University of Reading

Potatoes & Drought Stress: Improving Methods and Investigating the Utility of Canopy Traits to Inform Irrigation

Submitted for the qualification of Doctor of Philosophy in Crop Science

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Abstract

As an extremely drought susceptible crop, potato production is expected to be severely affected by climate change, due to the reduced seasonal rainfall predicted in many areas. To maintain or improve potato yields under these conditions, drought tolerant cultivars must be developed, irrigation infrastructure must be improved, and irrigation systems must become more efficient. However, potato is an under researched crop relative to the nutritional value it contributes to the food system. Of the research that has been conducted, many pot studies have likely been affected by the confounding effects of small pots on plant morphophysiology. Therefore, this thesis presents the first investigations of this effect in potato, demonstrating the cultivar-specific confounding effects of small pots on potato morphology. Further data show this is primarily a result of inadequate irrigation under supposedly well-watered conditions, diminishing the relative effects of water-restriction. These results provide methodological recommendations for further potted potato research, which were utilised in further experiments. These showed that canopy temperatures, but not SPAD values, reliably increase due to water-restriction in potted potato. It was also found that canopy temperatures return to baseline with the resumption of well-watered conditions, implying the potential for canopy temperatures to be integrated into a temperature-controlled irrigation system. Thus, the final study presented here investigates the efficacy of such a system. This system was limited by its sample size and by infection of the plants with blight, but it shows that the challenges threatening potato production may be mitigated with further investigation and refinement of similar systems.

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Dominic Hill

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Chapter 1: Dominic Hill (DH) produced all text.

Chapter 2: DH produced all text, tables, and figures. David Nelson (DN) provided expertise and reviewed the text. John Hammond (JH) provided supervision and reviewed the text. Luke Bell (LB) provided supervision, reviewed the text, and obtained project funding. All authors contributed to the article and approved the submitted version.

Chapter 3: DH grew all the experimental plants, collected all data, and produced all text, tables, and figures. DN provided expertise and reviewed the text. JH provided supervision and reviewed the text. LB provided supervision, reviewed the text, and obtained project funding. All authors contributed to the article and approved the submitted version.

Chapter 4: DH grew all the experimental plants, collected data, and produced all text, tables, and figures. DN provided expertise and procured the seed tubers. JH provided supervision and reviewed the text. Lorenzo Conte (LC) assisted with growing the experimental plants and collected data. LB provided supervision, reviewed the text, and obtained project funding.

Chapter 5: DH grew all the experimental plants, collected data, and produced all text, tables, and figures. DN provided expertise and procured the seed tubers. JH provided supervision and reviewed the text. Antreas Koryzis (AK) assisted with growing the experimental plants and collected data. LB provided supervision, reviewed the text, and obtained project funding.

Chapter 6: DH grew all the experimental plants, collected data, and produced all text, tables, and figures. DN provided expertise and procured the seed tubers. JH provided supervision and reviewed the text. LB provided supervision, reviewed the text, and obtained project funding.

Chapter 7: Dominic Hill (DH) produced all text.

Supplementary Material: DH produced all text, tables, and figures.

Chapter 1: Introduction to Thesis

1.1 Aims and Objectives

This project initially aimed to assess the impact of soil organic matter content on potato cultivar performance, and its efficacy in reducing the impacts of heat and drought stress. This was intended to be investigated primarily with the use of two multispectral imaging sensors, Phenospex PlantEye F500s (Phenospex, Heerlen, Netherlands), that the University of Reading had recently invested in. However, early in the COVID-19 pandemic, while the literature review presented in Chapter 2 of this thesis was being written (Hill *et al.*, 2021), it became clear that this would be impractical. To take advantage of the immobile PlantEye, experimental plants needed to be grown in pots in the glasshouses at the Crop and Environment Laboratory at the University. However, the research presented in the review made it clear that there was no consensus in the literature on how to properly grow potato for pot experiments, nor on how to impose meaningful drought stress on potato (Hill *et al.*, 2021). Further research was unable to provide solutions to these problems, as many established protocols and recommendations are impractical for potato.

One of the first decisions a crop scientist must make when planning a glasshouse or controlled environment experiment is the size of the pot in which to grow the experimental plants. The literature recommends that, to prevent the confounding effects of small pots on experimental plants, “an appropriate pot size is one in which the plant biomass does not exceed 1 g L^{-1} [of substrate]” (Poorter *et al.*, 2012). This is impractical for potato, which has been recorded to produce over 1,000 g of dry matter alone (Wheeler and Tibbitts, 1987), suggesting a minimum pot size of 1,000 L. This decision is further complicated by the difficulty of imposing appropriate drought stress on potted plants (Turner, 2019), possibly due to the inadequate water holding capacity of small pots to prevent drought stress under supposedly well-watered conditions (Sinclair *et al.*, 2017). Therefore, the first aim of this project became to understand the relationships between pot size, water availability, and potato morphology in potato and the first objective was to develop a more appropriate pot size recommendation for drought experiments in potato.

The evidence presented in Chapter 3 demonstrated that small pots confound and diminish the effects of water-restriction in potato, but that these effects reduced in pots ≥ 20 L. However, it remained unclear how pot binding, i.e., the confounding effects of small pots on plant morphophysiology, could be effectively mitigated in potato. To find solutions to this problem, the root cause of pot binding needed to be understood. It has been suggested that pot binding results from the inability of small pots to hold sufficient volumes of water to prevent drought stress between periods of irrigation under supposedly well-watered conditions (Sinclair *et al.*, 2017). This "water availability" hypothesis of pot binding is particularly relevant for potato, which is unusually productive under well-watered conditions (Sun *et al.*, 2015) but is extremely susceptible to drought stress (Schafleitner *et al.*, 2009). Therefore, the second aim of this project was to investigate the water availability hypothesis of pot binding in potato. The objectives became to develop practical methods for mitigating the effects of pot binding in potted potato experiments and, secondarily, to assess the validity of the measurements collected with the PlantEye by comparison with established, low-tech methods.

In the meantime, the method used in Chapter 5 was developed to allow the efficient phenotyping of potato while circumventing these issues. Troughs were constructed with sufficient volumes, and installed with soil moisture probes, to ensure that water was not systemically limited under intendedly well-watered conditions. The troughs were also palletised to allow for their movement from the glasshouse compartment in which they were grown to the PlantEye and back. These experiments aimed to understand potentially remotely sensible responses of potato to drought stress. Uniquely, this included investigating the effects of the resumption of irrigation on these traits, with the objective of identifying those traits which could be utilised to inform irrigation of potato in the field. To investigate the reliability of the two most promising responses, there was also the objective to replicate these experiments in the field

Finally, once canopy temperature had been identified as a trait capable of informing irrigation in potato, the experiment presented in Chapter 6 was designed. The results in Chapter 5 demonstrated that canopy temperatures increased rapidly under water restriction, returned to baseline quickly after resumption of irrigation, and could be detected as significant with a difference of < 2 °C. Therefore, we aimed to use this response to completely control an irrigation system for potatoes grown under a rain-out shelter. Due to the complexity associated with measuring canopy temperature continuously over an entire growing season, this experiment was necessarily limited in terms of sample size, plot area, and power. However, the objective was simply to assess whether the concept of temperature-controlled irrigation could be feasible in an agricultural setting, and to understand the advantages and disadvantages of doing so with wired leaf temperature probes.

1.2 References

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Chapter 2: Morphophysiology of Potato (*Solanum tuberosum*) in Response to Drought Stress: Paving the Way Forward

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2.1 Abstract

The cultivated potato (*Solanum tuberosum* L.) is currently the third most important food crop in the world and is becoming increasingly important to the local economies of developing countries. Climate change threatens to drastically reduce potato yields in areas of the world where the growing season is predicted to become hotter and drier. Modern potato is well known as an extremely drought susceptible crop, which has primarily been attributed to its shallow root system. This review addresses this decades old consensus, and highlights other, less well understood, morphophysiological features of potato which likely contribute to drought susceptibility. This review explores the effects of drought on these traits and goes on to discuss phenotypes which may be associated with drought tolerance in potato. Small canopies which increase harvest index and decrease evapotranspiration, open stem-type canopies which increase light penetration, and shallow but densely rooted cultivars, which increase water uptake, have all been associated with drought tolerance in the past, but have largely been ignored. While individual studies on a limited number of cultivars may have examined these phenotypes, they are typically overlooked due to the consensus that root depth is the only significant cause of drought susceptibility in potato. We review this work,

particularly with respect to potato morphology, in the context of a changing climate, and highlight the gaps in our understanding of drought tolerance in potato that such work implies.

2.2 Introduction

2.2.1 Potato Cultivation

The cultivated potato, *Solanum tuberosum*, originated in the New World, where its wild relatives can still be found from the southern USA (38°N) to Argentina and Chile (41°S) (Spooner *et al.*, 2004). Potato cultivation began in South America around 8,000 years ago (Lutaladio and Castaldi, 2009), resulting in the many thousands of landraces still grown by Andean smallholders (Bradshaw and Ramsay, 2009). Potatoes were first introduced to Europe in the 16th century by Spanish conquistadors during the Columbian exchange (Lutaladio and Castaldi, 2009). By the end of that century, potatoes had been introduced into the United Kingdom (UK) and Ireland, where they had a transformative effect on society, helping to feed the industrial revolution (Bradshaw and Ramsay, 2009). Records of potato breeding in Europe begin around a hundred years later in 1807 (Bradshaw and Ramsay, 2009), but overreliance on a few cultivars and clonal propagation resulted in the infamous destruction of the Irish potato crop by late blight in 1845 (Lutaladio and Castaldi, 2009). A concerted effort to produce resistant, high-yielding cultivars followed, some of which are still grown today (Lutaladio and Castaldi, 2009).

Between 2012 and 2016, potato rose from the fourth (Monneveux, Ramírez and Pino, 2013) to the third (Aliche *et al.*, 2018) most important food crop in the world, behind only rice and wheat (Camire, Kubow and Donnelly, 2009). As of 2017, potatoes are grown on about 19.3 million hectares globally, with an estimated total yield of 388 million tonnes (FAOSTAT, 2018). Over half of the global potato harvest now comes from developing countries (Mackay, 2009), where potatoes are an important source of employment, income and nutrition (Lutaladio and Castaldi, 2009). The production of potatoes in developing countries increased from 1.5 million to 21.1 million tonnes in the half century between 1961 and 2011 (FAOSTAT, 2018). Potato is a favoured crop in developed and developing countries alike as it yields more food, more efficiently than any other crop (Lutaladio and Castaldi, 2009). Approximately 85% of the biomass of a potato plants is edible: much higher than the 50% of edible biomass from cereals (Lutaladio and Castaldi, 2009). Consequently, potatoes are the most productive food crop per unit area in the world, yielding 5600 kcal/m^3: over double that of wheat (2300 kcal/m^3) (Renault and Wallender, 2000).

2.2.2 Potato Water Use in a Changing Climate

Potatoes are a relatively water-efficient crop, producing more calories per unit water used than any other crop (Sun *et al.*, 2015). A kilogram of potatoes requires only 105 l kg^{-1} of water to produce, compared to 1408 l kg^{-1} for rice, 1159 l kg^{-1} for wheat and 710 l kg^{-1} for maize (Renault and Wallender, 2000). However, in the UK and the United States of America (USA), potatoes are often supplemented with additional water, particularly in the drier areas of eastern England (Daccache *et al.*, 2011), and the warmer southern states (Byrd *et al.*, 2014) respectively. In some regions of the Mediterranean, including southern Italy, irrigation for early crops is essential to obtain marketable yields (Cantore *et al.*, 2014). In a typical dry year, maincrop potatoes in the UK need between 143 and 313 mm of irrigated water, depending primarily on the agroclimatic zone and secondarily on the local soil available water content (Knox, Weatherhead and Bradley, 1997). In the exceptionally dry year of 2018, the minimum estimated irrigation requirement for maincrop potatoes in the UK increased to 154 mm (Knox and Hess, 2019). These irrigation requirements are higher than most other crops grown in the UK including sugar beet, 0 to 253 mm; cereals, 0 to 82 mm; carrots, 0 to 258 mm; and strawberries, 0 to 132 mm (Knox, Weatherhead and Bradley, 1997). Only apple orchards are estimated to require a greater volume of irrigated water in a typical dry year than potatoes, needing 114 to 364 mm, depending on agroclimatic zone and soil type (Knox, Weatherhead and Bradley, 1997). In the southern state of Florida in the USA, potato production typically uses 10 mm of water every 24-36 hours between flowering and harvest, totalling around 610 mm (Byrd *et al.*, 2014).

Existing irrigation infrastructure for potato includes rain guns/sprinklers, furrow irrigation, and drip tape (Djaman *et al.*, 2021). In the UK, rain gun irrigation is the most prevalent method, accounting for 72% and 67% of the total irrigated area in 2001 and 2005, respectively (Weatherhead, 2007). However, this method is relatively inefficient due to water loss through evaporation, wind (Bavi *et al.*, 2009), and canopy interception (Zhou *et al.*, 2018). Drip irrigation has been shown to be a more efficient alternative for potato crops (Waddell *et al.*, 1999; Starr, 2005; Wang, Kang and Liu, 2006; Patel and Rajput, 2007; Zhou *et al.*, 2018) without significant yield reductions (Yang *et al.*, 2023). This method offers advantages such as reduced workload for growers and the ability to remain in place throughout the growing season. Subsurface drip tape installation has demonstrated positive effects on root development, nitrogen uptake, and leaching reduction (Yang *et al.*, 2023). The use of drip irrigation systems presents an opportunity for more targeted water application, both in terms of timing and location, to meet specific crop water requirements more efficiently.

Despite their water efficiency, this high water requirement makes potatoes are extremely susceptible to drought stress throughout their life cycle (Schafleitner *et al.*, 2009). The susceptibility of potato to drought has primarily been attributed to its shallow root system (van Loon, 1981), with cultivar root length being correlated with yield under drought condition (Lahlou and Ledent, 2005); and canopy characteristics (Aliche *et al.*, 2018), with stem-type canopy cultivars performing better under drought conditions than leaf-types (Schittenhelm, Sourell and Löpmeier, 2006). These characteristics can result in dramatically decreased yields under drought conditions, with one study reporting an 87% decrease in tuber number in the cultivar Désirée, which was unable to maintain stem height and leaf number under drought stress, both characteristics of stem-type cultivars (Luitel *et al.*, 2015).

As potatoes are such a drought susceptible crop (Schafleitner *et al.*, 2009), climate change represents a real threat to potato production in the UK and around the world. Regional climate changes are being brought about by global warming and its effects on weather systems at planetary, regional and local levels (Arnelf and Reynard, 1996). The specific effects of a global increase in average temperature on local weather patterns are unpredictable but, the incidence of extreme and adverse weather conditions are likely to increase, with significant effects on crop production (Harkness *et al.*, 2020). In the UK, precipitation is likely to be redistributed throughout the year, with droughts in the summer and extreme rainfall in the winter both becoming more frequent (Rial-Lovera, Davies and Cannon, 2017). Climate change has been predicted to slightly increase potato production in the UK as higher temperatures speed plant development and lengthen the growing season (Daccache *et al.*, 2011). However, due to water unavailability, the land area suited to unirrigated potato production in the UK is predicted to decrease by 74-95%, depending on future emissions (Daccache *et al.*, 2012). Historically rainfed areas will have to be irrigated in the future, increasing water demand and production costs much more than small increases in water use by already irrigated areas (Daccache *et al.*, 2012). Current irrigation infrastructure will be insufficient to meet peak water supply needs in ~50% of years (Daccache *et al.*, 2011), leading to reduced yields, increased costs and possible crop failures (Daccache *et al.*, 2012).

2.2.3 Potato Research

Despite the global popularity of potato, and its importance as a source of employment, income, and nutrition, there is a distinct lack of recent morphophysiological potato research. In the case of drought, the majority of studies investigating its effects on root growth (Tourneux *et al.*, 2003; Lahlou and Ledent, 2005; Mane *et al.*, 2008), canopy growth (Jefferies and Mackerron, 1987; Jefferies, 1993a; Deblonde and Ledent, 2001; Lahlou, Ouattar and Ledent, 2003), and yield (Levy, 1986b; Jefferies and Mackerron, 1989) are at least 10 years old. There have been recent studies, published in the last five years, observing the effects of drought on the morphophysiology of potato (Aliche *et al.*, 2018; Chang *et al.*, 2018; Michel *et al.*, 2019; Pourasadollahi *et al.*, 2019), but such studies are limited in the scientific literature. Unlike in other crops, including tomato (Susič *et al.*, 2018), grape (Zovko *et al.*, 2019), and maize (Asaari *et al.*, 2019), there has been even less research investigating the effects of drought on potato using modern phenotyping methods, such as multispectral, hyperspectral or three-dimensional imaging. In a review published in 2013 regarding drought tolerance in potato, the mean year of publication for citations that demonstrated the measurement of drought-related phenotypic responses in potato was 2001 (Monneveux, Ramírez and Pino, 2013).

The reasons for the recent disinterest in morphophysiological research in potato are unclear but may result from a feeling within the field that the effects of a specific abiotic stress on the morphophysiology of potato have been completely elucidated, or a shift in focus to the molecular and genetic components underlying these traits and responses, which have previously been reviewed (Obidiegwu *et al.*, 2015). In the case of potato and drought, the majority of morphophysiological studies were published between the late 1980s and early 2000s (MacKerron and Jefferies, 1986; Jefferies and Mackerron, 1987, 1989, 1993; Deblonde and Ledent, 2001; Lahlou, Ouattar and Ledent, 2003; Tourneux *et al.*, 2003; Lahlou and Ledent, 2005), establishing a consensus regarding the effects of drought stress on potato. While these studies form the foundation of the field, they were obviously limited by contemporary technology. These studies primarily focused on traits that could be easily measured at the time, including tuber number (MacKerron and Jefferies, 1986; Deblonde and Ledent, 2001), plant height (Deblonde and Ledent, 2001; Tourneux *et al.*, 2003), and dry matter metrics (Jefferies and Mackerron, 1987, 1993). Thus, a revivification of the field that takes advantage of modern cultivars, novel agricultural practices, and high-throughput phenotyping techniques is called for, making use of innovative methodologies, including the functional phenomics pipeline (York, 2019), to investigate potato morphophysiology with unprecedented precision.

Functional phenomics, the study of plant phenotypes as they relate to plant function under specific environmental conditions, aims to address the significant knowledge gap between the ever-advancing field of plant genetics and plant morphophysiology, which remains a limiting factor in our understanding of plant performance in an agronomic setting (York, 2019). Recent advances in imaging technologies at a range of wavelengths make this process orders of magnitude more practical as high-throughput phenotyping (HTP) platforms allow the generation of vast quantities of spectral data with much lower temporal and manual input (Kim *et al.*, 2020). Previous research investigating a specific phene, an individual genetically-determined phenotypic trait (York, Nord and Lynch, 2013) for example, canopy openness, relied on manual measurements of variables including stem height, individual leaf area, and stem and leaf dry weights (Schittenhelm, Sourell and Löpmeier, 2006). Now, a properly calibrated multispectral sensor could capture this data in seconds, alongside measures of chlorophyll conductance, water status, and vegetation indices (Kim *et al.*, 2020).

By accelerating the rate at which desirable phenes can be identified, investigated, and understood, HTP platforms have the potential to relieve the current bottleneck in plant breeding cycles (Araus and Cairns, 2014). This is essential as a doubling of global crop production is predicted to be necessary by 2050 (Tilman *et al.*, 2011), an increase which current crop yield improvement rates will be unable to meet (Ray *et al.*, 2013). HTP platforms will be an important tool in the process of accelerating crop improvement rates, although there is a risk that the generation of such vast datasets will shift the breeding cycle bottleneck from phenotyping to data analysis (Cobb *et al.*, 2013). However, advances in machine learning and data mining will likely alleviate this problem, elucidating relationships between agronomically relevant variables and compound indices which are currently too abstract to investigate (Araus and Cairns, 2014). Presently, simple regression analysis of the data captured by HTP platforms can also be used to discover discrete phenes that associate with agronomic traits under specific environmental conditions (York, 2019). However, due to the stigmatisation of data mining for hypothesis-generation, and obvious conceptual reasons, a broader understanding of crop morphophysiology as it relates to a specific environmental stress is necessary. Thus, this review is an attempt to synthesise the field as it stands, paving the way forward for morphophysiological potato research that takes advantage of developments in functional phenomics.

2.3 Methodology

An initial literature search was conducted with Web of Science, using the search terms “*Solanum tuberosum*” and “drought”. “*Solanum tuberosum*” was used instead of “potato”, or any variation thereof, to exclude references to sweet potato, *Ipomoea balatas*. The results of this search (*n* = 520) were then filtered using the Web of Science agronomy category to exclude the many biochemical, genetic, and physiological studies that have been well-covered elsewhere (Obidiegwu *et al.*, 2015). The remaining references (*n* = 110) were further filtered using Web of Science categories to exclude proceedings papers and book chapters, leaving only primary research articles (*n* = 105). The further exclusion of studies where the effects of drought on potato morphophysiology were, for our purposes, confounded by the experimental manipulation of other variables, including plant nutrition and ambient temperature, was based on the title and abstract of each paper (*n* = 23). This search found few but mostly recent studies. Thus, the remaining references included were found either as references in the papers returned by the Web of Science search, or by using the “Cited by…” hyperlink for the older papers on Google Scholar (*n* = 70). These references were subject to the same inclusion and exclusion criteria as the initial search.

As stated previously, there has been little recent research regarding the morphophysiology of potato under drought-stressed conditions, particularly concerning the investigation phenotypes that are hypothesised to confer any level of drought-tolerance. The results of the literature search ranged from 1958 to 2020 with a mean publication year of ~2001. A large number of the references reviewed (*n* = 18) were published in the ten years between 1986 and 1995, inclusive, when interest in in potato morphology was being extensively studied by the household names of potato research including R. A. Jefferies, D. Levy, C. D. van Loon, D. K.

MacKerron, and P. C. Struik. However, twenty-five references were found that were published between 2011 and 2020, inclusive, fourteen of which were published in the last five years. These more recent studies are often investigating various genetic and biochemical markers of drought but warrant inclusion here as they also include relevant measurements, for our purposes, including tuber fresh weight, number and dry matter, which remain inescapable due their commercial significance.

2.4 Effects of Drought on Potato

2.4.1 Effects of Drought on Potato Growth

Drought is technically a purely meteorological term that describes a prolonged period of time with little or no rain (Solh and Van Ginkel, 2014). From a biological perspective, the definition of drought is expanded to include its effects on plant life. Drought in this context is still a period of little or no rain, but one which leads to a soil moisture deficit and, consequently, a reduction of water potential in affected plant tissues (Mitra, 2001). In agriculture, drought may be considered as a period of water shortage that leads to a moisture deficit in the soil and drought stress in a crop, preventing the crop from reaching its maximum genetic potential yield (Mitra, 2001). Drought stress is a crop’s response to drought and includes the morphological and physiological adaptations that occur when plants perceive the loss of enough water to maintain pre-drought growth (Cheruth *et al.*, 2009). The effects of drought on potato, discussed below, are technically the result of a plant-initiated response to an environmental change which causes the plant to prioritise survival and reproduction over optimum growth and yield.

Drought may further be defined in terms of onset and duration with respect to a crop's life cycle. Intermittent drought describes one or more periods of an inadequate water supply for optimum growth that occur at any time throughout the growing season (Neumann, 2008). After intermittent drought, soil moisture is restored allowing normal growth to resume. This differs from terminal drought, which also describes a period of inadequate water supply for optimal growth, but one from which there is no replenishment of soil moisture within the crop's life cycle (Neumann, 2008). Terminal drought causes a progressive decline in soil moisture and, depending on its severity and duration, may result in reduced yields and even early plant death (Neumann, 2008).

Drought stress occurs when plants lose, or perceive the loss of, enough water to maintain optimal growth (Cheruth *et al.*, 2009). Plants generally respond to moderate drought stress with the closure of stomata to reduce further water loss via evapotranspiration (Keskin, Tumer and Birinci, 2010). This response also reduces gas exchange through the stomata, limiting CO_2 availability for photosynthetic assimilation (Cornic, 2000; Pourasadollahi *et al.*, 2019). Stomatal closure was previously believed to be a primarily hydraulic response to a decrease in leaf water potential caused by an excessive loss of water by evapotranspiration, regardless of root water potential or soil moisture (Kramer, 1988). However, in many plants, including potato, stomatal closure occurs before any drop in leaf water potential is detectable (Jefferies and Mackerron, 1989; Davies and Zhang, 1991). While hydraulic mechanisms likely do have some role in regulating stomatal conductance (Davies and Zhang, 1991), chemical processes have been shown to regulate stomatal conductance even before any detectable change in leaf water potential (Davies and Zhang, 1991).

Abscisic acid (ABA) has been identified as a key molecule involved in root-to-shoot signalling of a drought stress and as an important regulator of stomatal conductance in wheat (Ali, Jensen and Mogensen, 1998), maize (Bahrun *et al.*, 2002) and soybean (Liu, Jensen and Andersen, 2003). Potato roots tips have been shown to produce ABA as a response to a moderate decrease in soil moisture (Liu *et al.*, 2005). A linear relationship between xylem-borne ABA, the concentration of which is increased by ABA production in the roots, and stomatal conductance has been observed at mild soil water deficits in potato (Liu *et al.*, 2005). This suggests that chemical root-to-shoot signalling has an important role in stomatal conductance even before detectable decreases in leaf water potential. But, the relationship between ABA and stomatal conductance is less significant at severe soil water deficits (Liu *et al.*, 2005), implying the presence of other unknown mechanisms involved in regulating stomatal conductance in potato. The relationship between ABA and drought tolerance in potato has recently been confused further by evidence that suggests that, only one of two drought-tolerant cultivars, Gwiazda is hypersensitive to ABA signalling, closing its stomata significantly earlier when treated directly with ABA, compared to both, the drought-tolerant cultivar, Tajfun and, the drought-susceptible cultivar, Oberon (Boguszewska-Mańkowska *et al.*, 2018). This suggests the presence of multiple mechanisms contributing to the drought tolerance or susceptibility of potato cultivars (Boguszewska-Mańkowska *et al.*, 2018), some of which remain unknown.

It is also possible that, at higher moisture deficits the above effects reduce a potato plant's ability to mount an appropriate adaptive response to prevent further water loss. More severe drought stress, or desiccation, has increasingly significant effects on plant cell structure and function as water loss increases (Cheruth *et al.*, 2009). Intense drought stress can cause damage to cellular structure by reducing turgor pressure (Moore *et al.*, 2008), decreasing enzymatic activity involved in ATP production and carbon fixation (Farooq *et al.*, 2009), and ultimately plant death (Munné-Bosch and Alegre, 2004). While drought stress is an undesirable response in agronomic terms, it is important to note that drought stress facilitates adaptive mechanisms which evolved as prophylaxes against the above effects at the expense of maximum yields (Basu *et al.*, 2016).

The effects of drought on potato growth vary greatly depending on the cultivar-specific canopy and root characteristics described below. The effects of drought stress on potato also depend on abiotic factors including the duration, timing (Jefferies and Mackerron, 1993) and severity (Stark *et al.*, 2013) of water stress, the implementation of which has never been standardised, as shown in Tables 1, 2 and 3. Existing soil moisture (Jefferies and Mackerron, 1993), nutrient availability (Saravia *et al.*, 2016) and evaporative demand (Jefferies and Mackerron, 1993) further complicate the effects of drought on potato growth. However, drought represents one of the most essential biological challenges to all crop species (Shao *et al.*, 2009). Thus, the effects of drought on fundamental potato plant growth are relatively consistent with small differences between cultivars of primarily agronomic significance (Schittenhelm, Sourell and Löpmeier, 2006).

2.4.2 Effects of Drought on Below Ground Growth in Potato

The effects of drought on below ground potato growth are well studied, but these studies often find seemingly contradictory results (Table 1). Drought has been shown to increase maximum root depth (Steckel and Gray, 1979; Lahlou and Ledent, 2005) which, logically, allows potato plants access to deeper soil water (Stalham and Allen, 2004). Total root length, on the other hand, has been found to decrease (Albiski *et al.*, 2012), remain consistent, and increase (Boguszewska-Mańkowska, Zarzyńska and Nosalewicz, 2020) in response to water stress. Similarly, root dry mass has been observed to increase (Tourneux *et al.*, 2003; Lahlou and Ledent, 2005; Anithakumari *et al.*, 2011), decrease (Lahlou and Ledent, 2005; Mane *et al.*, 2008) and remain constant (Mane *et al.*, 2008) under drought conditions. Stolon number has also been found to both increase (Lahlou and Ledent, 2005) and decrease (Haverkort, Van De Waart and Bodlaender, 1990) due to drought stress.

These apparent contradictions are likely due to differences between cultivar genotype x environment (GxE) responses to drought (Rudack *et al.*, 2017; Boguszewska-Mańkowska, Zarzyńska and Nosalewicz, 2020), which become exaggerated with increasing water stress (Epstein and Grant, 1973). Experimental variation, including differences in drought severity, duration and timing; location, soil type, and tuber physiological age, contribute to these conflicting results (Steckel and Gray, 1979; Obidiegwu *et al.*, 2015). Root growth in potato is also particularly susceptible to soil compaction which reduces root depth and density (Stalham *et al.*, 2007), preventing potato from more extensively foraging for water and nutrients (White *et al.*, 2005). Due to the unpredictable effects of these factors on root growth during drought and challenges in quantifying root growth accurately, it may be more productive to focus on above ground growth to reduce water stress.

Root responses to drought are clearly highly varied in potato, but the underlying mechanisms that sense water scarcity are common across other plant species. Generally, plants sense local water scarcity through several transmembrane proteins, with a diverse range of downstream effects, including facilitating Ca2+ influx (Lamers, van der Meer and Testerink, 2020). These effects then trigger the range of architectural changes in root structure described above through root cell division, elongation, and differentiation, coordinated by root apices (Gupta, Rico-Medina and Caño-Delgado, 2020). In addition to these responses, plants also attempt to limit water loss through stomata through the closure of guard cells, mediated by decreased turgor pressure (Gupta, Rico-Medina and Caño-Delgado, 2020). As evapotranspiration is the primary method by which plants dissipate thermal energy (Gates, 1964), the investigation of canopy temperatures may also prove useful in understanding the response of potato to drought.

Table 1. A summary of the effects of drought stress on key physiological root traits in potato and the range of methodologies by which these variables were manipulated.

Reference	**(Albiski et al., 2012)**	**(Anithakumari et al., 2011)**	**(Haverkort et al., 1990)**	**(Lahlou & Ledent, 2005)**	**(Mane et al., 2008)**	**(Tourneux et al., 2003)**
Observations	Decreased root length	Increased root dry mass	Decreased stolon number	Increased root depth, increased root dry mass (Remarka, Nicola & Monalisa), decreased root dry mass (Désirée), increased stolon number	Decrease root dry mass (Ccompis), no effect on root dry mass (Sulla)	Increased root dry mass
Cultivar	SY-C.1, SY-C.2, SY-C.3, SY-C.14, SY-C.28, SY-C.29, SY-C.31, SY-C.46, SY-C.52, SY-C.53, SY-C.54, SY-C.55, SY-C.56, SY-C.57, SY-C.58, SY-C.59, SY-C.60, SY-C.61	A random subset of the C × E diploid potato mapping population	Radosa, Bintje	Remarka, Dérirée (field and greenhouse); Nicola, Monalisa (field only)	Sullu (subsp. *andigenum*), Ccompis (subsp. *andigenum*)	Alpha, Waycha (subsp. *andigenum*), Luky (subsp. *andigenum*), Ajahuiri (*Solanum ajanhuiri*), Janko Choquepito (*Solanum curtilobum*), CIP 382171.10 (subsp. *tuberosum* × subsp. *andigenum*)
Culture Method	In vitro	In vitro	Field & Pots	Field (Remarka, Désirée, Nicola & Monalisa) Pots (Remarka & Désirée)	Field	Pots
Drought Conditions	Six variations of growth media containing 0, 2, 4, 6, 8 or 10 % (w:v) sorbitol to create graduated water potentials between -0.58 MPa (least sever water stress) to -2.5 MPa (most severe water stress).	Water potential of growth media lowered to -0.7 MPa by the addition of polyethylene glycol (PEG) for 7 weeks. 3 of 7 replicates were then allowed to recover for 4 weeks.	Irrigated to field capacity when soil moisture exceeded 100 kPa.	Rainfed in the field. Irrigated to field capacity when soil moisture dropped below -0.8 MPa in the pots.	Irrigated as controls until 45 days after planting when irrigation was completely suspended for 59 days (unclear if drought was terminal or intermittent).	Plants irrigated as controls until being subjected to either intermittent drought (gradual decline in water supply for five weeks, and one week with no water supply followed by full restoration of water supply) or terminal drought (same as intermittent drought but with no restoration of water supply) at tuberization.
Control		Plants were grown in the same growth media in the absence of PEG. Water potential unclear.	Irrigated to maintain soil moisture levels at "near field capacity" constantly.	Irrigated with 20 mm five times throughout the season in the field. Irrigated to field capacity when soil moisture dropped below -0.3 MPa in the pots.	Irrigated to maintain soil moisture between 0 and -0.02 MPa.	Irrigated to field capacity twice per week.

2.4.3 Effects of Drought on Above Ground Growth in Potato

Canopy growth is one of the most drought sensitive biological processes in plants (Shao *et al.*, 2009) and is a result of the irreversible elongation of many individual plant cells (Lockhart, 1965). This process is reliant on the maintenance of high turgor pressure, which stretches plant cell walls causing cell expansion and thus plant growth (Szabolcs, 1999). Consequently, when the fraction of transpirable soil water falls below a species-specific threshold, leaf growth ceases (Schafleitner, 2009). In most crops, leaf growth stops when the transpirable soil water drops below 40-50% (Weisz, Kaminski and Smilowitz, 1994), but in potato, leaf growth declines with 60% of transpirable water remaining in the soil, highlighting its sensitivity to drought stress (Weisz, Kaminski and Smilowitz, 1994). Thus, the first noticeable effect of drought stress on potato is reduced leaf growth (Jefferies and Mackerron, 1987), resulting in potato canopy growth being more affected by drought stress than root growth (Boguszewska-Mańkowska, Zarzyńska and Nosalewicz, 2020). Drought also typically decreases both the individual leaf area (Jefferies, 1993b; Kesiime *et al.*, 2016) and number of green leaves (Deblonde and Ledent, 2001) in potato, as well as reducing potato stem number (Lahlou, Ouattar and Ledent, 2003; Chang *et al.*, 2018) and height (Deblonde and Ledent, 2001; Chang *et al.*, 2018), although the latter is less affected in early cultivars (Deblonde and Ledent, 2001). Through these mechanisms, the evidence for which is summarised in Table 2, drought reduces the photosynthetic area of the canopy: the primary determinant of productivity in potato (Allen and Scott, 1980).

Table 2. A summary of the effects of drought stress on key physiological canopy traits in potato and the range of methodologies by which these variables were manipulated.

Reference	(Aliche et al., 2018)	(Chang et al., 2018)	(Deblonde & Ledent, 2001)	(Jefferies, 1993)	(Jefferies & Mackerron, 1987)	(Lahlou et al., 2003
Observations	Decreased canopy growth rate, fewer new leaves, premature leaf shedding	Decreased stem length	Reduced leaf number, reduced stem height	Reduced individual leaf area	Reduced leaf growth	Reduced stem number
Cultivar	103 commercial cultivars	Chubaek, Superior, Jayoung	Eersteling, Jaerla, Krostar Eersteling, Claustar, Bintje, Nicola, Désirée	19 commercial cultivars	Maris Piper, Record, Désirée, Pentland Crown, Pentland Dell, Pentland Squire	Remarka, Dérirée (field and greenhouse); Nicola, Monalisa (field only)
Culture Method	Field	Field	Field	Field	Field	Field (Remarka, Désirée, Nicola & Monalisa) Pots (Remarka & Désirée)
Drought Conditions	Rainfed.	Rainfed until emergence and then totally deprived of water until tuberisation. After tuberization, plants were irrigated when indicators of drought stress were visible (“wilting and growth retardation”).	Deprived of water by a plastic sheet at 50% emergence for 8 weeks.	Deprived of water by a mobile rain shelter from emergence to harvest.	Deprived of water by polythene sheeting laid over the plants from emergence to harvest.	Rainfed in the field. Irrigated to field capacity when soil moisture dropped below -0.8 MPa in the pots.
Control	Rainfed plus irrigated with roughly 15 to 30 mm of water on 14 occasions.	Predominantly rainfed, irrigated with trickle irrigation between May and June during a dry period. Plants were irrigated when indicators of drought stress were visible (“wilting and growth retardation”).	Rainfed only or rainfed plus 37 and 35 mm of irrigation in 1996 and 1996, respectively.	Rainfed plus sprinkler irrigation to maintain a soil moisture deficit of <25 mm.	Rainfed plus trickle irrigation to maintain a soil moisture deficit of <30 mm.	Irrigated with 20 mm five times throughout the season in the field. Irrigated to field capacity when soil moisture dropped below -0.3 MPa in the pots.

In a recent and comprehensive study investigating the effects of drought on 103 cultivars of potato, the response of canopy growth to drought stress was found to be highly variable (Aliche *et al.*, 2018). Generally, naturally occurring periods of drought reduced canopy growth regardless of drought timing (Aliche *et al.*, 2018). This is a logical result of a lack of water inhibiting plant growth: the product of high turgor pressure forcing cell expansion (Szabolcs, 1999). Early drought was found to slow canopy growth, increasing the time taken for plants to reach optimum canopy cover (Aliche *et al.*, 2018). Later drought had a greater effect on maximum canopy cover due to reduced new leaf formation and early shedding of mature leaves (Aliche *et al.*, 2018). Early drought has also recently been demonstrated to slow canopy development by reducing stem length by 75 to 78%, further increasing time to full canopy cover (Chang *et al.*, 2018). This result was replicated over two growing seasons, and in one of the two study years, stem thickness and stem number were also found to be significantly decreased by drought (Chang *et al.*, 2018). The lack of statistical significance in the first trial year was likely due to a shorter drought period which, crucially, ended before emergence when stem number is effectively fixed, baring the death of individual stems (Chang *et al.*, 2018). The significant results from the following year corroborate older findings regarding the negative effects of drought on stem length (Deblonde and Ledent, 2001) and number (Lahlou, Ouattar and Ledent, 2003) in potato.

Another recent study found that drought significantly reduced the leaf area index (LAI) of three cultivars, Karaka, Moonlight, and Russet Burbank, subjected to drought for the duration of the life cycle (Michel *et al.*, 2019). Droughted plants were irrigated with a fifth of the volume of water supplied to well-watered plants to prevent early senescence (Michel *et al.*, 2019). Each cultivar was affected similarly, with drought stress reducing LAI from the end of the first month after planting (Michel *et al.*, 2019). Except for cv. Karaka, LAI started to decline earlier under drought conditions compared to well-watered conditions (Michel *et al.*, 2019), reducing leaf area duration (LAD) and thus the total radiation intercepted throughout the life cycle: the primary determinant of dry biomass production in potato (Allen and Scott, 1980). This finding was recently corroborated in the cultivars Desirée and Karú INIA, where water restriction was found to have a greater negative effect on tuber yield than high temperatures, due to the effects of drought stress on LAD (Ávila-Valdés *et al.*, 2020). LAI was also found to decrease in the cv. Banba under drought conditions, although it is unclear how LAI or LAD were affected in this cultivar over the course of the life cycle (Pourasadollahi *et al.*, 2019). These findings corroborate previous work that suggested that LAD, rather than the maximum LAI at a single point, was most strongly associated with biomass production, most of which is partitioned to tubers (Jefferies and Mackerron, 1993). The differences seen in LAI between cultivars may be due to differences in canopy architecture (Michel *et al.*, 2019), which will be discussed later in this review.

The timing of drought has varying effects on different cultivars, particularly with respect to maturity classes (Aliche *et al.*, 2018; Chang *et al.*, 2018). As late maturing cultivars generally require longer to reach exponential canopy growth and full canopy cover, compared to early maturing cultivars (Aliche *et al.*, 2018), late droughts are effectively earlier in the life cycle of late cultivars. This may be indicative of an ability of late maturing cultivars to recover after late droughts by delaying achievement of full canopy cover, which has previously been suggested (Romero *et al.*, 2017). By taking longer to achieve full canopy cover, the relatively large canopies of late maturing cultivars may be less affected by the canopy reduction effects of late drought, allowing these cultivars to recover post-drought and compensate for lost growth. The relatively large canopies of late maturing cultivars have been demonstrated to persist for much longer than similarly droughted early maturing cultivars, increasing LAD, which likely accounts for the significantly higher yields found in late maturing cultivars under drought stress (Aliche *et al.*, 2018).

The cumulative effects of drought stress on above ground potato growth are a result of a reduction in the rate of photosynthesis within the leaves (Pieters and El Souki, 2005). Drought stress affects photosynthesis by limiting ribulose bisphosphate (RuBP) production (Tezara *et al.*, 1999). RuBP production is affected by reduced adenosine triphosphate (ATP) synthesis, which is inhibited by the high intracellular ionic concentration caused by the low relative water content of leaves during drought stress (Lawlor, 2002). Reduced photosynthetic carbon assimilation leads to the unavailability of substrates required for respiration and plant growth (Flexas *et al.*, 2006). Reduced CO_2 concentration in the mesophyll due to stomatal closure may also decrease photosynthetic rate in plants during drought (Cornic, 2000).

The relative effects of stomatal closure and reduced RuBP production under drought conditions are debated (Parry *et al.*, 2002). Decreased RuBP production has been suggested as the primary effect of drought on growth in soybean (Vu, Allen and Bowes, 1987) and sunflower (Tezara *et al.*, 1999). In several other species, including common vine grape and common bean, stomatal conductance has been suggested as the factor limiting plant growth under drought (Bota, Medrano and Flexas, 2004). The growth limiting effect of drought stress may be species, or even cultivar, specific and vary depending on relative soil water content (Bota, Medrano and Flexas, 2004) and remains unstudied in potato.

Canopy temperature has also widely been identified as an effective trait for selecting drought-tolerant cultivars and therefore measuring water stress in many species (Chaudhuri and Kanemasu, 1985; Chaudhuri *et al.,* 1986; Hatfield, Quisenberry and Dilbeck, 1987; Blum *et al.*, 1989; Stark, Pavek and McCann, 1991; Mahmud *et al.*, 2016; Anderegg *et al.*, 2021). Previous research has shown that water-restriction can significantly increase canopy temperatures in potato. For example, high-frequency deficit irrigation to 50% pot capacity resulted in an average increase of 2.3°C ± 0.7°C at 1 pm across five cultivars (Mahmud *et al.*, 2016). Field studies with cv. Unica demonstrated even larger increases of ~4°C between 3-4 pm under water-restricted conditions (Rinza *et al.*, 2019). This rise in canopy temperature is attributed to reduced transpiration rates caused by stomatal closure in response to soil moisture deficits (Fuchs, 1990). Consequently, canopy temperature serves as a valuable indicator of both drought stress and periods of reduced yield accumulation in potato plants.

Leaf greenness, measured as SPAD units, has emerged as another important trait for assessing drought stress and improving crop yields (Monteoliva, Guzzo and Posada, 2021). Water-restriction has been shown to increase leaf greenness in potato, with severe water-restriction in one pot experiment pots resulting in an increase of ~10 SPAD units for cv. Unica (Ramírez *et al.*, 2014). Pot experiments with cvs. Sarnav, Unica, and Désirée have also found increases of ~5 SPAD units within 10 days of water-restriction (Rolando *et al.*, 2015). These findings have been further supported by recent studies on six cultivars under short- and long-term water-restriction, although with less consistent differences between cultivars and treatments (Li *et al.*, 2019). It has been suggested that the effects of water-restriction on leaf greenness are associated with reduced leaf growth in drought-susceptible cultivars, suggesting that chlorophyll concentrations rise due to reductions in leaf area (Rolando *et al.*, 2015).

2.5 Effects of Drought on Potato Yield

As the primary outcome of agronomic significance, the effects of drought on tuber yield have been more extensively studied. Tuber yields after drought stress have been quantified in several ways, including total fresh tuber mass (Lahlou, Ouattar and Ledent, 2003; Carli *et al.*, 2014), total tuber dry matter (Deblonde, Haverkort and Ledent, 1999), marketable tuber yield (Steckel and Gray, 1979; Cantore *et al.*, 2014) and tuber number (Deblonde and Ledent, 2001). In general, all these metrics are reduced by drought (Obidiegwu *et al.*, 2015), with some exceptions (Nadler and Heuer, 1995). This review will focus on tuber fresh mass and tuber dry matter and concentration as these are the most economically relevant measures of yield.

2.5.1 Effects of Drought on Fresh Tuber Mass

Fresh tuber yields are primarily dependent on tuber dry matter and water content (Jefferies and Mackerron, 1993). Tuber water content and radiation interception are the morphophysiological traits most affected by drought stress in potato (Jefferies and Mackerron, 1987). Fresh potato tubers have a water content of around 80%, with a small amount of variation between cultivars (Navarre, Goyer and Shakya, 2009). This makes fresh tuber mass highly vulnerable to drought stress, having been shown on more than one occasion to be a greater contributor to yield loss than tuber number (Struik and Van Voorst, 1986; Carli *et al.*, 2014). The vast majority of previously reviewed evidence showing significantly decreased fresh tuber mass after drought (Obidiegwu *et al.*, 2015).

Total water deprivation from emergence to harvest can reduce relative tuber water content of Maris Piper by 69%, compared to potatoes irrigated with sufficient water to maintain a soil water deficit of no greater than 30 mm (Jefferies and Mackerron, 1989). The effects of drought on fresh tuber mass appear to be highly cultivar-dependent (Lahlou, Ouattar and Ledent, 2003). Fresh tuber yield reductions in a single study ranged from 11% to 44% in Désirée and Remarka respectively (Lahlou, Ouattar and Ledent, 2003). In this study, field grown potatoes were not totally deprived of water, receiving 148 mm of effective rainfall across the season, which may account for lower yield losses than those observed in totally water deprived Maris Piper (Jefferies and Mackerron, 1989). Many different protocols have been used to assess the effects of drought stress on potato tubers, as shown in Table 3, making it difficult generalise the effects of drought stress on potato, even within cultivars.

Table 3. A summary of the effects of drought stress on key physiological tuber traits in potato and the range of methodologies by which these variables were manipulated.

Reference	**(MacKerron & Jefferies, 1986)**	**(Martin et al., 1992)**	**(Painter & Augustin, 1976)**	**(Steckel & Gray, 1979)**	**(Tourneux et al., 2003)**
Observations	Decrease in tuber number	No effect on processing quality, no effect on prevalence of internal or external defects	Increase in prevalence of misshapes	Reduced tuber dry matter	Decreased dry matter concentration (Up-to-Date & Troubadour), increased dry matter concentration (Alpha)
Cultivar	Maris Piper	Russet Burbank	Russet Burbank	King Edward, Pentland Crown, Majestic, Maris Piper	Alpha, Waycha (subsp. *andigenum*), Luky (subsp. *andigenum*), Ajahuiri (*Solanum ajanhuiri*), Janko Choquepito (*Solanum curtilobum*), CIP 382171.10 (subsp. *tuberosum* × subsp. *andigenum*)
Culture Method	Greenhouse plots	Field	Field	Field	Pots
Drought Conditions	Twenty-two treatments with varying lengths (8 to 40 days) of total water deprivation at either 50% emergence, tuber initiation or small tuber stage.	Six treatments trickle irrigated with 30 mm when SMD reached 50 mm. Irrigation was removed and rain was excluded at various points during the season with varying severity.	Four water treatments. 1) Soil moisture was depleted to 25% before irrigation during early tuber set and then depleted to 65% before irrigation for the remained of the season. 2) Soil moisture was depleted to 65% before irrigation. 3) Soil moisture was depleted to 75% before irrigation. 4) Soil moisture was depleted to 85% before irrigation. How much water was given is unclear but assumed to restore field capacity.	Deprived of water by a mobile rain shelter from emergence to harvest, except for one bout of irrigation with 25 mm of water at the time of tuber formation.	Plants irrigated as controls until being subjected to either intermittent drought (gradual decline in water supply for five weeks, and one week with no water supply followed by full restoration of water supply) or terminal drought (same as intermittent drought but with no restoration of water supply) at tuberization.
Control		Three treatments trickle irrigated with either 20 mm of water when soil moisture deficit reached 30 mm, 30 mm when SMD reached 50 mm or 50 mm when SMD reached 50 mm.		Rainfed plus irrigation to field capacity when the soil moisture deficit reached 25 mm.	Irrigated to field capacity twice per week.

(Continued)

Table 3. Continued

Reference	(Jefferies & Mackerron, 1987)	(Jefferies & Mackerron, 1989)	(Jefferies & MacKerron, 1993)	(Lahlou et al., 2003)	(Lefèvre et al., 2012)	(Levy, 1986)	(Luitel et al., 2015)
Observations	Decreased total dry matter, increased dry matter concentration	Reduced tuber water content	Reduced tuber dry matter concentration	Reduced fresh tuber yield	Increased tuber water content	No effect on prevalence of misshapes, increase in prevalence of misshapes (Kondor)	Decreased tuber number
Cultivar	Maris Piper, Record, Désirée, Pentland Crown, Pentland Dell, Pentland Squire	Maris Piper	21 commercial cultivars	Remarka, Dérirée, Nicola, Monalisa	21 Andean cultivars	Blanka, Kondor, Draga, Monalisa, Alpha, Désirée, Romano, unnamed clone, Cara	Five CIP clones, the German cultivar NPI-106, Désirée
Culture Method	Field	Field	Field	Field (all), Pots (Remarka & Désirée)	Outdoor controlled plots	Field	Field
Drought Conditions	Deprived of water by polythene sheeting laid over the plants from emergence to harvest.	Deprived of water by a mobile rain shelter from emergence to harvest.	Deprived of water by a mobile rain shelter from emergence to harvest.	Rainfed in the field. Irrigated to field capacity when soil moisture dropped below -0.8 MPa in the pots.	As control but irrigation stopped for 58 days, 86 days after planting.	Irrigated every 3 to 4 days to replace either 0.64 to 0.89 or 0.40 to 0.67 times water lost to evapotranspiration.	Irrigated once soon after planting, then total water deprivation.
Control	Rainfed plus trickle irrigation to maintain a soil moisture deficit of <30 mm.	Rainfed plus trickle irrigation to maintain a soil moisture deficit of <30 mm.	Rainfed plus sprinkler irrigation to maintain a soil moisture deficit of <25 mm.	Irrigated with 20 mm five times throughout the season in the field. Irrigated to field capacity when soil moisture dropped below -0.3 MPa in the pots.	Deprived from rainfall by a plastic rain shelter and 60 cm below-ground barrier. Drip irrigated to maintain a soil water potential between 0 and -0.02 MPa.	Irrigated every 3 to 4 days to replace water lost by evapotranspiration.	Rainfed and furrow irrigated when soil moisture dropped below 8% to maintain “ideal moisture conditions (8-16%)”.

In contrast, some Andean potato cultivars have been demonstrated to significantly increase tuber water content during drought stress (Lefèvre *et al.*, 2012). This may be due an adaptive drought response that increases tissue K^+ concentrations, which improve osmotic regulation of tuber water content (Khosravifar *et al.*, 2008). K^+ supplementation has been shown to promote sucrose storage despite lower assimilate production due to drought stress which may further contribute to osmotic regulation and tuber fresh weight in drought tolerant landraces (Allison, Fowler and Allen, 2001). These Andean cultivars are the exception and represent a subspecies of cultivated potato, *Solanum tuberosum* subsp. *andigenum*, genetically distinct from commercially cultivated cultivars (Raker and Spooner, 2002). However, the Andean population is an important source of genetic variation for use in commercial *Solanum tuberosum* subsp. *tuberosum* breeding programs (Sukhotu and Hosaka, 2006). As maintaining tuber water content is a key trait associated with yield maintenance under drought conditions (Jefferies and Mackerron, 1989), these cultivars may prove useful in the future.

2.5.2 Effects of Drought on Total Tuber Dry Matter

Tuber dry matter correlates with, and is used as a proxy for, yield and quality in potato (Dull, Birth and Leffler, 1989). As total dry matter production in potato is proportional to total intercepted radiation (Allen and Scott, 1980), drought stress indirectly reduces tuber dry matter production by reducing the photosynthetic area of the canopy (Jefferies and Mackerron, 1989). Dry matter concentration is clearly highly dependent on tuber water content (Jefferies and Mackerron, 1987) and is most commonly used as an index of tuber quality, especially for processing cultivars (Pritchard and Scanlon, 1997). Low dry matter concentrations in processing cultivars are also associated with higher production costs (Pritchard and Scanlon, 1997). Total tuber dry matter is a more important marker for total yield as it indicates the efficiency of assimilate translocation into tubers (Jovanovic *et al.*, 2010). As such economically significant markers, total dry matter and dry matter content have been extensively investigated in potato. An early study found consistent decreases in tuber dry matter, after total post-emergence water deprivation, in cultivars Pentland Dell, Majestic, Maris Piper and King Edward (Steckel and Gray, 1979). These represent a range of reputed drought sensitivities, including the drought tolerant Pentland Crown, and drought susceptible King Edward (Steckel and Gray, 1979). The decreases in total dry matter due to drought stress were remarkably similar between these two cultivars: 15.2 to 7.0 and 15.5 to 6.7 t ha^{-1} respectively (Steckel and Gray, 1979). However, the reported drought tolerance of Pentland Crown was found to be due to its ability to maintain dry matter in marketable tubers, defined as >40 mm in length (Steckel and Gray, 1979). This showed a need to investigate many variables in many cultivars to fully understand the effects of drought stress on total dry matter, especially in the context of marketable output.

Nineteen cultivars of potato, totally deprived of water from emergence to harvest, had a 52% higher tuber dry matter concentration, on average, than plants irrigated to maintain a maximum soil moisture deficit of 25 mm (Jefferies and Mackerron, 1993). The drought stressed plants also had, averaged across all cultivars, 44% less tuber dry matter than the irrigated plants (Jefferies and Mackerron, 1993). This supports the suggestion that increased tuber dry matter concentration is likely a function of reduced water content after drought, rather than of higher dry matter production (Jefferies and Mackerron, 1989). However, while every cultivar showed an increased dry matter concentration after drought, not all cultivars had reduced total dry matter; in cultivars Baillie, Duke of York and Ulster Sceptre changes in total dry matter were statistically insignificant (Jefferies and Mackerron, 1993). The authors propose this results from already low total dry matter in these cultivars under irrigation but, Draga, the second lowest yielding cultivar with irrigation, did show significant reductions in total dry matter due to drought stress (Jefferies and Mackerron, 1993). This hypothesis was disputed by later evidence that demonstrated that some cultivars have the potential to produce relatively high dry matter yields under drought stress, despite performing relatively poorly under well-watered conditions (Steyn *et al.*, 1998). Any inherent differences in drought tolerance of these cultivars, which could account for the insignificant changes, were not acknowledged. Baillie, Duke of York and Ulster Sceptre are classified respectively as “medium-to-high”, “medium” and “high” drought resistant cultivars by the European Cultivated Potato Database (The European Cultivated Potato Database, 2008, 2011, 2018) and so should be expected to maintain total tuber dry matter yields under drought stress.

These results contrasted with previous research which found that, while dry matter concentration significantly decreased in cultivars Up-to-Date and Troubadour, dry matter concentration in the cultivar Alpha increased under intermittent drought stress (Levy, 1983). This may be a demonstration of the cultivar’s ability to prevent water loss by evapotranspiration due to the low surface area of its relatively small canopy (Tourneux *et al.*, 2003). However, in this experiment, a “white course net” was used to reduce the infection of subject plants with aphid-borne potato viruses (Levy, 1983). This method had previously been demonstrated to reduce available solar radiation by 18% (Marco, 1981), which may have disproportionately countered the purported advantages of large canopies with respect to drought tolerance (Schittenhelm, Sourell and Löpmeier, 2006).

Grafting experiments have shown that potato scions have a greater effect on the relative partitioning of dry matter into tubers than root stock (Jefferies, 1993a). Dry matter was preferentially partitioned into the canopy in Cara scion grafts, compared to Désirée, corresponding with greater canopy expansion, but lower tuber dry matter, under drought stress (Jefferies, 1993a). In contrast, a positive association between stem length and tuber dry matter has also been shown under conditions of total water deprivation (Deblonde and Ledent, 2001). This relationship was weak ($R^2 = 0.53$; significant at $P < 0.1$), and only observed in one of the two trial years (Deblonde and Ledent, 2001). The experimental design also included using a “strong plastic sheet” to exclude rainfall from the droughted plots which was placed directly on the soil surface (Deblonde and Ledent, 2001). Holes were cut in the sheet for the plants at 50% emergence but, the weight of this sheet could have had a stunting effect on stems emerging later, potentially confounding the relationship between stem length and tuber dry matter. Regardless, these data could be evidence of a dominant effect of canopy architecture on drought tolerance in potato, but the nature of this relationship and the trade-offs between relative assimilate partitioning, canopy radiation interception and evapotranspiration remain unknown.

2.5.3 Effects of Drought on Potato Quality

There is little previous research directly observing the effects of drought stress on physical defects in potato. This is perhaps because the primary measure of quality in processing cultivars is dry matter concentration (Pritchard and Scanlon, 1997) which has been covered above. However, structural defects have been shown to occur after even short periods of drought stress (van Loon, 1986). Intense periods of drought stress followed by heavy rainfall or irrigation during tuber bulking result in higher rates of misshapen tubers than continuous drought stress throughout the bulking phase (van Loon, 1986). Secondary growth can occur after tuber water potential drops to -500 kPa for as little as three days as intra-tuber irregularities in the conversion of assimilates into storage products causes variable growth rates across the tuber (Moorby, Munns and Walcott, 1975). Why tubers grow uniformly before drought stress but irregularly after it is unknown, but drought stress may induce irregular intra-tuber maturation patterns which, when resupplied with water, lead to faster rates of tuber bulking in less mature areas of the tuber.

The effects of drought stress on secondary growth in potato may be confounded by the effects of temperature, which has been demonstrated to cause secondary growth regardless of drought stress (Bodlaender, Lugt and Marinus, 1964). This study also found no secondary growth in plants grown at 16°C which were subject to repeated bouts of total water deprivation, lasting several weeks, followed by an unknown amount of water (Bodlaender, Lugt and Marinus, 1964). It’s unclear whether these results are because of drought stress on secondary growth or the effects of temperature on variables not measured in this experiment; slower evaporation rate, slower growth and altered water-use efficiency could all confound the effects of low temperature and drought stress on secondary growth.

The effects of drought stress on the prevalence of misshapen tubers in potato may be cultivar dependent. The previous research is unclear on the cultivar/s used, but a more comprehensive analysis of nine cultivars found no association between drought intensity and the prevalence of misshapen tubers, except in the cultivar Kondor (Levy, 1986b). Kondor showed significantly higher rates of misshapen tubers under moderate and severe drought conditions compared to plants with an “adequate” water supply, ~39%, ~42% and ~23% respectively, (Levy, 1986b). This response was only seen in the spring, not in the summer when the rate of misshapen tubers was <5% across all water treatments but average and maximum temperatures were higher (Levy, 1986b), further complicating the relationships between drought stress, temperature and tuber quality in potato.

The suggestion that the effects of drought on tuber quality are highly cultivar dependent is supported by research in the cultivar Russet Burbank, where no significant differences were found in tuber processing quality or the rates of internal and external defects across fourteen different irrigation protocols (Martin *et al.*, 1992). These protocols included irrigated only, rainfed only and irrigated and rainfed plots as well as early, middle and late drought conditions (Martin *et al.*, 1992). In both middle drought protocols, there was only a slight increase in external defects, which occurred at an average rate of 11.8% compared to an average of 7.3% in control plots (Martin *et al.*, 1992). These middle droughted plots were maintained with soil water deficits of 88 mm and 135 mm after tuber initiation for the majority of tuber bulking, compared to an average of 50 mm across control watering protocols (Martin *et al.*, 1992). These results are also supported by evidence in Russet Burbank, which found a slight increase in misshapen tubers, but only with severe drought, where available soil moisture was reduced to 25% during early tuber bulking (Painter and Augustin, 1976). The differences were again very small with “bottlenecks and dumbbells” rising from ~12% of tubers in less severely droughted plants to ~15% in severely droughted plants (Painter and Augustin, 1976).

It has been difficult to discriminate the effects of drought stress, temperature, and cultivar-environment interactions on structural defects in potato. While there is evidence that specific cultivars do respond to drought stress by producing misshapen tubers (Levy, 1986b), the differences in prevalence of misshapes between drought stressed and irrigated plants are small, often insignificant and may occur only with very severe drought conditions (Painter and Augustin, 1976; Levy, 1986b; Martin *et al.*, 1992). Evidence in this area is limited and there has been little recent work investigating the effects of drought stress on structural defects in potato. This may be because a consensus seems to have been reached that temperature is the primary cause of structural defects in potato (Sparks, 1958; Bodlaender, Lugt and Marinus, 1964; Levy, 1986b; van Loon, 1986; Struik, Geertsema and Custers, 1989) but, with temperatures and incidences of drought set to rise this relationship may need more up-to-date analysis.

2.6 Biological Strategies to Reduce the Effects of Drought Stress in Potato

The above literature outlines the general effects of drought stress on the cultivated potato, *S. tuberosum*. However, as already noted, there are many important differences between potato cultivars, not least in terms of drought tolerance. Drought tolerance in potato is mediated by complex, often poorly understood, relationships between a range of physiological and morphological variables which are affected by both genotype and environment (Spitters and Schapendonk, 1990). These variables include cultivar maturity class (Deblonde, Haverkort and Ledent, 1999; Tourneux *et al.*, 2003; Aliche *et al.*, 2018), genetics (Schafleitner *et al.*, 2007; Anithakumari *et al.*, 2011, 2012) and morphology (Steckel and Gray, 1979; van Loon, 1981; Schittenhelm, Sourell and Löpmeier, 2006; Iwama, 2008). Here we primarily focus on potato morphology with the intention of informing future research exploiting recent developments in multispectral, three-dimensional imaging and high-throughput phenotyping platforms.

2.6.1 Drought Escape versus Drought Tolerance

The growing season of potatoes is primarily determined by local temperature ranges throughout the year (Haverkort, 1990). To avoid the winter frosts (Sukumaran and Weiser, 1972), the UK's lowland growing season typically begins between late-March and early-April, and ends around the end of September (Haverkort, 1990). This long season increases the probability that a period of, at least, mild drought stress will occur. Three primary biological strategies have emerged to mitigate the effects of drought on potato yields: drought escape, tolerance and avoidance (Kooyers, 2015).

2.6.1.1 Drought Escape

Drought escape, the simplest of these strategies, involves the rapid progression of a plant through its life cycle, decreasing the probability that drought will occur at any stage before the plant can reproduce (Muthoni and Kabira, 2016). In potato agriculture, this may be achievable by the use of early maturing cultivars which have shorter life cycles than second-early and maincrop cultivars (Griffith *et al.*, 1984). Using early maturing cultivars to escape late season drought in a Mediterranean climate has been suggested based on crop modelling (Haverkort and Goudriaan, 1994). Experimental evidence has shown that the early maturing cultivars, Russet Norkotah (Stark *et al.*, 2013), Blanka and Monalisa (Levy, 1986a), can escape drought stress when it occurs late in the season. However, early season droughts are much more damaging to early cultivars than those with longer life cycles, which are better able to recover once soil water is replenished (Deblonde, Haverkort and Ledent, 1999). Early cultivars also produce lower overall yields than later cultivars under favourable conditions (Levy, 1986a; Stark *et al.*, 2013) and mild drought stress (Deblonde, Haverkort and Ledent, 1999). The use of drought escape for maintaining yields during drought stress is therefore an inherently high-risk, low-reward strategy while the onset, or absence, of drought within the growing season remains unpredictable.

2.6.1.2 Drought Tolerance and Avoidance

Drought tolerance in crop species is the ability of a plant to maintain biomass, growth or yield when exposed to drought (Tardieu, Simonneau and Muller, 2018). This vague definition has previously been used to include drought escape, described above (Tardieu, Simonneau and Muller, 2018), and drought avoidance, which involves preventing drought stress in the plant tissue despite a droughted environment (Kooyers, 2015). The difference between drought tolerance and avoidance can be considered one of scale rather than kind.

Drought tolerance is the ability of plants to weather periods of drought stress through physiological adjustments, including increased osmoprotectant production, osmotic regulation and sugar accumulation (Kooyers, 2015). Drought avoidance is the ability of plants to withstand drought through morphological adjustments, including increased root growth, stomatal closure and increased root:shoot ratio (Kooyers, 2015). Both involve increasing water use efficiency (WUE) and can be difficult to distinguish. One review of drought avoidance strategies in herbaceous populations describes root growth in response to drought as an example of both drought tolerance and drought avoidance (Kooyers, 2015). Unlike drought escape, drought tolerance and avoidance strategies are more likely to be linked, as morphological responses must be triggered by physiological changes in signalling. For example, in potato, stomatal conductance (a drought avoidance trait) responds to abscisic acid accumulation in the leaves (Tekalign and Hammes, 2005). Therefore, in this review drought tolerance and drought avoidance strategies will both be referred to as drought tolerance.

2.6.2 Potato Plant Architecture and Drought Tolerance

2.6.2.1 Potato Root Architecture and Drought

As stated previously, the susceptibility of potato to drought stress has been at least partially attributed to its shallow root system (van Loon, 1981). The primary function of all plant roots is to take up water and dissolved nutrients from the soil (Zwieniecki, Thompson and Holbrook, 2002). It follows that some metric describing plant roots would therefore be an important predictive factor for plant growth or tuber yield, particularly under conditions where water is limited (Manschadi *et al.*, 2008). In potato, cultivars that are more tolerant of drought stress have previously been shown to have deeper rooting systems (Steckel and Gray, 1979; Zarzyńska, Boguszewska-Mańkowska and Nosalewicz, 2017) or higher root dry weights (Lahlou and Ledent, 2005; Iwama, 2008).

Previous research primarily suggests root depth is the metric most strongly associated with drought tolerance (Steckel and Gray, 1979; Lahlou and Ledent, 2005; Puértolas *et al.*, 2014). In the seminal field study observing yields of potato cultivars with known differences in root morphology, deeper rooting cultivars, illustrated in Figure 1, were observed to maintain significantly higher yields under drought stress (Steckel and Gray, 1979). However, the authors suggest that the differences in root depth between cultivars were too small (~100 mm) to account for the differences in drought tolerance. A later field experiment corroborated these findings with different cultivars (Lahlou and Ledent, 2005). They found a significant positive correlation between cultivar root depth at 78 DAP and a drought tolerance index, expressed as a ratio of cultivar tuber dry mass under drought to tuber dry mass with irrigation. Despite this, differences in root depth were similarly small, R^2

was low (0.50), and there was no correlation between root depth and yield under irrigated conditions.

Later, (Puértolas *et al.*, 2014)) suggested that high root density at depths of >40 cm was more important to drought tolerance than root depth alone, shown in Figure 1. They suggest that small differences in rooting depth can account for the differences in yield seen in previous experiments as dense roots in deeper soil strata have greater access to ground water and thus are responsible for a disproportionate amount of water uptake. This was supported by data showing that the deepest 5% of total root length accounted for over half of the water uptake of the cultivar Cara under prolonged drought conditions (Stalham and Allen, 2004). Root growth has also been shown to be preferentially maintained over shoot growth under drought conditions, further supporting the importance of root length for drought tolerance (Jefferies, 1993a).

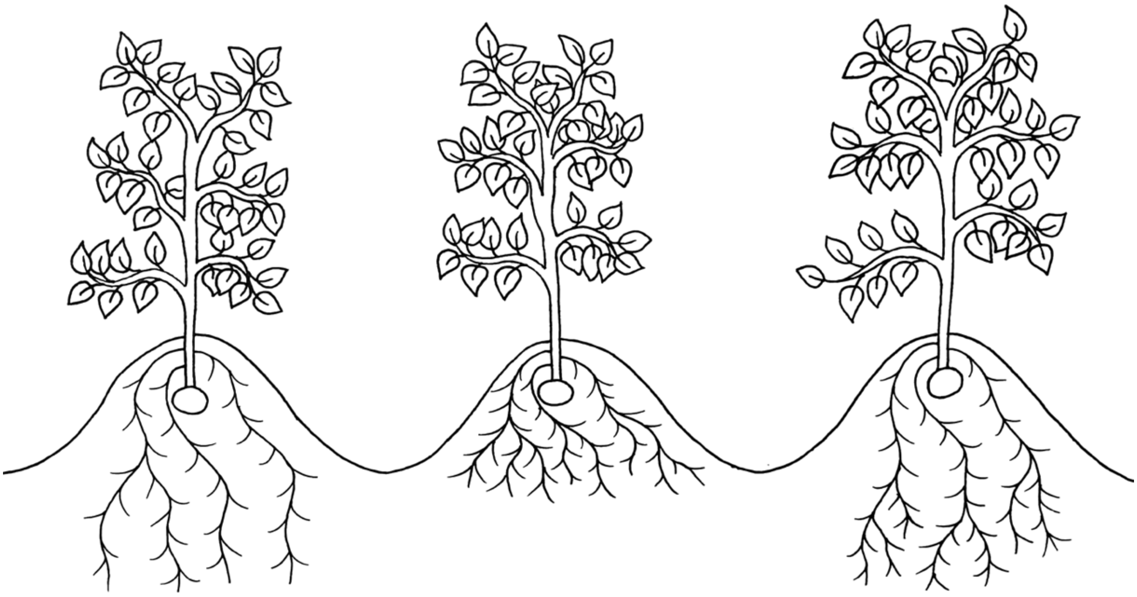

Figure 1. An illustration of three root morphotypes which have been suggested as improving drought tolerance in potato: deep roots (left), dense roots in shallow soil strata (middle) and dense roots in deep soil strata (right).

Root dry mass has been shown as both positively (Lahlou and Ledent, 2005) and negatively (Tourneux *et al.*, 2003) associated with tuber yields under drought stress. Tourneux et al. (2003) experiments showed a highly significant negative correlation between root dry mass and yield, suggesting a trade-off between root production and tuber bulking which favours the former under drought conditions. In contrast, Lahlou & Ledent (2005) found a weak positive correlation between tuber yield and root dry mass under drought stress ($R^2 = 0.34$) and propose that the conflicting results may be due to differences in the cultivars used between the experiments.

However, the assumption that the relative drought sensitivity of potato compared to other crops is due to its comparably shallow root system has been questioned (Iwama, 2008). High intra-crop variability in root length makes it unclear which crops have the deepest and densest roots (Iwama, 2008). This may be particularly true for potato, as potato cultivars have been shown to have highly variable root systems which react differently to drought stress (Tourneux *et al.*, 2003; Lahlou and Ledent, 2005). While this may be the case, a comprehensive comparison of root characteristics in a range of field grown crops found potato had the lowest total root length per unit area of any of the observed crops (21 km m^{-2}), less than one quarter that of wheat (86 km m^{-2}) (Yamaguchi, Tanaka and Tanaka, 1990). However, this study used only one potato cultivar, Danshakuimo (Irish Cobbler), which has been demonstrated to produce particularly shallow and short root systems, with low total dry weights (Iwama, 2008), when compared to several other cultivars (Iwama, 1998).

Due to the above associations between cultivar root length and drought tolerance, it has been suggested that root length and vigorous root growth should be prioritised as a selection criteria for breeding new, drought tolerant cultivars (Iwama, 2008; Puértolas *et al.*, 2014). Root pulling resistance has been identified as a potential measure to select for root length in potato, and has been shown to positively associate with yield under drought conditions (Ekanayake and Midmore, 1992). This may be due to tolerant cultivars being better able to maintain, or improve, root proliferation under drought conditions, as has been shown in maize (Westgate and Boyer, 1985). The ability of cultivars to increase their root:shoot ratio under drought conditions has also been associated with drought tolerance, although its effects on yield have not yet been observed (Jefferies, 1993a).

2.6.2.2 Potato Canopy Architecture and Drought

The relationships between canopy characteristics of potato cultivars and drought tolerance are less well understood (Schittenhelm, Sourell and Löpmeier, 2006). Most research on potato canopy traits is more concerned with the effects of drought on the canopy, which have been considered above, rather than the effects of canopy traits on drought tolerance. This is understandable as drought stress affects all plants by limiting stable photosynthetic productivity at the chloroplast, leaf and canopy levels (Jones and Corlett, 1992). However, potato canopies have an important role in regulating evapotranspiration (Vos and Groenwold, 1989), dry matter partitioning (Jefferies, 1993a) and tuber yield (Schittenhelm, Sourell and Löpmeier, 2006) under drought conditions.

In the absence of drought or disease, the productivity of potato is linearly related to its capacity to intercept solar radiation (Allen and Scott, 1980). Thus, vigorous early canopy growth creating maximal ground coverage before tuberisation has been suggested as a selection criteria to improve yield (Moll and Klemke, 1990). However, when season-long water availability cannot be guaranteed, these canopy characteristics may become suboptimal. In the absence of drought, the optimum LAI for tuber production has been placed at 4.6 (Harper, 1963), although some variation between cultivars exists (Gordon, Brown and Dixon, 1997). However, under drought conditions, optimum total leaf area index becomes dependent on the trade-off between maximising photosynthesis and minimising evapotranspiration (Schittenhelm, Sourell and Löpmeier, 2006).

Compared to other crops, stomatal control of evapotranspiration rate in potato is highly conservative (Sadras and Milroy, 1996). The early closure of stomata in potato contributes to its drought sensitivity by reducing photosynthesis and assimilate production, thus reducing canopy growth and yield (Dalla Costa *et al.*, 1997). Leaf thickness has been proposed as a drought tolerance associated trait in potato (Schittenhelm, Sourell and Löpmeier, 2006) as it may improve stomatal regulation of evapotranspiration (Chaves *et al.*, 2002). This relationship has not been shown experimentally in potato, but has been shown in other agricultural species including wheat (Hameed *et al.*, 2002), olive (Bacelar *et al.*, 2004) and mulberry (Guha *et al.*, 2010).

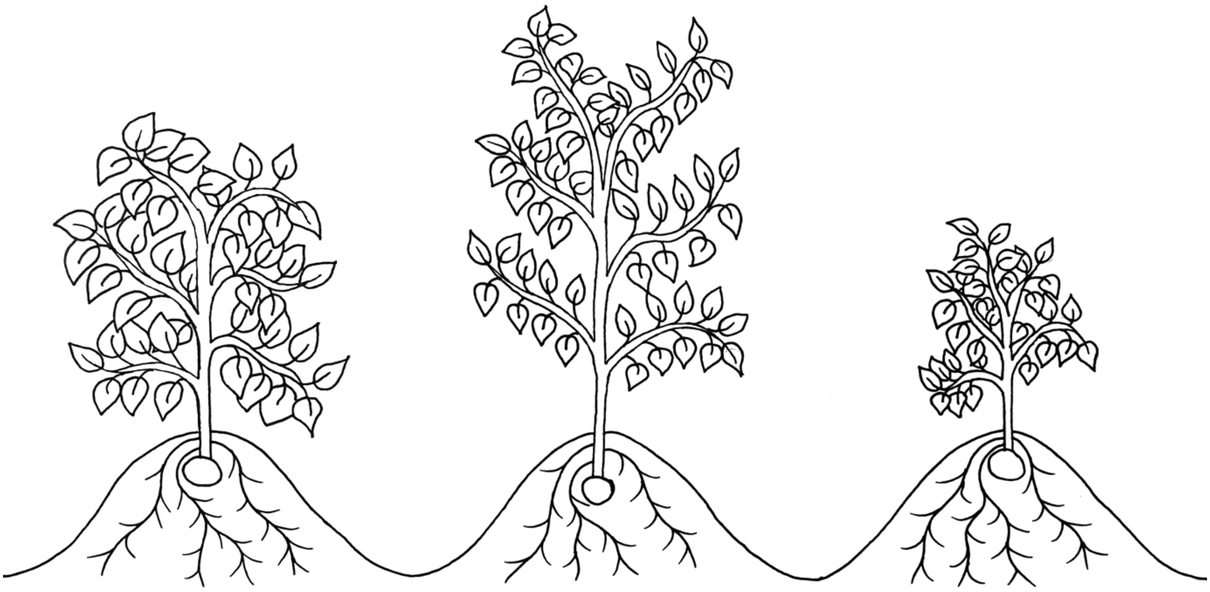

Figure 2. An illustration of three canopy architectures, two of which have been suggested as improving drought tolerance in potato: open “stem-type” canopies, e.g. cv. Tomba, which may improve light penetrance and interception (middle), and very small canopies, e.g. cv. Alpha, which may reduce evapotranspirative water loss (right). A dense “leaf-type” canopy, e.g. cv. Procudent, which has been suggested to be detrimental to potato yields under drought is also illustrated (left).

Small canopies may also contribute to drought tolerance in potato by reducing the surface area available for water loss by evapotranspiration (Tourneux *et al.*, 2003), shown in Figure 2. The cultivar Alpha has been shown to produce very small canopies characterised by an average height of 10 cm, consisting of 2.5 stems with only 8.5 leaves on the main stem (Tourneux *et al.*, 2003). This would appear to be a negative strategy for productivity considering the association between yields and solar radiation interception (Allen and Scott, 1980). However, yields in the cultivar Alpha were unaffected by drought stress, even when its water supply was incrementally decreased for five weeks followed by total water deprivation until plant death (Tourneux *et al.*, 2003). This result is not merely a function of Alpha maintaining already low yields under well-watered conditions, as may be the case in Baillie, Duke of York and Ulster Sceptre (Jefferies and Mackerron, 1993). The yield of Alpha under both irrigated and drought conditions was comparable to other cultivars under irrigation, including the cultivar Waycha which produces a significantly larger canopy than Alpha (Tourneux *et al.*, 2003). This suggests reducing evapotranspiration through methods excluding stomatal closure my contribute to maintaining yields under drought stress in potato.

Leaf density has also been associated with drought tolerance in potato, with less dense stem-type canopies performing increasingly better than denser leaf-types as drought severity intensifies (Schittenhelm, Sourell and Löpmeier, 2006). Cultivars described as stem-type have relatively small leaf:stem ratios compared to those described as leaf-types (Schittenhelm, Sourell and Löpmeier, 2006), illustrated in Figure 2. But, despite having a sparser canopy, the stem-type cultivar Tomba has been shown to produce higher yields under drought stress than Procudent, a leaf-type cultivar (Schittenhelm, Sourell and Löpmeier, 2006). Leaf-types produce larger leaves than stem-types which, while increasing radiation interception in the short-term, can lead to self-shading (Schittenhelm, Sourell and Löpmeier, 2006). This results in photosynthetic inactivity in the lower leaves, which may be responsible for the yield losses of leaf-types under drought conditions (Schittenhelm, Sourell and Löpmeier, 2006). Stem-types have also been shown to compensate for their small leaf size by producing large open canopies (Schittenhelm, Sourell and Löpmeier, 2006), favoured in other crops for improved light penetration (Duvick and Cassman, 1999; Murchie *et al.*, 1999). However, it may be that the drought tolerance of Tomba is more a function of a large root mass (Schittenhelm, Sourell and Löpmeier, 2006) than its stem-type canopy architecture. The later hypothesis may have been supported by a more recent study, where Tomba was found to be the most drought tolerant cultivar out of seventeen, despite having the highest water consumption under well-watered and drought stressed conditions (Meise *et al.*, 2019). Maintaining a high water consumption, even under water-restricted conditions, seems more consistent with the maintenance of a large root mass than with a stem-type canopy architecture, although, as the experiment took place in 5 l pots (Meise *et al.*, 2019), this may not have been the case and canopy architecture cannot be ruled out as a causal factor. In another experiments, the leaf-type cultivar Konyu-2 out yielded others with similar root systems but lower leaf:stem ratios (Deguchi *et al.*, 2010). This was attributed to the unique ability of Konyu-2 to preferentially partition dry matter into leaves over stems, allowing it to achieve an optimal leaf area index even under drought conditions (Deguchi *et al.*, 2010). Due to the significant effects of root characteristics on drought tolerance outlined above and the difficulty in controlling these variables, the optimal canopy architecture for drought tolerance in potato remains unclear.

2.7 Conclusion

Despite its status as the most profitable crop produced in many countries, particularly in the UK and central Europe (Petrenko and Searle, 2016), many morphophysiological processes of potato remain unstudied. As climate change increases the risk of summer droughts in many parts of the world (Daccache *et al.*, 2012), an understanding of modern cultivar-environment interactions will be needed on which to base further research. Until recently, high profit margins have masked inefficiencies in potato production (Taylor *et al.*, 2018), and perhaps reduced the emphasis on fundamental and actionable research investigating potato production. This review has highlighted the many gaps that remain in the understanding of key morphophysiological processes in potato. It is well documented that potato is a highly susceptible to drought stress (van Loon, 1981; Schafleitner, 2009; Aliche *et al.*, 2018) but the relative effects of premature stomatal closure and reduced RuBP production on photosynthetic rate in potato remain unknown, as do the mechanisms by which stomatal conductance is regulated during severe drought stress. This has made it difficult to evaluate the optimum canopy structures for high yields under drought conditions. An understanding of the role of stomatal conductance as a drought stress response is essential for evaluating the potential trade-off in canopy size between small canopies, which reduce water loss by transpiration (Tourneux *et al.*, 2003) and large canopies, which maximise radiation interception (Allen and Scott, 1980). The optimum potato canopy for assimilate partitioning may also factor into this trade off, as scion grafts dominate partitioning under drought stress (Jefferies, 1993a). These knowledge gaps may not have been investigated based on the assumptions that potatoes will continue to be profitable regardless and that drought tolerance in potato is adequately understood. Much of the research cited in this review states clearly and with conviction that shallow root systems are the primary cause of the drought susceptibility of potato (van Loon, 1981; Ekanayake and Midmore, 1992; Zarzyńska, Boguszewska-Mańkowska and Nosalewicz, 2017; Aliche *et al.*, 2018; Chang *et al.*, 2018). While root depth is associated with drought tolerance, the authors of studies investigating the relationship between root depth and drought tolerance suggest that the correlations are too weak and the effect sizes too small to account for the variation in drought tolerance seen between cultivars (Steckel and Gray, 1979; Lahlou and Ledent, 2005). In contrast, the effects of drought stress on canopy growth in potato are much more variable than on its effects on root growth (Boguszewska-Mańkowska, Zarzyńska and Nosalewicz, 2020). Thus, screening for drought tolerant cultivars by observing the canopy architecture of potato under drought stress will likely be faster, more convenient, and higher resolution than the less sensitive, delicate, and labour-intensive process of measuring root growth (Zarzyńska, Boguszewska-Mańkowska and Nosalewicz, 2017). However, unlike in other crops (Susič *et al.*, 2018; Asaari *et al.*, 2019; Zovko *et al.*, 2019), very little work has been conducted with potato that utilises modern phenotyping methods, such as multispectral, hyperspectral or three-dimensional imaging. These technologies present an opportunity to better understand the effects of drought stress on potato and will be a useful to accelerate the screening of drought tolerant cultivars.

Similarly, tuber quality has been almost entirely attributed to high temperatures in the field (Bodlaender, Lugt and Marinus, 1964), despite evidence in specific cultivars to the contrary (Painter and Augustin, 1976). High inter-cultivar variability in drought tolerance has been repeatedly demonstrated in potato (Steckel and Gray, 1979; Levy, 1983; Sprenger *et al.*, 2015; Aliche *et al.*, 2018), making it difficult to generalise the observed effects of drought on one or a few cultivars to the commercial population. Studies investigating tens of cultivars are extremely valuable (Jefferies, 1993b; Jefferies and Mackerron, 1993; Luitel *et al.*, 2015; Aliche *et al.*, 2018), but remain scarce due to the obvious logistical problems associated with large scale field trials. This highlights the need for a greater understanding of specific phenotypic traits, with respect to drought tolerance, which may be generalisable between cultivars with similar morphologies. Enhancing drought-protective morphological traits may then become the focus of breeding programs within *Solanum tuberosum* subsp. *tuberosum*, and novel traits observed in *Solanum tuberosum* subsp. *andigenum* may be introduced into commercial cultivars. Many of the new cultivars already produced by breeding programmes in recent decades will also need to be investigated with respect to drought tolerance. Much of the research cited here is now relatively old and would benefit from a rejuvenation of interest in drought tolerance in potato, which is becoming increasingly important as the climate changes in favour of drier growing seasons in many places.

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Chapter 3: Small pots confound and diminish the effects of water-restriction on potato (*Solanum tuberosum* L.) morphophysiology

3.1 Abstract

Recent advancements in imaging technologies have facilitated the development of high-throughput platforms, which have greatly reduced the financial and temporal costs of plant phenotyping studies and brought the amount of data they generate in line with molecular and genetic approaches. These platforms have allowed researchers to maximise their sample sizes, which are now primarily limited by spatial constraints, particularly in controlled environments. This has pushed researchers towards growing plants in very small pots, increasing statistical power but with unknown costs on experimental validity. In this study, we investigated the interactive effects of small pots, i.e., pot binding, and water-restriction on the morphophysiology and yield of two potato cultivars with contrasting maturities, Maris Piper (late maturing) and Charlotte (early maturing) using a multispectral 3D scanning high-throughput phenotyping system. Small pots were found to directly, but differentially, confound the morphology of these potato cultivars, including a reduction in the plant height of Charlotte, but no effect on the height of Maris Piper. Charlotte was significantly shorter (by ~70%) than Maris Piper in 2.5 and 5 L pots, respectively, but there were no significant differences in plant height between cultivars in pot sizes ≥ 10 L. Similar results were found with digital biomass (DB), which was significantly greater in Maris Piper compared to Charlotte in 2.5 and 5 L

pots. In 10 L pots, Charlotte had an 33.9% greater DB than Maris Piper, while the differences between cultivars in 20 and 40 L pots were not significant. This highlights the need to consider pot size in experimental designs that utilise high-throughput phenotyping platforms for data collection, especially when comparisons are being made, e.g., in terms of drought tolerance, between cultivars. The results of this study suggest that small pots (≤ 5 L) are inappropriate for investigating the effects of water-restriction on potato and for screening cultivars for drought tolerance under these conditions.

3.2 Introduction

As a drought-sensitive crop (van Loon, 1981), potato production is likely to be adversely affected by the changes in precipitation and fresh-water availability predicted to result from climate change (Daccache *et al.*, 2012; George *et al.*, 2017). To combat this, researchers must continue to investigate the genetic, physiological, and morphological basis of drought tolerance in potato and other crops. Traits which are shown to associate with yield under drought conditions can be targeted for selection by plant breeders, producing more drought-tolerant cultivars. However, the experimental conditions under which potato crops are studied are inconsistent within the literature, and there are conflicting opinions on what constitutes drought (Hill *et al.*, 2021).

The quantitative analysis of plant morphology has been a significant bottleneck in potato breeding, as the measurement of even simple phenotypic traits, sometimes called phenes (York, 2019), was – until recently – destructive, costly, and time consuming (Furbank and Tester, 2011). Advances in imaging sensors and image processing now allow for the non-destructive, relatively cheap, and high-throughput phenotyping (HTP) of many crops. In potato, RGB sensors have been coupled with unmanned aerial vehicles (UAVs) to measure individual plant height and total ground cover on a field-wide scale (de Jesus Colwell *et al.*, 2021). More sophisticated multispectral imaging sensors have also been deployed with UAVs to assess simulated hail damage to potato crops at the field level (Zhou *et al.*, 2016). Magnetic resonance imaging has also been used to non-destructively measure tuber volume in potted potato plants under varying water conditions (Musse *et al.*, 2021).

UAVs are increasingly becoming an effective phenotyping tool on the field-wide scale, but trade-offs remain between throughput and resolution, dimensionality, and environmental control. In many controlled-environment studies, the increased screening capacity of HTP platforms has necessitated a shift to very small pots, as space is often a limiting resource (Turner, 2019). However, small pots are known to affect plant growth and development by a poorly understood process known as “pot binding” (Sinclair *et al.*, 2017). For example, when three cultivars of oilseed rape (*Brassica napus* L.) were grown in 6 L and 0.22 L pots, the relative rankings of several phenotypic traits in response to drought conditions differed significantly between the pot sizes (Dambreville *et al.*, 2016). Importantly for HTP platforms, drought conditions were observed to induce an increased root:shoot ratio, an effect only seen in the larger pots (Dambreville *et al.*, 2016). This demonstrates the susceptibility of HTP platforms to systemic errors, caused by pot binding effects, which may have a greater effect on plant growth and development than the experimental conditions being imposed.

Previous work has aimed to provide guidelines for scientists to help avoid the confounding effects of pot binding (Poorter *et al.*, 2012; Sinclair *et al.*, 2017; Turner, 2019). The canonical recommendation for pot experiments is that the ratio of dry plant biomass to substrate volume should not exceed 1 g L^{-1} (Poorter *et al.*, 2012). As potatoes have been observed to produce over a kilogram of dry matter in controlled environments (under a 24-hour photoperiod) (Wheeler and Tibbitts, 1987), 1,000 L pots would be required to ensure this advice was adhered to. This is obviously impractical for controlled-environment phenotyping experiments, where space is at a premium (Turner, 2019).

The mechanisms behind pot binding are debated but are thought to be a product of interactions between plant roots and their environment. As potatoes have a relatively small root system (van Loon, 1981), the recommendations described above may be excessive for this crop. However, many studies investigating the effects of drought on potato have used pot sizes that would likely have caused pot binding effects. Several studies used pots between 5 and 10 L (Rykaczewska, 2015; Meise *et al.*, 2019; Qin *et al.*, 2019; Orsák *et al.*, 2020; Dorneles *et al.*, 2021; Mthembu *et al.*, 2022), but there are examples of “6-inch” (~2 L) and 4.7 L pots being used (Rolando *et al.*, 2015; Gervais *et al.*, 2021). Two earlier studies, conducted when manual data collection restricted sample sizes, used large 48 L pots (Lahlou, Ouattar and Ledent, 2003; Lahlou and Ledent, 2005). However, these square pots were only 30 cm deep, less than half the depth required for unrestricted root growth

(Stalham and Allen, 2001). In two other recent studies investigating the effects of drought on potato root system architecture, plants were grown in 100 cm tall, galvanised-steel cylinders (Zarzyńska, Boguszewska-Mańkowska and Nosalewicz, 2017; Boguszewska-Mańkowska, Zarzyńska and Nosalewicz, 2020). This creative design theoretically allows for the unrestricted root growth of all but the deepest rooting cultivars (Stalham and Allen, 2001), preventing the most severe effects of pot binding, but at the cost of replication.

In addition to the unknown effects of pot binding in potato, the interpretation of these studies is further complicated by the lack of consistent protocols for imposing or preventing drought stress (Hill *et al.*, 2021). In nominally well-watered pots, soil moisture content has varied between experiments from 60% (Meise *et al.*, 2019) to 90% (Qin *et al.*, 2019) of field capacity (FC), with inter-irrigation periods ranging from 24 hours (Gervais *et al.*, 2021) to 2 days (Li *et al.*, 2019). The lack of consistency is well-demonstrated by the recent use of 60% FC as both a “moderate water restriction” treatment (Qin *et al.*, 2019) and a well-watered control treatment (Meise *et al.*, 2019).

This is further complicated by the reporting of “FC” in previous experiment. FC is defined by the Soil Science Society of America as, “the content of water, on a mass or volume basis, remaining in a soil 2 or 3 days after having been wetted with water and after free drainage is negligible” (Soil Science Glossary Terms Committee, 2008). FC is not an appropriate term for pot experiments, as moisture in potted substrates is not affected by the suction from underlying soil due capillary action (Kirkham, 2005). Pot capacity (PC) is the proper term in this context (Turner, 2019) and is defined similarly as the amount of water, mass or volume, remaining in a pot after irrigation to saturation and subsequent cessation of drainage (Kirkham, 2005).

Field or pot available water is the amount of water lost from the substrate between FC or PC, respectively, and the permanent wilting point (Soil Science Glossary Terms Committee, 2008). The permanent wilting point is the maximum amount of water contained within a substrate at which plants begin to wilt beyond the possibility of recover (Soil Science Glossary Terms Committee, 2008). As plants are adapted to different water environments, the permanent wilting point is species specific and has been estimated to be between -0 6 MPa and -1 0 MPa in potato (Vos and Haverkort, 2007).

Pot size confounds these water dynamic in pot experiments (Passioura, 2006). Substrate at the top of tall pots experiences greater forces of suction than substrate in shorter pots, due increased capillary action from to the greater volume of soil underneath (Turner, 2019). This may lead to a greater redistribution of water from the top of the pot to the bottom, where the substrate is more likely to become over-saturated and drip out from any drainage holes. Layers of substrate at the bottom of small pots may also become hypoxic as a result of this process (Passioura, 2006).

Comparing the internal water dynamics between pot sizes is beyond the scope of this study, as a complex array of sensors would need to be installed to measure substrate water availability throughout each pot size. Bespoke pots would also be required to control for differences in pot height while varying pot volume.

This inconsistency may contribute to the confounding effects of pot binding through a mechanism described by the "water availability" hypothesis (Sinclair *et al.*, 2017). This hypothesis predicts that pot binding is primarily, if not entirely, a result of the inability of substrates in small pots to hold enough water to prevent drought-stress, between irrigation periods, in well-watered plants. In a recent 5 L pot study, cessation of irrigation had significant effects on net CO2 fixation, stomatal conductance, water use efficiency, and shoot temperature within 24 hours, in early and late maturing potato genotypes (Dorneles *et al.*, 2021). This suggests that even daily watering may not be sufficient to prevent drought stress in well-watered potato plants in pots of this size.

In this study, we aimed to develop a more appropriate guideline for potato pot experiments by investigating the relationships between pot size, water availability, and potato morphology. We hypothesised that morphophysiological evidence of drought stress (i.e., the relative intra-cultivar differences between well-watered and water-restricted plants), in two cultivars of potato (Maris Piper and Charlotte), would be negatively associated with pot size, due to pot binding. Further, we predicted that any evidence of drought stress, intentional or otherwise, would be more pronounced in the less drought-tolerant cultivar, Charlotte.

3.3 Materials and methods

A pot experiment was carried out in the Crop and Environment Laboratory glasshouses (51°43’71”N, 0.94°18’12”W) at the University of Reading, UK. Seed tubers of two potato cultivars, Charlotte and Maris Piper, were supplied by Branston Ltd (Branston, UK), from their growers’ seed stocks. On 17th December

2020 six pre-spouted seed tubers of each cultivar were planted individually in 2.5, 5, 10, 20, and 40 L pots, giving a total of sixty plants. All pots were filled with a 2:1, by volume, mixture of peat compost and vermiculite, and a single seed tuber was planted in each. Planting depth could not be consistent between each pot size, so each seed tuber was planted at a depth of one third the depth of its pot.

All pots were watered beyond saturation three days per week until 2nd March 2021, 75 days after planting (DAP). After this date, which coincided with flower bud formation (FBF) and thus tuber initiation onset (Li *et al.*, 2019), two water regimes were imposed. Half the plants of each cultivar, in each pot size, remained well-watered for the duration of the experiment. The other half were drought stressed with a long-term water restriction (Li *et al.*, 2019). This water restriction was imposed by providing droughted plants with 50% of the average estimated daily evapotranspiration of the plants prior to FBF, grouped by pot size and cultivar.

To make this estimate, each pot was watered beyond saturation on 26th February 2021 (71 DAP), indicated by excess water flowing from the drainage holes of each pot. 48 hours later, after the dripping had halted completely, each system (plant, pot, and substrate) was weighed with a KERN HCB 50K100 hanging scale (Kern & Sohn GmbH, Balingen, Germany). All systems were reweighed after a further 48 hours with the same equipment, before the next watering. The difference between the two weights was taken as the water lost from each system over the 48-hour period and was halved to give the volume of water lost from each system per day.

At 75 DAP, droughted plants were watered with 50% of the average of these measurements, grouped by pot size and cultivar, multiplied by the number of days since the previous watering. Well-watered plants continued to be watered beyond saturation. Both groups of plants were watered on the same day, three days per week, for the remainder of the experiment (Li *et al.*, 2019).

From the 15th of January (29 DAP) to 16th April, the plants were scanned with two PlantEye F500 multispectral 3D scanners (Phenospex, Heerlen, Netherlands), which have previously been used to measure “high-temperature-induced” morphophysiological changes in potato (Lazarević *et al.*, 2022). Scans took place at 36, 43, 63, 78, 92, and 105 DAP. Integrated software (Phena; Phenospex, Heerlen, Netherlands) generated 3D point clouds of the plants each time they were scanned, which were used by HortControl (Phenospex, Heerlen, Netherlands), to calculate morphological parameters, including digital biomass, plant height, and average greenness (Lazarević *et al.*, 2021). The PlantEye has a capture range of 1.1 m, which capped the measurements of plant height (Figure 1). This, combined with the self-shading of lower leaves by leaves closer to the sensor, constitutes a significant limitation of the phenotyping platform and, therefore, the results presented here. This systematic error is discussed further in the discussion (Section 3.5.1), and is highlighted in the relevant figure legends.

Figure 1. A photograph of a potato plant (Maris Piper) that has grown too tall to be accurately phenotyped by the PlantEye sensor. This was a common occurrence with plants grown in ≥ 20 L pots after 63 DAP.

Plants were topped on 30th April, 134 DAP, and all tubers were harvested on 5th May. Infected stems were removed before this date due to infections with, and to prevent the spread of, blackleg (*Pectobacterium atrosepticum*) (Supplementary Table S1). After harvesting, total fresh tuber yield was measured for each plant.

Statistical analysis was conducted with three- (yield) and four-way ANOVAs in R Studio (RStudio Team, 2020). The grouping factors pot size, cultivar, and treatment were included in all ANOVAs, with DAP included for all non-yield dependent variables. Pots were arranged in blocks within the glasshouse by pot size. Both these blocks and the pots within them were rearranged randomly after each scan to prevent order effects. Residual analysis was performed prior to all ANOVAs to ensure that the assumptions of these tests were met. Q-Q plots were produced from the residuals of each dependent variable to visually check for normality. These results were then corroborated statistically with Shapiro-Wilk tests ($p < 0.05$). Variables with non-normal distributions, as indicated by either of these tests, were transformed to improve normality. All the data presented here are based on the original (plant height) or back-transformed (digital biomass and greenness average) values, except for the ANOVA results. Percentage differences were calculated for comparisons between pot sizes, cultivars, and DAP, while differences between well-watered and water-restricted plants were calculated as percentage changes from the former to the later. Levene's tests were used to check for equality of variance between the groups of each variable ($p < 0.05$).

3.4 Results

3.4.1 Fresh Tuber Yield

Mean $\log_{10}$ fresh tuber yield (FTY) was significantly affected by pot size ($p < 0.001$), cultivar ($p < 0.001$), and drought treatment ($p = 0.032$), but there were no significant interactions between any of these main effects (Table 1). The grouping factor with the greatest effect on FTY was pot size, with the largest pots (40 L) producing a 137.8% greater FTY than the smallest pots (2.5 L). The relationship between FTY and pot size was exponential, with each increase in pot size being associated with a significant ($p < 0.05$) increase in FTY (Figure 2). The difference between cultivars was smaller but still significant ($p < 0.001$), with Maris Piper producing 23.4% more FTY than Charlotte. Finally, treatment had a significant ($p = 0.032$) effect on FTY overall, with water-restriction being associated with a 10.4% decrease in FTY.

Table 1. Main effects and interaction terms for a three-way ANOVA on the fresh tuber yield ($\log_{10}$-transformed) of two potato cultivars (Maris Piper and Charlotte), grown in different size pots (2.5, 5, 10, 20, and 40 L) and subjected to well-watered or water-restricted treatments. Significant p-values (< 0.05) are indicated with an asterisk (*).

Source of Variation	DFn	DF	F	*p*
Pot Size (PS)	4	37	126.277	0.000*
Cultivar (C)	1	37	22.465	0.000*
Treatment (T)	1	37	4.947	0.032*
PS x C	4	37	1.219	0.319
PS x T	4	37	0.292	0.881
C x T	1	37	0.484	0.491
PS x C x T	4	37	0.351	0.842

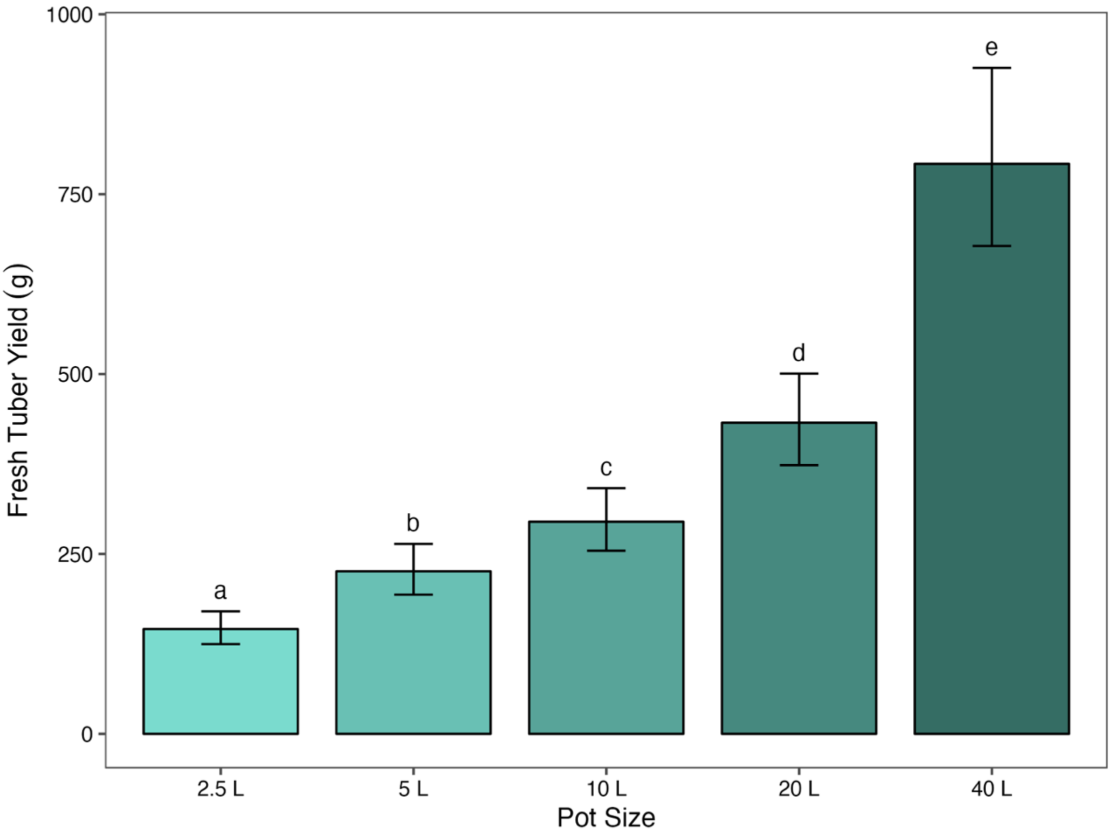

Figure 2. Mean (back-transformed from log10) fresh tuber yield (FTY) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent FTY across both cultivars and water treatments within each pot size (n = 12) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$).

Although there were no statistically significant interactions, there were differences in the relationships between the effect of water-restriction on FTY and pot size between the two cultivars (Figure 3). The decrease in FTY between treatments increased linearly with pot size in Maris Piper, but this relationship was not observed in Charlotte. Water-restriction was associated with a FTY reduction of 3.4, 7.6, 16.8, 21.7, and 15.8% in Maris Piper relative to their well-watered controls, in 2.5, 5, 10, 20, and 40 L pots respectively. The difference in FTY between water-restricted and well-watered controls of Charlotte resulted in FTY decreases of 15.7, 6.7, 7.3, 4.2, and 15.3%, with respect to the same pot sizes.

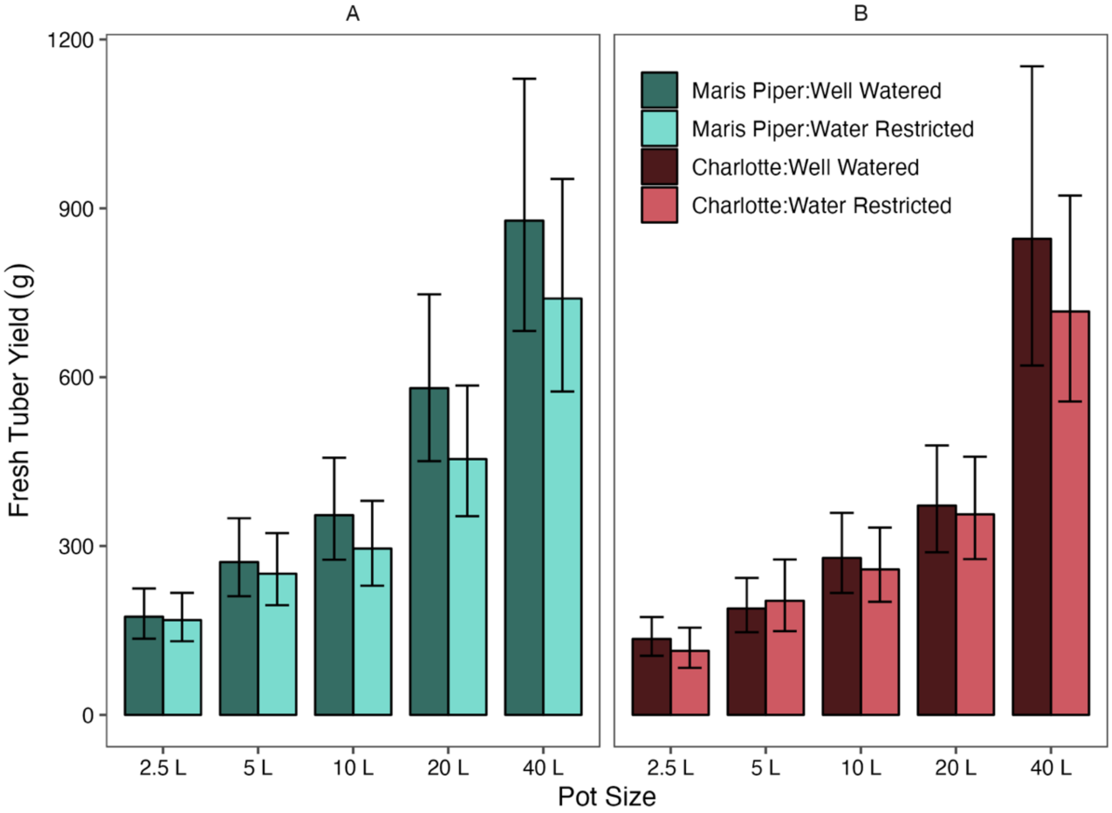

Figure 3. Mean (back-transformed from log10) fresh tuber yields (FTY) of two potato cultivars, Maris Piper (A) and Charlotte (B), under well-watered (dark bars) or water-restricted (light bars) conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent FTY for each combination of pot size, cultivar, and water treatment (n = 3) ± 95% CIs.

3.4.2 Digital Biomass

Mean (√) digital biomass (DB) was significantly affected by pot size ($p < 0.001$), cultivar ($p < 0.001$), treatment ($p < 0.001$) and DAP ($p < 0.001$) (Table 2). Across both cultivars DB was significantly ($p < 0.05$) higher in 5 L pots than in 2.5 L pots, with a difference of 25.4%. Db in 10 L pots was also significantly ($p < 0.05$) higher than in 5 L pots, with a difference of 41.2%. DB in 20 and 40 L pots was significantly ($p < 0.05$) lower than in 10 L pots, with a difference of 20.2% between 10 and 20 L pots. The significant ($p < 0.001$) interaction between pot size and cultivar suggests that these differences in DB between small and large pot sizes were largely associated with the effect of pot size primarily on the cultivar Charlotte (Figure 4), with the DB of Maris Piper remaining similar across pot sizes. The DB of Maris Piper was 80.1 and 92.1% greater than that of Charlotte in 2.5 and 5 L pots, respectively. In 10 L pots, this was reversed, with Charlotte having an 33.9% greater DB than Maris Piper, while no significant differences between cultivars in 20 and 40 L pots were observed.

Table 2. Main effects and interaction terms of a four-way ANOVA for digital biomass (sqrt-transformed), plant height, and greenness average (squared) of two potato cultivars (Maris Piper and Charlotte), grown in different size pots (2.5, 5, 10, 20, and 40 L) and subjected to well-watered or water-restricted treatments. Data were collected relative to days-after-planting (DAP). Significant p-values (< 0.05) are indicated with an asterisk (*).

Source of Variation	DFn	DF	Digital Biomass	Plant Height	Greenness Average
Pot Size (PS)	4	222	0.000*	0.000*	0.000*
Cultivar (C)	1	222	0.000*	0.000*	0.000*
Treatment (T)	1	222	0.000*	0.001*	0.002*
DAP	5	222	0.000*	0.000*	0.000*
PS x C	4	222	0.000*	0.000*	0.176
PS x T	4	222	0.035*	0.041*	0.047*
C x T	1	222	0.118	0.039*	0.374
PS x DAP	20	222	0.000*	0.000*	0.000*
C x DAP	5	222	0.000*	0.000*	0.001*
T x DAP	5	222	0.706	0.353	0.001*
PS x C x T	4	222	0.111	0.334	0.188
PS x C x DAP	20	222	0.001*	0.000*	0.000*
PS x T x DAP	20	222	0.532	0.782	0.386
C x T x DAP	5	222	0.986	0.885	0.705
PS x C x T x DAP	20	222	0.818	0.517	0.001*

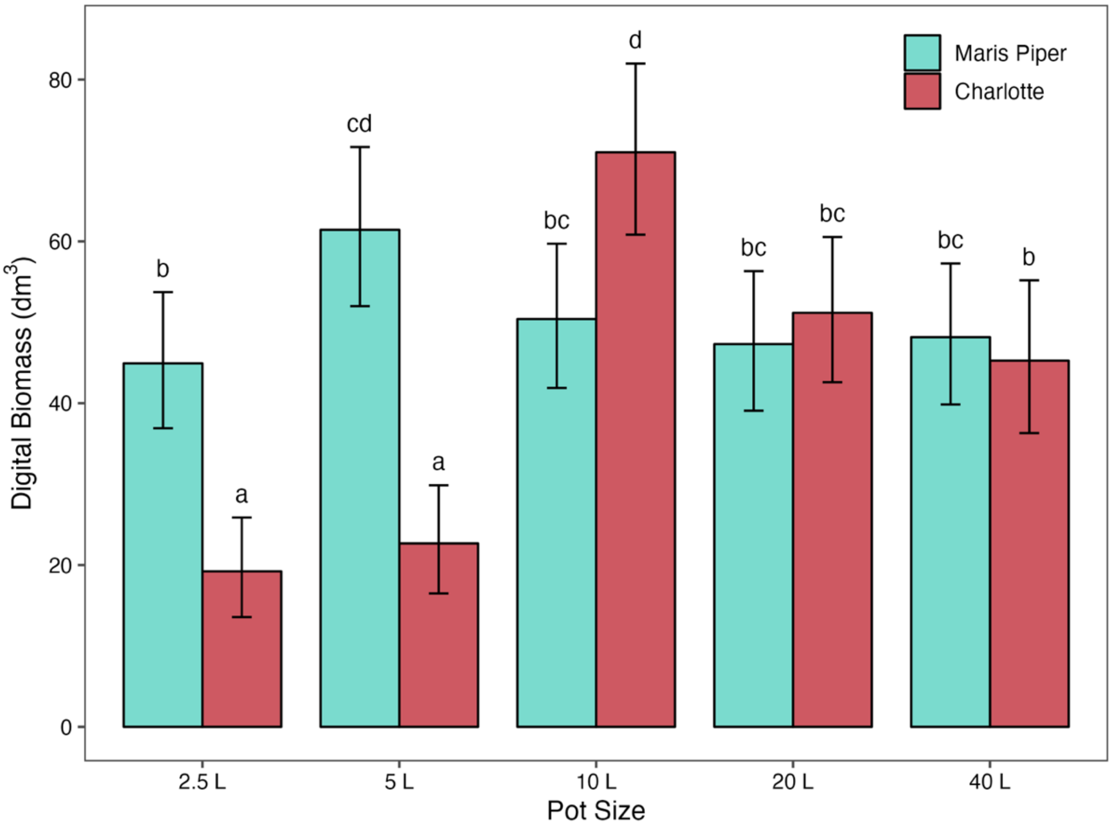

Figure 4. Mean (back-transformed from sqrt) digital biomass (DB) of two potato cultivars, Maris Piper (green) and Charlotte (pink), grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent DB across both water treatments and all measurement dates within each combination of pot size and cultivar (n = 36) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$). **N.B. Measurements of digital biomass were confounded in ≥ 10 L pots due to self-shading caused by leaves close to the PlantEye obsuring lower leaves.**

The significant (p = 0.001) interaction between pot size, cultivar, and DAP shows that the DB of Maris Piper was relatively unaffected by pot size, while the DB of Charlotte was significantly stunted when grown in 2.5 and 5 L pots (Figure 5). This interaction also shows that the onset of senescence occurred earlier, and progressed faster, in Charlotte when grown in 2.5 and 5 L pots, compared to both Maris Piper in the same pot sizes and both cultivars in all larger pot sizes.

The interaction between pot size and treatment also had a significant ($p = 0.035$) effect on DB (Table 2). Water restriction was associated with DB reductions of 23.7, 12.9, 35.0, 33.0, and 17.9% in 2.5, 5, 10, 20, and 40 L pots, respectively (Figure 6). These differences were only significant ($p < 0.05$) in 10 and 20 L pots.

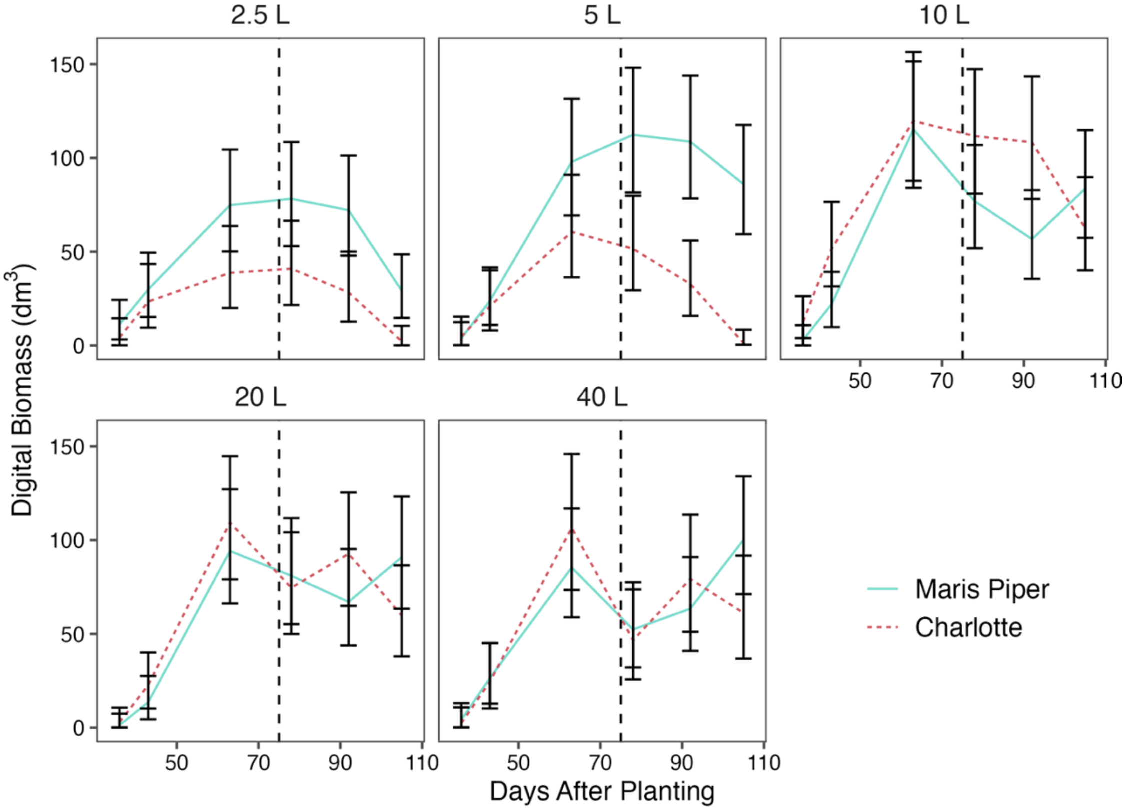

Figure 5. Mean (back-transformed from sqrt) digital biomass (DB) of two potato cultivars, Maris Piper (solid green) and Charlotte (dashed pink), grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent DB across both water treatments within each combination of pot size, cultivar, and DAP (n = 6) ± 95% CIs. The vertical dashed line indicates the onset of water-restriction. Compact letters denoting significant differences between groups can be found in Supplementary Table S2. **N.B. Measurements of digital biomass were confounded in ≥ 10 L pots due to the self-shading caused by leaves close to the PlantEye obsuring lower leaves.**

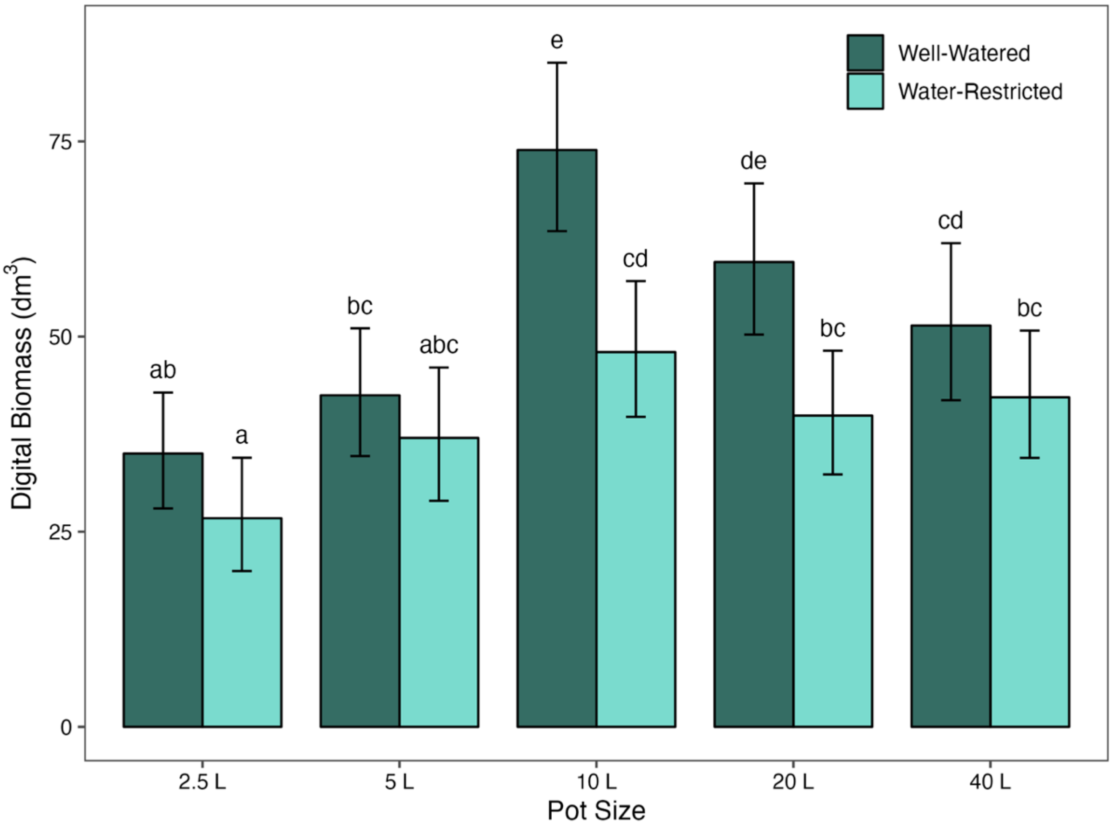

Figure 6. Mean (back-transformed from sqrt) digital biomass (DB) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered (dark green) or water-restricted (light green) conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent DB across both cultivars and all measurement dates within each combination of pot size and water treatment (n = 36) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$). **N.B. Measurements of digital biomass were confounded in ≥ 10 L pots due to the self-shading caused by leaves close to the PlantEye obsuring lower leaves.**

3.4.3 Plant Height

Mean plant height (PH) was also significantly affected by pot size ($p < 0.001$), cultivar ($p < 0.001$), treatment ($p = 0.001$), and DAP ($p < 0.001$) (Table 2). The PH of each pot size fell into one of three significantly ($p < 0.05$) different groups (Figure 7). 2.5 and 5 L pots were statistically similar and produced the shortest plants. PH was highest in 10 L pots, reaching an average of 601.4 mm. This was 34.3% higher than PH in 5 L pots and 13.5% higher than 20 L pots, which reached 525.5 mm. PH was not significantly different in 20 and 40 L pots, with a difference of 19.6 mm between these two pot sizes.

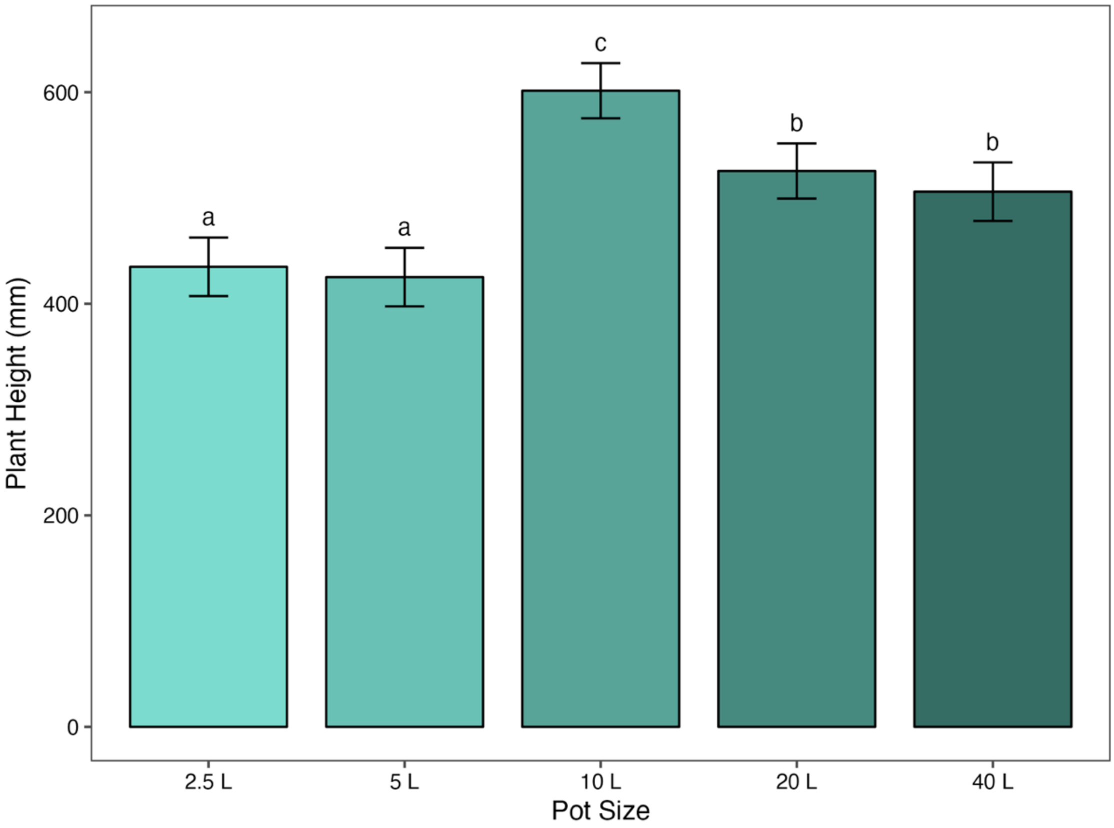

Figure 7. Mean plant height (PH) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent PH across both cultivars, water treatments, and all measurement dates within each pot size (n = 72) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$). **N.B. Plant height measurements for plants grown in ≥ 20 L pots are systematically low as plants in these pot sizes grew beyond the scanable area of the PlantEye after 63 DAP.**

The PH of Maris Piper was significantly ($p < 0.05$) higher than that of Charlotte. Across all pot sizes and both treatments, the PH of Charlotte was 436.4 mm, 24.9% shorter than Maris Piper (560.7 mm). The significant ($p < 0.001$) interaction effect between pot size and cultivar showed that this difference between the cultivars was related to the disproportionate stunting effect of small pot sizes (≤ 5 L) on Charlotte (Figure 8).

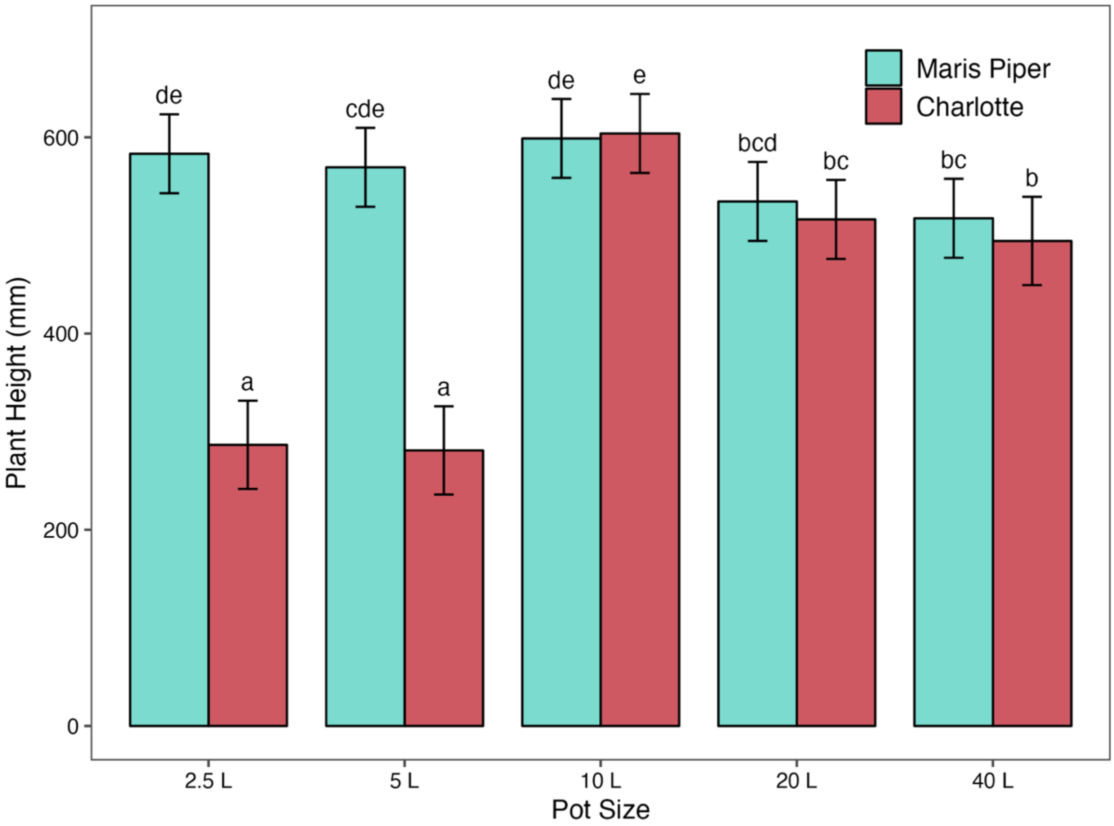

Figure 8. Mean plant height (PH) of two potato cultivars, Maris Piper (green) and Charlotte (pink), grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent PH across both water treatments and all measurement dates within each combination of pot size and cultivar (n = 36) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$). **N.B. Plant height measurements for plants grown in ≥ 20 L pots are systematically low as plants in these pot sizes grew beyond the scanable area of the PlantEye after 63 DAP.**

There were no significant differences in PH between Maris Piper and Charlotte grown in pots ≥ 10 L, but Charlotte was 68.2 and 67.8% shorter than Maris Piper grown in 2.5 and 5 L pots, respectively. The significant ($p = 0.001$) interaction between pot size, cultivar, and DAP further demonstrated that small (≤ 5 L) pots had a greater stunting effect on Charlotte than Maris Piper across the whole lifecycle

(Figure 9). PH of Charlotte was significantly ($p < 0.05$) lower than that of Maris Piper from 63 DAP to the end of the experiment in 2.5 and 5 L pots, but the two cultivars were not significantly different on any date in the larger pot sizes (Figure 9).

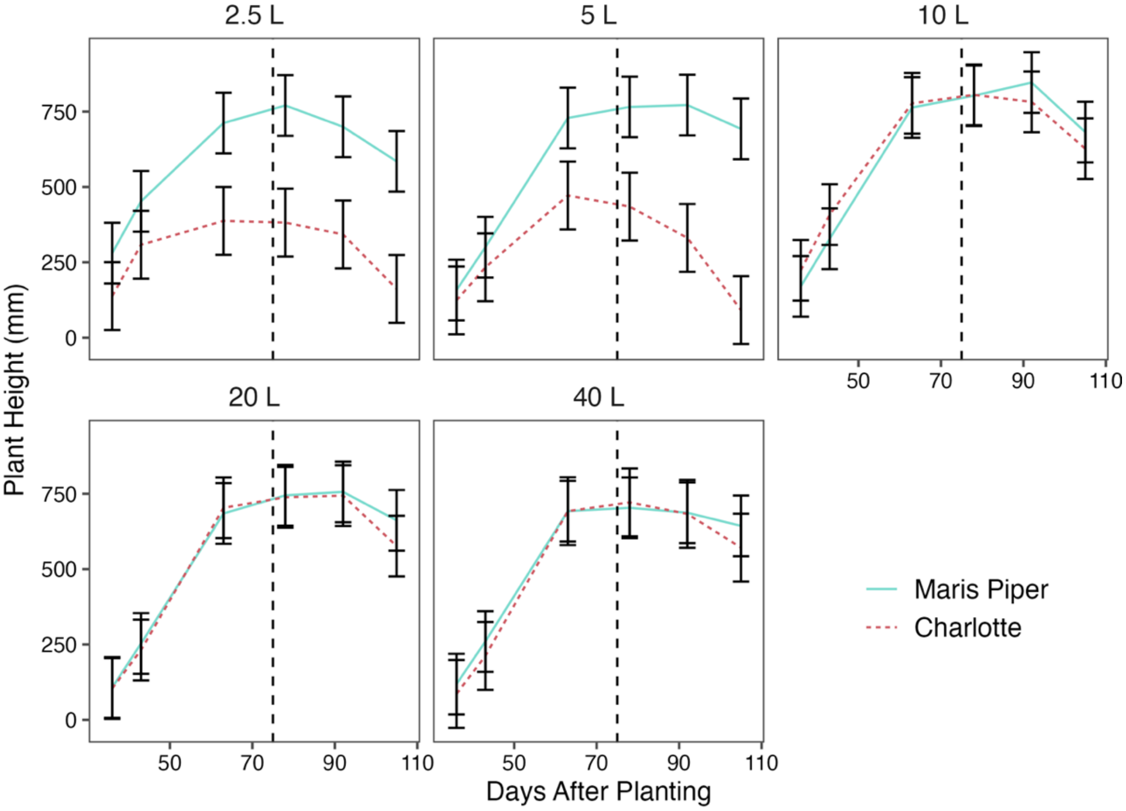

Figure 9. Mean plant height (PH) of two potato cultivars, Maris Piper (solid green) and Charlotte (dashed pink), grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent PH across each water treatment within each combination of pot size, cultivar, and measurement date (n = 6) ± 95% CIs. The vertical dashed line indicates the onset of water-restriction. Compact letters denoting significant differences between groups can be found in Supplementary Table S3. **N.B. Plant height measurements for plants grown in ≥ 20 L pots are systematically low as plants in these pot sizes grew beyond the scanable area of the PlantEye after 63 DAP.**

Water restriction was associated with a small (6.0%) but significant ($p < 0.001$) reduction in overall PH. The reduction in PH associated with water restriction was only significant ($p < 0.05$) for plants grown in 10 L pots, where water-restricted plants (561.8 mm) were 12.3% shorter than well-watered plants (641.0 mm) (Figure 10). Water-restriction was associated with a small increase in PH of 2.0% in 5 L pots: PH decreased with water-restriction in all other pot sizes, although not significantly.

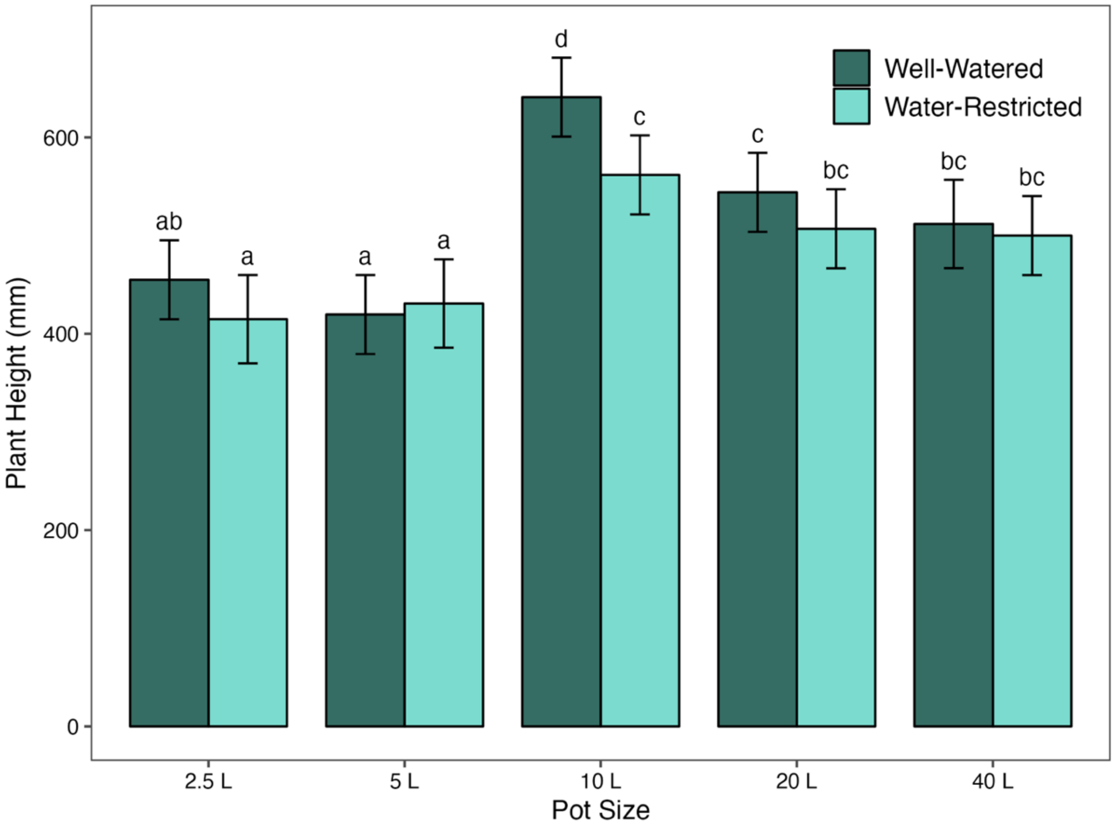

Figure 10. Mean plant height of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered (dark green) or water-restricted (light green) conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent PH across each cultivar within each combination of pot size, water treatment, and measurement date (n = 6) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$). **N.B. Plant height measurements for plants grown in ≥ 20 L pots are systematically low as plants in these pot sizes grew beyond the scanable area of the PlantEye after 63 DAP.**

3.4.4 Greenness Average

The mean (^{2}) 'greenness average' index (greenness) was significantly affected by all four experimental variables (Table 2). Mean greenness increased with pot size, although the differences between pot sizes were not all significant (Figure 11). The differences in greenness between large (≥ 10 L) pot sizes were small, but greenness was 24.5 and 27.7% higher in 40 L pots versus 2.5 and 5 L pots, respectively. There was a significant ($p < 0.001$) interaction between pot size and DAP that suggests the effect of pot size on greenness was related to plant longevity (Figure 12).

Between 43 and 92 DAP, the differences in greenness between pot sizes were small, but by 105 DAP all plants in 2.5 and 5 L pots were dead, yet plants in the larger pot sizes had only recently started to senesce. By 92 DAP, plants in 2.5 L and 5 L pots already had significantly ($p < 0.05$) lower greenness than plants in 10 L pots, which had significantly ($p < 0.05$) lower greenness than plants 40 L pots. The greenness of plants in 20 L pots was not significantly different from that of plants in either 10 or 40 L pots at 92 DAP.

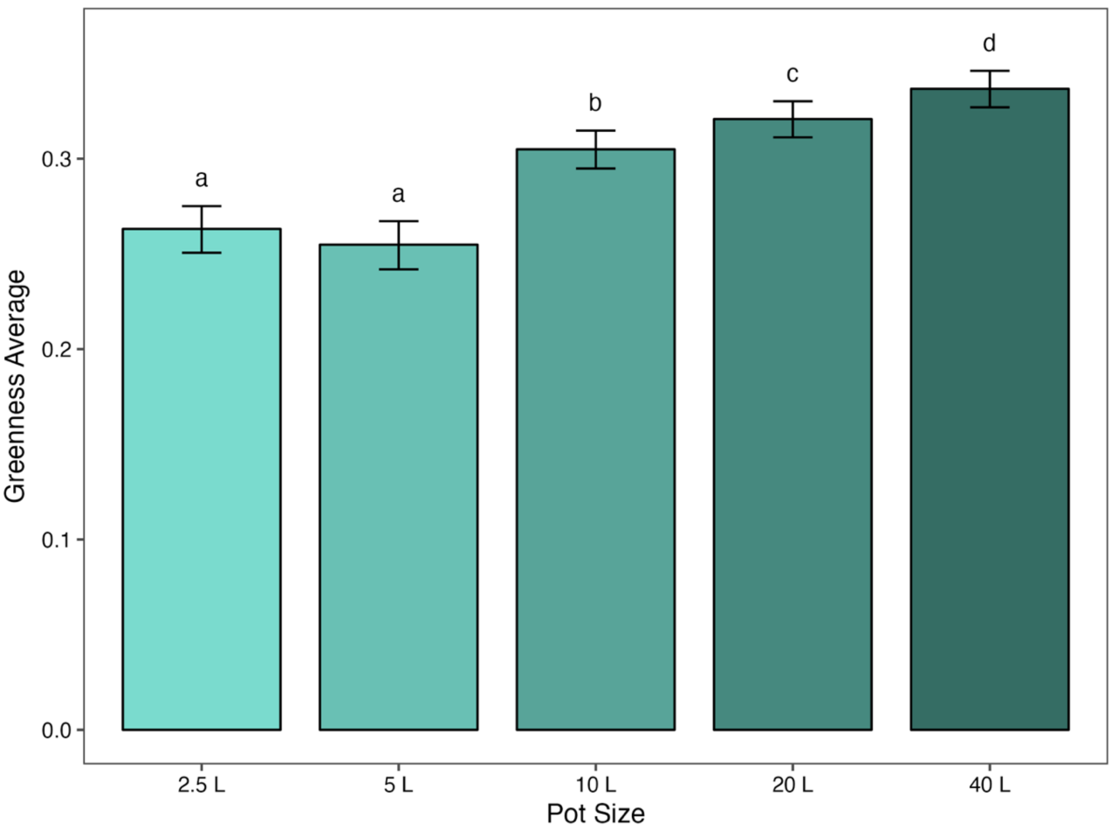

Figure 11. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent greenness across each cultivar, water treatment, and measurement date within each pot size (n = 72) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$).

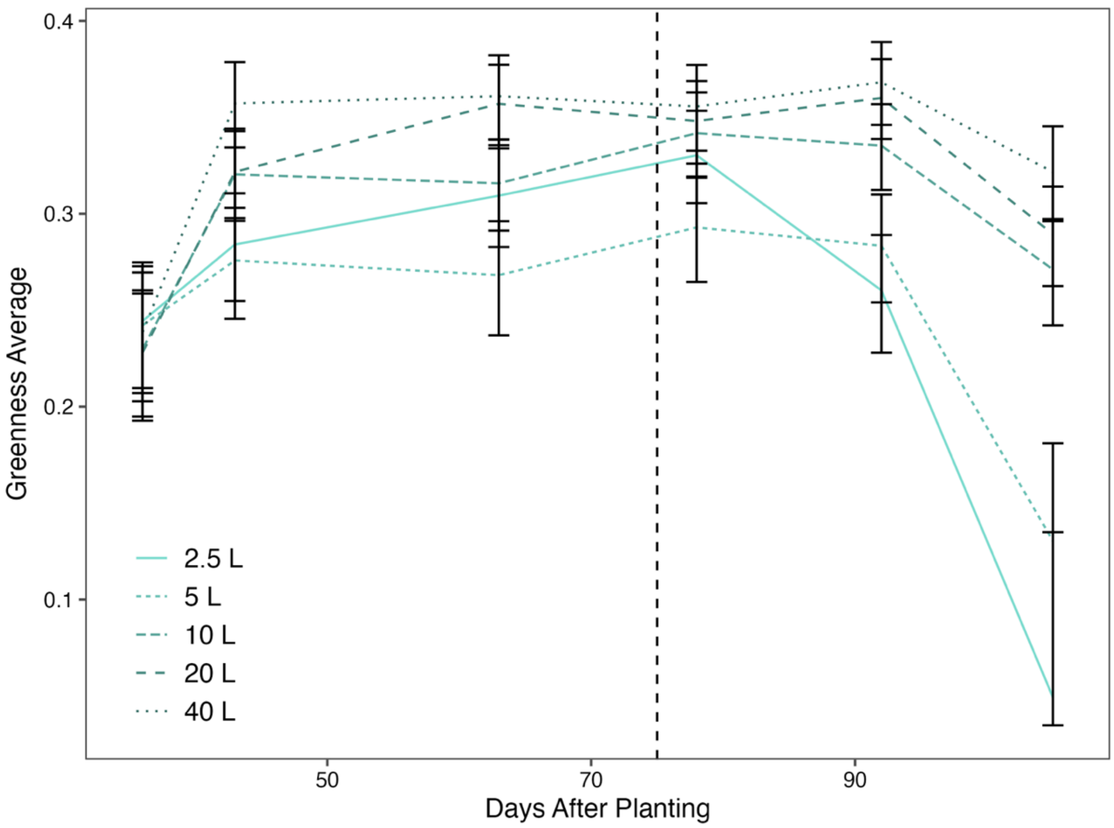

Figure 12. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots (solid light to dotted dark green), from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent greenness across each cultivar and water treatment within each combination of pot size and measurement date (n = 12) ± 95% CIs. Compact letters denoting significant differences between groups can be found in Supplementary Table S4.

Greenness was significantly ($p < 0.001$) affected by cultivar (Table 2). The overall greenness of Maris Piper was 6.7% higher than that of Charlotte. Again, this difference appears to be the result of a difference in longevity and acceleration of senescence, as Maris Piper was able to maintain greenness to 92 DAP, versus 78 DAP for Charlotte (Supplementary Figure S1). However, the interaction between pot size, cultivar, and DAP shows that the greenness of Maris Piper was only significantly ($p < 0.05$) higher than that of Charlotte at 105 DAP in 20 and 40 L, but that longevity was similar between both cultivars in 2.5 and 5 L pots (Figure 13). Although the greenness of Maris Piper was 20.0% higher than of Charlotte at 105 DAP in 10 L pots, this difference was not significant.

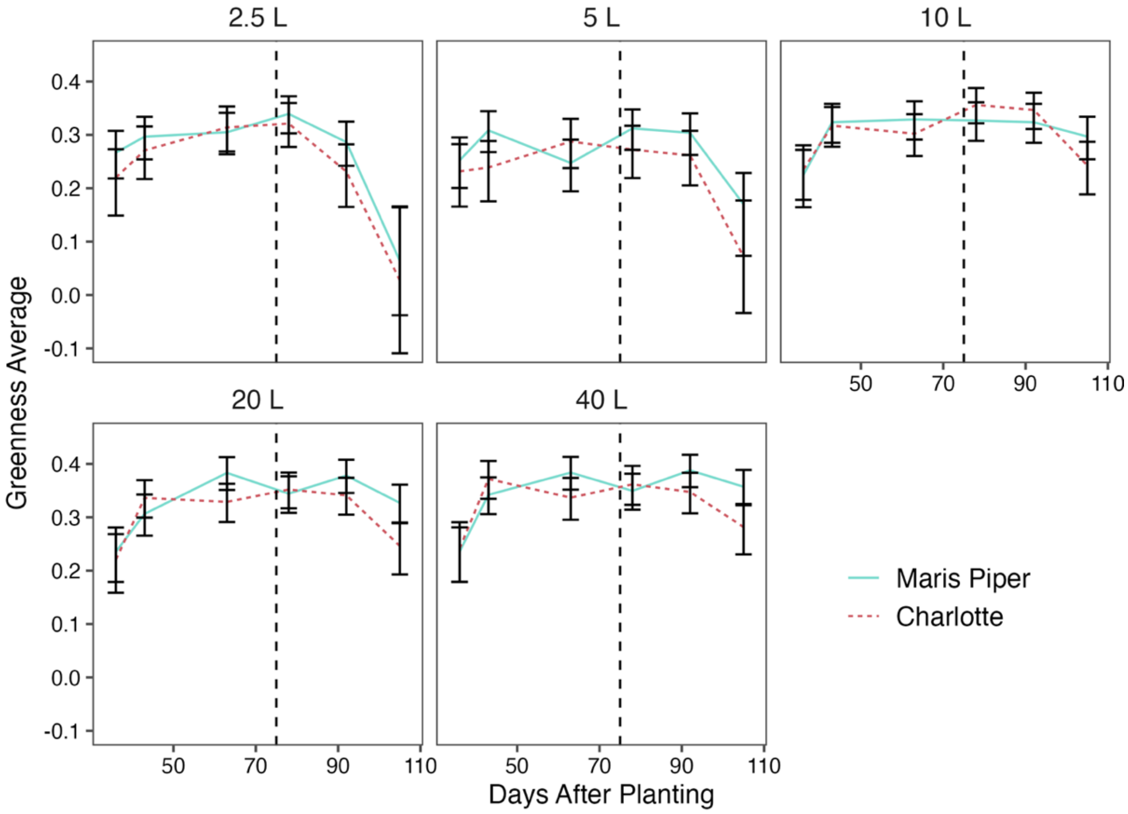

Figure 13. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars., Maris Piper (solid green) and Charlotte (dashed pink), grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent greenness across each water treatment within each combination of pot size, cultivar, and measurement date (n = 6) ± 95% CIs. Compact letters denoting significant differences between groups can be found in Supplementary Table S5.

Overall, water-restriction was associated with a small, but significant (p = 0.002), increase in greenness of 4.0%. Analysis of the significant (p = 0.001) interaction between treatment and pot size showed that water-restriction was associated with the greatest greenness increase in 2.5 and 5 L pots, but that this difference was only significant ($p < 0.05$) in 5 L pots (Figure 14). There was also a significant ($p = 0.001$) interaction between treatment and DAP (Table 2). The increase in greenness from well-watered to water-restricted plants grew from 0.6% to 4.0% between the measurement dates immediate before and after the imposition of water restriction, although the latter difference remained insignificant (Supplementary Figure S2). The only significant difference between the treatment groups occurred at 43 DAP, 32 days before the onset of water restriction.

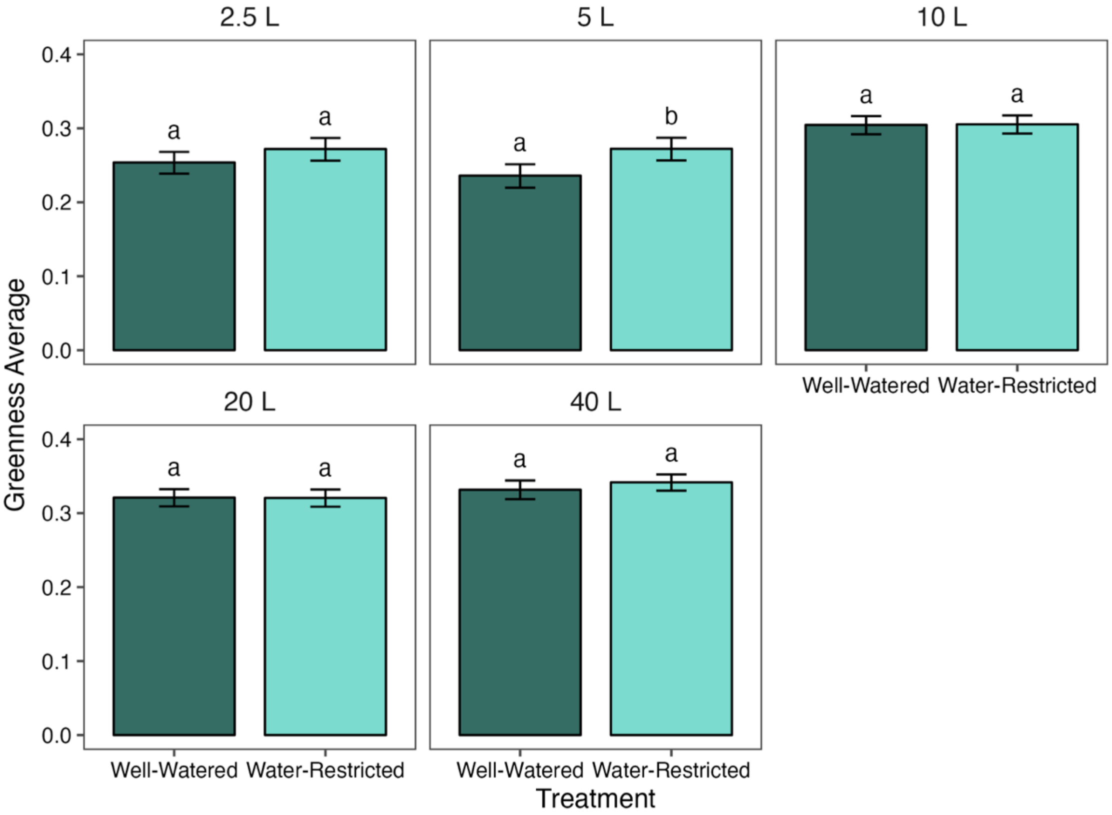

Figure 14. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered (dark green) and water-restricted (light green) conditions, in 2.5, 5, 10, 20, and 40 L pots. Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent greenness across each cultivar and measurement date within each combination of pot size and treatment ($n = 6$) ± 95% Cis. Means with different letters within each facet were significantly different by Tukey's test ($p < 0.05$).

3.5 Discussion

The data support the hypothesis that pot binding can confound the effects of water-restriction on the morphology of potato over time, particularly in pots ≤ 5 L (Figures 4, 8, and 13). The use of such small pots in previous research has been encouraged by the development of HTP platforms (Turner, 2019), and the necessary trade-off between replication and pot size that exists in controlled environments. The decision of many researchers to prioritise replication in controlled environment studies is understandable, as the statistical power of experiments can be limited by small sample sizes. Use of smaller pots therefore enables greater replication in limited space. The use of large pots is more expensive and labour intensive, especially if they require moving for data collection under HTPs. However, the confounding effects of small pots on the relationships between water-restriction and potato morphology, demonstrated here, were large enough to significantly affect responses under water restriction.

3.5.1 Small pots diminish the effects of water-restriction

In this experiment, the effect of water-restriction on yield increased with pot size. Tuber water content is one of the two morphophysiological traits, along with radiation interception, most affected by drought (Jefferies and Mackerron, 1993). As potato tubers have a water content of ~80% (Navarre, Goyer and Shakya, 2009), potato fresh tuber yields are extremely vulnerable to drought. Thus, a decrease in fresh tuber yield due to water-restriction is probably the most consistently observed effect of drought on potato (Struik and Van Voorst, 1986; Jefferies and Mackerron, 1989; Lahlou, Ouattar and Ledent, 2003; Carli *et al.*, 2014; Obidiegwu *et al.*, 2015; Hill *et al.*, 2021). Our data support the “water availability” hypothesis of pot binding, which predicts that small pots hold an insufficient volume of water to prevent drought-stress between irrigation periods, even in supposedly well-watered controls (Sinclair *et al.*, 2017). We found that, particularly in the late-maturing Maris Piper, the yield reduction associated with water-restriction increased with pot size, suggesting that the unintended drought-stress occurring in well-watered plants in small pots was diminished in larger pots, allowing for a greater yield differential between treatments (Figure 3). This relationship was less clear with Charlotte, although this is likely due to the death of three water-restricted plants of this cultivar. As there were no significant interactions between pot size, cultivar, and treatment, it would have been inappropriate to statistically investigate this relationship further.

These data show that greater yields allow for greater differences between treatment groups, which remains consistent with our hypothesis that the effect size of water-restriction on potato would be negatively associated with pot size. Regardless of the mechanism, these results show that pot binding (i.e., the confounding effects of small pots on plant morphophysiology) can be an important systemic error in controlled environment potato research. Depending on the pot size used, conflicting conclusions can be drawn regarding the effects of water-restriction on potato. For example, the effect of water-restriction on digital biomass and plant height was insignificant in small (≦ 5 L) pots, but significant in at least one larger pot size. This could foreseeably be overcome with appropriate sample sizes, but this is unlikely to be possible in controlled environments (Turner, 2019).

Water-restriction was associated with an overall reduction in digital biomass (plant volume) and plant height. These results corroborate previous research on the deleterious effects of water-restriction on biomass (Schittenhelm, Sourell and Löpmeier, 2006; Alhoshan *et al.*, 2019; Alvarez-Morezuelas *et al.*, 2022) and plant height (Deblonde and Ledent, 2001; Luitel *et al.*, 2015; Chang *et al.*, 2018). Biomass and height are useful traits for selection and crop management as both are important determinants of yield in potato (Jefferies, 1995; Tourneux *et al.*, 2003; Schittenhelm, Sourell and Löpmeier, 2006) and can be phenotyped, accurately and remotely, with UAV imaging (de Jesus Colwell *et al.*, 2021).

However, the relationships between pot size and the effects of water-restriction on digital biomass and plant height were confounded by the size of the plants in larger pots. Our results show that both biomass and height peaked in well-watered 10 L pots, but this was not really the case. Potato stems can exceed 1 m in length and many cultivars produce canopies with greater-than-optimum leaf area indices (Schittenhelm, Sourell and Löpmeier, 2006). This can cause self-shading (Schittenhelm, Sourell and Löpmeier, 2006), which not only obscures photosynthetic biomass from solar radiation, but also obscures biomass from imaging sensors. Therefore, measurements of digital biomass in large pots were confounded, as large leaves towards the top of the stems shaded lower leaves from the view of the PlantEye (Figure 1). Plant height was also affected by this error and appeared not to exceed ~750 mm, but this is due to the PlantEye sensor having a capture range of 1.1 m, which was reduced further by the size of the largest pots.

Thus, the results for plant height in plants grown in ≥ 20 L pots after 63 DAP presented here are generally systemically low. Figures presenting digital biomass data also show an unrepresentative variety in from 63 DAP due to the real variations in the degrees of self-shading occurring and the growth of plants beyond the height of the sensor. This occurred because of the natural variability in canopy architecture between the plants and the resulting interactions between the plants and the PlantEye sensor.

Once plants grew beyond the height of the sensor, measurements of plant height were capped at ~750 mm (~1.1 m PlantEye capture range - pot height) and thus are consistently low for plants grown in ≥ 20 L pots from 63 DAP. However, the extent to which digital biomass was affected was related to the position and size of the highest measurable leaves. Where leaves were relatively large and close to the sensor, the degree of self-shading from the perspective of the PlantEye was high, resulting in unrepresentatively low measurements of digital biomass. Conversely, once these leaves had grown beyond the sensor, the degree of self-shading temporarily decreased and measurements of digital biomass increased, until the next most basal leaf grew high or large enough to obscure the rest of the plant. These effects account for the apparent maximum plant height of ~750 mm in this study (Figure 9), and the variability in the results reported for digital biomass of plants grown in ≥ 20 L pots from 63 DAP (Figure 5).

This further highlights the importance of considering logistical factors that could confound the results of phenotyping experiments in further research, beyond the confounding effects of small pot sizes.

This was not an issue with plants in ≤ 10 L pots, where mean plant height peaked at 60 cm. In these pot sizes, the effects of water-restriction on biomass and height were only significant in the largest pot size, 10 L. Treatment had such a small effect on plant height in 5 L pots, that water-restricted plants were 2.6% taller than supposedly well-watered plants. When compared to the 12.3% decrease in plant height due to water-restriction in 10 L pots, this demonstrates, more clearly than yield, the diminishing effect of small pots on the severity of water-restriction.

We cannot definitively state that the insignificant differences between treatments can be explained by the “water availability” hypothesis of pot binding, as testing this directly was beyond the scope of this experiment. We do, however, caution researchers against using very small (≤ 5 L) pots when investigating the effects of water-restriction on potato morphology, as it decreases the likelihood of observing significant effects due to pot binding effects. This is an important area for future research, as creative irrigation protocols may be able to overcome the diminishing effects of small pots on the relative severity of water-restriction in potato.

The relationship between pot size and water-restriction was reversed for greenness, with the only significant increase in greenness occurring in 5 L pots. Greenness did increase with water-restriction in all other pot sizes, but these differences were not significant. This small effect size may account for the varied effects of water-restriction on greenness seen in previous research. Most of these studies observed an increase in greenness with water-restriction (Ramírez *et al.*, 2014; Rolando *et al.*, 2015; Rudack *et al.*, 2017; Li *et al.*, 2019), although this result is not always consistent across genotypes or growth stages (Mthembu *et al.*, 2022).

We are only aware of a single study in which greenness consistently decreased because of water-restriction (Anithakumari *et al.*, 2012), although it's important to note that this work investigated diploid potato cultivars and not cultivated tetraploids.

Chlorophyll content has been suggested as an effective target for selection of drought-tolerant crops and genotypes under water-restricted conditions (Monteoliva, Guzzo and Posada, 2021). In our study, the effects of water-restriction on greenness were measured by the PlantEye sensor, rather than the more common SPAD-meter method used in the research referenced above. It's currently unclear how strongly correlated these measures are, but a previous model based on green (550 nm) and red (700 nm) band imaging of individual potato leaves has been shown to correlate highly (R2 = 0.88) with SPAD-meter measurements (Borhan *et al.*, 2017).

This shows the utility of multispectral imaging for chlorophyll content determination, but further research is needed to assess the validity and reliability of the method used here. SPAD-meters are rapid, non-destructive, and highly suitable for small plot areas (Borhan *et al.*, 2017), but require leaf contact, manual data collection, and can be susceptible to point sampling anomalies. Remote imaging sensors share these advantages, with the potential to improve sampling rate and whole-plant accuracy (Liu, Liu and Sun, 2020).

3.5.2 Cultivars respond differently to small pot sizes

Small pots (≤ 5 L) were associated with a significant stunting effect on Charlotte, but not Maris Piper (Figures 3 and 7). This was demonstrated with both biomass and plant height, which were significantly lower in Charlotte than Maris Piper in equivalent pot sizes, and Charlotte in larger pot sizes. Similar confounding effects of pot size on plant growth under well-watered and water-restricted conditions have been demonstrated in oilseed rape, where the ranking of cultivars' total dry weight was dependent on the pot size used (Dambreville *et al.*, 2016). Due to the large number of potato cultivars and the expense of field trials, the selection of genotypes for further research has been based, at least in part (Haas *et al.*, 2020), on the display of desirable or contrasting responses to water-restriction in pot experiments (Meise *et al.*, 2019; Gervais *et al.*, 2021). Biomass and plant height are important selection criteria for drought tolerance in potato (Jefferies, 1995; Tourneux *et al.*, 2003; Schittenhelm, Sourell and Löpmeier, 2006), but screening for these traits based on HTP experiments in small pots may lead to the inappropriate selection of cultivars for further investigations and field trials.

The cause of this interaction between pot size and cultivar is unclear but may be related to the contrasting maturity and drought-tolerance of the two cultivars. Maris Piper, being the less determinant and more drought-tolerant cultivar may have been better able to maintain above-ground biomass accumulation under water-restricted conditions, which occurred regardless of the intended treatment in small pots. The production of a large canopy has previously been shown as advantageous under water-restricted conditions, as total radiation interception is less affected by the reduction of biomass associated with long-term water-restriction (Schittenhelm, Sourell and Löpmeier, 2006). Further research is needed to elucidate whether the stunting of specific genotypes is consistent with the "water availability" hypothesis of pot binding.

3.6 Conclusion

Small pots were associated with a reduction in the effect of water-restriction on potato biomass and height, two important selection criteria for drought tolerance in potato (Jefferies, 1995; Tourneux *et al.*, 2003; Schittenhelm, Sourell and Löpmeier, 2006). Small pots also confounded the effects of water-restriction on potato biomass and height by disproportionately stunting the development of the early-maturing cultivar, Charlotte, compared to the later-maturing Maris Piper. Thus, small pots likely confound the process of screening potato cultivars for morphological traits associated with drought tolerance under water-restriction, which may lead to the inappropriate selection of cultivars for further research or breeding. This is particularly important for the design of HTP platforms, which typically prioritise replication over pot size due to the spatial limitations associated with controlled environments (Turner, 2019). Elucidating the cause of this pot binding effect is beyond the scope of this experiment, which aimed to develop a more appropriate guideline for potato pot experiments than the 1 g L^{-1} biomass:substate ratio that is typically recommended (Poorter *et al.*, 2012). As this study was limited by the self-shading of plants in large pot sizes, we cannot recommend a pot size that allows plants to develop in a way that is representative of those grown the field. Further research is needed to assess pot binding hypotheses, such as the "water availability" hypothesis (Sinclair *et al.*, 2017), in potato, as creative irrigation protocols, based on continuous pot weight measurements or unrestricted access to water, may alleviate the confounding effects of small pots on potato morphophysiology. However, the results of this study clearly demonstrate that small pots (≤ 5 L) are inappropriate for investigating the effects of water-restriction on potato and for screening cultivars for drought tolerance under these conditions.

3.7 References

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Chapter 4: Investigating the Water Availability Hypothesis of Pot Binding: Small Pots and Infrequent Irrigation Confound the Effects of Drought Stress in Potato (*Solanum tuberosum* L.)

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4.1 Abstract

To maximise the throughput of novel, high-throughput phenotyping platforms, many researchers have utilised smaller pot sizes to increase the number of biological replicates that can be grown in spatially limited controlled environments. This may confound plant development through a process known as “pot binding”, particularly in larger species including potato (*Solanum tuberosum*), and under water-restricted conditions. We aimed to investigate the water availability hypothesis of pot binding, which predicts that small pots have insufficient water holding capacities to prevent drought stress between irrigation periods, in potato. Two cultivars of potato were grown in small (5 L) and large (20 L) pots under polytunnel conditions and were subjected to three irrigation frequencies: every other day, daily, and twice daily. Plants were phenotyped with two Phenospex PlantEye F500s and canopy and tuber fresh mass and dry matter were measured. Increasing irrigation frequency from every other day to daily was associated with a significant increase in fresh tuber yield, but only in large pots. This suggests a similar level of drought stress occurred between these treatments in the small pots,

supporting the water availability hypothesis of pot binding. Further increasing irrigation frequency to twice daily was still not sufficient to increase yields in small pots but caused an insignificant increase in yield in the larger pots, suggesting some pot binding may be occurring in large pots under daily irrigation. Canopy temperatures were significantly higher under each irrigation frequency in the small pots compared to large pots, which strongly supports the water availability hypothesis as higher canopy temperatures are a reliable indicator of drought stress in potato. Digital phenotyping was found to be less accurate for larger plants, probably due to a higher degree of self-shading. The research demonstrates the need to define the optimum pot size and irrigation protocols required to completely prevent pot binding and ensure drought treatments are not inadvertently applied to control plants.

4.2 Introduction

In the last two decades, the rapid development of plant phenotyping technologies has alleviated a significant bottleneck in our understanding of, and ability to select for, desirable traits in important agricultural crops. Historically, the measurement of even simple phenotypic traits was often destructive, expensive, and time-consuming (Furbank and Tester, 2011). Now, non-destructive plant phenotyping can occur over agriculturally relevant areas and timescales, with comparatively low financial and labour costs (Pieruschka and Schurr, 2019).

Researchers have begun to combine these high-throughput phenotyping platforms (HTPPs) with controlled environments to understand how predicted climate scenarios might affect crops in the future (Langstroff *et al.*, 2022). However, while the phenotyping bottleneck has been released, controlled environments with the requisite precision to maintain forecast conditions remain spatially limited. This has led to a trade-off between biological replication and the representativeness of laboratory-grown plants to their field-grown relatives.

To maximise replication in controlled environments and other confined spaces, many researchers utilise smaller pot sizes. However, small pots may confound plant development through a poorly understood process called “pot binding”. The “water availability” hypothesis of pot binding suggests that all plants in small pots are inadvertently drought stressed, as the small volumes of substrate hold insufficient amounts of freely extractible water to prevent this stress between irrigation periods (Sinclair *et al.*, 2017).

If this process occurs in an experiment aiming to investigate the effects of water deficits on plant development, then pot binding will covertly increase the drought stress severity of both the water deficit treatment and the supposedly well-watered control treatment. As the severity of survivable drought stress is limited by the minimum volume of water available for transpiration (Turner, 2019), pot binding will therefore decrease the difference in water deficit between treatments.

This is particularly problematic for large crops with high water requirements, including potato (*Solanum tuberosum*). While potato has a high water-efficiency (Sun *et al.*, 2015), it requires high volumes of water for efficient growth (Knox, Weatherhead and Bradley, 1997; Byrd *et al.*, 2014; Knox *et al.*, 2018) and is extremely susceptible to drought stress (Schafleitner, Gutierrez and Legay, 2009).

According to the water availability hypothesis, this increases the susceptibility of potato to pot binding, relative to the experimental pot size.

Previous research has aimed to provide guidelines to both impose meaningful drought stress (Turner, 2019) and prevent pot binding (Poorter *et al.*, 2012) in pot experiments. The established recommendation to prevent pot binding, based on a meta-analysis of 65 studies, is that the ratio of dry plant biomass to substrate volume should not exceed 1 g L^{-1} (Poorter *et al.*, 2012). As potato has been recorded to produce over 1,000 g of dry matter in controlled environments (Wheeler and Tibbitts, 1987), the recommendation would require a minimum pot volume of 1000 L, which is impractical for phenotyping experiments.

Previous research has aimed to create a more realistic recommendation for pot experiments with potato (Chapter 3). Five pot sizes (2.5, 5, 10, 20, and 40 L) were used to investigate the confounding effects of pot size on water-restriction in potato and the practicalities of using larger pot sizes for phenotyping experiments. It was found that pots ≤ 5 L were inappropriate for investigating the effects of water-restriction on potato, primarily due to a strong drought-independent stunting effect observed in one of two cultivars evaluated, which was not seen in larger pot sizes. Large 40 L pots were also found to be impractical for controlled-environment studies where pots must be manually moved for phenotyping.

Here we investigate the water availability hypothesis in potato and assess whether the effects of pot binding on potato morphophysiology could be mitigated in practical pot sizes by reducing the inter-irrigation period. We also validate the specific phenotyping methods used by comparing their results with low-tech, established (Elsayed *et al.*, 2021; Ninanya *et al.*, 2021; Mthembu *et al.*, 2022), and accurate methods.

4.3 Materials and Methods

4.3.1 Plant Material and Growing Conditions

A pot experiment was carried out at the Crop and Environment Laboratory (51°26'13"N 0°56'32.5"W) at the University of Reading, UK. Thirty pots of each size, 5 and 20 L, were filled with a 2:1 by volume mixture of John Innes No. 2 compost and sharp sand (Jubilee Building Supplies, Bracknell, UK). Each pot was fertilised with either 19.5 g (5 L pots) or 78.0 g (20 L pots) of Osmocote Pro (3-4 Mo). On 1st June 2023, pre-sprouted seed tubers of both *Solanum tuberosum* cvs. Maris Piper and Charlotte were planted in individual pots, with 15 tubers planted into 5 L pots and 15 tubers planted into 20 L pots for each cultivar. All plants were grown outside and uncovered from planting until 28 days after planting (DAP) when they were moved under an open-ended polytunnel. Before being covered, all plants were grown under rainfed conditions with supplementary hand-watering to saturation when rainfall was insufficient. Once covered, all plants were irrigated to saturation daily with a manual irrigation system, until the start of the treatment conditions. Mean ambient temperatures and relative humidities for the data collection period are summarised in Table 1.

On 3rd July (32 DAP), plants from each pot size and cultivar were randomly assigned to one of three water treatments: irrigation to saturation every other day ($T_{1/2}$), irrigation to saturation daily (T_1), or irrigation to saturation twice daily (T_2). Each treatment comprised of 5 pots per pot size and cultivar. The maximum water lost from each pot size had previously been measured gravimetrically at 6-, 18-, 24-, and 48-hours post-saturation, at the cessation of excess runoff from each pot. From 3rd July to 4th August (64 DAP), all plants were automatically irrigated with the irrigation volumes in Table 2. After 4th August, irrigation was withdrawn, the plant canopies were harvested, and the tubers were left to mature in situ until 18th August (78 DAP).

Table 1. Mean ambient temperatures (T) and relative humidities (RH) in the field between 27th June and 4th August 2023. Ambient temperature and relative humidity were retrieved from the University of Reading Atmospheric Observatory (N 51°26'29.2" W 0°56'16.0").

	T (°C)	RH (%)
Mean	17.0	71
S.E.	0.3	2

Table 2. The three water treatments in this experiment were imposed with an automatic irrigation system that provided water to the pots at either 48-, 24-, or 18- and 6-hour intervals. Irrigation volumes were calculated gravimetrically as the maximum water lost from each pot size over the relevant time intervals. These conditions were imposed from 3rd July to 4th August, after a period of uniform well-watered conditions from planting.

Treatment / Irrigation Frequency	5 L pots	20 L pots
Every Other Day ($T_{1/2}$)	400 ml at 12:00	2,800 ml at 12:00
Daily (T_1)	400 ml at 12:00	2,800 ml at 12:00
Twice Daily (T_2)	400 ml at 12:00 + 200 ml at 18:00	2,800 ml at 12:00 + 800 ml at 18:00

4.3.2 Non-Destructive Data Collection

Between 27th June and 4th August, average canopy temperature and SPAD values were recorded for each plant at least three times per week. Canopy temperatures were measured with an AIR-801 infrared thermometer with a resolution of 0.1°C (ATP Instrumentation, Ashby-de-la-Zouch, UK) and SPAD values were measured with SPAD-502Plus (Konica-Minolta, Tokyo, Japan). Three leaves, each from distinct levels within the canopy, were sampled and averaged to give an accurate estimate for the whole canopy (Víg *et al.*, 2012). Canopy levels were standardised by measuring the terminal leaflet on the third highest fully expanded leaf (Gervais *et al.*, 2021), followed by the terminal leaflets on the fifth and seventh leaves. To control for order effects, particularly on canopy temperature, measurements were taken from the highest canopy level of each plant first, followed by the second level, followed by the third. All measurements were taken between 10:00 and 12:00, to prevent the onset of irrigation from confounding the results.

On 13th July (42 DAP), a subsample of three plants per group (treatment x pot size x cultivar) were scanned with two PlantEye F500 multispectral 3D scanners (Phenospex, Heerlen, Netherlands). It was anticipated that plants in the 20 L pots would grow too large to be accurately phenotyped by the PlantEye sensor. Therefore, subsamples of three plants per group were scanned for both pot sizes to maximise coverage for each plant and to maintain balance across the groups. PlantEye scanners have previously been used to measure “high-temperature-induced” (Lazarević *et al.*, 2022) and drought-related (Chapter 3) morphophysiological changes in potato. The PlantEye measured reflectance of five wavelengths: red (620–645 nm), green (530–540 nm), blue (460–485 nm), near-infrared (820–850 nm), and infrared (940 nm). The integrated software, Phena (Phenospex, Heerlen, Netherlands), generated 3D point clouds of the plants by triangulating adjacent points. These point clouds were then used by the HortControl software (Phenospex, Heerlen, Netherlands) to calculate morphological parameters, including digital biomass, greenness index (greenness), hue, normalised difference vegetation index (NDVI), plant senescence reflectance index (PSRI), leaf angle, and light penetration depth. Vegetation indices were calculated in HortControl as ratios of the reflectance of relevant wavelengths, e.g., greenness was calculated as $\frac{(2 \times R_{Green} - R_{Red} - R_{Blue})}{(R_{Green} + R_{Red} + R_{Blue})}$, where R equals reflectance. Morphological parameters were calculated from the spatial distribution of triangles within the point clouds (Lazarević *et al.*, 2021). Due to the high correlations between certain variables, e.g., digital biomass, leaf area, and leaf area index, only the previously stated variables were analysed.

4.3.3 Destructive Data Collection

All plant canopies were harvested on 4th August 2023, 64 DAP. Fresh canopy biomass was measured immediately post-harvest. Canopies were then individually bagged and oven-dried at 60 °C for at least 72 hours. The canopies were then reweighed for the calculation of canopy dry matter percentage. Tubers were left to mature *in situ* for an additional 14 days, after which they were counted and weighed. Subsamples of three representative tubers per plant were sliced into 5 mm cross-sections, and oven-dried at 60 °C for at least 72 hours. The sliced tubers were then reweighed to calculate tuber dry matter content.

4.3.4 Statistical Analysis

All statistical analyses were completed in RStudio (RStudio Team, 2020). For each relevant dependent variable, a linear model was generated with either the formula treatment x pot size x cultivar or treatment x pot size x cultivar x sample date, depending on whether that variable was measured once or over time. QQ plots and Shapiro-Wilks test of normality were used on the residuals of each model to check that the assumption of normality was met. Shapiro-Wilks tests were also used to check the assumption of normality by groups. The assumption of homogeneity of variance was checked using Levene's test with the same formula as each respective model. Average canopy temperature and average canopy SPAD were assessed for normality with histograms and QQ plots exclusively as the sample sizes for these variables were too high to be accurately assessed for normality with Shapiro-Wilks tests (Lumley *et al.*, 2002). If any of these assumptions were not met, signified by a *p*-value ≤ 0.05 or a non-normal QQ plot, the data were transformed, and the tests of normality and homogeneity of variance were reassessed.

Once these assumptions were met, a three or four-way ANOVA was run on the model for each variable. Average canopy temperature and canopy SPAD were assessed with repeated measures ANOVAs, with sample date as a within-subjects factor and plant numbers as unique identifiers. The data for these assessments were found to violate the assumption of sphericity, due to the high number of repeat measurements, so an appropriate correction was applied (Haverkamp and Beauducel, 2017). The Greenhouse-Geisser (GG) correction was selected as it has recently been demonstrated to be more conservative than Huynh-Feldt adjustments (Blanca *et al.*, 2023). The results presented in Table 4 reflect these corrections. Any significant interactions, signified by a *p*-value ≤ 0.05, were decomposed into simple three and/or two-way interactions and simple main effects, all with appropriate Bonferroni adjustments. When the assumptions of the ANOVA were met, the overall error term from each ANOVA was used for all further analysis of that dependent variable.

All data presented here refer to estimated marginal means that were extracted from the linear model for the respective dependent variable with the “emmeans” package in R. These means, ± 95% CIs were then used to represent the data graphically with the “ggplot2” package. Any data that required transformation to meet the assumptions of the relevant statistical tests were back transformed with the inverse function before being represented graphically. Compact letters were calculated from the estimated marginal means and 95% CIs with the “multcomp” package. Means not sharing any letter are significantly different by the Tukey-test at the 0.05% level of significance (Piepho, 2018). To ensure the consistency of language and comparisons, differences between means are presented here as absolute values and percentage differences, i.e., the difference between the two means divided by their average.

To compare the two methods of measuring biomass, digitally and gravimetrically, the data were split into two groups based on pot size. The data were then filtered to exclude the two plants from each group that were not scanned on 13th July. Both measurements were then assessed for normality with Shapiro-Wilks tests and QQ plots. Once normality was assured, correlation coefficients and *p*-values for each pot size were calculated with the Pearson method.

4.4 Results

4.4.1 Manual Tuber Measurements

4.4.1.1 Pot size significantly affects fresh tuber yield, but irrigation treatment only has a significant effect in larger pots

Mean fresh tuber yield (FTY) was significantly affected by pot size ($p < 0.001$) and cultivar ($p < 0.001$), but not by treatment ($p = 0.081$) (Table 3, Supplementary Table S1). There was also a single significant interaction effect between pot size and treatment ($p = 0.003$) on FTY. Pot size had the greatest effect of the three grouping factors; there was a large (836.8 g, 117.1%) difference in FTY across all plants in 20 L pots ($\bar{x}$ = 1132.8 g) compared to 5 L pots ($\bar{x}$ = 296.0 g). The difference between cultivars was much smaller (71.8 g, 13%); the mean FTY of all Maris Piper plants ($\bar{x}$ = 683.1 g) was slightly higher than that of Charlotte ($\bar{x}$ = 611.3 g).

Table 3. Main effects and interaction terms of a three-way ANOVA for fresh tuber yield (sqrt(g)), mean tuber mass (log10(g)), tuber dry matter (%), fresh canopy biomass (log10(g)), canopy dry matter (log10(%)) of two potato cultivars (Maris Piper and Charlotte), grown in one of two pots sizes (5 and 20 L) and subjected to every other day, daily, or twice daily irrigation treatments. Significant *p*-values (< 0.05) are indicated in bold.

		Fresh Tuber Yield (sqrt(g))		Mean Tuber Mass (log10(g))		Tuber Dry Matter (%)		Fresh Canopy Biomass (log10(g))		Canopy Dry Matter (log10(%))	
Effect	DF	F	p	F	p	F	p	F	p	F	p
Treatment (T)	47	2.7	0.081	0.7	0.486	0.7	0.499	27.2	**0.000**	286.4	**0.000**
Pot Size (PS)	47	2069.6	**0.000**	39.4	**0.000**	14.5	**0.000**	3621.2	**0.000**	17.8	**0.000**
Cultivar (C)	47	19.5	**0.000**	0.1	0.733	100.8	**0.000**	40.4	**0.000**	6.7	**0.003**
T x PS	47	6.8	**0.003**	0.4	0.660	1.1	0.334	3.4	**0.043**	32.5	**0.000**

T x C	47	1.0	0.372	0.1	0.926	1.1	0.329	0.2	0.840	1.3	0.270
PS x C	47	2.7	0.106	3.1	0.082	9.7	**0.003**	3.2	0.078	3.2	0.051
T x PS x C	47	2.2	0.127	0.4	0.694	2.1	0.132	0.2	0.828	0.5	0.620

When grouped by pot size, treatment had a significant effect on FTY in 20 L pots ($p = 0.001$), but not in 5 L pots ($p = 1.000$). The response to treatment in 20 L pots was dose-dependent (Figure 1). Each increase in irrigation frequency was associated with an increase in FTY, but only the difference between pots watered every other day and pots watered daily or twice daily was significant ($p < 0.05$). There was a small (63.0 g, 5.8%) difference in FTY between 20 L pots watered every other day ($\bar{x} = 1053.7$ g) and pots watered daily ($\bar{x} = 1116.8$ g), and a larger (114.7 g, 9.8%) difference between the latter and pots watered twice daily ($\bar{x} = 1231.4$ g).

The analysis of tuber number demonstrated a lack of within-group normality due to the incongruently consistent tuber number within a single group (every other day, 5 L, Charlotte). In this group, all but one replicate produced 10 tubers, the other produced 7, causing a significantly non-normal distribution ($p < 0.001$) that was not present across the whole sample or within any other groups. Further statistical analysis was discarded, but simple summary statistics showed only pot size had a noticeable effect on tuber number; plants in 5 L pots produced an average of 10 tubers, compared to 25 tubers in 20 L pots. When grouped by either cultivar or treatment, average tuber number per plant was within one tuber for each group.

There was sufficient variation in FTY within the non-normal group (5 L pots, Charlotte, irrigation every other day) that further analysis of mean tuber mass was appropriate. Mean tuber mass was only significantly affected by pot size ($p < 0.001$), with no statistically significant interactions (Table 3). There was a small (13.4 g, 35.8%) difference between the pot sizes; the mean tuber mass of all plants in 20 L pots (x̄ = 44.0 g) was slightly higher than that in 5 L pots (x̄ = 30.6 g).

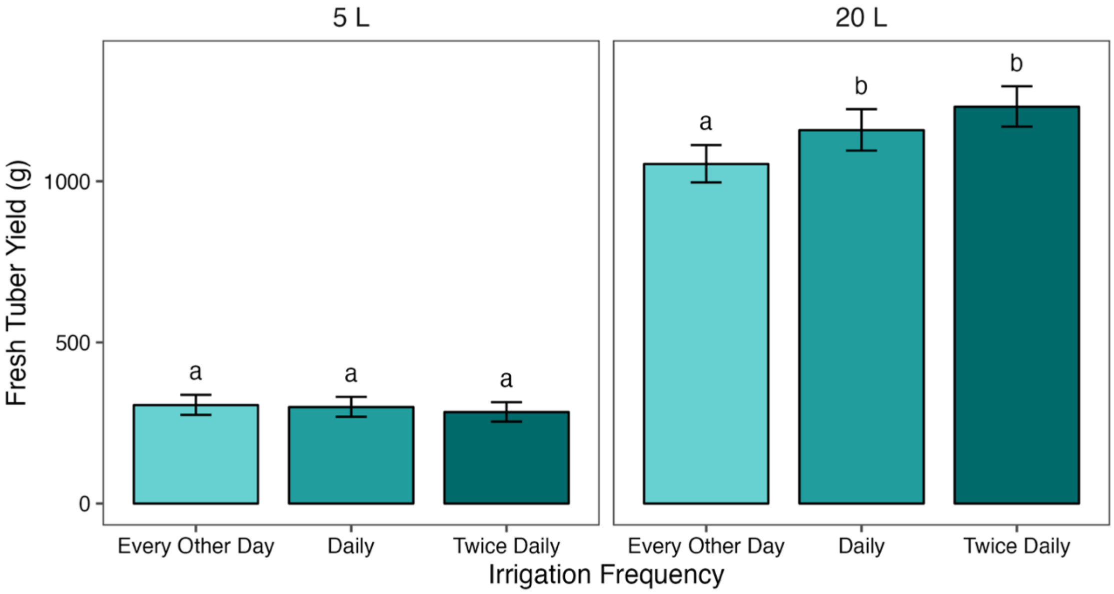

Figure 1. Mean fresh tuber yields (FTY) from potato plants grown in two pot sizes, 5 and 20 L, under three different water treatments: watered to capacity every other day, daily, and twice daily. Plants were grown under an open-ended polytunnel between 1st June and 4th August 2023. Tubers were harvested on 18th August 2023, 78 days after planting. Means represent FTY across two cultivars of potato, Maris Piper and Charlotte, (n = 10) ± 95% CIs. Means with different letters within each panel were significantly different by Tukey's test ($p < 0.05$).

In summary, mean fresh tuber yield (FTY) was significantly affected by pot size and cultivar, but not by treatment (Table 3, Supplementary Table S1). Pot size had the greatest effect on FTY, with an 836.8 g (117.1%) difference between FTY in 5 and 20 L pots; FTY was greater in the latter. Treatment had a significant effect exclusively in 20 L pots, with each increase in irrigation frequency being associated with an increase in FTY, although the difference in FTY between plants irrigated daily and twice daily was not significant in this pot size (Figure 1). Mean tuber mass was also significantly affected by pot size, but the effect was small (13.4 g, 35.8%).

4.4.1.2 Pot size significantly affects tuber dry matter in Maris Piper, but not in Charlotte

Mean tuber dry matter percentage (TDM%) was significantly affected by pot size ($p < 0.001$) and cultivar ($p < 0.001$), but not by treatment ($p = 0.499$). There was also a significant interaction between pot size and cultivar ($p = 0.003$) (Table 3). Cultivar had the greatest effect of the three grouping factors; there was a small (3%, 14.3%) difference in TDM% between Maris Piper (x̄ = 20.7%) and Charlotte (x̄ = 18.0%). The difference between pot sizes was smaller (1.2% 6.5%); the mean TDM% of all plants in 5 L pots (x̄ = 20%) was very slightly higher than of those in 20 L pots (x̄ = 19%).

The interaction effect between pot size and cultivar demonstrated a difference in the effect of pot size on TDM% between the two cultivars. There was no significant ($p = 1.000$) difference in the TDM% of Charlotte between the 5 L (x̄ = 18.0%) and 20 L pots (x̄ = 17.9%), but there was a significant ($p < 0.001$) difference (2.3%, 10.9) between Maris Piper in 5 L (x̄ = 21.9%) and 20 L pots (x̄ = 19.6%). TDM% in Maris Piper was also significantly higher than that of Charlotte in both 5 L ($p < 0.001$) and 20 L ($p < 0.001$) pots.

4.4.2 Manual Canopy Measurements

4.4.2.1 Increasing irrigation from every other day to daily significantly increases canopy biomass, but further increases have no significant effect

Fresh canopy biomass was significantly affected by pot size ($p < 0.001$), cultivar ($p < 0.001$), and treatment ($p < 0.001$) (Table 3). There was also a significant interaction between pot size and treatment ($p = 0.043$). Pot size had the greatest effect on canopy biomass, with a very large (934.1 g, 191.9%) difference between 5 L (x̄ = 173.3 g) and 20 L pots (x̄ = 953.7 g). Maris Piper (x̄ = 444.8 g) produced heavier (73.3

g, 18.0%) canopies than Charlotte ($\bar{x}$ = 371.5 g) and each increase in irrigation frequency was associated with an average increase in fresh biomass, although these were not always significant.

Analysis of the interaction effect between pot size and treatment showed that, when averaged across the two cultivars, canopy biomass increased significantly ($p < 0.05$) between $T_{1/2}$ and T_1 in both 5 L and 20 L pots, with a difference of 45.1 g (27.3%) and 129.0 g (13.9%) between treatments, respectively. In the 5 L pots, there was no significant difference in biomass between the T_1 ($\bar{x}$ = 187.4 g) and T_2 ($\bar{x}$ = 195.1 g) treatments. This effect was consistent in the 20 L pots, where there was no significant difference between the T_1 ($\bar{x}$ = 1010.3 g) and T_2 ($\bar{x}$ = 993.4 g) treatments (Figure 2).

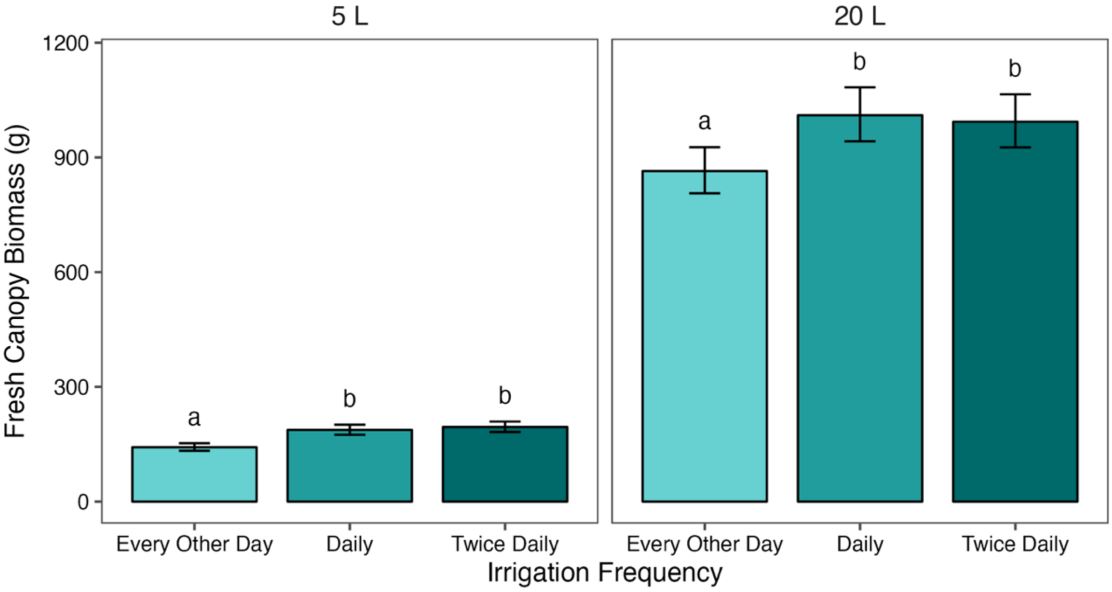

Figure 2. Mean fresh canopy biomass of potato plants grown in two pot sizes, 5 and 20 L, each under three different water treatments: watered to capacity every other day, daily, and twice daily. Plants were grown under an open-ended polytunnel between 1st June and 4th August 2023. Canopies were harvested on 4th August, 64 days after planting. Means represent canopy biomass across two cultivars of

potato, Maris Piper and Charlotte, (n = 10) ± 95% CIs. Means with different letters within each facet were significantly different by Tukey's test ($p < 0.05$).

4.4.2.2 Pot size significantly affects canopy dry matter in Charlotte, but not in Maris Piper

Canopy dry matter percentage (CDM%) was significantly affected by all three grouping factors (pot size, $p < 0.001$; cultivar, $p < 0.001$; treatment, $p = 0.003$) and there was a significant interaction effect between pot size and cultivar ($p < 0.001$) (Table 3). When averaged across all treatments, pot size had a significant effect on the CDM% of Charlotte ($p < 0.001$), but not on that of Maris Piper ($p = 0.590$). The CDM% of Maris Piper in 5 L ($\bar{x} = 10.5\%$) and 20 L pots ($\bar{x} = 10.8\%$) were within 1%, while that of Charlotte was significantly lower in 20 L pots ($\bar{x} = 6.7\%$) than in 5 L pots ($\bar{x} = 8.3\%$). Cultivar had the greatest effect on CDM%, with an absolute difference of 3.1% (34.7% difference), compared to a 0.8% (8.7% difference) between the pot sizes. Treatment had a smaller effect, with an absolute difference of 0.5% (5.7% difference) between plants irrigated every other day and daily, and 0.3% (3.6% difference) between the latter and twice daily irrigation. Again, there was a significant difference in canopy dry matter percentage between irrigation every other day and twice daily ($p < 0.05$), but not between either of those frequencies and daily irrigation ($p > 0.05$).

4.4.2.3 Each increase in irrigation frequency was associated with a significant decrease in canopy temperature

Canopy temperature was significantly affected by treatment ($p < 0.001$), pot size ($p < 0.001$), cultivar ($p = 0.001$), and sample date ($p < 0.001$) and there was a significant four-way interaction between cultivar, pot size, treatment, and sample date ($p = 0.006$). When this interaction effect was broken down by cultivar, there was a significant ($p < 0.001$) three-way interaction between pot size, sample date, and treatment in both Maris Piper and Charlotte. When each cultivar was grouped by pot size, there was a significant ($p < 0.001$) interaction between treatment and sample date in all four groups. Significant effects of treatment were only seen on specific sample dates, which varied between the groups of cultivar and pot size (Figures 3 & 4).

Across all other factors, canopy temperature demonstrated a dose-dependent response to treatment, as each increase in irrigation frequency was associated with a significant decrease in canopy temperature ($p < 0.05$). Plants irrigated every other day ($\bar{x}$ = 18.7 °C) were 0.6 °C warmer than those irrigated daily ($\bar{x}$ = 18.0 °C). Plants irrigated daily were also 0.3 °C warmer than those irrigated twice daily ($\bar{x}$ = 17.7 °C). This relationship was consistent within each pot size, although canopy temperatures within each treatment were significantly higher in 5 L pots compared to 20 L pots ($p < 0.05$).

Plants irrigated every other day in 5 L pots ($\bar{x}$ = 19.3 °C) were 1.3 °C warmer than those under the same conditions in 20 L pots ($\bar{x}$ = 18.0 °C). Plants irrigated both daily and twice daily were both 0.4 °C warmer in 5 L pots ($\bar{x}$ = 18.2 °C and 17.9 °C, respectively) compared to 20 L pots ($\bar{x}$ = 17.8 °C and 17.5 °C, respectively). Canopy temperatures in each group of pot size and treatment were significantly different from all other groups ($p < 0.05$), except for irrigation every other day in 20 L pots and daily irrigation in 5 L pots.

This relationship between canopy temperature, treatment, and pot size was similar between the cultivars. There was a larger difference in canopy temperature between plants irrigated daily and every other day in 5 L pots compared to 20 L pots, in both Maris Piper (Δx̄ = +1.2 °C and +0.2 °C, respectively) and Charlotte (Δx̄ = +0.9 °C and +0.2 °C, respectively). The temperature differences between plants irrigated twice daily and daily were more consistent, with a 0.3 °C increase in canopy temperature in Maris Piper and a 0.4 °C increase in temperature in Charlotte, both regardless of pot size.

The relationships between canopy temperature, treatment, pot size, and cultivar were particularly evident when grouped by sample date (Figures 3 & 4). The difference in canopy temperature between plants irrigated every other day and daily was frequently much larger, and more likely to be significant, in 5 L pots than 20 L pots, regardless of cultivars.

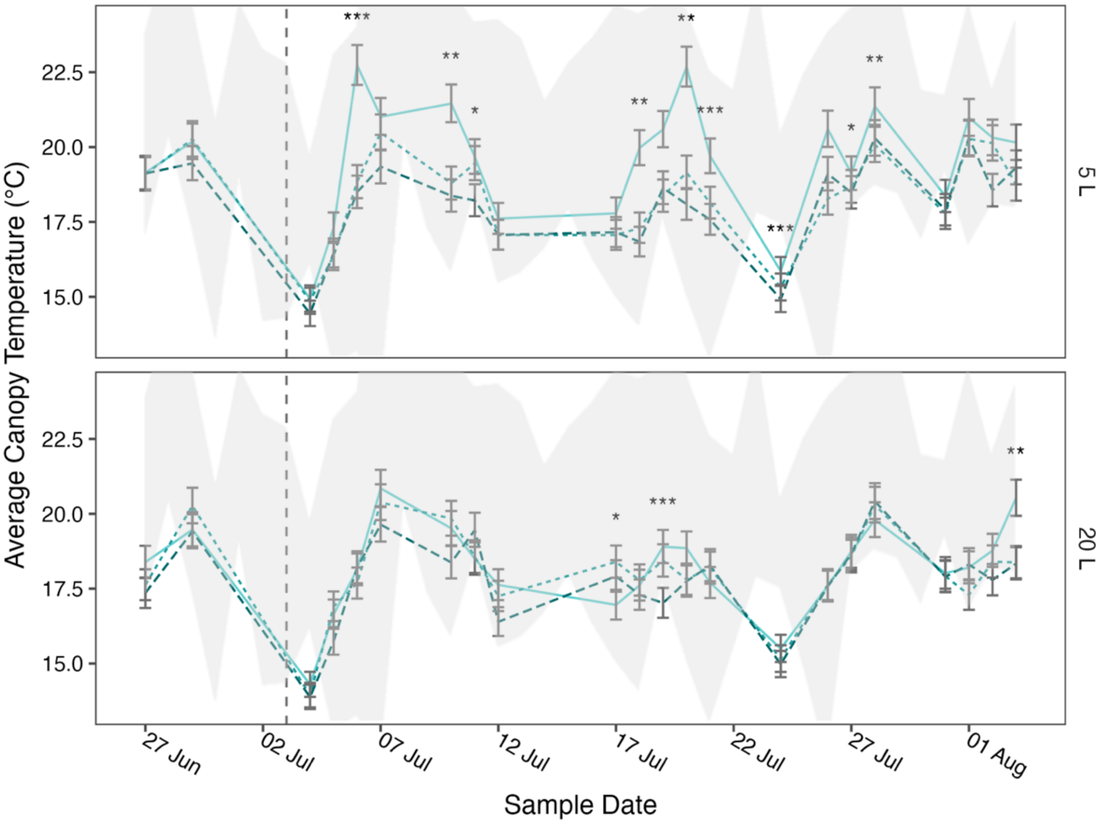

Figure 3. Mean canopy temperature of potato (cv. Maris Piper) over time, grown in

two pot sizes, 5 (top) and 20 L (bottom), each under three water treatments: watered to capacity every other day (solid line), daily (dotted line), and twice daily (dashed line). Plants were grown under an open-ended polytunnel between 1st June and 4th August 2023. Canopy temperature was measured between 27th June and 4th August. The different irrigation frequency treatments commenced on 3rd July 2023 (vertical dashed line). Daily ambient temperature ranges are shown by the grey ribbon. Means represent canopy temperature averaged across three canopy levels: top, middle, and bottom, (n = 5) ± 95% CIs. Means with asterisks above were significantly affected by treatment according to main effects analysis grouped by pot size, cultivar, and sample date with a Bonferroni p-value adjustment (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

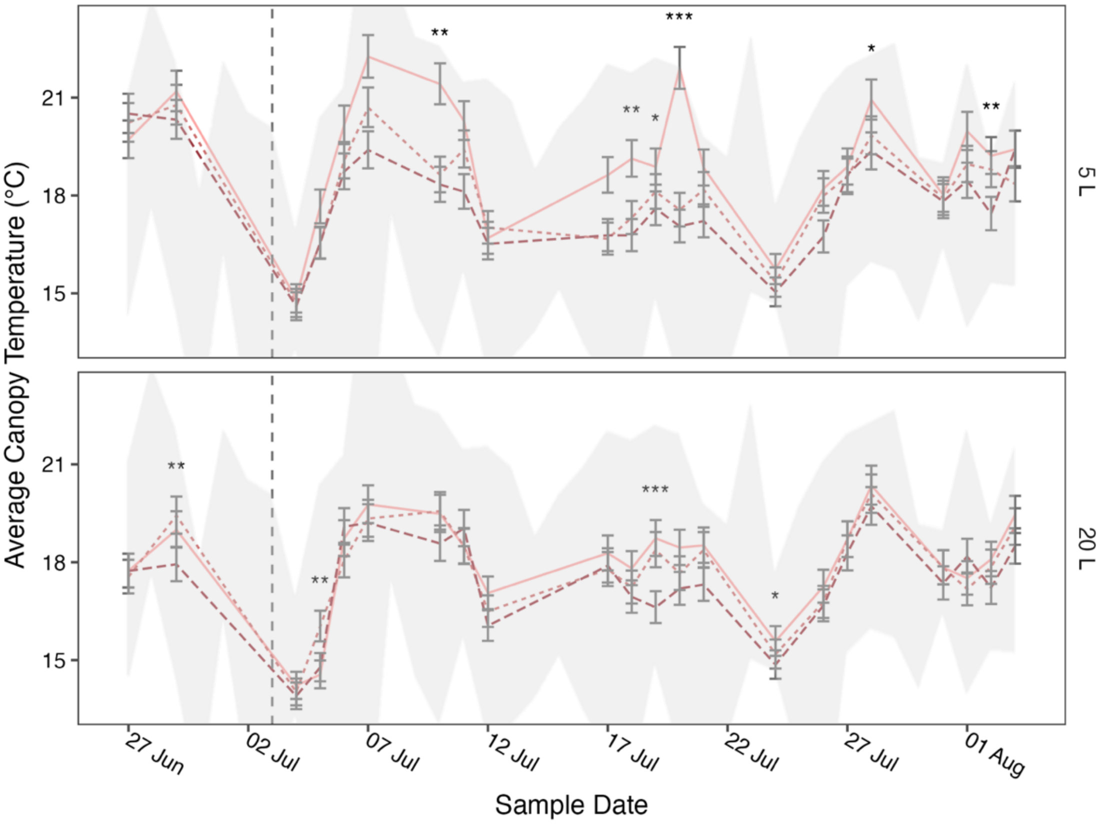

Figure 4. Mean canopy temperature of potato (cv. Charlotte) over time, grown in two pot sizes, 5 (top) and 20 L (bottom), each under three water treatments: watered to capacity every other day (solid line), daily (dotted line), and twice daily (dashed line). Plants were grown under an open-ended polytunnel between 1st June and 4th August 2023. Canopy temperature was measured between 27th June and 4th August. The different irrigation frequency treatments commenced on 3rd July 2023 (vertical dashed line). Daily ambient temperature ranges are shown by the grey ribbon. Means represent canopy temperature averaged across three canopy levels: top, middle, and bottom, (n = 5) ± 95% CIs. Means with asterisks above were significantly affected by treatment according to main effects analysis grouped by

pot size, cultivar, and sample date with a Bonferroni p-value adjustment (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

In summary, canopy temperature was significantly affected by treatment, pot size, cultivar, and sample date, with a significant interaction effect between all four factors (Table 4). Across all other factors, canopy temperature demonstrated a dose-dependent response to treatment, as each increase in irrigation frequency was associated with a significant decrease in canopy temperature (Figure 5). This relationship was consistent within each pot size, although canopy temperatures within each treatment were significantly higher in the smaller pots. All these groups were significantly different from one another, except for plants irrigated every other day in 20 L pots and every day in 5 L pots. When grouped by sample date, the difference in canopy temperature between plants irrigated every other day and daily was more likely to be significant in the smaller pots; this effect was consistent between the cultivars.

4.4.2.4 *Average canopy SPAD values were not affected by pot size or irrigation frequency*

Average canopy SPAD was significantly affected by cultivar ($p < 0.001$) and sample date ($p < 0.001$) but not by pot size ($p = 0.502$) or treatment ($p = 0.612$) (Table 4, Supplementary Table S1). The four-way interaction was not significant ($p = 0.779$), but there was a significant interaction between pot size, cultivar, and sample date ($p = 0.001$). When grouped by sample date, there were significant ($p < 0.05$) interactions between pot size and cultivar on thirteen of the twenty-two sample dates. Post-hoc pairwise comparisons for each sample date with a significant interactions demonstrated that Charlotte had consistently higher SPAD values in both pot sizes, Charlotte in 5 L pots began the experiment with significantly ($p < 0.05$) higher SPAD values than Charlotte in 10 L pots and Maris Piper in both pot sizes, and Maris Piper ended the experiment with significantly ($p < 0.05$) lower SPAD values than the other three groups (Figure 5).

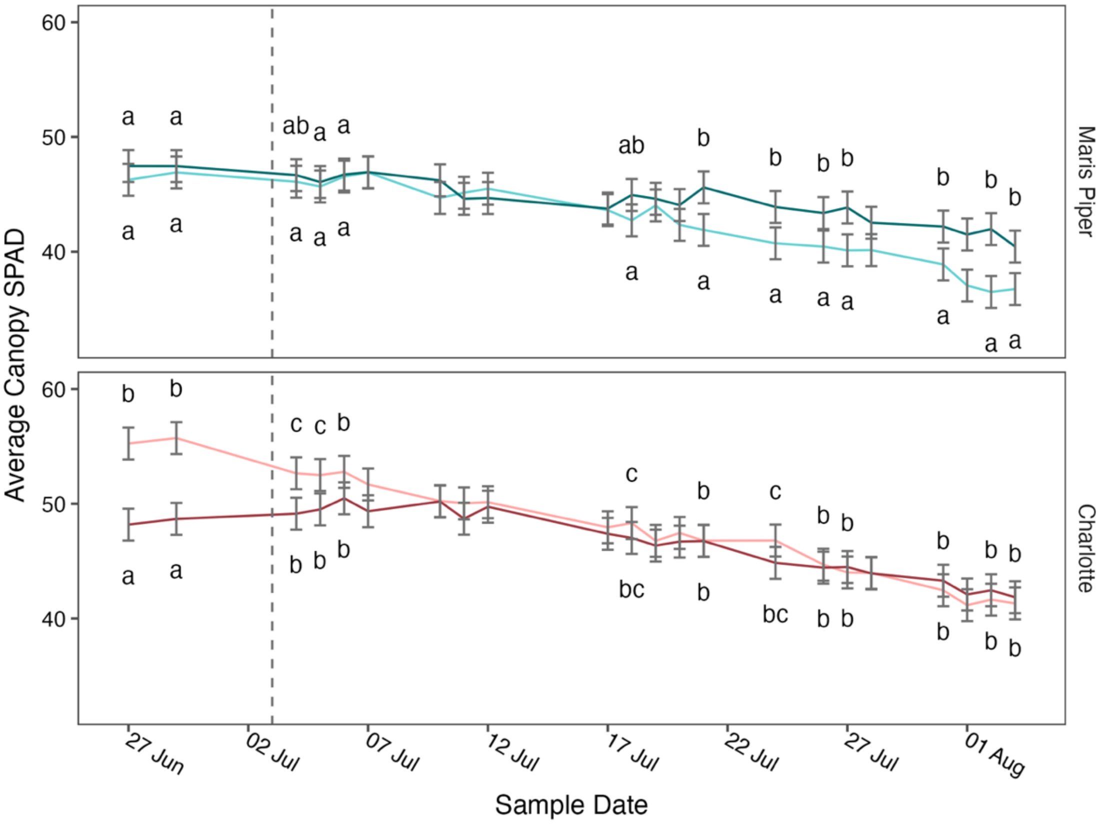

Figure 5. Mean canopy SPAD values of two cultivars of potato, Maris Piper (top facet) and Charlotte (bottom facet) over time, grown in two pot sizes, 5 (light lines) and 20 L (dark lines), across three water treatments: watered to capacity every other day, daily, and twice daily. Plants were grown under an open-ended polytunnel between 1st of June and 4th of August 2023. SPAD values were measured between 27th June and 4th August. The different irrigation frequency treatments commenced on 3rd July 2023 (vertical dashed line). Means represent average SPAD values measured at three canopy levels per plant: top, middle, and bottom, (n = 5) ± 95% CIs. Compact letters were removed for sample dates with an insignificant ($p > 0.05$) interaction between pot size and cultivar. Within each sample date, means with different letters were significantly different by Tukey's test ($p < 0.05$).

Table 4. Main effects and interaction terms of a four-way ANOVA for canopy temperature (°C) and average canopy SPAD of two potato cultivars (Maris Piper and Charlotte), grown in one of two pots sizes (5 and 20 L) and subjected to every other day, daily, or twice daily irrigation treatments. Temperature and SPAD values were sampled between 27th June and 4th August with a handheld laser thermometer and SPAD meter, respectively. Significant *p*-values (< 0.05) are indicated in bold.

		Canopy Temperature (°C)		Average Canopy SPAD	
Effect	DF	F	p	F	p
Treatment (T)	48	50.5	**0.000**	0.5	0.612
Pot Size (PS)	48	71.9	**0.000**	0.5	0.502
Cultivar (C)	48	13.5	**0.001**	84.6	**0.000**
Sample Date (SD)	421.68	481.6	**0.000**	106.0	**0.000**
T x PS	48	13.1	**0.000**	0.0	0.952
T x C	48	0.2	0.795	1.2	0.306
PS x C	48	0.0	0.942	15.7	**0.000**
T x SD	421.68	10.1	**0.000**	1.3	0.188
PS x SD	421.68	20.3	**0.000**	8.4	**0.000**
C x SD	421.68	7.7	**0.000**	3.0	**0.001**
T x PS x C	48	0.2	0.786	1.6	0.220
T x PS x SD	421.68	8.9	**0.000**	0.8	0.743
T x C x SD	421.68	2.4	**0.001**	1.0	0.422
PS x C x SD	421.68	8.1	**0.000**	2.9	**0.001**
T x PS x C x SD	421.68	2.1	**0.006**	0.8	0.779

4.4.3 PlantEye Measurements

4.4.3.1 Irrigation frequency had a significant effect on digital canopy biomass in both pot sizes, but only in Maris Piper

Digital canopy biomass was also significantly affected by pot size ($p < 0.001$), cultivar ($p < 0.001$), and treatment ($p = 0.003$). In contrast to fresh canopy biomass, there was also a marginally significant interaction between all three grouping factors ($p = 0.05$), and significant interactions between each pair of factors (Table 5, Supplementary Table S1). Again, pot size had the greatest effect, with a difference of 163.23 dm^3 (138.1%) in digital biomass between 5 L ($\bar{x} = 36.60$ dm^3) and 20 L pots ($\bar{x} = 199.83$ dm^3).

When grouped by cultivar, there was a significant interaction between pot size and treatment on digital canopy biomass in Maris Piper (*p* = 0.003) but not in Charlotte (*p* = 0.246). When grouped further by pot size, treatment had a significant effect on Maris Piper in both 5 L (*p* = 0.004) and 20 L pots (*p* = 0.002). There was no interaction between pot size and treatment in Charlotte as the effect of treatment on digital canopy biomass was insignificant in 20 L pots (*p* = 1.000).

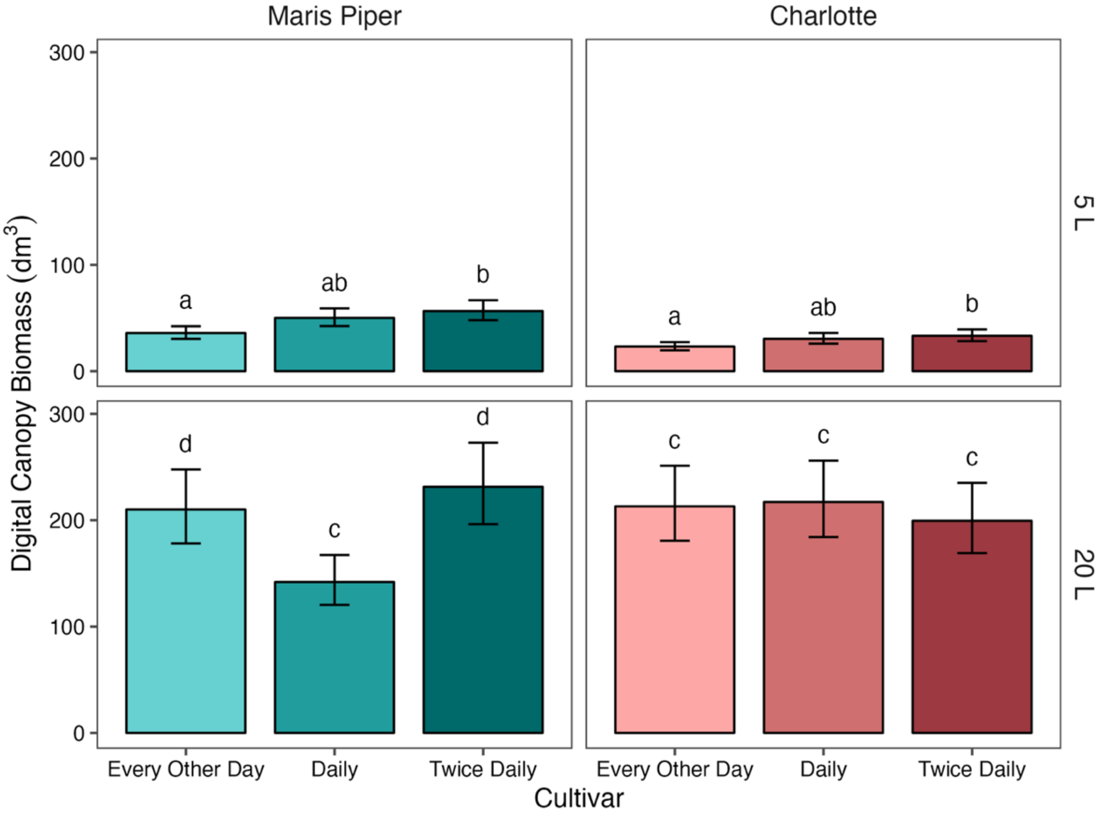

Figure 6. Mean digital canopy biomass of two cultivars of potato, Maris Piper and Charlotte, grown in two pot sizes, 5 and 20 L, each under three water treatments: watered to capacity every other day, daily, and twice daily. Plants were grown under an open-ended polytunnel between 1st June and 4th August 2023. Canopies were scanned on 13th July, 42 days after planting. Means represent digital biomass, measured by HortControl (Phenospex, Heerlen, Netherlands), in decilitres cubed (n = 5) ± 95% CIs. Means with different letters within each cultivar were significantly different by Tukey's test (*p* < 0.05).

4.4.3.2 Digital biomass was significantly correlated with fresh canopy biomass, but only in smaller pots

In 5 L pots, there was a significant, strong, positive correlation between fresh canopy biomass and digital canopy biomass (*r* (16) = 0.780, *p* < 0.001). However, in 20 L pots, this correlation was not significant (*r* (16) = 0.015, *p* = 0.952) (Figure 7). A similar correlation was found between manual and digital measurements of plant height. In 5 L pots there was a significant positive correlation (*r* (16) = 0.896, *p* < 0.001), but in 20 L pots, this correlation was not significant, (*r* (16) = -0.390, *p* = 0.110).

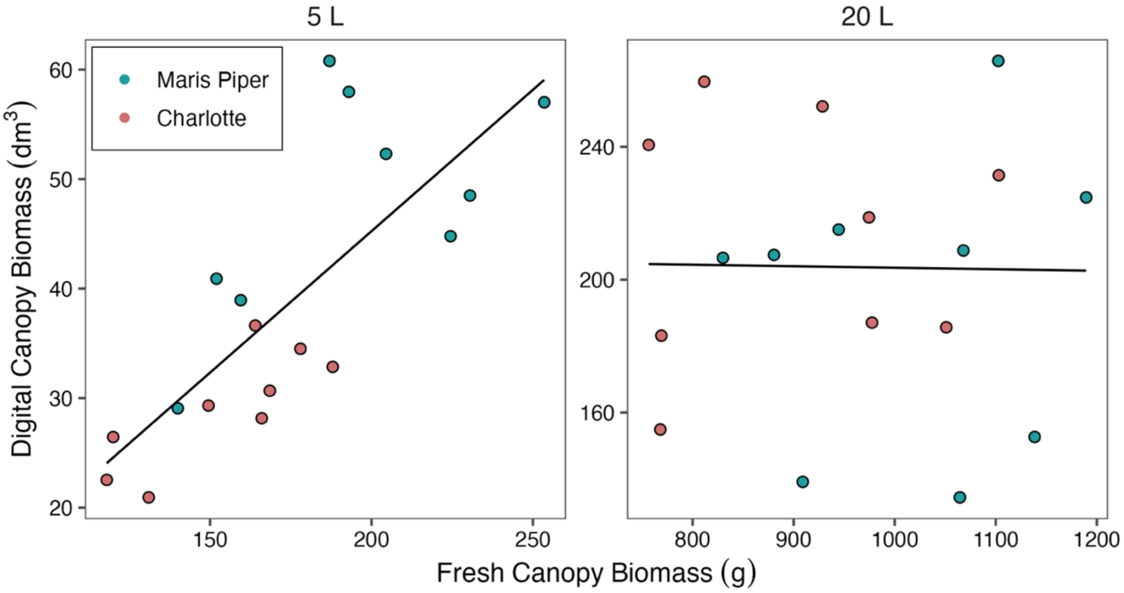

Figure 7. Correlations between digital canopy biomass and fresh canopy biomass for two cultivars of potato, Maris Piper (green) and Charlotte (pink), in two pot sizes, 5 L (left panel; *r* (16) = 0.780, *p* < 0.001) and 20 L (right panel; *r* (16) = 0.015, *p* = 0.952), under three water treatments: watered to capacity every other day, daily, and twice daily. Plants were grown under an open-ended polytunnel between 1st June and 4th August 2023. Digital canopy biomass was measured by HortControl (Phenospex, Heerlen, Netherlands) on 13th of July, 42 days after planting, and canopies were harvested and weighed on 4th of August, 64 days after planting.

4.4.3.3 Average greenness was significantly correlated with SPAD values, but only in smaller pots

Average greenness was significantly affected by pot size ($p < 0.001$) and cultivar ($p = 0.001$), but not by treatment ($p = 0.896$) (Table 5). There was also a significant three-way interaction between all three grouping factors ($p < 0.001$). When grouped by cultivar, there was a significant interaction between pot size and treatment in Charlotte ($p < 0.001$) but not Maris Piper ($p = 0.204$). The effect of treatment on Charlotte was only significant in 20 L pots ($p < 0.001$). Within this group, average greenness was significantly ($p < 0.05$) higher with irrigation every other day compared to daily and twice daily, which were not significant different from each other. Overall, Maris Piper ($\bar{x} = 0.33$) had a slightly (0.04 index units, 4.5%) higher average greenness than Charlotte ($\bar{x} = 0.31$) and plants in 20 L pots ($\bar{x} = 0.35$) had a higher (0.06, 17.7%) average greenness than those in 5 L pots ($\bar{x} = 0.29$). Overall, there was no significant correlation between average greenness and average canopy SPAD values (r (16) = -0.213, $p = 0.213$). When broken down by pot size, there was a significant, very strong, negative correlation between these two variables in 5 L pots (r (16) = -0.864, $p < 0.001$), but not in 20 L pots (r (16) = 0.344, $p = 0.162$).

4.4.3.4 Irrigation had significant effects on average hue, but only for Charlotte in small pots and Maris Piper in large pots

Average hue was significantly affected by all three grouping factors (pot size, $p < 0.001$; cultivar, $p < 0.001$; treatment, $p = 0.001$) and there was a significant three-way interaction ($p = 0.002$) (Table 5). When grouped by cultivar, there were significant interactions between pot size and treatment in both Maris Piper ($p < 0.001$) and Charlotte ($p = 0.012$). Treatment had a significant effect on Charlotte in

5 L ($p = 0.008$) and on Maris Piper in 20 L pots ($p < 0.001$), but not on Charlotte in 20 L pots ($p = 0.788$) or on Maris Piper in 5 L pots ($p = 0.696$). However, the differences in average hue between treatments within these groups were very small (≤ 5.3%).

4.4.3.5 Irrigation frequency significantly affected average NDVI, but only in large pots

Whole-plant average NDVI was significantly affected by treatment ($p < 0.001$) and pot size ($p < 0.001$), but not by cultivar ($p = 0.280$) (Table 5). There was a significant three-way interaction between all three grouping factors ($p < 0.001$). When grouped by cultivar, there were significant two-way interactions between treatment and pot size in both Maris Piper ($p = 0.048$) and Charlotte ($p < 0.001$). Within each cultivar, the effect of treatment was only significant in 20 L pots (Maris Piper, $p < 0.001$; Charlotte, $p < 0.001$). However, the percentage differences between treatments within these groups were small (< 5%).

4.4.3.6 Average PSRI was consistently affected by irrigation frequency in both pot sizes and cultivars

Whole-plant average PSRI was significantly affected by treatment ($p = 0.001$), pot size ($p < 0.001$), and cultivar ($p < 0.001$) and there was a significant three-way interaction between all three grouping factors ($p = 0.002$) (Table 5). When grouped by cultivar, there were significant interactions between treatment and pot size in both Maris Piper ($p < 0.001$) and Charlotte ($p < 0.001$). Within each cultivar, the effect of treatment was significant in both cultivars in both pot sizes (Maris Piper: 5 L, $p < 0.001$; 20 L, $p < 0.001$; Charlotte: 5 L, $p < 0.001$; 20 L, $p < 0.001$). The percentage differences between these groups were large, but the absolute differences between significantly different groups were still small (< 0.03 index units).

4.4.3.7 Leaf angle was not affected by irrigation frequency, pot size, or cultivar

Leaf angle was not significantly affected by any of the grouping factors and there were no significant interactions (Table 5).

4.4.3.8 Light penetration depth ranking of each cultivar was different between the pot sizes

Light penetration depth was significantly affected by treatment (p = 0.013) and pot size (p < 0.001), but not by cultivar (p = 0.179) (Table 5). There was a single significant interaction between pot size and cultivar (p = 0.001); the effect of cultivar was significant in 5 L pots (p = 0.004), but not in 20 L pots (p = 0.262). The difference in light penetration depth between Maris Piper and Charlotte was 82.49 mm (46.7%) in 5 L pots, compared to only 36.61 mm (11.7%) in 20 L pots. Light penetration depth was shorter for Charlotte in 5 L pots ($\bar{x}$ = 135.52 mm) compared to Maris Piper ($\bar{x}$ = 218.01 mm), but longer in 20 L pots (Charlotte, $\bar{x}$ = 331.47 mm; Maris Piper, $\bar{x}$ = 294.86 mm). Light penetration depth also increased with increasing irrigation frequency, but the only significant difference occurred between every other day and twice daily irrigation (p < 0.05).

Table 5. Main effects and interaction terms of a three-way ANOVA (analysis of variance) for digital canopy biomass average greenness, average hue, average NDVI, average PSRI, leaf angle, light penetration depth of two potato cultivars (Maris Piper and Charlotte), grown in one of two pots sizes (5 and 20 L) and subjected to every other day, daily, or twice daily irrigation treatments. Data were collected on 13th July 2023 with two PlantEye F500 multispectral 3D scanners (Phenospex, Heerlen, Netherlands), and were processed by HortControl (Phenospex, Heerlen, Netherlands). Significant *p*-values (< 0.05) are indicated in bold.

		Digital Canopy Biomass (log10(dm^{3}))		Average Greenness		Average Hue		Average NDVI	
Effect	DF	F	p	F	P	F	P	F	P
Treatment (T)	24	7.5	**0.003**	0.1	0.896	10.3	**0.001**	11.2	**0.000**
Pot Size (PS)	24	1356.7	**0.000**	225.2	**0.000**	121.3	**0.000**	296.4	**0.000**
Cultivar (C)	24	18.0	**0.000**	14.9	**0.001**	50.0	**0.000**	1.2	0.280
T x PS	24	10.6	**0.001**	8.3	**0.002**	14.4	**0.000**	2.7	0.085
T x C	24	3.6	**0.041**	23.9	**0.000**	2.4	0.115	18.4	**0.000**
PS x C	24	40.2	**0.000**	43.1	**0.000**	41.1	**0.000**	4.4	**0.047**
C x PS x T	24	3.4	**0.050**	16.1	**0.000**	8.5	**0.002**	19.1	**0.000**

		Average PSRI		Leaf Angle (°)		Light Penetration Depth (mm)	
Effect	DF	F	P	F	P	F	P
Treatment (T)	24	915545.6	**0.000**	0.7	0.524	5.3	**0.013**
Pot Size (PS)	24	10753737.9	**0.000**	0.7	0.422	67.9	**0.000**
Cultivar (C)	24	4427915.0	**0.000**	2.9	0.100	1.9	0.179
T x PS	24	1279031.5	**0.000**	1.7	0.206	1.5	0.235
T x C	24	210004.8	**0.000**	0.3	0.717	1.3	0.282
PS x C	24	3646813.9	**0.000**	1.0	0.335	12.9	**0.001**
C x PS x T	24	751822.7	**0.000**	0.8	0.477	0.5	0.585

4.5 Discussion

4.5.1 Fresh tuber yield, but not fresh canopy biomass, support the water-availability hypothesis of pot binding

4.5.1.1 Fresh Tuber Yield

This experiment aimed to investigate the water availability hypothesis of pot binding in potato. The hypothesis states that pot binding, i.e., the confounding effects of small pots on plant morphophysiology, is primarily a result of an unintentional drought stress experienced by purportedly well-watered plants (Sinclair *et al.*, 2017). Pot binding is thought to occur when the water holding capacity of a potted substrate is insufficient to prevent drought stress between irrigation periods (Turner, 2019). Previous research has suggested that pot binding can be mitigated by providing plants with 1 L of substrate for every gram of dry biomass that a plant is expected to produce (Poorter *et al.*, 2012). As potato has been observed to generate over 1,000 g of dry biomass (Wheeler and Tibbitts, 1987), this recommendation is impractical for this crop in most controlled environmental facilities.

To test this hypothesis, we grew two cultivars of potato, Maris Piper and Charlotte, in two pot sizes, 5 and 20 L, each under one of three water treatments: irrigation to saturation twice daily (T_2), daily (T_1), or every other day ($T_{1/2}$). If pot binding is a product of water unavailability under T_1 conditions, then morphophysiological indicators of drought stress should be observed in both $T_{1/2}$ and T_1 plants. This effect should be mitigated by increasing the pot size (Poorter *et al.*, 2012; Turner, 2019) or by increasing the irrigation frequency (Sinclair *et al.*, 2017; Turner, 2019).

Therefore, we hypothesised that there would be greater similarities in traits known to be affected by drought stress between T_1 and $T_{1/2}$ treatments in the smaller pots. We also hypothesised that this effect would be mitigated in the larger pots, and by increasing the frequency of irrigation from T_1 to T_2. We assessed several morphophysiological indicators of drought stress that have previously been shown to affect potato, including tuber yield, canopy biomass, canopy and tuber dry matter (Obidiegwu *et al.*, 2015; Hill *et al.*, 2021), average canopy temperature (Stark, Pavek and McCann, 1991; Ninanya *et al.*, 2021) and SPAD values (Li *et al.*, 2019).

Fresh tuber yield and canopy biomass are the two morphological traits most sensitive to water-restriction in potato (Jefferies and Mackerron, 1987, 1993). As plant tissue growth is primarily a result of cell elongation (Shao *et al.*, 2009), which is driven by high turgor pressure (Lockhart, 1965), water deficits result in reduced growth of many tissues. Canopy biomass is also particularly affected by water-restriction in potato, compared to other crops. Leaf growth in most crop species ceases when the fraction of transpirable soil water drops below 40-50%; in potato leaves, growth is negligible once the available soil water reaches 60% (Weisz, Kaminski and Smilowitz, 1994).

In this experiment, fresh tuber yield was significantly reduced by decreasing irrigation frequency from T_1 to $T_{1/2}$, but only in the larger, 20 L pots. In the 5 L pots, there was no meaningful difference in tuber yield between T_1 and $T_{1/2}$ (Figure 1). This is consistent with the water availability hypothesis of pot binding, as the difference in yield between the hypothetically well-watered and intentionally drought stressed plants was minor compared to the yield difference in the large pots. This also supports previous research, which found that potato yield reductions associated with water-restriction increased with pot size (Chapter 3).

Increasing irrigation from T_1 to T_2 was not sufficient to increase fresh tuber yield in 5 L pots (Figure 1), suggesting that saturation twice per day in very small pots is still insufficient to prevent pot binding in potato. It could be suggested that yields in the small pots were limited by the pot volume, rather than drought stress. However, there was no evidence that this was the case as yields in the small pots were very low (296.0 g) and the tubers occupied only a small amount of the pot volume. There was also a further, albeit not significant, increase in yield between T_1 and T_2 in 20 L pots. This suggests that, while larger pots may prevent pot binding well enough to detect significant yield differences between $T_{1/2}$ and T_1, they may not eliminate it altogether under daily irrigation.

4.5.1.2 Fresh Canopy Biomass

In contrast with fresh tuber yield, canopy biomass was similarly affected by water restriction in the two pot sizes, with a significant decrease in biomass between T_1 and $T_{1/2}$ occurring in both. This finding is inconsistent with the water availability of pot binding, as are the percentage differences in biomass between T_1 and $T_{1/2}$ in the two pot sizes. Canopy biomass was more effected by water-restriction in 5 L pots (27%), compared to 20 L pots (16%) (Figure 2). Increasing irrigation frequency from T_1 to T_2 had an insignificant effect on canopy biomass in both pot sizes, although it was associated with a slight increase in biomass in 5 L pots, which suggests that T_1

might be unable to maintain maximum canopy biomass accumulation in the smaller pots.

It is not clear why fresh tuber yield and canopy biomass are affected differently by water restriction in the two pot sizes. It is possible that, due to extremely limited water availability, yield was maintained at the expense of biomass under $T_{1/2}$ in the smaller pots. This seems unlikely as both fresh tuber yield and canopy biomass have previously been shown to decrease in 4.7 L pots from a similar treatment to $T_{1/2}$ (irrigation to saturation every other day) to a treatment that restricts water even further (Rolando *et al.*, 2015).

4.5.1.3 Canopy and Tuber Dry Matter

Both canopy and tuber dry matter percentages were primarily affected by cultivar, with pot size and treatment having small effects. Maris Piper had a significantly higher dry matter concentration than Charlotte in both the canopy and tubers (Table 2). Dry matter content is known to vary between potato cultivars (Navarre, Goyer and Shakya, 2009), and is related to cultivar maturation. Researchers have previously defined maturation in potato as the point of maximum dry matter accumulation (Sabba *et al.*, 2007), which, in the absence of stress, is dependent on life cycle length. Late maturing cultivars, including Maris Piper, can delay senescence for longer than early cultivars, including Charlotte, facilitating greater radiation interception and photosynthesis over time (Aliche *et al.*, 2019). This allows dry matter production to continue for longer in late maturing cultivars, which accounts for the differences observed here.

The canopy and tuber dry matter percentages of both cultivars were relatively unaffected by treatment (Table 2), with a significant but small decrease in the former with increasing irrigation frequency and no effect in the latter. Above- and below-ground dry matter accumulation responses to water-restriction are known to vary greatly between cultivars (Hill *et al.*, 2021). However, the differences observed between the cultivars were significantly confounded by pot size, both in the canopy and tubers. The tuber dry matter of Charlotte was identical in both pot sizes, whereas that of Maris Piper was significantly higher in the smaller pots compared to the larger pots. Above ground, this was completely reversed, as the canopy dry matter content of Maris Piper being unaffected by pot size and an association between small pots and higher dry matter concentration in Charlotte. This suggests that something other than water availability is causing the confounding effects of small pots on potato morphophysiology.

Previous research in tall but narrow 11.8 L pots (⌀ = 10 cm) has shown that both self- and reciprocally grafted potato canopies elicit greater control over dry matter accumulation than root stocks (Jefferies, 1993). This, coupled with the small and non-interactive effects of water-restriction in both pot sizes, suggests that pot binding may have a confounding effect on potato canopies that is not related to inadvertent drought stress. The cause of this is beyond the scope of this experiment, but previous research with five cowpea cultivars in 11, 17, and 76 L pots, suggested that small pots were associated with greater root abscisic acid production, and downstream reductions in canopy and root biomass, even under well-watered conditions (Ismail, Hall and Bray, 1994). Similar findings have also been found in tomato, where shoot growth was restricted in small pots despite "great care" (Turner, 2019) to maintain consistent water and nutrient availability between pot sizes (Hurley and Rowarth, 1999).

4.5.2 Average canopy temperatures support the water-availability hypothesis and suggest pot binding can be mitigated by increasing irrigation frequency, but SPAD values suggest pot binding might also be due to root restriction

4.5.2.1 Canopy Temperature

To provide an indication of drought stress during the experiment, average canopy temperature was measured throughout. In plants, canopy temperature is kept within the lethal limits for a particular species through transpiration (Gates, 1964). As relatively cool groundwater is taken up by the roots and moved through the plants to the leaves, it absorbs the excess thermal energy generated by solar radiation from the surrounding tissue and removes it from the plant by evaporating through the stomata (Lin *et al.*, 2017). Even tiny amounts of transpiration can dissipate significant amounts of thermal energy and cool plant canopies by a few degrees (Gates, 1964).

Plants roots respond to water scarcity through alterations in root architecture (Hill et al., 2021), in part to maintain this mechanism of canopy cooling. External water scarcity is thought to be sensed by several transmembrane proteins with a diverse range of mechanisms, including facilitating Ca2+ influx (Lamers, van der Meer and Testerink, 2020). Architectural changes are then affected through a coordinated response in the root apex in terms of cell division, elongation, and differentiation (Gupta, Rico-Medina and Caño-Delgado, 2020). In addition to attempting to increase water uptake, plants also respond to water scarcity by reducing water loss. Under well-watered conditions, plant stomata remain open to facilitate gaseous exchange between the internal and external environments for photosynthesis. This process also allows water vapour to leave plant tissues in a process called evapotranspiration, which enables the canopy cooling described above.

Evapotranspiration also creates negative pressure as water vapour exits the stomata, which cascades down the plant to the roots, where it is the primary driver of water uptake (Steudle, 2000). When the amount of water lost through evapotranspiration exceeds the roots' ability to uptake water from the soil, turgor pressure is decreased across plant tissues. This initially occurs in the guard cells that surround the stomata, as these cells are physically closest to where water loss is occurring. Therefore, these cells rapidly and automatically respond to water scarcity by closing stomata due to their internal structure (Gupta, Rico-Medina and Caño-Delgado, 2020). This mechanism conserves water but at the expense of both photosynthetic assimilation of carbon and canopy cooling through the mechanism described above.

Measuring potato canopy temperatures under well-watered conditions was evaluated as a method of evaluating drought tolerance between cultivars over 30 years ago (Stark, Pavek and McCann, 1991). The method was successful in potato and other crops, as canopy temperature and water use are negatively correlated under well-watered conditions (Keener and Kircher, 1983; Chaudhuri and Kanemasu, 1985), and canopy temperature and drought susceptibility are positively correlated under water-restricted conditions (Blum *et al.*, 1989; Stark, Pavek and McCann, 1991).

With recent advancements in remote sensing, ground and aerial measurements of canopy temperature have been investigated as methods of estimating drought stress in potato (Rud *et al.*, 2014). Previous research has demonstrated that canopy temperature can be integrated within a water stress index that is strongly correlated with stomatal conductance (Rud *et al.*, 2014). This index was also shown to increase under water-restricted conditions compared to well-watered controls (Rud *et al.*, 2014).

Across this experiment, each increase in irrigation frequency was associated with a significant decrease in canopy temperature (Supplementary Figure S1). The differences between the treatments were smaller than previously suggested (Gates, 1964), varying by ~1 °C above or below the daily irrigation treatment (T_1). This is related to climatic conditions, as the mean ambient air temperature at 09:00, one hour before canopy temperatures were measured, was only 17.8 ± 0.5 °C. Within each treatment, canopy temperature was consistently higher in the smaller pots than in the larger pots (Supplementary Figure S1). Again, the differences were small (< 1.5 °C) due to the low potential evapotranspiration early in the photoperiod.

The difference between T_1 and $T_{1/2}$ (every other day) treatments was significantly larger in the smaller pots compared to the larger pots (Supplementary Figure S1). This seems contrary to the prediction of the water availability hypothesis of pot binding. If pot binding was a result of the relative inability of daily irrigation to maintain potential evapotranspiration in small pots, then canopy temperatures should be more similar between T_1 and $T_{1/2}$ in smaller pots than in larger pots. However, the high canopy temperatures under $T_{1/2}$ conditions in 5 L pots shows that this treatment produces much more severe drought stress than the same treatment in 20 L pots. Importantly, canopy temperatures decreased relative to T_1 under the T_2 (twice daily) treatment in both pot sizes, suggesting that neither pot size could sustain potential evapotranspiration under T_1 conditions.

The only combinations of pot size and treatment that were not significantly different from each other were T_2 in 5 L pots and $T_{1/2}$ in 20 L pots. This shows that the effects of pot binding do result from water unavailability can be mitigated to some extent by increasing irrigation frequency. However, canopy temperatures under $T_{1/2}$ conditions in 20 L pots were still significantly higher than under T_1 and T_2 conditions, which demonstrates that maintaining adequate water availability for maximum transpiration is not possible in 5 L pots with twice daily watering to saturation.

Canopy temperatures in both cultivars responded similarly to each treatment, with Maris Piper being significantly warmer under each (Supplementary Figure S1). This is a result of Maris Piper being a later maturing cultivar than Charlotte. Late cultivars produce larger canopies (Hill *et al.*, 2021) and thus require greater volumes of water to maintain potential transpiration (Fandika *et al.*, 2016). The canopy temperature of Maris Piper was also more affected by water-restriction from T_1 to $T_{1/2}$ than Charlotte in 5 L pots (+1.2 °C versus +0.9 °C), but similarly affected by water-restriction in 20 L pots (+0.2 °C in both). This highlights the necessity of considering cultivar specific water requirements when selecting experimental pot sizes to prevent pot binding in potato.

When broken down by sample date, canopy temperature was affected by treatment more frequently in the 5 L pots (Figures 3 & 4). Although the differences across the whole experiment were typically significant, the effects of treatment on canopy temperature were only significant on 3 or 4 days in 20 L pots in Maris Piper and Charlotte, respectively. This contrasts with the 9 and 6 days where treatment had a significant effect on canopy temperature in 5 L pots in same cultivars, respectively. This is indicative of the extreme drought stress experienced by plants under $T_{1/2}$ conditions in 5 L pots, which have previously been used as a well-watered control condition (Li *et al.*, 2019; Chapter 3).

As the differences in canopy temperatures between treatments were significant across the experiment, it is likely that significant differences on individual sample dates may have been more frequent with greater sample sizes. This demonstrates the potential utility of canopy temperature as a metric by which potato irrigation systems can be controlled. If slight differences between canopy temperatures in the field and a concurrent or historical well-watered population can be detected, then irrigation could be scheduled when canopy temperatures begin to rise.

4.5.2.2 Average SPAD values

SPAD meter readings are a reliable proxy for chlorophyll content (Borhan *et al.*, 2017), and have been shown to be very strongly correlated with chlorophyll content in wheat, rice, and soybean, $R^2 = 0.93$ (Monje and Bugbee, 1992); and *Arabidopsis thaliana*, $R^2 = 0.98$ (Ling, Huang and Jarvis, 2011). In potato, SPAD values have been closely approximated with a computer imaging technique (Borhan *et al.*, 2017), demonstrating the possibility of crop water- and nutrient-management with remote measures of canopy greenness.

Potato SPAD values have previously been shown to increase due to water-restriction (Ramírez *et al.*, 2014; Rolando *et al.*, 2015; Rudack *et al.*, 2017; Li *et al.*, 2019), probably due to decreasing leaf water contents increasing chlorophyll concentrations (Rolando *et al.*, 2015; Gervais *et al.*, 2021). However, more recent work has shown that the effects of water deficits on chlorophyll content in potato varies greatly depending on cultivar and growth stage (Mthembu *et al.*, 2022).

For example, chlorophyll content in the *Solanum tuberosum* cv. Panamera increased with water-restriction during tuber initiation but decreased with water-restriction in the vegetative, tuber bulking, and maturation stages. In contrast, chlorophyll content in the cv. Bikini increased under water-restriction in every growth stage other than tuber initiation (Mthembu *et al.*, 2022). This variability may explain why no effect of treatment was observed here, with no interactions between treatment or any other grouping factor.

Average canopy SPAD was affected by an interaction between cultivar, pot size, and sample date (Table 4). Charlotte was greener than Maris Piper, demonstrating the variability in SPAD values between cultivars, regardless of treatment, previously observed (Mthembu *et al.*, 2022). It is unclear why Charlotte in 5 L pots had significantly higher SPAD values than Charlotte in 20 L pots at the beginning of sampling. However, after 9 days the difference between pot sizes in Charlotte had disappeared, and SPAD values in both pot sizes remained similar for the duration of sampling (Figure 5).

In Maris Piper, average SPAD values decreased at a faster rate in the larger pots (Figure 5). This was associated with the faster rate of senescence observed with Maris Piper in the 5 L pots, an effect that has been observed before (Chapter 3). It is unlikely that early senescence in Maris Piper is a result of water unavailability in smaller pots, as senescence did not occur at a faster rate in the water-restricted plants. Instead, it is possible that the early onset of senescence was a product of nutrient unavailability, another proposed cause of pot binding (Poorter *et al.*, 2012).

Root volume restriction in aerated liquid culture has previously been observed to reduce chlorophyll content and cause early senescence in alder (*Alnus glutinosa*) seedings (Tschaplinski and Blake, 1985) reduced leaf water potential due to an imbalanced root/shoot ratio (Tschaplinski and Blake, 1985). Research with starfruit (*Averrhoa carambola*) has also shown reduced leaf water potential and photosynthetic rate with root restriction, but this effect was compounded by water-restriction (Ismail and Noor, 1996). Root restriction also increased the rate of maturation in starfruit.

If an imbalanced root/shoot ratio is a component of pot binding, then increasing irrigation frequency to mitigate water unavailability will be limited in its capacity to alleviate pot binding in small pots. As average canopy SPAD values were unaffected by treatment in this experiment, it is possible that chlorophyll content was more affected by root restriction than water-unavailability. This would explain why Maris Piper was more affected than Charlotte here, as the former produced larger canopies in both pot sizes. However, as neither leaf water potential or root/shoot ratios were measured here, further research is needed to assess the relative effects of root- and water-restriction on leaf chlorophyll content in potato.

4.5.3 Digital phenotyping is less valid for larger plants with high self-shading

In this study, digital phenotyping tools (PlantEye F500 & HortControl) were used to measure canopy biomass and height. The measurements produced with these tools were compared to manual measurements of canopy biomass and plant height, which are established methods of assessing the effects of water-restriction in potato (Elsayed *et al.*, 2021; Ninanya *et al.*, 2021; Mthembu *et al.*, 2022). There was a significant positive, correlation between digital and manual measures of both canopy biomass (Figure 7) and height, but only in 5 L pots. In the 20 L pots, there was no clear relationship between digital and manual measurements of canopy biomass or plant height.

Previous experiments have found positive correlations between PlantEye measurements of leaf area and manually collected reference measurements, including in soybean, $R^2 = 0.89$ to 0.91 (Manavalan *et al.*, 2021); peanut, $R^2 = 0.94$; cowpea, $R^2 = 0.93$; and pearl millet, $R^2 = 0.86$ (Vadez *et al.*, 2015). However, these studies focussed on early plant growth to maximise the sample size. The authors suggested that overlapping leaves may result in inaccurate measurements of leaf area and digital biomass for more mature plants, or crops with high leaf area indices (Vadez *et al.*, 2015; Manavalan *et al.*, 2021).

Here, the plants were scanned at 42 DAP, by which time those in 20 L pots may have exceeded the threshold leaf area index (LAI) of 1.5, above which digital phenotyping of leaf area and biomass becomes increasingly inaccurate (Vadez *et al.*, 2015). This cannot be confirmed as LAI was not measured manually and the digital measurements are invalid, at least in 20 L pots. Leaf overlap, or self-shading, is known to occur in leaf-type cultivars of potato (Schittenhelm, Sourell and Löpmeier, 2006) and explains the discrepancy in accuracy of digital biomass measurements between the pot sizes found here and previously (Chapter 3). Further research is needed to define more accurate LAI thresholds for valid digital phenotyping of morphological traits in potato and other crops. However, digital phenotyping of canopy biomass and plant height, at least with the platform used here, is inaccurate in mature potato plants in pots ≥ 20 L.

Leaf angle was also measured in this study with digital phenotyping tools, and was found to be unaffected by treatment, pot size, and cultivar, with no significant interactions (Table 5). This contradicts previous research, which has suggested leaf angle is a secondary trait with potential as an indicator of drought tolerance under water-restricted conditions (Mulugeta Aneley, Haas and Köhl, 2023). In potato, leaf angle has been shown to be ~5° higher in water-restricted plants than control plants during the day light period, with treatment having a significant effect on leaf movement: an integration of leaf angle throughout the diurnal cycle (Mulugeta Aneley, Haas and Köhl, 2023). A positive correlation between leaf angle and the independently verified drought tolerance of twenty potato cultivars has also been observed (Köhl, Aneley and Haas, 2023).

Similar results have been found in wheat (Lonbani and Arzani, 2011) and soybean (Martynenko *et al.*, 2016). It's possible that the high degree of self-shading present in potato confounded the measurements of leaf angle by the PlantEye, as is the case with leaf area and digital biomass (Vadez *et al.*, 2015; Manavalan *et al.*, 2021). However, it should be noted that the significant effect of treatment on leaf movement in potato was observed with a previous model (F400) of PlantEye (Mulugeta Aneley, Haas and Köhl, 2023). This experiment was conducted in 30 L pots, suggesting pot size may also have a confounding on leaf angle in potato. However, in the absence of a direct comparison between plants in 20 and 30 L pots, or with field-grown plants, the cause of the null result observed here remains unclear.

4.6 Conclusion

We investigated the water availability hypothesis of pot binding, i.e., the confounding effects of small pots on plant morphophysiology, in potato. We assessed whether these effects could be mitigated in practical pot sizes for high-throughput phenotyping platforms by reducing the inter-irrigation period. The validity of digital measurements of plant morphology were also assessed by comparison with established, low-tech methods. The analysis of fresh tuber yield, but not fresh canopy biomass, supported the water availability hypothesis of pot binding. Increasing irrigation frequency from every other day to daily was only associated with a significant increase in fresh tuber yield in the larger pots, suggesting a similar intensity of drought stress under both treatments in the smaller pots. Further increasing the irrigation frequency from daily to twice daily was insufficient to significantly increase fresh tuber yields in both pot sizes but did cause an insignificant increase in fresh tuber yield in the large pots, suggesting daily irrigation might not be sufficient to completely prevent pot binding even in larger pot sizes. Canopy biomass appeared to be less affected by pot binding as reducing irrigation from daily to every other day significantly reduced biomass in both pot sizes. There was a small increase in biomass when irrigation frequency was increased to twice daily in the small pots, but this was not significant and therefore does not strongly support the water availability hypothesis. Canopy temperatures were significantly higher in the small pots under each irrigation frequency, which strongly supports the water availability hypothesis as higher canopy temperature is a reliable indicator of drought stress in potato. The canopy temperatures of Maris Piper, a late maturing cultivar, were more affected than those of the early maturing cultivar, Charlotte, in small pots, highlighting the importance of considering cultivar-specific water requirements when selecting experimental pot sizes. Canopy temperatures were similar between twice daily irrigation in small pots and irrigation every other day in large pots and were reduced in large pots with increasing irrigation frequency. This suggests that increasing irrigation frequency might be unable to prevent pot binding due to water unavailability in small pots, but that increasing irrigation frequency is able to mostly mitigate pot binding in large pots. Further research is needed to define the optimum pot size and irrigation protocol to completely prevent pot binding for phenotyping experiments. Digital phenotyping was found to be less valid for larger plants, probably due to a higher degree of self-shading. We found significant positive correlations between digital and manual measurements of canopy biomass and plant height, but only in small pots. Further research should attempt to define an appropriate leaf area index threshold for valid digital phenotyping in potato.

4.7 References

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Chapter 5: Investigating the Utility of Potato (*Solanum tuberosum* L.) Canopy Temperature and Leaf Greenness Responses to Water-Restriction for the Improvement of Irrigation Management

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5.1 Abstract

Traits that rapidly respond to stress in important agricultural crops have the potential to provide growers with actionable feedback. E.g., traits that respond to water-restriction could inform irrigation systems by identifying crop water status and requirements in real-time. This would be particularly useful for potato, which is extremely susceptible to drought. We conducted two pot experiments and one field experiment to evaluate the utility of two traits, canopy temperature and leaf greenness, for informing irrigation management in potatoes. We also evaluated the efficacy of Phenospex PlantEye F500 sensors for the remote sensing of leaf greenness. We found that canopy temperatures of the cvs. Maris Piper (Spring Pot Experiment, +0.8°C; Autumn Pot Experiment, +5.3°C) and Désirée (Autumn Pot Experiment, +2.5°C) increased with water-restriction and that the canopy temperatures of Maris Piper return to baseline within three days after the resumption of well-watered conditions. We also found that these responses varied between cultivars, with predictable outcomes based on reported and corroborated drought tolerance ratings. We found inconclusive evidence of leaf greenness

increasing due to water-restriction (Spring Pot Experiment, +0.8°C; Autumn Pot Experiment, +5.3°C) and found no evidence that post-drought recovery periods return this trait to baseline. However, leaf greenness measurements from the Phenospex PlantEye F500 were moderately to strongly correlated with SPAD values, suggesting this tool might be useful in the screening of drought-tolerant cultivars in the future.

5.2 Introduction

Recent advances in plant phenotyping platforms have alleviated a significant bottleneck in our ability to understand useful traits in important agricultural crops (Furbank and Tester, 2011). The collection of phenotypic data has historically been destructive, expensive, and time-consuming, but now researchers are able to collect these data on agriculturally relevant scales (Furbank and Tester, 2011). These advancements have not only increased the sample sizes available to crop scientists, but also vastly expanded the range of traits available for phenotyping. Of the many categories of traits now available for phenotyping, the most successfully exploited have been integrative traits (Araus et al., 2008). These are traits, such as plant yield, that are affected at multiple levels of plant organisation over the whole life cycle (Araus et al., 2002). However, many such traits, including harvest index and light interception efficiency, have little remaining potential for further improvement (Long, Marshall-Colon and Zhu, 2015).

As a result of these technological advancements and the diminishing returns from the selection of desirable integrative traits, novel traits are being phenotyped in a range of crop species. The most investigated of these traits include canopy temperature, stomatal conductance, senescence, fruit colour, carbon isotope discrimination, light interception, leaf area and water potential, chlorophyll content and fluorescence, and various root traits (Yol, Toker and Uzun, 2015). Some of these traits are also being assessed for potential positive associations with yield maintenance under abiotic stress. For example, phenotyping the so-called "stay-green" effect, i.e., chlorophyll content maintenance, has been extensively reviewed as a potential method for improving yields under drought conditions (Monteoliva, Guzzo and Posada, 2021).

A subset of these phenotypic traits might therefore prove useful in providing growers with actionable feedback from their crops. For example, a trait that is found to reliably respond to drought stress could theoretically be used to inform and improve irrigation management, potentially reducing the substantial yield penalties (Jefferies and Mackerron, 1993) and irrigation costs associated with drought (Daccache *et al.*, 2012). This is particularly relevant for potato, which is extremely susceptible to drought (Schafleitner, Gutierrez and Legay, 2009), due to its relatively high water-requirements (Knox, Weatherhead and Bradley, 1997) and shallow root system (van Loon, 1981). A comprehensive review of phenotyping the effects of drought on potato has previously been published (Hill *et al.*, 2021), but most of the studies reviewed focused on the selection of beneficial traits for breeding stress tolerance, rather than the potential for actionable plant-feedback investigated here.

We propose that the utility of such a trait should be defined by 1) the convenience and accuracy with which it can be measured on agriculturally relevant scales; 2) the rate, intensity, and reliability of its response to a relevant stress; 3) the practicability and cost/benefit ratio of an appropriate intervention; and 4) the rate, intensity, and reliability of its response to that intervention. With respect to convenience of measurement, advances in multi- and hyperspectral imaging, unmanned aerial vehicles, and image processing have made automatic remote sensing synonymous in the literature with "easy to measure" (Araus and Cairns, 2014). The suitability of a trait for remote sensing significantly narrows down those that might prove useful in increasing the efficiency of agricultural systems through a plant-feedback approach.

In the case of drought stress, previous research has highlighted canopy temperature (Chaudhuri and Kanemasu, 1985; Chaudhuri *et al.*, 1986; Hatfield, Quisenberry and Dilbeck, 1987; Blum *et al.*, 1989; Stark, Pavek and McCann, 1991; Mahmud *et al.*, 2016; Anderegg *et al.*, 2021) and, more recently, leaf greenness (Ramírez *et al.*, 2014; Rolando *et al.*, 2015; Bai and Purcell, 2019; Li *et al.*, 2019; Anderegg *et al.*, 2021; Monteoliva, Guzzo and Posada, 2021) as effective traits with respect to the selection of drought tolerant cultivars. Canopy temperature indices have also been investigated as methods of measuring water stress (Rud *et al.*, 2014) and for controlling irrigation systems in potato (Rinza *et al.*, 2022). Within this research, both canopy temperature and leaf greenness have been measured with remote sensing technologies (Bai and Purcell, 2019; Rinza *et al.*, 2019), satisfying the first of our criteria for screening candidate traits for a plant-feedback based agricultural system.

This research has also demonstrated the rate, intensity, and reliability of the responses of potato canopy temperature and leaf greenness to water-restriction, satisfying the second criteria. Water-restriction (high-frequency deficit irrigation to 50% pot capacity) has been shown to increase canopy temperatures in five cultivars of potato, by an average of 2.3°C ± 0.7°C, at 1 pm (Mahmud *et al.*, 2016). Similar results have since been found with the cv. Unica in the field, for which water-restriction was associated with an increase in canopy temperatures by ~4°C between 3-4 pm (Rinza *et al.*, 2019). Canopy temperatures are known to rise with increasing soil moisture deficits due to the reduced transpiration rates associated with stomatal closure (Fuchs, 1990). This process preserves plant water status under drought conditions but has downstream effects on carbon assimilation, increasing survivability at the cost of yield in agricultural species. Canopy temperature is therefore useful not only for estimating drought stress, but also for indicating periods of reduced yield accumulation.

Leaf greenness, as a proxy for chlorophyll content, has been proposed as an important trait for improving crop yields in the future, particularly under drought stress (Monteoliva, Guzzo and Posada, 2021). Increases in leaf greenness during periods of water-restriction are associated with reduced leaf growth in drought susceptible potato cultivars, suggesting that chlorophyll concentrations increase under water-restriction due to reductions in leaf area (Rolando *et al.*, 2015). Severe water-restriction protocols in pots have been associated with an average increase in leaf greenness of ~10 SPAD units with the cv. Unica (Ramírez *et al.*, 2014). Smaller, but still significant, increases in leaf greenness due to water-restriction were also observed in the field. In another pot experiment, increases in leaf greenness, of ~5 SPAD units, were observed within 10 days of water-restriction in the cvs. Sarnav, Unica, and Désirée (Rolando *et al.*, 2015). These results have more recently been corroborated in six cultivars observed under short- and long-term water-restriction, although with less consistent differences in leaf greenness between the cultivars and treatments (Li *et al.*, 2019).

However, very little research has investigated the practicability and cost/benefit ratios of basing irrigation management on these responses, or the rate, intensity, and reliability of the responses of these traits to irrigation. Before the former can be evaluated, the latter must be understood to prevent systems being designed where the relevant intervention is not effective. Therefore, we aim to assess the effects of water-restriction and, uniquely, well-watered recovery periods on the canopy temperature and leaf greenness of potato, both in the glasshouse and in the field. As droughts are predicted to become more frequent in many areas, we also aim to understand the effects of repeated water-restriction cycles on these traits. We hypothesised that water-restriction would be associated with increases in canopy temperature and leaf greenness, as seen in previous research, and that these responses would be stronger in less drought tolerant cultivars. We also hypothesised that these increases in canopy temperature and leaf greenness would be reversed with the resumption of well-watered conditions as transpiration and leaf expansion resume. Secondarily, we aimed to assess the utility of a remote sensing technology, Phenospex PlantEye F500 sensors, for measuring leaf greenness in potato and to quantify the relationship between these measurements and SPAD values.

5.3 Materials and Methods

5.3.1 Plant Material and Growing Conditions

Two pot experiments (spring and autumn) and one field experiment (summer) were carried out at the Crop and Environment Laboratory (N 51°26’13.0” W 0°56’31.0”) at the University of Reading, UK. Both pot experiments were conducted in twelve bespoke plywood troughs (1140 x 300 x 412 mm, L x W x H; Figure 1). Each trough was filled with 148 L of a 2:1 by volume mixture of John Innes No. 2 compost and sharp sand (Jubilee Building Supplies, Bracknell, UK). Each trough was fertilised with 576 g of Osmocote Pro (3-4 Mo). Ambient temperature and relative humidity during the summer field experiment were retrieved from the University of Reading Atmospheric Observatory (N 51°26'29.2” W 0°56'16.0”) and were measured manually on each sample date in the glasshouse (Table 1).

For the pot experiments, the cv. Maris Piper was selected due to its popularity with UK growers, driven by its high yield and resistance to *Globodera rostochiensis* (Buckley, 2015). Melody and Désirée were selected for the spring and autumn pot experiments, respectively, for to their comparable maturity classes with Maris Piper and purportedly higher drought tolerance (Agriculture and Horticulture Development Board, 2023; Science and Advice for Scottish Agriculture, 2023). Pentland Javelin was selected for the summer field experiment to represent earlier maturing cultivars and inferred lower drought tolerance than Maris Piper (Hill et al., 2021). This was deemed necessary to cover a greater variety of the cultivated potato germplasm and to allow the detection of differences between the responses measured here in the relatively uncontrollable field environment.

Table 1. Ambient mean temperatures (T) and relative humidities (RH) in the field between 12th May and 8th August 2022, and in the glasshouse between 6th June and 4th July 2022 and 23rd November and 17th December 2022 for the spring and autumn pot experiments, respectively. Ambient temperature and relative humidity during the summer field experiment were retrieved from the University of Reading Atmospheric Observatory (N 51°26'29.2" W 0°56'16.0") and were measured manually on each sample date in the glasshouse.

	Spring Pot Experiment		Autumn Pot Experiment		Summer Field Experiment	
	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
Mean	21.7	67	17.5	79	17.1	63
S.E.	0.8	1	1.5	2	0.4	1

5.3.2 Spring Pot Experiment

On 31st March 2022, eighteen pre-sprouted seed tubers of both *Solanum tuberosum* cvs. Maris Piper and Melody were planted at a depth of 10 cm, with three tubers in each trough. All plants were grown under a glasshouse from planting to harvesting, 123 days after planting (DAP). Throughout the experiment, irrigation was controlled by a GP2 data logger and controller (Delta-T Devices, Cambridge, UK). Soil moisture was measured with four WET150 multi-parameter soil sensors (Delta-T Devices, Cambridge, UK).

Each WET150 sensor was buried at a depth of 30 cm, at a 60° angle relative to the soil surface. One sensor for each combination of treatment and cultivar was buried in a sentinel trough. To impose the two treatment conditions, well-watered and water-restricted, each trough was connected to one of two irrigation loops. Each loop was independently controlled by the GP2, based on the soil moisture content measured by the WET150 sensors. Both loops could supply each trough with 12 L of water per hour through two drippers per plant.

Figure 1. A photograph of the spring pot experiment on 22nd April 2022 (22 DAP) showing the twelve bespoke plywood troughs in which the experimental plants were grown. Each trough contained three plants and was irrigated through six 2 L hour^{-1} drippers attached to one of two irrigation loops: well-watered or water-restricted. Each trough was palletised and could be disconnected from the irrigation loops to be moved into the adjacent glasshouse compartment to be scanned by two Phenospex PlantEye F500 multispectral 3D scanners (Phenospex, Heerlen, Netherlands).

Before the onset of water-restriction, the GP2 was programmed to check all the probes every hour for a soil moisture content (SMC) reading of < 36% ($A_0 = 1.32$, $A_1 = 8.70$). If this condition was met by both probes within a treatment, dripper irrigation for each trough under that treatment was initiated automatically. The GP2 would then recheck each sensor every minute for an SMC of ≥ 36%. Once this condition was met by both probes, irrigation would automatically stop. An SMC of 36% was chosen as the irrigation threshold based on the WET150 readings at 80% pot capacity (Turner, 2019), which was calculated gravimetrically.

These conditions were maintained until 8th June 2022 (69 DAP) when 50% flower bud formation was reached, which coincides with tuber initiation (Li *et al.*, 2019). On this date, the irrigation loop for the water-restricted troughs was manually turned off (Turner, 2019). The well-watered troughs remained under the same conditions as above. Irrigation for water-restricted troughs was reinitiated on 13th June and the plants were allowed to recover until 17th June (78 DAP). The second drought period lasted from then until 24th June (85 DAP), after which all troughs were well-watered until harvest on 4th July (95 DAP).

5.3.3 Autumn Pot Experiment

On 12th September 2022, eighteen pre-sprouted seed tubers of both *Solanum tuberosum* cvs. Maris Piper and Désirée were planted at a depth of 10 cm, with three tubers in each trough. Due to the observation of slightly abnormal plant development in the spring pot experiment (longer stems, which required staking), all plants were grown outside and uncovered from planting until 65 DAP, before being moved into the glasshouse. Before being covered, all plants were grown under rainfed conditions, which was sufficient to maintain a well-watered environment.

Once moved into the glasshouse, plants were grown under lights with a 16-hour photoperiod, to reduce variability in photosynthetically active radiation between this and the earlier experiments. Irrigation was controlled with the same method as above. In this experiment, all troughs were under the same well-watered conditions from planting until 11th October 2023 (29 DAP). On this date, the irrigation loop for the water-restricted troughs was manually turned off and water-restricted conditions were maintained for the remainder of the experiment. The treatment for the well-watered troughs remained the same. The drought period lasted until 17th December 2023 (96 DAP), when all the plants were harvested.

5.3.4 Summer Field Experiment

On 12th May 2022, 40 pre-sprouted seed tubers of both *Solanum tuberosum* cvs. Maris Piper and Pentland Javelin were planted on the flat at a depth of 20 cm. The rows were manually ridged post-emergence to prevent stem lodging and tuber greening. The seed tubers were planted in one plot split into four blocks. Each block contained four rows, spaced at 90 cm on centre. Each row contained five plants of a single cultivar, planted 38 cm apart. Within each block, the two cultivars alternated between the rows and one row of each cultivar was assigned to each treatment: well-watered and water-restricted.

To mitigate order effects, the cultivar and treatment assignment for each row varied between the blocks. To mitigate edge effects, guard plants of the cv. Arran Victory were planted surrounding each block. This cultivar was selected as it produces purple tubers, which contrast the white tubers of Maris Piper and Pentland Javelin, preventing the guard plant tubers from being attributed to experimental plants.

All plants were grown under a rain-out shelter and irrigated via lines of 2 L hour^{-1} drippers, with one dripper per plant. These lines were supplied by one of two loops, which could be controlled independently to impose the two treatment conditions. Both were controlled manually to maintain well-watered conditions until 14th July (64 DAP). Irrigation for the water-restricted rows was then turned off until 18th July (68 DAP), after which it remained on until 3rd August (84 DAP). Water-restricted conditions were then maintained until the end of the experiment on 8th August (89 DAP).

5.3.5 Data Collection

Between 6th June and 4th July (spring pot experiment), 23rd November and 17th December (autumn pot experiment), and 5th July and 8th August (summer pot experiment), average canopy temperature and SPAD values were regularly recorded for each plant. Canopy temperatures were measured with an infrared laser thermometer (Scientific Laboratory Supplies, Nottingham, UK) and SPAD values were measured with SPAD-502Plus (Konica-Minolta, Tokyo, Japan).

For each of these measurements three terminal leaflets were sampled per plant, each from distinct levels within the canopy. These measurements were averaged across each plant to give an accurate estimate of temperature and SPAD for the whole canopy (Víg *et al.*, 2012). Canopy levels were defined as the third (Gervais *et al.*, 2021), fifth, and seventh highest fully expanded leaves on the main stem of each plant. In the two pot experiments, these leaves were marked with cable ties around the petioles, so the same leaflets could be measured throughout the experiment.

As canopy temperature was particularly affected by ambient temperature fluctuations, all measurements were taken from the highest canopy level of each plant first, followed by the second level, and finally the third. All measurements were taken from 10:00 to 12:00 to minimise the variation caused by ambient changes throughout the day.

On 6th, 10th, 17th, and 23rd June 2022, each trough was scanned with two PlantEye F500 multispectral 3D scanners (Phenospex, Heerlen, Netherlands). PlantEye scanners have previously been used to measure "high-temperature-induced" (Lazarević *et al.*, 2022) and drought-related (Chapters 3 & 4) morphophysiological changes in potato. Integrated software (Phena; Phenospex) generated 3D point clouds of the plants, which were used by HortControl software (Phenospex) to calculate morphological parameters, including digital biomass, plant height, leaf area index, light penetration depth, leaf angle, average greenness, average NDVI, and average NPCI. Due to the high correlations between certain variables, only the previously stated variables were analysed.

5.3.6 Statistical Analysis

All statistical analyses were conducted in RStudio (RStudio Team, 2020). Measurements for each plant within a trough (pot experiments) or row (field experiment) were grouped and averaged before analysis to prevent pseudo-replication. For each dependent variable, a linear model was formulated with treatment, cultivar, and sample date as interactive fixed effects and trough or row, depending on the experiment, as a random effect. Functions from the R package "easystats" (Lüdecke *et al.*, 2022) were used to assess whether each model met the following assumptions of ANOVA testing: homogeneity of variance, normality of residuals, and a lack of significant outliers. Homogeneity and normality were assessed both statistically and visually, as large sample sizes are often unable to be accurately assessed with statistics alone (Lumley *et al.*, 2002; Lüdecke *et al.*, 2022).

If any of these assumptions were not met, typically signified by a p-value > 0.05, the data were transformed, and the tests of normality and homogeneity of variance were reassessed. Once these assumptions were met, ANOVA testing was run on each model. ANOVA testing was selected as it allows for the comparison of effects across multiple independent variables, which included treatment, cultivar, and sample date here. Each experiment was analysed separately as comparing responses to water-restriction in different environments is beyond the scope of these experiments.

In the spring pot experiment, one group (well-watered, Melody, sampled on 20th June) had exceptionally low variance in canopy temperature. This resulted in the model violating the assumptions of ANOVA testing so two models were constructed, either including or excluding this group. Only the latter met the assumptions of ANOVA testing, but the results between the two ANOVAs did not differ in terms of significance, so results of the former are presented here.

All data presented are estimated marginal means. These were extracted, along with upper and lower 95% confidence limits, from each model with the "emmeans" package in R (Lenth, 2023). These means ± CIs were plotted with the "ggplot2" package (Wickham, 2016). Any data that required transformation, as described above, were back transformed with the inverse function in R before being plotted with "ggplot2". Compact letters were calculated from the estimated marginal means and CIs with the "multcomp" package (Hothorn, Bretz and Westfall, 2008). Means not sharing any letter are significantly different by the Tukey-test at the 0.05% significance level (Piepho, 2018).

5.4 Results

5.4.1 Spring Pot Experiment

5.4.1.1 Average Canopy Temperature

Water-restriction was associated with a slightly (+0.4°C, 1.7%) higher average canopy temperature across both cultivars and all sample dates in the spring pot experiment, but this difference was not statistically significant (Table 2). Although the overall difference in canopy temperature between the treatments was small, the overall temperature increase between the treatments for Maris Piper was larger (+0.8°C, 3.5%). The overall canopy temperature of Melody was unaffected. There was a significant interaction effect between treatment and sample date on canopy temperatures (p = 0.010). Post hoc analysis showed that water-restriction was associated with significant increases in the canopy temperatures of Maris Piper relative to those of the well-watered control group on 10th (+3.9°C, 16.8%), 17th (+2.7°C, 10.4%), and 24th June (+1.8°C, 9.4%). All three of these dates occurred during the water-restricted periods. The canopy temperatures of Melody were not significantly affected by water-restriction on any sample dates (Figure 2). There were almost no differences in canopy temperature between the cultivars overall (0.6%), or under well-watered (1.2%) or water-restricted (2.3%) conditions. Sample date had a strongly significant effect on canopy temperature (p < 0.001), due to fluctuations in ambient temperature throughout the experiment. There were no other significant or marginal interactions.

Table 2. Main effects and interactions terms of three-way ANOVAs for average canopy temperatures (°C) and average canopy SPAD values of two potato cultivars (Maris Piper and Melody), grown in 148 L troughs, inside a glasshouse, under either well-watered or water-restricted conditions. Canopy temperature and SPAD values were sampled between 6th June (67 DAP) and 4th July 2022 (95 DAP) with a handheld laser thermometer and a SPAD meter, respectively.

		Average Canopy Temperature			Average Canopy SPAD		
Effect	DenDF	F	p	Sig.	F	p	Sig.
Treatment (T)	8	0.94	0.361		0.35	0.568	
Cultivar (C)	8	0.11	0.747		1.04	0.339	
Sample Date (SD)	118	123.61	0.000	***	36.47	0.000	***
T x C	8	0.98	0.351		0.53	0.487	
T x SD	118	2.18	0.010	*	0.49	0.943	
C x SD	118	0.69	0.785		2.16	0.011	*
T x C x SD	118	0.92	0.548		0.51	0.930	

Significant *p*-values are indicated at the following levels: $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

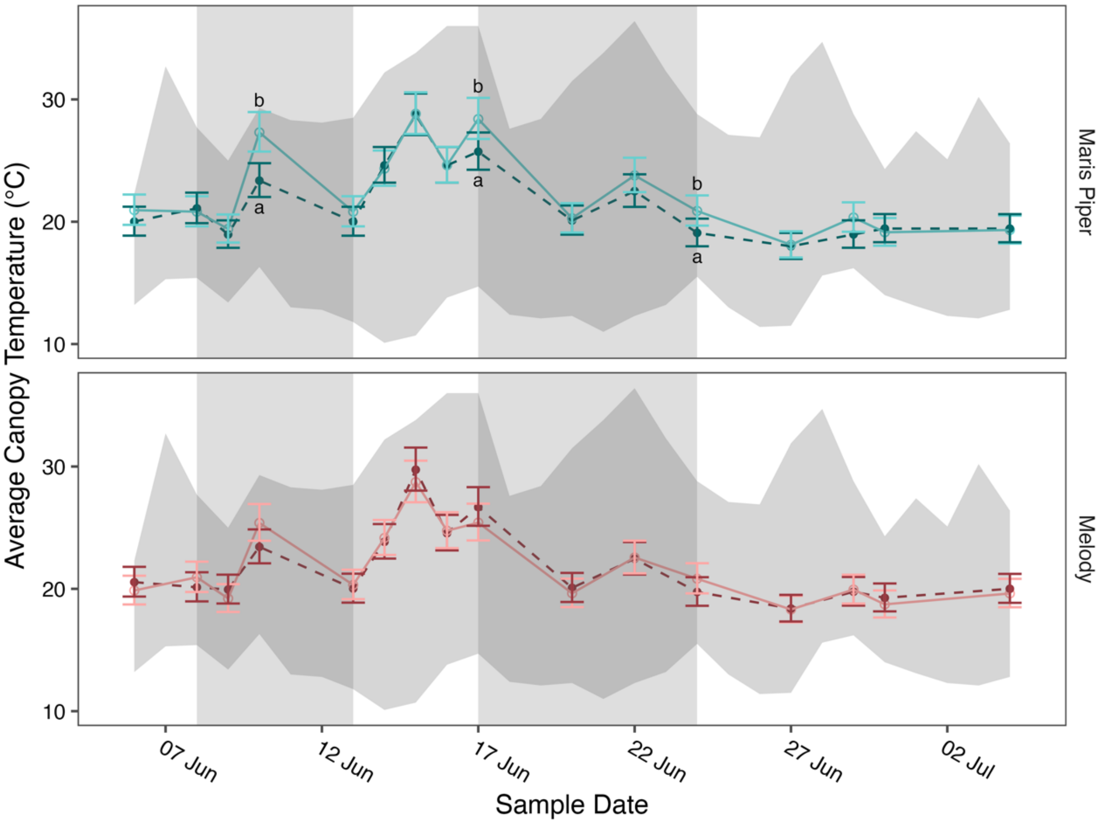

Figure 2. Mean canopy temperatures of potato (cvs. Maris Piper and Melody) over time, grown in 148 L troughs under either well-watered (dashed line) or water-restricted (solid line) conditions. Plants were grown in the glasshouse between 31st March and 4th July 2022 (95 DAP). Canopy temperatures were measured between 6th June and 4th July 2022. Water-restricted conditions were imposed between 8th June and 13th June and again between 17th June and 24th June (vertical shaded bars). Outside of these dates, water-restricted plants were well-watered. Means represent canopy temperatures averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3) ± CI. Means with different letters within each facet and sample date were significantly different by Tukey's test ($p < 0.05$). Daily ambient temperature ranges are shown by the grey ribbon. Letters denoting non-significant differences were removed for readability.

5.4.1.2 Average Canopy SPAD

Water-restriction was associated with a very slightly (0.5%) higher average canopy SPAD value across both cultivars and all sample dates, but this difference was not statistically significant (Table 2). SPAD values increased with water-restriction in Maris Piper (+0.6 SPAD units, 1.6%), but not in Melody. Unlike with canopy temperature, there was not a significant interaction effect between treatment and sample date on canopy SPAD. Whilst the main and interaction effects of treatment on canopy SPAD were all insignificant, canopy SPAD values for water-restricted Maris Piper did noticeably increase (3.4%) after the first period of water-restriction. The difference between the treatments decreased over time but the relationship was maintained for the duration of the experiment. Post hoc analysis showed that these differences were not significant on any of the sample dates (Figure 3).

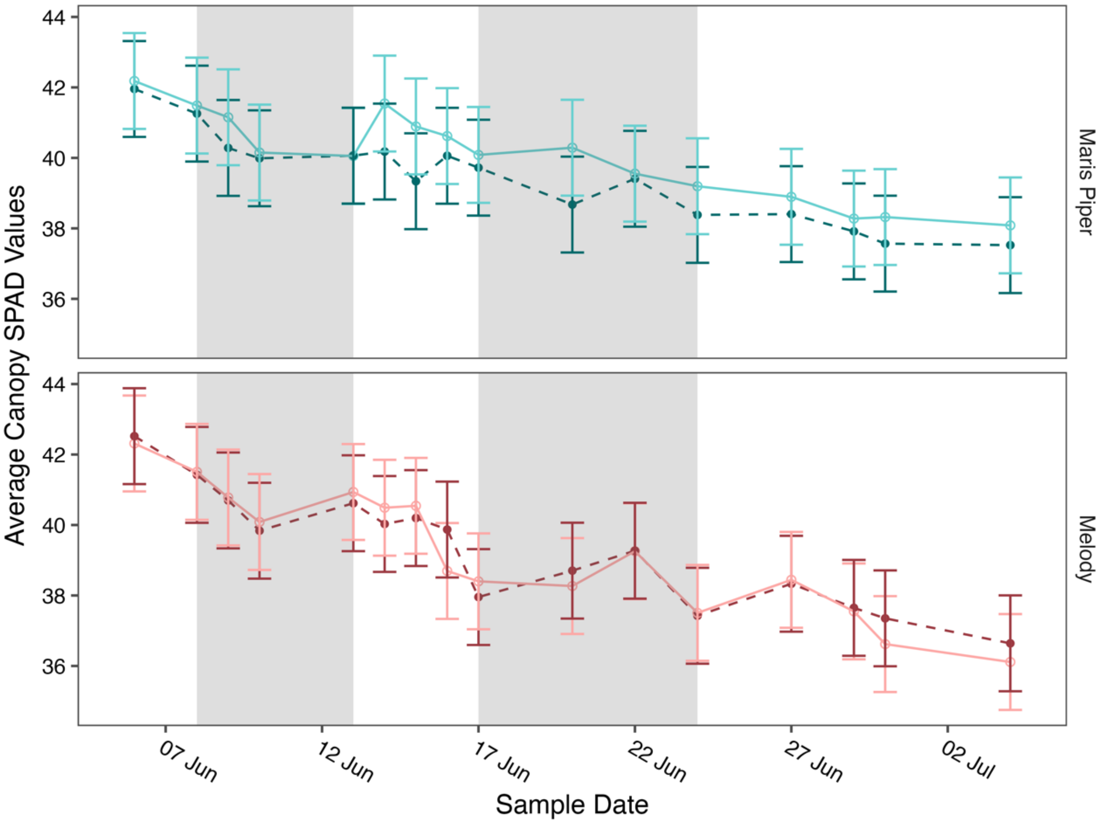

Figure 3. Mean canopy SPAD values of potato (cvs. Maris Piper and Melody) over time, grown in 148 L troughs under either well-watered (dashed line) or water-restricted (solid line) conditions. Plants were grown in the glasshouse between 31st March and 4th July 2022 (95 DAP). Canopy SPAD values were measured between 6th June and 4th July 2022. Water-restricted conditions were imposed between 8th June and 13th June and again between 17th June and 24th June (shaded areas). Outside of these dates, water-restricted plants were well-watered. Means represent canopy SPAD values averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3) ± CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test ($p < 0.05$). Letters denoting non-significant differences were removed for readability.

There was a small difference in canopy SPAD between the cultivars (1.0%), but there was a significant interaction effect between cultivar and sample date on canopy SPAD (p = 0.011). Post hoc analysis demonstrated that Maris Piper had slightly, but significantly, higher canopy SPAD values than Melody on 17th June (4.5%) and 4th July (3.9%).

5.4.1.3 Phenospex PlantEye F500s

None of the variables measured by the Phenospex PlantEye F500s were significantly affected by water-restriction across the whole experiment (Supplementary Table S1). There was a marginally insignificant effect of treatment on NDVI (p = 0.051). Post hoc analysis revealed this to be a result of a significant (5.9%) increase in the NDVI of well-watered Maris Piper, relative to the water-restricted Maris Piper, on the last sample date. Melody remained unaffected by treatment on all sample dates (Supplementary Figure S1). The overall effect of treatment on the greenness index was insignificant (p = 0.226). However, there was a significant difference in the greenness of Maris Piper between the treatments on the last sample date. Water-restriction was associated with a large (12.0%) increase in the greenness of Maris Piper on this date. There was also a moderate to strong, significant correlation between average greenness as measured by the PlantEye sensors and the average canopy SPAD values recorded on the same sample dates: 6th, 10th, and 17th June 2022 (r (34) = 0.68, $p < 0.001$; Figure 4). When the two cultivars were analysed separately, the correlations for both cultivars remained significant but was stronger in Melody than Maris Piper (Maris Piper, r (17) = 0.57, p = 0.013; Melody, r (17) = 0.84, p < 0.001; Figure 4).

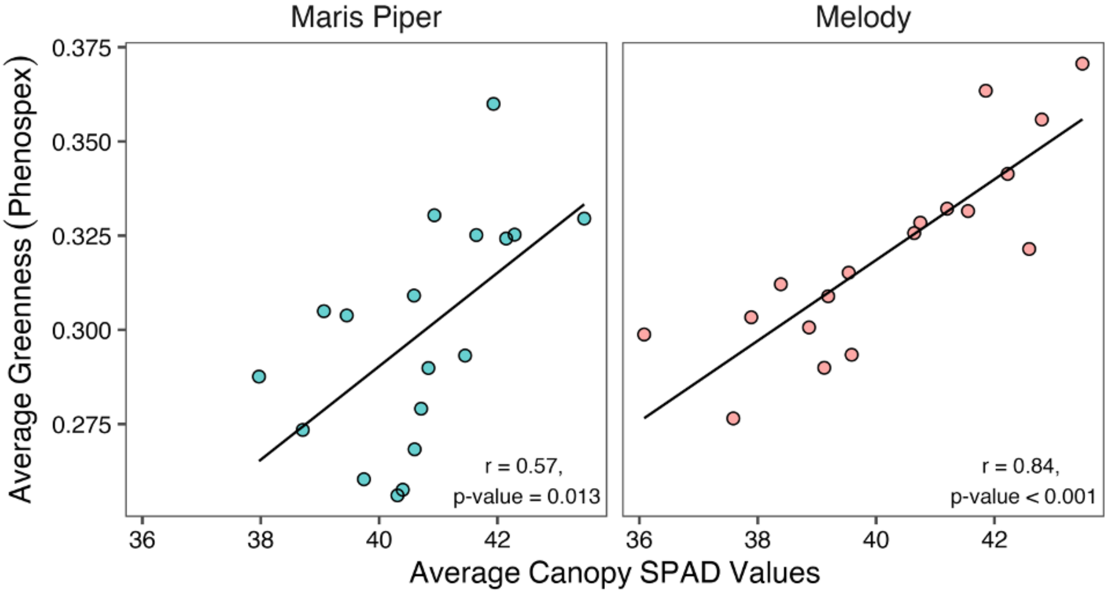

Figure 4. Correlation between digital average greenness and average canopy SPAD values for two cultivars of potato, Maris Piper and Melody, grown in 148 L troughs under either well-watered or water-restricted conditions (Maris Piper, r (17) = 0.57, p = 0.013; Melody, r (17) = 0.84, p < 0.001; Combined, r (34) = 0.59, p < 0.001). Plants were grown in the glasshouse between 31st March and 4th July 2022 (95 DAP). Digital average greenness and average canopy SPAD values were measured on 6th, 10th, and 17th June 2022. Points represent digital average greenness from three plants per trough (n = 3) and canopy SPAD values averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3). Digital average greenness was measured by HortControl (Phenospex, Heerlen, Netherlands).

There was a significant interaction effect between treatment and sample date on light penetration depth (*p* = 0.025). Post hoc analysis showed that this was a result of the substantial and significant (39.8%) increase in light penetration depth associated with water-restriction in Melody on the last sample date (Supplementary Figure S1). Post hoc analysis showed that there were no other significant differences between treatments for any of the other digitally measured variables (Supplementary Figures S1 & S2). In contrast with treatment, there were significant differences between the cultivars in half of the digitally measured variables. Digital biomass, height, leaf area index, and NPCI were all significantly affected by cultivar (*p* = 0.009, 0.017, 0.010, and 0.011, respectively).

5.4.1.4 Fresh Tuber Yield

Water-restriction was associated with a large and significant (48.7%) reduction in fresh tuber yield per trough across both cultivars (*p* < 0.001; Table 3). There was a large and significant (21.9%) difference in the mean fresh tuber yields of Melody (2,366.2 g) and Maris Piper (1,898.6 g) under well-watered conditions. However, water-restriction was associated with similar yield decreases in Melody (48.9%) and Maris Piper (48.4%), accounting for the lack of an interaction effect (Figure 6).

Table 3. Main effects and interactions terms of a two-way ANOVA for the fresh tuber yield of two potato cultivars (Maris Piper and Melody), grown in 148 L troughs, inside a glasshouse, under either well-watered or water-restricted conditions. Plants were harvested on 4th July 2022 (95 DAP).

		Fresh Tuber Yield (g)		
Effect	DenDF	F	p	Sig.
Treatment (T)	8	121.24	0.000	***
Cultivar (C)	8	13.71	0.006	**
T x C	8	1.59	0.243	

Significant p-values are indicated at the following levels: p < 0.05, *; p < 0.01, **; p < 0.001, ***.

5.4.2 Autumn Pot Experiment

5.4.2.1 Average Canopy Temperature

Water-restriction was associated with a (+3.9°C, 9.5%) higher average canopy temperature across both cultivars and all sample dates in the autumn pot experiment. This difference was found to be statistically significant (*p* = 0.001; Table 4). There was also a significant interaction effect between treatment and sample date ($p < 0.001$). Post hoc analysis showed that water-restriction was associated with significantly higher canopy temperatures in Maris Piper on all but the first two sample dates (+4.2°C, 10.9%; +6.7°C, +16.9%; +7.1°C, 20.1%; +5.2°C, 13.6%; +5.4°C, 14.4%; +8.0°C, 20.6%). Similar results were found with Désirée, where water-restriction was associated with significantly higher temperatures on three (+3.7°C, 8.8%; +4.3°C, 9.9%; +5.5°C, 13.0%) of the eight sample dates (Figure 5). The overall increase in canopy temperature across the experiment was higher in Maris Piper (+5.3°C, 13.6%) than in Désirée (+2.5°C, 5.8%).

There was a small (2.5°C, 5.8%) difference in the average canopy temperatures of the two cultivars across both treatments and all sample dates (Table 4). Désirée was observed to be slightly warmer than Maris Piper, a difference that was found to be significant ($p = 0.007$). Compared to the spring pot experiment, there was a large difference in canopy temperature between the cultivars under the well-watered treatment (9.8%), but not the water-restricted (2.5%) conditions, with Désirée being warmer than Maris Piper under both. There was also a significant interaction effect between cultivar and sample date on canopy temperature ($p = 0.011$). Post hoc analysis showed that the average canopy temperature of Désirée was significantly warmer than that of Maris Piper on all but the first sample dates.

Table 4. Main effects and interactions terms of three-way ANOVAs for average canopy temperatures (°C) and average canopy SPAD values of two potato cultivars (Maris Piper and Désirée), grown in 148 L troughs, inside a glasshouse, under either well-watered or water-restricted conditions. Canopy temperature and SPAD values were sampled between 23rd November and 17th December 2022 with a handheld laser thermometer and a SPAD meter, respectively.

		Canopy Temperature			Canopy SPAD		
Effect	DenDF	F	p	Sig.	F	p	Sig.
Treatment (T)	8	28.95	0.001	***	3.90	0.084	
Cultivar (C)	8	12.74	0.007	**	2.76	0.136	
Sample Date (SD)	55	5.13	0.000	***	265.46	0.000	***
T x C	8	4.06	0.079		0.02	0.904	
T x SD	55	4.48	0.001	***	3.16	0.007	**
C x SD	55	2.93	0.011	*	1.90	0.087	
T x C x SD	55	0.81	0.584		1.31	0.262	

Significant *p*-values are indicated at the following levels: $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

5.4.2.2 Average Canopy SPAD

Water-restriction was associated with a small (6.9%) increase in average canopy SPAD values across both cultivars and all sample dates (Table 4). This increase was found to be marginally statistically insignificant ($p = 0.084$). However, there was a significant interaction between treatment and sample date ($p = 0.007$). Post hoc analysis demonstrated that water-restriction was associated with significant increases in canopy SPAD on 15th December 2022 (11.9%) for Maris Piper and on 7th (11.6%) and 15th December (16.2%) for Désirée (Figure 5). There was a small (4.0%) difference in average canopy SPAD values between the two cultivars across both treatments and all sample dates, with that of Maris Piper being slightly higher than that of Désirée. Post hoc analysis showed that the canopy SPAD of Maris Piper was only significantly higher than that of Désirée on 7th and 15th December.

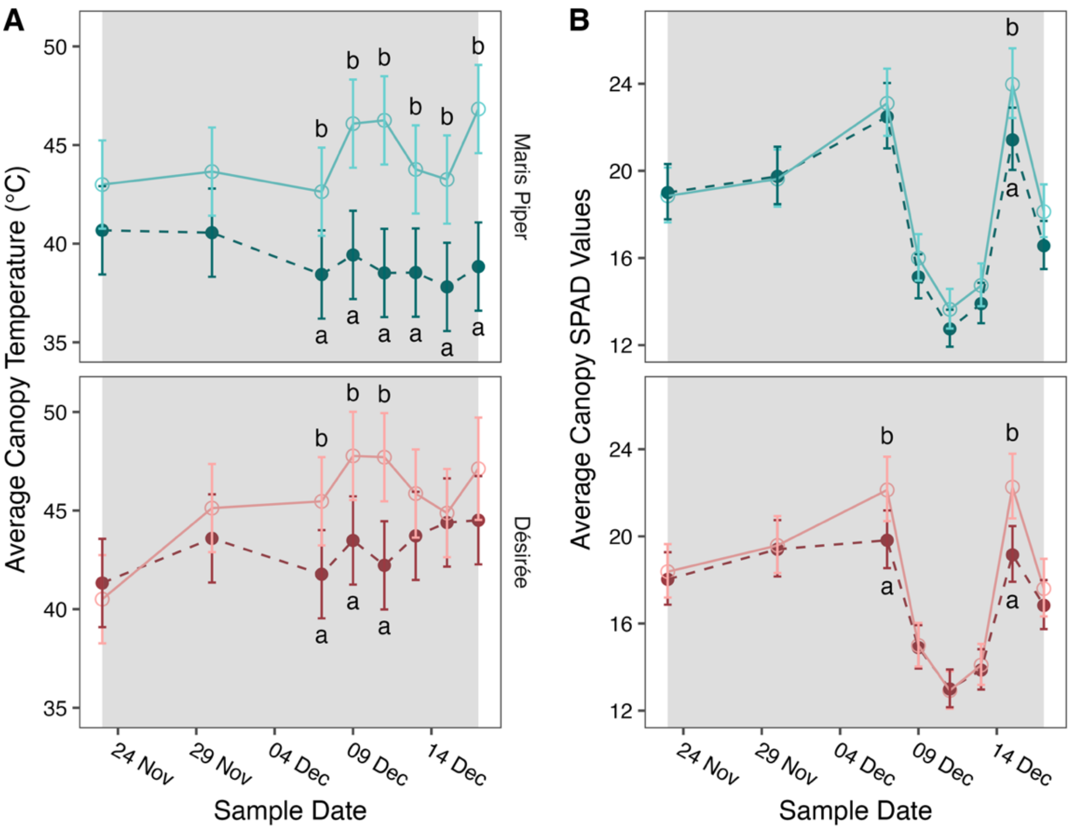

Figure 5. Mean canopy temperatures (A) and SPAD values (B) of potato (cvs. Maris Piper and Melody) over time, grown in 148 L troughs under either well-watered (dashed line) or water-restricted (solid line) conditions. Plants were grown in the glasshouse between 12th September and 17th December 2022 (95 DAP). Canopy temperatures and SPAD values were measured during a single water-restriction period, imposed between 23rd November and 17th December 2022 (shaded area). Means represent canopy temperatures and SPAD values averaged across three canopy levels: top, middle, and bottom, from three plants per trough (n = 3) ± CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test ($p < 0.05$). Letters denoting non-significant differences were removed for readability. Ambient temperature is not included as it was confounded by the nearby presence of growth lights.

5.4.2.3 Fresh Tuber Yield

Results for fresh tuber yield in the autumn pot experiment were consistent with those for the spring pot experiment. Water-restriction was associated with a very large and significant (70.9%) reduction in fresh tuber yield per trough across both cultivars ($p < 0.001$; Table 5). Unlike in the previous experiment, there was a much smaller (and non-significant;17.3%) difference in well-watered fresh tuber yield between Maris Piper (1,792.0 g) and Désirée (2,130.6 g). Interestingly, there was only a very small (5.8%) difference in the fresh tuber yield of Maris Piper between the pot experiments, despite the latter being conducted after the northern hemisphere summer. Water-restriction had a similar effect on both cultivars, being associated with large and significant (69.1% and 72.6%, respectively) decreases in fresh tuber yield in Maris Piper and Désirée (Figure 6).

Table 5. Main effects and interactions terms of a two-way ANOVA for the fresh tuber yield of two potato cultivars (Maris Piper and Désirée), grown in 148 L troughs, inside a glasshouse, under either well-watered or water-restricted conditions. Plants were harvested on 17th December (96 DAP).

		Fresh Tuber Yield (g)		
Effect	DenDF	F	p	Sig.
Treatment (T)	8	129.16	0.000	***
Cultivar (C)	8	1.11	0.323	
T x C	8	0.29	0.604	

Significant *p*-values are indicated at the following levels: $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

5.4.3 Summer Field Experiment

5.4.3.1 Average Canopy Temperature

Water-restriction was not associated with a significant increase in average canopy temperature in the field across both cultivars and all sample dates (Table 6). There were no significant interaction effects between temperature and either of the other two grouping factors. Post hoc analysis demonstrated that there was a single significant difference between the treatments in Pentland Javelin on the 19th July 2022 (Figure 6).

Table 6. Main effects and interactions terms of three-way ANOVAs for average canopy temperatures (°C) and average canopy SPAD values of two potato cultivars (Maris Piper and Pentland Javelin), grown in the field, inside a rain-out shelter, under either well-watered or water-restricted conditions. Canopy temperature and SPAD values were sampled between 5th July (55 DAP) and 8th August 2022 (89 DAP) with a handheld laser thermometer and a SPAD meter, respectively.

		Canopy Temperature			Canopy SPAD		
Effect	DenDF	F	p	Sig.	F	p	Sig.
Treatment (T)	12	1.24	0.286		0.02	0.898	
Cultivar (C)	13	0.04	0.853		3.73	0.077	
Sample Date (SD)	90	145.48	0.000	***	50.71	0.000	***
T x C	13	0.55	0.471		0.00	0.989	
T x SD	90	0.65	0.754		0.22	0.990	
C x SD	90	2.04	0.068		7.81	0.000	***
T x C x SD	90	1.03	0.411		0.33	0.918	

Significant *p*-values are indicated at the following levels: $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

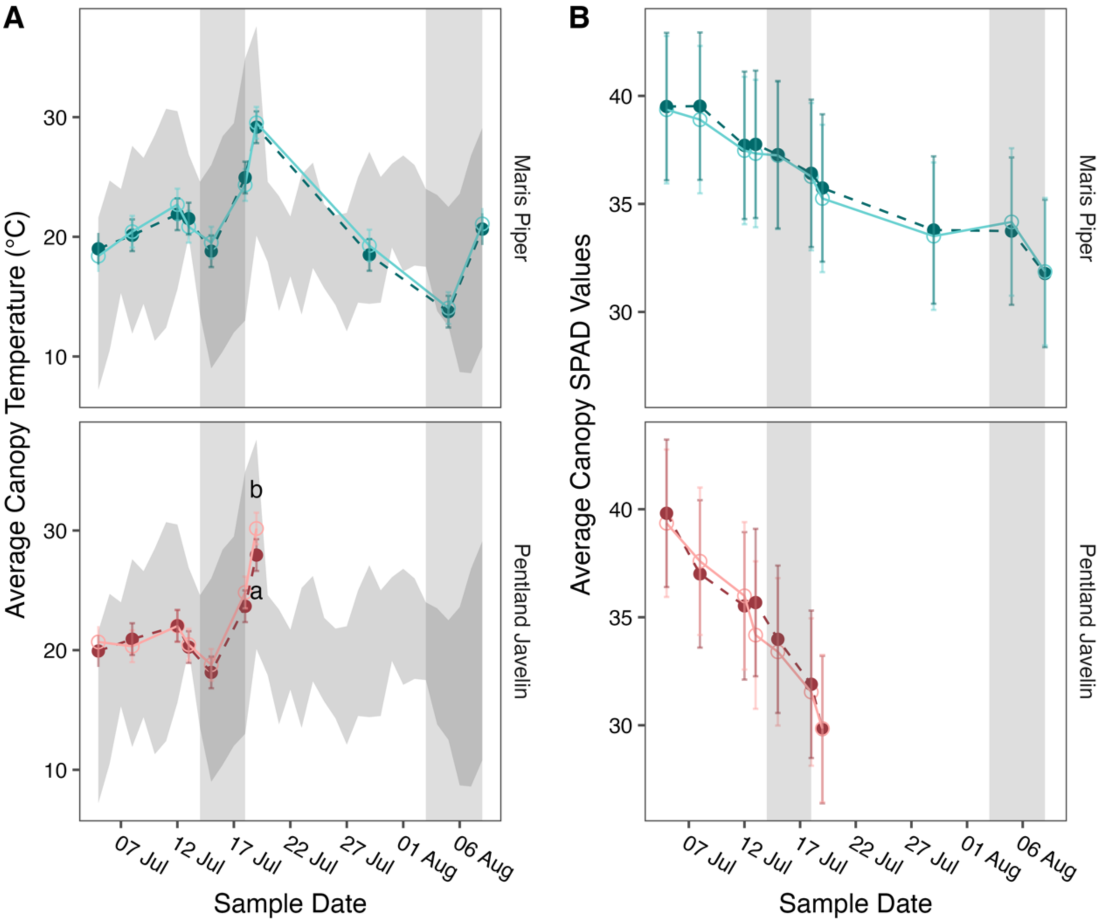

Figure 6. Mean canopy temperatures (A) and SPAD values (B) of potato (cvs. Maris Piper and Pentland Javelin) over time, grown under a rain-out shelter in the field between 12th May and 8th August 2022 (89 DAP). All plants were grown under well-watered conditions, with irrigation supplied by dripper lines, until 14th July (64 DAP). Subsequently, each row of plants was subjected to either well-watered conditions for the duration of the experiment (dashed lines) or two water-restricted periods (vertical grey bars) between 14th July (64 DAP) and 18th July (68 DAP) and between 3rd August (84 DAP) and 8th August (89 DAP) (solid lines). Between these periods, the water-restricted plants were well-watered and allowed to recover from drought stress. After these periods of water-restriction, all plants were harvested, and fresh tuber yields for each row were measured. Canopy temperature and SPAD values were sampled between 5th July (55 DAP) and 8th August 2022 (89 DAP). Daily ambient temperature ranges are shown by the grey ribbon. Means represent canopy temperatures and SPAD values averaged across three canopy levels: top, middle, and bottom, from five plants per row (n = 4) ± CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test ($p < 0.05$). Letters denoting non-significant differences were removed for readability.

5.4.3.2 Average Canopy SPAD

Water-restriction had no effect on average canopy SPAD values in the field across both cultivars and all sample dates (Table 6). There was a significant interaction effect between cultivar and sample date on canopy SPAD in the field but, this was due to the 100% mortality of Pentland Javelin. Post hoc analysis showed no differences in SPAD values between the treatments on any sample dates (Figure 6).

5.4.3.3 Fresh Tuber Yield

Water-restriction was associated with a large and highly significant (29.1%) decrease in fresh tuber yield per row in the summer field experiment ($p < 0.001$; Table 7). Similar results were found within each cultivar. Water-restriction was associated with a larger decrease in the fresh tuber yield of Pentland Javelin (41.3%) than in Maris Piper (24.1%), although both yield reductions were significant (Figure 6). Across the two water treatments, there was a very large and highly significant (90.1%) difference in fresh tuber yield between the two cultivars ($p < 0.001$).

Table 7. Main effects and interactions terms of a two-way ANOVA for the fresh tuber yield of two potato cultivars (Maris Piper and Pentland Javelin), grown in the field, inside a rain-out shelter, under either well-watered or water-restricted conditions. Plants were harvested on 8th August (89 DAP).

	Fresh Tuber Yield (g)			
Effect	DenDF	F	p	Sig.
Treatment (T)	8	42.49	0.000	***
Cultivar (C)	8	296.00	0.000	***
T x C	8	1.12	0.310	

Significant *p*-values are indicated at the following levels: $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.

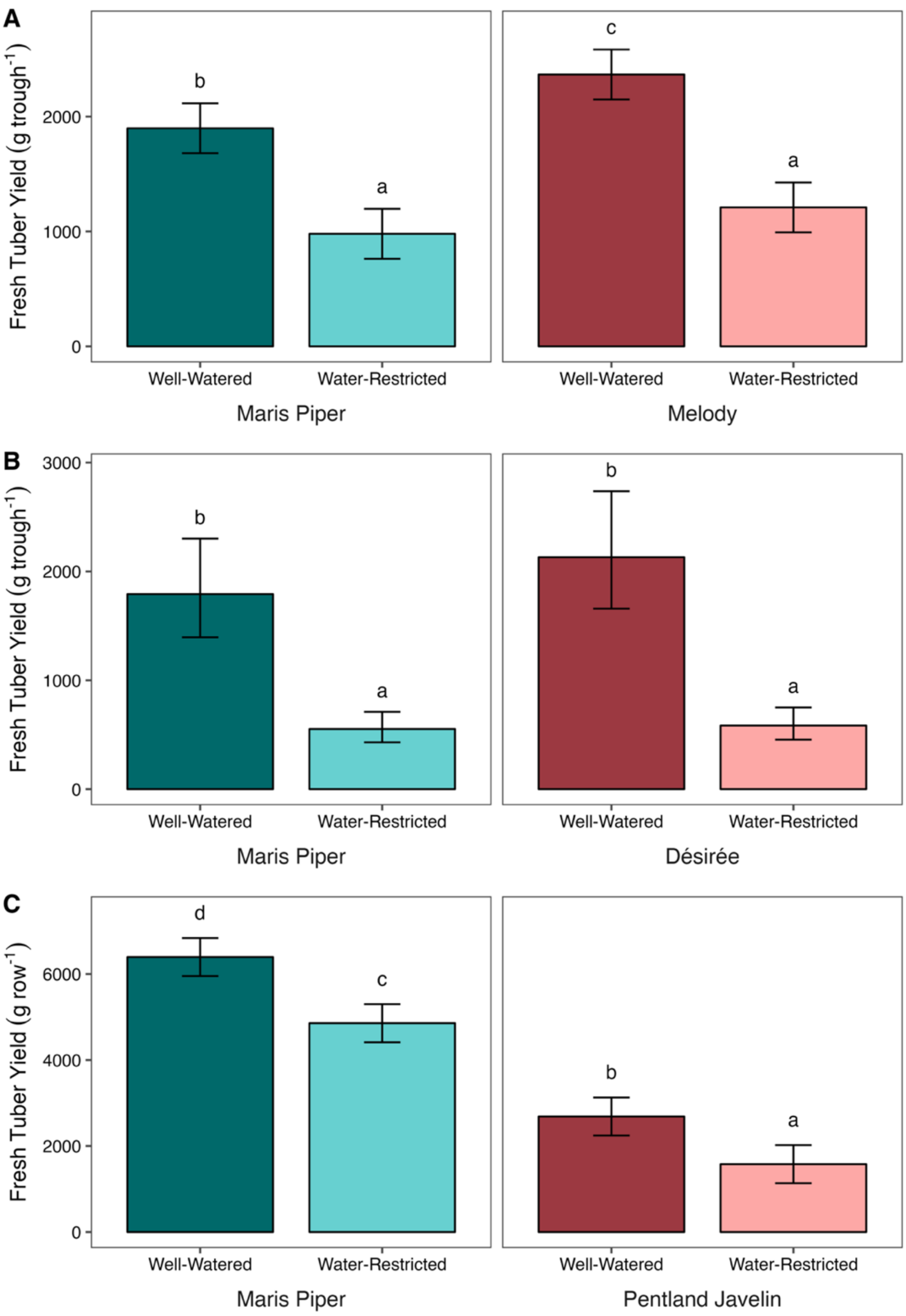

Figure 6. Mean fresh tuber yields of potato (cvs. Maris Piper, Melody, Désirée, and Pentland Javelin), grown in either (A & B) 148 L troughs, inside a glasshouse, or (C) in the field, inside a rain-out shelter, both under either well-watered (dark bars) or water-restricted (light bars) conditions. Plants were grown in the glasshouse between (A) 31st March and 4th July 2022 (95 DAP) or (B) 12th September and 17th December 2022 (96 DAP). Plants were grown in the field (C) between 12th May and 8th August 2022 (89 DAP). Tubers were harvested on (A) 4th July (95 DAP), (B) 17th December (96 DAP), and (C) 8th August 2022 (89 DAP). Means represent FTY averaged across (A & B) three plants per trough or (C) five plants per row (A & B, n = 3; C, n = 4) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$).

5.5 Discussion

5.5.1 Canopy temperatures were reliably increased under water-restriction and returned to baseline after irrigation resumed

In both pot experiments, water-restriction was reliably associated with an increase in the average canopy temperature of Maris Piper. In the spring, the overall increase in canopy temperature of Maris Piper due to water-restriction was relatively small: 0.8°C. However, on individual sample dates during both water-restriction periods, we observed significant increases in canopy temperatures of 3.9°C, 2.7°C, and 1.8°C. These differences returned to baseline by the first sample date after both water-restriction periods. Similar results were found in the autumn pot experiment, where the canopy temperature of Maris Piper increased significantly due to water-restriction by 4.2°C, 6.7°C, 7.1°C, 5.2°C, 5.4°C, and 8.0°C after the first two sample dates. Canopy temperature responses to water-restriction in the other cultivars, Melody and Désirée, were less reliable. There were no significant differences in the canopy temperatures of Melody on any sample dates during the spring pot experiment. Significant increases in canopy temperature due to water-restriction were only observed on three sample dates for Désirée in the autumn pot experiment, although the temperature increases on these sample dates were comparable to those of Maris Piper. In the summer field experiment, canopy temperatures were not reliably affected by water-restriction across or within the sample dates for either cultivar.

We hypothesised that the canopy temperatures of Melody and Désirée would respond less strongly to water-restriction than that of Maris Piper, due to the latter's relative drought susceptibility. According to the AHDB potato variety database, the drought tolerance ratings of Maris Piper, Melody, and Désirée are 3, 5, and 7 out of 9, respectively (Agriculture and Horticulture Development Board, 2023). The European Cultivated Potato Database (ECPD) concurs, classifying the drought tolerance of Maris Piper as “low to medium” and Désirée as “high to very high” (Science and Advice for Scottish Agriculture, 2023). The ECPD has no Information on the drought tolerance of Melody. However, the extent to which the canopy temperatures of Melody were unaffected by water-restriction was not expected. Also unexpectedly, the reduction in the effect size of water-restriction on Désirée towards the end of the autumn pot experiment was not a result of decreasing canopy temperatures under water-restriction. Rather, the canopy temperatures of Désirée under well-watered conditions increased after 11th December. This was likely due to early senescence, which has previously been reported in Désirée under long photoperiods and high temperatures (Demagante and Vander Zaag, 1988), similar to those Désirée experienced here.

The design of the spring pot experiment accounted for the small overall effect of water-restriction on the canopy temperatures of Maris Piper. This study aimed to evaluate the utility of traits including canopy temperature for a plant-feedback irrigation system. Therefore, it was necessary to include well-watered periods in the water-restricted treatment to investigate whether these traits would provide useful evidence of both stress and recovery. In this experiment, canopy temperature was shown to respond predictably to both water-restriction and subsequent well-watered conditions (Figure 2). After a significant temperature increase due to water-restriction on the last sample date of the second water-restricted period, the canopy temperature of Maris Piper returned to that of the well-watered control group within three days. However, the ambient temperature on the last sample date of the first water-restriction period was relatively cool, reducing the need for canopy cooling by transpiration, and thus was not driving a difference in canopy temperature between the treatments. Therefore, the rate with which canopy temperatures are restored post-drought remains unclear, and likely depends on factors including ambient temperature, irrigation rate, and cultivar.

To understand the size of the effect of water-restriction on canopy temperatures in potato, it was also important to investigate the effects of a single, terminal period of water-restriction on this crop. Water-restriction was associated with a much larger, 3.9°C, overall increase in canopy temperatures in this experiment, compared to the spring pot experiment. As was predicted due to the relative drought tolerances of the cultivars used, the canopy temperatures of Maris Piper were more affect by water-restriction than that of Désirée, with overall average increases of 5.3°C and 2.5°C, respectively. The difference in effect size between the two pot experiments presented here highlights the confounding effects that non-standardised growing conditions and drought protocols can have on potato morphophysiology (Hill *et al.*, 2021).

Intermittent drought stress is more analogous to conditions in the field (Turner, 2019), especially for potato, which is typically irrigated irregularly with booms or rain guns (Daccache *et al.*, 2012). However, the results presented here demonstrate the difficulty with detecting meaningful effect sizes in canopy temperature due to intermittent water-restriction. This is compounded by the high variance in canopy temperatures related to fluctuations in ambient temperature, both between and within sample dates. Post hoc analysis can be targeted at individual sample dates to mitigate the effects of the former issue. The latter could be addressed in future research with the use of imaging technologies that can phenotype multiple plants concurrently. Previous research has used infrared cameras to sample the canopy temperature of multiple potato plants in parallel and allowed for the detection of a significant difference between treatments of < 1°C (Rinza *et al.*, 2019). Significant *p*-values are perhaps over relied upon (Greenland *et al.*, 2016) but, if statistical models are to be used to control crop irrigation systems, then some method of detecting meaningful deviations from well-water canopy temperatures must be defined.

The results presented here from the pot experiments are consistent with previous research that showed reduced canopy temperature depressions (CPD) in potato due to high-frequency deficit irrigation (Mahmud *et al.*, 2016). CPD was defined as the difference between ambient air temperature and average canopy or leaf temperatures and is thus a measure of the cooling effect of transpiration. This effect was consistent across all five cultivars investigated but varied in magnitude throughout the day and between the cultivars. For example, the differences in CPD between the treatments were smallest at 8 am, when the ambient temperatures were cool, and greatest at 1 pm, when they peaked. The more drought tolerant cultivars, CIP 393371.58 and CIP 396244.12, were also found to have smaller differences in CDP between the treatments, which is consistent with our findings. Similar results have also been found by another study on the cv. Unica, with the greatest differences in canopy temperature between treatments occurring at 3 – 4 pm (Rinza *et al.*, 2019). Other studies have investigated the utility of screening canopy temperatures to detect drought tolerant potato cultivars (Stark, Pavek and McCann, 1991; Ninanya *et al.*, 2021) or to control potato irrigation systems with temperature-based crop water-stress indices (Rinza *et al.*, 2022). However, we are not aware of any studies that investigated both the effects of water stress and subsequent recovery on potato canopy temperatures.

Understanding the effects of water-restriction on potato canopy temperatures is further complicated by moving from the glasshouse to the field. The results from our summer field experiment were not consistent with those found in the pot experiments, or with previous research (Mahmud *et al.*, 2016; Rinza *et al.*, 2019). Canopy temperatures were not reliably affected by water-restriction across or within the sample dates. It's likely that the less homogenous conditions of the field experiment were partly responsible for this. Variations in ambient temperatures, relative humidity, and soil water holding capacity are greater in the field compared to the relatively controlled conditions of the glasshouse, and therefore increase the canopy temperature variance within each treatment. In this experiment, extremely high ambient temperatures for the region may also have confounded the effects of water-restriction on potato canopy temperatures.

The first water-restriction period during the summer field experiment coincided with an "unprecedented extreme heatwave", where ambient temperatures exceeded 40°C for the first time on record in the UK (Met Office National Climate Information Centre, 2022). This could account for the lack of temperature differences between the treatment groups, as it’s likely that all the plants experienced significant heat stress, which would have confounded the effects of drought stress (Hill *et al.*, 2021). It’s also possible that the irrigation system was unable to provide sufficient volumes of water to prevent drought stress in the well-watered plants, due to the extreme requirements for evapotranspiration caused by the heatwave. High ambient temperatures were certainly responsible for the 100% fatality rate observed in Pentland Javelin after 19th July 2022 (Figure 6). Early maturing cultivars, including Pentland Javelin, are known to be less robust to heat and drought stress due to their smaller root systems and the reduced capacity to recover associated with greater determinacy (Hill *et al.*, 2021).

The issue of greater environmental variability in the field could be overcome with larger scale, remote sampling of canopy temperature. This approach has previously been successfully implemented in cotton, where sixteen infrared thermocouples were used to detect elevated canopy temperatures in cotton (Peters and Evett, 2008; O’Shaughnessy and Evett, 2010). These thermocouples were attached to a centre pivot irrigation boom to remotely collect canopy temperatures across large plots of field-grown cotton. Water use efficiency was significantly improved compared to manual irrigation with this method (O’Shaughnessy and Evett, 2010). However, centre pivot irrigation is not used in potato, at least in the UK, and it’s unlikely remote sensing of canopy temperature could be used to inform irrigation management with sprinkler irrigation. Drip irrigation would be more suitable but would require complex infrastructure to differentially control irrigation within the field. Field-wide drip irrigation management would be more feasible but may only be commercially viable in areas with limiting water availability. This may soon become the case in the UK, where water availability is predicted to limit potato production in 50% of years by 2050 (Daccache et al., 2011).

5.5.2 Water-restriction was associated with small increases in SPAD which were maintained for the duration of the spring pot experiment

In these experiments, overall average canopy SPAD values (leaf greenness) had similar relationships with water-restriction as canopy temperature. In both the pot experiments, water-restriction was associated with a small overall increase in leaf greenness. As with temperature, this difference was larger across the single water-restriction period of the autumn pot experiment (+6.9%) than across the intermittent water-restriction periods of the spring pot experiment (+0.5%). However, neither of these differences were found to be significant. In the spring pot experiment, there was a noticeable increase in the leaf greenness of Maris Piper immediately after the first water-restriction period. This relative increase, compared to the well-watered Maris Piper, was maintained until the end of the experiment. Post hoc testing showed that this difference was not significant on any sample dates. The overall difference in leaf greenness between the treatments was also not significant in the autumn pot experiment ($p = 0.084$). However, there were three dates on which leaf greenness was significantly higher with water-restriction than without: one for Maris Piper and two for Melody. There was also a large U-shaped dip in leaf greenness for both cultivars in the autumn pot experiment between 7th and 15th December 2022. As with temperature, water-restriction had no effect on leaf greenness in the summer field experiment.

To our knowledge, the experiments presented here are the first to assess the utility of leaf greenness as a trait to inform irrigation management through its response to cycles of well-watered and water-restricted conditions. This is certainly the case for potato but may also be true for all agricultural crops. Earlier research in potato has demonstrated that, in cultivars where leaf greenness increases under drought stress, it typically remains elevated for 20 to 50 days (Rolando et al., 2015; Li et al., 2019). However, these experiments were designed to assess the utility of leaf greenness as a marker of drought tolerance in large panel breeding programmes (Rolando et al., 2015; Li et al., 2019), and therefore were only interested in the initial response. While the latter did include a “cyclical” water-restriction treatment, the well-watered recovery periods were not differentiated from the water-restriction periods in the final analysis, as this was beyond the scope of the experiment. However, our initial findings suggest that leaf greenness does not return to baseline after well-watered conditions are restored to previously water-restricted plants. Therefore, we cannot recommend leaf greenness as a useful trait for irrigation management in potato.

Previous research has found much stronger evidence of a positive effect of water-restriction on leaf greenness than that observed here. In a study on the potato cv. Unica, a “stay-green” effect was observed under the most severe water-restriction treatments in both the glasshouse and the field (Ramírez *et al.*, 2014). This size of this stay-green effect, defined as the maintenance of SPAD values over time, appeared to be positively correlated with the severity of water-restriction. The differences in leaf greenness between treatments over time were found to be significant on all sample dates in that experiment, but it’s unclear whether each of the less severe treatments were significantly different from the control. In a subsequent study on three potato cultivars, significant short-term increases in leaf greenness were consistently observed under water-restricted conditions (Rolando *et al.*, 2015) that were comparable with the moderate water-restriction used previously (Ramírez *et al.*, 2014). In the latter experiment, greater short-term increases in leaf greenness due to water-restriction were associated with reductions in tuber yield, suggesting maintenance of leaf greenness under water-restriction is associated with drought tolerance in potato.

In a more recent leaf greenness study on six potato cultivars with similar maturities but varying drought tolerances, four cultivars demonstrated higher leaf greenness under both short- and long-term water-restriction compared to well-watered conditions (Li *et al.*, 2019). One cultivar (Favorita) showed smaller increases in leaf greenness under both water-restricted treatments towards the end of the experiment. In another cultivar (Atlantic), leaf greenness decreased under both water-restricted treatments relative to control conditions. The authors concluded that leaf greenness increases were consistently and negatively associated with drought tolerance under both water-restricted conditions. This conclusion was reached despite the observations of Atlantic, which maintained leaf greenness under water-restricted conditions but produced low yields under all conditions. The authors suggested that the effects of water-restriction on very drought susceptible cultivars may be inconsistent with less susceptible cultivars, or that the growing conditions confounded these results.

In the spring pot experiment, a small but insignificant increase in the leaf greenness of Maris Piper was observed after the first water-restriction period and a stay-green effect was observed in the water-restricted group for the duration of the experiment. Melody exhibited greater maintenance of leaf greenness under water-restricted conditions, remaining consistent with the well-watered group. Melody is known to be more drought tolerant than Maris Piper (Agriculture and Horticulture Development Board, 2023) and this was reflected in the fresh tuber yields of Maris Piper and Melody under water-restriction observed here (Figure 6). Therefore, these findings provide tentative support to the hypothesis of this research and evidence from previous work, but the lack of statistical significance observed here must be noted. In the autumn experiment, the larger increase in leaf greenness due to water-restriction was observed in the Désirée. This cultivar is purported to be more drought tolerant than Maris Piper (Agriculture and Horticulture Development Board, 2023; Science and Advice for Scottish Agriculture, 2023), and was shown to produce higher fresh tuber yields under water-restriction (Figure 6). This result is inconsistent with our hypothesis and contradicts the evidence from previous research, including the spring pot experiment. However, there was a large confounding effect on the leaf greenness measurements in this experiment, the cause of which is unclear. U-shaped dips in SPAD values of a similar magnitude have been observed before, but these were not consistent between treatment groups (Li *et al.*, 2019). It's probable that some systemic physiological effect, e.g., pot binding (Sinclair *et al.*, 2017), or an environmental change was therefore responsible for the inconsistent leaf greenness results found here. This issue will be discussed in detail in the following section.

Unlike with canopy temperature, there is no evidence that leaf greenness returns to baseline post-drought. Previous research has demonstrated that in cultivars where leaf greenness increases under drought stress, it typically remains elevated for 20 to 50 days (Rolando *et al.*, 2015; Li *et al.*, 2019). It was hypothesised that this occurred due to the imposition of long-term water-restriction without recovery (Rolando *et al.*, 2015) or because of insufficient recovery periods. This finding is supported by the evidence presented here from the spring pot experiment, where leaf greenness increased in Maris Piper after water-restriction and remained elevated for the duration of the experiment.

Exactly why water-restriction was associated with such inconsistent effects on leaf greenness is unclear. The allocation of nitrogen (N) to chlorophylls is strongly affected by N supplementation conditions (Makino and Osmond, 1991), but this was considered in the experimental design. Slow-release fertiliser and conservative irrigation protocols were used to minimise the confounding effects of leeching on N availability. N availability could have been greater under well-watered conditions due to the faster dissolution of the fertiliser, but SPAD values were generally higher under water-restricted conditions. Therefore, differences in N availability are unlikely to have contributed to the inconsistent effects of water-restriction on leaf greenness observed here.

Previous research has shown that the severity and duration of water-restriction has a strong effect on SPAD values in potato (Ramírez et al., 2014). In pots, only the most severe water-restricted conditions, 30% of transpired water replaced daily by drip irrigation or partial rootzone drying, were associated with a significant increase in SPAD values. The two less severe water-restricted conditions, 60 and 45%, did not cause significantly different SPAD values from controls. Similar results were also found in the field, with the addition of the largest effects occurring on the last sample date. It’s therefore possible that our water-restricted conditions were not severe enough to observe significant differences in SPAD values. The confounding effects of ambient light on SPAD values are discussed below.

5.5.3 Phenospex PlantEye F500 measurements of greenness were strongly correlated with canopy SPAD values

None of the variables measured by the Phenospex PlantEye F500s in the spring pot experiment were found to be significantly affected by water-restriction. There were significant increases in NDVI and average greenness in Maris Piper (Supplementary Figure S2) and in light penetration depth in Melody (Supplementary Figure S1) on the final sample dates for the PlantEye. For Maris Piper, the increases in NDVI and greenness occurred in the well-watered plants and not in the water-restricted plants, although greenness was trending up for both treatments. It’s unclear why this occurred, but it did coincide with an increase in the average canopy SPAD values of well-watered Maris Piper two days earlier (Figure 3). Anomalous fluctuations in canopy SPAD values were observed in both pot experiments and have been observed in previous research in pots (Li *et al.*, 2019). Our as-yet unpublished data from previous experiments has shown that small pots can have important and significant confounding effects on potato (Chapter 3, Figures 4 & 8; Chapter 4, Figure 1), which are likely associated with inadvertent drought stress caused by the insufficient water-holding capacity of small substrate volumes. However, this was considered in the design of the troughs and the irrigation protocol used in these experiments, which should have been sufficient to prevent water-availability related pot binding (Sinclair *et al.*, 2017; Turner, 2019).

Phenospex report that data from their PlantEye F500 sensors are unaffected by ambient light conditions (*PlantEye F500 - Multispectral 3D laser scanner for plant phenotyping*, 2018), although this has not been independently verified. However, there was a moderate to strong correlation between average greenness as measured by the PlantEye F500s and average canopy SPAD values (r (34) = 0.68, p < 0.001; Figure 4). SPAD values have previously been shown to decrease under greater ambient light intensities in tobacco (Nauš *et al.*, 2010), soybean, and rice (Xiong *et al.*, 2015), due to intracellular light-dependent chloroplast movement. Therefore, it’s likely that variation in ambient light conditions were associated with the fluctuations in average greenness and canopy SPAD observed here and in previous research (Li *et al.*, 2019), rather than a systemic error.

5.6 Conclusions

These experiments demonstrate for the first time that potato canopy temperatures rapidly return to baseline with the resumption of well-watered conditions. Taken with the support that these experiments provide to previous research, showing that water-restriction is associated with increases in canopy temperatures of potato (Mahmud *et al.*, 2016; Rinza *et al.*, 2019), we have shown that direct measurements of canopy temperatures have potential for informing irrigation systems in potato. We found that this response is cultivar-dependent, as the canopy temperatures of the more drought tolerant cvs., Melody and Désirée, were less affected by water-restriction than those of Maris Piper. Further research should therefore include a range of potato cultivars with contrasting maturities and drought tolerance ratings. Extremely high temperatures during the field experiment also dramatically confounded the effects of water-restriction. Thus, more research is needed to assess the utility of canopy temperature for plant-feedback irrigation systems in the field. Our results for leaf greenness in Maris Piper and Désirée provide weak support for previous research (Ramírez *et al.*, 2014; Rolando *et al.*, 2015; Li *et al.*, 2019), showing much smaller increases in average canopy SPAD values than those observed before. Uniquely in potato, we have shown that the resumption of well-watered conditions did not return the leaf greenness of Maris Piper to baseline. Therefore, this research suggests that leaf greenness is a more useful trait for selecting drought-tolerant cultivars than for a plant-feedback irrigation system. The moderate to strong correlation observed between the Phenospex PlantEye F500s and the SPAD-502Plus measurements of leaf greenness suggest the former may be useful in this screening process.

5.7 References

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Chapter 6: Canopy Temperature Differences Can Be Used to Control Irrigation in Potato (*Solanum tuberosum* L.)

6.1 Abstract

Due to its extreme drought susceptibility, potato production in the UK is at risk from the predicted redistribution of rainfall away from the summer growing season associated with climate change. To prevent yield losses, more efficient methods of irrigation for potato must be developed. In this experiment, irrigation for three independent blocks of potato was triggered when the average canopy temperature in a block exceeded that of the adjacent well-watered reference block by 1.5 °C. We found that this threshold was sufficient to prevent a significant increase in average canopy temperatures between the temperature-controlled and well-watered blocks. It was also sufficient to prevent a significant change in fresh tuber yield or dry matter content. The total volume of water supplied to each temperature-controlled block was highly variable, suggesting that further research will be required to optimise the efficiency and reliability of such a system.

6.2 Introduction

Due to the extreme drought susceptibility of potato plants (Schafleitner *et al.*, 2009), a changing climate is a significant threat to potato production in the UK and around the world. While it has been predicted that warmer ambient temperatures might increase the rate of plant development and lengthen the UK growing season

(Daccache *et al.*, 2011), potato yields may be become limited by water scarcity (Daccache *et al.*, 2012). Climate models suggest that annual rainfall in the UK will be relatively unaffected (±10%) by a medium emissions scenario, but that rainfall will be redistributed from the summer growing season (-10 – 40%, depending on emissions) to the winter (+10 – 30%) (Rial-Lovera, Davies and Cannon, 2017). Under a high emissions scenario, the land area suitable for unirrigated potato production in the UK might decrease by up to 95% (Daccache *et al.*, 2012), increasing the water use and associated costs for potato production (Daccache *et al.*, 2012). However, water resources are already unsustainably exploited in the areas most at risk of reduced precipitation (Daccache *et al.*, 2012). Current irrigation infrastructure might be inadequate to provide sufficient irrigation volumes in 50% of years from 2050 (Daccache *et al.*, 2011). Therefore, emissions must be reduced, irrigation infrastructure improved, and/or irrigation efficiency increased to maintain potato yields in the UK and similar agroclimatic regions.

Irrigation for potato crops is typically provided by rain guns/sprinklers, furrow irrigation, or drip tape (Djaman *et al.*, 2021). Rain gun irrigation is the most used method in the UK, accounting for 72 and 67% of the total irrigated area in 2001 and 2005, respectively (Weatherhead, 2007). However, rain gun irrigation is relatively inefficient, as water is lost to evaporation, wind (Bavi *et al.*, 2009), and canopy interception (Zhou *et al.*, 2018). Drip irrigation has been demonstrated to irrigate potato crops more efficiently (Waddell *et al.*, 1999; Starr, 2005; Wang, Kang and Liu, 2006; Patel and Rajput, 2007; Zhou *et al.*, 2018) without incurring significant yield reductions (Yang *et al.*, 2023). Drip irrigation also has the advantage of reducing the workload for growers as, unlike rain gun irrigation, it can be left in situ after installation for the remainder of the growing season. Drip tape is typically installed under the soil surface, which has positive effects on root development, nitrogen uptake and leaching (Yang *et al.*, 2023), running down the length of each row after branching off from a central pressurised water source. This presents the as-yet untapped potential for irrigation to be targeted more specifically, both in terms of when and where crop water requirements are highest.

Valves at the water source already allow growers to manually control the duration and flow rate of drip irrigation but this could be controlled remotely by real-time estimates of evapotranspiration requirements. This approach has been trialled, using a method which automatically estimated and provided crop water requirements based on canopy temperature measurements (Evett *et al.*, 2002), which are known to correlate positively with soil moisture deficit (Fuchs, 1990). This approach was shown to maintain high yields while increasing water use efficiency in maize, but not soybean, compared to a traditional manual irrigation schedule (Evett *et al.*, 2002). Precision could be further increased by the addition of valves upstream of independent sectors of drip tape, allowing for the targeting of water resources to specific areas with the field. As far as we are aware, this has not been investigated with a drip irrigation system. However, a similar method utilising spatially variable centre pivot irrigation has been investigated in cotton (O'Shaughnessy and Evett, 2010). There, irrigation was triggered for a field sector once local canopy temperatures had remained above a time temperature threshold (TTT; > 28 °C for > 452min in 24 hours). Both cotton yields and irrigation water use efficiency were either maintained or increased under TTT irrigation compared to manual irrigation, under full irrigation and three deficit irrigation regimes (O’Shaughnessy and Evett, 2010).

The TTT method used was based on single readings of canopy temperature for each sector, which were extrapolated to estimate a diurnal canopy temperature curve (Peters and Evett, 2008a). This may not account for the large temperature fluctuations we have previously observed in potato (Chapter 5), nor does it account for any stress experienced under supposedly well-watered conditions. Therefore, we aimed to control irrigation for temperature-controlled blocks of potato, based on effectively continuous measurements of the temperature differences (ΔT) between the temperature-controlled blocks and a well-watered reference sample. We hypothesised that triggering irrigation for temperature-controlled blocks when ΔT was > 1.5 °C and ceasing irrigation when ΔT returned to < 1.5 °C, would maintain yields in potato, without increasing water use.

6.3 Materials and Methods

6.3.1 Plant Material and Growing Conditions

A field experiment was carried out at the Crop and Environment Laboratory (51°26'12.3"N 0°56'32.7"W) at the University of Reading, UK. On 25th April 2023, 120 pre-sprouted seed tubers of the *Solanum tuberosum* cv. Maris Piper were planted on the flat at a depth of 20 cm. The rows were manually ridged post-emergence to prevent stem lodging and tuber greening. Seed tubers were planted in one plot split into six blocks (Figure 1). Each block contained four rows, spaced at 60 cm on centre. Within each block, each row contained five plants, planted 33 cm apart.

Blocks 1, 4, and 5 were randomly assigned to the well-watered treatment and blocks 2, 3, and 6 were assigned to the temperature-controlled treatment. To mitigate edge effects, guard plants of the cv. Sarpo Mira were planted to surround each block. This cultivar was chosen because it produces red tubers, which are easily distinguishable from the white tubers of Maris Piper. Sarpo Mira was also selected as it has an independent resistance rating of 7 (foliage) and 9 (tubers) out of 10 to blight (Science and Advice for Scottish Agriculture, 2023), which was affecting a nearby commercial potato crop at the time of planting.

Figure 1. A to-scale representation of the experimental plot layout overlayed on a satellite image of the Crop and Environment Laboratory (51°26'12.3"N 0°56'32.7"W) at the University of Reading, UK. The insert (right) shows the experimental plot rotated by 161° to show the blocks in ascending order (not to scale). Well-watered plots are shown in dark green, temperature-control blocks in light turquoise, and guard plants in purple. Irrigation lines are shown in white. The location of the commercial potato crop that was the source of blight infection is highlighted in red. Imagery ©2024 Getmapping plc, Infoterra Ltd & Bluesky, Maxar Technologies, The GeoInformation Group; Map data ©2024 Google

All plants were grown under a rain-out shelter (Figure 2) from 26th June (62 DAP) and were irrigated via lines of 2 L hour^{-1} drippers, with one dripper per plant. These lines were supplied by four loops, which were independently controlled by a GP2 data logger (Delta-T Devices, Cambridge, UK). Lines within the well-watered blocks were supplied by a single loop, whereas each temperature-controlled block was supplied independently. All loops were manually controlled to maintain well-watered conditions until 14th July (80 DAP), after which two automatic irrigation programmes were imposed by the GP2. The GP2 was programmed to irrigate the well-watered blocks at 10:00, 12:00, 14:00, and 16:00 for 30 minutes, providing 4 L plant^{-1} day^{-1}. This irrigation volume was based on the gravimetrically calculated water requirements of a nearby potato pot experiment (Table 1; Chapter 4, Table 1). Ambient temperature and relative humidity during the canopy temperature sampling period were retrieved from the University of Reading Atmospheric Observatory (N 51°26'29.2" W 0°56'16.0"; Table 2).

Table 1. Gravimetric calculations of potato water use from a concurrent, nearby pot experiment (Chapter 4, Table 1). Volumes were calculated by measuring the maximum mass lost from saturated 20 L pots over the stated time periods. This calculated amount was rounded up to 4 L day^{-1} to simplify the programming of irrigation software (Delta-T Devices, Cambridge, UK) and to account for the greater volume of soil available to plants in the field.

Treatment	Water Lost After 18 Hours (6 pm – 12 pm)	Water Lost After 6 Hours (12 pm – 6 pm)	Total Water Lost Over 24 Hours
Twice Daily (T_2)	2,800 ml	800 ml	3,600 ml

The GP2 also independently triggered irrigation for each temperature-controlled block if the average canopy temperature of that block was 1.5°C higher than that of the well-watered blocks. This condition was assessed every 10 minutes. The canopy temperature of each block was measured with two MT2-05 leaf temperature sensors (Delta-T Devices, Cambridge, UK), wired into the GP2. Each sensor was attached to a different plant on the terminal leaflet on the third highest fully expanded leaf (Gervais *et al.*, 2021). The sensors were moved fortnightly, if necessary, to maintain this position as the plants grew throughout the experiment.

Figure 2. A photograph of the open-ended polytunnel used as a rainout shelter for the experimental plot. The photograph was taken from the north end of the polytunnel, with block 6 in the foreground. The polytunnel was erected on 26th June (62 DAP) to prevent light filtration from affecting early plant development. Gutters were installed along the long sides of the polytunnel and directed downhill to prevent rainwater that landed on the polytunnel from affecting the experiment.

Table 2. Mean ambient temperatures (T) and relative humidities (RH) in the field between 14th June and 30th October 2023. Ambient temperature and relative humidity were retrieved from the University of Reading Atmospheric Observatory (N 51°26'29.2" W 0°56'16.0").

	T (°C)	RH (%)
Mean	16.3	79
S.E.	0.3	1

These treatments were maintained until the plants had senesced to the point where meaningful temperature data collection became impossible on 30th October (189 DAP). The whole irrigation system was then turned off and the blocks were left to dry out until the tubers were harvested between 15th and 17th November (205-207 DAP). Tubers were harvested by row within each block and were immediately washed and weighed to give fresh tuber mass. A subsample of five representative tubers per row were selected for dry matter analysis. These tubers were weighed, cut into 5 mm slices, oven-dried at 60 °C for at least 72 hours, and reweighed.

6.3.2 Statistical Analysis

All the descriptive statistics, statistical analysis, and graphical representations presented here were produced in RStudio (R Core Team, 2023). Daily average block temperatures were calculated with functions available in base R and plotted with the "ggplot2" package (Wickham, 2016). To prevent pseudo-replication, fresh tuber yield and dry matter content measurements were grouped by block before further analysis. Linear models were then constructed for the average block temperatures, fresh tuber yields, and dry matter contents, with treatment as a fixed effect. The package “easystats” (Lüdecke *et al.*, 2022) was used to assess homogeneity of variance and normality of residuals, and to check for outliers. Each variable tested had one outlier, but these were only significant due to the low sample size and were not deemed to be a problem for further analysis. These models were then subjected to ANOVA testing with base R functions. Estimated marginal means were calculated from each model with the “emmeans” package (Lenth, 2023). The package “multcomp” (Hothorn, Bretz and Westfall, 2008) was used to generate compact letter displays from the models, which were plotted with the estimated marginal means with “ggplot2”. Means not sharing any letter are significantly different by the Tukey-test at the 0.05% significance level (Piepho, 2018).

6.4 Results

6.4.1 Water Use of Temperature-Controlled Blocks Varied Greatly

Irrigation for the well-watered blocks was initiated a total of 430 times across the experiment. As each irrigation period lasted for 30 minutes and provided each plant with 2 L^{-1} $hour^{-1}$, the total irrigation volume provided to the well-watered blocks was 430 L $plant^{-1}$. Irrigation for the water-restricted blocks was initiated much less frequently: 376 times for block 2; 309 times for block 3; and 63 times for block 6. However, for two of the three temperature-controlled blocks, irrigation remained on for longer than the in the well-watered blocks: 1,055 hours for block 2; 299 hours for block 3; and 42 hours for block 6. This provided each of these blocks with 2,110, 598, and 84 L $plant^{-1}$, respectively (Table 3).

6.4.2 Irrigation Maintained Canopy Temperatures Between Treatments

Both daily and overall canopy temperatures were very similar between the blocks (Figure 3). The average daily canopy temperatures of the temperature-controlled blocks were still noticeably higher than those of the well-watered blocks, particularly during the final month of the experiment. However, the overall average temperature of the temperature-controlled blocks was only 0.5°C (2.8%) warmer than that of the well-watered blocks (Figure 4). This is well below the 1.5°C irrigation threshold used to trigger irrigation for the temperature-controlled blocks. This overall temperature difference was not found to be statistically significant (F = 5.57, $p = 0.078$).

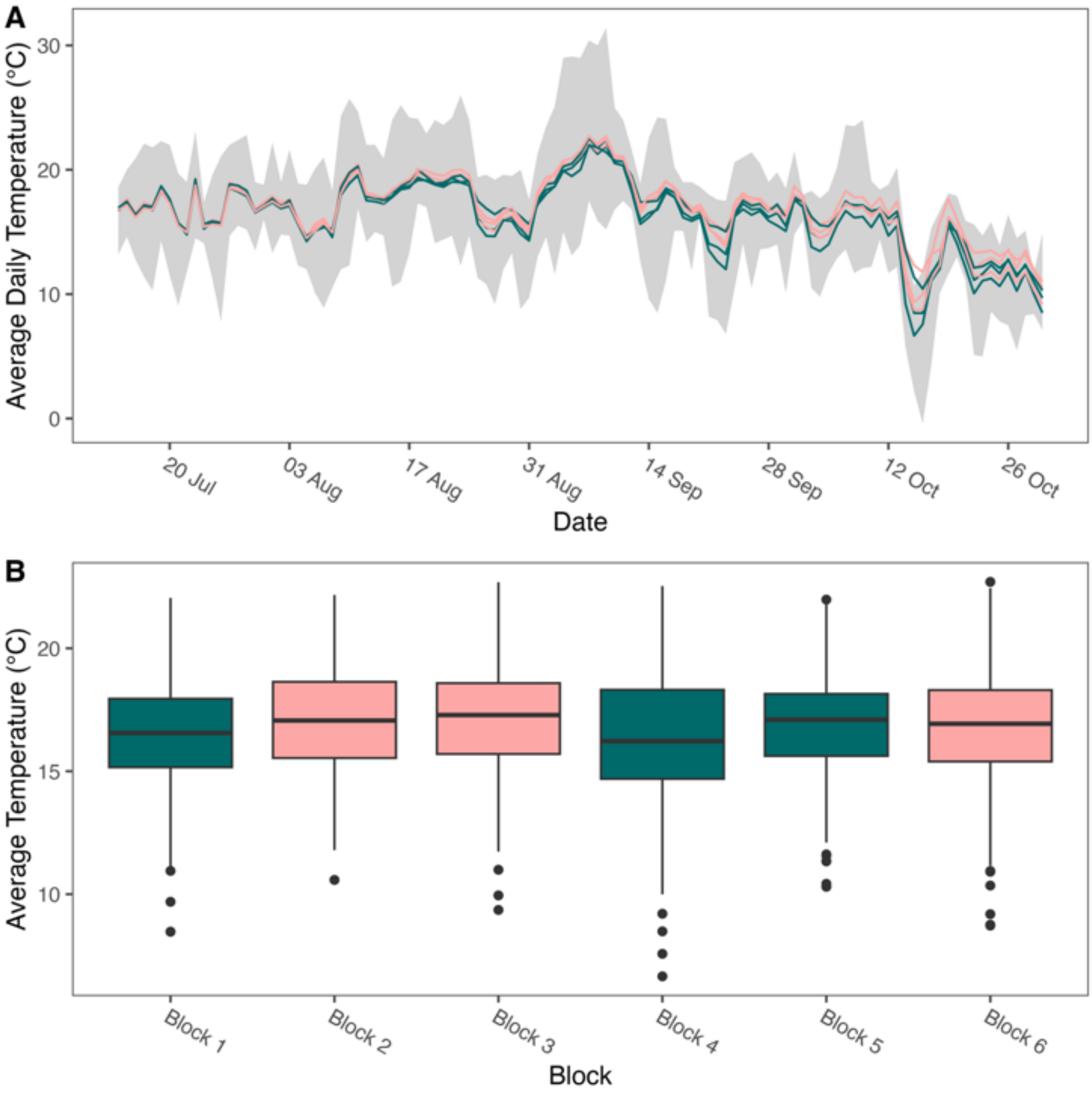

Figure 3. (A) Average daily temperatures of Maris Piper potato leaves in each block (n = 2), recorded at 10-minute intervals with MT2-05 leaf temperature sensors from 14th July (80 DAP) to 30th October (189 DAP). Well-watered blocks are shown in green and temperature-controlled blocks in pink. The grey ribbon shows the daily ambient temperature range, bounded by the minimum and maximum daily temperatures for each day within the sample period. (B) Boxplots of the average temperatures of Maris Piper potato leaves within each block (n = 2) across the entire sample period. Well-watered blocks are shown in green and temperature-controlled blocks in pink.

6.4.3 Fresh Tuber Yield Decreased in Temperature-Controlled Blocks

There was a larger difference in fresh tuber yield between the treatments (Figure 4). The well-watered blocks produced an average yield of 17.8 kg on an area of 4.0 m^2 (extrapolated to 44.9 t ha^{-1}). The average yield of the temperature-controlled blocks was 13.3 kg on the same area (33.6 t ha^{-1}), 4.5 kg (25.1%) lighter than that of the well-watered blocks. This equates to an average yield loss of 225 g $plant^{-1}$, 4.5 kg $block^{-1}$, or 11.3 t ha^{-1} if extrapolated with the same plant spacing used here. However, this difference in fresh tuber yield was not found to be statistically significant (F = 3.15, p = 0.151).

There was a negative correlation between fresh tuber yield and canopy temperature across all the blocks (Figure 4) though this relationship was found to be insignificant (R = -0.64, p = 0.168). These data show that one of the temperature-controlled blocks produced a much lower fresh tuber yield than another the other blocks. This block (block 2) produced 6.7 kg less of fresh tuber mass than the average of the other two blocks under this treatment (Table 3). The fresh tuber yields of the well-watered blocks were more like each other than those of the temperature-controlled blocks. There was a smaller difference in dry matter content between the treatments. The average dry matter content in the well-watered blocks was 24.2%, compared to 24.8% in the temperature-controlled blocks. Again, this difference was not found to be statistically significant (F = 1.76, p = 0.255).

Table 3. Mean total water use, average temperature, fresh tuber yield, and dry matter content of each block in the experiment. Total water use was calculated from recordings of when irrigation was on for each block, multiplied by the per-plant dripper rate of 2 L hour^{-1}. Total irrigation depth was calculated by multiplying total water use by the number of plants per block (20) and dividing by block area (3.96 m^2). Average daily irrigation depth was calculated by dividing Total Irrigation Depth by the number of days the temperature-controlled irrigation system was active (110 days). Average temperatures were calculated as the average temperature of two MT5-02 leaf temperature sensors within each block, across the course of the sample period (14th July, 80 DAP, to 30th October, 189 DAP). The fresh tuber yield of each block was measured between 15th and 17th November (205-207 DAP) and average tuber dry matter content was measured with a subsample of 20 tubers per block.

Block	Treatment	Total Water Use (L plant^{-1})	Total Irrigation Depth (mm)	Average Daily Irrigation Depth (mm)	Average Temp. (°C)	Fresh Tuber Yield (kg)	Dry Matter Content (%)
1	WW	430	2,172	20	16.4	15.5	24.2
2	TC	2,110	10,657	97	17.0	8.9	24.0
3	TC	598	3,020	27	17.0	15.3	25.5
4	WW	430	2,172	20	16.2	19.4	24.5
5	WW	430	2,172	20	16.7	18.4	23.7
6	TC	84	424	4	16.7	15.8	25.0

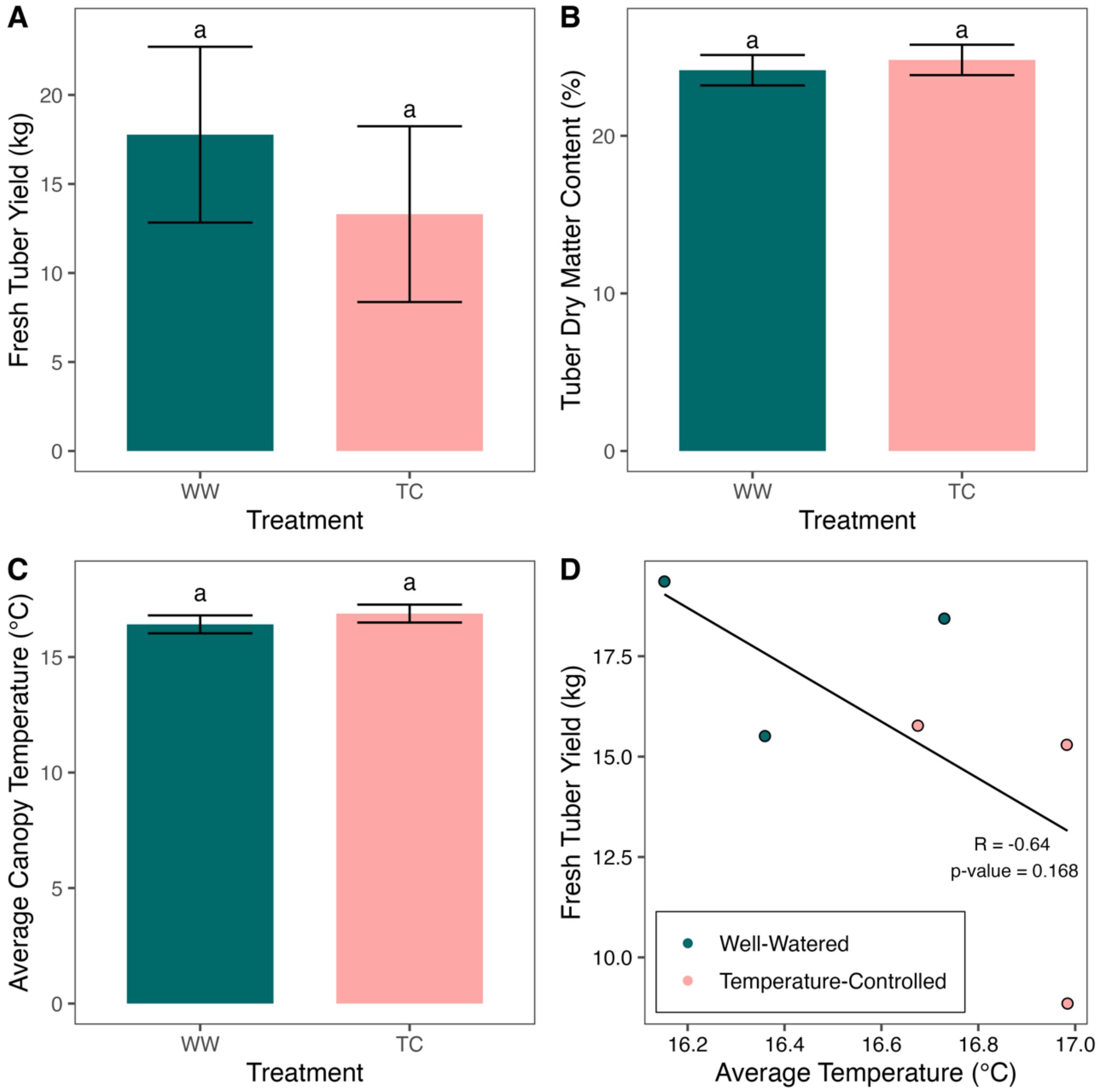

Figure 4. (A) Average fresh tuber yields of potato, cv. Maris Piper, grown under a open-ended polytunnel between 25th April and 15th November 2023. Means represent the fresh tuber yields, harvested between 15th and 17th November, of the well-watered (green) and temperature-controlled (pink) blocks (n = 3) ± 95% CIs. (B) Average tuber dry matter content of a subsample of 20 tubers per block. Means represent the dry matter content of the subsample of tubers harvested between 15th and 17th November, of the well-watered (green) and temperature-controlled (pink) blocks (n = 3) ± 95% CIs. (C) Average canopy temperatures measured every 10 minutes between 14th July (80 DAP) to 30th October (189 DAP) by two MT5-02 leaf temperature sensors in each block. Means represent the average canopy temperature of the well-watered (green) and temperature-controlled (pink) blocks (n = 3) ± 95% CIs. For (A), (B), and (C), means with different letters within each facet were significantly different by Tukey's test ($p < 0.05$). (D) Correlation between fresh tuber yield and average canopy temperature in the well-watered (green) and temperature-controlled (pink) plots ($r(4) = -0.64$, $p = 0.168$).

6.5 Discussion

This research does not clearly support the hypothesis that controlling irrigation with a threshold ΔT value of 1.5 °C between a temperature-controlled and well-watered reference sample significantly reduces water use without incurring a yield penalty. However, the lack of a significant loss of yield in this experiment suggests that programming larger ΔT values could further reduce water use without affecting yield. The threshold of 1.5 °C was based on as-yet unpublished data from our previous research on the effects of water-restriction on canopy temperature in potato (Chapter 5, Figure 2). The smallest significant difference in canopy temperature between well-watered and water-restricted samples in those experiments was 1.8 °C, so it was determined that 1.5 °C would prevent a significant difference in canopy temperature, and thus drought stress. However, partial root zone drying (PRD) providing 50% of total water used in full irrigation has previously been shown not to reduce potato tuber yields (Yactayo *et al.*, 2013). PRD has also been shown to increase marketable yield by 15% in potato, by improving tuber size distribution (Jensen *et al.*, 2010). These studies show that moderate water restrictions only impose minor drought stress on potato and suggest that a larger ΔT could have been used. This would have resulted in a higher severity of drought stress within the plots but might have reduced water use without incurring a significant yield penalty.

Irrigation in the temperature-controlled blocks was triggered less frequently, but typically remained on for longer, than in the well-watered blocks. Irrigation volumes in the temperature-controlled blocks were sufficient to prevent a significant increase in overall canopy temperature (Figure 4). Despite the greater average water use, the temperature-controlled blocks produced a lower (4.5 kg, 25.1%) average fresh tuber yield than the well-watered blocks. This difference was not found to be significant, and there was no difference in dry matter content between the treatments (Figure 4). The decrease in fresh tuber yield under temperature-controlled irrigation was primarily due to the relatively low yield of block 2 compared to the other temperature-controlled blocks (Table 3). Fresh tuber yields were also underestimated in all blocks due to the rotting of tubers associated with blight infection. Blight was first observed in a commercial aeroponic seed potato crop ~40 m away from this experiment. The disease spread first to the blocks closest to the commercial crop, and therefore had the greatest effect on blocks 1 and 2 (Figure 1). Quantifying this effect was impossible as the affected tubers had mostly disintegrated by the harvesting date.

The water use of each temperature-controlled block ranged from 84 to 2,110 L plant^{-1} in blocks 6 and 2, respectively (Table 3). Block 2 was certainly overwatered, which likely contributed to the observed yield losses. We hypothesise that this was due to higher canopy temperatures in block 2, caused by lower transpiration rates in plants with blight infections. The effects of blight on canopy temperatures have not been investigated in potato, but citrus blight has been associated with reduced diurnal transpiration rates in citrus trees (Syvertsen, Bausher and Albrigo, 1980). While the causal agent of citrus blight is unknown, it’s logical that any disease that causes tissue damage is likely to decrease transpiration rates and therefore canopy temperatures (Fuchs, 1990). Overwatering of potato in the field is known to reduce fresh tuber yields through nutrient leeching and root tissue hypoxia (Rens *et al.*, 2022). Increasing soil water levels from “low” to “high” has also been shown to increase the percentage stem area infected with the fungal pathogen black dot (*Colletotrichum coccodes*) from 7.8 to 30.1% and 3 to 29% in the spring and summer, respectively (Cummings and Johnson, 2014). Humid conditions are also known to increase the transmissibility of late blight (Fry, 2008), which may also have contributed to the high yield losses in blocks 1 and 2 observed here. This could be mitigated in future experiments by utilising the programmable safety conditions for the GP2, e.g., limiting the total daily irrigation for the temperature-controlled blocks to that of the well-watered blocks. This has been an effective strategy in cotton, were temperature-controlled irrigation was limited to 20 mm per dose (O’Shaughnessy and Evett, 2010).

The slight elevation gradient of this experimental plot may have contributed to this difference through the downhill percolation of irrigation water. This could have been prevented by splitting the blocks over several independent plots, but this demonstrates an important disadvantage of using wired probes for such experiments. The MT2-05 probes used here are supplied with a 5 m cable, restricting the possible area of the experimental plot. Thermal imaging has been preferred in previous research on canopy temperature responses to water-restriction in potato (Rud *et al.*, 2014; Rinza *et al.*, 2019, 2022; Elsayed *et al.*, 2021), perhaps for this reason. This previous research has demonstrated that thermal imaging of canopy temperatures can be utilised in a crop water-stress index (CWSI) that compares sampled canopy temperatures to empirically, theoretically, or statistically estimated canopy temperatures of a non- and fully-transpiring canopy (Rud *et al.*, 2014). This method has been shown to significantly reduce water use in both drip and furrow potato irrigation systems but has only been shown to maintain fresh tuber yields with a moderate CWSI threshold of 0.4 under furrow irrigation (Rinza *et al.*, 2022).

This method would be improved by the development of remote thermal imagery sensing and better integration between this and irrigation system controllers. However, wired probes currently have the advantages of sensing remotely and almost continuously (Figure 3). This, and the integration between the probes and the data logger, allowed canopy temperatures to inform the irrigation system in real time, which is not possible with the handheld infrared sensors used previously (Elsayed *et al.*, 2021; Rinza *et al.*, 2022). A similar disadvantage is implicit with the temperature-time threshold (TTT) method of irrigation, which aims to maintain canopy temperatures within optimal limits for enzymatic activity (Peters and Evett, 2008b). This method estimates local canopy temperatures throughout the day based on a single daily measurement. However, this method utilises empirically supported estimates of daily temperature fluctuations based on these single readings and has been demonstrated to produce higher yields than manual irrigation in cotton under a centre pivot system (O’Shaughnessy and Evett, 2010).

Future research should aim to combine the strengths of the CWSI irrigation methods, the TTT method, and the ΔT method used here. The CSWI method has demonstrated the effectiveness of thermal imagery for collecting canopy temperature data on agriculturally relevant scales. The TTT method has shown that maintaining canopy temperatures within the narrow range that is optimal for enzyme activity can effectively be applied to automatic irrigation systems. Our method provides preliminary support for irrigation scheduling based on the ΔT from a well-watered reference population. In future research, machine learning could be used with field-scale thermal imagery to develop a model of optimum canopy temperatures under well-watered conditions. Significant deviations in canopy temperature could then be used to trigger irrigation, with the possibility of targeting irrigation where it’s most required through pivot irrigation or isolated drip-tape runs.

6.6 Conclusion

We aimed to control irrigation for three temperature-controlled blocks of potato based on a temperature difference (ΔT) threshold between these blocks and three well-watered blocks, as measured by wired leaf temperature probes. We hypothesised that triggering irrigation at a ΔT of > 1.5 °C, and ceasing irrigation at ≤ 1.5 °C, would reduce water use across the growing season, without incurring a significant yield penalty. This experiment did not support this hypothesis as water use was significantly higher in the temperature-control blocks, although we did not observe a significant yield penalty. It’s likely that the ΔT threshold, while sufficient to prevent a significant increase in canopy temperature across the experiment, was too low to prevent overwatering. This could have contributed to the increased incidence of blight observed in the most overwatered block, accounting for the associated yield losses. The experimental design was limited due to each block being planted on the same plot, highlighting a disadvantage with the use of wired probes to measure canopy temperatures. However, these probes allowed us to uniquely control irrigation with continuous canopy temperature measurements. Further research should aim to optimise this method, by investigating a more appropriate ΔT threshold, and by integrating thermal imagery into a similar system of irrigation control as that used here. Machine learning may also be used to develop a model of optimum canopy temperatures under well-watered conditions, and trigger irrigation based on significant deviations from this model.

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Chapter 7. Overall Discussion and Conclusions

7.1 Overall Discussion

Potato has been described as under researched relative to the nutritional value it currently provides to the global food system (Manners and van Etten, 2018). This is well evidenced by the key papers reviewed in Chapter 2, where the mean year of publication was 2001 (Hill *et al.*, 2021). Many of the important references in Chapters 3 (oilseed rape) and 6 (cotton) investigated other crops, as little or no research on the processes described in these chapters were available in potato. However, potato should have a prominent role in the global food strategies of the future, considering its superlatively high calorie production relative to its land and water use (Renault and Wallender, 2000; Sun *et al.*, 2015). This aim will be impossible without future research taking full advantage of novel phenotyping techniques, particularly with respect to traits which have the potential to improve the extremely high drought susceptibility of potato (Schafleitner, Gutierrez and Legay, 2009; Monneveux, Ramírez and Pino, 2013). Unfortunately, much of the previous research investigating the responses of potato phenotypes to drought has neglected to consider important and well-known, if poorly understood, systemic confounding factors (Poorter *et al.*, 2012; Sinclair *et al.*, 2017; Turner, 2019).

Chapters 3 and 4 of this thesis have significantly advanced the understanding of one such factor, pot size, in potato research. In Chapter 3, small pots (≤ 5 L), which have been used extensively in potato phenotyping research, were shown to confound the relationships between water-restriction and the morphophysiology of two potato cultivars with contrasting maturities. This was particularly evident with digital measurements of plant height and biomass, traits that are important morphological markers of drought tolerance in potato (Schittenhelm, Sourell and Löpmeier, 2006) and are easily measured with, primarily canopy-focussed, novel phenotyping technologies. These traits were found to be stunted exclusively in small pots, but only in one of the two cultivars – a previously unknown effect in potato (Chapter 3, Figures 3 & 7). This effect could have led to systemic errors in cultivar selection for breeding programs aimed at improving drought tolerance in potato. This experiment also showed that the effects of water-restriction on potato yields, height, and digital biomass were diminished in small pots. However, the design of this experiment was limited both in terms of statistical power and in its ability to elucidate the causes of pot binding in potato.

Chapter 4 aimed to address these limitations by focusing on only two pot sizes with greater biological replication and by comparing the relative effects of three irrigation frequencies on potato morphophysiology. Specifically, this experiment aimed to investigate the water availability hypothesis of pot binding in potato for the first time. This predicts that the water holding capacity of small pots is insufficient to prevent drought stress between daily waterings (Sinclair *et al.*, 2017). We found evidence to support the water availability hypothesis in both pot sizes, but the effects were stronger in the smaller pots. Canopy temperatures, a reliable indicator of drought stress in plants (Fuchs, 1990), were significantly higher under daily, versus twice daily, irrigation in both cultivars and pot sizes. Therefore, some level of drought stress must have occurred in both pot sizes under daily irrigation.

However, larger pots were associated with significantly lower canopy temperatures under each irrigation frequency. Together, these results suggest that, while pot binding might occur in 20 L pots, even with twice daily irrigation, it can be mitigated by increasing the pot size or irrigation frequency. These results were supported by those for fresh tuber yield, although daily irrigation is probably sufficient to prevent pot binding affecting yield in 20 L pots. These results support the water availability hypothesis of pot binding and caution against the use of pots ≤ 20 L for potato, particularly with daily irrigation.

Chapters 3 and 4 will be important for future phenotyping research in potato, as the resulting papers are the first to demonstrate the confounding effects of pot size on the relationships between water-restriction and plant development in potato. However, the pot size and irrigation frequency where further increases in either do not significantly reduce the effects of pot binding were not found, except perhaps with respect to canopy biomass (Chapter 4, Figure 2). Therefore, the results from these chapters do not provide strong evidence for the use of a particular protocol for potato phenotyping experiments, but rather show that pot size and irrigation frequency are important considerations that cannot simply be gleaned from previous research. Both experiments showed that the PlantEye phenotyping platform at the University of Reading is inappropriate for measuring many traits of potato in large pots. This is not intended as a criticism of Phenospex or the University, but rather demonstrates that defining protocols base on these experiments would also be inappropriate. However, the PlantEye was extremely unreliable throughout this project and scanning had to be abandoned on several sample dates during the experiments presented in Chapters 3, 4, and 5. Pot size, irrigation frequency, and even the specific phenotyping technologies used in future research, should be carefully considered and optimised for the specific crop being phenotyped and the dependent and independent variables of interest.

Generally, these chapters do suggest that increasing pot size and/or irrigation frequency will reduce the effects of pot binding in potato. However, it's important to note that there are considerations beyond the scientific when designing a pot experiment such as these. The largest pots used in Chapter 3 were 40 L, weighing between 32 and 40 kg depending on the soil moisture content. It was possible to move these from the site of the experiment to the phenotyping platform manually, but other researchers should consider their own capacity when designing similar experiments. 20 L pots were the largest used in Chapter 4, as it's unlikely that many researchers would consider larger pots, unless they can be moved mechanically or phenotyped in situ. It is for these reasons that the troughs used in Chapter 5 were developed. The troughs were constructed with a volume of 148 L, providing 49.3 L per plant, and were palletised to facilitate movement between the experimental glasshouse compartment and the phenotyping platform. This design reduced the probability of pot binding as much as possible, while allowing for easy movement of the plants. The troughs were also designed to conform with established principles for imposing and maintaining water-restriction in pots (Turner, 2019) and irrigated as frequently as necessary to maintain optimum soil moisture contents. This does not guarantee that pot binding was completely prevented but, it was certainly minimised.

Chapter 5 presents the most comprehensive recent research available on the response of canopy temperatures to water-restriction in commercially relevant cultivars of potato in the UK (Goffart *et al.*, 2022). This chapter also elucidated the effects of resuming irrigation on canopy temperatures and SPAD values in potato for the first time. The results showed that canopy temperatures, but not SPAD values, reliably returned to baseline after the recommencement of well-watered conditions. This finding has important implications for the future of irrigation management in potato, where irrigation efficiency must improve to prevent the predicted yield losses under a changing climate (Daccache *et al.*, 2012). Previous research has investigated the utility of canopy temperatures, integrated into crop water stress indices (CWSI), to control irrigation in potato (Rud *et al.*, 2014; Rinza *et al.*, 2022). However, absolute canopy temperature differences are more useful for growers who are both more likely to directly measure canopy temperatures than calculate CWSI and are primarily interested in delaying irrigation for as long as possible to reduce the associated costs. However, significant differences in canopy temperatures were only observed under glasshouse conditions. The field trial reported in Chapter 5 coincided with record temperatures in the UK, so basing irrigation on canopy temperatures under these conditions cannot be recommended.

The experiments in Chapter 5 were also limited by the inconsistency of seed tuber availability throughout 2022. As the most popular commercial cultivar in the UK (Goffart *et al.*, 2022), quality Maris Piper seed was available throughout the year, but Melody seed was unavailable at the time of planting for the autumn pot experiment; therefore, Désirée was chosen for its comparable maturity class and drought tolerance. This could be interpreted as an advantage, as the responses to drought of different potato cultivars are highly variable (Hill *et al.*, 2021). However, the pot experiments aimed to assess the reliability of canopy temperature and SPAD value responses to water-restriction. Therefore, it would have been preferable to utilise Melody throughout. In the field, it was more relevant to assess the responses of cultivars in different maturity classes to assess the suitability of each for the experiments being planned for Chapter 6. Maris Piper is a late maturing cultivar, so Pentland Javelin was selected to represent early cultivars. However, the "unprecedented extreme heatwave" in July 2022 (Met Office National Climate Information Centre, 2022), resulted in a 100% mortality rate in Pentland Javelin, probably due to the smaller root systems and reduced recovery capacity of early maturing cultivars (Hill *et al.*, 2021). It would be inappropriate to interpret this single result as a warning of future early potato crop failures, but it does demonstrate that further research on the absolute heat and drought stress tolerances of cultivars representing all maturity classes is urgently required.

A novel method of controlling irrigation for potato was trialled in Chapter 6. This temperature-controlled method triggered irrigation for blocks of potato when the difference in canopy temperatures between the experimental and well-watered control blocks exceeded a threshold of 1.5 °C. This method successfully prevented any yield losses in the experimental blocks, relative to the well-watered controls, but the total water use of the experimental blocks varied dramatically. Previous research has demonstrated that canopy temperatures can be used to control irrigation systems, most notably in potato (Rud *et al.*, 2014; Rinza *et al.*, 2022) and cotton (Hatfield, Quisenberry and Dilbeck, 1987; O'Shaughnessy and Evett, 2010). However, the temperature-controlled method presented in Chapter 6 has the unique advantage of sampling canopy temperatures every ten minutes, compared to the maximum sampling rate of once per day in the previous research. Unfortunately, the experiment was limited by a severe blight infection, which confounded the canopy temperature measurements, particularly in the overwatered block 2. Unfavourable weather conditions also pushed back the construction of the rain-out shelter, delaying the initiation of the experimental irrigation system. Despite these limitations, and those outlined in Chapter 6, this experiment shows the potential of near-continuous measurements of canopy temperature to inform irrigation management in potato for the first time. Substantial further research is required, including the integration of thermal imaging into a remote and continuous canopy temperature measurement system, to maximise the area over which canopy temperatures can be sampled. Machine learning should also be investigated as a method of defining the canopy temperature ranges of well-watered populations, to remove the requirement for a reference sample, and to optimise the temperature differential threshold. Finally, sectional systems should be investigated that target irrigation at specific areas of the field with higher evapotranspiration requirements, to further increase the efficiency of potato irrigation.

7.2 Conclusions

Potato is the third most important food crop in the world (Aliche *et al.*, 2018) as it yields yield more food, more efficiently than all other crops (Lutaladio and Castaldi, 2009). However, historically high profit margins have masked inefficiencies in potato production (Taylor *et al.*, 2018) and contributed to the underrepresentation of potato in the crop science literature (Manners and van Etten, 2018). This is concerning, as potato is an important source of nutrition in the developing world (Lutaladio and Castaldi, 2009) and is predicted to be severely affected by climate change in the UK (Daccache *et al.*, 2011, 2012) – an important potato producing country (Goffart *et al.*, 2022). Therefore, this thesis documents a program of research that was necessarily developed from the ground up, to make the best use of the limited methodologies and previous research available for potato research.

The lack of established methodologies in the literature (Hill *et al.*, 2021) provided an opportunity to empirically evaluate previous methodologies, which might not be available to researchers working with more well-researched crops. This project initially aimed to understand the effects of drought stress on potato by utilising a novel phenotyping platform that required plants be grown in pots; therefore, the most important methodological variables to investigate were irrigation protocols and pot size. As these have the potential to confound drought research in all crops, some previous literature was available to provide a framework for further investigation (Poorter *et al.*, 2012; Sinclair *et al.*, 2017; Turner, 2019), which was novel in potato.

The investigations into pot size, and later irrigation frequency, were limited in their ability to recommend a specific methodology to eliminate pot binding in potato. However, it can be stated that pots ≤ 5 L are entirely inappropriate for potato phenotyping experiments, due to the cultivar-specific stunting effects observed in such pots (Chapter 3). Subsequently, evidence was found that pot binding occurs in pots ≤ 20 L, even with twice daily irrigation (Chapter 4). This may seem trivial, but many recent potted potato experiments have been conducted in pots ≤ 10 L (Rolando *et al.*, 2015; Rykaczewska, 2015; Meise *et al.*, 2019; Qin *et al.*, 2019; Orsák *et al.*, 2020; Dorneles *et al.*, 2021; Gervais *et al.*, 2021; Mthembu *et al.*, 2022). Fortunately, the results presented here demonstrate that the effects of pot binding can be mitigated, if not eliminated, by increasing pot size and/or irrigation frequency. When increasing pot size is impossible, e.g., in spatially limited controlled environments, frequent, i.e., at least twice per day, irrigation and monitoring of soil moisture content are therefore recommended for potted potato experiments.

Gaining a better understanding of pot binding in potato allowed the design of subsequent experiments to mitigate these systemic effects. Using bespoke, palletised troughs, and by controlling irrigation with continuous measurements of soil moisture content, it was discovered that canopy temperatures, but not SPAD values, reliably increase with water-restriction. Canopy temperatures were also found, for the first time, to return to those of control plants after the resumption of well-watered conditions. This suggested that canopy temperatures could be used to control irrigation in potato, allowing water to be supplied when the crop needs it and saved when it does not. However, these results were found exclusively under glasshouse conditions and not in the field. It’s likely that this was due to the confounding effects of an “unprecedented extreme heatwave” which caused record temperatures in the UK (Met Office National Climate Information Centre, 2022). Further research should therefore aim to investigate the effects of cyclical water-restriction on the canopy temperatures of potato in the field, specifically the effects immediately after the return of irrigation.

Finally, this discovery was utilised to control an irrigation system for potato. The system used leaf temperature probes to sample the average canopy temperatures of six blocks of potato and triggered irrigation for an experimental block when the temperature of that block exceeded that of the paired control block by 1.5 °C. This method maintained fresh tuber yields and dry matter content, but water use between the experimental blocks varied greatly. The experiment was limited by biotic stress, caused by a blight infection from a nearby commercial crop, and the number and wire length of leaf temperature probes. However, given the necessity for improving the efficiency of irrigation for potato, this experiment is an important foundation for further research, which should focus on machine learning of optimal canopy temperatures in potato, the remote and continuous sensing of canopy temperatures in the field, and the optimisation of the temperature threshold used to trigger irrigation.

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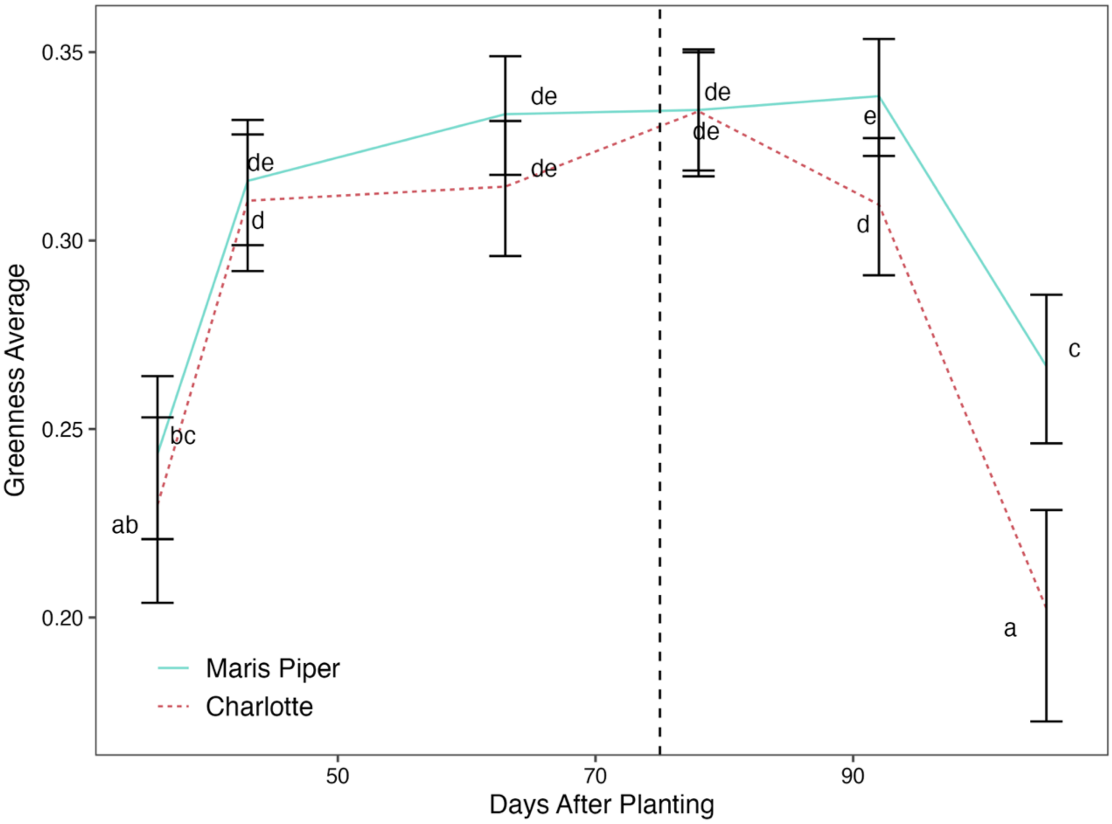
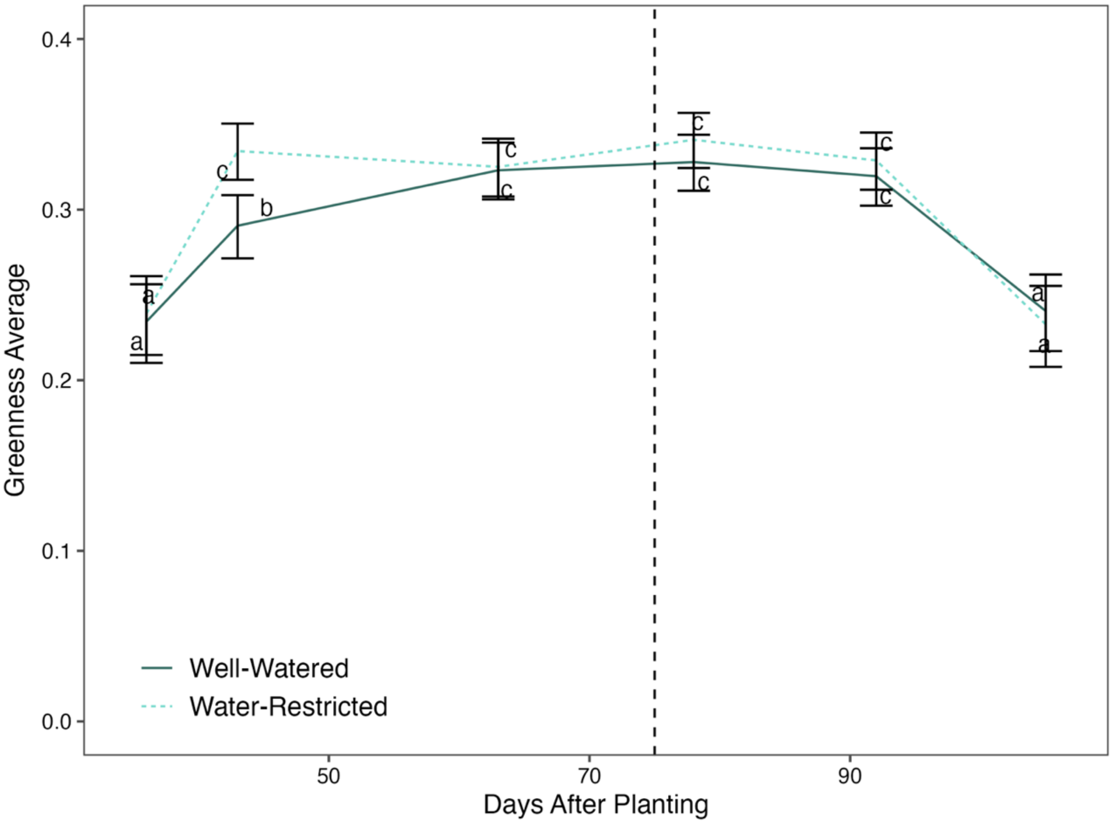
Supplementary Material

8.1 Chapter 3

8.1.1 Supplementary Figures



Supplementary Figure S1. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars, Maris Piper (solid green) and Charlotte (dashed pink), grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent greenness across each pot size and water treatment within each cultivar and measurement date (n = 30) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$).



Supplementary Figure S2. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered (solid dark green) or water-restricted (dashed light green) conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 day after planting. Means represent greenness across each pot size and cultivar within each combination of treatment and measurement date (n = 29) ± 95% CIs. The vertical dashed line indicates the onset of water-restriction. Means with different letters were significantly different by Tukey's test ($p < 0.05$).

8.1.2 Supplementary Tables

Supplementary Table S1. Three plants were removed from the sample and excluded from analysis due to blackleg infection.

Plant ID	Pot Size (l)	Cultivar	Treatment	Removed (DAP)
11	2.5	Charlotte	Water-Restricted	60
22	5	Charlotte	Water-Restricted	75

56	40	Charlotte	Water-Restricted	78

Supplementary Table S2. Mean (back-transformed from sqrt) digital biomass (DB) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 DAP. Means represent DB across both water treatments within each combination of pot size, cultivar, and sample date (n = 6) ± 95% CIs. Means with different letters within each pot size were significantly different by Tukey's test ($p < 0.05$).

Pot Size	Cultivar	DAP	Mean	SE	df	Lower CL	Upper CL	CLD
2.5 L	Maris Piper	36	11.28	0.54	222	3.20	24.29	abc
	Charlotte	36	4.22	0.61	222	0.09	14.50	ab
	Maris Piper	43	29.86	0.54	222	15.17	49.48	cd
	Charlotte	43	23.38	0.61	222	9.49	43.42	bcd
	Maris Piper	63	74.83	0.54	222	50.14	104.44	e
	Charlotte	63	38.76	0.61	222	19.99	63.69	de
	Maris Piper	78	78.24	0.54	222	52.94	108.47	e
	Charlotte	78	40.96	0.61	222	21.58	66.50	de
	Maris Piper	92	72.13	0.54	222	47.94	101.26	e
	Charlotte	92	28.29	0.61	222	12.71	50.04	cd
	Maris Piper	105	29.19	0.54	222	14.70	48.61	cd
	Charlotte	105	2.18	0.61	222	0.08	10.44	a
5 L	Maris Piper	36	3.82	0.54	222	0.15	12.41	ab
	Charlotte	36	4.68	0.61	222	0.17	15.34	ab
	Maris Piper	43	23.83	0.54	222	10.97	41.62	c
	Charlotte	43	21.00	0.61	222	8.00	40.16	bc
	Maris Piper	63	97.95	0.54	222	69.35	131.48	fg
	Charlotte	63	60.60	0.61	222	36.36	91.00	def
	Maris Piper	78	112.34	0.54	222	81.54	148.07	g
	Charlotte	78	51.56	0.61	222	29.44	79.83	cde
	Maris Piper	92	108.68	0.54	222	78.42	143.86	fg
	Charlotte	92	32.79	0.61	222	15.78	55.97	cd
	Maris Piper	105	85.99	0.54	222	59.34	117.55	efg
	Charlotte	105	1.30	0.61	222	0.38	8.37	a
10 L	Maris Piper	36	2.96	0.54	222	0.02	10.82	a
	Charlotte	36	12.67	0.54	222	3.96	26.31	ab

	Maris Piper	43	22.05	0.54	222	9.78	39.26	bc
	Charlotte	43	51.58	0.54	222	31.50	76.59	cd
	Maris Piper	63	115.30	0.54	222	84.06	151.46	f
	Charlotte	63	119.65	0.54	222	87.78	156.44	f
	Maris Piper	78	76.89	0.54	222	51.83	106.88	def
	Charlotte	78	111.65	0.54	222	80.95	147.28	f
	Maris Piper	92	56.70	0.54	222	35.53	82.80	d
	Charlotte	92	108.34	0.54	222	78.13	143.47	ef
	Maris Piper	105	83.62	0.54	222	57.38	114.79	def
	Charlotte	105	62.47	0.54	222	40.12	89.74	de
20 L	Maris Piper	36	1.34	0.54	222	0.17	7.44	a
	Charlotte	36	2.89	0.54	222	0.02	10.68	a
	Maris Piper	43	13.53	0.54	222	4.45	27.54	ab
	Charlotte	43	22.68	0.54	222	10.19	40.09	b
	Maris Piper	63	94.22	0.54	222	66.22	127.16	cd
	Charlotte	63	109.41	0.54	222	79.04	144.70	d
	Maris Piper	78	81.00	0.54	222	55.21	111.71	cd
	Charlotte	78	74.59	0.54	222	49.94	104.16	cd
	Maris Piper	92	67.07	0.54	222	43.83	95.24	cd
	Charlotte	92	92.71	0.54	222	64.95	125.40	cd
	Maris Piper	105	90.89	0.54	222	63.43	123.28	cd
	Charlotte	105	59.81	0.54	222	38.00	86.54	c
40 L	Maris Piper	36	4.17	0.54	222	0.22	13.05	a
	Charlotte	36	2.35	0.61	222	0.05	10.81	a
	Maris Piper	43	26.48	0.54	222	12.79	45.10	bc
	Charlotte	43	24.53	0.61	222	10.23	44.99	b
	Maris Piper	63	85.39	0.54	222	58.85	116.86	de
	Charlotte	63	106.57	0.61	222	73.42	145.88	e
	Maris Piper	78	52.35	0.54	222	32.10	77.52	bcd
	Charlotte	78	46.63	0.61	222	25.75	73.67	bcd
	Maris Piper	92	63.48	0.54	222	40.94	90.96	de
	Charlotte	92	79.28	0.61	222	51.11	113.61	de
	Maris Piper	105	100.14	0.54	222	71.20	134.02	e
	Charlotte	105	61.20	0.61	222	36.82	91.73	cde

Supplementary Table S3. Mean plant height (PH) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 DAP. Means represent PH across each water treatment within each combination of pot size, cultivar, and sample date (n = 6) ± 95% CIs. Means with different letters within each pot size were significantly different by Tukey's test ($p < 0.05$).

Pot Size	Cultivar	DAP	Mean	SE	df	Lower CL	Upper CL	CLD
2.5 L	Maris Piper	36	280.45	34.85	222	179.81	381.10	abc
	Charlotte	36	137.83	38.97	222	25.31	250.35	a
	Maris Piper	43	452.34	34.85	222	351.70	552.98	de
	Charlotte	43	308.21	38.97	222	195.69	420.74	abcd
	Maris Piper	63	712.05	34.85	222	611.40	812.69	fg
	Charlotte	63	387.43	38.97	222	274.91	499.95	cd
	Maris Piper	78	770.34	34.85	222	669.69	870.98	g
	Charlotte	78	381.79	38.97	222	269.27	494.31	cd
	Maris Piper	92	699.52	34.85	222	598.87	800.16	fg
	Charlotte	92	342.48	38.97	222	229.96	455.00	bcd
	Maris Piper	105	584.85	34.85	222	484.21	685.49	ef
	Charlotte	105	161.72	38.97	222	49.20	274.24	ab
5 L	Maris Piper	36	157.99	34.85	222	57.35	258.64	ab
	Charlotte	36	123.66	38.97	222	11.13	236.18	a
	Maris Piper	43	300.00	34.85	222	199.36	400.64	bcd
	Charlotte	43	233.19	38.97	222	120.67	345.71	abc
	Maris Piper	63	728.81	34.85	222	628.17	829.45	e
	Charlotte	63	471.63	38.97	222	359.11	584.15	d
	Maris Piper	78	765.43	34.85	222	664.79	866.07	e
	Charlotte	78	434.84	38.97	222	322.32	547.36	d
	Maris Piper	92	771.72	34.85	222	671.08	872.37	e
	Charlotte	92	330.91	38.97	222	218.39	443.44	cd
	Maris Piper	105	692.63	34.85	222	591.99	793.27	e
	Charlotte	105	91.44	38.97	222	-21.08	203.96	a
10 L	Maris Piper	36	170.13	34.85	222	69.49	270.77	a
	Charlotte	36	223.38	34.85	222	122.74	324.02	a
	Maris Piper	43	328.21	34.85	222	227.57	428.85	ab

	Charlotte	43	408.31	34.85	222	307.67	508.95	b
	Maris Piper	63	763.54	34.85	222	662.89	864.18	cde
	Charlotte	63	777.61	34.85	222	676.96	878.25	cde
	Maris Piper	78	802.78	34.85	222	702.14	903.42	de
	Charlotte	78	805.45	34.85	222	704.81	906.09	de
	Maris Piper	92	846.30	34.85	222	745.66	946.94	e
	Charlotte	92	782.20	34.85	222	681.56	882.85	cde
	Maris Piper	105	682.08	34.85	222	581.43	782.72	cd
	Charlotte	105	626.93	34.85	222	526.29	727.57	c
20 L	Maris Piper	36	107.30	34.85	222	6.66	207.94	a
	Charlotte	36	104.03	34.85	222	3.39	204.68	a
	Maris Piper	43	253.36	34.85	222	152.72	354.00	a
	Charlotte	43	231.75	34.85	222	131.10	332.39	a
	Maris Piper	63	684.68	34.85	222	584.03	785.32	bc
	Charlotte	63	703.57	34.85	222	602.93	804.21	bc
	Maris Piper	78	744.71	34.85	222	644.07	845.35	c
	Charlotte	78	738.34	34.85	222	637.70	838.98	bc
	Maris Piper	92	756.17	34.85	222	655.53	856.82	c
	Charlotte	92	743.82	34.85	222	643.18	844.47	c
	Maris Piper	105	661.88	34.85	222	561.24	762.52	bc
	Charlotte	105	576.47	34.85	222	475.83	677.11	b
40 L	Maris Piper	36	118.51	34.85	222	17.86	219.15	ab
	Charlotte	36	85.92	38.97	222	-26.60	198.44	a
	Maris Piper	43	259.89	34.85	222	159.25	360.54	b
	Charlotte	43	212.03	38.97	222	99.51	324.55	ab
	Maris Piper	63	692.26	34.85	222	591.62	792.90	c
	Charlotte	63	692.32	38.97	222	579.80	804.84	c
	Maris Piper	78	703.47	34.85	222	602.83	804.12	c
	Charlotte	78	721.45	38.97	222	608.93	833.97	c
	Maris Piper	92	687.10	34.85	222	586.46	787.74	c
	Charlotte	92	683.22	38.97	222	570.70	795.74	c
	Maris Piper	105	643.52	34.85	222	542.88	744.16	c
	Charlotte	105	571.37	38.97	222	458.85	683.89	c

Supplementary Table S4. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars, Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 DAP. Means represent greenness across each cultivar and water treatment within each combination of pot size and sample date (n = 12) ± 95% CIs. Means with different letters within each DAP were significantly different by Tukey's test ($p < 0.05$).

DAP	Pot Size	Mean	SE	df	Lower CL	Upper CL	CLD
36	2.5 L	0.24	0.01	222	0.21	0.27	a
	5 L	0.24	0.01	222	0.21	0.27	a
	10 L	0.23	0.01	222	0.19	0.26	a
	20 L	0.23	0.01	222	0.19	0.26	a
	40 L	0.24	0.01	222	0.20	0.27	a
43	2.5 L	0.28	0.01	222	0.25	0.31	ab
	5 L	0.28	0.01	222	0.25	0.30	a
	10 L	0.32	0.01	222	0.30	0.34	b
	20 L	0.32	0.01	222	0.30	0.34	b
	40 L	0.36	0.01	222	0.33	0.38	c
63	2.5 L	0.31	0.01	222	0.28	0.33	b
	5 L	0.27	0.01	222	0.24	0.30	a
	10 L	0.32	0.01	222	0.29	0.34	b
	20 L	0.36	0.01	222	0.34	0.38	c
	40 L	0.36	0.01	222	0.34	0.38	c
78	2.5 L	0.33	0.01	222	0.31	0.35	ab
	5 L	0.29	0.01	222	0.26	0.32	a
	10 L	0.34	0.01	222	0.32	0.36	b
	20 L	0.35	0.01	222	0.33	0.37	b
	40 L	0.36	0.01	222	0.33	0.38	b
92	2.5 L	0.26	0.01	222	0.23	0.29	a
	5 L	0.28	0.01	222	0.25	0.31	a
	10 L	0.34	0.01	222	0.31	0.36	b
	20 L	0.36	0.01	222	0.34	0.38	bc
	40 L	0.37	0.01	222	0.35	0.39	c
105	2.5 L	0.05	0.01	222	-0.04	0.13	a
	5 L	0.13	0.01	222	0.03	0.18	a
	10 L	0.27	0.01	222	0.24	0.30	b
	20 L	0.29	0.01	222	0.26	0.31	bc
	40 L	0.32	0.01	222	0.30	0.35	c

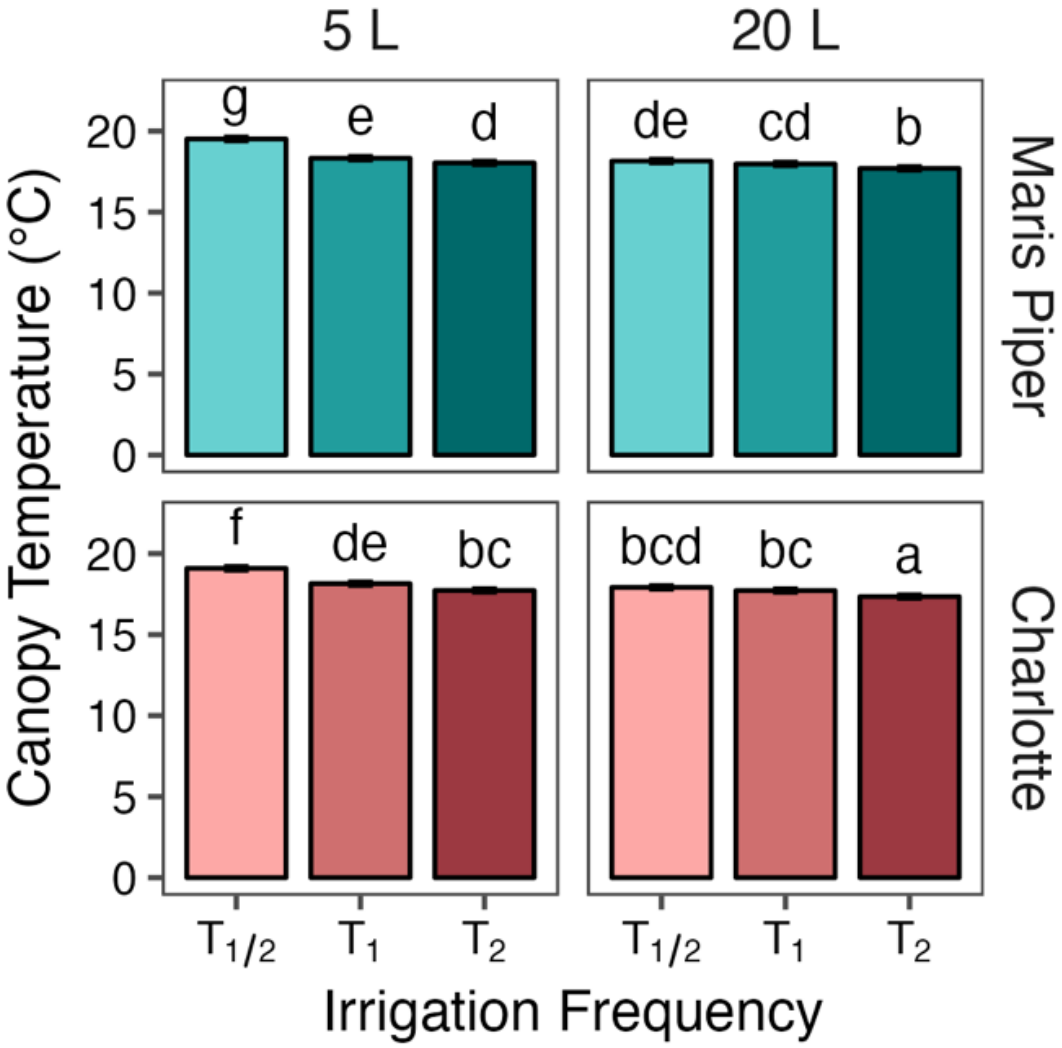
Supplementary Table S5. Mean (back-transformed from squared) average greenness index (greenness) of two potato cultivars., Maris Piper and Charlotte, grown under either well-watered or water-restricted conditions, in 2.5, 5, 10, 20, and 40 L pots, from 36 to 105 days after planting (DAP). Plants were grown under glasshouse conditions in a 2:1 peat:vermiculite mix and harvested 134 DAP. Means represent greenness across each water treatment within each combination of pot size, cultivar, and measurement date (n = 6) ± 95% CIs. Means with different letters within each pot size were significantly different by Tukey's test ($p < 0.05$).

Pot Size	Cultivar	DAP	Mean	SE	df	Lower CL	Upper CL	CLD
2.5 L	Maris Piper	36	0.27	0.01	222	0.22	0.31	bcd
	Charlotte	36	0.22	0.01	222	0.15	0.27	b
	Maris Piper	43	0.30	0.01	222	0.25	0.33	bcde
	Charlotte	43	0.27	0.01	222	0.22	0.32	bcd
	Maris Piper	63	0.30	0.01	222	0.26	0.34	cde
	Charlotte	63	0.31	0.01	222	0.27	0.35	de
	Maris Piper	78	0.34	0.01	222	0.30	0.37	e
	Charlotte	78	0.32	0.01	222	0.28	0.36	de
	Maris Piper	92	0.29	0.01	222	0.24	0.32	bcde
	Charlotte	92	0.23	0.01	222	0.17	0.28	bc
	Maris Piper	105	0.06	0.01	222	-0.11	0.17	a
	Charlotte	105	0.03	0.01	222	-0.04	0.16	a
5 L	Maris Piper	36	0.25	0.01	222	0.20	0.30	bcde
	Charlotte	36	0.23	0.01	222	0.17	0.28	bc
	Maris Piper	43	0.31	0.01	222	0.27	0.34	de
	Charlotte	43	0.24	0.01	222	0.18	0.29	bcd
	Maris Piper	63	0.25	0.01	222	0.19	0.29	bcde
	Charlotte	63	0.29	0.01	222	0.24	0.33	cde
	Maris Piper	78	0.31	0.01	222	0.27	0.35	e
	Charlotte	78	0.27	0.01	222	0.22	0.32	cde
	Maris Piper	92	0.30	0.01	222	0.26	0.34	cde
	Charlotte	92	0.26	0.01	222	0.21	0.31	bcde
	Maris Piper	105	0.17	0.01	222	0.07	0.23	ab
	Charlotte	105	0.07	0.01	222	-0.03	0.18	a
10 L	Maris Piper	36	0.22	0.01	222	0.16	0.27	a
	Charlotte	36	0.23	0.01	222	0.18	0.28	ab

	Maris Piper	43	0.32	0.01	222	0.29	0.36	cd
	Charlotte	43	0.32	0.01	222	0.28	0.35	cd
	Maris Piper	63	0.33	0.01	222	0.29	0.36	cd
	Charlotte	63	0.30	0.01	222	0.26	0.34	bcd
	Maris Piper	78	0.33	0.01	222	0.29	0.36	cd
	Charlotte	78	0.36	0.01	222	0.32	0.39	d
	Maris Piper	92	0.32	0.01	222	0.29	0.36	cd
	Charlotte	92	0.35	0.01	222	0.31	0.38	cd
	Maris Piper	105	0.30	0.01	222	0.25	0.33	abc
	Charlotte	105	0.24	0.01	222	0.19	0.29	ab
20 L	Maris Piper	36	0.24	0.01	222	0.18	0.28	a
	Charlotte	36	0.22	0.01	222	0.16	0.27	a
	Maris Piper	43	0.31	0.01	222	0.27	0.34	bc
	Charlotte	43	0.34	0.01	222	0.30	0.37	cde
	Maris Piper	63	0.38	0.01	222	0.35	0.41	e
	Charlotte	63	0.33	0.01	222	0.29	0.36	cd
	Maris Piper	78	0.34	0.01	222	0.31	0.38	cde
	Charlotte	78	0.35	0.01	222	0.32	0.38	cde
	Maris Piper	92	0.38	0.01	222	0.35	0.41	de
	Charlotte	92	0.34	0.01	222	0.30	0.37	cde
	Maris Piper	105	0.33	0.01	222	0.29	0.36	cd
	Charlotte	105	0.25	0.01	222	0.19	0.29	ab
40 L	Maris Piper	36	0.24	0.01	222	0.18	0.28	a
	Charlotte	36	0.24	0.01	222	0.18	0.29	a
	Maris Piper	43	0.34	0.01	222	0.31	0.37	bc
	Charlotte	43	0.37	0.01	222	0.33	0.41	c
	Maris Piper	63	0.38	0.01	222	0.35	0.41	c
	Charlotte	63	0.34	0.01	222	0.30	0.37	bc
	Maris Piper	78	0.35	0.01	222	0.31	0.38	c
	Charlotte	78	0.36	0.01	222	0.32	0.40	c
	Maris Piper	92	0.39	0.01	222	0.36	0.42	c
	Charlotte	92	0.35	0.01	222	0.31	0.38	bc
	Maris Piper	105	0.36	0.01	222	0.32	0.39	c
	Charlotte	105	0.28	0.01	222	0.23	0.32	ab

8.2 Chapter 4

8.2.1 Supplementary Figures



Supplementary Figure S1. Mean canopy temperatures of two potato cultivars, Maris Piper (top facets) and Charlotte (bottom facets), grown in two pot sizes, 5 (left facets) and 20 L (right facets), each under three different water treatments: watered to capacity every other day ($T_{1/2}$), daily (T_1), and twice daily (T_2). Plants were grown under an open-ended polytunnel between 1st June and 4th August 2023. Canopy temperature was measured between 27th June and 4th August. The different irrigation frequency treatments commenced on 3rd July 2023 (vertical dashed line). Means represent canopy temperature averaged across three canopy levels: top, middle, and bottom, and twenty-two sample dates (n = 110) ± 95% CIs. Means with different letters were significantly different by Tukey's test ($p < 0.05$).

8.2.2 Supplementary Tables

Supplementary Table S1. Mean fresh tuber yield, average tuber mass, tuber dry matter, fresh canopy biomass, canopy dry matter, digital biomass, canopy temperature, canopy SPAD values, average greenness, average hue, average NDVI, average PSRI, leaf angle, and light penetration depth of two cultivars of potato (Maris Piper and Charlotte), in two pot sizes (5 and 20 L), under three irrigation frequencies (every other day, $T_{1/2}$; daily, T_1; twice daily, T_2). Canopies were harvested and weighed on 4th August; tubers on 18th August 2023. Fresh tuber yield, average tuber mass, tuber dry matter, fresh canopy biomass, and canopy dry matter were measured manually. Canopy temperature and SPAD values were averaged across measurements sampled between 27th June and 4th August. The other variables were measured with two Phenospex PlantEye F500s on 13th July.

	Fresh Tuber Yield (g)				Average Tuber Mass (g)				Tuber Dry Matter (%)			
Treatment	Maris Piper		Charlotte		Maris Piper		Charlotte		Maris Piper		Charlotte	
	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L
$T_{1/2}$	338.0	1097.8	274.4	1010.6	28.4	44.3	29.4	42.2	22.4%	20.4%	18.6%	17.2%
T_1	308.6	1208.9	289.9	1109.6	31.0	48.0	34.9	42.5	21.4%	20.0%	18.0%	18.0%
T_2	295.2	1370.9	272.0	1099.4	28.6	53.5	32.0	42.7	21.8%	19.2%	17.6%	18.4%

	Fresh Canopy Biomass (g)				Canopy Dry Matter (%)				Digital Biomass (dm3)			
Treatment	Maris Piper		Charlotte		Maris Piper		Charlotte		Maris Piper		Charlotte	
	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L
$T_{1/2}$	162.9	923.3	124.3	809.2	11.2%	10.6%	9.2%	7.1%	35.9	210.1	23.2	213.1
T_1	210.8	1074.3	166.5	950.2	10.2%	11.2%	8.1%	6.6%	50.1	141.9	30.5	217.2
T_2	214.3	1061.4	177.6	929.6	10.0%	10.5%	7.7%	6.5%	56.6	231.4	33.3	199.4

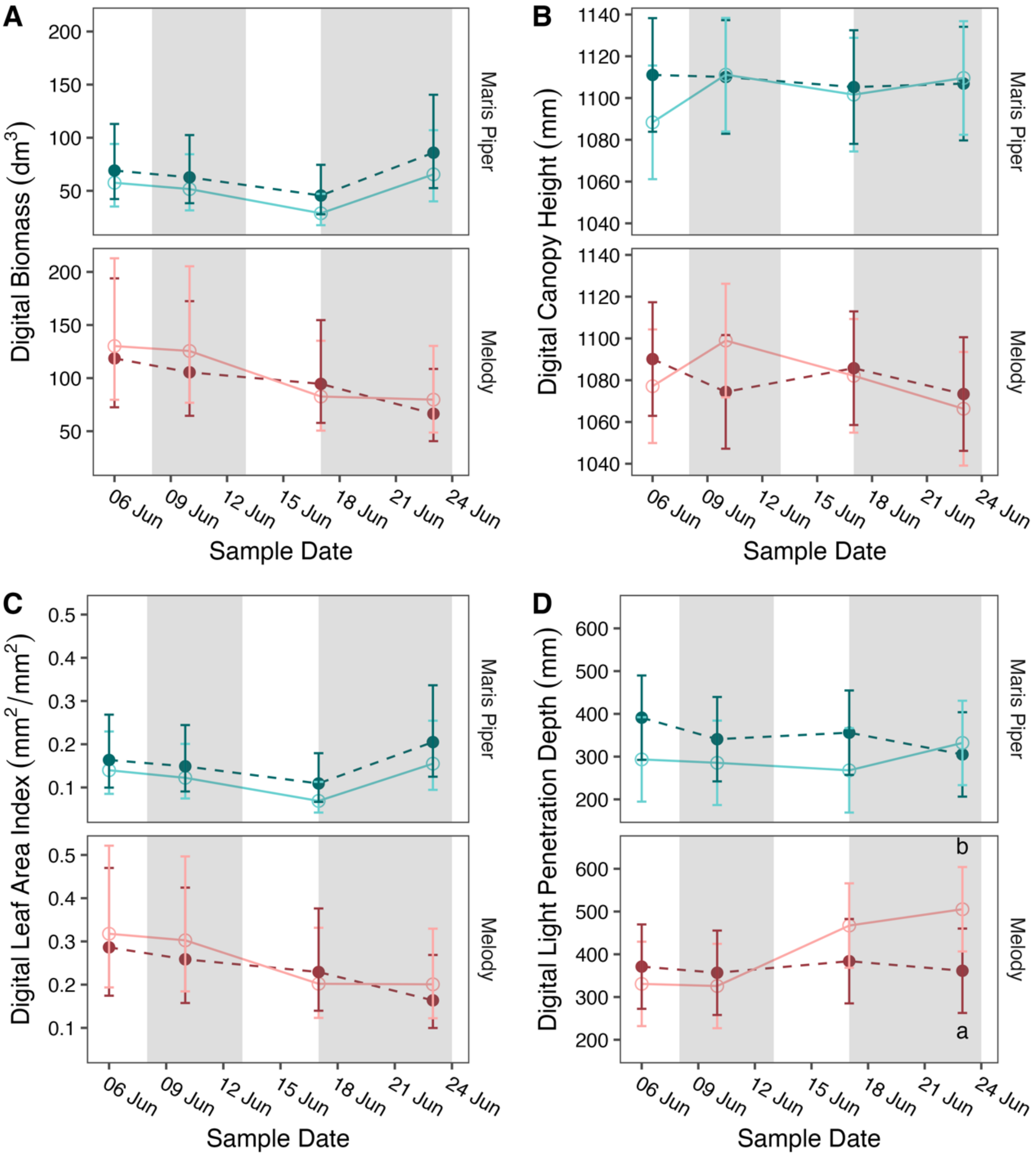
	Canopy Temperature (°C)				Canopy SPAD (SPAD Units)				Average Greenness (Index Units)			
Treatment	Maris Piper		Charlotte		Maris Piper		Charlotte		Maris Piper		Charlotte	
	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L
$T_{1/2}$	19.5	18.1	19.1	17.9	43.8	44.6	47.1	46.8	0.30	0.32	0.27	0.40
T_1	18.3	18.0	18.1	17.7	42.3	44.8	48.4	46.9	0.33	0.34	0.27	0.34
T_2	18.0	17.7	17.7	17.3	42.0	44.2	48.2	46.2	0.31	0.36	0.27	0.33

	Average Hue (Index Units)				Average NDVI (Index Units)				Average PSRI (Index Units)			
Treatment	Maris Piper		Charlotte		Maris Piper		Charlotte		Maris Piper		Charlotte	
	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L
$T_{1/2}$	118.76	114.02	125.05	114.96	0.69	0.71	0.69	0.76	0.00	0.02	-0.02	0.02
T_1	117.16	119.72	123.46	116.70	0.70	0.75	0.70	0.73	0.01	0.00	-0.01	0.01
T_2	117.01	113.54	120.96	116.43	0.69	0.73	0.69	0.72	0.01	0.02	0.00	0.01

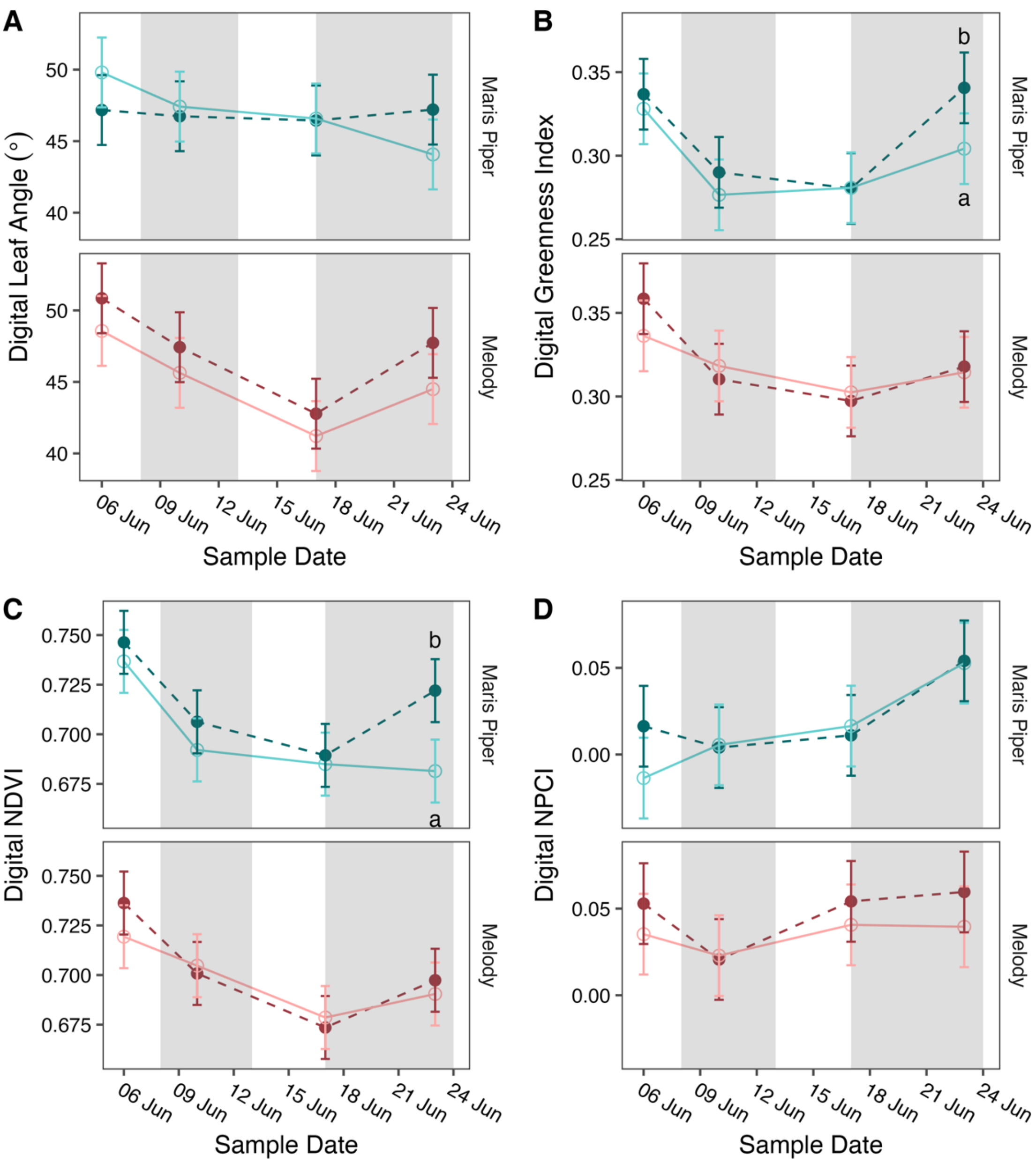
	Leaf Angle (°C)				Light Penetration Depth (mm)			
Treatment	Maris Piper		Charlotte		Maris Piper		Charlotte	
	5 L	20 L	5 L	20 L	5 L	20 L	5 L	20 L
$T_{1/2}$	40.20	41.40	41.47	43.69	197.89	290.45	121.49	381.20
T_1	42.32	41.62	41.70	44.37	191.61	247.47	122.32	281.59
T_2	41.91	41.08	42.66	41.28	264.54	346.65	162.76	331.63

8.3 Chapter 5

8.3.1 Supplementary Figures



Supplementary Figure S1. Means for digitally measured variables from the Phenospex PlantEye F500s of two potato cultivars (Maris Piper and Melody), grown in 148 L troughs under either well-watered or water-restricted conditions. Measurements were taken with the PlantEye on 6th, 10th, 17th, and 23rd June 2022. Means represent digital average greenness from three plants per trough (n = 3) ± CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test ($p < 0.05$). Letters denoting non-significant differences were removed for readability.



Supplementary Figure S2. Means for digitally measured variables from the Phenospex PlantEye F500s of two potato cultivars (Maris Piper and Melody), grown in 148 L troughs under either well-watered or water-restricted conditions. Measurements were taken with the PlantEye on 6th, 10th, 17th, and 23rd June 2022. Means represent digital average greenness from three plants per trough (n = 3) ± CIs. Means with different letters within each facet and sample date were significantly different by Tukey's test ($p < 0.05$). Letters denoting non-significant differences were removed for readability.

8.3.2 Supplementary Tables

Supplementary Table S1. Main effects and interactions terms of three-way ANOVAs for digitally measured variables from the Phenospex PlantEye F500s of two potato cultivars (Maris Piper and Melody), grown in 148 L troughs under either well-watered or water-restricted conditions. Measurements were taken with the PlantEye on 6th, 10th, 17th, and 23rd June 2022.

		Digital Biomass (dm3)			Height (mm)			LAI (mm2/mm2)			Light Penetration Depth (mm)		
Effect	DF	F	*p*	Sig.	F	*p*	Sig.	F	*p*	Sig.	F	*p*	Sig.
Treatment (T)	8	0.39	0.551		0.11	0.747		0.30	0.602		0.04	0.846	
Cultivar (C)	8	11.68	0.009	**	9.12	0.017	*	11.37	0.010	**	3.33	0.105	
Sample Date (SD)	32	32.43	0.000	***	0.44	0.723		4.09	0.018	*	1.60	0.215	
T x C	8	1.05	0.336		0.13	0.727		1.06	0.333		1.62	0.239	
T x SD	32	0.41	0.799		1.06	0.385		0.49	0.691		3.73	0.025	*
C x SD	32	5.23	0.002	**	0.65	0.591		4.24	0.015	*	2.55	0.079	
T x C x SD	32	0.29	0.883		0.34	0.798		0.06	0.981		0.87	0.472	

		Leaf Angle (°)			Average Greenness			Average NDVI			Average NPCI		
Effect	DF	F	*p*	Sig.	F	*p*	Sig.	F	*p*	Sig.	F	*p*	Sig.
Treatment (T)	8	1.86	0.210		1.72	0.226		5.26	0.051		1.83	0.213	
Cultivar (C)	8	1.15	0.315		4.78	0.060		2.54	0.150		10.93	0.011	*
Sample Date (SD)	32	15.36	0.000	***	24.30	0.000	***	37.52	0.000	***	9.42	0.000	***
T x C	8	2.13	0.183		0.72	0.422		2.18	0.178		0.21	0.661	
T x SD	32	2.00	0.140		1.39	0.270		2.07	0.131		1.08	0.377	
C x SD	32	6.12	0.003	**	2.98	0.052		1.10	0.370		3.73	0.025	*
T x C x SD	32	0.94	0.435		1.26	0.309		1.38	0.272		0.53	0.669	

Significant *p*-values are indicated at the following levels: $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***.