University of Reading

‘Apples in a Warmer World’®: UK Apple Productivity, Fruit Quality, and Climate Change.

For the Degree of Doctor of Philosophy

School of Agriculture, Policy, and Development

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Declaration

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

Adam John Peter

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Abstract

Future climate change will change the UK's top fruit production environment further. The impact of three modified temperature environments (Ambient, +2°C, and +4°C) on annual apple (*Malus domestica* Borkh.) production was investigated within a purpose-built field research facility in Kent. Fruit production showed bienniality: high in 2017, -19, -21; low in 2018, -20, -22, which was greatest in 'Fuji'. Analysis of data from fruit production over six years (2017-22) revealed unique temperature production responses across a genetically-diverse pool of 20 apple cultivars. A sequence of events triggered by seasonal temperature variables (Tmean, Tmax, and Tminmaxdiff) and crop load were primarily responsible for variation in yield and fruit quality. Temperature variables and crop load were negatively associated with floral bud production ($p<0.05$) in the subsequent season, which enhanced alternate bearing in the two warmer environments, causing an overall reduction in mean fruit yield across many cultivars ($p<0.05$). Fruit yield and fruit number per tree, sunlight, and precipitation were subsequently identified to affect fruit quality (firmness, soluble solids content (SSC), red colour coverage (RCC), dry matter content (DMC), and fruit weight). Warmer temperature environments had a positive effect on SSC and DMC, a negative effect on RCC, and a mixed effect on firmness ($p<0.05$) across most cultivars. These alterations in fruit quality had a minor effect on the subsequent storability of 'Gala' fruit. Differences in firmness and SSC were identified ($p<0.05$) amongst different treatments. However, reductions in RCC ($p<0.05$) substantially reduced the marketability of fruit from warmer environments. Warmer temperatures will influence many aspects of UK apple production, and cultivar selection will be key in mitigating negative effects of increased seasonal temperature. Crop management practices will also need to adapt to enhance resilience against lower winter chill, earlier fruit development, increased tree vegetative growth, and increased pest prevalence.

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Chapter 1: General Introduction

1.1 The Apple and its genetic diversity

The origins of the cultivated apple (*Malus domestica* Borkh.) have been traced back to its original Rosaceae wild ancestor (*Malus sieversii* (Ledeb.) M.Roem.) in central Asia (Harris et al., 2002). Seeds were likely dispersed by local megafauna in a mutualistic relationship spanning back to the Eocene, leading to diverse genetic clades and genotype hybridisation (Spengler, 2019). Early trans-Eurasian trading 2,000 years ago initiated the exchange of ancient *Malus* cultivars. Direct breeding, grafting techniques, and further hybridisation of these genomes accelerated the process of domestication for desired traits (Spengler, 2019). These traits have been optimised for their specific apple end use, which primarily includes dessert (for direct consumption), culinary (for cooking), cider (for cider production), and ornamental (no palatable fruits produced) (Morgan, 2013).

To date, roughly 7,500 *M. domestica* genotypes are documented (Elzebroek, 2008). Widespread diversity of cultivars presents a set of highly heterozygous *M. domestica* cultivars (Velasco et al., 2010). Whole genome sequencing advances over the past 15 years have aided understanding of genome and phenotype relationships, which is now being applied to modern breeding techniques (Velasco et al., 2010; Peace et al., 2019).

Commercial apple orchards typically graft scions of a cultivar on to rootstocks. Rootstocks aid early tree growth, regulate tree vigour and provide stress resistance (Marini and Fazio, 2018). In the early 1900's, a rootstock breeding programme in East Malling, Kent, produced a series of rootstocks (M1-16) that still form the basis of modern rootstock development today (Wang et al., 2019). M9, a dwarfing rootstock, is one of the most popular choices for commercial growers worldwide.

1.2 Apple fruit production: Overview

Apples are primarily produced in temperate climates in both northern and southern hemispheres. In production terms, it is estimated ~143 million tonnes of apples were produced worldwide in 2022 (FAOSTAT, 2023). China contributed the most, representing ~33% of total worldwide production. Numerous countries produce upwards of one million tonnes per annum, including several European nations (Poland, Russia, Italy, France, Ukraine, and Germany). The UK produced a comparatively smaller 556,000 t in 2022 (FAOSTAT, 2023).

Apples produced in the UK are primarily consumed within the UK. Only ~18,000 t (3%) were exported in 2019 (FAOSTAT, 2023). UK sales markets are dominated by year-round retailer sales. It is estimated that 80% of all fresh apples (including imports) are sold directly to supermarkets (BAP, 2021). This has seen a shift away from wholesale markets of the 1970s, as ‘in-house’ apple processing and packaging provided higher profit margins for retailers (Starkey and Carberry-Long, 1995). Supermarkets require a constant supply of fresh apples. Consequently, the UK is one of the largest importers of apple fruit in the world. Around 332,000 tonnes of fruit were imported in 2022 (DEFRA, 2023). This has created high levels of competition between domestic and imported produce (Axelson and Axelson, 2000; AHDB, 2021). Prices often remain relatively low, pressuring growers to produce abundant quantities of high-quality fruit to remain competitive. Closely coupled relationships between grower and supermarkets heavily favour large-scale suppliers and often exclude small-scale enterprises (Frances and Garnsey, 1996). Niche markets exist that can favour small to medium sized businesses, such as producing uncommon cultivars or selling direct to specialist small scale retailers.

Apple production in the UK consists of a mix of dessert, culinary and cider apple cultivars. Over recent years, growers have shifted towards producing a greater proportion of dessert cultivars. In 2014, dessert apple production represented 15.6% of total England and Wales orchard production (DEFRA, 2023). Some years later by 2021, this figure had increased to 19.2%. A handful of late-season bi-coloured cultivars dominate total production. However, production data from 2022 showed that dessert apple production decreased for the first time in 16 years. This has been attributed to primarily rising production costs and insufficient producer returns from retailers (The Grocer, 2023).

The cultivar ‘Cox’s Orange Pippin’, formerly the most popular dessert choice for growers, has been overtaken by ‘Gala’ and ‘Braeburn’ production over the last 20 years. Together these three cultivars (and their sports) occupy ~72% of all dessert apple production (DEFRA, 2023). These cultivars provide reliable yields, good long-term storage prospects, and are popular with consumers year-on-year. The remaining ~28% of domestic production is shared between various early-, mid-, and late-season cultivars (DEFRA, 2023). In recent years, the production of ‘club’ cultivars has increased, the most favoured of these being ‘Jazz’. Diversity in culinary cultivar sales is more limited - ‘Bramley’s Seedling’ is often the only choice available in UK supermarkets.

1.3 Apple fruit production: Basic agronomic principles for optimising yield and fruit quality

Generally, orchard profitability in UK production is driven by maximising the efficiency of producing abundant, high-quality fruit on an annual basis (AHDB, 2021; Tijero et al., 2021). Agronomic principles for achieving this depend on a wide range of external influences. Growers and agronomists must consider appropriate selection of planting system, cultivar, rootstock, and tree management strategies within a given geographic environment (Tijero et al., 2021).

Commercial apple cropping systems today are typically at a high tree planting density using dwarfing rootstocks to maximise light interception for high quality, uniform fruit (Robinson, 2008; Lordan et al., 2018). In high density planting systems, conical tree shapes are the most optimal for commercially important cultivars such as ‘Gala’ and ‘Fuji’ (Lordan et al., 2018). Tree architecture can be manipulated through pruning and thinning techniques. Removal of excess vegetative growth ‘little and often’ (rather than too much at one time) is beneficial for fruit growth and maintaining tree architecture (Lauri et al., 2002).

Key tree phenological events occur across the annual cycle, as is common amongst deciduous perennial tree fruit. Pome fruit have nine principal growth stages during seasonal development; bud development, leaf development, shoot development, inflorescence emergence, flowering, fruit development, fruit maturity, and senescence (Meier et al., 1994). These events are strongly associated with the environmental growing conditions, particularly temperature (Darbyshire et al., 2017). Crop management practices therefore require consideration of each tree phenological phase to ensure adequate cropping each production season.

Adequate crop protection is crucial for obtaining plentiful high-quality fruit. Apple cropping systems are vulnerable to a wide range of pests, disease, and weeds, varying in susceptibility by cultivar (Petkovsek et al., 2007). Integrated pest management (IPM) strategies have advanced over the past 40 years to provide a multidisciplinary, ecological approach to the management of pest populations (Blommers, 1994; Damos et al., 2015). Advances in plant breeding and increased understanding of how disease spreads in apple cropping systems have helped to mitigate disease incidence over recent years (Robinson, 2011; Luo et al., 2020).

1.4 Apple fruit quality and its importance

Regular oversupply within the worldwide fruit industry demands growers produce high quality fruit to remain competitive. ‘Fruit quality’ is a subjective term applied when evaluating produce. Apple quality is typically associated with intrinsic characteristics (physical and sensory) that lead a consumer to be satisfied with the product (Harker et al., 2003). Extrinsic properties (such as branding, packaging etc.) can also have a perceived impact on food quality from a consumer point of view (Ardeshiri and Rose, 2018).

Fruit quality attributes are determined by a mix of genetic, agronomic, and environmental factors (Musacchi and Serra, 2018). Genetic and agronomic factors can be managed effectively by growers, whereas environmental influences are largely driven by weather parameters such as temperature, light radiation, rainfall and humidity. Intrinsic fruit quality attributes for apples can be broadly represented by two categories: external (or ‘appearance’) and internal. External qualities include size, shape, colour and russeting. Internal qualities include (but are not limited to) texture (or ‘firmness’), starch content, soluble solids content (SSC), acidity, relative chlorophyll content, and dry matter (Musacchi and Serra, 2018).

Large genotypic diversity causes intrinsic quality trait variability among apple cultivars, making it objectively infeasible to state optimal quality for production. Specific marketing standards for apples (EU, No. 543/2011) aid in quantifying minimum requirements for certain cultivars, classifying produce into marketable classifications of Class I (‘good quality’), Class II (slight defects), or waste (unmarketable). These commercial standards primarily assess external qualities: size, structural integrity, and colour. This is satisfactory towards driving consumer purchasing, as external appearance is linked with a decrease in quality perception (Jaeger et al., 2018). However, there are few classifications that consider the internal qualities that contribute towards taste. A comprehensive review by Musacchi and Serra (2018) concluded a research gap was present in characterising ‘high’ fruit quality standards worldwide for every apple cultivar – particularly across the organoleptic and nutritional characteristics where few guidelines currently exist. Such factors should be important to growers and retailers. Evidence shows that improved organoleptic experience (e.g. ‘pleasant’ tasting) increases customer willingness to pay (Seppä et al. 2015) and nutritional value of apples is linked with numerous consumer health benefits (Goldberg, 2008).

1.5 Temperature effects on apple production

Perennial tree crops are cultivated in field environments and have commercial lifespans of 10+ years. Long lifespans subject trees to a wide range of biotic and abiotic factors that can affect crop development, growth, and yield. Exposure to certain environmental conditions throughout cultivation can produce a stress response that may influence aspects of crop production. Apple cultivation is typically well suited to temperate environments. However, suitable management strategies (such as appropriate cultivar and rootstock selection) can help mitigate the effect of certain stress-inducing environmental factors (Webster and Wertheim, 2003).

Temperature is the most important influence on the spatial distribution of plant species (Parker, 1963). As such, open environment temperatures can elicit a wide range of fruit tree responses throughout the annual life cycle, from spring bud burst to winter dormancy of new buds. Temperature has a direct influence on many physiological processes which can affect growth, development, and yield within a production season. While extreme temperatures can cause direct damage, fluctuations in non-extreme temperatures still influence the rates of respiration, photosynthesis, and transpiration of apple trees (Landsberg and Jones, 1981). Field studies have shown that increased seasonal temperature (i.e. the temperature during active fruit development) is associated with increased fruit growth (Warrington et al., 1999), but reduced fruit retention and yield (Atkinson et al., 1998) dependent on the cultivar. Additionally, warmer weather is associated with increased tree shoot growth for the cultivar ‘Fuji’ (Kweon et al., 2013).

Low temperatures control dormancy induction in autumn (Faust et al., 1997; Heide and Prestrud, 2005). Winter temperatures influence the subsequent season’s bud break (Naor et al., 2003) through accumulation of winter chill units. Winter chill requirements for apple can range anywhere from 400 to 2900 hours below 6°C (Hauagge, 2007; Hawerroth et al., 2013). Insufficient chill units accumulated during warmer winters can deepen dormancy (Cook et al., 2017) and cause irregular and late bud break and flowering (Powell, 1985). Delayed dormancy through insufficient winter chill has also been shown to decrease yields and fruit quality in perennial fruit crops (Saure, 2011; Atkinson et al., 2013). As this issue presents multiple knock-on effects, management strategies in many temperate regions aim to prevent prolonged dormancy. This includes the introduction of dormancy breaking chemicals.

Freezing temperatures have a wide range of impacts throughout the production season. Freezing and frost events are thought to be the single biggest abiotic cause of loss across all horticultural crops (Rieger, 1989). Freezing temperatures have a direct impact on multiple plant organs. The scale of impact is dependent on the timing of the frost event, and the stage of development at which the organ is at. Overwintering organs become increasingly more frost-sensitive in the run-up to flowering (Szalay et al., 2019). Prolonged soil frosts can reduce water uptake in spring, causing delayed tree growth and development, as well as xylem damage and dieback (Beikircher et al., 2016). This evidence highlights that the extent of damage from freezing temperatures is highly dependent on timing. A review by Vitasse et al. (2014) concluded that the overall risk of freeze injuries to temperate trees is ‘low’ and confined to just spring as the trees exit winter dormancy. Frost protection solutions are utilised to mitigate frost damage during this narrow window, including chemical growth regulators, sprinkler irrigation systems and wind machine operations.

1.6 Future climate change predictions

The Intergovernmental Panel on Climate Change (IPCC) is a major international consortium that regularly assesses the scientific understanding of climate change impacts and future predictions. Assessments have concluded unequivocally that warming of the global climate due to human activity has occurred over the past 70 years and is predicted to continue throughout the 21st century (IPCC, 2022). Average global surface temperature increased by 1.1°C between 1880 and 2020 (IPCC, 2023). Warming temperature trends between 1980 to 2008 generally exceeded one standard deviation of historic year to year variability (Lobell et al., 2011). Increased land surface temperatures, warmer oceans, higher sea levels, and a reducing cryosphere are being driven primarily by high atmospheric greenhouse gas presence. Future climate change scenarios (across both a global and regional scale) produce simulated climate predictions based on low to high confidence intervals. Global surface temperature is likely to increase by an average of 2°C above the 1850-1900 mean by 2100, exhibiting non-uniform variability between decades (IPCC, 2023). Future changes in precipitation have high confidence scenarios based on latitudinal location. It is likely that many high latitudinal locations will witness an increase in annual mean precipitation, whereas many mid-latitudinal locations will likely witness a decrease (IPCC, 2014). Extreme precipitation events will also likely increase in occurrence and severity.

Future climate change will have a profound impact on weather patterns in the UK. UKCP18 climate projections predict greater interannual mean temperature variability (Kennedy-Asser et al., 2021). All UK regions will likely see an increase in extreme weather event occurrences, including drought (Burke et al., 2010) and intense precipitation (Madsen et al., 2014). By current UK heatwave definitions (which vary by region), heatwaves will increase in frequency and by range occurring throughout May to September (Sanderson and Ford, 2016). Whilst all UK regions will see warmer temperatures, Southern regions will see greater increases compared to Northern (Kennedy-Asser et al., 2022).

1.7 Climate change impacts on global crop productivity

Future climate change impacts on agriculture will be severe and have a great influence on food production and security (Mahato, 2014). It represents a credible threat to sustaining global crop productivity at rates necessary to keep up with demand (Lotze-Campden, 2011; Lobell and Gourdji, 2012). There will likely be large disparities in crop climate change impacts, but heat stress will adversely affect most production regions (Deryng et al., 2014). Warming over the past 50 years is thought to have already reduced productivity of many staple food crops across Europe, Africa, and Australia (Ray et al., 2019). Crop yields of main arable crops (e.g. wheat) have generally decreased since 1980 which is attributed to warmer weather – offsetting yield gains attained from technological advancement (Lobell et al., 2011). A further 1-3°C average annual temperature increase is projected to reduce global crop yields by 3-12% by 2050 (Knox et al., 2012; Wing et al., 2021), and 11-15% by 2100 (Wing et al., 2021). Crops will likely be affected directly (e.g. altered physiology) and indirectly (e.g. altered environments). For example, crops planted in the UK will see increased plant evapotranspiration, which will in turn affect soil water availability (Watts et al., 2015). Whilst long-term projections will likely have negative impacts on crop productivity, certain scenarios may provide more favourable conditions. For example, earlier maturing wheat influenced by warmer environments may avoid the peak of summer heat and drought stress (Semenov, 2007).

1.8 Climate change impacts on perennial tree crops

The phenological life cycle of established perennial crops compared to annual crops presents unique challenges in response to anticipating future climate change impacts. This is especially true when the value of perennial horticultural crops is derived not only from the quantity, but also the quality of the harvested product (Glenn et al., 2013). Despite the low flexibility of woody crops (i.e. the time taken to establish orchards), it is predicted that perennial cropping systems will have greater resilience to future climate change compared to annual systems (Medda et al., 2022). Positive effects on tree growth and development are expected from higher CO_2 concentrations (Maracchi et al., 2005; Glenn et al., 2013; Medda et al., 2022). As a result, yields across some temperate regions in Northern Europe may increase (Olsen et al., 2011). Furthermore, it is predicted that perennial cropping systems will play a useful role in climate change mitigation strategies by serving as carbon sinks (Malhotra, 2017; Ledo et al., 2020).

However, a wide range of negative impacts are predicted across a wide spectrum of perennial crops, to the extent that they will likely outweigh the positive effects (Glenn et al., 2013; Medda et al., 2022). Warmer temperatures are linked with negative effects on tree phenology, physiological processes, and with a greater presence of pests and disease (Glenn et al., 2013; Rai et al., 2015; Medda et al., 2022). Greater frequency of extreme weather events (such as heatwaves and drought) will increase the incidence of crop heat stress and depleted soil water availability, and so will negatively affect tree growth and development (Maracchi et al., 2005; Oleson et al., 2011; Malhotra, 2017). Warmer seasonal temperature will shorten growing periods which will reduce fruit yields (Malhotra, 2017). A recent study by Meza et al. (2023) concluded expected future land suitability for global perennial crop production. Depending on the climate change scenario, substantial restructuring of global production may be required; Northern hemisphere perennial crop regions will generally see increased land suitability, whereas Southern hemisphere will see less due to lack of suitable land to migrate towards. With regards to fruit quality, perennial fruits and vegetables will have altered quality attributes in response to temperature and CO_2 changes, with post-harvest quality generally reduced (Mattos et al., 2014).

1.9 Climate change impacts on apple production

The direct impact of various climate change scenarios on long-term apple production is relatively unknown at a field scale in comparison to annual crops. Long-term climate change responses cannot be obtained from studies of short-term effects (De Boeck, 2015). In addition, apple trees cultivated within irrigated pots and placed under polythene or glass are subject to higher levels of drought stress compared to field environment substrates (Treder et al., 1996). The setup and maintenance of environment response studies on the apple crop therefore have high time and financial costs.

Warmer production season months will likely have a profound impact on advancing phenology. Studies on apple phenology and climate change typically focus on analysing temperature and fruit production associations based on historic data. For example, data from the National Fruit Collections in Brogdale, Kent, has shown that a 1.5°C increase in mean temperature at this site has advanced apple flowering date by 18 days over the past 50 years (Hadley, personal comm.). A recent study by Kunz and Blanke (2022) concluded several findings regarding apple phenology and temperature. Seasonal temperature increases of 1.7°C were correlated with advancing flowering by 11-14 days, fruit maturation by 4-12 days (depending heavily on cultivar), and leaf canopy duration by 6-10 days. Similar flowering observations have been made elsewhere in Europe, including in Romania where flowering has advanced by ~14 days over the past 50 years (Chitu and Palinaenu, 2020). In the Southern hemisphere, advanced full bloom over the past 40-50 years has also been noted in South African apple production regions (Grab and Craparo, 2011). Other studies report seasonal phenological events from bud break to leaf fall are modelled to occur earlier in locations all around the world (Reivero et al., 2016; Cho et al., 2020; El Yaacoubi et al., 2020). A major concern for growers in temperate climates is the effect of late-seasonal frosts on earlier-flowering trees, especially for early-flowering cultivars with poor frost tolerance (Szalay et al., 2019). However, various studies downplay the overall effect of frost events, claiming it is feasible that frost damage will remain the same as present day levels despite accelerated phenology (Eccel et al., 2009; Pfleiderer et al., 2019).

Evolution of winter dormancy mechanisms in apple (and many other temperate plants) enables tolerance of low temperature stress throughout winter (Horvath et al., 2003). Accumulation of winter chill (i.e. the amount of time below a certain temperature threshold) enables release from plant dormancy, which optimises reproductive development and subsequent crop yield in apple (Saure, 2011; Atkinson et al., 2013). Insufficient chill accumulation during mild winters results in altered budbreak and flowering phenology (Petri and Leite, 2003) which subsequently negatively influences fruit yield, floral initiation, fruit quality attributes, and disease resistance (Atkinson et al., 2013; Rai et al., 2015). Based on future climate change predictions, studies have determined negative influences of reduced winter chill in regions such as the UK (Else and Atkinson, 2010), the Mediterranean (Funes et al., 2016), and Iran (Ahmadi et al., 2019). Winter chill reduction has already impacted apple production in Northern India over the past few decades – production has had to relocate to higher altitudes to ensure an abundant, good quality crop each year (Basannagari and Kala, 2013; Pramanick et al., 2015). Chilling requirements are species- and cultivar- specific (Samish, 1954), and therefore insufficient winter chill effects can be mitigated through appropriate cultivar selection.

The effects of increased occurrence of extreme climate events, such as heatwaves and drought, will likely invoke physiological responses in apple trees depending on the timing (Bindi and Oleson, 2011; Rai et al., 2015). High temperatures and drought will also increase damage to apple fruit (Rai et al., 2015). Mild water stress in summer can influence vegetative growth and dormancy in the following season (Fernandez et al., 2020). Precipitation differences during bud-break and flowering can also influence later stages of fruit development (Cho et al., 2020).

Less is known about the direct effect of climate change induced warmer seasonal temperatures on field-scale apple production. Suguira et al. (2013) commented on how taste and textural apple attributes in Japan have likely changed over the past 40 years of warming. Whilst field studies have measured the effects of varied temperature treatments on apple production (for example Atkinson et al., 1998), the effects of future climate change scenarios on apple fruit yields and quality are relatively unknown.

1.10 The National Fruit Collection Trust's (NFCT) 'Apples in a Warmer World'® project

The NFCT aims to inform and educate the public about work undertaken within the National Fruit Collections (NFC), based at Brogdale, Kent, United Kingdom. The NFC, owned by DEFRA (Department for Environment, Food, and Rural Affairs) and curated by University of Reading, hosts a living collection of over 3,500 fruit tree cultivars. The NFC and NFCT help to develop understanding of fruit genetic diversity by describing traits that are beneficial now and into the future. Between 2011 and 2022, the Trust developed and oversaw the 'Apples in a Warmer World'® project. This long-term project investigated the effects of climate change (specifically warmer temperature and variation in rainfall) on diverse apple cultivars using a unique experimental field system. Across an original scope of ten production seasons (to replicate commercial systems), the project aimed to better understand climate effects of phenology, growth, yield, and quality of apples in a UK context. The main aim was to aid growers, both commercial and amateur, in identifying which genetic traits are most resilient to future climate change impacts on apple production. Unfortunately, due to circumstances beyond the NFCT's control (see Chapter 2), the project fieldwork was concluded in 2022, several years earlier than planned.

1.11 Project findings from Lane (2022)

The first three years of the 'Apples in a Warmer World'® investigation demonstrated several conclusions based on the first three years' worth of production data (Lane, 2022). The main findings were as follows:

- The temperature treatments affected seasonal development of apple across every cultivar (i.e. advanced phenology). Sensitivity to temperature varied by developmental phase and cultivar. For example in 2019, 'Gala' phenological development occurred earlier at every measured interval in Plus4 conditions compared to Ambient; bud burst by four days, full flowering date by nine days, and harvest date by 22 days.

- The temperature treatments affected net photosynthetic rate, with photosynthetic rate declining within warmer environments. In 2018, a 2.2°C increase in mean June-August temperature reduced net photosynthetic activity (A_{max}) by 3-4 µmol CO_2^{-2} s^{-1}. Cultivars differed quantitatively, with differences between the early-, mid-, and late-season cultivars.
- Yield parameters (total fresh weight per tree, total fruit number, and fruit weight) were often affected by mean temperature during the fruit development period, although cultivars differed in that response. There were yearly variations in yield parameters, with evidence of large variation among years present within many cultivars.
- Tree vegetative growth parameters (trunk growth, shoot extension, and pruning weight) were positively associated with an increase in temperature, with sensitivity varying between cultivars. There was also considerable variation between years (2018 and 2019) in this regard. In 2018, Mean annual tree trunk growth increased from 2.2cm (Ambient) to 3.2cm (Plus4). In comparison, 2019 tree trunk growth did not differ between temperature treatments.
- Rainfall variation had limited effects on apple development and yield parameters. Drought treatments had a slight effect on net photosynthetic rate and vegetative growth.

Based on just two years of modified production environment data (plus one ‘baseline’ year), it was clear that conclusions on long-term environmental effects on production variables (such as fruit yield and quality) could not be determined. Therefore, the experimental data compiled for this thesis continued many of the experimental outputs performed by Lane (2022) to better understand these longer-term environmental effects on apple. Additionally, it was important to expand the range of experimental outputs. For example, the use of controlled atmosphere storage is of substantial importance to the UK apple industry. Therefore, increased experimental scope increased commercial relevance of the results obtained.

1.12 Hypotheses

The overall hypotheses to be evaluated within this study partly form a continuation of work started in Lane (2022), but with some additional cultivars included. These hypotheses are listed below:

***H_1* – Changes in seasonal temperature will alter the fruit yield and quality of a range of diverse apple cultivars.**

***H_0* – Changes in seasonal temperature will have no effect on the fruit yield and quality of a range of diverse apple cultivars.**

***H_1* – The effects of changes in seasonal temperature on fruit yield and quality differ between a diverse range of apple cultivars.**

***H_0* – The effects of changes in seasonal temperature on fruit yield and quality do not differ amongst a diverse range of apple cultivars.**

Subsequent experimental hypotheses are outlined in each individual chapter covering dependant variables relating to fruit yield (Chapters 3 and 4) and fruit quality (Chapters 5 and 6). The full data set from 2017-2022, i.e., including results from Lane (2022), was studied to test several of these hypotheses.

Chapter 2: Materials and Methods

2.1 Continuation of the long-term experiment

This thesis forms a continuation of research within the National Fruit Collections Trust's (NFCT) 'Apples in a Warmer World'® long-term research project analysing the effects of modified field environment regimes on UK apple production. As such, much of the methodology replicates and experimental work continues work described in Lane (2022). Relevant materials and methods for the current study are reported here. For information on the initial setup, troubleshooting, and validation of environmental modifications (temperature and rainfall), please refer to Lane (2022).

2.2 The Experimental System

2.2.1 Facilities

The experimental facility, completed in May 2017 and decommissioned in November 2022, was based at a 0.7 hectare site at Brogdale, Kent, UK (51.296107, 0.881629). The facility consisted of three triple-span tunnels where apple trees were cultivated in the natural soil (soil type = clay with flint) (Figure 2.1). The polytunnel structure (HayGrove Ltd., Ledbury, UK) was covered by 200μm Lumisol diffuse plastic (British Polythene Industries Ltd., Rushden, UK) to enable modified temperature regimes with high ambient light transmission of 69.9% (with no significant differences between treatments).

Use of these triple span tunnels allowed the manipulation and regulation of climatic conditions. Trees were cultivated under nine different climatic treatments; a combination of three unique temperature and three rainfall regimes based on possible climate change scenarios (Figure 2.2). Each of the triple-span polytunnels was regulated by ventilation to provide a unique temperature regime utilising solar radiation to warm the tunnels; ambient (replicating outside temperature), +2°C [nominal] and +4°C [nominal]. These temperature uplifts were maximum uplifts in the early years of the investigation, but later on the maxima were increased (see below) but the treatments are referred to as +2°C and +4°C throughout. A TomTech T100 monitoring system (TomTech Ltd., Derby, UK) manipulated tunnel temperature through altering tunnel vent position in response to temperature logs. The treatment differences were an average compared to ambient conditions, with deviations of ~1-2°C.

Each span of the triple-span tunnel provided one of three rainfall regimes: 100% (replicating outside), 80% (simulating drier conditions) and 120% (simulating wetter conditions). The position of each rainfall regime was the same in each triple-span polytunnel; 80% in the west, 100% in the centre and 120% in the east (Figure 2.2). Rainwater was collected via guttering and re-distributed through overhead sprinklers above the trees. Irrigation was monitored and controlled by a Mi-4 Heron controller (Heron Electric Company Ltd., Littlehampton, UK).

Polytunnel fabric covered all sides of the +2°C (Plus2) and +4°C (Plus4) tunnels, whereas in the ambient tunnel the sides were normally left open (closed during rainfall events) to avoid warming. This means that the Plus2 and Plus4 tunnels were (loosely speaking) more ‘closed’ systems compared to ambient. This presented differences in exposure to certain abiotic (e.g. wind) and biotic (e.g. insect pollinators) factors.

In 2020, an additional weather recording system (Metos, Pessl Instruments GmBH, Peterborough, UK) was installed within the facility, with stations placed within each individual tunnel span (nine in total). Whereas the TomTech system provided one reading per triple-span tunnel, these instruments indicated environmental differences at three different locations (north, middle, and south). Monitoring of the facility occurred on a regular basis, with any faults reported and dealt with in a timely manner.

No further additional hardware was installed within the facility beyond Lane’s (2022) initial study. Section 7.1 put forward suggestions for future improvements to the facility. These included the use of thermic polythene film or artificial heating to increase winter temperature uplifts in warmer treatments, and free-air CO_2 enrichment (FACE) systems to evaluate the effects of elevated CO_2 concentrations.

Figure 2.1. *Aerial view (from the south) of the temperature treatment polytunnels; Plus2 (left), Plus4 (centre), and Ambient (right).*

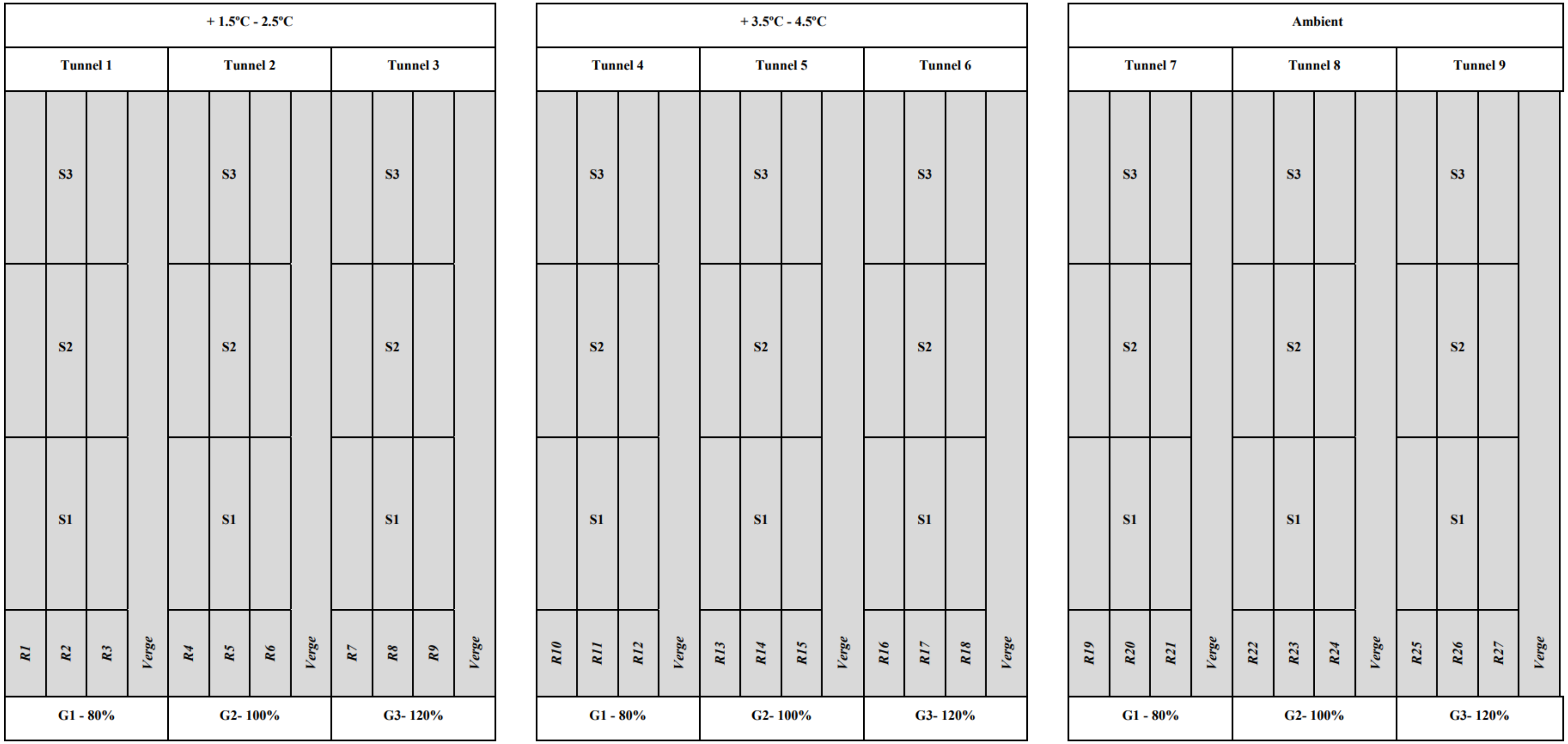


Figure 2.2. *Plan of the layout and treatments design of the 'Apples in a Warmer World'® tunnels from Lane (2022), showing regimes (°C), tunnel numbers (T1-9), pseudo blocks (S1-3), tree rows (R1-27), grassed verge areas, and rainfall treatments (80, 100, or 120%) in irrigation timing groups (G1-3). The overall polytunnel site area was 66m x 84m.*

2.2.2 Trees and cultivars

A total of 21 different apple cultivars (Table 2.1) were incorporated within the trial during the project. These were selected for their commercial importance, seasonality, or for a specific phenotypic trait. Budwood for all trees was cultivated identically on site based within the National Fruit Collections' nursery. Scion budwood was grafted on Malling 9 (M9) rootstock with a 'Golden Delicious' interstock. The M9 rootstock was chosen for its commercial importance and dwarfing nature, with an interstock used to aid successful scion grafting. Once grafted, the trees were left to propagate for two years before planting in the orchard. The trees were planted at 1.5m x 1.5m spacing and planted alongside a 2m wooden stake to aid leader shoot growth. Metal wiring surrounded each stake to prevent damage from herbivorous pests. Across each modified environment, trees were planted across three staggered rows with a grass verge separating each rainfall regime within each polytunnel.

Table 2.1*. All cultivars incorporated in the Apples in a Warmer World climate change investigation, highlighting identification, pollination group (1-7), harvest seasonality (-Early, -Mid, or -Late), use (Dessert or Culinary), genetic trait (reasoning for selection). Most cultivars were planted out in 2014 (those with data for 2017-22) but five were established later.*

Cultivar	Accession Number	Pol. Group	Season	Use	Genetic Trait	Trees Planted	Years w/ data
Braeburn	1964-033	4	L	D	Commercial	2016	2019-22
Beverly Hills	1974-357	4	E	D	Low chill	2018-19	2020-22
Bramley's Seedling (LA)(3n)	1974-341	3	L	C	Commercial	2014	2017-22
Cox's Orange Pippin (LA)	2000-008	7	L	D	Fruiting mid	2014	2017-22
Discovery (EMLA 1)	1973-189	6	E	D	Fruiting early	2014	2017-22
Edward VII	1921-015	1	L	C	Flower late	2014	2017-22
Fuji	1963-019	4	L	D	Standards	2014	2017-22
Gala (LA 69A)	1976-144	6	L	D	Standards	2014	2017-22
George Cave (LA 70A)	1979-160	4	E	D	Diversity	2014	2017-22
Golden Delicious (LA 65A)	1974-346	5	L	D	Fruiting late	2014	2017-22
Granny Smith (LA 73A)	1976-145	2	L	D	Fruiting late	2018-19	2020-22
Jolyne	1950-167	5	M	D	Growth habit	2014	2017-22
Jonathan (EMLA 1)	1979-164	6	L	D	Growth habit	2014	2017-22
Kandile	1957-076	4	L	D	Growth habit	2014	2017-18
King of the Pippins	1972-030	7	M	D	Standards	2014	2017-22
Lappio	1958-130	5	L	D	Growth habit	2014	2017-22
Stark's Earliest (LA 68A)	1979-186	6	E	D	Flower early	2014	2017-22
Tropical Beauty	1961-087	4	L	D	Low chill	2018-19	2020-22
Winter Banana	1921-094	5	L	D	Low chill	2018-19	2020-22
Winter Pearmain	1946-107	7	L	D	Growth habit	2014	2017-22
Yellow Bellflower	1953-140	5	L	D	Low chill	2014	2017-22

Much of the primary planting was completed in 2014 before the environment controlling system was introduced. Some trees failed to establish, with some cultivars prone to canker. For some cultivars, only a few replicate trees were missing, but in other cases the cultivar was replaced by another. The majority of these later plantings were made in winter 2018/19, with even further gaps filled by winter of 2020/21. By the end of the trial, over 95% of planned tree plots had plantings.

2.2.3 Tree management and agronomy

Trees were managed in accordance with commercial practice where possible. Protocols were designed and implemented by Fruit Advisory Services Team (FAST) LLP, based on site at Brogdale Farm.

Integrated pest management (IPM) strategies were applied across all treatments and facilitated by FAST LLP. This included regular crop-walking, an annual pesticide programme, introduction of natural predators (*Coccinella* spp.), and more. Additional reactive seasonal insecticide applications were applied to mitigate periods of increased pest pressure from aphid species (*Eriosoma lanigerum*) and (*Dysaphis plantaginea*).

Artificial commercial hives of *Bombus terresteris* were introduced to each modified temperature environment, placed on a raised platform in the middle of each triple-span polytunnel. This aided pollination to mitigate the effects of advanced flowering in the earlier flowering cultivars, as well as mitigating the benefits of external pollinator presence in the more 'open' Ambient treatment.

Root pruning was not carried during the investigation. All trees were subject to summer (most years) and winter pruning (every year) according to commercial practice.

2.2.4 Statistical design

The statistical design of the orchard was completed by the NFCT and University of Reading in 2014. It was clear early on in concept that a randomised block design would not be possible given the constraints of the environment regulating system and relatively small size of the orchard. A mixed-model approach with a split-block design was decided as a good compromise for the statistical design. For each of the nine environment treatments, six replicate trees per cultivar were assigned across three pseudo blocks (S1-3, S1 = south, S2 = centre, S3 = north). Two replicates were randomly assigned positions within each pseudo block. The implementation of pseudo-blocking was designed to minimise the effects of any environmental variation throughout the length of the tunnels. For example, readings in 2017 highlighted temperature differences (~0.1-0.5°C) between several locations within each polytunnel (Lane, 2022).

2.2.5 Tree condition and health from 2020 onwards

As the trial had been operational for several years, variation in the condition and health of trees at the start of this current study was present between treatments (2020 onwards). This presented complications during data analysis, providing extra variables to consider.

As mentioned previously, there was variation in the age of trees planted. By 2020, age varied from seven years, to less than one. Differences in tree age presented variation in tree physiology, growth, fruit yield and fruit quality between replicates. This was less problematic for certain cultivars. For example, most replicates of 'Granny Smith' were planted in 2018-19. Therefore, despite being planted later than most trees, there was little tree age difference between treatments and replicates. Some cultivars however had replicates planted across a more staggered timeframe.

Certain insect pests had established annual infestation patterns during the spring and summer of each season. The most notable of these was woolly apple aphid (WAA) (*Eriosoma lanigerum*). This brown/black sap-sucking pest fed and colonised around the thinner sections of apple tree bark during the summer months. The main symptoms included waxy white secretions that covered branches, as well as galls on branch feeding locations. Wounds often split, implicating tree health from exposure to canker-causing fungi and bacteria. Widespread infestations occurred in the heat temperature treatments, particularly within the Plus2 tunnel. The ambient tunnel had little infection. This may be due to more favourable environmental conditions and more suitable levels of protection within warmer tunnels (e.g. protection from wind). In late 2020, a health check-up of trees showed ~20% of all Plus2 trees and ~10% of Plus4 tunnel trees had some form of WAA damage (Appendix 2.1). Some cultivars showed higher levels of susceptibility, for example over 50% of all Stark's Earliest trees in Plus2 were at least somewhat affected. Infected trees showed signs of altered vegetative growth (e.g. low leaf bud development) which had a subsequent impact on fruit yield and quality. A more intensive pesticide spraying regime was introduced during the peak of WAA populations to help mitigate spread. However, it was unlikely that without extensive tree grubbing, infestations would likely persist. In 2020, the decision was made to not grub and replace any WAA damaged trees. At the point of decision making of the longer-term trial, it would not have been cost-effective to re-plant new trees – especially for the more susceptible cultivars.

Other insect pests such as rosy apple aphid (*Dysaphis plantaginea*), green apple aphid (*Aphis pomi*) and apple rust mite (*Aculus schlechtentali*) were seasonal pests that generally appeared on trees during the summer months. Damage typically affected new vegetative growth and caused minor additional fruit waste come harvest. Any major infestations were reported to farm management where the issue was addressed.

Fungal and bacterial disease were present on a minor level. Such diseases included apple scab (*Vanturia inaequalis*), apple canker (*Nectria* spp.) and brown rot (*Monilina fructigena*). Best practice advice was adhered to from farm management to help mitigate spread and severity of disease.

With all the above considered, it was decided to remove data for some trees from certain analyses. For example, data from younger trees (planted within one to three years) were discounted from yield analyses if the majority of a cultivar's tree population had been planted much earlier (2014-15). Trees with notable pest damage in a given year were removed from the analysis if yield patterns appeared anomalous – tree data from previous years was still included. Fruit utilised for fruit quality analysis were also not sampled from infected or visibly damaged trees. Whilst this system was not perfect (pest infections were not strictly quantified throughout the study), it mitigated the impact of pest influence within data analyses.

2.2.6 Early termination to the longer-term experiment

In February 2022, a succession of winter storms named 'Dudley' (16-17 February), 'Eunice' (18 February) and 'Franklin' (21-22 February) inflicted significant structural damage to the experimental facility based at Brogdale. The metal polytunnel framework, venting system, plastic polytunnel sheeting, irrigation system, temperature sensors, communications hub, and several trees all suffered catastrophic damage across all three triple-span tunnels (Figure 2.3). The scale of damage meant that the cost of repairs were far too great for the NFCT to fund. Consequently, it was announced in 2022 that the 'Apples in a Warmer World'® modified environment facility would not be able to continue experimental work going forward. Some fieldwork observations relating to yield were able to continue for the 2022 season, albeit with no temperature or rainfall uplifts; the polythene was removed, and ambient environments provided to all the trees from late February 2022. In November 2022, the remaining structures were decommissioned, and experimental work concluded.

***Figure 2.3**. Severe facility damage inflicted by Storms Dudley, Eunice and Franklin, February 2022. Top left: Trees uprooted in Plus2. Top right: Polytunnel roof ripped off in Plus2. Bottom left: Buckled and dislodged metalwork and detached irrigation sprinklers in Plus4. Bottom right Polytunnel fabric ripped from roof and side of Ambient.*

2.3 Data Collection

2.3.1 Overview

Data collection for this study was a mix of unique experiments and continuing longer-term experiments. For longer term studies, much of the same methodology has been carried over from Lane (2022) with a few alterations. Table 2.2 shows which experimental work concluded with Lane (2022), continued from Lane (2022), and was unique to this study (Peter). Phenology, fruitlet thinning, yield, pruning, and some fruit quality analyses observations were collected over at least five years. Photosynthetic rate, extension growth, and tree girth observations concluded with Lane (2022) (two to three years of data). Further fruit quality analyses and storability experiments were conducted across 2020 and 2021.

Table 2.2. *Experimental work conducted across six production seasons within the 'Apples in a Warmer World'® trial, indicating which production seasons were associated with each experiment (x) and individual (Lane or Peter).*

Experimental Work	**2017 (Lane)**	**2018 (Lane)**	**2019 (Lane)**	**2020 (Peter)**	**2021 (Peter)**	**2022 (Peter)**
Phenology (Bud Break, Flowering)	x	x	x	x	x	
Fruitlet Thinning	x	x	x	x	x	x
Photosynthetic Rate	x	x				
Fruit Maturity	x	x	x	x	x	
Yield	x	x	x	x	x	x
Extension Growth	x	x	x			
Tree Girth	x	x	x			
Tree Pruning (Summer)	x	x	x		x	
Tree Pruning (Winter)	x	x	x	x	x	x
Fruit Quality (Firmness, SSC)	x	x	x	x	x	
Fruit Quality (RCC, DMC)				x	x	
'Gala' Storability				x	x	
Flower Cluster Counts				x	x	x

The sections below provide a brief overview of the data collection undertaken within each part of experimental work listed in Table 2.2. These sections refer to results and analyses specific to this thesis. Methodology and application of results relevant to yield (Chapters 3 and 4), fruit quality (Chapter 5), and storability (Chapter 6) are described in more detail in those chapters.

2.3.2 Phenology

Recording of when a developmental stage occurred tracked the rate of seasonal development between cultivars and treatments. Continuation of analysis from Lane (2022) in each production season was crucial to understand how variation in weather variables affected the timing of important orchard seasonal milestones. The developmental stages are based on the BBCH scale of 'pome fruit' identification key (Meier et al., 1994). These are as follows:

- Bud break. One stage. Principal growth stage 0, Code 07 ('Beginning of bud break: first green leaf tips just visible'). Starting from March W1, the date of 50% an individual tree's buds reaching this stage of development was recorded. This was assessed three times a week until all trees had reached this requirement. There was a degree of compromise in this methodology as it would be impractical to count individual buds on each tree.

- Flowering. Four stages. Principal growth stage 6, codes 61 (‘Beginning of flowering: about 10% of flowers open), 65 (‘Full flowering: at least 50% of flowers open’), ‘80% flowering’ (non-BBCH stage) and ‘90% petal fall’ (non-BBCH stage). Starting from March W4, the dates that these flowering stages were reached were recorded. This was observed three times a week until all trees met these requirements. There was a degree of compromise in this methodology as it would be impractical to count individual buds on each tree. Trees that flowered unevenly may have also had more inaccurate estimates.

Phenology and temperature variation were not analysed directly as part of this study. Instead, potential associations with altered phenology were tested within several experimental analyses (Chapters 4, 5, and 6). The mean dates (2018-21) for bud break and full flowering can be seen in Appendix 2.2.

2.3.3 Fruitlet Thinning

Fruitlet thinning is a common commercial practice that lowers crop load and enhances fruit development. Within May and June each season, each individual tree was thinned to a commercial standard fruit load, as determined by FAST guidelines. A typical figure of ~120 fruitlets per tree was targeted, with slight reductions dependent on cultivar (e.g. ‘Bramley’s Seedling’). This was designed to serve a balance between commercial practice, and to not cancel out potential modified environment effects.

In terms of data collection, all fruit removed was counted and weighed.

2.3.4 Fruit Maturity

As a fruit matures, starch compounds begin to break down to simpler polysaccharides. Therefore, the concentration of starch remaining is indicative of fruit ripeness. Starch levels are often assessed to determine optimal harvest time in commercial practice. Optimal harvest time can depend on the cultivar and what purpose the fruit will be utilised for (AHDB, 2021). The process of monitoring starch levels is relatively simple, as detailed in Appendix 2.3. Two to three weeks before an expected harvest date (for each cultivar x treatment) and regularly thereafter, a subsample of fruit was harvested from each replicate for starch testing. Once an average of 50% Starch Index (S/I) had been reached (also known as the ‘tree-ripe’ stage), the cultivar x treatment was eligible for harvest. This 50% S/I figure does not imply optimal maturity for some cultivars, however a standard measure target across all cultivars enabled a fair comparison when analysing impacts of modified environments.

2.3.5 Yield

Once a cultivar x treatment reached 'tree ripe' stage, all trees were harvested within one to three days. For each tree, all fruit was picked, including waste on the ground. Total fruit was counted and weighed to determine the total fresh weight (kg) for a tree. Fruit used for maturity testing also contribute towards total fresh weight totals. A subsample from up to six replicates of cultivar x treatment was then used for grading. This categorised fruit in to either Class I fruit, Class II fruit, or waste. Up to twenty Class I or II (depending on availability) fruit were then randomly selected for fruit quality and dry matter assessments. Given the wide range of cultivars grown, the harvest season typically spanned the period from early July ('Stark's Earliest') to early November ('Braeburn').

2.3.6 Fruit Quality

A series of fruit quality measurements were applied to ten fruits across each cultivar x treatment within one to two days of harvest. Fruit quality tests are conducted in commercial practice to ensure crops meet government and retailer varietal marketing standards. The tests selected were quick and convenient to complete across 60 possible cultivar x treatment combinations. More in depth fruit quality tests, such as titratable acid analysis, required more time and resources to complete and so were not conducted. Each fruit was subject to the following non-destructive and destructive tests:

- Weight (g). Using electronic scales, the weight of each fruit was taken.
- Firmness (kg). Using a FT 327 Fruit Pressure Tester, (Effegi Ltd., London, UK) an 11mm probe measured the flesh firmness of two opposite sides of fruit. An average reading was then taken. Firmness was an indicator of fruit perishability by damage and is also a popular trait for consumers (firmness contributes to the 'crunchiness' of a fruit).
- Soluble Solids Content (SSC) (%Brix). SSC is a measure of soluble solids within an aqueous solution. Using a PAL-1 refractometer (Agato Ltd., Bristol, UK), a few ml of juice from each apple was used to determine the Brix percentage of sugar present.
- Red Colour Coverage (RCC) (%). For red and bi-coloured fruit cultivars, an estimate of red colour surface coverage (%) was noted by eye. For green fruit, the fruit was assessed for whether the skin was primarily green or yellow. Minimum marketable standards typically exist for RCC, as it is a popular trait for consumers.

- Dry Matter Content (DMC) (%). Using ten additional apples, segments from each fruit were removed and placed in an oven at 70°C for 24 hours to determine average DMC for apples within a cultivar x treatment.

2.3.7 'Gala' Storability

This unique experiment assessed whether modified temperature environments had an impact on fruit quality attributes during and after controlled atmosphere (CA) storage. Due to time and resource constraints, and commercial relevance, this was conducted for the cultivar 'Gala' only. The initial plan was to repeat experiments over three years. However, due to the 2022 storm damage to facilities, the experiment was conducted over two years only (2020 and 2021).

Fruit utilised for storage experiments were harvested in a different manner to those harvested for standard fruit quality assessments. 100-120 fruit were harvested from a select few replicate trees across each modified environment treatment (n=9) once maturity tests showed 85% average starch coverage. This pre-ripened stage harvest of fruit maturity is standard commercial practice for long term storage, as fruit continues to mature gradually once in store.

Fruit were stored in CA facilities based at the Produce Quality Centre, East Malling, Kent. Fruit were placed in self-contained units where environmental conditions were regulated and monitored across six to seven months. Fruit were removed from CA storage and fruit quality tested at intervals of six weeks. An additional set of assessments were conducted to a set of samples before entering CA storage. This served as a baseline round of assessments. The following fruit quality attributes were tested on each fruit:

- Weight (g)
- Firmness (kg)
- Soluble Solids Content (SSC) (%Brix)
- Red Colour Coverage (RCC) (%)
- Starch Index (S/I) (%)

Twenty fruit were subject to these tests at each removal stage. Ten fruit were tested one day after removal (allowing sufficient time for fruit to reach room temperature), and the remaining ten fruit assessed seven days later ('shelf-life' tests).

2.4 Environmental Data

2.4.1 Temperature overview

The three modified temperature environments (Ambient, Plus2, and Plus4) were initiated on 1st November 2017, and terminated on 17th February 2022. Apple production results from 2017 served as baseline assessments whilst the modified environment system was set up. Yield results were obtained for 2022 to test for 'legacy' effects of the modified treatments. Temperatures in all three regimes were monitored and recorded by the TomTech system on an hourly basis. The programmed software responded to these hourly weather logs for the Plus2 and Plus4 regimes through closing side-vents (increasing temperature uplift) or opening side-vents (reducing temperature uplift) when necessary. Initial issues were found with the temperature monitoring system throughout 2016-19, including temperature logging, frosts, tunnel damage, and missing data. These issues were mitigated and reported on in Lane (2022).

2.4.2 Temperature values

Mean, minimum, maximum and minmaxdiff (difference between minimum and maximum) temperature for each year, month, and modified temperature treatment are displayed in Table 2.3.

The passive venting system was successful in manipulating temperature. Annual temperature in the Plus2 and Plus4 environments ranged from 0.4 to 0.8 °C, and 1.0 to 1.8 °C warmer than Ambient, respectively. This meant that specific temperature uplifts were approximate average design values, with deviations of ~1-3°C depending on the time of day. This was due to the passive monitoring system's dependence on solar radiation; temperature uplifts in Plus2 and Plus4 environments were only possible during daylight hours. During night hours the temperatures among the three environments were relatively comparable. Periods of the year with shorter daylengths (autumn and winter) therefore had little temperature uplift. For example, mean temperature between Ambient and Plus4 varied by only 0.2°C in winter, compared to 2.1°C in summer.

In May 2020, a software update was applied to the TomTech system that allowed greater temperature uplift within the modified environments. The new rules were as followed:

- Plus2 regime: Up to +3°C (up from +2°C originally) uplift compared to ambient at any one time if previous 24h temperature mean < +2°C.
- Plus4 regime: Up to +6°C (up from +4°C originally) uplift compared to ambient at any one time if previous 24h temperature mean < +4°C).

Whilst the software upgrade didn't affect the night temperatures issue (the passive system still required reliance on solar radiation), it meant that overall average temperatures were closer to the design values from daytime temperature uplifts. For example, Plus4 and Ambient annual temperature difference in both 2020 and 2021 was ~+0.4°C greater than previous years (Table 2.3). During summer months, this update often meant that Plus4 was ~+6°C warmer than Ambient to compensate for cooler hours within the 24-hour monitoring period. For example, June mean daily temperature uplifts between Ambient and Plus4 were generally ~3-4°C in 2020, compared to ~1-2°C in 2019 (Figure 2.4).

An important note is that the Ambient regime also received minor temperature uplifts from outside conditions as an effect of the polytunnel structure which provided partial cover. Whilst not ideal, the polytunnel structure was required for regulating rainfall and to provide the same light transmission as the warmer treatments. As reported in Lane (2022), mean annual Ambient tunnel temperature was 0.79°C warmer than true outside conditions.

Table 2.3*. Average mean, minimum, maximum and minmaxdiff temperature (°C) for each month, year (2017-22), and meteorological season (spring, summer, autumn, and winter) across the three treatments (Ambient, Plus2 and Plus4). Temperature uplifts were non-operational in both 2017 (until November) and 2022 (from March).*

Year	Tmean (°C)			Tminimum (°C)			Tmaximum (°C)			Tminmaxdiff (°C)		
2017	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
JAN	3.4	3.4	3.4	0.0	0.0	0.0	6.7	6.7	6.7	6.7	6.7	6.7
FEB	6.9	6.9	6.9	4.2	4.2	4.2	9.6	9.6	9.6	5.4	5.4	5.4
MAR	9.7	9.7	9.7	5.8	5.8	5.8	13.4	13.4	13.4	7.6	7.6	7.6
APR	9.6	9.6	9.6	4.5	4.5	4.5	15.0	15.0	15.0	10.6	10.6	10.6
MAY	13.4	13.4	13.4	8.7	8.7	8.7	18.2	18.2	18.2	9.7	9.7	9.7
JUN	17.4	17.4	17.4	12.2	12.2	12.2	23.1	23.1	23.1	10.7	10.7	10.7
JUL	18.4	18.4	18.4	13.7	13.7	13.7	23.1	23.1	23.1	9.4	9.4	9.4
AUG	17.6	17.6	17.6	12.2	12.2	12.2	22.9	22.9	22.9	10.7	10.7	10.7
SEP	14.4	14.5	14.6	10.2	10.2	10.4	19.8	20.3	21.3	8.8	9.4	10.2
OCT	13.1	13.3	13.4	9.5	9.4	9.7	N/A	N/A	N/A	N/A	N/A	N/A
NOV	6.3	6.4	6.5	3.9	3.7	4.1	5.8	7.5	9.7	5.3	7.3	9.2
DEC	5.2	5.2	5.2	2.2	1.9	2.3	7.8	9.5	11.8	5.6	7.5	9.5
2018	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
JAN	6.2	5.9	6.3	3.1	2.7	3.0	9.0	10.3	12.1	5.9	7.6	9.1
FEB	3.1	3.0	3.6	0.3	-0.1	0.3	6.2	7.0	8.3	5.9	7.1	8.0
MAR	5.8	5.9	6.5	3.1	2.7	3.2	9.1	9.8	11.1	6.1	7.1	7.9
APR	11.2	11.7	12.5	7.4	7.3	7.9	16.1	17.0	18.7	8.7	9.7	10.8
MAY	13.7	15.0	15.7	8.9	9.4	9.9	19.1	21.0	22.9	10.2	11.7	12.9
JUN	16.6	17.9	18.5	11.8	12.0	12.5	21.4	23.1	25.5	9.5	11.1	13.0
JUL	20.8	21.9	23.1	14.6	14.9	15.6	27.4	29.6	31.2	12.9	14.6	15.6
AUG	18.2	19.0	20.1	13.4	13.5	14.3	23.5	25.3	27.0	10.1	11.8	12.7
SEP	14.9	15.2	16.0	10.6	10.1	10.8	19.9	21.3	22.9	9.3	11.2	12.1
OCT	12.2	12.3	13.0	8.4	7.8	8.4	16.4	17.9	19.3	8.0	10.1	10.9
NOV	8.6	8.3	8.6	6.0	5.3	5.8	11.2	12.1	12.6	5.3	6.8	6.8
DEC	7.4	6.9	7.2	4.9	3.8	4.3	9.8	10.1	10.5	4.9	6.3	6.2
2019	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
JAN	4.3	4.0	4.4	1.7	0.7	1.2	6.7	7.6	8.3	5.0	6.8	7.2
FEB	7.0	6.9	7.4	3.6	2.9	3.3	11.4	12.8	13.9	7.8	9.9	10.6
MAR	8.7	8.8	9.0	5.0	4.8	4.9	12.3	13.5	13.7	7.3	8.7	8.8
APR	9.4	10.5	11.0	5.2	5.3	5.7	14.2	17.1	17.8	9.0	11.8	12.1
MAY	12.7	13.9	14.6	7.6	8.0	8.4	18.1	20.5	21.9	10.5	12.5	13.5
JUN	16.3	17.6	17.5	11.6	12.0	12.1	20.9	23.1	23.3	9.4	11.1	11.3
JUL	19.3	20.5	21.2	13.8	14.3	14.7	25.0	27.2	28.5	11.1	12.9	13.8
AUG	18.6	19.4	20.1	13.5	13.5	13.9	24.6	26.5	28.0	11.1	13.0	14.2
SEP	15.5	16.1	16.8	11.0	10.8	11.0	20.6	22.5	24.1	9.6	11.7	13.1
OCT	11.7	12.1	12.5	8.4	8.4	8.7	15.0	16.6	17.6	6.6	8.2	8.9
NOV	7.3	7.3	7.7	4.4	4.2	4.6	10.1	10.8	11.6	5.6	6.5	7.0
DEC	6.9	6.6	6.9	4.1	3.7	4.0	9.4	9.2	9.4	5.3	5.5	5.4
2020	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
JAN	6.8	6.6	6.8	4.5	4.2	4.5	9.1	9.0	9.2	4.6	4.8	4.8
FEB	6.9	6.9	7.2	4.1	3.9	4.2	10.0	10.2	10.7	5.9	6.4	6.5
MAR	7.4	7.4	7.6	4.2	4.0	4.3	11.0	11.5	11.7	6.8	7.5	7.4
APR	11.2	12.5	13.3	5.7	6.1	6.4	17.4	20.1	21.4	11.7	14.1	15.0
MAY	14.3	16.0	17.3	8.2	8.8	9.4	20.7	23.5	25.9	12.6	14.6	16.5
JUN	16.7	18.5	19.7	11.4	12.0	12.4	22.4	25.4	28.0	11.0	13.4	15.6
JUL	18.1	19.8	20.7	12.9	13.3	13.6	23.5	26.5	28.9	10.6	13.2	15.3
AUG	19.8	21.3	22.2	15.5	15.9	16.2	25.2	28.1	30.4	9.7	12.2	14.2
SEP	15.8	16.7	17.4	11.9	12.0	12.5	20.3	23.0	24.9	8.4	11.0	12.5
OCT	11.4	11.8	12.2	9.3	9.3	9.6	14.4	16.3	17.5	5.1	7.1	7.8
NOV	9.6	9.7	9.9	6.3	6.2	6.5	12.7	14.0	14.3	6.3	7.8	7.9
DEC	6.1	6.0	6.3	4.0	3.8	4.0	8.3	9.1	9.7	4.3	5.4	5.7
2021	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
JAN	4.1	4.0	4.4	2.1	1.8	2.0	6.1	7.1	7.9	3.9	5.3	5.8
FEB	5.7	5.7	6.2	3.4	3.2	3.5	8.1	9.7	10.2	4.7	6.5	6.7
MAR	7.4	8.2	8.7	3.5	3.4	3.7	11.3	13.9	15.2	7.7	10.5	11.5
APR	6.7	8.2	9.2	2.1	2.4	2.8	11.8	14.7	17.2	9.6	12.3	14.3
MAY	11.5	13.0	13.4	7.1	7.5	7.6	16.4	19.5	21.0	9.3	12.0	13.4
JUN	17.3	18.8	19.1	12.6	13.0	13.1	22.0	25.0	26.0	9.5	12.0	12.9

Year	Tmean (°C)			Tminimum (°C)			Tmaximum (°C)			Tminmaxdiff (°C)		
JUL	18.0	19.6	21.2	13.7	13.9	15.2	23.0	26.1	28.7	9.4	12.2	13.5
AUG	17.0	18.2	19.3	13.3	13.3	14.0	21.5	24.4	26.6	8.2	11.1	12.6
SEP	16.8	17.8	18.6	12.3	12.2	12.7	21.9	24.8	26.8	9.7	12.5	14.0
OCT	12.5	12.9	13.3	9.0	8.8	9.0	15.8	18.0	18.9	6.8	9.2	9.9
NOV	8.1	8.2	8.5	5.6	5.3	5.5	10.8	12.5	13.0	5.2	7.2	7.5
DEC	7.4	7.2	7.4	5.0	4.7	4.9	9.4	9.7	10.2	4.4	5.1	5.3
2022	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
JAN	5.3	5.2	5.5	2.4	1.9	2.2	8.3	9.6	10.0	6.0	7.7	7.8
FEB	7.9	7.9	8.0	4.5	4.3	4.4	11.0	11.9	12.1	6.5	7.6	7.7
MAR	8.4	8.4	8.4	3.9	3.9	3.9	11.6	11.6	11.6	7.7	7.7	7.7
APR	11.0	11.0	11.0	4.8	4.8	4.8	13.8	13.8	13.8	9.0	9.0	9.0
MAY	14.8	14.8	14.8	9.0	9.0	9.0	19.0	19.0	19.0	10.0	10.0	10.0
JUN	17.9	17.9	17.9	10.2	10.2	10.2	21.2	21.2	21.2	10.9	10.9	10.9
JUL	21.7	21.7	21.7	13.1	13.1	13.1	25.9	25.9	25.9	12.8	12.8	12.8
AUG	21.5	21.5	21.5	13.9	13.9	13.9	25.0	25.0	25.0	11.6	11.6	11.6
SEP	16.4	16.4	16.4	11.1	11.1	11.1	19.7	19.7	19.7	8.6	8.6	8.6
OCT	14.6	14.6	14.6	10.1	10.1	10.1	17.9	17.9	17.9	7.8	7.8	7.8
ALL[1]	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
JAN	5.0	4.8	5.1	2.3	1.9	2.1	7.6	8.4	9.0	5.3	6.5	6.9
FEB	6.3	6.2	6.6	3.3	3.1	3.3	9.4	10.2	10.8	6.0	7.1	7.5
MAR	7.9	8.1	8.3	4.2	4.1	4.3	11.5	12.3	12.8	7.2	8.2	8.5
APR	9.9	10.6	11.1	4.9	5.0	5.4	14.7	16.3	17.3	9.8	11.2	12.0
MAY	13.4	14.4	14.9	8.2	8.6	8.8	18.6	20.3	21.5	10.4	11.8	12.7
JUN	17.0	18.0	18.4	11.6	11.9	12.1	21.8	23.5	24.5	10.2	11.6	12.4
JUL	19.4	20.3	21.1	13.6	13.8	14.3	24.7	26.4	27.7	11.0	12.5	13.4
AUG	18.8	19.5	20.1	13.6	13.7	14.1	23.8	25.4	26.7	10.2	11.7	12.6
SEP	15.7	16.1	16.6	11.2	11.1	11.4	20.4	22.1	23.5	9.1	10.9	11.9
OCT	12.6	12.8	13.2	9.1	9.0	9.3	15.9	17.3	18.2	6.9	8.5	9.1
NOV	8.0	8.0	8.5	5.3	5.0	6.5	11.1	12.3	11.7	5.6	7.1	6.9
DEC	6.6	6.4	6.3	4.1	3.6	4.5	8.9	9.5	9.1	4.9	6.0	6.3
18-21[2]	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
SPR	10.0	10.9	11.6	5.7	5.8	6.2	14.8	16.8	18.2	9.1	11.0	12.0
SUM	18.1	19.4	20.2	13.2	13.5	14.0	23.4	25.9	27.7	10.2	12.4	13.7
AUT	12.0	12.4	12.9	8.6	8.4	8.8	15.8	17.5	18.6	7.1	9.1	9.9
WIN	6.0	5.8	6.2	3.4	3.0	3.3	8.6	9.3	10.0	5.2	6.3	6.7
17-21[3]	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**	**Amb**	**Plus2**	**Plus4**
2017	11.3	11.4	11.4	7.3	7.2	7.3	15.7	15.9	16.2	8.5	8.7	9.0
2018	11.6	12.0	12.7	7.7	7.5	8.0	15.8	17.1	18.6	8.1	9.6	10.5
2019	11.5	12.0	12.5	7.5	7.4	7.7	15.7	17.3	18.2	8.2	9.9	10.5
2020	12.0	12.8	13.4	8.2	8.3	8.6	16.3	18.1	19.4	8.1	9.8	10.8
2021	11.1	11.9	12.5	7.5	7.5	7.9	14.9	17.2	18.5	7.4	9.7	10.6

[1] 1 Jan 2017 – 31 Oct 2022 inclusive [2] 1 Mar 2018 – 28 Feb 2022 [3] 1 Jan 2017 – 31 Dec 2021

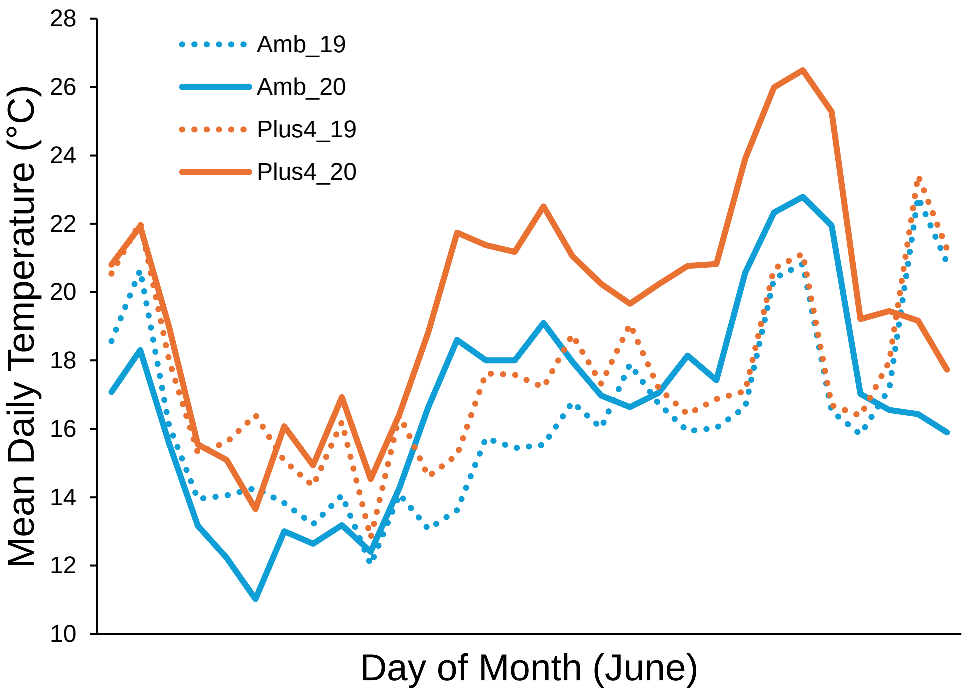


***Figure 2.4**. Average daily temperature (°C) during June for 2019 (dotted) and June 2020 (solid) within Ambient (blue) and Plus4 (orange) environments, displaying how temperature treatment uplift compares after venting software modifications in May 2020.*

2.4.3 Missing data

All weather data between 1st January to 31st October 2017, and 17th February to 31st October 2022, was sourced from 'Faversham' MetOffice Weather Station database, located at Brogdale, Kent ~400m away from the trial site. These time periods coincide with those when the modified temperature environments were inactive.

Intermittent communications interruptions and faults with the TomTech systems caused instances of missing temperature and rainfall data. Missing data between 2017-19, and in 2022, was replaced with 'Faversham' MetOffice weather data. Plus2 and Plus4 environment mean, minimum and maximum temperatures were estimated using long-term averages cf. the ambient tunnel from the remaining data.

Data from 'Faversham' MetOffice Weather Station was unavailable between 2020 and 2021. This was due to COVID-19 pandemic restrictions which prevented the affiliated volunteers from accessing and uploading weather station data to the MetOffice database. Missing data during this period was instead sourced from 'Manston' MetOffice weather station database, located ~30km away from the trial site. Whilst this sizeable distance was not ideal, this was the closest weather station with regular, hourly available data. Temperatures for all three modified environments were calculated based on validated regression models (Appendix 2.4).

In total, 134 days out 1,461 between 1st January 2018 and 31st December 2021 required the use of MetOffice weather station data. Lengthy outages of Tomtech temperature data occurred within January 2018 (23 days), June 2018 (30 days) and September-October 2020 (46 days).

2.4.4 Irrigation system and issues

As for the temperature modification system, the setup, testing, and validation of the irrigation system was described in Lane (2022).

The irrigation modification systems introduced on 1st November 2017 (same date as temperature uplift introduction) was successful at manipulating rainfall to achieve desired specifications for each treatment (80%, 100%, and 120% rainfall based on ambient condition monitoring). Total ambient rainfall for production seasons 2017-2021 (the years where treatments were applied) is provided in Figure 2.5.

Issues with the irrigation system became apparent over time. During project conception, it was envisaged (based on future climate change scenarios) by the NFCT board that 20% more or less of the total rainfall would be sufficient for simulating ‘drought’ and ‘excess rainfall’ conditions. However, with hindsight, this appeared to not be the case. During the production seasons there were exceptional dry periods (e.g. July 2018 when it was also very hot), and so rainfall differences between treatments were negligible. And when rainfall was in excess, all treatments had excess rainfall. During the investigation, it was likely that more severe changes (e.g. -40% and +40% rainfall), perhaps combined with much greater rainfall storage, would be required to simulate effects of differences in drought and excess rainfall among the treatments.

This issue was further compounded by the facility itself. The polytunnel was designed to be as water-tight as possible, but leaks from the plastic coverings and guttering became more frequent over time from wear and tear. For example, high winds on several occasions caused tearing of polytunnel plastic, allowing excess ambient rainfall in to the facility. Tear repairs would often require waits of several weeks or even months for labour availability to resolve the issue. Expanding and contracting of the metal guttering over time also allowed for gaps to eventually appear at joints, resulting in further leaks.

Moreover, no barriers were introduced between the soils within and outside of the facility. As such, soil water from outside the experimental system was likely introduced. As tree roots were not confined to the area within the facility as well, it is possible that trees surrounding the fringes of the polytunnels had greater access to water resources than those further within.

Due to the more closed nature of Plus2 and Plus4 polytunnels compared to Ambient, moisture was more susceptible to becoming trapped. During night hours, moisture was prone to condense on the cool polytunnel roofs, resulting in water cycling back to the trees. The net effect of this likely increased the total rainfall received by Plus2 and Plus4 compared to Ambient.

During 2020 and 2021, PhD candidate Catherine Chapman conducted soil component analysis as part of a study investigating the effects of temperature on soil nutrient availability. As part of her studies, soil water content was analysed at four different time intervals within a single year's timeframe. The results confirmed inconsistencies in soil water content (SWC) throughout the three polytunnels. It was expected that 120% rainfall treatments should have had the greatest SWC, and 80% rainfall treatments to have the least. However, this was not the case with sporadically different measurements within the cropping system. For example, September 2021 readings showed that (on average) Ambient had much greater SMC than the two warmer treatments (Appendix 2.5). The data also confirmed suspicions that fringe tree plots had greater access to water compared to inner plots. For example, Tunnels 1, 4, and 7 generally showed greater SWC compared to Tunnels 2, 5, and 8 despite lower irrigation input.

With the above problems considered, as well as Lane's (2022) conclusions that rainfall treatments were having minimal impacts on results, it was decided that the analyses undertaken within this current study would not consider the rainfall treatments in detail, and instead focus solely on the impact of varied temperature regimes.

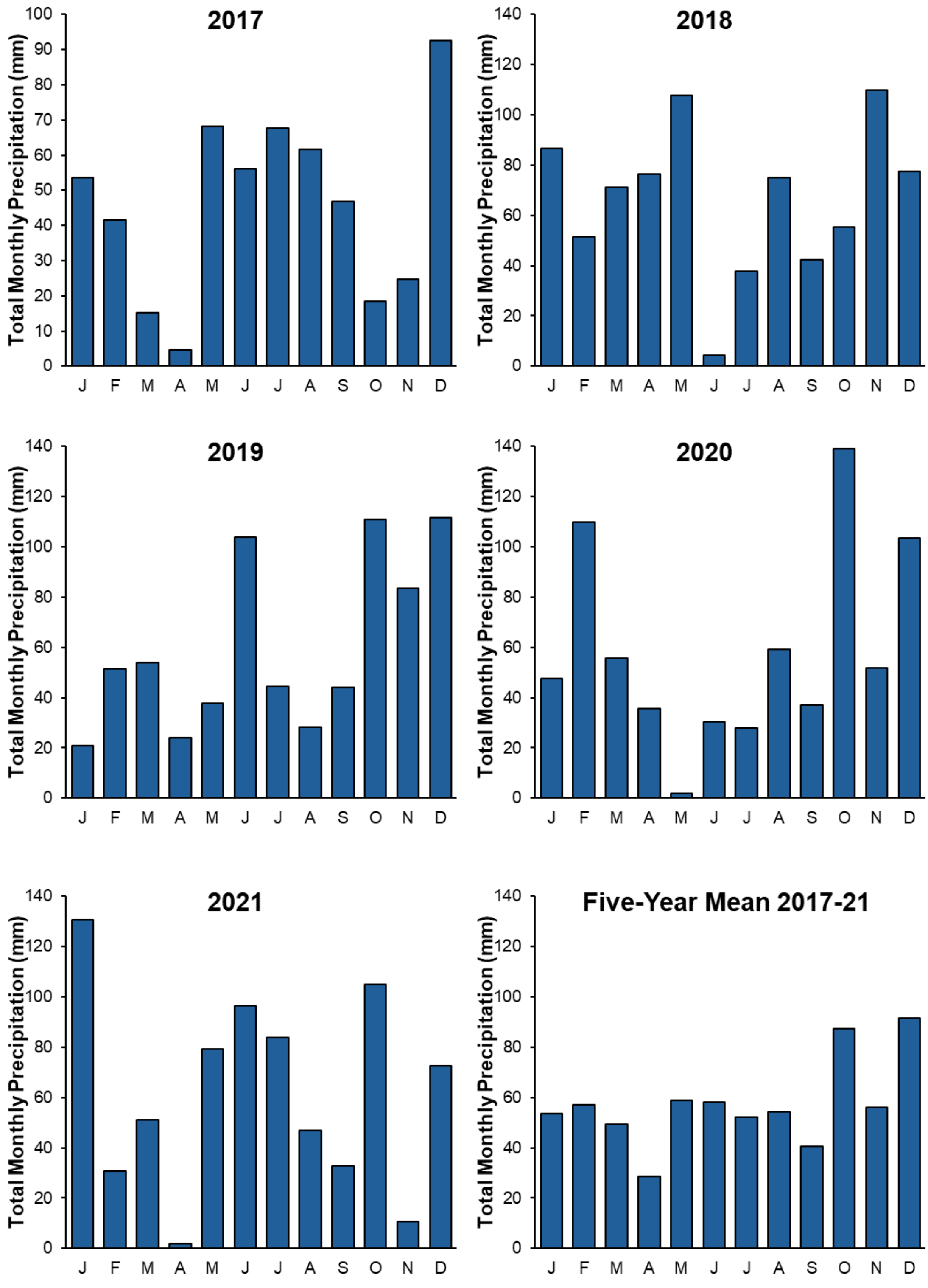


Figure 2.5. *Total precipitation and five-year mean (2017-21) for each month of the trial that was collected and redistributed within the modified environments via sprinkler irrigation. Treatments 1, 4, and 7 received 80% of monthly totals. Treatments 2, 5, and 8 received 100% of monthly totals. Treatments 3, 6, and 9 received 120% of monthly totals.*

2.5 Statistical Analysis

Details of the statistical methods used are described in the following chapters (Chapters 3 to 6).

All statistical analyses were performed in RStudio v22.03 to v24.01 (Posit, Boston, USA).

Chapter 3: The influence of seasonal temperature on yield of apple

3.1 Introduction

Seasonal climate, weather, and their influence on apple fruit yield is broad and complex. Up to three quarters of long-term UK apple yield variation can be explained by meteorological factors (Beattie and Folley, 1978). Its impact can be direct or indirect dependent upon the weather's association with other factors of the environment affecting yield. Additionally, practical tree management strategies influence plant growth responses to weather. Seasonal temperature, the subject of this investigation, represents temperature within a specific temporal scale: from 'bud burst' (i.e. when plant tissue growth initiates from fruiting buds, signalling the end of ecodormancy (Lang et al., 1987)) to fruit maturation and harvest. This time period incorporates a wide range of annual plant phenological growth stages. The influence of temperature within each growth stage, and its subsequent effect on fruit yield, has been shown to fluctuate throughout the growing season.

The importance of temperature in transitioning annual fruiting bud physiology from endormancy to ecodormancy through winter chill unit accumulation is well documented in deciduous fruit crops (Faust et al., 1997; Saure, 2011). Insufficient winter chill can lead to altered yield-determining factors within the forthcoming growing season, such as delayed bud break, lower flower quality and impaired fruit set (Saure, 2011; Atkinson et al., 2013). This can have secondary effects on yield parameters, such as altered fruit size and fresh weight (Oukabli et al., 2003; Saure, 2011). Studies have documented and modelled the risk of climate change induced alterations to winter chill accumulation, and its potentially negative net effect on fruit yield parameters, within existing apple production systems and current commercially important cultivars in the UK and Europe (González Noguer, 2022). Apple production hubs outside of Europe including in Australia (Parkes et al., 2020), India (Pramanick et al., 2015) and Morocco (El Yaacoubi et al., 2014) are also threatened by future winter chill accumulation alteration in response to climate change.

More rapid phenological development over the past 50 years is associated with an average increase in temperature, and this trend is expected to continue with future climate change throughout Europe (Chmielewski and Rötzer, 2001; Menzel et al., 2006; El Yaacoubi et al., 2014, Chitu and Paltineanu, 2020). Results from Lane (2022) indicate that the Plus4 modified temperature environment accelerated bud burst date by ~2-7 days, full flowering date by ~1-3 weeks, and harvest date by ~1-4 weeks, dependent on cultivar (Appendix 2.2). This advancement may have consequences for yield. Earlier flowering may potentially lead to greater risk of flower bud damage from frost events (Pramsohler et al., 2012; Hoffmann and Rath 2013) and altered pollinator temporal synchrony (Wyver et al., 2023). Fruit yield variation in relation to earlier harvest date is less understood. Christodoulou and Culham (2021) found that a 10% increase in Growing Degree Days led to a 1% increase in fruit weight. Furthermore, their results demonstrated that fruit growth plateaus in later stages of development, specifically highlighting that a two-week earlier sampling did not substantially alter fruit size across 12 different cultivars.

Temperature during early fruitlet development after flowering is thought to influence yield output at harvest (Jackson and Hamer, 1980). Apple fruit development is split in to two phases: cell division (0 to 40 days after fertilisation) followed by cell expansion (~40 days after fertilisation to fruit maturity) (Blanpied and Wilde, 1968; Pratt, 1988; Atkinson et al., 1998). Fruit growth in the cell division phase is significantly enhanced in warmer growing environments (Warrington et al., 1999). Potential maximum fruit size is set by 50 days after full bloom in the case of ‘Royal Gala’ (Stanley et al., 2015). However, fruit growth sensitivity to temperature during this phase can be effectively managed through agronomic practices, such as cultivar selection, rootstock selection and managing crop load (Marini et al., 2014). Additionally, warmer temperatures during this period may exacerbate natural ‘June drop’, resulting in fewer fruits being retained through to maturity (Grauslund and Hansen, 1975; Atkinson et al., 1998).

Global surface temperatures have increased by up to 1°C in the period between 1950 and 2020 (Parmesan et al., 2022). Studies based on analysing historic long-term yield and meteorological data variation have modelled future climate change effects on apple production. Li et al. (2019) modelled meteorological yield under two climate change scenarios (RCP 4.5 and RCP 8.5) based on 1990-2019 reference data from 28 apple growing counties in Shaanxi, China. The models showed increased yield under more severe climate change scenarios (by 2.43-2.78 t/ha). They concluded that the positive effects of climate change were greater than the negative effects. Conversely, 24 years of reference data (1985-2009) from Kullu Valley, India, indicated that a rise of 1.2°C was associated with yield losses of 0.4 t/ha (Sen et al., 2015).

It is clear from the literature that climate change induced temperature patterns will be highly influential on yield-determining parameters of apple. However, the overall combined impact on fruit yield in the field in the UK is unclear. This chapter addresses whether varied seasonal temperature through application of sustained modified temperature field environments have a significant impact in determining apple fruit yield across a range of diverse cultivars. The following null hypotheses were tested:

H_0 - Increase in seasonal temperature does not influence apple fruit yield per tree, total fruit per tree or mean fruit weight per tree.

H_0 - Diverse cultivars studied showed the same response to temperature.

3.2 Materials and Methods

The modified environments and cultivars studied are described in Chapter 2. For consistency, much of the raw data collection related to fruit yield replicates methodology from Lane (2022). Yield parameters were measured and recorded across all cultivars (14 between 2017-19; 20 between 2020-22). Agronomic practices that may have potentially impacted yield were applied to all treatments within a similar timeframe to avoid creating management bias, including winter and summer tree pruning, pesticide application, tree planting and more. Fruitlet thinning occurred each year in June (post-petal fall) to coincide with natural 'June drop'. Trees were thinned to two fruitlets per cluster across all treatments and cultivars to replicate commercial standards. In high-bearing trees, entire clusters were removed. Thinning aimed to achieve 120-150 fruitlets per tree (estimated by eye). Priority in the fruitlet thinning workflow was given to early cultivars in warmer treatments to ensure thinning occurred at an optimal time.

3.2.1 Data collection

The methods used to determine yield and yield components for this study are described in Chapter 2.3.

Prior to harvest, sample fruits from each temperature treatment and cultivar were assessed for Starch Index Score (SIS). Fruit were harvested from each cultivar - treatment combination at the 'tree ripe' stage (50% SIS). This was the same across all cultivars and years as a standard. Harvest date was when 50% SIS was achieved. Harvesting of fruit occurred in one round of picking. Data on total fruit number, total fresh weight and mean individual fruit weight were collected for every tree.

Modified environment data utilised for this study (temperature and rainfall) was described in Chapter 2.4. Where gaps in data were present, these were replaced as set out in Chapter 2.4.3.

3.2.2 Analysis assumptions

Analysing the direct impact of temperature on apple fruit yield within a field environment was challenging given complex environmental variable interactions. It is highly feasible that environmental variables not incorporated within the analysis may have been influential on determining yield outputs.

As mentioned in Chapter 2, comparison of varied rainfall treatments (ambient, +20%, -20%) was not incorporated into the data analysis. Instead, inter-year variation of total ambient seasonal rainfall (80% flowering date to harvest date) was included in multiple linear regression models alongside seasonal temperature.

It was assumed that pollination services did not vary among the temperature treatments. Evidence for this is provided in Appendix 2.6, where a study in 2021 concluded little variation in percentage fruit set between the three temperature regimes.

It was assumed that there was little difference in winter chill accumulation between treatments in all years. Evidence for this was provided in Lane (2022), where a study in 2019 concluded 10 out of 12 cultivars received adequate winter chill units across all treatments. Whilst it is possible optimal chill accumulation was not met across all years, similar winter mean and minimum temperatures (Table 2.3) would likely mean few differences between treatments.

Whilst the majority of trees of 15 cultivars were planted in 2013, new tree plantings were incorporated throughout the duration of the six-year investigation to fill gaps where other cultivars failed to establish or were diseased. For these cultivars, newly-planted trees were not incorporated in to the yield analysis until fruit-bearing maturity was reached (typically 3-4 years). Five extra cultivars (‘Beverly Hills’, ‘Braeburn’, ‘Granny Smith’, ‘Tropical Beauty’ and ‘Winter Banana’) were transplanted in 2018/19. Data collection for these cultivars was initiated in 2020. Trees with substantial pest infestation (typically *Eirosoma lanigerum* or *Dysaphis plantaginea*) or other diseases (those with symptoms synonymous with canker infections) were omitted from the analyses.

3.2.3 Statistical analysis

The data analysis was split in to two sections. First, differences in yield parameters between temperature treatments were determined. Secondly, relationships were studied between seasonal temperature and yield, with key cultivars identified as showing high yield sensitivity from increased seasonal temperature.

Yield parameters were subject to Analysis of Variance (ANOVA). The unit of observation was the tree. Observations were grouped by factors year (n=6), temperature regime (n=3) and cultivar (n=14-20, dependent on year).

Standardised fresh weight yield values for each tree were calculated for each cultivar (Z) based on its six-year range of results. These standardised datasets permitted cross-cultivar analysis. This was conducted using the following equation:

$$Z = \frac{x - \mu}{\sigma}$$

where x = total fruit fresh weight for a tree (kg), μ = mean cultivar fresh weight (kg) and σ = standard deviation.

Annual cross-cultivar yield (2017-22) comparison was conducted utilising mean standardised fresh weight values. One-way ANOVA compared cross-varietal standardised fresh weight values for each temperature regime and year. Post-hoc Tukey tests determined statistically unique means from across all treatment x year combination.

Mean seasonal temperature (°C) for each cultivar x temperature environment combination was calculated from mean daily temperatures (°C) from 80% flowering date to harvest date.

To identify relationships between modified environment parameters (temperature and rainfall only) and standardised yield, a three-step process was used. First, mean standardised yield (unit of observation = tree) was regressed against mean seasonal temperature for each cultivar, with trendlines grouped by temperature treatment. Secondly, further ANOVA plus paired t-tests were utilised to determine significant differences ($p<0.05$) between temperature treatment trendline gradients of the response of standardised yield to temperature for each cultivar. Thirdly, multiple linear regression models determined significant model weights of four different plant phenology-based temperature parameters on mean standardised fresh weight per treatment x year; 1) 1 January to bud burst, 2) bud burst to 80% flowering 3) 80% flowering to 'mid-season' (halfway date between 80% flowering and harvest date) and 4) 'mid-season' to harvest date. These analyses were performed on cultivars that showed at least slight differences in coefficients from the previous analyses. In addition, total precipitation (mm) within the same four phenological phases were incorporated in the model to determine if there was a significant effect of inter-annual variation in rainfall on fresh weight.

All data analysis was carried out using RStudio v2022.10.0. All work was conducted using packages "agricolae", "correlation", "emmeans", "ggplot2", and "ggRmisc".

3.3 Results

3.3.1 Yield parameter results overview

3.3.1.2 Harvested apple fresh weight

Six years of yield output data from this investigation showed significant differences (p<0.001) (Appendix 3.1) in apple fresh weight yield per tree (kg) between temperature treatments, cultivars, and year (Figure 3.1). Fruit yields varied greatly, between nil (e.g. 'Fuji' in all temperature regimes in 2018) to 31.3kg per tree ('Golden Delicious', 2021, Plus4). 'Beverly Hills', 'Granny Smith', 'Tropical Beauty' and 'Winter Banana' provided low yields throughout, whereas 'Gala' provided the greatest mean yield of 17.7kg per tree. 'Stark's Earliest' provided comparatively consistent yield across the three temperature treatments, varying between 6.5 and 14.9kg per tree. Many cultivars showed great variation in yield between years. For example, 'Fuji' yielded well in 2017, 2019 and 2021, but provided negligible fruit yield in 2018, 2020 and 2022; 'Golden Delicious', 'King of the Pippins' and 'Lappio' also showed higher and lower yields in those odd and even years, respectively.

Note that the 2017 results provided a baseline year before temperature treatments were applied.

The 2018 results provided the first results with the temperature modified. Twelve out of 14 cultivars exhibited joint highest yields in ambient conditions compared to the Plus4 treatment, with eight cultivars producing lower yields in Plus4 (p<0.01). Two cultivars ('Fuji' and 'King of the Pippins') produced low yields across all treatments, with 90-99% less yield in 2018 compared to 2017 baseline results.

Generally, 2019 replicated the 2017 results: comparing specific temperature treatments between years gave similar harvested fresh weight. Three cultivars ('Discovery', 'Stark's Earliest', and 'Yellow Bellflower') produced significantly more yield (p<0.05) in Ambient compared to Plus4. Eight showed no significant difference between Ambient and Plus4 yield. From 2019 onwards, Plus2 yield was generally lower compared to the other two treatments. This is thought to be due to greater seasonal pest pressure from 'woolly apple aphid' (*Eirosoma lanigerum*) and 'rosy apple aphid' *Dysaphis plantaginea*), which proved challenging to mitigate.

Yield results in 2020 typically replicated yield patterns witnessed in 2018: six out of 14 established cultivars produced significantly higher (p<0.05) harvested fresh weight in Ambient than Plus4. Two cultivars ('Lappio' and 'Winter Pearmain') produced significantly more yield (p<0.05) in Plus4 than Ambient. The five additional cultivars from later plantings harvested from 2019-20 onwards provided comparatively low yields, ‘Braeburn’ being the highest yielding of the five.

Yield differences in 2021 were reflective of 2019 yields, however there were a few differences for several cultivars. Five out of 14 originally established cultivars produced significantly greater yields ($p<0.05$) in Plus4 compared to Ambient. Infestations of *D. plantaginea* were especially severe within Plus2. This likely contributed towards 12 cultivars producing significantly less ($p<0.05$) fresh weight compared to Plus4.

Modified temperature environments were removed in early 2022. Despite this, patterns seen in previous years persisted; seven out of 14 original trial cultivars produced significantly higher ($p<0.05$) yields in Ambient compared to Plus4. One (later-established) cultivar (‘Braeburn’) produced significantly higher ($p<0.05$) yields within Plus4 compared to Ambient.

3.3.1.2. Total harvested apple fruit

Mean harvested fruit number per tree varied significantly ($p<0.001$) (Appendix 3.2) between treatments, dependent on cultivar and year (Figure 3.2). Patterns mimicked biennial trends seen in Figure 3.1, with generally higher fruit numbers within 2017, -19, and -21, and lower fruit numbers in 2018, -20, and 22. In the baseline season (2017), most cultivars produced no significant difference in fruit number between tunnels pre-temperature modification. 2017 was the first season where crop load was managed through fruitlet thinning, replicating commercial practice.

In the first two temperature modified production seasons (2018 and 2019), several cultivars produced significantly different ($p<0.05$) fruit quantities. 2018 was a challenging cropping year: several cultivars produced their lowest annual crop across all three treatments. ‘Bramley’s Seedling’, ‘Cox’s Orange Pippin’, ‘Discovery’, ‘Fuji’, ‘King of the Pippins’, ‘Lappio’ and ‘Winter Pearmain’ saw 80-90% reduced crop quantity across all treatments when compared to adjacent year’s harvests (2017 and -19). However, four out of 14 cultivars showed more resilience in Ambient, harvesting more ($p<0.05$) fruit compared to Plus4. These differences were present despite crop load being managed the same way between treatments in both 2017 and 2018. No cultivars showed significant differences in numbers of fruits between the Plus2 and Plus4 treatments. In 2019, two cultivars (‘Discovery’ and ‘Yellow Bellflower’) produced significantly more fruit ($p<0.05$) in Ambient compared to both Plus2 and Plus4. Substantial fruitlet thinning was required to reduce fruit numbers to ~120 fruit at harvest within each treatment. Many cultivars show slight, statistically insignificant reductions in fruit within Plus4.

The subsequent biennial cycle (2020 and 2021) produced more severe differences between treatments. In 2020, four out of 14 originally established cultivars produced fewer fruit in Plus4 compared to Ambient. Three cultivars produced fewer mature fruits fruit in both 2018 and 2020 ('Jonathan', 'Stark's Earliest' and 'Yellow Bellflower'). One cultivar produced significantly fewer ($p<0.01$) fruit in Ambient compared to Plus4. In 2021, insufficient fruitlet thinning in Plus2 and Plus4 treatments produced undesirably high harvested fruit numbers. In extreme cases, certain cultivars had 350+ fruits harvested per tree in Plus4 ('Gala' and 'Golden Delicious'). As such, in eleven out of 14 originally established cultivars significantly more ($p<0.05$) fruit were harvested in Plus4 than Ambient.

2022 repeated 2020 trends albeit more severely; Ambient produced significantly more fruit ($p<0.05$) than Plus4 for six cultivars, and ten cultivars compared to Plus2. Numbers of fruit in Plus2 and Plus4 in 2021 were much greater than the target load after June thinning.

3.3.1.3. Mean individual fruit weight

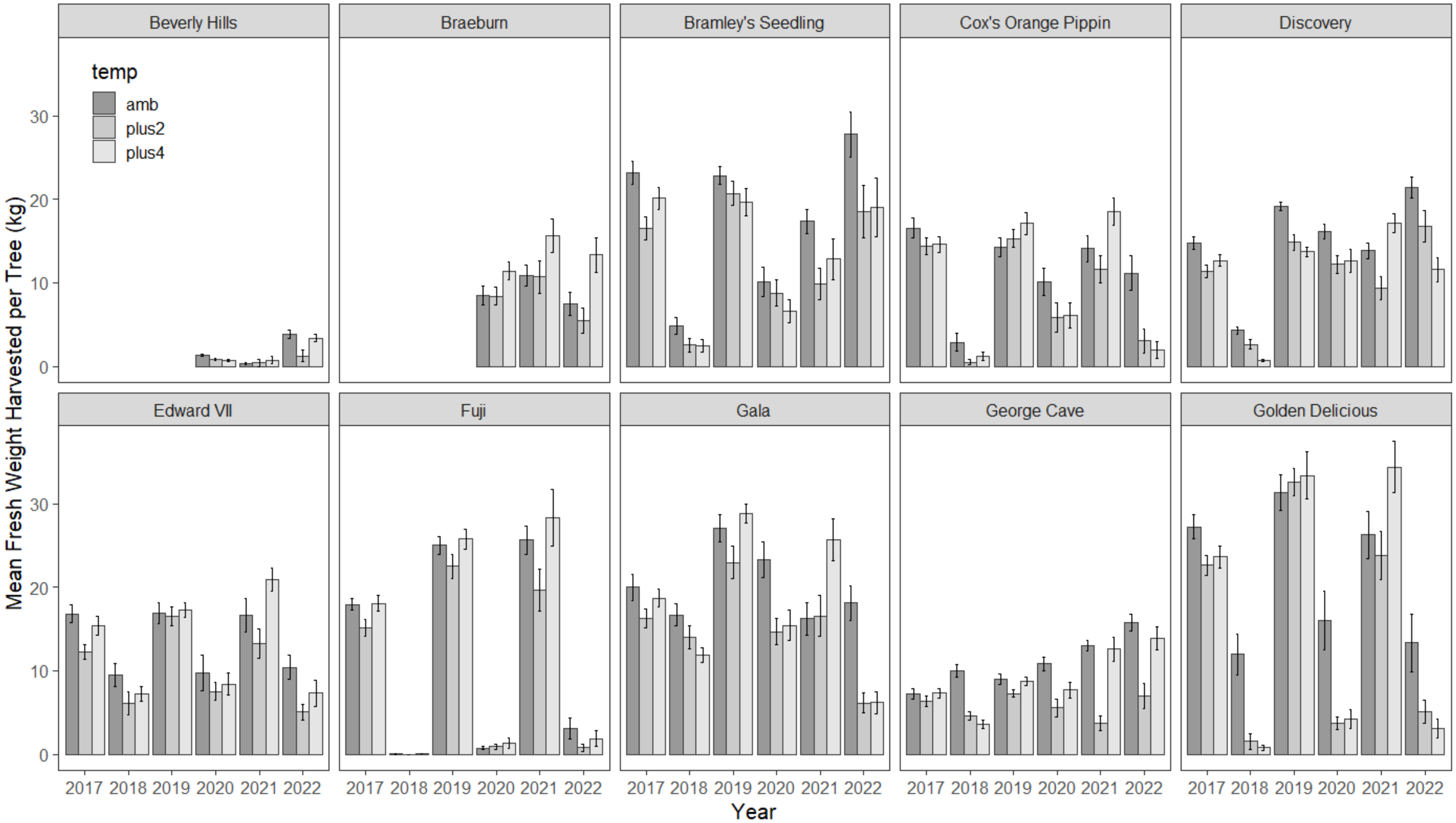
Mean individual fruit weight is the mean harvested fruit fresh weight per tree divided by fruit number per tree. Fruit weight also significantly varied ($p<0.001$) (Appendix 3.3) between treatments, depending on cultivar and year (Figure 3.3).

In 2017 (the baseline year), mean individual fruit weight differed between treatments in eleven out of 14 tested cultivars despite little differences between treatments in other yield parameters.

2018 saw significant treatment differences within several cultivars. Fruit in five out of 14 cultivars had significantly greater ($p<0.01$) mean weight in Plus4 than Ambient. In 2019, fruit was generally heaviest in Plus4 despite comparable fruit loads. Three cultivars saw statistically heavier ($p<0.05$) fruit in Plus4 compared to Ambient.

Fruit weight response to temperature in 2020 was more mixed between cultivars: four saw the greatest ($p<0.05$) fruit weight in Ambient; and two cultivars in Plus4. 'Gala' produced comparable fruit weight between treatments despite 40% less crop load in Plus4.

2021 produced the greatest treatment differences (in which year fruit numbers in Plus4 and Plus2 were many more than in Ambient). Nine cultivars produced significantly heavier ($p<0.05$) fruit in Ambient compared to Plus4.



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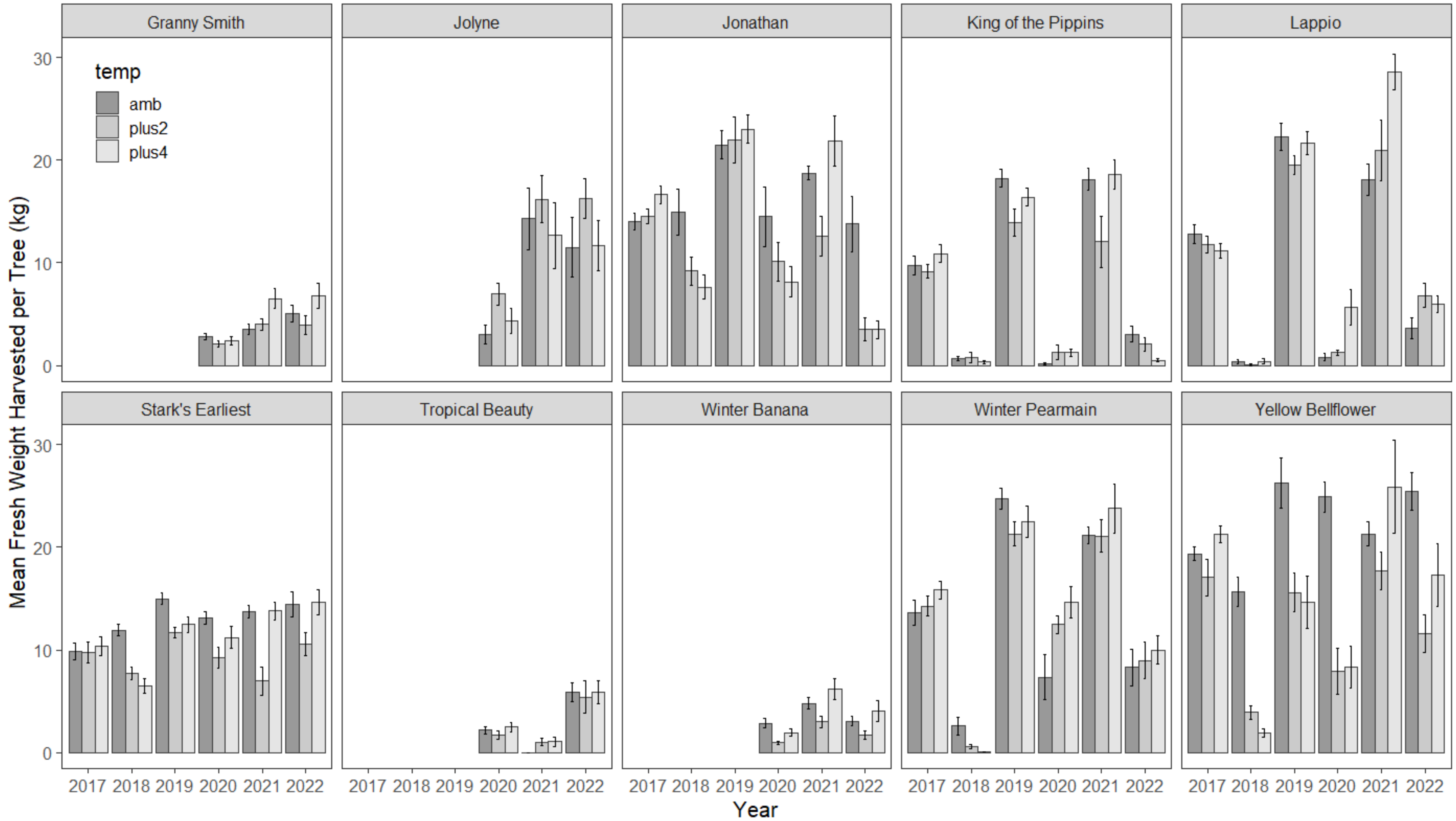
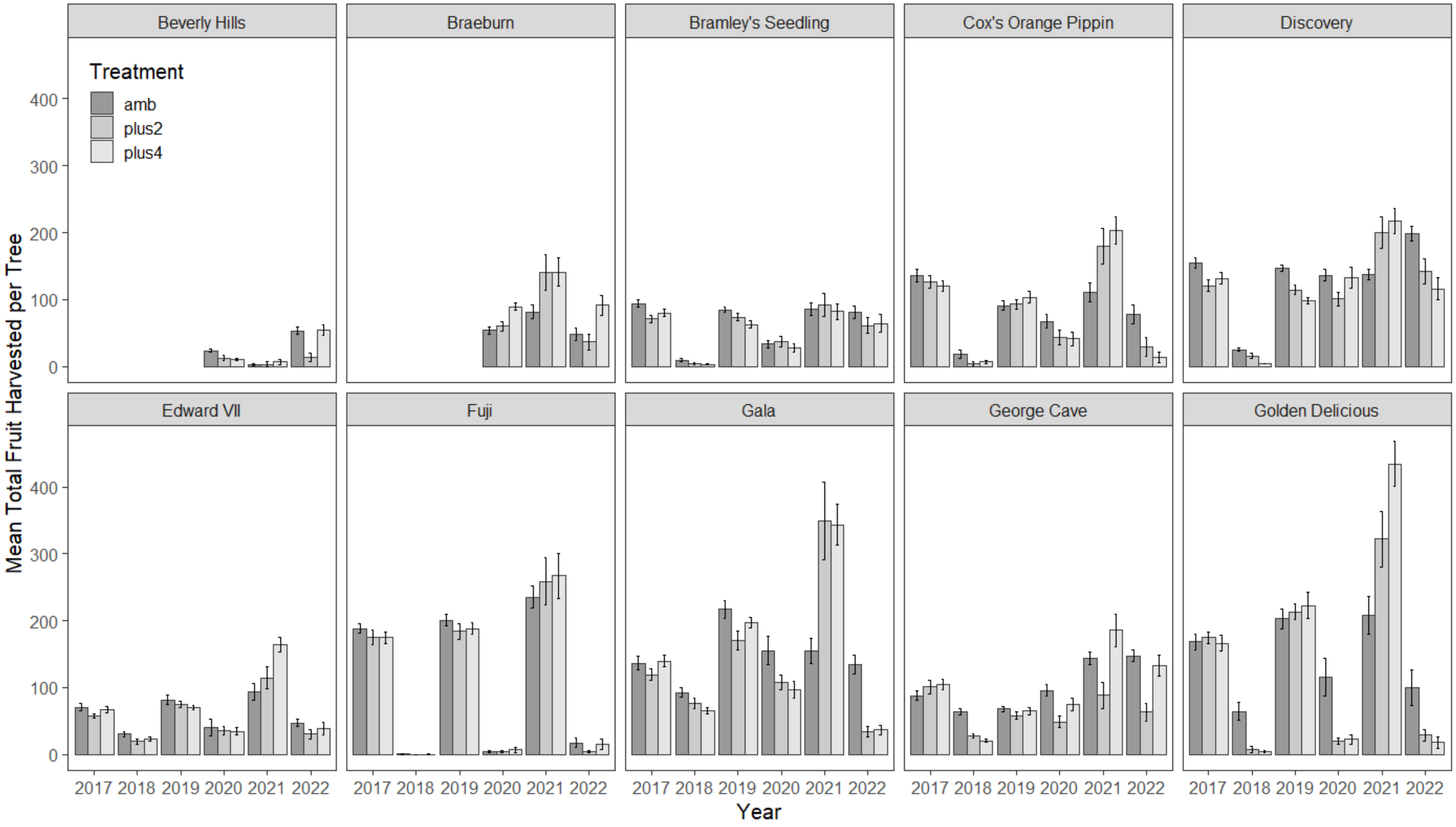


Figure 3.1. *Mean apple fruit fresh weight harvested per tree (kg) (± mean SE) for each cultivar (2017-22) and modified temperature environment (Ambient, Plus2, and Plus4).*



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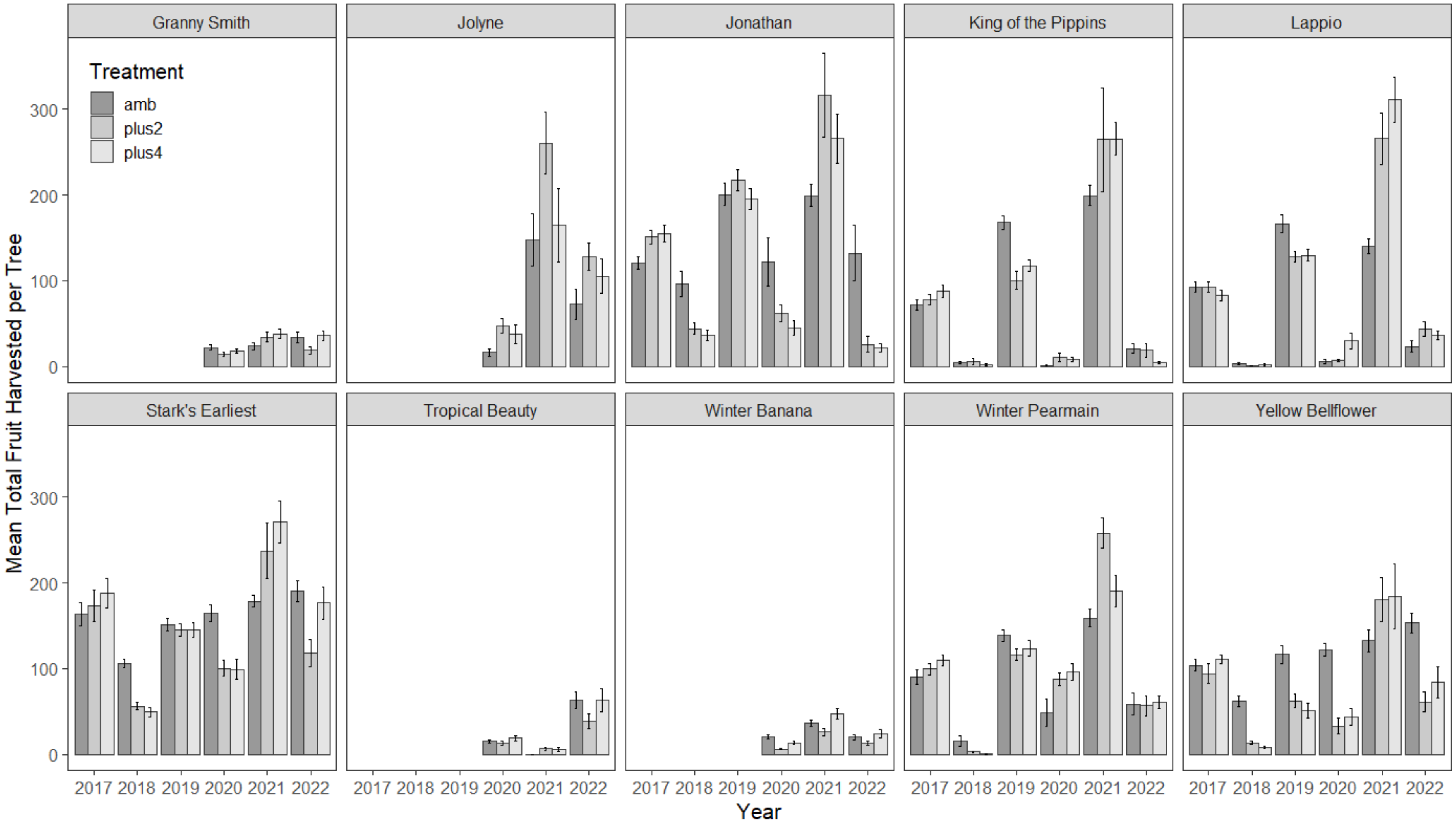
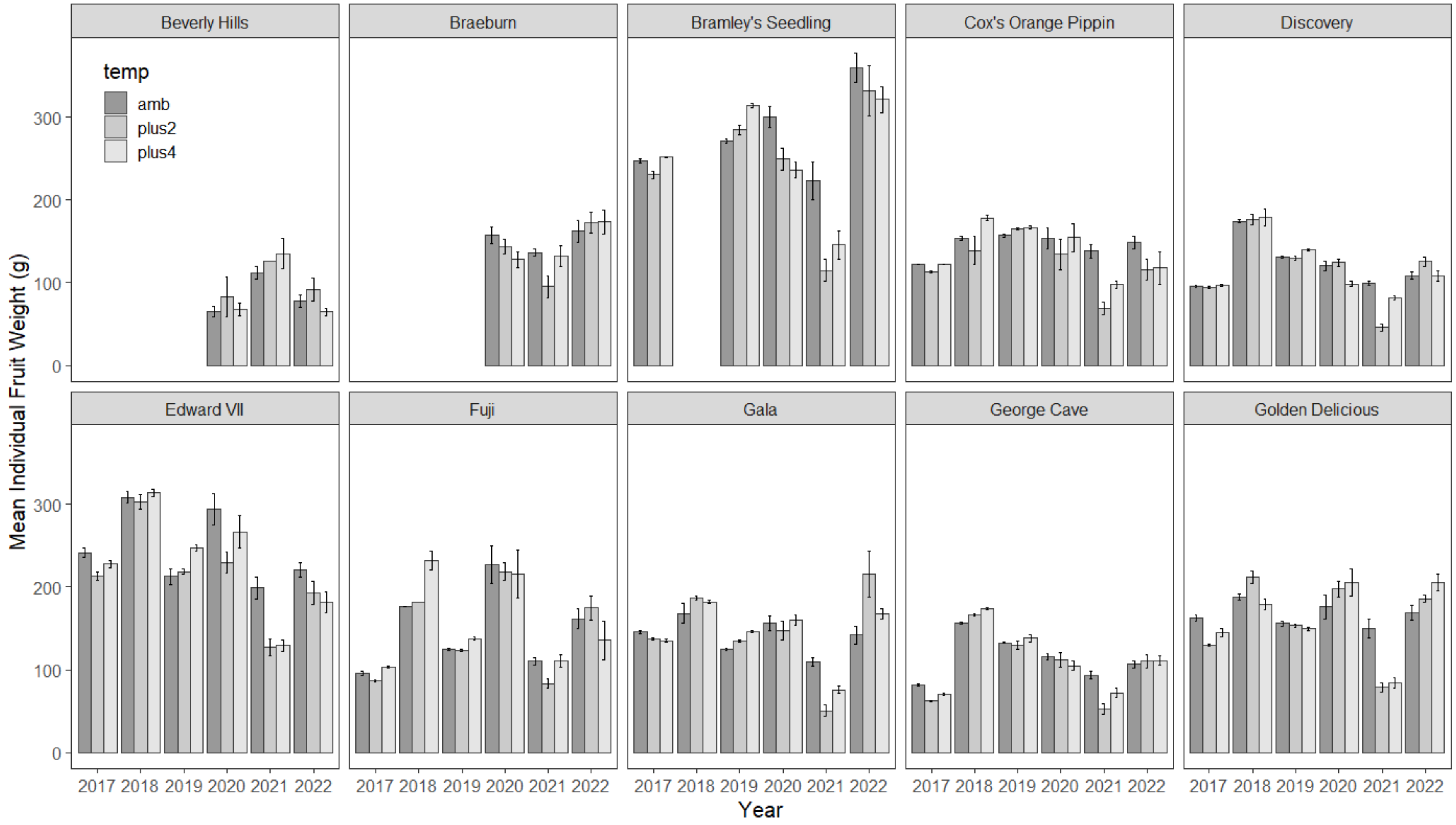


Figure 3.2. *Mean number of apple fruits harvested per tree (± mean SE) for each cultivar (2017-22) and modified temperature environment (Ambient, Plus2, and Plus4).*



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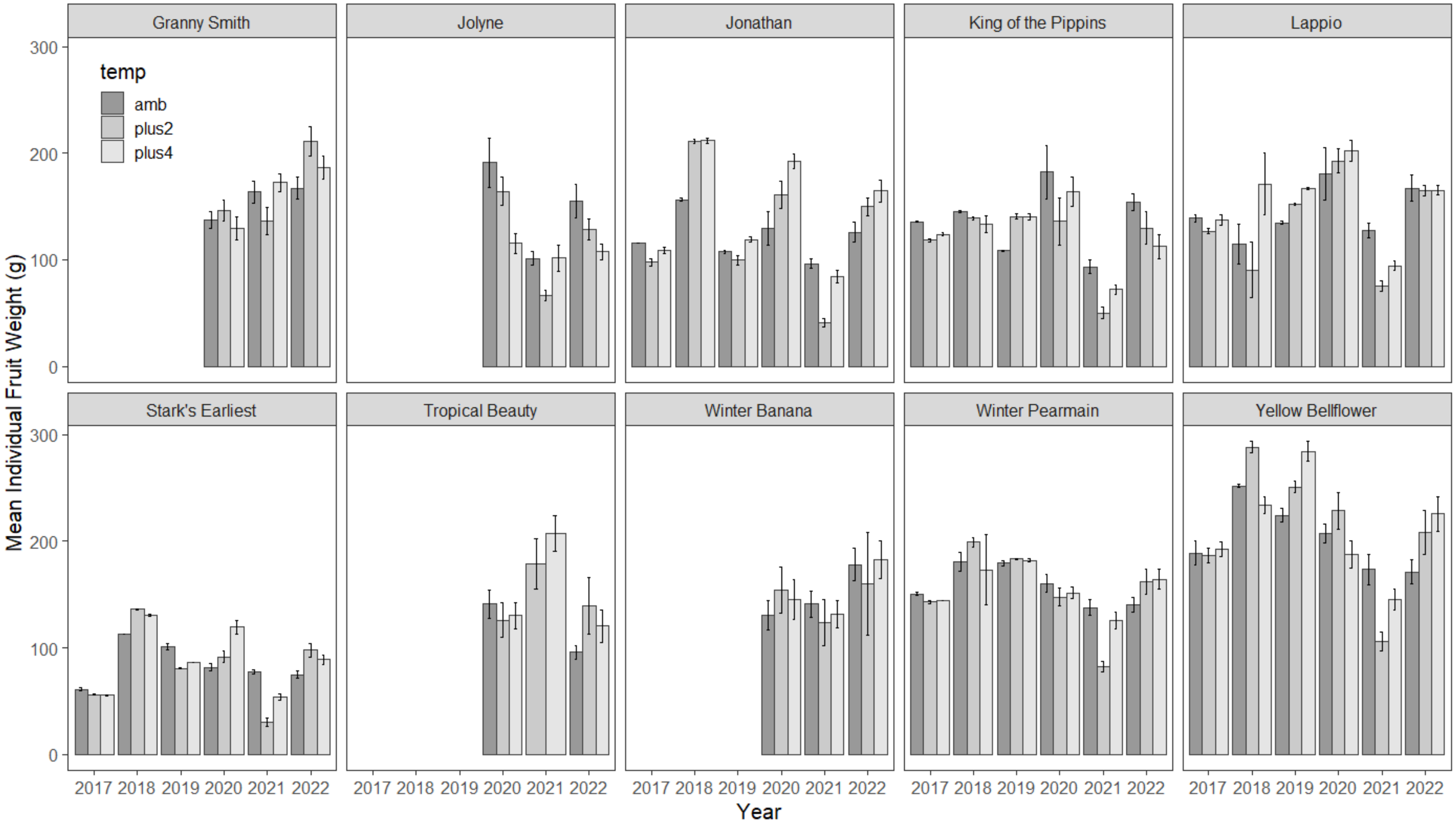


Figure 3.3*. Mean individual apple fruit weight (g) (± mean SE) for each cultivar (2017-22) and modified temperature environment (Ambient, Plus2, and Plus4).*

Overall, the results provided distinct and consistent yield patterns between production seasons (Figure 3.4). Yield values were standardised relative to each cultivar's long-term range from six years of data (2017-2022). This permitted a cross-cultivar comparison of the response of modified temperature environment across all years. Mean standardised harvested fresh weight yield, meaned across all 14-20 cultivars (dependent on year), varied within each year among temperature treatments and among years within temperature treatments (Figure 3.4).

All treatments produced positive standardised yield in the odd years (2017,-19,-21), and negative standardised yield in the intervening years (2018,-20,-22) with the exception of Ambient in 2022. Two-way ANOVA (Appendix 3.4) with post-hoc Tukey tests of each year x treatment combination (n=15) showed that Plus2 standardised yield was lower than Ambient ($p<0.05$) across every production year. Plus4 yield was significantly lower than Ambient across each 'low-yield' production year (2018,-20,-22), whereas mean Ambient and Plus4 yields within 2017 and 2019 did not differ, but Plus4 yield was significantly greater in 2021 (the only production year where this was true). In 2022 (when seasonal temperature was the same between treatments) recorded the only instance where a treatment (Ambient) achieved a mean standardised yield above the mean within an 'off' year. Cultivars in Plus4 also generally achieved greater yields compared to other 'off' years, with significantly greater mean standardised yield than in either 2018 or 2020. The reduced standardised yield differences between 2020 and -22 were likely due to the incorporation of six new cultivars, the majority of which did not vary greatly in yield between years (Figure 3.1).

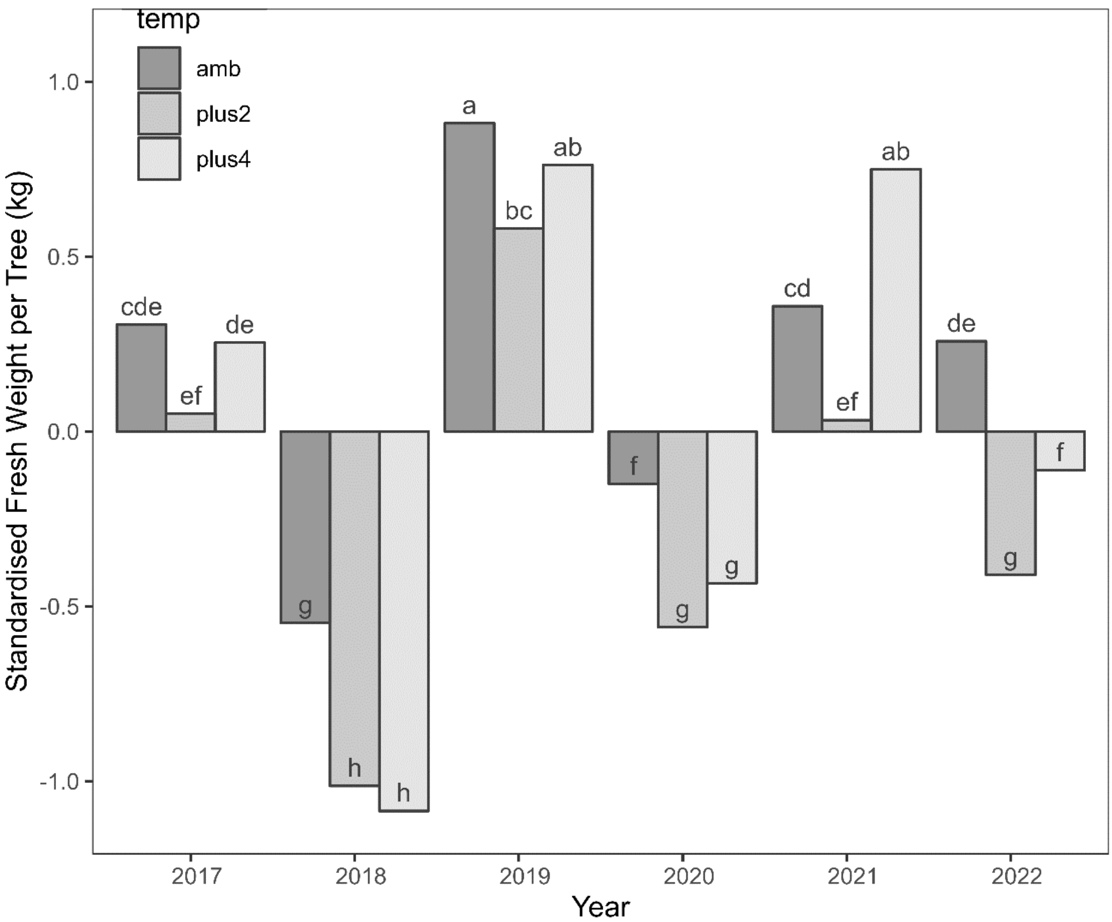


Figure 3.4. *Mean standardised harvested apple fruit yield per tree (kg) for each temperature regime (2017-22) with Tukey's post-hoc tests indicating significant differences between variables (a-g). Each value represents the mean standardised fresh weight of 14-20 cultivars (dependent on year). Standardised yields (z = (x-μ)/σ) were specific for each cultivar's long-term range.*

3.3.2 Analysing the effect of seasonal temperature on apple fruit yield

Linear regression analysis was applied to determine the relationship between mean seasonal temperature (°C) and apple fruit fresh weight harvested per tree (kg) across 20 cultivars from all four modified environment production years combined (Figure 3.5). Data from all treatments were combined to assess the overall relationship. All 15 of the cultivars established at the outset with four years (2018-21) of modified environment yield data showed a significant ($p<0.05$) relationship between variables. The most responsive cultivar was 'Golden Delicious', with a modelled 6.42kg per tree reduction in fruit yield for every 1°C increase in seasonal temperature. The coefficient of determination (R^2) was particularly low across most cultivars despite the significant regressions. For example, 'Lappio' had an R^2 value of 0.04, indicating very high yield variation within tree populations at each seasonal temperature value. The strongest R^2

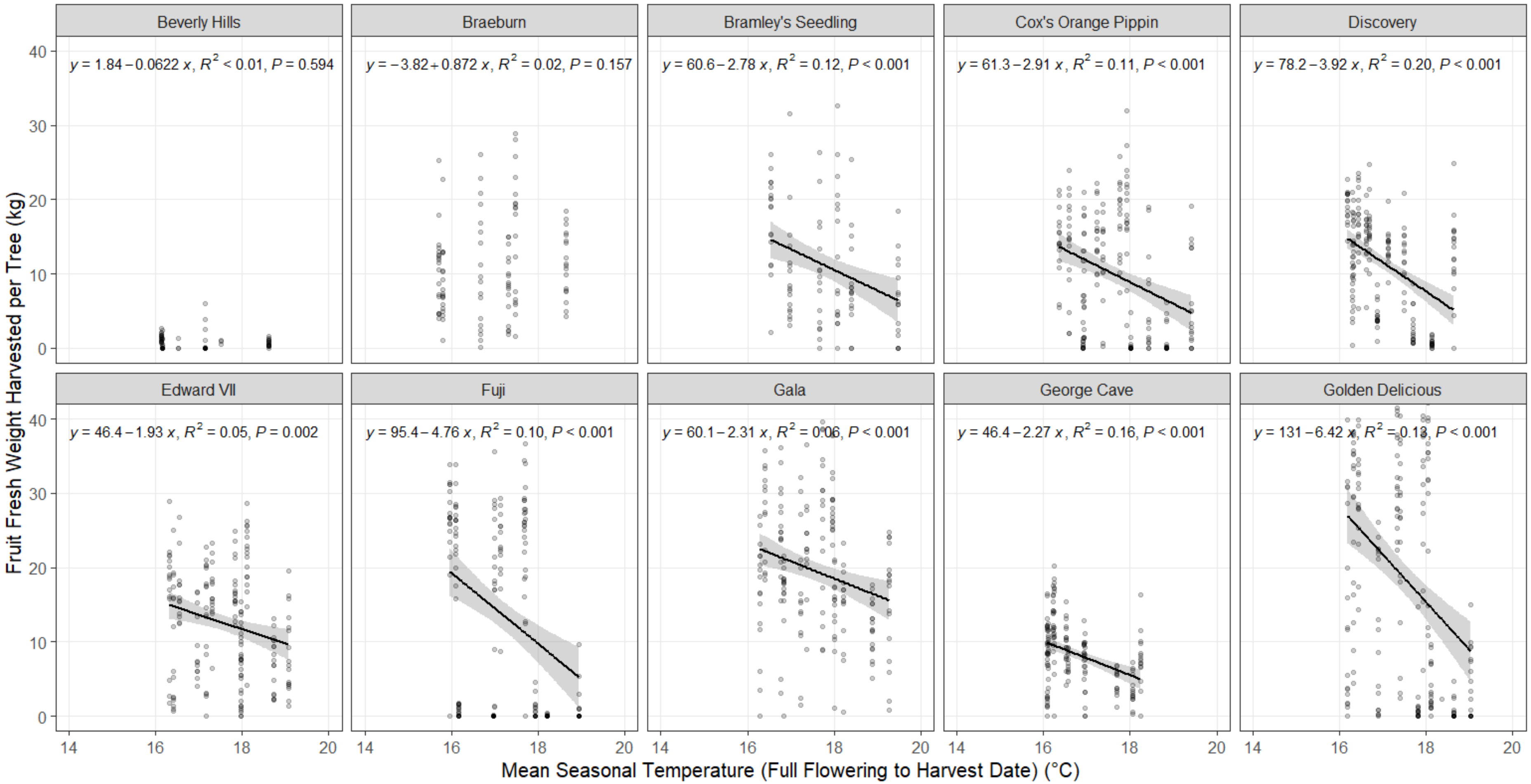
result was seen in 'Yellow Bellflower' at 0.39, indicating potentially the strongest relationship between the two variables out of all cultivars across the four years.

The five remaining cultivars ('Beverly Hills', 'Braeburn', 'Granny Smith', 'Tropical Beauty, and 'Winter Banana') showed no significant relationships between seasonal temperature and yield based on two years (2020-21) of modified environment yield data.

Applying years (2018-21) as independent groups within the regression analysis enabled assessment of how seasonal temperature from each year may have influenced standardised yield for each modified environment production season (Figure 3.6). Overall, a mix of both positive and negative relationships were found, varying among cultivars and also among years within cultivars. 14 out of 20 cultivars expressed a significant ($p<0.05$) relationship between variables. Out of a possible 64 regressions, 16 were negative ($p<0.05$) and six were positive ($p<0.05$). The majority of negative relationships were found in 2018 and 2020, and positive in 2019 and 2021. Three cultivars ('Discovery', 'Stark's Earliest', and 'Yellow Bellflower') produced negative ($p<0.05$) relationships in (2018 and 2019). Two cultivars ('Bramley's Seedling' and 'Stark's Earliest') produced exclusively negative responses. One cultivar ('Braeburn') produced exclusively positive responses. One cultivar ('Fuji') showed very little variation between years in the response slope to seasonal temperature (but yield differed among years). The alternation in standardised yield for the mean of all cultivars between positive in odd years (2017,-19,-21) and negative in even years (2018,-20,-22) (Fig. 3.4) and the high variation in the response of standardised yield to temperature in many cultivars (Fig. 3.5) reflected diverse responses to temperature within several cultivars among the years (Fig. 3.6). For example, 'Cox's Orange Pippin', 'Gala', 'Golden Delicious', and 'Jonathan' showed positive responses in 2019 and 2021, but negative in 2018 and 2020.

Applying modified temperature treatments as independent groups within the regression analysis enabled assessment of whether coefficients between seasonal temperature and standardised fresh weight differed among the three treatments (Figure 3.7). Relationships were overwhelmingly negative across all temperature treatments: 43 out of a possible 57 trendlines show a significant ($p<0.05$) negative relationship between seasonal temperature and yield. The occurrence of significant relationships differed between treatments. In Ambient, eleven out of 19 cultivars showed significant relationships, compared to 17 out of 19 cultivars for Plus4. The two cultivars which did not show a relationship in Plus4 were 'Beverly Hills' and 'Braeburn'. The six cultivars that produced a significant relationship in Plus4, but not in Ambient, were 'Edward VII', 'Gala', ‘George Cave’, ‘Golden Delicious’, ‘Granny Smith’, ‘King of the Pippins’, and ‘Stark’s Earliest’.

Temperature ranges within Ambient generally varied less than within Plus4, and regression slopes were often more severe in Ambient compared to Plus2 and Plus4. For example, ‘Discovery’ had a slope of -2.32 kg per tree in fruit yield per 1°C in Ambient, compared to -0.63 in Plus4 (Figure 3.5). However, R^2 values were generally stronger in Plus4 regressions, indicating the linear models were better at explaining the variance in Plus4 compared to Ambient.



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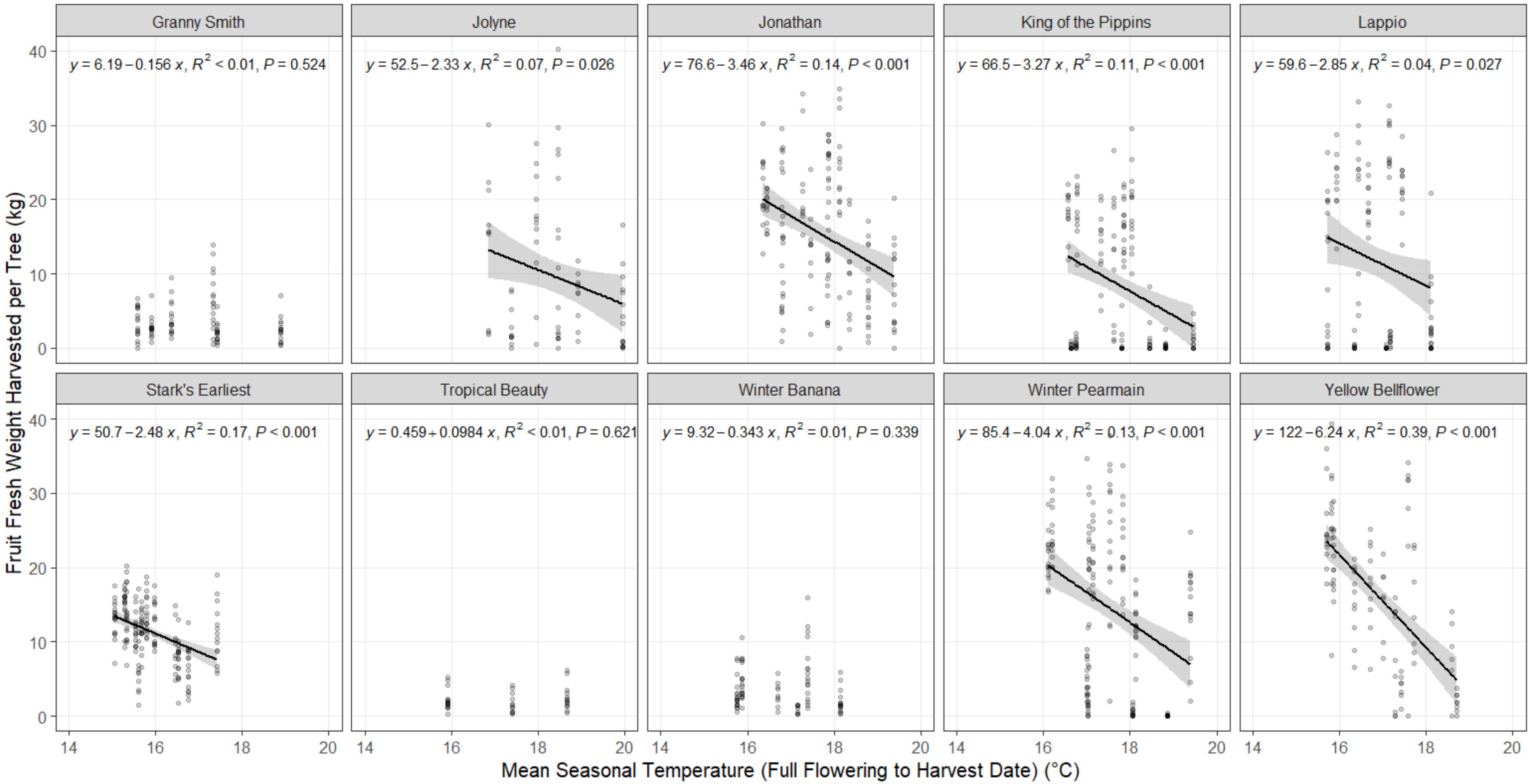
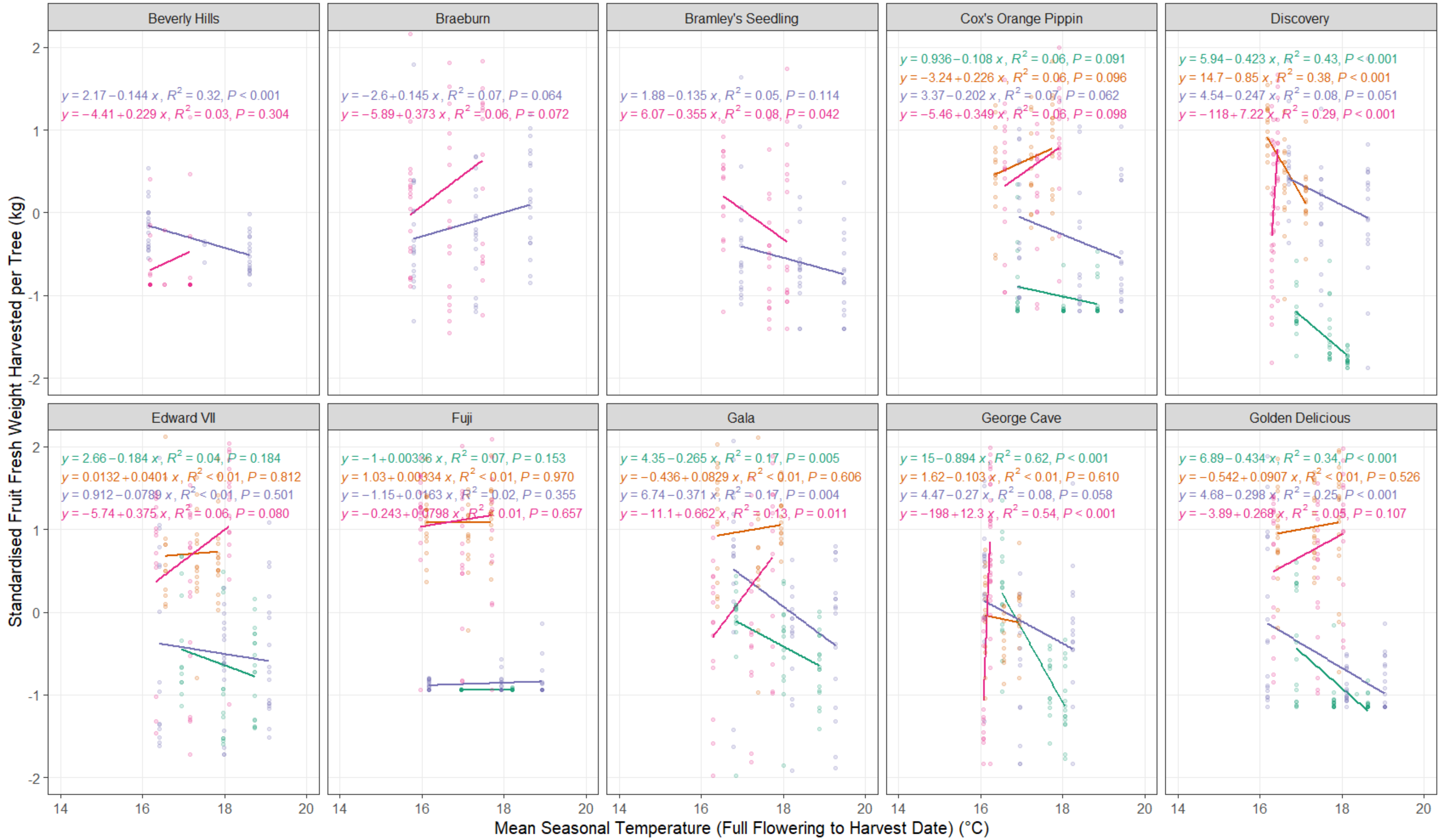


***Figure 3.5**. Linear regressions of the effect of mean seasonal temperature (°C) on standardised apple fruit fresh weight harvested per tree (kg) across 20 apple cultivars (2018-21). Each point represents the value for one tree in a production season. The grey shading indicates mean ± standard error.*



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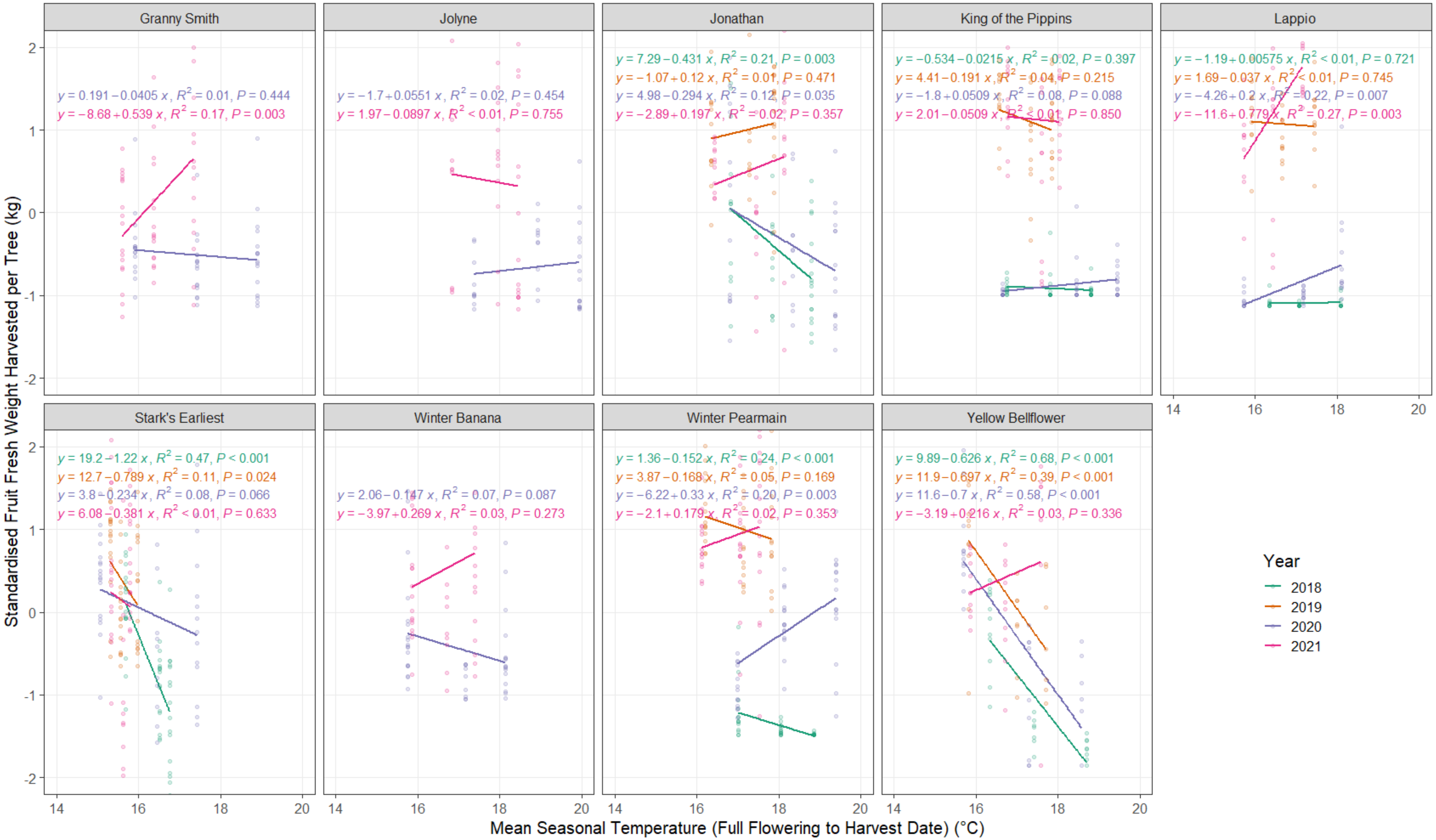
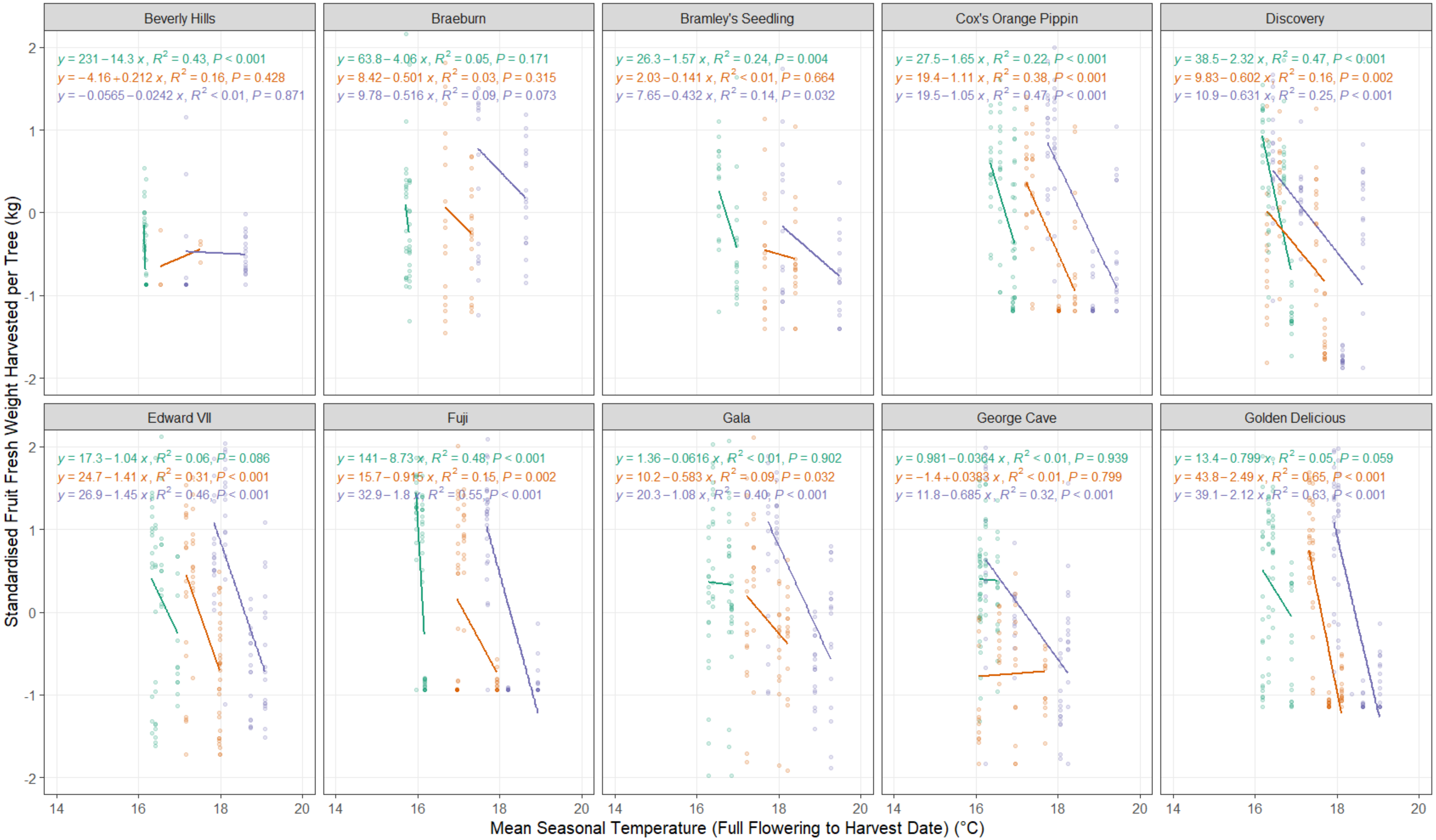


***Figure 3.6**. Linear regression analysis of the effect of mean seasonal temperature (°C) on standardised apple fruit fresh weight harvested per tree (kg) across 20 apple cultivars for each of the production seasons with modified temperature environments (2018-21).*



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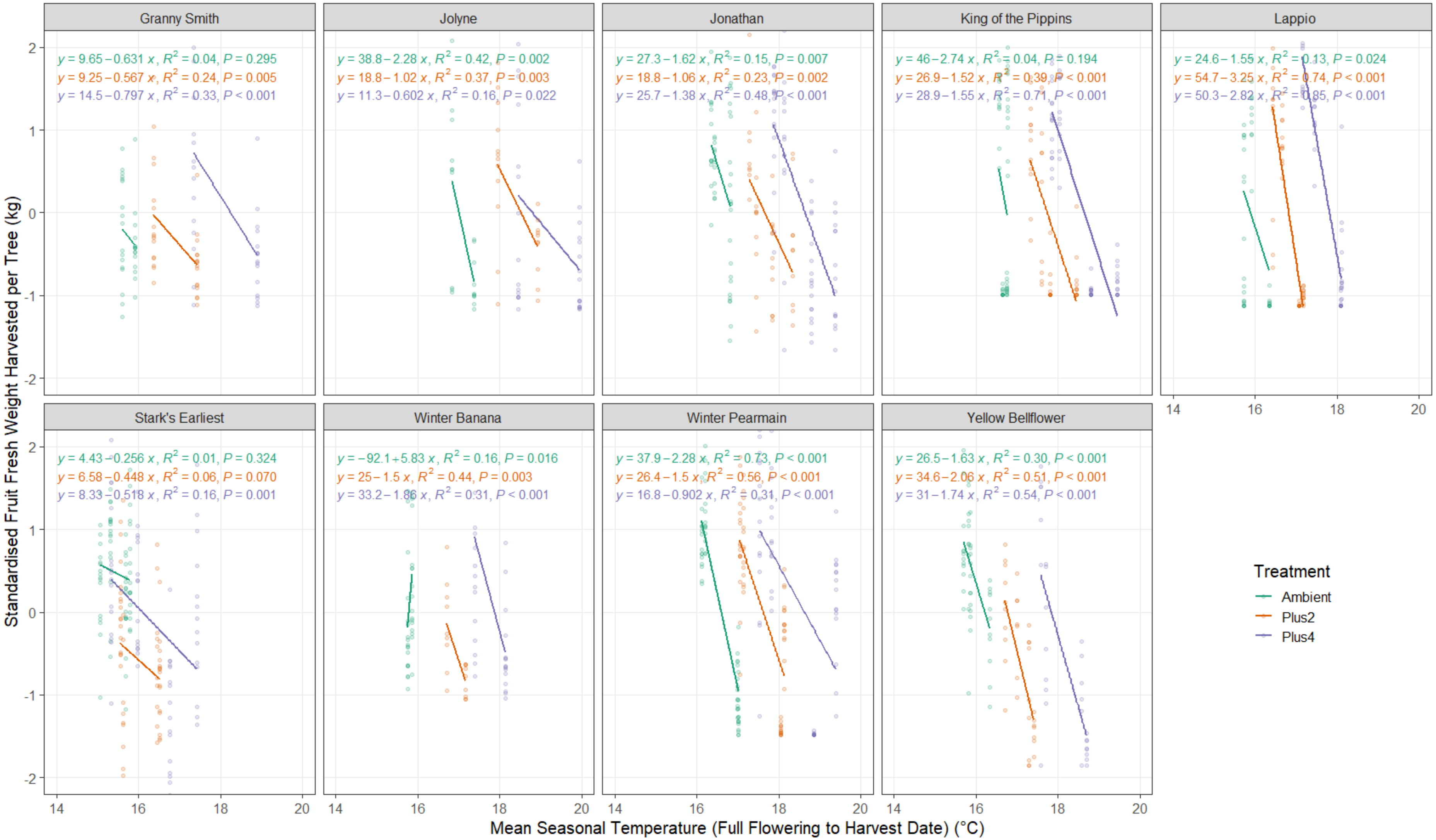


***Figure 3.7**. Linear regressions of the effect of mean seasonal temperature (°C) on standardised fruit fresh weight harvested per tree (kg) across 20 cultivars (2018-21) for each temperature regime (Ambient, Plus2, and Plus4). Each point represents the value for one tree in a production season.*

The use of ANOVA and paired t tests identified significant differences in the slopes of regression models listed in Figure 3.7. Several cultivars were identified as having significantly ($p<0.05$) shallower slopes in Ambient than in the Plus2 and Plus4 temperature treatments (Table 3.1). These cultivars were ‘Golden Delicious’, ‘Lappio’, and ‘Winter Banana’. The strongest difference between Ambient and warmer environment slopes was seen in ‘Golden Delicious’, with t ratios of 3.71 (Amb-Plus2) and 3.27 (Amb-Plus4). Four cultivars (‘Beverly Hills’, ‘Discovery’, ‘Fuji’, and ‘Winter Pearmain’) had steeper regression slopes in Ambient compared to Plus2 and Plus4. None of the remaining cultivars showed significant differences in regression slopes between Ambient and warmer environments, but the non-significant positive t-ratios in seven of them (e.g., ‘Gala’ Amb – Plus4) indicated possible minor differences in slope between treatments.

Multiple linear regression (MLR) analysis was applied to determine yield response to temperature for four specific phenological periods within seasons. Based on findings from Table 3.1, yield results from three cultivars (‘Golden Delicious’, ‘Lappio’ and ‘Winter Banana’) were utilised for regression analysis based on significant treatment differences in regression coefficients between seasonal temperature and standardised fresh weight. Specifically, Plus4 regression slopes were significantly more negative than Ambient for these three cultivars, indicating greater yield sensitivity in response to warmer temperature compared to other cultivars where coefficients do not differ between treatments. The overall model was relatively successful ($f=15.4$, adj. $R^2=0.89$, $p<0.001$) (Table 3.2). However, the results indicated that three out of four temperature variables contributed no significant model weight ($p>0.05$) in determining harvested fresh weight. The two significant results ($p<0.05$) indicated that raised mean temperature within the final months of fruit development contributed towards a decrease in yield ($t=-3.84$, $p=0.002$). Additionally, lower minimum temperatures were also related to reduced yield within this phase of fruit development ($t=3.90$, $p=0.002$). Similarly, total precipitation (mm) during late fruit development also contributed significantly ($p<0.05$) towards model weight ($t=-2.49$, $p=0.03$).

The MLR analysis was applied to an expanded pool of cultivars (Table 3.3). The seven cultivars selected were those which provided positive t ratios, whether or not significant, between Ambient and warmer environment treatments in Table 3.1 because all these cultivars indicated greater yield sensitivity to increased seasonal temperature. It also increased the sample size from 30 to 84.

This amended MLR model (f=19.4, adj. R^2=0.61, p<0.001) showed that temperature across three of the four phases (1st January to bud burst, full flowering to 'mid-season', and 'mid-season' to harvest) contributed significant model weights (p<0.05) (Table 3.3). All three temperature parameters (Tmean, Tmin, and Tmax) had a significant influence on yield; Tmean (t=3.10, p=0.003) provided a positive t value, whereas Tmin (t=-2.45, p=0.015) and Tmax (t=-3.17, p=0.002) t values were negative. Tmean from full-flowering to 'mid-season' (t=-2.36, p=0.019) negatively influenced yield, whereas as Tmax (t=2.18, p=0.030) positively influenced yield. Likewise, Tmean from 'mid-season' to harvest (t=-2.69, p=0.008) was also negatively associated with yield, whereas Tmin (t=3.39, p<0.001) within this period was positively influential. Precipitation from three time periods – 1st January to bud burst (t=-2.87, p=0.005), full flowering to 'mid-season' (t=3.45, p<0.001), and 'mid-season' to harvest (t=-2.49, p=0.004) had significant effects on yield (negative, positive, negative, respectively). No modified environment parameters from bud burst to full flowering contribute significantly to the model.

Table 3.1. *Comparison of linear regression slopes (b) from Figure 3.5 for 19 cultivar x temperature treatment (Ambient, Plus2, Plus4) combinations using ANOVA and paired t-tests. Significant (p<0.05) positive t ratios (bold) indicate potential difference in yield response to temperature in Plus4 compared to Ambient.*

Cultivar	Treatment Contrast	b	se	df	t ratio	P
Beverly Hills	Amb – Plus2	-14.531	4.402	72	-3.301	0.004**
	Amb – Plus4	-14.295	4.383	72	-3.261	0.005**
	Plus2 – Plus4	0.236	0.436	72	0.542	0.851
Braeburn	Amb – Plus2	-3.554	3.549	101	-1.001	0.578
	Amb – Plus4	-3.540	3.528	101	-1.003	0.577
	Plus2 – Plus4	0.014	0.527	101	0.027	0.999
Bramley's Seedling	Amb – Plus2	-1.433	0.634	92	-2.261	0.067
	Amb – Plus4	-1.141	0.570	92	-2.003	0.117
	Plus2 – Plus4	0.292	0.372	92	0.784	0.714
Cox's Orange Pippin	Amb – Plus2	-0.540	0.432	183	-1.249	0.426
	Amb – Plus4	-0.598	0.404	183	-1.481	0.302
	Plus2 – Plus4	-0.058	0.244	183	-0.239	0.969
Discovery	Amb – Plus2	-1.718	0.422	186	-4.071	<0.001***
	Amb – Plus4	-1.688	0.400	186	-4.226	<0.001***
	Plus2 – Plus4	0.029	0.217	186	0.135	0.9901
Edward VII	Amb – Plus2	0.375	0.581	178	0.645	0.795
	Amb – Plus4	0.412	0.544	178	0.757	0.730
	Plus2 – Plus4	0.037	0.367	178	0.101	0.994
Fuji	Amb – Plus2	-7.810	1.424	172	-5.495	<0.001***
	Amb – Plus4	-6.930	1.411	172	-4.903	<0.001***

Cultivar	Treatment Contrast	b	se	df	t ratio	P
	Plus2 – Plus4	0.890	0.340	172	2.617	0.026*
Gala	Amb – Plus2	0.521	0.544	177	0.958	0.605
	Amb – Plus4	1.023	0.494	177	2.073	0.098
	Plus2 – Plus4	0.502	0.331	177	1.520	0.284
George Cave	Amb – Plus2	-0.074	0.545	165	-0.137	0.990
	Amb – Plus4	0.648	0.529	165	1.226	0.440
	Plus2 – Plus4	0.723	0.207	165	3.492	0.002
Golden Delicious	Amb – Plus2	**1.688**	**0.456**	**204**	**3.706**	**<0.001*** **
	Amb – Plus4	**1.322**	**0.404**	**204**	**3.272**	**0.004** **
	Plus2 – Plus4	-0.366	0.354	204	-1.035	0.555
Granny Smith	Amb – Plus2	-0.064	0.795	91	-0.081	0.996
	Amb – Plus4	0.166	0.776	91	0.214	0.975
	Plus2 – Plus4	0.230	0.280	91	0.821	0.691
Jolyne	Amb – Plus2	-1.264	0.831	68	-1.521	0.288
	Amb – Plus4	-1.679	0.765	68	-2.195	0.079
	Plus2 – Plus4	-0.415	0.439	68	-0.947	0.613
Jonathan	Amb – Plus2	-0.554	0.659	146	-0.841	0.678
	Amb – Plus4	-0.240	0.600	146	0.400	0.916
	Plus2 – Plus4	0.314	0.369	146	0.853	0.671
King of the Pippins	Amb – Plus2	-1.227	1.583	150	-0.775	0.719
	Amb – Plus4	-1.195	1.563	150	-0.765	0.725
	Plus2 – Plus4	0.031	0.359	150	0.087	0.996
Lappio	Amb – Plus2	**1.699**	**0.592**	**121**	**2.869**	**0.013***
	Amb – Plus4	**1.276**	**0.536**	**121**	**2.382**	**0.049***
	Plus2 – Plus4	-0.424	0.454	121	-0.933	0.621
Stark’s Earliest	Amb – Plus2	0.192	0.411	178	0.466	0.887
	Amb – Plus4	0.261	0.359	178	0.727	0.748
	Plus2 – Plus4	0.070	0.272	178	0.257	0.964
Winter Banana	Amb – Plus2	**7.337**	**2.700**	**81**	**2.718**	**0.021***
	Amb – Plus4	**7.691**	**2.584**	**81**	**2.977**	**0.011***
	Plus2 – Plus4	0.354	0.957	81	0.370	0.928
Winter Pearmain	Amb – Plus2	-0.782	0.324	167	-2.410	0.045*
	Amb – Plus4	-1.380	0.284	167	-4.853	<0.001***
	Plus2 – Plus4	-0.598	0.248	167	-2.411	0.045*
Yellow Bellflower	Amb – Plus2	0.426	0.584	101	0.730	0.746
	Amb – Plus4	0.105	0.477	101	0.220	0.974
	Plus2 – Plus4	-0.322	0.489	101	-0.658	0.788

Key: b = slope, se = mean standard error, df = degrees of freedom, t ratio = difference between sample means divided by the standard error of the difference of two treatment groups. Key for significance: * p<0.05, ** P<0.01, *** p<0.001, otherwise NS (p>005)

***Table 3.2**. Multiple linear regression results detailing significant model weights of the effects of four developmental phases and four weather variables (mean, minimum and maximum temperature, and total precipitation) on standardised mean apple fruit fresh weight (kg) for the combined results of three cultivars ('Golden Delicious', 'Lappio' and 'Winter Banana'), across three modified temperature environments between 2018 and 2021 (n=30). Cultivars were selected based on significant findings from Table 3.1. Final model residuals are inclusive of variables with p values of <0.1 only.*

Development Phase	**Weather Variable**	**Coefficients**			
		b	**se**	**t**	**P**
	Constant	-0.865	9.660	-0.09	0.930
1st Jan to Bud Burst	Tmean (°C)	1.284	1.779	0.721	0.483
	Tmin (°C)	-1.206	1.292	-0.934	0.367
	Tmax (°C)	0.248	0.486	0.510	0.619
	Total Ppt (mm)	-0.007	0.007	-1.035	0.319
Bud Burst to Full Flowering	Tmean (°C)	-1.616	1.570	-1.029	0.322
	Tmin (°C)	0.834	0.876	0.953	0.358
	Tmax (°C)	0.685	0.633	1.081	0.299
	Total Ppt (mm)	0.001	0.010	0.106	0.917
Full Flowering to 'Mid-Season'	Tmean (°C)	2.021	1.427	1.416	0.180
	Tmin (°C)	-1.241	0.816	-1.521	0.152
	Tmax (°C)	-0.921	0.700	-1.316	0.211
	Total Ppt (mm)	**0.019**	**0.007**	**2.674**	**0.019***
'Mid-season' to Harvest	Tmean (°C)	**-4.205**	**1.095**	**-3.840**	**0.002****
	Tmin (°C)	**3.465**	**0.889**	**3.899**	**0.002****
	Tmax (°C)	1.155	0.572	2.019	0.065
	Total Ppt (mm)	**-0.013**	**0.005**	**-2.485**	**0.027***

Final model residuals: se = 0.760, df = 24, adj. R^2 = 0.340, f = 3.993, p=0.009

Key: b = slope, se = mean standard error, df = degrees of freedom.

Key for significance: * p<0.05, ** p<0.01, *** p<0.001

***Table 3.3**. Multiple linear regression results detailing significant model weights of the effects of four developmental phases and four environmental variables (mean, minimum and maximum temperature, and total precipitation) on standardised mean apple fruit fresh weight (kg) for the combined results of seven cultivars ('Edward VII', 'Gala', 'Golden Delicious', 'Lappio', 'Stark's Earliest', 'Winter Banana', and 'Yellow Bellflower'), across three modified temperature environments between 2018 and 2021 (n=72). Cultivars were selected based on those that had positive t-ratios between treatments in Table 3.1. Final model residuals are inclusive of variables with p values of <0.1 only.*

Development Phase	Weather Variable	Coefficients			
		b	se	t	P
	Constant	1.470	2.164	0.679	0.499
1st Jan to Bud Burst	Tmean (°C)	**1.826**	**0.589**	**3.098**	**0.003****
	Tmin (°C)	**-1.051**	**0.429**	**-2.451**	**0.015***
	Tmax (°C)	**-0.539**	**0.170**	**-3.173**	**0.002****
	Total Ppt (mm)	**-0.008**	**0.003**	**-2.866**	**0.005****
Bud Burst to Full Flowering	Tmean (°C)	0.594	0.585	-1.015	0.311
	Tmin (°C)	-0.496	0.303	-1.635	0.104
	Tmax (°C)	-0.125	0.275	-0.455	0.650
	Total Ppt (mm)	0.001	0.003	0.120	0.904
Full Flowering to 'Mid-Season'	Tmean (°C)	**-0.883**	**0.373**	**-2.364**	**0.019***
	Tmin (°C)	0.221	0.255	0.866	0.388
	Tmax (°C)	**0.408**	**0.187**	**2.178**	**0.030***
	Total Ppt (mm)	**0.009**	**0.002**	**3.545**	**<0.001*****
'Mid-season' to Harvest	Tmean (°C)	**-0.963**	**0.359**	**-2.686**	**0.008****
	Tmin (°C)	**0.692**	**0.204**	**3.389**	**<0.001*****
	Tmax (°C)	0.274	0.185	1.482	0.140
	Total Ppt (mm)	**-0.005**	**0.002**	**-2.956**	**0.004****

Final model residuals: se = 0.598, df = 180, adj. R^2 = 0.598, f = 24.76, p<0.001

Key: b = slope, se = mean standard error, df = degrees of freedom.

Key for significance: * p<0.05, ** p<0.01, *** p<0.001

Additional MLR was undertaken utilising six of the seven cultivars as Table 3.3 (i.e., excluding 'Stark's Earliest'), except this time analysing contrasting model weights of mean temperature from individual months (January to October) on standardised apple fruit yield (Table 3.4). The model (f=19.95, adj. R^2=0.75, p<0.001) identified three out of ten months that contributed significantly to the model: February (t=2.02, p=0.05), July (t=2.71, p=0.009), and August (t=-2.17, p=0.04). The remaining seven months did not contribute significant model weight (p>0.05).

Table 3.4. *Multiple linear regression results detailing significant model weights of the effects of mean temperature (°C) from each month of development from January to October on standardised mean apple fruit fresh weight (kg) for the combined results of six cultivars ('Edward VII', 'Gala', 'Golden Delicious', 'Lappio', 'Winter Banana', and 'Yellow Bellflower'), across three modified temperature environments between 2018 and 2021 (n=84). Cultivars selected all had positive t-ratios between treatments in Table 3.1. NB: The cultivar 'Stark's Earliest' was excluded due to its early seasonality (typically harvested in July). Final model residuals are inclusive of variables with p values of <0.1 only.*

Month	**Coefficients**			
	b	**se**	**t**	**P**
Constant	4.123	4.549	0.906	0.369
January	0.643	0.446	1.444	0.155
February	**1.375**	**0.682**	**2.017**	**0.049***
March	-0.272	0.413	-0.658	0.513
April	0.595	0.404	1.473	0.146
May	-0.537	0.484	-1.109	0.272
June	0.340	0.618	0.550	0.584
July	**0.970**	**0.358**	**2.712**	**0.009****
August	**-1.888**	**0.867**	**-2.171**	**0.036***
September	0.563	0.627	0.898	0.373
October	-0.852	0.823	-1.035	0.305

Final model residuals: se = 0.463, df = 62, adj. R^2 = 0.708, f = 50.13, p<0.001

Key: b = slope, se = mean standard error, df = degrees of freedom.

Key for significance: * p<0.05, ** p<0.01, *** p<0.001

3.4 Discussion

Results from this chapter elaborate further on findings from Lane (2022) on the effects of modified temperature environments on apple fruit yield. First, the results identified consistent differences in apple fruit yield (kg), fruit number per tree, and mean fruit weight between temperature treatment and year, dependent on the cultivar (Figures 3.1 to 3.7). Secondly, the results indicate that seasonal temperature had contrasting effects on yield parameters, dependent on cultivar (Tables 3.1 and 3.2). Where differences were significant ($p<0.05$), yield response to warmer temperature was more often negative than positive. Therefore, there is sufficient evidence to reject the null hypothesis: hence, increase in seasonal temperature did affect apple fruit yield per tree, total numbers of fruit per tree, and mean fruit weight.

In total, 19 out of 20 cultivars showed at least one significant difference in a parameter of yield between modified temperature environments and year (Appendices 3.1 to 3.3); the exception being 'Tropical Beauty'. This demonstrated high levels of yield variation within the six years of study across almost all cultivars. Given the consistent biennial cycle of yield variation (a 'high-cropping' year, followed by a 'low-cropping' year) across many cultivars and treatments, it is likely that alternate bearing patterns were exhibited throughout the duration of the study. Alternate bearing patterns may have affected all three yield parameters, as yield, fruit number and individual fruit weight are intrinsically linked (Atkinson et al., 1998).

Trends in alternate bearing are consistent throughout the six years of data collection – harvested fresh weight in 'on' years (2017, -19 and -21) were higher, and lower in 'off' years (2018, -20 and -22). Alternate bearing varies in severity by cultivar (Singh 1948). As such, it is impossible to accurately account for the impact of alternate bearing within environment-yield modelling without thorough investigations for individual cultivars. The risk of alternate bearing impacts within the trial was high – traditional cultivars are generally more prone to alternate bearing habits compared to more modern ones bred from the 20th Century onwards (Jonkers 1979). However, more modern cultivars, such as the regular bearing 'Gala', also displayed evidence of yield variation between years. Additionally, specifically in the case of 'Gala', inter-year yield parameter variation appears more pronounced in Plus4 than Ambient. The literature shows that environment can be influential on yield variation between years (Monselise and Goldschmidt, 1982; Kofler et al., 2022). This consequently raises the question of whether modified temperature was a factor towards enhancing alternate bearing patterns. Thus, studies into alternate bearing and intra-year yield will be explored in more detail within this thesis (here and in the following chapter).

The presence of alternate bearing was most apparent within the fruit number parameter. Several cultivars produced an 80-90% reduction in fruit number across all treatments in 2018 compared to ‘on’ years. For four cultivars, this reduction was more severe in warmer treatments compared to Ambient. This included the commercially important ‘Gala’, where Plus4 produced 30% fewer fruit compared to Ambient. Such results match those seen in studies such as Atkinson et al. (1998)’s UK field study, where raised temperature environment under polytunnel led to 56% decrease in total ‘Cox’s Orange Pippin’s’ fruit at harvest with a 1°C average rise in seasonal temperature. This was attributed to negative associations between temperature and fruit retention, as modified temperature was applied during flowering (May). However, in the case of ‘Cox’s Orange Pippin’, the results from this study indicate sufficient fruit retention within ‘on’ years across all treatments despite a 1-3°C increase in seasonal temperature in warmer treatments. An investigation during ‘off’ years would however be required to confirm whether fruit retention differs between alternate years.

Multiple linear regression (MLR) revealed that temperature during late fruit development was potentially the most sensitive period for determining yield (out of all temperature parameters investigated). Temperature during this period had significant model weight across all three MLR’s (Tables 3.2 to 3.4). The MLR relating to three suspected temperature sensitive cultivars (‘Golden Delicious’, ‘Lappio’ and ‘Winter Banana’) revealed that temperature during late fruit development was the only aspect of temperature to influence yield (Table 3.2). The positive relationship between minimum temperature and yield during this period was the strongest out of all of the MLR models. Cool weather during fruit growth adversely affects yield (Jackson and Hamer, 1980; Jackson et al., 1982). Reduction in cell expansion is known to be a contributing mechanism for this (Atkinson et al., 1998). Curiously, mean temperature was negatively associated with yield, potentially indicating that ‘mild’ conditions (i.e. not too hot or cold) may have provided optimal conditions for yield. Warmer July weather, yet cooler August weather, also had a positive relationship with yield (Table 3.4), providing further inconsistencies.

Two of the MLR’s suggest that raised temperatures between January and bud burst (typically mid-March) may be influential in determining yield (Tables 3.3 and 3.4). Raised mean temperatures then had a positive relationship with yield. This contradicts several studies that have demonstrated that increased Winter temperature has a negative impact on yield. Beattie and Folley (1978) demonstrated that mild temperatures in February to April were related to poor 'Cox's Orange Pippin' yields in the subsequent season. Other studies related to winter temperature effects are related to winter chill unit accumulation, where yield parameter sensitivity is associated with insufficient winter chill (El Yaacoubi et al., 2020). It is possible that a positive relationship between winter temperatures and yield provided further evidence that winter chill accumulation was satisfactory across all treatments (as discussed in Lane (2022)), or at least not limited enough to have a direct impact on yield. A negative relationship between minimum temperature and yield (Table 3.3) provided some evidence that low temperatures during this period are still beneficial, possibly for achieving sufficient winter chill accumulation.

Inconsistent significant relationships were found between early fruit development temperature and yield – a negative relationship with Tmean, and a positive relationship with Tmax (Table 3.3). Increased temperatures are typically associated with higher yields through raised cell division in early fruit development (Warrington et al., 1999), so a positive relationship would be expected with Tmean as with Tmax.

The MLR models highlighted mixed effects of inter-annual variation in rainfall on yield. Rainfall had a positive relationship on yield during early fruit development, whereas it had a negative effect during late fruit development. Similar studies have found positive associations between rainfall and yield. Li et al. (2018) identified that June to August precipitation affected yield positively within apple production systems in China. Deficit irrigation (i.e. insufficient water) reduced cell and fruit expansion during this period (Naor, 2012), which is linked with reducing fruit size and yield (Warrington et al., 1999). Raised temperatures are linked to higher rates of evapotranspiration, increasing apple crop water requirements (Allen et al., 1998). Hence, as a consequence the severity of water stress in Plus4 may have been more severe than in Ambient in dry years (2018 and-20). However, this factor would be difficult to evaluate given differences in alternate bearing habits among treatments. Espinoza-Meza et al. (2023) demonstrated that 50% irrigation deficits in Mediterranean climates significantly reduced Fuji yield and fruit size. Furthermore, supplementary irrigation is known to increase fruit size in 'Cox's Orange Pippin' (Atkinson et al., 1998). The results from the current investigation indicate that RCP UK projections of "hotter, drier summers" may lead to reduced yields within rainfed apple production systems.

Overall, the modelling showed some inconsistent effects of seasonal weather on yield. Difficulties in modelling the effects of seasonal temperature and rainfall on yield parameters were likely compounded by the inter-year variation in weather. As noted in

Table 2.3, 2018 and 2020 production seasons were on average warmer than 2019 and 2021. 2018 and 2020 also experienced lower summer rainfall in comparison to 2019 and 2021. Due to likely alternate bearing influences on yield between years, analyses showed that lower yields were generally correlated with warmer and drier weather, and higher yields with cooler and wetter weather. This therefore highlights the need for long-term data to model accurately the relationships between perennial fruit crop production and the parameters of weather.

Variation in fruit set and retention is widely known to be a determining factor of yield at harvest. Whilst not explicitly assessed as part of this chapter, a study by Stephens (2022) investigated fruit set differences between temperature treatments (Appendix 3.5). Conducted over one season in 2021 (a biennial ‘on’ year), Stephens found no significant differences ($p<0.05$) in fruit set among temperature treatments across five tested cultivars. High pre-blossom temperatures are linked with reduced fruit set potential (Jackson and Hamer, 1980), whilst higher post-blossom temperatures have been linked with both increasing (Jackson and Hamer, 1980) and reducing (Grauslund, 1975) fruit set dependent on the cultivar. The evidence from Stephens (2022) suggests no effect of increased temperature, however the overall number of fruit harvested was much higher in the warmer treatments. This was most likely due to insufficient fruitlet thinning in Plus2 and Plus4 compared to Ambient (resulting in increased crop load) in specifically 2021, rather than differences in fruit set. A similar study in a biennial ‘off’ year may have potentially produced different results, as alternate bearing patterns are known to influence fruit set (Monselise and Goldschmidt, 1982).

Biennial bearing habits were thought to have been established prior to treatment application in 2017 (Lane, 2022). In theory, the alternate bearing behaviour may have been established before the investigation began in 2017, or during the period of study (2017-2022), or both. Whilst in others, it may have been a consequence of the warmer years (e.g. 2018) and/or warmer temperature treatments. The investigations within the next chapter will elaborate on the role of temperature in potentially influencing this alternate bearing patterns between temperature treatments.

3.5 Conclusions

Altered seasonal temperature regimes had a varied impact on the parameters of fruit yield (harvested yield and fruit number per tree, and mean fruit weight) across the range of diverse apple cultivars. Significant differences ($p<0.05$) were detected among all three temperature regimes for all three yield parameters (harvested fresh weight, total fruit and mean fruit weight). In 16 out of the 20 cultivars in the study, significant differences in harvested fresh weight yield per tree were found among the modified temperature environments. The effects of warming on these yield parameters were more often negative than positive. Moreover, the warmer temperature treatments produced greater year-to-year variation in these three yield parameters.

The linear relationships between seasonal temperature and standardised fresh weight yield within each temperature treatment did not statistically differ significantly in slope among regimes in the majority of tested cultivars, despite the greater range of seasonal temperature in the two warmer treatments. The influence of temperature within each stage of seasonal temperature tested was mixed – alternate bearing instead may be the main driver of yield determination. The importance of seasonal rainfall was highlighted within several stages of fruit development.

Trends in alternate bearing were consistent throughout the six-year trial with a higher cropping year (2017,-19,-21) followed by a low cropping year (2018,-20,-22). However, the scale of variation differed between biennial cycles and temperature treatment. Biennial yield variation was more pronounced in the warmer treatments for many trial cultivars, despite all treatments receiving largely the same management. This may mean that sustained exposure to warmer temperature environments can propagate alternate bearing, just not specifically seasonal temperature. In the next chapter, the role of varied temperature is assessed for its influence specifically on inter-annual variation in yield.

Chapter 4: The impact of modified climate environment on inter-year production variability of apple

4.1 Introduction

Inter-year apple production variability has been an historic obstacle within the perennial crop production industry. Repeated cycles of a high-crop production season followed by a low-crop production season (referred to as ‘alternate bearing’ or ‘biennial bearing’) is documented across a wide range of crop genera including *Prunus* (apricots), *Mangifera* (mango), *Persea* (avocado) and more (Sharma et al., 2019). Biennial bearing within *Malus* crops (apples) has been historically reported within a wide range of cultivars used in worldwide production (Jonkers, 1979; Monselise and Goldschmidt, 1982). However, more advanced breeding and crop management techniques introduced over the course of the 20th Century are attributed to effectively mitigating the presence of alternate bearing within commercial practice (Jonkers, 1979; Monselise and Goldschmidt, 1982; Koutinas et al., 2014). In more susceptible cultivars, the high production season is referred to as the ‘on’ year, and the low production season the ‘off’ year.

The onset of alternate bearing in apple is widely known to be caused by varied floral bud differentiation between alternate production years. Reproductive buds differentiate from spur or lateral terminal vegetative buds in the season prior to bloom – a process that typically starts three to six weeks after full bloom when vegetative extension growth reduces (Pratt, 1988). However, the exact biological mechanisms involved with determining floral bud differentiation in perennial crops are still undetermined. It is a complex phenomenon with a mix of genetic, physiological, crop management and environmental factors likely all contributing to a certain extent (Monselise and Goldschmidt, 1982; Koutinas et al., 2014; Sharma et al., 2019).

Advances in genomic studies have revealed more about the metabolic pathways involved with alternate bearing. Genomic regions associated with hormones are more likely associated than just flowering transcriptomes alone (Monselise and Goldschmidt, 1982; Guitton et al., 2012). In apple, Kofler et al. (2022) revealed possible key proteomic differences between ‘on’ and ‘off’ year floral bud differentiation in the cultivar ‘Gala’. However, no causal relationships have been determined. For the time being, cultivar genetic make-up has been proposed as the most important determinant of onset and duration of apple floral bud initiation (Krasniqi et al., 2013; Kofler et al., 2019). Different cultivars have demonstrated differences in key physiological processes. Cultivars with greater transpiration rate and stomatal conductance may inhibit flowering, though the exact biomechanisms for this are unknown (Elsysy et al., 2019). Scion carbohydrate storing capacity is linked with alternate bearing susceptibility (Monselise and Goldschmidt, 1982; Lordan et al., 2019; Jupa et al., 2021). Rootstock choice is also known to affect alternate bearing patterns due to rootstock influences on plant physiological processes due to differences in root structure and xylem transport efficiency (Jupa et al., 2021). Individual rootstocks have been identified as being associated with enhanced alternate bearing, such as 'Malling 9' (M9) (Kviklys et al., 2016).

Outside of cultivar and rootstock choice, crop management strategies in preventing and mitigating alternate bearing are well documented. The basic principles focus on the reduction of developing fruiting buds within the 'on' year (i.e. managing crop load), as heavy cropping can lead to reduced yields in the subsequent season (Jonkers, 1979). Crop load was identified as the secondary determinant of floral bud initiation, with a crop load mediated factor likely delaying its onset (Kofler et al., 2019). Heavy bearing years in susceptible cultivar groups (such as 'Delicious') will likely induce a low bearing year (Monselise and Goldschmidt, 1982). Historic 'Cox's Orange Pippin' UK yields have noted heavy cropping is also linked with reduced flowers of smaller size, as well as reduced fruit set (Buszard and Schwabe, 1995). With crop load managed effectively, presence of developing fruiting buds should not inhibit floral bud initiation for the following season (Elsysy et al., 2019). Methods to reduce crop load include hand and chemical thinning of fruiting buds at either the blossom or fruitlet stage. This consequently reduces carbohydrate consumption for fruit development, beneficial for both subsequent season fruit bud differentiation as well as improving fruit set (Lordan et al., 2019). Sufficient reduction of excess vegetative growth (pruning) may influence alternate bearing as wood growth vigour can influence floral bud initiation physiological processes (Jupa et al., 2021). Excessive early cropping is also linked with stronger alternate bearing patterns (Krasniqi et al., 2013).

The role of seasonal temperature on fruit yield is discussed in Chapter 3. However, the role of pre-seasonal temperature on fruit yield is also influential on determining both the quantity and quality of apple fruiting buds. For the purposes of this study, 'pre-seasonal' refers to any time period before flower pollination that may impact fruit bud production. Kofler et al. (2019) proposed heat accumulation as the third determinant (after genotype and crop load) associated with the onset of floral bud initiation. Within the UK, higher than average temperatures during this period are thought to be of overall benefit to flowering in the subsequent season. This is attributed to induction of earlier floral initiation, allowing more time for successful bud development (Abbott et al., 1975).

However, high temperature extremes are linked with reduced floral initiation (Abbott et al., 1973; Caprio and Quamme, 1999). Greater sinusoidal daily temperature fluctuations between high and low temperatures throughout summer have been reported to greatly reduce flower cluster production (Abbott et al., 1973). High diurnal temperature differences are also associated with reduced or even eliminated flowering in litchi crops (Menzei et al., 1989). This idea was developed further by Heide et al. (2020) who determined that both consistently low (12°C) and high (27°C) temperatures were associated with altering growth cessation and subsequently inhibition of floral initiation in the apple cultivar ‘Elstar’. Similarly, frost events are linked with reduced floral initiation (Monselise and Goldschmidt, 1982). Reduction of other plant stresses, such as heat stress (through increased light shading), are also associated with alleviating alternate bearing patterns in the susceptible cultivar ‘Golden Delicious’ (Juillion et al., 2022). In the period between fruit harvest and dormancy, favourable climatic conditions for photosynthesis may lead to more ‘vigorous flowering’ in the subsequent season (Ferree et al., 2015).

Temperature within the apple dormancy phase (typically November to March in the UK) can influence yield variation. As mentioned in Chapter 3, the role of insufficient winter chill accumulation in affecting flowering and yield is well documented within the literature. High chill requirement cultivars may become more unviable in response to future climate change, whereas low to medium chill cultivars will likely be resilient (Deldago et al., 2021). Excluding winter chill requirements, yield sensitivity to temperature within specific time periods during dormancy have also been identified. Raised temperatures throughout autumn are linked with reduced fruit set (Lordan et al., 2019). In the UK, Beattie and Folley (1977) linked raised pre-blossom temperatures with reduced yield in ‘Cox’s Orange Pippin’. Additionally, raised February to March temperatures have also been specifically associated with reduced fruit set, thus reducing subsequent yield (Jackson et al., 1982). These patterns are also seen in other geographic regions. For example, January to March minimum temperature is an important parameter in apple yield modelling in Kullu Valley, India (Sen et al., 2015).

There is some evidence that reduced water stress benefits floral bud initiation in apple (Koutinas et al., 2014; Sharma et al., 2019). However, other than temperature and rainfall, associations between floral bud initiation and environment are uncommon within the literature. Krasniqi et al. (2013) did not find any aspect of agroclimate (including temperature) affected alternate bearing, whereas genetics and crop management practices did, as was replicated and reported in Kofler et al. (2019).

The impact of future climate change scenarios on alternate bearing patterns in apple is relatively unexplored. Prior results in this thesis (Chapter 3) indicate that there may be differences in alternate bearing patterns between three temperature treatments across a wide range of cultivars within the long-term 'Apples in a Warmer World'® experiment. Therefore, the hypotheses were as followed:

H_0: Modified temperature will have no direct influence on the long-term yield variation observed within the study.

H_1: Modified temperature will be determined to have a direct influence on the long-term yield variation observed within the study.

4.2 Materials and Methods

4.2.1 Overview

It was clear from the literature that the physiological mechanisms that underpin alternate bearing patterns in *Malus* are complex and not fully understood. As such, a study to properly investigate the causes of alternate bearing within this trial would have required extensive data collection on the environmental response of plant physiological outputs (e.g. evapotranspiration, vegetative growth, flower bud initiation etc.). The presence of alternate bearing already established in trees before treatment application (such as through inappropriate tree management for specific cultivars) also meant that a study into the direct cause of it was also implausible. Discovery and observation of alternate bearing patterns within the study were also anecdotal (i.e. the study did not originally aim to assess alternate bearing differences between treatments). However, given the yield trends identified in Chapter 3 of this thesis, it was highly feasible that differences in modified temperature environments may have influenced alternate bearing. Therefore, this study aimed to specifically identify associations between temperature and alternate bearing patterns throughout the long-term study. As long-term rainfall and tree phenology data were well documented throughout this study, these variables were also assessed for any potential associations with yield variation.

The analysis was split in to three parts. First, differences in alternate bearing patterns between modified temperature environment were identified for each of the 20 study cultivars. As reported in Chapter 3, alternate bearing habits were established across many cultivars before treatment environments were applied in 2017. However, it was also noted that the severity of alternate bearing appeared consistently enhanced within the two warmer regimes compared to Ambient. Therefore, those cultivars that presented different alternate bearing patterns between treatments were then put forward for further analysis. Secondly, differences in floral bud production between alternate production years were observed to further validate alternate bearing differences between treatments. This would provide further evidence that potential seasonal temperature effects on inter-year yield variation (such as fruit set and fruit retention) were likely low or negligible impact. Thirdly, associations between recorded trial environmental parameters (temperature and rainfall) and alternate bearing patterns were analysed. Yield variation associations with other management and production parameters (e.g. phenological timings, crop load) were also analysed due to their significance within the literature.

4.2.2 Raw data collection and methodology

The collection of yield data over a six-year period was described in Chapter 3.

Flower cluster data was collected over a three-year period (2020-22) to observe differences between biennial production seasons (at least one ‘on’ year and ‘off’ year). The methodology replicated that of Lane (2022). Each tree (at point of full flowering) had individual flower clusters counted and categorised in one of three categories; >15 (indicating ‘normal’ bearing), <15 (indicating ‘low’ bearing), and zero (indicating no fruit bearing).

The analyses focussed on determining whether four temperature variables – Tmean (mean temperature, °C), Tmin (minimum temperature, °C), Tmax (maximum temperature, °C) and Tminmaxdiff (difference between minimum and maximum temperature, °C) – were associated with variation with yield parameters. Yield and temperature associations from ‘on’ years (2017, 2019, and 2021) and ‘off’ years (2018, 2020, and 2022) were tested independently as individual sample subsets. This was primarily to avoid bias from alternate bearing habits being present across most cultivars before treatment application.

Once cross-cultivar temperature associations were determined, regression analysis was then applied at the cultivar level to determine how genotype response differs. The same regression analysis was applied to both ‘on’ and ‘off’ year datasets to compare response within alternate seasons.

Finally, further correlation and regression analysis determined associations between non-temperature related parameters and yield variation. These variables were chosen based on study data availability and whether the literature review suggests they were influential on alternate bearing patterns. These variables were crop load (kg total harvested fruit per tree) from the previous production season, flowering and harvest dates from the previous production season, and total seasonal rainfall from both the previous and current production season.

4.2.3 Weather data

Weather data was obtained as described in Chapter 2. The mean values for temperature parameters across tested time periods are listed in Table 4.1.

Table 4.1. *Six-year (2017-22) temperature comparison between the three modified temperature environments (Ambient, Plus2 and Plus4). Each temperature (Tmean, Tmin, Tmax, and Tmmdiff) and period (Feb-Apr, May-Oct, and Nov-Jan) combination represent the parameters compared within the yield variation analysis (NB: each cultivar's 'May-Oct' temperature values were specific to the full-flowering to harvest date period only in the analyses. The May-Oct values provided below are a basic summary of temperature within this period).*

Year	Period	Treatment	Tmean (°C)	Tmin (°C)	Tmax (°C)	Tmmdiff (°C)
2017-18	Feb-Apr	Amb	8.82	4.84	12.77	7.93
		Plus2	8.82	4.84	12.77	7.93
		Plus4	8.82	4.84	12.77	7.93
	May-Oct	Amb	15.72	11.06	21.71	10.03
		Plus2	15.78	11.05	21.75	10.08
		Plus4	15.80	11.14	21.83	10.15
	Nov-Jan18	Amb	5.88	3.08	8.29	5.69
		Plus2	5.83	2.76	9.79	7.56
		Plus4	6.00	3.12	11.89	9.29
2018-19	Feb-Apr	Amb	6.78	3.64	10.57	6.93
		Plus2	6.92	3.37	11.35	7.98
		Plus4	7.61	3.86	12.79	8.94
	May-Oct	Amb	16.08	11.28	21.29	10.01
		Plus2	16.88	11.28	23.04	11.76
		Plus4	17.74	11.92	24.79	12.87
	Nov-Jan19	Amb	6.76	4.17	9.23	5.05
		Plus2	6.35	3.27	9.89	6.62
		Plus4	6.74	3.72	10.46	6.74
2019-20	Feb-Apr	Amb	8.39	4.62	12.65	8.03
		Plus2	8.81	4.36	14.47	10.11
		Plus4	9.22	4.67	15.13	10.46
	May-Oct	Amb	15.69	10.97	20.69	9.72
		Plus2	16.59	11.15	22.74	11.59
		Plus4	17.12	11.45	23.91	12.46
	Nov-Jan20	Amb	7.02	4.35	9.51	5.16
		Plus2	6.83	4.04	9.64	5.60
		Plus4	7.12	4.35	10.05	5.71
2020-21	Feb-Apr	Amb	8.51	4.65	12.81	8.15
		Plus2	8.90	4.63	13.96	9.33
		Plus4	9.39	4.95	14.62	9.66
	May-Oct	Amb	16.02	11.53	21.08	9.55

Year	Period	Treatment	Tmean (°C)	Tmin (°C)	Tmax (°C)	Tmmdiff (°C)
		Plus2	17.33	11.88	23.79	11.91
		Plus4	18.25	12.27	25.92	13.65
	Nov-Jan21	Amb	6.56	4.14	8.98	4.84
		Plus2	6.53	3.89	10.01	6.12
		Plus4	6.84	4.15	10.60	6.45
2021-22	Feb-Apr	Amb	6.61	3.00	10.42	7.42
		Plus2	7.42	3.00	12.86	9.86
		Plus4	8.07	3.35	14.30	10.95
	May-Oct	Amb	15.49	11.30	20.11	8.81
		Plus2	16.70	11.43	22.94	11.51
		Plus4	17.45	11.93	24.64	12.72
	Nov-Jan22	Amb	6.89	4.31	9.47	5.17
		Plus2	6.86	3.94	10.60	6.67
		Plus4	7.13	4.18	11.04	6.86
2022	Feb-Apr	Amb	9.11	4.38	12.16	7.78
		Plus2	9.13	4.31	12.42	8.11
		Plus4	9.17	4.33	12.50	8.16
	May-Oct	Amb	17.80	11.23	21.47	10.29
		Plus2	17.80	11.23	21.47	10.29
		Plus4	17.80	11.23	21.47	10.29

4.2.4 Statistical analysis

Mean Alternate Bearing Indices (ABI) were calculated for each originally established trial cultivar (n=14) x modified temperature environment (n=3) combination (unit of observation = one tree, n=11 to 18 trees per cultivar x temperature environment combination). This was obtained through the following formula (as per Monselise and Goldschmidt, 1982):

$$Mean\ ABI = \frac{\left(\frac{a_2 - a_1}{a_2 + a_1} + \frac{a_3 - a_2}{a_3 + a_2} + \frac{a_4 - a_3}{a_4 + a_3} + \frac{a_5 - a_4}{a_5 + a_4} + \frac{a_6 - a_5}{a_6 + a_5}\right)}{5}$$

where a_n = mean fresh weight per tree for each successive year (1=2017 to 6=2022). Mean ABI = 0 is no alternate bearing, mean ABI = 1 is complete alternate bearing.

Differences in mean ABI across each cultivar and treatment were calculated using one-way ANOVA with post-hoc Tukey testing. This was performed using R packages “stats” and “agricolae”.

Pearson’s correlation analysis was performed to determine associations between temperature parameters from specific pre-seasonal and seasonal time periods and yield (mean harvested fresh weight per tree). This was performed using the R package “correlation”. Statistical significance of correlations was determined using post-hoc Bonferroni testing. To compare the environmental responses in a cross-cultivar manner, all seasonal parameters were standardised relative to each cultivar’s long term six-year range (as performed in Chapter 3). The temperature periods “Previous November to January” and “Previous February to April” were also standardised relative to their long-term ranges, however these values were universal across all cultivars. Correlation analysis was performed on ‘on’ and ‘off’ year datasets independently.

Additionally, Pearson’s correlation analysis was performed between temperature parameters and ABI, as well as between other production variables and yield parameters.

All regression analyses were performed using the R packages “ggplot2” and “ggpubr”.

4.3. Results

4.3.1 Overview

Mean six-year harvested fruit fresh weight per tree (kg) varied by cultivar and temperature environment, ranging from 0.83kg (‘Beverly Hills’, Plus2) to 22.5kg (‘Yellow Bellflower’, Ambient). Statistical differences between treatments are described in Chapter 3 (Figure 3.1).

Mean Alternate Bearing Index (ABI) varied among both cultivars and temperature treatments in the 15 cultivars studied in all six years. (five remaining cultivars have only three-year yield observations 2020 to 2022) (Table 4.2). Mean five-year ABI ranged from 0.16 (‘George Cave’, Ambient) to 0.95 (‘Fuji’, Plus2). The largest difference in ABI among treatments was seen in ‘Yellow Bellflower‘, with a difference of 0.48 between Ambient (0.15) and Plus4 (0.63). The smallest overall difference between the three treatments was seen in ‘Fuji’ (0.01). Ten out of 15 cultivars showed statistically significantly greater ($p<0.05$) mean ABI in a warmer temperature environment compared to Ambient. Out of those 10 cultivars, six cultivars had statistically similar means of ABI between Plus2 and Plus4. Three cultivars had the highest ABI exclusively in Plus4, one exclusively in Plus2. Two cultivars (‘Jolyne’ and ‘Lappio’) showed the highest ABI in Ambient. Only three cultivars (‘Fuji’, ‘King of the Pippins’, and ‘Winter Pearmain’) showed no differences in ABI between treatments. The 10 cultivars with statistically greater ABI within warmer environments (‘Bramley’s Seedling’, ‘Cox’s Orange Pippin’, ‘Discovery’, ‘Edward VII’, ‘Gala’, ‘George Cave’, ‘Golden Delicious’, ‘Jonathan’, ‘Stark’s Earliest’, and ‘Yellow Bellflower’) were put forward for further analysis to evaluate the effect of modified temperature on alternate year yield variation.

***Table 4.2**. Mean harvested fruit fresh weight per tree (kg) and alternate bearing index (ABI) for 20 apple cultivars across three modified temperature environments (Ambient, Plus2, and Plus4) based on long-term observations. (ABI figures not considered for more recently planted cultivars (2) due to inconsistent fruiting in young trees). One-way ANOVA and post-hoc Tukey tests indicate differences (a-c) in ABI between modified temperature environments.*

	Mean Six-Year (2017-2022) Harvested Fresh Weight per Tree (kg) (±SE) Temperature Environment			Mean Five-Year (2018-2022) Alternate Bearing Index (±SE) Temperature Environment		
Cultivar	**Ambient**	**Plus2**	**Plus4**	**Ambient**	**Plus2**	**Plus4**
Beverly Hills[2]	1.86 (± 0.27)	0.83 (± 0.26)	1.62 (± 0.27)	NA	NA	NA
Braeburn[2]	8.93 (± 0.73)	8.18 (± 0.92)	13.46 (± 1.03)	NA	NA	NA
Bramley's Seedling[1]	17.53 (± 1.06)	12.82 (± 0.98)	13.37 (± 1.08)	0.49 (± 0.03) b	0.63 (± 0.03) a	0.64 (± 0.03) a
Cox's Orange Pippin[1]	11.37 (± 0.75)	8.59 (± 0.81)	9.73 (± 0.89)	0.58 (± 0.04) b	0.77 (± 0.04) a	0.74 (± 0.04) a
Discovery[1]	14.88 (± 0.66)	11.15 (± 0.66)	11.39 (± 0.65)	0.32 (± 0.03) b	0.45 (± 0.03) a	0.49 (± 0.04) a
Edward VII[1]	13.32 (± 0.75)	10.00 (± 0.65)	12.74 (± 0.72)	0.31 (± 0.03) b	0.46 (± 0.03) a	0.45 (± 0.03) a
Fuji[1]	12.02 (± 1.16)	9.57 (± 1.08)	12.50 (± 1.40)	0.94 (± 0.01) a	0.95 (± 0.01) a	0.94 (± 0.02) a
Gala[1]	20.14 (± 0.84)	14.89 (± 0.86)	17.66 (± 1.00)	0.21 (± 0.03) b	0.29 (± 0.03) b	0.40 (± 0.03) a
George Cave[1]	11.26 (± 0.42)	5.71 (± 0.40)	9.02 (± 0.55)	0.16 (± 0.01) c	0.35 (± 0.04) a	0.25 (± 0.02) b
Golden Delicious[1]	20.33 (± 1.44)	14.96 (± 1.37)	16.38 (± 1.62)	0.62 (± 0.04) b	0.82 (± 0.02) a	0.85 (± 0.02) a
Granny Smith[2]	3.81 (± 0.36)	3.34 (± 0.38)	5.25 (± 0.60)	NA	NA	NA
Jolyne[1]	9.60 (± 1.64)	13.50 (± 1.31)	9.59 (± 1.48)	0.57 (± 0.07) a	0.30 (± 0.06) b	0.40 (± 0.05) ab
Jonathan[1]	16.33 (± 0.89)	11.87 (± 0.89)	13.51 (± 0.97)	0.32 (± 0.03) b	0.39 (± 0.04) b	0.56 (± 0.03) a
King of the Pippins[1]	8.37 (± 0.93)	6.49 (± 0.80)	8.08 (± 0.86)	0.90 (± 0.02) a	0.87 (± 0.03) a	0.92 (± 0.01) a
Lappio[1]	9.87 (± 1.17)	10.09 (± 1.13)	12.21 (± 1.27)	0.88 (± 0.03) a	0.84 (± 0.03) ab	0.77 (± 0.03) b
Stark's Earliest[1]	13.18 (± 0.35)	9.36 (± 0.41)	11.57 (± 0.47)	0.13 (± 0.01) b	0.24 (± 0.02) a	0.23 (± 0.02) a
Tropical Beauty[2]	2.69 (± 0.47)	2.72 (± 0.62)	3.15 (± 0.53)	NA	NA	NA
Winter Banana[2]	3.56 (± 0.31)	1.88 (± 0.29)	4.08 (± 0.55)	NA	NA	NA
Winter Pearmain[1]	12.96 (± 1.01)	13.16 (± 0.90)	14.60 (± 1.02)	0.65 (± 0.03) a	0.58 (± 0.04) a	0.57 (± 0.04) a
Yellow Bellflower[1]	22.49 (± 0.83)	12.81 (± 0.98)	15.15 (± 1.53)	0.15 (± 0.02) c	0.44 (± 0.04) b	0.63 (± 0.05) a

[1] Trees primarily planted in 2013. [2] Trees primarily planted 2017 onwards (yields for 2020-22 only)

4.3.2 Differences in biennial flower cluster quantity among modified temperature environments

Flower cluster production varied greatly among cultivars, temperature environments, and years (Table 4.3). In 2020, the first biennial 'off' year, the majority of cultivars had incidences of 'low' bearing trees. The most notable lower bearing cultivars were 'Edward VII' and 'Golden Delicious', consisting of ~50% of trees across all temperature environments with <15 flower clusters (FC). Several cultivars differed in numbers of trees among the three temperature environments. This was because of high pest pressure in the warmer treatments – trees with substantial pest damage were removed from the analysis. Therefore, differing population sizes may make for unfair bearing comparisons between treatments at the cultivar level. However, combining all cultivar data revealed that proportionally, the Plus2 environment (66%) produced 11% fewer 'normal' bearing trees compared to Ambient (77%). Plus4 (70%) produced 7% fewer compared to Ambient (Figure 4.1). Additionally, both Plus2 and Plus4 (6%) produced a greater proportion of trees with 0FC compared to Ambient (1%). The main contributing cultivars for this observation were 'Cox's Orange Pippin', 'Edward VII', 'Golden Delicious', and 'Yellow Bellflower' which had multiple incidences of 0FC in the warmer environments. The remaining cultivars showed similar levels of 'normal' bearing among treatments.

Data from 2021 (the biennial 'on' year) showed that all temperature treatments had high levels of 'normal' bearing, ranging from 93% (Ambient and Plus2) to 97% (Plus4) (Figure 4.1). Plus4 had proportionally the lowest amount of 'low' bearing trees (2-3%), whereas Ambient had the highest (7-8%). As such, there were minor differences between treatments at the cultivar level in 2021 (Table 4.3).

Data from 2022 (the second biennial 'off' year) showed differences compared to 2020. Overall, there was a greatly reduced proportion of 'normal' bearing trees in Plus2 (70%) and Plus4 (69%) compared to Ambient (91%) (Figure 4.1). At the cultivar level, more cultivars showed differences between treatments when compared to 2020. The most notable were 'Cox's Orange Pippin', 'Edward VII', 'Gala', 'Golden Delicious', 'Jonathan' and (to a lesser extent) Yellow Bellflower.

The cultivars 'Discovery', 'George Cave', and 'Stark's Earliest' (all early harvesting cultivars) showed little variation among treatments and years.

Overall, fewer flower clusters were produced in the warmer temperature environments across several cultivars. However, this seems to vary even when comparing only the biennial 'off' years, as shown by a greater selection of cultivars that exhibited fewer flower clusters in 2022 compared to 2020.

***Table 4.3**. Flower cluster (FC) count data (2020-22) from apple cultivars with significant alternate bearing index (ABI) differences among three modified temperature regimes (Ambient, Plus2, and Plus4). Total FC's were counted per tree (n=11-18 per treatment) and categorised in to either 'normal' (>15), 'low' (between 1 and 15) or no (0) bearing.*

		2020 ('off' year)				2021 ('on' year)				2022 ('off' year)			
Cultivar	**Temp Env.**	**Total**	**>15FC**	**1-15FC**	**0FC**	**Total**	**>15FC**	**1-15FC**	**0FC**	**Total**	**>15FC**	**1-15FC**	**0FC**
Cox's Orange Pippin	Amb	18	14	4	0	18	15	3	0	18	15	2	1
	Plus2	16	9	5	2	18	16	2	0	18	6	10	2
	Plus4	17	9	6	2	18	18	0	0	17	5	7	5
Discovery	Amb	17	15	1	1	17	16	0	1	17	17	0	0
	Plus2	15	15	0	0	17	17	0	0	17	17	0	0
	Plus4	16	16	0	0	18	17	1	0	17	17	0	0
Edward VII	Amb	18	9	9	0	18	14	4	0	18	17	1	0
	Plus2	18	7	9	2	18	16	2	0	18	12	5	1
	Plus4	18	10	8	0	18	18	0	0	18	8	10	0
Gala	Amb	17	16	1	0	18	18	0	0	17	16	0	1
	Plus2	16	14	2	0	17	16	1	0	17	11	6	0
	Plus4	17	14	3	0	17	17	0	0	16	14	2	0
George Cave	Amb	18	15	3	0	18	18	0	0	18	18	0	0
	Plus2	16	12	3	1	17	13	3	1	16	15	0	1
	Plus4	17	15	1	1	18	15	1	1	17	17	0	0
Golden Delicious	Amb	18	9	8	1	18	15	3	0	18	10	8	0
	Plus2	13	3	10	0	18	18	0	0	16	6	9	1
	Plus4	15	5	5	5	18	18	0	0	15	3	11	1
Jonathan	Amb	18	15	3	0	18	18	0	0	18	17	1	0
	Plus2	15	10	5	0	18	17	1	0	18	10	8	0
	Plus4	14	11	4	0	18	17	0	1	15	8	6	1
Stark's Earliest	Amb	18	15	3	0	18	18	0	0	18	18	0	0
	Plus2	12	11	1	0	17	16	1	0	14	13	1	0
	Plus4	15	14	1	0	18	18	0	0	18	18	0	0
Yellow Bellflower	Amb	12	11	1	0	17	16	1	0	12	12	0	0
	Plus2	11	6	2	3	17	17	0	0	16	15	1	0
	Plus4	11	5	6	1	15	15	0	0	14	11	3	0

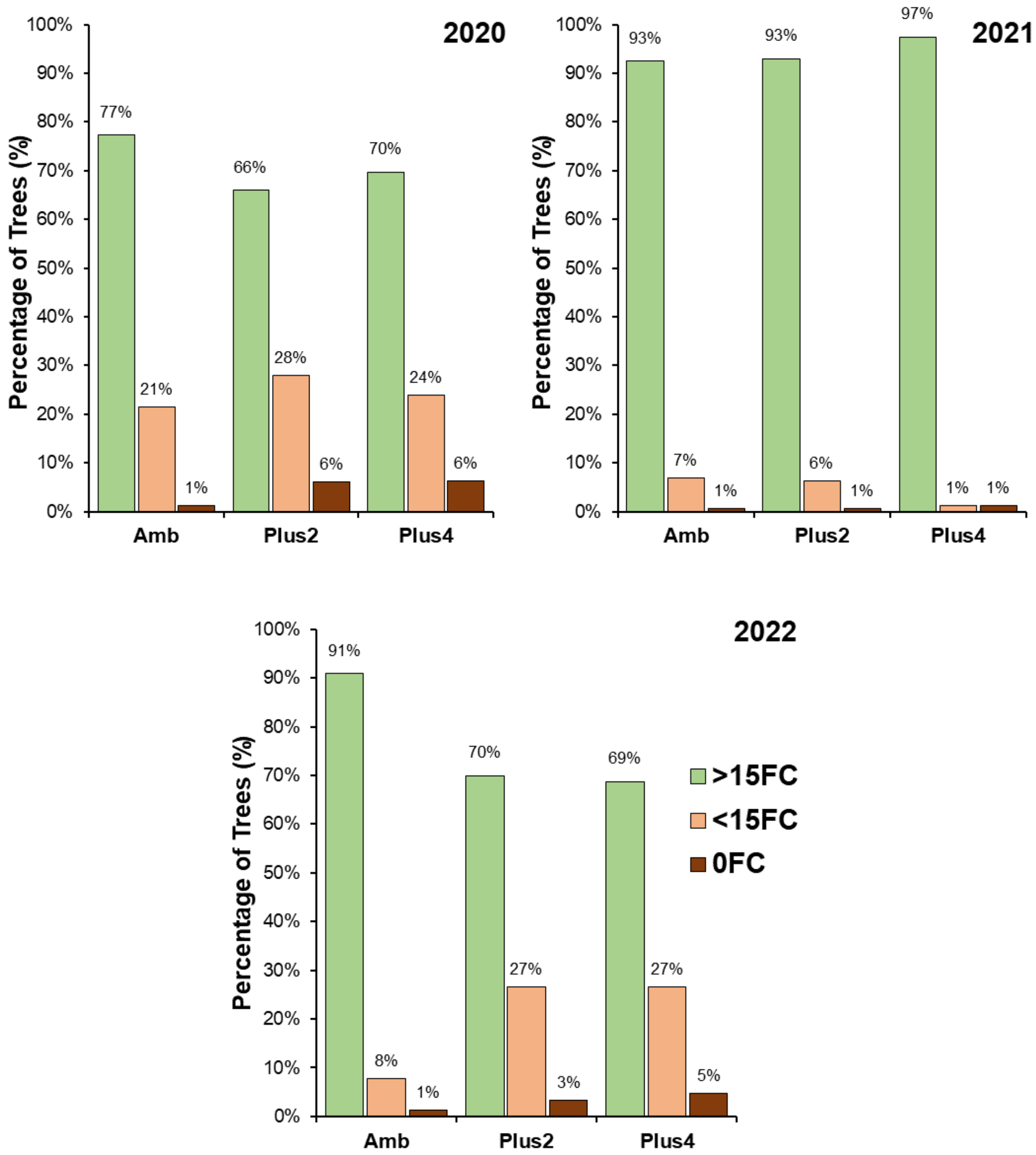


Figure 4.1*. The proportion of apple trees with 'normal' (>15), 'low' or no (0) flower cluster (FC) production across a cross-cultivar population (those listed in Table 4.2) for three production years (2020, 2021, and 2022).*

4.3.3 Associations between temperature parameters and long-term yield variation

Pearson's correlation analysis identified differences in associations between standardised temperature parameters and standardised yield (harvested fruit fresh weight per tree (kg)) between 'on' and 'off' year datasets (Table 4.4). In biennial 'on' years, two current seasonal temperature parameters (Tmean and Tmin) were negatively associated ($p<0.05$) with influencing fruit yield. However, biennial 'off' year yields were highly associated ($p<0.001$) with November to January temperature; positively with Tmin ($t=5.22$, $r=0.49$) and negatively with Tminmaxdiff ($t=-6.31$, $r=-0.56$). Additionally, a positive association (p<0.05) was seen with Nov-Jan Tmean (t=4.14, 0.40). The majority of correlations produced p values >0.999, highlighting that previous summer temperature and February to April temperature had minimal association with either 'on' and 'off' year yields. The results highlight how November to January (only) temperature was highly associated with 'off' year, but not 'on' year yield; and current season temperature (only) was highly associated with 'on' year, but not 'off' year yield.

Table 4.4*. Pearson's Correlation between standardised temperature variables from four time series (preceding summer, November to January, February to April, and current production season (cultivar specific – full-flowering to harvest) and standardised mean fruit fresh weight per tree (kg) within alternate bearing 'on year' (2017,19,21) and 'off' year (2018,20,22) using cross-cultivar data from 10 apple cultivars.*

	Temp	'On' Year (2017, 19 and 21)				'Off' Year (2018, 20 and 22)			
Time Period	**Variable**	**r**	**t**	**df**	**p**	**r**	**t**	**df**	**P**
Previous Summer (May-Oct) [1]	Tmean	-0.01	-0.01	58	>0.999	-0.13	-1.20	88	>0.999
	Tmin	-0.02	-0.18	58	>0.999	0.06	0.56	88	>0.999
	Tmax	0.02	0.15	58	>0.999	-0.28	-2.78	88	>0.999
	Tminmaxdiff	0.03	0.23	58	>0.999	-0.23	-2.23	88	>0.999
Previous Nov to Jan [1]	Tmean	0.39	3.19	58	>0.999	0.40	4.14	88	**0.037***
	Tmin	0.17	1.30	58	>0.999	0.49	5.22	88	**<0.001*****
	Tmax	0.01	0.08	58	>0.999	-0.33	-3.26	88	0.654
	Tminmaxdiff	-0.06	-0.49	58	>0.999	-0.56	-6.31	88	**<0.001*****
Previous Feb to Apr	Tmean	0.07	0.68	88	>0.999	0.31	3.11	88	>0.999
	Tmin	0.05	0.47	88	>0.999	0.33	3.30	88	0.654
	Tmax	0.16	1.53	88	>0.999	-0.02	-0.20	88	>0.999
	Tminmaxdiff	0.13	1.27	88	>0.999	-0.23	-2.26	88	>0.999
Current Season (Cultivar specific, Full- Flowering to Harvest Date)	Tmean	0.43	4.27	82	**0.024***	-0.48	-4.10	55	0.090
	Tmin	0.43	4.32	82	**0.020***	-0.38	-3.09	55	>0.999
	Tmax	0.34	3.27	82	0.723	-0.44	-3.65	55	0.385
	Tminmaxdiff	0.17	1.55	82	>0.999	-0.35	-2.73	55	>0.999

***significant at <0.001, **significant at <0.01, *significant at <0.05

[1] 2017 data (i.e. May 2016 – Jan 2017) N/A

Pearson correlation analysis revealed significant associations between several temperature variables and year-to-year yield variation (ABI) (Table 4.5). Previous summer Tmax (t=4.75, r=0.36) and Tminmaxdiff (t=4.36, r=0.34) were positively associated (p<0.01) with increased ABI. Three November to January temperature variables were highly associated (p<0.001) with greater ABI, these were Tmin (t=-5.54, r=-0.41), Tmax (t=0.46, r=0.24), and Tminmaxdiff (t=8.68, r=0.58). Finally, Tminmaxdiff from the current production season was associated (t=4.09, r=0.36, p<0.05) with raised

ABI. As with the yield-temperature correlation analyses (Table 4.3), February to April temperature variables had little association with ABI.

***Table 4.5**. Pearson's Correlation between standardised temperature variables from four time series (preceding summer, November to January, February to April, and current production season (cultivar specific)) and standardised two-year alternate bearing index (ABI) based on fruit yield (harvested fresh weight per tree, kg) data from 2017 to 2022 using cross-cultivar data from 10 apple cultivars. ABI values were standardised according to each cultivar's long-term range.*

Time Period	Temp variable	r	t	df	P
Previous Summer (May-Oct)	Tmean	0.28	3.55	148	0.180
	Tmin	0.17	2.13	148	>0.999
	Tmax	0.36	4.75	148	**0.002****
	Tminmaxdiff	0.34	4.36	148	**0.009****
Previous Nov-Jan	Tmean	-0.25	-3.09	148	0.828
	Tmin	-0.41	-5.54	148	**<0.001*****
	Tmax	0.46	6.34	148	**<0.001*****
	Tminmaxdiff	0.58	8.68	148	**<0.001*****
Previous Feb-Apr	Tmean	0.09	1.05	148	>0.999
	Tmin	0.01	0.08	148	>0.999
	Tmax	0.24	3.05	148	0.967
	Tminmaxdiff	0.28	3.61	148	0.148
Current Season (Cultivar specific, Full Flowering to Harvest Date)	Tmean	0.21	2.27	112	>0.999
	Tmin	-0.06	-0.59	112	>0.999
	Tmax	0.31	3.40	112	0.327
	Tminmaxdiff	0.36	4.09	112	**0.029***

***significant at <0.001, **significant at <0.01, *significant at <0.05

The November to January Tminmaxdiff (°C) was the temperature variable which provided the strongest correlation with yield in 'off' years (Table 4.4) and with mean ABI across all years (Table 4.5). Hence, regression analysis was performed between November to January Tminmaxdiff (°C) and harvested fruit fresh weight per tree (kg) for 'off' year (Figure 4.2) and 'on' year (Figure 4.3) results independently across each of the 10 study cultivars.

Within the 'off' year analysis, yields were logarithmically transformed as this provided better R^2 values compared with linear regression and better described the observations. The analyses (Figure 4.2) showed a significant negative logarithmic relationship ($p<0.05$) within six out of the 10 tested cultivars. The remaining four cultivars ('George Cave', 'Gala', 'Jonathan', 'Stark's Earliest') provided similar trendlines but did not reach significance ($p=0.061$ to 0.103). 'Yellow Bellflower' provided the greatest response of yield to Tminmaxdiff (y=78.3-34x, R^2=0.65), followed by 'Discovery' (y=58-25.5x, R^2=0.51) and 'Golden Delicious' (y=47.8-22x, R^2=0.61). On the linear scale in Figure 4.2 it can be seen that the majority of cultivars showed a steeper response between 5 and 7°C, with a shallower response from ~7°C onwards. Many cultivars showed a 50% reduction in yield, or more, with a 2-3°C increase in mean November to January Tminmaxdiff.

In contrast to the 'off' year analyses (Figure 4.2), the two years of 'on' year yield results (2019 and 2021) provided no relations with November to January Tminmaxdiff in all ten tested cultivars (Figure 4.3).

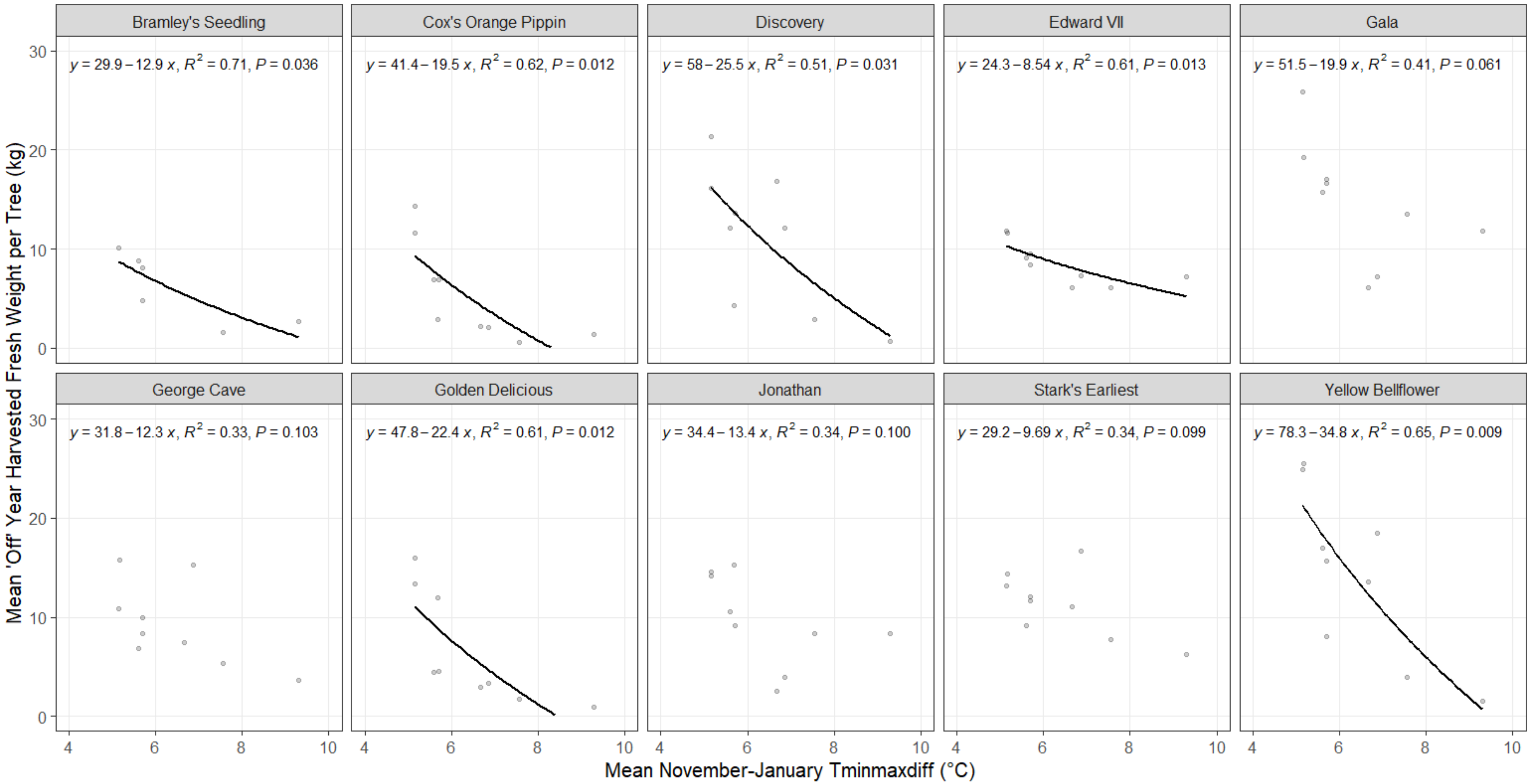


Figure 4.2. *Logarithmic (y = ln(x)) regressions of mean 'off' year (2018, 2020, and 2022) harvested fruit fresh weight per tree (kg) against mean November-January minimum-maximum temperature difference (Tminmaxdiff) (°C) in 10 apple cultivars.*

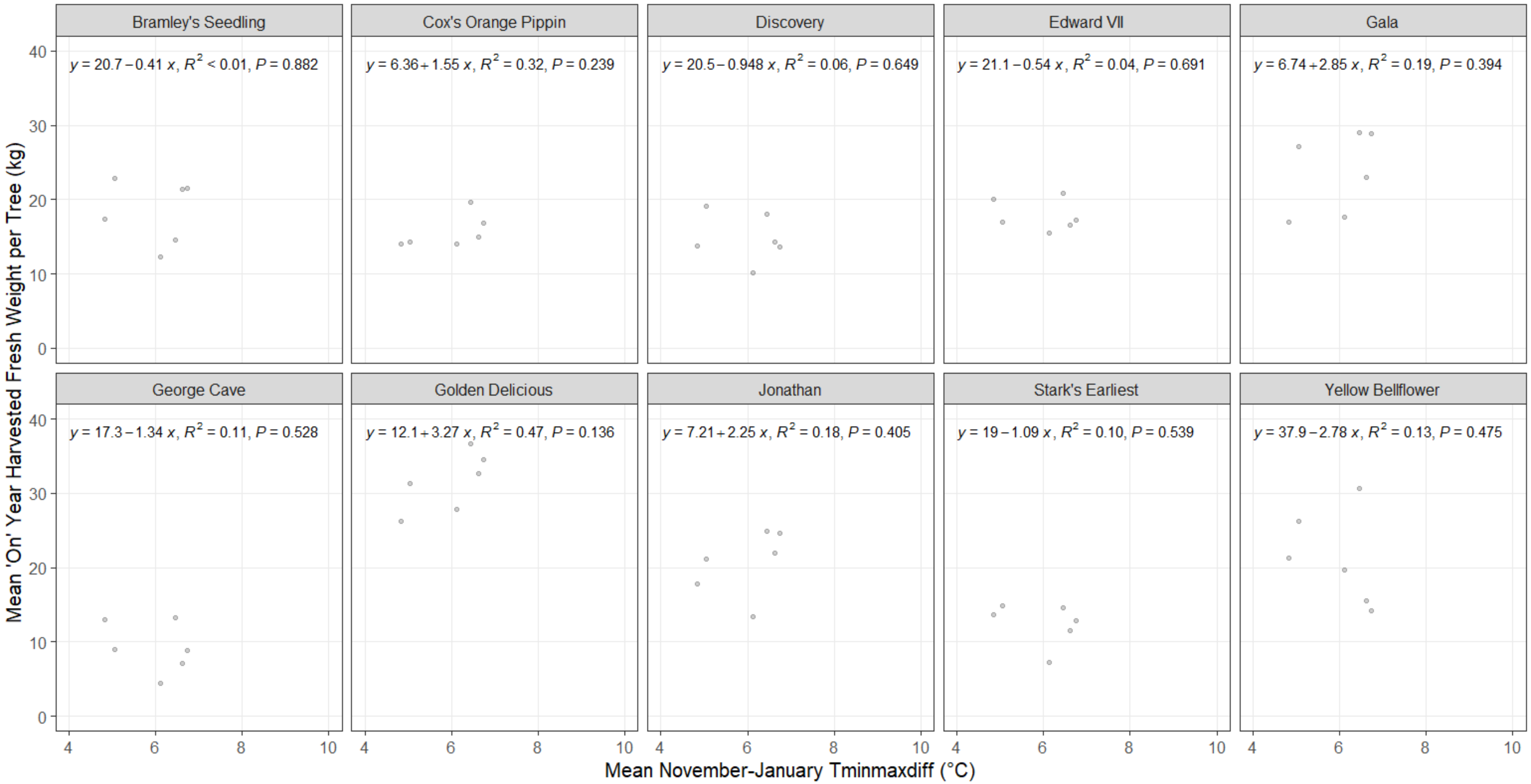


***Figure 4.3**. Linear regression of 'on' year (2017, 2019, and 2021) harvested fruit fresh weight per tree (kg) against mean November-January minimum-maximum temperature difference (Tminmaxdiff) (°C) in 10 apple cultivars.*

The temperature within certain months of the preceding production season (April to August, during primary floral initiation) was found to be associated with yield variation (Table 4.6). May Tmin and May Tmean from the preceding production season were highly negatively associated ($p<0.001$) with ‘off’ year yield; April Tmean was the only other temperature variable associated with ‘off’ year yield ($p<0.05$). Three June temperature variables (Tmean, Tmin, and Tmax) were all positively associated ($p<0.01$) with an increase in two-year ABI, as were July Tmin ($p<0.05$), and May Tmin ($p<0.001$). The latter was the only one-month temperature variable associated with both ‘off’ year yield (negatively) and ABI (positively). There were no significant associations between these 20 temperature variables and ‘on’ yea yield, therefore these results do not appear in Table 4.6.

***Table 4.6**. Pearson’s Correlation between ‘off’ year fruit yield (2018, 2020, 2022) or ABI (2017 to 2022) and temperature variables within each month of the preceding production season during primary floral initiation (April to August). ABI values were standardised according to each cultivar’s long-term range.*

Month	Temp Variable	‘Off’ Year Yield r	t	df	P	Two-Year ABI r	t	df	P
April	Tmean	-0.40	-4.07	88	**0.003****	0.14	1.67	148	>0.999
	Tmin	-0.24	-2.31	88	>0.999	0.08	0.99	148	>0.999
	Tmax	-0.35	-3.54	88	0.206	0.15	1.89	148	>0.999
	Tminmaxdiff	-0.21	-2.01	88	>0.999	0.12	1.48	148	>0.999
May	Tmean	-0.47	-5.04	88	**<0.001*****	0.22	2.72	148	>0.999
	Tmin	-0.54	-6.00	88	**<0.001*****	0.37	4.77	148	**0.001****
	Tmax	-0.22	-2.17	88	>0.999	0.16	2.03	148	>0.999
	Tminmaxdiff	-0.05	-0.47	88	>0.999	0.06	0.75	148	>0.999
June	Tmean	-0.18	-1.67	88	>0.999	0.39	5.22	148	**<0.001*****
	Tmin	0.01	0.10	88	>0.999	0.37	4.84	148	**0.001****
	Tmax	-0.26	-2.48	88	>0.999	0.36	4.66	148	**0.002****
	Tminmaxdiff	-0.32	-3.12	88	0.788	0.30	3.76	148	0.08
July	Tmean	-0.04	-0.40	88	>0.999	0.29	3.66	148	0.113
	Tmin	0.01	0.02	88	>0.999	0.32	4.12	148	**0.021***
	Tmax	0.01	0.04	88	>0.999	0.25	3.21	148	0.538
	Tminmaxdiff	0.01	0.05	88	>0.999	0.21	2.56	148	>0.999
August	Tmean	-0.05	-0.47	88	>0.999	0.01	0.08	148	>0.999
	Tmin	0.34	3.35	88	0.385	-0.14	-1.76	148	>0.999
	Tmax	-0.09	-0.86	88	>0.999	0.07	0.79	148	>0.999
	Tminmaxdiff	-0.26	-2.52	88	>0.999	0.21	2.58	148	>0.999

***significant at <0.001, **significant at <0.01, *significant at <0.05

Given the findings in Table 4.6, ‘off’ year yield was plotted against previous year May Tmin (Figure 4.4), plus ABI against June Tmean (Figure 4.5) in linear regressions across each of the ten study cultivars. Mean May temperature in the previous year had a significant negative effect ($p<0.05$) on ‘off’ year yield for five out of ten cultivars. Three out of these five cultivars were early cultivars (‘Discovery’, ‘George Cave’, and ‘Stark’s Earliest’). The strongest relationships were seen in ‘Discovery’ ($y=93.1-10.2x$, $R^2=0.82$, $p<0.001$) and ‘Yellow Bellflower’ ($y=102-11x$, $R^2=0.64$, $p=0.009$). Four of the remaining five cultivars also showed negative, albeit not significant, responses. Warmer June Tmean increased ABI ($p<0.05$) in four cultivars (‘Edward VII’, ‘Gala’, ‘Stark’s Earliest’, and ‘Yellow Bellflower’); all four of these cultivars are harvested late in the season. The remaining six cultivars also showed positive, but not significant, responses. The results suggest that ‘Yellow Bellflower’ may be the cultivar most severely impacted by mean June temperature, with a 0.127 increase in ABI with every 1°C mean increase. It was also the only cultivar to show a significant response within both Figure 4.4 and Figure 4.5, highlighting potential yield responses to both May and June temperatures in the previous year.

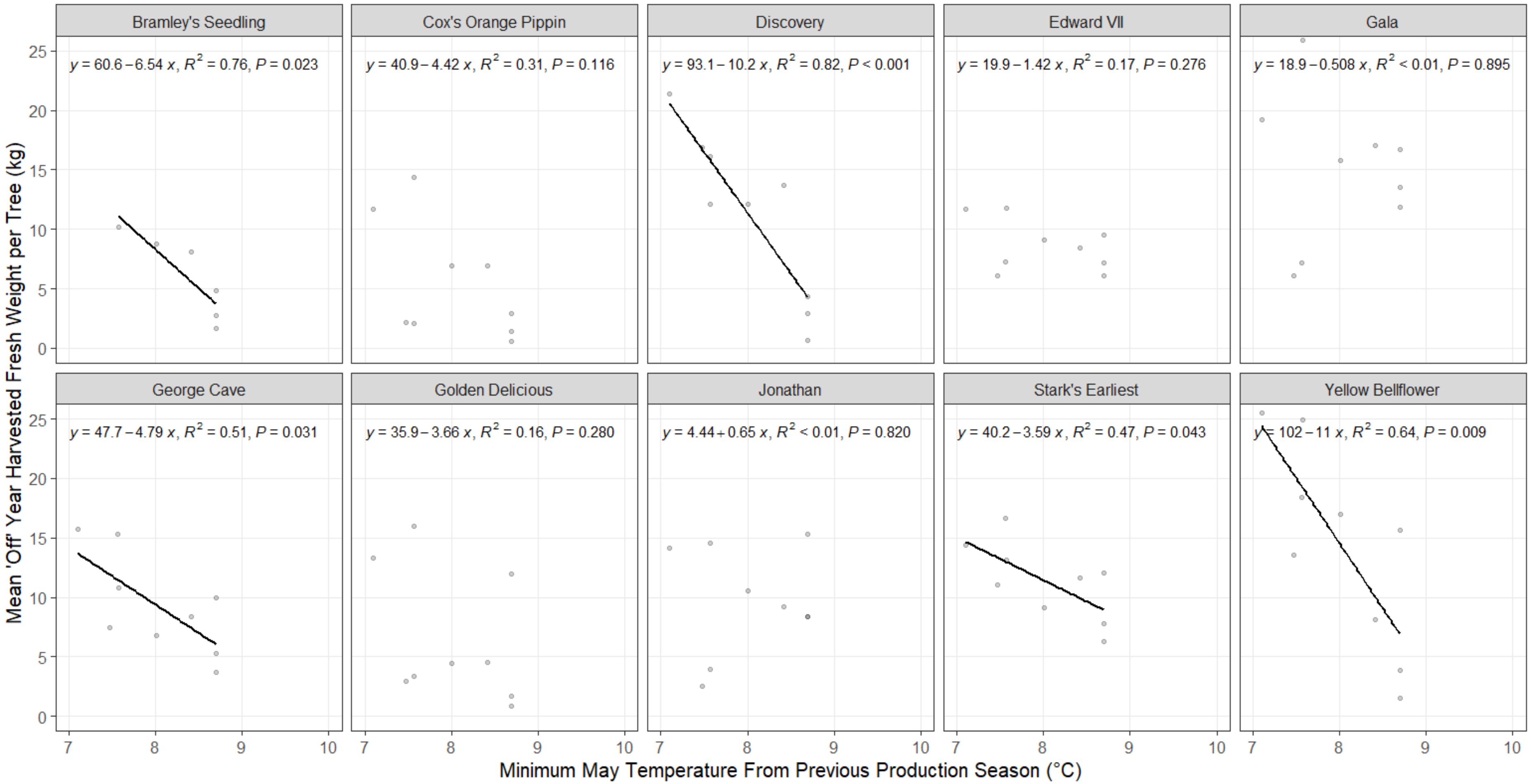


Figure 4.4. *Linear regression analysis between mean 'off' year (2018, 2020, and 2022) harvested fresh weight per tree (kg) and mean minimum May temperature in the previous year (°C) across ten apple cultivars.*

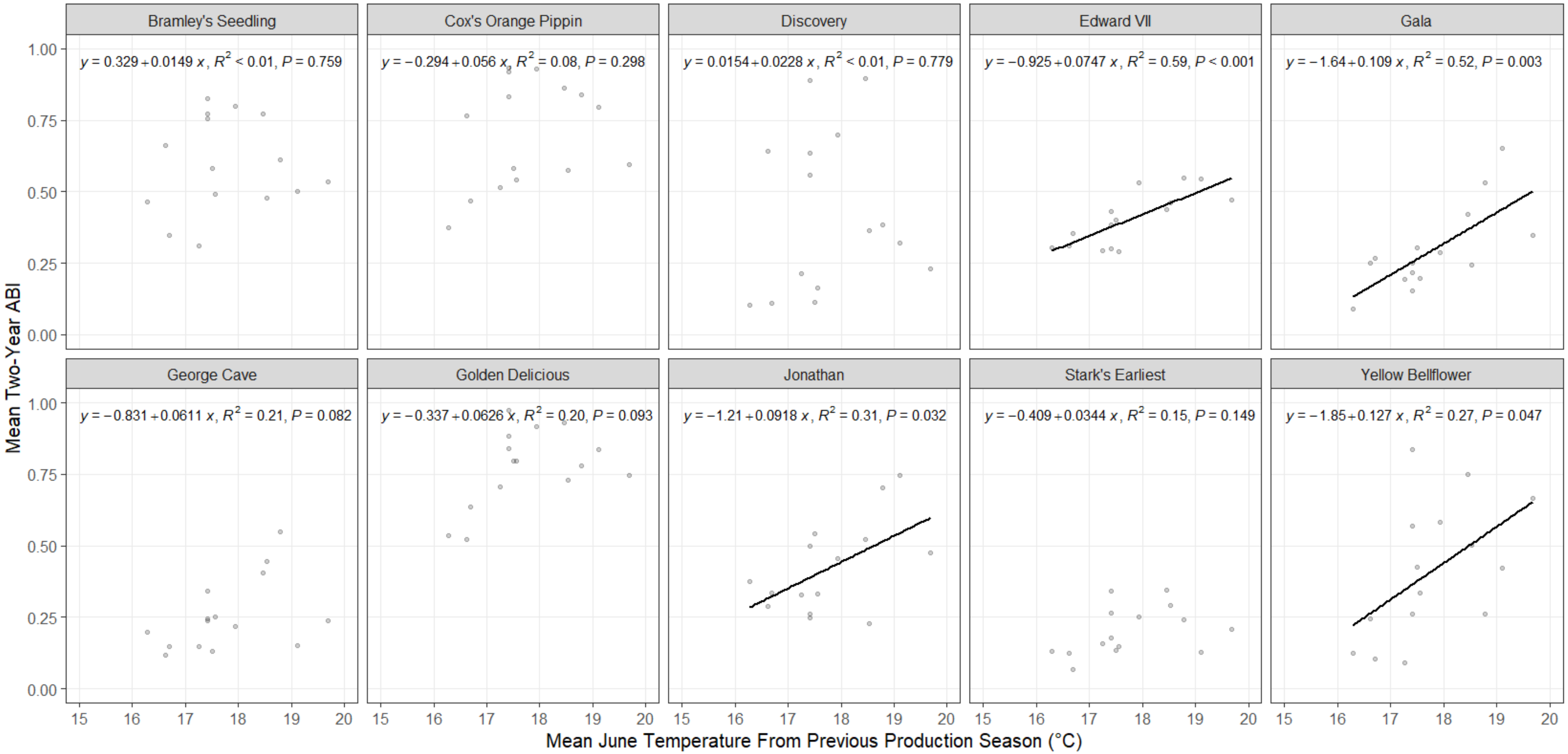


Figure 4.5. *Linear regression analysis between mean alternate bearing index (ABI) and mean June temperature in the previous year (°C) across ten apple cultivars.*

4.3.4 Associations between non-temperature related parameters and long-term yield variation

Pearson's Correlation analysis between select standardised production variables (previous production season flowering and harvest date, current seasonal rainfall (mm), and previous seasonal rainfall (mm)) and yield parameters revealed no significant associations (Table 4.7). The strongest, but insignificant, correlation was seen between 'on year' standardised yield and total seasonal rainfall in the current season (r=-0.41, t=-3.27, p=0.896).

***Table 4.7**. Pearson Correlation data between four standardised non-temperature related variables and three standardised yield parameters ('on' year yield, 'off' year yield, and two-year alternate bearing index (ABI)). The four variables consist of the timing of two apple phenological events (full-flowering and harvest date) in the previous production season, and two rainfall variables (total seasonal rainfall from the previous and current production season).*

Yield Variable	Prod. variable	r	t	df	P
'On' year standardised yield (kg)	FF date	0.20	1.80	76	>0.999
	H date	-0.07	-0.60	76	>0.999
	Rainfall (current)	-0.41	-3.27	52	0.896
	Rainfall (prev)	0.07	0.65	76	>0.999
'Off' year standardised yield (kg)	FF date	-0.13	-0.97	52	>0.999
	H date	-0.07	-0.47	50	>0.999
	Rainfall (current)	-0.21	-1.57	52	>0.999
	Rainfall (prev)	0.27	2.00	52	>0.999
Two-Year ABI	FF date	0.07	0.84	130	>0.999
	H date	-0.01	-0.12	128	>0.999
	Rainfall (current)	0.10	1.04	106	>0.999
	Rainfall (prev)	0.18	2.05	130	>0.999

***significant at <0.001, **significant at <0.01, *significant at <0.05

Linear regression analysis identified significant (p<0.05) relationships between crop load (standardised harvested fruit yield per tree) from the previous production season, and standardised 'off' year yield (standardised harvested fresh weight per tree (kg)) from the subsequent production season across five out of nine test cultivars (Figure 4.6). Within each of the five late-season cultivars ('Cox's Orange Pippin', 'Edward VII', 'Gala', 'Golden Delicious', and 'Jonathan'), only, significant relations were detected; the regression slopes ranged between -0.64 and -0.82. This equates to an increase of 1 standard deviation above mean crop load in the preceding season ('on year') reducing harvested fresh weight yield by -0.64 to -0.82 standard deviations in the following 'off' year. Three early harvesting cultivars (‘Discovery’, ‘George Cave’, and ‘Stark’s Earliest’) showed no relationship between crop load and subsequent seasonal yield.

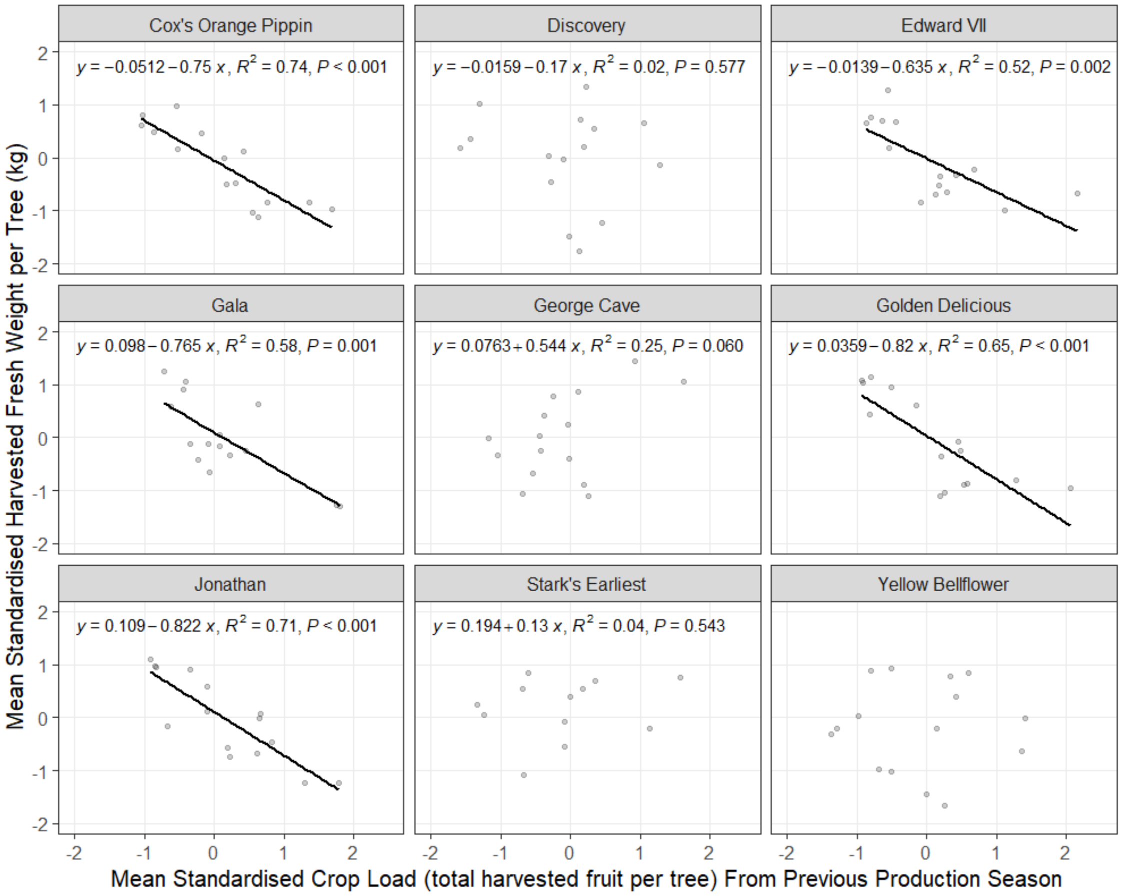


Figure 4.6. *Linear regression analysis between standardised crop load (kg) in the previous production season (‘on year’) and standardised harvested fresh weight (kg) in the current season (‘off’ year’) across nine cultivars.*

4.4 Discussion

The results from Chapter 3 of this thesis revealed that the three modified temperature environments (Ambient, Plus2, and Plus4) altered year-to-year yield variation across many study cultivars. As such, an increase in seasonal temperature was associated with a reduction in average apple fruit yield. Results from this chapter have further refined these differences. First, using the alternate bearing index as the main indicator, 10 out of 15 cultivars studied had significantly enhanced alternate bearing patterns within a warmer treatment compared to Ambient (Table 4.2). Secondly, alternate bearing patterns were likely to have been caused by reduced floral cluster production in warmer environments in the 'off' years for many of these cultivars (Figure 4.1). Thirdly, temperature from specific time periods were identified as being associated within inter-year variation in fruit yield and alternate bearing. Therefore, there is sufficient evidence to reject the null hypothesis of this study. Specifically, changing the temperature regime affected inter-annual variation in fruit yield in many of the cultivars investigated.

Differences in alternate bearing patterns were found among the diverse cultivars studied. This supports the consensus that genetic background (i.e. cultivar choice) is highly influential in determining alternate bearing susceptibility (Monselise and Goldschmidt, 1982; Krasniqi et al., 2013 Kofler et al., 2019). The influence of genetic background over environmental influence was best demonstrated by results from two widely known biennial cultivars; 'Fuji' and 'Golden Delicious'. Environment modification potentially influenced an ABI shift of 0.23 from Ambient to Plus4 for 'Golden Delicious', whereas no shift was observed in 'Fuji' (Table 4.2); 'Fuji' had the greatest ABI at c. 0.94 (Table 4.2) with virtually no yield in 'off' years across all treatments (Chapter 3) even in Ambient. The cultivar 'Gala', a regular-bearing cultivar, showed a considerable ABI shift of 0.19 between Ambient and Plus4. As 'Golden Delicious' is a parent cultivar of 'Gala', this may highlight that those cultivars associated with the 'Delicious' family observe similar responses between environment and 'off' year floral bud initiation (as demonstrated in Monselise and Goldschmidt (1982)).

Temperature from the previous November to January was consistently associated with 'off' year yield parameters throughout the study. This is reflected in Table 4.4, Table 4.5, and Figure 4.2. A positive relationship between Tmin and 'off' year yield implies that a milder Winter is associated with increasing both floral initiation and subsequent fruit yield. Stronger negative associations were present between November to January Tminmaxdiff and 'off' year fruit yield, however. This implies that increased daily temperature fluctuations within this period also reduce 'off' year fruit yield. High Tmax on its own was not statistically associated with reducing yield. The negative logarithmic relationship between November to January Tminmaxdiff and ‘off’ year yield was relatively consistent among all ten cultivars, albeit not always significant (Figure 4.2). This means that regular-bearing cultivars (e.g. ‘Discovery’, ‘Gala’) responded to warming similarly to the more biennial ones (e.g. ‘Cox’s Orange Pippin’, ‘Golden Delicious’) despite differences in the physiological processes shown to contribute towards enhanced alternate bearing patterns (Elsysy et al., 2019; Jupa et al., 2021; Kofler et al, 2022). The literature generally provides little evidence that temperature during autumn to early winter has a significant bearing on yield, but Lordan et al. (2019) commented on how raised autumn temperatures were associated with lower fruit set. Studies into temperature effects on fruit yield during this period generally refer to winter Chill accumulation as the likely contributing factor. However, given that increased Tmean and Tmin were associated with increasing yield in the current study, it seems unlikely that insufficient winter Chill requirements are responsible. This observation is supported by Lane (2022) who found that winter Chill requirements were met sufficiently for the majority of study cultivars.

The results show that the associations between fruit yield and temperature differ between ‘on’ and ‘off’ years (Table 4.4). There were no significant associations between ‘on year’ fruit yield and any temperature parameter, from any period of time, before the start of the current cropping season. This may imply physiological differences in response to environment between ‘on’ and ‘off’ years within this selection of apple genotypes. This would agree with Kofler et al. (2022) who determined proteomic differences (differences in specific protein abundance) between ‘on’ and ‘off’ year production seasons for the cultivar ‘Gala’. The causal relationships for these differences are yet to be determined in the literature. Based on the findings in Table 4.4, there is potential for the agro environment to contribute to those reported proteomic differences.

The influence of large daily temperature fluctuations on reducing perennial fruit yields have been reported from various sources, but not before or within the early tree dormancy period (Abbott et al., 1973; Menzei et al., 1989). Additionally, exposure to more extreme temperatures (i.e. high deviations from mean temperatures) can reduce floral initiation and subsequent fruit yield (Caprio et al., 1999; Heide et al., 2020). Several significant results suggest that Tminmaxdiff, particularly from the early tree dormancy period (November to January), was the most influential temperature parameter affecting subsequent seasonal fruit yield: it had the strongest association out of any temperature and time period combination in Table 4.4 ($t=-6.31$, $r=-0.56$). However, given the absence of literature to support this statement, it’s feasible that this correlation is not evidence of causality. A potential reason could be the modified environment facility's tendency to trap cool area in the more enclosed environment treatments (Plus2 and Plus4) during winter. Consequently, Plus2 and Plus4 treatments had a lower average Tmin in November to January compared to Ambient across most years (Table 4.1). As yield variation in the Plus2 and Plus4 was consistently greater than Ambient, and Tmean, Tmax and Tminmaxdiff all provided significant correlations in this period with the subsequent 'off' year yields, it is clear that temperature then has an effect but less clear which if any aspect of temperature is critical.

Several significant associations were found between temperature parameters of specific months in the preceding season and fruit yield in the subsequent season (Table 4.6). Floral bud differentiation from vegetative buds typically begins three to six weeks after full bloom (Pratt, 1988). Full-bloom dates varied extensively by cultivar, typically ranging from mid-April (Stark's Earliest) to the end of May ('Braeburn', 'Edward VII'). For most cultivars, three to six weeks post-full bloom fell within the months of May and June. As such, the results show that previous season May and June temperature may be associated with 'off' year fruit yield. May Tmean and Tmin were negatively associated with 'off' year yield, and June Tmean, Tmin and Tmax were positively associated with ABI. This evidence therefore suggests that an increase in temperature during these time periods may lead to greater fruit yield variation between two years. This disagrees with research that suggests that warmer average temperatures (but not sustained 'hot' conditions of +27°C) during this period are beneficial for floral bud initiation (Heide et al., 2020)

The influence of crop load on subsequent year fruit yield was demonstrated in several cultivars (Figure 4.6). This agrees well with the literature as heavier crop loads are linked with increased alternate bearing patterns through reducing fruit yield in the subsequent fruiting season (Jonkers, 1979; Monselise and Goldschmidt, 1982). For those cultivars with the strongest associations in Figure 4.6 ('Cox's Orange Pippin', 'Gala', 'Golden Delicious', and 'Jonathan') the evidence may suggest crop load was a stronger inducer of alternate bearing patterns than modified temperature. This would agree with several studies that came to similar conclusions (Krasniqi et al., 2013; Kofler et al., 2019). In 2021, crop load was (on average) much higher in Plus4 compared to Ambient (Appendix 4.2). This was likely due to insufficient fruitlet thinning during this production season, therefore likely a main contributing factor to increased Plus4 alternate bearing in 2022. However, results from other 'on' years (2017 and 2019), show that crop load in Ambient was equal or even greater than Plus4 in some instances. Despite this, crop load is still substantially lower in Plus4 in subsequent 'off' years (Appendix 4.2). Fewer 'Cox's

Orange Pippin' and 'Golden Delicious' trees also exhibited 'normal' bearing in 2020 in Plus4 compared to Ambient (Table 4.3). Therefore, crop load may not be the main contributing factor responsible for increased alternate bearing differences within certain cultivars and years.

No significant associations were found between the previous season's flowering date and subsequent season fruit yield, nor between previous season's harvest date and subsequent season fruit yield (Table 4.7). This was the case for both 'on' and 'off' year sample subsets. This is despite flowering dates being accelerated by 1-2 weeks compared to Ambient in Plus4, and harvest date by 1-3 weeks (dependent on cultivar) (Lane, 2022). The literature suggests that earlier flowering dates triggered by increased heat accumulation may be beneficial for floral bud initiation as it increases the window for it to occur (Heide et al., 2020; Jupa et al., 2021). The effect of earlier cropping on subsequent fruit yield is more mixed. There is evidence that earlier cropping may be beneficial for subsequent flowering (Williams et al., 1980; Ferree et al., 2015) or detrimental (Krasniqi et al., 2013). Nonetheless, no influence of the timing of phenological events on affecting alternate bearing (as a likely consequence of raised heat accumulation) could be determined within this study. Based on analysis from the same table, total precipitation in the previous production was found to have an insignificant impact on fruit yield in the subsequent season. Supplementary irrigation is thought to encourage floral bud initiation (Koutinas et al., 2014). It is therefore possible that rainfall needs were generally sufficient, or that irregular distribution of soil moisture content across treatments (Appendix 2.5) was responsible for the lack of correlation within the analysis.

Genotypic traits likely played a role in determining yield variation. For example, the harvesting seasonality of apple cultivars may have influenced yield response to environment. Responding similarly as numerous late-harvesting cultivars, three early harvesting cultivars - 'Discovery', 'George Cave', and Stark's Earliest – all observed enhanced ABI patterns in warmer treatments compared to Ambient. However, there were key differences within certain yield variability responses. First, the three cultivars did not express differences in low flower cluster production between treatments (Table 4.3). This doesn't necessarily imply that warmer treatments produced identical quantities of flower clusters, just that early-harvesting cultivars were not as severely reduced as late-season ones. Secondly, 'George Cave' and 'Stark's Earliest' did not produce a statistically significant relationship between November to January Tminmaxdiff and 'off' year yield (Figure 4.2). Finally, all three cultivars showed no statistically significant relationship between previous year crop load and subsequent yield (Figure 4.6). Additional correlation analysis between yield variation and study parameters concerning early-harvesting cultivars, only, detected no unique significant associations (Appendix 4.3). November to January temperature was found to be influential, as was also the case for the majority of late-season cultivars.

Of the cultivars studied, ‘Yellow Bellflower’ fruit yield appears to be highly responsive to temperature, perhaps more so than any other study cultivar. The cultivar’s ‘off’ year yield variation was significantly associated with May, June and November to January temperature from the previous production season. Its selection in the long-term study was based on its low chill characteristics and unique ‘drooping’ style branch growth. Due to their late incorporation to the study, mature tree yield data on other low chill cultivars (e.g. ‘Winter Banana’) was unavailable. Therefore, it cannot be concluded on whether all low chill cultivars behave in the same manner.

The methodology of managing crop load within the study may have contributed towards yield differences between treatments. Commercial cultivars in the UK have recommended guidelines for achieving optimal target numbers of fruit per tree (AHDB, 2021). Whilst this is mainly to optimise marketable fruit production, the guidelines also help ensure regular bearing is maintained between years. For example, 95 fruits are recommended for ‘Cox’s Orange Pippin’ and ‘Discovery’, and 115 for ‘Gala’ on mature, dwarfing rootstocks. For practicality reasons, all trees were fruitlet thinned to an equal target value (~120 fruits). Therefore, this may highlight that ‘Gala’ was thinned more appropriately than ‘Cox’s Orange Pippin’. This is a potential reason for why ‘Cox’s Orange Pippin’ was so heavily influenced by crop load between years (Figure 4.6). In commercial environments, thinning would usually consist of a mix of flower and hand thinning (AHDB, 2021). As no flower thinning occurred, this would likely also contribute towards enhancing alternate bearing patterns. The timing of fruitlet thinning may be a key factor in determining bearing differences between temperature treatments. All trees were fruitlet thinned by hand throughout late-May to late-June. The development of floral bud initiation likely occurred earlier in Plus4 compared to Ambient because of earlier flowering (1-2 weeks). Therefore, it is possible that Plus4 fruit were thinned too late in the season. Kofler et al. (2019) proposed that a crop load mediated factor was responsible for initiating floral bud differentiation in ‘Gala’. In Plus4 trees, this delayed onset may have reduced bud differentiation by narrowing the time window in which it occurs compared to Ambient.

Based on the results, genomic traits, temperature variation, and crop load probably all contributed somewhat towards yield and ABI differences among the modified temperature treatments. However, these effects varied among the diverse cultivars. Being a field experiment, there are likely numerous unquantified variables that contributed towards yield variation between environment treatments. For example, whilst trees with substantial historic damage from pests were removed from the sampling subsets, varying severity of more seasonal pest damage (e.g. from species such as *Dysaphis plantaginea*) remained part of the sample sets. Anecdotally, the Plus2 and Plus4 treatments were more susceptible to regular seasonal pest damage than Ambient. As presence of pests can affect subsequent fruit bearing (AHDB, 2021), it is highly feasible that this contributed towards reduced fruit bearing.

The list of external variable effects on fruit bearing is vast, including other environmental variables unquantified here such as humidity, crop nutrition, soil water availability and more (Sharma et al., 2019). There are also other management choices that may have contributed towards exacerbated alternate bearing problems such as M9 rootstock selection, a rootstock that has been associated with enhancing ABI across numerous cultivars (Kviklys et al., 2016). Therefore, a main recommendation for further work would be to perform controlled-environment experiments in an attempt to replicate and/or validate findings relating to genomic, temperature, and crop load effects on apple yield variation. A similar recommendation was proposed by Kofler et al. (2019), where it was recommended that investigations into floral bud initiation require specific experimental setups in controlled-environment experiments. It is unlikely that results from this study could ever be replicated given the novel methodology. For example, it was realised in hindsight that trees had acquired severe alternate bearing habits given the lack of fruit thinning prior to modified temperature treatment application. However, it would be feasible test individual parameters, such as November to January temperature impacts on floral bud production, given the right experimental controlled environment setup.

4.5 Conclusions

The results from this study explored the complex nature of how external environment and tree management potentially affected yield variation within a wide range of apple cultivars. Certain cultivars produced enhanced alternate bearing under different year-round temperature modification treatments. This was likely partly due to differences in the quantity of floral clusters produced under the warmer temperature regimes compared to ambient conditions.

Temperature parameters from particular periods of time before flowering were associated with effects on apple fruit yield and its inter-annual variation. Mean temperature from the previous April and May, as well as November to January Tmin and Tminmaxdiff, were strongly associated with influencing ‘off’ year fruit yield. Additionally, temperature from the previous April, May, June, and November to January were associated with the two-year alternate bearing index (ABI). Temperature from the current season was associated with ‘on’ year yield and ABI, matching findings from Chapter 3 that seasonal temperature affected fruit yield. Temperature yield responses varied greatly between cultivars, highlighting the importance of cultivar selection. Crop load was found to be highly negatively associated with subsequent year fruit yield for some apple cultivars.

This medium- to long-term field study has demonstrated the complex nature of how perennial fruit crops respond to environment. As such, analysis of the long-term data has detected several significant associations between modified temperature environment and apple fruit yield. However, these correlations do not necessarily imply causation. It is recommended that further study, primarily through the use of controlled environment experiments, be used to potentially replicate and validate significant temperature and yield associations. Nonetheless, it is clear that apple fruit yield is affected by temperature in the previous season, as well as that in the current season; that alternate bearing was often greater with an increase in temperature (varying by cultivar); floral bud initiation (through altered flower cluster production) was affected by long-term temperature modification which subsequently affected apple fruit yield; crop load in one year affected fruit yield in the subsequent season for several cultivars; and altered flowering dates, altered harvest date, and varied rainfall were not associated with affecting long-term yield variation within this study.

Chapter 5: The influence of seasonal temperature on fruit quality of apple

5.1 Introduction

‘Fruit quality’ is a subjective term applied whilst evaluating fruit produce. The quality of apple fruit is typically associated with intrinsic characteristics (physical and sensory) that lead a consumer to be satisfied with the product (Harker et al., 2003). Extrinsic properties (such as branding, packaging etc.) also have a perceived impact on food quality from a consumer point of view (Ardeshiri and Rose, 2018).

Fruit quality attributes are determined by a mix of genetic, agronomic, and environmental factors. Genetic and agronomic factors can be managed effectively by growers, whereas environmental influences are largely driven by climatic parameters such as temperature, light radiation parameters, and humidity (Musacchi and Serra, 2018). Intrinsic fruit quality attributes of apples can be broadly represented by two categories: external (or ‘appearance’) and internal. External qualities include size, shape, colour and russeting. Internal qualities include (but are not limited to) texture (or ‘firmness’), starch, soluble solids content (SSC), acidity, chlorophyll absorbance index, and dry matter content (Musacchi and Serra, 2018).

Wide genotypic diversity causes intrinsic quality trait variability among apple cultivars (Mignard et al., 2021). This therefore makes it objectively infeasible to specify ‘optimal’ quality for apple production as a whole. Specific apple marketing standards (for example, EU No. 543/2011) aid in quantifying minimum requirements for certain cultivars. These classify produce into marketable classifications of Class I (‘good quality’), Class II (slight defects) and waste (unmarketable). These commercial standards primarily assess external qualities: size, structural integrity, and colour. This is satisfactory towards driving consumer purchasing, as poor external appearance is linked with a decrease in quality perception (Jaeger et al., 2018). However, few classification systems consider internal qualities that contribute towards taste.

A comprehensive review by Musacchi and Serra (2018) concluded a research gap was present in characterising ‘high’ fruit quality standards worldwide for every apple cultivar – particularly across organoleptic and nutritional characteristics where few guidelines currently exist. Such factors should be important to growers and retailers. Evidence shows that improved organoleptic experience (e.g. ‘pleasant’ tasting) has been shown to increase customer willingness to pay (Seppa et al., 2015) and the nutritional value of apples is linked with numerous consumer health benefits (Goldberg, 2003).

Climate-change-induced temperature increase will directly affect photosynthetic plant rates, causing alterations in sugars, organic acids, flavonoid content, firmness, and antioxidant activity (Moretti et al., 2010). The effects of weather on individual apple fruit quality attributes have been studied for over a century. For example, Brooks and Fisher (1926) concluded that apple fruits exposed to direct sunlight had greater sugar content compared to those shaded. Extremely high temperatures are linked with a greater proportion of waste fruit within an apple production system. Fruit flesh temperatures >40°C are linked with increased incidence of watercore, sunburn, and texture reduction (Ferguson et al., 1999). A large-scale field study of climate effects on 'Fuji' in China concluded that fruit quality parameters were 'positively' affected by warmer temperatures (Zhang et al., 2018). However, they also noted that different fruit quality attributes were impacted distinctly by different meteorological factors. Therefore, it is important to consider each attribute individually, rather than as a collective 'fruit quality' indicator.

Fruit firmness is an important intrinsic fruit quality parameter for both industry and consumer. Within the industry, monitoring firmness is an important metric both pre-harvest and post-harvest, especially when optimising fruit for long-term storage. Pre-harvest firmness assessments are integral for optimising harvest date, whereas post-harvest assessments are an integral part of continuous fruit quality store monitoring procedures. Optimal values are dependent on a range of factors, including cultivar, storage length, end use, etc. From a consumer perspective, firmness is one quantitative measure related to the sensory property of texture, which is a critical component for evaluating quality of apple fruit (Brookfield et al., 2011). Historic temperature shifts over the past 50 years are thought to have negatively influenced tree physiological processes (cell division and expansion) that determine fruit firmness (Ornelas-Paz et al., 2018). Warmer environments during early fruit development are also linked with decreased firmness at harvest (Warrington et al., 1999).

Soluble solids content (SSC) is a method of quantifying the concentration of soluble sugars in an apple, typically through use of a refractometer (Musacchi and Serra, 2018). It was found to be a key consumer trait in apple due to a close association with the taste of 'sweetness' (Harker et al., 2003). Several studies have investigated the effect of changing climates on SSC. Long-term observations between 1970-2010 in Japan determined that 'Fuji' SSC has increased by 0.20-0.28%Brix per decade as a result of warming weather (Sugiura et al., 2013). Higher seasonal temperature is also positively associated with SSC in China (Zhang et al., 2018). Temperature during early fruit development is particularly influential: Warrington et al. (1999) demonstrated higher SSC when 'Braeburn' and 'Fuji' fruit were exposed to maximum temperatures of 22°C compared to 13°C. However, other studies have produced inconclusive or even negative associations between temperature and SSC. For example, Lee et al. (2023) demonstrated that several elevated temperature scenarios all resulted in reduced SSC in ‘Fuji’. SSC is also highly influenced by management practices: Iwanami et al. (2023) concluded that crop load was a better indicator of SSC than seasonal temperature.

The colouration of an apple fruit is dependent on the cultivar. Concentrations of anthocyanin, chlorophyll and carotenoid pigments are typically responsible for pigmentation across all angiosperm species, with their biosynthetic pathways highly determined by environmental factors such as light radiation parameters and temperature (Reinbothe and Reinbothe, 1996). Anthocyanin accumulation is the primary pigment responsible for red colouration in fruit. Its biosynthesis in apple is known to be heavily regulated by temperature (Lin-Wang et al., 2011). Colour accumulation occurs during late fruit development and maturity. Cooler night-time temperatures are linked with more optimal anthocyanin production (Curry, 1997). In the UK, the two most popular grown cultivars (‘Gala’ and ‘Braeburn’) are ‘bicoloured’. Current marketing standards recommend that certain strains of these cultivars should have at least 50% red colour coverage (RCC) (EU, No. 543/2011). Failure to meet these minimum standards will result in classification below Class 1. Inadequate ‘Gala’ RCC because of higher temperatures is already an issue in several apple production areas such as the Mediterranean (Iglesias et al., 2008). Consequently, new strains of these cultivars have been developed that are more resilient to temperature effects on red colour accumulation (Argenta et al., 2023; Iglesias et al., 2016). UK growers may potentially need to adopt these sports to replace existing cultivar strains should marketability standards remain the same in the face of future climate change.

Dry matter content (DMC) is the remaining content of an apple after all the moisture is removed. DMC has been studied to be positively associated with consumer preference of ‘Royal Gala’ apples (Palmer et al., 2010). DMC is a more-recently-adopted industry fruit quality parameter for apple. As such, less is understood about its response to environment. However, DMC and SSC are known to be highly positively correlated in other fruits such as plum and peach (Scalisi and O’Connell, 2021). Increased sunlight interception is linked with increased apple fruit DMC, however excessive radiation may inhibit DMC accumulation through sunburn damage (Corelli-Grappadelli and Lakso, 2002).

Traditionally, the UK’s temperate climate does not require the use of irrigation to attain a high proportion of marketable fruit (Faust, 2000). However, with “hotter, drier summers” expected with future climate change, irrigation needs will likely increase. A literature review documented that deficit irrigation studies over the past 40 years have both ‘positive’ and ‘negative’ impacts on fruit quality parameters (Musacchi and Serra, 2018). For example, deficit irrigation was found to positively influence firmness, SSC, and DMC (Mplelasoka et al., 2001) and increase colour coverage (Mills et al., 1996) for the cultivar ‘Braeburn’. However, Musacchi and Serra (2018) also mention numerous studies where deficit irrigation negatively impacts frequency of fruit disorders, damage, and nutritional interference.

The relationship between meteorological climate and fruit quality is complex and can be influenced by grower management practices. The literature suggests that future climate change will influence a wide range of fruit quality metrics used within industry. However, there are few studies that compare the direct effect of raised temperature regimes on apple production within the same spatial and temporal field environment. As part of the ‘Apples in a Warmer World’s’® long-term investigation into the effect of future climate scenarios on UK apple production, this study investigated how three different seasonal temperature regimes impacted a range of fruit quality metrics utilised within the UK apple industry. The hypotheses were as follows:

***H_0*: Modified temperature environments will have no effect on the outcome of tested fruit quality parameters across a range of apple cultivars.**

***H_1*: Modified temperature environments will have an effect on the outcome of tested fruit quality parameters across a range of apple cultivars.**

5.2 Materials and Methods

5.2.1 Overview

The study was split in to three sections that determined the influence of varied temperature regimes on five fruit quality parameters. Fruit quality assessments and data collection relating to weather, environment and production were conducted over a five-year period (2017-2021) of the ‘Apples in a Warmer World’® long-term study. Firstly, data from 10-16 cultivars (see Table 2.1) were compared among the three different temperature regimes. Secondly, the direct relationships between seasonal temperature and fruit quality parameters were explored. Thirdly, multivariate modelling incorporating weather, environment and production variables was applied to determine what was most influential on fruit quality parameters within the study environment.

5.2.2 Fruit quality assessments

Data relating to fruit quality were obtained from sixteen trial cultivars over the course of five apple production seasons (2017-2021); 2017 served as a baseline year where all trees were subject to the same environment.

The fruit quality metrics studied were selected based on methods that were cost-effective and produced reliable, quick, and easily replicable results. This ensured identical fruit quality measurement standards were maintained between cultivars and treatments across each 3-4 months of seasonal fruit harvest activities. Methodology replicated the tests performed within a commercial environment, such as those listed within the AHDB's "Apple Best Practice Guide", 2021.

Fruit were harvested as described in Chapter 3. At the time of harvest, up to 20 Class 1 fruit were randomly sampled from each temperature and rainfall treatment combination for each trial cultivar (nine environment treatments in total). Class 1 fruit refers to samples of 'good quality' – perfectly sound flesh, minimal defects, and only slight russeting (EU, No. 543/2011). In instances where 20 treatment Class 1 fruit were unobtainable, Class 2 fruit were used. All such Class 2 fruit were free from major defects. Ten sample fruits were randomly selected for weight, firmness, SSC, and red colour coverage (RCC) analysis, with the remaining ten used for dry matter analysis. Fruit quality assessments were processed within 6-12 hours of harvest. This allowed fruit to 'cool' to room temperature without the compromise of natural post-harvest fruit changes.

Each individual apple fruit was weighed using a calibrated weight scale (to the nearest 0.001g). Fruit weight and size are heavily positively correlated variables. Due to the incorporation of diverse apple cultivars, unique fruit shapes skew maximum fruit diameter. Therefore, as this study compared treatments at the cross-cultivar level, fruit size was an inappropriate measure for comparison. Fruit weight was a more accurate measure and also allowed for greater precision. For the purpose of this study, fruit weight and size were interchangeable terms given their strong association with one another.

Firmness readings (kg) were obtained invasively through use of a mounted FT-327 handheld penetrometer (Effegi) with 11mm probe. Fruit were prepared for this analysis by removing small slices of fruit skin on opposite sides (~30-40mm wide, ~3-5mm thick) of the fruit, and applying pressure using a 11mm probe attached to the penetrometer for two seconds. Readings were obtained on both sides of the fruit, with the average obtained from both observations recorded. The unit for measurement was kg, replicating methodology used within UK industry (AHDB, 2021).

SSC (%Brix) readings were obtained using a calibrated PAL-1 3810 Digital Pocket Refractometer (Atago, Tokyo 105-0011, Japan) (NB: %Brix and °Brix are interchangeable units where 1°Brix is equal to 1%Brix).

The RCC (%) of fruit was estimated by eye as equipment to measure red colour more accurately was not available for this study. The same individual performed colour assessments throughout the trial for consistency purposes.

DMC (%) observations were obtained from the second group of ten sampled fruits. Two segments from opposite sides of each fruit were placed within an oven at >100°C for 24-48 hours to remove all water content. DMC was calculated on the fresh weight basis by dividing post-oven fruit segment weight by original weight.

Various aspects of growing environment, including temperature, are linked with effects on external and internal apple fruit damage and disorders (Musacchi and Serra, 2018). As the methodology concerned the performance of Class 1 fruit only, little consideration was given to the effect of modified temperature regime on external fruit damage. Internal fruit disorders discovered during fruit quality assessments concerned a negligible quantity of samples. These sampled fruits were discarded and removed from the analysis.

5.2.3 Meteorological and production data

Weather data (2017-21) was sourced as documented in Chapter 2.4. To replicate studies within other chapters of this thesis, rainfall treatments were not incorporated in to the fruit quality data analysis, for the reasons given in Chapter 2.4. That is, it was assumed that the small variation in rainfall across all modified temperature environments had no effect on fruit quality.

As with any missing observations for temperature and rainfall, sunlight hours data (01/01/2017-31/12/2021) was sourced from long-term hourly data observations from Manston Airport's MetOffice weather station (~30km from the cultivation site). Although a difference is to be expected between Manston's and Brogdale's sunlight hours, Manston was the closest historic weather database with hourly data; and inter-annual differences are expected to be similar.

Growing degree days (GDD) were calculated from 1st January to harvest date for each cultivar and temperature treatment. This time period replicates commercial practice. GDD was calculated using the following formula:

$$GDD = \left[\frac{Tmax + Tmin}{2}\right] - Base\ Temp$$

The base temperature estimate was 6°C across all cultivars.

Yield results (total fruit and fresh weight per tree) from each cultivar and temperature treatment were collected over a six-year period (2017-22). Mean values were based on total harvested trees (n=10-18 trees per treatment depending on cultivar).

The total amount of vegetative growth removed from each tree was recorded annually (2017-21). This typically involved a full winter prune (February-April) followed by a lighter summer prune (June-July) where necessary.

Flowering and harvest dates were sourced from trial phenological recordings (2017-2021). ‘Full flowering’ represents the average date for a cultivar and temperature treatment combination reaching BBCH66 ‘80% full inflorescence’. Harvest date represented the date a cultivar and temperature treatment combination reached 50% on the starch index scale (also known as ‘tree-ripe’ stage).

Other production and environment variables were utilised within the fruit quality data analysis. All categoric, independent, and dependent variables used for the analysis are described in Table 5.1.

***Table 5.1**. Description of all variables used in the cross-cultivar apple fruit quality analysis.*

Variable name	Unit	Type	Variable Description
Cultivar	-	Categoric	Cultivar (16 in total)
Year	-	Categoric	Year of assessment (2017-21)
Temp	-	Categoric	Modified temperature environment (Amb, Plus2 or Plus4)
Trait	-	Categoric	Selected cultivar trait for analysis (eight total)
Seasonality	-	Categoric	Early, mid, or late season harvesting
Tmean	°C	Independent	Mean seasonal temperature (full flowering to harvest date)
Tmin	°C	Independent	Mean daily minimum seasonal temperature (full flowering to harvest date)
Tmax	°C	Independent	Mean daily maximum seasonal temperature (full flowering to harvest date)
Tminmaxdiff	°C	Independent	Mean daily minimum maximum daily seasonal temperature difference (full flowering to harvest date)
GDD	-	Independent	Total growing degree days (Jan 1st to harvest date)
SunHours	-	Independent	Total seasonal daylight hours (full flowering to harvest date)
Ppt	mm	Independent	Total seasonal precipitation (full flowering to harvest date)
Yield_FW	kg	Independent	Mean fresh weight harvested per tree
Yield_TF	-	Independent	Mean total fruit number harvested per tree
Pruning	kg	Independent	Mean total vegetative growth removed per tree
FFdate	-	Independent	Mean full flowering date per tree (day of year)
Hdate	-	Independent	Harvest date (day of year)
FQfirmness	kg	Dependent	Mean firmness (2017-21)
FQssc	%Brix	Dependent	Mean soluble solids content (2017-21)
FQweight	g	Dependent	Mean fruit sample weight (2020-21)
FQcolour	%	Dependent	Mean red colour coverage (where applicable, 10 cultivars) (2020-21)
FQdm	%	Dependent	Mean dry matter content (2020-21)

5.2.4 Statistical analysis

Mean values based on five years (firmness, SSC, fruit weight; 2017-21) or two years (RCC and DMC; 2020-21) of assessments were calculated across sixteen apple cultivars except for RCC which was only applicable to 10 of the 16 cultivars. The cultivars ‘Braeburn’ and ‘Jolyne’ provided data for 2020 and 2021 only. Four more recently-incorporated trial cultivars (‘Beverly Hills’, ‘Granny Smith’, ‘Tropical Beauty’, and ‘Winter Banana’) were excluded from the analysis. This was due to immature trees producing highly variable fruit quality across all treatments. One-way ANOVA results were conducted to determine statistically significant differences between the temperature treatment means using the R package “stats”.

Regression analyses between seasonal temperature (independent variable) and firmness, SSC, RCC and DMC (dependent variables) were modelled for each applicable cultivar. The variable 'fruit weight' was excluded from analysis to avoid overlap with Chapter 3. Data was used from all modified temperature environments. The data points for each cultivar represent mean seasonal temperature values between 'full-flowering' and 'harvest' dates against mean fruit quality values within a single fruit production season. A linear regression line ($y = a + bX$), coefficient of determination (R^2), and statistical significance (p) were calculated for each cultivar and fruit quality variable. This was conducted using the R package "ggplot2".

Further regression analyses modelled the effect of seasonal temperature (x) on a pool of cross-cultivar values for firmness, SSC, RCC and DMC (y). As fruit quality outputs naturally vary among apple genotypes (Mignard et al., 2021), y axis values were standardised ($z = (X – \mu) / \sigma$) relative to the total variation within the inter-annual cultivar population. Comparing environment and production responses within each cultivar's five-year range allowed adequate comparison between different genotypes. An additional set of regression analyses demonstrated how three different component periods of fruit development and maturation (early-, mid-, and late-season) compared in fruit quality temperature responses (see Table 2.1 for classification of cultivars). For this analysis, seasonal temperature was also standardised ($z = (X – \mu) / \sigma$) relative to the total temperature variation within each cultivar. This provided a more proportional comparison of fruit quality response between the three different periods of the season. This was conducted using the R package "ggplot2".

Multiple linear regression analyses assessed the strength of the relationship between two seasonal weather variables (temperature and precipitation) from four phenology phases (1st January to bud burst, bud burst to full-flowering, full-flowering 'mid-season', and 'mid-season' to harvest date) across five fruit quality parameters. Standardised values relative to each cultivar's long-term variation were again utilised across all variables. 'Mid-season' represented the date halfway between full-flowering and harvest date. Model results revealed overall model strength from all predictor variables combined, as well as identifying the importance of each individual predictor through highlighting statistical significance ($p<0.05$ indicating a significant relationship). This was conducted using the R package "ggplot2".

Linear multivariate analysis was used to determine the influence of recorded trial variables on individual fruit quality parameters (see Table 5.1). Correlation analysis determined the strength of correlations between each predictor and response variable using the R package “correlation”. Principal Component Analysis (PCA) was conducted using the R package “factoextra” to explore independent and dependent variable relationships in reduced dimensionality. Partial Least Squares Regression (PLSR) was conducted using the package “mdatools” to determine the influence of independent variables (Table 5.1) on fruit quality parameters. Due to large quantities of missing data between 2017-19, PLSR models were imposed on each individual fruit quality parameter instead of a ‘pool’ of dependent variates. Model weights and loadings were calculated for each independent and dependent variate combination. Full cross validation was used to assess models. Subsequent Variable Importance in Projection (VIP) scores were calculated to indicate the model impact strength of the dependent variables. A VIP threshold score of 1.0 or above was used to determine the main independent variable factors affecting fruit quality parameters, as performed in Zhang et al. (2018).

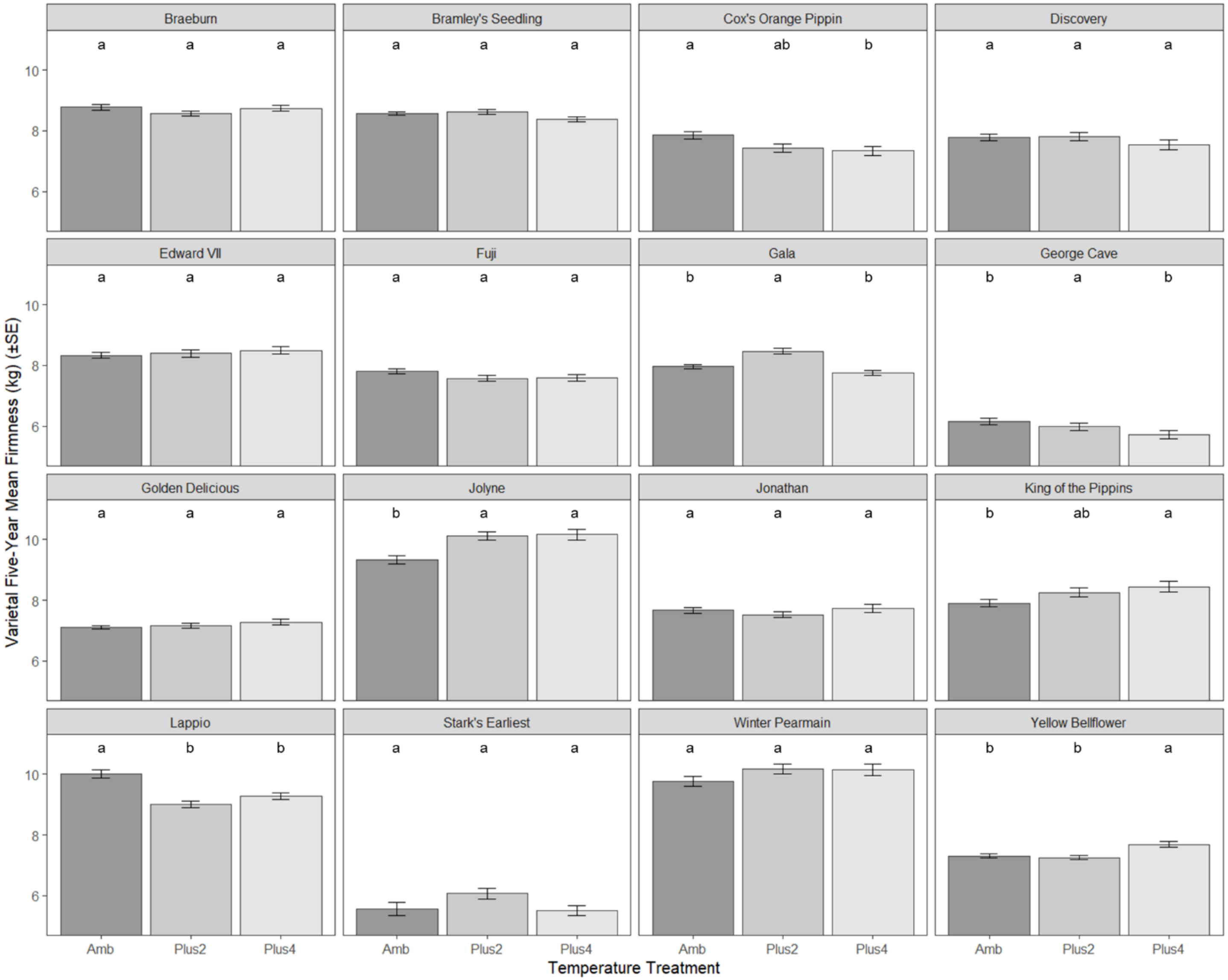
5.3 Results

5.3.1 Overview of apple fruit quality assessments

Figure 5.1 presents five-year mean values (2017-21) for firmness (5.1a) and SSC (5.1b) and two-year mean values (2020-21) for sample fruit weight (5.1c), RCC (5.1d) and DMC (5.1e) across 10 (RCC, 5.1d) to 16 (5.1a, b, c, e) trial cultivars and three temperature treatments. Fruit quality values varied considerably by cultivar. As such, there was considerable cross-cultivar range across all fruit quality parameters. Mean firmness ranged from 5.5kg ('Stark's Earliest', plus4) to 10.2kg ('Lappio', Ambient) (Figure 5.1a). Mean SSC ranged from 11.4%Brix ('Stark's Earliest', Plus4) to 14.4%Brix ('Golden Delicious', Plus2) (Figure 5.1b). Mean fruit weight varied from 103.6g ('Stark's Earliest', Ambient) to 334.9g ('Bramley's Seedling', Ambient) (Figure 5.1c). Mean estimated RCC varied from 15.2% ('Winter Pearmain', Plus4) to 89.3% ('Braeburn', Ambient) (Figure 5.1d) for those cultivars where RCC is a cultivar feature (10 out of 16 cultivars). Finally, DMC ranged from 13.0% ('Stark's Earliest', Ambient) to 18.6% ('Lappio', Plus2) (Figure 5.1e).

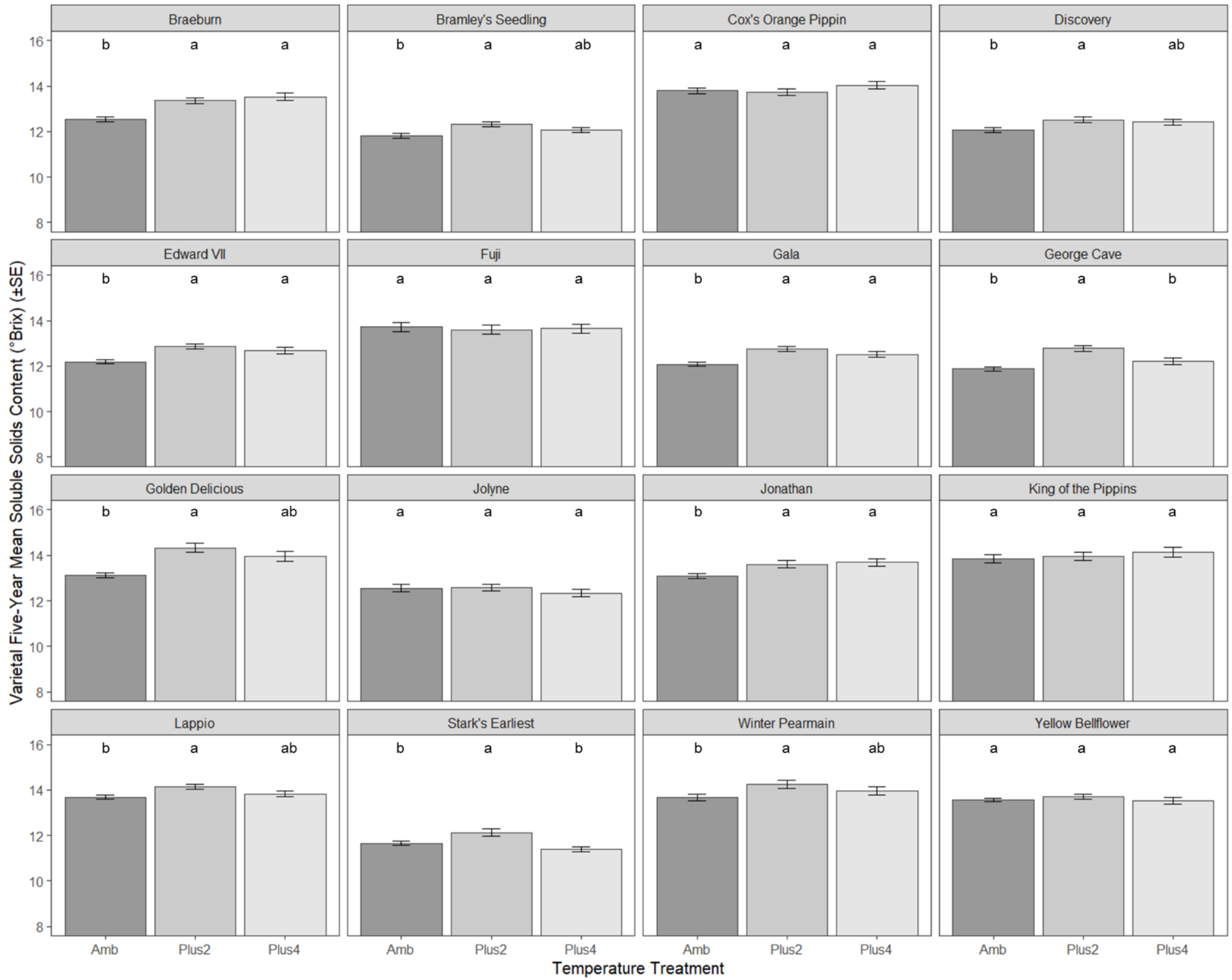
One-way ANOVA results (Appendix 5.1) showed significant differences among the three temperature treatments within each mean fruit quality metric. For fruit sample weight, ten out of 16 cultivars produced significant differences among treatments ($p<0.05$); eight producing heavier fruit in Ambient compared to the warmer treatments. For fruit firmness, seven out of 16 cultivars produced significant differences among treatments ($p<0.05$); three cultivars produced firmer fruit in Ambient, four produced firmer fruit within warmer treatments. For SSC, 12 out of 16 cultivars showed significant differences among treatments ($p<0.05$). All 12 of these cultivars showed greater SSC in warmer treatment conditions compared to Ambient. For estimated RCC, eight out of ten cultivars produced significant differences among treatments ($p <0.05$). All eight cultivars showed greater RCC within Ambient conditions. Lastly for DMC, only one cultivar produced significant differences among treatments ($p<0.05$); 'Braeburn' fruit produced in warmer treatment conditions had greater DMC than in Ambient.

a



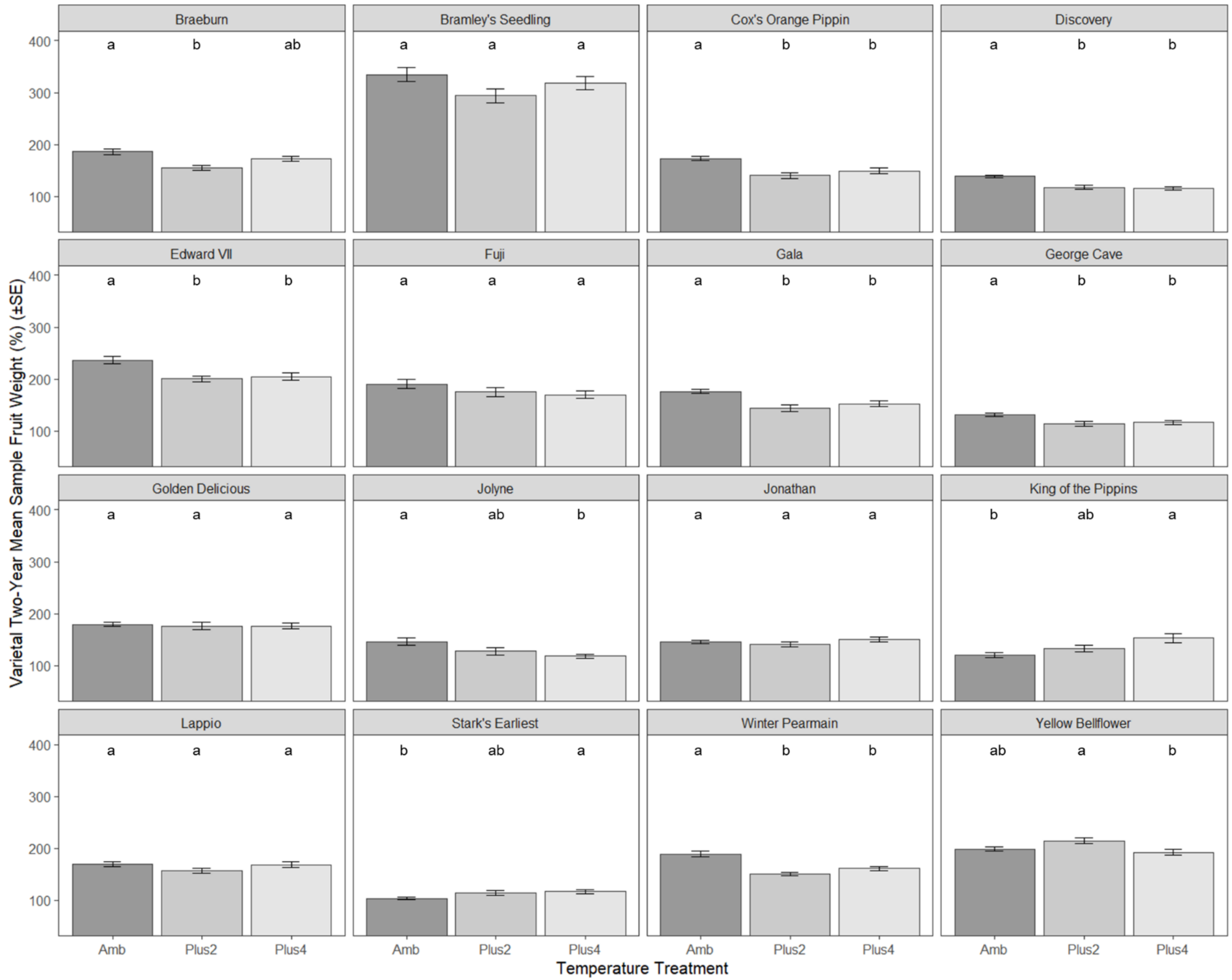
Braeburn
Bramley's Seedling
Cox's Orange Pippin
Discovery
Edward VII
Fuji
Gala
George Cave
Golden Delicious
Jolyne
Jonathan
King of the Pippins
Lappio
Stark's Earliest
Winter Pearmain
Yellow Bellflower
Varietal Five-Year Mean Firmness (kg) (±SE)
Temperature Treatment
Amb
Plus2
Plus4

b

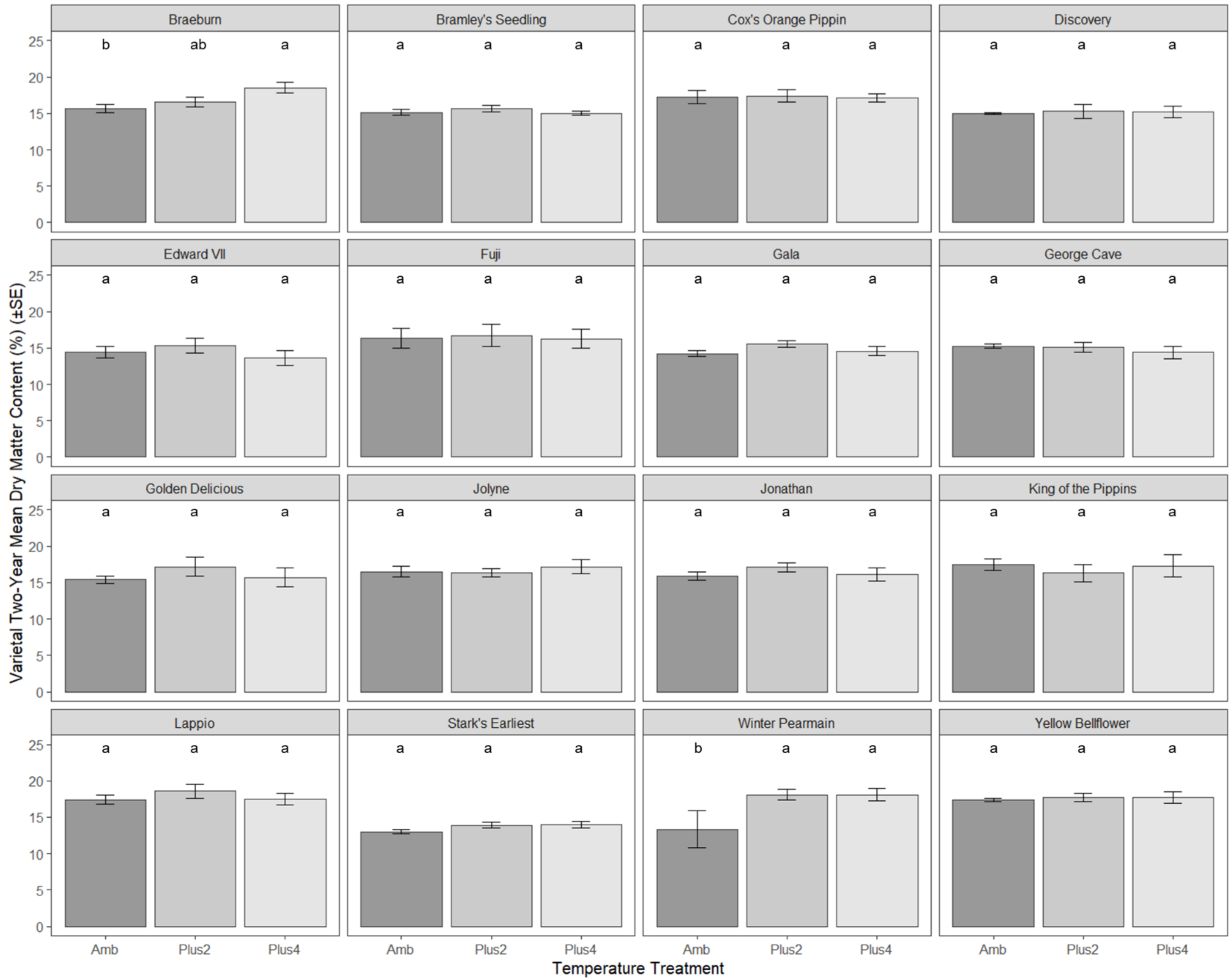


Braeburn
Bramley's Seedling
Cox's Orange Pippin
Discovery
Edward VII
Fuji
Gala
George Cave
Golden Delicious
Jolyne
Jonathan
King of the Pippins
Lappio
Stark's Earliest
Winter Pearmain
Yellow Bellflower
Varietal Five-Year Mean Soluble Solids Content (°Brix) (±SE)
Amb
Plus2
Plus4
Temperature Treatment

c


Braeburn
a b ab
Bramley's Seedling
a a a
Cox's Orange Pippin
a b b
Discovery
a b b
Edward VII
a b b
Fuji
a a a
Gala
a b b
George Cave
a b b
Golden Delicious
a a a
Jolyne
a ab b
Jonathan
a a a
King of the Pippins
b ab a
Lappio
a a a
Stark's Earliest
b ab a
Winter Pearmain
a b b
Yellow Bellflower
ab a b
400
300
200
100
Varietal Two-Year Mean Sample Fruit Weight (%) (±SE)
Amb
Plus2
Plus4
Temperature Treatment

d


Braeburn
Bramley's Seedling
Cox's Orange Pippin
Discovery
Edward VII
Fuji
Gala
George Cave
Golden Delicious
Jolyne
Jonathan
King of the Pippins
Lappio
Stark's Earliest
Winter Pearmain
Yellow Bellflower
b ab a
a
b
0 5 10 15 20 25
Varietal Two-Year Mean Dry Matter Content (%) (±SE)
Amb Plus2 Plus4
Temperature Treatment

e

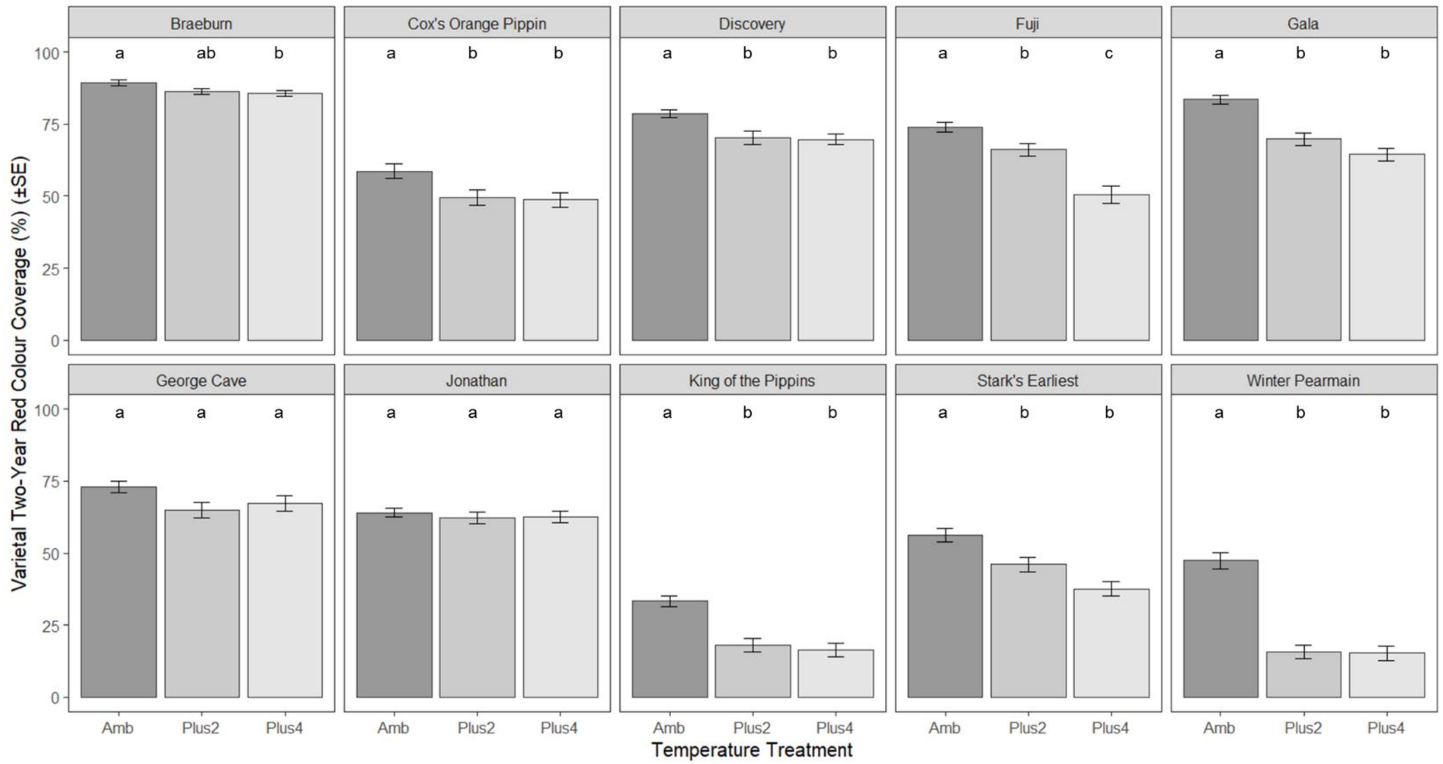


***Figure 5.1**. Overview of mean (±SE) quality measurements of apple fruits (a: Firmness, b: SSC, c: Fruit Weight, d: DMC, e: RCC) across three temperature environments (Ambient, Plus2, and Plus4) and up to 16 apple cultivars. Values represent mean temperature treatment assessment over five years of tests (2017-21). One-way ANOVA results (a-c) indicate significant differences ($p<0.05$) between treatments. In 2017 all three tunnels were maintained at close to ambient.*

5.3.2 Apple fruit quality and seasonal temperature

Mean fruit quality values from each year were compiled from each temperature treatment and plotted against seasonal temperature (Figure 5.2) to determine whether a significant linear regression could be detected for each of the 16 study cultivars. The value of seasonal temperature was calculated separately for each cultivar in each temperature regime between the dates of full flowering and harvest for each year. The response of firmness to seasonal temperature across the contrasting cultivars was mixed (Figure 5.2a). Two cultivars ('Discovery' and 'George Cave', both early-harvesting cultivars) showed significant negative ($p<0.01$) relations, whereas four late-season cultivars showed significant positive ($p<0.05$) relations. The most sensitive relationship was seen in 'Winter Pearmain', where firmness increased by 1.16kg for every 1°C increase in seasonal temperature. 'Gala' and 'Braeburn', commercially important cultivars in the UK, showed no significant trends in firmness with temperature with comparatively little variation in firmness detected.

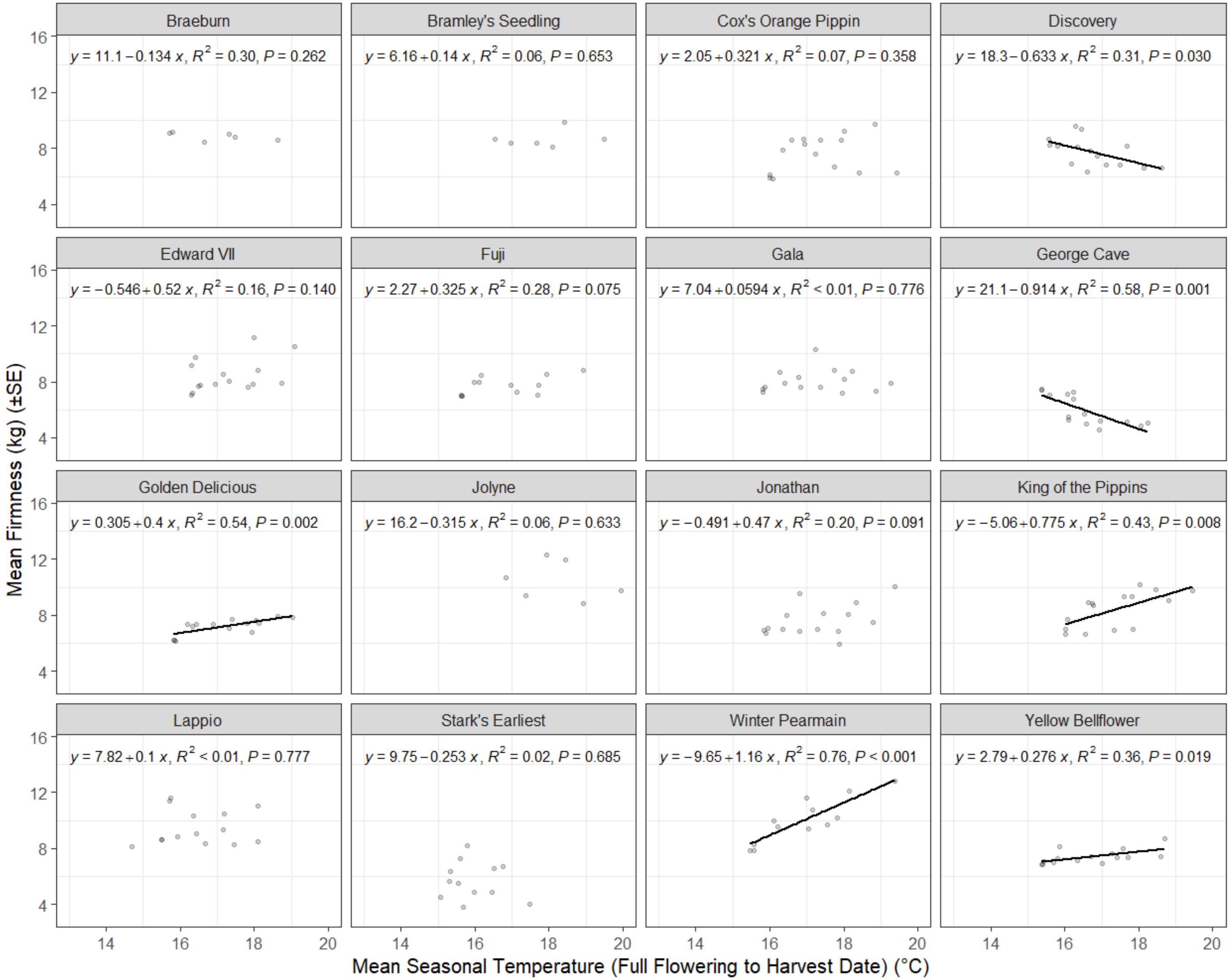
The relationship between seasonal temperature and SSC was consistently positive across all 16 cultivars, albeit not often significantly so (Figure 5.2b). Only the three cultivars 'Discovery', 'George Cave' and 'Golden Delicious' showed significant (positive) linear relations ($p<0.05$), although those in 'Jonathan' and 'Stark's Earliest' approached significance ($0.10>p<0.05$). The most responsive relationship was seen in the early-harvesting cultivar 'Discovery', where SSC increased by 1.21%Brix for every 1°C increase in seasonal temperature. Not one of the 16 cultivars showed any suggestion of a non-significant negative relation.

Seasonal temperature and RCC relations were less consistent across cultivars (Figure 5.2c), possibly due to the limited number of observations (only two years of data). Nonetheless, three significant negative ($p<0.05$) relations were detected among the 10 applicable cultivars: 'Fuji', 'King of the Pippins' and 'Winter Pearmain'. 'Winter Pearmain' was the most susceptible cultivar with a decrease of 19.3% in RCC for every 1°C increase in seasonal temperature. The observations for five other cultivars also concurred with a decline in RCC with increase in temperature, but in two ('Discovery' and 'Jonathan') the raw data showed (non-significant) increases in RCC.

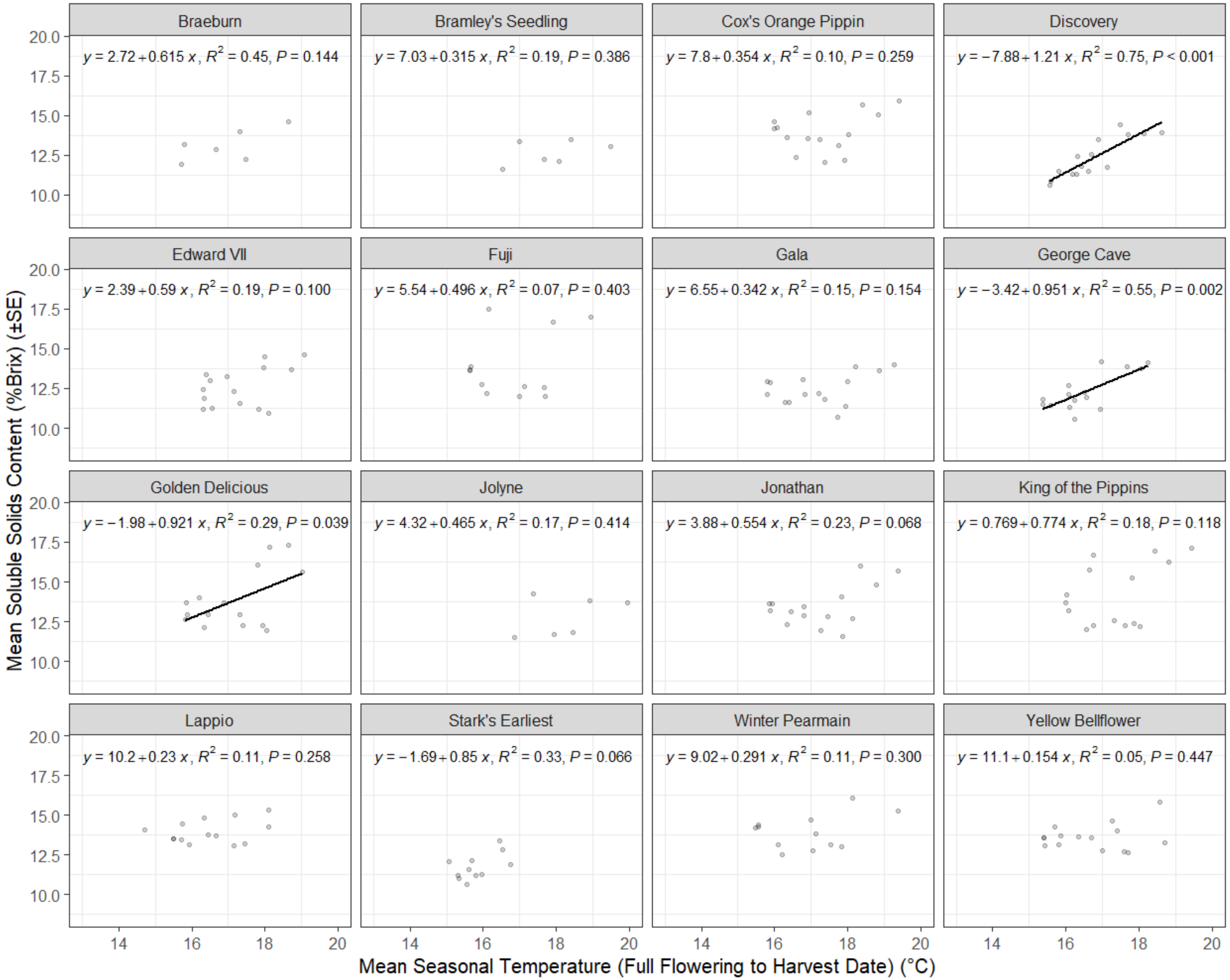
The response of DMC to seasonal temperature, like SSC, was consistently positive across all 16 cultivars studied (Figure 5.2d). However, only the responses in two cultivars ('Discovery' and 'Winter Pearmain') achieved significance ($p<0.05$) whilst that in 'Stark's Earliest' was almost significant ($0.10>p<0.05$). As with RCC, the non-significant (yet broadly similar) trends in the majority of cultivars may have been due to the limited number of observations (only two years of data).

Overall, no cultivar showed significant responses of all four aspects of fruit quality to temperature, but one (‘Discovery’) did so for three (all but RCC). At the other extreme, ten cultivars (‘Braeburn’, ‘Cox’s Orange Pippin’, ‘Bramley’s Seedling’, ‘Edward VII’, ‘Fuji’, ‘Gala’, ‘Jolyne’, ‘Jonathan’, ‘Lappio’, ‘Stark’s Earliest’) showed no significant response to temperature for any of the aspects of fruit quality assessed in Figure 5.2. In some cases at least this may have been due to high variability when considering each cultivar alone (e.g., ‘Fuji’ in Figure 5b, d). For this reason, the response of fruit quality to temperature was investigated further - with all cultivars combined.

a

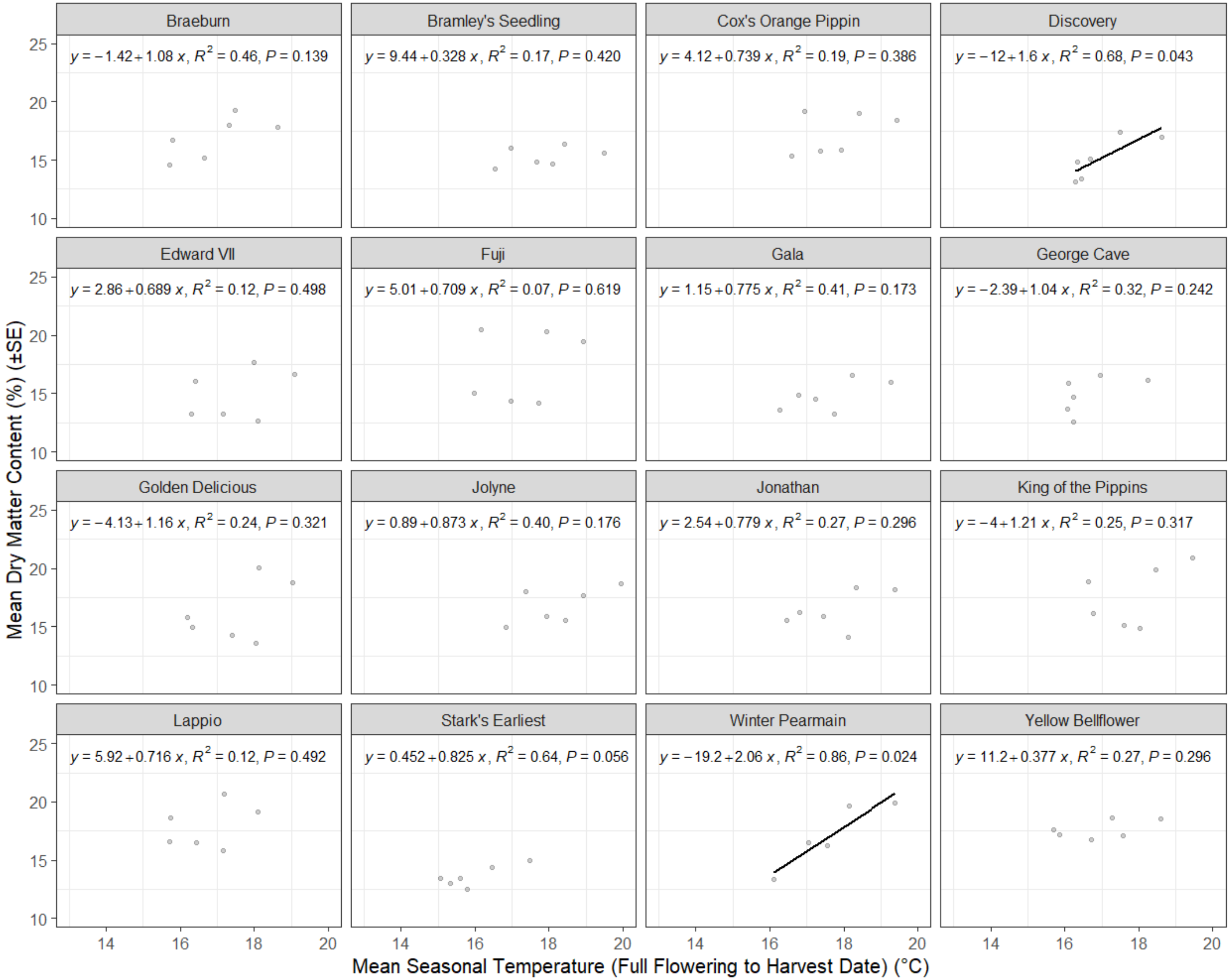

Braeburn
y = 11.1 − 0.134 x, R2 = 0.30, P = 0.262
Bramley's Seedling
y = 6.16 + 0.14 x, R2 = 0.06, P = 0.653
Cox's Orange Pippin
y = 2.05 + 0.321 x, R2 = 0.07, P = 0.358
Discovery
y = 18.3 − 0.633 x, R2 = 0.31, P = 0.030
Edward VII
y = −0.546 + 0.52 x, R2 = 0.16, P = 0.140
Fuji
y = 2.27 + 0.325 x, R2 = 0.28, P = 0.075
Gala
y = 7.04 + 0.0594 x, R2 < 0.01, P = 0.776
George Cave
y = 21.1 − 0.914 x, R2 = 0.58, P = 0.001
Golden Delicious
y = 0.305 + 0.4 x, R2 = 0.54, P = 0.002
Jolyne
y = 16.2 − 0.315 x, R2 = 0.06, P = 0.633
Jonathan
y = −0.491 + 0.47 x, R2 = 0.20, P = 0.091
King of the Pippins
y = −5.06 + 0.775 x, R2 = 0.43, P = 0.008
Lappio
y = 7.82 + 0.1 x, R2 < 0.01, P = 0.777
Stark's Earliest
y = 9.75 − 0.253 x, R2 = 0.02, P = 0.685
Winter Pearmain
y = −9.65 + 1.16 x, R2 = 0.76, P < 0.001
Yellow Bellflower
y = 2.79 + 0.276 x, R2 = 0.36, P = 0.019
Mean Firmness (kg) (±SE)
Mean Seasonal Temperature (Full Flowering to Harvest Date) (°C)

b



Braeburn
y = 2.72 + 0.615 x, R² = 0.45, P = 0.144
Bramley's Seedling
y = 7.03 + 0.315 x, R² = 0.19, P = 0.386
Cox's Orange Pippin
y = 7.8 + 0.354 x, R² = 0.10, P = 0.259
Discovery
y = −7.88 + 1.21 x, R² = 0.75, P < 0.001
Edward VII
y = 2.39 + 0.59 x, R² = 0.19, P = 0.100
Fuji
y = 5.54 + 0.496 x, R² = 0.07, P = 0.403
Gala
y = 6.55 + 0.342 x, R² = 0.15, P = 0.154
George Cave
y = −3.42 + 0.951 x, R² = 0.55, P = 0.002
Golden Delicious
y = −1.98 + 0.921 x, R² = 0.29, P = 0.039
Jolyne
y = 4.32 + 0.465 x, R² = 0.17, P = 0.414
Jonathan
y = 3.88 + 0.554 x, R² = 0.23, P = 0.068
King of the Pippins
y = 0.769 + 0.774 x, R² = 0.18, P = 0.118
Lappio
y = 10.2 + 0.23 x, R² = 0.11, P = 0.258
Stark's Earliest
y = −1.69 + 0.85 x, R² = 0.33, P = 0.066
Winter Pearmain
y = 9.02 + 0.291 x, R² = 0.11, P = 0.300
Yellow Bellflower
y = 11.1 + 0.154 x, R² = 0.05, P = 0.447
Mean Soluble Solids Content (%Brix) (±SE)
Mean Seasonal Temperature (Full Flowering to Harvest Date) (°C)
10.0
12.5
15.0
17.5
20.0
14
16
18
20

c



Braeburn
y = −1.42 + 1.08 x, R² = 0.46, P = 0.139
Bramley's Seedling
y = 9.44 + 0.328 x, R² = 0.17, P = 0.420
Cox's Orange Pippin
y = 4.12 + 0.739 x, R² = 0.19, P = 0.386
Discovery
y = −12 + 1.6 x, R² = 0.68, P = 0.043
Edward VII
y = 2.86 + 0.689 x, R² = 0.12, P = 0.498
Fuji
y = 5.01 + 0.709 x, R² = 0.07, P = 0.619
Gala
y = 1.15 + 0.775 x, R² = 0.41, P = 0.173
George Cave
y = −2.39 + 1.04 x, R² = 0.32, P = 0.242
Golden Delicious
y = −4.13 + 1.16 x, R² = 0.24, P = 0.321
Jolyne
y = 0.89 + 0.873 x, R² = 0.40, P = 0.176
Jonathan
y = 2.54 + 0.779 x, R² = 0.27, P = 0.296
King of the Pippins
y = −4 + 1.21 x, R² = 0.25, P = 0.317
Lappio
y = 5.92 + 0.716 x, R² = 0.12, P = 0.492
Stark's Earliest
y = 0.452 + 0.825 x, R² = 0.64, P = 0.056
Winter Pearmain
y = −19.2 + 2.06 x, R² = 0.86, P = 0.024
Yellow Bellflower
y = 11.2 + 0.377 x, R² = 0.27, P = 0.296
Mean Dry Matter Content (%) (±SE)
Mean Seasonal Temperature (Full Flowering to Harvest Date) (°C)

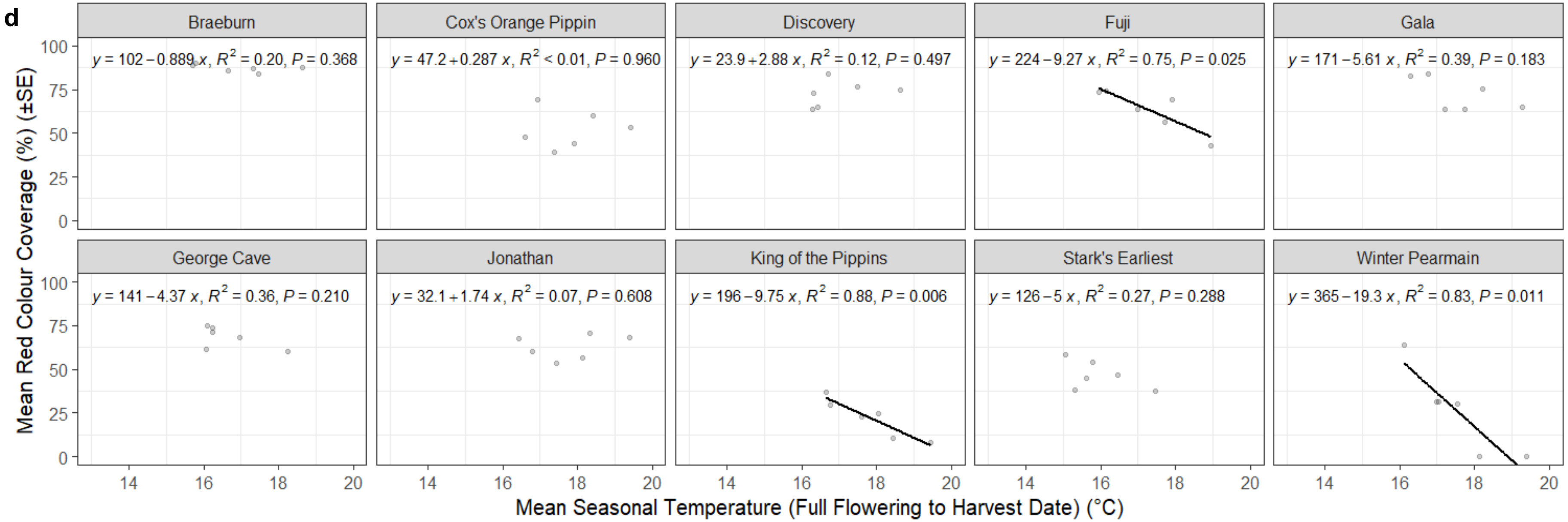


Figure 5.2. *Linear regressions between four aspects of apple fruit quality (a: Firmness, b: SSC, c: DMC, d: RCC) and mean seasonal temperature (°C) across 10 (d) to 16 (a, b, and c) cultivars. Each data point represents the mean for one temperature regime for a cultivar in a specific fruit production season (a, b; 2017-2021: c, d, 2020-21).*

5.3.3 The effect of seasonal temperature on cross-cultivar apple fruit quality

The relationship between seasonal temperature and each of the fruit quality attributes first standardised for each cultivar and then combined in a single analysis was mixed (Figure 5.3); variability about the fitted lines was sometimes considerable. Mean standardised fruit quality values were calculated for the combination of each cultivar (n=16), temperature treatment (n=3), and year (n=5) combination. Fruit quality parameters values were standardised relative to the cultivar range across the five years of the study (all treatments included), where zero is the mean score for a cultivar and the value one represents one standard deviation from the cross-treatment mean. Values from each cultivar were then pooled together to create a cross-cultivar fruit quality parameter database (n=76-240). Mean seasonal temperature spanned a wide range within this database from a low of 14.3°C (‘Stark’s Earliest’, Ambient, 2017) to a high of 19.9°C (‘Jolyne’, Plus4, 2020) across the five-year period (2017-21).

Firmness produced the greatest range of standardised values; a minimum of -1.62 to a maximum of 2.09 (Figure 5.3a). The next widest were SSC (Figure 5.3b) and DMC (Figure 5.3d) with similar standardised value ranges; -1.25 to 1.81 and -1.54 to 1.61, respectively. RCC produced the narrowest range, from -1.07 to 1.50 (Figure 5.3c). Significant linear relations ($p<0.001$) were detected between seasonal temperature and each of apple fruit firmness, SSC, RCC, and DMC (Figure 5.3). Whilst the responses were significant for each variable, the coefficient of determination was relatively low; R^2 values ranged from 0.06 (firmness) to 0.22 (RCC and DMC). Firmness, SSC, and DMC provided positive linear relations with mean seasonal temperature, whereas that for RCC was negative.

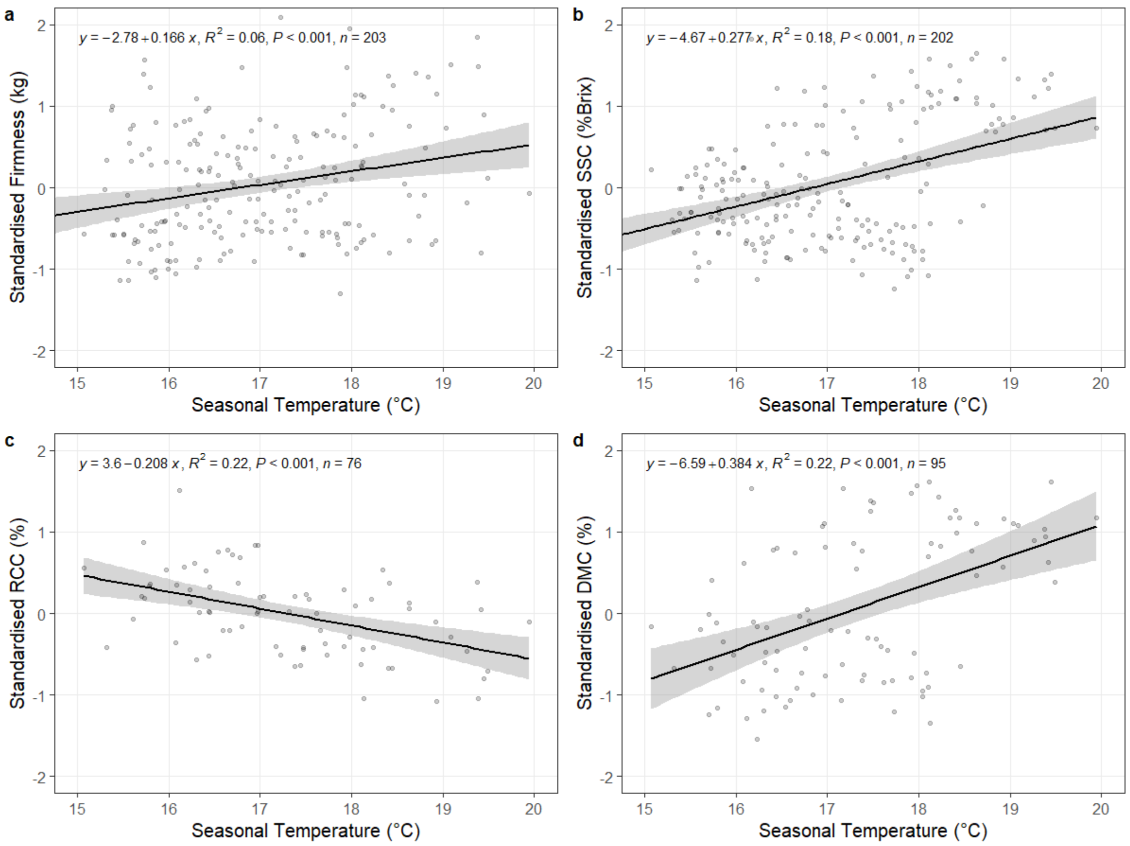


***Figure 5.3**. Linear regressions (±mean SE) between mean seasonal temperature (°C) and four standardised (see text) apple fruit quality parameters of 10-16 cultivars (a: Firmness, b: SSC, c: RCC, d: DMC). Each data point represents one temperature regime value for one cultivar in one fruit production season (2017-2021).*

The seasonalities of the cultivars (early-, mid-, or late-fruiting) provided contrasting cross-cultivar fruit quality responses to seasonal temperature (Figure 5.4). Linear regression analysis showed that early-fruiting cultivars (n=3) demonstrated a negative relation between fruit firmness and seasonal temperature ($p<0.001$), whereas both the mid-season (n=2) and the late-season (n=11) cultivars showed positive relations ($p<0.001$). All three seasonal groups of cultivars showed positive relations ($p<0.001$) between SSC and seasonal temperature (Figure 5.4b) and between DMC and seasonal temperature (Figure 5.4d). However, in the case of SSC the early-fruiting cultivars appeared more sensitive than mid- and late-season cultivars with a steeper regression slope (0.66, 0.31 and 0.35 %Brix °C^{-1}, respectively) whilst for DMC the mid-season cultivars were the most sensitive with a gradient of 1.71 %DMC °C^{-1} with shallower gradients of 1.00 and 1.09 %DMC °C^{-1} for the early- and late-season cultivars, respectively. RCC showed no significant relations with temperature ($p>0.05$) within any seasonality, but the non-significant relations were positive for early- and mid-season cultivars but negative for late-season cultivars.

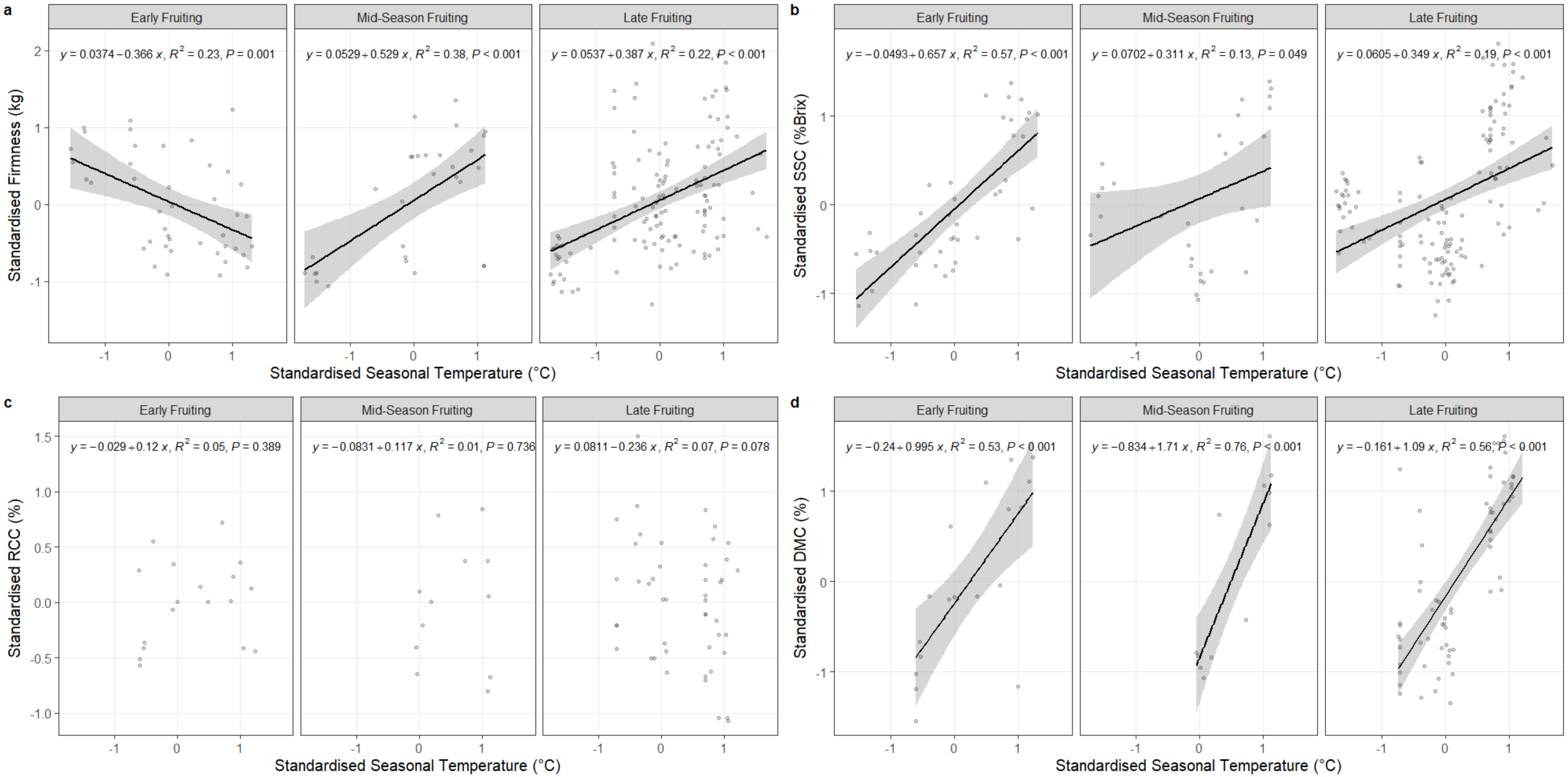


Figure 5.4. *Linear regressions (±mean SE) between standardised apple fruit quality parameters (a: Firmness, b: SSC, c: RCC, d: DMC) and standardised seasonal temperature (°C) for 10-16 cultivars split by their harvesting seasonality (early, mid or late season, see Table 2.1). Each data point represents one mean temperature regime for one cultivar from one specific fruit production season (2017-2021).*

Given these apparent differences in the responses of fruit quality to temperature with fruit harvesting dates, multiple linear regression analysis was conducted to investigate the influence of weather in different phases of the growing season (split by four different phenological phases) on individual fruit quality attributes across the standardised data. The models overall provided variable explanations of the variance (Table 5.2) with adjusted R^2 values ranging from 0.30 (firmness) to 0.73 (DMC). Significant responses were found between the weather in at least one phenological phase and each fruit quality attribute.

Furthermore, the models suggest the relationship between weather and fruit quality is complex; certain variables are associated both positively and negatively, depending on the phenological phase, with individual fruit quality attributes. For example, the SSC of the fruit at harvest was increased by greater rainfall between both 1st January and bud burst, and also between bud burst and full flowering, but reduced by warmer temperature between full flowering and mid-season; and SSC was increased by warmer temperature between bud burst and full flowering and between full flowering and mid-season but reduced by warmer temperature between mid-season and harvest.

Of the five attributes of fruit quality assessed in this way, SSC was the only one to be affected significantly by the environment (temperature or rainfall or both) during every one of the four phases of phenology from 1st January to harvest. The weather during the final phase of fruit development from mid-season and harvest affected four of the five attributes of fruit quality significantly, the exception being DMC. Fruit DMC was affected by the weather significantly between full flowering and mid-season by both temperature (positively) and by rainfall (negatively).

***Table 5.2**. Multiple linear regression models describing the effect of eight seasonal weather variables (four seasonal temperature, four seasonal precipitation values) on five cross-cultivar standardised apple fruit quality parameters. Final model residuals are inclusive of variables with p values of <0.1 only.*

		Environ. Variable	Phenology Phase	Coefficients			
				b	S.E.	t	P
FRUIT FIRMNESS (kg)		(Constant)		0.011	0.043	0.266	0.790
	Final model	Mean Temperature (°C)	1^{st} Jan to BB (1)	0.005	0.101	0.049	0.961
	residuals:		**BB to FF (2)**	**-0.202**	**0.058**	**-3.467**	**<0.001*****
	S.E = 0.602		FF to Mid-Season (3)	-0.043	0.066	-0.651	0.516
	df = 198		**Mid-Season to HD (4)**	**0.164**	**0.061**	**2.685**	**0.008****
	f = 22.23	Total Precipitation (mm)	**1^{st} Jan to BB (1)**	**0.361**	**0.058**	**6.197**	**<0.001*****
	adj. R^2 = 0.296		BB to FF (2)	0.002	0.063	0.027	0.978
			FF to Mid-Season (3)	-0.076	0.097	-0.786	0.433

		Environ. Variable	Phenology Phase	Coefficients			
				b	S.E.	t	P
			Mid-Season to HD (4)	**0.118**	**0.056**	**2.101**	**0.037***
FRUIT SSC (%Brix)		(Constant)		**0.067**	**0.031**	**2.193**	**0.030***
	Final model residuals:	Mean Temperature (°C)	1st Jan to BB (1)	0.084	0.072	1.156	0.249
	S.E = 0.432		**BB to FF (2)**	**0.320**	**0.042**	**7.676**	**<0.001*****
	df = 195		**FF to Mid-Season (3)**	**0.225**	**0.047**	**4.768**	**<0.001*****
	f = 60.43		**Mid-Season to HD (4)**	**-0.115**	**0.044**	**-2.611**	**0.009****
	adj. R^2 = 0.641	Total Precipitation (mm)	**1st Jan to BB (1)**	**0.222**	**0.042**	**5.319**	**<0.001*****
			BB to FF (2)	**0.104**	**0.045**	**2.324**	**0.021***
			FF to Mid-Season (3)	**-0.169**	**0.070**	**-2.428**	**0.016***
			Mid-Season to HD (4)	-0.022	0.040	-0.557	0.578
FRUIT WEIGHT (g)		(Constant)		0.068	0.102	0.660	0.511
	Final model residuals:	Mean Temperature (°C)	**1st Jan to BB (1)**	**0.387**	**0.114**	**3.397**	**0.001****
	S.E = 0.381		BB to FF (2)	0.073	0.124	0.592	0.555
	df = 92		FF to Mid-Season (3)	0.088	0.052	1.689	0.095
	f = 53.21		**Mid-Season to HD (4)**	**-0.236**	**0.069**	**-3.416**	**<0.001*****
	adj. R^2 = 0.623	Total Precipitation (mm)	1st Jan to BB (1)	-0.01	0.116	-0.877	0.383
			BB to FF (2)	0.153	0.077	1.984	0.050
			FF to Mid-Season (3)	0.051	0.095	0.540	0.591
			Mid-Season to HD (4)	**-0.153**	**0.048**	**-3.173**	**0.002****
FRUIT RCC (%)		(Constant)		0.060	0.117	0.153	0.610
	Final model residuals:	Mean Temperature (°C)	1st Jan to BB (1)	0.122	0.145	0.840	0.403
	S.E = 0.399		BB to FF (2)	0.237	0.143	1.662	0.101
	df = 73		FF to Mid-Season (3)	-0.066	0.060	-1.091	0.279
	f = 25.23		**Mid-Season to HD (4)**	**-0.406**	**0.084**	**-4.830**	**<0.001*****
	adj. R^2 = 0.393	Total Precipitation (mm)	1st Jan to BB (1)	0.182	0.135	1.351	0.181
			BB to FF (2)	**0.256**	**0.101**	**2.536**	**0.014***
			FF to Mid-Season (3)	0.130	0.120	1.079	0.285
			Mid-Season to HD (4)	-0.078	0.058	-0.139	0.890
FRUIT DMC (%)		(Constant)		0.019	0.133	0.140	0.889
	Final model residuals:	Mean Temperature (°C)	1st Jan to BB (1)	0.253	0.149	1.700	0.093
	S.E = 0.478		BB to FF (2)	-0.304	0.161	-1.890	0.062
	df = 91		**FF to Mid-Season (3)**	**0.228**	**0.067**	**3.378**	**0.001****
	f = 83.8		Mid-Season to HD (4)	0.081	0.089	0.904	0.368
	adj. R^2 = 0.726	Total Precipitation (mm)	**1st Jan to BB (1)**	**-0.356**	**0.150**	**-2.367**	**0.020***
			BB to FF (2)	-0.040	0.100	-0.396	0.693
			FF to Mid-Season (3)	**-0.420**	**0.123**	**-3.412**	**<0.001*****
			Mid-Season to HD (4)	-0.097	0.063	-1.532	0.129

5.3.4 Multivariate analysis of factors affecting apple fruit quality

Pearson's correlation analysis between 18 study variables was conducted using the cross-cultivar standardised data set (Table 5.3). The five fruit quality parameters differed in their correlation strength with meteorological and production variables. Individual fruit weight, firmness, SSC, and DMC were positively correlated with every temperature variable ($p<0.05$). RCC negatively correlated with two temperature variables (Tmax and Tminmaxdiff). Individual fruit weight, SSC and DMC positively correlated with total sunlight hours ($p<0.001$); however, firmness was negatively correlated ($p<0.001$). Individual fruit weight, SSC and DMC were negatively associated with total seasonal precipitation ($p<0.001$).

Production variables were also highly influential on fruit quality parameters. Individual fruit weight, firmness, SSC, and DMC were negatively associated with total fruit harvested per tree ($p<0.001$). Tree pruning was positively associated with individual fruit weight, RCC and DMC ($p<0.001$) and negatively with firmness ($p<0.001$). Later flowering dates were negatively influential on individual fruit weight and DMC ($p<0.001$), but positively on firmness ($p<0.001$). Later harvest dates shared no correlation with any fruit quality variable.

Significant correlations were found among the five fruit quality variables. Individual fruit weight was positively correlated with all four other variables ($p<0.05$). SSC and DMC values were highly positively correlated ($p<0.001$). Firmness was also positively correlated with SSC ($p<0.05$). Other than with fruit weight, RCC was not correlated with any other fruit quality variable.

***Table 5.3**. Pearson's correlation matrix of all independent and dependent variables within the cross-cultivar apple fruit quality analysis (n= 41 to 191 depending on variable combination). Statistically significant ($p<0.05$) correlations are highlighted in the bottom half of the table. The key for weather and production variables is listed in Table 5.1.*

Variable	Tmin	Tmax	Tmin-maxdiff	GDD	Sun Hours	Ppt	Yield FW	Yield TF	Pruning	FF Date	Harvest Date	Fruit Weight	RCC	Firmness	SSC	DMC
Tmean	0.82	0.92	0.71	0.59	-0.03	-0.46	-0.39	-0.39	0.13	0.19	-0.16	0.62	-0.13	0.32	0.47	0.77
Tmin	.	0.60	0.31	0.50	-0.26	-0.09	-0.41	-0.33	-0.12	0.45	0.06	0.26	0.05	0.45	0.37	0.25
Tmax	.	.	0.90	0.60	0.05	-0.58	-0.33	-0.33	0.19	0.02	-0.25	0.50	-0.27	0.25	0.44	0.73
Tminmaxdiff	.	.	.	0.54	0.14	-0.64	-0.20	-0.22	0.32	-0.16	-0.34	0.39	-0.32	0.13	0.35	0.63
GDD	.	.	.	.	0.14	-0.41	-0.30	-0.33	0.05	-0.07	0.37	0.57	0.01	0.21	0.47	0.69
SunHours	.	.	.	.	.	-0.60	-0.29	-0.65	0.70	-0.69	-0.19	0.76	0.03	-0.45	0.32	0.84
Ppt	.	.	.	.	.	.	0.32	0.58	-0.56	0.41	0.27	-0.75	0.03	0.09	-0.54	-0.86
Yield FW	.	.	.	.	.	.	.	0.80	0.02	0.01	0.06	-0.44	0.18	-0.26	-0.71	-0.73
Yield TF	.	.	.	.	.	.	.	.	-0.42	0.29	0.15	-0.78	-0.06	0.00	-0.68	-0.83
Pruning	.	.	.	.	.	.	.	.	.	-0.55	-0.38	0.66	0.69	-0.44	-0.03	0.43
FFDate	.	.	.	.	.	.	.	.	.	.	0.27	-0.50	0.04	0.37	-0.06	-0.57
HarvestDate	.	.	.	.	.	.	.	.	.	.	.	0.11	0.22	0.08	0.07	0.05
FQ Weight	.	.	.	.	.	.	.	.	.	.	.	.	0.29	-0.27	0.73	0.73
FQ Colour	.	.	.	.	.	.	.	.	.	.	.	.	.	-0.22	-0.05	-0.09
FQ Firm	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.14	-0.05
FQ SSC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.93

(Figure continued below).

Significance																
Variable	**Tmin**	**Tmax**	**Tmin-maxdiff**	**GDD**	**Sun Hours**	**Ppt**	**Yield FW**	**Yield TF**	**Pruning**	**FF Date**	**Harvest Date**	**Fruit Weight**	**RCC**	**Firmness**	**SSC**	**DMC**
Tmean	+ ***	+ ***	+ ***	+ ***	ns	- ***	- ***	- ***	ns	+ **	- *	+ ***	ns	+ ***	+ ***	+ ***
Tmin	.	+ ***	+ ***	+ ***	- ***	ns	- ***	- ***	ns	+ ***	ns	+ *	ns	+ ***	+ ***	+ *
Tmax	.	.	+ ***	+ ***	ns	- ***	- ***	- ***	+ *	ns	- ***	+ ***	- *	+ ***	+ ***	+ ***
Tminmaxdiff	.	.	.	+ ***	+ *	- ***	- **	- **	+ ***	- *	- ***	+ ***	- **	+ *	+ ***	+ ***
GDD	.	.	.	.	ns	- ***	- ***	- ***	ns	ns	+ ***	+ ***	ns	+ **	+ ***	+ ***
SunHours	.	.	.	.	.	- ***	- ***	- ***	+ ***	- ***	- **	+ ***	ns	- ***	+ ***	+ ***
Ppt	.	.	.	.	.	.	+ ***	+ ***	- ***	+ ***	+ ***	- ***	ns	ns	- ***	- ***
Yield FW	.	.	.	.	.	.	.	+ ***	ns	ns	ns	- ***	ns	- ***	- ***	- ***
Yield TF	.	.	.	.	.	.	.	.	- ***	+ ***	+ *	- ***	ns	ns	- ***	- ***
Pruning	.	.	.	.	.	.	.	.	.	- ***	- ***	+ ***	+ ***	- ***	ns	+ **
FFDate	.	.	.	.	.	.	.	.	.	.	+ ***	- ***	ns	+ ***	ns	- ***
HarvestDate	.	.	.	.	.	.	.	.	.	.	.	ns	ns	ns	ns	ns
FQ Weight	.	.	.	.	.	.	.	.	.	.	.	.	+ *	+ *	+ ***	+ ***
FQ Colour	.	.	.	.	.	.	.	.	.	.	.	.	.	ns	ns	ns
FQ Firm	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+ *	ns
FQ SSC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+ ***

* Significant at p<0.05, ** Significant at p<0.01, *** Significant at p<0.001, ns No significance, + Positive correlation, - Negative correlation

Principal component analysis (PCA) transformed the list of independent and dependent variables (Table 5.1) into two principal components that explained 92.9% of total variation. Dependent variables, generally, did not correlate highly with either principal component. Therefore, as the PCA provided little insight in to the associations of fruit quality variables, this analysis (Appendix 5.2) is not considered further.

Partial Least Square Regression (PLSR) models were calibrated and cross-validated for each of the five fruit quality parameters based on 11-12 independent variables from 2-5 years of study data (depending on available data). Model overviews and variable coefficients are listed in Table 5.4. Models differed in their strength to explain fruit quality parameter variance. The strongest models calibrated were those for SSC (c.v. RMSE = 0.38, ycumexpvar = 75.0%), DMC (c.v. RMSE = 0.39, ycumexpvar = 85.2%), and fruit weight (c.v. RMSE = 0.39, ycumexpvar = 73.7%). In contrast, the models for RCC (c.v. RMSE = 0.41, ycumexpvar = 37.8%) and firmness (c.v. RMSE = 0.56, ycumexpvar = 42.7%) were less successful.

Variables that contributed significant model weight ($p<0.05$) were identified within each fruit quality parameter PLSR model. For firmness, these were sunlight hours ($t=-4.63$, $p<0.001$), precipitation ($t=-4.75$, $p<0.001$) and minimum temperature ($t=2.81$, $p<0.01$). For SSC, seven out of 12 variables contributed significant model weight namely total harvested fruit per tree ($t=-10.49$, $p<0.001$), annual pruning weight ($t=-9.23$, $p<0.001$), harvested fresh weight per tree ($t=-9.19$, $P<0.001$), precipitation ($t=-5.44$, $p<0.001$), sunlight hours ($t=3.59$, $p<0.001$), mean temperature ($t=2.96$, $p<0.01$), maximum temperature ($t=2.13$, $p<0.05$), and harvest date ($t=2.01$, $p<0.05$). For sample fruit weight, only yield parameters contributed significantly. These were total harvested fruit ($t=-4.50$, $p<0.001$) and harvested fresh weight per tree ($t=3.78$, $p<0.001$). RCC had only two variables contributing significantly – min-max temperature difference ($t=-2.19$, $p<0.05$) and harvested fresh weight per tree ($t=2.10$, $p<0.05$). in contrast, the DMC model identified eight out of the 11 variables tested as significant contributors. These were precipitation ($t=-5.43$, $p<0.001$), total harvested fruit per tree ($t=-5.35$, $p<0.001$), mean temperature ($t=4.38$, $p<0.001$), minimum temperature ($t=3.25$, $p<0.01$), harvested fresh weight per tree ($t=-2.86$, $p<0.01$), sunlight hours ($t=2.62$, $p<0.05$), maximum temperature ($t=2.59$, $p<0.05$), and min-max temperature difference ($t=2.13$, $p<0.05$).

The variable importance for projection (VIP) analysis (Figure 5.5) from these PLSR models identified several variables across each fruit quality parameter that scored >1 (i.e. indicating high influence in determining dependent variable values). For firmness, these were sunlight hours (1.80), minimum temperature (1.36), flowering date (1.26), and annual pruning (1.17). For SSC, these were total harvested fruit per tree (1.46), harvested fresh weight per tree (1.44), annual pruning weight (1.19), and precipitation (1.18). For fruit sample weight, these were precipitation (1.64), total harvested fruit (1.41), sunlight hours (1.25), and harvested fresh weight (1.01). For RCC these were precipitation (1.69) harvested fresh weight per tree (1.62), and min-max temperature difference (1.40). For DMC, these were precipitation (1.73), sunlight hours (1.23), total harvested fruit (1.19), and growing degree days (1.14).

Table 5.4*. Partial Least Squares Regression (PLSR) model overviews and corresponding coefficients for the effect of meteorological and production variables on five apple fruit quality parameters. All variables were standardised relative to long-term data variation.*

PLSR Model Overview	Mean STD Predictor Var.	Coefficient	S.E.	t	P
Firmness (kg)	Mean Seasonal Temperature (°C)	- 0.049	0.058	- 0.85	0.396
	Minimum Seasonal Temperature (°C)	**0.183**	**0.065**	**2.81**	**0.006****
df = 143	Maximum Seasonal Temperature (°C)	- 0.043	0.063	- 0.68	0.499
ncomp = 4	Seasonal Min-Max Temperature (°C)	0.027	0.066	0.40	0.691
Cal R^2 = 0.427	Growing Degree Days	0.141	0.089	1.57	0.118
C.v. R^2 = 0.362	**Total Seasonal Sunlight Hours**	**- 0.468**	**0.101**	**- 4.63**	**<0.001*****
Cal RMSE = 0.530	Total Seasonal Precipitation (mm)	0.027	0.104	0.26	0.792
C.v. RMSE = 0.559	**Total Fresh Weight per Tree (kg)**	**- 0.351**	**0.074**	**- 4.75**	**<0.001*****
Xcumexpvar = 74.4%	Total Fruit Harvested per Tree	0.120	0.073	1.64	0.103
Ycumexpvar = 42.7%	Total Annual Tree Prunings (kg)	- 0.016	0.085	- 0.19	0.852
	Tree Flowering Date	- 0.166	0.090	- 1.83	0.069
	Tree Harvest Date	- 0.065	0.100	- 0.65	0.518
SSC (%Brix)	**Mean Seasonal Temperature (°C)**	**0.058**	**0.020**	**2.96**	**0.004****
	Minimum Seasonal Temperature (°C)	- 0.001	0.032	- 0.04	0.965
df = 142	**Maximum Seasonal Temperature (°C)**	**0.417**	**0.020**	**2.13**	**0.035***
ncomp = 4	Seasonal Min-Max Temperature (°C)	0.037	0.025	1.47	0.143
Cal R^2 = 0.750	Growing Degree Days	- 0.021	0.035	- 0.62	0.539
C.v. R^2 = 0.724	**Total Seasonal Sunlight Hours**	**0.125**	**0.035**	**3.59**	**<0.001*****
Cal RMSE = 0.363	**Total Seasonal Precipitation (mm)**	**- 0.200**	**0.037**	**- 5.44**	**<0.001*****
C.v. RMSE = 0.382	**Total Fresh Weight per Tree (kg)**	**- 0.258**	**0.028**	**- 9.19**	**<0.001*****
Xcumexpvar = 82.4%	**Total Fruit Harvested per Tree**	**- 0.256**	**0.024**	**- 10.49**	**<0.001*****
Ycumexpvar = 75.0%	**Total Annual Tree Prunings (kg)**	**- 0.337**	**0.037**	**- 9.23**	**<0.001*****
	Tree Flowering Date	0.030	0.044	0.69	0.492
	Tree Harvest Date	**0.066**	**0.033**	**2.01**	**0.046***
Fruit Weight (g)	Mean Seasonal Temperature (°C)	0.028	0.155	0.18	0.857
	Minimum Seasonal Temperature (°C)	0.045	0.137	0.33	0.743
df = 62	Maximum Seasonal Temperature (°C)	- 0.158	0.180	- 0.88	0.383
ncomp = 6	Seasonal Min-Max Temperature (°C)	0.156	0.178	0.88	0.382
Cal R^2 = 0.737	Growing Degree Days	- 0.096	0.109	- 0.89	0.376
C.v. R^2 = 0.626	Total Seasonal Sunlight Hours	- 0.140	0.140	- 1.00	0.320
Cal RMSE = 0.328	Total Seasonal Precipitation (mm)	- 0.331	0.185	- 1.79	0.078

PLSR Model Overview	Mean STD Predictor Var.	Coefficient	S.E.	t	P
C.v. RMSE = 0.391	**Total Fresh Weight per Tree (kg)**	**0.460**	**0.122**	**3.78**	**<0.001*** **
Xcumexpvar = 96.3%	**Total Fruit Harvested per Tree**	**- 0.551**	**0.123**	**- 4.50**	**<0.001*** **
Ycumexpvar = 73.7%	Tree Flowering Date	- 0.033	0.092	-0.36	0.723
	Tree Harvest Date	0.250	0.137	1.83	0.072
RCC (%)	Mean Seasonal Temperature (°C)	- 0.069	0.104	- 0.65	0.519
	Minimum Seasonal Temperature (°C)	- 0.169	0.161	- 0.98	0.333
df = 48	Maximum Seasonal Temperature (°C)	- 0.103	0.074	- 1.41	0.166
ncomp = 3	**Seasonal Min-Max Temperature (°C)**	**0.228**	**0.104**	**- 2.19**	**0.033***
Cal R^2 = 0.375	Growing Degree Days	0.045	0.128	0.36	0.718
C.v. R^2 = 0.201	Total Seasonal Sunlight Hours	0.036	0.085	0.47	0.640
Cal RMSE = 0.364	Total Seasonal Precipitation (mm)	- 0.342	0.171	- 1.92	0.060
C.v. RMSE = 0.412	**Total Fresh Weight per Tree (kg)**	**0.331**	**0.153**	**2.10**	**0.041***
Xcumexpvar = 66.9%	Total Fruit Harvested per Tree	0.004	0.078	0.00	0.999
Ycumexpvar = 37.8%	Tree Flowering Date	0.144	0.126	1.10	0.276
	Tree Harvest Date	0.115	0.113	1.04	0.301
DMC (%)	**Mean Seasonal Temperature (°C)**	**0.180**	**0.041**	**4.38**	**<0.001*** **
	Minimum Seasonal Temperature (°C)	**0.146**	**0.045**	**3.25**	**0.002*** *
df = 62	**Maximum Seasonal Temperature (°C)**	**0.087**	**0.034**	**2.59**	**0.012***
ncomp = 3	**Seasonal Min-Max Temperature (°C)**	**0.102**	**0.048**	**2.13**	**0.037***
Cal R^2 = 0.852	Growing Degree Days	0.033	0.054	0.60	0.549
C.v. R^2 = 0.816	**Total Seasonal Sunlight Hours**	**0.089**	**0.034**	**2.62**	**0.011***
Cal RMSE = 0.347	**Total Seasonal Precipitation (mm)**	**- 0.230**	**0.042**	**- 5.43**	**<0.001*** **
C.v. RMSE = 0.387	**Total Fresh Weight per Tree (kg)**	**- 0.150**	**0.053**	**- 2.86**	**0.006*** *
Xcumexpvar = 80.5%	**Total Fruit Harvested per Tree**	**-0.198**	**0.037**	**- 5.35**	**<0.001*** **
Ycumexpvar = 85.2%	Tree Flowering Date	- 0.003	0.045	- 0.07	0.942
	Tree Harvest Date	0.038	0.043	0.88	0.380

Key: df = Degrees of freedom, ncomp = Number of model components, Cal R^2 = Calibrated model coefficient of determination, C.v. R^2 = Cross-validated coefficient of determination, Cal RMSE = Calibrated model root mean square error, C.v. RMSE = Cross-validated root mean square error, Xcumexpvar = Predictor variable accumulation of variance, Ycumexpvar = Response variable cumulative accumulation of variance. Key for significance: * p<0.05, ** P<0.01, *** p<0.001, otherwise NS (p>005).

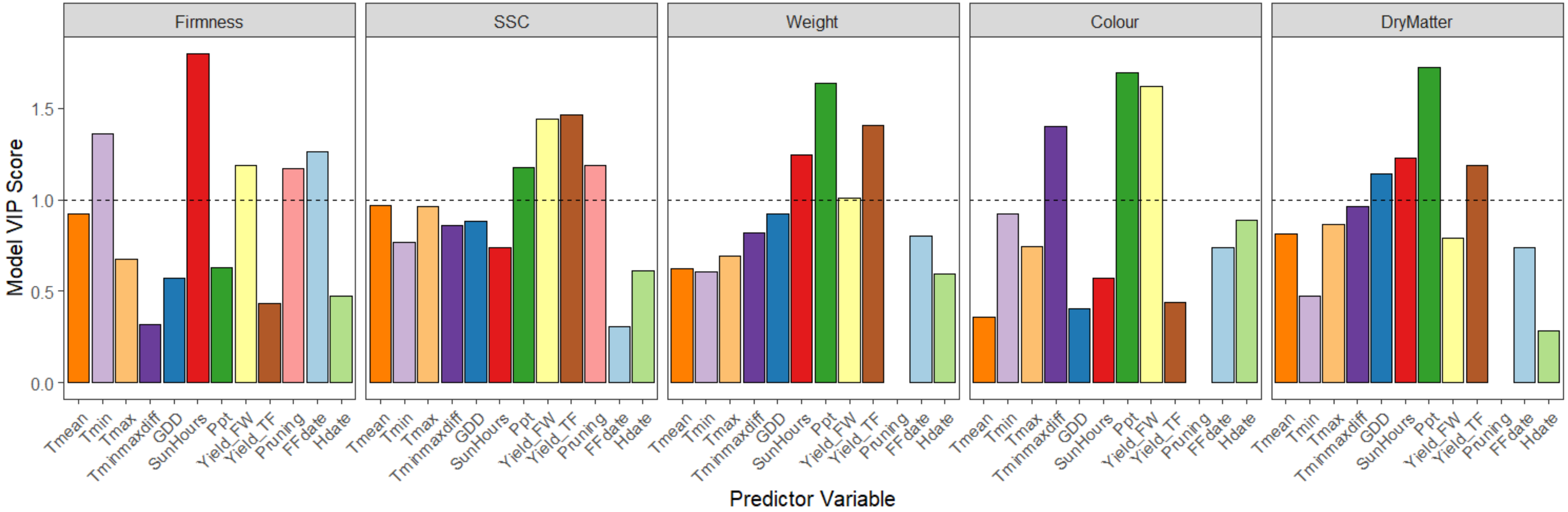


Figure 5.5. *Variable importance in projection (VIP) of 12 trial predictor variables affecting five apple fruit quality parameters. A VIP threshold score >1 indicates high influence in determining the value of that fruit quality variable. All variables were standardised relative to long-term data variation.*

5.4 Discussion

Research shows that the quality of fresh fruit and vegetable crops can be affected directly and indirectly by climate-change induced rising temperatures (Moretti et al., 2010). Future climate variation is expected to influence fruit quality within multiple geographic apple production regions (Sugiura et al., 2013; Zhang et al., 2018; El Yaacoubi et al., 2020). The long-term data (2017-2021) reported here demonstrated that the implementation of different temperature regimes had statistically significant mixed effects on five different fruit quality parameters across a range of diverse apple cultivars. The several different analytical approaches used provided a consistent conclusion that temperature affected apple fruit quality, but also identified several important points of detail - not the least being that the cultivars differed to some extent in their responsiveness of fruit quality to temperature; that these differences were associated with the earliness of harvesting of the cultivar; and that during the annual cycle of fruit production temperature had a varied effect amongst attributes of fruit quality. The analyses also determined the significant influence of other external variables relating to other meteorological and production variables on fruit quality. However, whether these changes are beneficial to the UK fruit industry or not is likely to be dependent on future fruit marketability standards and consumer trends. Each fruit quality variable's response to the modified temperature environments is discussed individually below.

5.4.1 Apple fruit firmness

Historic temperature shifts in the 20th and 21st centuries are thought to have influenced physiological processes in apple trees that determine fruit firmness values (Ornelas-Paz et al., 2018). Responses of fruit firmness to raised temperature within this study differed: some cultivars showed reduced firmness ('Cox's Orange Pippin', 'George Cave', and 'Lappio') and others raised firmness ('Gala', 'Jolyne', 'King of the Pippins', and 'Yellow Bellflower'). The remaining nine cultivars showed no significant differences between treatment.

The initial linear regression analyses suggested that firmness was not influenced consistently by seasonal temperature across this selection of apple cultivars – in that only six out of 16 trial cultivars produced significant relations (Figure 5.2), four of which were positive and two negative. However, once the cultivars were classified by the seasonality of fruit harvest the analyses of these combined standardised datasets showed that early-fruiting cultivars had a negative relation between fruit firmness and temperature whereas this relation was positive for mid- and late- fruiting cultivars (Figure 5.4). Relationships with all temperature parameters (Tmean, Tmin, Tmax and

Tminmaxdiff) were significant and positive (Table 5.3). Moreover, fruit firmness and temperature relations were significant during the periods from bud burst to full flowering (positive) and from mid-season to harvest (negative) (Table 5.2); i.e., positive effects of temperature on subsequent firmness during initial fruit development but negative during late fruit development. All of which suggests that the effect of warmer temperature on apple fruit firmness in future UK production seasons will depend not just on the extent of warming but the stage of fruit development when it occurs. However, bud burst to flowering is typically a shorter timeframe compared to mid-season to harvest (~30-50 days and 90-120 days, respectively), so temperature effects in the shorter bud burst to flowering period may not be as relevant to growers as those from mid-season to harvest; and in whole season temperature models. The negative associations during early reproductive phases replicate findings in Atkinson et al. (1998) where warmer seasonal temperature treatments were associated with reduced firmness in ‘Cox’s Orange Pippin’. Warrington et al. (1999), where numerous cultivars (including the cultivars ‘Braeburn’ and ‘Fuji’ studied here) exhibited reduced firmness when exposed to temperatures >19°C (compared to 13°C) during full bloom. In both studies, reduced firmness was attributed to an effect of raised temperatures inducing greater cell division in early fruit development.

Fruit size and firmness were negatively correlated with temperature in other studies (Blanpied et al., 1978; De Salvador et al., 2006). Data from the current study may contradict those findings in that there was a significant positive correlation between fruit firmness and weight (Table 5.3). A significant positive association was found between firmness and SSC, which matched findings from De Salvador et al. (2006). Firmness and DMC had previously been observed as being positively associated (Palmer et al., 2010). Firmness showed no association with RCC. Apple colour has been studied to be an indicator of internal fruit quality in ‘Fuji’ (Ku et al., 2019). However, it is not thought that the anthocyanin accumulation that is responsible for RCC is linked with decreased firmness in apple. Warmer temperature environments showed significantly reduced RCC in ‘Fuji’ yet did not differ in firmness when compared to Ambient (Figure 5.1), therefore providing further evidence that firmness is not associated with anthocyanin accumulation.

Five independent variables (Tmin, SunHours, Yield_FW, Pruning, and FFdate) scored a VIP score above 1 which indicated high influence on fruit firmness. The relationship between total sunlight hours and firmness is thought to be tenuous, however: Robinson et al. (1983) suggested that light exposure effects on fruit firmness are an indirect result of direct light exposure effects on increasing fruit size and advanced maturity. Total sunlight hours also scored a VIP >1 for the fruit weight PLSR model, so this may support Robinson et al.'s (1983) suggestion.

The PLSR model also suggests significant negative associations between firmness and fruit fresh weight per tree. This is synonymous with Opara et al. (1997) where fruit firmness was higher in trees with reduced crop loads. Chapter 3 of this thesis concluded that alternate bearing patterns are enhanced within the warmer temperature treatments. Therefore, the implication of this suggests that fruit firmness will vary more between years in warmer environments. Data within Figure 5.1 supports this suggestion; the standard error of the mean was greater in Plus4 than Ambient for many of the cultivars.

The degree of fruit maturity at harvest can affect apple firmness. Later harvest dates are associated with either reduced or unaffected firmness dependent on the cultivar (DeEll et al., 1999). All fruit were picked at the same maturity index (50% starch/index score). However, in practice, fruit maturity indices vary by cultivar. Whilst it is feasible that earlier harvest date could affect maturity and firmness, the PLSR data contradicts this: harvest date was not associated with fruit firmness (Table 5.4). Conversely, flowering date scored highly within the VIP analysis (Figure 5.5) despite not quite reaching statistically significance within the PLSR model. As earlier flowering date is heavily associated with increased temperature (Lane, 2022), there is evidence within the multivariate analysis that temperature during reproductive phases is highly influential on fruit firmness at harvest.

Total precipitation appeared to have a small impact on fruit firmness. The cross-cultivar multiple linear regression showed that firmness was positively associated with winter and late-season rainfall (Table 5.2). However, the multivariate correlation, PLSR, and VIP analyses suggested that the two variables were not strongly associated with each other.

With regard to trait responses to environment, cultivar seasonality affected firmness in response to raised temperature. Early-harvesting cultivars were generally negatively influenced, whereas mid to late season cultivars were generally positively influenced. The most extreme cases were 'George Cave' (early) and 'Winter Pearmain' (late). A mean seasonal temperature increase of 1°C reduced 'George Cave' apple firmness by 0.914kg, whereas it increased 'Winter Pearmain' firmness by 1.16kg.

To conclude, the response of apple fruit firmness to seasonal environment was complex and varied considerably among cultivars. The evidence both within this study and the literature suggests that increased temperature has more of an indirect influence on firmness rather than direct. However, temperature specifically during flowering (~20-30 days of the season) may have a direct influence due to altered fruit growth patterns. Poor

PLSR model results indicate that temperature variables alone are not sufficient to predict firmness.

5.4.2 Apple soluble solids content and dry matter content

The genetic diversity of apple cultivars and the climate are both known to influence apple nutritional content (Mignard et al., 2022). Within this study, the response of soluble solids content (SSC) and dry matter content (DMC) to the raised temperature regimes were remarkably similar. First, the Pearson's correlation of 0.93 within the cross-cultivar analysis of this study confirmed that SSC and DMC were intrinsically linked (Table 5.3). Second, correlation data with independent variables showed that SSC and DMC correlated in similar ways with other weather and production variables. Recent studies with other fruiting crops, in stone fruit (Scalisi and O'Connell, 2021) and cucumber (Valverde-Miranda et al., 2021), have also shown that SSC and DMC are highly positively correlated. Therefore, this discussion evaluates SSC and DMC together.

The raised temperature regimes increased SSC (Figures 5.3 and 5.4) and no individual cultivar had significantly greater values of SSC or DMC within the Ambient tunnel (Figure 5.1). Additionally, none of the cultivars showed significantly greater values of SSC or DMC in Plus4 compared to Plus2. 'Braeburn' was the only cultivar to show treatment differences in DMC. The cultivar linear regressions for both SSC and DMC (Figure 5.2) are also consistently positive (even if not always significantly so). Hence the cross-cultivar relation between each of SSC and DMC with temperature is clearly positive. However, environmental responses varied between cultivars (Figure 5.2) which likely contributed towards low coefficient of determination values for both SSC and DMC in Figure 5.3 (0.18 and 0.22 respectively).

The multiple linear regression analysis (Table 5.2) produced differences between SCC and DMC in temperature response within the different seasonal phases of phenology. Both SSC and DMC were related positively with temperature from full flowering to mid-season. However, whereas DMC was not affected significantly by temperature during other phases of development SSC was affected positively by temperature during early season plant growth and negatively as the fruit matured. These results are indicative of the complex relationship between temperature and SSC, where studies have shown positive (Sugiura et al., 2013) and negative effects (Lee et al., 2023) in the cultivar 'Fuji'.

The multivariate analysis revealed both SSC and DMC were statistically sensitive to a large proportion of independent variables. Both SSC and DMC represented the best performing PLSR models (Table 5.4) of the five fruit quality parameters with 75% and 85.2% of total variance explained, respectively. The effect of yield parameters (total harvested fruit and harvested fresh weight) produced the strongest t value out of all variables for SSC (e.g., t = -10.5 for SSC in response to total harvested fruit). This strong negative association suggests crop load may have more effect on SSC and DMC than temperature. The VIP scores provide further evidence of this: no temperature variables passed the >1.0 threshold, whereas yield variables did (Figure 5.5). A recent study by Iwanami et al. (2023) drew similar conclusions, suggesting tree-dependent variation in SSC could be explained by varied crop load in 'Fuji'. They also highlighted the importance of solar radiation. This was reflected too here in the PLSR VIP analysis for both SSC and DMC (Figure 5.6). Greater quantity of sunlight hours have historically been linked with higher apple sugar content (Brooks and Fisher, 1926) through greater fruit starch accumulation (Iwanami et al., 2023).

Both the MLR and PLSR analyses highlight the inconclusive and complex relationship between SSC and precipitation, as commented on by Musacchi and Serra (2018). Precipitation was negatively related to SSC and to DMC in the PLSR analyses (Table 5.4). In the MLR analyses (Table 5.2), SSC was positively related to precipitation from 1st January to full flowering but then negatively until mid-season whilst DMC was negatively related to precipitation from 1st January to bud burst and from full flowering to mid-season. Overall, precipitation after flowering generally reduced both SSC and DMC in the MLR analyses, in agreement with the PLSR analyses.

5.4.3 Apple fruit red colour coverage

Warmer temperatures are linked with reduced anthocyanin accumulation, responsible for the buildup of RCC in apple (Lin-Wang et al., 2011; Iglesias et al. 2016). The results from this study generally replicated these findings. Out of the ten applicable cultivars, eight produced significantly less mean fruit RCC within the warmer temperature regimes compared to ambient conditions (Figure 5.1). 'Fuji' and 'Gala', two internationally-important-commercial cultivars, saw mean colour reductions of ~20-30% when cultivated under +4°C conditions. This reduction potentially threatens their marketability in the future as climate changes. Dependent on the cultivar strain, current UK and EU government specifications dictate a minimum requirement of 30-50% RCC for Class 1 fruit. Therefore, these results suggest a much greater proportion of 'Fuji' and 'Gala' fruits may be unmarketable in the UK under warmer conditions.

The direct relationship between seasonal temperature and RCC is unclear for most cultivars based on the linear regression analysis (Figure 5.2). 'Fuji', 'King of the Pippins' and 'Winter Pearmain' showed clear significant negative relationships, but the other seven cultivars did not despite the significant treatment differences shown in Figure 5.1.

Similar to dry matter content, this could be due to large standard error of the mean from low sample size (n=6 based on two years of data). With more years of data, a more accurate linear regression might be produced across all cultivars. In the case of Fuji (a significant relation), RCC reduced by 9.27% for every increase of 1°C in mean seasonal temperature. The cross-cultivar regression analysis further confirmed that seasonal temperature had a significant negative relation with RCC across the pool of apple cultivars (Figure 5.3).

Mean temperature in late fruit development was highly negatively related with RCC (Table 5.2). The temperature sensitivity during this phase matches findings where cooler night-time temperatures were linked with enhanced anthocyanin production as apple fruits matured (Curry, 1997). No other seasonal temperature interval was significantly related with RCC. This suggests that temperature during flowering and early fruit development may be irrelevant to red colour accumulation. Further evidence for this was provided by the PLSR results in which seasonal Tminmaxdiff was one of only three variables modelled to affect RCC (Table 5.4).

Correlations amongst the dependent variables suggests that RCC is positively correlated with fruit weight only (Table 5.3). The literature suggests that the direct influence of colour accumulation on other fruit quality variables is tentative. Therefore, it's feasible this correlation arose from the effects of temperature on each variable. Fruit weight is generally negatively associated with temperature. As colour accumulation is also negatively correlated with temperature, this matches the association found.

Total seasonal precipitation was found to influence RCC negatively in the PLSR model with a VIP value >1 (Figure 5.6). However, the MLR identified precipitation between bud burst and flowering to be positively influential on RCC. All other seasonal time periods had no effect. Previous studies have found precipitation to have inconclusive effects on RCC. Deficit irrigation field studies at various seasonal timings on the cultivar 'Braeburn' found reduced irrigation to have a positive, negative, or non-significant effect on RCC (Mills et al., 1996; Mpelasoka et al., 2001). Therefore, the results from this study further highlight the complex and inconclusive relationship between precipitation and RCC.

5.4.4 Apple fruit weight

The effect of the three temperature regimes on fruit weight was analysed as part of Chapters 3 and 4 relating to yield. It was found that fruit weight was more varied in warmer environments between years as a likely consequence of enhanced alternate bearing patterns. Fruit sampled within this study were not reflective of true average fruit size, due to the selection of Class 1 fruit for quality analyses. However, the measurement of fruit weight was important for comparisons with other fruit quality metrics, as documented within the literature (Mpelasoka et al., 2001; De Salvador et al., 2006; Musacchi and Serra, 2018). This study agreed with those findings - fruit weight was significantly correlated with three out of four other fruit quality parameters (SSC, DMC, and RCC) in the Pearson's Correlation matrix (Table 5.3).

The influence of temperature on sample fruit weight throughout the study was mixed. The MLR analysis identified late-season temperatures as being strongly and negatively associated with fruit weight. A non-significant positive association was present during the period from full flowering to mid-season. Temperature during this fruit growth period has a positive effect on final fruit size (Warrington et al., 1999; Stanley et al., 2015), but no significant association was found in this study's MLR. Late-season precipitation was found to influence fruit size negatively. This contradicts studies where reduced water availability during fruit maturity is linked with reducing fruit size at harvest (Reid and Kalcsits, 2020).

The PLSR model provided further evidence that temperature had an insignificant direct effect on fruit weight – no temperature variables had a significant impact (Table 5.4). However, precipitation had the highest VIP score of any variable, negatively influencing fruit weight. Based on the literature, it is unlikely that increased seasonal rainfall is directly reducing fruit weight. Instead, it is more likely that yield parameters are the main influencers. This is for two reasons. First, total harvested fruit and harvested fresh weight produced the largest t values within the PLSR model by a considerable margin (-4.50 and 3.78, respectively). Secondly, the wettest seasons of the trial (2019 and 2021) coincided with high-bearing seasons of many cultivars' alternate bearing cycles. It is also worth noting that 2018 and 2020 were considerably drier years, when cultivars were typically in their low-bearing season. Further analysis would be required to determine this. However, given the evidence available here this is a feasible explanation.

The cross-cultivar data shows eight out of 16 cultivars having statistically greater fruit weight within the Ambient regime. This replicates the findings from Chapter 3, where increased temperatures were associated with lower and more variable mean fruit sample weight over a six-year period of the long-term study. In the case of 'Gala', mean fruit weight was ~20% less in Plus4 compared to Ambient. One cultivar ('King of the Pippins') produced statistically greater fruit sample weight in Plus4 despite a much greater mean standard error indicating high variation over six years of data.

5.5 Conclusions

The implementation of modified temperature environments across a five-year period had a significant effect on the outcome of the fruit quality parameters of Class 1 apple fruit assessed here. However, these effects varied across the 10-16 diverse apple cultivars. Seasonal temperature was generally positively associated with mean SSC and DMC, negatively associated with mean RCC and fruit weight, and both positively and negatively associated with firmness depending on cultivar. Fruit quality response to changes in seasonal temperature also varied by trait - early season cultivars generally responded differently and more sensitively compared to mid- and late-season cultivars.

The study was conducted in a field environment. Multiple measured independent variables other than temperature were found to influence the outcome of fruit quality parameters. Precipitation and sunlight hours were two other meteorological factors that were highly influential on fruit quality outcomes. However, yield parameters were identified as being the most influential factor determining several fruit quality parameters – even more than direct seasonal temperature variables. This study therefore provides evidence that enhanced varied inter-year yield patterns (as a possible indirect consequence of raised temperature, as demonstrated in Chapters 3 and 4) are highly influential in determining fruit quality.

It cannot be concluded that fruit quality is ‘improved’ by increase in seasonal temperature as the term is subjective across a cross-cultivar sample population. The definition of ‘high-quality’ fruit varies dependent on a desired individual cultivar specification from a grower or industrial entity. The information within this study, which covers a range of diverse cultivars, should aid growers in what to expect in relation to certain fruit quality parameters with an increase in seasonal UK temperatures.

Chapter 6: The impact of modified temperature regimes on storability of ‘Gala’ fruit

6.1 Introduction

Apples possess a long shelf life compared to most other fruit crops. This can be exploited to extend fruit availability to consumers through appropriate storage practices. The primary objective of apple fruit storage is to regulate the natural process of fruit ripening. This constitutes a series of physiological, biochemical and organoleptic changes that contribute towards the development of a softened, edible fruit with desirable fruit quality attributes (Brady, 1987; Prasanna et al., 2007). Apple is a climacteric fruit, meaning the process of ripening continues after removal from its parent plant (Kader, 1999). The presence of ethylene, a plant hormone, is one of the main driving factors towards triggering or accelerating the metabolic pathways involved with fruit ripening (Brady, 1987). Other abiotic factors, including temperature, humidity, volatiles and gases (e.g. oxygen and carbon dioxide), regulate fruit ripening (Prange et al., 2005; Paul and Pandey, 2014).

Technological advancements in fruit and vegetable storage capabilities have progressed significantly over the past 50-70 years primarily through development of controlled atmosphere (CA) storage and ripening-controlling inputs. Controlled atmosphere storage regulates the internal atmosphere in which fruit are maintained over an extended period. Typically, CA chambers reduce O_2, temperature and humidity, whilst increasing CO_2 concentrations, to inhibit fruit respiration (Rama and Narasimham, 2003). This practice is applied to many types of fruits and vegetables, enabling a global year-round supply of fresh produce.

Storage in CA environments has become a tool of critical importance to the UK fresh produce economy. In 2022, 78% of all UK dessert apple production by tonnage was provided by three late-season cultivars, namely ‘Gala’ (55%), ‘Braeburn’ (16%), and ‘Cox’s Orange Pippin’ (7%) (DEFRA, 2023). Production of these three cultivars were worth over £150m to the UK economy, with the majority of produce allocated for long-term CA storage to extend the supply season to the following spring. Only 5% of dessert apple produce was exported, meaning the remaining 95% was likely targeted for domestic use.

Optimal CA storage conditions are dependent on the cultivar or strain and desired extended seasonality of produce. Post-harvest storage processes initially start with of a period of pre-cooling to remove excess heat from fruit. Commercial ethylene and oxygen scrubbers may also be applied around this point, as excess presence of these compounds in CA storage is linked with accelerated ripening and high respiration rates (Johnson, 1997). In the case of long-term storage (~6 months) of ‘Gala’, it is recommended that fruit is placed in environmental conditions of 0.5°C, 3-5% carbon dioxide (CO_2) and 1-2% oxygen (O_2) (AHDB, 2021). The monitoring of fruit quality attributes in store on a regular basis is essential for ensuring continuous high fruit marketability.

The stage of maturity at harvest is critical for determining storage life and quality – immature fruits are more subject to mechanical damage, whilst overripe fruits will quickly become soft and mealy (Kader, 1999). Studies have determined optimal harvest date for specific cultivars based on how different harvest dates affect storability. Later harvest dates are associated with reduced firmness at harvest (Ingle et al., 2000; Konopacka and Plocharski, 2004; Kvikliene et al., 2006). However, high firmness at harvest can be mitigated during storage (Ingle et al., 2000), with earlier harvested fruit losing a greater proportion of firmness during storage (Kvikliene et al., 2006). Soluble solids content (SSC) are positively associated with later harvest dates. However, similar to firmness, harvest date has little impact on SSC after six months of storage (Ingle et al., 2000; Kvikliene et al., 2006). These studies also demonstrated that harvest date had little impact on storage disorder incidence, citing weather as being a more important influence on fruit quality. However, these studies concern the effect of maturity on storability within one production environment. The literature is less clear on how accelerated maturity impacts fruit quality. Lysiak et al. (2020) investigated the effect of longitude, latitude, and microclimate on ‘Jonagold’ storability within several European environments in the same production year. Harvest date varied by ~2 weeks between growing regions. They found that differences in microclimate and harvest date had little impact on ‘Jonagold’ storability, with only flavanol variables displaying evidence of microclimate effects.

The development of storage disorders can drastically alter the marketability of fruit. Apples supplied to the market need to be free from internal and external disorders and should have limited potential to develop these during the period from retailing to consumption (AHDB, 2021). Storage disorder prevalence can vary based on cultivar, pre-harvest conditions, and storage conditions. Delaying CA storage after harvest is associated with mitigating disorders (DeEll and Ehsani-Moghaddam, 2012) In the case of ‘Gala’, the most notable disorders from long-term storage include senescent scald (skin browning), senescent breakdown (flesh browning), skin necrosis, lenticel blotch pit, and bitter pit. Disorders are associated with other negative fruit quality traits. For example, Argenta et al. (2023) found that flesh browning was associated with accelerated ripening and softer fruit.

Future climate change is expected to have an economic impact on long-term apple storage. Increased ambient temperatures will lead to greater storage postharvest losses and increased storage costs (James and James, 2010). This is especially true for apple due to its especially low temperature requirements (typically 1-3°C) compared to other long-term stored crops (Lesinger et al., 2020).

An overview of the main effects of seasonal weather on apple fruit quality is provided in Chapter 5. However, post-harvest effects of the production environment may also affect fruit storability. Fruit quality and subsequent storability are mainly determined by pre-harvest factors such as growing environment (including weather) and cultural practices (Ferguson et al., 1999). Warming weather trends over recent years have already impacted upon the selection of cultivars for long-term storage within certain geographic regions (Iglesias et al., 2008, Iglesias et al., 2016). Increased ambient temperatures are also linked with more rapid softening of fruits (Johnson, 1997).

The prevalence of storage disorders is also linked with pre-harvest climate. Cooler seasonal temperatures are associated with increased scald and flesh browning during storage (Ferguson et al., 1999; Marc et al., 2020; Argenta et al., 2023). However, a cool period before harvest is linked with reduced scald incidence (Nikitin and Makarkina, 2019; Marc et al., 2020). High seasonal rainfall is associated with increased flesh browning (Argenta et al., 2023) and scald (Nikitin and Makarkina, 2019). Storage disorders originating from pathogens (such as black rots) are predicted to increase in prevalence due to rising temperatures and humidity (Weber, 2009). However, it should be noted though that disorder incidence is dependent on cultivar choice.

There is a gap in research in how storability varies within a fruit population sourced within the same spatial and temporal environment, specifically at the same 'maturity' at harvest, but after different production temperatures. This study investigated how modified temperature environments, and so earlier maturity, in the field affected the storability of 'Gala' fruit stored in commercial CA conditions. The hypotheses for this study were as followed:

***H_0*: Modified temperature regimes and accelerated harvest dates will have no impact on the long-term storability of 'Gala' apple fruit.**

***H_1*: Modified temperature regimes and accelerated harvest dates will have a significant effect on the long-term storability of 'Gala' apple fruit.**

6.2 Materials and Methods

6.2.1 Overview

This study investigated the effect of modified temperature environment on long-term storability of the cultivar 'Gala (LA 69A)' (accession number: 1976 – 44). The experiment was carried out with fruit harvested in 2020 and in 2021. A third-year's study was planned in 2022, but this was not possible due to the storm damage to the tunnels (Chapter 2). Five fruit quality attributes were assessed during 6-7 months of controlled atmosphere storage. Fruit were sampled from trees cultivated under three unique temperature regimes (Ambient, Plus2 and Plus4). Each temperature regime consisted of three replicates, sourced from each of the 'rainfall' treatments. Likewise with other studies in this project, the effect of varied rainfall on storability was not analysed given its negligible impact on production and fruit quality (more information in Chapter 2).

Fruit were harvested at 85% Starch Index (S/I) score across all treatments, replicating industry standard procedures specific to 'Gala' (AHDB, 2021). Harvest date varied with temperature treatment each year (Table 6.1). All three 'rainfall' treatments within a temperature regime were harvested at the same time. Samples of 120 (2020) and 100 (2021) fruit were drawn from 10-18 healthy trees (i.e. trees free from pest and disease) from each regime. Fruit were placed in CA storage at the Produce Quality Centre, East Malling, Kent. Nets containing 20 fruit each were placed in self-contained units where environmental conditions were continuously regulated and monitored. Fruit were not treated with a scrubber or any commercial ethylene-reducing inputs before initiating CA storage. Nets were acclimatised in refrigerated store conditions (~1°C) for two days before CA conditions were manually established using N2 flush and CO_2 addition. Fruit were removed and fruit quality monitored at roughly six-week intervals. Manual re-establishment of CA conditions were applied to remaining nets after fruit removal. An initial set of fruit quality assessments was conducted (n=10) for each environment treatment (therefore n=30 across each temperature treatment), followed by an identical set of tests seven days later (known as 'shelf-life' tests). Fruit were stored in 'room-temperature' conditions (~18-20°C) to emulate normal consumer storing conditions. Any instances of acquired storage disorders on fruit were recorded in both initial and shelf-life tests.

Unforeseen complications arose within both years of the study in relation to achieving optimal storage procedures. Consequently, there were distinct methodological differences between years in relation to storage conditions:

- 2020 – Access to controlled atmosphere storage was delayed by 26 days after initial harvest. Fruit were kept within an industrial non-atmospheric controlled cold store at ~5°C until CA facilities became available. All treatments were exposed to pre-CA cold storage as a standard. This was 26 days for Ambient and Plus4, and 20 days for Plus2; Plus2 fruits were placed in CA storage six days sooner due to the logistics of transferring fruit between the two experimental sites. CA storage conditions replicated those recommended for optimal long term 'Gala' storage, viz. 0.7°C, 1% O_2, 5% CO_2 (AHDB, 2021). An extra round of fruit quality tests occurred before the start of CA storage to determine the effect of non-CA storage on fruit.
- 2021 – CA facilities were available immediately after harvest, with fruit placed in CA storage immediately after a 48-hour cooling period after harvest. However, the precise desired CA temperature for Gala was not available (and so the 2020 storage environment could not be replicated). Temperature was maintained at 1.5-2.0°C, rather than the optimal 0.7°C for long term 'Gala' storage; the atmosphere was 1% O_2, 5% CO_2 again.

Given these differences in storage environments (as well as production environments) between years, analyses were conducted within each year separately.

***Table 6.1**. Fruit harvest and collection dates after storage and corresponding periods days post-harvest (dph) for 'Gala' fruit quality testing across each field treatment. Each collection represents a round of sampling from storage for fruit quality assessments (n=30) plus an additional sample set (n=30) for shelf-life testing.*

2020	Harvest date (HD)	CA storage start date (CA Start)	1st collection (OCT)	2nd collection (DEC)	3rd collection (JAN)	4th collection (MAR)
Ambient	10 Sep	6 Oct (26 dph)	29 Oct (49 dph)	7 Dec (88 dph)	18 Jan (130 dph)	1 Mar (172 dph)
Plus2	25 Aug	14 Sep (20 dph)	29 Oct (65 dph)	7 Dec (104 dph)	18 Jan (146 dph)	1 Mar (188 dph)
Plus4	19 Aug	14 Sep (26 dph)	29 Oct (71 dph)	7 Dec (110 dph)	18 Jan (152 dph)	1 Mar (194 dph)

2021	Harvest date (HD)	1st collection (OCT)	2nd collection (DEC)	3rd collection (JAN)	4th collection (MAR)
Ambient	10 Sep	29 Oct (48 dph)	14 Dec (94 dph)	25 Jan (136 dph)	15 Mar (185 dph)
Plus2	31 Aug	29 Oct (58 dph)	14 Dec (104 dph)	25 Jan (146 dph)	15 Mar (195 dph)
Plus4	25 Aug	29 Oct (64 dph)	14 Dec (110 dph)	25 Jan (152 dph)	15 Mar (201 dph)

Fruit quality assessments replicated (for the most part) those performed in Chapter 5 for both the initial ('day0') and shelf-life ('day7') testing. Each individual apple fruit was weighed using a calibrated weight scale (to the nearest 0.001g). Firmness readings (kg) were obtained invasively through use of a calibrated Effegi handheld penetrometer with 11mm probe. Fruit were prepared for analysis through removing small slices of fruit skin on opposite sides (~30-40mm wide, ~3-5mm thick) of the fruit, and applying pressure using a 11mm probe attached to the penetrometer for two seconds. Readings were obtained on both sides of the fruit, with an average reading obtained from both observations. The unit for measurement was kg, replicating the UK industry methodology (AHDB, 2021). The SSC (%Brix) readings were obtained using a calibrated Atago Digital Pocket Refractometer PAL-1 3810. Red colour coverage (%) of fruit were estimated by eye as equipment to accurately measure red colour was not available. The same individual performed colour assessments throughout the trial for consistency purposes. Starch/Index (S/I) score (%) analysis was performed by cutting an apple in half and exposing the flesh to solution containing 4% potassium iodide/1% iodine. The fruit were then allowed to dry for ~30 minutes. The S/I score represented the proportion of flesh-stained blue by the solution. This process enabled assessment of the maturity or 'ripeness' of the apple as it provides an effective indicator of starch breaking down to simpler carbohydrates. This assessment was performed at each storage interval until fruit were well ripened (~10-20 S/I %). Results at each storage interval were compared against minimum fruit standards for four fruit quality metrics (Table 6.2). Failure to meet these criteria warranted the fruit 'unmarketable'.

***Table 6.2**. List of minimum market specification for fruit quality attributes using two specification bodies as an example.*

FQ Parameter	Specification Body	Minimum Requirements for Class I
Weight (g)	EU No. 543/2011	**90 g** (special requirements for 70-90 g)
Firmness (kg)	WFL Qualytech	**6.2 kg** (mean), **5.8 kg** (minimum)
SSC (%Brix)	WFL Qualytech	**11.5 %Brix**
Red Colour Coverage (%)	EU No. 543/2011	**33 – 50 %** (dependent on 'Gala' strain)

Agronomic practices differed between years with summer pruning applied in 2021 to remove excess vegetative growth on trees across all treatments, but not in 2020. However, both production years followed a full winter prune. Fruitlet thinning was applied in June of both production years. Integrated pest management strategies were implemented in both years, with extra insecticide applications during notable outbreaks of Woolly Apple Aphid (*Eriosoma lanigerum*) and Rosy Apple Aphid (*Dysaphis plantaginea*). No further insecticide applications occurred 14 days before harvest to minimise residues on harvested fruit.

6.2.2 Weather data

Seasonal weather parameters for 'Gala' from full flowering to 'tree ripe' maturity date are listed in Table 6.3 for both 2020 and 2021. Mean seasonal temperature (°C) in 2020 varied from 16.8°C (Ambient) to 19.3°C (Plus4). The range of mean seasonal temperature in 2021 was narrower, from 16.3°C (Ambient) to 17.7°C (Plus4). Seasonal weather was notably warmer, drier and sunnier in 2020 compared to 2021. Mean seasonal temperature differed by 0.40°C in Ambient, 1.00°C in Plus2 and 1.53°C in Plus4 between years. Minimum temperature did not vary much between treatments and year (11.7 to 12.3°C). Maximum temperature (°C) varied by ~4-5°C between Ambient and Plus4 treatments in both years. Maximum temperature was ~1-2°C warmer in 2020 compared to 2021. Mean seasonal precipitation was 188mm in Ambient, 167mm in Plus2, and 151mm in Plus4 more in 2021 than 2020. Both 'spring' and 'summer' mean seasonal temperature were warmer in 2020 than 2021. Spring rainfall was particularly low in 2020 (<100mm across all treatments), whereas in 2021 weather was particularly wet (~200m). Summer rainfall was similar between years, with 2021 being slightly wetter.

***Table 6.3**. Weather data for each year and modified temperature regime. Seasonal weather (temperatures, sun hours and total precipitation) represents data between full flowering date and harvest date. 'spring' refers to early fruit development (April to June), and 'summer' late fruit development up until harvest (July to September).*

Year	Temp Treat.	Tmean (°C)	Tmin (°C)	Tmax	Sun Hours	Total ppt (mm)	'Spring' Tmean (°C)	'Summer' Tmean (°C)	'Spring' Ppt (mm)	'Summer' Ppt (mm)
2020	Ambient	16.78	11.80	22.25	1113	175.2	15.47	18.07	78.4	108.2
	Plus2	18.22	11.93	25.20	1065	156.8	16.38	20.09	63.0	93.8
	Plus4	19.26	12.28	27.45	1044	155.2	17.28	21.32	56.2	100.0
2021	Ambient	16.28	12.01	21.03	721	362.8	16.14	16.38	224.2	138.6
	Plus2	17.22	11.69	23.68	717	323.4	15.54	18.87	194.0	135.4
	Plus4	17.73	11.88	25.16	704	306.6	15.49	19.99	175.8	135.0

6.2.3 Statistical analysis

One-way ANOVA compared statistical difference between mean fruit quality values of each temperature treatment (df=2) at each assessment interval and year. Post-hoc Tukey tests were utilised to classify differences between treatments. This was conducted using the "agricolae" package in RStudio. Fruit firmness (kg), SSC (%Brix) and S/I score (%) values were assessed on how variables change over days post-harvest (dph). Fruit weight and red colour coverage were likewise analysed at each interval for correlation analyses; however, the experiment did not analyse how these variables changed over time.

Correlation analyses among the fruit quality variables (weight, colour, firmness, SSC, and S/I score) was conducted using the "Hmisc" package in RStudio.

Correlation analyses among each of the five fruit quality parameters and two tree yield parameters (total fruit harvested and total harvested fresh weight (kg)) were conducted using the "Hmisc" package in R. Values were sourced from long-term yield data (see Chapter 3).

6.3 Results

6.3.1 Comparison of fruit quality attributes among treatments

Fruit quality assessments were performed at harvest and at four dates throughout 'Gala' fruit storage to determine differences between modified temperature environments. The study was performed across two separate fruit production seasons (2020 and 2021), and monitored the change in firmness (kg), soluble solids content (SSC, %Brix) and starch/index score (%) across 6-7 months of controlled atmosphere (CA) storage. Weight (g) and red colour coverage (%) were also monitored to determine differences between treatments.

Some 'Gala' fruit failed to meet marketable standards for UK consumers (Table 6.4): specifically, 2020 fruit from Plus4 at all testing dates due to inadequate red colour coverage; and 2021 fruits from all three regimes at harvest and those from ambient stored until October due to low SSC values.

Significant differences in fruit weight were identified among production temperature treatments in eight out of the 11 testing times for the 2020 and 2021 fruits (Table 6.4); the three times that did not see significant differences were CA Start 2020, MAR 2020, and MAR 2021. In 2020, post-hoc Tukey testing revealed Plus4 fruit were not significantly lighter than Plus2 or Ambient fruit at any test. Plus2 fruit were the lightest in four out of six occasions: Harvest Date ($f=11.2$, $p<0.01$), OCT ($f=25.3$, $p<0.001$), DEC ($f=20.5$, $p<0.001$) and JAN ($f=18.3$, $p<0.001$). Fruit weight in 2021 was generally lower than 2020 across all treatments. Ambient fruit from 2021 were heavier than those from the two warmer treatments at all times, significantly so in three out of the five tests: Harvest Date ($f=62.6$, $p<0.001$), DEC ($f=9.99$, $p<0.001$) and JAN ($f=12.6$, $p<0.001$). Overall, there was high biennial variation in fruit weight, with fruit weight generally highest from Plus4 in 2020, and from Ambient in 2021.

Red colour coverage differed vastly between years and among treatments. In 2020, mean red colour coverage ranged from 59.8-67.8% in Ambient, 33.7-49.1% in Plus2, and 19.3-30.0% in Plus4. Post-hoc Tukey testing revealed Ambient to have significantly greater red colour coverage than both warmer temperature treatments across every testing date where applicable ($f=24.9$-37.5, $p<0.001$). Plus2 fruit also showed greater red colour coverage than Plus4 fruit at all sampling times ($p<0.001$). In contrast in 2021, no significant differences were detected among temperature treatments at any interval ($f=0.67$-1.58, $p>0.05$) with extreme values varying only from 49.8 to 59.0%.

Differences in firmness, SSC and S/I score were identified among temperature treatments at harvest in both 2020 and 2021. In 2020, there were significant differences between Plus2 and Plus4 treatments for S/I score (f=5.92, p<0.01). Firmness and SSC did not differ significantly among temperature treatments at harvest in 2020. The 2021 study found significant differences between temperature treatments for firmness (f=62.6, p<0.001) and SSC (f=20.7, p<0.001). Tukey post-hoc testing revealed that Ambient fruit were 1.84kg (19.6%) firmer than Plus2 fruit, and 1.58kg (16.4%) firmer than Plus4 fruit at harvest. Plus2 fruit had greater SSC than Ambient and Plus4, however Ambient and Plus4 were similar. These results highlight some differences in fruit quality attributes between temperature treatments at the optimal harvest time for the storage of 'Gala' (85% S/I score), with inter-annual variation present within temperature treatments.

Harvested fruit in 2020 were stored in temporary non-CA conditions until CA facilities were available (20-26 days post-harvest). Fruit quality assessments performed after this temporary storage period (at CA Start) found significant differences between treatments for firmness (f=25.8, p<0.001) and S/I score (f=5.51, p<0.01).

Table 6.4*. Mean 2020 (top) and 2021 (bottom) values for 'Gala' apple fruit quality attributes during CA storage for each modified temperature production regime at each testing interval with one-way ANOVA results and statistically significant differences (p<0.05) indicated. Mean values below marketable standards (Table 6.2) are displayed emboldened in red.*

Test Date	Treatment	Weight (g)	Colour (%)	Firmness (kg)	SSC (%Brix)	S/I score (%)
Harv.	Amb	N/A	N/A	10.24 a	11.58 a	83.60 a
Date	Plus2	152.75 b	33.67 a	10.67 a	12.12 a	85.77 a
2020	Plus4	176.78 a	**19.33** b	10.48 a	11.98 a	77.12 b
CA	Amb	173.33 a	59.83 a	8.95 b	12.84 a	49.50 b
Start	Plus2	155.77 a	43.39 b	10.31 a	13.07 a	65.54 a
	Plus4	169.35 a	**19.67** c	9.27 b	13.43 a	51.33 b
OCT	Amb	174.17 a	64.17 a	8.53 b	13.19 b	27.50 a
	Plus2	157.00 b	49.00 b	9.20 a	14.24 a	28.17 a
	Plus4	177.50 a	**29.17** c	8.28 b	14.14 a	26.83 a
DEC	Amb	160.27 b	66.17 a	8.44 b	13.21 b	28.33 a
	Plus2	150.93 c	49.17 b	8.95 a	14.50 a	30.67 a
	Plus4	168.67 a	**28.83** c	8.56 ab	14.07 a	21.00 b
JAN	Amb	167.63 a	67.83 a	8.18 a	13.53 b	26.33 a
	Plus2	157.30 b	46.33 b	8.52 a	14.62 a	23.33 a
	Plus4	172.27 a	**30.00** c	7.47 b	14.10 ab	25.83 a
MAR	Amb	170.40 a	65.00 a	7.98 a	13.79 a	N/A
	Plus2	153.90 a	38.00 b	7.84 a	14.42 a	N/A
	Plus4	163.00 a	**24.83** c	7.25 b	14.31 a	N/A

Test Date	Treatment	Weight (g)	Colour (%)	Firmness (kg)	SSC (%Brix)	S/I score (%)
Temperature Treatment One-Way ANOVA (df=2) F-statistic and significance						
Harv	-	11.19**	6.81*	1.49	1.72	5.92**
CA St	-	2.36	27.51***	25.75***	2.246	5.51**
OCT	-	25.32***	25.46***	10.77***	10.66***	0.08
DEC	-	20.49***	34.51***	3.97*	9.64***	6.63**
JAN	-	18.33***	24.91***	9.56***	7.91***	0.37
MAR	-	2.20	37.53***	5.22**	2.90	N/A

Test Date	Treatment	Weight (g)	Colour (%)	Firmness (kg)	SSC (%Brix)	S/I score (%)
Harv.	Amb	126.81 a	55.18 a	11.24 a	9.55 b	N/A
Date	Plus2	97.15 b	59.00 a	9.40 b	11.14 a	N/A
2021	Plus4	105.00 b	50.18 a	9.66 b	10.08 b	N/A
OCT	Amb	122.67 a	52.67 a	10.39 a	11.30 b	40.00 a
	Plus2	104.19 b	56.83 a	10.13 ab	12.20 a	22.00 b
	Plus4	115.82 a	53.50 a	9.81 b	11.70 ab	17.17 b
DEC	Amb	125.80 a	55.33 a	10.00 a	11.90 ab	14.33 a
	Plus2	108.27 b	56.83 a	9.63 ab	12.17 a	9.83 b
	Plus4	112.10 b	49.83 a	9.56 b	11.40 b	6.83 b
FEB	Amb	125.38 a	54.18 a	10.16 a	11.72 a	N/A
	Plus2	101.39 b	54.18 a	9.60 b	12.18 a	N/A
	Plus4	109.72 b	52.00 a	9.78 a	11.66 a	N/A
MAR	Amb	112.37 a	52.50 a	10.13 a	11.90 ab	N/A
	Plus2	99.28 a	57.83 a	9.71 a	12.46 a	N/A
	Plus4	111.98 a	52.67 a	9.82 a	11.62 b	N/A
Temperature Treatment One-Way ANOVA (df=2) F-statistic and significance						
Harv	-	22.08***	1.58	62.63***	20.67***	N/A
OCT	-	7.84***	0.31	4.63*	5.91**	20.58***
DEC	-	9.99***	0.91	3.90*	3.48*	13.36***
FEB	-	12.56***	0.10	7.24**	1.64	N/A
MAR	-	2.94	0.67	2.98	4.35*	N/A

* Significant at p<0.05 ** Significant at p<0.01 *** Significant at p<0.001. N/A data unavailable.

Firmness decreased and SSC values increased during 5-7 months in CA storage within each temperature treatment in 2020 but much less so and with treatment differences in 2021 (Table 6.5). The final round of fruit quality assessments occurred in March of both years after 6-7 months of storage.

The largest change in 2020 for both firmness and SSC was observed in Plus4 fruit (-30.8% and +19.5% from harvest, respectively) over the course of 194 days. Ambient fruit firmness and SSC changed the least (-22.1% and +19.1%, respectively) over the course of 172 days (22 fewer days post-harvest than Plus4). In 2021, Ambient fruit observed the biggest change for both firmness and SSC (-9.88% and +24.6% from harvest, respectively).

In 2021, firmness degradation differed among treatments when compared to 2020. By March 2021, Plus4 2020 fruit had softened 0.97kg (8.75%) more than Ambient, and 0.4kg (4.52%) more than Plus2. The Plus4 fruit had accumulated 0.12%Brix (0.37%) more SSC than Ambient, and 0.03%Brix (0.47%) more than Plus2 fruit. In 2021 harvested fruit, Ambient fruit softened less than 2020 (-9.88%), whereas Plus2 and Plus4 fruit increased in firmness (+3.30% and +1.66%, respectively). For SSC, Ambient fruit accumulated 1.03%Brix (12.8%) more than Plus4, and 0.81%Brix (9.33%) more than Plus2. However, overall Ambient SSC remained lower than Plus2. The results indicate inter-year variation among treatments in firmness and SSC change during storage.

Table 6.5*. Changes in 'Gala' fruit Firmness (kg) and Soluble Solids Content (SSC, %Brix) from harvest date (HD) to final assessment in March (MAR, 6-7 months of controlled atmosphere (CA) post-harvest [PH] storage).*

Year	**Test**	**Treat.**	**Days HD to MAR**	**Value at harvest**	**Value post-storage**	**Change in value**	**% change**	**% per day PH**
2020	Firmness	Amb	172	10.24	7.98	-2.26 kg	- 22.07 %	- 0.128 %
		Plus2	188	10.67	7.84	-2.83 kg	- 26.52 %	- 0.141 %
		Plus4	194	10.48	7.25	-3.23 kg	- 30.82 %	- 0.159 %
	SSC	Amb	172	11.58	13.79	2.21 %Brix	+ 19.08 %	+ 0.111 %
		Plus2	188	12.12	14.42	2.30 %Brix	+ 18.98 %	+ 0.101 %
		Plus4	194	11.98	14.31	2.33 %Brix	+ 19.45 %	+ 0.100 %
2021	Firmness	Amb	185	11.24	10.13	-1.11 kg	- 9.88 %	- 0.053 %
		Plus2	195	9.40	9.71	0.31 kg	+ 3.30 %	+ 0.017 %
		Plus4	201	9.66	9.82	0.16 kg	+ 1.66 %	+ 0.008 %
	SSC	Amb	185	9.55	11.90	2.35 %Brix	+ 24.61 %	+ 0.133 %
		Plus2	195	11.14	12.46	1.32 %Brix	+ 11.85 %	+ 0.061 %
		Plus4	201	10.08	11.62	1.54 %Brix	+ 15.28 %	+ 0.076 %

6.3.2 Correlation analysis

Pearson's correlation analysis revealed that several fruit quality variables were correlated at three different storage timepoints (Table 6.6). Individual fruit weight was negatively correlated with firmness ($p<0.001$) and positively correlated with SSC ($p<0.001$). Weight was also negatively correlated with colour, except in March, and S/I score ($p<0.05$). Correlations with fruit weight were the strongest within the analysis: -

0.64 with firmness in December and 0.47 with SSC in December. Correlations between some variables altered during storage. For example, S/I score was negatively correlated with SSC at harvest (-0.46) yet positively correlated in December (0.27). Firmness and SSC also show this shift from a positive correlation at harvest to negative in December and March. Correlations between certain variables became stronger with a period in storage – especially between harvest date and December. For example, the correlation between weight and SSC increased from 0.35 at harvest to 0.47 and 0.46 in December and March, respectively. Overall, correlation analysis indicated that some fruit quality variables were intrinsically linked, and these associations became stronger as fruit matured in CA storage.

Table 6.6. *Correlations among 'Gala' fruit quality parameters with data sourced from three assessment intervals across long-term CA storage (Harvest Date, December and March); n = 54 (9 environments x 2 years x 3 test dates).*

HARV. DATE	**Colour**	**Firmness**	**SSC**	**S/I Score**
Weight	-0.45***	0.21*	0.35***	-0.30*
Colour	-	-0.03	-0.16	-0.07
Firmness	-	-	0.15*	-0.08
SSC	-	-	-	-0.46***
DECEMBER	**Colour**	**Firmness**	**SSC**	**S/I Score**
Weight	-0.17*	-0.64***	0.47***	-0.25*
Colour	-	0.16*	-0.06	0.38***
Firmness	-	-	-0.24**	0.31**
SSC	-	-	-	0.27**
MARCH	**Colour**	**Firmness**	**SSC**	**S/I Score**
Weight	-0.14	-0.63***	0.46***	N/A
Colour	-	0.34***	-0.13	N/A
Firmness	-	-	-0.45***	N/A
SSC	-	-	-	N/A

* Significant at p<0.05 ** Significant at p<0.01 *** Significant at p<0.001. N/A data unavailable.

Some yield parameters were significantly correlated with some fruit quality parameters throughout storage, others not at all test dates, and in some cases not at all (Table 6.7). Total fruit number per tree was correlated negatively with fruit weight, firmness and SSC at harvest and post-storage in March. Total fruit number was negatively associated with S/I score, but only mid-storage in December (-0.76, p<0.001). Total fresh weight per tree did not correlate with fruit quality parameters at any stage. Red colour coverage did not correlate with yield parameters at any stage. The results highlight high influence of crop load (i.e. total fruit per tree) on certain fruit quality parameters both at harvest and after long-term storage.

Table 6.7*. Correlations among 'Gala' fruit quality parameters and two yield parameters (total fruit harvested per tree and total fresh weight per tree) with data sourced from three assessment intervals across long-term CA storage with sample population (n) indicated at each test and variable].*

Stage	Yield (per tree)	Weight	Colour	Firmness	SSC	S/I Score
Harv	n	15	15	18	18	18
	Total fruit	-0.80***	0.43	-0.63**	-0.47*	0.29
	Total fresh weight	-0.35	-0.06	-0.23	-0.37	0.25
DEC	n	18	18	18	18	18
	Total fruit	-0.72***	0.11	0.41	-0.74***	-0.76***
	Total fresh weight	-0.11	0.07	-0.04	-0.42	-0.28
MAR	n	18	18	18	18	N/A
	Total fruit	-0.69**	0.37	0.61**	-0.66**	N/A
	Total fresh weight	0.05	0.36	0.13	-0.32	N/A

* Significant at $p<0.05$ ** Significant at $p<0.01$ *** Significant at $p<0.001$. N/A data unavailable.

6.3.3 Shelf-life analysis

Seven-day shelf-life testing between CA start to March revealed that fruit firmness continued to soften, and generally a higher proportion of SSC accumulated within every phase for 2020 fruits (Figure 6.1). The rate of firmness degradation was greater (1.5-3.3kg depending on treatment) where fruit had no exposure to CA conditions (CA Start) or a short exposure (in October). Firmness degradation stabilised for assessments between December and March. Plus4 fruit generally accumulated greater SSC in comparison to Ambient with the greatest increase over the 7 days for fruits at harvest (2021) or in September (2020).

Shelf-life testing results for the 2021 fruits generally showed less change across all treatments than 2020. Opposite trends were seen among treatment combinations within results for each of firmness and SSC. Changes in firmness for Ambient and Plus2 fruits were marginal (~±0.2kg) over the 7 days, whereas Plus4 fruits often softened slightly more (±0.5) – except in October when values rose. Changes in SSC immediately after harvest were greater than after CA storage; Ambient and Plus4 fruits after periods of CA storage showed little change whereas in Plus2 fruit SSC accumulated comparatively more.

Overall, changes in firmness and SSC over the seven day shelf life period were somewhat inconsistent between years and production temperature treatment, but ambient fruit generally showed less change than those from Plus2 and Plus4, and SSC increased the most in the 7 days after harvest or in September in both years.

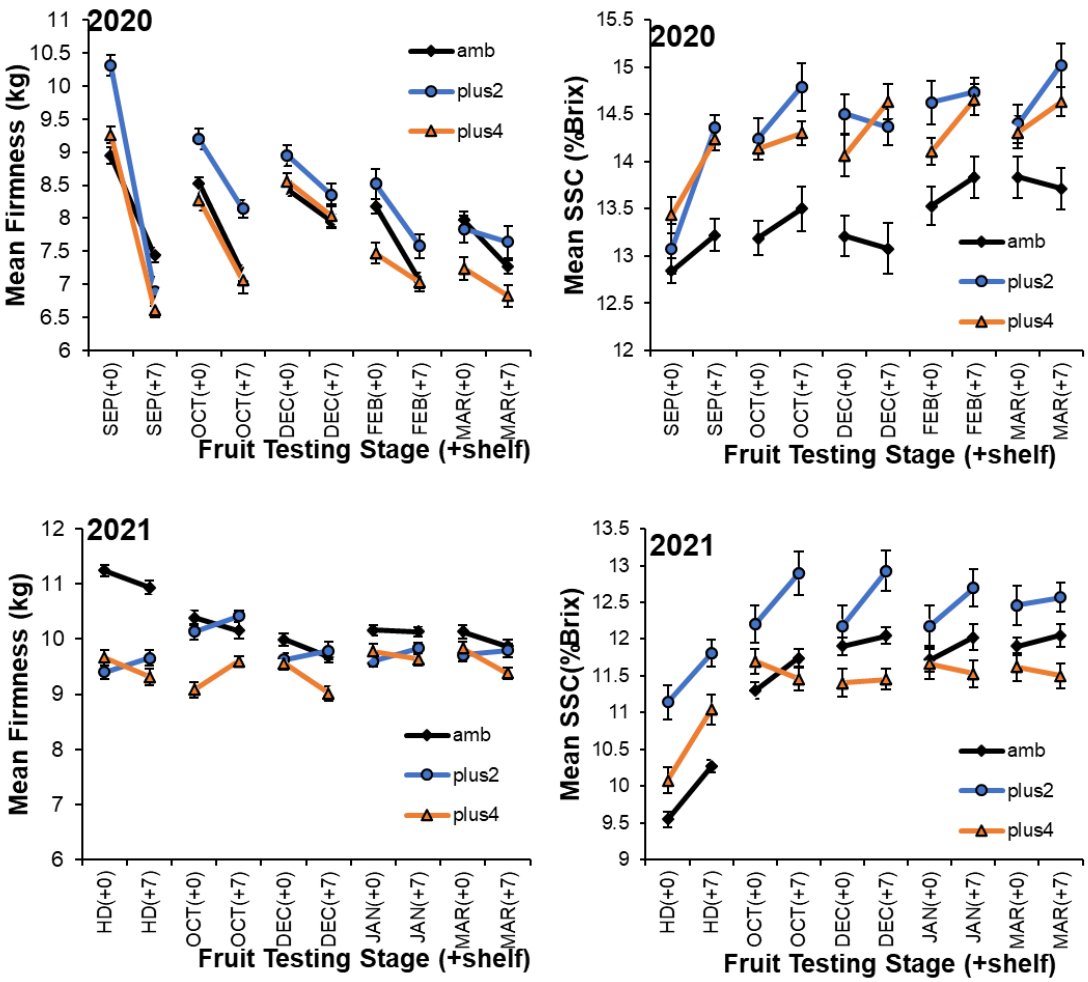


***Figure 6.1**. Changes in mean (±SE) firmness and SSC of 'Gala' fruit across a seven-day shelf period at room temperature after removal from CA storage in 2020/21 (top) and 2021/22 (bottom) (note: shelf-life testing was not conducted at harvest in 2020).*

6.3.4 Storage disorder incidence

Storage disorder incidences on 2020 fruit were infrequent across all treatments, only affecting <1% of all fruit (data not shown). However, in 2021 under slightly warmer CA conditions (and cooler, wetter production conditions) the prevalence of storage disorders was much more apparent by December (Figure 6.2). After 11-13 weeks of CA storage, Ambient (25%) and Plus4 (23.3%) fruit had comparable levels of storage disorders present, whereas Plus2 fruit appeared less affected (11.7%). After a further 12-13 weeks in storage the proportion of affected fruit increased greatly for both Ambient (62.1%) and Plus4 (65%) treatments, with Plus2 fruit remaining more resilient to acquiring storage disorders (30%). Storage disorders were primarily senescent scald (~90% of disorders). This was typically characterised by light-medium brown patches of skin, with a browning of the flesh underneath in more severe cases. The remaining fruit with storage disorders showed skin necrosis (~5%), russeting (~5%), or lenticel breakdown (~2%). A minority of fruit (<1%) exhibited multiple disorders. There were very few cases of fungal rot disorder (~0.1%) across both years of study.

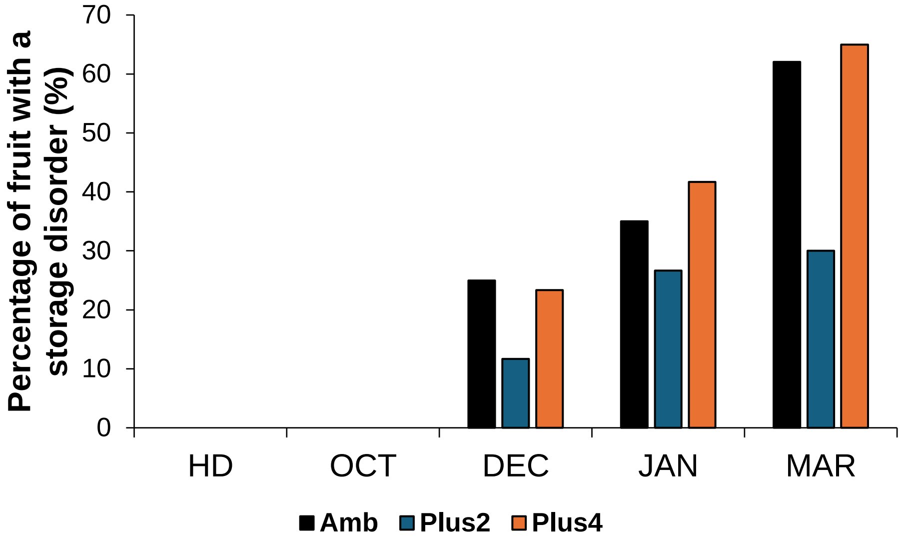


***Figure 6.2**. Proportion of 2021 'Gala' fruit across each temperature treatment and assessment interval affected by a storage disorder.*

6.4 Discussion

Fruit quality parameters are heavily influenced by environmental and agronomic conditions, which contribute towards year-to-year variation (Musacchi and Serra, 2018). The difference in 'Gala' fruit quality attributes at harvest (85% Starch Index) between 2020 and 2021 revealed high variation between the two study years. This variation affected overall fruit marketability, though for different reasons, between years. Fruit from all treatments in 2020 were of 'good' weight (~150g), retained adequate firmness, had suitable SSC, and remained free from storage disorders throughout all six to seven months of storage; but red colour coverage in the warmest treatment fell below minimum standards. Fruit from all treatments in 2021 retained high firmness and red colour coverage across all treatments, but SSC did not meet minimum standards at harvest and only became marginally marketable after three months in CA storage under ambient production conditions. Inadequate SSC accumulation was more severe for Ambient fruit in 2021 than for both Plus2 and Plus4, with one month in CA storage enough for Plus2 and Plus4 fruit to cross the marketing threshold. Additionally, the increase in the presence of storage disorders rendered ~50% of Ambient and Plus4 2021 fruit unmarketable after six months of CA storage.

Differences between temperature treatments were found at each storage assessment time. Whilst this might be expected as harvest dates differed among treatments, the 2020 results reveal that it was not due to different maturity levels at each assessment if the starch index score is used as an indicator of maturity. However, the results for 2021 fruit differ in that Ambient fruit did not ripen in storage as quickly as warmer treatments. Overall, while the 2020 fruit results show that Plus4 and Plus2 fruits softened more rapidly compared to Ambient, there were no significant firmness or SSC differences among treatments after 6-7 months of storage. Firmness in 2021 was also similar among treatments. These findings match those of Lysiak's et al. (2020) and Ingle et al. (2000) where weather and different harvest dates were found to have little impact on the storability of two different apple cultivars. Both firmness and SSC met minimum marketability standards after 6-7 months of storage across all treatments in the current study. Evidence of more rapid softening in Plus4 fruit in 2020 (but not 2021) could have resulted in unmarketable fruit if the experiment had extended beyond 6-7 months storage. However, given that commercial 'Gala' CA fruit storage (without an ethylene scrubber) typically ends in late-March, this would be largely irrelevant.

In general, mean fruit weight was 30-40% lower in 2021 compared to 2020. This may have been due to enhanced alternate bearing patterns for yield across all treatments, as discussed in Chapters 3 and 4. Alternate bearing patterns were more severe in Plus2 and Plus4 treatments with 2020 being a ‘low-cropping’ year and 2021 ‘high-cropping’. This was reflected in significantly lighter Plus2 and Plus4 fruit in 2021. Fruit weight was more similar among treatments in 2020, with Plus4 fruit weight occasionally significantly heavier than Ambient. Correlation analysis revealed that greater crop load (total fruit number per tree) was associated with reductions in the weight of each fruit, SSC, and S/I score; both negative and positive associations were found with firmness. Heavy cropping trees are typically associated with raised SSC (De Salvador et al., 2006). The evidence from this study contradicts these findings, with crop load being negatively associated with SSC across all three testing times during CA storage. The SSC was generally lower across all treatments in 2021 compared to 2020, and this coincided with the ‘high-cropping’ season in 2021 where crop load was especially high. Therefore, the negative correlations between crop load and SSC may be non-causal with the main driving factor for SSC being the difference in seasonal weather, which would match the findings from Kvikliene et al. (2006).

The importance of fruit size and weight in determining other fruit quality variables is reflected in the literature, as well as Chapter 5. Results from this study revealed that the mean weight of fruits was closely associated with other fruit quality variables, specifically colour, firmness, SSC, and S/I score. Weight had a strong negative relation with firmness, but positive with SSC. Greater fruit weight was also associated with a lower S/I score which may have contributed towards the increase in SSC (Musacchi and Serra, 2018). Indeed, S/I score and SSC showed a strong negative correlation here. Firmness also correlated highly with other fruit quality variables in December and March assessments – positively with SSC and negatively with S/I score. These correlations replicate the findings of Kvikliene et al. (2006) where lower fruit firmness was associated with more mature fruit. Overall, the correlation analyses indicate that both mean fruit weight and firmness were key fruit quality indicators, not only directly but also indirectly through their correlations with SSC and S/I score, within long-term CA storage.

A very low incidence of storage disorders was detected during the CA storage of 2020 fruits. Conversely, the incidence of storage disorders was high with stored 2021 fruit with over 50% of fruit damaged after 6-7 months of CA storage. There was little difference between Ambient and Plus4 2021 fruit, with Plus2 fruit less susceptible. Seasonal temperature was much cooler in 2021 than 2020. This is likely to have been a contributing factor (Argenta et al., 2023; Ferguson et al., 1999). However, the Plus4 seasonal temperature was higher in 2021 compared to Ambient in 2020 when disorder incidence was low, implying that temperature may not be the only factor involved. The much greater precipitation throughout the 2021 growing season (more than double 2020) is also likely to have been a contributing factor (Argenta et al., 2023; Nikitin and Makarkina, 2019). Storage disorders are also associated with greater fruit weight (Argenta et al., 2023). This was not the case here for comparisons between the two years, 2021 fruits were lighter and had a high incidence of storage disorders, but the reduced fruit weight from the Plus2 regime compared to Ambient and Plus4 may have been a factor contributing towards the former's reduced incidence of disorders.

The methodology in 2020 cf. that in 2021 may have inadvertently reduced storage disorder incidence. Delayed CA storage by up to 30 days after harvest is associated with lower scald incidence (DeEll and Ehsani-Moghaddam, 2012). Additionally, a period of cool weather before harvest can replicate this effect (Marc et al., 2020; Nikitin and Makarkina, 2019). Fruit in 2020 were placed in temporary non-CA controlled refrigerated conditions (~5°C) for 20-26 days after harvest, whereas 2021 fruit were placed into CA conditions after a period of 48-hour cooling. However, the reported benefits to disorder reduction cited above often enhance the rate of fruit softening (DeEll and Ehsani-Moghaddam, 2012; Konopacka et al., 2004). The results from this study replicated these findings with 2020 fruit losing a greater proportion of firmness over time compared to 2021. The warmer CA temperature (1.5-2°C in 2021 cf. 0.7°C in 2020) may well have also contributed towards greater disorder incidence in 2021. Fruit ripened faster in 2021 compared to 2020, probably as a consequence of this warmer than optimum temperature for long-term storage (AHDB, 2021). Increased disorder prevalence coincided with S/I score declining below 15% in December 2021. Based on the evidence from this study, it is likely both seasonal weather conditions and the differences in storage methodology contributed towards the variation in disorder occurrence between study years. Nonetheless, there is little evidence to suggest that fruit production in the different temperature regimes affected the occurrence of storage disorders to any substantial extent.

Differences in red colour coverage among treatments contrasted between the two study years. In 2020, Ambient fruit (59-68%) had significantly greater coverage than Plus2 (38-49%) and Plus4 (19-30%). Effective red-pigment anthocyanin production is linked with cooler night conditions as fruits mature (Gonda et al., 2006; Blankenship et al., 1987). Earlier ripening is likely to have provided fruit in the warmer treatments with fewer ripening days with cool night temperatures compared to Ambient. Seasonal temperature in 2021 was generally cooler than in 2020, which may have contributed to the reduced differences among treatments in red colour and the greater values overall in 2021. However, another influence was likely to be canopy shading. Greater vegetative shoot growth in the warmer treatments, as reported in previous chapters, is likely to have reduced sunlight exposure. Increased shading is associated with less red colour accumulation on apple (Gonda et al., 2006; Takos et al., 2006). All treatments received vegetative summer pruning in 2021, but not in 2020. This meant that shading was reduced in 2021, and the greater red colour accumulation may have been a consequence of this.

The seven-day shelf-life testing of ‘Gala’ fruit provided further evidence of adequate marketability across all treatments in terms of firmness and SSC. The results reveal few consistent differences in the changes over the seven days among treatments. Plus4 fruit firmness was the closest to falling below the 5.8-6.2 kg market threshold in March 2020, but these fruit were harvested first and so this might be expected. The steepest changes for both attributes were seen in September 2020, where fruit had been kept in temporary non-CA storage prior to testing, which highlights the importance of rapid entry into CA stores to delay fruit ripening. This agrees with the findings of DeEll and Ehsani-Moghaddam (2012).

Certain limitations, primarily the need to rely on semi-commercial facilities run for the benefit of other users, within this study prevented it from providing a definitive answer to the effect of modified temperature production regimes on subsequent fruit storability. First, the methodology differed slightly between years such that, due to circumstances beyond control, neither year of study provided optimal storage conditions for ‘Gala’ (in 2020 there was a delay to starting CA; in 2021 the storage temperature was c. 0.8-1.3°C warmer than optimal). Secondly, a third year of study would have been helpful in forming conclusions and testing associations given the vast heterogeneity among the 2020 and 2021 results. Thirdly, firmness and SSC are important commercial parameters for store monitoring procedures, but do not provide a ‘full picture’ of overall fruit quality. Studies of this topic typically analyse factors such as titratable acidity, mineral content, flavanol content, and much more. Taste testing procedures with trained professionals would have also provided key consumer analysis on whether treatments differed in their flavour profiles.

6.5 Conclusions

Exposure of 'Gala' fruit production to different modified temperature regimes had mixed direct and indirect effects on long-term fruit storability. Seasonal temperature uplifts of up to +4°C accelerated harvest by 16-22 days at optimal harvest maturity (85% S/I) and appeared to affect fruit quality early on in CA storage. The indirect effects of the temperature treatments on alternate bearing probably affected the quality of fruit and subsequent storability through effects on mean fruit weight. However, after six to seven months in CA storage, any differences in fruit quality from the effects of the three different fruit production temperature regimes were minimised. Consequently, and in particular there was little or no difference in the overall marketability of 'Gala' fruit among the modified temperature regimes after storage.

Study methodology and pre-harvest conditions were likely to have been the main factors that influenced fruit quality and storage disorder incidence. Modified seasonal temperature had little or no impact on the development of disorders during long-term CA storage.

Further years of study and consistent methodology would be required to confirm relationships between modified temperature environment and 'Gala' storability.

Chapter 7: General Discussion

7.1 Summary of key findings

Six years of data collection within the 'Apples in a Warmer World'® investigation has shown how the implementation of three unique modified field temperature regimes had both direct and indirect effects on a wide range of apple production variables across diverse cultivars. The experimental system provided four production seasons (2018-21) of modified environments, after an initial 'baseline' year (2017), with a further 'legacy' year (2022).

Long-term responses relating to global environmental changes often cannot be derived from the study of short-term effects, which therefore warrants experiments on longer time scales (De Boeck et al., 2015). A further three years of study from Lane's (2022) initial work has provided more clarity on apple production parameters' responses to modified temperature. For example, six years of data has encapsulated three complete alternate bearing cycles for most cultivars. However, it would be disingenuous to describe the findings as based on 'long-term' data, given that perennial crop cycles are much longer (around 10-20 years in the case of apple commercial systems) than annual crop systems.

The chapters in this thesis aimed to address the effect of modified temperature regimes, primarily through uplift of seasonal temperatures, on apple production parameters relating to yield and fruit quality. The analyses initially detected differences in yield between three temperature regimes: Ambient, +2°C ('Plus2'), and +4°C ('Plus4') (Chapter 3). Treatment differences were present amongst many of the diverse apple cultivars studied. Linear relationships between seasonal temperature and yield appeared overwhelmingly negative, though not quite always so, at a superficial level. However, after further investigation, the response of yield to varied seasonal temperature within each temperature treatment was shown to be similar. Only a small proportion of cultivars ('Golden Delicious', 'Lappio', and 'Winter Banana') demonstrated differences in yield temperature response amongst treatments. Relationships between seasonal temperature and yield showed mixed effects of different periods' temperature on yield, with evidence that February, July, and August temperature may have been more influential than that in other months. However, relationships were inconsistent and varied greatly between cultivars.

Fresh weight yield per tree across all treatments experienced some level of alternate bearing. In theory, the alternate bearing behaviour may have been established before the investigation began in 2017, or during the period of study (2017-2022), or both. The wide range of cultivars included variation in the tendency to bienniality. Hence, some cultivars may have had the behaviour established before 2017 whilst in others it may have been a consequence of the warmer years (e.g. 2018) and/or the warmer temperature treatments (Chapter 4). Analysis of flower cluster production (at time of flowering) revealed that warmer regimes were producing fewer floral clusters in biennial 'off' years (2018, -20, and -22). Subsequent detection of significant associations of cultivar, management, and environment effects on yield variation revealed potential explanations for differences between treatments. The causes of alternate bearing and its exacerbation are complex and not fully understood. However, cultivar selection, management practices, and environment are thought to be the main drivers (Monselise and Goldschmidt, 1982; Kofler et al., 2019). Temperature associations were found with yield during the periods of floral bud initiation (May-June) and early plant dormancy (November-January), providing possible explanations to why differences in alternate bearing were detected between the unique modified environments.

In addition to yield, several fruit quality parameters showed differences between modified temperature environments (Chapter 5). The analyses showed clear associations between seasonal temperature and fruit quality response: Soluble Solids Content (SSC) and Dry Matter Content (DMC) were positively influenced, Red Colour Coverage (RCC) and Fruit Weight were negatively influenced, and firmness showed mixed influences dependent on the cultivar. However, multivariate analysis suggested that the overall influence of seasonal temperature variables may not have been as high as other meteorological aspects, such as precipitation and sunlight hours. Additionally, the models determined that at least one yield parameter was highly influential on each of the five tested fruit quality parameters. Associations between yield parameters and fruit storability (Chapter 6) further demonstrated the high influence of inter-annual yield variation on the subsequent season's production metrics. Therefore, combining evidence from all four chapters presents a possible sequence of events that explains some of the variance seen in results throughout the trial (Figure 7.1).

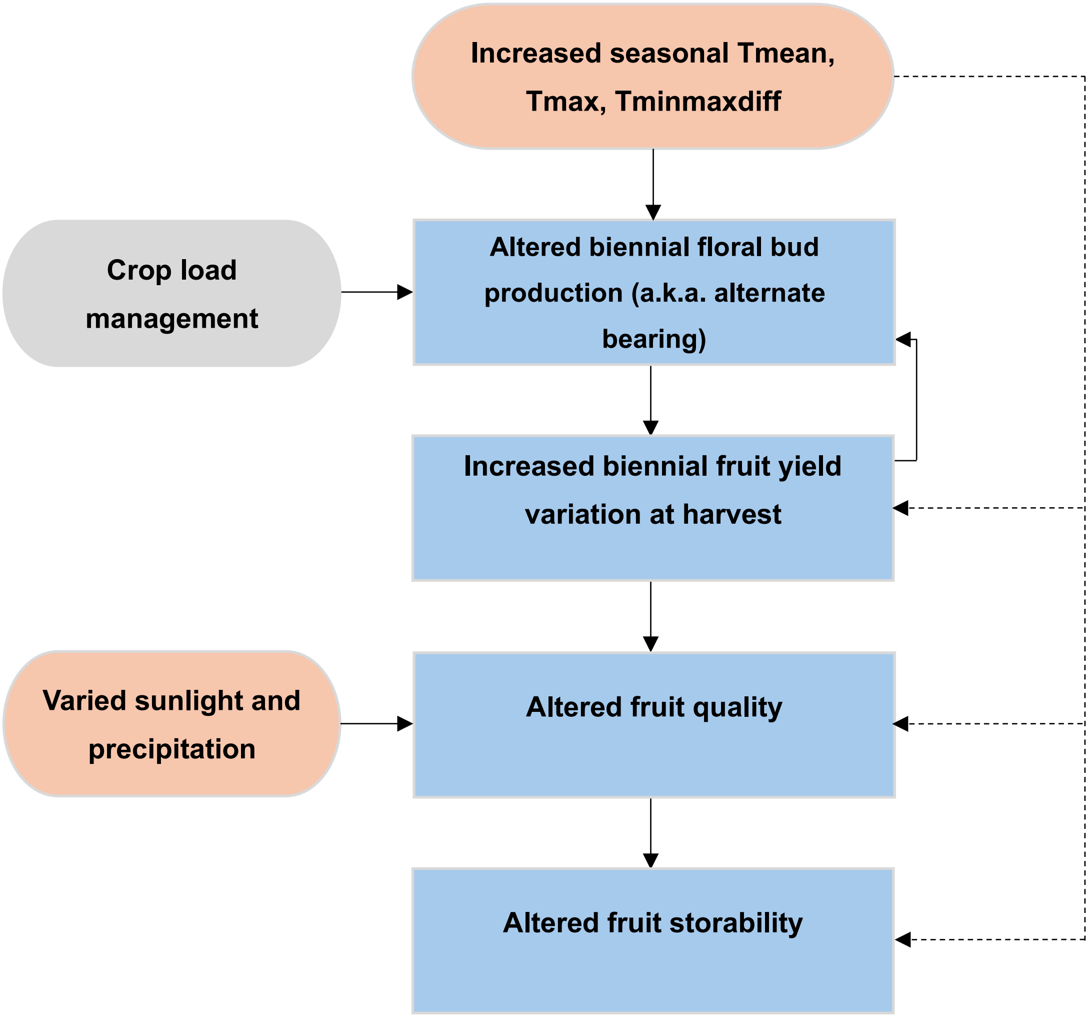


Figure 7.1*. Simplified flowchart on how key variables (blue = production, orange = meteorological, grey = crop management) influenced apple production parameters across a wide range of cultivars within six years (2017-22) of data collection within the NFCT's 'Apples in a Warmer World' investigation. Solid lines indicate strong direct influences, with dotted lines indicating weaker influences.*

The flowchart in Figure 7.1 is highly generalised: the impacts listed are not applicable to all cultivars studied and it does not encapsulate a 'full' range of variables (both quantified and unquantified) that may have had influence on one or several flowchart phases. Additionally, the flowchart does not speculate on specific mechanisms (e.g. molecular or physiological) that are involved in influencing each stage. What it does attempt is to explain the potential role of varied temperature between the three modified environments within the six years of experimental data collection. The sequence begins with the choice of cultivar, crop load management, and raised temperature variables influencing the altered production of floral clusters produced during pollination. This then translated in to altered yield at harvest. The feedback loop of crop load on the subsequent year's yield (i.e. a high yielding year inducing a low yielding year) is well documented in perennial crops (Jonkers, 1979; Monselise et al., 1982), so this is reflected in the flowchart. Altered yields then directly affected fruit quality (amongst other meteorological factors such as sunlight and precipitation), which then subsequently affected fruit quality attributes related to storability in ‘Gala’ (the only cultivar in which storability was studied). At each stage, direct associations were found with temperature (dotted lines). However, it was shown that greater variation could be explained by the sequence of events initiated by altered floral bud production.

7.2 Comparison of treatment effects on selected cultivars

The effects of modified temperature environment on fruit production responses varied by apple cultivar and genetic trait. The summary tables below encapsulate data from all four chapters and provide a brief description of temperature effects on measured production variables related to phenology, yield, tree growth, and fruit quality for seven notable apple cultivars between the two extreme tunnels (Ambient and Plus4). These were ‘Gala’ (key commercial cultivar), ‘Braeburn’ (introduced later into the study), ‘Discovery’ (early-harvesting), ‘Bramley’s Seedling’ (culinary), ‘Golden Delicious’ (influenced by seasonal temperature in this study), ‘Fuji’ (not heavily influenced by temperature in this study), and ‘Cox’s Orange Pippin’ (heritage, highly referenced in UK studies). For each cultivar, a brief verdict is provided on the influence of warmer weather based on results.

7.2.1 'Gala'

***Table 7.1**. Overview of key horticultural industry parameters based on six years of results from the 'Apples in a Warmer World'® study for the cultivar 'Gala'.*

Production Variable	Ambient Mean	Plus4 Mean	Change Amb to Plus4
Mean Seasonal Temperature (°C) [1]	16.57	18.76	↑ 2.19 °C
Mean Winter Temperature (Nov-Mar) (°C) [1]	6.75	7.05	↑ 0.30 °C
Full-flowering date [1]	28th APR	20th APR	↓ 8 Days
Harvest date [1]	26th SEP	31st AUG	↓ 27 Days
Harvested Fresh Weight per tree (kg) [2]	20.14 (± 0.80)	17.66 (± 0.96)	↓ 12.31 % (- 2.48kg)
Harvested Fruit per tree [2]	150 (± 6.76)	146 (± 11.50)	↓ 2.67 % (- 4 fruit)
Mean Fruit Weight (g) [2]	143.09 (± 3.31)	144.27 (± 3.71)	↑ 0.82 % (1.28g)
Alternate Bearing Index (ABI) [2]	0.21 (± 0.02)	0.40 (± 0.02)	↑ 90.47 % (0.19)
Total Annual Prunings Removed (kg) [2]	4.16 (± 0.70)	4.60 (± 0.44)	↑ 10.57 % (0.44kg)
Firmness (kg) [1]	8.13 (± 0.07)	7.80 (± 0.09)	↓ 4.06 % (- 0.33kg)
Soluble Solids Content (SSC) (%Brix) [1]	12.08 (± 0.10)	12.43 (± 0.16)	↑ 2.89 % (0.35%Brix)
Red Colour Coverage (RCC) (%) [3]	83.58 (± 0.90)	64.42 (± 1.39)	↓ 23.02 % (- 19.16%)
Dry Matter Content (DMC) (%) [3]	14.22 (± 0.41)	14.58 (± 0.60)	↑ 2.53 % (0.34 %)
CAS – Harvest Date	10th SEP	22nd AUG	↑ 19 Days
CAS – Firmness at Harvest [3]	10.74 (± 0.11)	10.08 (± 0.12)	↓ 6.15 % (- 0.66 kg)
CAS – Firmness in December (~3 months) [3]	9.29 (± 0.13)	9.21 (± 0.12)	↓ 0.86 % (- 0.08 kg)
CAS – Firmness in March (~6 months) [3]	9.07 (± 0.16)	8.45 (± 0.19)	↓ 6.84 % (- 0.62 kg)
CAS – SSC at Harvest [3]	10.56 (± 0.17)	11.03 (± 0.20)	↑ 4.45 % (0.47 %Brix)
CAS – SSC in December (~3 months) [3]	12.56 (± 0.15)	12.84 (± 0.21)	↑ 2.23 % (0.28 %Brix)
CAS – SSC in December (~6 months) [3]	12.91 (± 0.18)	12.92 (± 0.22)	↑ 0.08 % (0.01 %Brix)
CAS – 2020 Red Colour Coverage (RCC) (%)	63.01 (± 1.02)	26.55 (± 0.91)	↓ 58.84 % (- 36.46 %)
CAS – 2021 Red Colour Coverage (RCC) (%)	56.86 (± 1.09)	53.38 (± 1.44)	↓ 6.12 % (- 3.48 %)

[1] 4-year mean (2018-21), [2] 6-year mean (2017-22), [3] 2-year mean (2020-21), CAS = results from controlled atmosphere storage study

- **Substantial change in harvesting seasonality** (27 days earlier).
- **Moderate reduction in yield per tree** (-12.3%).
- **Substantial increase in alternate bearing habits** (90.5%).
- **Moderate increase in tree vegetative growth removal** (10.6%).
- **Minor reduction in firmness, minor increase in SSC**.
- **Substantial decrease in RCC** (-23.0%).
- **Minor changes in storability, though fruit generally still marketable 6+ months in CA storage.**
- Verdict: (See 'Gala' case study, below).

7.2.2 'Braeburn'

'Braeburn' is the UK's second most important commercial cultivar. In 2022, it was cultivated across 756ha, producing 33,600 tonnes of fruit valued at £24.7m to the UK economy (DEFRA, 2023). It shares many traits with 'Gala', including its high suitability for long-term storage. Within the long-term investigation, 'Braeburn' specimens were planted later than the original 15 cultivars (2017/18 compared to 2014). Consequently, only four years of yield data and two years of fruit quality data were obtained before data collection concluded. However, data from 'Braeburn' is unique due to its crop load management. Trees were appropriately fruitlet thinned throughout its development. Therefore, alternate bearing patterns were not established prior to data collection, an issue that was present in many other cultivars planted in 2013/14. Due to the cultivar's late harvesting seasonality, 'Braeburn' is notorious within the UK apple industry for not appropriately maturing before the end of the season. A summary of 'Braeburn' key production values based on two to four years of data is listed below, followed by bullet points describing key findings.

***Table 7.2**. Overview of key industry parameters based on two to four year study results for the cultivar 'Braeburn'.*

Production Variable	Ambient Mean	Plus4 Mean	Change Amb to Plus4
Mean Seasonal Temperature (°C) [1]	15.75	18.05	↑ 2.30 °C
Full-flowering date [2]	3rd MAY	20th APR	↓ 13 days
Harvest date [2]	3rd NOV	14th OCT	↓ 20 days
Harvested Fresh Weight per tree (kg) [3]	8.93 (± 0.73)	13.46 (± 1.03)	↑ 50.7 % (4.53kg)
Harvested Fruit per tree [3]	61 (± 5.25)	107 (± 9.27)	↑ 75.4 % (46 fruit)
Mean Fruit Weight (g) [3]	151.79 (± 5.81)	144.52 (± 7.73)	↓ 4.8 % (- 7.3 g)
Alternate Bearing Index (ABI) [1]	0.33 (± 0.05)	0.35 (± 0.05)	↑ 6.1 % (0.02)
Firmness (kg) [1]	8.78 (± 0.09)	8.74 (± 0.93)	↓ 0.5 % (- 0.04 kg)
Soluble Solids Content (SSC) (%Brix) [1]	12.55 (± 0.12)	13.53 (± 0.16)	↑ 7.8 % (0.98 %Brix)
Red Colour Coverage (RCC) (%) [3]	89.33 (± 1.00)	85.75 (± 1.08)	↓ 4.0 % (- 3.58 %)
Dry Matter Content (DMC) (%) [3]	15.65 (± 0.56)	18.54 (± 0.60)	↑ 18.8 % (2.89 %)

[1] 3-year mean (2019-21), [2] 2-year mean (2020-21), [3] 4-year mean (2019-22)

- **Substantial change in harvesting seasonality** (20 days earlier).
- **Substantial increase in yield** (50%) **and fruit number** (75%) **with little compromise on mean fruit weight.**
- **Greater inter-annual variation in fruit quality parameters** (especially firmness).
- **Moderate increase in SSC, substantial increase in DMC, minor reduction in RCC**.

- **Verdict**: Although the trees were only planted in 2016-18, production responses of ‘Braeburn’ to warmer temperatures appear very positive. Substantially higher volumes of fruit (>100 per season) were produced within the four years (2019-22) of assessments compared to Ambient (61 per season). This potentially indicates that the time required for ‘Braeburn’ to produce high yielding trees is reduced under warmer weather. Fruit maturation of ‘Braeburn’ was achieved each season in warmer regimes, which typically did not occur in Ambient. This is likely responsible for the substantial increase in both SSC and DMC in Plus4. However, accelerated ripening may be detrimental to ‘Braeburn’s’ suitability for long-term storage. Therefore, storability studies are required to assess the commercial viability of this cultivar under warmer conditions.

7.2.3 'Bramley's Seedling'

Widely known as the UK's definitive culinary cultivar, 'Bramley's Seedling' is quite often the only culinary option available to UK consumers. Up until 2016 (where statistics on the cultivar were no longer reported), 'Bramley's Seedling' typically represented >95% of the UK's culinary apple market (DEFRA, 2023). Its desirable traits include high acidity (hence its culinary use), high tree vigour, and natural long-term storage potential.

Table 7.3*. Overview of key industry parameters based on two to six year study results for the cultivar 'Bramley's Seedling'.*

Production Variable	Ambient Mean	Plus4 Mean	Change Amb to Plus4
Mean Seasonal Temperature (°C) [1]	16.76	18.79	↑ 2.03 °C
Full-flowering date [3]	5th MAY	23rd APR	↓ 12 days
Harvest date [3]	3rd OCT	12th SEP	↓ 21 days
Harvested Fresh Weight per tree (kg) [3]	17.53 (± 1.06)	13.37 (± 1.08)	↓ 23.75 % (- 4.16 kg)
Harvested Fruit per tree [3]	64 (± 4)	53 (± 4)	↓ 16.64 % (- 11 fruit)
Mean Fruit Weight (g) [3]	329.59 (± 13.56)	340.01 (± 20.87)	↑ 3.16 % (10.42 g)
Alternate Bearing Index (ABI) [1]	0.49 (± 0.03)	0.64 (± 0.03)	↑ 30.05 % (0.15)
Total Annual Prunings Removed (kg)	3.27 (± 0.50)	3.55 (± 0.64)	↑ 8.57 % (0.28 kg)
Firmness (kg) [1]	8.62 (± 0.07)	8.34 (± 0.11)	↓ 3.23 % (- 0.28 kg)
Soluble Solids Content (SSC) (%Brix) [1]	11.91 (± 0.12)	12.28 (± 0.13)	↑ 3.09 % (0.37 %Brix)
Dry Matter Content (DMC) (%) [3]	15.13 (± 0.41)	15.03 (± 0.30)	↓ 0.69 % (-0.10 %)

[1] 4 year mean (2018-21), [2] 6-year mean (2017-22), [3] 2-year mean (2020-21)

- **Substantial change in harvesting seasonality** (21 days earlier).
- **Substantial decrease in yield** (-23.8%) **and moderate reduction in fruit number** (-16.6%), **resulting in slightly increased fruit weight** (3.2%).
- **Substantial increase in alternate bearing habits** (30.1%).
- **Slight changes in fruit quality attributes** (reduced firmness, increased SSC).
- **Verdict**: 'Bramley's Seedling' production responses to warmer environments were on the whole negative. The main negative effect was reduced crop load and increased alternate bearing patterns. Increases in fruit weight (and thus size) may exceed maximum supermarket requirements. Alterations in firmness and SSC may result in reduced storage potential. Increased SSC may alter the cultivar's unique highly acidic taste.

7.2.4 'Discovery'

As the UK's main early harvested commercial cultivar, 'Discovery' has historically symbolised the start of the UK's apple season. Up until 2015, it represented ~3-5% of the dessert apple market share (DEFRA, 2023). However, due to its low storage potential and the abundance of imported fruit, its commercial niche was no longer viable and thus its market share has reduced over the past 20 years. The majority of cultivars within this study consisted of late-season dessert cultivars, so there is interest in whether temperature responses of the early-seasonality trait differ.

***Table 7.4**. Overview of key industry parameters based on two to six year study results for the cultivar 'Discovery'.*

Production Variable	Ambient Mean	Plus4 Mean	Change Amb to Plus4
Mean Seasonal Temperature (°C) [1]	16.52	17.58	↑ 1.06 °C
Full-flowering date [1]	2nd MAY	21st APR	↓ 11 days
Harvest date [1]	12th AUG	26th JUL	↓ 17 days
Harvested Fresh Weight per tree (kg) [3]	14.88 (± 0.66)	11.39 (± 0.65)	↓ 23.50 % (- 3.50 kg)
Harvested Fruit per tree [3]	133 (± 6)	117 (± 8)	↓ 12.01 % (- 16 fruit)
Mean Fruit Weight (g) [3]	121.70 (± 3.05)	116.03 (± 3.77)	↓ 4.66 % (- 5.67 g)
Alternate Bearing Index (ABI) [1]	0.32 (± 0.03)	0.49 (± 0.04)	↑ 52.27 (0.17)
Total Annual Prunings Removed (kg)	2.30 (± 0.26)	2.75 (± 1.24)	↑ 19.53 % (0.45 kg)
Firmness (kg) [1]	7.57 (± 0.12)	7.36 (± 0.19)	↓ -2.82 % (0.21 kg)
Soluble Solids Content (SSC) (%Brix) [1]	12.44 (± 0.11)	12.82 (± 0.15)	↑ 3.02 % (0.38 %Brix)
Red Colour Coverage (RCC) (%) [3]	78.58 (± 1.39)	69.75 (± 1.88)	↓ 11.25 % (-8.84 %)
Dry Matter Content (DMC) (%) [3]	14.96 (± 0.09)	15.21 (± 0.82)	↑ 1.66 % (0.25 %)

[1] 4 year mean (2018-21), [2] 6-year mean (2017-22), [3] 2-year mean (2020-21)

- **Moderate change in harvesting seasonality** (17 days earlier).
- **Substantial decrease in yield** (-23.5%), **moderate decrease in crop load** (-12.0%).
- **Substantial increase in alternate bearing habits** (52.3%).
- **Substantial increase in annual vegetative growth removed** (19.5%).
- **Minor alterations in fruit quality** (firmness, SSC and DMC), **moderate reduction in RCC** (-11.3%).
- **Verdict**: The advancement of 'Discovery's' seasonality from August to late-July may provide a unique opportunity of getting fresh UK apples into the market two or so weeks earlier. Fruit quality parameters also appear relatively unaffected by warmer seasonal weather. However, the substantial impact of warmer weather on alternate bearing habits may indicate reduced ability of achieving a regular annual crop.

7.2.5 'Golden Delicious'

This golden-green coloured cultivar, discovered in the late 19th century in the USA, has been a popular supermarket cultivar over the past few decades. Its qualities include high sweetness, good storage potential, and high versatility (i.e. both dessert and culinary use). Whilst the cultivar is widely grown around the world in warmer climates, its lower tolerance to the UK's comparatively cooler temperate climate has contributed to lower commercial uptake within the UK. Therefore, there may be potential for 'Golden Delicious' to become a more viable commercial cultivar under projected warmer seasonal conditions.

Table 7.5. *Overview of key industry parameters based on two to six year study results for the cultivar 'Golden Delicious'.*

Production Variable	Ambient Mean	Plus4 Mean	Change Amb to Plus4
Mean Seasonal Temperature (°C) [1]	16.46	18.40	↑ 1.94 °C
Full-flowering date [1]	2nd MAY	21st APR	↓ 11 days
Harvest date [1]	26th SEP	17th SEP	↓ 9 days
Harvested Fresh Weight per tree (kg) [3]	20.33 (± 1.44)	16.38 (± 1.62)	↓ 19.43 % (- 3.95 kg)
Harvested Fruit per tree [3]	141 (± 11	144 (± 17)	↑ 2.19 % (3 fruit)
Mean Fruit Weight (g) [3]	166.78 (± 3.99)	154.15 (± 5.12)	↓ 7.57 % (- 12.63 g)
Alternate Bearing Index (ABI) [1]	0.62 (± 0.04)	0.85 (± 0.02)	↑ 37.38 % (0.23)
Total Annual Prunings Removed (kg)	1.65 (± 0.48)	2.41 (± 1.01)	↑ 46.14 % (0.76 kg)
Firmness (kg) [1]	7.32 (± 0.05)	7.54 (± 0.09)	↑ 3.03 % (0.22 kg)
Soluble Solids Content (SSC) (%Brix) [1]	13.18 (± 0.13)	14.29 (± 0.24)	↑ 8.39 % (1.11 %Brix)
Dry Matter Content (DMC) (%) [3]	15.39 (± 0.47)	15.68 (± 1.30)	↑ 1.91 % (0.29 %)

[1] 4 year mean (2018-21), [2] 6-year mean (2017-22), [3] 2-year mean (2020-21)

- **Slight change in harvesting seasonality** (9 days earlier).
- **Moderate-substantial reduction in yield** (-19.4%), **minor reduction in fruit weight** (-7.6%).
- **Substantial increase in alternate bearing habits** (37.4%).
- **Substantial increase in vegetative growth removed** (46.1%).
- **Minor increases in firmness and DMC, moderate increase in SSC** (8.4%).
- **Verdict**: As with other cultivars, temperature impacts on 'Golden Delicious' alternate bearing habits may potentially increase risk to achieving a high yielding crop each production year. However, impacts on fruit quality were largely positive. As 'Golden Delicious' is popular for its sweet taste, an increase in SSC may not compromise on this. Increases to firmness may also be beneficial, but this may have been caused by reduced mean fruit weight.

7.2.6 'Fuji'

Developed in Japan in the mid-20th Century, 'Fuji' has become one of the most widely cultivated apple cultivars in the world, produced highly intensively across Asia, North America, and South America. Its qualities include attractive appearance, pleasant flavour, and a low chill unit requirement. Due to these qualities, it is thought that 'Fuji' may be a suitable substitute for currently grown cultivars in future UK apple orchards.

Table 7.6*. Overview of key industry parameters based on two to six year study results for the cultivar 'Fuji'.*

Production Variable	Ambient Mean	Plus4 Mean	Change Amb to Plus4
Mean Seasonal Temperature (°C) [1]	16.08	18.13	↑ 2.05 °C
Full-flowering date [1]	30th APR	21st APR	↓ 9 days
Harvest date [1]	11th OCT	3rd OCT	↓ 8 days
Harvested Fresh Weight per tree (kg) [3]	12.02 (± 1.16)	12.50 (± 1.40)	↑ 4.02 % (0.48 kg)
Harvested Fruit per tree [3]	107 (± 10)	108 (± 13)	↑ 1.43 % (- 2 fruit)
Mean Fruit Weight (g) [3]	137.42 (± 5.26)	134.59 (± 5.33)	↓ 2.06 % (- 2.83 g)
Alternate Bearing Index (ABI) [1]	0.94 (± 0.01)	0.94 (± 0.02)	= = =
Total Annual Prunings Removed (kg)	1.15 (± 0.37)	1.44 (± 0.46)	↑ 25.22 % (0.29 kg)
Firmness (kg) [1]	8.11 (± 0.08)	7.81 (± 0.14)	↓ 3.70 % (- 0.30 kg)
Soluble Solids Content (SSC) (%Brix) [1]	13.77 (± 0.27)	13.57 (± 0.26)	↓ 1.40 % (- 0.19 %Brix)
Red Colour Coverage (RCC) (%) [3]	73.92 (± 1.58)	50.47 (± 2.89)	↓ 31.72 % (- 23.45 %)
Dry Matter Content (DMC) (%) [3]	16.35 (± 1.37)	16.26 (± 1.30)	↓ 0.55 % (-0.09 %)

[1] 4 year mean (2018-21), [2] 6-year mean (2017-22), [3] 2-year mean (2020-21)

- **Slight change in harvesting seasonality** (8 days earlier).
- **Minor changes in yield, fruit number, and mean fruit**.
- **No change in alternate bearing habits – ABI was severe across both treatments** (0.94).
- **Moderate increase in tree vegetative growth removed** (25.2%).
- **Minor reduction in firmness** (3.7%), **substantial reduction in RCC** (31.7%).
- **Verdict**: Alternate bearing habits were severe across all treatments with almost no yield in lean years even in ambient, and changes in seasonal temperature had little effect on exacerbating these habits like other cultivars in this study. The reduction in RCC indicates lower marketability of fruit produced in warmer environments compared to Ambient, especially for a cultivar famed for its 'pink' colour hues.

7.2.7 'Cox's Orange Pippin'

This 19th century cultivar is still regarded as the quintessential English apple. Before the adoption of current UK market leaders' 'Gala' and 'Braeburn', 'Cox's Orange Pippin' occupied ~40-60% of the domestic dessert apple market share throughout the late 20th and early 21st Century up until the 2010's (DEFRA, 2023). Its main qualities include its complex, aromatic taste and attractive appearance. Due to its characteristics and heritage status, it remains a key resource for apple breeding programmes. It is thought that 'Cox's Orange Pippin' is best suited for a relatively cool maritime climate. Therefore, the risks of a warming UK climate may undermine the production of this historic cultivar.

Table 7.7. *Overview of key industry parameters based on two to six year study results for the cultivar 'Cox's Orange Pippin'.*

Production Variable	Ambient Mean	Plus4 Mean	Change Amb to Plus4
Mean Seasonal Temperature (°C) [1]	16.70	18.48	↑ 1.78 °C
Full-flowering date [1]	2nd MAY	21st APR	↓ 11 days
Harvest date [1]	23rd SEP	10th SEP	↓ 13 days
Harvested Fresh Weight per tree (kg) [3]	11.37 (± 0.75)	9.73 (± 0.89)	↓ 14.40 % (- 1.64 kg)
Harvested Fruit per tree [3]	82 (± 6)	80 (± 8)	↓ 2.11 % (- 2 fruit)
Mean Fruit Weight (g) [3]	145.94 (± 3.13)	137.43 (± 4.66)	↓ 5.83 % (- 8.51 g)
Alternate Bearing Index (ABI) [1]	0.58 (± 0.04)	0.74 (± 0.04)	↑ 28.14 % (0.16)
Total Annual Prunings Removed (kg)	2.10 (± 0.87)	2.54 (± 0.63)	↑ 20.64 % (0.43 kg)
Firmness (kg) [1]	8.36 (± 0.09)	7.72 (± 0.16)	↓ 7.72 % (- 0.65 kg)
Soluble Solids Content (SSC) (%Brix) [1]	13.68 (± 0.13)	13.87 (± 0.18)	↑ 1.44 % (0.20 %Brix)
Red Colour Coverage (RCC) (%) [3]	58.67 (± 2.53)	48.67 (± 2.63)	↓ 17.05 % (- 10.0 %)
Dry Matter Content (DMC) (%) [3]	17.27 (± 0.82)	17.12 (± 0.56)	↓ 0.82 % (- 0.14 %)

[1] 4 year mean (2018-21), [2] 6-year mean (2017-22), [3] 2-year mean (2020-21)

- **Moderate change in harvesting seasonality** (13 days).
- **Moderate reduction in yield** (-14.4%), **minor reduction in fruit weight** (-5.8%).
- **Substantial increase in alternate bearing habits** (28.1%).
- **Potential substantial increase in tree vegetative growth** (20.6%, though with large degree of error).
- **Moderate reduction in firmness** (-7.8%) **and RCC** (-17.1%).
- **Verdict**: As a cultivar that is renowned for its fruit quality characteristics, the effects of warmer weather on both firmness and RCC may change consumer behaviour towards 'Cox's Orange Pippin'. However, taste testing may provide the best indicator of whether the cultivar retains its 'aromatic' taste. Greater alternate bearing may make annual yields less reliable under warmer seasonal environments.

7.3 Reviewing findings in the context of future climate change

As detailed in Lane (2022), emulating a commercial field environment for a perennial crop whilst modifying certain meteorological parameters was a challenging undertaking. The methodology of utilising passive solar radiation could only reasonably focus on uplifting temperature during spring and summer months, with more minor temperature uplifts achieved during the daytime of autumn and winter. Whilst increased growing season temperatures form one part of future climate change predictions, winters will also be warmer on average. That being said, the UKCP18 winter temperature projection range for 2070 (0.6 to 3.8°C) will not be as severe as the projected summer range (1.3 to 5.1°C) (MetOffice, 2022). Therefore, understanding the effects of growing season temperature uplifts are highly relevant in the context of future climate change impacts on fruit crops.

Associations between temperature and average yield parameters were more often negative than positive. Many cultivars showed a 10-15% mean annual reduction in harvested yield per tree. This would equate to a reduction of 206,000 – 308,000 tonnes in annual dessert apple production based on national statistics from 2022 (DEFRA, 2023). This loss in production would occur despite the experimental facility providing sufficient winter chill for most cultivars (Lane, 2022), which is typically regarded as the main threat to worldwide apple yields under future climate change predictions (Luedeling, 2012; Rai et al., 2015; Salama et al., 2015). As described in earlier sections, these yield reductions were generally caused by enhanced alternate bearing patterns within the warmer environments combined in some cultivars with a negative effect of temperature on yield within year. Increased risk of inter-annual production variability will cause greater uncertainty for growers to achieve a sufficient annual crop every year. Evidence from the later planted 'Braeburn' shows that with crop load patterns sufficiently managed throughout early tree growth and development, warmer weather may increase yields (+50.7%). However, inter-annual variation in yield parameters (as shown by mean standard error values in Tables 7.1 to 7.7) was still raised under warmer environments. The cultivar 'Fuji' also showed increased inter-annual yield parameter variation in warmer regimes despite similar alternate bearing patterns among treatments. Greater variation in quantity and size of apple will affect the proportion of fruit achieving Class I marketability (AHDB, 2021). Therefore, monitoring and management of crop load each year will be even more important to mitigate potential future climate change effects.

Warmer temperature environments were associated with altered fruit quality indicators, varying in severity by cultivar. Like yield parameters, increased inter-annual variation in fruit quality with warming was common, with the evidence from this study suggesting this was primarily due to variation in alternate bearing patterns between treatments (Chapter 5). However, cultivars showing low ABI treatment differences (e.g. 'Braeburn' and 'Fuji') generally showed greater variation in fruit quality metrics. Therefore, compounding varied yield effects, greater fruit quality variation will likely further negatively affect the proportion of an annual crop attaining high marketable standards. The most substantial effect of the warmer environments on fruit quality for the coloured or bicoloured cultivars was change in RCC. Optimal fruit size and colour are two extrinsic qualities highly valued by consumers (Harker et al., 2003; Carrillo-Rodriguez et al., 2013) and therefore retailers (Djekic et al., 2019). Additionally, a 'bad' experience will cause a consumer to stop buying a cultivar or brand for a period of time (Harker et al., 2003). Uniformity in fruit quality is therefore key for both consumers and retailers. Some aspects of this study have shown how warmer treatment effects can be mitigated. Preventing shading of fruits will enhance RCC in apple production systems (Jackson and Sharples, 1971; Musacchi and Serra, 2018). The effect of this can be seen in the 'Gala' storability study (Chapter 6). In that case, vegetative tree pruning was applied in summer 2021, but not in summer 2020, and treatment differences in RCC were present in 2020 but not in 2021.

Findings from Lane (2022) and pruning data from this current study have demonstrated that apple tree vegetative growth will likely increase under warmer temperatures. One explanation of this is that earlier fruit harvests provide a greater period of post-harvest shoot growth in autumn. Whilst shoot growth is always required, excessive vigorous growth is linked with reduced marketable yields, fruit quality, and subsequent years' cropping (Elfving, 1988). Apple shoot growth sensitivity is highest during early fruit development. Calderón-Zavala et al. (2022) showed that the highest shoot growth occurred within their hottest temperature treatment (33°C). Vegetative growth, crop load, and alternate bearing are known to affect one another due to consumption of plant resources (Monselise and Goldschmidt, 1982; Smith and Samach, 2013). The process of floral bud initiation occurs after extension growth of shoots, thus greater extension growth reduces plant resources for subsequent bud initiation (Monselise and Goldschmidt, 1982). Therefore, it is highly likely that (to a certain extent) greater vegetative growth contributed towards greater alternate bearing patterns in warmer environments. This is in addition to the direct effects on fruit quality as described in the previous paragraph. Future projected hotter spring and summers will consequently mean greater tree vegetative growth will be a common annual issue for growers to manage.

One aspect of the longer-term trial was to evaluate the response of genetic traits to warmer temperature environments. It became clear early on that the range of responses was highly specific to each cultivar. Some cultivar temperature responses, such as the alternate bearing patterns in ‘Gala’ and ‘Golden Delicious’, may have been linked by apple cultivar parentage (Monselise and Goldschmidt, 1982). However, differences in harvesting seasonality showed unique responses between early- and late-harvested cultivars. In general, early-harvested cultivars were more responsive to changes in seasonal temperature than late ones. The three early-harvested cultivars ‘Discovery’, ‘George Cave’ and ‘Stark’s Earliest’ displayed similar yield (Chapters 3 and 4) and fruit quality (Chapter 5) responses to seasonal temperature. The range of late-harvested cultivars displayed more varied responses. Differences in growth and development physiology between different harvesting seasonalities are likely responsible for this. Temperature is highly influential on early fruit growth through its impact on cell division and expansion (Atkinson et al., 1998; Warrington et al., 1999). Recent research has shown that differences in metabolic activity between early- and late-harvested cultivars contributed towards greater fruit size during early fruit development (Yue et al., 2023). Greater consumption of carbohydrates for fruit growth then induces greater alternate bearing patterns (Monselise and Goldschmidt, 1982). This perhaps explains why seasonal temperature did not alter yield of early-harvested cultivars within the current year (Chapter 3), but affected the subsequent season’s yield instead (Chapter 4). Increased May temperatures were associated with reduced yield in the subsequent season for all early-harvested cultivars, but only one late-harvested cultivar (‘Yellow Bellflower’). Additionally, differences in fruit growth patterns may be responsible for seasonal temperature and firmness relationship contrasts between early- and late-season cultivars (Chapter 5). Overall, increases in seasonal temperature, particularly in spring, may increase the variability of both yield and fruit quality in early-harvested cultivars more than later season cultivars.

Given the methodological difficulties in examining the effect of low chill accumulation within the facility (winter temperatures differed little among treatments), findings on this topic were not investigated within this study. However, the literature overwhelmingly references lack of winter chill unit accumulation as a detriment to fruit production (Chapter 3). The amount of chill units required varies by model, but broadly speaking traditionally bred temperate environment cultivars require 800-1200 chilling hours where temperatures are below 6°C (Haugge and Cummins, 1991). Southern areas of the UK will have warmer winters compared to northern regions (Murphy et al., 2009). With dessert apple production concentrated within southern England, there is considerable risk that cultivars with higher chill requirements (e.g. traditional ones such as ‘Cox’s Orange Pippin’) will not receive sufficient chill during warmer winters. Production of these at-risk cultivars may have to relocate to more northern latitudes or areas of higher altitude, an action which has already occurred in apple production hubs such as India (Singh et al., 2016) and China (Li et al., 2020). Adoption of lower chill requirement cultivars, or greater application of dormancy breaking inputs, may be required to overcome this in current UK dessert apple production areas.

The advancement of reproductive development through advanced accumulation of thermal time was well documented in Lane (2022). A further three years of phenology readings have refined when phenological events are expected for various cultivars and traits. The effect of raised temperature on flowering date was shown to vary greatly by genetic trait, which has also been documented in previous studies (Legave et al., 2013; Sapkota et al., 2021). Full-flowering date for the earliest flowering cultivar in Ambient ('Stark's Earliest') occurred seven days earlier in Plus4. Conversely, in the latest Ambient flowering cultivar ('Edward VII') flowering occurred 16 days earlier in Plus 4. Additionally, based on the findings amongst 20 cultivars, the range of dates in which full flowering occurred was much narrower under warmer conditions. For example, 14 out of 20 cultivars on average reached full flowering within a four-day period (18-21 April) under Plus4 conditions. Consequently, several factors will risk development of high-quality flower buds. Floral bud damage from late-spring frosts may cause substantial crop losses under warmer climate in many European countries (Unterberger et al., 2018; Dalhaus et al., 2020). However, UKCP18 projections predict fewer days of temperatures reaching below 0°C (Hanlon et al., 2021). Pfleiderer et al. (2019) suggested that earlier cultivars will be more susceptible to late frosts than later flowering ones. However, mean data from the current study indicates that later flowering cultivars (e.g. 'Braeburn' and 'Jolyne') will flower only a few days later than early flowering cultivars under Plus4 conditions. Therefore, even though late frosts may become more infrequent, the risk of damage may have enhanced severity as it will affect a much greater proportion of currently cultivated cultivars. Furthermore, a narrower and earlier range of apple flowering dates may potentially increase the desynchrony of apple cultivars and natural pollinators (Wyver et al., 2023).

The rainfall treatments utilised in this study were unlikely to have been sufficient to emulate changes in projected future rainfall. However, associations between annual rainfall variation and apple production metrics were demonstrated throughout the analyses. Yield was affected by rainfall over several fruit development phases (Chapter 3). Total seasonal rainfall was found to be highly influential on four out of five tested fruit quality parameters (Chapter 5). UKCP18 predictions expect greater precipitation extremes during seasonal crop production – greater periods of dry weather, and more intense rainfall events when it does rain (MetOffice, 2022). Additionally, rainfall intensity will increase in autumn, which coincides with late fruit development. Given the rainfall sensitivities described in this study, resilience to both droughty and waterlogged conditions will likely be required for optimising yield and fruit quality.

7.4 Implications for industry – 'Gala' case study

The cultivar 'Gala' is currently the most important commercially grown apple cultivar in the UK. In 2022, it was cultivated across 2,771ha (48.7% of all cultivated dessert apple area), producing 113,600 tonnes of apples (55.3% of all dessert apple production) valued at £111.3m (60.8% of all dessert apple value) (DEFRA, 2023). Several key traits of 'Gala' contribute towards its popularity. These traits include regular fruit bearing, successful growth in both temperate and warm environments, and pleasant, sweet taste. Perhaps the most important commercial trait is its tolerance of long storage periods, enabling an almost year-round supply (AHDB, 2021). The marketability of certain 'Gala' strains are known to be influenced by climate. For example, Iglesias et al. (2008) described how current strains were failing to meet marketable red colour accumulation caused by warmer conditions in Spain. It would therefore be reasonable to suggest that if 'Gala' production was undermined by future climate change, it would have serious ramifications for UK apple production given the time and expense it would require to establish commercial crop systems with alternative cultivars. Whilst other major apple production areas the UK imports from (e.g. France, South Africa etc.) may face similar climatic challenges, UK growers need to be proactive to ensure their product remains competitive within primarily domestic, but also global markets. The results and analysis obtained throughout the 'Apples in a Warmer World'® study may provide insights in to 'Gala' responses to increased seasonal temperature, and what growers can expect with the challenge of future climate change. The data in Table 7.1 provides an overview of mean values for key apple fruit parameters for this cultivar based on two-to-six-year data (dependent on the parameter).

The findings for 'Gala' demonstrate greater variation across 12 out the 15 quantitative parameters in the Plus4 environment compared to Ambient based on mean standard error values. As described in Figure 7.1, increased Plus4 production variability was likely driven by both direct and indirect effects of long-term temperature modification, particularly on alternate bearing patterns. Alternate bearing index (ABI), the main metric for measuring year-to-year yield variation, increased from 0.21 in Ambient to 0.40 in Plus4. This represents a 90.5% increase in mean inter-year yield variation in response to increased temperature. The discussion of alternate bearing results in Chapter 4 attributed this difference to a range of different environmental and crop management factors. These include temperature associations, crop load management, timing of thinning practices, and pest prevalence.

The changes in alternate bearing translate into an average annual yield loss (harvested fresh weight per tree) of 12.3% across a six-year period from 2017 to 2022 (17.66 kg per tree, down from 20.14kg). Applying this to mean UK production statistics of 'Gala' fruit for the same period (DEFRA, 2023) would equate to a mean production loss of 11,194t, valued at ~£17.33m to the UK economy each year.

Further disruption to crop value may be exacerbated by more variation among years in fruit quality. All five quality parameters of fruit weight, firmness, SSC, RCC, and DMC showed greater mean standard error in Plus4 compared to Ambient. As described in Chapter 5, fruit quality variation was likely driven by a mix of changes to temperature and of yield variation. Fruit uniformity is a key attribute for commercial market presentation (EU No 543/2011), meaning greater variation in fruit quality may lead to less fruit reaching adequate marketability standards for retailers. For example, less uniform fruit size may reduce the proportion of fruit achieving Class I standards (55-80mm diameter). Red colour coverage (RCC) was the most severely impacted fruit quality parameter – with Plus4 RCC reducing to 64.4% (down from 83.6%) at the tree-ripe stage of development. This translates to almost 20% less RCC compared to Ambient fruit. For fruit harvested for long-term controlled atmosphere (CA) storage, RCC reductions were most severe when trees were not sufficiently pruned in warmer conditions (only 26.6% RCC in 2020). As described in Chapter 6, more satisfactory summer pruning was likely influential in mitigating RCC differences between Ambient and Plus4 in 2021. However, with some 'Gala' strains requiring at least 25-50% coverage, lower RCC under warmer conditions may further reduce the proportion of fruit achieving minimum standards.

Other results indicate lesser impacts on 'Gala' fruit quality parameters. Firmness of 'Gala' may reduce under warmer environments, decreasing by 2.5% at tree-ripe stage harvest, and 6.2% at optimal storage harvest date. Loss of firmness is linked with accelerated ripening and reduced storability (Ornelas-Paz et al., 2018; Lee et al., 2023). As such, it is also associated with other fruit quality parameters, such as SSC and DMC (see Chapters 5 and 6). After six months of storage, firmness results still differed between the Ambient and Plus4 treatments. Reassuringly, firmness reductions may not be enough to greatly influence marketability. For example, WFL Qualytech minimum standards for firmness are 6.2kg for 'Gala'. With a Plus4 mean of 8.5kg, firmness was comfortably above this minimum figure after six to seven months of CA storage. However, the limitations of the study provide no indicator of fruit marketability beyond March (seven months post-harvest).

Raised Plus4 SSC at harvest may have beneficial commercial implications, particularly for fruit utilised for CA storage. Anecdotally, 2021 was a challenging seasonal growing climate for production of ‘Gala’ for UK growers. Levels of SSC were typically below average, as demonstrated by Ambient values at CAS harvest (10.6%Brix). Delayed onset of suitable SSC set back harvest dates of ‘Gala’ for the 2021 season. Plus4 fruit showed raised SSC in comparison around the optimal harvest date (11.0%Brix). With some standards (e.g. WFL-Qualytech) indicating minimum SSC of 11.5%Brix, this likely means that Plus4 fruit would be eligible for commercial markets at an earlier date than Ambient. This is especially true considering Plus4 fruit were harvested 19 days earlier in the season. Furthermore, SSC levels were very similar between Ambient and Plus4 by March, indicating overall ‘ripeness’ between treatments may be comparable. With additional results showing that the occurrence of storage disorders did not differ among treatments (Chapter 6), the results do not indicate that increased seasonal temperature greatly impacts overall ‘Gala’ storability. Further experimental work (e.g. further fruit quality parameter analysis, taste-testing panels) would help elaborate on these findings.

The influence of temperature greatly advanced seasonal phenological events of ‘Gala’, with full flowering accelerated by eight days (and note the limited winter warming in this study, Table 7.1), and ‘tree-ripe’ stage harvest by 20 days. Earlier apple flowering is linked with increasing susceptibility to frost damage under certain climate change scenarios, especially for early-flowering cultivars (Pfleiderer et al., 2019; Szalay et al., 2019). Several dessert apple production areas of Southern England (on average) have their last frost date in late April (Plantmaps, 2024). Therefore, under current climate conditions, earlier ‘Gala’ flowering may indeed be more susceptible to frost damage. However, ‘Gala’ is not currently considered an early-flowering cultivar. There are also mixed predictions that frost impacts on production will be reduced or remain the same when compared to the present day in response to future climate change (Eccle et al., 2009).

Results showed little evidence that advanced harvest date (27 days in the case of ‘Gala’) directly influenced overall yield (Chapter 4) and fruit quality (Chapter 6). However, a seasonal shift will require adaptation of grower operations in order to complete harvest activities earlier than usual. Cooling of ‘Gala’ fruit from the field to the store will also be more important. Greater harvest exposure to warm weather during late August opposed to September may affect the storability of harvested fruit (AHDB, 2021). Thus, an inability to react with suitable counter-measures might increase the volumes of waste fruit.

Warmer winters under future climate change are anticipated to reduce winter chill accumulation in the UK for a wide range of apple cultivars, leading to delayed bud burst, reduced flower quality, and reduced fruit set (Luedeling et al., 2013; Atkinson et al., 2013; González-Noguer, 2022). The modified field environment facility used within this study did not have the ability to maintain temperature uplifts throughout winter, consequently there is only a small uplift in winter temperature from Ambient to Plus4 (+0.30°C) compared to seasonal (April to October) temperature (+1.83°C). Consequently, the effects of potential low winter chill accumulation on 'Gala' were not investigated within this study. Generally, 'Gala' and its sports have relatively low chill requirements of 500-600 hours below 6 °C (dependent on model) (Hawerroth et al., 2013), and as such is widely cultivated in both temperate and moderate climates. It is therefore probable that 'Gala' production will remain largely unaffected by insufficient winter chill accumulation. Commercial defoliants (such as hydrogen cyanamide) are available to effectively induce dormancy early in the event of low chill winters (Abeba et al., 2012).

Differences in precipitation between modified environments generally did not have a significant impact on 'Gala' fruit production. In years where seasonal rainfall was low (such as 2018), associations with production parameters were found across all treatments. There was little evidence that increased temperature exacerbated drought effects within this study. With drier summers predicted under IPCC future climate change scenarios, the application of irrigation should still be considered irrespective of temperature.

An increase in temperature was associated with two aspects of 'Gala' vegetative growth; increased tree trunk growth (Lane, 2022) and increased vegetative growth removal. It is noted that the earlier harvests in Plus4 would have provided a longer period in autumn when assimilates were no longer being taken up by fruit. Increased vegetative growth will likely be an undesirable trait due to its competition with reproductive growth (Atkinson et al., 1998), associations with increasing alternate bearing (Monselise and Goldschmidt, 1982), and increased shading leading to reduced RCC (see Chapter 6). This may indicate that more thorough and frequent tree pruning and/or increased use of plant growth regulators will be required throughout the season to remove or prevent excess vegetative growth. This will likely increase seasonal tree management costs for growers, as well as further expose trees to potential pathogen infection in the case of greater tree pruning activity.

Other environmental variables not quantified within the study may also have a negative impact on production. Increased pest prevalence of two aphid species (*Eriosoma* *lanigerum* and *Dysaphis plantaginea*) in the Plus4 tunnel inflicted more severe ‘Gala’ tree and fruit damage compared to the Ambient environment. However, the methodology used within this study was an artificial environment. Aspects such as semi-closed polythene structures, trees planted in rows of three, and increased humidity all provide more suitable conditions for pest activity in comparison to standard commercial production. The effects of climate change on apple pests and pathogens are difficult to predict due to variation in expected weather patterns (Shuttleworth, 2021). Therefore, it is difficult to speculate on the impact of temperature effects on pest pressure within this study.

Increased incidence of extreme temperatures (>40°C) in the Plus4 environment had little effect on overall yield and fruit quality within this study. This was likely due to several methodological factors. These include slight polythene UV protection and increased tree shading from greater vegetative growth. The risk of increased frequency of extreme temperatures is however a sizeable concern due to its negative effects on crop physiology (Rosenzweig et al., 2014; Dreesen et al., 2012) and apple fruit quality (Dalhaus et al., 2020).

To conclude, this study has demonstrated that modified field temperature regime had an impact on a range of production parameters for the apple cultivar ‘Gala’. The results show that increased year-to-year variation may provide the greatest disruption to UK production, influencing yield, fruit quality, and perhaps storability. Many of these potential challenges described here can likely be managed effectively through increased monitoring and proactive tree management strategies, but this will be added expense to the grower. If managed effectively, there is potential that future raised seasonal temperatures may increase the quantity of abundant, high-quality UK ‘Gala’ fruit. Future work (e.g. field trials, controlled environment experiments etc.) is therefore required to determine whether management strategies can effectively produce high quality ‘Gala’ fruit in the face of future climate change without compromising profitability.

7.5 Key considerations for growers

The findings from this six-year study have indicated some key responses of apple cultivars to prolonged exposure to warmer seasonal environments. Many of these responses may have negative implications for commercial UK production. The following section will detail these responses, as well suggesting possible prevention measures and mitigation measures (i.e. limiting the issue once already present). The list is in chronological order of annual tree growth and development phases.

- **Low winter chill unit accumulation**

The main method for overcoming this will be appropriate cultivar selection, possibly through newly bred options with low winter chill requirements. Additionally, application of commercial dormancy breakers (such as defoliants) may be relied upon more often during more frequent mild winters.

- **Earlier flowering**

Greater accumulation of thermal time under warmer weather will advance flowering by one to two weeks across many cultivars (Appendix 2.2). Earlier flowering may equate to increased exposure to late-spring frosts in mid to late April. Therefore, frost protection solutions may be relied upon more often. Plant growth regulators may be a consideration to delay the timing of flowering. Additionally, early flowering may reduce synchrony with native pollinators during this time. Therefore, introduction of non-native pollinators (such as commercial *Bombus terrestris* colonies) may be required for sufficient pollination of apple crops.

- **Earlier seasonal pest infestations**

Anecdotal evidence from this trial demonstrated earlier annual appearance of seasonal fruit pests including woolly apple aphid (*Eriosoma lanigerum*) and rosy apple aphid (*Dysaphis plantaginea*). More thorough crop monitoring should focus on emergence of WAA nymphs on new shoot growth, and RAA adults on underside of leaves on new shoots. Congregating of ants on trees often provided hints of where RAA populations were abundant due to their symbiotic behaviour. Integrated pest management strategies should be robust in both preventing and reacting to aphid population booms. More sustainable control methods include establishment of natural predators (e.g. earwig species).

- **Greater tree vegetative growth**

Evidence from this study has demonstrated greater seasonal vegetative growth in response to warmer temperature. This increased growth was a common factor across the diverse selection of cultivars. Whilst this may be beneficial in some instances (e.g. see ‘Braeburn’ results), for established trees excess vegetative growth may divert plant nutrient use for optimal fruit growth and development and increase tree canopy shading. Additionally, desired tree architecture may be harder to maintain. Increased pruning activity of removing excess vegetative growth in both winter and summer should be considered. Care should be taken not to remove too much growth in one session in case of excess removal of the subsequent season's fruiting buds, heavily reduced tree vigour, and increased watershoot activity.

- **Fruit thinning timing**

The proliferation of alternate bearing activity within the warmer environments may have demonstrated that hand removal of excess fruitlets post fruit set (late-May to early June) may not have been sufficient in mitigating the issue. Earlier fruit thinning is therefore recommended, possibly at the blossom stage for more optimal results.

- **Earlier harvesting activity**

Earlier harvesting of fruit will shift the seasonality of certain cultivars by one to three weeks. Harvesting and post-storage operations will need to be planned earlier on within the season. Conversely, adoption of new cultivars that mature further on in the season (thus exploiting a greater season length) is a viable alternative option. However, there is a risk that these cultivars may not fully mature during cooler years.

- **Altered fruit quality**

The results show that increased seasonal temperature was generally associated with directly increased soluble solids content (SSC) and dry matter content (DMC), reduced red colour coverage (RCC), and mixed effects on firmness. Reduction in RCC was often the most drastic fruit quality parameter change across cultivars. Such changes may affect the proportion of harvested fruit meeting minimum required standards set by buyers. This is potentially further compounded by greater variation in fruit quality parameters under warmer environments. Fruit quality is highly influenced by seasonal conditions. Therefore, if for example the accumulation of RCC is already a current issue within a given location, adoption of climate resilient cultivars that can achieve sufficient RCC regardless of weather may be the best option in a warming environment.

- **Altered storability of 'Gala' fruit**

Storability studies of 'Gala' fruit revealed that fruit produced in warmer seasonal environments and stored in controlled atmosphere (CA) were still (generally) marketable in March of the following year. This is despite being harvested two to three weeks earlier in the season. However, this still consequently meant that fruit ripened earlier in the year compared to those produced in Ambient conditions. Therefore, viability of CA operations may need to become less reliant on 'Gala' to extend seasonality of produce later into the following year.

- **Drier summers, wetter autumns**

Appropriate cultivar and rootstock choice should consider resilience to both drought and waterlogged conditions. Sites of crop production should think more carefully over drainage properties and irrigation will likely be required for especially dry periods to optimise yield and fruit quality. Production on heavier particle soils (e.g. clay) should provide extra consideration for drainage during more intense rainfall events.

7.6 Future Research

The ‘Apples in a Warmer World’® study was originally conceived as a long-term study analysing the effects of varied temperature and rainfall regimes on apple production over a timespan of 10+ years. Unfortunately, severe storm damage to the experimental facility in early 2022 brought to a halt further experimental work regarding modified seasonal environments. The study captured six years of experimental data, which included a baseline year (2017), four subsequent seasons of modified temperature environment (2018-21), and one ‘legacy’ year (2022) where potential long-lasting effects of the modified environments were analysed. Considering the original project scope, continuation of that study would have added a further four or five years’ data with which to analyse the effect of seasonal temperature variation on apple production yet more thoroughly. In addition, new shorter-term studies were under consideration, particularly more severe pruning of trees (for reasons outlined above) and an in depth consideration of pest management strategies. Therefore, the continuation and development of the research reported here would be viable should similar modified environment experiments arise.

The ’Future Research’ section of Lane (2022) describes the rationale in which the modified environment facility could be improved upon to deliver important new studies related to future climate change impacts on apple production. These include the modification of rainfall regimes, the use of thermic polythene or artificial heat introduction to simulate low winter chill conditions, CO_2 uplift systems, and incorporation of cider apple cultivars. Whilst not acted upon within the subsequent three years of the longer-term study, incorporation of these aspects to the facility would have provided a better simulation of future climate change scenarios and thus should be recommended for future similar studies.

A field experiment of this scale and design provided limitations on independent replication of temperature treatments. For example, all Ambient trees were located at a more easterly location than the warmer treatments. Therefore, the credibility of findings from this study would be enhanced through independent replication of experimental treatments (Rogers et al., 2021). The most logical way of performing this would be through the use of controlled environment experiments to reduce uncontrolled extraneous variables affecting performance of dependent variable (Aziz, 2017). Use of controlled environment experiments have historically been used to assess temperature treatment effects on a wide range of apple production parameters. Based on the findings from this field study, seasonal temperature associations with enhanced alternate bearing patterns may have undermined variation in many other production variables. Therefore, controlled environment setups that investigate seasonal temperature effects on yield indicators should take precedence. Abbott et al. (1973) demonstrated how various temporal temperature treatments affected apple floral bud production using controlled environment chambers. An experimental setup akin to this should be sufficient for validating alternate bearing differences from the current study. For example, various November to January Tminmaxdiff treatments could be performed on potted apple trees to assess for differences in floral bud production.

In addition to floral bud production, weather is known to influence other plant physiological mechanisms that subsequently affect apple fruit yield. The influence of temperature on fruit set and retention has been observed during pollination (Tromp and Borsboom, 1994) and ‘June drop’ (Grausland and Hansen, 1975). Data on fruit set from each modified temperature environment was analysed in Stephens (2022) and confirmed no significant differences in fruit set (pre- ‘June drop’) between treatments in 2021 (Appendix 3.5). However, the establishment of alternate bearing patterns may influence fruit set during ‘off’ years (Monselise and Goldschmidt, 1982). Therefore, it is recommended to repeat data collection in alternate years to effectively define temperature effects on fruit set.

The fruit quality methodology utilised in this study was focused delivering on quick, reliable, and low-cost results across all cultivars (n=20), treatments (n=9) and replicates (n=10) (~1800 fruits total) across each production season, due to time and cost limitations. Data on further commonly analysed parameters including acids, flavonoids, ethylene, and other nutritional content variables would help refine the impacts of modified temperature environment on overall apple fruit quality (Musacchi and Serra, 2018). Information on treatment effects on fruit nutrition would also be potentially useful for public health reasons. The use of non-invasive spectroscopy analysis techniques, such as near infra-red (NIR) spectroscopy, are increasingly becoming a more viable way of analysing a wide range of fruit quality tests rapidly on apple (Grabska et al., 2023). Whilst an expensive option, spectroscopy techniques would enable rapid analysis of a wider range of analytical tests should the investigation be repeated. Additionally, studies have demonstrated the importance of consumer attitudes in determining the quality of apple produce (Harker et al., 2003; Péneau et al., 2006). Taste tests designed for an independent panel could compare consumer preferences of fruit sourced from the various treatments based on both extrinsic and intrinsic properties. Consumer preferences on extrinsic properties (size, shape, colour etc.) would be especially useful as these properties are most important at point of purchase (Harker et al., 2003). Traditional cultivars grown in the UK (e.g. ‘Cox’s Orange Pippin’) are widely known for unique flavour profiles, so understanding taste preferences between treatment fruit would be of high interest.

The previous section details agronomic practice recommendations to mitigate negative impacts of increased seasonal temperatures on apple production. These include (but are not limited to) earlier fruit thinning, more thorough vegetative growth pruning, and use of dormancy breakers. The recommendations are based on logic related to what is currently practiced in commercial environments (e.g. practices described in AHBD, 2021). However, scientific field trials would help improve their credibility. For example, trials that compare different methods of fruit thinning within modified temperature environments would (in theory) provide evidence for best thinning practice for preventing or mitigating alternate bearing patterns. As well as testing the findings from the current study, such results would go towards providing more specific best practice guidelines under warming climates.

A wide range of apple genotypes utilised in this study were highly responsive to seasonal temperature variation. A frequent recommendation was the selection of more modern, climate resilient cultivars for use within UK apple production systems. Therefore, field trials would be required to compare the performance of more modern ‘club’ cultivars (that have been selected for climate resilience) against current commercially important cultivars (e.g. ‘Gala’, ‘Braeburn’) under modified temperature environments.

The rootstock used (M9) was identical across all cultivars within this investigation. Physiological processes of rootstocks can depend heavily on growing environment, especially for dwarfing rootstocks such as M9 (Hatton, 1935; Marini and Fazio, 2018). Additionally, M9 rootstocks are associated with higher alternate bearing indices than other dwarfing and semi-dwarfing rootstocks (Kviklys et al., 2016). This highlights a need for integration of different rootstocks to understand how it may influence the performance of apple production parameters under varied temperature and rainfall regimes.

Storability studies revealed how modified temperature environments may have influenced long-term storage of the cultivar ‘Gala’. The two years of data showed high heterogeneity between two years of data, which was likely due to alternate bearing influences on fruit quality. Further years of study are required to further refine and validate associations between seasonal fruit production temperature and subsequent storability. Furthermore, similar studies should be performed on different cultivars (e.g. ‘Braeburn’) to confirm whether associations are specific to certain genotypes. Experimental setups that include different controlled atmosphere configurations should also be considered if optimal storage conditions may need to change to preserve fruit quality and extend seasonality (AHDB, 2021). Analysis on ‘shelf-life’ (change in fruit quality parameters over time spent in ambient conditions) of all apple cultivars will also provide further clarity on whether apple fruit in general degrade at an accelerated rate when sourced from warmer environments.

Overall, the results show that commercial production of apple will remain possible within Kent as the UK warms over the remainder of this century. Even though apple is a perennial crop, the dominant use of ‘Gala’ by the industry and the characteristics of that cultivar suggest that management practices can evolve with the expected time scale of changes to the environment. Research to support growers in that quest might use facilities similar to that developed at Brogdale, albeit with fewer cultivars (limited to those of commercial interest). Similar facilities are suggested in the light of the challenge posed by bienniality and the need to study tree management (especially the effect of fruit thinning and tree pruning at different times of the year) over several consecutive years to support reliable fruit production to produce high-quality, high yield fruit crops every year.

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Appendices

***Appendix 2.1**. Severity of Woolly Apple Aphid (WAA) (Eriosoma lanigerum) damage inflicted on each tree plot from a tree health survey in winter 2021, with cultivar shown. Plots in red indicate severe damage (substantial wax secretions, notable branch galls, reduced fruit yield), green (no visible WAA tree damage, though a still possible host) and grey (young trees, typically not affected by WAA within the facility).*

SOUTH

PLUS4 TUNNEL									PLUS2 TUNNEL								
Tunnel 6			Tunnel 5			Tunnel 4			Tunnel 3			Tunnel 2			Tunnel 1		
R18	R17	R16	R15	R14	R13	R12	R11	R10	R9	R8	R7	R6	R5	R4	R3	R2	R1
Fu	WP	Di	GS	Di	WP	GS	Br	Jol	Jol	Jon	GS	Ga	TB	GD	Fu	YB	BS
GS	Br	Ed	TB	La	Ga	GC	TB	BH	WB	Di	Br	GC	Di	WP	Jol	WB	Ed
BH	Jon	GC	Jol	GC	YB	WP	Ed	Ga	COP	YB	Ka	GS	BS	YB	GC	Ga	Jon
KoP	Ka	La	Ed	KoP	BS	KoP	Ka	YB	KoP	Fu	WP	BH	Br	Ed	GS	Di	La
GD	Ga	SE	Ka	WB	GD	SE	La	BS	BS	SE	Ga	Jol	WB	Fu	GD	SE	Br
TB	COP	BS	SE	COP	BH	Jon	Fu	Di	Ed	GD	TB	Ka	SE	KoP	WP	TB	COP
YB	WB	Jol	Jon	Br	Fu	WB	COP	GD	Di	La	GC	Jon	La	COP	BH	KoP	Ka
GC	YB	KoP	Ga	Ed	Jon	WB	GS	BH	GS	GC	KoP	Jol	TB	GD	La	GS	SE
WP	Ed	TB	Jol	Di	GD	Br	COP	TB	Jol	Di	TB	Di	GS	Ka	BH	Fu	TB
GS	Jon	Br	GC	COP	BH	Fu	SE	La	BS	Ga	WB	KoP	Ga	WB	COP	Ka	Ed
SE	BH	WB	BS	WB	TB	YB	Ka	BS	GD	Ed	Br	Jon	La	WP	GD	BS	YB
Fu	Ga	La	Ka	Br	GS	Di	GD	Ga	La	Jon	SE	SE	Br	COP	Jon	WB	Di
Jol	BS	GD	Fu	YB	KoP	Jol	Ed	GC	BH	WP	Ka	Fu	Ed	YB	Ga	GC	KoP
COP	Ka	Di	La	SE	WP	WP	KoP	Jon	COP	Fu	YB	BS	GC	BH	Jol	Br	WP
Ka	WB	BS	Br	COP	Ed	La	Ga	GS	Ga	Br	BS	WB	Jol	Ka	Ed	BH	Di
COP	GS	GD	BS	BH	Ka	TB	COP	BS	SE	La	WB	Br	GS	BH	BS	KoP	Fu
YB	Br	SE	WB	Ga	SE	Di	WB	GD	Ed	YB	GC	KoP	BS	COP	WB	COP	Jon
TB	GC	Fu	TB	Fu	Jol	Ed	Fu	Br	Di	COP	WP	YB	Fu	Di	TB	SE	WP
WP	Jol	BH	Di	La	GS	Jol	GC	Ka	TB	BH	Ka	GD	Jon	Ed	Jol	GS	YB
Jon	Ga	La	GC	KoP	Jon	SE	BH	YB	GD	Jol	Fu	Ga	La	WP	Ga	La	Ka
Ed	KoP	Di	YB	GD	WP	KoP	Jon	WP	GS	KoP	Jon	TB	GC	SE	GD	GC	Br
Ed	Br	YB	KoP	GC	Jon	SE	WP	KoP	Ka	WP	Ed	Ka	Di	WP	BH	GD	GC
Jon	BS	WP	SE	Br	TB	BS	GS	TB	Br	SE	COP	Fu	WB	BS	La	GS	Fu
Jol	GS	Ka	Fu	COP	Ga	Jol	BH	La	Jol	BH	GC	GC	GD	TB	Ga	Jol	KoP
TB	Fu	Di	Di	GS	WB	YB	COP	GD	Di	Jon	TB	YB	Br	GS	YB	WP	Jon
WB	COP	SE	YB	BS	GD	GC	Br	Ga	KoP	BS	Ga	La	BH	KoP	Di	Ka	Ed
La	Ga	GC	BH	WP	Jol	Di	Ed	Ka	GD	Fu	La	Jon	Ga	Jol	COP	SE	BS
BH	KoP	GD	Ed	La	Ka	Fu	Jon	WB	GS	WB	YB	Ed	SE	COP	TB	WB	Br
Ka	BH	KoP	WP	La	Jon	Ka	TB	La	BH	Ka	YB	Di	GS	La	BS	Di	Br
WB	GS	Fu	Di	SE	Br	Br	GC	Jol	TB	Ga	Fu	GD	Ed	KoP	SE	Ed	Fu
Ga	Di	Ed	GD	TB	WB	WP	BS	KoP	La	GS	Ed	Ka	YB	BH	GS	Ka	YB
WP	Jol	SE	BH	BS	Jol	COP	GS	Ga	GC	WB	WP	WP	COP	Br	GD	Jon	Ga
YB	TB	COP	GC	Ga	COP	YB	BH	Ed	KoP	Br	COP	Fu	Jol	WB	Jol	La	COP
BS	GD	Jon	GS	KoP	YB	Di	Fu	Jon	BS	Di	Jon	Ga	GC	SE	BH	WB	KoP
Br	La	GC	Ka	Ed	Fu	WB	GD	SE	Jol	SE	GD	Jon	TB	BS	WP	TB	GC
WP	SE	Ka	Jol	BS	Ka	SE	Ga	Br	Ga	SE	BH	WB	BS	Jol	GS	La	BS
GC	Jol	La	Ga	BH	Jon	TB	Ka	COP	Di	KoP	Ed	BH	GC	KoP	WB	GC	SE
Fu	COP	WB	Di	GC	KoP	Ed	La	Jol	GC	GD	Fu	YB	COP	GD	Br	Jol	GS
GD	GS	Jon	Ed	YB	TB	Jon	WB	BS	Br	Ka	BS	Ga	SE	La	BH	Ed	GD
Br	Ed	Di	COP	WB	Fu	BH	GD	GC	YB	COP	La	Ed	Jon	Fu	Fu	BS	Jol
YB	Ga	BH	GS	WP	SE	WP	Di	Fu	WP	WB	Jol	WP	Br	Ka	Jon	WP	BH
BS	TB	KoP	Br	GD	La	GS	KoP	YB	Jon	GS	TB	GS	Di	TB	GD	COP	WP

NORTH

Variety Key:	
Beverley Hills	BH
Braeburn	Br
Bramley's Seedling	BS
Cox's Orange Pippin	COP
Discovery	Di
Edward VII	Ed
Fuji	Fu
Ga	Ga
George Cave	GC
Golden Delicious	GD
Granny Smith	GS
Jolyne	Jol
Jonathan	Jon
Kandile	Ka
King of the Pippins	KoP
Lappio	La
Stark's Earliest	SE
Tropical Beauty	TB
Winter Banana	WB
Winter Pearmain	WP
Yellow Bellflower	YB

Colour Key:
NO WAA DAMAGE
WAA DAMAGE
NOT MATURE TREE

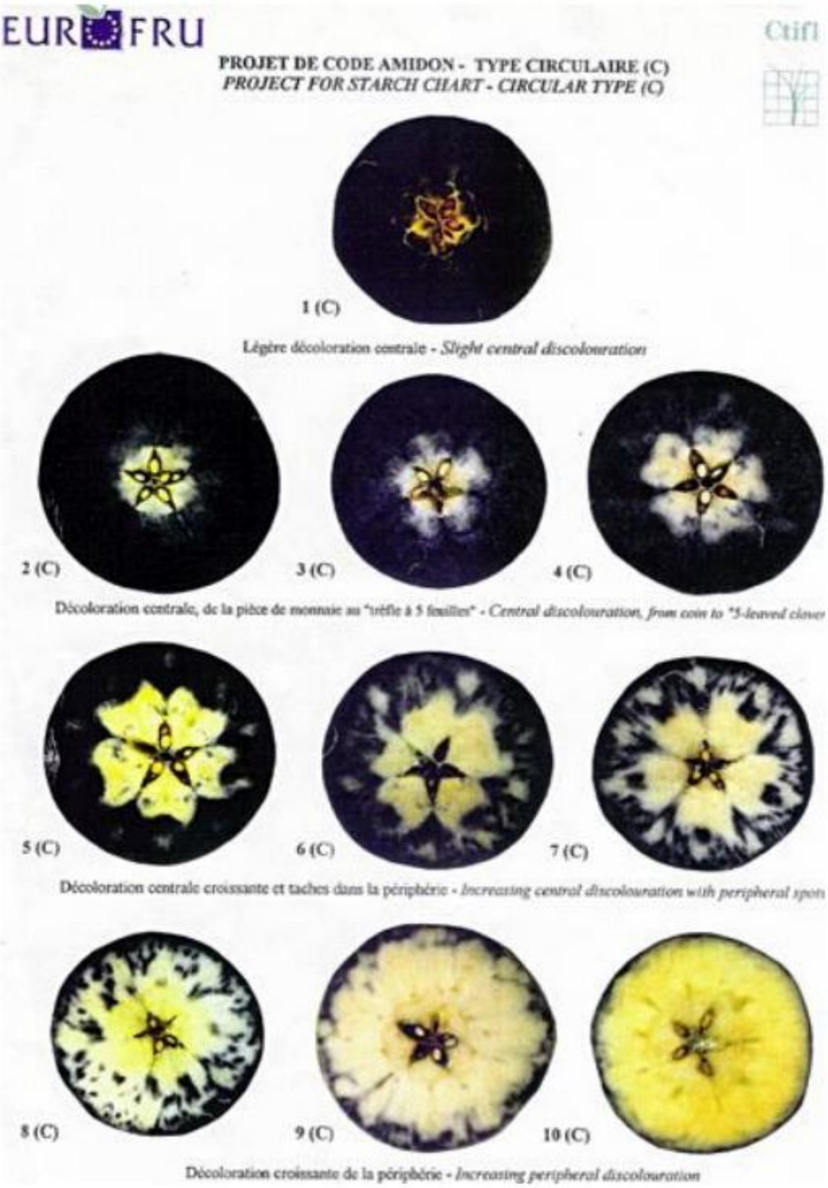
Appendix 2.2*. Mean full-flowering and harvest dates for each cultivar in each modified temperature regime (Ambient, Plus2 or Plus4) in four years (2018-21).*

	Full Flowering Date			Harvest Date		
Cultivar	**Ambient**	**Plus2**	**Plus4**	**Ambient**	**Plus2**	**Plus4**
Beverly Hills [1]	30 APR	19 APR	18 APR	10 AUG	6 AUG	6 AUG
Braeburn [1]	3 MAY	21 APR	20 APR	3 NOV	21 OCT	14 OCT
Bramley's Seedling	5 MAY	26 APR	23 APR	5 OCT	19 SEP	17 SEP
Cox's Orange Pippin	29 APR	24 APR	21 APR	23 SEP	12 SEP	10 SEP
Discovery	29 APR	23 APR	21 APR	12 AUG	28 JUL	26 JUL
Edward VII	13 MAY	2 MAY	28 APR	18 OCT	9 OCT	1 OCT
Fuji	29 APR	22 APR	21 APR	11 OCT	10 OCT	3 OCT
Gala	29 APR	22 APR	20 APR	26 SEP	7 SEP	31 AUG
George Cave	28 APR	20 APR	19 APR	7 AUG	27 JUL	23 JUL
Golden Delicious	2 MAY	23 APR	21 APR	26 SEP	18 SEP	17 SEP
Granny Smith [1]	3 MAY	22 APR	21 APR	2 NOV	26 OCT	13 OCT
Jolyne	10 MAY	4 MAY	22 APR	27 SEP	17 SEP	15 SEP
Jonathan	1 MAY	23 APR	23 APR	28 SEP	18 SEP	13 SEP
King of the Pippins	4 MAY	26 APR	23 APR	2 OCT	9 SEP	5 SEP
Lappio	28 APR	20 APR	18 APR	24 OCT	24 OCT	17 OCT
Stark's Earliest	25 APR	20 APR	18 APR	23 JUL	9 JUL	6 JUL
Tropical Beauty [1]	29 APR	19 APR	18 APR	21 OCT	15 OCT	9 OCT
Winter Banana [1]	1 MAY	21 APR	20 APR	31 OCT	23 OCT	21 OCT
Winter Pearmain	28 APR	18 APR	17 APR	22 SEP	7 SEP	4 SEP
Yellow Bellflower	26 APR	21 APR	19 APR	19 OCT	9 OCT	30 SEP

[1] Based on two years of data (2020-21) only

Appendix 2.3*. Protocol for fruit maturity assessments (from Lane, 2022).*

- Select two random apples from each cultivar replicate per tunnel, one from the top and one from the bottom of the tree (but not too low to avoid possible glyphosate damaged fruit).
- Ten apples minimum to be collected. If less replicates choose extra from replicates already in tunnel.
- Harvest 5 apples per tree 1 top, 2 middle, 2 lower. To achieve minimum 10 per cultivar.
- Make sure sampling represents the way the fruit is distributed on the tree. Generally the middle area should have the greater % of the crop, varying the sides of the tree as you go. When taking a random sample note the fruit that your eye is drawn to and then take the 3rd fruit away from it. Label each apple using a marker pen to show the individual tree it is from and record on record sheet. (added 16/8/2017).
- Grade and weigh.
- Starch/Iodine (S/I) test:
    - Cut each apple through the middle horizontally to reveal core.
    - Keep the end with the stalk.
    - Dip in iodine and potassium solution (1% Iodine, 4% Potassium Iodide).
    - Place face up in apple dimpled tray, leave samples for 20 minutes
    - Record colouration value from CTIFL maturity charts (see below).
    - Calculate average value, pick when tree ripe (50% mean S/I).



Scoring chart derived from CTIFL – Centre Technique Interprofessionel des Fruit et Légumes.

***Appendix 2.4**. Notes for conversion of "Manston" MetOffice weather data to Ambient, Plus2 and Plus4 values using regression analysis. Validation of models compiled using root mean square error (RMSE) analysis of each model. A total of 75 days-worth of daily mean temperature values (°C) within 2020 and 2021 were calculated using this method.*

Regressions:

- Initially, yearly regression equation (Ambient tunnel versus Manston data) calculated from 2019 data produced promising readings (R^2=0.98).
- Used 2020 data to validate model. Poor comparison – model strongly overestimated readings in winter, and underestimated readings in summer.
- Potentially a coefficient could be applied – however it was felt would it be overly complicated for its purpose.
- New plan – have six different models depending on time of year (Jan+Feb, Mar+Apr etc.) to estimate AMBIENT tunnel daily averages, based on Manston daily averages.
- Used 2018 and 2019 data for model creation, use 2020 data for validation again. Used RMSE to calculate goodness of fit (if <0.5, consider it fit for purpose).

PERIOD	MODEL	R^2	RMSE (2020 validation)
JAN-FEB	y = 1.0674x - 0.2737	0.961	0.18030
MAR-APR	y = 1.06x + 0.0983	0.9652	0.18079
MAY-JUN	y = 0.9879x + 1.1533	0.9364	0.08958
JUL-AUG	y = 1.0482x - 0.2512	0.94	0.07299
SEP-OCT	y = 1.1185x - 1.787	0.9641	0.10659
NOV-DEC	y = 1.089x - 0.8796	0.9378	0.09984

Nb: x = Manston daily average air temperature, y = Estimated Ambient tunnel daily average air temperature

- The above models appear relatively successful, with RMSE results <0.2 for every time period.
- The above regressions were used to estimate daily average temperatures for the ambient tunnel when there were gaps in both the TomTech data and Brogdale records.

CONVERTING FROM AMBIENT TO PLUS2 / PLUS4 – DAILY AVERAGE TEMPERATURE 2020/21 GAPS

- For missing 2020 data, weather data from 2021 used to linear regression model converting ambient average temperatures, in to plus2 and plus4 daily averages.

- Nb: NO VALIDATION OCCURING. This is because 2021 is only year (other than 2020) with altered venting software. Therefore, 2018-2019 temperature uplifts are irrelevant for modelling missing 2020 data.
- Similar to above, different models depending on the time of year (JAN-FEB, MAR- APR etc.).

Daily average Regressions (nb: only JAN-FEB and SEP-OCT models required):

PERIOD	AMB to +2 MODEL	R^2	AMB to +4 MODEL	R^2
JAN-FEB	y = 0.9782x – 0.0923	N/A	y = 0.9584 + 0.4919	N/A
MAR-APR	-	-	-	-
MAY-JUN	-	-	-	-
JUL-AUG	-	-	-	-
SEP-OCT	y = 1.0634x – 0.2534	0.986	y = 1.1123x – 0.3831	0.9598
NOV-DEC	-	-		-

Nb: x = Manston daily average air temperature, y = Estimated Ambient tunnel daily average air temperature.

Appendix 2.5*. Gravimetric soil water content (u, %) within the modified environment facility during the same one-week window in September 2021. Each cell corresponds to a particular tree near where the sample was taken. The colour scale indicates lowest SMC (red) to highest (green).*

SOUTH

AMBIENT									PLUS4 TUNNEL									PLUS2 TUNNEL								
Tunnel 9			Tunnel 8			Tunnel 7			Tunnel 6			Tunnel 5			Tunnel 4			Tunnel 3			Tunnel 2			Tunnel 1		
R27	R26	R25	R24	R23	R22	R21	R20	R19	R18	R17	R16	R15	R14	R13	R12	R11	R10	R9	R8	R7	R6	R5	R4	R3	R2	R1
											12.2															
																						11				
		16.4															14.7								10.6	
		15.8			17.4																					
																				9.61						
						12.2																				12.1
			9.6					17.4					11.4			13.1										
			14.1									17.3														
													15		15.1											
											8.86		12.3									10.4		11.4		
																				9.29						
															18.5											15.6
	12						11.4																			
																									11.4	
					18.5			17.4	9.52												9.06					
																							8.24			
																	9.4	22.8								
						10																				
	19							19		10.7																
																				12.9						
														9.6										8		
					14.8																					
																								12.6		
															13.7											
								13.9																		13.3
														12.5					11.9							
									11	10.4																
				14.1																		8.98				
														11.1												
							9.11												18.5		12.8					
									11.6																	
			10.7													11.5										
																	16.2									
12										12		8.98														
																		11.9								
		20.3																	15.4							
11.2								14.4														10.4				
	11.9																					16.3				

Appendix 3.1. *One-way ANOVA results comparing mean harvested fruit fresh weight per tree (kg) among the modified temperature environments (Ambient, Plus2, and Plus4) across each cultivar and year (2017-22).*

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
Beverly	2020	36	8.93	<0.001***	1.38 a	0.82 ab	0.67 b
Hills	2021	36	0.533	0.591			
	2022	36	2.123	0.134			
Braeburn	2020	50	2.57	0.087			
	2021	51	2.52	0.090			
	2022	51	6.163	0.004**	7.46 b	5.45 b	13.36 a
Bramley's	2017	40	6.148	0.005**	23.18 a	16.51 b	20.10 ab
Seedling	2018	45	2.459	0.097			
	2019	46	1.413	0.254			
	2020	46	1.325	0.276			
	2021	46	3.697	0.032*	17.33 a	9.84 b	12.85 ab
	2022	46	2.808	0.071			
Cox's	2017	38	1.334	0.276			
Orange	2018	44	2.782	0.073			
Pippin	2019	44	1.548	0.224			
	2020	45	2.230	0.119			
	2021	44	4.419	0.018*	14.08 ab	11.63 b	18.53 a
	2022	43	10.54	<0.001***	11.15 a	3.04 b	1.92 b
Discovery	2017	46	5.120	0.010**	14.79 a	11.35 b	12.65 ab
	2018	46	18.50	<0.001***	4.34 a	2.63 b	0.72 c
	2019	43	16.78	<0.001***	19.15 a	14.84 b	13.70 b
	2020	45	3.646	0.034*	16.10 a	12.21 b	12.63 ab
	2021	46	11.63	<0.001***	13.82 a	9.36 b	17.15 a
	2022	46	10.13	<0.001***	21.36 a	16.77 ab	11.59 b
Edward VII	2017	43	4.921	0.012*	16.83 a	12.29 b	15.36 ab
	2018	40	1.980	0.151			
	2019	40	0.143	0.868			
	2020	46	0.529	0.593			
	2021	46	5.129	0.010**	16.65 ab	13.25 b	20.88 a
	2022	46	3.919	0.027*	10.43 a	5.08 b	7.30 ab
Fuji	2017	47	3.683	0.033*	17.94 ab	15.15 b	18.07 a
	2018	47	0.698	0.503			
	2019	44	1.884	0.164			
	2020	47	0.484	0.619			
	2021	46	2.718	0.077			
	2022	46	1.374	0.263			
Gala	2017	38	2.173	0.128			
	2018	43	4.314	0.020*	16.67 a	14.01 ab	11.86 b
	2019	39	3.800	0.031*	27.08 ab	22.93 b	28.81 a
	2020	45	6.290	0.004**	23.31 a	14.66 b	15.45 b

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
	2021	44	5.657	0.007**	16.22 b	16.54 b	25.71 a
	2022	45	18.94	<0.001***	18.11 a	6.15 b	6.21 b
George	2017	31	0.940	0.402			
Cave	2018	40	33.80	<0.001***	10.00 a	4.60 b	3.57 b
	2019	37	2.507	0.095			
	2020	41	7.937	0.001**	10.83 a	5.47 b	7.68 b
	2021	41	24.20	<0.001***	13.05 a	3.72 b	12.59 a
	2022	41	12.05	<0.001***	15.74 a	6.98 b	13.93
Golden	2017	34	2.043	0.145			
Delicious	2018	48	16.76	<0.001***	11.95 a	1.53 b	0.81 b
	2019	50	0.200	0.819			
	2020	50	9.902	<0.001***	15.97 a	3.76 b	4.21 b
	2021	50	3.542	0.036*	26.25 ab	23.82 b	34.37 a
	2022	50	5.811	0.005**	13.34 a	5.10 b	3.05 b
Granny	2020	45	1.125	0.334			
Smith	2021	46	5.151	0.010**	3.48 b	4.03 b	6.51 a
	2022	46	2.171	0.126			
Jolyne	2020	33	2.442	0.103			
	2021	35	0.367	0.696			
	2022	35	1.154	0.327			
Jonathan	2017	35	2.773	0.076			
	2018	37	5.601	0.008**	14.94 a	9.20 ab	7.64 b
	2019	35	0.280	0.758			
	2020	34	2.374	0.108			
	2021	34	5.405	0.009**	18.73 ab	12.62 b	21.85 a
	2022	35	12.20	<0.001***	13.78 a	3.54 b	3.48 b
King of	2017	39	1.083	0.349			
the Pippins	2018	37	0.617	0.545			
	2019	37	3.980	0.027*	18.23 a	13.94 b	16.40 ab
	2020	35	1.792	0.182			
	2021	35	4.322	0.021*	18.11 ab	12.07 b	18.60 a
	2022	36	4.999	0.012*	3.05 a	2.06 ab	0.50 b
Lappio	2017	34	0.975	0.387			
	2018	29	1.062	0.359			
	2019	29	1.744	0.193			
	2020	29	6.492	0.005**	0.82 b	1.25 b	5.71 a
	2021	29	6.096	0.006**	18.11 b	20.96 b	28.56 a
	2022	28	2.535	0.097			
Stark’s	2017	38	0.123	0.885			
Earliest	2018	47	20.65	<0.001***	11.91 a	7.66 b	6.45 b
	2019	45	8.182	<0.001***	14.99 a	11.68 b	12.48 b
	2020	38	4.764	0.014*	13.13 a	9.21 b	11.20 ab
	2021	42	15.70	<0.001***	13.75 a	6.95 b	13.77 a
	2022	42	2.894	0.067			

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
Tropical	2020	39	0.908	0.412			
Beauty	2021	NA	NA	NA			
	2022	39	0.05	0.951			
Winter	2020	40	4.014	0.030			
Banana	2021	41	3.026	0.059			
	2022	40	1.684	0.199			
Winter	2017	44	1.281	0.288			
Pearmain	2018	39	6.759	0.003**	2.57 a	0.58 b	0.06 b
	2019	39	1.978	0.152			
	2020	41	5.455	0.008**	7.32 b	12.47 ab	14.64 a
	2021	42	0.763	0.473			
	2022	42	0.272	0.763			
Yellow	2017	24	2.357	0.116			
Bellflower	2018	23	46.95	<0.001***	15.64 a	3.89 b	1.91 b
	2019	22	7.79	0.003**	26.21 a	15.59 b	14.63 b
	2020	24	29.15	<0.001***	24.90 a	7.89 b	8.31 b
	2021	26	2.639	0.091			
	2022	26	12.35	<0.001***	25.48 a	11.54 b	17.29 b

Appendix 3.2. *One-way ANOVA results comparing mean fruit number harvested per tree among the modified temperature environments (Ambient, Plus2, and Plus4) across each cultivar and year (2017-22).*

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
Beverly	2020	36	9.831	<0.001***	23 a	12 ab	11 b
Hills	2021	36	0.522	0.598			
	2022	36	2.925	0.067			
Braeburn	2020	50	10.2	<0.001***	54 b	60 b	89 a
	2021	51	2.716	0.076			
	2022	51	5.852	0.005**	48 b	36 b	92 a
Bramley's	2017	40	4.672	0.015*	94 a	71 b	80 ab
Seedling	2018	45	4.063	0.024*	9 a	4 ab	3 b
	2019	46	4.553	0.016*	85 a	74 ab	63 b
	2020	46	0.507	0.605			
	2021	46	0.135	0.874			
	2022	46	0.877	0.423			
Cox's	2017	38	0.727	0.49			
Orange	2018	44	2.621	0.084			
Pippin	2019	44	0.785	0.462			
	2020	45	2.12	0.132			
	2021	44	5.938	0.005**	111 b	180 ab	203 a
	2022	43	8.389	<0.001***	77 a	28 b	13 b
Discovery	2017	46	4.179	0.022*	155 a	121 b	132 ab
	2018	46	18.38	<0.001***	24 a	16 b	4 c
	2019	43	21.45	<0.001***	147 a	115 b	98 b
	2020	45	2.596	0.086			
	2021	46	5.466	0.007**	138 b	200 a	217 a
	2022	46	6.983	0.002**	198 a	142 b	116 b
Edward VII	2017	43	2.483	0.095			
	2018	40	0.091	0.137			
	2019	40	1.471	0.242			
	2020	46	0.139	0.871			
	2021	46	7.208	0.002**	94 b	115 b	161 a
	2022	46	1.083	0.347			
Fuji	2017	47	0.642	0.531			
	2018	47	0.563	0.574			
	2019	44	0.929	0.402			
	2020	47	0.545	0.583			
	2021	46	0.357	0.702			
	2022	46	1.270	0.290			
Gala	2017	38	1.435	0.251			
	2018	43	4.467	0.017*	93 a	76 ab	65 b
	2019	39	3.692	0.034*	217 a	197 ab	170 b
	2020	45	3.986	0.026*	155 a	107 ab	96 b

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
	2021	44	8.902	<0.001***	154 b	349 a	343 a
	2022	45	30.24	<0.001***	134 a	34 b	36 b
George	2017	31	0.947	0.399			
Cave	2018	40	39.27	<0.001***	64 a	28 b	21 b
	2019	37	0.904	0.414			
	2020	41	6.437	0.004**	96 a	49 b	75 ab
	2021	41	6.509	0.004**	144 ab	88 b	185 a
	2022	41	11.90	<0.001***	148 a	63 b	132 a
Golden	2017	34	0.177	0.838			
Delicious	2018	48	16.39	<0.001***	64 a	9 b	5 b
	2019	50	0.403	0.671			
	2020	50	9.929	<0.001***	116 a	20 b	22 b
	2021	50	10.94	<0.001***	208 b	322 ab	434 a
	2022	50	6.764	0.003**	100 a	29 b	18 b
Granny	2020	45	1.853	0.169			
Smith	2021	46	2.259	0.116			
	2022	46	2.742	0.075			
Jolyne	2020	33	2.224	0.124			
	2021	35	2.193	0.127			
	2022	35	1.857	0.171			
Jonathan	2017	35	3.477	0.04*	121 b	150 ab	155 a
	2018	37	11.87	<0.001***	96 a	44 b	36 b
	2019	35	0.705	0.501			
	2020	34	5.362	0.009**	122 a	62 ab	45 b
	2021	34	3.174	0.05*	199 b	316 a	265 ab
	2022	35	11.1	<0.001***	132 a	26 b	22 b
King of	2017	39	1.614	0.212			
the Pippins	2018	37	0.613	0.547			
	2019	37	16.34	<0.001***	168 a	100 b	117 b
	2020	35	2.176	0.129			
	2021	35	1.100	0.344			
	2022	36	3.026	0.061			
Lappio	2017	34	0.84	0.441			
	2018	29	1.123	0.339			
	2019	29	7.289	0.003**	166 a	128 b	130 b
	2020	29	5.364	0.010*	6 b	7 b	30 a
	2021	29	12.8	<0.001***	141 b	266 a	311 a
	2022	28	2.093	0.142			
Stark's	2017	38	0.513	0.603			
Earliest	2018	47	38.55	<0.001***	106 a	56 b	49 b
	2019	45	0.214	0.808			
	2020	38	14.1	<0.001***	165 a	100 b	99 b
	2021	42	5.472	0.008**	179 b	237 ab	271 a
	2022	42	4.832	0.012*	191 a	118 b	176 ab

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
Tropical	2020	39	1.638	0.207			
Beauty	2021	NA	NA	NA			
	2022	39	1.433	0.251			
Winter	2020	40	8.17	0.001**	20 a	6 b	14 ab
Banana	2021	41	3.69	0.034*	36 ab	26 b	47 a
	2022	40	1.208	0.310			
Winter	2017	44	1.853	0.169			
Pearmain	2018	39	5.607	0.007**	16 a	3 b	1 b
	2019	39	2.484	0.097			
	2020	41	4.918	0.012*	49 b	88 a	96 a
	2021	42	9.561	<0.001***	159 b	258 a	191 b
	2022	42	0.029	0.972			
Yellow	2017	24	0.828	0.449			
Bellflower	2018	23	49.92	<0.001***	62 a	14 b	8 b
	2019	22	15.04	<0.001***	116 a	62 b	51 b
	2020	24	35.92	<0.001***	122 a	33 b	44 b
	2021	26	1.597	0.222			
	2022	26	14.86	<0.001***	153 a	61 b	84 b

***Appendix 3.3**. One-way ANOVA results comparing mean individual fruit weight (g) among the modified temperature environments (Ambient, Plus2, and Plus4) across each cultivar and year.*

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
Beverly	2020	35	0.411	0.666			
Hills	2021	9	0.747	0.501			
	2022	34	1.73	0.193			
Braeburn	2020	50	2.5	0.092			
	2021	51	4.183	0.021*	136.4 a	95.4 b	132.4 ab
	2022	46	0.212	0.81			
Bramley's	2017	40	13.19	<0.001***	246.7 a	230.6 b	251.6 a
Seedling	2018	41	34.6	<0.001***	566.2 b	621.4 b	760.4 a
	2019	46	31.6	<0.001***	271.0 c	285.0 b	313.9 a
	2020	41	7.937	0.001**	299.8 a	249.6 b	236.3 b
	2021	44	9.577	<0.001***	223.7 a	114.9 b	145.6 b
	2022	45	0.873	0.425			
Cox's	2017	38	56.98	<0.001***	122.4 a	113.3 b	121.9 a
Orange	2018	24	6.772	0.005**	154.0 b	138.8 b	177.9 a
Pippin	2019	44	7.955	0.001**	156.8 b	164.9 a	166.9 a
	2020	40	0.493	0.614			
	2021	44	24.64	<0.001***	137.9 a	69.5 c	97.5 b
	2022	27	1.985	0.157			
Discovery	2017	46	1.099	0.342			
	2018	44	0.116	0.891			
	2019	43	10.24	<0.001***	130.8 b	129.2 b	140.1 a
	2020	44	9.597	<0.001***	120.5 a	124.3 a	98.4 b
	2021	46	67.1	<0.001***	99.5 a	46.1 c	81.9 b
	2022	46	3.181	0.051			
Edward VII	2017	43	7.487	0.002**	241.3 a	212.9 b	228.0 ab
	2018	40	0.668	0.518			
	2019	40	12.5	<0.001***	212.7 b	218.6 b	247.7 a
	2020	44	3.281	0.047*	293.9 a	229.9 b	266.8 ab
	2021	45	15.04	<0.001***	198.6 a	127.2 b	129.3 b
	2022	44	2.744	0.075			
Fuji	2017	47	24.68	<0.001***	95.7 b	88.7 c	103.3 a
	2018	3	9.27	0.052			
	2019	44	26.2	<0.001***	124.4 b	122.9 b	137.6 a
	2020	22	0.08	0.923			
	2021	44	6.73	0.003**	110.3 a	83.5 b	110.3 a
	2022	25	1.187	0.322			
Gala	2017	38	10.08	<0.001***	145.8 a	136.8 b	135.0 b
	2018	43	1.75	0.186			
	2019	39	78.38	<0.001***	125.1 c	134.7 b	146.6 a
	2020	45	0.544	0.584			

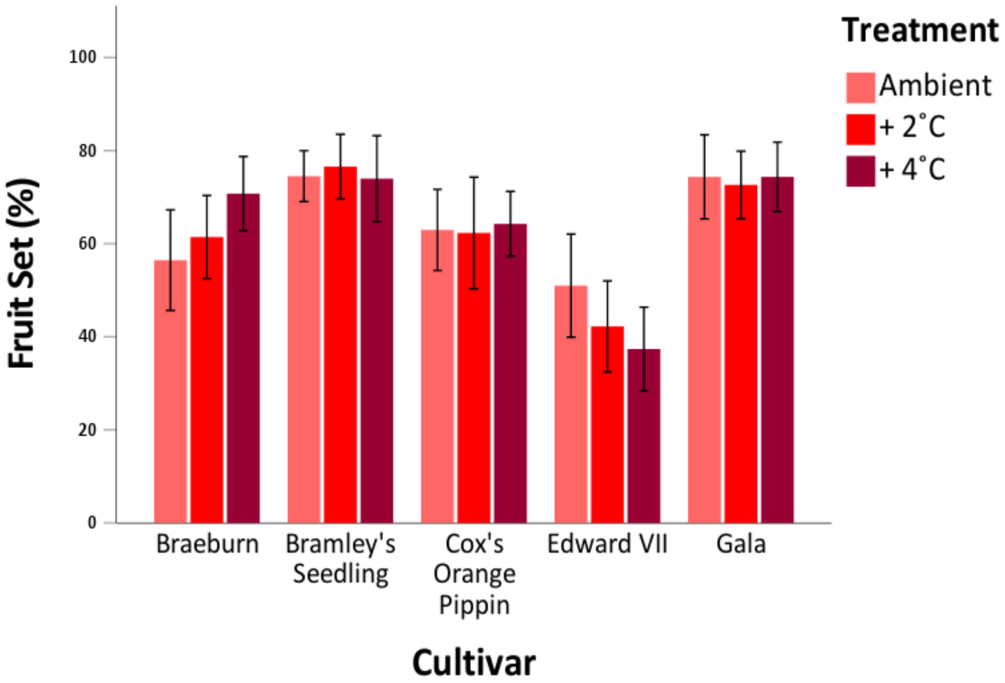
Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
	2021	43	28.19	<0.001***	109.5 a	50.7 c	75.9 b
	2022	42	5.373	0.008**			
George	2017	31	63.13	<0.001***	81.9 a	62.5 c	70.2 b
Cave	2018	40	31.73	<0.001***	156.4 c	166.3 b	173.7 a
	2019	37	1.466	0.244			
	2020	38	0.986	0.383			
	2021	39	14.14	<0.001***	93.6 a	52.9 b	71.9 b
	2022	37	0.216	0.807			
Golden	2017	34	14.83	<0.001***	162.6 a	129.6 c	145.3 b
Delicious	2018	32	7.858	0.002**	187.4 b	211.9 a	178.9 b
	2019	50	1.802	0.175			
	2020	41	1.3	0.284			
	2021	50	24.67	<0.001***	150.2 a	78.6 b	84.0 b
	2022	44	4.967	0.011*	168.7 b	185.9 ab	205.0 a
Granny	2020	45	0.765	0.471			
Smith	2021	45	3.034	0.058			
	2022	44	3.651	0.034*	167.0 b	211.3 a	186.8 ab
Jolyne	2020	31	7.471	0.002**	191.2 a	164.4 a	115.3 b
	2021	34	4.502	0.018*	101.5 a	66.6 b	101.7 a
	2022	35	5.148	0.011*	155.1 a	128.2 ab	107.5 b
Jonathan	2017	35	7.398	0.002**	115.7 a	97.7 b	108.9 a
	2018	37	180.7	<0.001***	156.4 b	211.2 a	211.7 a
	2019	35	12.94	<0.001***	107.5 b	99.6 b	119.1 a
	2020	33	7.382	0.002**	129.6 b	161.3 ab	192.1 a
	2021	33	30.48	<0.001***	96.4 a	41.0 b	84.3 a
	2022	31	4.141	0.026*	126.0 b	149.8 ab	164.5 a
King of	2017	39	51.72	<0.001***	135.9 a	118.2 c	123.7 b
the Pippins	2018	19	1.835	0.187			
	2019	37	63.99	<0.001***	108.6 b	140.5 a	140.3 a
	2020	17	1.231	0.317			
	2021	35	14.21	<0.001***	93.3 a	50.2 c	72.0 b
	2022	27	3.683	0.039*	153.9 a	129.8 ab	112.3 b
Lappio	2017	34	2.671	0.084			
	2018	8	2.421	0.151			
	2019	29	212.6	<0.001***	134.7 c	152.2 b	167.0 a
	2020	24	0.563	0.577			
	2021	29	24.71	<0.001***	127.4 a	75.3 c	94.5 b
	2022	28	0.025	0.975			
Stark's	2017	38	10.34	<0.001***	61.0 a	56.0 b	55.4 b
Earliest	2018	47	267.9	<0.001***	112.7 c	136.0 a	130.6 b
	2019	45	32.8	<0.001***	100.7 a	80.7 b	86.3 b
	2020	38	16.35	<0.001***	81.6 b	91.5 b	119.4 a
	2021	42	61.24	<0.001***	77.1 a	30.1 c	53.8 b
	2022	42	6.146	0.005**	74.8 b	97.7 a	89.0 ab

Cultivar	Year	df	f	p	Ambient	Plus2	Plus4
Tropical	2020	39	0.325	0.325			
Beauty	2021	13	0.759	0.759			
	2022	39	1.88	1.88			
Winter	2020	40	0.437	0.649			
Banana	2021	41	0.319	0.729			
	2022	40	0.208	0.813			
Winter	2017	44	11.08	>0.001***	150.6 a	143.2 b	144.3 b
Pearmain	2018	24	1.404	0.265			
	2019	39	1.108	0.340			
	2020	41	0.719	0.493			
	2021	42	17.36	<0.001***	137.7 a	82.5 b	125.8 a
	2022	42	1.768	0.183			
Yellow	2017	24	0.155	0.857			
Bellflower	2018	22	30.77	<0.001***	288.0 a	288.0 a	233.7 c
	2019	22	17.67	<0.001***	224.5 c	251.0 b	284.1 a
	2020	21	2.064	0.152			
	2021	25	8.428	0.002**	173.5 a	105.9 b	145.6 ab
	2022	26	2.977	0.069			

Appendix 3.4. *Two-way ANOVA comparing the effects of two factors (year (2017-22) and temperature treatment (Ambient, Plus2, and Plus4)) on cross-cultivar standardised fruit fresh weight per tree (kg). Results correspond with Figure 3.4 where post-hoc Tukey tests were applied to compare the year x temperature treatment combinations.*

Factor	**df**	**F**	**p**
Year	2	88.71	<0.001***
Temp	5	316.89	<0.001***
Year x Temp	10	12.84	<0.001***
(Residuals)	4381	-	-

Appendix 3.5. *Results from Stephens (2022) which investigated the effect of distance from introduced commercial beehives (m) on fruit set (%) from each temperature treatment (Ambient, Plus2, and Plus4) across five cultivars ('Braeburn', 'Bramley's Seedling', 'Cox's Orange Pippin'. 'Edward VII', and 'Gala'). The data was sampled in 2021 (a biennial 'on' year). The figure displays how fruit set (%) varied among cultivars and treatments (no significant differences were observed among treatments for each cultivar). The table displays Pearson's Correlation results between tree distance from introduced beehive (m) and fruit set (%). Several significant findings were found (p<0.05), varying by cultivar and treatment.*



Cultivar	Treatment	N	Pearson's Correlation	R^2
Braeburn	Ambient	18	0.465*	0.216
	+ 2˚C	17	- 0.107	
	+ 4˚C	18	- 0.778**	0.605
Bramley's Seedling	Ambient	17	- 0.268	
	+ 2˚C	13	- 0.318	
	+ 4˚C	15	- 0.443**	0.197
Cox's Orange Pippin	Ambient	15	- 0.450*	0.202
	+ 2˚C	11	- 0.583*	0.340
	+ 4˚C	17	- 0.254	
Edward VII	Ambient	14	- 0.327	
	+ 2˚C	18	- 0.249	
	+ 4˚C	17	0.48	
Gala	Ambient	15	0.045	
	+ 2˚C	15	- 0.10	
	+ 4˚C	16	- 0.318	

* Correlation is significant at the 0.05 level (1-tailed).
** Correlation is significant at the 0.01 level (1-tailed).

Appendix 4.1*. Corresponding one-way ANOVA results with degrees of freedom (df), F-statistic (f) and p-value (p) noted between mean alternate bearing index (ABI) of three modified temperature treatments (Ambient, Plus2 and Plus4) across 15 applicable apple cultivars (data in Table 4.2).*

	ABI		
Cultivar	**df**	**f**	**p**
Bramley's Seedling	234	6.44	0.002**
Cox's Orange Pippin	222	7.55	<0.001***
Discovery	234	6.19	0.002**
Edward VII	224	6.149	0.003**
Fuji	234	0.344	0.709
Gala	214	13.12	<0.001***
George Cave	193	12.81	<0.001***
Golden Delicious	244	20.66	<0.001***
Jolyne	70	4.743	0.01*
Jonathan	176	14.35	<0.001***
King of the Pippins	187	1.693	0.187
Lappio	154	3.494	0.033*
Stark's Earliest	209	10.65	<0.001***
Winter Pearmain	210	1.269	0.283
Yellow Bellflower	122	43.9	<0.001***

*Significant at p<0.05, **Significant at p<0.01, *** Significant at p<0.001

***Appendix 4.2**. Mean total harvested fruit per tree (kg) for each modified temperature environment (Ambient, Plus2, and Plus4), year (2017-2022), and 10 applicable apple cultivars.*

Cultivar	Treat.	2017	2018	2019	2020	2021	2022
Bramley's Seedling	Amb	94	9	85	33	86	81
	Plus2	71	4	74	37	92	61
	Plus4	80	3	63	28	82	64
Cox's Orange Pippin	Amb	135	18	91	67	111	77
	Plus2	127	4	93	43	180	29
	Plus4	120	7	103	41	203	13
Discovery	Amb	155	25	147	136	138	198
	Plus2	121	16	115	101	200	142
	Plus4	132	4	98	133	217	116
Edward VII	Amb	71	31	81	40	94	47
	Plus2	57	20	75	36	115	31
	Plus4	67	23	70	34	164	39
Gala	Amb	137	99	217	155	154	134
Gala	Plus2	119	76	170	107	349	34
	Plus4	139	65	197	96	343	36
George Cave	Amb	88	64	68	96	144	148
	Plus2	101	28	58	49	88	63
	Plus4	105	21	65	75	185	132
Golden Delicious	Amb	169	64	203	116	208	100
	Plus2	174	8	213	20	322	29
	Plus4	166	4	223	22	434	18
Jonathan	Amb	121	96	200	122	199	132
	Plus2	151	44	217	62	316	25
	Plus4	155	36	195	45	266	22
Stark's Earliest	Amb	163	106	151	165	179	191
	Plus2	173	56	145	100	237	118
	Plus4	188	49	145	99	271	176
Yellow Bellflower	Amb	104	62	116	122	133	153
	Plus2	94	14	62	33	181	61
	Plus4	111	8	51	44	184	84

Appendix 4.3. *Pearson's correlation analysis for early-harvesting cultivars only.*

		All Years				**'On' Year**				**'Off' Year**			
		r	**t**	**df**	**P**	**r**	**t**	**df**	**P**	**r**	**t**	**df**	**P**
Prev. Summer	Tmean	0.01	0.68	43	>0.999	-0.17	-0.70	16	>0.999	0.12	0.61	25	>0.999
	Tmin	0.27	1.81	43	>0.999	-0.08	-0.31	16	>0.999	0.37	2.01	25	>0.999
	Tmax	-0.04	-0.24	43	>0.999	-0.15	-0.60	16	>0.999	-0.09	-0.45	25	>0.999
	Tmmd	-0.01	-0.09	43	>0.999	-0.16	-0.65	16	>0.999	-0.03	-0.16	25	>0.999
Prev Nov to Jan	Tmean	0.06	4.97	43	0.008 **	0.48	2.16	16	>0.999	0.66	4.40	25	0.131
	Tmin	0.64	5.50	43	<0.001 ***	0.39	1.68	16	>0.999	0.71	5.04	25	0.025 *
	Tmax	-0.26	-1.74	43	>0.999	-0.20	-0.81	16	>0.999	-0.26	-1.33	25	>0.999
	Tmmd	-0.60	-4.87	43	0.012 *	-0.32	-1.36	16	>0.999	-0.65	-4.33	25	0.157
Prev Feb to Apr	Tmean	0.32	2.47	52	>0.999	-0.12	-0.61	25	>0.999	0.58	3.52	25	>0.999
	Tmin	0.18	1.33	52	>0.999	-0.07	-0.36	25	>0.999	0.49	2.84	25	>0.999
	Tmax	0.04	0.30	52	>0.999	-0.03	-0.15	25	>0.999	0.05	0.25	25	>0.999
	Tmmd	-0.06	-0.42	52	>0.999	0.01	0.06	25	>0.999	-0.23	-1.18	25	>0.999
Cur. Seas.	Tmean	-0.31	-2.11	43	>0.999	0.27	1.41	25	>0.999	0.43	-1.91	16	>0.999
	Tmin	-0.18	-1.19	43	>0.999	0.30	1.60	25	>0.999	-0.51	-2.40	16	>0.999
	Tmax	-0.27	-1.18	43	>0.999	0.19	0.99	25	>0.999	-0.33	-1.38	16	>0.999
	Tmmd	-0.20	-1.35	43	>0.999	0.04	0.20	25	>0.999	-0.14	-0.57	16	>0.999
Date	FF	0.47	3.35	40	>0.999	0.13	0.50	16	>0.999	0.57	3.27	22	>0.999
	Harv.	-0.01	-0.60	40	>0.999	0.06	0.25	16	>0.999	-0.12	-0.56	22	>0.999
Rain	Cur.	0.08	0.45	34	>0.999	-0.09	-0.35	16	>0.999	-0.61	-3.10	16	>0.999
	Prev.	0.05	0.29	40	>0.999	0.17	0.68	16	>0.999	0.33	1.66	22	>0.999

Appendix 5.1. *One Way ANOVA results amongst temperature treatment (Amb, Plus2, and Plus4) means of 5 apple fruit quality attributes (firmness, SSC, RCC, DMC and fruit weight) with statistically significant results highlighted.*

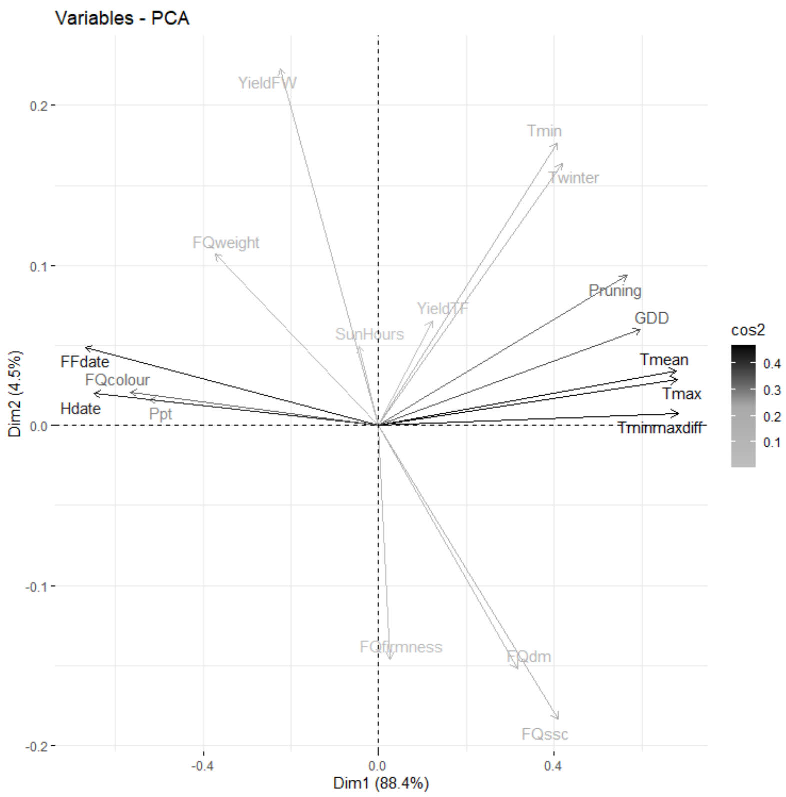
Cultivar	**Firmness (kg)**			**Soluble Solids Content (%Brix)**			**Red Colour Coverage (%)**			**Dry Matter Content (%)**			**Individual Fruit Weight (g)**		
	df	**f**	**P**	**df**	**f**	**P**	**df**	**f**	**P**	**df**	**f**	**P**	**df**	**f**	**P**
Braeburn	267	1.439	0.239	267	15.700	<0.001 ***	177	3.532	0.031 *	15	5.207	0.019 *	177	8.214	<0.001 ***
Bramley's Seedling	447	2.674	0.070	445	5.905	0.003 **	na	na	na	14	0.703	0.512	177	2.421	0.092
Cox Orange Pippin	432	4.219	0.015 *	417	1.286	0.277	177	4.656	0.011 *	15	0.032	0.969	177	10.41	<0.001 ***
Discovery	446	1.137	0.322	446	3.487	0.031 *	176	6.434	0.002 **	15	0.05	0.952	176	11.8	<0.001 ***
Edward VII	441	0.562	0.57	441	7.759	<0.001 ***	na	na	na	12	0.735	0.5	171	8.47	<0.001 ***
Fuji	335	1.945	0.145	335	0.098	0.907	155	27.04	<0.001 ***	11	0.031	0.97	155	1.598	0.206
Gala	447	19.34	<0.001 ***	447	8.948	<0.001 ***	177	26.18	<0.001 ***	15	1.825	0.195	177	9.413	<0.001 ***
George Cave	447	2.969	0.052	447	14.450	<0.001 ***	177	2.928	0.056	15	0.537	0.595	177	5.185	0.006 **
Golden Delicious	437	1.428	0.241	437	12.770	<0.001 ***	na	na	na	14	0.814	0.463	177	0.118	0.889
Jolyne	374	10.41	<0.001 ***	360	0.692	0.501	na	na	na	13	0.294	0.750	175	5.156	0.007 **
Jonathan	447	1.083	0.34	447	5.722	0.004	177	0.295	0.745	15	0.809	0.464	177	1.406	0.248

Cultivar	Firmness (kg)			Soluble Solids Content (%Brix)			Red Colour Coverage (%)			Dry Matter Content (%)			Individual Fruit Weight (g)		
	df	f	P	df	f	P	df	f	P	df	f	P	df	f	P
						**									
King of the Pippins	397	3.681	0.026 *	404	0.557	0.574	167	18.82	<0.001 ***	12	0.268	0.769	167	5.923	0.003 **
Lappio	363	18.38	<0.001 ***	363	4.587	0.011 *	na	na	na	14	0.69	0.518	166	2.584	0.079
Stark's Earliest	357	2.723	0.067	326	8.234	<0.001 ***	177	13.44	<0.001 ***	15	2.101	0.157	177	3.352	0.037 *
Winter Pearmain	356	1.743	0.176	356	2.963	0.053	177	51.18	<0.001 ***	12	4.257	0.040 *	177	19.05	<0.001 ***
Yellow Bellflower	440	10.17	<0.001 ***	440	0.778	0.46	na	na	na	15	0.111	0.896	170	4.907	0.008 **

*Significant at p<0.05, **Significant at p<0.01, *** Significant at p<0.001, na Not Available

Appendix 5.2*. Principal Component Analysis of variables used within multivariate apple fruit quality study.*

A principal component analysis (PCA) explained 92.9% of the cross-cultivar (14 cultivars, 2017-21) fruit quality database variance across the first two principal components. The first (PC1) accounted for 88.4% of the variance and was largely driven by three temperature parameters (Tmean, Tmax and Tminmaxdiff) with a squared cosine (cos2) value >0.4. The second PC2 (representing 4.5% of total variance) was largely driven by total fresh weight fruit yield per tree (YieldFW) and two further temperature parameters (Tmin and Twinter). With regard to fruit quality, DMC and SSC were more strongly associated with PC1 but fruit weight with PC2. Neither firmness nor RCC were strongly associated with either PC1 or PC2. Fruit SSC and DMC were correlated similarly with independent variables as displayed by their similar biplot loading location.



Principal Component Analysis (number of components = 2) loading biplot of all independent and dependent variables tested within the standardised cross-cultivar population apple fruit quality analysis. Darker gradient lines indicate a strong representation of the variable on a particular component (i.e. a higher Cos2 value). The key for the weather and production variables is listed in Table 5.1.