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Effect of Crop Load, Conditioning, Gradual Cooling, and Storage Temperatures on the Development of Multiple Physiological Disorders in Three Apple Cultivars in Norway.

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Abstract

Reducing food waste and post-harvest losses by minimizing physiological disorders is of importance worldwide and in Norway, especially in apple (*Malus domestica* Borkh). The main cultivar grown in Norway, 'Red Aroma', is susceptible to soft scald, a chilling injury, in storage. Soft scald is often controlled by storing fruit of sensitive cultivars in storage temperatures at or above 3°C, in addition to gradual cooling or conditioning. While soft scald is primarily affected by storage temperature, there are other factors that may affect incidence, including fruit size, and crop load. In this experiment, regulating crop loads were used as a way to ensure different fruit sizes and examine their effect on soft scald incidence. Two cultivars, 'Eden' and 'Fryd', were recently bred for the Norwegian market. Both cultivars have 'Honeycrisp' as a parent, which is susceptible to many physiological disorders, including soft scald. Determining if 'Eden' and 'Fryd' was susceptible to soft scald or other physiological disorders was therefore examined in a range of storage temperatures.

To obtain different fruit sizes, 'Red Aroma' trees were thinned to low, medium, or high crop loads and monitored throughout the season. Fruit were harvested at commercial harvest and exposed to 1 or 4°C. Separately, fruit from the three cultivars were exposed to six different temperature regimes for a gradual cooling experiment. Fruit were either placed in 7°C and temperatures were lowered by 1°C each day until they reached 1, 2, or 4°C, or placed directly at those three temperatures. Simultaneously, 'Eden' and 'Fryd' fruit were picked for a conditioning experiment and placed in six temperature regimes. Fruit were placed in 10 or 20°C and temperatures remained constant for one week before fruit were moved to 1 or 4°C, or fruit were placed directly in 1 or 4°C. All fruit were stored in regular atmosphere for nine or ten weeks, followed by a shelf-life period for 14 days at 20°C. Fruit quality (background color, firmness, soluble solids concentration, titratable acidity, and starch index) was monitored throughout storage, and fruit were assessed for physiological damage and fungal decay at the end of the shelf-life period.

Low crop load 'Red Aroma' fruit were largest throughout most of the growing season and at harvest. Unexpectedly, they were less mature at harvest than medium or high crop load fruit. However, fruit quality was overall similar for all three crop loads after cold storage and the shelf-life period. After the shelf-life period, no differences were found in soft scald incidence between crop loads. The differences in crop loads or fruit sizes may not have been great enough to influence soft scald susceptibility. Regardless of crop load, fruit stored in 1°C

had more soft scald than fruit stored in 4°C. This was also true of the ‘Red Aroma’ fruit from the gradual cooling experiment, where fruit stored in 4°C or gradually cooled from 7 to 4°C had less soft scald and similar fruit quality compared to all other temperature regimes. However, regardless of temperature regime, all fruit in the gradual cooling experiment had unmarketable firmness after nine weeks of cold storage, suggesting that short storage durations, harvesting at earlier maturities, and storage in 4°C or gradual cooling to 4°C may result in lower post-harvest losses in ‘Red Aroma’ fruit.

In contrast, ‘Eden’ and ‘Fryd’ fruit both maintained high fruit quality throughout cold storage, yet both cultivars were susceptible to multiple physiological disorders. Soft scald was not found in ‘Eden’ fruit and was only present in ‘Fryd’ fruit when conditioned at 20°C and stored at 1°C. Watercore was found in ‘Eden’ fruit at harvest and throughout storage and was not completely dissipated after the shelf-life period. Core browning was the most commonly found disorder in both cultivars. In ‘Eden’, core browning was minimized by storage at 1 or 2°C, with or without gradual cooling. Conditioning at either 1 or 4°C increased core browning incidence compared to direct storage. However, in ‘Fryd’, core browning was best controlled by storage at 4°C with conditioning or gradual cooling, although unacceptable amounts of core browning were found in each temperature regime. Other disorders, such as greasiness, bitter pit, and diffuse flesh browning were also found, but in small amounts. Fungal decay was limited in all experiments and unaffected by any treatment. In summary, physiological disorders, especially core browning, in ‘Eden’ and ‘Fryd’ and soft scald in ‘Red Aroma’ may have been caused by a variety of pre- and post-harvest factors and should be studied over multiple seasons, tree ages, locations, crop loads, and maturities at harvest.

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1 Introduction

Worldwide, 30% of all food produced for consumption is wasted (Gustavsson et al., 2011). As such, there is an increasing focus on reducing food waste, which will help achieve some of the UN's broader Sustainable Development Goals (SDG). Specifically, SDG 12.3 has a goal of halving worldwide food waste by 2030 throughout the supply chain, including through reducing post-harvest losses (UN General Assembly, 2015). Many countries also have specific goals in addition to those set out by the UN. In Norway, a further agreement on food waste reduction has been voluntarily created between private companies and the government, and potential further measures are currently being examined by the Norwegian government (One Planet, 2023). There is an immediate need to reduce food waste, which can be achieved in part by increasing regional production and decreasing post-harvest losses.

In apple (*Malus domestica* Borkh.) production, post-harvest losses have been shown to vary from 4-28% (Argenta et al., 2021). In Sweden, up to 10% of harvested fruit may be lost due to physiological disorders, with additional losses, sometimes up to 20%, due to fungal decay (Tahir, 2019). Thus, understanding ways to minimize physiological disorders and fungal decay in storage are critical parts of reaching the UN goal of reducing food waste.

1.1 Norwegian fruit production

Fruit production in Scandinavia is generally small-scale, with Norwegian apples accounting for only 13% of total apple consumption in the country (Svanes and Johnsen, 2019). In 2022, total apple production in Norway was 8,600 metric tons (Øie, 2023). Despite this low market share, Norwegian consumers prefer local apples, resulting in recent increased plantings and production (Svanes and Johnsen, 2019). Increasing production volumes provide more opportunities to maximize storage life of the fruit and reduce food waste.

Norwegian fruit production is unique from most other production systems in the world, due to the climatic factors, mainly the cool summers and long days during a short growing season (Svanes and Johnsen, 2019). As such, there are unique growing challenges, including limiting the cultivars that can be grown commercially. In Norway, 'Elstar' is among one of the latest ripening cultivars for commercial production, with harvest occurring in early to mid-October (Meland, 2009). In comparison, in a study on 'Elstar' fruit grown in the Netherlands, harvest occurred between the middle of September and early October (Schouten et al., 1998),

and ‘Elstar’ fruit grown in Italy can be harvested in early August (Südtiroler Apfel g.g.A., n.d.), showing a great variation in growing season between Norway and other production regions. Therefore, common cultivars harvested after ‘Elstar’ in Italy or Netherlands are unlikely to be commercially viable in Norway. Fruit quality is affected by many climatic factors and will therefore influence the storability of fruit. It is important to understand how best to store Norwegian fruit specifically, due to the unique cultivars and fruit quality compared to most other production regions.

The most commonly grown cultivars in Norway include ‘Red Aroma’, ‘Discovery’, ‘Summerred’, ‘Red Gravenstein’ and ‘Rubinstep’, which make up to 90% of the total production (Øie, 2023). Recent interest in new cultivars worldwide has resulted in many more cultivars being introduced in Norway as well (Frøynes et al., 2020), including ‘Eden’ and ‘Fryd’, which provide new challenges during production, storage, and distribution, as some of these cultivars have never been commercially grown before. Determining storage regimes for these new cultivars is of extreme importance to maximize their storability and minimize associated food waste.

1.2 Cultivars

1.2.1 ‘Eden’ and ‘Fryd’

Following recent worldwide interest in new apple cultivars, new cultivars have been introduced in Norway, including ‘Eden’ and ‘Fryd’, which are marketed for the Norwegian market. As ‘Eden’ and ‘Fryd’ are new cultivars, there are only preliminary harvest and storage recommendations available. Both cultivars were bred at Wageningen University and are crosses between ‘Honeycrisp’ and ‘SQ159’, commercially known as Magic Star® if grown conventionally, or Natyra® if grown organically (Fresh Forward Breeding & Marketing BV, 2022).

Limited post-harvest research is available on ‘SQ159’, however one study completed in Germany over four growing seasons did examine post-harvest quality of the fruit. The study found that the fruit developed no significant physiological disorders and was capable of being stored in controlled atmosphere (CA) for at least seven months while maintaining high firmness and soluble solids concentration (SSC) (Neuwald et al., 2016). Furthermore, ‘SQ159’ is marketed as having a long storage life (Fresh Forward Breeding & Marketing BV, n.d.). Although not mentioned in the published literature, an industry presentation suggested that ‘SQ159’ is susceptible to watercore (de Wild, 2022).

In contrast to ‘SQ159’, substantial research has been conducted on storage of ‘Honeycrisp’, as it has rapidly become one of the most widely grown apples in the US and is well known to be susceptible to many physiological disorders in storage. Research has shown that ‘Honeycrisp’ fruit maintain high quality in a variety of storage temperatures, durations, and atmospheres. However, a wide variety of disorders and problems, including bitter pit, chilling injury (in the form of soft scald, soggy breakdown, or low temperature breakdown), core browning, diffuse flesh browning, greasiness, internal CO_2 injury, leather blotch, lenticel blotch pit, vascular browning, and wrinkly skin have been shown to form in high percentages in various storage regimes (Al Shoffe et al., 2021, 2020; Watkins et al., 2004; Watkins and Mattheis, 2018). Preliminary research on two other cultivars that have ‘Honeycrisp’ as a parent has shown that one, ‘NY1’ grown in New York, is susceptible to core browning, vascular browning, greasiness and wrinkly skin (Brown et al., 2012), while ‘WA38’ grown in Washington may not be susceptible to most of the disorders common to ‘Honeycrisp’, although it can be greasy (Hanrahan et al., 2014). Therefore, it is possible that ‘Eden’ and ‘Fryd’ fruit may have inherited susceptibility to some of the disorders commonly found in ‘Honeycrisp’ fruit.

1.2.2 ‘Red Aroma’

The cultivar ‘Red Aroma’ is a strain of ‘Aroma’, which itself is a cross between ‘Ingrid Marie’ and ‘Filippa’. The cultivar was bred in Sweden and has been grown commercially since 1973. Currently, it is one of the most widely produced cultivars in Scandinavia (Tahir, 2006). It has good eating qualities and high yields (Meland, 2011). It is characterized as having yellow ground color, with red cover color and white to yellowish flesh (Tahir, 2006).

The fruit are commonly stored for a maximum of four to six months, as firmness is lost rapidly in storage. Additionally, ‘Red Aroma’ is chilling sensitive and can develop soft scald in temperatures below 3°C (Børve and Stensvand, 2016; Landfald, 1987). Large fruit have also been shown to have higher susceptibility to physiological disorders than small fruit (Weigl et al., 2023). The cultivar is also susceptible to senescent breakdown near the end of its storage period, and has limited resistance to fungal decay, making careful storage important (Børve and Stensvand, 2016; Landfald, 1987; Tahir, 2006).

1.3 Physiological disorders

Physiological disorders make up a significant proportion of losses in apple production. There are many physiological disorders that appear during storage of apples, and can be caused by many different factors, some of which may be cultivar specific. Although many physiological disorders only appear after storage has started and are influenced by factors in storage, it is common that pre-harvest factors may further dispose the fruit to a certain disorder. Generally, cultivar, rootstock, growing season, nutrient concentrations, and maturity of fruit at harvest can have significant effects on disorder development in storage. Disorder susceptibility is also influenced by cultivar and causes or symptoms may be cultivar specific as well (Freitas and Pareek, 2018; Sidhu et al., 2023; Wilkinson and Fidler, 1973). Symptoms of the same disorder may differ between cultivars, and different causes may result in similar symptoms. Descriptions of disorders can vary as well, making proper classification of disorders difficult in some instances (Watkins and Mattheis, 2018) Descriptions, causes, and methods of control for three relevant disorders in apples are described below.

1.3.1 Core browning

Core browning, also known as core flush or brown core, involves brown or pink discoloration of flesh in and around the core area, which can extend outwards past the coreline. Affected tissues are often soft and moist compared to unaffected tissue. Many commercially important cultivars are susceptible, including ‘Braeburn’, ‘Empire’, ‘Fuji’, ‘Gravenstein’ ‘Honeycrisp’, and ‘McIntosh’ (Meheriuk et al., 1994; Watkins and Mattheis, 2018).

There are many factors that can contribute to development of core browning, and it has been alternately defined as a chilling injury, CO_2 injury, or form of senescence (Meheriuk et al., 1994; Pierson et al., 1971; Snowdon, 1990), with recent publications defining it primarily as a chilling injury or CO_2 injury, suggesting exact causes are still unknown (Sidhu et al., 2023; Watkins and Mattheis, 2018). Delaying cold storage, long storage periods, and high water loss can all increase core browning development. Low storage temperatures and high CO_2 concentrations may also increase core browning, especially if stored in regular atmosphere (RA) storage; 1-methylcyclopropene (1-MCP), an ethylene inhibitor common in other countries, has been known to both increase or decrease core browning depending on the cultivar (Watkins and Mattheis, 2018; Wilkinson and Fidler, 1973). While primarily a post-harvest disorder, pre-harvest factors may influence incidence, including cold growing temperatures, low calcium content, early harvest, and presence of watercore. It has been

suggested that causes may even be region or season dependent (Smock, 1977; Wilkinson and Fidler, 1973).

1.3.2 Soft scald

Soft scald is a well-documented chilling injury that occurs in multiple cultivars, including 'Aroma', 'Golden Delicious', and 'Honeycrisp' (Landfald, 1987; Watkins and Mattheis, 2018). It is characterized by ribbon-like brown sections of the peel, with well-defined edges that increase in size over time and can, in severe cases, cover over half of the peel. It often partially extends into the skin, but only directly under already affected parts of the peel. Storage below 2°C aggravates disorder incidence in most susceptible cultivars, and temperatures above 3°C are often recommended to reduce disorder prevalence (Brooks and Harley, 1934; Plagge, 1926; Watkins et al., 2004; Watkins and Mattheis, 2018).

However, storage at 3°C alone may not be sufficient in years with high incidence, and has historically been impractical, as many cultivars are stored at temperatures below 3°C and other aspects of fruit quality decrease faster at these warmer temperatures (Harley and Fisher, 1931; Watkins et al., 2004). Many studies have implemented a period of delayed cooling after harvest in 10-20°C followed by lower final storage temperatures. Responses to this delayed cooling can be cultivar dependent, either increasing or decreasing soft scald incidence (Brooks and Harley, 1934; Watkins et al., 2004; Wilkinson and Fidler, 1973).

Commercial storage of 'Honeycrisp' in the US commonly employs a "conditioning" period of 10°C for one week before the fruit are placed in 3°C for the rest of the storage period (Watkins and Mattheis, 2018). This storage regime works well but is not always sufficient to completely eliminate soft scald in high incidence years, and can increase expression of bitter pit, another common physiological disorder (Al Shoffe et al., 2020). In 'Aroma' fruit, there is more variability, with delayed storage either reducing or having no effect on soft scald incidence. However, storage at 3-4°C generally decreases incidence compared to storage in colder temperatures (Børve and Stensvand, 2016; Landfald, 1987).

While the causes of soft scald are not fully known, there are many factors aside from storage temperature that can influence disorder development, including genetic predisposition, growing season, and maturity. In general, stress responses and metabolism may be different in fruit with soft scald than unaffected fruit, as a result of altered expression of multiple genes (Leisso et al., 2016). A gene region linked to soft scald incidence, and a specific group of alleles, or haplotype, that are associated with susceptibility to soft scald has been identified

(Miller et al., 2021). Two sets of this highly inheritable haplotype are present in ‘Honeycrisp’, meaning offspring of ‘Honeycrisp’, including ‘Eden’ and ‘Fryd’ are likely to inherit one copy and therefore be susceptible to soft scald as well. However, it was also noted that presence of the haplotype did not result in soft scald expression every year, due to external effects, such as maturity, location, and environmental factors (Howard et al., 2018; Miller et al., 2021).

Environmental factors have also been shown to have an impact on soft scald development as well. More specifically, climate factors such as rainfall, temperature, and relative humidity have been examined and increased sensitivity to soft scald at multiple times during the growing season have been suggested. Growing location also has an impact, with regional differences often being noted (Moran et al., 2020; Watkins et al., 2004; Wilkinson and Fidler, 1973). Variability between orchards and even between trees within an orchard has also been shown (Moran et al., 2009; Tong et al., 2003). Maturity has frequently been linked to soft scald, with advanced maturity or later harvests generally, but not consistently, increasing soft scald incidence (Brooks and Harley, 1934; Moran et al., 2010; Sjöstrand et al., 2023).

1.3.3 Watercore

Watercore is a disorder in which sorbitol accumulates in the inter-cellular spaces, resulting in a “glassy”, water-soaked, translucent appearance of the flesh. Many cultivars have some susceptibility to watercore, including ‘Fuji’, ‘Honeycrisp’, and ‘Red Delicious’. There are multiple types of watercore, defined in part by where in the flesh the symptoms start (DeLong et al., 2004; Faust et al., 1969; Snowdon, 1990; Watkins and Mattheis, 2018).

Stress watercore, due to stressful conditions during the growing season such as high temperatures, starts near the peel. The other two types of watercore are generally maturity-related and begin either around the vascular bundles of the coreline and expand outwards into the cortex (radial watercore), or near the carpel walls and expand throughout the core area before spreading past the coreline (block watercore) (Bowen and Watkins, 1997; Faust et al., 1969; Watkins and Mattheis, 2018). In some contexts, radial and block watercore are distinguished (Harker et al. 1999), but most studies do not distinguish between these two forms of maturity-related watercore.

Watercore occurs due to altered sorbitol transport into cells while the fruit is on the tree, resulting in an increase in sorbitol accumulation in the extracellular spaces, leading to the water-soaked appearance. Exact causes of this phenomenon are still unknown (Ferguson et al., 1999; Itai, 2015). There have also been multiple studies suggesting a relationship between

watercore and calcium. Often, but not consistently, these suggest sufficient calcium concentrations maintain membrane integrity or delay maturation, both of which can reduce watercore incidence and severity (Faust et al., 1969; Harker et al., 1999; Itai, 2015; Marlow and Loescher, 1984).

With few exceptions, watercore only develops on the tree, and does not increase in severity in storage, and if present in low amounts can often dissipate during storage. However, severe watercore can result in internal browning in the impacted tissue, described as watercore breakdown, and in some cultivars even tissue where watercore has dissipated can later develop watercore breakdown (Marlow and Loescher, 1984; Watkins and Mattheis, 2018). Multiple browning disorders have been associated with watercore, such as coreline browning, flesh browning, internal browning, brown watercore, and watercore breakdown (Argenta et al., 2002; Marlow and Loescher, 1984). It is likely some of these names describe the same disorder. Brown watercore has been described to co-occur with watercore breakdown (Smock, 1977), or has been used to presumably describe watercore breakdown (Argenta et al., 2002).

Environmental factors, fruit maturity, and calcium levels in the fruit play a large role in watercore development, impacting presence, morphology, and severity (Ferguson et al., 1999; Watkins and Mattheis, 2018). Block and radial watercore can be influenced by low temperatures during fruit maturation or throughout the whole season, and by advanced maturity or late harvest (Ferguson et al., 1999; Yamada et al., 1994; Yamada and Kobayashi, 1999). Incidence and severity have been shown to vary between growing regions and even within orchards and trees (Harker et al., 1999; Itai, 2015; Wang et al., 2023).

1.4 Major factors affecting storability of fruit

1.4.1 Crop load and fruit size

Crop load can be described as the number or weight of fruit per unit (commonly branch or tree) (Wünsche and Ferguson, 2005); here, crop load will refer to the number of fruit per tree. Crop load is found to affect many aspects of tree and fruit growth, including shoot growth, leaf morphology, creation of fruiting and vegetative buds, and biennial bearing. It can also impact storability of fruit by altering the physiology of fruit and susceptibility to both physiological disorders and decay (Wünsche and Ferguson, 2005).

Final fruit size is well correlated with crop load; low crop loads increase fruit size compared to high crop loads. Additionally, low crop loads or large fruit can result in larger fruit that mature sooner with more desirable quality at harvest (Kelner et al., 2000; Mészáros

et al., 2021; Wünsche and Ferguson, 2005). However, these fruit may not store well, due in part to advanced maturity. They can also be more susceptible to some storage disorders, including calcium related disorders (Link, 2000; Sidhu et al., 2023; Wünsche and Ferguson, 2005).

Calcium concentration per fruit often increases in larger fruit or in higher crop loads. Other mineral concentrations may also be affected by crop load, thereby influencing susceptibility to disorders and storability of the fruit. The relationship between calcium and crop load is particularly important, as fruit from low crop loads often have low levels of calcium and high levels of potassium, increasing bitter pit incidence in susceptible cultivars (Fallahi et al., 1985; Link, 2000; Wünsche and Ferguson, 2005). Fruit size has also been shown to be directly related to bitter pit incidence, with large fruit having higher levels of bitter pit than small fruit (Ferguson and Triggs, 1990; Perring and Jackson, 1975).

Light crop loads have also been shown to increase internal disorders in some cultivars, notably core browning and internal breakdown or browning in 'Cox's Orange Pippin' and 'Braeburn' (Johnson, 1992; Tough et al., 1998). In addition, increases in radial watercore, core rots, and some other forms of internal browning have been shown (Wünsche and Ferguson, 2005). Fruit size specifically has been shown to increase incidence of certain physiological disorders (Lee et al., 2013; Lidster et al., 1975), including soft scald and superficial scald in 'Aroma' (Weigl et al., 2023).

Most research on different crop loads involves effects on tree and fruit physiology, and comparatively little research follows the fruit through past time of harvest (Wünsche and Ferguson, 2005). Furthermore, studies that do follow fruit into storage often use small numbers of fruit, e.g. 15 fruit per replicate in Delong et al., (2006), or have contradicting results (Sidhu et al., 2023). Therefore, there is a relative lack of understanding on how crop loads and fruit size affect final fruit quality after storage and shelf-life.

1.4.2 Fruit quality, maturity, ripeness, and the interactions with storage length, temperature, and atmosphere

Quality is an important term with varying definitions depending on audience. For consumers, this often means external fruit coloration, flavor, texture, nutritional value, and absence of defects, while for producers and distributors external appearance, texture, and storability can be important (Kader, 2001, 1999; Musacchi and Serra, 2018). Specific standards in the EU and within Norway regulate and provide a minimum quality needed for fresh sale

(Comission Regulation (EC) No. 84/2004, 2004; Rosseland and Eidsvik, 2021). Fruit quality can be influenced by many pre- and post-harvest factors, such as cultivar, nutrient concentrations, and growing and storage temperatures. Fruit quality is largely determined at harvest and fruit quality is often associated and confused with maturity and ripeness (Abbott, 1999; Kader, 2001, 1999; Musacchi and Serra, 2018).

Maturity can be separated into two stages in fruit development leading to eventual ripening - physiological and horticultural maturity. Physiological maturity is when a fruit is able to continue developing even when detached from the tree, whereas horticultural maturity is when fruit have developed characteristics needed for final use. Fruit must be physiologically mature before harvest to ensure quality after storage (Kader, 1999; Toivonen, 2007).

Ripeness is when fruit has obtained characteristic quality and aesthetics to be accepted by consumers. This involves processes during late maturation through early stages of senescence that alter fruit quality, such as color, flavor, and texture. Fruit can be classified into two categories based on whether ripening can continue after harvest. Climacteric fruits are able be picked mature but unripe and can continue ripening after harvest; these fruit include apples. Climacteric fruit produce large amounts of ethylene and are more sensitive to it compared to non-climacteric fruits, and their ripening phase is concurrent with a sharp increase in ethylene production. Once this ripening phase has started in climacteric fruits, it often progresses quickly due to the autocatalytic nature of ethylene production (Kader, 1999; Toivonen, 2007).

There are many methods of measuring fruit quality both pre- and post-harvest, although these often measure ripeness (Abbott, 1999). Among the tests commonly used are determination of background color as a measurement of ripeness, firmness as a measurement of fruit texture, SSC and titratable acidity (TA) as measurements of eating quality, and starch content as a measurement of ripening phase. Desired values for each measurement are cultivar specific and depend on the intended use of the fruit. Fruit that will be marketed directly and sold ripe off the tree will have different desired measurement values than fruit intended for short term storage of a few months which in turn will have different desired measurement values than fruit intended for long term storage of a year or longer (Watkins, 2003).

Fruit quality, maturity, and ripeness interact with storage conditions such as temperature, atmosphere, and length, resulting in complex differences between fruit throughout and after storage. Storage conditions are generally designed to slow ripening and maintain fruit quality. Fruit are often harvested unripe, before the climacteric rise in ethylene production and subsequent rapid ripening. This allows the fruit to be stored for longer periods of time than if they were harvested ripe. However, this comes at a sacrifice of overall taste and eating quality,

as fruit harvested after the start of the climacteric rise often has the best aroma and flavor development (Fidler, 1973; Watkins, 2003). Regardless of harvest timing, there are cultivar specific differences in storage length as well. Early season cultivars commonly maintain quality for a shorter amount of time than late season cultivars, due to higher respiration rates in early season cultivars (Watkins, 2003).

One of the most important post-harvest factors to ensure a long storage life of fruit is storage temperature. Generally, lower storage temperatures increase the storage life of fruit, as long as the fruit are kept above the freezing point of tissues (-2°C). Low storage temperatures reduce the respiration rate of fruit, thereby reducing the rate of ripening. While the ripening rate is lower at 0°C than 4°C, many cultivars are chilling sensitive, meaning they develop chilling-induced disorders below a certain temperature (Fidler, 1973; Watkins, 2003). Often, the critical temperature for these cultivars is between 2-5°C. Therefore, while some cultivars are stored at -1 or 0°C, it is also common to store fruit from chilling sensitive cultivars at 3 or 4°C (Fidler, 1973; Watkins, 2003).

There is a compromise however between storage temperature and quality in chilling sensitive cultivars. While warmer temperatures reduces chilling disorders thus extending the storage life of the fruit, it also hastens ripening, which reduces the storage life of the fruit. Storage at 1-2°C in some cultivars represents a compromise between maintaining firmness and lowering chilling injury compared to storage at 0°C (Doerflinger et al., 2015). In contrast, some chilling sensitive cultivars are not cooled to their final storage temperature immediately after harvest. Instead, they are exposed to temperatures somewhere between field and final storage temperatures which can further reduce chilling injury (Watkins and Mattheis, 2018). As there are many variations of this delayed cooling which will be described further in this document, definitions to distinguish the two most relevant types are necessary.

Gradual cooling, or stepwise cooling, is distinguished as lowering the storage temperature multiple times until the final temperature is reached. For example, starting the storage temperature at 10°C for two days, lowering it to 4°C for two days, then finally lowering it to the final storage temperature, e.g. 1°C.

Conditioning, or preconditioning, is distinguished as keeping fruit for a period of time in one consistent temperature before lowering it to the final temperature. For example, storing fruit in 10°C for four days before placing the fruit in e.g. 1°C as the final storage temperature.

Storage atmosphere can also be an important aspect in maintaining fruit quality. Lowering the O_2 and raising the CO_2 concentration in the air has long been used to delay ripening by slowing respiration rates. Storing fruit in CA can limit respiration and ethylene

biosynthesis, thereby delaying ripening compared to storing fruit in RA. CA storage can also delay or eliminate development of certain physiological disorders, such as superficial scald (Prange and Wright, 2023; Watkins, 2017). However, CA storage can also increase sensitivity of fruit to other disorders, especially those affected by low O_2 or high CO_2. Specific storage recommendations are often made for cultivars susceptible to CO_2 injury when storing in CA (Contreras et al., 2014; Watkins and Mattheis, 2018).

1.5 Research goals and objectives

The overarching objective of these experiments was to gain knowledge of pre- and post-harvest factors that influence fruit quality and disorder development in storage of 'Red Aroma', 'Eden', and 'Fryd' fruit, thereby providing insight towards increasing storability of Norwegian grown fruit. Specific objectives were:

- Study the effect of different fruit sizes, achieved through different crop load levels, on maturity, fruit quality, and disorder development of 'Red Aroma' fruit at harvest and during storage, with particular emphasis on soft scald development.
- Study the effect of storage temperatures and gradual cooling on overall fruit quality and disorder development on chilling sensitive cultivars.
- Examine if gradual cooling to lower end temperatures is a viable alternative to storage at warmer temperatures for chilling sensitive cultivars, with focus on both fruit quality and disorder development.
- Study newly released cultivars ('Eden' and 'Fryd') stored in many different temperature combinations to assess their overall storage potential.
- Investigate which, if any, storage disorders 'Eden' and 'Fryd' are susceptible to, with particular focus on chilling injury and bitter pit.

2 Methods

2.1 Weather

Daily weather records were obtained from the weather station at NIBIO Ullensvang in south-eastern Norway. Growing degree days (GDD) were calculated by averaging the maximum and minimum daily temperatures then subtracting 10°C (base 10°C) from the date of full bloom until harvest (Tong et al., 2016). Dates of full bloom across the Ullensvang region for 'Eden' and 'Fryd' were 22 May and 21 May in 2022, respectively (Gasi et al., 2023). At NIBIO Ullensvang, 'Red Aroma' full bloom date was 18 May in 2022. Records were obtained for full bloom date for each of the past 10 years as well. When totaling accumulated GDD, 18 May (the date for full bloom of 'Red Aroma' at NIBIO Ullensvang in 2022) was used as the first measurements, and accumulated GDD was calculated up until 30 September, the latest harvest date in the present experiments.

2.2 'Red Aroma' crop load experiment

2.2.1 Thinning of the trees

A row of 'Red Aroma' trees located in Lofthus, Norway was selected for this experiment in early June 2022 with trees of uniform size. The trees were planted on M.9 rootstock in 2003 in a conventionally managed orchard. Twelve sections of seven trees each with a uniform crop load based on visual inspection were selected. Efforts were made to have all seven trees within a section be directly next to each other, however at times, sections included up to 10 trees with one to three trees excluded due to low crop loads.

In total, the 12 sections were selected and divided into a 3x4 design, with three pre-determined crop loads and four replicates. The three crop loads were assigned to each replicate, using the RANDOM equation in MS Excel (2021) to determine the order of crop load within each replicate. The three pre-defined crop loads were "High", "Medium", and "Low", and were 150 fruit per tree, 100 fruit per tree, and 50 fruit per tree, respectively (Table 2.1).

Table 2.1: Distribution of crop load level and replicate in the field. The crop load levels within each replicate consisted of 7 trees, totaling 84 trees overall.

Replication	Crop load level
1	High
1	Low
1	Medium
2	Low
2	High
2	Medium
3	Low
3	Medium
3	High
4	Medium
4	Low
4	High

The trees were monitored twice per week until after June drop to assess fruit growth and thinning timing, at which time they were thinned to the assigned uniform crop loads ± 5 fruit. When possible, fruit were thinned to one fruit per cluster, but in some instances two fruit per cluster remained spaced throughout the tree. If whole clusters were removed, efforts were made to spread out removal throughout the entire tree. Thinning started on 30 June and ended on 4 July.

2.2.2 Pre-harvest fruit assessments

When all thinning was completed, three representative fruit per tree were labeled for diameter measurements, with 252 fruit labeled in total. These diameter measurements were taken weekly until the day before harvest (Table 2.2) with digital calipers (SY295, Sylvac, Malleray, Czech Republic). If labeled fruit fell off the tree, another fruit of average diameter for the tree was labeled and used for all future measurements.

Table 2.2: Dates of diameter measurements and fallen fruit identification on ‘Red Aroma’ fruit from thinning until harvest in 2023.

Date	July				**August**					September		
	5	11	18	25	**1**	**9**	**15**	**22**	**29**	5	12	21
Fruit diameter measurements	x	x	x	x	x	x	x	x	x	x	x	x
Fallen fruit identification						x	x	x	x	x	x	x

Beginning on 9 August, fallen fruit within the general area of each replicate were cut in half longitudinally to determine potential causes of drop. Fruit were gathered from both sides of and underneath the trees within the replicate. Particular attention was paid to whether the likely cause of drop was mechanical, insect, physiological, or fungal damage. These damage assessments were completed weekly until harvest, on the same days as the fruit diameter measurements (Table 2.2).

2.2.3 Harvesting and placement of fruit in cold storage

Harvest occurred on 22 September 2022. Harvest timing was determined based on typical commercial harvesting windows and using a starch-iodine test to determine the amount of starch hydrolysis (Norsk landbruksrådgiving, 2020). Ten fruit per replicate and crop load were used for the starch-iodine test on 12 September and 10 fruit per crop load on 19 September.

Each tree was strip picked and all fruit was placed in boxes specific for each crop load level, replicate, and tree number. After harvest was completed, all fallen fruit was gathered and placed in boxes based on crop load and replicate, separate from the harvested fruit. All fruit was transported back to NIBIO Ullensvang and left in a storage hall overnight before fruit from each tree was sorted into three categories:

1. Small fruit (<60 mm in diameter)
2. Decayed, damaged, or otherwise unsellable fruit
3. Class 1 fruit (un-damaged and 60-95 mm in diameter).

No fruit greater than 95 mm in diameter were found. Fruit from three trees were excluded from further analysis because the fruit was visibly smaller prior to harvest than that on the other trees within the replicate. Total numbers of small, damaged, and class 1 fruit were counted per replicate, and the small or unsellable fruit were placed in cull bins.

After each tree was sorted, 240 class 1 fruit from all seven trees per crop load replicate were placed in labeled 6416-IFCO boxes with two layers of 30-count cardboard trays in each box. Half the boxes per crop load replicate were placed in a cold storage chamber at 1°C, while the other half were placed in cold storage at 4°C, all of which were stored for nine weeks. An additional 10 fruit per crop load were set aside for quality analyses that day.

2.2.4 Removal of fruit from cold storage and damage assessments

On 24 November, all fruit were removed from storage. The fruit were externally assessed for any physiological or pathological damage, with particular attention being paid to soft scald incidence. Additionally, any discoloration that wasn't clearly identifiable as soft or superficial scald was recorded separately as potential scald. When possible, rot was identified based on fungal signs. Ten undamaged fruit per crop load replicate and temperature were removed for assessment of background color, blush color, firmness, starch, SSC, TA, and damage registration (full quality analysis), and the remaining fruit were kept in 20°C for 14 days as a shelf-life simulation. After four and 11 days at 20°C, fruit were again visually assessed for external disorders, particularly soft scald.

After the shelf-life period, full quality analysis of 10 fruit samples were again taken from each crop load replicate at each temperature. The remaining fruit were examined for physiological and pathological damage incidence again. If soft scald was present, the fruit was cut to determine if the soft scald went into the flesh, and if so, how far. The approximate percentage of the surface covered by soft scald was also recorded. If fungal decay was found, it was sometimes cut to determine if fungal signs were present internally (e.g., core rot) that could help in identification. Some decayed fruit were left to incubate for two weeks in constant light at 20°C to see if any fungal signs developed to help with positive identification.

2.3 Gradual cooling of 'Red Aroma'

2.3.1 Harvesting

Harvest of 'Red Aroma' fruit from a commercial orchard in Utne, Norway occurred on 20 September 2022, the same week commercial harvesting of the cultivar began. Trees were on M.9 rootstock and planted in 2014 in a conventionally managed orchard. Only ripe, well-colored, undamaged, class 1 fruit were picked, and fruit were selected evenly throughout the orchard. Fruit were picked and immediately transferred into 6416-IFCO boxes with two layers of 30 fruit in each box; in total, 2,160 fruit were picked, with 30 additional fruit picked for quality analysis the same day.

2.3.2 Creation of temperature regimes and placement in cold storage

All fruit were placed in RA storage at NIBIO Ullensvang in either cold storage rooms or chambers the day they were harvested. Fruit were either placed directly into 1, 2, 4°C, or

placed into a 7°C cold chamber. After 24 hours, the temperature in the 7°C cold chamber was lowered one degree every day at 15:00 until the chamber reached 1°C seven days later. When the temperature in the cold chamber was lowered from 5°C to 4°C, six boxes were removed and placed with the fruit that went directly after harvest into 4°C. The same occurred with the fruit that would remain at 2°C and at 1°C when the cold chamber temperature reached 2°C and 1°C respectively. In total, there were six different temperature regimes, with three replicates in each temperature regime:

1. Constant 4°C (4°C)
2. Constant 2°C (2°C)
3. Constant 1°C (1°C)
4. Initial placement in 7°C where the temperatures were lowered by one degree per day until 4°C (7 – 4°C)
5. Initial placement in 7°C where the temperatures were lowered by one degree per day until 2°C (7 – 2°C)
6. Initial placement in 7°C where the temperatures were lowered by one degree per day until 1°C (7 – 1°C)

All fruit was stored for the same amount of total time. Samples of 10 fruit per temperature regime and replicate were taken for firmness, starch, and damage registration (partial quality analyses) after three and seven weeks of cold storage.

2.3.3 Removal from cold storage and damage assessment

All fruit were removed from cold storage after nine weeks and assessed for external damage before being placed in 20°C for 14 days for a shelf-life period. Damage was also assessed after the shelf-life period. The procedure for both damage assessments times was the same as described earlier (2.2.4). Samples of 10 fruit per replicate were also taken at removal from storage for full quality analysis, after seven days of the shelf-life period for partial quality analysis, and once more for full quality analysis after 14 days of the shelf-life period.

2.4 'Eden'

2.4.1 Harvesting, creation of treatments in gradual cooling and conditioning experiments, and placement in cold storage

Harvest of 'Eden' fruit from a commercial orchard in Velure, Norway occurred on 20 September. Trees were on M.9 rootstock and planted in 2019 in a conventionally managed orchard. Fruit were picked a few days before commercial harvesting began. In total, 4,320 fruit were picked and placed directly into 72 6416-IFCO boxes with two layers of 30 fruit in each. Only class 1 fruit were picked. In addition, one box of 30 fruit was set aside for quality analysis the same day. After harvest, the fruit was separated into two projects – gradual cooling and conditioning.

For the gradual cooling experiment, the procedure followed was the same as described in the 'Red Aroma' gradual cooling experiment (2.3.2). In total, there were six temperature regimes, with three replicates in each temperature regime:

1. Constant 4°C (4°C)
2. Constant 2°C (2°C)
3. Constant 1°C (1°C)
4. Initial placement in 7°C where the temperatures were lowered by one degree per day until 4°C (7 – 4°C)
5. Initial placement in 7°C where the temperatures were lowered by one degree per day until 2°C (7 – 2°C)
6. Initial placement in 7°C where the temperatures were lowered by one degree per day until 1°C (7 – 1°C)

All fruit in each temperature regime was stored together at NIBIO Ullensvang and all fruit was stored for the same amount of time.

Six temperature regimes were also created for the conditioning experiment. Six boxes each were placed directly into a RA cold storage room or chamber at 1°C or 4°C. Twelve of the remaining boxes were stored for one week in a room that averaged 10°C, while the other 12 were placed in a room that averaged 20°C. After one week, half the boxes from each of the two warmer temperatures were placed at 1°C, with the other half being placed in 4°C, in both cases for the remainder of their storage. The resulting temperature regimes, with three replicates each, were:

1. Constant 4°C (4°C)
2. Constant 1°C (1°C)
3. 10°C for one week followed by the remainder of storage at 4°C (10 – 4°C)
4. 20°C for one week followed by the remainder of storage at 4°C (20 – 4°C)
5. 10°C for one week followed by the remainder of storage at 1°C (10 – 1°C)
6. 20°C for one week followed by the remainder of storage at 1°C (20 – 1°C)

Fruit stored at the same final temperatures in conditioning and gradual cooling experiments were stored together. Throughout the storage period, samples of 10 fruit per replicate were taken for partial quality analysis from each temperature regime in both the gradual cooling and conditioning experiments after four and seven weeks of storage.

2.4.2 Removal from cold storage and damage assessment

After 10 weeks of storage, fruit from both the gradual cooling and conditioning experiments were removed from cold storage for external damage analysis and a 14 day shelf-life period at 20°C. The general process of external damage registration was the same as described earlier (2.2.4), with the addition of discolored (often grey) skin being labeled as potential external watercore. Ten fruit per replicate were taken for full quality analysis upon removal from cold storage. Additionally, 10 fruit samples were taken for partial quality analysis seven days of the shelf-life period.

Final analysis was completed after 14 days of the shelf-life period. This included taking 10 full quality analysis samples per temperature regime and external damage registration. In addition, each fruit was cut in half horizontally along the equator to assess internal damage. Watercore and core browning were assessed on a scale of 1-5 and 1-3, respectively. If a fruit was affected by multiple disorders or had disorders and fungal decay, all damage was recorded with the primary damage listed first.

2.5 ‘Fryd’

Harvest of ‘Fryd’ fruit occurred on 30 September from a commercial orchard in Hesthamar, Norway. Trees were planted in 2020 on M.9 rootstock in a conventionally managed orchard. Fruit were picked shortly before anticipated commercial harvesting. In total, 3,600 class 1 fruit were picked and transferred to 6416-IFCO boxes with two trays of 30 fruit each.

One additional box of 30 fruit were set aside for quality analysis on the same day. The fruit were separated into two projects – gradual cooling and conditioning.

The gradual cooling and conditioning experiments with 'Fryd' fruit had the same experimental set up as in 'Eden', with the same treatments as described in 2.4.1. Due to a lack of fruit in the orchard, the 1°C and 4°C fruit were shared between the two experiments. This meant there were 10 temperature regimes with three replicates each instead of 12 temperature regimes in 'Eden'. All 'Fryd' fruit from both experiments in each temperature were stored together at NIBIO Ullensvang, and analyses for both experiments were conducted on the same days.

Similar to the 'Eden' experiments, partial quality analysis was completed on 10 fruit samples per temperature regime for both 'Fryd' experiments three and seven weeks after harvest. All fruit were removed from cold storage after nine weeks and placed in 20°C for a 14 day shelf-life period at 20°C. Damage registrations at removal from cold storage and after the shelf-life period were completed as described previously (2.4.2). Full quality analysis was completed on 10 fruit per temperature regime at removal from cold storage, as well as after 14 days of the shelf-life period. Additionally, partial quality analysis was completed after seven days of the shelf-life period on 10 fruit per temperature regime.

2.6 General fruit quality analyses

Fruit quality analyses were standard across all experiments and completed based on the 10 fruit samples. Full quality analysis consisted of assessment of background color, cover color, firmness, starch content, SSC, TA, and damage registration. Partial quality analysis consisted of assessment of firmness, starch content, and damage registration. Fruit tested from cold storage were removed in the morning and allowed to warm up to room temperature before analysis in the afternoon the same day. Fruit were also assessed visually for background color on a scale from 1 (dark green) to 9 (dark yellow) (Tentation scale, Ctifl, France), and again for percent cover color on a scale from 1 (blush covering 0-15% of peel) to 9 (blush covering 90-100% of peel).

Fruit were then peeled with a hand-held peeler on the shade and sun side, and firmness (kg/cm^2) was measured on these sections using a digital penetrometer (FTA GS-25, Guss, Strand, South Africa) mounted with a 11.1 mm probe. After, fruit were cut in half equatorially, and an additional 1 cm thick equatorial slice of each fruit was cut and frozen. The calyx end of

the fruit was then dipped in an iodine-starch solution for starch hydrolysis analysis on a scale from 1 (100% starch) to 10 (0% starch) (Norsk landbruksrådgiving, 2020).

In December, the frozen slices from each sampling date were thawed and all 10 slices per replicate were juiced together for use determining SSC and TA content. SSC was determined using a digital refractometer (PR-32alpha, Atago, Tokyo, Japan). TA (% malic acid) were measured by titrating the same juice sample to pH 8.1 using a 0.1N NaOH solution (SP280 autotitrator, Mettler Toledo, Greifensee, Switzerland).

2.7 Watercore and core browning scales

During quality analysis of 'Eden' fruit, watercore was found both at harvest time and later in storage. Due to the presence of fruit with watercore at each sampling time, a scale specific to 'Eden' was created to better assess severity of watercore, rather than just incidence of watercore. This scale was based off one already in use for 'Fuji' (Figure 2.1; (Neuwald et al., 2012)) but adjusted for how watercore developed specifically in 'Eden'. Pictures were taken of individual fruit with varying severities of watercore and merged to create the scale.

Additionally, during quality analyses throughout storage of both 'Eden' and 'Fryd', core browning was found frequently enough to create an additional scale specific to core browning based on a severity from 1-3. Separate scales were created for 'Eden' and 'Fryd'.

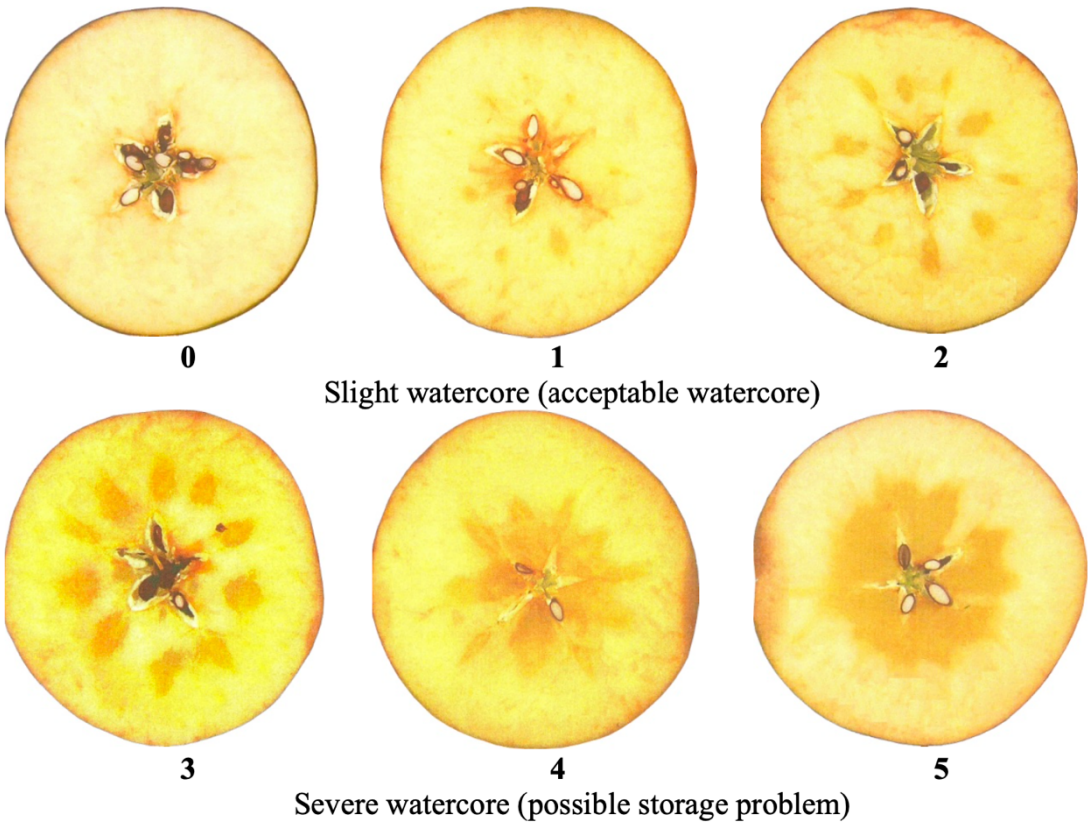

Figure 2.1: Watercore scale for 'Fuji' from 0-5, where 0 is no watercore, and 5 is 40% or more of the flesh is affected by watercore. Watercore between 0-2.5 at harvest can dissipate in storage. From Neuwald et al., 2012.

2.8 Statistics

Data was analyzed using the linear model and ANOVA procedures with the agricolae package (1.3-5; de Mendiburu F 2021) in R (version 4.2.1; R Core Team 2022), visualized in R Studio (2023; Posit Team). Damage incidence data was transformed using arcsine-square root transformation prior to analysis for sample sizes other than 100 to normalize the data. Significant differences were determined through analysis of variance and treatment means were separated using the Student Newman Keuls test at $\alpha = 0.05$. All data presented are non-transformed means.

3 Results

3.1 Weather conditions

Average daily temperature throughout the growing season ranged from a minimum of 7.5°C on 5 May to 20.1°C on 25 June (Figure 3.1). There were no frosts during this time period, with 2.9°C as the lowest temperature recorded on 3 May. The highest recorded temperature was 29.6°C on 24 June. Precipitation varied throughout the season, with a total of 380.6 mm. The average precipitation total for the past decade was 499.1 mm, and all but two years (2021 and 2017) had more total precipitation than in 2022. In the 30 days before harvest, average relative humidity was similar in 2022 compared to past years. However, there were only four of with relative humidity above 85% in the 30 days before harvest in 2022 compared to an average of 12.2 over the past decade. Additionally, in this same time period, there was less days of precipitation and less total precipitation in 2022 than the average of the last decade.

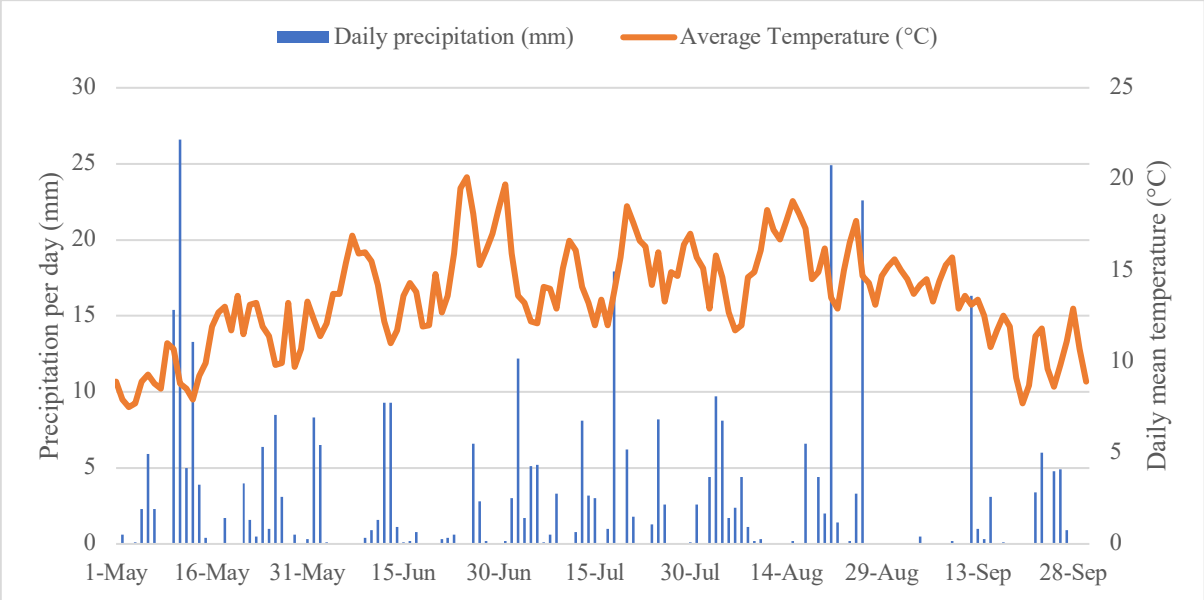

Figure 3.1: Daily average temperature (°C) and precipitation (mm) from 1 May to 30 September 2022 recorded at the NIBIO Ullensvang weather station in south-eastern Norway.

Bloom in 'Red Aroma' was among the earliest of the past ten years, on 18 May; other bloom dates were always 17 May or later, with the latest bloom date of 6 June in 2015. Bloom in 'Eden' and 'Fryd' measured in multiple orchards throughout the Ullensvang region was five days earlier than in 2021, as was true for 'Red Aroma' (20 May 2022 instead of 25 May 2021) (Gasi et al., 2023). As only two years of full bloom data was available for 'Eden' and 'Fryd'

as opposed to more than 10 years for 'Red Aroma' and bloom and harvest dates were overall similar for all three cultivars, only 'Red Aroma' data was used to calculate GDD.

GDD accumulation was rapid in May and early June, but was low throughout July, resulting in a delay in GDD accumulation as compared to other years (Figure 3.2). Only 10 days in July had a maximum temperature above 20°C, while 28 days had a minimum temperature below 15°C, and rainfall was recorded on 22 days throughout July. On 30 September, the last day of harvest in these experiments, accumulated GDD was lower than all but two other of the past ten years, at 629.5 GDD (Figure 3.2).

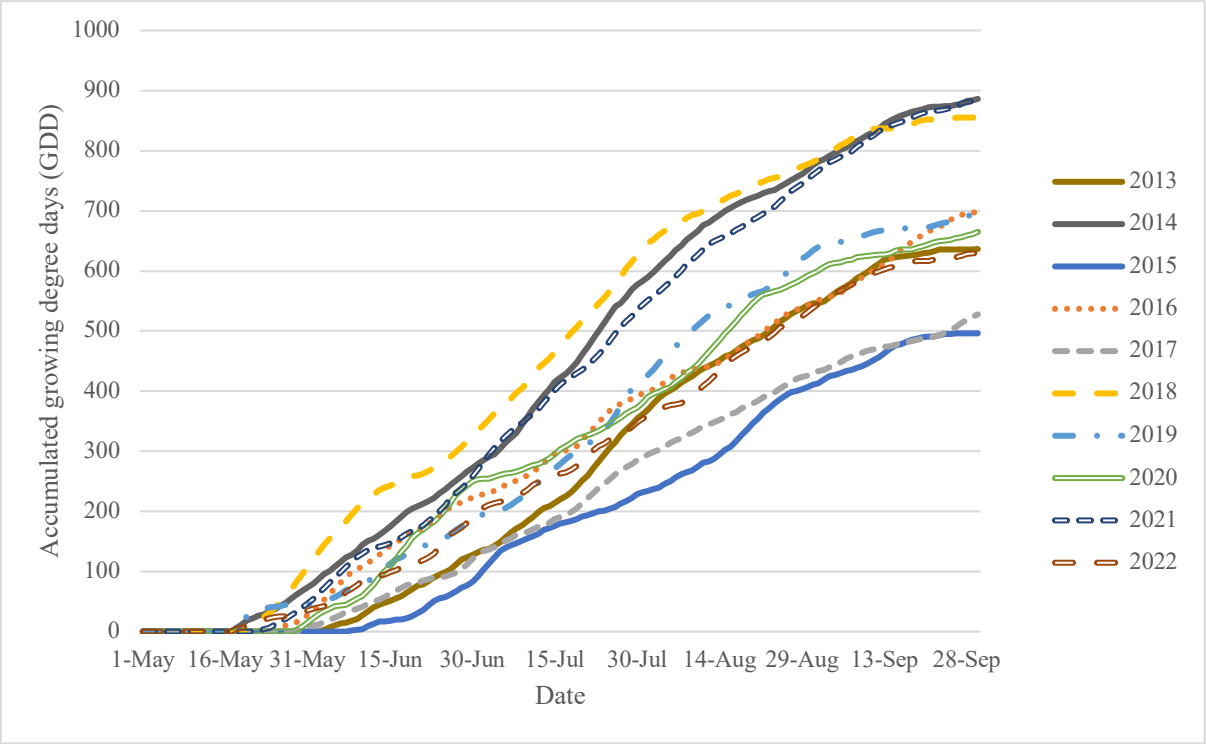

Figure 3.2: Accumulated growing degree days (GDD) over 10 years based of temperatures recorded at NIBIO Ullensvang. Growing degree days (GDD) were calculated by averaging the maximum and minimum daily temperatures then subtracting 10°C (base 10°C). GDD started the day after full bloom (DAFB), recorded each year as full bloom on 'Red Aroma' trees at NIBIO Ullensvang in south-eastern Norway.

3.2 'Red Aroma' crop load

3.2.1 Fruit measurements

Thinning of fruit was occurred from 30 June to 4 July. Directly after thinning, there were no differences in fruit size between treatments (P = 0.6484) or replicates (P = 0.2683). Fruit in all crop loads increased in size at each measurement time. The low crop load fruit was largest starting on 18 July (P = 0.0110) through the remainder of the growing season. Medium

crop load fruit was larger than high crop load fruit from 15 August (P <0.0001) onwards. At harvest, average size of low crop load fruit was 72.0 mm compared to 67.1 mm for medium crop load fruit and 64.1 mm for high crop load fruit (Figure 3.3). These differences were visible on the tree starting in August through harvest (Figure 3.4).

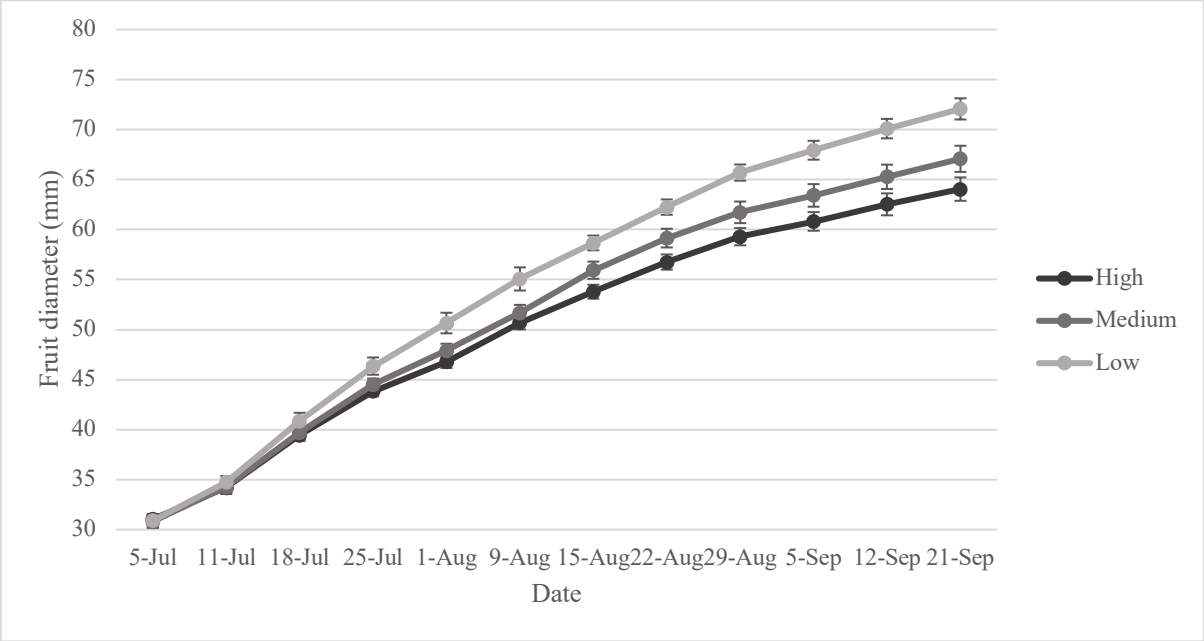

Figure 3.3: Effect of crop load on diameter of 'Red Aroma' fruit throughout the 2022 season. Fruit were measured once per week starting after hand thinning was completed (5 July) until the day before harvest (21 September). Mean of 4x21 fruit. Bars represent standard error (±SE).

Differences were found between replicates within each crop load, with the second replicate having the smallest fruit regardless of treatment at some measurement dates. These differences were only found on 25 July (P = 0.0292), 1 August (P = 0.0502), and 9 August (P = 0.0325), due to low crop load replicate 2 fruit being smaller than low crop load replicate 1 and 4. This trend continued throughout the growing season, including at harvest, and one of the trees that was excluded from storage was from replicate 2 of low crop load fruit (Figure 3.4).

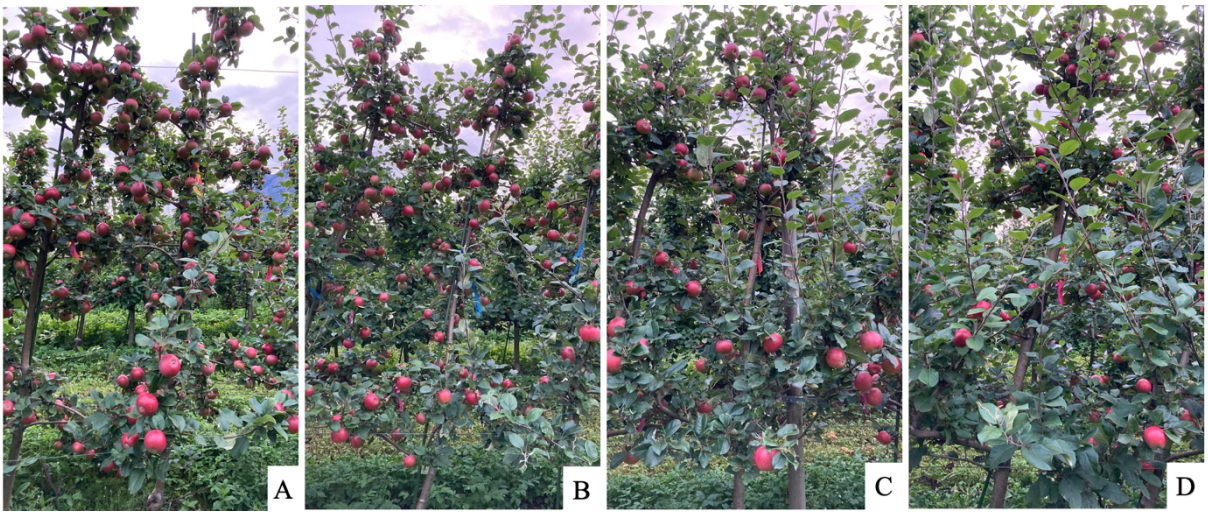

Figure 3.4: Three crop loads of 'Red Aroma' trees at harvest (Sept. 21, 2022) A: Typical high crop load tree. B: Typical medium crop load tree. C: Typical low crop load tree. D: Low crop load tree from replicate 2 that was excluded from storage due to small fruit.

3.2.2 Determination of fruit drop

When fallen fruit were first collected on 15 August, few fallen fruit were found, with no differences in totals between crop loads (P = 0.9624). The average number of fallen fruit increased on 12 September compared to all previous assessments and was highest at harvest on 22 September (P <0.0001). Core rot, sometimes identifiable as *Fusarium* spp., was the most commonly found fungal decay. The amount of core rot increased until harvest, with the harvest assessment having the highest amount of core rot (P <0.0001). Other fungal decay was infrequently found, with *Monillia* spp. and calyx-end rot found in the highest amounts. The amount of fallen fruit caused by other damage was highest at harvest compared to other sampling dates (P <0.0001). Additionally, 83% of the fallen fruit without fungal decay had no other noticeable causes for falling when assessed at harvest (Table 3.1).

Table 3.1: Causes of dropped 'Red Aroma' fruit at six sampling times prior to harvest in 2022. Mean of counts from 4x3 crop loads.

Date	Total fallen fruit	Core rot	Other rot	Other[1]
15 Aug.	2.5 c[2]	1.3 c	0.3	1.0 c
22 Aug.	3.8 c	2.1 bc	0.3	1.5 c
28 Aug.	6.1 c	3.9 b	1.0	1.2 c
5 Sept.	5.6 c	3.6 b	0.8	1.3 c
12 Sept.	9.8 b	4.1 b	1.0	4.8 b
22 Sept.	23.5 a	6.6 a	1.4	15.5 a
P-value	<0.0001	<0.0001	0.0593	<0.0001

[1]Including all physiological, insect, and mechanical damage
[2]Numbers with letters indicate significance between assessment dates according to Student Newman Keuls tests (P = 0.05).

Prior to harvest, differences between crop loads were only found on 22 August, where low crop load trees had less total fruit drop than medium or high crop load trees (P = 0.0341). At harvest, high crop load trees had 91% and 171% more fallen fruit than medium and low crop load trees respectively. Additionally, the highest numbers of fallen fruit with core rot were found in high crop load trees (P = 0.0455), but no differences were found between crop loads for other types of fungal decay (P = 0.4397). The highest number of fallen fruit without fungal decay were also found in the high crop load fruit, with no difference between the other two crop loads (P = 0.0063; Table 3.2).

Table 3.2: Effect of crop load on causes of dropped 'Red Aroma' fruit at harvest in 2022. Mean of counts from four replicates.

Crop load	Total fallen fruit	Core rot	Other fungal decay	Other
High	37.3 a[1]	9.3 a	2.0	26.0 a
Medium	19.5 b	5.5 b	1.3	12.8 b
Low	13.8 b	5.0 b	1.0	7.8 b
P-value	0.0014	0.0455	0.5397	0.0063

[1]Numbers with letters indicate significance between crop loads according to Student Newman Keuls tests (P = 0.05).

3.2.3 Number of fruit per tree and size of fruit per crop load

Total numbers of fruit per tree were different for each crop load, with the high crop load having the most fruit, and the low crop load having the least fruit per tree (P <0.0001). While there was no differences in the number of damaged or class 1 fruit between the medium and high crop loads, the medium crop load had less than half the number of small fruit compared to the high crop load (Table 3.3).

Table 3.3: Number of 'Red Aroma' fruit per tree at harvest in 2022. Class 1 fruit were all non-damaged fruit that were at least 60 mm in size. Small fruit were <60 mm. Mean of 4x7 trees.

Crop load	Small fruit	Damaged fruit[1]	Class 1 fruit	Total fruit
High	24.0 a[2]	8.7 a	96.4 a	129.1 a
Medium	9.4 b	9.3 a	90.4 a	108.6 b
Low	0.7 b	3.7 b	55.1 b	57.3 c
P-value	0.0026	0.0393	0.0001	<0.0001

[1]including all physiological, insect, and mechanical damage
[2]Numbers with letters indicate significance between crop loads according to Student Newman Keuls tests (P = 0.05).

Sizes of all quality samples were different for each crop load; high crop load fruit were 69.1 mm, medium crop load fruit were 70.9 mm, and low crop load fruit were 74.1 mm. Differences were found in weight of individual quality analysis fruit between all crop loads.

Low crop load fruit were heaviest, at 150.3 g, medium crop load fruit were 131.3 g, and high crop load fruit were lightest at 122.2 g (P <0.0001).

3.2.4 Quality analyses

At harvest, fruit were mature with starch values between 5.6 and 7.3. Low crop load fruit had more starch than the other two crop loads (P = 0.0112). There was no difference found in firmness between crop loads (P = 0.0628). Background color was green in all crop loads, without a yellow hue; cover coloration was high though, at 7 out of 9 in all crop loads. SSC was higher in low crop load fruit than high crop load fruit (P = 0.0486), but no differences were found in TA between crop loads (Table 3.4).

Table 3.4: Fruit quality at harvest (22 Sept. 2022) for 'Red Aroma' fruit from three different crop loads. Mean of 3x10 fruit.

Crop load	Background color[1]	Cover color[2]	Firmness (kg/cm^2)	Starch[3]	Soluble solids (%)	Titratable acidity (%)
High	3.2[4]	7.1	7.14	7.2 a	10.1 b	0.858
Medium	2.9	7.4	7.12	7.3 a	10.5 ab	0.865
Low	3.0	7.1	7.38	5.6 b	10.7 a	0.920
P-value	0.4715	0.2466	0.0628	0.0112	0.0486	0.0533

[1]Background color assessed on a scale of 1 (dark green) to 9 (dark yellow) according to Ctifl Tentation scale
[2]Cover color assessed on a scale of 1 (0-15% blush) to 9 (90-100% blush)
[3]Starch assessed on a scale of 1 (100% starch) to 10 (0% starch) after dipping in a starch-iodine solution.
[4]Numbers with letters indicate significance between crop loads according to Student Newman Keuls tests (P = 0.05).

After nine weeks of cold storage, starch had completely dissipated in all fruit regardless of crop load or storage temperature. Firmness decreased in cold storage, averaging 5.2 kg/cm^2 at removal from cold storage, as opposed to 7.2 kg/cm^2 at harvest (P <0.0001). Firmness was lower after the shelf-life period, at 4.1 kg/cm^2 (P <0.0001). At removal from cold storage, there was an effect of temperature on firmness, with 4°C fruit having higher firmness than 1°C fruit (P <0.0001), however there was no effect of crop load or the interaction between crop load and temperature on firmness. Average firmness for all crop loads in 1°C was above 5 kg/cm^2, while all crop loads in 4°C had average firmness below 5 kg/cm^2. After the shelf-life period, differences in firmness were present between crop loads, with medium crop load fruit maintaining higher firmness than low crop load fruit (P = 0.0339). No differences in firmness were found between temperatures, although the interaction between crop load and treatment showed that medium crop load fruit stored at 4°C had higher firmness than all other treatments except high crop load fruit stored at 1°C (P = 0.0093; Figure 3.5).

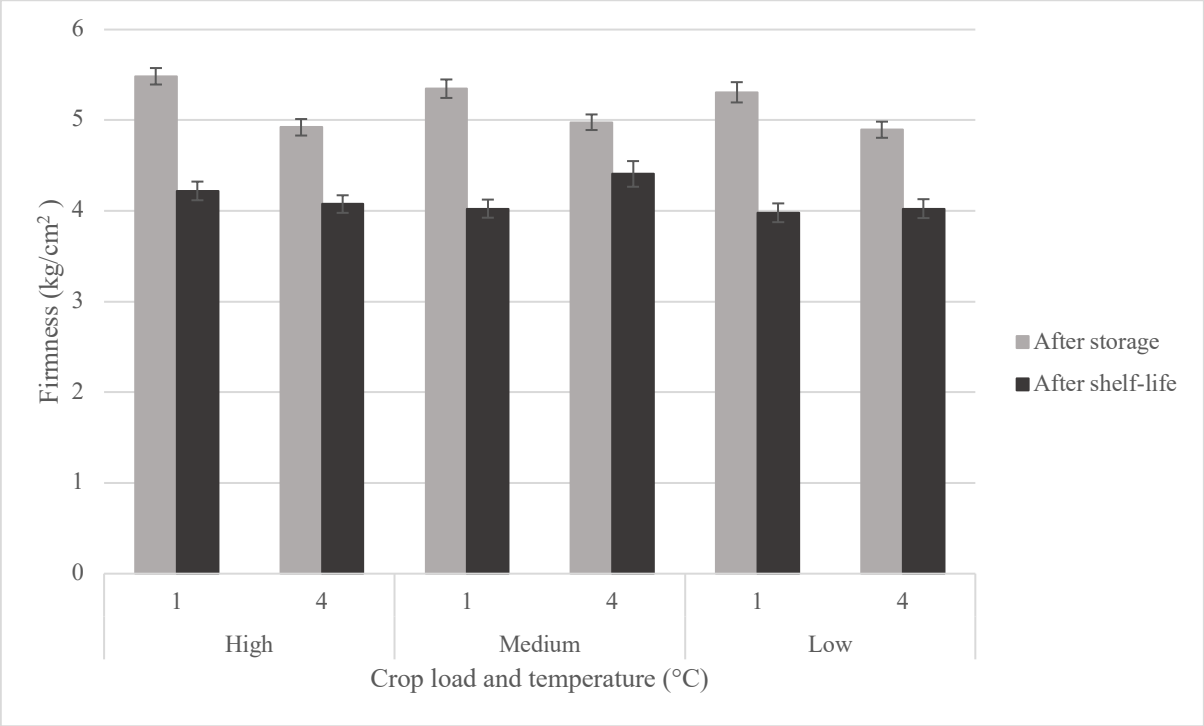

Figure 3.5: Firmness of 'Red Aroma' fruit from three different crop loads after nine weeks of cold storage at two temperatures in 2022. Sampling occurred directly after removal from cold storage and after a shelf-life period of 14 days at 20°C. Mean of 3x10 fruit. Error bars represent standard error (±SE).

Similar to at harvest, there was no difference between crop loads in background color after nine weeks of cold storage. After the shelf-life period, the 1°C high crop load fruit were less yellow than the other two crop loads stored at 1°C ($P = 0.0123$). There was no difference in background color in the 4°C fruit after the shelf-life period (Table 3.5).

Table 3.5: Fruit quality of 'Red Aroma' after nine weeks of cold storage and a 14 day shelf-life period at 20°C for different temperatures and crop loads in 2022. Mean of 3x10 fruit.

Storage conditions		After cold storage		
Temperature (°C)	Crop load	Background color[1]	Soluble solids content (%)	Titratable acidity (%)
1	High	4.6[2]	10.3	0.688 c
	Medium	4.5	10.4	0.733 b
	Low	5.2	10.2	0.776 a
	P-value	0.1187	0.8614	0.0041
4	High	5.3	9.7	0.688
	Medium	4.8	9.7	0.714
	Low	5.2	10.3	0.704
	P-value	0.7302	0.3910	0.8049
			After shelf-life period	
1	High	6.0 b	10.3	0.516 b
	Medium	6.4 a	10.1	0.535 b
	Low	6.6 a	10.0	0.600 a
	P-value	0.0123	0.0859	0.0377
4	High	6.2	9.3 b	0.505
	Medium	6.6	9.5 b	0.514
	Low	6.6	10.6 a	0.547
	P-value	0.4530	0.0132	0.7861

[1]Background color assessed on a scale of 1 (dark green) to 9 (dark yellow) according to Ctifl Tentation scale
[2]Numbers with letters indicate significance between crop loads at each sampling date according to Student Newman Keuls tests (P = 0.05).

SSC was not affected by sampling date (P = 0.1043), however when all sampling times were combined, low crop load fruit had lower SSC than the other two crop loads (P <0.0001). No differences were found between crop loads after cold storage. After the shelf-life period, low crop load fruit stored in 4°C had higher SSC than in the other two crop loads (P = 0.0132). Compared to at harvest, TA decreased after cold storage and the shelf-life period (P <0.0001). There were no differences between crop loads in fruit stored at 4°C, however in 1°C, low crop load fruit had the highest TA after both cold storage and the shelf-life period. Medium crop load fruit in 4°C had more TA than high crop load fruit after cold storage (P = 0.0041), but not after the shelf-life period (P = 0.0377; Table 3.5).

3.2.5 Assessment of physiological disorders and fungal decay

When fruit were removed from cold storage after nine weeks, limited external damages were observed. There was less than 3% total external damage, including less than 1% soft scald, regardless of crop load or storage temperature (P = 0.3978). Soft scald incidence in fruit stored at 1°C was higher at all subsequent sampling dates than at removal from cold storage, increasing from 0.3% at removal to 8.5% after the shelf-life period (P <0.0001). For all fruit

stored in 4°C, there was no difference in soft scald incidence between any sampling dates and overall incidence was below 0.5% (P = 0.7526).

After four, 11, and 14 days of the shelf-life period, 1°C fruit had higher soft scald incidence than 4°C fruit. No differences were found in soft scald incidence when comparing crop loads or crop loads and temperatures at any sampling time (Table 3.6). There were few other physiological disorders found, with senescent breakdown being the most common disorder other than soft scald. Senescent breakdown was present in 0.3% of the fruit after the shelf-life period (12 out of 3,478 fruit).

Table 3.6: Effect of storage temperature on soft scald incidence (%) on fruit from 'Red Aroma' trees of different crop loads at four assessment dates in 2022. Fruit were in cold storage for nine weeks before storage for 14 days at 20°C. Mean of 3x120 fruit after 10 weeks of cold storage and 3x110 fruit on the other sampling dates.

Crop load	Temperature (°C)	After cold storage for	After shelf-life at 20°C for		
		9 weeks	4 days	11 days	14 days
High	1	0.2	6.6	5.7	7.8
	4	0.2	0	0.7	0.3
Medium	1	0.2	5.9	7.1	9
	4	0.2	0.7	0	0.5
Low	1	0.6	3	3.2	8.8
	4	0	0.2	0	0
Significance					
	Crop load	NS[1]	NS	NS	NS
	Temperature	NS	****	****	****
	Crop load * Temperature	NS	NS	NS	NS

[1]NS, *, **, ***,**** Nonsignificant or significant at P ≤0.05, 0.01, 0.001, or 0.0001, respectively.

During the shelf-life period, the amount of fungal decay increased, with the highest percent after 14 days of the shelf-life period (P <0.0001). The percentage of fruit with fungal decay increased from 0.4% to 6.9% in the 1°C fruit (P <0.0001) and from 0.4% to 6.9% in the 4°C fruit (P <0.0001). However, there were no differences found between crop loads, temperatures, or crop loads and temperatures at any assessment date. The most common type of fungal decay found was core rot, sometimes identifiable as *Fusarium* sp..

3.3 Gradual cooling of 'Red Aroma'

3.3.1 Quality analyses

At harvest, fruit were mature and generally within the recommended harvesting window for 'Red Aroma', although SSC and starch index were both slightly lower than recommended (Norsk landbruksrådgiving, 2020). Firmness decreased in each temperature regime throughout the whole storage period, including during the shelf-life period (P = 0.0022). There was no consistent difference between temperature regime, however 4°C and 7 – 4°C fruit had the lowest firmness at multiple sampling times, including at removal from cold storage after nine weeks (Table 3.7). However, after two weeks at 20°C, the 1°C fruit were least firm (P = 0.0016). When differences between temperature regimes were found, they were small, with less than 0.5 kg/cm^2 separating all temperature regimes except at sampling after seven weeks of storage (Table 3.7).

Table 3.7: Effect of storage and gradual cooling on firmness (in kg/cm^2) of 'Red Aroma' fruit at five sampling times in 2022. Mean of 3x10 fruit. Firmness at harvest was 6.49 kg/cm^2.

Temperature regime	After cold storage for			After shelf-life at 20°C for	
	3 weeks	7 weeks	9 weeks	7 days	14 days
Direct 1°C	6.63[1]	5.65 ab	4.95 a	4.02	3.06 c
Gradual cooling 7°C to 1°C	6.74	5.61 ab	4.91 a	3.88	3.23 ab
Direct 2°C	6.71	5.78 a	4.90 a	4.15	3.28 ab
Gradual cooling 7°C to 2°C	6.90	5.49 ab	4.80 a	3.99	3.20 b
Direct 4°C	6.59	5.15 b	4.50 b	4.04	3.40 a
Gradual cooling 7°C to 4°C	6.88	5.16 b	4.52 b	4.23	3.37 ab
P-value	0.7919	0.0293	0.0070	0.6798	0.0016

[1]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

Starch degraded compared to harvest after three weeks of storage, with starch degradation being almost completed within seven weeks of storage (P <0.0001). There were no differences between temperature regimes at any sampling date. Background color was more yellow at each sampling time (P <0.0001), with no differences found between temperature regimes upon removal or after the shelf-life period. There was no difference in SSC compared to harvest at either sampling date (P = 0.8278) or between storage regimes at each sampling date (Table 3.8).

Table 3.8: Quality of 'Red Aroma' fruit in different temperature regimes after nine weeks of cold storage and a shelf-life of 14 days at 20°C in 2022. Mean of 3x10 fruit. Background color at harvest was 3.3, soluble solids content was 10.4%, and titratable acidity was 0.857%.

Temperature regime	Background color[1]		Soluble solids content (%)		Titratable acidity (%)	
	After cold storage	After shelf-life	After cold storage	After shelf-life	After cold storage	After shelf-life
Direct 1°C	5.2[2]	6.6	10.3	10.7	0.785 a	0.572
Gradual cooling 7°C to 1°C	5.1	6.4	10.5	10.3	0.786 a	0.552
Direct 2°C	5.3	6.2	10.3	10.4	0.764 ab	0.513
Gradual cooling 7°C to 2°C	5.0	5.9	10.3	10.3	0.766 ab	0.531
Direct 4°C	5.1	6.7	10.5	10.8	0.749 ab	0.526
Gradual cooling 7°C to 4°C	5.1	6.5	10.4	10.1	0.727 b	0.488
P-value	0.8843	0.7145	0.6622	0.2146	0.0251	0.0752

[1]Background color assessed on a scale of 1 (dark green) to 9 (dark yellow) according to Ctifl Tentation scale
[2]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

TA was highest in 1°C and 7 – 1°C fruit and lowest in 7 – 4°C fruit at removal from cold storage (P = 0.0251), although this difference was not maintained after the shelf-life period (P = 0.0752; Table 3.8). Compared to at harvest, TA was lower at removal from cold storage and decreased further after the shelf-life period (P <0.0001).

3.3.2 Assessment of physiological disorders and fungal decay

Upon removal from cold storage, few external damages were noted on the 'Red Aroma' fruit. There was 5.3% or less total external damage in each temperature regime, with no differences between temperature regimes (P = 0.0511). Soft scald incidence was low, with no difference between temperature regimes (P = 0.6231). Total damage increased during the shelf-life period and was higher after 14 days of the shelf-life period than at removal (P <0.0001; Figure 3.6A). This was due to an increase in both soft scald and fungal decay (P <0.0001 and P = 0.0005, respectively). There was no influence of temperature regime on amount of fungal decay, and overall incidence was low (Table 3.9). Multiple types of fungal decay were found, but core rot and blossom end rot were most common.

Table 3.9: Effect of storage temperature regime on percent of physiological disorders and fungal decay in 'Red Aroma' fruit in 2022. Fruit were stored for nine weeks before removal to 20°C for 14 days. Mean of 3x80 fruit.

Temperature regime	Total damage (%)	Fungal decay (%)[1]	Physiological disorders	
			Soft scald (%)[2]	Other (%)
Direct 1°C	24.6 a[3]	4.6	19.6 a	0.4
Gradual cooling 7°C to 1°C	12.5 b	3.3	8.3 b	0.8
Direct 2°C	16.7 ab	2.1	13.8 ab	0.8
Gradual cooling 7°C to 2°C	15.4 ab	2.5	12.5 ab	0.4
Direct 4°C	3.8 c	2.5	1.3 c	0.0
Gradual cooling 7°C to 4°C	8.8 b	6.7	1.7 c	0.4
P-value	0.0006	0.3834	0.0002	0.8335

[1]Fungal decay was identified by externally visible signs on the assessment day or after an additional 14 days at 20°C with constant light to promote fungal growth.
[2]Soft scald was determined by ribbon-like browning of the peel with clearly defined edges.
[3]Numbers with letters indicate significance between temperature regimes for each damage category according to Student Newman Keuls tests at P = 0.05.

After the shelf-life period, soft scald was the most common physiological disorder and was lowest in 4°C and 7 – 4°C fruit (P = 0.0002; Figure 3.6B). Soft scald incidence was over 10 times higher in the 1°C fruit than in the 4°C or 7 – 4°C fruit and was also higher than in the 7 – 1°C fruit. (P = 0.0002). Similarly, total damage was highest in 1°C fruit (P = 0.0006). Total damage was higher in 7 – 4°C fruit than 4°C fruit even though there was no significant difference between individual damage categories. There was no increase in soft scald incidence in 4°C or 7 – 4°C fruit between removal from cold storage after nine weeks and the shelf-life period, however an increase in soft scald incidence occurred in all other temperature regimes between the two sampling dates (P = 0.0286).

Figure 3.6: Soft scald on fruit of 'Red Aroma' in the gradual cooling experiment after nine weeks of cold storage and during a shelf-life period of 20°C. A: After seven days of the shelf-life period. B: After 14 days of the shelf-life period. The darker sections of the affected flesh have a secondary fungal infection.

3.4 ‘Eden’

3.4.1 Watercore

At harvest, watercore was found in 23% of the fruit used for quality analysis (seven out of 30 fruit total). The watercore ranged from very light, where less than 5% of the flesh was affected, to severe, where almost half of the fruit was affected. Most of the watercore was centered around the coreline area and expanded either outward towards the flesh or inwards to the core.

At sampling after four weeks of storage, 6% of the fruit used for quality analysis had watercore and 4.4% of the fruit had watercore after seven weeks of storage. Throughout this period, the watercore found was generally severe, and by October, browning of the watercore affected tissue was visible in one fruit. Throughout storage, the watercore found had similar patterns, allowing for the creation of a watercore scale specifically for ‘Eden’ (Figure 3.7), based off existing scales (Figure 2.1).

Figure 3.7: Watercore scale for ‘Eden’ fruit, where 0 was no watercore (not pictured), 1 was slight watercore (<2 small sections of watercore less than 1cm^2 each) and 5 was severe watercore (>40% of affected flesh and/or watercore-affected tissue that has turned brown). Brown tissue on opposite ends of each fruit was bruising from firmness tests.

The scale included values 0-5, and were defined as:

- Watercore 0: no watercore present.
- Watercore 1: One or a few instances (5%) of affected tissue that were generally close together. This watercore could either be in the flesh or the core area, but not both.
- Watercore 2: A few instances (10-20%) of affected tissue in either the core or the flesh, but not both.
- Watercore 3: Watercore spread out thinly in the flesh and core (20-30% affected tissue). If primarily in the flesh, affected tissue was spread out thinly throughout about half of the fruit. If primarily in the core, not all the core tissue was affected.
- Watercore 4: Affected tissue in much of the flesh and core (30-40%). If primarily in the flesh, affected tissue was dispersed throughout the whole fruit. If primarily in the core, the whole core was affected as well as the surrounding flesh outside the coreline.
- Watercore 5: Watercore present in much of the flesh and core (≥40% affected tissue). The whole core was affected and large sections of the flesh. This was sometimes externally visible. The affected tissues were watery and sometimes soft to the touch. Any browning of the affected tissue resulted in automatic watercore 5 classification.

3.4.2 Core browning

Core browning was found in 'Eden' fruit during quality analysis at each sampling time starting after seven weeks of storage. As such, a scale was created for what appeared to be characteristic levels of core browning in Eden (Figure 3.8). The scale included values 0-3, and was defined as:

- CB0: No core browning present.
- CB1: Slightly brown coloration of the core, with only one or two of the core sections affected.
- CB2: Slightly brown coloration of half or less of the core sections, sometimes barely expanding past the coreline.
- CB3: Dark brown coloration throughout the whole core, often expanding past the core area.

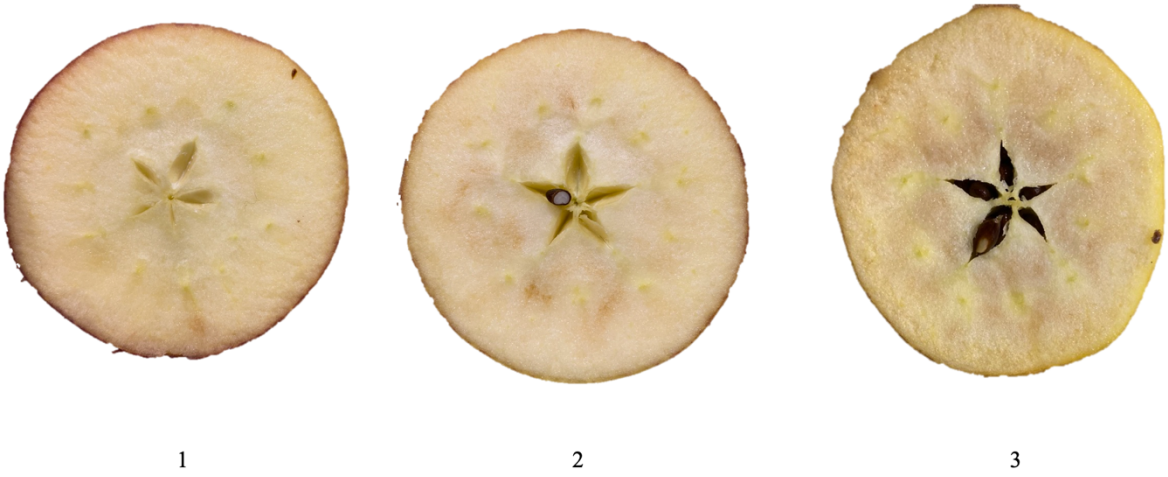

Figure 3.8: Core browning scale for 'Eden' fruit, where 0 was no core browning (not pictured), 1 was slight core browning in one or two sections of the core, 2 was slight core browning in half or more of the sections of the core, sometimes expanding past the coreline, and 3 was darker core browning in all core sections expanding well into the flesh.

3.4.3 Analyses at harvest

At harvest, the same fruit was used in quality analysis for both the gradual cooling and conditioning experiments. Fruit were mature, but less ripe than preliminary harvest recommendations (Fresh Forward Breeding & Marketing BV, 2022). Fruit had a starch value of 5.5. Background color was yellowish green and fruit had between 50-60% cover color.

3.4.4 Gradual cooling quality analyses

Firmness decreased throughout storage compared to at harvest and was lower after the shelf-life period than at removal from cold storage after 10 weeks, regardless of temperature regime ($P < 0.0001$). Differences between temperature regimes were only found at the end of the shelf-life period, where 1°C fruit had the highest firmness and 7 – 4°C fruit had the lowest firmness ($P = 0.0473$; Table 3.10). There were no differences in starch dissipation between

temperature regimes at any sampling date. Starch dissipation was almost completed after seven weeks of storage, and all starch had dissipated by removal from cold storage after 10 weeks.

Table 3.10: Effect of temperature regime on firmness (in kg/cm^2) of 'Eden' fruit in the gradual cooling experiment at five sampling times in 2022. Mean of 3x10 fruit per temperature regime. Firmness at harvest was 11.0 kg/cm^2.

Temperature regime	After cold storage for			After shelf-life at 20°C for	
	4 weeks	7 weeks	10 weeks	7 days	14 days
Direct 1°C	11.5[1]	10.8	9.6	8.5	8.4 a
Gradual cooling 7°C to 1°C	11.3	10.4	8.9	8.2	8.0 ab
Direct 2°C	10.7	10.4	8.8	8.1	7.3 ab
Gradual cooling 7°C to 2°C	10.9	10.6	8.8	8.7	7.8 ab
Direct 4°C	11.3	9.7	8.8	8.2	7.6 ab
Gradual cooling 7°C to 4°C	10.8	10.2	8.8	7.5	6.9 b
P-value	0.5056	0.5306	0.6901	0.0865	0.0473

[1]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

SSC was higher in fruit at harvest than in fruit after 10 weeks of cold storage or after the shelf-life period (P = 0.0002). There were no differences in SSC between temperature regimes after 10 weeks of cold storage or after the shelf-life period. Similarly, there was no differences in TA between any of the temperature regimes after 10 weeks of cold storage. After the shelf-life period, the 1°C and 4°C fruit had the lowest TA, while the 7 – 2°C fruit had the highest TA (P = 0.0004; Table 3.11). Regardless of temperature regime, TA decreased at each sampling time (P <0.0001).

Table 3.11: Effect of temperature regime on fruit quality of 'Eden' in the gradual cooling experiment at removal from cold storage after 10 weeks and after a 14-day shelf-life at 20°C in 2022. At harvest, background color was 5, soluble solids content was 11.8%, and titratable acidity was 0.846%. Mean of 3x10 fruit.

Temperature regime	Background color (1-9)[1]		Soluble solids content (%)		Titratable acidity (%)	
	At removal	After shelf-life	At removal	After shelf-life	At removal	After shelf-life
Direct 1°C	5.4[2]	8.9 a	11.1	11.2	0.665	0.493 cd
Gradual cooling 7°C to 1°C	5.5	8.8 a	10.9	11.1	0.679	0.521 bc
Direct 2°C	5.7	7.8 bc	11.0	10.7	0.649	0.542 b
Gradual cooling 7°C to 2°C	6.0	7.4 c	11.3	10.7	0.671	0.574 a
Direct 4°C	5.6	8.6 a	11.1	11.0	0.617	0.472 d
Gradual cooling 7°C to 4°C	5.4	8.0 b	11.0	10.9	0.627	0.539 b
P-value	0.7303	0.0002	0.6285	0.1431	0.0919	0.0004

[1]Background color assessed on a scale of 1 (dark green) to 9 (dark yellow) according to Ctifl Tentation scale
[2]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

Compared to harvest, background color was not different at removal from cold storage after 10 weeks but was more yellow after the shelf-life period ($P < 0.0001$). The 7 – 2°C fruit had the greenest background color after the shelf-life period ($P = 0.0002$). Greasy fruit were first noticed after four weeks of storage, with 44.4% of the fruit being greasy. The percent of greasy fruit was similar through 10 weeks of cold storage, but increased during the shelf-life period, with 93.3% of the fruit being greasy at the end of the shelf-life period ($P < 0.0001$). At removal from cold storage after 10 weeks, 4°C and 7 – 4°C fruit were greasiest, while 7 – 1°C fruit were least greasy ($P = 0.0262$), but this difference was not maintained after 7 or 14 days of the shelf-life period ($P = 0.2832$ and $P = 0.6797$, respectively).

3.4.5 Assessment of physiological disorders and fungal decay in the gradual cooling experiment

Upon removal from cold storage after 10 weeks, less than 5% of fruit had visible external disorders or fungal decay, and there were no differences in overall external damages between temperature regimes ($P = 0.8428$). Some fruit were noticeably discolored, with a slight grey-brown background color which appeared similar to external symptoms of severe watercore (Figure 3.9A). After the shelf-life period, most fruit had no externally visible damage, with the exception of externally visible watercore or senescent breakdown on some fruit (Figure 3.9B) and fungal decay. Fungal decay incidence was generally low, at 2.1% of fruit or less, and no differences were found between temperature regimes ($P = 0.6720$). However, total damage was high in most treatments after the shelf-life period, due mostly to internal physiological disorders (Table 3.12).

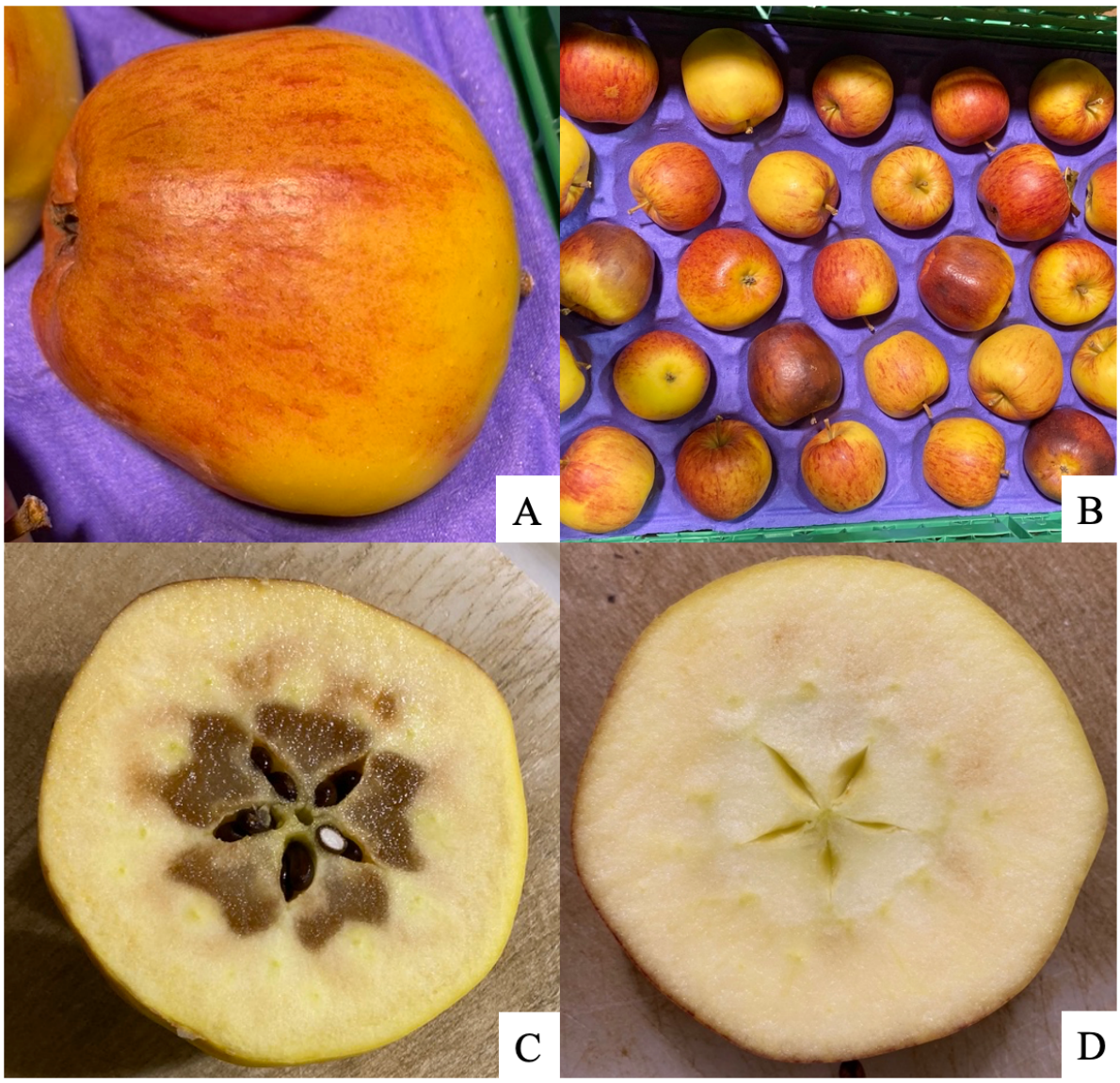

Figure 3.9: Fruit of ‘Eden’ in the gradual cooling experiment after 10 weeks of cold storage and a 14 day shelf-life period at 20°C in 2022. A: External brown discoloration of the ground color near the calyx end, labeled as externally visible watercore. B: A tray of 7 – 4°C fruit showing some fruit with external discoloration or senescent breakdown. C: Watercore breakdown and core browning in one fruit. The watercore areas are darker brown than the core browning areas. D: Diffuse flesh browning between vascular tissues without entering the core.

Across all temperature regimes, core browning accounted for the largest percentage of damages, although rates of incidence varied significantly. The 4°C and 7 – 4°C fruit had the most total damage of any temperature regime, due in part to higher incidences of watercore and diffuse flesh browning as well as higher core browning. Incidences of core browning were between 4-9 and 2-6 times higher than in the other temperature regimes, in 4°C and 7 – 4°C fruit respectively ($P = 0.0002$). No differences were found between the four other temperature regimes (Table 3.12).

Table 3.12: Effect of temperature regime on physiological disorders and fungal decay of 'Eden' fruit in the gradual cooling experiment in 2022. Fruit were stored for 10 weeks before removal and a shelf-life of 20°C for 14 days. Mean of 3x80 fruit.

Temperature regime	Total damage (%)	Fungal decay (%)[1]	Physiological disorders				
			Watercore (%)[2]	Core browning (%)[3]	Senescent breakdown (%)[4]	Diffuse flesh browning (%)[5]	Other (%)[6]
Direct 1°C	22.1 b[7]	2.1	0.4 b	7.5 c	2.1	0.0 b	10.4 a
Gradual cooling 7°C to 1°C	14.2 b	2.1	1.3 b	6.7 c	1.3	0.0 b	2.9 ab
Direct 2°C	21.3 b	0.8	0.8 b	15.8 c	0.8	0.0 b	2.9 ab
Gradual cooling 7°C to 2°C	16.3 b	0.8	0.8 b	9.6 c	2.1	0.4 b	2.5 ab
Direct 4°C	85.0 a	0.8	13.3 a	62.5 a	0.0	7.9 a	0.4 b
Gradual cooling 7°C to 4°C	66.7 a	0.4	16.7 a	37.5 b	0.4	10.4 a	1.3 b
P-value	0.0002	0.6720	0.0055	0.0002	0.2745	0.0089	0.0403

[1]Fungal decay was identified by externally or internally visible signs either on the assessment day or after an additional 14 days at 20°C with constant light to promote fungal growth.
[2]Watercore was defined as brown or green translucent areas of the flesh with a water-soaked appearance.
[3]Core browning was defined as browning of the flesh primarily between the core and coreline. In severe cases, it could extend past the coreline into the cortex.
[4]Senescent breakdown was defined as browning of the flesh originating near the peel, with affected skin being mealy, soft, and dry.
[5]Diffuse flesh browning was defined as browning in the cortex without affecting the vascular tissue or core area. Tissue stayed moist and firm.
[6]Including bitter pit, superficial scald, and internal corking
[7]Numbers with letters indicate significance between temperature regimes for each damage category according to Student Newman Keuls tests at P = 0.05.

Core browning was present in all temperature regimes at varying degrees of severity. CB1 was present in between 5-15% of fruit at each temperature regime, and there was no difference found between any of the temperature regimes (P = 0.3920). The highest amount of CB2 was found in 4°C fruit, at 20%, with 7 – 4°C also having high amounts of fruit with CB2 (P = 0.0004). Similarly, CB3 was highest in 4°C and 7 – 4°C fruit, at 27.5 and 20.8%, respectively, with no difference between the remaining temperature regimes (P = 0.0002; Figure 3.10).

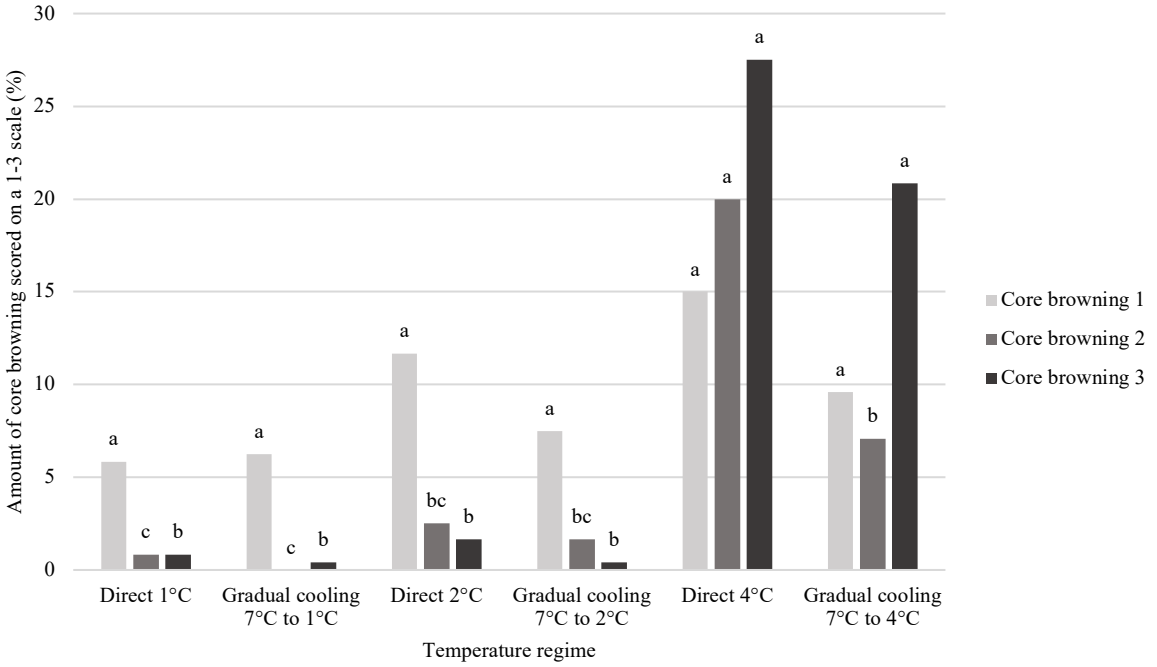

Figure 3.10: Effect of temperature regime on presence and severity of core browning in 'Eden' fruit in 2022 after 10 weeks of cold storage and a shelf-life of 14 days at 20°C. Core browning levels are based off of the previously described scale (Figure 3.8). Mean of 3x80 fruit. Significance between temperatures and core browning severity is according to Student Newman Keuls tests at P = 0.05.

Watercore was found mostly in the 4°C and 7 – 4°C treatments (Table 3.12), and in both temperature regimes the watercore was often accompanied by core browning as a secondary physiological disorder. In these fruit, the watercore was often present in the majority of the core area, with core browning visible in-between the watercore and spreading out into the flesh (Figure 3.9C). In all the fruit with watercore, the watercore had turned brown. Of the fruit with watercore, half of the 4°C fruit and 94% of the 7 – 4°C fruit had additional core browning. Furthermore, in the fruit with watercore that did not have core browning, most had senescent breakdown. Diffuse flesh browning was also present in this experiment, with the highest amount of affected fruit in the 4°C and 7 – 4°C treatments (P = 0.0089; Figure 3.9D). Furthermore, other physiological disorders were higher in 1°C fruit than in 4°C or 7 – 4°C fruit (P = 0.0403), mostly due to bitter pit, which had the highest incidence in 1°C fruit (P = 0.0188; Table 3.12).

3.4.6 Conditioning quality analyses

Fruit firmness was highest at harvest and decreased throughout storage (P <0.0001). Fruit lost firmness during the shelf-life period compared to at removal from storage (9.3 kg/cm^2), but there was no difference between firmness at seven days (8.5 kg/cm^2) or 14

days (8.0 kg/cm^2) of the shelf-life period (P <0.0001). When fruit from all storage and shelf-life period samplings were combined, there was no difference in firmness between the 1°C (9.6 kg/cm^2), 10 – 1°C (9.6 kg/cm^2), or 20 – 1°C (9.8 kg/cm^2) temperature regimes (P <0.0001). Fruit stored in 1°C with or without conditioning were firmer than 10 – 4°C and 20 – 4°C fruit (P <0.0001). Additionally, both 4°C (9.1 kg/cm^2) and 20 – 4°C (8.9 kg/cm^2) fruit were more firm than 10 – 4°C (8.2 kg/cm^2) fruit. The 10 – 4°C fruit were the least firm of all temperature regimes (P <0.0001). Fruit had less starch after four weeks of storage than at harvest, with all starch having dissipated after 10 weeks of storage (P <0.0001). There were no differences in starch dissipation between temperature regimes after four weeks of storage (P = 0.1660) or seven weeks of storage (P = 0.4086).

Compared to harvest, fruit became more yellow in storage, but no further yellowing occurred during the shelf-life period (P <0.0001). No differences were found between temperature regimes at removal from cold storage (P = 0.5003) or after the shelf-life period (P = 0.1561). SSC was highest at harvest and decreased throughout storage but did not further decrease during the shelf-life period (P = 0.0016). At removal from cold storage, 10 – 4°C and 20 – 4°C fruit had lower SSC than all other temperature regimes except for a difference between 20 – 4°C and 20 – 1°C fruit (P = 0.0006). After the shelf-life period, 10 – 4°C and 20 – 4°C fruit had lower SSC than all other temperature regimes (P = 0.0008). TA was highest at harvest and decreased in storage and in the shelf-life period (P <0.0001). There was no difference in TA between temperature regimes at removal from cold storage (P = 0.6839), but after the shelf-life period 4°C had significantly less TA than all other temperature regimes except 10 – 4°C (P = 0.0099; Table 3.13).

Table 3.13: Effect of temperature regime on fruit quality of 'Eden' in the conditioning experiment at removal from cold storage after 10 weeks and after a 14-day shelf-life at 20°C in 2022. At harvest, background color was 5, soluble solids content was 11.8%, and titratable acidity was 0.846%. Mean of 3x10 fruit.

Temperature regime	Background color[1]		Soluble solids content (%)		Titratable acidity (%)	
	At removal	After shelf-life	At removal	After shelf-life	At removal	After shelf-life
Direct 1°C	7.7[2]	8.1	11.6 a	11.2 a	0.633	0.528 ab
10°C conditioning before 1°C	8.2	8.9	11.4 ab	11.3 a	0.636	0.524 ab
20°C conditioning before 1°C	8.2	8.6	11.2 bc	11.1 a	0.620	0.546 a
Direct 4°C	8.1	8.3	11.4 ab	11.1 a	0.643	0.448 c
10°C conditioning before 4°C	8.4	8.8	10.8 d	10.3 b	0.615	0.459 bc
20°C conditioning before 4°C	8.7	8.4	11.0 cd	10.6 b	0.618	0.514 ab
P-value	0.5003	0.1561	0.0006	0.0008	0.6839	0.0099

[1]Background color assessed on a scale of 1 (dark green) to 9 (dark yellow) according to Ctifl Tentation scale
[2]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

Greasy fruit were first observed after four weeks of cold storage, with 51 % of fruit affected at removal from cold storage after 10 weeks. The amount of greasy fruit increased during the shelf-life period, with 87 % of fruit being greasy at the end of the shelf-life period (P <0.0001). The highest numbers of greasy fruit from all sampling times combined were found in the 10 – 4°C (99 %) and 20 – 4°C (90 %) temperature regimes (P = 0.0001). The percentage of greasy fruit in other temperature regimes was not different and ranged from 53 % to 64 % (P = 0.0001).

3.4.7 Assessment of physiological disorders and fungal decay in the conditioning experiment

When fruit were removed from cold storage, 6.6 % of fruit had externally visible damage, with no differences found between the temperature regimes (P = 0.2260). This damage was due to fungal decay or externally visible watercore (Figure 3.9A). After the shelf-life period, 1°C fruit had three times less total damage than any other temperature regime, while 10 – 4°C and 20 – 4°C had the most total damage (P <0.0001). Fungal decay was limited, and no differences between treatments were found (P = 0.2414; Table 3.14).

Table 3.14: Effect of temperature regime on physiological disorders and fungal decay of 'Eden' fruit in the conditioning experiment in 2022. Fruit were stored for 10 weeks before removal and a shelf-life of 20°C for 14 days. Mean of 3x80 fruit.

Temperature regime	Total damage (%)	Fungal decay (%)[1]	Total physiological disorders		
			Watercore (%)[2]	Core browning (%)[3]	Other (%)[4]
Direct 1°C	12.9 c[5]	5.0 a	1.3	1.3 c	5.4
10°C conditioning before 1°C	47.5 b	2.1 a	3.3	31.3 b	10.4
20°C conditioning before 1°C	46.8 b	2.1 a	0.4	40.5 b	2.2
Direct 4°C	66.7 b	0.8 a	9.6	54.2 b	2.1
10°C conditioning before 4°C	86.7 a	2.1 a	8.3	73.3 a	1.3
20°C conditioning before 4°C	90.4 a	6.3 a	1.3	79.2 a	0.4
P-value	<0.0001	0.2414	0.1796	<0.0001	0.0687

[1]Fungal decay was identified by externally or internally visible signs either on the assessment day or after an additional 14 days at 20°C with constant light to promote fungal growth.
[2]Watercore was defined as brown or green translucent areas of the flesh with a water-soaked appearance.
[3]Core browning was defined as browning of the flesh primarily between the core and coreline. In severe cases, it could extend past the coreline into the cortex.
[4]Including bitter pit, diffuse flesh browning, senescent breakdown, and internal corking
[5]Numbers with letters indicate significance between temperature regimes for each damage category according to Student Newman Keuls tests at P = 0.05.

Watercore was present in each temperature regime, although no differences between temperature regimes were found (P = 0.1796). When watercore was found, core browning or senescent breakdown was also commonly present in the same fruit (Figure 3.11A,B). No differences between temperature regimes were found for the amount of fruit with watercore and core browning (P = 0.2454) or watercore and senescent breakdown (P = 0.4410). With the exception of the 1°C fruit, the majority of the total damage in each temperature regime was due to core browning (Table 3.14).

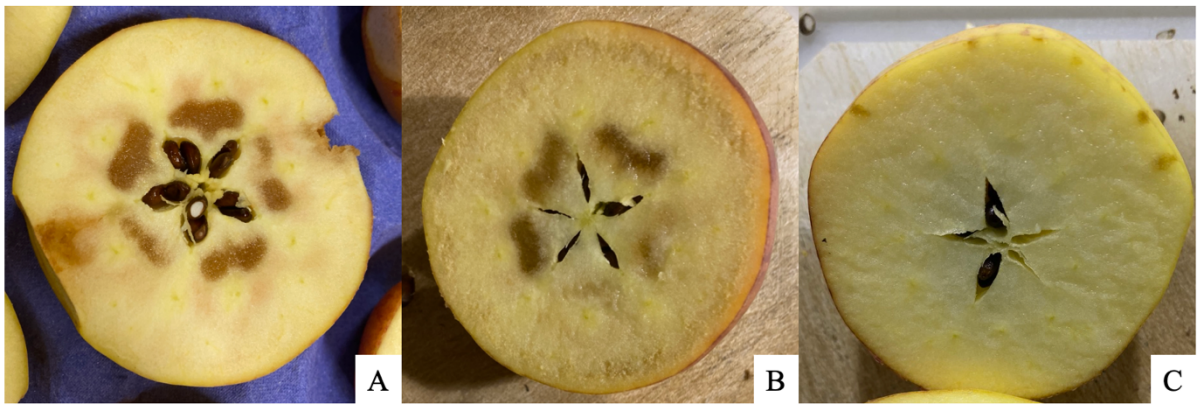

Figure 3.11: Fruit of 'Eden' from the conditioning experiment in 2022 after 10 weeks of cold storage and a 14-day simulated shelf-life at 20°C. A: Brown watercore with core browning 3 in unaffected areas. Holes are from firmness tests. B: Brown watercore with senescent breakdown near the peel. C: Bitter pit.

Fruit from 10 – 4°C and 20 – 4°C had higher amounts of core browning than any other temperature regime, while 1°C fruit had the lowest amount of core browning (P <0.0001). Fruit in 1°C had less CB1 than all other temperature regimes (P <0.0001), and less CB2 and CB3 then all fruit stored in 4°C regardless of whether they were conditioned or not. Excluding the 1°C temperature regime, CB1 was present in similar amounts across the remaining temperature regimes, ranging from 22.9% in 10 – 1°C to 33.3% in 10 – 4°C. While no differences in CB1 were found between any of the remaining temperature regimes, there were fewer fruit with CB2 in 10 – 1°C and 20 – 1°C than 10 – 4°C or 20 – 4°C (P = 0.0008). Furthermore, 10 – 1°C and 20 – 1°C had less CB3 than all fruit stored in 4°C regardless of whether they were conditioned or not (P = 0.0008). Despite fruit in 4°C having less total core browning than fruit in 10 – 4°C or 20 – 4°C, no differences between the three temperature regimes were found at any individual level of core browning (Figure 3.12).

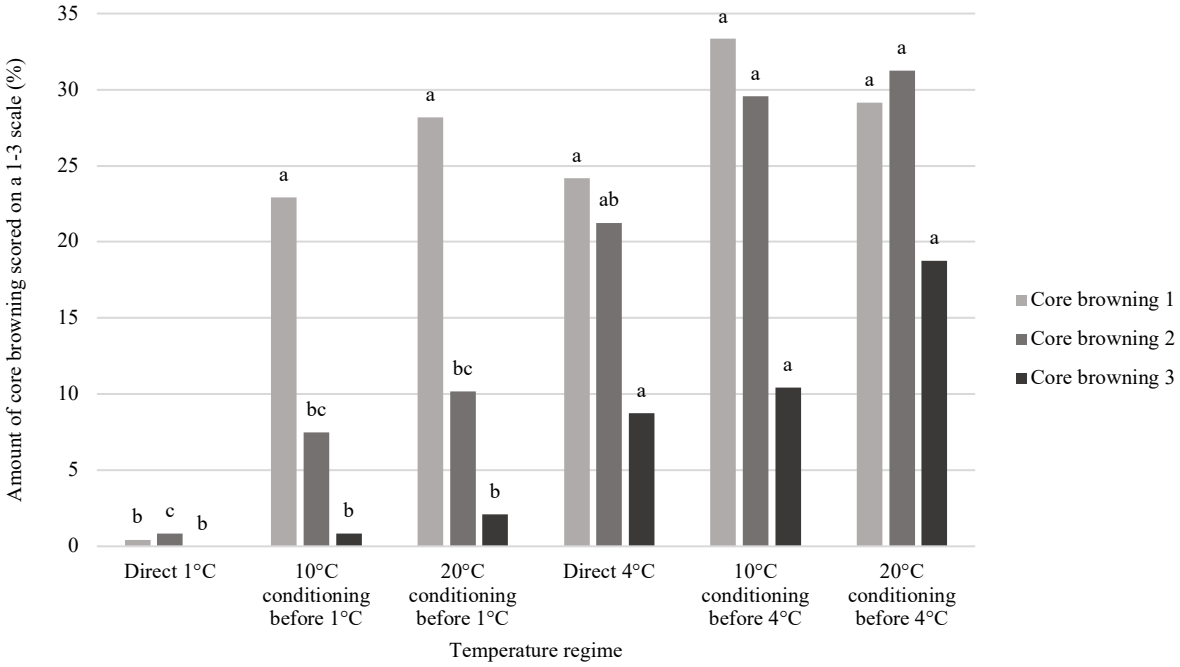

Figure 3.12: Effect of temperature regime on presence and severity of core browning in 'Eden' fruit from the conditioning experiment in 2022 after 10 weeks of cold storage and a shelf-life of 14 days at 20°C. Core browning levels are based off of the previously described scale (Figure 3.8). Mean of 3x80 fruit. Significance between temperatures and core browning severity is according to Student Newman Keuls tests at P = 0.05.

Some fruit (4.6%) from the 10 – 1°C treatment had slight senescent breakdown, and bitter pit was found occasionally, with 1°C fruit having more bitter pit (4.6%) than all other temperature regimes except 10 – 1°C (1.7%; P = 0.0067). All other temperature regimes had bitter pit in less than 1% of the fruit (Figure 3.11C). Diffuse flesh browning was only found in 4°C (0.8%) and 10 – 1°C (0.4%) fruit, with no differences between any of the temperature regimes (P = 0.2110).

3.5 'Fryd'

3.5.1 Core browning

Core browning was first observed after seven weeks of cold storage in some of the quality analysis samples. Symptoms varied but were generally minor (Figure 3.13). After removal from cold storage for nine weeks, the number of quality analysis fruit with core browning had increased. As such, a scale as created to help in determining severity of the core browning.

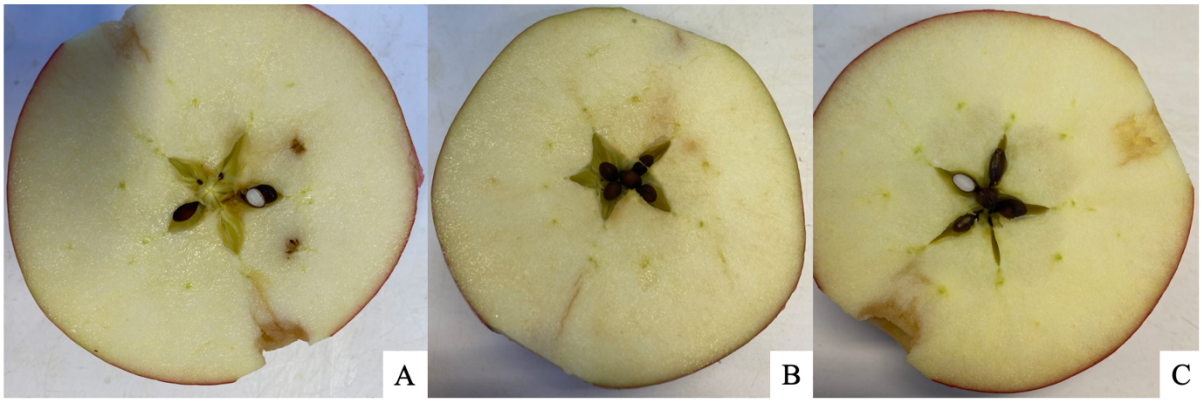

Figure 3.13: Fruit of 'Fryd' with core browning after seven weeks of cold storage in 2022. A: two small sections of three cavities along the coreline. B: slight browning around the top right carpel. C: slight core browning along the top half of the core.

The scale (Figure 3.14) was based on a 0-3 range but used different criteria than the core browning scale for 'Eden' due to different development patterns in the two cultivars. The scale was defined as:

- CB0: no core browning present
- CB1: Slightly brown coloration of the core, with generally half of less of the core sections affected.
- CB2: Dark brown coloration that affected half or less of the core sections, or slight brown coloration throughout all the core sections.
- CB3: Dark brown coloration throughout the whole core, which could be firm and dry, or spongy. Furthermore, the browning may have expanded past the core area.

In both CB2 and CB3, cavities were sometimes present within the core browning areas, and these were not recorded separately.

Figure 3.14: Core browning scale for ‘Fryd fruit, where 0 is no core browning (not pictured), 1 is slight core browning in a few sections of the core, 2 is slight core browning in all core sections or dark core browning in half or less of the core sections, and 3 is darker core browning in all core sections sometimes expanding into the flesh.

3.5.2 Analyses at harvest

At harvest, the same fruit were used in quality analysis for both the gradual cooling and conditioning experiments. Fruit were mature but relatively unripe, and were picked prior to the preliminary harvest recommendations (Fresh Forward Breeding & Marketing BV, 2022). Fruit were firm (8.9 kg/cm^2), with a green background color but 60-70% blush, and limited starch dissipation (2.2).

3.5.3 Gradual cooling quality analyses

Firmness decreased from harvest throughout nine weeks of storage, regardless of temperature regime, and continued decreasing throughout the shelf-life period ($P < 0.0001$). However, firmness was still high at removal from cold storage after nine weeks, with all temperature regimes loosing less than 1 kg/cm^2 when compared to harvest (Table 3.15). While differences between temperature regimes at each sampling date were inconsistent, the 7 – 4°C fruit had the lowest overall firmness, with no differences found between any of the other temperature regimes ($P = 0.0066$). Background color was more yellow at removal from cold storage than at harvest, progressing further after the shelf-life period ($P < 0.0001$). However, there were no differences between any of the temperature regimes at either sampling date (Table 3.15).

Table 3.15: Effect of temperature regime on fruit quality of 'Fryd' in the gradual cooling experiment at removal from cold storage and after a 14-day shelf-life at 20°C in 2022. At harvest, firmness was 8.9 kg/cm^2, background color was 3.1, soluble solids content was 10.7%, and titratable acidy was 1.003%. Mean of 3x10 fruit.

Temperature regime	Firmness (kg/cm^2)		Background color[1]		Soluble solids content (%)		Titratable acidity (%)	
	At removal	After shelf-life	At removal	After shelf-life	At removal	After shelf-life	At removal	After shelf-life
Direct 1°C	8.8[2]	6.9	6.5	6.5	11.4	12.0	0.806 a	0.503 a
Gradual cooling 7°C to 1°C	8.5	6.7	6.4	6.1	11.9	12.0	0.838 a	0.483 ab
Direct 2°C	8.2	7.1	6.7	6.3	12.3	12.4	0.736 b	0.459 b
Gradual cooling 7°C to 2°C	8.5	6.7	6.3	5.6	12.4	11.7	0.707 bc	0.456 b
Direct 4°C	8.0	7.2	6.9	5.4	12.1	11.8	0.710 bc	0.485 ab
Gradual cooling 7°C to 4°C	7.9	6.7	6.6	5.9	11.9	12.0	0.658 c	0.458 b
P-value	0.0883	0.2762	0.9692	0.6190	0.0646	0.0633	0.0001	0.0243

[1]Background color assessed on a scale of 1 (dark green) to 9 (dark yellow) according to Ctifl Tentation scale
[2]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

SSC increased from 10.7% at harvest to 12.0% after the shelf-life period ($P < 0.0001$). There were no differences in SSC between temperature regimes after storage or the shelf-life period (Table 3.15). TA was highest at harvest and decreased both in cold storage and after the shelf-life storage ($P < 0.0001$). The 7 – 1°C and 1°C fruit had the highest TA at removal from cold storage, with no difference between the two temperature regimes ($P = 0.0001$). After the shelf-life period, 1°C fruit still had higher TA than 2°C, 7 – 2°C, and 7 – 4°C fruit ($P = 0.0243$; Table 3.15).

Starch was degraded throughout storage starting after seven weeks until seven days of the shelf-life period ($P < 0.0001$). All starch had degraded by the end of the shelf-life period. There was less starch in 7 – 4°C fruit than 2°C fruit after three weeks of storage ($P = 0.0306$), and both the 7 – 4°C and 4°C fruit had less starch than either the 1°C or the 7 – 1°C fruit after seven and nine weeks of storage. During the shelf-life, there was no difference between storage regimes, as almost all the starch had dissipated after 7 days at 20°C (Table 3.16).

Table 3.16: Effect of storage temperature regime on starch dissipation of 'Fryd' fruit at four sampling times in 2022. Starch dissipation was scored on a scale from 1 (100% starch) to 10 (0% starch). At harvest starch was 2.2. Mean of 3x10 fruit per temperature regime. All starch had dissipated after 14 days of shelf-life.

Temperature regime	After storage for			After shelf-life at 20°C for
	3 weeks	7 weeks	9 weeks	7 days
Direct 1°C	2.2 ab[1]	5.9 b	6.2 b	9.7
Gradual cooling 7°C to 1°C	2.3 ab	5.9 b	6.4 b	9.7
Direct 2°C	1.9 b	6.9 ab	7.8 ab	9.9
Gradual cooling 7°C to 2°C	2.4 ab	6.8 ab	7.6 ab	9.8
Direct 4°C	2.4 ab	7.4 a	8.8 a	9.6
Gradual cooling 7°C to 4°C	2.8 a	7.9 a	8.8 a	9.8
P-value	0.0306	0.0072	0.0020	0.6555

[1]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

Greasy fruit were first noticed after seven weeks of cold storage, where 17.8% of the fruit were greasy. The level of greasiness increased after removal from cold storage, with 97.2% of the fruit being greasy at the end of the shelf-life period (P <0.0001). When comparing fruit from all harvest dates together, 7 – 4°C fruit were greasier than 1°C, 7 – 1°C, or 2°C fruit (P <0.0001), however throughout the shelf-life period there were no differences between the temperature regimes (After seven days of shelf-life P = 0.5162; after 14 days of shelf-life P = 0.6710).

3.5.1 Assessment of physiological disorders and fungal decay in the gradual cooling experiment

When fruit were removed from cold storage after nine weeks, each temperature regime had 5% or less fruit with external damages, including fungal decay, with no differences between temperature regimes (P = 0.8761). After the shelf-life period, the fruit still had few external damages. The percentage of fruit with fungal decay was still low, with no differences found between storage regimes (P = 0.4857; Table 3.17), and there were limited external physiological disorders. A few 7 – 2°C fruit had sunken irregular brown sections similar to lenticel breakdown or leather blotch, but without extending into the flesh, and extending well past the calyx end; these fruit did not have visible signs of bitter pit (Figure 3.15A).

Figure 3.15: Fruit of 'Fryd' from the gradual cooling experiment in 2022 after 10 weeks of cold storage and a 14-day simulated shelf-life at 20°C. A: Potential lenticel breakdown or leather blotch. B: core browning 3, brown watercore, and diffuse flesh browning.

In contrast to external damage, over half the fruit had internal damage, due mostly to core browning. Over 90% of the total damage in each temperature regime was due to core browning. While not different, the amount of core browning varied between temperature regimes, with values from 53.8% in 7 – 1°C to 90.4% in 1°C fruit (Table 3.17).

Table 3.17: Effect of temperature regime on physiological disorders and fungal decay of 'Fryd' fruit in 2022. Fruit were stored for 10 weeks before removal to 20°C for 14 days. Mean of 3x80 fruit.

Temperature regime	Total damage (%)	Fungal decay (%)[1]	Physiological disorders	
			Core browning (%)[2]	Other (%)[3]
Direct 1°C	92.1[4]	1.3	90.4	0.4 b
Gradual cooling 7°C to 1°C	55.8	1.7	53.8	0.4 b
Direct 2°C	81.3	0.4	80.8	0.0 b
Gradual cooling 7°C to 2°C	58.8	2.1	53.8	2.9 a
Direct 4°C	59.6	2.1	57.5	0.0 b
Gradual cooling 7°C to 4°C	65.0	1.7	63.3	0.0 b
P-value	0.0907	0.4857	0.0639	0.0059

[1]Fungal decay was identified by externally or internally visible signs either on the assessment day or after an additional 14 days at 20°C with constant light to promote fungal growth.
[2]Core browning was defined as browning of the flesh primarily between the core and coreline. In severe cases, it could extend past the coreline into the cortex.
[3]Including diffuse browning without associated core browning and brown shrunken lenticels
[4]Numbers with letters indicate significance between temperature regimes for each damage category according to Student Newman Keuls tests at P = 0.05.

Across all temperature regimes, CB1 was the most commonly found level of core browning. There was no difference in the amount of CB1 between treatments, but percentages ranged from 32.5% in 7 – 1°C to 55.0% in 2°C (P = 0.2976). CB2 was found in similar amounts to CB1 in 1°C fruit but was present in lower amounts in all other temperatures. Furthermore, 1°C had significantly higher CB2 than any other temperature regime (P = 0.0100). There was no difference in the amount of CB3 between any temperature regime (P = 0.1947; Figure 3.16). In all temperature regimes, about half the fruit with CB3 also had diffuse flesh browning, with between 35.3 and 81.8% of CB3 fruit also having diffuse flesh browning. It was assumed the core browning had started first and was the primary disorder, and the diffuse flesh browning was secondary. In a few extreme cases, these CB3 fruit had watercore and diffuse flesh browning as well (Figure 3.15B).

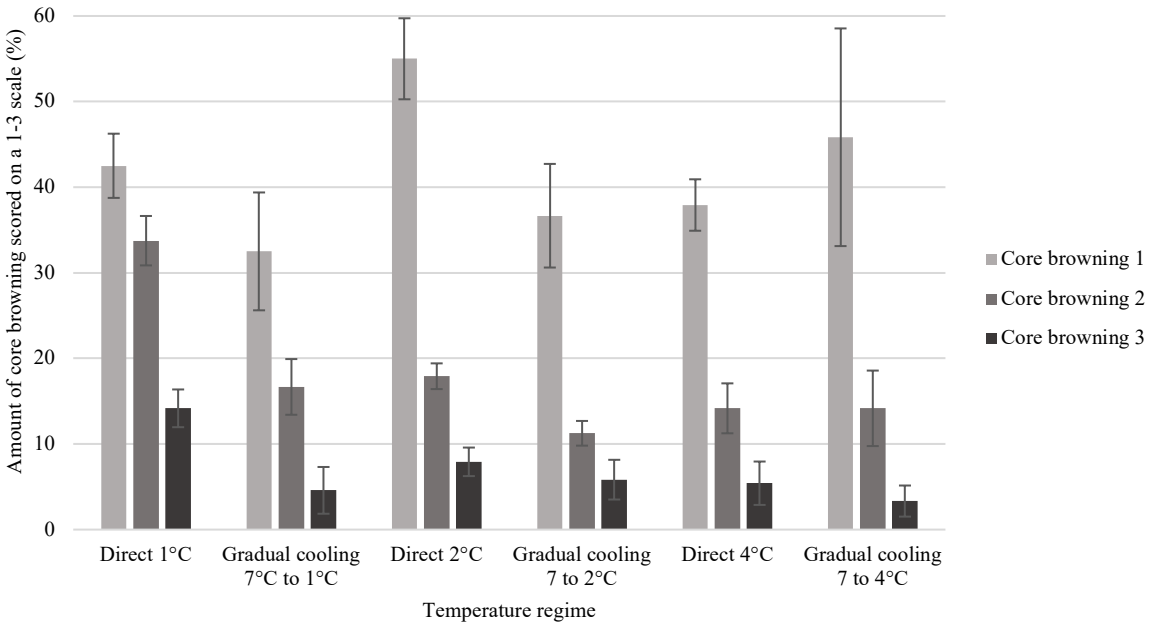

Figure 3.16: Effect of temperature regime on presence and severity of core browning in 'Fryd' fruit in 2022 after 10 weeks of cold storage and 14 days at 20°C. Core browning levels are based off of the previously described scale (Figure 3.14). Values are a mean of 3x80 fruit. Error bars represent standard error (±SE). Significance between temperatures and core browning severity is according to Student Newman Keuls tests at P = 0.05.

3.5.2 Conditioning quality analyses

Compared to at harvest, fruit firmness decreased starting after 7 weeks of cold storage and continued to decrease throughout the shelf-life period (P <0.0001). At removal from cold storage at nine weeks, 10 – 4°C and 20 – 4°C fruit were less firm than all other treatment regimes except 20 – 1°C, and 1°C fruit maintained the most firmness overall (P = 0.0004).

After seven days of the shelf-life period, all temperature regimes except 10 – 4°C were firmer than 20 – 4°C fruit (P = 0.0004), but after 14 days of the shelf-life period, only 4°C fruit were firmer (P = 0.0105). Similarly, 10 – 4°C fruit were less firm than 1°C, 10 – 1°C, and 4°C fruit after seven days of the shelf-life period (P = 0.0004), but after 14 days were only less firm than the 4°C fruit (P = 0.0105). All treatments lost less than 3 kg/cm^2 of firmness throughout the whole storage period, and treatments were within 1 kg/cm^2 of each other at the final sampling date (Table 3.18).

Table 3.18: Effect of temperature regime on firmness (in kg/cm^2) of 'Fryd' fruit from the conditioning experiment at five sampling times in 2022. At harvest firmness was 8.9 kg/cm^2. Mean of 3x10 fruit per temperature regime.

Temperature regime	After storage for			After shelf-life at 20°C for	
	3 weeks	7 weeks	9 weeks	7 days	14 days
Direct 1°C	9.0[1]	8.5 a	8.8 a	7.9 a	6.9 ab
10°C conditioning before 1°C	8.8	8.0 ab	8.2 b	7.5 ab	6.9 ab
20°C conditioning before 1°C	8.4	7.8 ab	7.5 bc	7.2 bc	6.7 ab
Direct 4°C	8.6	8.3 ab	8.0 b	8.0 a	7.2 a
10°C conditioning before 4°C	8.6	7.6 bc	7.2 c	6.9 cd	6.2 b
20°C conditioning before 4°C	8.7	7.3 c	6.9 c	6.5 d	6.4 b
P-value	0.2054	0.0021	0.0004	0.0004	0.0105

[1]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

Starch decreased gradually throughout storage, with some starch still present in the fruit at removal from cold storage after nine weeks, and all starch dissipating after 14 days of the shelf-life period (P <0.0001). After three and seven weeks of cold storage, 20 – 4°C fruit had the least starch of all temperature regimes and nearly all starch had dissipated after seven weeks (Table 3.19). At removal from cold storage, 1°C fruit had more starch than all other temperature regimes, and 10 – 1°C fruit had more starch remaining than all but the 1°C and 4°C temperature regimes (P <0.0001). However, after seven days of the shelf-life period, there were no differences between any of the temperature regimes except for 4°C, which had more starch remaining than all temperature regimes other than 1°C (P = 0.0087; Table 3.19).

Table 3.19: Effect of temperature regime on starch (1-10 scale) of 'Fryd' fruit from the conditioning experiment at four sampling times in 2022. Starch dissipation was scored on a scale from 1 (100% starch) to 10 (0% starch). At harvest starch was 2.2. Mean of 3x10 fruit per temperature regime. All starch had dissipated after 14 days of shelf-life.

Temperature regime	After storage for			After shelf-life at 20°C for
	3 weeks	7 weeks	9 weeks	7 days
Direct 1°C	2.2 d[1]	5.9 d	6.2 d	9.7 ab
10°C conditioning before 1°C	2.8 d	7.6 c	8.2 c	9.9 a
20°C conditioning before 1°C	6.5 b	8.9 b	9.4 ab	10.0 a
Direct 4°C	2.4 d	7.4 c	8.8 bc	9.6 b
10°C conditioning before 4°C	4.0 c	9.0 b	9.6 ab	9.9 a
20°C conditioning before 4°C	7.6 a	9.6 a	9.8 a	10.0 a
P-value	<0.0001	<0.0001	<0.0001	0.0087

[1]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

From harvest through cold storage and the shelf-life period, background color became more yellow (P <0.0001). There were no differences at removal from cold storage between temperature regimes, but after the shelf-life period, 20 – 4°C fruit were more yellow than all other temperature regimes (P = 0.0262). SSC increased after cold storage as compared to at harvest (P <0.0001). At removal from cold storage, there was no significant difference in SSC between any of the temperature regimes (P = 0.1198). However, after the shelf-life period SSC was higher in 1°C fruit than 10 – 4°C fruit, with no other differences between temperature regimes. In contrast, TA decreased at each sampling time (P <0.0001). The 1°C and 10 – 1°C temperature regimes had significantly higher TA than all other temperature regimes at removal from cold storage, and 20 – 1°C and 4°C had significantly higher TA than the remaining two temperature regimes, 10 – 4°C and 20 – 4°C (P <0.0001). However, no differences between temperature regimes were found after the shelf-life period (P = 0.2892; Table 3.20).

Table 3.20: Effect of temperature regime on fruit quality of 'Fryd' in the conditioning experiment at removal from cold storage and after a 14-day shelf-life at 20°C in 2022. At harvest, firmness was 8.9 kg/cm^2, background color was 3.1, soluble solids content was 10.7%, and titratable acidy was 1.003%. Mean of 3x10 fruit.

Temperature regime	Background color[1]		Soluble solids content (%)		Titratable acidity (%)	
	At removal	After shelf-life	At removal	After shelf-life	At removal	After shelf-life
Direct 1°C	5.1[2]	7.2 b	11.4	12.0 a	0.806 a	0.503
10°C conditioning before 1°C	5.1	7.5 b	12.4	11.8 ab	0.769 a	0.484
20°C conditioning before 1°C	5.1	7.4 b	12.3	11.6 ab	0.701 b	0.454
Direct 4°C	5.4	7.0 b	12.1	11.8 ab	0.710 b	0.485
10°C conditioning before 4°C	5.2	7.2 b	11.8	11.4 b	0.637 c	0.461
20°C conditioning before 4°C	6.0	8.7 a	12.0	11.8 ab	0.626 c	0.465
P-value	0.6383	0.0262	0.1198	0.0263	<0.0001	0.2892

[1]Background color assessed on a scale of 1 (dark green) to 9 (dark yellow) according to Ctifl Tentation scale
[2]Numbers with letters indicate significance between temperature regimes at each sampling date according to Student Newman Keuls tests at P = 0.05.

Greasy fruit were first found after seven weeks of cold storage, with 38.9% of the fruit being greasy. There was no further increase in amount of greasy fruit at removal from cold storage after nine weeks, but during the shelf-life period, greasiness increased after both seven and 14 days, with 98.3% of all fruit being greasy at the end of the shelf-life period (P <0.0001). When comparing fruit from all harvest dates together, 10 – 4°C and 20 – 4°C fruit were greasier than fruit from the other temperature regimes (P <0.0001). However, by the end of the shelf-life period, there were no differences between the temperature regimes (P = 0.5464).

3.5.3 Assessment of physiological disorders and fungal decay in the conditioning experiment

Upon removal from cold storage, 'Fryd' fruit had few external disorders or damages, with no differences between temperature regimes (P = 0.7002). However, when fruit were removed for quality analysis after seven days of the shelf-life period, soft scald was observed on some fruit. At the end of the shelf-life period, soft scald was found on 26% of the 20 – 1°C fruit (Table 3.21). Two-thirds of fruit with soft scald had less than 25% of the skin visibly affected, with the soft scald extending at least 1mm into the flesh (Figure 3.17A). The rest of the soft scald covered between 25-50% of the peel of affected fruit, also extending into the flesh, with a few exceptions where the soft scald covered up to 75% of the peel and extended throughout most of the flesh (Figure 3.17B). Across all temperature regimes and both experiments with 'Fryd', 20 – 1°C was the only temperature regime where soft scald was found. Furthermore, fruit that had both soft scald and core browning were rare; only 5% of the fruit that had soft scald also had core browning. There were no other external physiological

disorders found, and total rot was below 7% for all treatments with no significant differences between treatments (P-value = 0.1642; Table 3.21).

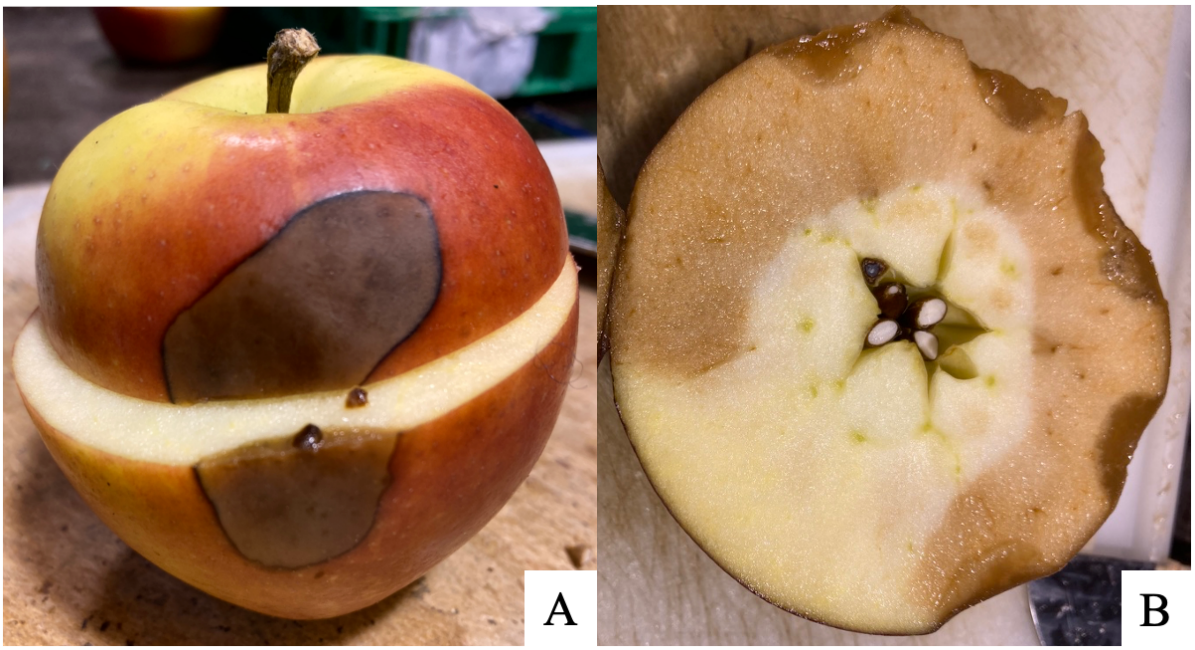

Figure 3.17: Fruit of 'Fryd' from the conditioning experiment in 2022 after 10 weeks of cold storage and a 14-day simulated shelf-life at 20°C. A: Soft scald covering 0-25% of the peel and extending into the flesh. The two "drops" visible on the flesh were seed halves. B: Soft scald covering 75-100% of the peel, extending throughout most of the flesh, and with secondary rot.

Total damage after storage was lowest in the 20 – 4°C fruit, and highest in the 1°C fruit ($P < 0.0001$). The 1°C fruit also had more core browning than any other temperature regime ($P < 0.0001$). The 4°C fruit had a significantly higher percent of core browning than all other temperature regimes except 1°C ($P < 0.0001$), but total damage was not different between 4°C and 20 – 1°C fruit due to the high percentage of soft scald in the 20 – 1°C fruit ($P < 0.0001$). No differences in total damage or core browning were found between 10 – 1°C, 10 – 4°C, or 20 – 4°C fruit. With the exception of the 20 – 1°C treatment, at least 75% of the total damage in each treatment was due to core browning (Table 3.21). Physiological disorders other than core browning or soft scald were found in small amounts, and no differences were found between temperature regimes ($P = 0.0613$).

Table 3.21: Effect of temperature regime on physiological disorders and fungal decay of 'Fryd' fruit in the conditioning experiment in 2022. Fruit were stored for 10 weeks before removal and a shelf-life period of 20°C for 14 days. Mean of 3x80 fruit.

Temperature regime	Total damage (%)	Fungal decay (%)[1]	Total physiological damage		
			Core browning (%)[2]	Soft scald (%)[3]	Other (%)[4]
Direct 1°C	92.1 a[5]	1.3	90.4 a	0.0 b	0.4
10°C conditioning before 1°C	35.8 cd	2.9	30.8 c	0.0 b	2.1
20°C conditioning before 1°C	52.7 bc	1.3	25.5 c	26.0 a	0.0
Direct 4°C	59.6 b	2.1	57.5 b	0.0 b	0.0
10°C conditioning before 4°C	42.1 cd	4.2	34.6 c	0.0 b	3.3
20°C conditioning before 4°C	27.9 d	6.7	20.8 c	0.0 b	0.4
P-value	<0.0001	0.1642	<0.0001	<0.0001	0.0613

[1]Fungal decay was identified by externally or internally visible signs either on the assessment day or after an additional 14 days at 20°C with constant light to promote fungal growth.
[2]Core browning was defined as browning of the flesh primarily between the core and coreline. In severe cases, it could extend past the coreline into the cortex.
[3] Soft scald was determined by ribbon-like browning of the peel with clearly defined edges.
[4]Including diffuse browning without associated core browning and brown shrunken lenticels
[5]Numbers with letters indicate significance between temperature regimes for each damage category according to Student Newman Keuls tests at P = 0.05.

When comparing the three levels of core browning, CB1 was highest in the 1°C and 4°C temperature regimes (42.5% and 37.9%, respectively) compared to any other temperature regime (P = 0.0002). Additionally, the other four temperature regimes were not significantly different from each other. Unlike CB1 however, 1°C fruit had over twice the amount of fruit with CB2 than the 4°C temperature regime. Amounts of CB2 were below 5% for all remaining temperature regimes, and while all lower than 1°C or 4°C temperature regimes, there was no difference between the others (P = 0.0008). CB3 was also present in all temperature regimes, but there were no difference in number of affected fruit between any of the temperature regimes (P = 0.3318; Figure 3.18). Diffuse flesh browning was often found with CB3 fruit as well, with between 35.3 and 100% of all CB3 fruit having diffuse flesh browning.

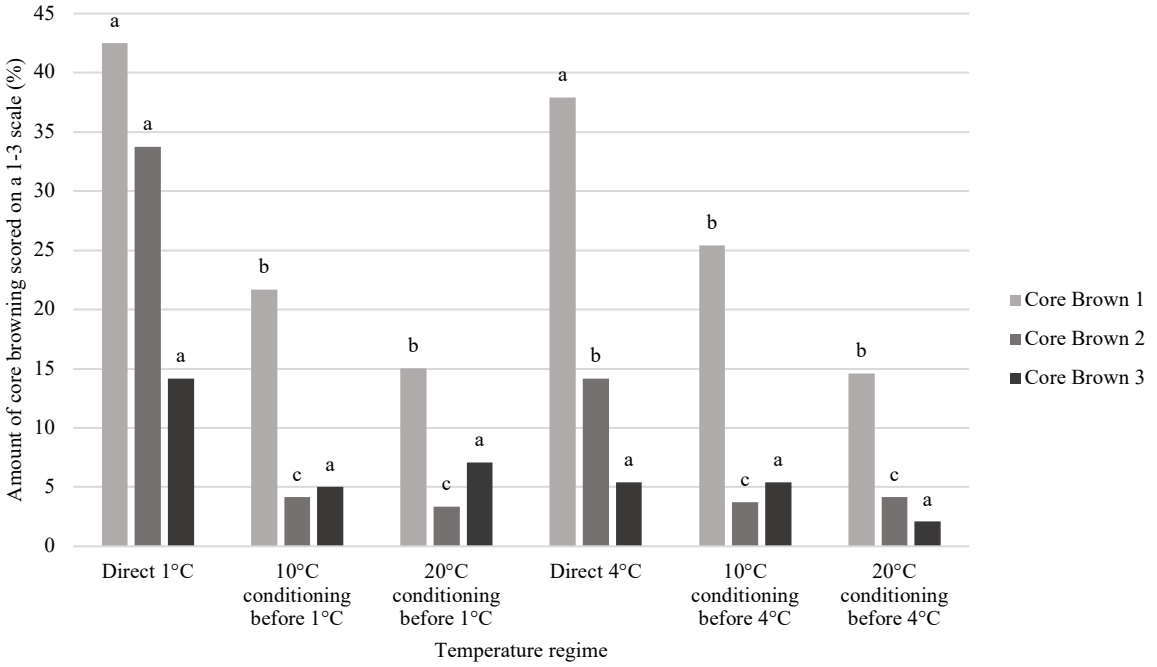

Figure 3.18: Effect of temperature regime on presence and severity of core browning in 'Fryd' conditioning fruit in 2022 after 10 weeks of cold storage and 14 days at 20°C. Core browning levels are based off of the previously described scale (Figure 3.14). Values are a mean of 3x80 fruit. Error bars represent standard error (±SE). Significance between temperatures and core browning severity is according to Student Newman Keuls tests at P = 0.05.

4 Discussion

4.1 'Red Aroma'

4.1.1 Effect of crop load on fruit size and count, fruit quality at harvest and after cold storage, and disorder incidence after cold storage

Thinning of 'Red Aroma' to three different crop loads resulted in fruits of different sizes throughout much of the growing season and at harvest. Low crop load fruit were first to reach marketable size (>60mm), followed by medium, then high crop load fruit. Fruit from low crop loads typically have more rapid growth than fruit from high crop loads, mostly due to more resource allocation. At harvest in the present experiment, low crop load fruit were heavier and larger than high crop load fruit, consistent with previous findings (Meland, 2011; Tahir et al., 2008; Wünsche and Ferguson, 2005).

Bloom thinning may have resulted in more differences in weight and size of 'Red Aroma' fruit in this experiment. Fruit can be up to 30% heavier at harvest if bloom thinning occurred as opposed to thinning after June drop (Link, 2000; Wünsche and Ferguson, 2005). In 'Aroma' and 'Summerred' fruit grown in Norway, blossom thinning was shown to increase

final fruit size compared to thinning at June drop (Meland, 2011; Meland and Kaiser, 2011), suggesting that if thinning in this experiment had been completed closer to bloom there may have been greater differences between the three crop loads.

At harvest in the current experiment, the number of fruit per tree in the low and medium crop loads were slightly above the pre-adjusted crop loads, while the high crop load had 20 fruit per tree fewer than planned. In a study in New Zealand on five year old 'Braeburn'/M26 trees, fruit were initially thinned to 180 fruit per tree, but at harvest only 160 fruit per tree were left, similar to what was found in high crop load trees in the present experiment (Wünsche et al., 2000). In overcropped trees, fruit drop has been shown to occur early in the season as a way to manage carbohydrate balance (Sharples, 1968; Wünsche and Ferguson, 2005). As the high crop load trees in the current experiment had the highest number of fallen fruit, often with no visible cause of drop, it could indicate the lack of adequate carbohydrate supply continued throughout the season, confirming that the high crop load treatment did in fact have too many fruit per tree.

Low crop load fruit were both larger and heavier than fruit from the medium or high crop loads. However, these differences were not as great as expected. There was less difference in fruit weights between crop loads in this experiment than in all three years of a previous experiment on crop loads of 'Aroma' fruit in Norway (Meland, 2011). Additionally, the three crop loads were only separated in diameter by 5mm, which, according to EU quality standards, would allow the fruit to all be packed together (Comission Regulation (EC) No. 84, 2004), suggesting the differences in size were relatively small.

In addition to being larger and heavier than the other crop loads, low crop load fruit in the current experiment were slightly less mature at harvest than the fruit from the other two crop loads. This was evidenced by low crop load fruit having higher amounts of starch and SSC, but similar background color, firmness, and TA compared to the medium and high crop load fruit. The delayed maturity was unexpected and in contrast to much of the research which suggests that maturity is advanced in low crop load fruit, as reviewed by Wünsche and Ferguson (2005). However, in Slovenia, low crop load 'Jonagold' fruit were shown to have delayed maturity compared to higher crop loads (Stopar et al., 2002). In 'Braeburn' from New Zealand and France, and 'McIntosh' from Massachusetts, no differences in starch patterns were found between high and low crop load fruit (Elgar et al., 1999; Greene and Autio, 1989; Kelner et al., 2000). In Norwegian studies, 'Elstar' fruit showed lower amounts of starch in low crop load fruit (Meland, 2009), however no differences in starch were found at harvest between hand thinned and unthinned fruit in 'Rubinstep' (Maas et al., 2020). In a Norwegian study on

‘Aroma’, when fruit were picked before starch had fully dissipated, lower crop loads had more starch (Meland, 2011). This variability in starch progression suggests that the typical effects of crop load on fruit quality may not always be expressed, especially in northern climates with a short, cool growing season and variable weather.

In contrast to starch content, SSC can be higher in low crop load fruit (Meland and Kaiser, 2011; Wünsche and Ferguson, 2005), as seen in the present experiment. In this experiment the lack of differences between crop loads in background color and TA at harvest was unexpected; in many cultivars low crop load fruit are often more yellow and have higher TA than high crop load fruit (Link, 2000; Serra et al., 2016; Wünsche and Ferguson, 2005). Prior studies in Norway have found no difference between crop loads on background color at harvest in ‘Rubinstep’ and ‘Aroma’ in Norway, suggesting factors other than crop load influence color development (Maas et al., 2020; Meland, 2011). Crop load effects on firmness may be variable, as some studies have shown low crop loads increase firmness (Greene and Autio, 1989; Meland and Kaiser, 2011; Tough et al., 1998), while others have shown inconsistent or no effect of crop loads on firmness (Link, 2000; Maas et al., 2020; Meland, 2011). This is similar to in the present experiment, where crop load did not have an effect on firmness at harvest and suggests factors other than crop load are more important in determining firmness at harvest (DeEll et al., 2001).

In storage in the current experiment, background color became more yellow, although differences between crop loads were only seen in 1°C after the shelf-life period, where high crop load fruit were greener than the other two crop loads. Background color can advance during storage regardless of crop load, as shown in ‘Braeburn’ fruit from New Zealand (Tough et al., 1998). In the present experiment, the further advancing of background color in low and medium crop load fruit at 1°C is unusual because fruit stored at 4°C advanced similarly regardless of crop load. It is possible the high crop load fruit at 1°C ripened slower than the other crop loads in storage.

Throughout storage, SSC remained similar to at harvest, with low crop load fruit generally having the highest SSC, as supported by other studies on ‘Braeburn’ in New Zealand and ‘Katja’ in Sweden (Elgar et al., 1999; Tahir et al., 2008). In the present experiment, TA declined during storage, regardless of crop load or temperature, as supported by similar findings in many cultivars grown in Germany (Ackermann et al., 1992; Link, 2000). However, low crop load fruit in the present experiment had higher TA than the other crop loads at each sampling time. This is consistent with previous research on multiple cultivars in Germany and ‘Braeburn’ in New Zealand (Link, 2000; Tough et al., 1998).

Firmness was highest at harvest in the current experiment and decreased throughout cold storage and the shelf-life period, regardless of storage temperature or crop load. Differences in firmness between crop loads were only found after the shelf-life period, with medium crop load fruit maintaining higher firmness than the low crop load fruit. The variability in firmness at harvest in different cultivars suggests this difference may be due in part to factors other than crop load. Furthermore, in the few studies where fruit of different crop loads were stored, firmness differences at harvest were not always maintained in storage, including on 'McIntosh' fruit from Massachusetts and 'Honeycrisp' fruit from Washington (Greene and Autio, 1989; Serra et al., 2016). This suggests that fruit quality of different crop loads at harvest may not be representative of fruit quality after storage and emphasizes the importance of continuing crop load experiments past harvest, as quality parameters can change between harvest and marketing.

Soft scald incidence was not different between the three crop loads, despite some differences in fruit size and maturity at harvest between crop loads in this experiment. Large fruit size has been shown to increase storage disorders in 'Red Aroma' fruit (Weigl et al., 2023), however in the present experiment, no difference between soft scald incidence was found between the three crop loads and therefore the different fruit sizes. This could be due to the limited differences in fruit size found in the present experiment. If larger differences in fruit size had been found between the three crop loads, it is possible the large fruit would have had higher disorder incidence.

Other cultivars have also been shown to have increased disorder incidence and severity in large fruit compared to small fruit. In 'Royal Gala' in Washington, large fruit were more susceptible to flesh breakdown and cracking than small fruit. These fruit were separated from bins of commercially harvested fruit (Lee et al., 2013). Additionally, large fruit are often more susceptible to bitter pit, due to lower calcium concentrations (Ferguson and Triggs, 1990; Perring and Jackson, 1975; Wünsche and Ferguson, 2005). However, studies on 'Cox Orange Pippin' in New Zealand have shown that regardless of fruit size, low crop load fruit had lower calcium concentrations and higher bitter pit incidence than high crop load fruit (Ferguson and Triggs, 1990; Ferguson and Watkins, 1992). This indicates that using crop load as a way to ensure different fruit sizes may not always result in differences in physiological disorders.

Increased maturity has been linked to increased soft scald susceptibility in multiple cultivars in Iowa and 'Honeycrisp' throughout the Northeast and Midwest US (Brooks and Harley, 1934; Tong et al., 2003; Watkins et al., 2005). In previous research on 'Aroma' fruit in Norway, more mature fruit at harvest had more total physiological damage after three

months of cold storage in RA (Knutsen et al., 2015), suggesting a link between maturity and soft scald incidence might be present for 'Aroma' fruit specifically. As such, if the low crop load fruit in this experiment had been as or more mature than the other crop loads, it is possible they would have developed more soft scald.

However, in the few articles that specifically examined soft scald in relation to crop load, no consistent effect of crop load was found on soft scald incidence. High crop loads have been linked to higher amounts of soft scald in 'Scifresh' fruit in one of two regions in New Zealand, and in 'Honeycrisp' fruit in Nova Scotia, but not in New York. The effect of crop load on soft scald incidence may be related to growing conditions as well, as Nova Scotia has a colder growing season than New York, and the association between crop load and soft scald incidence was only found in the colder growing region in New Zealand (Delong et al., 2006; Henriod et al., 2011; Robinson and Watkins, 2003). It is possible in the current experiment that another year with different growing conditions would have resulted in crop load having a larger effect on soft scald incidence in 'Red Aroma' fruit.

While many studies have found interactions between crop load and storage disorders aside from soft scald, these studies have been completed in warmer climates with longer growing seasons and as such may not be entirely applicable to fruit grown in a Norwegian climate (Ferguson and Watkins, 1992; Sharples, 1968; Wünsche and Ferguson, 2005). Unfortunately, few trials on crop loads in Nordic conditions assess the fruit after storage, or separate physiological disorders from overall decay.

Crop load did not have an effect on incidence of fungal decay in this experiment. Previous studies have shown that advanced maturity of fruit at harvest increases susceptibility to decay in storage in Scandinavian conditions (Knutsen et al., 2015; Tahir, 2006). However, in 'Katja' fruit after a storage period of 10 weeks, no effect of thinning was found on incidence of decay (Tahir et al., 2008). As crop loads seem to have variable effects on maturity and fruit quality in Nordic climates, low crop load fruit may not be more susceptible to fungal decay or physiological disorders than higher crop load fruit, as shown in the present experiment.

4.1.2 Effect of storage temperature on fruit quality and disorder development

In contrast to the limited effects of crop load on storability of 'Red Aroma' fruit, storage temperature impacted both fruit quality and disorder incidence in this experiment. After seven weeks of storage, 'Red Aroma' fruit in the gradual cooling experiment were below the required 5 kg/cm^2 firmness for commercial sale (Rosseland and Eidsvik, 2021), regardless of storage

temperature, and differences between storage regimes were only found after fruit were unmarketable. However, in the crop load experiment, 1°C fruit maintained acceptable firmness until after removal from cold storage, while 4°C fruit were too soft at removal from cold storage. Other studies on 'Aroma' have shown firmness is often close to or less than the requirement of 5 kg/cm^2 after four to six months of storage regardless of storage temperature (Børve and Stensvand, 2016; Sjöstrand et al., 2023; Tahir et al., 2007). Fruit in the present experiment were only stored for two months, suggesting these fruit may have been more mature at harvest and therefore less suitable to storage than those in the other studies (DeEll et al., 2001; Johnston et al., 2002).

Differences found in storage between temperatures in the present experiment were not maintained after the shelf-life period, and within each experiment, firmness varied by less than 0.6 kg/cm^2 between storage temperatures. Therefore, the differences that were present between temperature regimes may not be noticed by consumers, as 0.6 kg/cm^2 was the minimum threshold needed for a trained sensory panel to detect a difference in texture in multiple cultivars (Harker et al., 2002). As no gradual cooling regimes had lower firmness than 4°C, which is currently used for storage recommendations for 'Red Aroma' (Rosseland and Eidsvik, 2021), this suggests firmness would not be negatively affected by gradual cooling at any of the studied regimes.

Unlike temperature, no differences were found between temperature regimes on background color, or SSC in either the gradual cooling or crop load experiments. After cold storage TA was higher in 1°C than 7 – 4°C fruit in the gradual cooling experiment, or 4°C fruit in the crop load experiment, likely associated with lower respiration rates in colder temperatures, but differences were not maintained during the shelf-life period (Ackermann et al., 1992). Background color, SSC, and TA results were similar to what has been found in 'Aroma' in other studies (Børve and Stensvand, 2016; Sjöstrand et al., 2023).

When examining firmness, background color, SSC, and TA together, it suggests that there was no difference in overall quality between direct cooled fruit and gradually cooled fruit stored at 1, 2, or 4°C. In 'Aroma' fruit stored in 1 or 3°C for four to six months, no effect of temperature was found on overall quality, although these fruit were also too soft after removal and had unacceptable levels of damage (Børve and Stensvand, 2016). Additionally, gradually cooled or direct stored 'Aroma' fruit in 2°C CA storage for four to five months showed no differences in fruit quality, although storage in CA likely contributed to the maintenance of fruit quality (Sjöstrand et al., 2023). Temperatures between 1 and 4°C during storage, with or without gradual cooling appear to have minor but not impactful differences in short-term

storability of ‘Red Aroma’ fruit, and other factors, such as maturity at harvest, and physiological disorder development are likely to be of more importance.

Fruit stored at 1°C developed considerably more soft scald than fruit stored at 4°C in both experiments. Soft scald developing in 1°C with almost no development at 4°C is consistent with many other studies on ‘Aroma’ and other cultivars, clearly indicating it is a form of chilling injury, and emphasizing the importance of storing ‘Red Aroma’ fruit at temperatures above 1°C (Børve and Stensvand, 2016; Landfald, 1987; Plagge, 1926; Watkins et al., 2004; Watkins and Mattheis, 2018).

The effect of gradual cooling on overall damage depended on the final storage temperature, with gradually cooled fruit having lower, similar, or higher amounts of damage to direct stored fruit in 1, 2, or 4°C respectively. Gradual cooling before storage at 1°C resulted in less soft scald, similar to other studies on ‘Honeycrisp’ in the Northeast US (Al Shoffe and Watkins, 2018; Moran et al., 2010). A study on Swedish ‘Red Aroma’ fruit in CA storage showed gradual cooling to 2°C had inconsistent differences compared to direct storage in 2°C, although soft scald incidence was less than 7% in all years of the study (Sjöstrand et al., 2023).. As soft scald incidence was low, differences between the treatments were minimal, thereby limiting the utility of these results, especially as most fruit was stored in CA, and they did not compare storage at multiple final temperatures (Sjöstrand et al., 2023).

Fruit stored in 4°C had the lowest damage overall, with no differences between the other gradual cooling temperature regimes except 1°C, suggesting that 4°C is the optimal storage recommendation. However, gradual cooling before storage at 1, 2, or 4°C, or direct storage at 2°C seemed to be viable alternatives if 4°C is not an option. Storage at 4°C may be further preferable to gradual cooling because it may reduce overall damage and is easier to implement in a commercial setting than using a gradual cooling regime, especially if a storage room is filled with multiple cultivars or fruit harvested at multiple dates. However, the present research suggests that if there is a delay of a few days between harvest and placement in cold storage, there may be limited negative effects on overall storability and quality compared to if the fruit were placed directly in 4°C.

4.2 'Eden' and 'Fryd'

4.2.1 Effect of storage temperatures on quality analysis

At harvest, variation in maturity was present between fruit in both cultivars. Both 'Eden' and 'Fryd' are described as multiple-pick cultivars (Fresh Forward Breeding & Marketing BV, 2022), however almost every fruit was picked due to limited fruit numbers on the young trees. Furthermore, 'Fryd' fruit were likely picked too early, as suggested by preliminary recommendations for optimal maturity at harvest (Fresh Forward Breeding & Marketing BV, 2022). The early picking of 'Fryd' fruit may have increased disorder development and decreased fruit quality, as has been shown to occur in 'Honeycrisp' fruit from the Northeast US and Eastern Canada (Moran et al., 2010; Prange et al., 2011).

Fruit remained firm throughout storage in both 'Eden' and 'Fryd', although there was a general decrease in firmness between harvest and removal from cold storage for both cultivars. Firmness was well above the general 5-7 kg/cm^2 limit for firmness in other Norwegian cultivars (Rosseland and Eidsvik, 2021) and likely would have remained so if fruit had been stored longer, as firmness can be maintained for long periods of storage in both parental cultivars (Al Shoffe et al., 2021; Neuwald et al., 2016; Tong et al., 1999). In the gradual cooling experiment, there was no difference in firmness between any of the temperature regimes after cold storage. However, in the conditioning experiment, 10 – 4°C or 20 – 4°C fruit were less firm overall than 1°C fruit in both cultivars, implying there is a temperature at which firmness is lost more rapidly, even in cultivars with high firmness (DeEll et al., 2001). The range of differences in firmness was small (less than 1 kg/cm^2) in both cultivars regardless of storage temperature. Longer storage periods may accentuate these differences, especially in 'Eden' which lost more firmness after harvest than 'Fryd'. However, 10 weeks of storage appeared to have minimal negative effects on firmness in either cultivar whether fruit was gradually cooled or conditioned.

SSC decreased in 'Eden', and increased in 'Fryd' in storage, suggesting differences in maturity at harvest in the two cultivars. SSC has been shown to increase near harvest due to starch degradation in 'Glockenapfel' in Switzerland (Ackermann et al., 1992); thus, if most of the starch is converted to sugars during storage instead of on the tree, SSC would increase. Furthermore, in fruit where climacteric ripening has started, SSC has been shown to decrease directly after storage (Ackermann et al., 1992). In both cultivars, there were no differences found between temperature regimes in the gradual cooling experiment, and differences found in the conditioning experiment for both 'Eden' and 'Fryd' were minimal.

By the end of the shelf-life period, background color was more yellow and TA decreased compared to harvest in both 'Eden' and 'Fryd', similar to what is found in studies on multiple cultivars (Ackermann et al., 1992; Watkins et al., 2004). The only differences in background color between temperature regimes in either cultivar were in the 'Eden' gradual cooling fruit after the shelf-life period. However, at this point all 'Eden' fruit were extremely yellow, suggesting they would be appropriate for commercial use regardless of the differences found between the regimes. In 'Eden', gradually cooled or conditioned fruit had higher TA than the corresponding direct cooled fruit, and TA was generally lower in 'Fryd' fruit from warmer storage temperatures. While not common to most cultivars, in some instances 'Honeycrisp' fruit has been shown to have lower TA at lower storage temperatures, perhaps due to maturity or varietal differences (Al Shoffe et al., 2021; Watkins et al., 2005).

When examining all assessed quality attributes together, the results suggest that there is no difference in overall quality between direct cooled fruit and gradually cooled fruit stored at any of the studied temperatures in either cultivar. While 1°C fruit was generally firmer, the differences may not be commercially important. Although no studies on storage temperature of 'Eden' or 'Fryd' have been conducted before, both parental cultivars have been shown to maintain quality in a range of storage temperatures. Specifically, 'SQ159' has been shown to have no changes in firmness, SSC, or TA after storage in 3°C compared of 1°C (Neuwald et al., 2016) and 'Honeycrisp' fruit had no changes in quality in a range of temperatures between 0-4°C, with or without a period of conditioning or gradual cooling (DeLong et al., 2004; Moran et al., 2010; Watkins et al., 2004).

4.2.2 Effect of temperature on soft scald

In 'Eden', no soft scald was found despite expected susceptibility due to 'Honeycrisp' parentage; in 'Fryd' it was only found in 20 – 1°C fruit (Howard et al., 2018; Miller et al., 2021). It is surprising that soft scald was only found in 20 – 1°C and not any of the other fruit stored in 1°C, especially as conditioning at 20°C has been shown multiple times to reduce soft scald incidence in 'Honeycrisp', even when fruit were stored between 0 and 2°C (Delong et al., 2006; DeLong et al., 2004; Watkins et al., 2004).

In contrast, studies on 'Jonathan' and 'Grimes Golden' in Iowa found that conditioning could increase soft scald incidence, and suggested that it was due to higher respiration in conditioned fruit (Brooks and Harley, 1934; Harley and Fisher, 1931). However, maturity at harvest was not measured in these studies, and it is likely they were more mature at harvest

than fruit used for modern long-term storage. More recently, industry handbooks noted broadly that delayed cooling can often increase soft scald due to increased respiration prior to storage, especially during ripening and the associated climacteric rise in respiration (Meheriuk et al., 1994; Pierson et al., 1971).

Even in 'Honeycrisp' conditioning has at times increased soft scald incidence, suggesting that susceptibility of 'Honeycrisp' fruit to soft scald may be related, albeit less so than in other cultivars, to respiration (Moran et al., 2010). It is possible that in the 'Fryd' experiment that conditioning fruit in 20°C increased respiration and when placed in 1°C, it was enough to result in chilling injury, while placement in 4°C was warm enough to prevent injury. Additionally, conditioning at 10°C or gradual cooling from 7°C may not have been warm enough to increase respiration to such a degree that it later induced chilling injury in 1°C. Based solely on soft scald development, storage at 1°C may be suitable for 'Fryd', as long as fruit are put in storage within a week of harvest; if there is a long delay between harvest and storage, 4°C would provide less risk of developing soft scald in storage. However, in the present experiments, 'Fryd', were picked before recommended commercial harvest and was relatively immature (Fresh Forward Breeding & Marketing BV, 2022). Previous studies have shown that soft scald incidence in 'Honeycrisp' from the Northeast US is higher at later harvests or in fruit with advanced maturity (Moran et al., 2010; Tong et al., 2003; Watkins et al., 2005). If the 'Fryd' fruit in the present experiments had been harvested at a later maturity, such as at a starch of 6 instead of 2.2, it is possible that soft scald would have been expressed in more temperature regimes.

Moreover, weather conditions may have impacted the relative lack of soft scald development in all three cultivars in the present experiments, as 2022 was a year with low precipitation. Soft scald has been linked to moisture throughout the season in 'Honeycrisp' in the Northeast US and Eastern Canada, and 'Frida' in Sweden, with wetter seasons, especially during specific times of the growing season, increasing soft scald incidence (Lachapelle et al., 2013; Moran et al., 2009; Sjöstrand et al., 2023). Soft scald incidence was shown to be negatively correlated with precipitation in the 30 days before harvest in 'Honeycrisp' over seven years in Maine and five years in Ontario (Moran et al., 2009). However, another study on 'Honeycrisp' over three years in Quebec and Nova Scotia and eight years in Ontario found that there was no effect of weather 30 days before harvest on soft scald incidence (Lachapelle et al., 2013).

A recent study on 'Aroma' fruit over three seasons and 'Frida' fruit over two seasons in Sweden showed that high relative humidity 28 days before harvest was associated with higher

soft scald incidence in storage in ‘Frida’ fruit, or when pooling data from both ‘Frida’ and ‘Aroma’, but not in ‘Aroma’ alone (Sjöstrand et al., 2023). While in the present experiments average relative humidity within a month of ‘Red Aroma’ and ‘Eden’ harvest was similar to other years in the last decade, there were only four days with relative humidity greater than 85%, as opposed to 12 days on average. Additionally, during the month before ‘Red Aroma’ and ‘Eden’ harvest, precipitation was recorded on fewer days than the average over the past decade. Total precipitation in the month before harvest in 2022 was also half of the average in the past decade. The comparative lack of precipitation near harvest in 2022 compared to prior years may have resulted in less soft scald susceptibility in ‘Red Aroma’, ‘Eden’, and ‘Fryd’. However, no conclusive trends should be drawn off one year of data, especially as there appears to be significant varietal and regional differences in correlations between weather conditions and soft scald incidence (Lachapelle et al., 2013; Moran et al., 2009; Sjöstrand et al., 2023).

4.2.3 Effect of storage temperature on core browning

Even though no and limited soft scald were found in ‘Eden’ and ‘Fryd’ respectively, 48.3% of ‘Eden’ fruit and 45.7% of ‘Fryd’ fruit were damaged, primarily due to core browning. Affected fruit were firm and maintained good external quality, with the exception of greasiness. As there were no external indicators of core browning in ‘Eden’ or ‘Fryd’, disordered fruit may not be able to be identified and sorted out during packing, proving problematic for the industry. Additionally, core browning incidence and severity increases rapidly after removal from cold storage (Pierson et al., 1971; Snowdon, 1990; Watkins and Mattheis, 2018). This suggests that quick marketing of the fruit is important, especially in lots where core browning has been detected in quality samples.

Core browning incidence was affected by temperature regime in ‘Eden’ and ‘Fryd’, but the same temperature regimes did not reduce browning in both cultivars, as cooler temperatures generally reduced core browning in ‘Eden’ but increased core browning in ‘Fryd’. Core browning incidence is likely affected by multiple pre- and post-harvest factors, and causes may be cultivar specific (Meheriuk et al., 1994; Watkins and Mattheis, 2018). Exact causes of core browning are hard to define, as it has alternately been considered a chilling injury, CO_2 injury, and a form of senescence (Meheriuk et al., 1994; Pierson et al., 1971; Snowdon, 1990).

In the present experiments, core browning of ‘Fryd’ was highest at temperatures below 4°C, especially without conditioning or gradual cooling, suggesting it may have been a form of low temperature injury. A recent review article on multiple types of internal browning

classifies core browning as a chilling injury (Sidhu et al., 2023). Core browning in 'Gravenstein', 'James Grieve', and 'Bramley's Seedling' in Norway and 'McIntosh' in Ontario has been shown to decrease with storage temperatures above 1°C, or with gradual cooling (Chu, 1999; Landfald, 1956). In a study on 'Honeycrisp' fruit from New York and Pennsylvania over three seasons, fruit were either unconditioned or conditioned for a week at 10°C and placed in either 0.5 or 3°C for two to six months of storage. While core browning incidence was low in all years of the study, it was only found in unconditioned fruit regardless of storage temperature (Al Shoffe et al., 2020). In all three studies, the authors suggested causes of core browning included more than just chilling injury or susceptibility to cold temperatures (Al Shoffe et al., 2020; Chu, 1999; Landfald, 1956). In the present experiments, core browning in 'Fryd' was likely caused by factors other than just low temperatures, as storage at 4°C with conditioning, which is commonly used to prevent chilling injury (Watkins et al., 2004; Watkins and Mattheis, 2018), still resulted in over 20% damage.

In contrast, in the present experiments, 'Eden' fruit stored in 1 or 2°C without conditioning had less core browning than fruit stored in 4°C. Core browning has been reported to increase at higher storage temperatures in 'Cox's Orange Pippin' in the UK and in one of four years for 'Braeburn' in British Columbia (Lau, 1998; Wilkinson and Fidler, 1973). Additionally, German grown 'Elstar' fruit stored in CA only had core browning at temperatures averaging 8.7°C rather than 2.0 or 3.5°C, and New York grown 'Empire' fruit stored in CA at 3°C had more or less core browning compared to fruit stored in CA at 0 or 0.5°C depending on the year (Köpcke, 2015; Watkins and Liu, 2010). There is clearly a variable effect of temperature on core browning incidence, and both high and low storage temperatures have been able to control disorder development depending on the year and cultivar. These conflicting associations are similar to the differences seen in the present experiments between 'Eden' and 'Fryd', and indicate that while storage temperatures may affect disorder development, core browning is unlikely to be exclusively a chilling injury (Watkins and Mattheis, 2018).

While less commonly mentioned, core browning has also been considered a form of senescence (Snowdon, 1990; Wilkinson and Fidler, 1973), or separated into two distinct forms: senescent and non-senescent (Smock, 1977; Watkins and Liu, 2010). This is supported by a study in Norway which found that 'Gravenstein', 'Bramley's Seedling', and 'James Grieve' fruit stored for five to seven months, until overripe, all developed core browning. However, other factors, including high CO_2 levels (>5%) and low storage temperatures (1°C) also influenced core browning development (Landfald, 1956). Core browning in 'Empire' in New

York and ‘Macoun’ in Maine has been shown to increase or decrease with later harvesting dates and advancing maturity, depending on the year, suggesting maturity affects but does not solely cause core browning in these cultivars (James et al., 2010b; Moran and McManus, 2005).

Both CA storage and 1-MCP have been shown to reduce core browning in cultivars such as ‘Empire’, ‘Fuji’, and ‘Honeycrisp’ (Argenta et al., 2000, 2010; Lee et al., 2019; Nock and Watkins, 2013; Watkins and Liu, 2010; Watkins and Nock, 2012). CA and 1-MCP both reduce the rate of ripening and delay the initiation of senescence, thereby reducing the rate of core browning development (Argenta et al., 2000). However, 1-MCP does not consistently decrease core browning, including in ‘Honeycrisp’ (Al Shoffe et al., 2021; Nock and Watkins, 2013). These results suggest that harvest timing, maturity, and ripeness may have different effects on core browning depending on year and cultivar, and therefore that senescence may impact, but is not the only cause of, core browning (Watkins and Mattheis, 2018).

In the present experiments, some ‘Eden’ fruit had senescent breakdown, including fruit that also had core browning, suggesting the core browning could be a form of senescence. Additionally, both dry and moist types of core browning were found, which have previously been described as senescent and non-senescent core browning, respectively (Smock, 1977). This is further supported by non-conditioned fruit and fruit stored at 1°C having lower incidences and severity of core browning, as colder storage temperatures decrease respiration and slow ripening (Wilkinson and Fidler, 1973). In contrast to ‘Eden’, minimal senescent breakdown was found in ‘Fryd’, which corresponds to the early harvest and less mature fruit and suggests the core browning in ‘Fryd’ is not senescence related. The early harvest of ‘Fryd’ may still help explain the high core browning incidence however, as early harvests have also been generally associated with non-senescent core browning (Watkins and Mattheis, 2018; Wilkinson and Fidler, 1973).

Despite the lack of CA storage in the present experiment, core browning can also be affected by CO_2 concentrations (Meheriuk et al., 1994; Watkins and Mattheis, 2018; Wilkinson and Fidler, 1973). Studies has shown high CO_2 concentrations (5-12%) in CA increase core browning in ‘Cox’s Orange Pippin’ and ‘Laxton’s Superb’ in the UK (Wilkinson and Fidler, 1973). Additionally, core browning in ‘Braeburn’ has been associated with CO_2 injury in New Zealand and Germany, and is considered a form of Braeburn browning disorder (Büchele et al., 2023; Elgar et al., 1999). However, as CA storage in <4% CO_2 decreases core browning compared to RA storage in many cultivars, including ‘McIntosh’ in Ontario, ‘Empire’ in New York, and ‘Fuji’ in New York and Washington, (Argenta et al., 2000, 2010; Chu, 1999; Lee et

al., 2019; Watkins and Liu, 2010), it is unlikely to be exclusively caused by high CO_2 (Watkins and Mattheis, 2018).

Specifically, both core browning and CO_2 injury has been reported to affect 'Honeycrisp' fruit from New York and Pennsylvania in RA storage, especially if fruit were unconditioned (Al Shoffe et al., 2020). In agreement with this, research in Michigan on 'Honeycrisp' showed that conditioning reduced CO_2 injury incidence in both RA and CA storage for one to six months in the years where core browning was found in meaningful amounts (Contreras et al., 2014).

Therefore, it is possible that CO_2 injury could occur in both 'Eden' and 'Fryd' in RA storage, especially as 'Honeycrisp' is also susceptible to CO_2 injury in RA storage (Al Shoffe et al., 2020; Contreras et al., 2014). However, in the present experiments, it is unlikely that the core browning seen was exclusively due to CO_2 injury. The percentage of affected fruit was much higher in the present experiments than in other studies with CO_2 injury in RA storage (Al Shoffe et al., 2020; Contreras et al., 2014), and while some of the affected core tissue had cavities, there were no symptoms of CO_2 injury in the cortex of any fruit in the present experiments (Snowdon, 1990). Additionally, susceptibility to CO_2 injury is usually greatest within the first few weeks to a month of storage (Argenta et al., 2000; Wilkinson and Fidler, 1973), while in the present experiments, core browning developed only after one month of storage. CO_2, storage temperatures, harvest timing, and maturity at harvest all likely contributed to core browning in 'Eden' and 'Fryd', along with other pre-harvest factors.

While less examined, crop load in 'Braeburn' has been shown to influence core browning, with higher crop loads reducing core browning incidence. As the 'Eden' and 'Fryd' trees used in the present experiments were young, the crop loads were relatively low. It is therefore possible that increased crop loads as the trees mature will reduce core browning incidence in these fruit.

Additionally, growing season and region may impact core browning development (Smock, 1977; Watkins and Mattheis, 2018). Cool growing seasons and cool temperatures in the month before harvest have been shown to increase susceptibility of 'Braeburn' fruit to flesh browning, including core browning (McCormick et al., 2021). In separate studies on 'Empire', no core browning was detected in two seasons in Ontario grown fruit, while it was found for three seasons in New York (DeEll et al., 2005; Watkins and Liu, 2010). Furthermore, in the New York study, core browning incidence varied between orchards within the same growing region (Watkins and Liu, 2010). In 'Honeycrisp' grown in Washington over three seasons, no core browning was detected, while fruit from one season in Ontario had core browning, but

only in the 1-MCP treated fruit. Ontario has a colder, wetter, and shorter growing season than Washington, providing further support towards cold growing seasons increasing core browning development (Serban et al., 2019). As 2022 was a cool growing year with low GDD, it is therefore possible that core browning in 'Eden' and 'Fryd' was associated with low growing temperatures in the present experiments.

While storage temperatures may have provided the easiest method of avoiding core browning in 'Eden' and 'Fryd' in the present experiments, they were unlikely the only causes of core browning, or the only method to minimize incidence. Core browning in 'Eden' seems to have been associated with storage in warmer temperatures (4°C) or conditioning, along with senescence and over ripe fruit after the shelf-life period, and perhaps CO_2 concentrations. In contrast, core browning in 'Fryd' seems to have been associated with storage in colder temperatures (1 or 2°C) or lack of conditioning or gradual cooling, along with early harvest. Both cultivars may have been affected by low crop loads, or the cool growing season, among other pre-harvest factors. Storage in RA instead of CA may have also impacted the early and significant development of core browning.

4.2.4 Effect of storage temperature on watercore

Watercore was found in both 'Fryd' and 'Eden', but instances in 'Fryd' were rare. Both parent cultivars have shown susceptibility to watercore, although there has been little dedicated research on this (de Wild, 2022; Delong et al., 2006). As watercore susceptibility is likely inheritable (Itai, 2015), finding watercore in 'Eden' and 'Fryd' was not surprising. Watercore seemed to dissipate during storage in 'Eden' fruit, as is common in other cultivars (Clark et al., 1998; Marlow and Loescher, 1984; Neuwald et al., 2012). It is unusual that watercore incidence after the shelf-life period was highest in the fruit stored at 4°C, as watercore commonly dissipates faster at warmer storage temperatures or delayed CA storage, both of which result in increased respiration rates (Köpcke, 2015; Neuwald et al., 2012; Wilkinson and Fidler, 1973).

No visible watercore breakdown was noticed in 'Eden' fruit. However, it is unknown if any tissue previously affected by watercore later developed watercore breakdown that was classified as core browning. As watercore occurred primarily in the core area, watercore breakdown would also occur in the core area, like core browning. Watercore breakdown can result in browning and cavity formation of affected flesh (Watkins and Mattheis, 2018), similar

to the core browning with cavities found in the present experiments. Watercore breakdown has been suggested to occur simultaneously with brown watercore (Smock, 1977), similar to the core browning and watercore sometimes found in the present experiments. When fruit were cut after the shelf-life period, watercore breakdown was not specifically identified, although transparent brown tissue was identified as brown watercore. Therefore, it is possible that when core browning and brown watercore were identified in the same 'Eden' fruit, it was a form of watercore breakdown. In 'Fuji' fruit, watercore breakdown has been reported to not appear until after three months of RA storage (Tanaka et al., 2020). However, compared to other cultivars, 'Fuji' is known for being relatively resistant to watercore breakdown and watercore-associated flesh browning (Bowen and Watkins, 1997; Tanaka et al., 2020), suggesting 'Eden' fruit in the present experiments may still have developed watercore breakdown.

Many storage disorders have been associated with watercore, such as watercore breakdown, brown watercore, internal browning, and flesh browning in many cultivars, and CO_2 injury in 'Fuji' (Argenta et al., 2000; Marlow and Loescher, 1984; Tanaka et al., 2020). Combined with watercore affecting but not being the only cause of many of these browning disorders, nomenclature and determination of watercore-related storage disorders is often confusing (Marlow and Loescher, 1985; Tanaka et al., 2020). In the present experiments, there were few overall cases of identifiable watercore after the shelf-life period. All fruit with brown watercore were classified as severe, with a rating of 5 (Figure 3.7) regardless of how much tissue was affected, two factors which may have limited proper classification and further analysis of watercore. Therefore, while in the present experiments storage in 4°C seemed to delay watercore dissipation, it is most important to note that both cultivars, and especially 'Eden', are susceptible to watercore and that not all watercore dissipated after two months of RA storage, therefore reducing the storability and quality of the fruit.

While the association between temperature, maturity, and watercore is often debated, with temperature either being related to watercore independently of maturation or only due to the effect of temperature on maturation rate, both temperature and maturity are well known to affect maturity-related watercore incidence (Itai, 2015; Marlow and Loescher, 1984; Yamada and Kobayashi, 1999). Often, cooler seasons or growing regions have been shown to increase watercore incidence. For example, in two growing regions in New Zealand, the cooler growing region saw higher incidences of watercore than the warmer region in two seasons (Harker et al., 1999)(Harker *et al.*, 1999). In Japan, young 'Himekami' and 'Fuji' trees in pots were exposed to different temperatures for one month prior to harvest. The fruit exposed to colder temperatures had more and earlier watercore development, regardless of whether the

temperatures were constant or had day and night fluctuations, (Yamada et al., 1994; Yamada and Kobayashi, 1999). In the present experiments, it is possible that the cool Norwegian climate, and the cool growing season in 2022 affected watercore incidence, and warmer growing years may result in less watercore development.

Watercore can even be orchard specific within a region. In Germany, two orchards of 'Gloster' fruit within 15 km of each other had varying levels of watercore within the same season – from 53.1% to 9.6% - supporting between orchard differences (Köpcke, 2015). If fruit in the present experiments had been picked from another orchard, it is possible watercore incidence would have been altered.

Maturity-related watercore, as the name suggests, has long been known to increase with advancing fruit maturity before harvest in many cultivars (Bowen and Watkins, 1997; Itai, 2015; Marlow and Loescher, 1984). During a nine-week harvesting period in New Zealand, watercore incidence in 'Fuji' and severity increased with advancing maturity and later harvesting (Bowen and Watkins, 1997). Among control measures for reducing watercore in storage, monitoring fruit for watercore presence and harvesting when watercore is first seen are often cited as most important (Meheriuk et al., 1994; Pierson et al., 1971; Snowdon, 1990; Watkins and Mattheis, 2018). It is therefore likely that the watercore in 'Eden' in the present experiments was related to maturity and would have been reduced if the fruit had been picked earlier.

Related to maturity, light crop loads on young trees have been shown to have higher watercore incidence than mature trees. Most of this research was completed on standard trees in the 1940s or earlier, however high density 'Fuji' plantings show the association holds true for modern orchards (Marlow and Loescher, 1984; Şerban et al., 2019). As the 'Eden' and 'Fryd' trees in the present experiments were only in their third and third leaf, they may have been more susceptible to watercore than mature trees will be.

Calcium concentrations have been related to watercore development in multiple cultivars and locations, including 'Red Mill' fruit in the UK and 'Fuji' fruit in New Zealand (Bowen and Watkins, 1997; Itai, 2015; Sharples, 1967). In New Zealand grown 'Fuji', fruit without watercore sometimes had higher calcium concentrations than fruit with watercore. It was suggested that lower calcium concentrations could be involved in earlier development of watercore (Bowen and Watkins, 1997). Watercore incidence is related to changes in membrane integrity as maturity develops and ripening increases. Membrane integrity is also influenced by calcium concentration (Itai, 2015; Marlow and Loescher, 1984). However, associations between watercore and calcium are not always consistent (Itai, 2015), and in the present

experiments, it is unknown if calcium concentrations affected watercore development or not as mineral analyses were not performed.

4.2.5 Effect of storage temperature on other disorders

Both ‘Eden’ and ‘Fryd’ fruit started to become greasy in storage, and almost all fruit of both cultivars were greasy by the end of the shelf-life period, regardless of temperature regime. This is well documented to occur in ‘Honeycrisp’ fruit as well, and is not consistently influenced by storage temperature, although it has been shown to increase with conditioning. It can also be associated with late harvests and long-term storage, e.g., five months. It is surprising that the fruit in the present experiments were greasy despite the short storage period and the early harvest of ‘Fryd’, however in the ‘Honeycrisp’ studies, the shelf-life period was only seven days or less instead of 14, and fruit were from mature trees (Al Shoffe et al., 2021; Delong et al., 2006; DeLong et al., 2004; Watkins et al., 2005). While no association between greasiness and tree age has been investigated, many physiological disorders have been shown to decrease in severity once trees reach maturity, including on ‘Aroma’ trees in Sweden, where fruit from 6-20 year old trees had better storability than from four year old trees (Tahir et al., 2007). If fruit from the present experiments had been subjected to a shorter shelf-life period, or were from older trees, it is possible the percentage of greasy fruit would have been reduced.

Bitter pit was found in ‘Eden’ fruit, but not in ‘Fryd’ fruit in the current experiment, and ‘Eden’ fruit stored in 1°C had the highest incidence of bitter pit. This is of interest as incidence in ‘Honeycrisp’ is often worse at warmer storage temperatures, especially when a conditioning period is used (Al Shoffe et al., 2021; Watkins et al., 2004). Incidence was generally low in the present experiments, even in the 1°C temperature regime, but it confirms ‘Eden’ is susceptible to the disorder. The bitter pit that was seen may be due in part to the low crop loads of the young trees, tree age itself, or improper calcium nutrition, as all can influence incidence and severity of the disorder (Ferguson and Watkins, 1992; Watkins and Mattheis, 2018).

Diffuse flesh browning was only found in meaningful amounts in ‘Eden’ in 7 – 4°C fruit and 4°C fruit from the gradual cooling experiment, but not 4°C fruit from the conditioning experiment. In ‘Fryd’, diffuse flesh browning was rarely found by itself but was often found in association with severe core browning (CB3). In ‘Fryd’, the diffuse flesh browning may have been a continuation of core browning (Snowdon, 1990). Storage temperatures appeared to affect development in storage in ‘Eden’, with low storage temperatures minimizing

development. This is different from diffuse flesh browning in 'Cripps Pink', where diffuse flesh browning is considered a chilling injury and incidence can be reduced by storage at 3°C instead of 0°C (James et al., 2008; Moggia et al., 2015). In 'Honeycrisp' in the Northeast US and Canada, no effect of storage temperature or conditioning was found in one experiment, but in another 0°C was used to induce diffuse flesh browning as opposed to storage at 3°C (Tong et al., 2016; Watkins et al., 2005). Contrasting results in 'Cripps Pink', 'Honeycrisp' and 'Eden' suggest that responses to storage temperatures may be cultivar specific.

Susceptibility of fruit to diffuse flesh browning may also be due to pre-harvest factors. Weather and growing region in both 'Cripps Pink' and 'Honeycrisp' has been linked to diffuse flesh browning incidence, with cold growing seasons or regions generally increasing diffuse flesh browning susceptibility in both cultivars. Limited accumulation of GDD (less than 1100) at harvest is linked to increased development of diffuse flesh browning in 'Cripps Pink' in Australia (James et al., 2010a; James and Jobling, 2009). In 'Honeycrisp' in the Northeast US, minimum temperatures below 15°C and maximum temperatures below 20°C around 70 days after full bloom (DAFB), were observed in years and locations with high levels of diffuse flesh browning. The high amount of diffuse flesh browning was associated with a rapid drop in temperature around 70 DAFB (Tong et al., 2016). In the present experiments, 20 days in July (70 DAFB was 9 July) saw maximum temperatures below 20°C and all but two days had minimum temperatures below 15°C. Additionally, there was a lower GDD accumulation at the end of the season than average in the past decade. There could be a relationship between low temperatures during the growing season and diffuse flesh browning susceptibility in 'Eden' and 'Fryd' as there is in 'Honeycrisp' or 'Cripps Pink' (James et al., 2010a; James and Jobling, 2009; Tong et al., 2016).

4.3 Conclusions and continuation of experiments

In the present experiments, while some conclusions can be drawn about storability of 'Eden', 'Fryd', and 'Red Aroma' and their expression of certain physiological disorders, all analysis was completed on fruit from one season only. To achieve more meaningful conclusions, experiments could be continued through future seasons. As growing seasons and weather conditions have been shown to influence expression of many physiological disorders, determining if disorder development (i.e., soft scald, watercore, and diffuse flesh browning) in 'Eden', 'Fryd', or 'Red Aroma' is also influenced by growing season and weather in

Norwegian conditions would be of importance. Furthermore, using fruit from multiple regions, or orchards, may provide a more robust picture of disorder severity throughout Norway.

Harvesting the fruit at different maturities could also influence disorder development and fruit quality in storage. As commercial harvest timings are still being created for 'Eden' and 'Fryd' this provides an opportunity to study both harvest timing and temperature regimes simultaneously to maximize fruit storability. Of particular interest is whether core browning could be related to maturity at harvest or ripening during storage in 'Eden' or 'Fryd', especially as 'Fryd' were harvested too early. Additionally, watercore incidence and severity in 'Eden' could be considered when determining optimal harvest timing.

No effect of crop load or fruit size was found on quality or soft scald incidence in the present study. Greater extremes between crop load (i.e., 25 fruit per tree vs. 200 fruit per tree) or using other methods to determine crop load (i.e., tree cross sectional area) could exaggerate differences between the crop loads during the season and result in greater differences in fruit quality or soft scald incidence in storage. Additionally, using different sized fruit from one tree, or trees of similar crop loads, could result in differences in disorder development not attainable by different crop loads.

In 'Red Aroma' fruit in the present experiments, soft scald was highest when fruit were stored at 1°C. As minimal differences in fruit quality were found between the different temperature regimes, the results of these experiments support the continued use of 4°C for commercial storage of 'Red Aroma' to reduce soft scald incidence. If storage at 4°C is not feasible, gradual cooling before storage at a range of temperatures may still reduce soft scald incidence compared to direct storage at 1°C. Additional research in years with higher soft scald incidences could provide further support for gradual cooling.

When considering storage of the three cultivars, future research could examine multiple temperatures in combination with CA storage. In 'Aroma' in Norway and Sweden, CA storage has been shown to increase storability by better maintaining quality, and reducing disorder and decay incidence (Børve and Stensvand, 2016; Knutsen et al., 2015; Sjöstrand et al., 2023). As the 'Red Aroma' fruit in the current experiments were all unmarketable by the end of November, CA could be helpful in reliably extending the storage life of the fruit, especially at 4°C with or without gradual cooling. Gradual cooling and storage in 2°C was less effective than storage in 4°C in RA in reducing soft scald in 'Red Aroma' the present experiments, and storage in 2°C in CA with or without gradual cooling did not eliminate soft scald incidence in Swedish fruit either (Sjöstrand et al., 2023).

Storage in CA could also be beneficial for 'Eden' and 'Fryd' fruit, as it may reduce core browning, greasiness, and senescent browning, extending the storage life of these cultivars as well. While storage in 1°C limited disorder development in 'Eden', in 'Fryd' all tested temperature regimes resulted in unacceptable levels of damage. Therefore, storage at specific temperatures alone may not be enough to reduce disorder development sufficiently. Additionally, causes of core browning seemed to be different for 'Eden' and 'Fryd', being related to warmer storage temperatures, CO_2 concentration, and senescence in 'Eden', but colder storage temperatures, lack of gradual cooling or conditioning, and early harvest in 'Fryd'. Distinguishing between the types of core browning seen in the two cultivars could also help determine what storage conditions to expose fruit to. It is clear that future research needs to be completed on storage temperatures of both 'Eden' and 'Fryd'.

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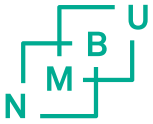

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