POSTHARVEST COLD STORAGE AFFECTS FLOWER LIFE AND PHYSIOLOGY OF POTTED TULIPS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

May 2018

by

Isabel Anne Branstrom

ABSTRACT

Postharvest cold storage is a common practice by potted tulip growers to respond to quality and timing demands. Plants are placed into cold storage at a marketable stage and kept there until it is time for sale. There are still questions about how this storage impacts flower quality. The focus of this research was to determine how temperature and duration of postharvest cold storage affect carbohydrate status and flower life of potted tulips, and how tulips respond once out of storage. Changes associated with senescence such as lipid peroxidation and carbohydrate status were studied in order to characterize tepal senescence. There were changes in plants held at warmer temperatures for longer durations that demonstrate continued development in postharvest storage. It was concluded that best flower quality resulted from storage for ≤ 1 week at all temperatures. For durations > 3 weeks, best flower quality resulted from storage at 1ºC.

BIOGRAPHICAL SKETCH

Isabel Branstrom is from South Florida. Thanks to her mother, the artist, and father, the plant scientist, she grew up in an environment that made her a natural born horticulturist. After attending an arts-focused middle and high school, she decided to focus on science in college, always keeping in mind that the two subjects are not mutually exclusive. Totally enchanted by physiology and the intricacies of plants, she chose to pursue a M.S. at Cornell University. The author is so thankful for the time she was given to study the postharvest physiology of tulips. It gave her the freedom to grow as a scientist and strengthened her love of plants and people.

Dedicated to my mom and dad. The plant nerds who started it all.

ACKNOWLEDGMENTS

The author would like thank Bill Miller first and foremost. This project would not have been possible without the support, advice and laughs he provided. Thank you for always taking the time to answer continuous questions about science and life – whether in the office or over beers at The Barn. The author is incredibly grateful to have had such a wonderful advisor throughout this process.

Thank you also to Tim Setter for his help and advice, especially in regard to pesky enzyme protocols, and to Rose Harmon for providing reassurance, answering the same question multiple times, and for always being ready with a hug. Oh, and for helping the author power through a daunting number of samples.

The author would also like to thank the friends that made Ithaca a home. Transitioning from Florida to New York did not seem like the easiest task, but, as it turns out, if you have kind, smart and loving friends around you...it is. Thank you for tables full of good food, music shows, dance parties, and your support along the way.

Finally, the author would like to thank her family for the freedom and love they have always provided. She will forever be grateful for a family whose love transcended the 1,300 miles that separated them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Introduction

Tulips (*Tulipa* sp.) have a longstanding importance worldwide. They are the flowers that were exchanged for entire properties in the 17th century, and caused one of the first economic bubbles (Christenhusz et al., 2013). Hundreds of cultivars are available today for cut flowers, potted plants and use in the landscape. Due to the diversity available there are tulips for all uses and many climatic zones, and thus their popularity does not wane. It was reported that in 2017 the largest producer, the Netherlands, produced 2 billion blooms (Dutch News, 2017). The Netherlands is also responsible for the large export of bulbs to other countries, of which the largest markets outside the EU are Japan and the USA (Buschman, 2005).

Much research has been focused on control of flowering in tulips through forcing. Forcing allows growers to mimic the natural cold period that initiates flowering so that blooms coincide with the largest periods of demand. This period usually extends from early January to May, with emphasis on holidays such as Easter and Mother's Day (De Hertogh, 1989). However, another critical stage that affects tulip quality is post-greenhouse handling. There is little information on how this period of storage specifically affects physiology of tulip plants, and subsequent flower quality after storage. Thus, the purpose of this study is to determine how postharvest cold storage affects carbohydrate pools, oxidative stress and flower life.

Background

The tulip is one of the major flowering bulbs in the Liliaceae family. It's center of origin is in Central Asia, near the Tien Shan and Pamir Alai mountain ranges (Pavord, 2008). Tulips were naturalized westward (Afghanistan, Iran and Turkey), to the north and to the northeast

(Siberia, Mongolia, and China) by various tribes and traders (Christenhusz et al., 2013). Tulip bulbs were first brought to Europe by traders from Istanbul in the 17th century, where they spread rapidly (Christenhusz et al., 2013). People recognized the value, variety and potential of tulips and began enthusiastically breeding them. By the end of the 19th century, a strong tulip market had been established, dominated by the Dutch, but fueled by a worldwide enchantment with tulips (Pavord, 2008).

This enchantment does not wane today as the tulip continues to be one of the most important flower crops across the globe. Tulips have great diversity in flowering time, growth, and flower shape (De Hertogh and Le Nard,1993). This diversity allows cultivars to be suited for different uses such as cut flowers, potted plants or landscape use. There are currently approximately 6,000 registered cultivars of tulips (Christenhusz et al., 2013). Tulips are generally divided into 15 groups based on flowering time or flower form (Pavord, 2008). These groups include Single Early, Double Early, Triumph, Darwin Hybrid, Single Late, Lily-flowered, Fringed, Viridiflora, Rembrandt, Parrot, Double Late, Kaufmanniana, Fosteriana, Greigii. This collection arose from the 15th group, Species, which is comprised of wild tulips.

The Netherlands accounts for 87% of worldwide production and is the leading exporter of bulbs (Buschman, 2005). In order to meet the year-round demand for tulips, North American growers import hundreds of millions of bulbs a year. In 2011, the U.S. Census Bureau reported bulbs worth $160 million dollars were imported into the US. This accounted for 10.4% of all ornamental plant imports. With this sort of investment in the crop, it is important to avoid losses whenever possible.

Tulips are a type of geophyte – a plant in which the perennial buds are underground on a storage organ (bulb, tuber, rhizome, etc.). Tulip bulbs are surrounded by a thin tunic and usually

have two to six fleshy scales attached to a basal plate that produces roots. Buds, located at the base of the inner scales, become the above ground organs (apical buds) and daughter bulbs (lateral buds) used for multiplication. Tulips can reproduce two ways - via these bulbs or via seed, but it will take 5-7 years before a bulb capable of flowering is formed from seed (Pavord, 2008). This sexual reproduction leads to new varieties and is used for breeding, but asexual reproduction is exclusively used in production.

Tulips function on an annual replacement cycle. It is critical for tulips to receive a cold period to promote rooting, shoot elongation, flower induction and daughter bulb enlargement. In the fall, flower meristem induction and root growth occurs rapidly as soil temperatures decrease. The apical bud, which has already differentiated, starts to elongate slowly as temperatures drop even more during the winter. When temperatures start to rise in the spring, plant growth increases rapidly. Daughter bulbs begin to grow and mother-bulb scales begin to shrivel. At the end of spring, aerial organs senesce, daughter-bulb growth ceases and the bulb enters an apparent dormant state.

It is possible for growers to mimic the natural cycle of tulip bulbs in controlled environments, known as forcing, which has had a great impact on tulip flower availability in the marketplace. Considerable research has been conducted to establish protocols for tulip forcing in potted tulips. De Hertogh (1989) suggests first that bulbs are checked for disease and mechanical damage and then placed in a well-ventilated facility at 17°C until they are precooled or planted and directly placed in the greenhouse for non-precooled bulbs. Flower differentiation is expressed at warm temperatures (>17°C). Bulbs are then planted in well-drained medium with a pH of 6.0- 7.0 and placed into cold storage ($\leq$ 9°C) 14 to 24 weeks. Morphological markers are often used when forcing tulips (Dole, 2003), and are largely cultivar dependent (De Hertogh,

1989). It is common to hold plants at 9ºC until sufficient root growth has occurred, then at 5ºC until shoots are about 1 inch (2.54 cm) and then down to 0-2ºC to retard shoot growth. After this period of cold storage, plants are moved into warmer temperatures (17-20°C or lower for non-precooled bulbs) to promote rapid growth and flowering.

Postharvest handling and marketing is another important stage in tulip plant production, and the focus of this research. This is the period after forcing, when the plants are at a marketable stage but must be kept at that stage to prevent losses or early senescence. In the "Holland Bulb Forcers Guide," there are two short paragraphs covering this topic. De Hertogh and Springer (1977) have established that marketable plants at the green bud, or bud color stage, should be moved to 0-2°C and held until time of sale. It is not fully understood how this period of storage specifically affects the physiology of potted tulips. It is possible that changes in carbohydrate metabolism or possible oxidative stress occur and have subsequent effects on flower quality after storage. By characterizing how different temperatures and durations of storage impact tulip flower senescence, a greater understanding of the effects of postharvest storage can be gained and used to prevent losses.

Changes Associated with Postharvest Cold Storage

Postharvest cold storage is a method growers use to keep plants at a marketable stage, and deal with the separation between growing areas and market areas. Ornamental plants must be timed and maintained in order to account for transportation over long distances. Plants are grown in the greenhouse under optimal conditions and transferred to storage coolers or rooms. Often storage conditions are sub-optimal in regards to temperature, humidity, water and light (Ferrante et al., 2014). This transition can have damaging effects on the plants and lead to a number of

disorders including leaf yellowing, color loss, flower abscission, wilting and fungal growth. Understanding the physiological changes in plants due postharvest storage provides insight into possible ways to manipulate these conditions to help prevent these disorders and maintain potted tulip quality during this critical period.

Energy Status

Changes in the status of biomolecules used to produce chemical energy, strongly influence plant growth and development. Sugars derived from photosynthesis are used as an energy source as well as important substrates and signal molecules in metabolism. Sugar status is one of the most important factors that affects the life of a plant, and understanding how this status changes can give insight into how postharvest storage influences overall quality in potted tulips.

In tulips, most research has been focused on carbohydrate status of the bulb in response to forcing. Exposing bulbs to low temperatures after exposure to warm temperatures triggers subsequent growth of the shoot and daughter bulbs. This period of low temperature ranges from 12 to 16 weeks at 5 to 9°C, depending on cultivar. Shoots of non-cooled bulbs (held at 17°C) elongated slowly compared to cooled bulbs (Lambrechts et al., 1992). If non-stored bulbs make it to the point of flowering, there are severe disorders. This can be attributed to the slow mobilization of carbohydrates from bulb scales due to low activity of amylase and other enzymes that are critical in the breakdown of large polysaccahrides such as starch (Lambrechts et al., 1994). Cooling enhances enzyme activity in the bulb, which is accompanied by mobilization of carbohydrates such as starch, fructose, and sucrose from bulb scales to above ground (Lambrechts et al., 1994). As the flower stalk elongates, sucrose content of the bulb internodes

decreases, while glucose content and invertase activity increases. Starch content in the internodes decreases initially, and then increase (Lambrechts et al., 1994).

The current study focuses on the carbohydrate status of above ground organs after this period of flower stalk elongation. Despite proper cooling and carbohydrate partitioning, disorders still occur at this point due to unfavorable conditions of postharvest storage. One of these conditions is lack of light, which causes chlorophyll to degrade. Without light, the last step in chlorophyll biosynthesis – the conversion of 8-aminolevulinic acid to protochlorophyllide by NADH protochlorophyllide reductase – cannot occur (Zhang and Zhou, 2013). In long periods of darkness, chlorophyll degradation occurs due to changes in the expression of genes associated with chlorophyll catabolism in *Arabidopsis* (Liebsch and Keech, 2016). This change often favors the process of senescence in periods of energy starvation, thus leading to common postharvest disorders such as leaf and bud senescence. During 2 weeks of postharvest storage in the dark and light at 4°C, there was no change in chlorophyll concentration in lower leaves of potted lilies (Ranwala and Miller, 2000). However, once the plants were transferred to 22°C, plants held in the dark lost 66% of all chlorophyll in their lower leaves. This shift from 4°C to 22°C triggered leaf senescence, and was characterized by this loss of chlorophyll as well as depletion of energy sources (Ranwala and Miller, 2000).

In dark postharvest storage, plants continue to respire and reduce energy sources in the plant with no replenishment from current photosynthesis. This can have detrimental effects on overall plant quality as this lack of resources leads to various disorders and poor growth. In potted lilies, plants held in the dark for 2 weeks at 4°C gradually lost about 50% of total soluble carbohydrates (glucose, fructose and sucrose) in their lower leaves (Ranwala and Miller, 2000). This gradual loss continued once plants were transferred to 22°C, indicating a depletion in

energy status during storage. Supplemental light also significantly increased the level of total soluble carbohydrates in plants held in the dark and 4°C (Ranwala and Miller, 2000). Higher levels of carbohydrates play a role in delaying senescence in species such as oleanderleaf protea *(Protea nerifolia)* and carnation (*Disanthus caryophyllus)* (McConchie et al., 1991; Heide and Oydvin, 1969) where it's possible they provide a source to maintain energy levels.

Proper temperature is also one of the most important factors of postharvest storage that influences energy status and quality of plants (Ferrante et al., 2014). Low temperatures are often used to reduce postharvest respiration rates, and change the activity of various pathways and reduce overall carbohydrate metabolism (Taiz and Zeiger, 2006; ap Rees et al., 1988). Warmer temperatures stimulate respiration and decrease carbohydrate pools. These effects were seen in potted lilies held at 4°C in the light, which upon being transferred to 22°C, experienced a rapid loss in soluble carbohydrates and metabolic activities (Ranwala and Miller, 2000). Therefore, ornamental species are generally held at the lowest tolerable temperature in order to slow respiration and maintain quality (Ferrante et al., 2014).

Oxidative stress

Low temperatures slow growth and metabolism and have the potential to keep plants at a marketable quality (Nell, 1993). However, too low temperature, even at non-freezing levels, can disrupt cellular functioning, integrity and lead to detrimental oxidative stress (Prasad et al., 1994; Kerdnaimongkol et al, 1997). On the cellular level, these disorders are largely due to the production of reactive oxygen species such as the superoxide anion (O_2•-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH). These species react with lipids, proteins, DNA and RNA causing significant damage to these important cellular components.

Reactive oxygen species (ROS) are a natural product of aerobic plant metabolism. They are produced via the reduction of molecular oxygen usually due to exposure to high energy or electron transfer reactions such as those that occur in the chloroplasts, mitochondria and plasma membrane as part of respiration and photosynthesis (Taiz and Zeiger, 2006). Reactive oxygen species have benefits when in non-toxic amounts. These include serving as signals for stress responses due to drought, salinity, chilling, heavy metals and pathogens (Sharma et al., 2012). There are molecules that scavenge for reactive oxygen species and keep them in check. These include the antioxidative enzymes superoxide dismutase, peroxidase and catalase, which are upregulated in response to rising ROS levels (Sharma et al., 2012). In order to measure reactive oxygen species in plant systems, levels of these ROS scavengers are often measured as well as levels of lipid peroxidation. Damage occurs when the level of ROS exceeds the level of defense mechanisms. At this point, ROS cause damage via peroxidation of lipids, oxidation of proteins, and inhibition of enzymes, nucleic acids and eventual programmed cell death (Sharma et al., 2012).

It is possible that unfavorable conditions of postharvest storage can induce a stress response and create a damaging ratio of reactive oxygen species. In potted 'Star Gazer' lily, there was a gradual loss in superoxide dismutase (SOD) activity in leaves of dark-held plants at 4°C, which continued upon transfer to 22°C (Ranwala and Miller, 2000). A significant decrease in catalase (CAT) activity after transfer to 22°C and increased lipid peroxidation suggests that oxidative stress plays a role in dark, low-temperature induced leaf senescence (Ranwala and Miller, 2000).

There are still questions about the extent and role of this damage in potted tulips, especially in regards to flowers, and more specifically peal, senescence. There has been

extensive work done in cut flower systems. In cut hemerocallis *(Hemerocallis* sp.) flowers, H_2O_2 increases over the senescence period and a progressive increase in SOD (Chakrabarty et al., 2009). In gladiolus (*Gladiolus* sp.) tepals, there was a decrease in ascorbate peroxidase, which could be the prerequisite for flower senescence and eventual increase in H_2O_2 (Hossain et al., 2006). In cut tulips Azad et al. (2008) proposed that this prerequisite was instead intercellular energy depletion, which was maintained with supplemental sucrose. The current study aims to consider these two factors with a focus on potted tulips.

Methods to Enhance Postharvest Life

There is a strong influence of sub-optimal storage conditions on potted plant metabolism that can lead to a decline in quality. Current postharvest methods focus on ways to manipulate conditions or plant functioning in order to slow metabolism and delay degenerative processes. By merely slowing these processes, and not totally impairing them, it is possible for plants to recover once out of storage (Ferrante et al., 2014).

Postharvest Storage

An optimal postharvest storage environment is important to maintain quality of flowering potted plants. In production, plants are put into postharvest storage once they have been grown in the greenhouse and are waiting to be sent to market. Temperature, light, and duration all influence the physiological functioning and the ultimate performance of a plant. Therefore, an effective way to maintain plant quality is to manipulate the postharvest storage environment to conditions that best help the species maintain flower life, and overall quality.

Temperature is the most important factors of postharvest storage (Ferrante et al., 2014). In a study of postharvest temperature on potted carnations, flower and plant longevity were greatly reduced when held at 24°C versus 18°C or 21°C. Plants held at 18°C lived nearly twice as long than those held at 24°C (Leonard, Nell and Barrett, 1995). In 'Meijikatar' and 'Meirutral' potted roses (*Rosa* sp.), plants held at 4°C had the longest flower life compared to those held at 16°C and 24°C during postharvest evaluation. There were increased incidents of flower abscission and wilting in plants held at warmer temperatures, which largely contributed to the shorter flower life. Increased leaf abscission was also observed (Cushman et al., 1998).

As mentioned previously, holding plants at this lowest tolerable temperature slows metabolism, which in turn delays postharvest disorders due to stress, but also makes it possible to recover after storage (Ferrante et al., 2014). Low temperature also plays another important role by reducing water loss due to increased evaporation and transpiration. The most effective approach has been to rapidly cool plants and keep them at optimal temperatures (Reid and Jiang, 2012).

Duration of storage is another critical factor of postharvest storage as it determines how long plants are exposed to unfavorable conditions. Longer periods of storage generally lead to shorter plant longevity. This was observed in 'Cotillion' potted carnations, where plant longevity was reduced by 25% as storage duration increased from 3 to 9 days. Plants held for 13 days had the shortest plant longevity (Leonard, Nell and Barrett, 1995). Longer storage increased incidents of postharvest disorders, which was observed in potted lilies. 'Donau' lily plants held for 20 days experienced greater leaf abscission than those held for 0 or 10 days (Funnell and Heins, 1998).

Plant Growth Regulators

Postharvest treatment with growth regulators is often beneficial. In many ethylene sensitive plants, ethylene action inhibitors such as 1-methylcyclopropene (MCP) and silver thiosulfate (STS) are used (Ferrante, et al., 2014). However, tulip senescence is not associated with ethylene production (Sexton, 2000). Therefore, plant growth regulators such as gibberellins and cytokinins target other pathways and improve postharvest quality.

The combination of GA_{4+7} and 6-benzyladenine (BA), sprayed when plants were at a marketable stage improved flower longevity and reduced leaf yellowing in potted tulips (Kim and Miller, 2007; Kim and Miller, 2009). This effect, however, was largely dependent on cultivar and senescence type (Kim and Miller, 2009). There were three categories based on senescence symptoms: wilting, wilting-abscission and abscission (without wilting). Flower longevity of wilting-type cultivars was enhanced with GA_{4+7}/BA treatments over 10 mg L^{-1} and that of wilting-abscission-type cultivars was only enhanced with 50 mg L^{-1} applications. In abscission-type cultivars, application of GA_{4+7}/BA at any concentration had no effect on flower longevity (Kim and Miller, 2009).

It was proposed that the overall effect of this treatment is due to enhanced photosynthetic activity, and subsequent higher carbohydrate levels, by the GA_{4+7}/BA application (Kim and Miller, 2009) – possibly due to photosynthetic stimulating effect of cytokinins (He et al., 2005). It was also proposed that this treatment created a shift in source-sink allocation so that tepals and/or leaves become a stronger sink for carbon. Kim and Miller (2009) also proposed that the differences observed in senescence types could possibly be attributed to this shift in source-sink allocation. In abscission-type cultivars more carbohydrates could be directed to tepals and flowers of without the GA_{4+7}/BA treatment, increasing fresh weight and making tepals more susceptible to abscission.

Ranwala et al. (2003) observed unfavorable stem elongation due to the effect of GA in potted lilies. However, Kim and Miller (2009) only observed this elongation in only one of the twenty potted tulips cultivars studied. In all senescence cultivar types studied by Kim and Miller (2009), the GA_{4+7}/BA application reduced leaf yellowing. There was a similar delay in leaf yellowing with the application of thidiazuron (TDZ), another cytokinin (Jiang et al., 2009).

Experimental Overview

The current study is focused on the effects of postharvest storage on carbohydrate status, oxidative stress and flower life in potted tulips. It has two parts; the first part focuses on the direct effect of postharvest storage temperature and duration on whole-plant carbohydrate status and flower life. It is still unclear how potted tulips respond to postharvest storage on a metabolic level, and if there is the same trend of continued respiration and depleted carbohydrate pools that leads to poor quality and premature senescence that was in other species. Ultimately, the first part aims to break down any possible connections between temperature and duration of postharvest storage, carbohydrate metabolism and flower life. By understanding these connections, it is possible to determine optimal conditions of postharvest storage that maintain plant quality.

The second part focuses on subsequent effects of postharvest storage on flowers and aims to characterize senescence by looking at carbohydrate status and oxidative stress. It aims to understand how potted tulips respond once out of postharvest storage and how senescence proceeds from this point. As in other species, it is possible that oxidative stress and carbohydrate status play roles in tulip flower senescence. This section also explores qualities of tulip tepals

and how methods to study oxidative stress, specifically antioxidative enzymes, are not always universal, or easy.

These experiments take a closer look at physiology of potted tulips, and the effect of postharvest storage on plant functioning and senescence. By studying the connection between temperature, duration of storage, energy status and stress the effects of postharvest storage will be elucidated and potentially used to prevent crop losses.

References

Azad A.K., Ishikawa, T., Sawa, Y. and H. Shibata. 2008. Intracellular energy depletion triggers programmed cell death during petal senescence in tulip. Journal of Experimental Botany. 59(8): 2085-2095.

Buschman, J.C.M. 2005. Globalisation – Flower – Flower Bulbs – Bulb Flowers. Acta Horticulturae. 673: 27-33.

Chakrabarty, D., Verma, A.K. and S.K. Datta. 2009. Oxidative stress and antioxidant activity as the basis of senescence in *Hemerocallis* (day lily) flowers. Journal of Horticulture and Forestry. 1: 113-119.

Christenhusz, M.J.M., Govaerts, R., David J.C., Hall, T., Borland, K., Roberts, P.S., Tuomisto, A., Buerki, S., Chase, M.W. and M.F. Fay. 2013. Tiptoe through the tulips – cultural history, molecular phylogenetics and classification of *Tulipa* (Liliaceae). Botanical Journal of the Linnean Society. 172: 280-328.

Cushman, L.C., Pemberton, H.B., Miller, Jr., J.C., and J.W. Kelly. 1998. Interactions of flower stage, cultivar and shipping temperature and duration affect pot rose performance. HortScience. 33(4): 736-740.

De Hertogh, A. and G. Springer. 1977. Part II: Suggestions on the use and marketing of bulb flowers and plants. Holland Technical Service Bulletin 4.

De Hertogh, A. 1989. Holland Bulb Forcer's Guide, 4th Edition. Hillegom, The Netherlands: International Flower-Bulb Center. B2-B19.

De Hertogh A. and M. Le Nard. 1993. The Physiology of Flower Bulbs. Amsterdam, The Netherlands: Elsevier Science Publishers. 617-682.

Dole, J.M. 2003. Research approaches for determining cold requirements for forcing and

flower of geophytes. HortScience. 38(3): 341-346.

Ferrante, A., Trivellini, A., Scuderi, D., and D. Romano. 2014. Post-production physiology and handling of ornamental potted plants. Postharvest Biology and Technology. 100: 99-108.

Funnell, K.A., and R.D. Heins. 1998. Plant growth regulators reduce postproduction leaf yellowing of potted Asiflorum lilies. HortScience. 33(6): 1036-1037.

Heide, O.M. and J. Oydvin. 1969. Effects of 6-Benzylamino-purine on keeping quality and respiration of glasshouse carnations. Horticultural Research. 9(1): 26.

He, P., Osaki, M., Takebe, M., Shinano, T. and J. Wasaki. 2005. Endogenous hormones and expression of senescence-related genes in different senescent types of maize. Experimental Botany. 56: 1117-1128.

Hossain, Z., Mandal, A.K.A., Datta, S.K., and A.K. Biswas. 2006. Decline in ascorbate peroxidase activity – a prerequisite for tepal senescence in gladiolus. Journal of Plant Physiology. 163: 186-194.

Jiang, C.Z., Macnish, L.W., King, A., Yi, M and M.S. Reid. 2009. Thidiazuron, a non-metabolized cytokinin, shows promise in extending the life of potted plants. Acta Horticulturae: IXth International Symposium on Postharvest Quality of Ornamental Plants. 847: 59-65.

Kim, H.J and W.B. Miller. 2007. Effects of GA_{4+7} and benzyladenine application on postproduction quality of 'Seadov' pot tulip flowers. Postharvest Biology and Technology. 47: 416-421.

Kim, H.J and W.B. Miller. 2009. GA_{4+7} plus BA enhances postproduction quality in pot tulips. Postharvest Biology and Technology. 51: 272-277.

Kerdnaimongkol, K., Bhatia, A., Joly, R.J., and W.R. Woodson. 1997. Oxidative stress and diurnal variation in chilling sensitivity of tomato seedlings. Journal of American Society of Horticulture Science. 122: 485-490.

Lambrechts, H., Franssen, J.M. and C. Kollöffel. 1992. The 4-methylene-glutamine:asparagine ratio in the shoot of tulips bulbs cv. Apeldoorn as a criterion for their dry-storage duration at 5°C. Scientia Horticulturae. 52: 105-112.

Lambrechts, H., Rook, F. and C. Kollöfeel. 1994. Carbohydrate status of tulip bulbs during cold-induced flower stalk elongation and flowering. Plant Physiology. 104: 515-520.

Leonard, R.T., Nell, T.A and J.E. Barrett. 1995. Effects of production and postproduction factors on longevity and quality of potted carnations. Acta Horticulturae VIth International Symposium on Postharvest Physiology of Ornamental Plants. 405: 356-361.

Liebsch, D. and O. Keech. 2016. Dark-induced leaf senescence: new insights into a complex light-dependent regulatory pathway. New Phytologist. 212(3): 563-570.

McConchie, R., Lang, N.S. and K.C. Gross. 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. Journal of the American Society for Horticultural Science. 116(6): 1019-1024.

Nell, T.A. 1993. Flowering potted plants: Prolonging shelf performance: Postproduction care & handling. Ball Publishing, Batavia, IL.

Pavord, A. 2008. Bulb. New York, NY: Octopus Books.

Prasad, T.K., Anderson, M.D., Martin, B.A and C.R. Stewart. 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. Plant Cell. 6: 65-74.

Ranwala, A.P. and W.B. Miller. 2000. Preventative mechanisms of gibberellin$_{4+7}$ and light on low-temperature-induced leaf senescence in *Lilium* cv. Star Gazer. Postharvest Biology and Technology. 19: 85-92.

Ranwala, A.P, Legnani, G. and W.B. Miller. 2003. Minimizing stem elongation during spray applications of gibberellin$_{4+7}$ and benzyladenine to prevent leaf chlorosis in Easter lilies. HortScience. 38: 1210-1213.

ap Rees, T., Burrell, M.N., Entwistle, T.G., Hammond, J.B., Kirk, D. and N.J Kruger. 1988. Effects of low temperature on the respiratory metabolism of carbohydrates by plants. Symposium of the Society of Experimental Biology. 42: 377-393.

Reid, M.S. and C.Z. Jiang. 2012. Postharvest biology and technology of cut flowers and potted plants. Horticultural Reviews. 40(1): 1-54.

Sexton, R., Laird, G., and W.G. van Doorn. 2000. Low temperature sensing in tulip (*Tulipa gesneriana* L.) is mediated through increased response to auxin. Journal of Experimental Botany. 51: 587-594.

Sharma, P., Jha, A.B., Dubey, R.S. and M. Pessarakli. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanisms in plants under stressful conditions. Journal of Botany. 2012: 1-26.

Taiz, L. and E. Zeiger. 2006. Plant Physiology, 4th Edition. Sunderland, MA: Sinauer Associates, Inc.

Zhang, H. and C. Zhou. 2013. Signal transduction in leaf senescence. Plant Molecular Biology. 82(6): 539-545

CHAPTER 2: INFLUENCE OF POSTHARVEST STORAGE ON CARBOHYDRATE STATUS AND FLOWER LIFE OF POTTED TULIPS

Abstract

The focus of this research was to determine how temperature and duration of postharvest cold storage affected carbohydrate status and flower life of potted tulips. At the bud color stage, forced, potted plants were moved from the greenhouse at 17ºC into dark storage at 1, 4 and 7ºC and were held for 1, 2, 4 or 7 weeks (Year 1) or 1, 3, or 5 weeks (Year 2). After cold storage, plants were moved into a postharvest evaluation room at 20ºC (12 hr of light at 8-12 µmol/m^{-2}/sec^{-1}) to assess flower life. Total soluble carbohydrates (glucose, fructose, sucrose) and starch were measured in the buds, leaves and stems of 'Saigon' and 'Strong Gold' (Year 1) and tepals, floral organs, leaves and stems of 'Spryng,' 'van Eijk' and 'Yellow Flight' (Year 2). In all cultivars, plants stored at 7ºC for 7 (Year 1) or 5 (Year 2) weeks had the shortest flower longevity. Plants held at 1ºC showed the least change in carbohydrate status, and the longest flower life. As duration and temperature of storage increased, the total soluble carbohydrates in buds and tepals increased in most cultivars. The lowest levels of total soluble carbohydrates was observed in plants held at 7ºC for 7 (Year 1) or 5 (Year 2) weeks. Warmer temperatures and longer durations led to a decrease in flower life as plants continued to develop while in storage. It was concluded that best flower quality resulted from storage for $\leq$ 1 week at all temperatures. For durations > 3 weeks, best flower quality resulted from storage at 1ºC and storage at 4ºC or 7ºC should be avoided if possible.

Introduction

Postharvest storage is used to maintain plant quality before transport to market. The most important factors that influence quality of plants during storage and transport are temperature, relative humidity and light (Ferrante et al., 2014). However, genotype plays a large role in determining a plant's ability to withstand sub-optimal conditions and still maintain quality. Therefore, it is important to understand the responses of each species to storage in order to determine ways to maintain plant quality. This experiment focuses on the response of potted tulips to temperature and duration of postharvest storage and the effect on overall plant quality. It also explores the possible effect of carbohydrate status on flower life once plants are out of storage.

Generally, quality is determined by appearance and marketability of the plant. This encompasses color, shape and size. However, preferable appearance is largely species dependent. Some plants such as poinsettia (*Euphorbia pulcherrima)* are grown for foliage, so responses such as leaf drop are critical in determining quality (Wang and Blessington, 1990). For flowering species like the tulips used in this study, flower appearance is critical in deciding quality. Müller et al. (1999) determined that rose flowers can be considered "healthy" when they are half to fully open with no symptoms of senescence. The definition of quality can also extend into less visually-based parameters such as stress resistance and longevity (Ferrante et al., 2014). The current study focuses on flower longevity, or how long a flower does not display signs of senescence, as a determining factor of plant quality.

Temperature is a critical parameter that influences metabolism and overall plant quality (Ferrante et al., 2014). It is an important factor in postharvest storage because of its correlation with respiration and ethylene production (Nowak and Rudnicki, 1990). By holding plants at the

lowest tolerable temperature, respiration and ethylene production are reduced(Reid, 1991). However, the lowest tolerable temperature is species dependent. For potted tulips, the "Holland Bulb Forcer's Guide" states an optimum temperature is from 0-2°C (De Hertogh, 1989). The current study examines this range and warmer temperatures to get a clear picture of how optimal and sub-optimal temperatures affect potted tulip physiology.

Duration of storage is also important, and for each species there is a minimum and maximum duration of tolerable storage. According to De Hertogh (1989), when properly stored at low temperatures, potted tulips can be held for up to 4 weeks with no effect on marketable appearance. This period is much longer compared to other species, such as potted roses, which can only remain in storage for up to three days without significant reduction in plant longevity and overall quality (Cushman et al., 1998).

For tulips, changes in metabolism due to temperature and duration of postharvest storage are not fully understood. There is some information offered by the "Holland Bulb Forcer's Guide," but questions about the effect of storage parameters still remain. By examining carbohydrate status and flower life, the effect of postharvest storage on energy status and plant quality, and the connection between these two, can be explored.

The goal of this experiment was to study physiological changes in potted tulips that had been held in postharvest cold storage. The specific stage studied was when plants are at the marketable bud color stage after forcing and growing out in the greenhouse. The ultimate objective was to determine how a period of postharvest storage affects carbohydrate pools and flower life of potted tulips and their ultimate marketability.

Materials and Methods

Plant Material

The experiment was conducted over two years. In the first year, two cultivars were used: 'Saigon' and 'Strong Gold.' In Year 2, cultivars 'Spryng,' 'van Eijk' and 'Yellow Flight' were used. Bulbs were stored at 17ºC, potted and placed in a cooler at 9ºC lowered to 1ºC as rooting progressed, for 16 weeks. Plants were then moved into the greenhouse and grown at 17ºC.

Postharvest Cold Storage

At bud color stage in the greenhouse, plants were divided into groups for storage at 1, 4 or 7ºC for 1, 2, 4 or 7 weeks (Year 1) and 1, 3 or 5 weeks (Year 2). In Year 1, each pot contained 6 plants. One plant was harvested for carbohydrate analysis on the day plants were removed from cold storage and the remaining plants were used for flower life analysis and averaged per pot. In Year 2, each pot contained 5 plants.

Flower life was measured over the subsequent days after storage in a growth chamber at 20ºC (12h of light at 8-12 µmol/m^{-2}/sec^{-1}). Whole plant carbohydrate status was measured directly after plants came out of cold storage. One plant was harvested for carbohydrate analysis on the day plants were removed from cold storage and the remaining plants were used for flower life analysis and averaged per pot. The other two plants were harvested for tentative enzyme assays. Plants to be stored were moved out of the greenhouse and placed in a cardboard box, which was closed to reduce excessive water loss. These boxes were placed in a dark cooler at the appropriate temperature.

Flower life

The pots with the remaining intact plants were placed in a growth chamber at 20ºC with 12 hours of light (7-12 µmol/m^2/s) and 12 hours of darkness. Buds were observed daily and tagged as each opened and senesced. Final senescence was identified as the point when there was a significant color change in tepals, which was also accompanied by petal curling and signs of wilting (Figure 2.1). Flower life was found by subtracting senescence date from opening date. Plants were watered as needed.

Carbohydrate Analysis

In Year 1 of the experiment three tissues were sampled: entire stem, all leaves, and flower bud. In Year 2, entire stem, all leaves, tepals and remaining floral organs were sampled. Samples were taken directly after plants were removed from cold storage. Fresh weight was recorded and samples were frozen at -80ºC until freeze drying. Dry weight was recorded after freeze-drying. Freeze dried tissue was ground using a Wiley Mill.

To extract carbohydrates, 3 mL of 80% ethanol was added to 50 mg of ground sample. 100 µL of lactose (4 mg/mL) was added as an internal standard and the sample incubated at 70ºC. After 5 minutes, the samples were vortexed and placed back in incubation at 70ºC for 25 min. The samples were then removed and centrifuged at 4,000 RPM for 10 min. The supernatant was poured into a clean test tube. 3 mL of 80% ethanol was added to the remaining sample, and incubated at 70ºC for 30 min and centrifuged as above. The supernatants were combined and the sequence repeated one more time. The 9 mL of supernatant was poured onto an ion-exchange column with a 1 mL layer of cation exchange resin (Dowex 50W, hydrogen form) and a 1 mL layer of anion exchange resin (Amberlite IRA-67, acetate form) to remove amino acids, organic

acids and the like that may interfere with analysis. After the supernatant had flowed through, 3 mL of 80% ethanol was added and drained into the sample. The final samples were held at 4 ºC in 10 mL glass test tubes until drying on a Buchler Rotary Evapo-Mix using a vacuum. The samples in the 18 mL glass test tubes were placed on the machine at 50ºC for approximately 45 min, or until all the ethanol had evaporated. Dried samples were held at -20ºC until analysis.

After extraction, carbohydrates were dissolved in HPLC grade water and subjected to ion chromatography. A Dionex CarboPac PA1 column (035391, Thermo Fisher, Waltham, MA) and guard (043096, Thermo Fisher, Waltham, MA) delivering 25 µL/injection and a 1mL/min flow rate. Carbohydrates were measured as anions in NaOH (200 mM isocratic) using pulsed electrochemical detection.

The remaining insoluble debris after carbohydrate extraction was air dried and used for starch analysis. 4 mL of Na-acetate buffer (100mM, pH 4.5) was added to the debris. The tubes were vortexed and placed in a boiling water bath for 30 minutes. After cooling, 1 mL of amyloglucosidase (A7255, Sigma; 50 units of enzyme per mL). This enzyme cleaves starch into glucose. The starch digest tubes were then vortexed and incubated for 2 days at 50ºC.

After incubation, samples were centrifuged for 10 minutes at 4,000 RPM and appropriate aliquots added to tubes. A glucose oxidase, peroxidase and *o*-dianisidine solution was made using 5 units of glucose oxidase (G61255, Sigma) 1 unit of peroxidase (P8125, Sigma) and 0.04 mg of *o*-dianisidine (D3252, Sigma) per 5 mL of this solution, which was then added to each tube. Samples were then incubated for 30 minutes at 30ºC. After incubation, 1 mL of HCl (1M) was added to each tube to stabilize the color.

Absorbance was determined at 450 nm to determine glucose concentration. A calibration curve was created using a set of glucose standards (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 µmol). From final absorbance, mg starch/g dry weight was calculated.

Statistical Analysis

A completely randomized design was used. Regression assumptions were checked and transformations were made to the data if necessary. Means were compared by two-way analysis of variance using R (Version 1.0.153).

Results

Flower Life

In both Year 1 and Year 2, temperature and weeks of storage interacted to affect flower life in all cultivars (Table 2.1, Table 2.2). As postharvest storage duration increased, flower life decreased and in most cases this effect was even more significant at warmer temperatures.

Carbohydrate Status

In Year 1, there were differences in glucose levels in all tissues in both 'Saigon' and 'Strong Gold' (Figure 2.2). At 7 weeks, buds of plants held at 7ºC had the highest level of glucose in 'Saigon,' while plants held at 1ºC had the highest level in 'Strong Gold.' As duration and temperature of storage increased, there was a general increase in glucose levels in the stem.

Plants held at 7ºC for 7 weeks had the highest level of fructose in the buds of both cultivars, and in the leaves and stems of 'Saigon' (Figure 2.3). Fructose levels in the buds, leaves and stems of 'Strong Gold' plants held at 1ºC and 4ºC generally remained consistent for plants stored for all durations.

Plants held at 1ºC for all durations had the highest level of sucrose in all tissues (Figure 2.4). As duration of storage increased up to 7 weeks, there were lower levels of sucrose in all tissues compared to the levels at 1, 2 or 4 weeks.

There were changes in total soluble carbohydrate (TSC) levels in plants held for more than 2 weeks in all tissues in both cultivars (Figure 2.5). In the buds of 'Saigon,' plants held for 7 weeks had the lowest level of TSC when held at 1ºC, while 'Strong Gold' plants held for 7 weeks had the lowest level at 7ºC. TSC levels in the leaves of plants held for 7 weeks at 4ºC and 7ºC were lower than those in plants held for 1, 2 or 4 weeks.

Starch levels were low in all tissues and in both cultivars, although buds initially contained 4 to 5-fold more starch than leaves and stems (Figure 2.6). As duration of storage increased, starch levels in the buds of both cultivars generally decreased. Plants held at 4ºC and 7ºC for 7 weeks had higher levels of starch in the leaves than those held at 1ºC.

In Year 2, glucose levels in the floral organs (all flower parts except tepals) increased as duration of storage increased for plants held at 4ºC and 7ºC in 'Spryng' and 'Yellow Flight' (Figure 2.7). In tepals of 'Yellow Flight,' glucose levels decreased as duration of storage increased. Plants held at 7ºC for 5 weeks had the lowest level of glucose in the leaves of 'Spryng' and 'van Eijk.' There were less consistent trends observed in the stems, but in general plants held for 5 weeks at 4ºC or 7ºC had lower levels than those held for 1 or 3 weeks.

As duration of storage increased, fructose levels increased in the floral organs and tepals of all cultivars (Figure 2.8). Fructose levels were consistently lowest in the tepals of plants held at 1ºC in all cultivars. For plants held at all temperatures and in all three cultivars, fructose levels in the leaves were lower in plants held for 1 week than those not held in storage. However,

fructose levels in the stems of all cultivars were higher in plants held for 1 week than those not held in storage.

Sucrose levels were consistently highest in all tissues in all cultivars for plants held at 1ºC (Figure 2.9). Plants held for 1 week at 1ºC had the highest level of fructose. Once storage increased to 3 weeks, the sucrose level was either higher or lower depending on temperature, tissue type and cultivar. Sucrose levels in the floral organs, leaves and stems of plants held at 4ºC or 7ºC in most cultivars were lower when held for 5 weeks versus 3 weeks. Sucrose levels in the tepals of plants held at 4ºC or 7ºC in most cultivars were higher when held for 5 weeks versus 3 weeks.

Total soluble carbohydrate levels (TSC) were higher in the floral organs and stems of plants held for 1 week at all temperatures in all three cultivars than those of plants not held in storage (Figure 2.10). As duration of storage increased, TSC levels in the tepals of 'Spryng' and 'Yellow Flight' increased. TSC levels remained relatively consistent in the leaves of plants held for all durations at all temperatures in all three cultivars. The only exception is a substantially lower level in plants held at 4ºC or 7ºC for 3 weeks than those held at 0, 1, or 5 weeks.

Starch levels were very low in all tissues of plants held for all durations and at all temperatures in 'Spryng,' 'van Eijk' and 'Yellow Flight' (Figure 2.11). The highest levels were observed in the floral organs and stems of each cultivar. As duration and temperature of storage increased, starch levels in the floral organs decreased linearly. Plants held at 1ºC consistently had the lowest level of starch in the floral organs in all three cultivars and for every duration of storage. Plants held for 5 weeks generally had higher levels of starch in the tepals than those held for 0, 1 or 3 weeks when held at mostly all temperatures. Starch levels were highest in the leaves of 'Yellow Flight' plants held for 5 weeks in all temperatures. In 'van Eijk' and 'Yellow Flight,'

starch levels in the stems of plants held for 1 week at all temperatures were lower than those not held in storage.

Effect of Carbohydrate Status on Flower Life

The relationship between flower life and total soluble carbohydrate and starch levels resulting from different storage temperature and duration was analyzed. In Year 1, there was a significant relationship between total soluble carbohydrate status of the buds and flower life of 'Saigon' plants held at 4ºC and 7ºC, and those held for 7 weeks (Figure 2.12). Starch levels in the buds and flower life also had a significant relationship in 'Saigon' and 'Strong Gold' plants held at 4ºC and 7ºC, and 'Strong Gold' plants held for 4 weeks (Figures 2.13 and 2.15). In the leaves, total soluble carbohydrate levels and flower life had a significant relationship in 'Strong Gold' plants held for 1 week (Figure 2.14). Starch levels in the leaves and flower life of 'Saigon' plants held at 4ºC and for 4 and 7 weeks (Figure 2.13) and 'Strong Gold' plants held at all temperatures and for 4 weeks (Figure 2.15) also had a significant relationship. Levels of starch in the stem and flower life of 'Saigon' plants held at 7 ºC and for 2 weeks were significantly related (Figure 2.13).

In Year 2, there was a significant relationship between total soluble carbohydrate levels in the tepals and flower life of 'Spryng' and 'van Eijk' plants held at 4ºC (Figures 2.16 and 2.18), and 'Yellow Flight' plants held at 7ºC (Figure 2.20). In 'Spryng,' flower life of plants held at 4ºC was significantly related to starch levels in the tepals (Figure 2.17). In floral organs, there was a significant relationship between total soluble carbohydrate status and flower life of 'van Eijk' and 'Yellow Flight' plants held for 5 weeks (Figures 2.18 and 2.20), as well as those held at 1ºC and 7ºC in 'Yellow Flight' (Figure 2.20). Starch levels of the floral organs had a

significant relationship with the flower life of 'Spryng' plants held at 4ºC and 7ºC (Figure 2.17) and 'van Eijk' plants held at 7ºC (Figure 2.19), and 'Yellow Flight' plants held for 5 weeks (Figure 2.21). The total soluble carbohydrate status of leaves significantly related to the flower life of 'van Eijk' plants held 1ºC (Figure 2.18) and 'Yellow Flight' plants held at 7ºC (Figure 2.20). The starch status of leaves also related to the flower life of 'Spryng' and 'van Eijk' plants held at 4ºC (Figures 2.17 and 2.19), as well as 'Yellow Flight' plants held in storage at 7ºC (Figure 2.21). In the stem, total soluble carbohydrate levels significantly related to the flower life of 'Spryng' plants held at 7 ºC (Figure 2.16) and 'van Eijk' plants held at all temperatures (Figure 2.18). In the stems of 'van Eijk' plants held for 3 weeks (Figure 2.19), starch level significantly related to final flower life.

Discussion

Flower life

Increasing duration (0 to 7 weeks) of storage and temperature (1-7 ºC) significantly decreased flower longevity in both years, and in all cultivars. This reduction in flower life could be attributed to continued plant development in storage, which was more pronounced in plants held for longer periods of time and at warmer temperatures. Although it is not completely clear what levels of carbohydrates lead to a longer flower life, there are increased levels of fructose in plants held in storage for greater durations at warmer temperatures that link to continued growth and development.

It is not clear that any period of postharvest storage should be avoided, because higher flower life was observed in 'Spryng,' "van Eijk' and 'Yellow Flight' plants held for 1 week in Year 2. This could be attributed to the fact that even at 7ºC, postharvest storage was cooler than

the growth chamber at 20ºC. However, it is clear that periods greater than 1 week should be avoided if possible due to the decrease in flower life observed in all cultivars. If longer periods must be used, temperatures greater than 4ºC should be avoided and temperatures of 1ºC are preferred.

Carbohydrate Analysis

Understanding the stage at which plants are placed into postharvest storage is critical in understanding the changes in carbohydrate status. Because plants are at the "bud color stage" this puts them at a stage prior, but still close to, anthesis. The primary goal of postharvest storage is to keep plants at this stage, so flowers open when plants arrive at the consumer's home. A negative effect of postharvest storage is development past from this "bud color" stage.

Flowering is largely a response to environmental factors such as light, temperature and relative humidity (Reid, 2005). The stage from mature bud to flower opening is one of the most active growth periods in plant development as petals expand, and floral organs extend. At this point the bud functions as a strong sink, with carbohydrates being translocated to it from source organs such as the leaves (Rogers and Munne-Bosch, 2016). Warm temperature and duration of postharvest storage can contribute to flower opening and development. It is the differences in responses to these environmental factors, as well as differences in development timing among cultivars that contribute to carbohydrate status and flower lives observed in this study.

In Year 1, the total soluble carbohydrate status of both 'Saigon' and 'Strong Gold' plants remained constant when held at 1ºC. There were changes associated with plants held at warmer temperatures in all tissues, and especially in plants held for 7 weeks. Keeping plants at the lowest tolerable temperature contributed to reduced metabolism and respiration, and is a possible

explanation for these differences (Ferrante et al., 2014). Starch levels were low compared to total soluble carbohydrate levels in both cultivars, but were highest in the buds.

The largest changes in carbohydrate pool sizes occurred in the buds. There was a general decrease in sucrose as temperature and storage duration increased in both cultivars as well as an increase in fructose in plants held at 7ºC for 7 weeks. Fructans have been identified as an important reserve carbohydrate that occur concurrently in the storage organs of many geophytes, including tulips (Ranwala and Miller, 2008). Therefore, this increase in fructose could be associated with fructan mobilization from the bulb. It may also be attributed to increased respiration, which was associated with warmer temperatures as well as tepal expansion in tulips (Collier, 1997).

The stems of both 'Saigon' and 'Strong Gold' had the most soluble carbohydrates, with glucose as the most abundant. This abundance was also observed by Collier (1997) in tepals. Stems also contained higher levels of sucrose, especially at cooler temperatures and fructose at warmer temperatures. The differences in these sugars could be could be due to their role in flower development and the translocation through the stem to the bud, with possible reserve carbohydrates coming from the bulb. Collier (1997) also observed higher levels of glucose and sucrose as plants move towards anthesis, and it is possible these high levels were maintained when plants were kept at cooler temperatures.

In Year 2, there was a higher level of fructose in the stem at warmer temperatures and longer durations of storage. Fructans serve as reserve carbohydrates in the stem of lettuce plants, and are eventually used for seed development (Lee and Sugiyama, 2006). These increased levels of fructose were much higher in 'Spryng' and "van Eijk,' while levels of sucrose were higher in 'Yellow Flight.' Again, this increase may be associated with fructan mobilization from the bulb

and continued growth and development in postharvest storage as temperatures increase and flowers move through anthesis (Collier, 1997).

'Yellow Flight,' in general, was a shorter and slower growing cultivar. Therefore, general differences in cultivar may also have had an effect on carbohydrate status and response to postharvest environment. "Spryng' and 'van Eijk' may have been more sensitive to the environment, respiring more, and progressing towards anthesis at a faster rate than 'Yellow Flight.'

Total soluble carbohydrates levels in the stem of 'Spryng' were higher at 1ºC when plants were held in storage for longer durations, which can potentially be attributed to maintenance of carbohydrate pools at cooler temperatures. Starch levels were much lower in the leaves and stems of 'Spryng' compared to 'van Eijk' and 'Yellow Flight,' and were highest in the floral organs of all cultivars. The floral organs of tobacco plants accumulate starch hours prior to anthesis (Ren, 2007). This starch accumulation at warmer temperatures in tulip floral organs may also demonstrate preparation for anthesis.

Effect of Carbohydrate Status on Flower Life

The level and translocation of carbohydrates is considered to be an important factor affecting the development of rose and many cut flowers (Khayat and Zieslin, 1989). In the current study, there was a significant negative relationship between total soluble carbohydrate levels in the buds and flower life in 'Saigon' (Figure 2.12) in Year 1 and tepals of 'Spryng' (Figure 2.16), 'van Eijk' (Figure 2.18) and 'Yellow Flight' (Figure 2.20) in Year 2. This relationship was largely seen in plants held at warmer temperatures, for longer periods of time. As total soluble carbohydrates increased in the buds, flower life decreased. Increased levels of

total soluble carbohydrates in the bud suggest greater mobilization from source organs or possibly even the bulb. Because the bud and tepals are a sink at the point when the tulips in this study are placed in storage, it is possible that greater accumulation of carbohydrates means greater progression towards anthesis. Brown bornia (*Boronia megastigma)* experiences a decrease in carbohydrate status in flower buds once plants have committed to anthesis (Day et al., 1995). Greater progression towards anthesis suggests that when plants come out of storage they will already be closer to senescence, and thus their flower life is shorter.

There was a significant relationship between starch level and flower life in the flower organs of all cultivars. Higher levels of starch levels in the bud (Year 1), or flower organs (Year 2) were correlated with longer flower lives of 'Saigon' (Figure 2.13), 'Strong Gold' (Figure 2.15), 'Spryng' (Figure 2.17), 'van Eijk' (Figure 2.19) and 'Yellow Flight' (Figure 2.21). Because starch is an important indicator of source and sink relationships (Ho, 1998), this accumulation demonstrates the sink demand of the flower organs due to the stage of development and proximity to anthesis (Ren, 2007). There were higher levels of starch in the early stages of lily flower development, and decreased as the flower developed (De Sousa Santos et al., 2016). It is possible that the observed correlation between flower life and starch in the flower organs is an indicator of flower stage. Flowers with higher levels of starch in their tepals were at an earlier stage of development, and father away from senescence than others. Thus, they had a longer flower life once out of storage.

Total soluble carbohydrate levels in the leaves and stems was positively related to flower life in 'Strong Gold' in Year 1, and 'Spryng' and 'van Eijk' in Year 2. Again, these effects were largely seen in plants held at warmer temperatures, for longer periods of time. Increased levels of total soluble carbohydrates in the leaves were correlated with increased flower life in 'Strong

Gold' (Figure 2.14). Total soluble carbohydrate levels in the stem were positively correlated with longer flower life of 'van Eijk' plants held at all temperatures (Figure 2.18). Starch in the stem also played a significant role in affecting flower life in 'Saigon' (Figure 2.13) as well as 'Strong Gold' (Figure 2.15) and 'van Eijk' (Figure 2.19), where higher levels were correlated with longer flower life.

These relationships suggest that increased energy reserves in source organs such as the leaves and stem can lead to a longer flower life. This trend was observed in oleanderleaf protea *(Protea nerifolia)* and carnation (*Dianthus caryophyllus)* (McConchie et al., 1991; Heide and Oydvin, 1969). It is possible that these reserve carbohydrates provide a source to maintain energy levels in plants and sustain the plant while in postharvest storage. Lower levels could lead to energy starvation and rapid senescence.

These relationships, however, were not observed or significant in all cultivars. In 'Strong Gold' buds, increased total soluble carbohydrate levels in the bud were correlated with longer flower life (Figure 2.14). Higher levels of total soluble carbohydrates in the leaves of 'van Eijk' plants held at 1ºC (Figure 2.18), and 'Yellow Flight' leaves held at 7ºC (Figure 2.20) were correlated with shorter flower life. Higher levels of starch in the leaves were also correlated with shorter flower life in 'Yellow Flight' (Figure 2.21). These differences can possibly be attributed to differences in cultivars' response to postharvest environment and time it takes to progress towards anthesis. 'Yellow Flight' plants were slower growing, and therefore it is possible that any increase in carbohydrate was a sign of change in metabolism and therefore progress towards anthesis. The reasons behind these differences in cultivar responses are still unclear but could be attributed to genotypic factors. These factors and how they relate to carbohydrate status and flower life should continue to be explored.

Conclusion

There are changes in carbohydrate status and flower life that are associated with temperature and duration of postharvest storage. These changes differ among cultivars, but in general plants held at cooler temperatures had the least change in carbohydrate pools during storage and had the longest flower life after storage. Storage at warmer temperatures lead to an increase in fructose in most plant tissues. This is a potential signal of fructan mobilization from the bulb as well as continued growth and development, which can exhaust carbohydrate pools and lead to early senescence.

Changes in carbohydrates are also related to the stage of development. Because plants are at the "bud color stage" this puts them at a stage prior, but still close to, anthesis. This is a period of active growth and buds become a strong sink as petals expand, and floral organs extend. Plants progress towards this period of anthesis due to responses to the postharvest environment. At warmer temperatures, they are more likely to flower. And at these warmer temperatures there is a greater accumulation of carbohydrates in the bud tissues. This accumulation of total soluble carbohydrates was correlated with shorter flower life. On the other hand, increased levels of total soluble carbohydrates in source organs such as leaves and stems were correlated with longer flower life.

This study demonstrated that despite varying level of carbohydrates, keeping plants at the lowest tolerable temperature (1ºC) for periods less than 3 weeks accomplishes this for most potted tulip cultivars.

References

Collier, D.E. 1997. Changes in respiration, protein and carbohydrates of tulip tepals and *Alstromeria* petals during development. Journal of Plant Physiology.150:446-451.

Cushman, L.C., Pemberton, H.B., Miller, Jr., J.C., and J.W. Kelly. 1998. Interactions of flower stage, cultivar and shipping temperature and duration affect pot rose performance. HortScience. 33(4): 736-740.

Day, J.S., Loveys, B.R., Aspinall, D. 1995. Cytokinin and carbohydrate changes during flowering of *Bornia megastigma.* Australian Journal of Plant Physiology. 22: 57-65.

De Hertogh, A. 1989. Holland Bulb Forcer's Guide, 4th Edition. Hillegom, The Netherlands: International Flower-Bulb Center. B2-B19.

De Sousa Santos, M.N., Mapeli, A.M. and M.M. Tolentino. 2016. Carbohydrate metabolism in floral structures of *Lilium pumilim* in different development stages. Ciencia Rural. 46(7): 1142-1144.

Ferrante, A., Trivellini, A., Scuderi, D., and D. Romano. 2014. Post-production physiology and handling of ornamental potted plants. Postharvest Biology and Technology. 100: 99-108.

Heide, O.M. and J. Oydvin. 1969. Effects of 6-Benzylamino-purine on keeping quality and respiration of glasshouse carnations. Horticultural Research. 9(1): 26

Ho, L.C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annual Reviews of Plant Physiology and Plant Molecular Biology. 39: 355-378.

Khayat, E. and N. Zieslin. 1989. Translocation of ^{14}C-carbohydrate content and activity of enzymes of sucrose metabolism in rose petals at different night temperatures. Physiologia Plantarum. 76: 581-585.

Lee, O. and N. Sugiyama. 2006. Changes in carbohydrate composition in lettuce flower stalks during development. Journal of Horticultural Science and Biotechnology. 81(5): 928-932.

McConchie, R., Lang, N.S. and K.C. Gross. 1991. Carbohydrate depletion and leaf blackening in *Protea neriifolia*. Journal of the American Society for Horticultural Science. 116(6): 1019-1024.

Müller, R., Stummann, B.M., Andersen, A.S and M. Serek. 1999. Involvement of ABA in postharvest life of miniature potted roses. Plant Growth Regulation. 29: 143-150.

Nowak, J. and R.M. Rudnicki. 1990. Postharvest handling and storage of cut flowers, florist greens, and potted plants. Portland, OR: Timber Press.

Ranwala, A.P. and W.B. Miller. 2008. Analysis of nonstructural carbohydrates in storage organs of 30 ornamental geophytes by high-performance anion-exchange chromatography with pulsed amperometric detection. New Phytologist. 180: 421-433.

Reid, M.S. 1991. Effects of low temperatures on ornamental plants. Acta Horticulturae: V International Symposium on Postharvest Physiology of Ornamental Plants. 298: 215-224.

Reid, M.S. 2005. Flower development: from bud to bloom. Acta Horticulturae: VIIIth International Symposium on Postharvest Physiology of Ornamental Plants. 669: 105-110.

Ren, G., Healy, R., Klyne, A.M., Horner, H.T., James, M.G. and R.W. 2007. Thornburgh. Transient starch metabolism in ornamental tobacco floral nectaries regulates nectar composition and release. Plant Science. 173: 277-290.

Rogers, H. and S. Munné-Bosch. 2016. Production and scavenging of reactive oxygen species and redox signaling during leaf and flower senescence: similar but different. Plant Physiology. 171: 1560-1568.

Wang, Y.T and M. Blessington. 1990. Growth of four tropical foliage species treated with

paclobutrazol or uniconazole. HortScience, 25(2): 202-204.

Table 2.1. Effect of storage temperature and weeks of storage on flower life of 'Saigon' and 'Strong Gold' in Year 1.

		Flower Life (Days)	
Weeks of Storage	Temperature (ºC)	'Saigon'	'Strong Gold'
0		10.9	13.4
1	1	11.1	13.1
	4	11.1	12.5
	7	11.3	11.8
2	1	10.9	12.0
	4	10.9	12.4
	7	10.5	10.1
4	1	10.6	11.4
	4	10.3	9.9
	7	7.6	7.3
7	1	9.2	9.3
	4	7.4	6.0
	7	3.0	1.9
Temperature (T)		***	***
Weeks of storage (W)		***	***
T x W		***	***

NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively

Table 2.2. Effect of storage temperature and weeks of storage on flower life of 'Spryng,' 'van Eijk' and 'Yellow Flight' in Year 2.

		Flower Life (Days)		
Weeks of Storage	Temperature (ºC)	'Spryng'	'van Eijk'	'Yellow Flight'
0		7.2	7.4	11.0
1	1	7.5	6.8	10.2
	4	7.7	7.6	10.9
	7	8.2	7.4	11.2
3	1	5.6	5.4	8.1
	4	6.2	5.1	7.7
	7	4.6	5.0	8.1
5	1	5.7	4.4	6.1
	4	2.8	3.4	8.1
	7	0.8	1.0	3.5
Temperature (T)		***	***	***
Weeks of storage (W)		***	***	***
T x W		***	***	***

NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively

Figure 2.1. Typical appearance of a tulip flower at senescence.

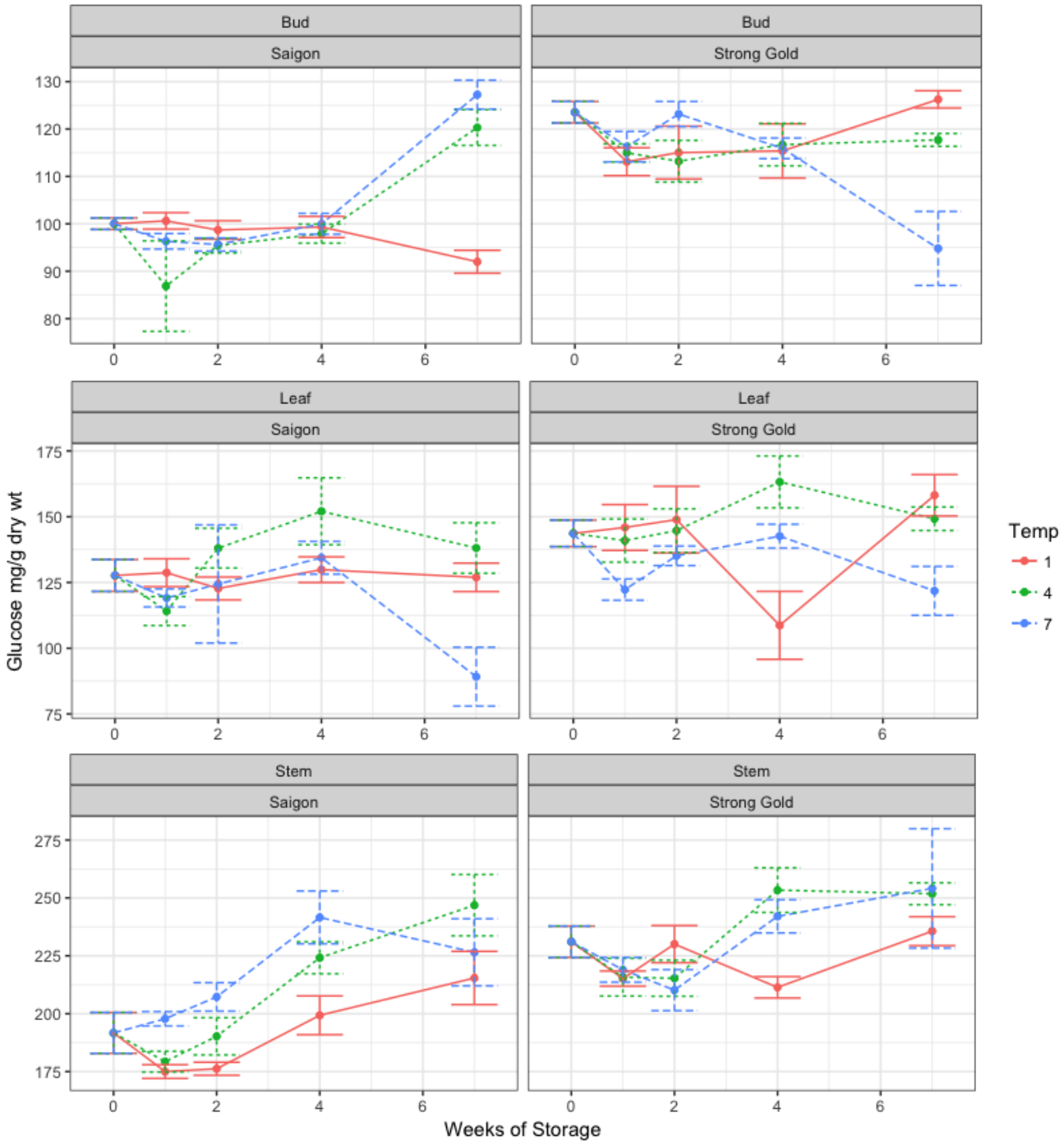


Figure 2.2. Effects of length of 1, 4 or 7ºC cold storage on glucose level of tulips in Year 1. L to R: 'Saigon,' 'Strong Gold.' Top to Bottom: Bud, leaf, stem. Data are means of 6 replicates, ± SE.

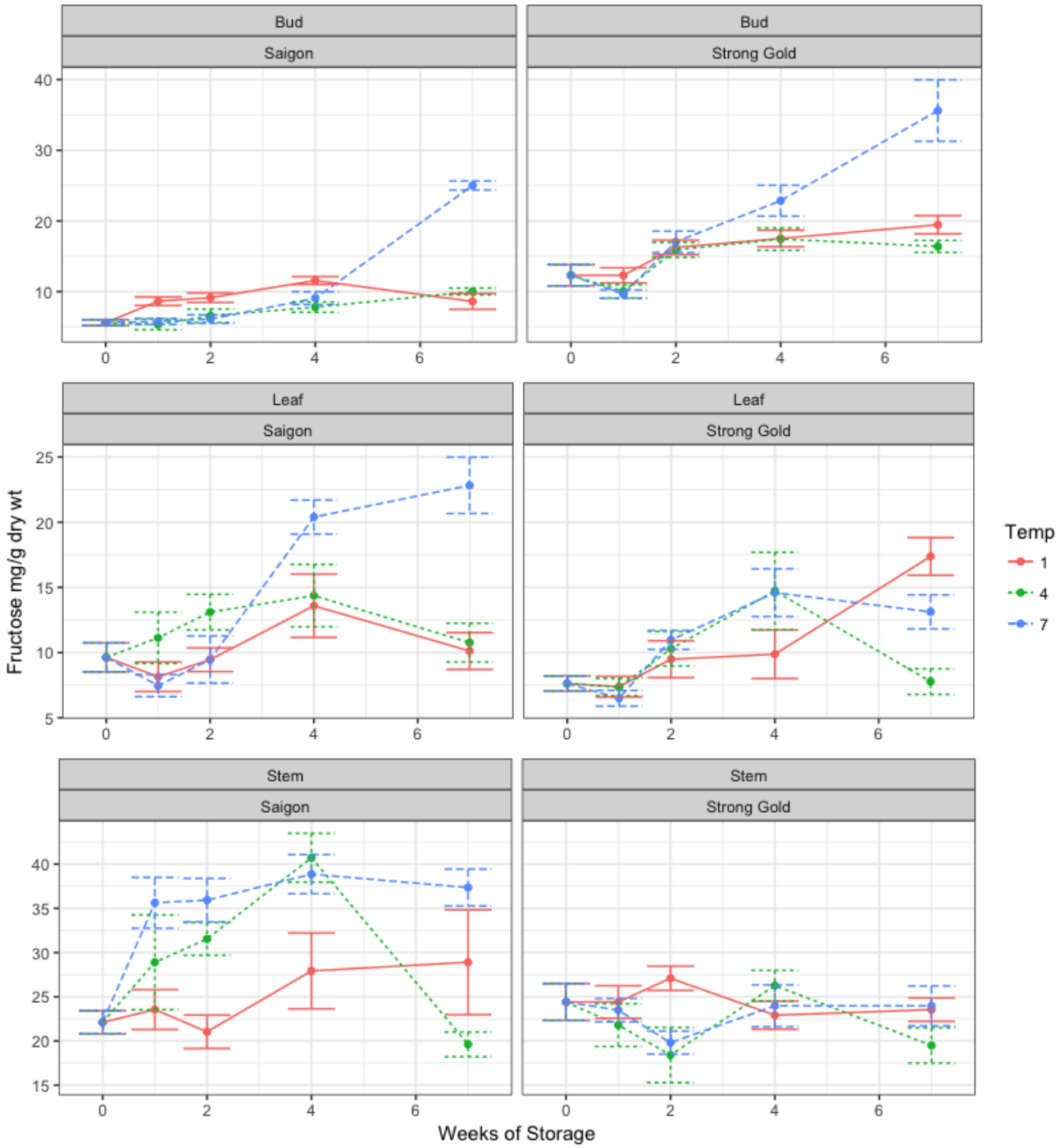


Figure 2.3. Effects of length of 1, 4 or 7ºC cold storage on fructose level of tulips in Year 1. L to R: 'Saigon,' 'Strong Gold.' Top to Bottom: Bud, leaf, stem. Data are means of 6 replicates, ± SE.

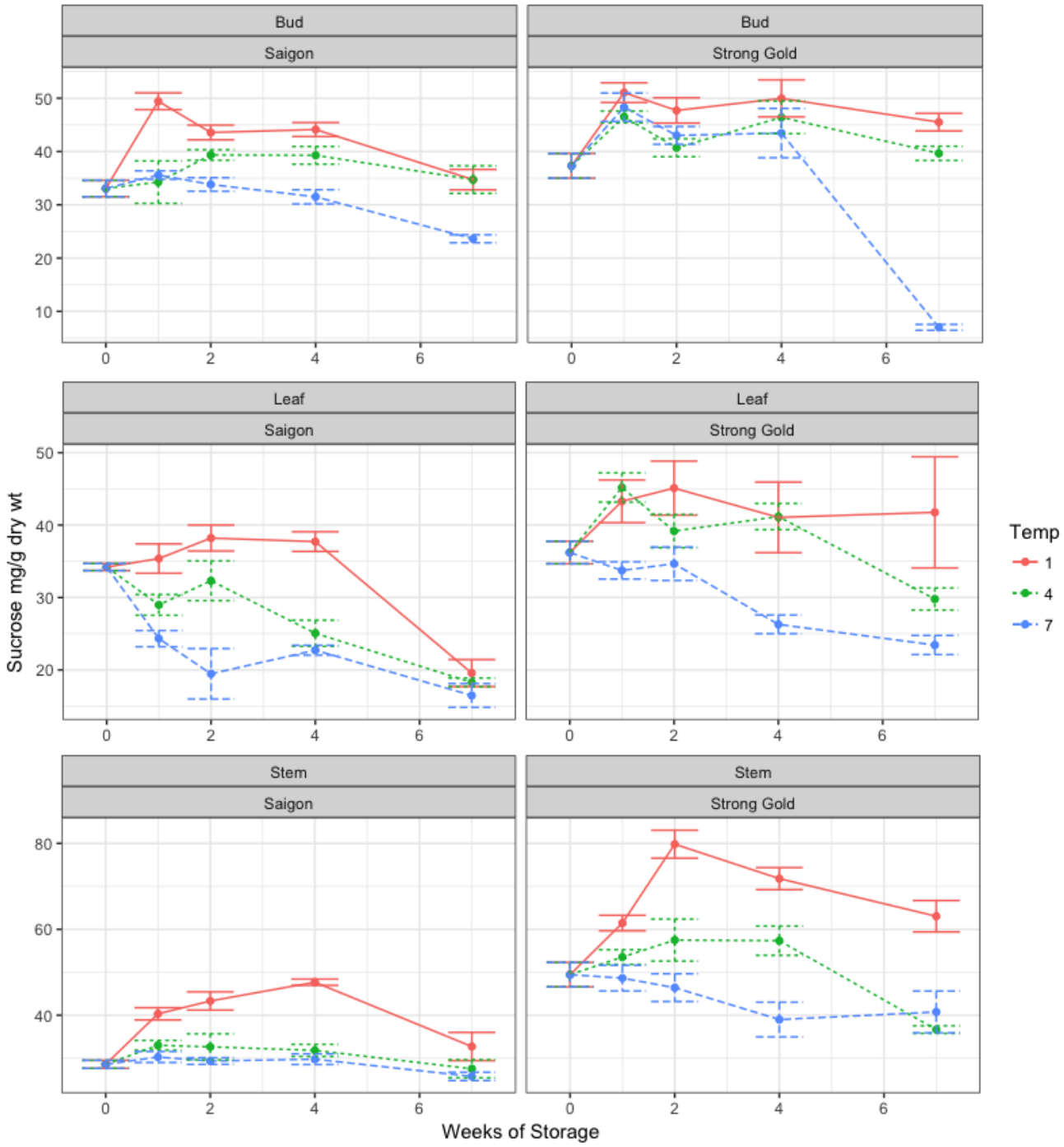


Figure 2.4. Effects of length of 1, 4 or 7ºC cold storage on sucrose level of tulips in Year 1. L to R: 'Saigon,' 'Strong Gold.' Top to Bottom: Bud, leaf, stem. Data are means of 6 replicates, ± SE.

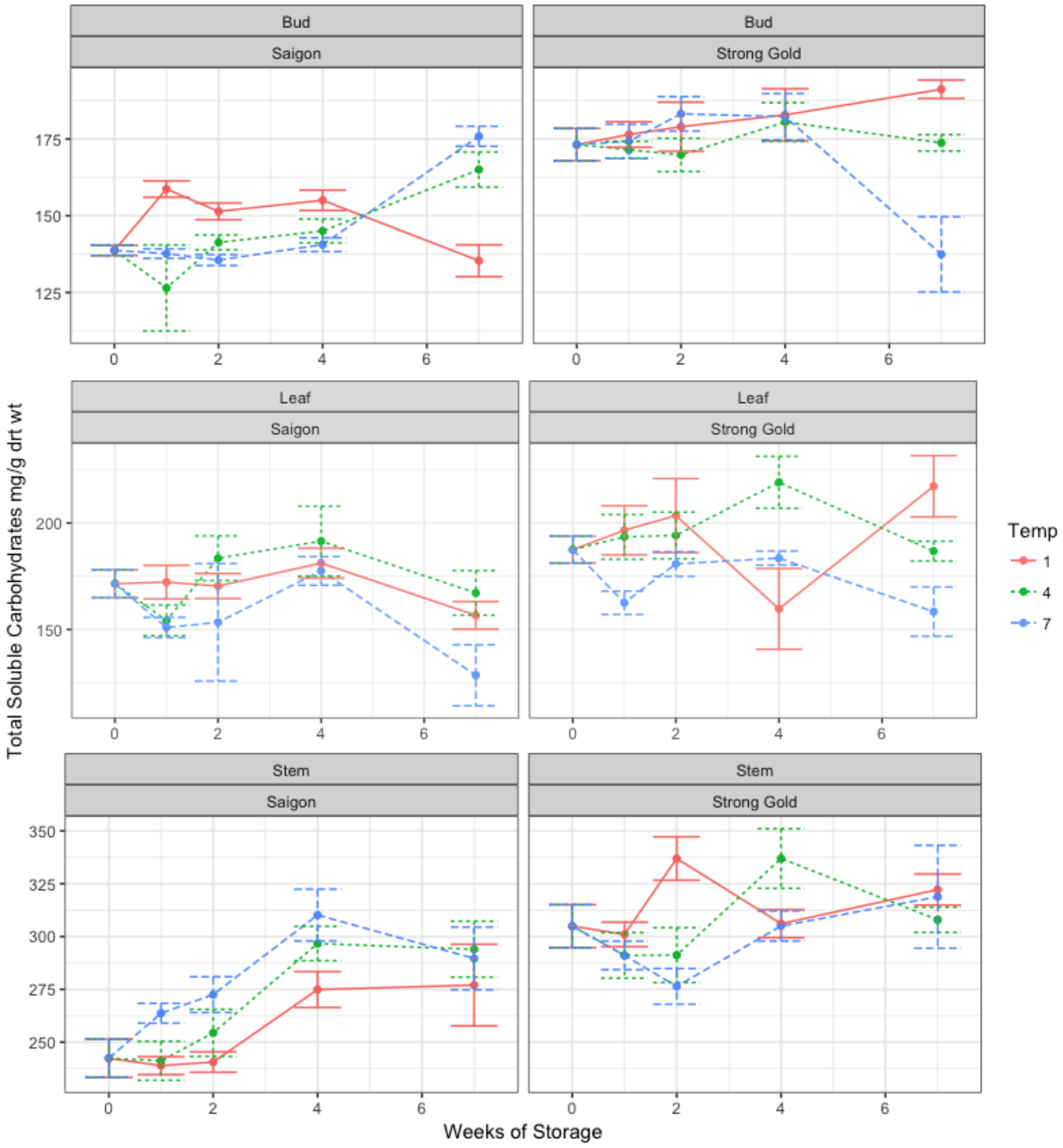


Figure 2.5. Effects of length of 1, 4 or 7ºC cold storage on total soluble carbohydrate level of tulips in Year 1. L to R: 'Saigon,' 'Strong Gold.' Top to Bottom: Bud, leaf, stem. Data are means of 6 replicates, ± SE.

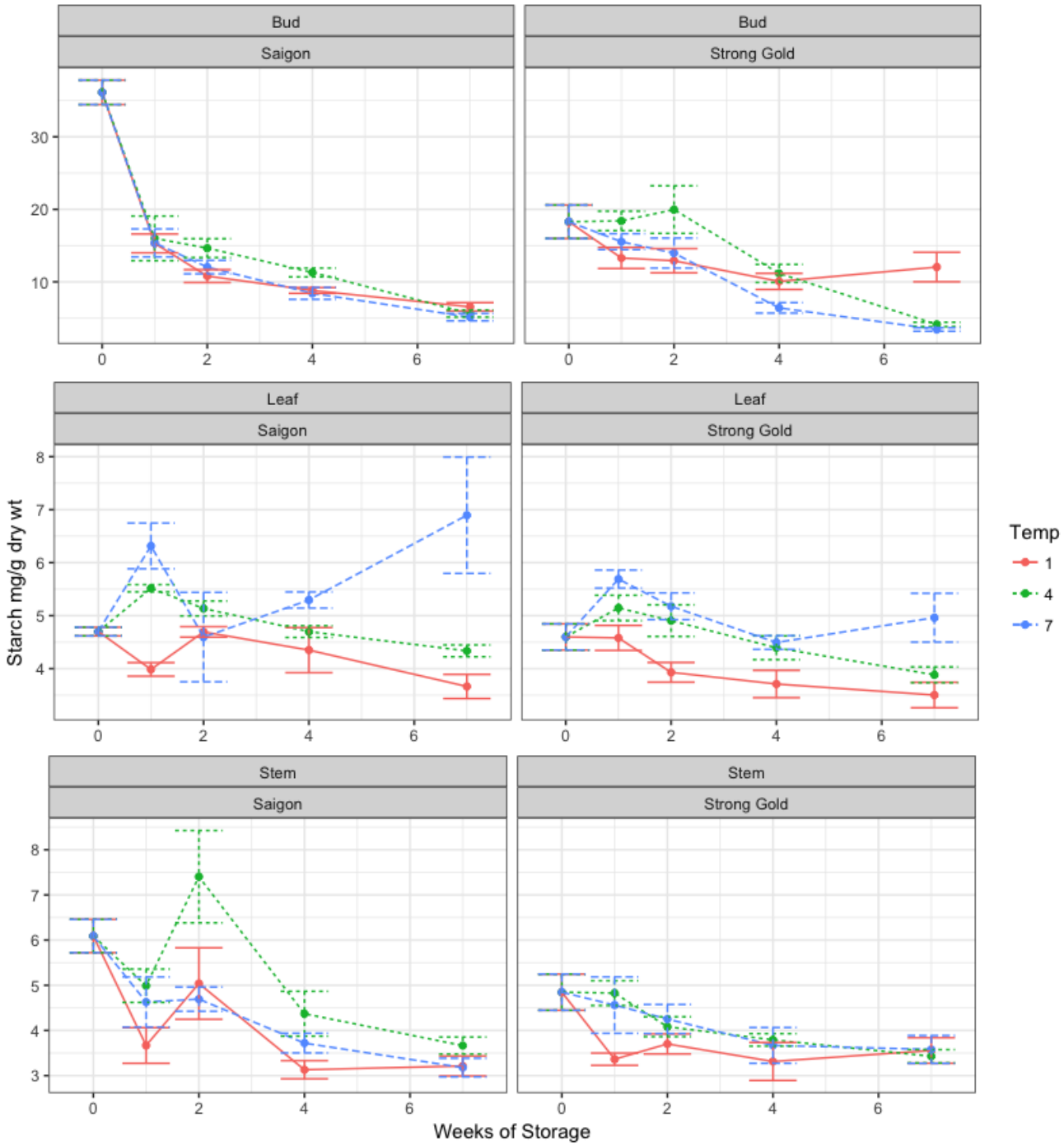


Figure 2.6. Effects of length of 1, 4 or 7ºC cold storage on starch level of tulips in Year 1. L to R: 'Saigon,' 'Strong Gold.' Top to Bottom: Bud, leaf, stem. Data are means of 6 replicates, ± SE.

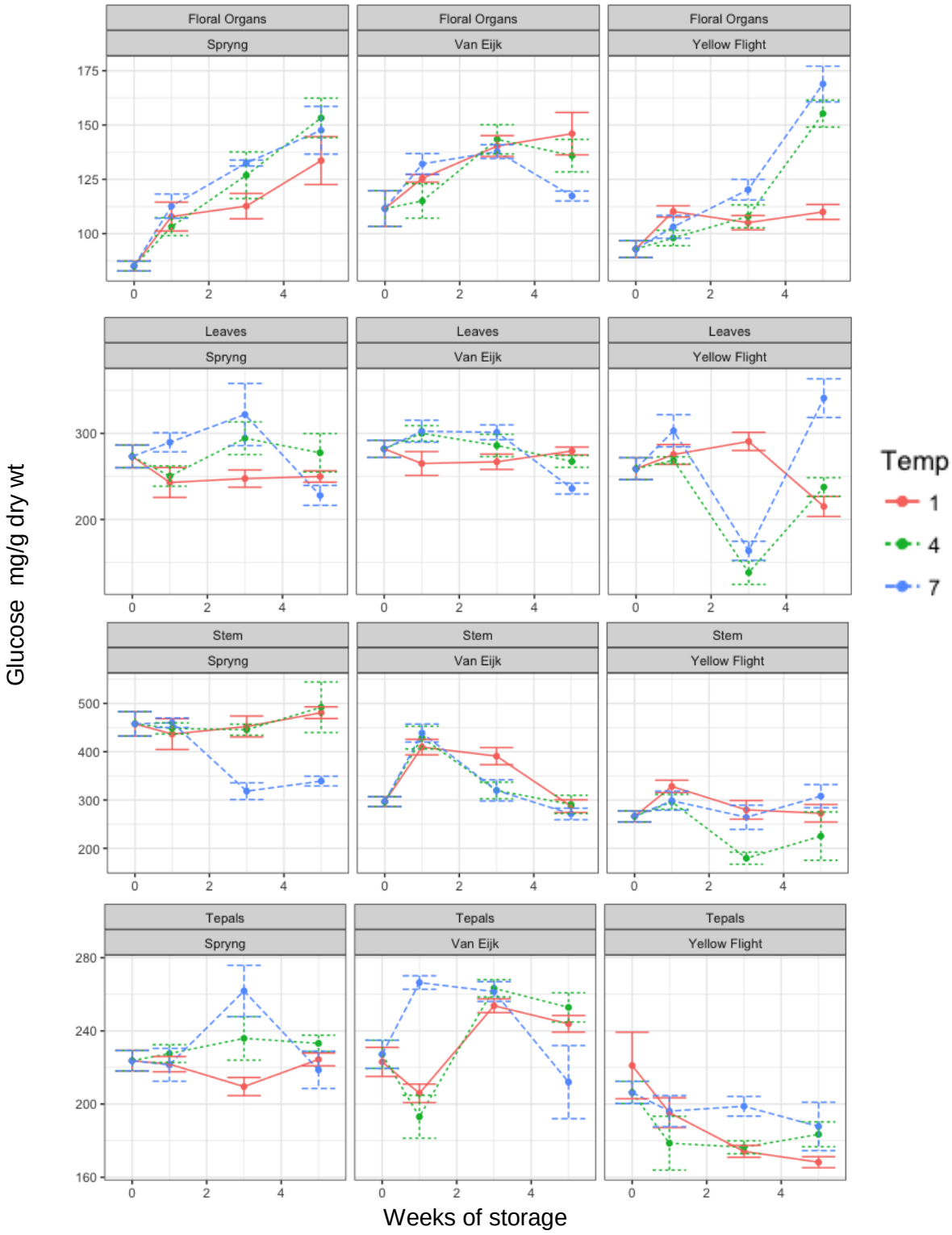


Figure 2.7. Effects of length of 1, 4 or 7ºC cold storage on glucose level of tulips in Year 2. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Top to Bottom: Floral organs, leaves, stem and tepals. Data are means of 6 replicates, ± SE.

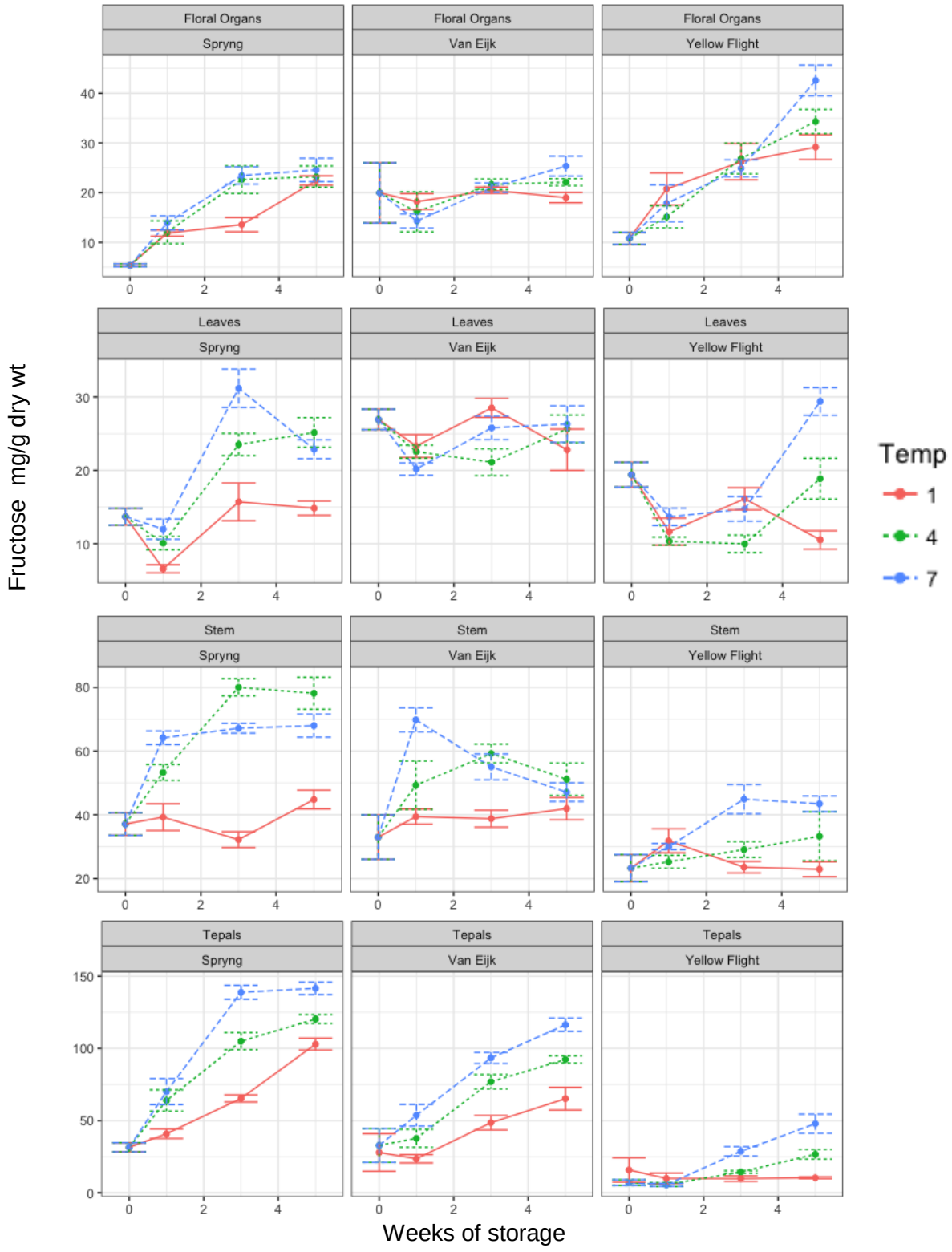


Figure 2.8. Effects of length of 1, 4 or 7ºC cold storage on fructose level of tulips in Year 2. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Top to Bottom: Floral organs, leaves, stem and tepals. Data are means of 6 replicates, ± SE.

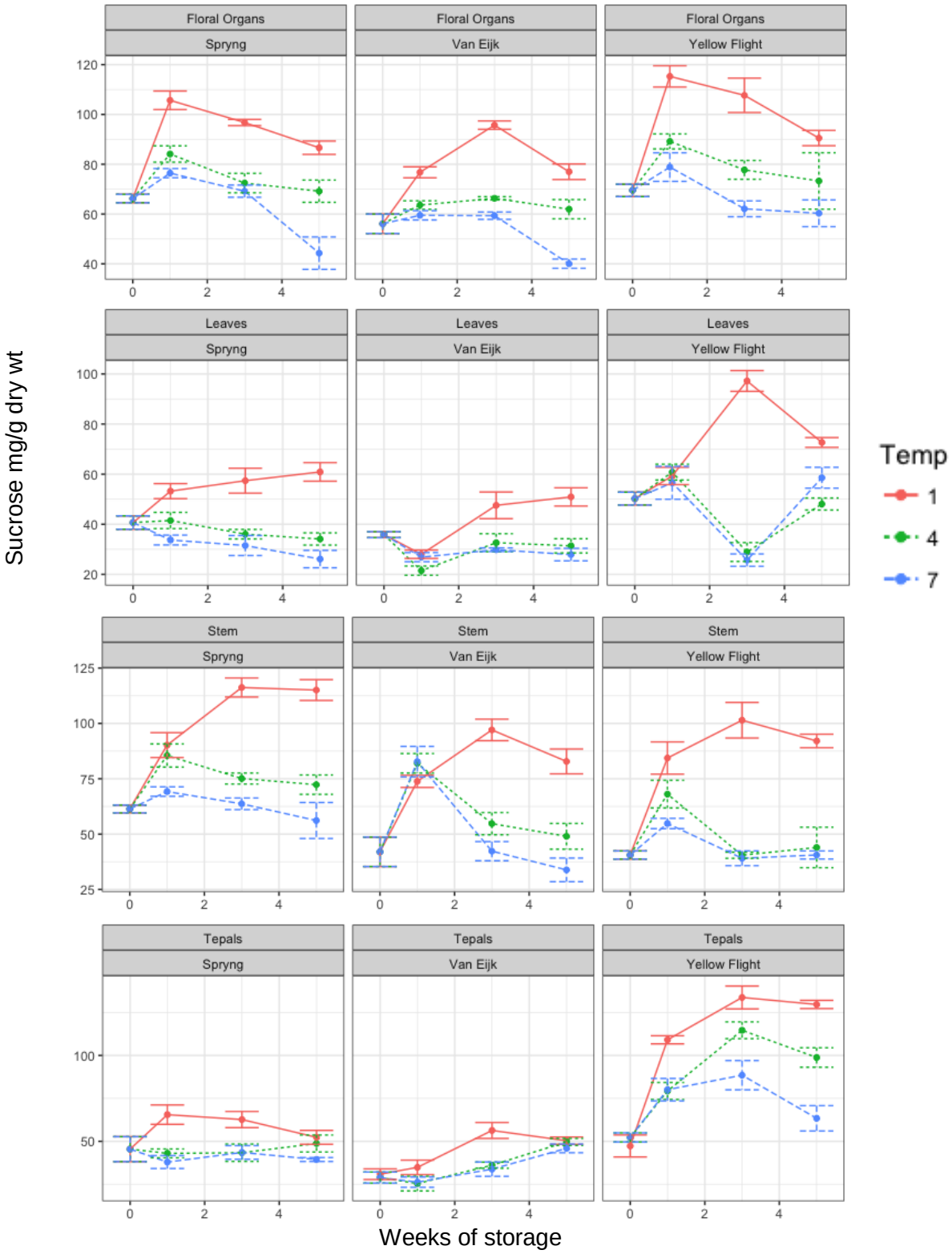


Figure 2.9. Effects of length of 1, 4 or 7ºC cold storage on sucrose level of tulips in Year 2. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Top to Bottom: Floral organs, leaves, stem and tepals. Data are means of 6 replicates, ± SE.

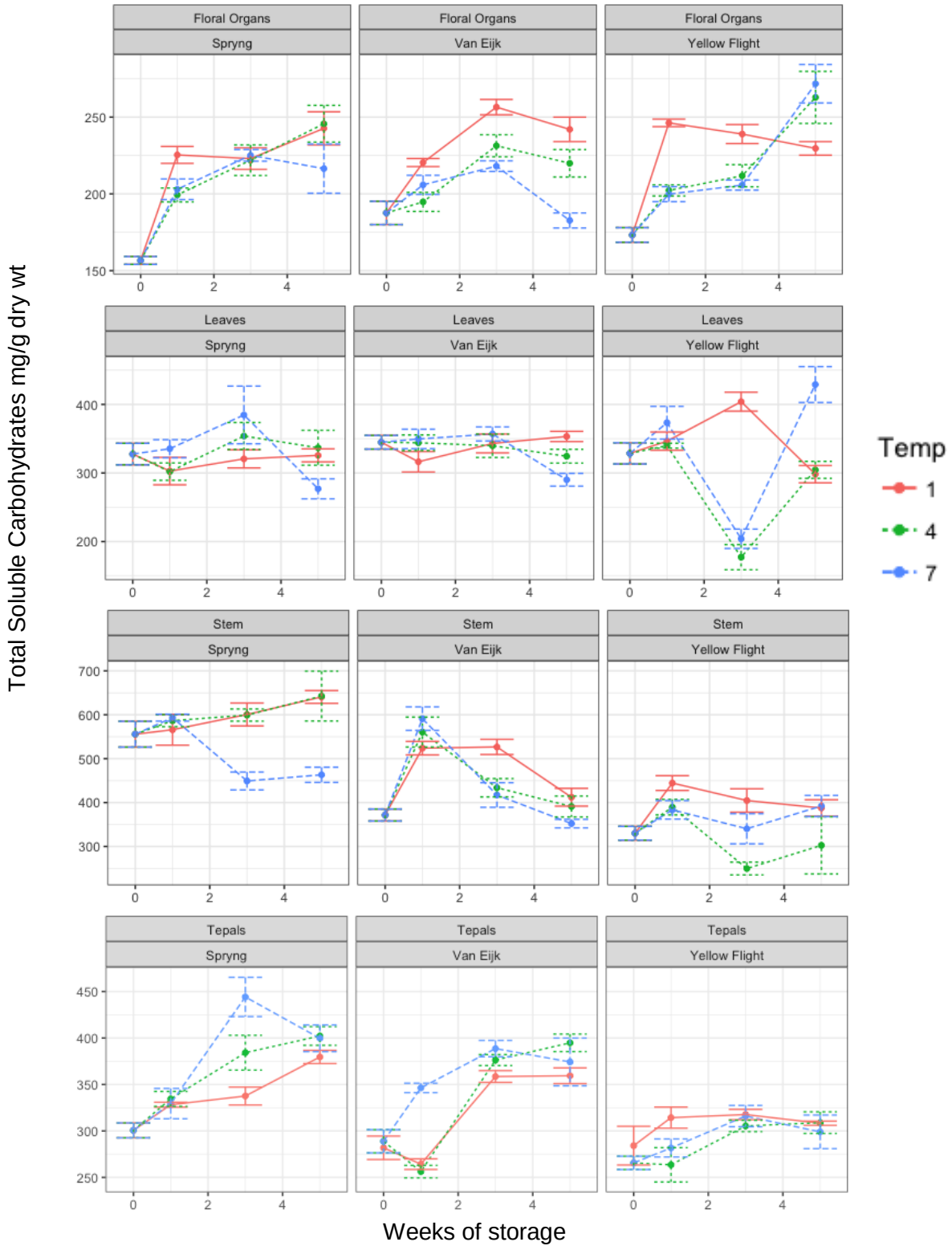


Figure 2.10. Effects of length of 1, 4 or 7ºC cold storage on total soluble carbohydrate (TSC) level of tulips in Year 2. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Top to Bottom: Floral organs, leaves, stem and tepals. Data are means of 6 replicates, ± SE.

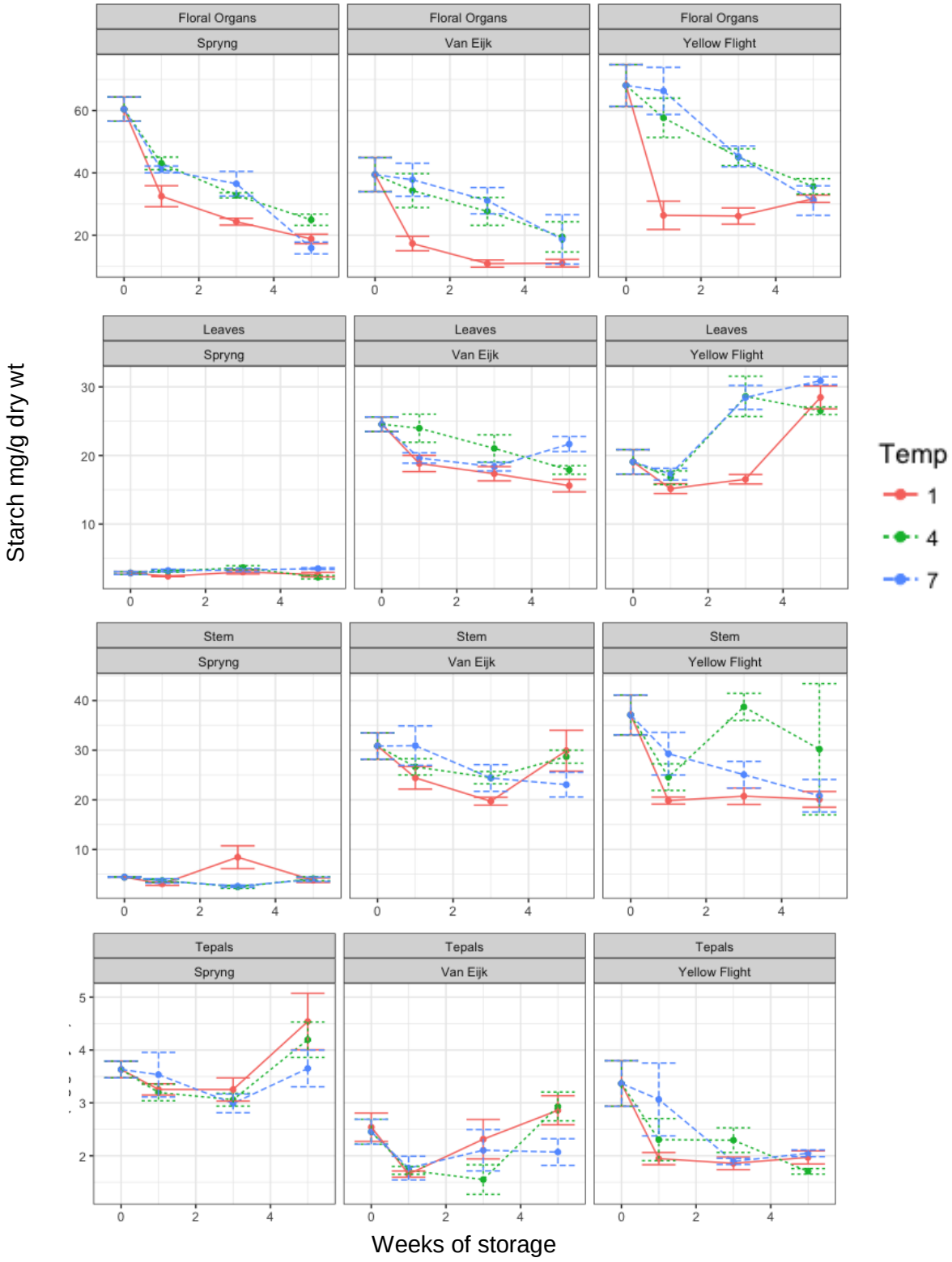
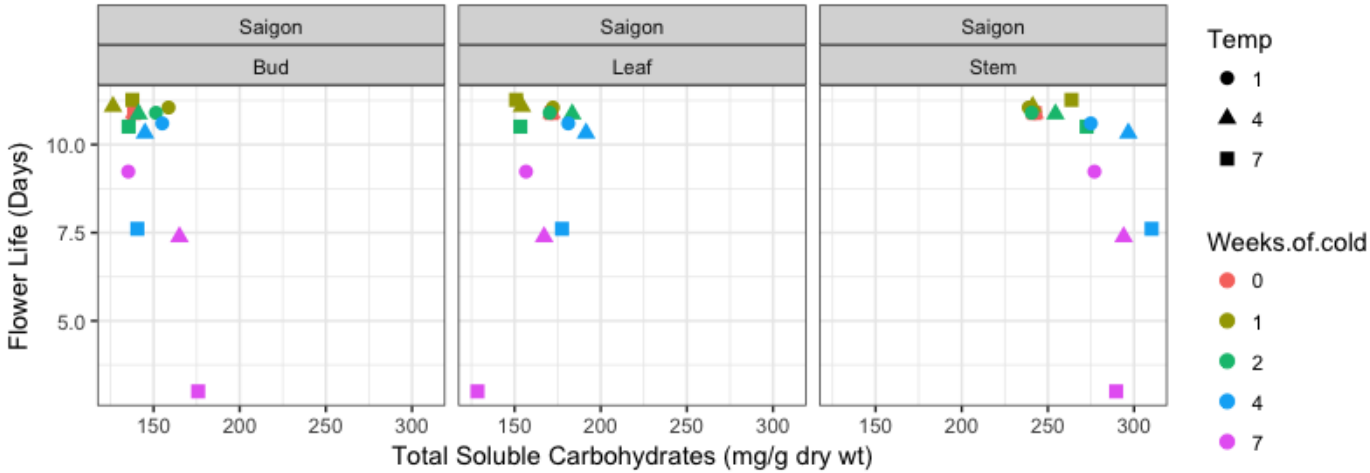
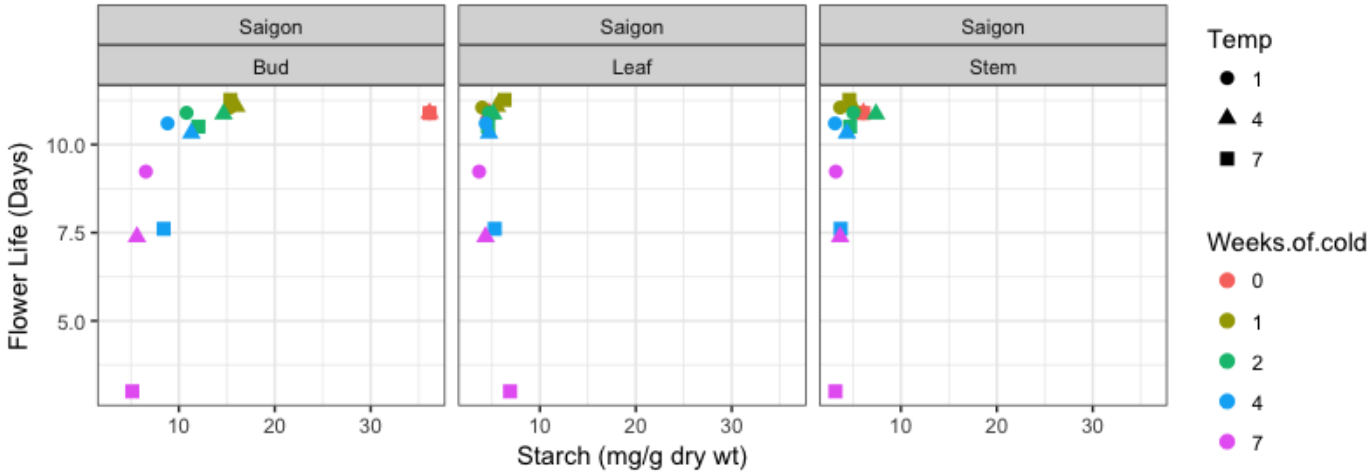


Figure 2.11. Effects of length of 1, 4 or 7ºC cold storage on starch level of tulips in Year 2. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Top to Bottom: Floral organs, leaves, stem and tepals. Data are means of 6 replicates, ± SE



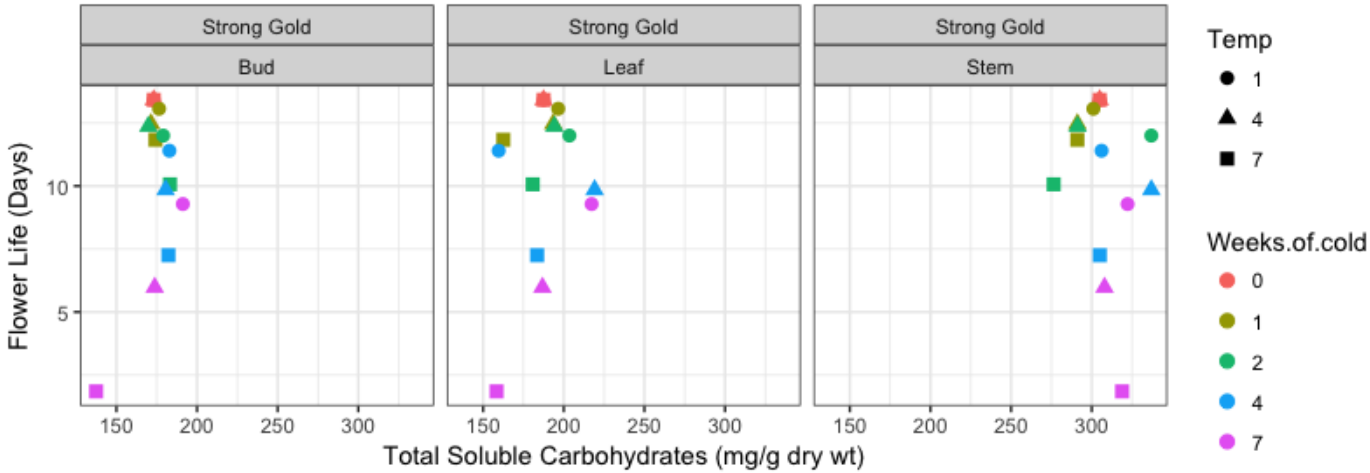
Tissue	Temperature (ºC)	TSC[y] (mg/g dry wt)	Weeks of Storage	TSC[y] (mg/g dry wt)
Bud	1	NS	1	NS
	4	***	2	NS
	7	***	4	NS
			7	***
Leaves	1	NS	1	NS
	4	NS	2	NS
	7	NS	4	NS
			7	NS
Stem	1	NS	1	NS
	4	NS	2	NS
	7	NS	4	NS
			7	NS

Figure 2.12. Relationship between initial total soluble carbohydrate (TSC[y]) level and flower life and the significance of this relationship in 'Saigon' plants in Year 1. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 2, 4 or 7 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively
[y]TSC = total soluble carbohydrates, the sum of sucrose, glucose and fructose



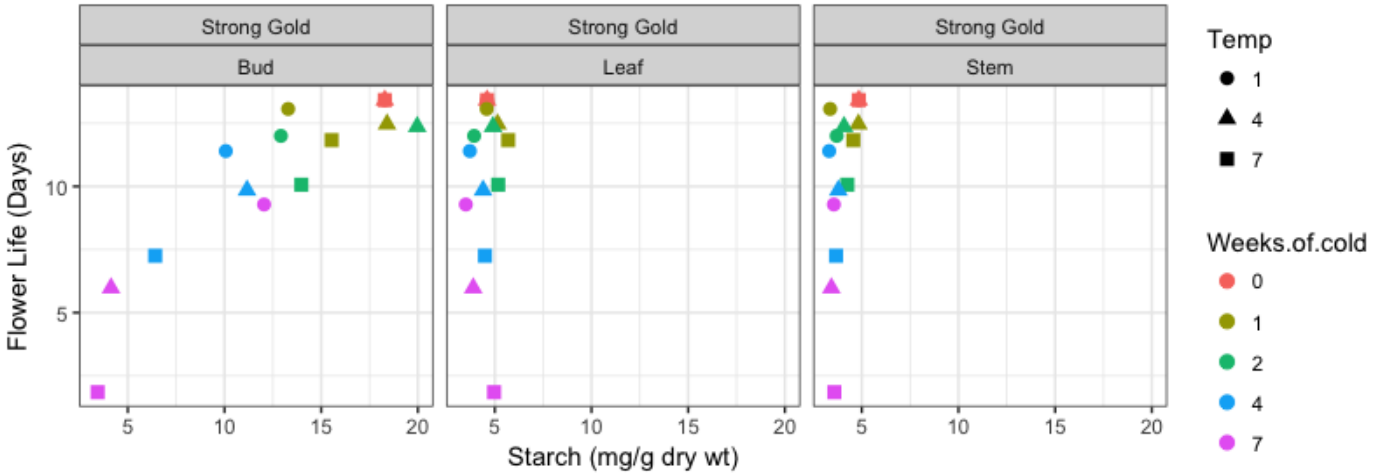
Tissue	Temperature (ºC)	Starch (mg/g dry wt)	Weeks of Storage	Starch (mg/g dry wt)
Bud	1	NS	1	NS
	4	***	2	NS
	7	**	4	NS
			7	NS
Leaves	1	NS	1	NS
	4	***	2	NS
	7	NS	4	*
			7	*
Stem	1	NS	1	NS
	4	NS	2	*
	7	**	4	NS
			7	NS

Figure 2.13. Relationship between initial starch level and flower life and the significance of this relationship in 'Saigon' plants in Year 1. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 2, 4 or 7 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively



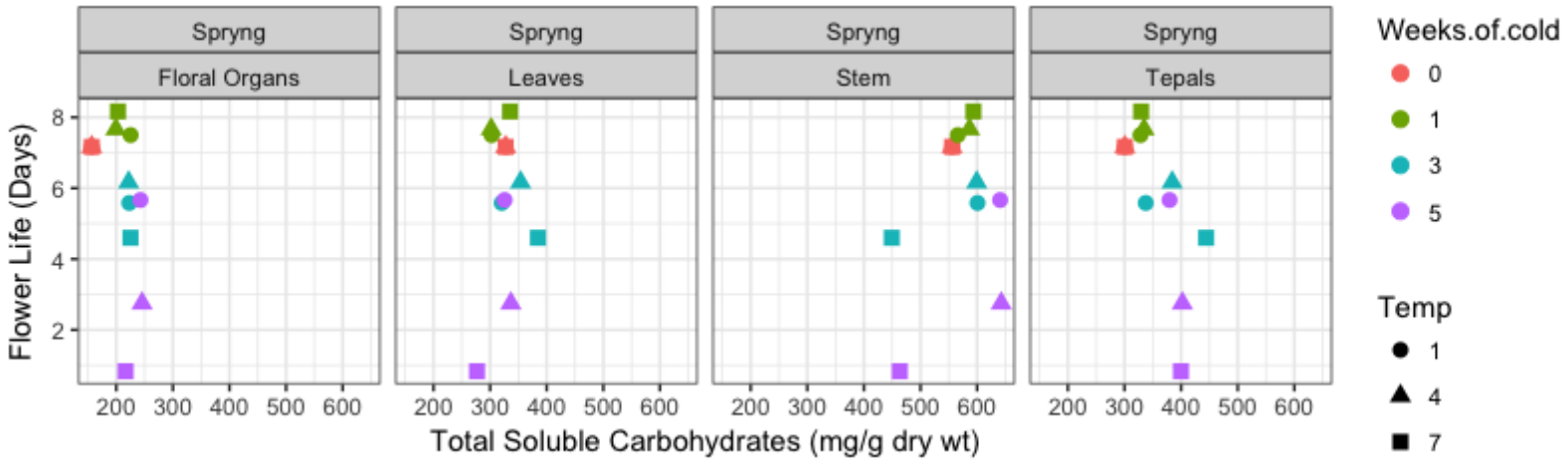
Tissue	Temperature (ºC)	TSC[y] (mg/g dry wt)	Weeks of Storage	TSC[y] (mg/g dry wt)
Bud	1	NS	1	NS
	4	NS	2	NS
	7	NS	4	NS
			7	**
Leaves	1	NS	1	**
	4	*	2	NS
	7	*	4	NS
			7	NS
Stem	1	NS	1	NS
	4	NS	2	NS
	7	NS	4	NS
			7	NS

Figure 2.14. Relationship between initial total soluble carbohydrate (TSC[y]) level and flower life and the significance of this relationship in 'Strong Gold' plants in Year 1. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 2, 4 or 7 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively
[y]TSC = total soluble carbohydrates, the sum of sucrose, glucose and fructose



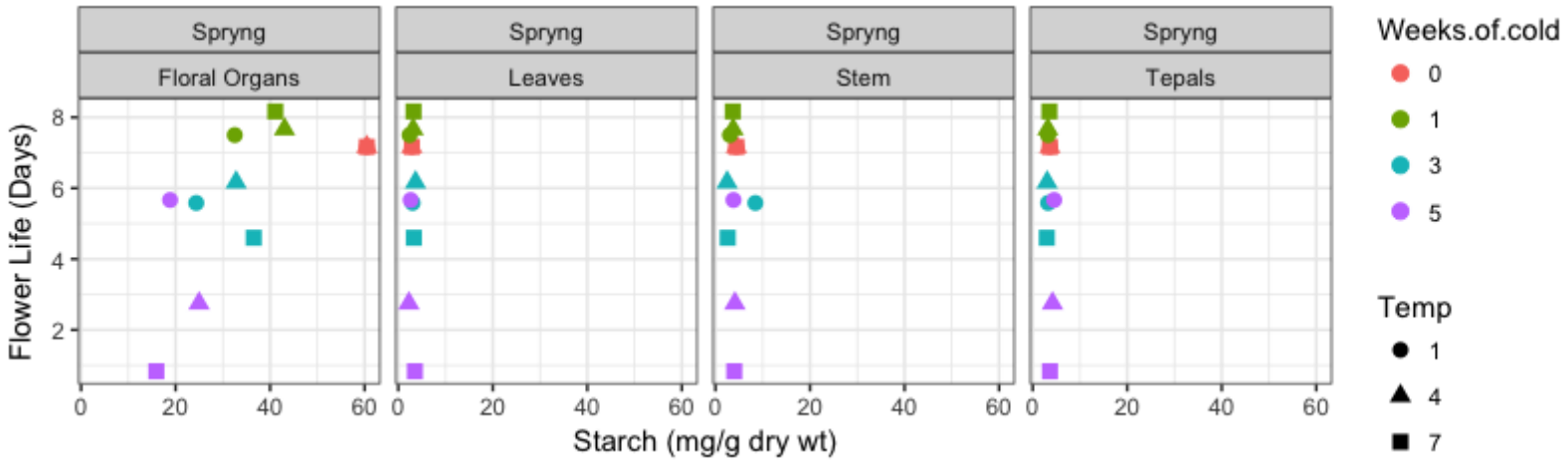
Tissue	Temperature (ºC)	Starch (mg/g dry wt)	Weeks of Storage	Starch (mg/g dry wt)
Bud	1	NS	1	NS
	4	***	2	NS
	7	***	4	**
			7	NS
Leaves	1	*	1	NS
	4	**	2	NS
	7	*	4	*
			7	NS
Stem	1	NS	1	NS
	4	***	2	NS
	7	NS	4	NS
			7	NS

Figure 2.15. Relationship between initial starch level and flower life and the significance of this relationship in 'Strong Gold' plants in Year 1. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 2, 4 or 7 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively



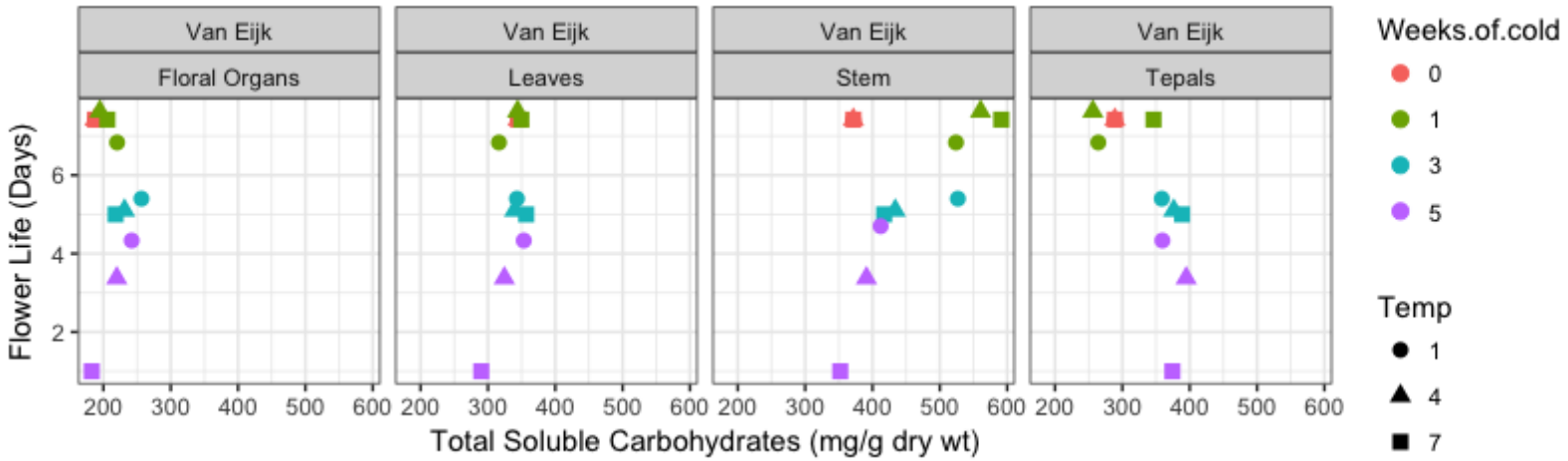
Tissue	Temperature (ºC)	TSC[y] (mg/g dry wt)	Weeks of Storage	TSC[y] (mg/g dry wt)
Floral	1	NS	1	NS
Organs	4	NS	3	NS
	7	NS	5	NS
Leaves	1	NS	1	NS
	4	NS	3	NS
	7	NS	5	NS
Stem	1	NS	1	NS
	4	NS	3	NS
	7	**	5	NS
Tepals	1	NS	1	NS
	4	***	3	NS
	7	NS	5	NS

Figure 2.16. Relationship between initial total soluble carbohydrate (TSC[y]) level and flower life and the significance of this relationship in 'Spryng' plants in Year 2. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 3 or 5 weeks).
NS, *, **, *** denotes nonsignificant or significant at P < 0.05, 0.01, or 0.001 respectively
[y]TSC = total soluble carbohydrates, the sum of sucrose, glucose and fructose



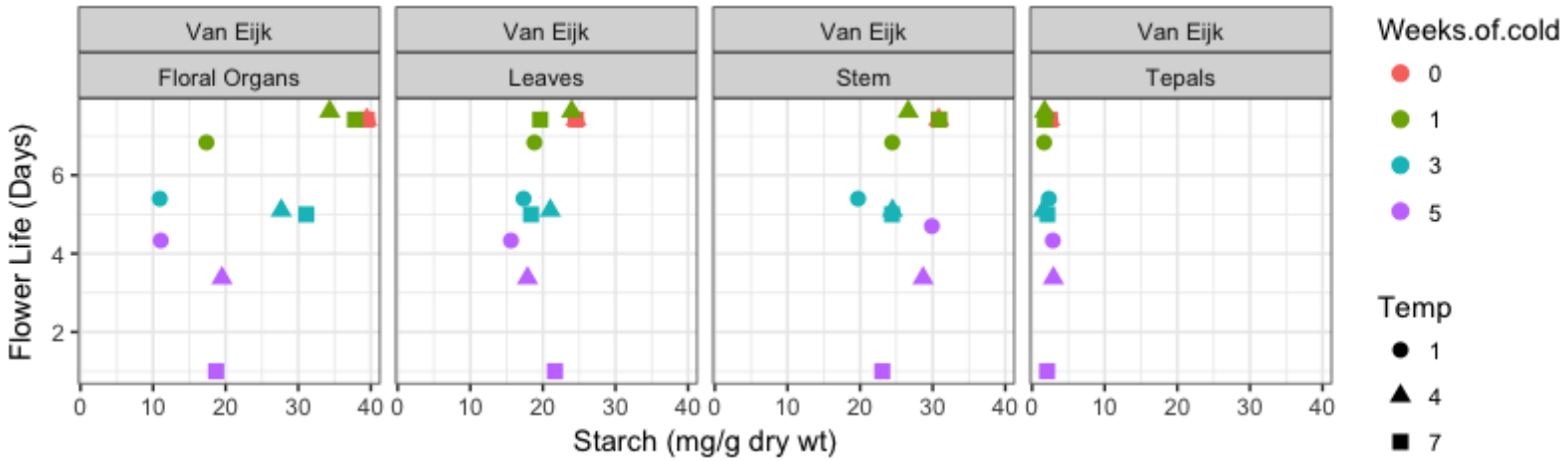
Tissue	Temperature (ºC)	Starch (mg/g dry wt)	Weeks of Storage	Starch (mg/g dry wt)
Floral	1	NS	1	NS
Organs	4	*	3	NS
	7	***	5	NS
Leaves	1	NS	1	NS
	4	*	3	NS
	7	NS	5	NS
Stem	1	NS	1	NS
	4	NS	3	NS
	7	NS	5	NS
Tepals	1	NS	1	NS
	4	**	3	NS
	7	NS	5	NS

Figure 2.17. Relationship between starch level and flower life and the significance of this relationship in 'Spryng' plants in Year 2. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 3 or 5 weeks).
NS, *, **, *** denotes nonsignificant or significant at P < 0.05, 0.01, or 0.001 respectively



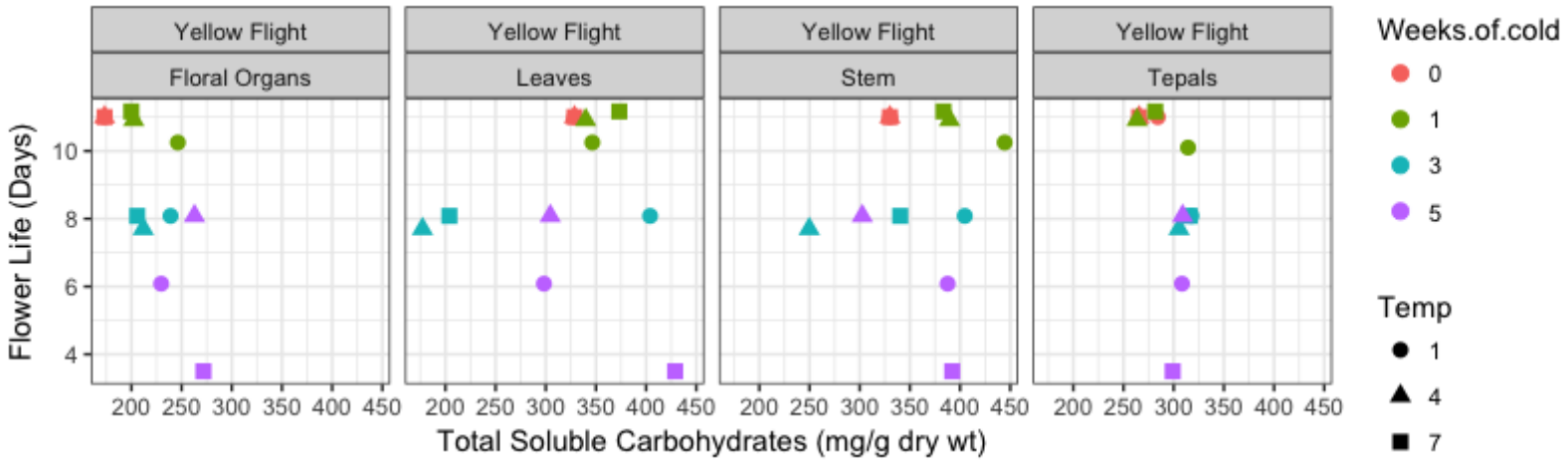
Tissue	Temperature (ºC)	TSC[y] (mg/g dry wt)	Weeks of Storage	TSC[y] (mg/g dry wt)
Floral	1	NS	1	NS
Organs	4	NS	3	NS
	7	NS	5	*
Leaves	1	**	1	NS
	4	NS	3	NS
	7	NS	5	NS
Stem	1	*	1	NS
	4	***	3	NS
	7	**	5	NS
Tepals	1	**	1	NS
	4	***	3	NS
	7	NS	5	NS

Figure 2.18. Relationship between initial total soluble carbohydrate (TSC[y]) level and flower life and the significance of this relationship in 'van Eijk' plants in Year 2. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 3 or 5 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively
[y]TSC = total soluble carbohydrates, the sum of sucrose, glucose and fructose



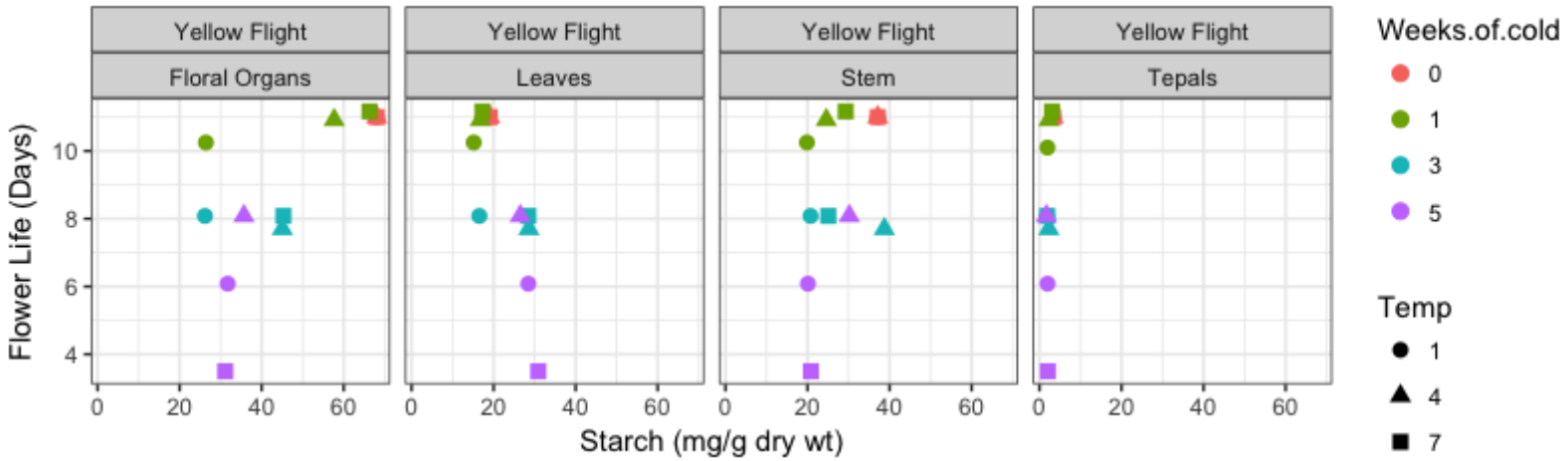
Tissue	Temperature (ºC)	Starch (mg/g dry wt)	Weeks of Storage	Starch (mg/g dry wt)
Floral	1	NS	1	NS
Organs	4	NS	3	NS
	7	*	5	NS
Leaves	1	NS	1	NS
	4	*	3	NS
	7	NS	5	NS
Stem	1	NS	1	NS
	4	NS	3	*
	7	NS	5	NS
Tepals	1	NS	1	NS
	4	NS	3	NS
	7	NS	5	NS

Figure 2.19. Relationship between initial starch level and flower life and the significance of this relationship in 'van Eijk' plants in Year 2. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 3 or 5 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively



Tissue	Temperature (ºC)	TSC[y] (mg/g dry wt)	Weeks of Storage	TSC[y] (mg/g dry wt)
Floral	1	*	1	NS
Organs	4	NS	3	NS
	7	***	5	*
Leaves	1	NS	1	NS
	4	NS	3	NS
	7	**	5	NS
Stem	1	NS	1	NS
	4	NS	3	NS
	7	NS	5	NS
Tepals	1	NS	1	NS
	4	NS	3	NS
	7	*	5	NS

Figure 2.20. Relationship between initial total soluble carbohydrate (TSC[y]) level and flower life and the significance of this relationship in 'Yellow Flight' plants in Year 2. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 3 or 5 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively
[y]TSC = total soluble carbohydrates, the sum of sucrose, glucose and fructose



Tissue	Temperature (ºC)	Starch (mg/g dry wt)	Weeks of Storage	Starch (mg/g dry wt)
Floral	1	NS	1	NS
Organs	4	NS	3	NS
	7	NS	5	*
Leaves	1	NS	1	NS
	4	NS	3	NS
	7	***	5	NS
Stem	1	NS	1	NS
	4	NS	3	NS
	7	NS	5	NS
Tepals	1	NS	1	NS
	4	NS	3	NS
	7	NS	5	NS

Figure 2.21. Relationship between initial starch level and flower life and the significance of this relationship in 'Yellow Flight' plants in Year 2. Different shapes and colors represent different postharvest storage treatments: shapes represent storage temperatures (1, 4 and 7ºC) and colors represent storage durations (0, 1, 3 or 5 weeks).
NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively

CHAPTER 3: CHARACTERIZING TULIP SENESCENCE AFTER POSTHARVEST STORAGE

Abstract

Flower appearance is one of the main factors determining the overall quality of potted tulips. Postharvest cold storage is used to maintain plant quality prior to marketing, but there are still questions about subsequent effects of this period of storage on flower life. The focus of this research was to understand how potted tulips respond once out of postharvest storage and how senescence proceeds from this point. At the bud color stage, forced, potted plants (cultivars 'Spryng,' 'van Eijk' and "Yellow Flight') were moved from the greenhouse at 17ºC and placed into dark storage at 1ºC for 1, 3 or 5 weeks. After cold storage, plants were moved into a postharvest evaluation room at 20ºC (12h of light at 8-12 µmol/m^{-2}/sec^{-1}) to assess flower life, changes in carbohydrate status and lipid peroxidation. Changes in total soluble carbohydrates (glucose, fructose, sucrose), starch, and malondialdehyde (MDA) an indicator of membrane integrity, were measured in the tepals. In 'Spryng,' plants stored for 3 weeks had longer flower life than those stored for 0, 1 or 5 weeks. 'van Eijk' plants stored for 3 weeks had the longest flower life. 'Yellow Flight' plants stored for 1 or 5 weeks had longer flower lives than those stored for 3 weeks. After 8 days out of storage, when most plants were deemed senesced, total soluble carbohydrate levels in tepals decreased. In all three cultivars, plants not held in storage had increasing levels of MDA as days in the growth chamber increased. Plants held at 5 weeks had the highest level of MDA at day 0 in the growth chamber. This could illustrate a subsequent effect of cold storage on plants that caused plants held for 1, 3 and 5 weeks to respond differently once out of storage, while plants not held in storage progressed normally through senescence in the growth chamber.

Introduction

Flower longevity is a critical factor that determines the marketable quality of potted ornamental plants. Due to the precise control of flower senesce due to physiological or environmental cues (Reid and Jiang, 2012), the postharvest environment plays a role in early senescence. The main focus of this experiment was to determine the subsequent effects, if any, of postharvest cold storage on tulip flower senescence. It aims to understand how potted tulips respond once out of postharvest cold storage and how senescence proceeds from this point. The main factors studied were changes in carbohydrate status and lipid peroxidation. The challenges experienced when measuring enzymatic activities of tulip petal senescence are also reported.

Flower senescence is often associated with a simple trigger such as pollination, ethylene production or photoperiod. Senescence of petal tissue is often initiated by pollination or lack of stigma receptivity. These events lead to hormone signaling (ethylene, ABA and cytokinins), redox signaling and nutrient remobilization to the developing ovary. In this event, lower levels of antioxidative enzymes have been observed leading to eventual oxidative damage (Roger and Munné-Bosch, 2016). Organ death is a result of nutrient remobilization and this oxidative damage. Despite these findings, the cascade of senescence events is still not completely understood and is highly variable depending on species (Reid and Jiang, 2012). In the case of ornamental geophytes, including tulips, the role of ethylene can probably be eliminated due to insensitivity (Woltering and Van Doorn, 1988; Sexton, 2000; Azad et al., 2008).

There are many changes associated with petal senescence observed in many ornamental species. Including changes in pigmentation, loss of cellular protein, ultrastructural changes, as well as changes in sugar levels and enzymatic activities.

Changes in Pigmentation

The onset of flower senescence is readily visible due to changes in color. The prominent pigments that contribute to flower color are anthocyanins, carotenoids and betalins. Anthocyanins are the largest and most diverse group and range from red to blue depending on pH (Rani and Singh, 2014). Changes in pigmentation can be attributed to a variety of causes including changes in environment, pH and biochemical changes (Yoshida et al, 1995; Azad et al., 2008). Exposure to high temperature and low light reduced pigment content in the petals of *Arabidopsis*, largely due to the downregulation of genes that encode for anthocyanin biosynthesis (Gonzalez, 2009). As 'Ile de France' tulip flowers began to wilt 12 days after opening. The petals began to lose pigment and by Day 14 petals began to detach from the receptacle (Azad et al., 2008).

Loss of Cellular Protein

Proteins are essential biochemical molecules involved in plant growth and development. These biomolecules are lost from the petals of ornamental geophytes during flower senescence. As lily flowers (*Lilim longiflorum)* progress through senescence, protease activity increased leading to protein degradation (Battelli et al., 2011). Application of protease inhibitors delays senescence (Van Doorn and Woltering, 2008). Similarly, degradation of proteins by proteasomes was upregulated in senescing 'Dutch Master' daffodil (*Narcissus psedonarcissus)* petals (Bieleski and Reid, 1992). In tulip tepals, protein concentration increased through development, but as soon as the flower opened in the greenhouse there was an observed 24% loss in protein concentration. By the end of senescence there was a further 18% loss (Collier, 1997).

Ultrastructural Changes and Membrane Stability

Changes in cellular structure and membrane stability are characteristic of the degradation and disassembly stage of flower senescence (Yoshida, 2003). Major events that occur include increase in vacuole size, organelle loss and collapse of the tonoplast surrounding the vacuole (Van Doorn and Woltering, 2004). Plugging of plasmodesmata is also an ultrastructural change during senescence of lily petals (Battelli et al., 2011).

Cell membranes are also a site of ultrastructural change during petal senescence. Increasing levels of free radical oxygen species (ROS) lead to degradation of fatty acids and increased membrane permeability. In petals, the main source of ROS production are likely the peroxisomes, mitochondria and apoplast (Roger and Munné-Bosch, 2016). Lipid peroxidation has been observed in senescing iris (*Iris versicolor)* 'Mme Florent Stepman' lilac (*Syringa vulgaris),* lily and carnation (*Dianthys caryophyllus)* tepals and petals where increased oxidative stress occurs (Jedrzejuk et al., 2016; Song et al., 2014; Shan and Zhao, 2015; Ahmad and Tahir, 2016).

Lipid peroxidation in plant tissues is determined by measuring malondialdehyde (MDA) levels using thiobarbituric acid- reactive-substances (TBARS). MDA is produced via autooxidation and degradation of fatty acids in cells. The secondary end product of this oxidation reacts with thiobarbituric acid (TBA) via an acid-catalyzed addition of electrons and yields a pinkish-red chromagen with a maximum absorbance at 532nm (Kappus, 1985).

Sugar Levels

The changes observed in sugar levels in senescing plant tissues are often associated with respiration rates. In developing day lily flowers, respiration increased on a whole-flower basis

(Lay-Yee at al., 1992). The maximum rate occurred when the flower was fully open. In flower tulips, a rapid increase in respiration per unit dry-mass was observed before flower opening and maximum respiration was observed at flower opening. As tepals progressed through senescence, there was a decrease in respiration (Collier, 1997).

In tulip tepals specifically, intra-cellular energy depletion is one of the main causes of programmed cell death. Increased levels of exogenous sugars increase the life of cut tulips, drawing a connection between the importance of carbohydrate status and delaying senescence. Supplemental sucrose maintained ATP synthesis and delayed DNA degradation as well as the translocation of degenerative biomolecules (Azad et al., 2008).

Enzymatic Activities

To deal with elevated levels of ROS, there is often an increase of ROS scavenging molecules in plant tissues. These include enzymes such as superoxide dismutase, catalase and ascorbate peroxidase. As plants progress towards senescence, these ROS scavenging enzymes play an important role in regulating the process.

Increased levels of antioxidative enzymes in the early stages of senescence delay ROS accumulation. However, due to the fact that petal senescence is generally a non-reversible process, the activity of these enzymes cannot keep up with ROS production and lead to eventual organ death. In gladiolus petals, decreased levels of ascorbate peroxidase are assumed to be a trigger for flower senescence, leading to increased levels of endogenous H_2O_2 levels (Hossain et al., 2006). In iris petals, ascorbate peroxidase and superoxide dismutase activity had decreased by the time the petals started to wilt, however, catalase activity increased (Bailly et al., 2001).

Superoxide dismutase plays a role in reducing superoxide ions to hydrogen peroxide, where they are then converted to H_2O molecules by peroxidase (Taiz and Zeiger, 2006). Other studies in tulip tepals measured the activity of ascorbate peroxidase, catalase and concentration of H_2O_2. The activity of these antioxidative enzymes were highest in tepals on day 9 of flower development, after the flower had opened and subsequently decreased after day 9 as the flower progressed through senescence (Azad et al., 2008). There were efforts made in the current study to measure the activity of superoxide dismutase due to its role in sequestering ROS, and presence in the petal tissues of other species such as carnation, lilac and 'Luoyang Red' tree peony (*Paeonia suffruticosa)* (Droillard and Paulin, 1990; Jedrzejuk et al., 2016; Shi et al., 2016). However, there were issues encountered, which will be explored later.

It is possible that changes in energy status and oxidative stress are associated with tulip flower senescence, and this effect could be exacerbated by the postharvest storage environment. Although the exact signal cascade has yet to be identified, there is observed antioxidative enzyme activity in tulip tepals and programmed cell death is triggered by intracellular energy depletion in tulips (Azad et al., 2008). By looking at carbohydrate status and lipid peroxidation in tepals, this experiment continues to explore the physiological changes associated with senescence in potted tulips.

The goal of this experiment was to characterize how potted tulips progress through senescence and determine whether postharvest cold storage affects this progression. The specific stage studied was when plants were at the marketable bud color stage after forcing and growing out in the greenhouse. The ultimate objective was to determine how a period of postharvest storage affects carbohydrate status, lipid peroxidation and ultimate marketability of potted tulips once they are out of storage.

Materials and Methods

Plant Material

Three cultivars were used: 'Spryng,' 'van Eijk,' and 'Yellow Flight.' Bulbs were stored at 20ºC, potted and placed in a cooler at 9ºC for 16 weeks. Plants were then moved into the greenhouse at 17ºC for forcing prior to experimentation.

At the bud color stage in the greenhouse, plants were divided into 5 groups for storage at 1ºC for 0, 1, 3 or 5 weeks. There were a total of 60 pots per cultivar, and 15 pots per treatment. In each treatment, 5 pots were used for carbohydrate analysis, 5 for lipid peroxidation and 5 for antioxidative enzyme analysis. There were 5 plants per pot. One flower was sampled per pot on each sampling day. Stored plants were moved out of the greenhouse and then placed in a box, which was closed to reduce excessive water loss. These boxes were placed in a dark cooler at 1ºC. After 1, 3 or 5 weeks, the plants were removed from the cooler and placed in the growth chamber at 20ºC with 12 hours of light (7-12 µmol/m^2/s) and 12 hours of dark for analysis. Plants were observed for senescence daily and collected as described below.

Postharvest Cold Storage

One temperature (1ºC) and three time durations (0, 1, 3 and 5 weeks) were used for cold storage in this experiment. Plants were taken out of storage and placed in the growth chamber at 20ºC with 12 hours of light (7-12 µmol/m^2/s) and 12 hours of dark. Carbohydrate status, lipid peroxidation and flower life was evaluated after postharvest cold storage after 0, 4, 8 and 12 days in the growth chamber. Attempts were made to study anti-oxidative enzymes, but were ultimately unsuccessful due to protocol issues. Tepals were used because they display the first sign of flower senescence (Rani and Singh, 2014).

Flower Life

One plant per pot was selected for flower life analysis in the growth chamber. Buds were observed daily and tagged as each opened and senesced. Final senescence was identified as the point when there was a significant color change in tepals, which was also accompanied by petal curling and signs of wilting. Plants were watered as needed.

Carbohydrate Analysis

Samples were taken after 0, 4, 8 and 12 days in the growth chamber. Fresh weight was taken and samples frozen at -80ºC until freeze drying. Dry weight was taken after freeze-drying. Freeze dried tissue was ground using a Wiley Mill.

To extract carbohydrates, 3 mL of 80% ethanol was added to 50 mg of ground sample. 100 µL of lactose (4 mg/mL) was added as an internal standard and the sample incubated at 70ºC. After 5 minutes, the samples were vortexed and placed back in incubation at 70ºC for 25 min. The samples were then removed and centrifuged at 4,000 RPM for 10 min. The supernatant was poured into a clean test tube. 3 mL of 80% ethanol was added to the remaining sample, and incubated at 70ºC for 30 min and centrifuged as above. The supernatants were combined and the sequence repeated one more time. The 9 mL of supernatant was poured onto an ion-exchange column with a 1 mL layer of cation exchange resin (Dowex 50W, hydrogen form) and a 1 mL layer of anion exchange resin (Amberlite IRA-67, acetate form) to remove amino acids, organic acids and the like that may interfere with analysis. After the supernatant had flowed through, 3 mL of 80% ethanol was added and drained into the sample. The final samples were held at 4 ºC in 10 mL glass test tubes until drying on a Buchler Rotary Evapo-Mix using a vacuum. The

samples in the 18 mL glass test tubes were placed on the machine at 50ºC for approximately 45 min, or until all the ethanol had evaporated. Dried samples were held at -20ºC until analysis.

After extraction, carbohydrates were dissolved in HPLC grade water and subjected to ion chromatography. A Dionex CarboPac PA1 column (035391, Thermo Fisher, Waltham, MA) and guard (043096, Thermo Fisher, Waltham, MA) delivering 25 µL/injection and a 1 mL/min flow rate. Carbohydrates were measured as anions in NaOH (200 mM isocratic) using pulsed electrochemical detection.

The remaining insoluble debris after carbohydrate extraction was air dried and used for starch analysis. 4 mL of Na-acetate buffer (100mM, pH 4.5) was added to the debris. The tubes were vortexed and placed in a boiling water bath for 30 minutes. After cooling, 1 mL of amyloglucosidase (A7255, Sigma; 50 units of enzyme per mL). This enzyme cleaves starch into glucose. The starch digest tubes were then vortexed and incubated for 2 days at 50ºC.

After incubation, samples were centrifuged for 10 minutes at 4,000 RPM and appropriate aliquots added to tubes. A glucose oxidase, peroxidase and *o*-dianisidine solution was made using 5 units of glucose oxidase (G61255, Sigma) 1 unit of peroxidase (P8125, Sigma) and 0.04 mg of *o*-dianisidine (D3252, Sigma) per 5 mL of this solution, which was then added to each tube. Samples were then incubated for 30 minutes at 30ºC. After incubation, 1 mL of HCl (1M) was added to each tube to stabilize the color.

Absorbance was determined at 450 nm to determine glucose concentration. A calibration curve was created using a set of glucose standards (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 µmol). From final absorbance, mg starch/g dry weight was calculated.

Lipid Peroxidation Products

Malondialdehyde (MDA) was used to indicate lipid peroxidation using the protocol established by Heath and Packer (1968). MDA in the supernatant was determined as thiobarbituric acid-reactive substances (TBA). Frozen tissue (1 g fresh weight) was ground using liquid nitrogen and 10 mL of 80% ethanol added. Samples were placed in a water bath at approximately 23ºC for 30 minutes. Samples were then centrifuged at 4,000 RPM for 10 min. At this point two tubes were prepared per sample and designated "TBA-" (solution lacking TBA) or "TBA+" (solution that contained TBA). 400 µL of sample was added to the "TBA-" solution containing only 3.6 mL 20% TCA and to the "TBA+" solution containing 2 mL of 0.5% TBA in 20% TCA. Samples were then incubated for 25 minutes at 95ºC and centrifuged at 4,000 RPM for 1 minute to clarify the solution. Samples were read at 532 nm and 600 nm. Due to interference of pigments (largely anthocyanins) in the tepals, modifications were made using the protocol established by Hodges et al. (1999). The absorbance of the "TBA- solution" was subtracted from the absorbance of the "TBA+ solution" of the same sample in order to correct for compounds other than MDA that absorb at 532 nm. After this difference was calculated, the absorbance at 600nm was subtracted from the absorbance at 532 nm for each sample to account for nonspecific turbidity. The results were expressed as nmol of MDA g^{-1} of fresh weight.

Attempted Extraction and Assay of Anti-Oxidative Enzymes

One gram fresh weight of petal tissue was taken for each sample and frozen at -80ºC until use. Extractions were carried out on ice as described by Ranwala and Miller (2000). One gram fresh weight tissue was ground using liquid nitrogen and 10 mL/g of 50 mM K-phosphate buffer (pH 7.5) containing 1% insoluble PVPP was added. Samples were then stirred, and homogenate

was filtered through Miracloth. This filtrate was centrifuged and the supernatant desalted with a PD-10 column equilibrated with 50 mM K-phosphate buffer (pH 7.5).

The desalted supernatant was used for superoxide dismutase (SOD) analysis. The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the protocol established by Dhindsa (1981). A 3mL reaction mixture was made that contained 50 mM K-phosphate buffer (pH 7.0), 13 mM methionine. 0.1 mM EDTA, 75 of NBT, 20 µM riboflavin and 0 to 200 µL of enzyme extract. 100 µL of riboflavin (20 µM) was added last as the tubes were placed 30 cm below an 18W fluorescent lamp. The reaction began when the light was turned on and was allowed to run for 10 minutes. Absorbance was then read at 560 nm.

It is necessary to have a substrate of the SOD-catalyzed reaction. Therefore the reaction mixture included a superoxide anion generator in order to measure the activity of SOD. In this protocol, riboflavin generates the superoxide anion radicals in the presence of methionine and light. Riboflavin is excited by light to a triplet stage, where it then activates oxygen and methionine by energy transfer. Singlet oxygen reacts with methionine to produce a number of toxic materials (Lee et al., 2006). Methionine contains a sulfur atom with 4 non-binding electrons that reacts with electrophilic singlet oxygen. This reaction can result in denaturation and aggregation of the protein (Min and Boff, 2002). NBT can be reduced by the anions produced in this reaction and lead to the production of formazan, a chromogen, which is then measured at 560 nm. The activity of SOD in samples of unknown activity was then determined according to the extent to which the superoxide anion was destroyed and the production of formazan was decreased.

Because SOD inhibits the oxidation of a substrate with the superoxide anion, it was expected that with more SOD there would be less reduction of NBT and less color change, or production of formazan. In order to have a completely linear enzymatic reaction in which the only limiting factor is the enzyme itself, it was established that 20µM of riboflavin (100 µL) and 75µM of NBT (in a 3 mL reaction mixture) was appropriate. Unexpected reactions occurred when experimenting with this amount of enzyme extract, and comparing boiled versus living samples. The highest absorbance was observed in the blank samples (no enzyme extract) which was to be expected. However, a similar reaction was expected when samples were boiled, which did not result.

Samples were boiled for 30 minutes and 0 to 200 µL added to the reaction mixture. This boiling was expected to denature superoxide dismutase and any NBT inhibiting substances. The absorbance of the boiled samples was less than that of the living, demonstrating less inhibition, but regardless, it still appeared that a reaction was occurring (Figure 3.1).

It is still not certain why this reaction was occurring, but it is speculated that substrates, as well as characteristics of SOD and tulip tepals themselves all play a role. First, the methionine-riboflavin mixture has been identified as strong microbicidal complex that decreased the enzymatic activities of catalase, peroxidase and SOD (Lee et. al, 2006). If the activity of the enzyme itself is being decreased by substrates, this could be a serious contributing factor to the unsuccessful assay in tulip tepals. Another compounding factor is that SOD levels may be generally low in tulip tepals. Previous studies have focused on the activity of catalase and ascorbate peroxidase in tepals (Azad et al., 2009), but not SOD.

The results observed in living samples could be due to the inhibiting effect of something other than SOD, or in conjunction with SOD. Tulip tepals are heavily pigmented. This was an

issue while assaying MDA in tulip tepals, but that was overcome using the protocol established by Hodges et al. (1999). Pigment molecules as well as α-tocopherol and β-carotene play a role in detoxifying ROS (Lee et al., 2006). It is possible these molecules are present in tulip tepals and are one of the reasons the inhibition of NBT was observed in living samples. These molecules could have endured boiling, or these molecules could have been denatured during boiling. In that case, the difference observed between the blanks and boiled samples is merely due to the different extract to reaction mixture volumes. Less reaction mixture led to greater absorbance, and this absorbance decreased as volume of enzyme extract increased.

Due to time constraints, further work on antioxidative enzymes was not explored any further. However, further research should be done to understand the role, if any, of SOD in tulip tepals and how to appropriately measure this antioxidative enzyme.

Statistical Analysis

A completely randomized design was used. Regression assumptions were checked and transformations were made to the data if necessary. Means were compared by one-way analysis of variance using R (Version 1.0.153).

Results

Flower life

In 'Spryng,' plants held for 3 weeks had the longest flower life (Table 3.1). 'Yellow Flight' plants held for 1 and 5 weeks both had the longest flower life (Table 3.1). However, the practical significance of these differences is minimal. The largest difference was between 'Yellow Flight' plants held for 3 weeks and those held for 1 or 5 weeks, and was only 1.5 days.

Carbohydrate Status

When examining the initial effect of cold storage on carbohydrates (Day 0), increasing duration of cold storage reduced glucose levels in the tepals of most cultivars (Figure 3.2) and increased fructose levels (Figure 3.3). There was an inconsistent change in tepal sucrose levels, but plants stored for 1 or 3 weeks showed the highest amounts (Figure 3.4). There was little to no consistent effect of storage duration on total soluble carbohydrate levels (Figure 3.5). Starch levels were low in the tepals of all three cultivars (Figure 3.6). Starch levels in the tepals of 'Spryng' showed little change as storage length increased, while they increased in 'van Eijk' and decreased in 'Yellow Flight.'

During postharvest senescence, or the period after Day 0 in the growth chamber, there were changes associated with almost all carbohydrate levels as duration of storage and days in the growth chamber increased. Glucose levels in the tepals tended to decrease as days in the growth chamber increased in all three cultivars (Figure 3.2). Non-stored plants had less change in glucose levels, while plants held for 5 weeks showed a rapid decrease in glucose levels from Day 4 to 12.

Fructose levels increased as senescence progressed (Figure 3.3). This trend was observed in all cultivars and for almost all storage durations. By Day 12 there were decreases in fructose levels in 'Spryng,' and 'van Eijk' tepals in plants stored for 3 or 5 weeks, and well as 'Yellow Flight' plants held for 5 weeks.

Sucrose levels in the tepals tended to decrease during postharvest senescence (Figure 3.4). In 'Yellow Flight,' plants held for 1 week in storage consistently had the highest level of

sucrose in the tepals. In most cultivars as days in the growth chamber increased, plants stored for 5 weeks consistently had the lowest level of sucrose in the tepals.

The summation of these three sugars is represented by total soluble carbohydrates (TSC). In all three cultivars, storage duration and days in the growth chamber all affected the level as plants progressed through senescence (Figure 3.5). In general, TSC increased in the tepals of non-stored plants. 'Spryng' and 'Yellow Flight' plants stored for 5 weeks had decreasing levels of TSC during senescence whereas with 'van Eijk,' TSC increased (Figure 3.5). Depending on cultivar, plants stored for 1 or 3 weeks had slight increases, decreases, or no change.

Starch levels were very low in the tepals of all three cultivars as plants progressed through senescence in the growth chamber (Figure 3.6). There was little change associated with 'Spryng' and 'van Eijk' plants. Starch levels in 'Yellow Flight' tepals were high in plants stored for 0 or 1 week, but this level decreased by day 4 in the growth chamber.

Lipid peroxidation

Malondialdehyde (MDA) levels as measured at the end of cold storage, increased as duration of cold storage increased (Figure 3.7). Plants held in storage for 5 weeks had the highest level in all three cultivars.

As plants progressed through senescence between days 4-12, MDA levels increased linearly or quadratically in non-stored plants in all three cultivars (Figure 3.7). Conversely, MDA in plants stored for 5 weeks generally decreased as time in the growth chamber increased in all three cultivars, while plants held for 1 or 3 weeks showed different and inconsistent changes.

Discussion

The relationship between carbohydrate status and senescence appears to vary among cultivar, but during postharvest storage the levels of these carbohydrates changed. ‘Spryng’ and ‘Yellow Flight’ plants held in storage for 5 weeks had a lower concentration of total soluble carbohydrates. In both of these cultivars, plants held for 0 weeks in storage also increased to the highest level of total soluble carbohydrates after Day 4 in the growth chamber. This could be attributed to increases in fructose and glucose in the tepals after this point. It is possible that a period of storage at 1ºC slows respiration rates relative to the rates of non-stored plants. In tulip tepals, respiration rates were highest before flower opening and decreased as tepals senesce (Collier et al., 1997). Plants that were not exposed to this period of cool temperatures continue to progress naturally through senescence, and thus we see this increased period of respiration before opening. It is possible that there may have been subsequent effects of cold storage on tulip tepals that led to slower metabolism and accumulation of sugars in the tepals.

In this study, there is a relationship between tulip tepals and energy demand, depletion, and senescence. In many plant tissues, sugar levels are correlated with respiration rates. This is thought to be because of substrate availability to continue to maintain energy sources (Azcón-Bieto et al., 1983). In all three cultivars after Day 8, there were decreases in total soluble carbohydrate levels. Day 8 was the point where ‘Spryng’ and ‘van Eijk’ plants were deemed senesced. ‘Yellow Flight’ plants were deemed senesced only approximately one day after. Tepals acting as a strong sink could have possible led to the initially high levels of carbohydrates at this point. However, as respiration increased there was relatively poor recovery of carbohydrates and amino acids in senescing tulip tepals (Collier, 1997). As substrates for producing energy sources

are depleted and not replaced, ATP and other energy sources also decrease. Azad et al. (2008) observed that it was this decrease in energy sources that led to rapid programmed cell death and senescence in tulip tepals.

In regards to MDA and oxidative stress, there was an effect of duration of cold storage on MDA levels. In all three cultivars, plants held for 0 weeks in storage had increasing levels of MDA as days in the growth chamber increased and plants progressed through senescence. In all cultivars, plants held in cold storage for 5 weeks initially had the highest level of MDA at 0 days in the growth chamber. This could once again point at a subsequent effect of cold storage on plants that caused plants held for 1, 3 and 5 weeks to respond differently once out of storage, while plants not held in storage progressed naturally through senescence.

The observed higher levels of MDA in plants held in storage for 5 weeks could be also be associated with a response to chilling. This sort of response was observed in soybean leaves of cold acclimated and non-acclimated plants, where both plants had increased levels of MDA during a chilling period. However, when recovering, cold acclimated plants had lower levels of MDA than non-acclimated plants (Yadegari et al., 2007). As senescence proceeded in the growth chamber, levels of MDA generally decreased in the tepals of these plants. It is possible that longer cold storage provides some sort of "acclimation" for plants, and causes an upregulation in cold acclimation proteins provide stability to cell membranes (Chen et al., 1991). The increases observed in fructose may also be associated with this response as cryoprotection has been suggested as role of fructans (Pontis, 1989). However, more research needs to be done on this response in tulips, and tepals specifically.

Conclusion

Senescence is a complex cascade of events in tulip teals. It is associated with energy depletion, changes in sugar status and oxidative stress (Azad et al., 2008). In the current study, postharvest storage caused changes in carbohydrate status and lipid peroxidation in the tepals of 'Spryng,' 'van Eijk,' and 'Yellow Flight.' These responses could be associated with a chilling response, but further research should be done to explore this possibility. Although these changes reflect different physiological responses among treatments, there were minimal effects on flower life.

References

Azad, A.K, Ishikawa, T., Sawa, Y. and H. Shibata. 2008. Intracellular energy depletion triggers programmed cell death during petal senescence in tulip. Journal of Experimental Botany. 59: 2085-2095.

Ahmad, S.S. and I. Tahir. 2016. Increased oxidative stress, lipid peroxidation and protein degradation trigger senescence in *Iris versicolor* L. flowers. Physiology and Molecular Biology of Plants. 22(4): 507-514.

Azcón-Bieto, J., Lambers, H. and D.A. Day. 1983. Effect of photosynthesis and carbohydrate status on respiratory rates and the involvement of the alternative pathway in leaf respiration. Plant Physiology. 72: 598-603.

Bailly, C., Corbineau, F. and W.G. Van Doorn. 2001. Free radical scavenging and senescence in *Iris* tepals. Plant Physiology and Biochemistry. 39: 649-656.

Batelli, R., Lombardi, L., Rogers, H.L., Picciarelli, P., Lorenzi, R. and N. Ceccarelli. 2011. Changes in ultrastructure, protease and caspase-like activities during flower senescence in *Lilium longiflorum.* Plant Science. 180: 716-725.

Bieleski, R.L. and M.S. Reid. 1992. Physiological changes accompanying senescence in ephemeral daylily flower. Plant Physiology. 98: 1042-1049.

Chen, Y.M., Liu, H.F and C.Y. Lin. 1991. Chilling stress effect on the growth: Mitochondrial activity and protein synthesis in etiolated mungbean seedlings. Taiwania. 36: 277-290.

Collier, D.E. 1997. Changes in respiration, protein and carbohydrates of tulip tepals and *Alstromeria* petals during development. Journal of Plant Physiology.150: 446-451.

Dhindsa, R., Plumb-Dhindsa, P. and T. Thorpe. 1981. Membrane permeability and lipid

peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental Botany. 32(126): 93-101.

Droillard, M.J. and A. Paulin. 1990. Isozymes of superoxide dismutase in mitochondria and peroxisomes isolated from petals of carnation (*Dianthus caryophyllus)* during senescence. Plant Physiology. 94: 1187-1192.

Ferrante, A., Trivellini, A., Scuderi, D., and D. Romano. 2014. Post-production physiology and handling of ornamental potted plants. Postharvest Biology and Technology. 100: 99-108.

Gonzalez, A. 2009. Pigment loss in response to the environment: A new role for the WD/bHLH/MYB anthocyanin regulatory complex. New Phytologist. 182: 1-3.

Heath, R. and L. Packer. 1968. Photoperoxidation in Isolated Chloroplasts. Archives of Biochemistry and Biophysics. 125: 189-198.

Hodges, D.M., DeLong, J.M., Forney, C.F. and R.K. Prange. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 207: 604-611.

Hossain, Z., Mandal., A.K.A. Datta, S.K. and A.K. Biswas. 2006. Decline in ascorbate peroxidase activity – a prerequisite factor for tepal senescence in gladiolus. Journal of Plant Physiology. 163: 186-194.

Jedrzejuk A., Lukaszewska, A., Rabiza-Swider and E. Skutnik. 2016. Low temperature forcing reduces oxidative stress in lilac flowers. Horticulture and Environmental Biotechnology. 57(6): 625-632.

Kappus, H. 1985. Lipid peroxidation: mechanisms, analysis, enzymology and biological relevance. In H. Sies, Oxidative stress. London, UK: Academic Press. 273-310.

Lay-Yee, S.M. Stead, A.D. and M. Reid. 1992. Flower senescence in daylily (*Hemerocallis)*.

Physiologia Plantarum. 65: 455-459.

Lee, M.H., Der-Syh Tzeng, D. and F.S. Hsu. 2006. Photodynamic effects of methionine-riboflavin mixture on antioxidant proteins. Plant Pathology Bulletin. 15: 17-24.

Min, D.B. and J.M Boff. 2002. Chemistry and reaction of singlet oxygen in foods. Comprehensive Reviews in Food Science and Food Safety. 1: 59-72.

Pontis, H.G. 1989. Fructans and cold stress. Journal of Plant Physiology. 124: 148-150.

Rani, P. and N. Singh. 2014. Senescence and postharvest studies of cut flowers: A critical review. Tropical Agricultural Science.37(2): 159-201.

Ranwala, A.P. and W.B. Miller. 2000. Preventative mechanisms of gibberellin$_{4+7}$ and light on low-temperature-indiced leaf senescence in *Lilium* cv. Star Gazer. Postharvest Biology and Technology. 19: 85-92.

Reid, M.S. and C.Z. Jiang. 2012. Postharvest cut flowers and potted plants. In R. Kamenetsky and H. Okubo, Ornamental Geophytes: From Basic Science to Sustainable Production. Boca Raton, FL: CRC Press. 333-361.

Rogers, H. and S. Munné-Bosch. 2016. Production and scavenging of reactive oxygen species and redox signaling during leaf and flower senescence: Similar but different. Plant Physiology. 171: 1560-1568.

Sexton, R., Laird, G., and W.G. van Doorn. 2000. Low temperature sensing in tulip (*Tulipa gesneriana* L.) is mediated through increased response to auxin. Journal of Experimental Botany. 51: 587-594.

Shan, C. and X. Zhao. 2015. Lanthanum delays the senescence of *Lilium longiflorum* cut flowers by improving antioxidant defense system and water retaining capacity. Scientia Horticulturae. 197: 516-520.

Shi, J., Shi, G. and Z. Tian. 2016. Effect of exogenous hydrogen peroxide or ascorbic acid on senescence in cut flowers of tree peony (*Paeonia suffruticosa* Adr.). Horticulture Science and Biotechnology. 90(6): 689-694.

Song, L., Liu, H., You, Y., Sun, J., Yi, C., Li, Y., Jiang, Y. and J. Wu. 2014. Quality deterioration of cut carnation flowers involves antioxidant systems and energy status. Scientia Horticulturae., 170: 45-52.

Taiz, L. and E. Zeiger. 2006. Plant Physiology, 4th Edition. Sunderland, MA: Sinauer Assocaites, Inc.

Van Doorn, W.G. and E. Woltering. 2004. Senescence and programmed cell death: Substance or semantics? Journal of Experimental Botany. 55: 2147-2153.

Van Doorn, W.G. and E.J. Woltering. 2008. Physiology and molecular biology of petal senescence. Journal of Experimental Botany. 55: 2147-2153.

Woltering, E. J., and W. G. van Doorn. 1988. Role of ethylene in senescence of petals – morphological and taxo- nomical relationships. Journal of Experimental Botany 39:1605–1616.

Yadegari, L.Z., Heidari, R. and J. Carapetian. 2007. Influence of cold acclimation on proline, malondialdehyde (MDA), total protein and pigments contents in soybean (*Glycine max)* seedlings. Journal of Biological Sciences. 7(8): 1436-1441.

Yoshida, K., Kondo, T., Okazaki, Y. and K. Katou. 1995. Cause of blue petal color. Nature. 373: 291.

Yoshida, K. 2003. Molecular regulation of leaf senescence. Current Opinion in Plant Biology. 6: 79-84

Table 3.1. Effect of weeks of storage on flower life of ‘Spryng,’ ‘van Eijk’ and ‘Yellow Flight’ plants held at 1ºC

	Flower Life (Days)		
Weeks of Storage	‘Spryng’	‘van Eijk’	‘Yellow Flight’
0	8.4	8.1	8.9
1	7.5	7.1	9.3
3	8.8	8.1	7.9
5	8.7	7.6	9.3
Weeks of Storage (W)	*	*	**

NS, *, **, *** denotes nonsignificant or significant at $P < 0.05$, 0.01, or 0.001 respectively

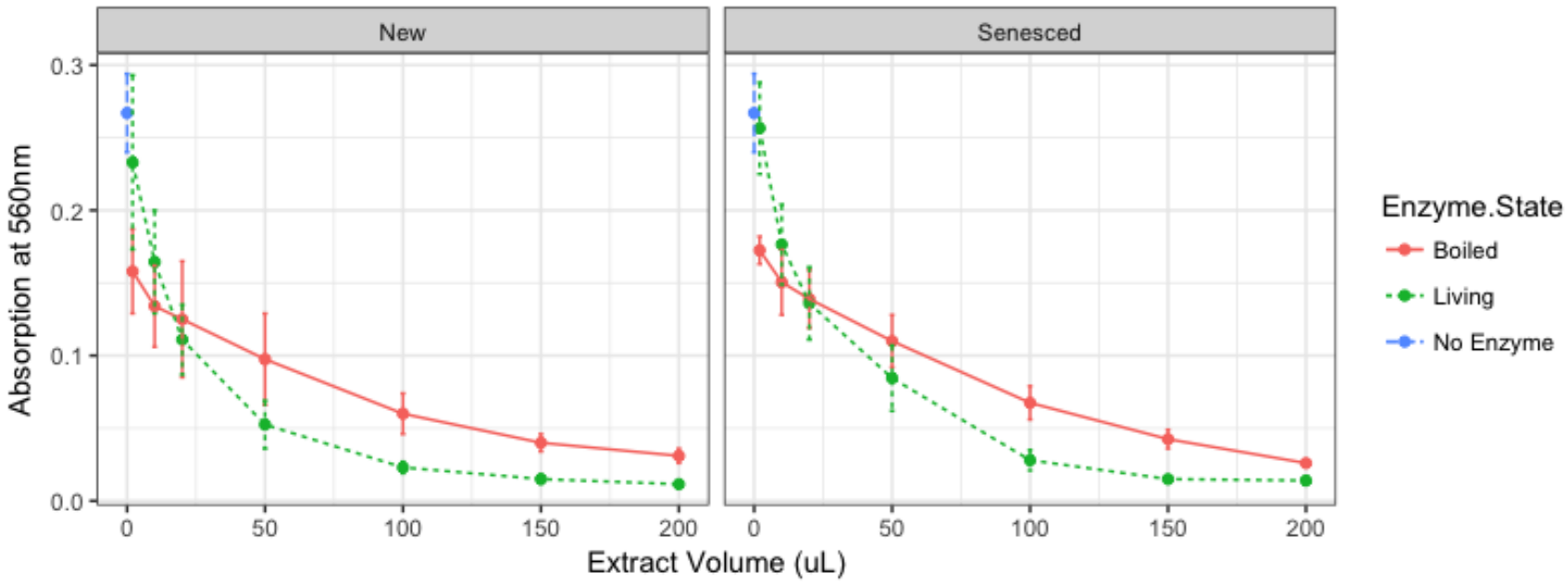


Figure 3.1. Differences in absorbance at 560 nm of living and boiled tepal samples when trying to establish a protocol to measure superoxide dismutase (SOD).

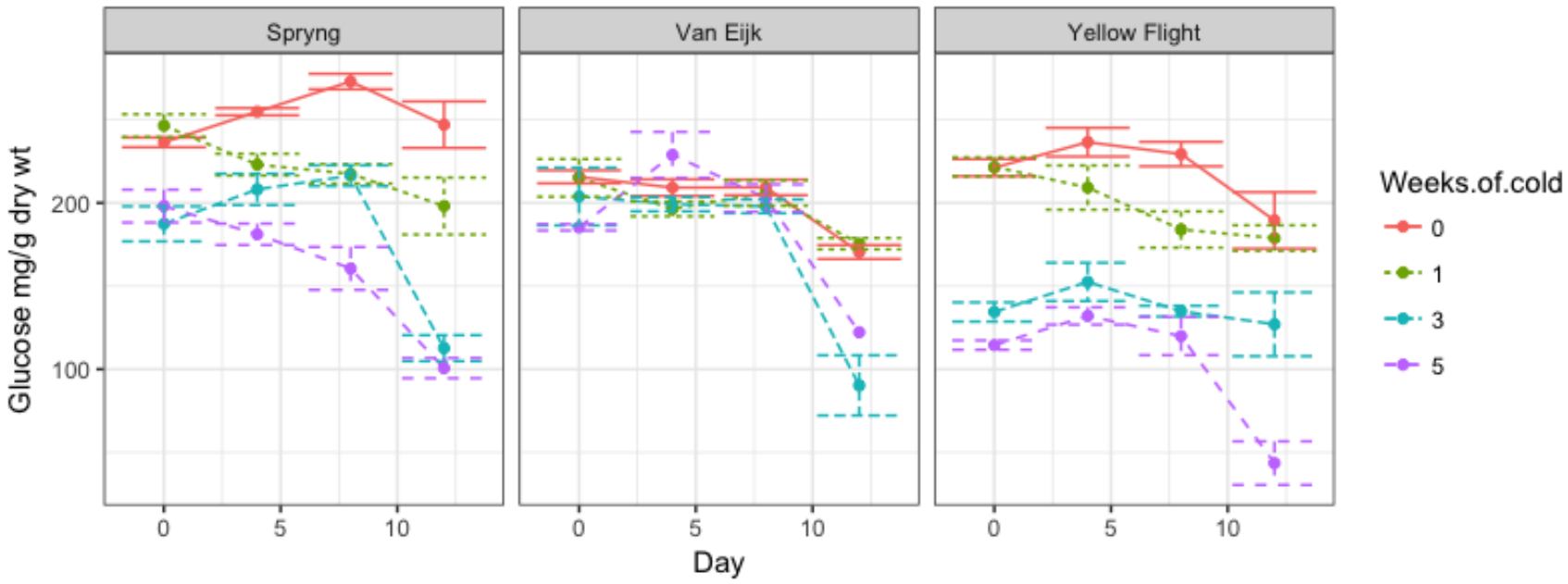


Figure 3.2. Change in glucose level of tulip tepals over the course of 0, 4, 8 and 12 days in the growth chamber in plants stored at 1ºC for 0, 1, 3 and 5 weeks. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Data are means of 5 replicates, ± SE.

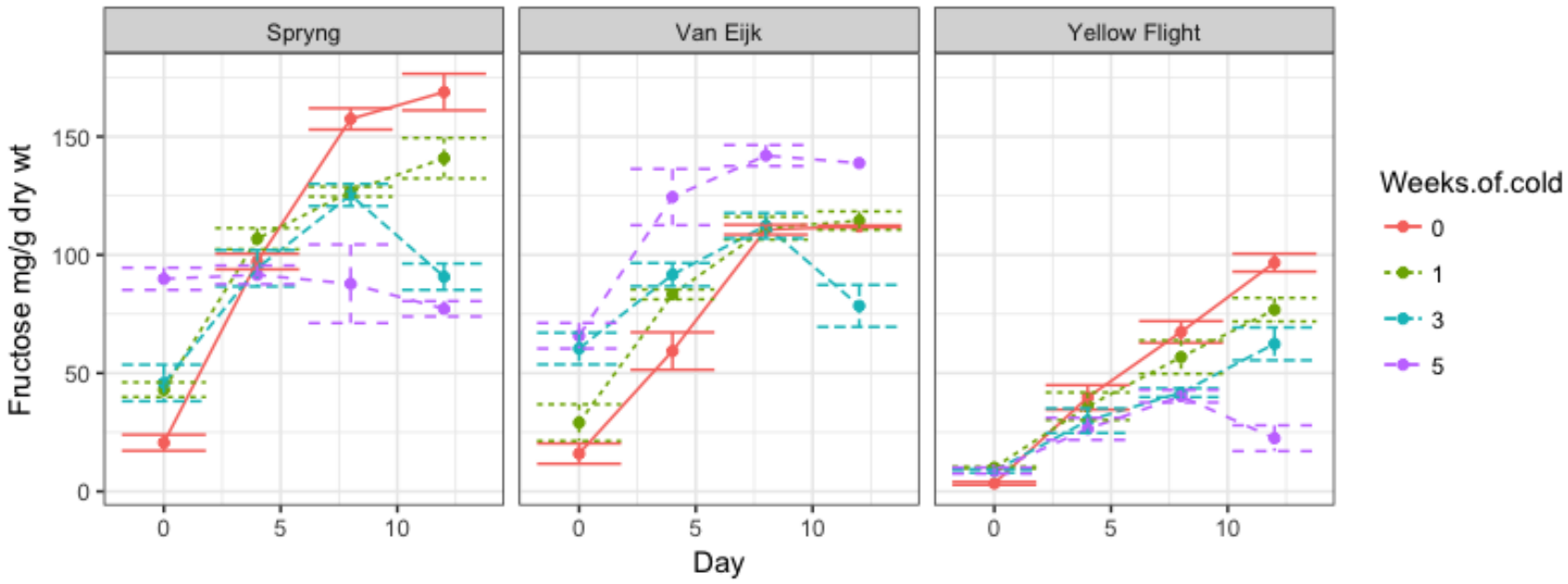


Figure 3.3. Change in fructose level of tulip tepals over the course of 0, 4, 8 and 12 days in the growth chamber in plants stored at 1ºC for 0, 1, 3 and 5 weeks. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Data are means of 5 replicates, ± SE.

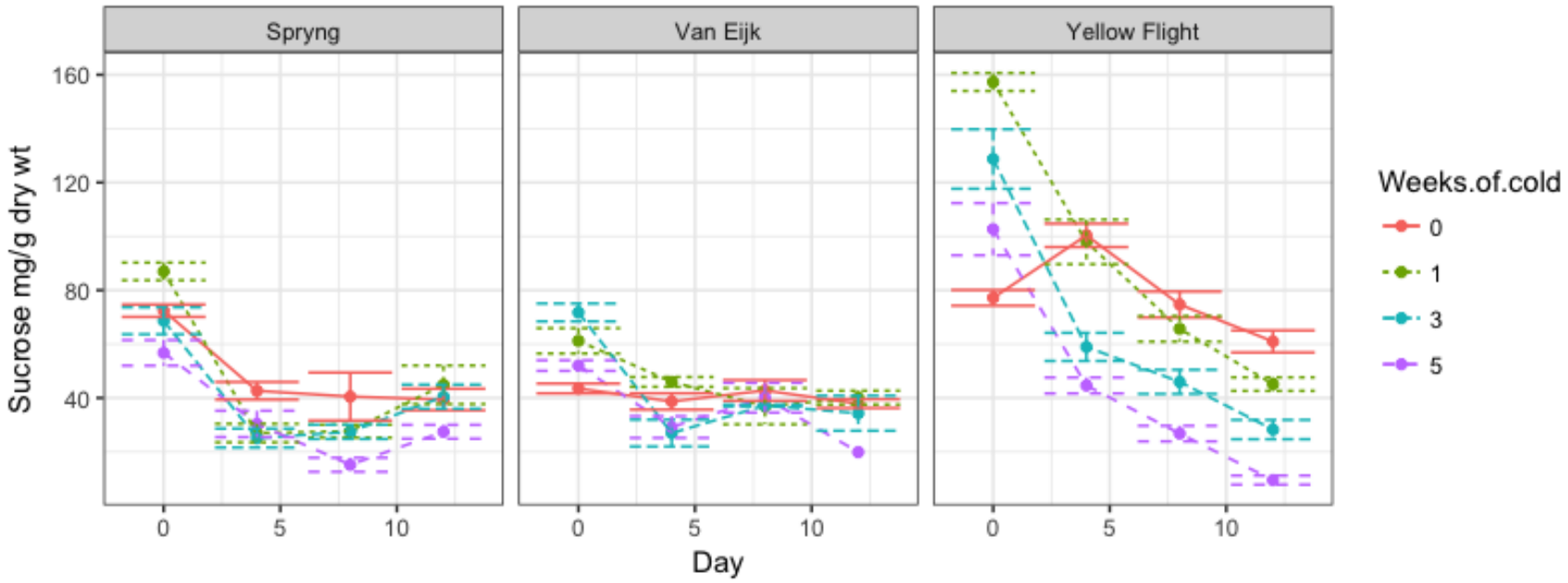


Figure 3.4. Change in sucrose level of tulip tepals over the course of 0, 4, 8 and 12 days in the growth chamber in plants stored at 1ºC for 0, 1, 3 and 5 weeks. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Data are means of 5 replicates, ± SE.

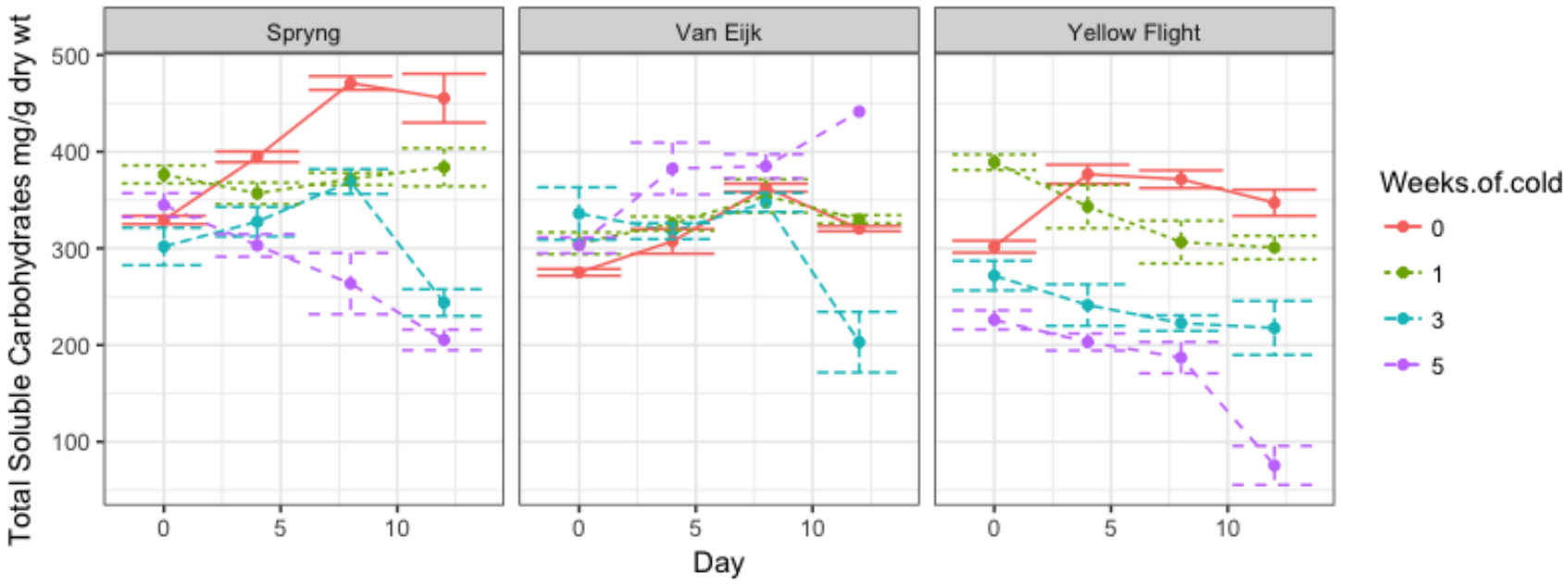


Figure 3.5. Change in total soluble carbohydrate (TSC) level of tulip tepals over the course of 0, 4, 8 and 12 days in the growth chamber in plants stored at 1ºC for 0, 1, 3 and 5 weeks. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Data are means of 5 replicates, ± SE.

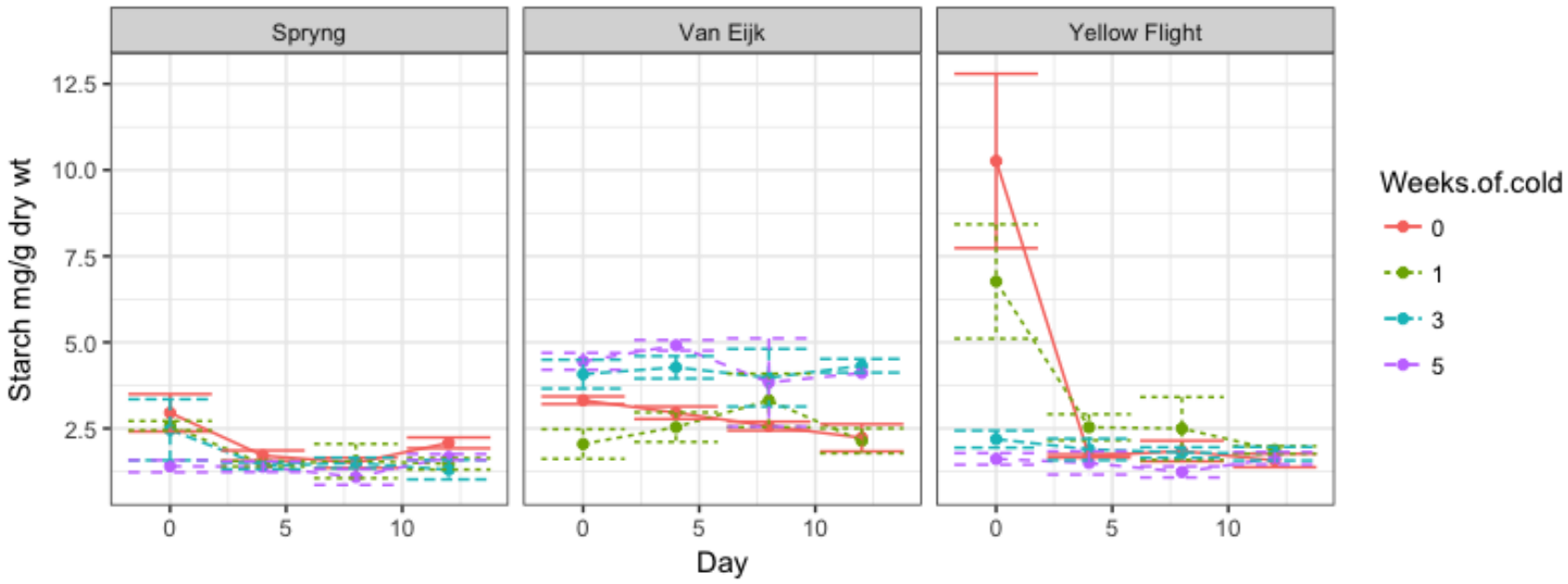


Figure 3.6. Change in starch level of tulip tepals over the course of 0, 4, 8 and 12 days in the growth chamber in plants stored at 1ºC for 0, 1, 3 and 5 weeks. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Data are means of 5 replicates, ± SE.

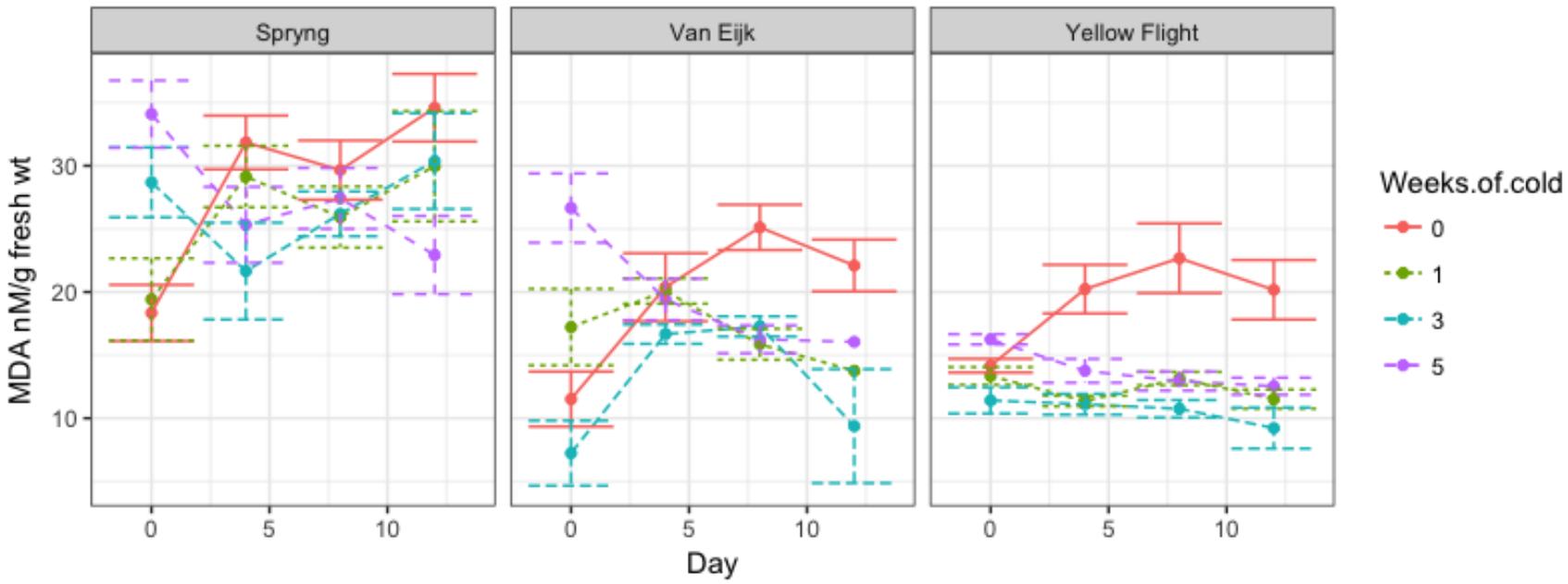


Figure 3.7. Change in malondialdehyde (MDA) level of tulip tepals over the course of 0, 4, 8 and 12 days in the growth chamber in plants stored at 1ºC for 0, 1, 3 and 5 weeks. L to R: 'Spryng,' 'van Eijk' and 'Yellow Flight.' Data are means of 5 replicates, ± SE.