 1200 µmol m⁻² s⁻¹, and readings recorded.

Chlorophyll fluorescence parameters other than the maximum quantum efficiency of photosystem II (Fv/Fm) are significantly influenced by high temperatures (Midmore and Prange, 1992). Therefore, in this study, the data on all chlorophyll fluorescence parameters were recorded. These were the minimum fluorescence (Fo), the maximum fluorescence (Fm), the variable fluorescence (Fv) (Fv = Fm – Fo) and the maximum quantum efficiency of photosystem II (Fv/Fm) as described by Baker and Rosenqvist (2004).

7.3.3 The diurnal respiratory measurements

Respiratory measurements were conducted to investigate the influence of an episode of high temperature on the diurnal respiratory response of leaves of different leaf age on the main shoot of single-stemmed potato plants. Respiration measurements were conducted on the terminal leaflets of the seventh and the thirteenth leaf of six randomly selected potato plants in Expt. 4. The measurements were made at intervals of two hours from 7.00 am to 7.00 pm then at 11.00 pm and at 1.00 am on two different days (39 and 43 DAP) during the high-temperature period. Sunset during the data collection period was between 5.21 to 5.30 pm. Therefore, measurements that were conducted at 7.00 pm were made when the potato plants had been in the dark for about one and a half hours. Measurements that were conducted from 7.00 pm to 1.00 am were referred to as the night-time and those from 7.00 am to 10.00 am as the morning. Measurements were made on two treatments: the control (22 °C) and the 30 °C treatment. The leaves were tagged before the high temperatures were applied.

7.4 Data analysis

The high temperature response of the leaf photosynthetic rates, chlorophyll fluorescence parameters and respiration rates were subjected to one-way analysis of variance (ANOVA) as described in Chapter 4 Section 4.5 except for the analysis of the interaction between the leaf position and temperature which was subjected to a two-way analysis of variance. In each
case, the significance of differences between means were tested using Least Significant Difference (LSD) and reported at 5 % level of probability unless otherwise specified.

7.5 Results

7.5.1 The impact of an episode of high temperature on the photosynthetic performance of the terminal leaflet of the seventh leaf

The high temperature improved the photosynthetic rates per unit area of the terminal leaflet of the seventh leaf during the high temperature period in both experiments and after the end of the high temperature period in Expt. 2 (Fig. 7.2). In Expt. 2, the photosynthetic rates of the terminal leaflets of the seventh leaf were 14 to 19 % high in the high temperature treatment relative to the control both during and after the high temperature period except on day 44 and 52 after planting in which the rates were same as in the control. In Expt. 4, the photosynthetic rates per unit area of the terminal leaflet of the seventh leaf was 10-17 % higher in the high temperature treatment than in the control on approximately half the occasions when measured during the high temperature period. The photosynthetic rates on the terminal leaflet of the seventh leaf in the high temperature treatment were the same as the control after the plants were transferred back to cooler conditions.
Figure 7.2: Photosynthetic rates per unit area of the terminal leaflet of the seventh leaf of potato plants as influenced by 9-day episode of high temperatures applied shortly after tuber initiation. The data were collected before the high temperatures were applied, during the high-temperature period and after the end of the high temperatures. Bars are LSD at 5 % level of probability. NS = Not significant.

7.5.2 The influence an episode of high temperature on the leaf chlorophyll fluorescence of the terminal leaflet of the seventh leaf

Chlorophyll fluorescence parameters of the terminal leaflet of the seventh leaf measured during the high-temperature period in Expt. 2 were influenced differently by the high temperatures (Fig. 7.3). The maximum quantum efficiency of photosystem II (Fv/Fm) per unit area of the terminal leaflet of the seventh leaf was generally unaffected by temperature except for one occasion when the 30 °C treatment was higher than the control (45 DAP) and
one when it was lower (48 DAP). Similarly, the maximum (Fm) and the variable (Fv) fluorescence were also unaltered by the higher temperatures except for one occasion when they were lower than the control (44 DAP) and one when they higher (46 DAP). The minimum fluorescence (Fo) of the plants exposed to high temperature was lower than the control at the beginning of the high temperature period (45 and 46 DAP) but was higher with longer exposure to the high temperatures. Lack of a significant negative impact of the high temperatures on the maximum quantum efficiency of photosystem II (Fv/Fm) is consistent with improvement on the photosynthetic performance of the terminal leaflet of the seventh leaf in 7.5.2 above.
Figure 7.3: The leaf chlorophyll fluorescence parameters of the terminal leaflet of the seventh leaf as influenced by a 9-day episode of the high temperatures. The data were measured during the high-temperature period in Expt. 2: (A) Maximum efficiency of PSII (Fv/Fm); (B) Minimum fluorescence (Fo); (C) Variable fluorescence (Fv) and (D) Maximum fluorescence (Fm). Bars are LSD at 5% level of probability. NS = Not significant.
7.5.3 The influence of the age of the leaf on the photosynthetic performance of potato leaves

Photosynthetic rates per unit leaf area of the terminal leaflets differed among leaves at different positions (Fig. 7.4). The newly expanded leaves had higher photosynthetic rates per unit area than the older leaves. Leaves at the fifth position had the highest photosynthetic performance. Those at the third and the seventh position had similar performance while leaves at the eleventh position had the lowest photosynthetic rates of the terminal leaflets.

![Expt. 2 Before high temperatures were applied](image)

*Figure 7.4: Photosynthetic performance of the terminal leaflets of potato leaves of different ages on the main shoot of plants grown at 22 °C. The data were collected on three different days (on day 18, 19 and 21 after planting) before the high temperatures were applied in Expt. 2. Bars are LSD at 5% level of probability. Leaves are numbered from the apex of the plant.*

7.5.4 The influence of an episode of high temperatures on the photosynthetic performance of potato leaves of different ages

The high temperatures tended to reduce the photosynthetic rates of the older leaves more than of the younger leaves (Fig. 7.5; Fig. 7.6) although this was not consistent over time. In Expt. 2, there was lower photosynthesis in the terminal leaflets of leaves at the seventh, eleventh and fifteenth position in the 30 °C treatment than in the control on the second day.
(45 DAP) of the high temperature period (Fig. 7.5). However, photosynthetic performance of the older leaves in the 30 °C treatment was the same or higher than in the control by day 6 of the high temperatures. In Expt. 4, the terminal leaflet of the fifteenth leaf had less photosynthesis towards the end of the high temperature period (six and nine days after the start of the high temperature episode) but there was higher photosynthetic rate in the terminal leaflet of the eleventh leaf on day 9 of the high temperatures (Fig. 7.6).
Figure 7.5: Photosynthetic rate per unit leaf area of the terminal leaflets of potato leaves of different ages as influenced by a 9-day episode of high temperatures. The data were collected on three different days (on day 45, 47 and 49 after planting) during the high-temperature period of Expt. 2. Day 45 after planting was day the 2nd day of the high temperature. Bars are LSD at 5 % level of probability. Asterisk (*) shows significant means. Leaves are numbered from the apex of the plant.
Figure 7.6: Photosynthetic rate per unit leaf area of the terminal leaflets of potato leaves of different ages as influenced by a 9-day episode of high temperatures. The data were collected on three different days (on day 41, 44 and 47 after planting) during the high-temperature period of Expt. 4. Day 41 after planting was the 3rd day of the high temperature. Bars are LSD at 5% level of probability. Asterisk (*) shows significant means. Leaves are numbered from the apex of the plant.
7.5.5 The effect of an episode of high temperature on the diurnal respiration of potato leaves of different ages

The terminal leaflets of both the seventh and the thirteenth leaf had similar diurnal respiratory rates per unit leaf area whether plants were grown at 22 or 30 °C (Fig. 7.7). However, except for the night-time (7.00 pm to 1.00 am) dark respiration of the terminal leaflet of the seventh and thirteenth leaf on day 39 after planting, the terminal leaflets of both leaves in the high temperature treatment had higher morning (7.00 am to 10.00 am) on 39 and 43 DAP and night-time (43 DAP) dark respiration rates than the control (Fig. 7.8). None of the treatments had any significant differences during the midday to evening period.

The morning dark respiration rates of the terminal leaflets of plants in 30 °C on 39 DAP was between 7.2 and 8.9 µmol CO₂ m⁻² s⁻¹ (seventh leaf) and; 4.9 and 8.2 µmol CO₂ m⁻² s⁻¹ (thirteenth leaf). The night-time rates were about the same (6.0 µmol CO₂ m⁻² s⁻¹) for both leaves. In the control, the night-time and morning respiratory rates in the terminal leaflets of both leaves were between 6.0 and 4.0 µmol CO₂ m⁻² s⁻¹.

The data are presented twice to emphasis two points: the diurnal respiratory performance of leaves of different ages of potato plants grown at two different temperatures (Fig. 7.7) and how the two temperature conditions influenced the diurnal respiratory performance of the two leaves (Fig. 7.8).
Figure 7.7: The diurnal respiratory rates per unit leaf area of the terminal leaflet of leaves of different ages of potato plants grown at 22 °C and 30 °C as influenced by leaf position. The data were collected on two different days (on 39 and 43 DAP) during the high-temperature period in Expt. 4. Bars are LSD at 5 % level of probability. NS = Not significant. Leaves are numbered from the apex of the plant.
Figure 7.8: The diurnal respiration rates per unit area of the terminal leaflets of leaves of different ages potato plants as influenced by 30 °C. The data were collected on two different days (on 39 and 43 DAP) during the high-temperature period. Bars are LSD at 5% level of probability. NS = Not significant. Leaves are numbered from the apex of the plant.
7.5.6 The photosynthesis at cooler conditions of a leaf that emerged during episode of the high temperatures

The terminal leaflets of the third leaf that emerged during the 9-day episode of 26 or 30 °C had higher photosynthetic rates per unit leaf area than the control. Three days after the end of the 9-day episode of high temperature in Expt. 2 (56 DAP), the terminal leaflet of the leaf that emerged during high temperatures had 26 % (30 °C) and 15 % (26 °C) higher photosynthetic rates per unit leaf area than the 19.6 µmol CO₂ m⁻² s⁻¹ in the control (p = 0.05). Two weeks later (70 DAP), the photosynthetic rate per unit area of the terminal leaflet of the third leaf that emerged at 30 °C was still higher (27 %) than the 8.9 µmol CO₂ m⁻² s⁻¹ in the control (p = 0.05).

Figure 7.9: Photosynthetic rate per unit leaf area at 22 °C of the terminal leaflet of the third leaf that emerged during the 9-day episode of 26 or 30 °C in Expt. 2. The data were collected at three different days after the end of the high-temperature period (on day 56, 63 and 70 after planting). Bars are LSD at 5 % level of probability.
7.6 Discussion

Based on the photosynthetic and respiratory performance of the potato plants exposed to the 9-day episode of high temperature applied shortly after tuber initiation, there were four key findings. (1) The 9-day episode of the high temperatures tended to increase the net photosynthetic rate per unit area of the terminal leaflet of the seventh leaf during the high temperature period and in Expt. 2, the increase persisted when plants were grown back at cooler base temperatures; (2) In contrast, the 9-day episode tended to reduce the net photosynthetic rates in the terminal leaflets of the older leaves (leaves at the leaf position below the seventh leaf) although this was not consistent; (3) Leaves that emerged during the high temperature period had higher photosynthetic rates than the control plants when plants were grown back at cooler temperatures and (4) The high temperatures increased the morning (7.00 am to 10.00 am) and night-time (7.00 pm to 1.00 am) dark respiration rates.

An increase in net photosynthesis of a single leaf under high temperature is consistent with the results of Wolf et al. (1990b), Basu and Minhas (1991) and Lafta and Lorenzen (1995). However, the long term photosynthetic performance of a leaf level before, during and after an episode of high temperature is new. Also new is the photosynthetic performance at cooler conditions of leaves that emerged during the high temperature period, the influence of the high temperatures on the photosynthetic performance of potato leaves at different leaf position on the main stem and the influence of the high temperatures on the diurnal respiration of leaves of different age. These are important components of how an episode of high temperatures alters the total plant carbon production and hence the available carbon for tuber growth.

The photosynthetic response of the potato plants cv. Royal Blue to the 9-day episode of the high temperatures was dependent on leaf age. There was an increased net photosynthetic rate on the terminal leaflet of the seventh leaf during the high temperature period and after plants were grown at cooler conditions following the episode of the high temperatures (Fig. 7.2). Further, even the terminal leaflet of the leaf that emerged during the high temperature period also had higher photosynthetic rates than the control (Fig. 7.9). This is also consistent with the chlorophyll fluorescence data that showed that unaffected maximum quantum efficiency of photosystem II (Fv/Fm) of the terminal leaflet of the seventh leaf (Fig. 7.3). However, immediately plants were exposed to the high temperatures, the older leaves

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showed a reduction in the photosynthetic rates although this was not consistent over time. The negative impacts of the high temperatures on the photosynthetic efficiency of older leaves means that it is erroneous to use measurements from a single leaf to make inferences about whole plant photosynthetic responses in potato plants. This may explain the findings of Lafta and Lorenzen (1995) who found increased leaf level photosynthetic rates but tuber dry matter were still reduced to a similar extent as those of Timlin et al. (2006) who related the reduction in tuber growth to reduced canopy photosynthesis at high temperatures. However, neither studies considered the important impact of a change in plant leaf area under high temperature. This is an important finding because there are even studies in which varietal resistance to high temperatures in potato plants has been evaluated purely on single leaf photosynthetic responses without the information on the whole plant carbon production and tuber growth (Wolf et al., 1990b). Moreover, the impact of the high temperatures on the leaf level photosynthetic performance of potato leaves were conflicting in previous studies (Wolf et al., 1990b; Hancock et al., 2014). The conflicting results could be partly attributed to measurements being made on leaves of different age without consideration for the contribution of leaf age to the photosynthetic response of potato plants exposed to high temperatures (Dwelle et al., 1983; Reynolds et al., 1990; Prange et al., 1990). In the present study, the response of photosynthesis and respiration varied among young, mature and old potato leaves under high temperatures.

In this study, the 9-day episode of high temperatures impaired the whole plant carbon gain hence the available carbon for plant and tuber growth (Chapter 5 Section 5.5 and Chapter 6 Section 6.5). The potato plants used in these experiments were single stemmed comprising only the main shoot. Leaves on the main shoot were mostly the fully expanded and mature leaves. Thus, the main shoot was the main photosynthetic surface and the source of sucrose export to the rest of the plant including the tubers. However, not only did the high temperature treatments reduce photosynthesis in the older leaves (Fig. 7.5; Fig. 7.6), they also reduced leaf area on the main shoots both at the end of the high temperature period and at the final sampling while the lateral shoots grew rapidly after the end of the high temperature period relative to the control (Chapter 5 Section 5.5.8). Thus, there is a combined impact of a reduced leaf area and reduced net photosynthesis per unit leaf area. In addition to inhibition of photosynthesis, the high temperatures increased the morning (7.00 am to
(10.00 am) and night-time (7.00 pm to 1.00 am) respiration rates; at periods during the diurnal cycle when the carbon production from photosynthesis was limited or zero (Dwelle et al., 1983; Timlin et al., 2006). Thus, understanding impact of high temperature on whole plant carbon production need considerations on the whole-diurnal cycle. Measurements targeted only at particular periods of the day such as mid-day might not capture the changes in the dynamics that are influenced by high temperatures such as increased respiration rates only at certain times.

The proportion of leaves on the lateral shoots compared to the main shoot was more in the high temperature treatments than in the control (Chapter 5 Section 5.5.8). However, the young unfolded leaves are photosynthetically inefficient and need more carbon to attain full expansion (Smillie, 1962; Geronimo and Beevers, 1964; Hardwick et al., 1968; Weerakoon et al., 2000; Amthor, 2012). Therefore, it is likely that net carbon production at the whole plant level was depressed even further in the high temperature treatments relative to the control. However, further examination of the contribution of the lateral leaf growth to whole plant photosynthetic performance of potato plants is needed. This is because tuber dry matter were reduced in Expt. 1 by high temperature (Chapter 5 Section 5.5.2) even though the growth of the lateral shoots compensated for decreased main shoot leaf area to maintain whole plant leaf area (Chapter 5 Section 5.5.8.2).

The photosynthetic and respiratory responses of the potato plants exposed to high temperature in the present study add significantly to our understanding of the whole carbon balance. Canopy photosynthesis is influenced by the photosynthetic rates of individual leaves and the available leaf area (Peng and Krieg, 1991; Fleisher et al., 2006). Previous studies have illustrated a reduction in canopy photosynthesis in potato plants grown at high temperatures (Fleisher et al., 2006; Timlin et al., 2006) but the contribution of the individual leaves to the whole canopy photosynthesis was not defined.

### 7.7 Conclusions

a. The 9-day episode of the high temperatures reduced photosynthesis in older leaves and increased respiration rates during hours of the day when photosynthesis contributed less to the whole plant carbon gain.
b. By contrast, with the direct effect of the 9-day episode of the high temperatures, the photosynthetic performance was improved at cooler conditions in the leaves that emerged during the high temperature period.

c. Further study is needed to understand the diurnal photosynthetic and respiration response of the potato plants at high temperatures and how this might be influenced by leaf age, repeated episodes, cooler conditions following an episode of the high temperatures, and the contribution of main shoot and lateral shoots to the whole plant carbon production.
CHAPTER EIGHT

Influence of an episode of high temperatures before or after tuber initiation on starch and sugar distribution in potato plants
Influence of an episode of high temperatures before or after tuber initiation on starch and sugar distribution in potato plants

Abstract

Little is known about how episodes of high temperatures might influence the distribution of sucrose and starch in potato plants. It was hypothesized that an episode of high temperatures before tuber initiation would have less effect on starch and sugar distribution in potato plants than an episode after initiation. In this chapter, the impact of a 9-day episode of high temperatures applied before (Expt. 1) or after (Expt. 2) tuber initiation on the distribution of sucrose and starch in potato plants (cv. Royal Blue) was investigated. In both experiments, potato plants were grown at 22 °C. Then, at 35 days after planting (DAP) (Expt. 1) or 43 DAP (Expt. 2), the plants were transferred to high temperature treatments at either 26 °C or 30 °C and grown for nine days before being returned to 22 °C for 26 days. At the end of the experiment (and at the end of the high temperature episode in Expt. 2) the plants were harvested and divided into different organ types. The samples were then dried and ground. Then analysed through UHPLC (sugars) or calorimetrically (starch). Starch and sucrose content in the tubers was reduced to a similar extent whether the high temperatures were applied before or after tuber initiation. There was no significant accumulation of starch or sucrose in the main stems of the high temperature treatments relative to the control either at the end of the high temperature episode (Expt. 2) or at the final sampling (Expt. 1 and 2). High amounts of sucrose accumulated in the leaves of the high temperature treatments in Expt. 1. In contrast, the main shoot leaves of the high temperature treatments in Expt. 2 had lower sucrose and starch content than in the control. The reduction in starch and sucrose content in the tubers is inconsistent with the proposed roles of reduced starch synthase and increased gibberellic acid in the carbon partitioning of potato under high temperatures. In this study, the reduced starch content in the tubers was more consistent with insufficient whole plant carbon occasioned by the reduced leaf area. In Expt. 1, less number of cells in each tuber might have reduced the rate of starch deposition hence the accumulation of more starch and sucrose in the leaves. However, this need further investigations.
8.1 Introduction

Little is known about the impact of episodes of high temperatures on the distribution of sugars and starch within the potato plant. In Chapter 5, tuber dry matter was reduced to a similar extent when potato plants (cv. Royal Blue) were exposed to a 9-day episode of 26 and 30 °C temperatures before or after tuber initiation. The tuber dry matter in Chapter 5 was reduced due to less whole plant carbon occasioned by the reduction in leaf area but continued shoot growth.

In previous studies, the reduction in tuber dry matter in potato plants grown at high temperatures has been linked to the inhibition of starch synthase or the production of higher levels of gibberellin. Starch synthase controls the conversion of sucrose into starch in the potato tubers hence starch deposition and tuber growth (Hawker et al., 1979; Mares and Marschner, 1980; Engels and Marschner, 1986). The inhibition of starch synthase has been shown to cause a reduction in the amount of starch deposited into the tubers hence less tuber dry matter (Mares and Marschner, 1980; Krauss and Marschner, 1984; Nielsen et al., 1994; Miyazawa et al., 1999). Gibberellins, on the other hand, not only inhibit starch synthase, but also promote shoot growth that diverts the carbon away from the tubers (Lovell and Booth, 1967; Booth and Lovell, 1972; Mares et al., 1981). Menzel (1983) found high amounts of gibberellins and reduced tuber dry matter in potato cv. Sebago plants grown at 35/30 °C than at 20/15 °C D/N temperatures. Thus, the association of gibberellic acid with tuber reduction in potato plants grown at high temperatures (Menzel, 1980, 1983, 1985).

Inhibition of starch deposition in the tubers is usually accompanied by lower starch content and high levels of sucrose in the tubers (Mares and Marschner, 1980; Engels and Marschner, 1986; Wolf et al., 1990a, 1991; Geigenberger et al., 1998). In the shoot, more sucrose or starch may accumulate in the leaves as a result of the impaired starch deposition in the tubers (Basu and Minhas, 1991; Lafta and Lorenzen, 1995). However, these understandings are based on experiments in which plants or the tubers were under persistent influence of high the temperatures throughout the period of the experiment (Krauss, 1981; Menzel, 1985; Lafta and Lorenzen, 1995; Timlin et al., 2006). Further, only tuberized potato plants were used in these experiments. Little is known about the distribution of carbon within potato plants which might be exposed to an episode of high temperatures before initiation and whether the responses might be explained based on starch synthase inhibition or higher gibberellic acid.
It was hypothesized that an episode of high temperatures before tuber initiation would have less effect on starch and sugar distribution in potato plants than an episode after initiation. In this chapter, the influence of nine days of high temperatures applied shortly before or after tuber initiation on the distribution of sugars and starch within the potato plants was studied.

8.2 Materials and methods
Analysis of starch and water-soluble carbohydrates of potato plants was conducted at a laboratory located at the Centre for Biosecurity and Food Security, Murdoch University, Perth (32° 4' S; 115° 50' E) Western Australia. The water-soluble carbohydrates were estimated through ultra-high-performance liquid chromatography (UHPLC) and starch estimated colorimetrically using the phenol-sulphuric method.

8.2.1 Chemicals
Standards and reagents used in this analysis were obtained from Sigma, Australia. These were glucose (99.5 % GC), fructose (99 %), sucrose (99.5 % GC), maltose monohydrate, starch (starch from corn), acetonitrile HPLC grade (99.9 %), ammonia solution (35 %), methanol HPLC grade (99.9 %), phenol (ACS reagent, 99 %), hydrochloric acid (ACS reagent, 37 %), and sulphuric acid (Aldrich, 99.9 %). The laboratory was supplied with Double Distilled Water (DD-water).

8.2.2 Plant samples
The potato plant samples for the analysis were obtained from Expt. 1 and 2. These experiments investigated the impact of a 9-day episode of 26 °C and 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation on potato plants. The potato plants were grown, managed and sampled as described in Chapter 5. Stolons, tubers, main stems, lateral stems, main shoot petioles, main shoot leaves and lateral shoot leaves were sampled and processed for the analysis as described in Chapter 4. The samples were collected at the final sampling (Expt. 1 and 2) as well as at the end of the high temperature period in Expt. 2. Water-soluble carbohydrates were analysed in the tubers from all treatments: the control (22 °C) and the two high-temperature treatments (26 °C and 30 °C). The water-soluble carbohydrates from other plant parts were only analysed for the control and the 30 °C treatments. Starch
was analysed only on the tuber, the main stem and the main shoot leaf samples for the same
treatments used in the analysis for the water-soluble carbohydrates.

8.2.3 Estimation of the water-soluble carbohydrates

A total of 240 samples: 60 (Expt. 1) and 180 (Expt. 2) were analysed for the water-soluble carbohydrates. Each sample consisted of either four (Expt. 1) or 6 (Expt. 2) biological replicates.

8.2.3.1 Standard curves for the water-soluble carbohydrates

Four different sugar standards were prepared. One (1) mg ml\(^{-1}\) stock solutions for fructose, glucose and maltose were prepared by dissolving 25 mg of each dry sugar in 25 ml of 50 % aqueous methanol in three separate 25-ml volumetric flasks. A stock solution of 10 mg ml\(^{-1}\) sucrose was prepared by dissolving 500 mg of the dry sugar in 50 ml of 50 % aqueous methanol in a 50-ml volumetric flask. The standards were held in the volumetric flasks with plastic stoppers and kept in the dark at 4 °C.

On the day prior to the analysis of samples, dilution series of the standards were made in 50 % aqueous methanol. The concentrations used in this study were 0.1, 0.3, 0.5, 0.7 and 1.0 mg ml\(^{-1}\) for each of fructose, glucose, maltose and sucrose. Two additional concentrations, 1.5 and 2.0 mg ml\(^{-1}\), were included for the sucrose standard. The dilutions were carried in 50-ml plastic vials with screw-tops. The diluted standards in the plastic vials were also held in the dark at 4 °C.

8.2.3.2 Extraction of the water-soluble carbohydrates

The water-soluble carbohydrates were extracted from the plant samples using a method adapted from Duarte-Delgado et al. (2015). In this method, 500 mg of the dry-powder of the plant material was weighed into separate 50-ml screw-top glass vials using an analytical weighing balance (Sartorius Model No. R200D). Then, 8 ml of 50 % aqueous methanol was added to the sample. One 50-ml vial with only 8 ml of the 50 % aqueous methanol was also included as a blank sample. The vials were transferred to a digital ultrasonic cleaner (Model No. PS-20A) and the mixtures cleaned for 5 mins at 25 °C. The vials were then transferred to an 80 °C water bath and extracted for 60 mins at 17 revolutions per minute (rpm). The extracts were cooled at room temperature (22 °C) for 30 mins then transferred into 2-ml centrifuge vials and centrifuged for 30 mins at 6000 rpm. The clear extracts were filtered through 0.22
µm filters into 2-ml HPLC vials using 3-ml syringes. The filtered extracts were kept at in the dark at 4 °C until they were analysed.

The extraction of the water-soluble carbohydrates from the 240 samples took 6 days. To deal with any drift in the extraction method that might arise because of the different extraction periods, the samples were completely randomized. Further, one tuber sample was used as a quality control (QC) sample. This sample was included in each extraction batch. The samples extracted for the water-soluble carbohydrates had no technical replicates; the extraction was conducted only on the biological replicates.

8.2.3.3 Separation and quantification of the water-soluble carbohydrates

A Waters™ Acquity Ultra High-Performance Liquid Chromatography (UHPLC) system equipped with a 20 µL loop and a High-Resolution Mass Detector (Waters™ Q-Tof Premier Micromass Technology) was used to simultaneously separate and analyse the sugars. The system was run at 0.13 ml min⁻¹ using a Shodex NH₂P-40 2B, 4 µM, 2.0 × 50 mm analytical column protected by a Shodex Asahipa NH₂P-50G 2A, 5 µm, 2.0 × 10 mm guard column. The column temperature was 35 °C and the pressure was 250 psi. The sample temperature was 4 °C. An isocratic mobile phase of 75 % aqueous acetonitrile and 0.05 % NH₃ was used. The injection volume was 2.0 µL.

Mass Lynx v4.1 software was used for data acquisition and peak integration. The sugar samples and the standards were randomly injected into the UHPLC system. The column was conditioned first before the sugar samples were injected into the system. A blank sample containing only the DD-water followed by two runs from the 1 mg ml⁻¹ quality control (QC) sucrose standard were used to condition the column. The 1 mg ml⁻¹ QC sucrose was also injected after every 10 samples for a system stability check. This followed an optimization experiment that showed that the system was slightly variable (Appendix B).

The sample run time was 15 mins. This allowed 10 minutes for cleaning the column and 5 mins for injecting the sugar sample. An isocratic solution of 90 % aqueous acetonitrile was injected for 5 mins followed by a 75 % aqueous acetonitrile solution for 5 mins. The sugar sample was injected during the last 5 mins.
8.2.4 Estimation of starch

Starch analysis was conducted only for the tuber, the main stem and the main shoot leaf samples from both experiments (Expt. 1 and 2). The tuber samples were from all the treatments while the main stem and the main shoot leaf samples were only from the control and the 30°C treatment as described for the water-soluble carbohydrates.

8.2.4.1 Glucose standard curve for the estimation of starch

Stock solution containing 1 mg ml⁻¹ glucose standard was prepared by dissolving 50 mg of glucose in 50 ml of 0.7 M HCl in a 50-ml volumetric flask. A glucose dilution series was prepared using 0.7 M HCl to give: 0.02, 0.04, 0.06, 0.08 and 0.1 mg ml⁻¹. The dilutions were made in duplicates one day before the plant samples were analysed and kept in the dark at 4°C.

8.2.4.2 Hydrolysis of the samples

Starch from the potato samples was hydrolysed using a method adapted from Haebel et al. (2008). About 50 mg (tuber) or 200 mg (stem or leaf) sample was mixed with 20 ml of 0.7 M HCl in a 50-ml screw-top glass vial. A blank sample with only 20 ml of 0.7 M HCl and two starch samples containing 20 and 50 mg of the starch standard were also prepared. The mixture was then cleaned in an ultrasonic cleaner for 5 mins. The vial was transferred to a 99 ± 1°C water bath and hydrolysed for 3 hours. The hydrolysate was cooled at room temperature for 30 mins. Two millilitres of the cooled hydrolysate were transferred into a 2-ml centrifuge vial and kept in the dark at 4°C until they were analysed for the total glucose content.

The hydrolysis was conducted over 6 days. During each hydrolysis, three samples: two starch samples of 20 and 50 mg of the starch standard and one from the QC tuber sample (maintained during the analysis of the water-soluble carbohydrates) were included in each batch. These were used to check for drift in the hydrolysis method and adjustments were made accordingly.

8.2.4.3 Determination of the glucose in the hydrolysate

The glucose concentration of the hydrolysate was estimated colorimetrically using UV-VIS Spectrophotometer (Beckman Instruments, Australia Pty Ltd). The method was adapted from Dubois et al. (1956); Albalasmeh et al. (2013). Two millilitres of the hydrolysate were centrifuged for 15 mins at 6000 rpm. One millilitre of the supernatant was pipetted into 50-
ml graduated plastic vial with a screw cap and diluted 50 times by adding 49 ml of DD-water. One millilitre of the diluted hydrolysate was pipetted into 15-ml Pyrex test tube. One millilitre of the glucose standard concentrations was also pipetted into separate 15-ml Pyrex test tubes. Working under the fume hood, 1 ml of 5 % fleshly prepared phenol was added, followed by 5 ml of concentrated sulphuric acid. The test tubes then were vortexed at 2000 rpm for 30 seconds (s) and cooled at room temperature for 30 mins. To the cooled samples, 6 ml of DD-water was added and the samples vortexed for 30 s. The test tubes were left to equilibrate at room temperature for 15 mins. The absorbance of each sample was read at 490 nm.

8.3 Calculations

Only sucrose was detected in the analysis of the water-soluble carbohydrates.

8.3.1 Starch and sucrose concentration

The starch and sucrose concentration in the plant samples was calculated in grams per a hundred grams of the dry matter (g 100 g⁻¹ dry matter) of the analysed plant part as follows:

\[
\text{Starch or sucrose (g 100 g}^{-1}\text{ dry matter of sample)} = \frac{\text{GS} \times V \times \text{DF}}{\text{WS}} \times 100
\]

Where:

\(\text{GS} = \text{Starch or sugar concentration (Absorbance divided by the slope of the curve) (mg ml}^{-1}\)\)

\(V = \text{Volume of the sample (ml)}\)

\(\text{DF} = \text{Dilution factor}\)

\(\text{WS} = \text{Weight of the sample extracted (mg)}\)

8.3.2 Starch and sucrose content

The starch and sucrose content in the plant parts was calculated in grams per plant as follows:

\[
\text{Sucrose or starch content (g plant}^{-1}\) = GSS \times \text{TDM}
\]

Where:
GSS = Starch or sucrose concentration of the plant part (g g\(^{-1}\) dry matter)

TDM = Total dry matter per plant of the plant part (g plant\(^{-1}\))

8.4 Data analysis

Each sample had four (Expt. 1) or six (Expt. 2) biological replicates. The samples analysed for starch also had three technical replicates. The water-soluble carbohydrates were only assessed on the biological replicates because of cost limitation. All the reported data are the means of the biological replicates (sucrose) or of the combined biological and the technical replicates (starch). The analysis of variance (ANOVA) was conducted through the General Linear Model (GLM) procedure of the SAS Software. Significant means were compared using Least Significant Difference (LSD) and reported at 5 % level of probability unless otherwise specified.

8.5 Results

8.5.1 Tuber sucrose concentration and content

At the final sampling of Expt. 1, the concentration and content of sucrose in the tubers was not different among treatments except for plants in the 26 °C treatment which had 12 % lower concentration and 63 % lower content of sucrose than in the control (Fig. 8.1; Fig. 8.2).

In Expt. 2, there was 28 % (26 °C) and 45 % (30 °C) lower sucrose concentration and 92 % (30 °C) lower sucrose content in the tubers of the high temperature treatments than in the control at the end of the high temperature period. At the final sampling, tubers in all treatments were not significantly different in sucrose concentration. The tubers in the 30 °C treatments were 30.7 % lower in sucrose content than the control. However, sucrose content in the tubers in the 30 °C treatment had increased 16-fold in the period between the end of the high temperature and the final sampling. In the control and the 26 °C treatments, the sucrose content in the tubers was only doubled and tripled, respectively, during the same period.

Compared the two experiments at the final sampling, sucrose concentration and content were only reduced relative to the control in the tubers of the 26 °C treatment in Expt. 1. In
Expt. 2, sucrose concentration was not significantly affected among treatments. It was only the sucrose content in the 30 °C treatment that was low relative to the control.

Figure 8.1: Sucrose concentration (g 100 g⁻¹ dry matter) of tubers as influenced by a 9-day episode of 26 °C or 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation. Data at the end of the high temperature period of Expt. 2 (top) and at the final sampling of Expt. 1 and 2 (bottom). Bars are LSD at 5 % level of probability.
Figure 8.2: Sucrose content (g plant\(^{-1}\)) of tubers as influenced by a 9-day episode of 26 °C or 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation. Data at the end of the high temperature period of Expt. 2 (top) and at the final sampling of Expt. 1 and 2 (bottom). Bars are LSD at 5 % level of probability.

### 8.5.2 Tuber starch concentration and content

In Expt. 1, plants in all treatments had the same starch concentration in the tubers at the final sampling but the starch content in the tubers of the high temperature treatments were 26 % (26 °C) and 33 % (30 °C) lower than in the control (Fig. 8.3; Fig. 8.4).

In Expt. 2, there was 7 % (26 °C) and 21 % (30 °C) lower starch concentration and 87 % (30 °C) less starch content in the tubers of plants in the high temperature treatments than in the control at the end of the high temperature period. At the final sampling, starch concentration and content were still less in the tubers of the high temperature treatments relative to the control. The high temperatures had 16 % (26 °C) and 5 % (30 °C) lower starch concentration.
and 29 % (26 °C) and 36 % (30 °C) lower starch content in the tubers than in the control. However, starch concentration in the 30 °C treatment increased by 26 g 100 g⁻¹ of tuber dry matter between the end of the high temperature period and at the final sampling. In the control and the 26 °C treatment, the increase was only by 10.6 and 12.4 g 100 g⁻¹ of tuber dry matter respectively.

Comparing the two experiments at the final sampling, starch concentration was only reduced relative to the control, in the tubers of high the temperature treatments in Expt. 2. However, starch content was lower in the tubers of all the high temperature treatments in both experiments and the reduction was to a similar extent.

![Figure 8.3: Starch concentration (g 100 g⁻¹ dry matter) of tubers as influenced by a 9-day episode of 26 °C or 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation. Data at the end of the high temperature period of Expt. 2 (top) and at the final sampling of Expt. 1 and 2 (bottom). Bars are LSD at 5 % level of probability.](image)
In Expt. 1, the main shoot leaves of the 30 °C treatment were 65 % higher in sucrose concentration and 47 % higher in sucrose content relative to the control at the final sampling. However, plants in all treatments were statistically the same in both the concentration and content of sucrose in the main stems (Fig. 8.5; Fig. 8.6).

In Expt. 2, plants in all treatments were statistically the same in the sucrose concentration and content in both the main shoot leaves and the main stems at the end of the high temperature period.
temperature period. At the final sampling, sucrose concentration in the main shoot leaves was not significantly different among treatments. However, the sucrose content in the main shoot leaves was 55% lower in the 30 °C treatment than in the control. The main stems of plants in the 30 °C treatment was 57% lower in concentration and 55% lower in content of sucrose than in the control.

Overall, relative to the control, more sucrose accumulated in the main shoot leaves of the high temperature treatments in Expt. 1. The main stems were not significantly different among treatments. In Expt. 2, there was less sucrose content in the main shoot leaves of the high temperature treatments. Further, the main stems had both lower sucrose concentration and content.
Figure 8.5: Sucrose concentration (g 100 g⁻¹ dry matter) of the main shoot leaves and the main stems as influenced by a 9-day episode of 26 °C or 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation. Data at the end of the high temperature period of Expt. 2 (top) and at the final sampling of Expt. 1 and 2 (bottom). Bars are LSD at 5 % level of probability.
Figure 8.6: Sucrose content (g plant\(^{-1}\)) of the main shoot leaves and the main stems as influenced by a 9-day episode of 26 °C or 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation. Data at the end of the high temperature period of Expt. 2 (top) and at the final sampling of Expt. 1 and 2 (bottom). Bars are LSD at 5 % level of probability.

### 8.5.4 Starch concentration and content in the main shoot leaves and stems

In Expt. 1, there was 13.4 % higher starch concentration but 29.4 % lower starch content in the main shoot leaves of plants in the 30 °C treatment than in the control at the final sampling (Fig. 8.7; Fig. 8.8). Starch concentration in the main stems was not significantly different among treatments but the main stems of plants in the 30 °C treatment had 12.4 % lower starch content than in the control.
In Expt. 2, plants in all treatments were not significantly different in either starch concentration or content of the main shoot leaves or the main stems at the end of the high temperature period. At the final sampling, plants in the 30 °C treatment had 5 % higher starch concentration and 38 % less starch content in the main shoot leaves than in the control. In the main stems, there was 5 % lower starch concentration in the 30 °C treatment than in the control. However, starch content in the main stems was not significantly different among treatments.

**Figure 8.7:** Starch concentration (g 100 g⁻¹ dry matter) of the main shoot leaves and the main stems as influenced by a 9-day episode of 26 °C or 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation. Data at the end of the high temperature period of Expt. 2 (top) and at the final sampling of Expt. 1 and 2 (bottom). Bars are LSD at 5 % level of probability.
Figure 8.8: Starch content (g plant⁻¹) of the main shoot leaves and the main stems as influenced by a 9-day episode of 26 °C or 30 °C applied shortly before (Expt. 1) or after (Expt. 2) tuber initiation. Data at the end of the high temperature period of Expt. 2 (top) and at the final sampling of Expt. 1 and 2 (bottom). Bars are LSD at 5 % level of probability.

8.5.5 Sucrose concentration and content in other plant parts

In Expt. 1, although the significance was only marginal (p = 0.07), there was substantially higher (35 %) sucrose concentration and content (49 %) in leaves of the lateral shoots of plants in the 30 °C treatment than in the control at the final sampling (Table 8.1). Further, there was substantially lower (61 %) sucrose content in the stolons of the 30 °C treatment than in the control at the final sampling. However, the sucrose concentration and content in the lateral
stems and the main shoot petioles, and the sucrose concentration in the stolons was not significantly different in all treatments.

In Expt. 2, treatments had no significant differences in the sucrose concentration and content in the stolons, lateral stems, leaves on the lateral shoots, and the main shoot petioles at the end of the high temperature period except for the sucrose concentration in the lateral stems which was 44% high than in the 30 °C treatment than in the control (Table 8.2). At the final sampling, treatments still had no significant differences in the sucrose concentration and content in the stolons, lateral stems, leaves on the lateral shoots and the main shoot petioles except for the sucrose concentration in the stolons which was 38% less in the 30 °C treatment than in the control (Table 8.3).
Table 8.1: Sucrose concentration (mg g⁻¹ dry matter) and content (mg plant⁻¹) at the final sampling of Expt. 1 in the stolons, lateral stems, leaves of the lateral shoots and main shoot petioles of potato plants exposed to a 9-day episode of 30 °C shortly before tuber initiation.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Sucrose concentration (mg g⁻¹ dry matter)</th>
<th>Sucrose content (mg plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stolons</td>
<td>Lateral stems</td>
</tr>
<tr>
<td>22 °C</td>
<td>690</td>
<td>400</td>
</tr>
<tr>
<td>30 °C</td>
<td>380</td>
<td>700</td>
</tr>
<tr>
<td>LSD 5 %</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant; Means with the different letters within a column are significantly different at the level of probability showed in bracket.

Table 8.2: Sucrose concentration (mg g⁻¹ dry matter) and content (mg plant⁻¹) at the end of the high temperature period of Expt. 2 in the stolons, lateral stems, leaves of the lateral shoots and main shoot petioles of potato plants exposed to a 9-day episode of 30 °C shortly after tuber initiation.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Sucrose concentration (mg g⁻¹ dry matter)</th>
<th>Sucrose content (mg plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stolons</td>
<td>Lateral stems</td>
</tr>
<tr>
<td>22 °C</td>
<td>145</td>
<td>32a</td>
</tr>
<tr>
<td>30 °C</td>
<td>100</td>
<td>57b</td>
</tr>
<tr>
<td>LSD 5 %</td>
<td>NS</td>
<td>11 (5 %)</td>
</tr>
</tbody>
</table>

NS = Not significant; Means with the different letters within a column are significantly different at the level of probability showed in bracket.
Table 8.3: Sucrose concentration (mg g\(^{-1}\) dry matter) and content (mg plant\(^{-1}\)) at the final sampling of Expt. 2 in the stolons, lateral stems, leaves of the lateral shoots and main shoot petioles of potato plants exposed to a 9-day episode of 30 °C shortly after tuber initiation.

<table>
<thead>
<tr>
<th>Temp</th>
<th>Sucrose concentration (mg g(^{-1}) dry matter)</th>
<th>Sucrose content (mg plant(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stolons</td>
<td>Lateral stems</td>
</tr>
<tr>
<td>22 °C</td>
<td>210a</td>
<td>67</td>
</tr>
<tr>
<td>30 °C</td>
<td>130b</td>
<td>43</td>
</tr>
<tr>
<td>LSD 5 %</td>
<td>70 (5 %)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant; Means with the different letters within a column are significantly different at the level of probability showed in bracket.
8.6 Discussion

Based on the analysis of sucrose and starch concentration and content of plant samples of potato plants exposed to the 9-day episode of high temperatures shortly before (Expt. 1) or after (Expt. 2) tuber initiation, there were four main findings: (1) Starch content in the tubers was reduced to a similar extent whether the high temperatures were applied before or after tuber initiation; (2) The concentration and content of sucrose in the tubers was either unaltered or suppressed but not increased in plants that were exposed to the 9-day episode of the high temperatures either before or after tuber initiation; (3) There was higher sucrose concentration and content in the leaves of plants that were exposed to the 9-day episode of the high temperature before tuber initiation. In contrast, sucrose concentration and content was not different at the end of the high temperature but the content was lower at the final sampling in the main shoot leaves of plants that were subjected to the 9-day episode of the high temperatures after tuber initiation and (4) The concentration and content of starch or sucrose in the main stems was either unaltered or suppressed but not increased in the high temperature treatments either before or after tuber initiation. These results have the following implications.

The reduction in starch content in the tubers at the end of the high temperature period and at the final sampling in these experiments are not attributable to the inhibition of starch synthase. Starch synthase is responsible for the conversion of sucrose into starch in potato tubers hence tuber growth (Hawker et al., 1979; Müller-Röber et al., 1992; Geigenberger et al., 2004; Baroja-Fernández et al., 2009). In previous studies, temperatures above 25 °C have been shown to inhibit starch synthase activity in both grains and tubers (Krauss and Marschner, 1984; Keeling et al., 1993; Jenner, 1994; Keeling et al., 1994). However, while the inhibition of starch synthase has been shown to reduce the amount of starch in the tubers, this inhibition also caused the sucrose in the tuber to increase (Mares and Marschner, 1980; Krauss and Marschner, 1984; Geigenberger et al., 1998). This was not seen in the present study; sucrose concentration content or concentration was either unchanged or reduced. Thus, the information on the change in carbohydrate composition of the tubers supports the idea proposed in earlier chapters that the reduction in tuber dry matter was not primarily due to the inhibition of starch synthase.
After high temperature exposure, the total available photosynthate supply was reduced due to the small leaf area (Chapter 5 Section 5.8.2) and less net photosynthesis per unit area (Chapter 7 Section 7.5.4; 7.5.5). However, shoot growth after the end of the high temperature period was the same in the control and in the high temperature treatments (Chapter 5 Section 5.5.3). This means that less carbon was available for tuber growth. This is supported by the results of the sugar and starch analysis reported in this chapter. In Expt. 2, there was less sucrose and starch in the tubers of the high temperature treatment at the end of the high temperature period (Fig. 8.1; Fig. 8.2; Fig. 8.3; Fig. 8.4). Further, neither sucrose nor starch accumulated in the main shoot leaves or in the main stems at the end of the high temperature period (Fig. 8.5; Fig. 8.6; Fig. 8.7; Fig. 8.8). This is consistent with the idea that there was insufficient whole plant carbon available for growth and that this was most likely responsible for the reduced tuber growth. At the final sampling of Expt. 2, there was still evidence of insufficient whole plant carbon production which might have limited tuber growth. Relative to the control, tubers in the high temperature treatments still had less starch and sucrose. The concentration and content of starch or sucrose in the main shoot leaves and stems was also unchanged or suppressed at the final sampling of Expt. 2 except for the slight increase in the starch concentration in the main shoot leaves (Fig. 8.5; Fig. 8.6; Fig. 8.7; Fig. 8.8).

At the final sampling of Expt. 1, the data also indicated scarcity in the amount of carbon partitioned to the tubers. The tubers in the high temperature treatments had less sucrose and starch content relative to the control. Further, stolons in the high temperature treatment had lower sucrose content relative to the control. However, the main shoot leaves of the high temperature treatment had high sucrose and starch concentration and content relative to the control except for the starch content. In addition, sucrose concentration in the leaves of the lateral shoots was increased while no sugar or starch accumulated in the main stems of the high temperature treatments. This not only means that the tubers in the high temperature treatments had insufficient carbon for growth, accumulation of sucrose in the leaves also suggests that there were restrictions in the rate of starch deposition into the tubers. This was perplexing because the feedback mechanism occurred in plants that had no visible tubers during the high temperature period. The accumulation of sucrose or starch in the leaves is usually related to tuberized plants under persistent influence of high temperatures (Lorenzen, 1989; Basu and Minhas, 1991; Lorenzen and Ewing, 1992). Nösberger and Humphries (1965)
observed that a reduction in the growth of the tubers increased the amount of sugar and starch deposits in the stems and leaves. This raises questions on what could have been the additional mechanism that led to the reduction in tuber growth especially in plants that had been exposed to the 9-day episode of the high temperatures before tuber initiation.

It is possible that tubers in high temperature treatments of Expt. 1 had fewer cells for starch deposition due to their initiation when the supply of photosynthates was reduced. Studies indicate that tubers with fewer cells expand less rapidly due to the insufficient storage capacity for carbon (Plaisted, 1957; Reeve et al., 1973; Xu et al., 1998b). The total number of cells in each tuber is dependent on the total available photosynthate and tuber growth stage. Chen and Setter (2003, 2012) found that elevated CO₂ not only increased the whole plant dry matter in potato cv. Katahdin plants grown at 700 than at 350 µmol CO₂ mol⁻¹. They also showed that the number of cells in each tuber per plant was 67 % higher relative to the control when the potato plants were exposed to elevated CO₂ for two weeks from the tuber initiation stage. The tubers in plants exposed to elevated CO₂ for two weeks at tuber bulking stage only had 18 % more cells per plant relative to the control (Chen and Setter, 2012). Further, they showed that the total number of cells in the tubers was reduced by 40 % while their average cell volume by 30 % when the plants were heavily shaded (150 µmol m⁻² s⁻¹) at tuber initiation relative to unshaded conditions (600 µmol m⁻² s⁻¹). The shade was imposed for two weeks. Imposing the shade also for two weeks at tuber bulking led to 75 % reduction in the number of cells and 34-55 % less cell volume. The higher reduction in the number of cells in each tuber when the shade was applied at tuber bulking compared to tuber initiation might have been due to the differences in the tuber weights. The total number of cells in each tuber was calculated by multiplying the cell number per gram fresh weight in a sample by whole tuber fresh weight (Chen and Setter, 2012). However, not only are tubers at the initiation stage smaller in volume when compared to tubers at later stages of growth, the tuber cell division also stops after tubers are only 0.8 cm in diameter and only enlargement proceeds until the maturity (Xu et al., 1998b). This means that tubers at the initiation have more cells per volume compared with the tubers at later stages of growth.

In Expt. 1, plants had no visible tubers during the high temperature period unlike in Expt. 2 where the high temperatures were applied after the presence of tubers was confirmed. All tubers in Expt. 1 were initiated after the end of the high temperature period when there was
less whole plant carbon occasioned by the reduced leaf area and less might have been again available for the tubers due the continued shoot growth. Thus, the newly initiated tubers may have had fewer cells and or reduced cell volume which in turn may have contributed to impaired starch deposition hence the feedback mechanism. However, the findings of Chen and Setter (2003, 2012) were based on a control of low irradiance (600 µmol m$^{-2}$ s$^{-1}$) compared to field conditions (1500-2000 µmol m$^{-2}$ s$^{-1}$) and the data were only collected at the end of the experiments. Further investigations are needed on the relationship of the cell number and volume with episodes of high temperature and at the different stage of tuber growth.

8.7 Conclusions
a. A 9-day episode of high temperatures either before or after tuber initiation reduced starch content in the tubers.

b. The reduction in starch content in the tubers was consistent with a mechanism of reduced carbon supply to the tubers occasioned by reduced less leaf area (Chapter 5 Section 5.8.2) combined with reduced net photosynthesis (Chapter 7 Section 7.5.4; 7.5.5) and the continued shoot growth (Chapter 5 Section 5.5.3).

c. Tuber growth rates in plants exposed to an episode of the high temperatures before tuber initiation might have been exacerbated by fewer cells in the tubers. However, this require further investigations.
Influence of auxins on the physiology, growth and tuber yields of potato plants exposed to an episode of high temperature
Influence of auxins on the physiology, growth and tuber yields of potato plants exposed to an episode of high temperature

Abstract
While the literature suggests that gibberellins control the growth responses in potato plants grown at high temperatures, the responses of potato plants exposed to episodes of high temperatures in previous Chapters were more consistent with the known effects of auxins. Consequently, it was hypothesized that exogenously applied auxins would overcome the negative impact of a 9-day episode of high temperature on the growth of potato plants and tubers. Two experiments were conducted: Expt. 4 and 5. Expt. 4 investigated the impact of an auxin (IAA) and auxin inhibitor (TIBA) while Expt. 5 evaluated the influence of four concentrations of IAA (0, 5, 15, 45 µM) on potato plants exposed to a 9-day episode of 30 °C. In both experiments, plants were grown at 22 °C before and after the end of high temperature period. The 30 °C temperature and the PGRs were applied shortly after tuber initiation on the same day. Exogenously applied TIBA had the same impact on plant and tuber growth in potato plants in the control (22 °C) as the 9-day episode of 30 °C on the No-PGRs plants. Plant and tuber growth were similar with or without the application of IAA in plants exposed to the 9-day episode of 30 °C. Both IAA and TIBA increased the net photosynthetic rates in the seventh leaf tagged before the high temperatures were applied and the chlorophyll concentration index in the same seventh leaf and in the third leaf that emerged during the high temperature period. In conclusion, TIBA impaired plants and tuber growth in a similar manner to the 9-day episode of 30 °C but IAA did not overcome the impact of the 9-day episode of 30 °C on plant and tuber growth of potato plants. It is likely that auxin inhibition is partly responsible for the growth responses in potato plants exposed to an episode of high temperatures.

9.1 Introduction
Previous studies concluded that gibberellins stimulate shoot growth which diverts carbon away from tubers in potato plants grown at high temperatures. Menzel (1980) found that potato cv. Sebago grown at 32/18 °C or 32/28 °C day and night (D/N) temperatures responded similarly as those that were treated with gibberellic acid (GA). Menzel (1983) extracted
gibberellins from potato cv. Sebago plants and found higher gibberellin concentrations in the crude extracts from the buds in plants that were grown at 35/30 °C than at 20/15 °C D/N. However, the evidence that links GA to the growth responses of potato plants at high temperatures is still inconsistent and leaves open the involvement of a different mechanism. For example, there are reports that the tuber dry matter is reduced even without improvement on shoot dry matter in potato plants grown at high temperatures (Lafta and Lorenzen, 1995).

Firstly, the question of the production of tubers at high temperatures needs further clarification. In Chapter 5 Section 5.5.7 and Chapter 6 Section 6.5.3, there were fewer tubers per plant only at the end of the high temperature period in the high temperature treatments. At the final sampling, all treatments had the same number of tubers or the high temperature treatments had slightly more number of tubers per plant than the control after plants had been grown back at cooler temperatures for more than 26 days. The literature, however, shows two contrasting findings on the effect of GA on the production of tubers in potato plants. There are some studies that indicate more but small-sized tubers with GA (Kumar and Wareing, 1974; Struik et al., 1989b; Šimko, 1994; Bou-Torrent et al., 2011; Roumeliotis et al., 2012; Herman et al., 2016) while there are other studies that show fewer number of tubers in potato plants treated with GA (Lovell and Booth, 1967; Menzel, 1980; Sharma et al., 1998; Hartmann et al., 2011). At high temperatures, potatoes are usually shown to have fewer number of tubers per plant than the control (Menzel, 1980; Midmore and Prange, 1992) but in these studies, potato plants were not only exposed to long term high temperatures, the plants were also never transferred to cooler conditions after the end of the high temperature period. This contrasts to experiments in the current study in which potato plants were exposed to a 9-day episode either before or after tuber initiation and grown back to cooler base temperatures at the end of the high temperature period.

The second question relates to the elongation of the main shoot in potato plants exposed to high temperatures. The results of Chapter 5 Section 5.5.5 and Chapter 6 Section 6.5.7 showed that shoot elongation was impaired at the end of an episode of the high temperatures. Plants exposed to an episode of high temperature were of equal height or taller than the control at the final sampling but at the end of the high temperature period, they were shorter. In the literature, there is a strong evidence that links GA to elongated shoots in the potato plants.
(Kumar and Wareing, 1972, 1974; Balamani and Poovaiah, 1985; Little and MacDonald, 2003; Hartmann et al., 2011) and the same stimulation of shoot elongation has also been observed in potato plants grown at high temperatures (Lovell and Booth, 1967; Menzel, 1980; Carrera et al., 2000; Rykaczewska, 2015). However, this was only in the plants that were grown under the long-term high temperatures without exposure to cooler conditions after the end of the high temperature period.

Lastly is question of stimulation of the shoot growth in potato plants exposed to high temperatures. In Chapter 5 and 6, the results showed that plants exposed to an episode of high temperatures had less growth on the main shoots at both the end of the high temperature period and at the final sampling. However, the lateral shoots grew rapidly after the end of the high temperature period. Such an influence of the high temperature on branching in potato plants has been demonstrated before (Fleisher et al., 2006). However, even Fleisher et al. (2006) did not observe these in cooler conditions after the end of the high temperature period but only in plants that were exposed to a consistent influence of the high temperatures. Further, such a shift in the shoot architecture of potato plants has never been demonstrated in any GA related studies (Lovell and Booth, 1967; Booth and Lovell, 1972; Sharma et al., 1998) nor in high temperatures studies where the reduction in tuber growth is linked to stimulated shoot growth (Menzel, 1980, 1983, 1985; Timlin et al., 2006).

The impaired dry matter and elongation on the main shoot at the end of high temperature period and the resumption of shoot elongation combined with the rapid growth on lateral shoots when plants were grown back at cooler conditions following the end of the episode of the high temperatures are more like the response to an auxin effect. Auxins are known to control apical dominance (Cline, 1994; Kieber and Schaller, 2014) and disruption of the production of auxins or stimulation of cytokinins, decrease the apical dominance and promote lateral bud development (Skoog and Thimann, 1934; Thimann, 1937, 1939; Sachs and Thimann, 1967; Kumar and Knowles, 1993; Herman et al., 2016). Ravindra et al. (2016) found that the main shoot in the potato cv. Kufri Chipsona 3 was stimulated when treated with either GA or Naphthalene Acetic Acid (NAA-Auxin). In other plant species, Van Overbeek (1938) associated dwarfness in maize with lower auxin production. Given that the impaired shoot elongation during the episode of the high temperatures in Chapter 5 and 6 was temporary and plants experienced an outgrowth of the laterals when plants were grown back
in cooler conditions, it is proposed that these responses were the result of auxin inhibition during the high temperature episode.

A number of studies have implicated the involvement of auxins in plant and tuber growth of potato plants. Harmey et al. (1966) found enhanced tuberization in potato cv. British Queen stem cuttings dipped in a growth medium containing 2 mg of indole-3-acetic acid (IAA) L$^{-1}$ supplemented with sucrose but tuberization was inhibited in the medium containing GA. Ponnampalam and Mondy (1986) found decreased levels of total glycoalkaloid (TGA) and lower nitrate-nitrogen in the tubers of potato cv. Katahdin and Kennebec that were foliar sprayed with 10 µM of IAA. Wang et al. (2009) sprayed potato cv. Zhongshu 3 plants with a 2 g of a gibberellic acid inhibitor L$^{-1}$ (Chlorocholine chloride, CCC) and found not only improved tuber yields but also increased IAA and Zeatin content in the potato leaves. Farhan et al. (2010) reported 14.8 % improved tuber yields when the foliage of potato cv. Pentland Crown was sprayed with a solution containing 45 mg L$^{-1}$ of Naphthalene Acetic Acid (NAA) and 4.5 mg L$^{-1}$ of 6-Benzyl Amino Purine (BAP-cytokinin). However, little information is available on the possible involvement of an auxin mechanism in potato plants exposed to high temperatures. There is also little information on how auxins might influence the whole plant performance of potato in terms of leaf production, growth and carbon partitioning. Further, studies involving GA always attribute the response of the potato plant directly to the gibberellins. However, there are cases where the responses could be auxin-linked. Booth and Lovell (1972) attributed both the decrease in the starch and the increase in sugars the tubers of the potato cv. Majestic plants to GA but the shoot apices of the plants were removed which means auxin metabolism was altered.

Different concentrations of IAA have been used to investigate the effect of auxins on both tuber and shoot growth in potato plants. Kumar and Wareing (1974) placed the basal ends of stem cuttings of induced potato cv. Andigena and Majestic in an advanced stage of tuber development in nutrient solutions containing 0.1, 1, 10 and 30 parts per million (ppm) of IAA and found increased tuber fresh weights at lower concentrations while concentrations more than 10 ppm completely inhibited tuber growth. Ponnampalam and Mondy (1986) found decreased total glycoalkaloid and nitrate nitrogen in the tubers of potato plants cv. Katahdin and Kennebec whose leaves were sprayed twice with 10 µM (micromolar) of IAA at 60 and 67 DAP. In an in Vitro experiment, Roumeliotis et al. (2012) found tuberization in the potato cv.
Bintje explants whose basal parts had been treated with 80 µM of an auxin inhibitor, 2,3,5-triiodobenzoic acid (TIBA).

It was hypothesized that exogenously applied auxins would overcome the negative impact of a 9-day episode of high temperature on the growth of potato plants and tubers. This chapter firstly investigated the influence of an auxin (IAA) and an auxin inhibitor (TIBA) on plant and tuber growth of potato exposed to a 9-day episode of 30 °C. Then, it evaluated the influence of four concentrations of IAA on potato plants exposed to high temperature.

9.2 Materials and methods
Two experiments were conducted. Expt. 4 investigated the influence of IAA and TIBA while Expt. 5 evaluated the impact of four concentrations of IAA on potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Expt. 4 was conducted from March to June 2017 and Expt. 5 from July to September 2017.

9.2.1 Experimental design and treatment structure
Expt. 4 was a 2 × 3 factorial with two levels of temperatures (the control (22 °C) and a treatment involving the exposure of plants to high temperatures) and three levels of PGRs (No-PGRs, IAA and TIBA). Expt. 5 was 2 × 4 factorial with the same two temperatures but with four levels of IAA concentration (0, 5, 15 and 45 µM). The temperatures were the main plot factors while the PGRs were the subplots.

Potato cv. Royal Blue plants were grown in a glasshouse at 22 °C before and after the end of the high temperature period in both experiments (Expt. 4 and 5). In both experiments, the high temperature treatments were applied for a 9-day episode of 30 °C after the presence of tubers was confirmed and immediately after plants had been treated with the PGRs at 37 days after planting (DAP) (Expt. 4) and at 41 DAP (Expt. 5). At the end of the nine days, the plants in the 30 °C were transferred back to 22°C and grown together with the control for another 23 (Expt. 4) or 20 (Expt. 5) days before the final sampling.

9.2.2 Glasshouse conditions and plant management
The glasshouses set up and management was as described in Chapter 4. In Expt. 4, the solar duration ranged from 12 hours in March (at planting) to 10 hours in June (at the final
sampling). In Expt. 5, the solar duration ranged from 10 hours and 20 minutes in July (at planting) to 12 hours in September (at the final sampling). The mean relative humidity during the high temperature period in the 30 °C treatment was 51.6 % (Expt. 4) and 61 % (Expt. 5). In the control (22 °C), the mean relative humidity for the duration of the experiment was 70 % (Expt. 4) and 61 % (Expt. 5). The growth medium, planting bags, planting and subsequent plant management in both experiments were as described in Chapter 4. In each experiment, six extra potato plants were used to check for the presence of tubers before the high temperatures were applied.

9.2.3 The application of the plant growth regulators

The PGRs in Expt. 4 were applied at the rate of 10 µM corresponding to 1.75 ppm for IAA and 80 µM corresponding to 39.98 ppm for TIBA (Roumeliotis et al., 2012). In Expt. 5, the following concentrations of IAA were used: 5, 15 and 45 µM corresponding to 0.875, 2.62 and 7.87 ppm (Kumar and Wareing, 1974).

The different PGRs were weighed and dissolved in 10 ml 1N NaOH. Then the solutions of the different PGRs were transferred to 1-Litre measuring cylinder and diluted with DDI (double deionized water). Similar amounts of DDI water containing only the 10 ml 1 N NaOH was also prepared for the No-PGRs (Expt. 4) and the “0” IAA concentration (Expt. 5). All solutions were prepared 5 hours before being applied and were kept in the dark at 2 to 4 °C.

To minimise any impact of the direct solar radiation on the PGRs after application, the application was conducted one hour after sunset. During the application of the PGRs, all the plants were first moved outside the glasshouse and divided into two temperature treatment groups: the 22 °C and the 30 °C (the high temperature treatment group). In each group, there were three (Expt. 4) or four (Expt. 5) treatments. The foliage of six plants per treatment in each group was sprayed to the point of run off with 400 ml of the plant growth regulators (Expt. 4) or the different concentration of IAA (Expt. 5) using a 500-ml handheld sprayer. It is possible that some of the solution also entered the growth medium.

Immediately after application of the PGRs, the high temperature treatment groups in both experiments were moved to another glasshouse and the high temperature started. The 22 °C treatment pots were moved back to the glasshouse already maintained at 22 °C. To minimise the chances of wet plants with different PGRs treatments coming into contact, plants with
the same PGR were kept close together but not in contact until the following day. On the following day, when all plants were dry, plants in all treatments were completely randomized within and between benches of the glasshouse. Plants from different treatments on the same bench were placed as close as possible but not in contact. Further randomization was conducted every three days during the high temperature period and weekly after plants were returned to the control condition.

9.3 Measurements

9.3.1 Non-destructive measurements
Photosynthetic measurements were only carried out in Expt. 4. They were conducted on the terminal leaflet of the seventh leaf from the apex which was tagged a day before the high temperature treatment commenced. These measurements commenced the day before the high temperatures were applied and continued daily through the high temperature period with a final measurement made before the final sampling. The net photosynthetic rates were recorded using the photosynthetic equipment (LCpro + System, Serial No. 32125, ADC BioScientific Ltd. UK) and an open leaf chamber as described in Chapter 7 Section 7.3.1.

Leaf chlorophyll concentration index (CCI) was measured on the terminal leaflets of two different leaves: the seventh leaf from the apex (as described above) and the third leaf that emerged after treatments were commenced. The CCI measurements were conducted at the end of the high temperature period and at the final sampling in Expt. 4. The measurements were conducted using the SPAD chlorophyll meter CCM-200 Plus (Apogee Instruments, Inc. USA) at the same time of the day as the photosynthetic measurements.

The length of the main shoot above the seventh leaf that was tagged a day before the high temperature was applied and the number of the newly emerged leaves above the seventh leaf was recorded at the end of the high temperature period and at the final sampling in Expt. 4.

9.3.2 Destructive measurements
Only one destructive sampling was conducted in Expt. 4 and 5. This was the final sampling at the end of both experiments. During data collection six plants per treatment were sampled for leaf area, the plant height, and the dry weights following the methods described in
Chapter 4 Section 4.3. Plants were also sampled for the total number and the different size categories of tubers.

9.4 Calculations and data analysis

In Expt. 4, the effects due to temperature, PGRs treatment and their interaction were subjected to two-way analysis of variance (ANOVA) as outlined in Chapter 4. Where significant difference was found, the means were compared using Least Significant Difference (LSD) and reported at 5% level of probability unless otherwise specified.

In Expt. 5, linear or curvilinear regression models were used to test the effect of IAA concentration on the leaf area, the number of tubers and their size distribution and the dry matter variables (tuber, below ground, leaf, stem, shoot, and whole plant) using the SAS procedure Proc REG. The significance of the regression coefficients was also tested. Improvement of regression terms was checked through the R-squared ($R^2$). Only the regression models with improved terms are reported. No data required transformation even though the different size category of the tubers, the dry matter and leaf area on the lateral shoots and the senesced leaves on the main shoot were analysed as percentages of the total number of tubers per plant, the dry matter or leaf area on the main shoot and the dry matter of the whole plant leaf, respectively.

9.5 Results

9.5.1 Air temperatures during the high-temperature period

In Expt. 4, the air temperatures during the high temperature period in the 30 °C treatment ranged from 29 to 31.7 °C with a mean of 30.3 °C (Fig. 9.1). In the control (22 °C) treatment, the day and night temperatures ranged from 21 to 22.2 °C with a mean of 21.5 °C.

In Expt. 5, the day temperature differed from the night temperature due to the malfunction of an air conditioner in one glasshouse. The mean night-time (7.00 pm to 7.00 am) and day-time (7.00 am to 7.00 pm) temperatures were 24.5 °C and 29 °C, respectively. The day/night fluctuation allowed a better comparison to a field situation but contrasted to all the other experiments in this study. The control (22 °C) treatment in Expt. 5 had similar day and night air temperatures during the high temperature period. These temperatures ranged from 21.1 to 22.7 °C with a mean of 21.5 °C (Fig. 9.1).
Figure 9.1: The mean air temperatures of the different treatments averaged over the nine days of the high temperature episode in Expt. 4 and 5.

9.5.2 Tuber dry matter

In Expt. 4, the tuber dry matter at the end of the experiment was influenced independently by temperature \( (p = 0.0001) \) and plant growth regulators \( (p = 0.0069) \); there was no significant interaction effect between them (Fig. 9.2). Plants in the 30 °C treatment had 31.8 % less tuber dry matter than in the control. The tuber dry matter per plant in the No-PGRs (39.4 g plant\(^{-1}\)) and the IAA-treated plants (35.9 g plant\(^{-1}\)) were not significantly different but the TIBA-treated plants had 18.7 % less tuber dry matter per plant than the No-PGRs. The tuber dry matter per plant in the 30 °C treatment was the same with or without IAA application. The tuber dry matter in the TIBA-treated plants in control (22 °C) was reduced in a similar manner as those of the No-PGRs in the 30 °C treatment.

In Expt. 5, neither the IAA concentrations \( (p = 0.30) \) nor the interaction between the temperature and the IAA concentration \( (p = 0.35) \) significantly influenced the tuber dry matter at the end of the experiment. Only the temperature significantly \( (p = 0.02) \) influenced the
tuber dry matter per plant; potato plants in the 30 °C treatment had 12.6 % less tuber dry matter than the control (Fig. 9.2). When the untreated treatments (treatments with 0 µM IAA concentration) were compared, the 30 °C treatment had 24.5 % less tuber dry matter than the control.

Figure 9.2: Tuber dry matter per plant as influenced by IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Data at the end of Expt. 4 and 5. Error bars are LSD at 5 % level of probability.
9.5.3 The number of tubers per plant

In Expt. 4, both the temperature \((p = 0.03)\) and the plant growth regulators \((p = 0.02)\) significantly influenced the total number of tubers per plant but not the interaction between them \((p = 0.9)\) at the end of the experiment (Fig. 9.3). All plants in the 30 °C treatment had 23 % more tubers per plant than in the control. The IAA-treated plants had 33 % more tubers than the No-PGRs plants but the number of tubers per plant in the TIBA treatment were not significantly different from the No-PGRs plants. Plants treated with either IAA or TIBA had the same total number of tubers per plant as those in the 30 °C treatment.

In Expt. 5, there was no significant effect of either the temperature \((p = 0.15)\), the IAA concentration \((p = 0.52)\) or the interaction between temperature and the IAA concentration \((p = 0.42)\) on the total number of tubers per plant.

![Graph showing the total number of tubers per plant](image)

**Figure 9.3:** The total number of tubers per plant as influenced by the IAA and TIBA (Expt. 4) and the IAA concentration (Expt. 5) in potato plants exposed to 9 days of 30 °C shortly after tuber initiation. Data at the end of Expt. 4 and 5. Error bars LSD at 5 % level of probability.

9.5.4 Size distribution of tubers per plant

In Expt. 4, temperature \((p = 0.01)\) and the plant growth regulators \((p = 0.007)\) significantly influenced the size distribution of tubers per plant in the potato plants at the end of the experiment (Fig. 9.4). Plants in the 30 °C treatment had 38 % fewer tubers with the diameter of greater than 2.5 cm than the control. The IAA and TIBA-treated plants had 45 % fewer tubers with the diameter of greater than 2.5 cm than the No-PGRs plant. The number of
tubers with the diameter greater or less than 2.5 cm in either IAA or TIBA-treated plants in the control was not different from those in the 30 °C treatment.

In Expt. 5, there was no significant effect of the IAA concentration on the size distribution of tubers. Neither did temperature nor the interaction between temperature and the IAA concentration have any significant influence on the size distribution of tubers (Fig. 9.4).

![Figure 9.4: Percent size distribution of tubers per plant as influenced by IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Tubers were categorised based on diameter (D) ≤ 2.5 or ≥ 2.5 cm. The classification of tubers was based on their diameter (D) at the widest part. Data at the end of Expt. 4 and 5. Error bars are LSD at 5 % level of probability.](image)
9.5.5 Whole plant, shoot and below ground dry matter

In Expt. 4, high temperature significantly reduced the whole plant \((p = 0.002)\) and the below ground dry matter \((p = 0.0001)\) but not the shoot dry matter at the end of the experiment (Fig. 9.5). The PGRs treatments also significantly influenced the whole plant \((p = 0.0001)\) and the below ground dry matter \((p = 0.0023)\) but also the shoot dry matter \((p = 0.0001)\). However, there were no significant interactions of temperature and PGRs treatments. The 30 °C treatment had 17 % less whole plant and 30 % less below ground dry matter than the control. The TIBA-treated plants had 33 % less whole plant, 40 % less shoot and 28 % less below ground dry matter than the No-PGRs plants while those of the IAA-treated plants were not different from the No-PGRs treatments. The whole plant and below ground dry matter in plants exposed to the 9-day episode of 30 °C was the same with or without IAA application. The whole plant and below ground dry matter in TIBA-treated plants in the control was also not different from those of the No-PGRs plants in the 30 °C treatment.

In Expt. 5, the temperature marginally \((p = 0.06)\) reduced the whole plant but significantly \((p = 0.03)\) affected the below ground dry matter per plant at the end of the experiment. The IAA concentrations had no significant effects on the whole plant \((p = 0.34)\) or the below ground \((p = 0.30)\) dry matter per plant and caused a marginal \((p = 0.09)\) increase on the shoot dry matter per plant. The interaction of temperature and the IAA concentration had no significant impact on the whole plant \((p = 0.18)\) or the below ground dry matter per plant \((p = 0.33)\) but significantly reduced the shoot \((p = 0.006)\) dry matter per plant. The 30 °C treatments had 8.4 % less whole plant and 11.6 % less below ground dry matter than the control. The shoot dry matter per plant remained unchanged with increased concentration of IAA in the high temperature treatments. In the control, there was less shoot dry matter per plant with increased concentration of IAA.
Figure 9.5: Whole plant, shoot and below ground dry matter per plant as affected by IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Data at the end of Expt. 4 and 5. Error bars are LSD 5 % level of probability.

9.5.6 Main and lateral shoot dry matter

In Expt. 4, the main shoot dry matter at the end of the experiment was influenced only by the plant growth regulators \( p = 0.0001 \) while the lateral shoot dry matter only by the temperatures \( p = 0.0001 \) (Fig. 9.6). Plants treated with TIBA had 38 % less main shoot dry matter than the No-PGRs or the IAA-treated plants. There was 76 % more dry matter in lateral shoots compared to the main shoots of plants in the 30 °C treatment than in the control.
In Expt. 5, the main shoot dry matter at the end of the experiment was influenced by the interaction between temperature and the IAA concentration ($p = 0.0049$). There was no change in the main shoot dry matter per plant with increased concentration of IAA in the high temperature treatments. However, there was less main shoot dry matter per plant with increased concentration of IAA in the control. Plant treated with 45 µM of IAA in 30 °C had 26% more dry matter on main the shoots than in the control.

Figure 9.6: Main shoot dry matter and the lateral shoot dry matter as a percent of the main shoot dry matter per plant as influenced by IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-days of 30 °C applied shortly after tuber initiation. Data at the end of Expt. 4 and 5. Error bars are LSD at 5% level of probability.

9.5.7 Leaf emergence

In Expt. 4, the influence of temperature on leaf emergence was only significantly ($p = 0.01$) at the end of the high temperature period and only the plant growth regulators ($p = 0.0009$) at the final sampling (Fig. 9.7). At the end of the high temperature period, the number of
emerged leaves per plant was 7 % higher in the 30 °C treatment than in the control. At the final sampling, the number of emerged leaves in the IAA and No-PGRs plants were not significantly different but TIBA-treated plants had 15 % fewer emerged leaves than either the IAA-treated or the No-PGRs plants.

**Figure 9.7:** Emergence of leaves per plant above the seventh leaf as influenced by the IAA and TIBA in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Data at the end of high temperature period and at the final sampling of Expt. 4. Error bars are LSD at 5 % level of probability.

### 9.5.8 Shoot elongation above the seventh leaf

In Expt. 4, the length of the main shoot above the seventh leaf at the end of the high temperature treatment was reduced by temperature ($p = 0.0001$). The interaction between the temperature and the plant growth regulators also significantly ($p = 0.01$) influenced shoot elongation (Fig. 9.8). Plants treated with either IAA or TIBA in the 30 °C treatment had shorter lengths than plants in the control at the end of the high temperature period. Further, the IAA-treated plants had shorter lengths than the TIBA-treated plants in 30 °C treatment.

At the final sampling, the elongation of the main shoot was influenced by the temperature ($p = 0.0013$) and the plant growth regulators ($p = 0.0001$). Plants in the 30 °C treatment had 17.2 % shorter shoot length above the seventh leaf than the control. The shoot length above the seventh leaf in plants that were treated with IAA in the control was not different from the No-PGRs treatment in the same control. However, shoot length above the seventh leaf in the
TIBA-treated plants was reduced to a similar extent whether plants were in the control or at the high temperatures.

Figure 9.8: Shoot elongation per plant above the seventh leaf tagged before high temperatures were applied as influenced by IAA and TIBA in potato plants exposed to 9 days of 30 °C applied shortly after tuber initiation in Expt. 4. Data at the end of the high temperature period and at the final sampling of Expt. 4. Error bars are LSD at 5 % level of probability.

9.5.9 Elongation of the main shoot

In Expt. 4, the main shoot length at the end of the experiment was influenced independently by the temperature ($p = 0.0002$) and the plant growth regulators ($p = 0.0001$) (Fig. 9.9); there was no significant interaction. Plants in the 30 °C treatment were 10 % shorter than the control (Fig. 9.9). The TIBA-treated plants were 17 % shorter than the No-PGRs plants.

In Expt. 5, neither temperature ($p = 0.11$), IAA concentration ($p = 0.28$) nor the interaction between temperature and IAA concentration ($p = 0.53$) had any significant effect on elongation of the main shoot in the potato plants.
Figure 9.9: The length of the main shoot per plant as influenced by IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-day of 30 °C applied shortly after tuber initiation. The data at the end of Expt. 4 and 5. Error bars are LSD at 5 % level of probability.

9.5.10 Biomass partitioning ratios

In Expt. 4, the high temperature increased the proportion of the dry matter partitioned to the shoot by 23 % ($p = 0.0001$) but decreased the proportion partitioned to the tubers by 17 % ($p = 0.0001$) at the end of the experiment (Fig. 9.10). TIBA-treated plants partitioned 12 % more dry matter to the shoot ($p = 0.046$) and 8 % less dry matter to the tubers ($p = 0.0001$) compared to the No-PGRs plants. The IAA-treated plants partitioned the same amount of dry matter to the shoot and tuber as the No-PGRs plants. However, there was no significant interaction between the temperature and the PGRs.

In Expt. 5, there was no significant influence of the temperature, the IAA concentration or the interaction between the temperature and the IAA concentration on dry matter partitioning in potato plants at the end of the experiment.
Figure 9.10: The proportion of dry matter partitioned to the shoot and tubers as influenced IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Data at the end of Expt. 4 and 5. Error bars are LSD at 5 % level of probability.

9.5.11 Leaf growth and expansion

9.5.11.1 Leaf growth

In Expt. 4, only the PGRs treatments had significant impact on the whole plant ($p = 0.0001$) and the main shoot ($p = 0.0001$) leaf dry matter per plant at the end of the experiment (Fig. 9.11). By contrast, temperature and the interaction of temperature and the PGRs did not influence the whole plant and main shoot leaf dry matter per plant. The TIBA-treated plants had 37.4 % less whole plant and 39.3 % less main shoot leaf dry matter than the No-PGRs treatments. The IAA and the No-PGRs plants had statistically similar whole plant and main shoot leaf dry matter in all the temperature treatments.

The proportion of biomass in the lateral shoots leaves compared to the main shoot leaves was significantly influenced by temperature ($p = 0.0001$). About 72 % more dry matter was accumulated in the leaves of the lateral shoots compared to the main shoots in the potato
plants in the 30 °C treatment than in the control. The plant growth regulators also significantly 
\( p = 0.04 \) influenced the dry matter accumulated in the lateral leaves. All plants in the control 
were similar in the dry matter partitioned to the lateral shoot leaves. However, in 30 °C 
treatment, the TIBA-treated plants partitioned 25 % more dry matter on the lateral shoot 
leaves compared to the main shoot leaves than the No-PGRs plants. In contrast, in the 30 °C 
treatment, the IAA-treated plants partitioned 34 % less dry matter on lateral shoot leaves 
compared to the main shoot leaves than the No-PGRs plants.

In Expt. 5, neither temperature \( p = 0.80 \), IAA concentration \( p = 0.18 \) nor the interaction 
between temperature and the IAA concentration \( p = 0.69 \) significantly affected the whole 
plant and the main shoot leaf dry matter or the biomass partitioned on the leaves of the 
latent shoots compared to the main shoot leaves.
Figure 9.11: Whole plant and main shoot leaf dry matter per plant and the percent of dry matter partitioned on leaves of the lateral shoots compared to the main shoot leaves as influenced by IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Data at the end of Expt. 4 and 5. Error bars are LSD at 5 % level of probability.

9.5.11.2 Leaf expansion

In Expt. 4, all plants in the 30 °C treatment had 29 % less whole plant leaf area, 44 % less main shoot leaf area and 83 % more leaf area on the lateral shoots than on the main shoot compared to the control ($p = 0.0001$) at the end of the experiment (Fig. 9.12). Plants treated with IAA were not statistically different in the whole plant and main shoot leaf area compared to the No-PGRs plants. However, the TIBA-treated plants had 47.5 % less whole plant and 39.3
% less main shoot leaf area than the No-PGRs plants. Plants treated with either the IAA or TIBA had were not significantly different from the No-PGRs plants in terms of the leaf area on the lateral shoots compared to the main shoot, but the TIBA-treated plants had 52 % more leaf area on the lateral shoots compared to the main shoot than the IAA-treated plants ($p = 0.04$). The interaction of the temperature and the PGRs was not significant for the whole plant ($p = 0.8$) or the main shoot ($p = 0.4$) leaf area. However, the IAA-treated plants in 30 °C had less leaf area on the lateral shoots compared to the main shoot than either the No-PGRs or TIBA-treated plants ($p = 0.07$). In 22 °C, none of the PGRs treatments was significantly different from the No-PGRs plants.

In Expt. 5, Plants in 30 °C treatment had 26 % less whole plant leaf area ($p = 0.002$), 27 % less main shoot leaf area ($p = 0.001$) and 42 % more leaf area on the lateral shoots compared to the main shoots ($p = 0.07$) than in control. However, the concentration of IAA or the interaction of temperature and the IAA concentration was not significant.
At the end of the experiment
Expt. 4
Whole plant leaves

At the end of the experiment
Expt. 5
Whole plant leaves

Leaf area (cm² plant⁻¹)

Main shoot leaves

Lateral shoot leaf area as % of main shoot leaf area

Percentage plant⁻¹

No-PGRs IAA TIBA

PGRs

22 °C 30 °C

30 °C → y = -7.9 × 10⁻³x² + 0.4x + 1.3
R² = 0.18, p = .046 (x²), p = .044 (x)

IAA Concentration (μM)

22 °C 30 °C

9.5.11.3 Leaf senescence

In Expt. 4, plants in the 30 °C treatment had 64 % more senesced main shoot leaves than in the control temperature (p = 0.0001) (Fig. 9.13). The main shoots in the TIBA-treated plants were 29 % more senesced than the No-PGRs plants (p = 0.03).
In Expt. 5, the 30 °C treatments had 32 % more senesced main shoot leaves than control ($p = 0.0006$) (Fig. 9.13). However, IAA concentration or the interaction of temperature and IAA concentration was not significant.

![Diagram showing senesced main shoot dry matter as a percent of the whole plant leaf dry matter]

**Figure 9.13:** Senesced main shoot dry matter per plant as a percent of the whole plant leaf dry matter as influenced by IAA and TIBA (Expt. 4) and IAA concentration (Expt. 5) in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation. Data at the end of Expt. 4 and 5. Error bars are LSD at 5 % level of probability.

### 9.5.12 Leaf chlorophyll concentration index

In Expt. 4, at the end of the high temperature period the PGRs-treated plants in 30 °C had greener terminal leaflet of the seventh leaf than the No-PGRs plants in the same 30 °C
treatment ($p = 0.08$). At the final sampling, plants in the 30 °C treatment had 15.4 % less green terminal leaflet of the seventh leaf than in the control ($p = 0.0002$).

At the end of the high temperature period in Expt. 4, the CCI in the terminal leaflet of the third leaf was the same with or without IAA application in 30 °C treatment. However, the CCI in the terminal leaflet of the third leaf in the TIBA-treated plants in the control was not different from the No-PGRs plants in 30 °C treatment. At the final sampling, the terminal leaflet of the third leaf in the TIBA-treated plants was 36 % less green in the 30 °C treatment than in the control ($p = 0.04$).

Figure 9.14: Leaf chlorophyll concentration index (CCI) in the terminal leaflet of the third leaf that emerged during the high temperature period and of the seventh leaf tagged before the high temperatures were applied in potato plants exposed to a 9-day episode of 30 °C applied shortly after tuber initiation in Expt. 4. Error bars are LSD at 5 % level of probability.
9.5.13 Leaf photosynthesis

In 30 °C treatment, the IAA and TIBA-treated plants had higher photosynthesis per unit area of the terminal leaflet of the seventh leaf both during the high temperature period and after plants were grown back at cooler conditions; except at the beginning of the high temperature treatment (IAA and TIBA-treated plants) and 64 DAP (in TIBA-treated plants) (Fig. 9.15). At the control condition, the IAA-treated plants had slightly higher photosynthesis than the Non-PGRs plants during the high temperature period. Six days before the final sampling, the rates in the IAA-treated plants were slightly lower than in the No-PGRs plants. The TIBA-treated plants initially had a slightly higher photosynthesis at the beginning of the high temperatures than the No-PGRs plants. However, the rates in the TIBA-treated plants were lower than in the No-PGRs plants both during the high temperature period and after the end of the high temperature period. Overall, IAA was beneficial during the high temperature period and after plants were exposed to cooler base temperatures following an episode of the high temperatures. TIBA was only beneficial during the high temperature period.
Figure 9.15: Photosynthetic performance of the terminal leaflet of the seventh leaf as influenced by IAA and TIBA in potato plants in the control (22 °C) or in the high temperature (30 °C) treatment. Data before the high temperatures were applied, during the high temperature and after the end of the high temperatures in Expt. 4. Error bars are LSD at 5 % level of probability.
**9.6 Discussion**

Expt. 4 and 5 were conducted to investigate the role of auxin supply in the responses of the potato plants to an episode of high temperature. There were four findings: (1) The auxin inhibitor (TIBA) reduced plant and tuber growth in the control (22 °C) to a similar extent as the 9-day episode of 30 °C; (2) Auxin (IAA) application did not improve dry matter partitioning to the tubers nor the growth of the potato plants that were exposed to the 9-day episode of the high temperature; (3) Both the auxin and auxin inhibitor increased the chlorophyll concentration index in the seventh leaf (emerged before the high temperature was applied) and in the third leaf that emerged during the high temperature and (4) The auxin and the auxin inhibitor increased the net photosynthetic performance of the terminal leaflet of the seventh leaf of the potato plants exposed to the 9-day episode of the high temperature.

The similarity of the extent to which plant growth was reduced in the plants at 22 °C when treated with TIBA to the plants that were exposed to the 9-day episode of 30 °C is consistent with the hypothesis that negative impacts of an episode of high temperatures on tuber growth could be associated with an auxin inhibition (Chapter 5). The dry matter in the tubers, whole plant, below ground plant parts and on the main shoot, the leaf area on the whole plant and on main shoot, the main shoot length, the total number of tubers per plant and the size distribution of tubers (small and large tubers) all responded quantitatively or qualitatively in the TIBA treated plants at 22 °C the same as in the No-PGRs plants exposed to the 9-day episode of 30 °C (Fig. 9.2 to Fig. 9.12).

The reduced tuber growth in the TIBA-treated plants in the control and the No-PGRs plants in the 30 °C treatment was most likely due to reduced leaf growth. The whole plant and main shoot leaf area in the TIBA-treated plants at 22 °C was reduced to a similar extent as those of the No-PGRs plants exposed to the 9-day episode of 30 °C (Fig. 9.12). There was a further reduction in leaf area in the TIBA-treated plants exposed to the 9-day episode of 30 °C. This means that the high temperatures exacerbated the negative impact of the auxin inhibitor on leaf growth. This is consistent with reports of Sakata et al. (2010) in which low levels IAA was found in the anthers of barley plants and in *Arabidopsis* grown at 29 °C than at 20 °C D/N although further clarity is needed because there are other studies that found increased levels of IAA in the anthers of cotton and in *Arabidopsis* exposed to high temperatures (Gray et al., 1998; Franklin et al., 2011; Min et al., 2014). In this study, however, the reduced leaf area
means that less photosynthate was available for tuber growth in TIBA-treated plants at 22 °C as well as those of the high temperature treatment. This is consistent with the results of Chapter 5 and 6 in which the losses in tuber dry matter under the episode of the high temperatures were more clearly related to the reduced leaf area than to the inhibition of starch synthase or the production gibberellins.

Sink capacity in terms of the number of tubers per plant was also consistent with results in Chapter 5 and 6. Slightly or significantly more tubers per plant were produced in all the high temperature treatments and with the plant growth regulators relative to the No-PGRs plants at 22 °C (Fig. 9.3; Fig. 9.4). This means that the reduced tuber dry matter in both the TIBA-treated plants at 22 °C and the high temperature treatment was not due to lack of enough sinks for the deposition of starch.

While the application of TIBA had a similar effect on potato growth, morphology and yield as did the high temperature treatment, the IAA treatment did not overcome the impact of a high temperature episode. In the study presented here, the potato plants were treated with 10 µM (Expt. 4) or 0, 5, 15 and 45 µM (Expt. 5) concentration of IAA. The PGRs was applied on the foliage and it is also likely that some of the solution went into the growth mix since the foliage was sprayed to the point of run off. Auxins are produced at the growing tip of the plant and move basipetally through the stems where they inhibit the growth of the lateral buds (Snow, 1929; Goldsmith, 1977; Aloni et al., 2006). This has been demonstrated in experiments in which suitable amounts of auxins applied on decapitated plants inhibits the lateral buds (Skoog and Thimann, 1934; Fanu, 1936; Thimann, 1939). Perhaps there would have been some responses with the right concentration (Kumar and Wareing, 1974), site, method and frequency of the application (Ponnampalam and Mondy, 1986) and or even with interaction with other PGRs such as gibberellic acid and cytokinins (Kumar and Wareing, 1972; Davies et al., 1986; Kumlay, 2014). However, due to time limitation, it was not possible to optimize on the method of application and or any of the factors mentioned above.
9.7 Conclusions

The similarity in the tuber, whole plant, below ground and main shoot dry matter; the leaf area of the whole plant and on main shoot; the main shoot length, and the total number of tubers per plant and the size distribution of tubers (small and large tubers) per plant between the TIBA treatment in the control temperature and the plants exposed to the 9-day episode of the high temperature is consistent with the hypothesis that reduced auxin production plays a role in the response of potato to a high temperature. However, addition of auxin did not overcome the effects of the high temperature treatment. Further research is needed to determine the role of auxins in potato plants exposed to an episode of high temperatures both for understanding the mechanism of action and for potential agronomic intervention.
CHAPTER TEN

General discussion
10.1 Overview

This study is based on the premise that potato crops will over time experience higher growth temperatures, but more importantly, increased exposure to episodes of temperatures above the threshold for optimum growth. The overall objective of this study was to develop an understanding of how episodes of high temperatures influence physiology, growth and tuber yields in potato plants. The approach of the present study contrasts with previous reports which were based on the application of persistent high temperature treatments. The aim was also to determine how growth stage and agronomic interventions, such as exogenous application of plant growth regulators, altered this response. To achieve this objective, the study included the follow five aims:

a. To determine the frequency and duration of episodes of high temperatures experienced during the critical period of tuber bulking in the main potato growing regions of Western Australia.

b. To investigate the impact of an episode of high temperature applied shortly before or after tuber initiation on canopy development, photosynthetic gain and dry matter partitioning, especially to the tubers.

c. To determine the influence of varying duration of high temperatures shortly after tuber initiation on canopy development and dry matter partitioning to the tubers.

d. To establish how an episode of high temperature applied shortly before or after tuber initiation altered the distribution of sucrose and starch within the potato plant.

e. To determine the influence of an exogenous auxin or auxin inhibitor application on canopy development and tuber growth in potato plants exposed to an episode of high temperature shortly after tuber initiation.

Potatoes grown in Western Australia experience frequent episodes, varying in duration, of high temperatures above the 25 °C critical threshold during the tuber bulking period (Chapter 3). Two consecutive days of over 25, 30 and 35 °C temperatures were experienced during tuber bulking in all regions except in Albany, Forrest Grove, and Pinjarra (Chapter 3 Section 3.4.3). Further, two consecutive days of temperatures over 25 °C occurred 3 to 8 times in all
the regions. Seven consecutive hot days above 30 °C occurred only once every few years except in Albany and Pinjarra.

The glasshouse experiment showed that tuber dry matter was reduced to a similar extent when a 9-day episode of 26 and 30 °C was applied shortly before or after tuber initiation (Chapter 5 Section 5.5.2). The reduction in tuber growth occurred after the end of the high temperature period even in plants that had no visible tubers during the high temperature period. In plants that had been exposed to the episode of the high temperature before tuber initiation, there was less starch content in the tubers but more sucrose content in the leaves. Nonetheless, the reduction in the starch content in the tuber could not have been due to inhibition of starch synthase (Chapter 8 Section 8.5.1 and 8.5.2). The total number of tubers per plant was the same in all treatments or were slightly more in the high temperature treatments. However, more small-sized tubers (tubers with diameter of less than 2.5 cm at the widest part) were produced in the high temperature treatments (Chapter 5 Section 5.5.7).

Leaf area on the main shoots was reduced by approximately 40% both at the end of the high temperature period and at the final sampling (Chapter 5 Section 5.5.8). In addition, the net photosynthesis (measured between 12.00 and 2.00 pm during the high temperature period) was also reduced more in the terminal leaflets of the older leaves than in younger ones of plants exposed to high temperature. There was increased morning (7.00 am to 10.00 am) and the night-time (7.00 pm to 1.00 am) dark respiration rate regardless of leaf age in plants exposed to high temperature (Chapter 7 Section 7.5.4 and 7.5.5). These three responses to the high temperature episode combined to reduce the available photosynthate for both plant and tuber growth. However, the main shoot continued to grow after the end of the high temperature period largely due to the rapid expansion of the lateral shoots (Chapter 5 Section 5.5.3). The lateral shoot was therefore a competitive sink after the high temperature episode that would have further reduced the photosynthate available for tuber growth.

Tuber dry matter was linearly and negatively correlated with duration of high temperatures (Chapter 6 Section 6.5.2). There was less tuber dry matter with longer duration of 30 °C temperatures applied shortly after tuber initiation. This means that even a few days exposure to the 30 °C temperatures subsequently reduced tuber dry matter. The tuber dry weights were also positively related to the main shoot leaf dry matter and area (Chapter 6 Section 6.5.10). The lower the leaf area the lower was the tuber dry matter. At the end of the high
temperature period, there were fewer tubers per plant in treatments with longer duration of the high temperatures. However, at the final sampling, plants in all treatments had the same number of tubers per plant. Which means that more tubers were initiated at cooler conditions in plants that had been exposed to longer duration of high temperatures. Further, more small-sized tubers (tubers with diameter of less than 2.5 cm at the widest part) were produced in plants exposed to durations of high temperatures of more than three days (Chapter 5 Section 5.5.7; Chapter 6 Section 6.5.3).

The auxin inhibitor applied to plants at the control temperature (22 °C) caused similar effects as the 9-day episode of the high temperature. However, the exogenously applied auxins did not overcome the negative impact of a 9-day episode of 30 °C on plant and tuber growth (Chapter 9 Section 9.5.2 to 9.5.11). Interestingly, there was an increase in the leaf chlorophyll concentration index and in photosynthetic rates per unit leaf area of the terminal leaflet of the seventh leaf with the exogenous application of either the auxin or the auxin inhibitor (Chapter 9 Section 9.5.12 to 9.5.13).

10.2 What led to the reduced plant and tuber growth

10.2.1 Less carbon available for tuber growth

After the end of the high temperature period, the main shoot leaf area of plants in the high-temperature treatments was only 60 % of that of the control. Thus, there was evidently a rapid and substantial decrease in availability of photosynthate in the high temperature treatments relative to the control during the critical period of tuber growth. The depressed supply of photosynthates is supported by lower net photosynthesis (measured during the high temperature period between 12.00 and 2.00 pm) on the terminal leaflets of older leaves and increased dark respiration rates in the morning (7.00 am to 10.00 am) and at night-time (7.00 pm to 1.00 am) in the high temperature treatment relative to the control (Chapter 7 Section 7.5.5). Moreover, the shoot dry matter was the same in plants in all treatments at the end of the high temperature period and at the final sampling. This means that shoot continued to accumulate dry matter at unchanged rate both during the high temperature period and after plants were grown at cooler conditions following exposure to the high temperatures. Thus, there were four compounding effects that depress photosynthate
availability for tubers in the high temperature treatments. This is consistent with the reduced tuber dry matter (Chapter 5 Section 5.2) and the linear relationship between the tuber dry matter and leaf area (Chapter 6 Section 6.5.2 and 6.59). It is also reflected in the lower starch and sucrose content in the tubers combined with the lower sucrose concentration and content in the main shoot leaves, the main stem and the stolons of the high temperature treatments relative to the control demonstrate that less carbon was available for plant and tuber growth (Chapter 8 Section 8.5.3; 8.5.4 and 8.5.5).

A further possible factor contributing to the reduced tuber growth, especially in Expt. 1, was reduction in tuber demand occasioned by fewer number of cells per tuber. Tubers with fewer number of cells expand less rapidly due the insufficient storage space for carbon (Plaisted, 1957; Reeve et al., 1973; Xu et al., 1998b). The number of cells in each tuber is dependent on the supply of photosynthates to the tuber (Chen and Setter, 2003, 2012). In Expt. 1 the tubers were initiated after the end of the high temperature period. In Expt. 2, the high temperatures were applied after tubers had been formed. In both experiments, however, there was reduced supply of photosynthates to the tubers due to reduced leaf area, lower net photosynthetic rates in older leaves, higher respiration over the day regardless of the leaf age and the continued shoot growth at unchanged rate. Therefore, even though the total number of tubers per plant was unchanged in both experiments, the tubers in Expt. 1 might have had less number of cells than in Expt. 2. This means that the high temperature treatment in Expt. 1 might have had reduced sink demand. Reduced sink demand is associated with accumulation of starch and sucrose on the potato leaves (Nösberger and Humphries, 1965; Basu and Minhas, 1991; Lafta and Lorenzen, 1995). This is consistent with the results of Expt. 1 where high sucrose accumulated in the leaves of the main and lateral shoots of plants in the high temperature treatment relative to the control (Chapter 8 Section 8.5.3 and 8.5.5). In contrast, in Expt. 2, the level of sucrose in the leaves was unchanged or was less in the high temperature treatments relative to the control both at the end of the high temperature period and at the final sampling. Therefore, the hypothesis that the high temperature restricts the number of cells initiated and hence limits the sink strength of tubers formed before or after high temperature treatment warrants further testing.
10.2.2 Inhibition of starch synthase was not associated with the reduction of tuber growth caused by the episode of high temperatures

Inhibition of starch synthase was linked in previous studies to reduced tuber growth in potato plants grown at high temperatures. However, this is based on studies in which only the tubers were exposed to the high temperatures (Krauss and Marschner, 1984; Geigenberger et al., 1998) or the plants were grown at cooler temperatures and exposed to the long-term persistent influence of high temperatures (Lafta and Lorenzen, 1995). Further, previous studies demonstrate that more sucrose usually accumulates in the tuber whenever starch deposition is inhibited by the inability of starch synthase to convert sucrose into starch (Krauss and Marschner, 1984; Geigenberger et al., 1998). By contrast, in the current study, tuber growth was reduced even in plants that had no tubers during the exposure to high temperatures, and then experienced optimal temperatures during tuber growth. The suppression of tuber growth continued even after the plants were growing back at 22 °C; a temperature that would be expected to promote optimum starch synthase activity (Krauss and Marschner, 1984; Mohabir and John, 1988) and hence tuber growth (Nakaseko et al., 1970; Randeni and Caesar, 1986). Further, neither the concentration nor the content of sucrose of tubers in the high temperature treatments was greater than the control (22 °C), either at the end of the high temperature episode of Expt. 2 or at the final sampling of Expt. 1 and 2. These suggest a different mechanism by which high temperature inhibits tuber growth than the inactivation of starch synthase.

10.2.3 Importance of an auxin mechanism

It has been proposed that higher levels of gibberellins are produced in potato plants grown at high temperatures and that this stimulates shoot growth, thus diverting carbon away from the tubers (Menzel, 1985). Menzel (1983) found higher content of gibberellins in the crude extract of the terminal buds (terminal buds with young leaves of less than 4 cm long) of the potato cv. Sebago plants grown at 35/30 °C than at 20/15 °C D/N. Gibberellins are known to impair tuber initiation, leading to fewer tubers per plant, and stimulate stem elongation (Lovell and Booth, 1967; Menzel, 1980, 1983, 1985). However, in Menzel (1980, 1983, 1985) plants were not exposed to an episode of high temperature as occurs in the field. Experiments involved exposures of the plants to persistent high temperatures and the part of the shoot
that is stimulated by the gibberellins acid was never reported. The results of the present study do not support the hypothesis put forward by Menzel (1985).

Results of the current experiments are consistent with those of Fleisher et al. (2006) who showed that it was the lateral shoots that increased under high temperatures, although treatments used by Fleisher et al. (2006) were under persistent influence of high temperatures. In fact, the current study showed that elongation of the main shoot was impaired during the high temperature period but recovered when plants were transferred back to 22 °C (Chapter 5 Section 5.5.5). This pattern is not consistent with a gibberellin effect triggered during a high temperature treatment. Further, the shoot dry matter in the high temperature treatments was not higher than the control either at the end of the high temperature period nor at the final sampling, but the tuber dry matter was still reduced (Chapter 5 Section 5.5.4 and Chapter 6 Section 6.5.5). This is consistent with the work of Lafta and Lorenzen (1995) who demonstrated that tuber dry matter was still reduced even when the shoot dry matter is not increased. Finally, in the current study, all plants had the same number of tubers or slightly more tubers were initiated in the high temperature treatments relative to the control both at the end of the high temperature period and at the final sampling (Chapter 5 Table 5.2 and Chapter 6 Section 6.5.3) whereas an increase in GA levels due to high temperature would have been expected to stimulate shoot growth but result in fewer tubers.

The shorter plants after the end of the high temperature period and the resumption of active shoot elongation, including expansion of the lateral shoots, when plants were returned to cooler conditions is more consistent with a response to reduced auxin levels (Sachs and Thimann, 1967; Phillips, 1975). The response of control plants (22 °C) in the present study to the exogenous application of an auxin inhibitor; 2,3,5-triiodobenzoic acid (TIBA) supports this idea. Plants grown at 22 °C responded similarly to those exposed to 30 °C. If high temperature accelerates the degradation of auxin, as suggested by the effect of the TIBA treatment at 22 °C, the application of auxins to plants exposed to 30 °C was expected to overcome the impact of the 9-day episode of the high temperature (Chapter 9 Section 9.5.2 to 9.5.11). The auxin treatments failed to do that in the present study, which either indicates that the right rate of auxin was not applied or that auxin acts in concert with some other growth substance. Further research could investigate the degradation of auxins at high temperatures.
10.3 Limitation of the current study and future work

10.3.1 Exploring the influence of high temperatures on potato plants beyond the high temperature period

In contrast to previous studies in which the influence of high temperatures was only reported at the end of the high temperature period, this study demonstrated that the high temperatures continued to influence the net photosynthetic rates, canopy development and tuber growth even after the end of high temperature period. This needs to be emphasised in studies of the impact of temperatures in the potato plants.

10.3.2 Understanding the impact of repeated episodes on potato plants and whether the potato plants acclimate to high temperature

Field-grown potato plants are repeatedly exposed to episodes of varying duration of high temperatures (Chapter 3). This study investigated the influence of only a single episode of high temperatures. Although there was a linear relationship between tuber dry matter, leaf area and the duration of the 30 °C temperatures, little is known about the responses in tuber and leaf growth should plants be exposed to a second or third episode of high temperatures after having returned to cooler temperature conditions. Do potato plants acclimate to high temperatures? Acclimatory response of potato plants to an environmental stress has been demonstrated with drought stress. Banik et al. (2016) found that three potato cultivars: Fv12246-6, Vigor and Russet Burbank were more tolerant to 7 to 10 days of drought stress (10 % less moisture) after exposure to short term drought cycles lasting for about 5 to 7 days than when the plants were exposed directly to the drought stress. Other plant species have been shown to acclimate well at high temperatures. Li et al. (1991) found positive relationship in plant dry weight, pod set, pot weight and yield if common beans were first grown at 25/18 °C D/N and acclimated at 37 °C D/N for 24 hours before being exposed to 50 °C. Improved photosynthetic performance, PSII, Rubisco activity, Rubisco activation state, chlorophyll content and carotenoid content has also been demonstrated in maize that were first acclimated to 50 °C then exposed to 41 °C than those that grew at 25 °C then exposed to temperatures of over 35 °C (Sinsawat et al., 2004). Thus, acclimation to high temperatures may have a major influence on the impact of high temperature in the field where plants are typically exposed to repeated episodes.
10.3.3 Exploring field studies in which the day and night temperatures are variable, multi-stemmed potato plants are growing in the canopy, and the diurnal change in temperature is gradual

It has been suggested that high night-time temperatures are more deleterious to tuber growth than the high day-time temperatures (Driver and Hawkes, 1943). Gregory (1956) postulated that tubers had greater growth when the night-time temperatures were 20 °C than 26 °C. Plants in the present study were exposed to constant day and night temperatures before the high temperature treatment, during the high temperature period and after the end of the high temperature treatment. Only in Expt. 5 were the night temperatures cooler than the day temperatures during the high temperature period. However, tuber dry matter in Expt. 4 were reduced to a similar extent as those in Expt. 5, although plants in Expt. 4 were exposed to hotter night temperatures (30/30 °C) than in Expt. 5 (30/25 °C) D/N temperatures. Further investigation is required to establish the extent to which cooler night temperature might alter the responses of potato plants to an episode of the high temperatures. High night temperature will diminish the available photosynthate for shoot and tuber growth and hence warrants further study since limited photosynthate availability appears to be the primary mechanism by which high temperature treatments acted in the present study.

Field grown potato plants are usually multi-stemmed; with the number of shoots ranging from one to more than five shoots per tuber (Oliveira, 2000; Shayanowako et al., 2014). The number of stems directly influence the distribution of leaf area hence the total intercepted radiation and the dry matter generated (Fleisher et al., 2011). Multi-stemmed potato plants become a consideration especially in field conditions in which plants are usually in canopies. In the present study, however, only single-stemmed plants were used to maintain uniform plants since the number of shoots growing from a single tuber can be highly variable.

In field conditions, plants experience the gradual change in the diurnal temperatures. The soil and air temperatures increases gradually from sunrise up to a maximum which could be about 12.00 to 3.00 pm then cools down as the sun approaches sunset (Parton and Logan, 1981). This gradual exposure to high temperatures can alter the response of plants. Law and Crafts-Brandner (1999) found that photosynthetic rates in wheat and cotton plants were acclimated to high temperatures when high temperatures were increased gradually rather than when there was an abrupt increase. In this study, plants were exposed instantly to high
temperatures. This might have occasioned the shock in the net photosynthetic rates that were observed in the high temperature treatments (Chapter 7 Section 7.5.4). Thus, the potato plants might have a different response in the field conditions.

### 10.3.4 Exploring the agronomic interventions including the application of plant growth regulators under variable temperature conditions

The understanding of how plant growth regulators influence plant morphology and physiology in potato grown under high temperatures may not only clarify the mechanisms of action, but also lead to useful methods of agronomic intervention. Gibberellic acid has been used successfully to alter growth in potato plants exposed to high temperatures (Menzel, 1980). However, its role is inconsistent with the responses of the potato plants exposed to an episode of high temperatures. Cytokinins may be involved or it could be the interaction between the auxins, gibberellins and cytokins in high temperature responses (Kumlay, 2014). However, the current study explored only the response of the potato plants to one PGRs: the auxin, IAA. Factors such as the concentrations used, timing of application, application method, site of application and or interaction with gibberellins and or cytokinins may influence the potato morphology and physiology hence the response to high temperatures. Thus, further investigation is suggested on the influence of high temperatures on the auxin metabolism in potato plants.

### 10.4 Conclusions

This study is the first to explain how an episode of high temperatures influences the physiology, growth and tuber yields of potato plants. Whereas previous studies suggested that high temperatures were more harmful after tuber initiations, the present study showed that tuber dry matter were suppressed to a similar extent whether episodes of high temperatures were applied shortly before or after tuber initiation. The suppression of tuber growth continued even when plants were exposed to cooler temperatures following an episode of the high temperatures. The suppression of tuber growth at high temperature was not consistent with the proposed inhibition of starch synthase or an increased production of gibberellic acid. Rather, under episodes of high temperatures, tuber growth was reduced because there was less available whole plant carbon. The reduction in whole plant carbon
availability was due to reduced leaf area, lower net photosynthesis and increased respiration rates at certain periods of the day. The reduced leaf area, lower net photosynthesis, increased respiration rates at certain period of the day and continued shoot growth at the same rate contributed to the less tuber growth.
References


enzymes to leaf nitrogen in rice, and their relationships to photosynthesis. *Plant Physiology*, **105**: 1231-1238.


Appendix A
Preliminary measurements on photosynthesis

1.1 Preamble
Preliminary measurements on photosynthesis were conducted alongside Expt. 2 to determine the width of the terminal leaflet that fitted the leaf chamber and the optimum light and the time of day at which potato leaves had maximum photosynthetic rate.

1.1.1 Adjusting for the smaller width of terminal leaflets
In photosynthetic systems, the photosynthetic measurements and respiratory rates are calculated as the rate of gas exchange per unit leaf area (LI-COR Inc, 2018). However, the leaves need to have fitted the leaf chamber to avoid erroneous measurements (Long et al., 1996). At times, leaves with smaller areas are unavoidable. Thus, the need for adjustments (Savvides and Fotopoulos, 2018). As was the case in this study.

The area of the leaf chamber of the photosynthetic equipment used in this study was based on bread leaf area of 6.25 cm² with square sides of 2.5 cm each. The lengths of the terminal leaflets of the potato leaves used in the photosynthetic measurements in this study fitted well with the leaf chamber; only the widths of the smaller leaflets could not fit. Thus, it was necessary to adjust for and correct for the photosynthetic readings. Similar adjustments targeted on the width of small leaves or leaflets has been conducted before (Long et al., 1996; Savvides and Fotopoulos, 2018).

To adjust for the photosynthetic readings of the smaller leaflets that did not fit the leaf chamber, the width of the smallest leaflet was first determined. Six leaflets that fitted well with the leaf chamber were destructive picked from the six plants. The width of these leaflets was then determined electronically using a portable laser leaf area meter (C1-202, CID Bio-Sciences, Inc. USA) and the mean calculated. This mean of the width was used to adjust for the photosynthetic reading of the smaller leaflets as follows:
Corrected $P_n = \frac{\text{Leaflet } P_n \times 3.57 \text{ cm}}{W (\text{cm})}$

Where:

Leaflet $P_n$ = The actual net photosynthetic ($P_n$) rate of the leaflet from the equipment.

3.57 cm = The width (cm) of the smallest leaflet that fitted with the leaf chamber.

$W$ = Actual width of the leaflet as measured on the intact plant.

1.1.2 Photosynthetic rate of potato leaves when exposed to different light regimes during data collection

The influence of different light regimes on the maximum photosynthetic response of potato leaves was investigated on six randomly selected potato in the control treatment on day 42 after planting in Expt. 2. There were three sources of light: ambient light as was determined by solar radiation within the glasshouse and two artificial lights: 1200 and 1500 µmol m$^{-2}$ s$^{-1}$ set from the photosynthetic equipment. Photosynthetic measurements were made from the fully expanded terminal leaflet of the seventh leaf. Results showed higher photosynthetic rate per unit area of the terminal leaflet of the seventh leaf when leaves were exposed to the two artificial lights from the photosynthetic equipment than when measurements were conducted at ambient lighting conditions of the glasshouse (Fig. A.1). But there were no significant differences between the artificial lights. The 1200 µmol m$^{-2}$ s$^{-1}$ was chosen for consideration in subsequent measurements. It was argued that the 1200 µmol m$^{-2}$ s$^{-1}$ light gave close to maximum photosynthetic yield from the potato leaflets. Further, repeated photosynthetic measurements were to be conducted in these experiments. Thus, it was argued that the 1200 µmol m$^{-2}$ s$^{-1}$ might pose minimal photodamage on the leaflets, if any, than if the leaflets were to be repeatedly shone with 1500 µmol m$^{-2}$ s$^{-1}$ or more light.
1.1.3 The time of day when photosynthetic rate of potato leaves was at maximum

To determine the time of day at which photosynthetic rate of potato leaves was at maximum, photosynthetic measurements were conducted on the terminal leaflets of the seventh leaf of six randomly selected potato plants at intervals of two hours from 8.00 am to 4.00 pm on 43 DAP. Two light regimes were used: ambient light; as was determined by solar radiation within the glasshouse and one artificial light intensity: 1200 µmol m\(^{-2}\) s\(^{-1}\) set from the photosynthetic equipment. Results showed that the photosynthetic rate of the potato leaves was stable between 10.00 am to 2.00 pm when leaves were exposed to 1200 µmol m\(^{-2}\) s\(^{-1}\) light (Fig. A.2). Measurements conducted under ambient light showed stable photosynthesis between 10.00 am to 12.00 pm. However, the rates declined after 12.00 pm. Under both light regimes, photosynthetic rate rose to a maximum quite early. This might have been due to the early sunrise during summer or the orientation of the glasshouse used in this study in relation to the solar radiation. The period between 12.00 to 2.00 pm was selected for conducting both photosynthetic and chlorophyll fluorescence measurements in subsequent experiments.

Figure A.1: Photosynthetic performance of the terminal leaflet of the seventh leaf of potato plants as influenced by different light regimes: ambient and two artificial light (1200 and 1500 µmol m\(^{-2}\) s\(^{-1}\)) from the photosynthetic equipment. The data were conducted on six control treatment plants (22 °C) at 12.00 pm Western Australia Time on 42 DAP. Bars are LSD at 5 % level of probability.
Figure A.2: Daytime photosynthetic response of the terminal leaflet of the seventh leaf of six potato plants exposed to two light regimes: ambient and an artificial light 1200 µmol m\(^{-2}\) s\(^{-1}\) from the photosynthetic equipment. Measurements were conducted on six randomly selected plants in the control treatment (22 °C) on 43 DAP in Expt. 2. Bars are LSD at 5 % level of probability.
Appendix B

Preliminary tests in sugar and starch measurements

1.1 Stability of the UHPLC system

Before plant samples were subjected to the UHPLC, two preliminary tests were conducted to determine the stability of the system and the recovery rate. The stability of the UHPLC system was investigated by randomly injecting three sucrose concentrations up to 10 times into the UHPLC system and determining the change in the peaks with the run-time. The sucrose concentrations used were: 0.1, 1 and 2 mg ml\(^{-1}\). The data on the peak area was then plotted against the number of injections and observed for the drift. The standard deviations of the peak areas were also calculated and plotted for every injection. The results showed some slight standard deviations of about 13, 4 and 2 % for the 0.1, 1 and 2 mg ml\(^{-1}\) sucrose concentrations, respectively (Fig. B.1). However, when the standard deviations were plotted against the injection number, it revealed that peak area in all concentrations increased with longer run-time (Fig. B.2). Therefore, there was need to adjust for this change in peak area with the machine run-time in the subsequent analysis. As such, 1.0 mg ml\(^{-1}\) sucrose concentration was included as a QC (quality control) sugar sample in the analysis of the water-soluble carbohydrate. This sample was injected after every 10 samples during the sugar analysis to allow correction.
Figure B.1: Ten injections of three different sucrose concentrations showing the drift in the UHPLC system.

Figure B.2: The drift in the different injections of three sucrose concentrations within a normal distribution with a width of two standard deviation (± 2 SD). Two standard deviations encompass 95% of the values.

1.2 Recovery of the sugars by the UHPLC

A recovery experiment was conducted by spiking an equal amount of the plant sample with different quantities of the sucrose standard. Five hundred milligram samples of the QC tuber were spiked with 0, 25, 50 and 75 mg of the sucrose standard. The samples were prepared in duplicates and were extracted and assayed for sucrose as described in Chapter 8 Section 8.2.3.1; 8.2.3.2; 8.2.3.3. There was a significant curvilinear relationship between the amount
sucrose standard added and the peak area obtained (Fig. B.3). Recovery was over 90 % when small quantities of the sucrose standard were added but less than 70 % when the spike was 75 mg.

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\% \text{ Recovery} = \frac{\text{Amount of sucrose recovered (g)}}{\text{Amount of sugar spiked (g)}} \times 100 \%
\]

Figure B.3: Recovery of the different amounts of sucrose standard spiked in 500 mg QC tuber sample.