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Rapid Leaf Senescence Symptoms Are Related to Carbohydrate Depletion in Cut Chrysanthemums, and Strategies for the Symptoms Reduction

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RAPID LEAF SENESCENCE SYMPTOMS ARE RELATED TO CARBOHYDRATE DEPLETION IN CUT CHRYSANTHEMUMS, AND STRATEGIES FOR THE SYMPTOMS REDUCTION

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ABSTRACT

Chrysanthemum (*Chrysanthemum ×morifolium*) is the second-largest exported cut flower worldwide; however, some cultivars exhibit rapid leaf senescence during their first week of vase life. This phenomenon negatively impacts consumer perception of plant quality, and its cause has been unknown. Experiments were performed in Colombia on cut chrysanthemums shipped to the U.S. for vase-life evaluation. After 10 d, the severity of leaf senescence symptoms was recorded. Experiments examined the effect of flower form [disbud (one flower per stem) versus spray (5-10 flowers per stem)], the effect of time of harvest (A.M. versus P.M.), and sugar sources (dextrose, fructose, mannitol, and sucrose) at different concentrations in vase solutions on senescence symptom development. Results showed that spray-form stems showed higher leaf senescence severity than disbud-form stems; morning harvest increased symptom severity compared to noon harvest; stems placed in a vase solution without sugars displayed more severe symptoms than stems treated with sugars in the vase solution except for mannitol treatments. Carbohydrate concentrations in leaves were collected throughout the experiment. Results showed starch, glucose, fructose, and sucrose reduction from harvest through storage and shipping until the stems were placed in vase solutions. In summary, the results demonstrate that the rapid leaf senescence of chrysanthemums is related to carbohydrate depletion in the leaves during the postharvest environment. Postharvest treatments such as hydration time and environment, the application of plant growth regulators, sucrose in the hydration solution, post-shipping holding solution, and vase solutions were evaluated. The most effective strategies for reducing leaf senescence

symptoms for most of the cultivars evaluated were afternoon harvesting, adding plant growth regulators, such as thidiazuron, and a mixture of cytokinins and gibberellic acid at 10 or 20 ppm in the hydration solution for 4 h after harvest, and the addition of 0.025 and 0.05 g/mL sucrose into vase solutions.

Keywords: chlorosis, cut flowers, marginal necrosis, plant growth regulator, source-sink relationships, sugar, vase life, veinal necrosis, wilting.

DEDICATION

To my grandfather and his wisdom. His advice and patience I will always treasure.

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CHAPTER ONE

LITERATURE REVIEW: CHRYSANTHEMUM CUT FLOWER LEAF SENESCENCE SYMPTOMS AND THEIR RELATIONSHIP WITH CARBOHYDRATE DEPLETION

Introduction

Chrysanthemum (*Chrysanthemum ×morifolium*) cut flower vase life varies substantially between farms and seasons. This variability has been related to physiological differences between cultivars, the greenhouse production environment, and the postharvest environment and duration. This literature review describes research that documents harvest and postharvest factors that affect chrysanthemum vase life and the relationship between chrysanthemum cut flower vase life performance and carbohydrate metabolism.

Colombia is one of the main chrysanthemums cut flower producers worldwide. Chrysanthemums are versatile cut flowers due to the wide range of flower colors, flower shapes, and flower forms. The two main product forms are disbud and spray. The disbud form has one flower/stem, and all the axillary flower buds are removed. The spray form has 5 to 10 flowers per stem and the main flower bud, while the axillary flowers are allowed to develop (Pulgarin Navarro 2021). The various flower shapes include button (small, dense, cushion flowers), cushion (compact, globe-shaped blooms), daisy flowers (disk florets surrounded by an array of ray florets), micro-daisy flowers, and decorative

all ray florets) or novelty flowers (outermost flowers are long ray florets and inner flowers are short ray florets).

Chrysanthemum harvest and post-harvest

Maintaining an optimal preharvest environment and implementing appropriate postharvest handling practices are essential for ensuring cut flower quality, longevity, and marketability. Growers must pay attention at every step of the postharvest chain, from harvest time to delivery to the consumer (Dole and Faust 2021). The industry typically expects 7-10 days of vase life despite the postharvest duration of up to 23 or 35 days for air or sea shipments, respectively (Pulgarin Navarro 2021). The following steps are the preharvest and postharvest events for chrysanthemum:

Preharvest and harvest environment. The preharvest environment includes the conditions and factors affecting plant growth and development before harvest, such as light intensity, temperature, plant nutrition, and soil moisture. The harvest environment refers to the conditions and activities involved in the process of gathering plants at their mature stage for commercial or consumption purposes. The preharvest environment affects product quality and performance in the postharvest environment.

The amount of light delivered during production affects postharvest performance (Kofranek and Halevy 1972). Chrysanthemums that received a light intensity of 240 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the flowering period had >14 d of vase life, while flowers that received 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the flowering period had 8 to 10 d vase life. This response was related to carbohydrate levels in the cut flower tissues, and higher light levels induced higher accumulation of carbohydrates (Seemann 1989). However, too

much light intensity may negatively affect the flower quality of some species. It has been reported that light intensities > 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the last three weeks before harvest causes photooxidative stress on plants that decreases vase life on chrysanthemums (Kofranek and Halevy 1972; Moe 1975).

Temperatures above 27 °C or below 15 °C during the last three weeks before harvest resulted in poor postharvest performance of cut roses (*Rosa rubiginosa*), cultivars Zorina, Garnette, and Baccara due to high respiration rates causing high carbohydrate consumption at high temperatures or reduced water uptake under low temperature conditions (Moe 1975). Soil moisture conditions before harvest are essential to reduce postharvest wilting; therefore, a common practice by growers is to irrigate plants immediately before harvest to maintain turgor pressure in the postharvest environment (Pulgarin Navarro 2021).

Recommendations for the diurnal time of harvest are conflicting. Harvesting just after sunrise was not recommended due to the increased risk of physically damaging leaves due to their increased brittleness (Pulgarin Navarro 2021). Cutting flowers during the morning, around 2 h after sunrise, allows for the cutting of more turgid stems. Still, at this time of the day, flowers may be wet due to condensation that occurs during the night, and humid or wet flower tissue is to be avoided for disease management purposes (Hidalgo et al. 2011). Water-stress-sensitive cultivars are recommended to be harvested first each day. In contrast, afternoon harvest is recommended for flowers that need to open after harvest or must be stored for an extended period due to the higher carbohydrate content in the plant in the afternoon (Ahmad et al. 2014).

The stage of flower development at the time of harvest varies with the market and cultivar. Flowers to be stored and shipped are cut with fewer open buds than those harvested and sold directly to the final customer (Dole and Faust 2021). Colombian growers report that chrysanthemum cultivars with large petals and intense colors must be harvested before they open. Otherwise, petals will be damaged during postharvest processes because petals are susceptible to discoloration and/or blackening. Flowers harvested too open will have a shorter vase life, while flowers harvested immaturely will generally not open well or not develop good pigmentation. Harvesting flowers at earlier stages of development requires higher carbohydrate supplies to open the flowers in the postharvest environment.

Harvest. Colombian chrysanthemum cut flowers are produced in plastic greenhouses with no climate-control conditions. After cuttings are rooted, they are planted directly into ground bed until harvested (Pulgarin Navarro 2021). The harvest process is done manually by grasping each stem and pulling the entire plant out of the soil. After the roots are exposed, the stem is cut and grouped into bunches or placed directly in buckets (Hidalgo et al. 2011; Pulgarin Navarro 2021). For bunches, approximately ten stems are tied with elastic bands and placed inside a plastic sleeve and then dropped into buckets.

Transport between greenhouse and postharvest area. After harvest, stems are transported to the packing house in buckets containing a commercial hydration solution that contains a pH stabilizer that keeps a 3.5-4.5 pH and a biocide (Hidalgo et al. 2011). Biocides such as sodium hypochlorite and calcium hypochlorite are commonly used, and

citric acid is the most common acidifier (Dole and Faust 2021). Buckets contain as many as 200 stems. During cloudy days, buckets may be devoid of solution since the transpiration rates are low. Buckets are moved using special carts or monorail systems. Most farms have transport procedures that guarantee <1 h from harvest to delivery to the packing house to minimize respiration and maximize postharvest longevity (Hidalgo et al. 2011; Ahmad et al. 2014).

Hydration. Once in the packing house, stems are transferred to buckets containing a hydration solution. For chrysanthemums, hydration solutions contain the same compounds as the transport solutions. Reducing water pH between 3.5 and 4.5 and adding biocides reduces the risk of stem occlusion by bacteria (van Doorn W 1989). Water loss of >7% can be recovered by placing stems in 10 cm of water for 1 h at 20˚C, 60% relative humidity (RH), and a PPFD of 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Van Meeteren and Van Gelder 1999). Less than one hour of hydration can cause wilting and leaf-yellowing symptoms in some cultivars (Van Meeteren and Van Gelder 1999; Hidalgo et al. 2011).

Adding sugars into postharvest hydration solutions is recommended for chrysanthemum and, for instance, sucrose at concentrations between 0.02 and 0.05 g/mL with a 12 h exposure time to increase sugar concentration inside stems before storage have been proven to reduce the risk of carbohydrate depletion during the vase life (Mayak et al. 1973a); however, this practice is not used in commercial production. For some cut flower species plant growth regulators are added to the hydration solution to reduce senescence, e.g., cytokinins and gibberellic acid, and inhibit ethylene perception, e.g., silver thiosulfate; however, these compounds are not used for chrysanthemum.

Processing and packaging. After hydration, stems are moved from a bucket with the hydration solution to buckets without the solution for ~30 min. This allows free water to evaporate from the stems before packaging since free water contributes to botrytis growth and can reduce the strength of the cardboard shipping boxes. The stems are combined into bouquets with other chrysanthemum cultivars and other species and then packed dry in cardboard boxes (Pulgarin Navarro 2021). The time required to make flower bunches and bouquets is typically up to 60 min. These processes occur in packing houses without climate control, so temperatures vary throughout the day, but are typically 10-25 °C.

Cooling. After packaging, boxes are moved into a refrigerated cooler at 3-5 °C and placed in forced-air cooling systems for up to 1 h to remove the field heat. Staby and Reid (2005) reported that flowers must be kept at <9 °C to prevent quality losses during the vase life. Interruptions in the postharvest cold chain increase respiration rates and can cause premature flower opening, stem twisting (gravitropism), leaf and petal wilting, and flower petal shattering (Staby and Reid 2007).

Storage and shipping. Chrysanthemum cut flowers are shipped by sea or air. Air freight from South America typically arrives in Miami, where the USDA-Animal and Plant Health Inspection Service (APHIS) inspects the plants. Once inspected, the boxed flowers may be shipped to florists and retailers or processed into bouquets. Air-freight shipments typically require 5 to 10 d from harvest to delivery to wholesale and retail customers and 20 to 36 d for sea-freight shipments.

Carbohydrate metabolism of cut flowers

After harvest, chrysanthemums undergo various physiological changes that affect their vase life performance. Senescence symptoms such as leaf yellowing and wilting have been reported for different cultivars in different countries (Reyes-Arribas et al. 2001). The following sections provide a description of physiological processes relating to chrysanthemum cut flower vase life performance and carbohydrate metabolism.

Source-sink relationships. The source-sink relationship can be defined as the balance between plant organs responsible for taking up or creating resources (source) and the organs using those resources (sink) (Burnett 2019). The strength of a sink or source depends on the size of the organ and the rate at which a resource is used (source activity) (Burnett 2019). Sink strength changes over the life cycle of a plant as sinks develop and mature (Roitsch and Ehneß 2000).

After harvest, cut flowers require water and nutrients to continue functioning and surviving (Burnett 2019). Since the amount of carbohydrates in the flower bud at the time of harvest is insufficient to support respiration, and osmoregulation during vase life, carbohydrates must be imported (Marissen and La Brijn 1995). Leaves and stems are the sources to supply flower buds with carbohydrates. Carbohydrates are transported to flower buds mainly via phloem vessels, while carbohydrate uptake from the vase primarily occurs via the xylem vessels (Roitsch and Ehneß 2000). If carbohydrate concentrations inside the plant are higher than that required for flower opening, the excess carbohydrates can be exchanged for other compounds used for plant osmoregulation.

Methods of altering source-sink relationships. Plant hormones regulate ethylene production and are involved in senescence and maintaining the quality and freshness of the flowers (Iqbal et al. 2017). The main hormones involved in these responses are ethylene perception inhibitors, cytokinins, and gibberellins.

Ethylene inhibitors. Ethylene is involved in the development of plant organs and promotes, inhibits, or induces plant senescence (Iqbal et al. 2017). Chlorophyll degradation and leaf abscission, desiccation, and necrosis represent the visual symptoms of leaf senescence (Ferrante and Francini. 2006). Chrysanthemums have been reported as sensitive to ethylene that causes premature wilting and yellowing of leaves and flowers (Pardo Carrasco 2010). Ethylene inhibitors, such as silver thiosulfate (STS), 1-methylcyclopropene (1-MCP) and silver nitrate ($AgNO_3$), prevent ethylene-induced senescence and extend the postharvest longevity of chrysanthemum cut flowers (Dole et al. 2005; Mohamed 2012; Bhargava et al. 2015a).

Cytokinins. Cytokinins are plant hormones that regulate cell division and growth. It has been reported that the exogenous application of this plant hormone led to delayed senescence of plant leaves because it can modify source-sink relationships (Roitsch and Ehneß 2000). Benzyladenine (BA), a synthetic cytokinin, applied as a pulse treatment delayed leaf yellowing in cut solidago and alstroemeria flowers and chrysanthemums (Iqbal et al. 2017). Also, it was reported that there are products that stimulate cytokinin synthesis. Similarly, inoculation with microorganisms, such as *Bacillus subtilis*, can enhance plant growth by secreting cytokinins that promote cytokinin responses in the plant (Arkhipova et al. 2005).

Gibberellins. Gibberellins delay senescence and promote flower opening, which can help maintain the freshness and longevity of cut flowers (Iqbal et al. 2017). Gibberellins used as postharvest treatments prevent leaf yellowing in several cut flowers, e.g., pulse application of GA_3 reduced leaf symptoms on stock (*Matthiola incana*) (Ferrante et al. 2005; Ferrante et al. 2009) and chrysanthemum cut flowers (Florez-Roncancio et al. 1996; Mohamed 2012).

Water uptake disruption. Floral senescence is characterized by increased activity of RNase and other hydrolytic enzymes, ethylene production, vase solution viscosity, and membrane permeability, which generate ion loss and disrupt water uptake (Ferrante and Francini 2006). Senescence is a genetically programmed process regulated by phytohormones; however, water stress or ethylene exposure can accelerate it (Pardo Carrasco 2010). The combined effects of changes in membrane permeability and osmotic potential can reduce water retention. These effects decrease the turgor pressure and may cause irreversible wilting (Halevy and Mayak 1979).

Microbial growth. Bacteria and fungi can colonize the stem of cut flowers leading to stem blockage and wilting (Hidalgo et al. 2011). One study reported a slower sap flow five centimeters from the bottom of the stem compared to the base of the stem caused by vascular blockage related to bacterial growth (van Doorn W 1989).

Carbohydrate metabolism. Carbohydrate metabolism is regulated by cytokinins (Roitsch and Ehneß 2000), which were found to be involved in regulating sink strength, photosynthate partitioning, and phloem unloading (Burnett 2019). Long-distance transport of carbohydrates is done through the phloem sieve elements and is driven by

differences in pressure potential (Chang and Zhu 2017). Enzyme regulation in sucrose metabolism is important for phloem unloading and the import of sucrose into sink organs since the removal of sucrose from the phloem increases the gradient and thus enhances the flow toward sinks (Chang and Zhu 2017).

Carbohydrate status and the ethylene pathway are connected, e.g., increased endogenous carbohydrates in cut flowers reduce ethylene sensitivity (Zhou et al. 1998; Elgar et al. 1999; Ichimura et al. 2000a; Ichimura 2003). Nevertheless, while higher levels of endogenous carbohydrates reduce ethylene sensitivity in unrooted cuttings, they do not stop ethylene production (Rapaka et al. 2007a; 2007 b).

Cut flowers rely on stored carbohydrates for energy during the postharvest period, as they can only produce significant levels of carbohydrates through storage starch degradation (Roitsch and Ehneß 2000). The breakdown of carbohydrates can be influenced by various factors such as temperature, and humidity. High temperatures can speed up the breakdown of carbohydrates, while low temperatures can slow it down. High humidity can also accelerate the process of carbohydrate breakdown, while low humidity can cause the flowers to lose moisture (Iqbal et al. 2017).

It has been reported that the delivery of carbohydrates in the hydration, holding and vase solutions can delay senescence symptoms in cut flowers. Chrysanthemums have a large inflorescence that requires significant energy to maintain. After harvesting chrysanthemum cut flowers, they rapidly deplete carbohydrates due to their high respiration rate (Adachi et al. 1999).

Delivery of sugars during cut flower postharvest. Carbohydrates may be delivered in the hydration solution prior to storage/shipping or in the holding solutions after storage, and in the vase solution. Ichimura (2003) observed that adding sugar (glucose, fructose, sucrose) to the vase solution improved stem water uptake because of the decreased osmotic pressure of the solution and found that plants treated with sucrose in the vase solution increased water retention capacity compared to other monosaccharides, such as glucose and fructose, that produced a lower osmotic potential (Ichimura et al. 2000a; 2005).

Carbohydrates sources commonly evaluated are monosaccharides such as glucose, dextrose, fructose, and methyl glucoside and disaccharides such as sucrose and maltose. Halevy and Mayak (1979) concluded that the addition of non-metabolic sugars, such as mannitol, increased senescence due to their incapacity to enter at the respiration cycle as an energy supply. The optimum concentration of carbohydrates varies depending on the flowers being treated (Ichimura et al. 2000a; El-Ghait et al. 2012; Mashhadian et al. 2012; Jain 2014; Choudhari and Kulkarni 2018; Roshikanta et al. 2021).

Carbohydrate recommendations for various species. Most flowers benefit from 0.02 g/mL sucrose in the hydration solution. It has been shown that some flowers, such as chrysanthemum and gladiolus (*Gladiolus* sp.), benefit from higher concentrations, such as 0.04 to 0.06 g/mL sugar solutions (Mayak et al. 1973a; Zamani et al. 2011a). Other flowers, such as zinnias (*Zinnia elegans*) and coralbells (*Heuchera* sp.), are damaged when carbohydrate concentrations are greater than 0.01 g/mL (Stimart and Brown 1982a).

After shipment, holding solutions are provided for 12 to 24 h. Gladiolus and bird of paradise (*Strelitzia reginae*) stems are placed in a solution of 0.2 g/mL and 0.1 g/mL sucrose, respectively, 12 h before sale (Mayak et al. 1973b; Gendy and Mahmoud 2012), to promote flower opening. Holding solutions do not improve vase life of zinnias and coralbells (Mayak et al. 1973b; Stimart and Brown 1982b).

In vase solutions, 'flower foods' are formulated to provide sugar at a concentration of ~0.01 g/mL. Sugars concentration between 0.04 and 0.06 g/mL benefit chrysanthemum (Zamani et al. 2011b; Amin 2017a; 2017b), gerbera daisy (*Gerbera hybrida*) (De Silva et al. 2013), gladiolus (Mayak et al. 1973), and sweet pea (*Lathyrus odoratus*)(Ichimura 1998); sugar concentrations between 0.02 to 0.03 g/mL works well for lisianthus (*Eustoma grandiflorum*) (Ichimura 1998), lotus (*Nelumbo nucifera*) (Chathuri and Sarananda 2011) and rose (Ichimura et al. 2005). Sugar concentrations between 0.01 and 0.015 g/mL improve snapdragon (*Antirrhinum majus*) vase life (Ichimura 1998; Asrar 2012).

Senescence symptoms reported on cut flowers. Senescence-related symptoms include: de-greening of disk florets and sepals for cut flowers (Van Geest et al. 2016), brassicas (Tian et al. 1994) and *Arabidopsis thaliana* (Trivellini et al. 2012), rapid senescence of cut gladiolus spikes (Chore et al. 2020), leaf necrosis and yellowing on chrysanthemum (Adachi et al. 1999), protea (*Protea eximia* and *Protea neriljiolia*) (McConchie et al. 1991; Bieleski et al. 1992), petal blackening on roses (Ichimura et al. 2000a), christmas bells (*Sandersonia* sp.), iris (*Iris* sp.), carnations (*Dianthus caryophyllus*) (Van Doorn 2004), and lisianthus (Cavasini et al. 2018). The most

common leaf senescence symptom is the yellowing caused by chlorophyll degradation and impaired biosynthesis (Iqbal et al. 2017). Chlorophyll loss increases after ethylene exposure in chrysanthemum (Reyes-Arribas et al. 2001). Adding sugar to the holding (at least 1h with 4%-6% sucrose solution) and handling solutions (4%-6% sucrose into the vase solution) improves leaf performance and vase life (Zamani et al. 2011; Amin 2017a, 2017b). Hormone treatments applied as a 24 h pulse of 10 and 20 ppm of cytokinin (BA) and gibberellic acid respectively, showed a reduction in chrysanthemum leaf yellowing (El-Ghait 2012; Flórez-Roncancio et al., 1996). The addition of 1.1 and 2.2 ppm of thidiazuron (TDZ) in the vase solution also increases chrysanthemum vase life (Bhargava et al. 2015).

Wilting is a common senescence symptom related to stem plugging due to bacterial growth (da Costa et al. 2021), ethylene, and poor hydration after harvest (Halevy and Mayak 1979; Pardo Carrasco 2010). The use of biocides in the holding and handling solutions helps to prevent bacterial growth (Hidalgo et al. 2011). The most common biocides used are quaternary ammonium salts and chlorine compounds. Also, it has been shown that 1% sucrose in those solutions improves stem osmoregulation, which reduces wilting symptoms (Ichimura et al. 2000b).

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CHAPTER TWO

RAPID LEAF SENESCENCE SYMPTOMS ARE RELATED TO CARBOHYDRATE DEPLETION IN CUT CHRYSANTHEMUM

Abstract

Chrysanthemum (*Chrysanthemum ×morifolium*) is the second-largest exported cut flower worldwide that is cultivated for its variety of flower shapes, colors, and forms; however, the leaves of some commercial cultivars exhibit rapid leaf senescence during the first week of vase life. This characteristic negatively impacts consumer perception of plant quality. Thus, this problem is a concern for growers, and the cause of this phenomenon has not been known. Five experiments were performed to identify the cause of rapid leaf senescence in cut chrysanthemums that were commercially grown in Colombia and shipped to the U.S. for vase life evaluation. The first experiment evaluated leaf senescence symptoms severity (such as yellowing, necrosis, and wilting) of chrysanthemum cultivars grown with two flower forms: one terminal flower per stem (disbud) or 5-10 axillary flowers per stem (spray). Stems with the spray form showed leaf senescence symptoms, while stems from the disbud form did not display leaf senescence after 10 d vase life. The second experiment evaluated the time of harvest on leaf senescence, and it was observed that early morning harvest increased leaf senescence severity compared to noon harvest. The third experiment evaluated the effect of sucrose concentration (0, 0.1 or 0.2 g/mL) in the vase solution. Stems with no sucrose in the vase solution displayed severe leaf senescence symptoms, while stems with 0.1 or 0.2 g/mL sucrose presented a lower symptom severity after 10 d vase life. The fourth experiment

examined the effect of different sugars (dextrose, fructose, mannitol, and sucrose) at concentrations from 0 to 0.1 g/mL in the vase solution. The best results were obtained with 0.025 and 0.05 g/mL sucrose concentrations. In the fifth experiment, glucose, fructose, sucrose, and starch concentrations were measured in chrysanthemum leaves at the time of harvest, during postharvest hydration, after storage and shipment, and after 5 and 10 d of vase life. Results showed a trend in starch reduction and variation of the other sugars (glucose, fructose, and sucrose) over time. In summary, these experiments provide evidence that the rapid leaf senescence of chrysanthemums is related to carbohydrate depletion in the leaves during postharvest.

Keywords: chlorosis, cut flowers, marginal necrosis, source-sink relationships, vase life, veinal necrosis, wilting.

Introduction

Colombia has been producing cut flowers for more than 40 years with production mainly exported to the United States, Canada, The Netherlands, United Kingdom, Japan, Spain, Russia, and Poland, and valued at $1.5B USD in 2021 (Procolombia 2022). The main export flowers are rose (*Rosa ×hybrida*), carnation (*Dianthus caryophyllus*), and chrysanthemum. One of the major problems cut chrysanthemum growers have identified is the short postharvest vase life of some cut chrysanthemum cultivars due to rapid leaf senescence, which is defined as yellowing, necrosis, and/or wilting observed during the first 7 d of vase life (Hidalgo et al. 2011).

Chrysanthemum cut flower production starts with rooted cuttings planted in ground beds inside greenhouses and grown for 10 to 11 wks until the stems are harvested. A long-day photoperiod is provided during the first 4 wks, and s short-day photoperiod is provided to stimulate flowering during the last 6-7 wks of the crop. Three weeks after the start of short days, floral buds are removed to produce either spray or disbud forms. For the spray form, the terminal flower bud is removed, and 5-10 axillary flowers develop per stem. For the disbud form, all axillary flower buds are removed, and the terminal flower develops. Growers report that leaf senescence symptoms are mostly observed on spray-form chrysanthemums, while disbud forms are not susceptible.

Between four to five wks later, the harvest process is made manually, starting early morning until middle afternoon (between 6:30 am and 4 pm) (Hidalgo et al. 2011). Stems that are susceptible to wilting in the field are harvested first. The postharvest period begins when harvested stems are transported from the greenhouses to the packing room in buckets containing a pH stabilizer that keeps at 3.5-4.5 pH and a biocide (Hidalgo et al. 2011). In the packing room, the stems are transferred to another bucket filled with the same solution called the hydration solution and remain there for 4 to 12 h. After hydration, stems are processed, packaged, stored in a 3 to 5 °C room, and then shipped (Staby and Reid 2007). Upon delivery to the wholesale distributor or retailer, stems may be place in a holding solution for 4 to 24 h, before being sold (Halevy and Mayak 1979). The holding solution may or may not contain a carbohydrate source. Flower food packets are frequently attached to bunches for consumer use in the final vase solution. Flower food typically contains three components: a pH acidifier, a biocide, and

a carbohydrate source that supplies approximately 0.01 g/mL sugar. The carbohydrate source varies with market prices.

Carbohydrate depletion has been related to various senescence symptoms in different plant species such as de-greening of disk florets and sepals of chrysanthemum (Van Geest et al. 2016), sepals of broccoli (*Brassica oleracea*) (Tian et al. 1994), and inflorescence of arabidopsis (*Arabidopsis thaliana*) (Trivellini et al. 2012), rapid senescence of cut gladiolus (*Gladiolus palustris*) spikes (Chore et al. 2020), necrosis and yellowing of chrysanthemum leaves (Adachi et al. 1999) and protea leaves (*Protea eximia* and *P. neriljiolia*) (McConchie et al. 1991; Bieleski et al. 1992), and petal blackening on roses (Ichimura et al. 2000b), christmas bells (*Sandersonia* sp.), iris (*Iris* sp.), carnations (Van Doorn 2004), and lisianthus (*Eustoma grandiflorum*) (Cavasini et al. 2018).

It has been reported that the moment of harvest during the day can alter the postharvest performance of green leaves crops (Clarkson et al. 2005), carnation flowers (Verlinden and Garcia 2004), broccoli (Brassica oleracea) florets (Nishikawa et al. 2005), and portulaca (Portulaca grandiflora) cuttings (Rapaka et al. 2006) due to the changes of the plant endogenous carbohydrate concentrations during the day. Morning harvest can negatively impact postharvest longevity by having a lower carbohydrate supply than harvesting later in the day (Rapaka et al. 2006).

Also, it has been reported that the addition of sugars such as sucrose and dextrose in concentrations from 0.025 to 0.05 g/mL in the holding and vase solutions can improve vase life performance, e.g., 0.04 to 0.06 g/mL sugar benefits chrysanthemum (Zamani et

al. 2011b; Amin 2017a; Amin 2017b), gerbera daisy (*Gerbera hybrida*)(De Silva et al. 2013), gladiolus (Mayak et al. 1973), and sweet pea (*Lathyrus odoratus*)(Ichimura 1998); 0.02 to 0.03 g/mL sugar benefits lisianthus (Ichimura 1998), lotus (*Nelumbo nucifera*) (Chathuri and Sarananda 2011) and rose (Ichimura et al. 2005); 0.01 to 0.01.5 g/mL sugar benefits snapdragon (*Antirrhinum majus*) (Ichimura 1998; Asrar 2012). In contrast, cut roses treated with >0.025 g/mL sucrose in the vase solution resulted in necrotic leaves due to cell collapse (Markhart and Harper 1995).

The objective of this study was to determine if rapid leaf senescence in cut chrysanthemums may be related to carbohydrate depletion. Three strategies were used to manipulate the carbohydrate supply or demand: 1. flower form, 2. time of harvest, 3. the addition of different sugars at various concentrations to the vase solution. Also, nonstructural carbohydrates (NSC) concentration changes on leaves over time after harvest until the end of the vase life was measured.

Materials and methods

Chrysanthemum cut flowers were grown by commercial growers in Cundinamarca, Colombia, near the towns of Facatativa, Madrid, and Tenjo, and in Antioquia, Colombia, near Rionegro. Cultivars were chosen based on their high susceptibility to exhibiting rapid leaf senescence symptoms. The following cultivars were grown in Cundinamarca: Bomber Green (BG), Green Screen (GS), Peridot (P), and Shrek (S); the following cultivars were grown in Antioquia: Alligator (A), Lychee (L), Mark Twain (MT), Molly Purple (MP), Paintball Sunny (PS), WhatsApp (W), and Zumba (Z)

(Fig. 2.1). Except for Expt. 1, the cultivar L was grown in the disbud form, while A, BG, GS, MT, MP, P, PS, S, W, and Z were grown in the spray form. The cultivars A, BG, GS, P, S, and W have green flowers, L, MP, and Z have purple flowers, MT has white flowers, and PS has yellow flowers. A, BG, L, and Z have cushion flowers (compact, globe-shaped blooms), S has daisy flowers (disk florets surrounded by an array of ray florets), MP has micro-daisy flowers, MT, PS, and W have button flowers (small, dense, cushion flowers), and GS and P are decorative or novelty flowers (outermost flowers are long ray florets and inner flowers are short ray florets). Treatments applied at the greenhouse or before shipment, such as flower form and time of harvest, were made at the same farm at which the flowers were grown.

Plants were grown for 10-11 weeks in ground beds inside greenhouses covered with a single layer of polyethylene. Night-interruption lighting was provided during the first four weeks, while the remaining 6-7 weeks were under ambient photoperiods of ~12 h. The main flower bud was removed from the stem 4 wks prior to harvest in order to promote flower development in the leaf axils for the spray form stems. For the disbud form stems, the axillary flower buds were removed from the stems 4 wks before harvest. Spray form stems were harvested at commercial maturity, defined as at least five open axillary flowers/stem, and disbud stems were harvested at the same time as the spray form stems, 10-11 weeks after transplant. Stem length was >80 cm. Immediately after harvest, stems were placed in buckets that contained a 5 L hydration solution (2 mL/L Florissima 925, Florissima, Colombia) that provided a pH of 4.0 and contained 0.8% free chlorine. The stems were held in the hydration solution for 12 h, then packaged and

stored in cardboard boxes for 7 d in a cooler at 4 °C. Then the boxes were shipped for 3 d by airfreight to Clemson University (Clemson, SC, USA) for vase-life evaluation. Stems were recut at 5 cm from the vase before to be placed on vases with 800 ml of vase solution that was not refilled during the vase life evaluation. The vase solution varied with experiment. Vases were placed in a room with 12 h/d of light and a PPFD of 6-7 µmol.m-2.s-1, 19-21 °C air temperature, and 62%-66% relative humidity for 10 d.

Four leaf senescence symptoms (chlorosis, marginal necrosis, veinal necrosis, and wilting) were recorded on Day 10 in the vase using a visual scale from 0-7, where 0 = 0%; 1 = 1% -10%; 2 = 11%-20%; 3 = 21%-30%; 4 = 31%–50%; 5 = 51%-70%; 6 = 71%-99%; 7 = 100% of the leaf tissue was affected (Fig. 2.2).

Flower form (Expt. 1). Two flower form treatments were initiated 6 wks after transplant in the greenhouse. The spray form was implemented by removing the apical flower bud allowing 5 to 10 axillary flowers to develop per stem. The disbud form was implemented by removing all axillary flower buds in the leaf axils and leaving one apical flower per stem. Stems of both treatments were harvested at the same time 4 wks after bud removal. The cultivars used were BG, GS, P, and S. During harvest, stems were grouped in bunches of five, wrapped in a plastic sleeve, transported to the postharvest room, hydrated in buckets, packaged in cardboard boxes, stored in a cooler for 7 d, and then shipped for 3 d to Clemson University. For the vase life evaluation, each bunch was placed in a vase with 800 ml of water containing a commercial vase solution (10 ml/L FloraLife Express Universal 300, FloraLife, Walterboro, SC, USA). Each of the four senescence symptoms (Fig. 2) were rated for each stem per vase after 10 d vase life. This

experiment was conducted twice. For the first repetition, two vases were evaluated per treatment per cultivar for a total of 10 stems per cultivar per treatment. For the second repetition, four vases were evaluated per treatment per cultivar for a total of 20 stems per cultivar per treatment.

Time of harvest (Expt. 2). Spray forms of chrysanthemums were cultivated, harvested, and hydrated as in Expt.1 and then shipped to Clemson University for vase life evaluation. Stems were harvested between 0700 and 0800 HR, designated AM, or between 1200 and 1300 HR, designated PM. Upon arrival, stems were placed in vases with 800 ml of water containing a commercial vase solution (10 ml/L FloraLife Express Universal 300, FloraLife, Waltersboro, SC, USA). A bunch of five stems per treatment was sleeved and placed in one vase. Each of the four senescence symptoms (Fig. 2) were rated for each stem per vase after 10 d vase life. This experiment was conducted twice. Cultivars BG, GS, P, and S were used for the first repetition. Two vases per treatment and cultivar were evaluated for the first repetition for a total of 10 stems per cultivar. Cultivars A, BG, GS, L, MP, MT, P, PS, S, W, and Z were used for the second repetition per treatment. Four vases per treatment and cultivar were evaluated for the second repetition for a total of 20 stems per cultivar per treatment.

Sucrose in the vase solution (Expt. 3). Spray form stems of chrysanthemum were cultivated, harvested, and hydrated as described in Expt. 1 and then shipped to Clemson University for vase life evaluation. Four sucrose (pure granulated sugar) concentration solutions were prepared (0, 0.1, 0.2, and 0.3 g/mL). Stems were placed in vases with 800 ml of each sucrose concentration in addition to a commercial hydration solution (5 ml/L,

Floralife Hydraflor 100, FloraLife, Waltersboro, SC, USA) at a pH of 3.8. A bunch of five stems per treatment and cultivar were sleeved and placed in one vase. Each of the four senescence symptoms (Fig. 1) were rated for each stem per vase after 10 d vase life. This experiment was repeated two times. Cultivars A, BG, L, MP, MT, P, PS, S, W, and Z were used for the first repetition. Two vases per treatment and cultivar were evaluated for the first repetition for a total of 10 stems per cultivar per treatment. Cultivars BG, MT, P, PS, S, and W were used for the second repetition. Four vases per treatment and cultivar were evaluated for the second repetition for a total of 20 stems per cultivar per treatment.

Sugar sources and concentrations (Expt. 4). Spray form stems of chrysanthemum cultivars BG, P, S, and W were grown, harvested, and hydrated as described in Expt. 1 and then shipped by airfreight to Clemson University for a 7-d vase life evaluation. Stems were placed without sleeves into the vase. Four sugar sources were evaluated: dextrose, d- fructose (fructose), d-mannitol (mannitol), and sucrose (granulated or white sugar). Four solution concentrations were prepared for each sugar source (0.025, 0.050, 0.075, and 0.100 g/mL) along with a 0 g/mL control. Stems were placed in vases with 800 ml of each sugar solution, which also contained a commercial a hydration solution product (5 ml/L Floralife Hydraflor 100, FloraLife, Waltersboro, SC, USA) at a pH of 3.8. Five stems per treatment and cultivar were placed per vase, and four vases per treatment and cultivar were evaluated for a total of 20 stems per cultivar per treatment. Each of the four senescence symptoms (Fig. 1) were rated for each steam per vase after 7-d vase life. This experiment was performed once.

Changes of nonstructural carbohydrates after harvest (Expt. 5). Leaves were collected from spray form stems of chrysanthemum cultivars BG, GS, and P at five different times: immediately prior to harvest in the greenhouse, in the postharvest room after 12 h in the hydration solution, after shipment on Day 0, 4, and 10 in the final vase solution. All leaves were removed from one stem per cultivar, dried in a microwave oven for 7-8 minutes and ground for 1 min using an electric grinder until the particles were <2 mm. Sugar extraction was done by ethanol extraction (Protocol S1: sugar extraction, supplementary data, Landhäusser et al. 2018). Ground leaf tissue (30 mg) was placed in 2 ml microtubes. For each batch of samples, three more tubes were added, 30 mg of sucrose and d-fructose standards, and a blank. 1.5 mL of 80% ethanol was added to each microtube and shaken. Tubes were heated in a 90 °C bath for 10 min and then centrifuged (Landhäusser et al. 2018). The supernatant was transferred to a new microtube and dried for 4 h at 60 °C. 1 mL of deionized water was added to each tube. Then, microtubes were shaken by vortex, heated to 90 °C for 5 min, and shaken again. The tubes were centrifuged after returning to room temperature. The supernatant was used to determine glucose, fructose, and sucrose concentrations by measuring absorbance at 340 nm in a microplate reader (BioTek ELX800UV, US BioTek laboratories, USA) (Protocol S4: Quantification of glucose, fructose, and sucrose by enzyme, supplementary data (Landhäusser et al. 2018). Calculations for nonstructural carbohydrates (NSC) concentrations were performed following the final calculations of NSC concentrations were quantified by the enzyme method (Protocol S4, S6, Landhäusser et al. 2018).

Statistical design and analysis. A randomized incomplete blocks experiment design was used for all experiments with the stems as the experimental units and vases as blocks. Five stems per cultivar were placed on each vase, and the number of vases per treatment varied among experiments. Data were analyzed by ANOVA, and significant mean differences were analyzed by Fisher's least significant difference test ($\alpha = 0.05$).

Results and discussion

Flower form (Expt. 1). Chrysanthemum stems with the spray form showed higher leaf senescence severity across all four symptoms compared with the disbud form for all cultivars except S, which showed no differences between flower forms (Fig. 2.3). Shrek was the only cultivar that possesses a daisy-type flower, which has disk florets surrounded by an array of ray florets. The other three cultivars have decorate-type flowers, which only have ray florets (Fig. 2.1). The decorative flowers may have a higher carbohydrate demand during flower opening possibly related with a high number of flowers per stem (decorative flowers cultivars had 7 to 9 flowers, compared to other types of flowers with 5 to 7 flowers) or leaf size (decorative flowers had smallest leaves than the other type of flowers), but this would require additional experimentation to confirm.

Growers report that rapid leaf senescence is only observed on spray-form chrysanthemums, while disbud forms are not susceptible; however, different cultivars are grown in spray and disbud forms. This study is the first to grow the same cultivars simultaneously in both forms. The results demonstrate that a cultivar grown in the spray form with 5-10 axillary flowers is more susceptible to displaying rapid leaf senescence symptoms than a cultivar grown in the disbud form with a single, terminal flower. Figure

2.4 shows the symptoms observed on stems of cultivar P after 10 d in the vase. The higher flower count of the spray form appears to exceed the source capacity of the leaves more rapidly than the single flower disbud form (Burnett 2019). A previous study has reported that increasing the source size (leaf area) of cut chrysanthemum resulted in a larger sink size (flower size) (Carvalho et al., 2006).

Time of harvest (Expt. 2). Stems harvested in the morning between 0700 and 0800 HR showed higher chlorosis, marginal necrosis, veinal necrosis, and wilting severity for 8, 7, 8, and 8 of the 11 cultivars, respectively, than stems harvested between 1200 and 1300 HR (Fig. 2.5). Figure 2.6 displays these results for the cultivar S. Three cultivars (L, MP, Z) displayed no response to the harvest time or performed better with morning harvests. Interestingly, these cultivars all have purple flower petals (Fig. 1). Chrysanthemums with purple flowers are particularly susceptible to experiencing petal burn due to the petals warming to higher temperatures during sunny conditions.

Previous studies showed that diurnal changes in carbohydrate concentrations affect the postharvest longevity of leafy green crops (Clarkson et al. 2005), carnation flowers (Verlinden and Garcia 2004), broccoli (*Brassica oleracea*) florets (Nishikawa et al. 2005), and portulaca (*Portulaca grandiflora*) cuttings (Rapaka et al. 2006). For example, it has been reported for portulaca cuttings that total sugar (glucose, fructose, and sucrose) concentrations significantly increased in leaves daily from 0800 to 1200 HR and starch from 1200 to 1600 HR (Rapaka et al. 2006). Therefore, morning harvest can negatively impact postharvest longevity by having a lower carbohydrate supply than harvesting later in the day, related to carbohydrate production and accumulation during

the light period of the day. Higher endogenous carbohydrates in cut flowers reduce ethylene sensitivity and the resulting senescence symptom development (Elgar et al. 1999; Ichimura et al. 2000b; Ichimura et al. 2003).

Sucrose in the vase solution (Expt. 3). Sucrose concentrations of 0.1 and 0.2 g/mL in the vase solution resulted in reduced leaf senescence severity for all four symptoms for eight (A, BG, GS, L, MT, P, PS, S, and W) of the 10 cultivars, while the other two cultivars (MP, Z) showed reduced leaf senescence severity for three of the four symptoms (Fig. 2.7). Figure 2.8 visually demonstrates these results for the P. No adverse results were observed amongst the cultivars and sucrose concentrations. For the chlorosis, marginal necrosis, and wilting symptoms, six to eight of the 10 cultivars performed better or the same at 0.1 g/mL sucrose compared to 0.2 g/mL sucrose. For the veinal necrosis symptom, 9 of the 10 cultivars performed better or the same at 0.2 g/mL sucrose compared to 0.1 g/mL sucrose.

The addition of sugars in the vase solution allows flowers to maintain their metabolic processes, and thus the leaf tissues were maintained for an extended period. Research has shown that vase solutions containing sugars help to extend the vase life of cut flowers by several days; however, the optimal type and concentration of sugar sources vary with species. For example, the addition of sucrose in the vase at concentrations between 0.04 to 0.06 g/mL benefit chrysanthemum (Zamani et al. 2011b; Amin 2017a; Amin 2017b), gerbera daisy (De Silva et al. 2013), gladiolus (Mayak et al. 1973), and sweet pea (Ichimura 1998); sugar concentrations between 0.02 to 0.03 g/mL benefit lisianthus (Ichimura 1998), lotus (Chathuri and Sarananda 2011) and rose (Ichimura et al.

2005), while sugar concentrations between 0.010 to 0.015 g/mL improve snapdragon vase life (Ichimura 1998; Asrar 2012).

Our results demonstrate that sucrose concentrations (0.1 and 0.2 g/mL) provided in vase solutions reduced leaf senescence symptoms. The 0.2 g/mL solution increased the chlorosis, marginal necrosis, and wilting for four (L, MT, PS, and W), 5 (BG, L, MT, PS, and W) and three (MT, PS, and W) of the 10 cultivars (A, BG, L, MP, MT, P, PS, S, W, and Z), respectively, compared to the 0.1 g/mL treatment. This could be related to the accumulation of sugars in the margin of the leaves, causing cell collapse as has been reported for cut roses treated with sucrose concentrations >0.025 g/mL (Markhart and Harper 1992). Discoloration of the center of the P flowers was observed for sucrose treatments, more severe for 0.2 g/mL sucrose, possible related with over open flowers symptom (Fig. 2.8).

Sugar sources and concentrations (Expt. 4). Sucrose at 0.025 and 0.050 g/mL, followed by fructose at 0.025 g/mL and dextrose at 0.05 g/mL showed an improvement in leaf performance for all the cultivars evaluated (Fig. 2.9) as is shown in Fig. 2.10 for the cultivar W. Published studies of sugar evaluations such as glucose, fructose, and sucrose into vase solutions for roses at concentrations that vary between 0.025 and 0.1 g/mL each sugar shown an improvement in the cut flower stem performance during the vase life, reducing bent neck and leaves yellowing and wilting symptoms during vase life for different cut roses evaluated (Locke 2010; Rajya et al. 2022). For chrysanthemum, 0.02 and 0.04 g/mL sucrose vase solutions reduced senescence symptoms severity and increased vase life to 14 (El-Ghait et al. 2012; Jain 2014) and 10 d, respectfully

(Mashhadian et al. 2012). In contrast, mannitol caused an increase in all four senescence symptoms severity compared to the control (Fig 2.9). Halevy and Mayak (1979) concluded that the addition of non-metabolic sugars such as mannitol, increases senescence symptoms due to their incapacity to enter the respiration cycle as an energy supply.

Changes of nonstructural carbohydrates after harvest (Expt. 5). Leaf starch concentration decreased from harvest to the end of vase life for both cultivars (Fig. 2.11). Starch concentration of P dropped rapidly immediately after harvest, while GS decreased during storage. The concentration of the combined sugars (glucose + fructose; glucose + fructose + sucrose) went down during hydration, storage and shipping and then went up when sugar was supplied in the vase solution.

Sugar balance in the leaves after plants are harvested is not well understood; studies show leaves are not the primary source of soluble sugars in mum. Stems have a higher concentration because they serve as conduits for transporting sugars from the leaves, where photosynthesis occurs, to other parts of the plant for energy storage and use (Rajapakse). The constant concentration variation of sugars such as glucose, fructose, and sucrose could be caused by starch depletion, as starch is broken down into simpler sugars (glucose and fructose) due to enzymatic activity, the levels of these soluble sugars in the leaf tissue may increase to be stored, used as an immediate source of energy and also transform in sucrose, that this last one is transporter more efficiently to the sinks structures such as flowers (Taiz, et al 2015), but the exact role of sugars and how they are translocated in tissues are not clear. It has been reported that adding sugars into the vase

solution alters carbohydrate metabolism (Da Silva 2003), which could increase sugar concentrations in leaf tissue during the vase life.

In order to confirm sugar changes over time, repeating measurements on leaves and stems would be suggested.

Conclusions

This study demonstrates a high incidence of leaf senescence symptoms occur when there is a low carbohydrate supply at harvest (morning harvest), a high carbohydrate demand due to the flower form (spray flower form) or a low postharvest supply (no sugar in the vase solution). Veinal necrosis and chlorosis symptoms appear to be correlated with leaf senescence symptoms because their presence was similar among cultivars. Similarly, marginal necrosis and wilting symptoms appear to be correlated with leaf senescence symptoms. Sucrose and fructose at 0.025 and 0.05 g/mL in the vase solution were the most practical and effective means of improving leaf performance for cut flower chrysanthemum.

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Figure 2.1. Cultivars used for the different experiments: A. Alligator, B. Bomber Green, C. Green Screen, D. Lychee, E. Mark Twain, F. Molly Purple, G. Paintball Sunny, H. Peridot, I. Shrek, J. WhatsApp, and K. Zumba.

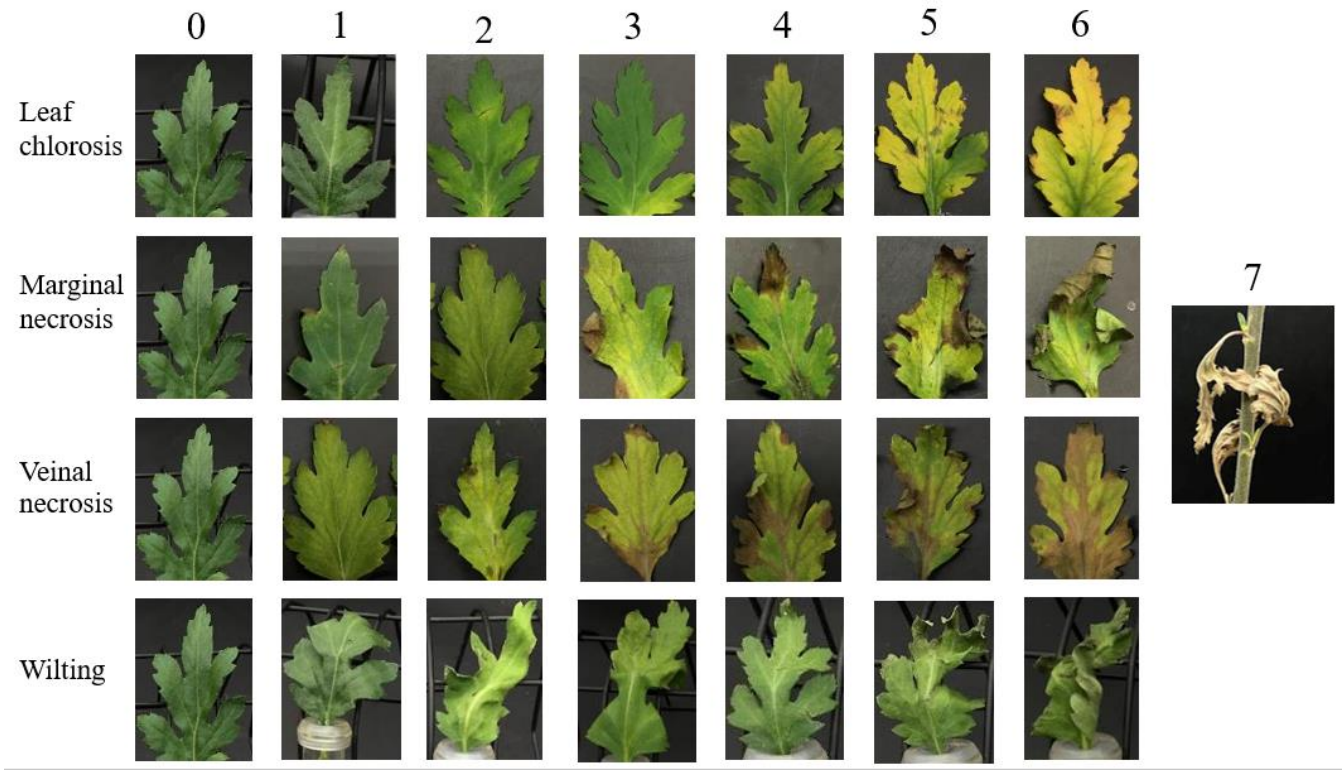

Figure 2.2. Leaf senescence progression scales for four symptoms (leaf chlorosis, marginal necrosis, veinal necrosis, wilting) of chrysanthemum during vase life evaluation after 10 d. Rating 7 (100% symptom severity) was not distinguishable among the four symptoms.

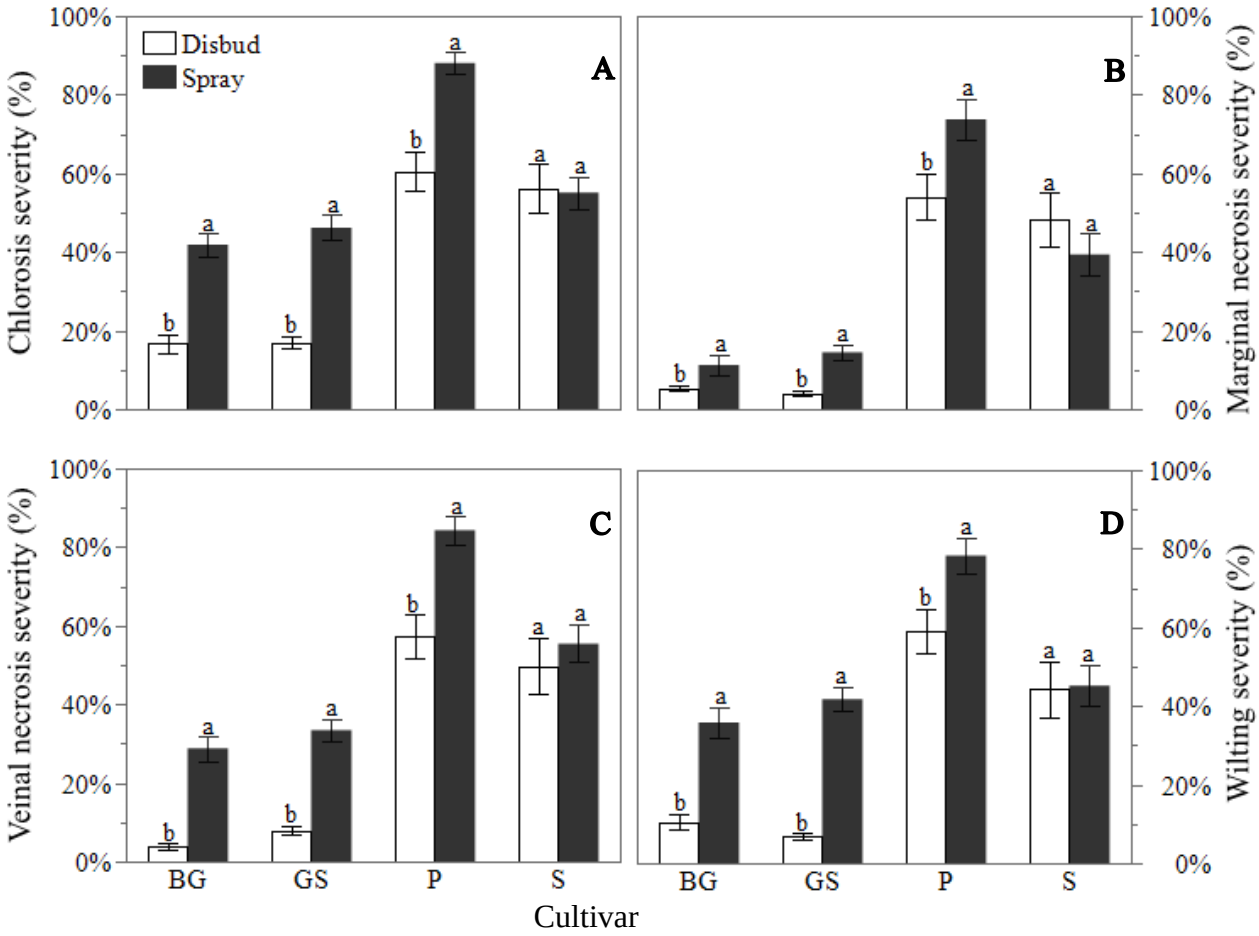

Figure 2.3. Effect of two flower forms on the leaf senescence symptom severity (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) on four chrysanthemum cultivars (Bomber Green (BG), Green Screen (GS), Peridot (P), and Shrek (S)) (Expt. 1). The disbud form is produced by removing the axillary flower buds and leaving one terminal flower, while the spray form is produced by removing the main flower bud and leaving multiple axillary flowers. Leaf senescence symptoms were recorded on day 10. Error bars = ±1SE. Different letters indicate significant differences within cultivar and symptom using Fisher's least significant difference test at $P<0.05$.

Figure 2.4. Chrysanthemum ‘Peridot’ grown in two forms: A. spray form (main flower bud removed, leaving multiple axillary flowers) and B. disbud form (axillary flower buds removed, leaving one terminal flower) (Expt. 1). Photos were taken at 10 d of vase life.

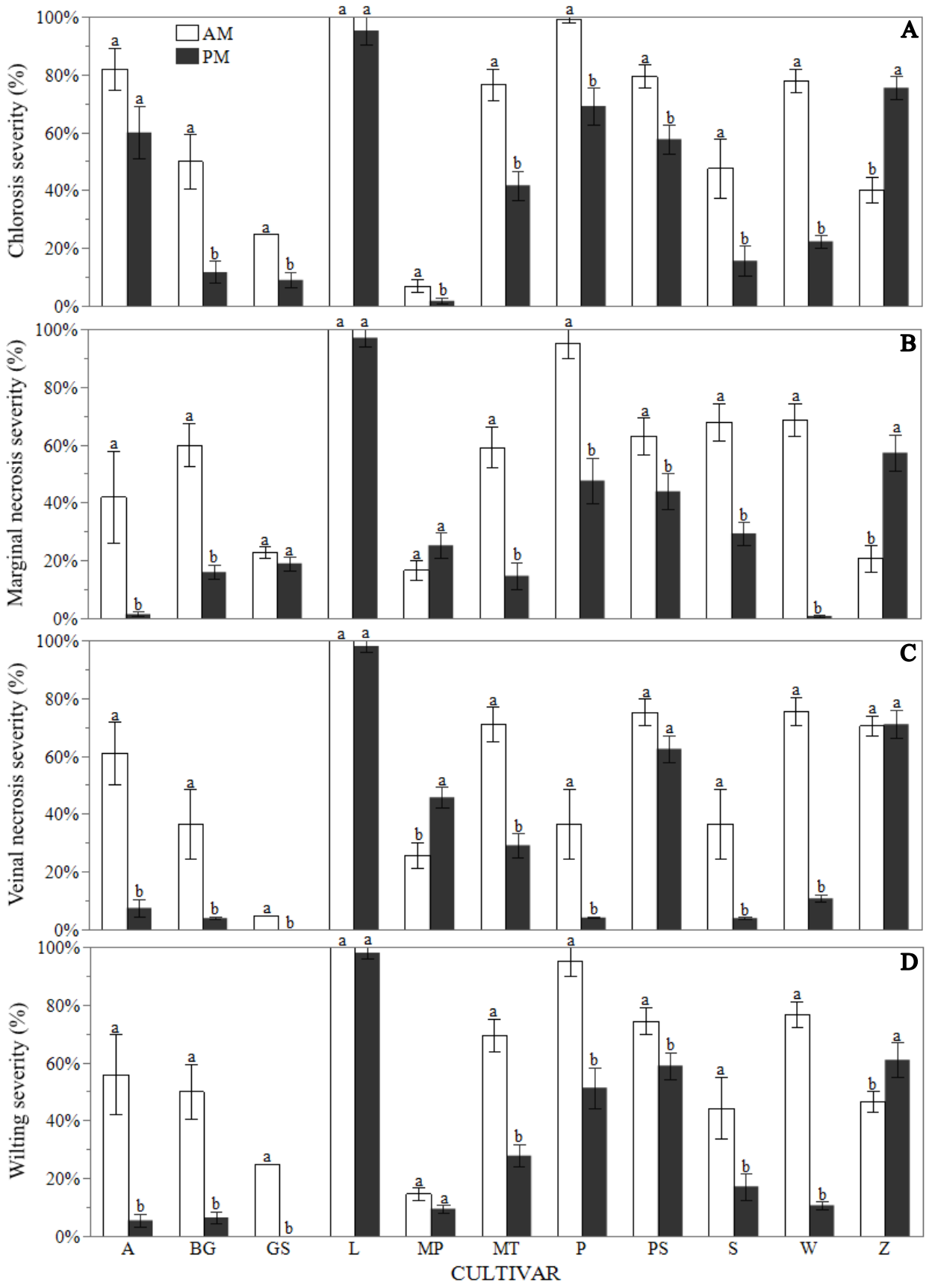
AM
PM
A
B
C
D
Chlorosis severity (%)
Marginal necrosis severity (%)
Veinal necrosis severity (%)
Wilting severity (%)
100%
80%
60%
40%
20%
0%
A
BG
GS
L
MP
MT
P
PS
S
W
Z
CULTIVAR

Figure 2.5. Effect of time of harvest, AM (stems harvested between 0700 and 0800 HR) vs. PM (stems harvested between 1200 and 1300 HR) on the leaf senescence symptom severity (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) on chrysanthemum cultivars Alligator (A), Bomber Green (BG), Green Screen (GS), Lychee (L), Molly purple (MP), Mark Twain (MT), Peridot (P), Paintball Sunny (PS), Shrek (S), WhatsApp (W), and Zumba (Z) (Expt. 2). Leaf symptoms were recorded on day 10. Error bars = ±1SE. Different letters indicate significant differences within cultivar and symptom using Fisher's least significant difference test at $P<0.05$.

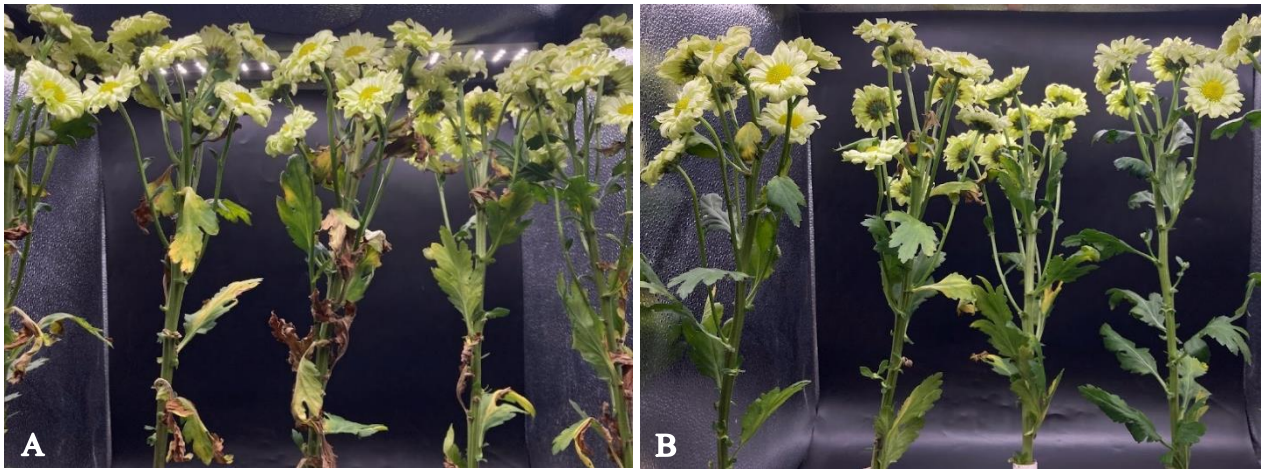

Figure 2.6. Chrysanthemum Shrek harvested at A. in the morning (0700-0800 HR) and B. in the afternoon (1200-1300 HR) (Expt. 2). Pictures were taken at 10 d vase life.

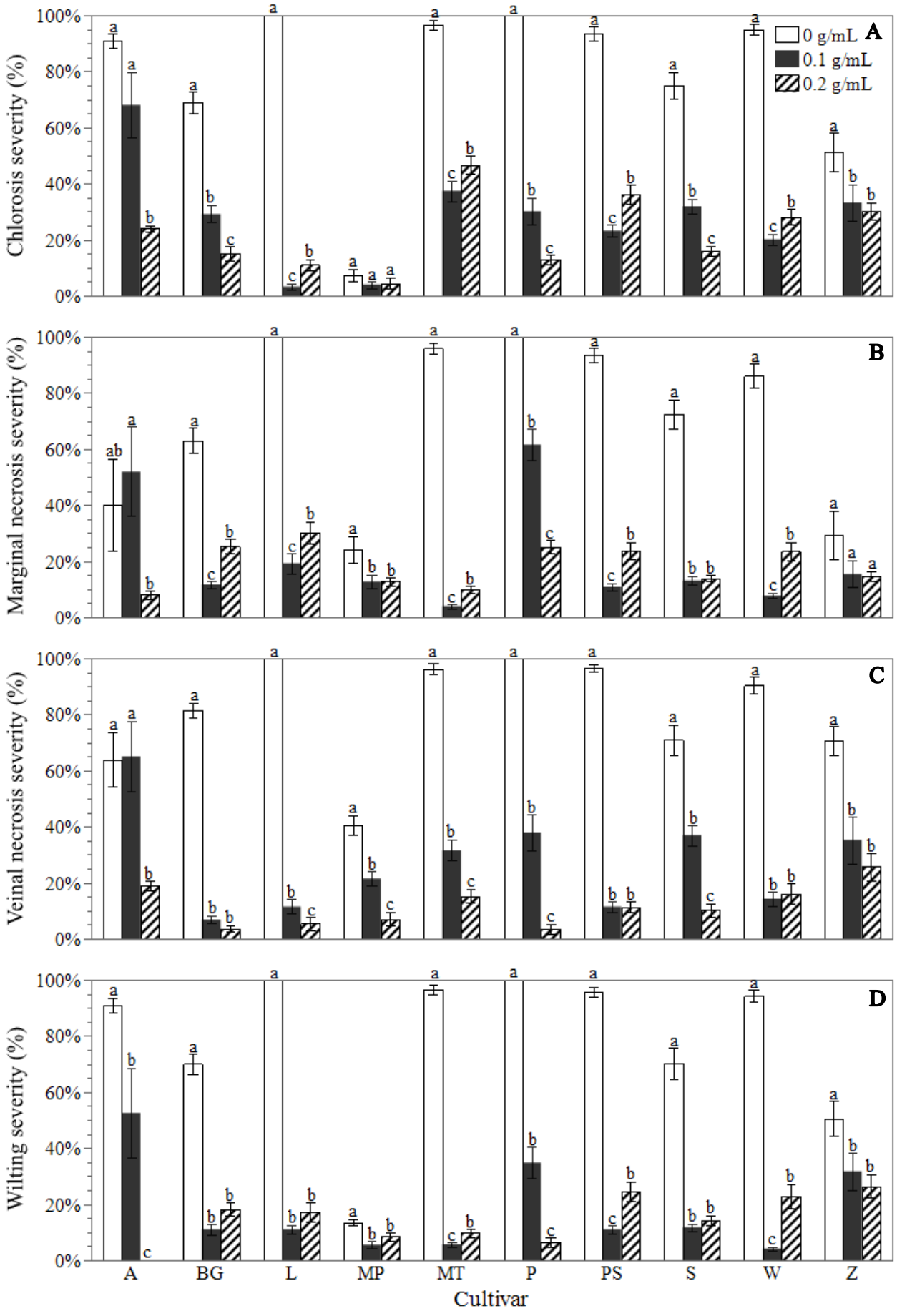
A
B
C
D
0 g/mL
0.1 g/mL
0.2 g/mL
Chlorosis severity (%)
Marginal necrosis severity (%)
Veinal necrosis severity (%)
Wilting severity (%)
100%
80%
60%
40%
20%
0%
A
BG
L
MP
MT
P
PS
S
W
Z
Cultivar

Figure 2.7. Effect of sucrose concentration in the vase solution (0, 0.1, and 0.2 g/mL) on the leaf senescence symptoms (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) severity on chrysanthemum cultivars Alligator (A), Bomber Green (BG), Lychee (L), Molly purple (MP), Mark Twain (MT), Peridot (P), Paintball Sunny (PS), Shrek (S), WhatsApp (W), and Zumba (Z). Leaf symptoms were recorded on day 10. Error bars = ±1SE. Different letters indicate significant differences within cultivar and symptom using Fisher's least significant difference test at $P<0.05$.

Figure 2.8. Chrysanthemum ‘Peridot’ placed into vase solutions at sucrose concentrations A. 0 g/mL or B. 0.1 g/mL. Pictures were taken after 10 d of vase life.

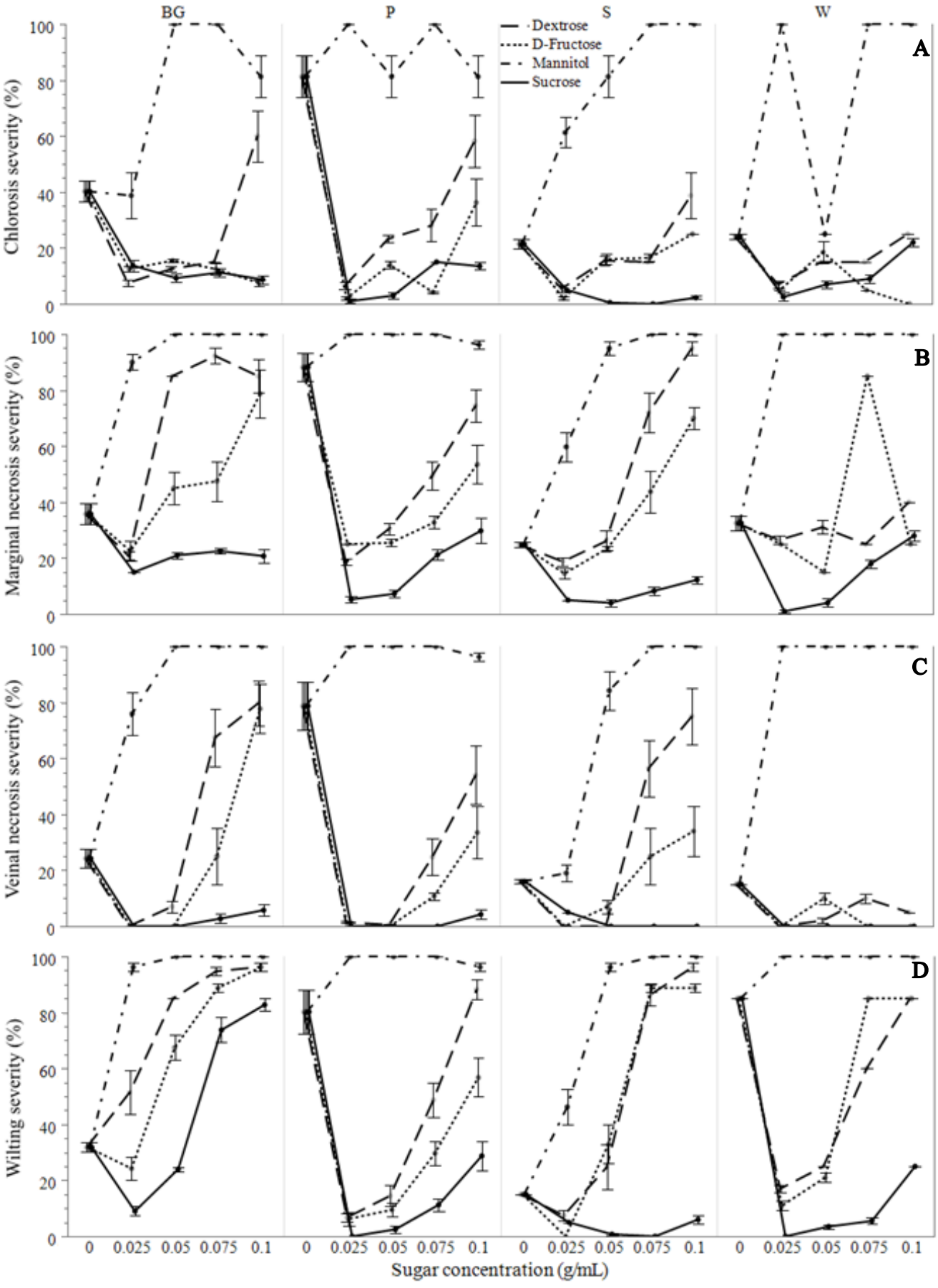
BG
P
S
W
Dextrose
D-Fructose
Mannitol
Sucrose
A
B
C
D
Chlorosis severity (%)
Marginal necrosis severity (%)
Veinal necrosis severity (%)
Wilting severity (%)
0
20
40
60
80
100
0
0.025
0.05
0.075
0.1
Sugar concentration (g/mL)

Figure 2.9. Effect of four sugar sources (dextrose, fructose, mannitol, sucrose) at 0, 0.025, 0.05, 0.075, 0.1 g/mL in the vase solution on the leaf senescence symptom severity (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) of chrysanthemum cultivars Bomber Green (BG), Peridot (P), Shrek (S) and WhatsApp (W). Leaf symptoms (chlorosis, marginal necrosis, veinal necrosis, and wilting) were recorded on day 10 in the vase. Error bars = ±1SE. Different letters indicate significant differences within cultivar and symptom using Fisher's least significant difference test at $P<0.05$.

Figure 2.10. Chrysanthemum ‘WhatsApp’ was placed into the vase with sucrose concentrations A. 0 g/mL or B. 0.025 g/mL. Pictures were taken after 7 d of vase life.

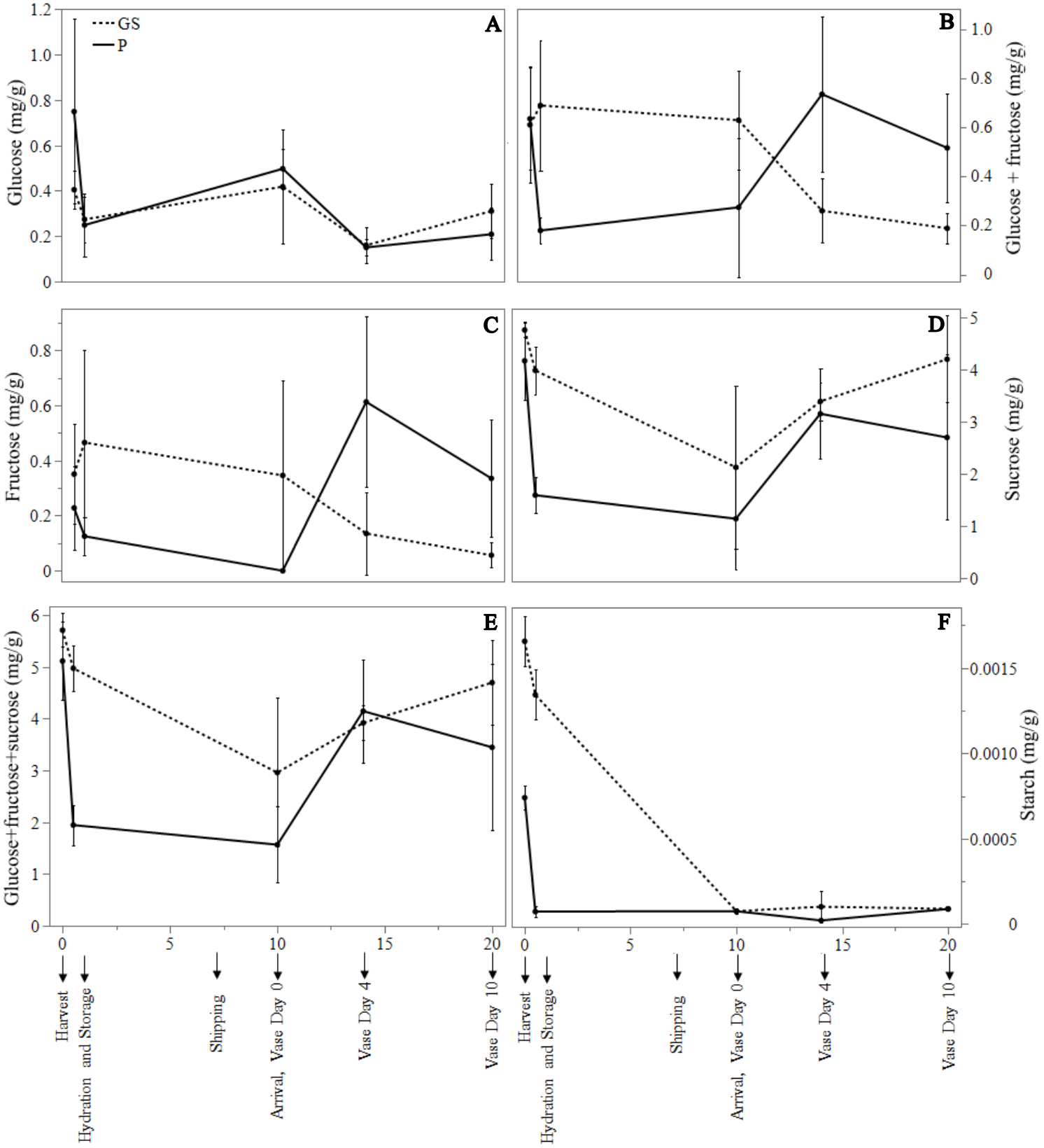
GS
P
A
B
C
D
E
F
Glucose (mg/g)
Glucose + fructose (mg/g)
Fructose (mg/g)
Sucrose (mg/g)
Glucose+fructose+sucrose (mg/g)
Starch (mg/g)
Harvest
Hydration and Storage
Shipping
Arrival, Vase Day 0
Vase Day 4
Vase Day 10

Figure 2.11. Leaf sugars (A. glucose, B. glucose plus fructose, C. fructose, D. sucrose, E. total sugars, F. starch). Measurements for cultivars Green Screen (GS), and Peridot (P) in the greenhouse during harvest (Field), after a 12 h hydration (Hydration), and after 10 days storage and shipment (Vase day 0), and 4 (Vase day 4) and 10 d (Vase day 10) in a vase solution containing control solution (10 ml/L FloraLife Express Universal 300, FloraLife, Waltersboro, SC, USA). Error bars = ±1SE.

CHAPTER THREE

EXPLORING PREHARVEST AND POSTHARVEST STRATEGIES FOR REDUCING LEAF SENESCENCE SYMPTOMS IN CUT CHRYSANTHEMUM

Abstract

Leaf senescence is the primary limitation to the postharvest longevity of cut flower chrysanthemum. The objective of this research was to evaluate preharvest and postharvest strategies to reduce leaf senescence severity as defined as chlorosis, marginal necrosis, veinal necrosis, and wilting. Preharvest treatments included the application of plant growth regulators (PGR), biorational products, and biocides. Chrysanthemum stems were harvested, placed in a hydration solution, stored, shipped, placed in a holding solution, and then placed in vase solutions for vase life evaluation. Postharvest treatments included hydration time and environment, PGR application, sucrose in the hydration solution, post-shipping holding solution, and vase solution. The most effective strategies for reducing leaf senescence were PGR application in the hydration solution for 4 h after harvest, and sucrose in the vase solution. Application of thidiazuron, and a mixture of benzyladenine and gibberellic acid at 10 or 20 ppm showed 35% to 45% reduction of the four leaf-senescence symptoms for cultivars Bomber Green, Green Screen, Peridot, Paintball Sunny, Shrek, and Zumba. Addition of 0.025 or 0.05 g/mL sucrose into the vase solution showed between 20% to 60% reduction on senescence symptoms on cultivar Bomber Green, WhatsApp, Green Screen and Peridot.

Keywords: chlorosis, marginal necrosis, plant growth regulator, sugar, vase life, veinal necrosis, wilting.

Introduction

Chrysanthemum (*Chrysanthemum ×morifolium*) is the second-largest exported cut flower worldwide; however, the leaves of some commercial cultivars exhibit rapid leaf necrosis during the first week in the vase (Dole and Faust 2021). This characteristic negatively impacts consumer perception of plant quality, which creates a risk of sales reduction. Chapter 2 demonstrated that carbohydrate depletion under preharvest and postharvest conditions increases the incidence of rapid leaf senescence, as observed from leaf chlorosis, necrosis, and wilting symptoms. The addition of carbohydrates such as sucrose or fructose in concentrations between 0.025 and 0.05 g/mL in the vase solution improved vase life performance for chrysanthemum (Ortiz 2023; Zamani et al. 2011; Amin 2017a, 2017b), gerbera daisy (*Gerbera ×hybrida*) (De Silva et al. 2013), gladiolus (*Gladiolus sp*.) (Mayak et al. 1973a), and sweet pea (*Lathyrus odoratus*) (Ichimura 1998).

Cytokinins are involved in carbohydrate metabolism by regulating sink strength, photosynthate partitioning, and phloem unloading (Roitsch and Ehneß 2000; Burnett 2019). Cytokinins can be exogenously applied as a synthetic plant growth regulator (PGR) (Burnett 2019) or as biorational products that stimulate endogenous cytokinin production, e.g., inoculation with *Bacillus subtilis* promotes cytokinin responses in lettuce (*Lactuca sativa*) (Arkhipova et al. 2005).

Also, the use of biorational such as salycilic (SA) wich is a signal molecule mediating that promote cell division and cell expansion, activating multiple pathways such as cytokinin pathways (Li et al. 2022).

This study aimed to evaluate preharvest and postharvest strategies to reduce leaf collapse in cut chrysanthemum stems. Preharvest treatments included PGR and biorational products. Postharvest treatments included hydration time and environment, PGR application, and sucrose in the hydration solution, post-shipping holding solution or vase solution.

Materials and methods

Plant production. Chrysanthemum cut flowers were grown by commercial growers in Cundinamarca, Colombia, South America near the towns of Facatativa, Madrid, and Tenjo, and in Antioquia near Rionegro. Spray form cultivars (stems with 5 to 10 axillary flowers) were chosen for their high susceptibility to exhibiting rapid leaf senescence symptoms. The following cultivars were grown in Cundinamarca: Bomber Green (BG), Green Screen (GS), Peridot (P), and Shrek (S), or in Antioquia: Alligator (A), Lychee (L), Mark Twain (MT), Molly Purple (MP), Paintball Sunny (PS), WhatsApp (W), and Zumba (Z). The cultivar L was grown in the disbud flower form, while A, BG, GS, MT, MP, P, PS, S, W, and Z were grown in the spray form. The cultivars A, BG, GS, P, S, and W have green flowers, L, MP, and Z have purple flowers, MT has white flowers, and PS has yellow flowers. A, BG, L, and Z have cushion flowers (compact, globe-shaped blooms), S has daisy flowers (disk florets surrounded by an array of ray florets), MP has micro-daisy flowers, MT, PS, and W have button flowers (small, dense, cushion flowers), and GS and P are decorative or novelty flowers (outer array of flowers are long, ray florets and inner flowers are short, ray florets) (Fig 2.1). Plants were grown in ground beds at a density of 92 or 96 plants/m^2 inside greenhouses covered

with a single layer of polyethylene. Night-interruption lighting was provided during the first four weeks after transplant, while the remaining weeks were under ambient photoperiods of ~12 h.

Postharvest. Stems were harvested at commercial maturity, defined as at least three open flowers/stems for stems grown in the spray form and a stem length of $\geq$80 cm. Immediately after harvest, stems were placed in buckets that contained a 5 L hydration solution (2 mL/L Florissima 925, Florissima, Colombia) adjusted to pH 4.0 and containing 0.8% free chlorine for 12 h. Then the stems were placed in cardboard boxes and stored for 7 d in a cooler at 4 °C. Boxes were then shipped by airfreight to Clemson University (10 d between storage and shipment) for 10 d vase-life evaluation. Stems were recut at 5 cm from the base before being placed in vases with 800 ml of vase solution that was not refilled during the vase life evaluation. The vase solution varied with experiment. Vases were placed in a room with a PPFD of 6-7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of 12 h/d, 19-21 °C air temperature, and 62%-66% relative humidity for 10 d. Holding solutions after shipment (carbohydrates, acid and biocide solutions) were used for experiment 7.

Experiments. Eight experiments were conducted. The first two preharvest experiments focused on treatments applied in the greenhouse before harvest, and these experiments were made at the same farm where the flowers were grown in Cundinamarca or Antioquia, such as PGRs and biorational products application. For the experiments made after harvest but before shipping the stems were grown and treated with standard methods during package, storage, shipment, and vase life. The remaining postharvest

experiments focused on treatments applied following shipment both harvest and postharvest processes made on the farm followed the commercial processes.

Preharvest PGR application (Expt. 1). Stems of the cultivars BG, GS, MT, P, PS, S, and W were treated once weekly in the greenhouse during the last 3 weeks of the 11-week crop cycle using a backpack pressure sprayer. For the first two experimental replications, cytokinin applications included benzyladenine (10 ppm BA) (0.5 mL/L Chrysal Viva, Chrysal Colombia S.A, Bogota, Colombia), thidiazuron (10 ppm TDZ) (0.025 g/L Thidiazuron 50 wp, DVA Agro Alemania, Colombia) or a mixture of cytokinins and gibberellic acid (10 ppm BA+ 10 ppm GA (GA_{4+7})) (0.56 mL/L Fascination, Valent BioSciences Corp., USA). For the third replication, BA, TDZ, and BA+GA were applied weekly at the same concentrations for 1, 2, or 3 weeks before stems were harvested. Five stems per cultivar per treatment were placed into each of two vases for each replication. For replication 3, flower deformation was recorded for individual flowers on a stem.

Preharvest biorational application (Expt. 2). Stems of the cultivars BG, GS, P, and S were treated once weekly in the greenhouse during the last three weeks of the 11-week crop cycle using a backpack pressure sprayer. Biorational products included salicylic acid (SA) (2 g/L salicylic acid, Soluciones & Solventes, Colombia) and *Bacillus subtilis* strain QST 713 (10 mL/L Cease, BioWorks, NY, USA). Ten stems per treatment per cultivar were evaluated and placed in two vases for repetition (five stems per vase). This experiment was conducted three times.

Postharvest hydration duration (Expt. 3). Stems of cultivars BG and S were harvested and transported to the postharvest room, where they were placed in buckets of hydration solution (2 mL/L Florissima 925, Florissima, Colombia) adjusted to pH 4.0 and 0.8% free chlorine for 0, 4, 8 or 24 h (room environmental conditions were 18.2 °C and 63.2% relative humidity). This experiment was performed once. Ten stems per treatment per cultivar were placed in two vases (five stems per vase).

Postharvest hydration environment (Expt. 4). Stems of cultivars BG, GS, P, and S were harvested and transported to the processing room where they were placed in a bucket with hydration solution (2 mL/L Florissima 925, Florissima, Colombia) adjusted to pH 4 and 0.8% free chlorine for 12 h inside the cooler at 4.1 °C and 65.7% relative humidity, and hydration solution temperature of 4.9 °C, or outside the cooler at 18.2 °C and 63.2% relative humidity and hydration solution temperature of 16.2 °C. Then the stems were packaged in cardboard boxes in their respective environment. Boxes were shipped together and upon arrival were placed in vases for the vase life evaluation. Ten stems per treatment per cultivar were placed in two vases (five stems per vase). This experiment was conducted once.

Postharvest PGR application in the hydration solution (Expt. 5). For the first replication, 10 ppm BA (0.5 mL/L Chrysal Viva, Chrysal Colombia S.A, Bogota, Colombia), 10 ppm TDZ (0.025 g/L Thidiazuron 50 WP, DVA Agro Alemania, Colombia), 10 ppm GA_3 (0.25 mL/L Progibb 40%, Valent BioSciences Corporation, USA), 10 ppm BA+ 10 ppm GA (GA_{4+7}) (0.56 mL/L Fascination, Valent BioSciences Corporation, USA), 10 ppm indole-3-butyric acid (IBA) (4 mL/L Hormodin 3, OHP, PA,

USA), 20 ppm silver thiosulfate (STS) (2.5 mL/L Chrysal AVB, Chrysal, USA) were mixed into water and pH adjusted to 3.8 with 2 mL/L citric acid, as hydration solution. For the second replication, 10, 20 and 40 ppm BA (0.50, 1.05, and 2.11 mL/L), 10, 20 and 40 ppm TDZ (0.025, 0.050, and 0.075 g/L, and 10, 20 and 40 ppm BA+GA (0.56, 1.11, and 2.22 mL/L) were used as PGR in the hydration solution prepared in equally that the first replication. Stems of cultivars BG, GS, P, and S were used for the first replication, and A, BG, L, MP, MT, P, PS, S, W, and Z were used for the second replication. Treatments were provided by placing stems in the respective treatments for 4 h in the postharvest room (16.7-18.2 °C; 63.2-64.9 % RH). Then, the stems were packaged, stored for 7 d, and shipped to Clemson University (3 d in route). Ten stems per treatment per cultivar were placed in two vases (5 stems per vase). This experiment was repeated three times.

Postharvest sucrose application in the hydration solution (Expt. 6). Three sucrose (pure granulated sugar) concentration solutions were prepared (0, 0.1, and 0.2 g/mL), and the pH was adjusted to 3.8 using citric acid. Stems of cultivars BG, GS, P, and S were placed in those solutions for 4 h, then packed, stored for 7 d, and shipped to Clemson University (3 d in route). Ten stems per treatment per cultivar were placed in two vases (5 stems per vase) to be evaluated. This experiment was conducted once.

Postharvest sucrose application in holding solution (*Expt. 7)*. At Clemson University, sucrose concentrations of 0, 0.1, and 0.2 g/mL were prepared in 5 L solutions, and pH was adjusted to 3.8 using a commercial hydration solution (5mL/L FloraLife Hydraflor 100, FloraLife, Walterboro, SC, USA). Shipped stems of cultivars MT, PS, and

W were unpacked and placed for 4 h for the first replication and 8 h for the second replication in the holding solution sucrose treatments. Then stems were placed in vases for the vase life evaluation (10 ml/L FloraLife Express Universal 300, FloraLife, Walterboro, SC, USA). Ten stems per treatment per cultivar were placed in two vases (5 stems per vase) to be evaluated. This experiment was conducted once.

Postharvest sucrose application in vase solution (Expt. 8). At Clemson University, five sucrose (pure granulated sugar) concentration solutions were prepared (0, 0.025, 0.050, 0.075, and 0.10 g/mL). Stems were placed in vases with 800 ml of each sucrose concentration solution; pH was adjusted to 3.8-4.3 using a commercial hydration solution (5 ml/L Floralife Hydraflor 100, FloraLife, Walterboro, SC, USA). Five stems per cultivar were placed per vase and 4 vases were used, for a total of 20 stems per treatment. This experiment was conducted twice. Cultivars BG, P, S, and W were used for the first replication, and BG, GS, and P were used for the second replication.

Data collection. Four leaf senescence symptoms (chlorosis, marginal necrosis, veinal necrosis, and wilting) were recorded on Day 10 in the vase using a visual scale from 0-7, where 0 = 0%; 1 = 1% -10%; 2 = 11%-20%; 3 = 21%-30%; 4 = 31%–50%; 5 = 51%-70%; 6 = 71%-99%; 7 = 100% of the leaf tissue was necrotic (Fig. 2.2). For Expt. 1, flower deformation data was recorded on Day 10 in the vase measuring the percentage of deformed or aborted flowers.

Statistical design and analysis. A randomized incomplete block experimental design was used for all experiments with the stems as the experimental units and vases as blocks. Five stems per cultivar were place in each vase, and the number of vases per

treatment varied among experiments. Data were analyzed by ANOVA, and significant mean differences were analyzed by Fisher's least significant different test ($\alpha = 0.05$).

Results and discussion

Preharvest PGR application (Expt. 1). For the first two repetitions preharvest application with PGRs showed lower senescence symptom severity compared to the control for all four symptoms for all cultivars tested, except S (Fig. 3.1). The lowest chlorosis, veinal necrosis, and wilting severity was observed for BG, GS, P, PS and S stems treated with 10 ppm TDZ. For cultivar MT the 10 ppm BA and 10 ppm BA+GA treatments showed less marginal necrosis than 10 ppm TDZ-treated stems. Flower abortion, short peduncles, and leaf marginal necrosis symptoms were observed in the greenhouse before stems were harvested for the 10 ppm BA and 10 ppm TDZ treatments for all the cultivars. Thus, improvement in leaf performance occurred at the expense of flower quality; therefore, the number of applications prior to harvest was evaluated in third replication of the experiment.

For the third repetition of Expt. 1, all symptom occurrence was reduced with applications of 10 ppm TDZ for all cultivars, except BG, for which 10 ppm BA reduced chlorosis and three applications of 10 ppm BA+GA reduced veinal necrosis (Table 3.1). Marginal necrosis symptom severity was reduced with two applications of 10 ppm BA and 10 ppm BA+GA on BG, and one application of 10 ppm TDZ on P. Marginal necrosis was reduced on GS with 10 ppm BA treatments, one or two applications of 10 ppm BA+GA, and one and three applications of TDZ. For BG, one and three applications of 10 ppm TDZ increased marginal necrosis severity compared to the control. Also, one and

three applications of 10 ppm BA+GA increased wilting compared to the control (Table 3.1).

Flower deformation and abortion was observed for all preharvest PGR treatments for all cultivars. Fig. 3.2 displays the range of results obtained for treatments made to P.

Previous work reported that concentrations between 2 and 12 ppm TDZ showed postharvest benefits, such as reduced leaf yellowing and senescence, improved maintenance of leaf chlorophyll, inhibition of abscisic acid (ABA) biosynthesis, and reduction in ethylene sensitivity (Ferrante et al. 2004; Uthairatanakij et al. 2007). It was reported that 20 ppm TDZ applications stimulate ethylene production in plants causing leaf abscission, thus TDZ is used for inducing cotton defoliation prior to harvest (Cathey 1986).

Exogenous BA application during flower formation inhibited flowering of arabidopsis (*Arabidopsis thaliana*) (Karunadasa et al. 2020). Also, peduncle elongation and smaller flowers were observed in the stems treated with BA+GA; these symptoms were also reported on arabidopsis treated with exogenous gibberellins (GA_(4+7))(Hedden 2001; Eriksson et al. 2006).

Preharvest biorational application (Expt. 2). Leaves treated with three weekly preharvest applications of *B. subtilis* and SA showed the same leaf senescence symptom severity for cultivars BG, GS, and S than control stems (Fig. 3.3); however, *B. subtilis* improved leaf performance for P. *B. subtilis* may enhance postharvest performance by the secretion of cytokinins such as zeatin riboside or volatile organic compounds that promote cytokinin responses such as delayed senescence (Arkhipova et al. 2005). Fig. 3.4

demonstrates the effect of three weekly applications of *B. subtilis* on P leaf senescence. Note that flower development of P was inhibited in a manner similar to the preharvest PGR treatments in Expt. 1.

Postharvest hydration duration (Expt. 3). Postharvest hydration in a commercial formulation of chlorine and citric acid for 0, 4, 8, and 24 h had no effect on senescence symptom severity (chlorosis, marginal necrosis, veinal necrosis, and wilting) during vase life (data not shown). It was previously reported that <1 h in the hydration solution (solution with pH around 4 and a biocide) can promote wilting and leaf-yellowing symptoms in some chrysanthemum cultivars (Van Meeteren and Van Gelder 1999; Hidalgo et al. 2011), but no effect was observed on S and BG in this study.

Postharvest hydration environment (Expt. 4). A 4.1 °C hydration and processing environment did not show consistent leaf vase life performance improvement over a warmer (18.2 °C) environment (Fig. 3.5). Hydration of stems inside the cooler (4.1 °C) did show a reduction in marginal necrosis for GS and S and less veinal necrosis and wilting on S compared to stems that were hydrated in the 18.2 °C environment (Fig. 3.6). In contrast, hydrating stems in the 4.1 °C cooler increased chlorosis and veinal necrosis severity on BG, and wilting severity on GS. Van Meeteren and Van Gelder 1999 reported that low temperature hydration (4 h hydration in a 5 °C solution) reduced risk of wilting and yellowing on chrysanthemum stems, which contrasts with the results observed here. The Van Meeteren and Van Gelder (1999) experiment provided a 24-h storage time while our experiment provided 10 d storage.

Postharvest PGR application in the hydration solution (Expt. 5). For the first two repetitions, TDZ and BA+GA treatments decreased all four senescence symptoms for all cultivars evaluated, except for P stems treated with BA+GA that showed the same veinal necrosis severity as the control (Table 3.2). Also, BA, GA and STS reduced all four symptoms severity for GS stems. BA reduced leaf chlorosis for GS, marginal necrosis for BG, GS, and P, veinal necrosis for BG and GS, and wilting for BG, GS and S. GA application decreased veinal necrosis and wilting severity for cultivars BG, GS and S, but pedicle elongation was observed during the vase life, which is not favorable for consumer perception. Stems treated with IBA showed the highest senescence symptom severity for BG and GS.

These results were consistent with the improvement in the leaf performance previously reported where exogenous postharvest applications of 10 ppm BA in the hydration solution and 2.2 ppm TDZ into the vase solution on chrysanthemum cut flowers reduced leaf yellowing and wilting (Mohamed 2012; Bhargava et al. 2015). Also, postharvest application of 20 ppm GA reduced leaf yellowing and increased vase life on chrysanthemum cut flowers (Florez-Roncancio et al. 1996, Mohamed 2012).

For the third repetition of Expt. 5, the best treatments from the first two replications were repeated. Results were consistent with the first two replications, where 10 ppm BA+GA and 10 ppm TDZ showed leaf senescence severity reduction compared to control stems for most of the cultivars including BG, P and S; however, BA at 10 ppm only showed benefit for BG (Table 3.3). Also, BA+GA at 20 ppm reduced symptom severity for all cultivars except for chlorosis and marginal necrosis on MT. The effect of

BA+GA at 40 ppm, TDZ at 20 and 40 ppm on symptoms severity reduction varied with cultivar, e.g., benefits were observed for BG, P, PS, S, but not for A and MT. All concentrations of BA increased symptom severity on A, MT, W and Z.

These results confirm that there is a benefit for applying TDZ or BA+GA on spray-form chrysanthemum cut flowers in the postharvest environment. Fig. 3.7 displays S stems that were hydrated for 4 h after harvest in 40 ppm TDZ or 20 ppm BA+GA.

Postharvest sucrose application in the hydration solution (Expt. 6). The addition of sucrose at 0.1 and 0.2 g/mL in the hydration solution for 4 h did not reduce leaf senescence symptom development during vase life (data not shown). Contrary to these results, the addition of sucrose into the hydration solution showed improvement into the water balance, wilting and yellowing reduction during chrysanthemum vase life (Durkin 1980). Also, 0.04 to 0.06 g/mL sucrose provided in the hydration solution for 12 h showed benefit on the chrysanthemum leaf performance (Mayak et al. 1973a).

Postharvest sucrose application in holding solution (*Expt. 7)*. Stems placed in 0.1 and 0.2 g/mL sucrose holding solutions for 4 h following transport reduced senescence symptom severity for all cultivars (Fig. 3.8). Reduced chlorosis and wilting for PS and W, and marginal necrosis for MT was observed on stems treated with 0.1 g/mL sucrose solution.

Sucrose holding solutions at the rate of 0.1 and 0.2 g/mL provided for 12 to 24 h after shipment have been reported for gladiolus (*Gladiolus sp*.) (Mayak et al. 1973) and bird of paradise (*Strelitzia reginae*) (Gendy and Mahmoud 2012). A reduction in leaf chlorosis was also reported in chrysanthemum cut flowers placed in a 0.2 g/mL sucrose

holding solution for 6 h (Choudhari and Kulkarni 2018). In some cases, the stems do not take up enough carbohydrates during a short treatment period (Halevy and Mayak 1979). Fig. 3.9 demonstrates the benefit of 0.1, and 0.2 g/mL sucrose holding solutions on MT.

Postharvest sucrose application in vase solution (Expt. 8). Stems that were placed into 0.025, 0.05, 0.075 and 0.1 g/mL sucrose solutions showed reduction in senescence symptoms severity (chlorosis, marginal necrosis, veinal necrosis and wilting) for all cultivars, except for marginal necrosis and wilting for GS and veinal necrosis on W (Fig. 3.10). The lowest four senescence symptoms severity were observed on stems that were placed into 0.025 g/mL sucrose vase solution, except for marginal necrosis on GS and all four symptoms on S. The lowest symptoms severity on S were observed on stems that were placed into 0.05 and 0.075 g/mL sucrose vase solutions. Fig. 3.11 displays the results of W stems placed in 0 or 0.025 g/mL sucrose vase solutions.

Multiple products, termed flower food, are added to vase solutions to improve vase life. These products typically include a sugar source at ~0.01 g/mL. The sugar will vary depending on market prices, but sucrose and dextrose are common. It has been reported that sucrose at concentrations from 0.04 to 0.06 g/mL improve chrysanthemum leaf performance by delaying yellowing and wilting symptoms (Zamani et al. 2011; Amin 2017a, 2017b), which support the results reported in this study that showed benefits from sucrose concentrations from 0.025 and 0.1 g/mL. Across all cultivars in this study, 0.025 g/mL sucrose in the vase solution yielded the best response.

Conclusions

This research demonstrates several strategies to reduce the severity of leaf senescence symptoms of chrysanthemum cut flowers. PGR application in the field showed a significant reduction in the leaf senescence symptoms, however, flower deformation or abortion was promoted. Adding PGR to the hydration solution immediately after harvest showed a significant reduction in the leaf senescence symptoms, The addition of carbohydrates into hydration, holding and vase solutions, e.g., sucrose concentrations between 0.025 and 0.10 g/mL in vase solutions, showed significant reduction in leaf senescence across all cultivars.

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Table 3.1. The effect of pre-harvest plant growth regulator (PGR) spray applications made to three chrysanthemum cultivars during the last 1, 2, or 3 weeks prior to harvest on four senescence severity symptoms (Expt. 1, third replication). The PGR treatments were 10 ppm benzyladenine (BA), 10 ppm benzyladenine and gibberellic acid (BA+GA), and 10 ppm thidiazuron (TDZ). The cultivars were: Bomber Green, Green Screen, and Peridot. Senescence severity was assessed at 10 d of vase life.

PGR treatment	Number of applications	Symptom severity				
		Chlorosis (%)	Marginal necrosis (%)	Veinal necrosis (%)	Wilting (%)	Flower deformation (%)
				Bomber Green		
Control	0	36 a[z]	5 bc	23 a	20 a	0 f
BA	1	5 c	5 bc	2 c	1 b	40 bcde
	2	1 c	1 d	3 c	0 b	53 abc
	3	0 c	5 bc	1 c	0 b	60 ab
BA+GA	1	23 b	4 bcd	14 b	17 a	30 cde
	2	4 c	3 cd	2 c	0 b	45 abcd
	3	1 c	7 b	3 c	0 b	60 ab
TDZ	1	0 c	13 a	0 c	5 b	20 ef
	2	0 c	4 bcd	0 c	1 b	60 ab
	3	0 c	11 a	0 c	0 b	65 a
				Green Screen		
Control	0	38 a	25 a	27 a	37 c	0 f
BA	1	27 b	11 c	11 cd	26 d	21 ef
	2	12 c	15 bc	6 def	20 d	44 bcd
	3	11 cd	9 c	7 de	20 d	56 ab
BA+GA	1	40 a	10 c	21 b	54 a	33 cde
	2	25 b	10 c	13 c	39 bc	35 bcde
	3	25 b	21 ab	15 c	48 ab	56 abc
TDZ	1	0 e	15 bc	4 ef	0 e	33 bcde
	2	0 e	21 a	1 ef	0 e	67 a
	3	3 de	13 c	0 f	1 e	68 a
				Peridot		
Control	0	97 ab	97 ab	86 b	92 ab	0 f
BA	1	100 a	100 a	100 a	100 a	36 e
	2	100 a	100 a	100 a	100 a	62 cd
	3	87 c	86 bc	86 ab	83 bc	76 bc
BA+GA	1	85 c	91 abc	72 b	84 bc	43 e

	2	42	d	36	e	20	c	8	e	61 d
	3	87	abc	84	c	77	b	75	c	82 b
TDZ	1	4	e	9	f	0	e	0	e	86 ab
	2	42	d	51	d	15	cd	25	d	100 a
	3	40	d	42	de	5	de	12	de	100 a

[z] Different letters indicate significant differences within cultivar, symptom and flower abortion using Fisher's least significant difference test at $P<0.05$.

Table 3.2. The effect of postharvest plant growth regulator (PGR) application in the hydration solution on four leaf senescence severity symptoms (chlorosis, marginal necrosis, veinal necrosis, and wilting) of four chrysanthemum cultivars after 10 d of vase life (Expt. 6, first two replications). The PGR treatments were 10 ppm benzyladenine (BA), 10 ppm benzyladenine plus 10 ppm gibberellic acid (BA+GA), 10 ppm gibberellic acid (GA), 10 ppm indole-3-butyric acid (IBA), 20 ppm silver thiosulfate (STS), and 10 ppm thidiazuron (TDZ).

PGR treatment		Symptom severity			
		Chlorosis (%)	Marginal necrosis (%)	Veinal necrosis (%)	Wilting (%)
			Bomber Green		
Control		70 b[z]	68 b	66 b	67 b
BA	10 ppm	55 bc	42 c	42 cd	41 cd
BA+GA	10 ppm	24 d	18 d	16 e	16 e
GA	10 ppm	35 cd	45 bcd	15 de	15 de
IBA	10 ppm	100 a	100 a	100 a	100 a
STS	20 ppm	66 abc	48 bc	62 abc	62 bc
TDZ	10 ppm	33 d	26 cd	18 e	18 e
			Green Screen		
Control		55 b	53 b	45 b	53 b
BA	10 ppm	19 d	23 cd	15 c	25 c
BA+GA	10 ppm	19 d	31 c	15 c	25 c
GA	10 ppm	22 cd	24 cd	15 c	21 c
IBA	10 ppm	100 a	100 a	100 a	100 a
STS	20 ppm	37 c	19 cd	27 c	27 c
TDZ	10 ppm	24 cd	15 d	15 c	15 c
			Peridot		
Control		100 a	100 a	100 a	100 a
BA	10 ppm	100 a	100 a	100 ab	100 a
BA+GA	10 ppm	82 b	80 bc	86 b	76 b
GA	10 ppm	97 ab	92 ab	83 ab	83 ab
IBA	10 ppm	100 ab	100 a	100 ab	100 ab
STS	20 ppm	100 ab	100 a	100 ab	100 ab
TDZ	10 ppm	59 c	70 c	53 c	48 c
			Shrek		
Control		69 a	65 a	64 a	61 a
BA	10 ppm	62 a	53 ab	55 ab	38 b
BA+GA	10 ppm	38 bc	26 c	16 d	15 c

GA	10 ppm	61 ab	28 bc	15 cd	15 bc
IBA	10 ppm	82 a	69 a	49 abc	49 ab
STS	20 ppm	57 abc	31 bc	31 bcd	31 bc
TDZ	10 ppm	33 c	26 c	20 d	15 c

[z] Different letters reflect differences within columns using Fisher's least significant difference test $P<0.05$.

Table 3.3. The effect of postharvest plant growth regulator (PGR) application in the hydration solution that showed an improvement on leaves performance during vase life, at concentrations of 10, 20 and 40 ppm each hormone, on the leaf senescence symptoms severity (chlorosis, marginal necrosis, veinal necrosis, and wilting) on eight chrysanthemum cultivars after 10 d of vase life (Expt. 6, third replication). The PGR treatments were 10, 20, or 40 ppm benzyladenine (BA), 10, 20 or 40 ppm benzyladenine plus gibberellic acid (BA+GA) and 10, 20 or 40 ppm thidiazuron (TDZ). The cultivars were Aligator, Bomber Green, Lyche, Molly Purple, Mark Twain, Peridot, Paintball Suny, Shrek, WhatsApp, and Zumba. Senescence severity was assessed at 10 d of vase life.

PGR treatment		Symptom severity			
		Chlorosis (%)	Marginal necrosis (%)	Veinal necrosis (%)	Wilting (%)
			Alligator		
Control		33 c[z]	2 b	13 de	15 cde
BA	10 ppm	57 ab	0 b	34 abc	25 cd
	20 ppm	70 a	0 b	23 bcd	27 c
	40 ppm	63 ab	26 a	38 ab	54 a
BA+GA	10 ppm	15 d	3 b	1 e	4 e
	20 ppm	16 d	4 b	1 e	3 e
	40 ppm	19 cd	5 b	2 e	3 e
TDZ	10 ppm	65 ab	0 b	40 a	33 bc
	20 ppm	55 b	2 b	20 cd	46 ab
	40 ppm	32 c	3 b	11 de	7 de
			Bomber Green		
Control		70 a	68 a	66 a	67 a
BA	10 ppm	55 ab	42 b	42 b	41 b
	20 ppm	46 bc	12 cd	24 bc	24 bc
	40 ppm	31 cde	12 cd	5 cd	10 c
BA+GA	10 ppm	23 def	18 cd	16 cd	15 c
	20 ppm	6 f	6 d	0 d	3 c

	40 ppm	7	f	1	d	1	d	9	c
TDZ	10 ppm	33	cd	26	bc	18	cd	18	c
	20 ppm	2	f	8	cd	2	cd	8	c
	40 ppm	8	ef	4	d	7	cd	15	c
						Mark Twain			
Control		65	de	40	de	60	c	55	c
BA	10 ppm	100	a	100	a	100	a	100	a
	20 ppm	100	a	100	a	100	a	100	a
	40 ppm	99	a	95	a	98	a	97	ab
BA+GA	10 ppm	42	f	9	f	20	e	16	e
	20 ppm	54	ef	27	ef	42	d	33	de
	40 ppm	77	cd	21	ef	49	cd	39	cd
TDZ	10 ppm	80	bc	61	cd	80	b	83	ab
	20 ppm	85	bc	71	bc	88	ab	80	b
	40 ppm	93	ab	88	ab	90	ab	92	ab
						Peridot			
Control		100	a	100	a	100	a	100	a
BA	10 ppm	100	a	100	a	100	a	100	a
	20 ppm	93	abc	95	a	94	ab	99	a
	40 ppm	100	a	100	a	100	a	100	a
BA+GA	10 ppm	82	bc	80	bc	86	ab	76	b
	20 ppm	33	e	51	d	34	d	58	c
	40 ppm	100	ab	100	a	100	a	100	a
TDZ	10 ppm	59	d	70	c	53	c	48	c
	20 ppm	29	e	69	c	21	d	55	c
	40 ppm	75	cd	91	ab	74	b	96	a
						Paintball Sunny			
Control		92	a	86	a	94	a	92	a
BA	10 ppm	95	a	82	a	87	ab	89	a
	20 ppm	94	a	75	a	80	abc	91	a
	40 ppm	89	a	68	ab	77	abc	88	a
BA+GA	10 ppm	36	e	12	d	26	e	27	d
	20 ppm	58	cd	40	c	46	de	44	cd
	40 ppm	55	d	26	cd	44	de	54	bc
TDZ	10 ppm	66	cd	35	cd	67	bc	64	b
	20 ppm	72	bc	35	cd	72	bc	64	b
	40 ppm	83	ab	45	bc	64	cd	59	bc
						Shrek			
Control		69	a	65	a	64	a	61	a
BA	10 ppm	62	ab	53	a	55	a	38	b

	20 ppm	39 cd	19 bc	9 bc	5 c
	40 ppm	33 cd	18 bc	11 bc	10 c
BA+GA	10 ppm	38 cd	26 b	16 bc	15 c
	20 ppm	9 e	8 c	1 c	1 c
	40 ppm	49 bc	18 bc	13 bc	8 c
TDZ	10 ppm	33 cd	26 b	20 b	15 c
	20 ppm	30 cd	23 b	0 c	6 c
	40 ppm	26 de	15 bc	0 c	10 c
				WhatsApp	
Control		68 bc	35 bc	69 abcd	77 ab
BA	10 ppm	73 abc	48 abc	73 ab	85 ab
	20 ppm	90 a	75 a	86 a	92 a
	40 ppm	81 ab	50 ab	70 ab	93 a
BA+GA	10 ppm	60 c	46 bc	50 d	54 c
	20 ppm	16 d	4 d	9 e	6 d
	40 ppm	70 bc	54 ab	65 bcd	79 ab
TDZ	10 ppm	58 c	30 bcd	79 ab	80 ab
	20 ppm	64 bc	40 bc	64 bcd	85 ab
	40 ppm	60 c	20 cd	52 cd	73 b
				Zumba	
Control		53 b	21 c	64 b	33 cd
BA	10 ppm	50 bc	21 c	50 bcd	41 bc
	20 ppm	85 a	63 a	85 a	75 a
	40 ppm	79 a	48 ab	84 a	57 ab
BA+GA	10 ppm	37 bcd	21 c	21 ef	6 e
	20 ppm	26 d	15 c	9 f	10 e
	40 ppm	29 d	21 c	8 f	10 e
TDZ	10 ppm	38 bcd	11 c	30 de	8 e
	20 ppm	43 bcd	27 bc	51 bc	27 cde
	40 ppm	35 cd	7 c	41 cd	16 de

[z] Different letters reflect differences within columns using Fisher's least significant difference test $P<0.05$.

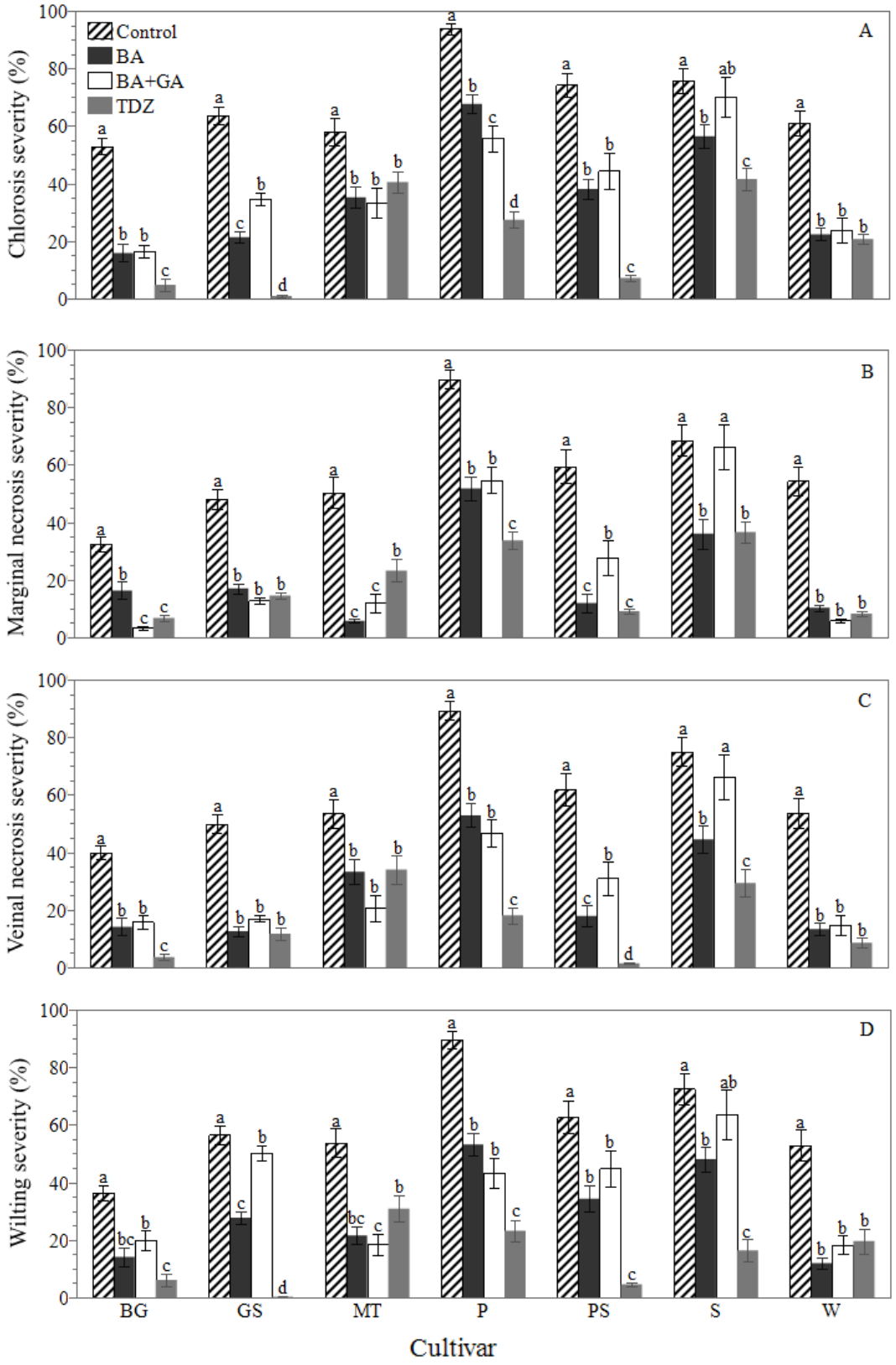
Control
BA
BA+GA
TDZ
A
B
C
D
Chlorosis severity (%)
Marginal necrosis severity (%)
Veinal necrosis severity (%)
Wilting severity (%)
0
20
40
60
80
100
BG
GS
MT
P
PS
S
W
Cultivar

Figure 3.1. Effect of three weekly preharvest spray applications of plant growth regulators on four leaf senescence severity symptoms (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) of seven chrysanthemum cultivars at 10 d of vase life (Expt. 1, first two replications). The plant growth regulator treatments were 10 ppm benzyladenine (BA), 10 ppm benzyladenine plus 10 ppm gibberellic acid (BA+GA), or 10 ppm thidiazuron (TDZ)]. The cultivars were Bomber Green (BG), Green Screen (GS), Mark Twain (MT), Peridot (P), Paintball Sunny (PS), Shrek (S), and WhatsApp (W). Error bars = ±1SE. Different letters indicate significant differences within cultivar and symptom using Fisher's least significant difference test at $P<0.05$.

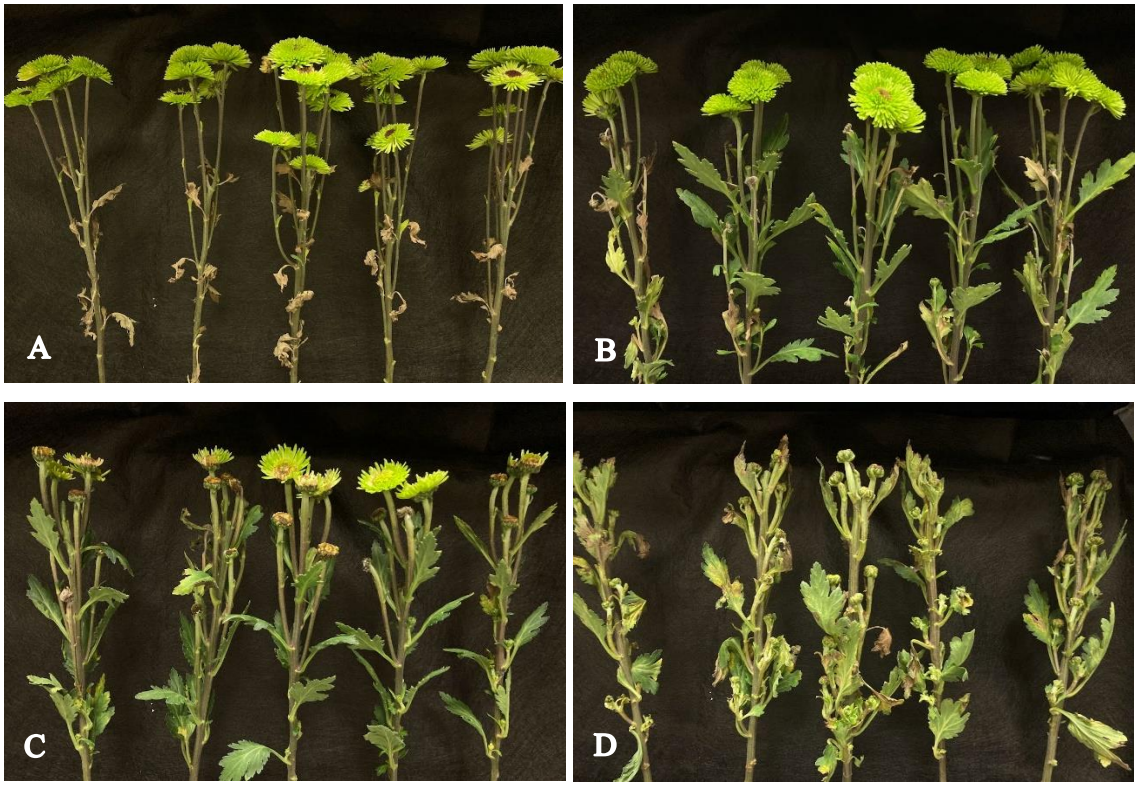

Figure 3.2. Preharvest treatment of chrysanthemum ‘Peridot’ cut flowers with selected weekly plant growth regulator applications: A. control, B. one application of thidiazuron (10 ppm), C. two applications of benzyladenine plus gibberellic acid (10 ppm each), D. three applications of thidiazuron (10 ppm). Pictures were taken after 10 d of vase life.

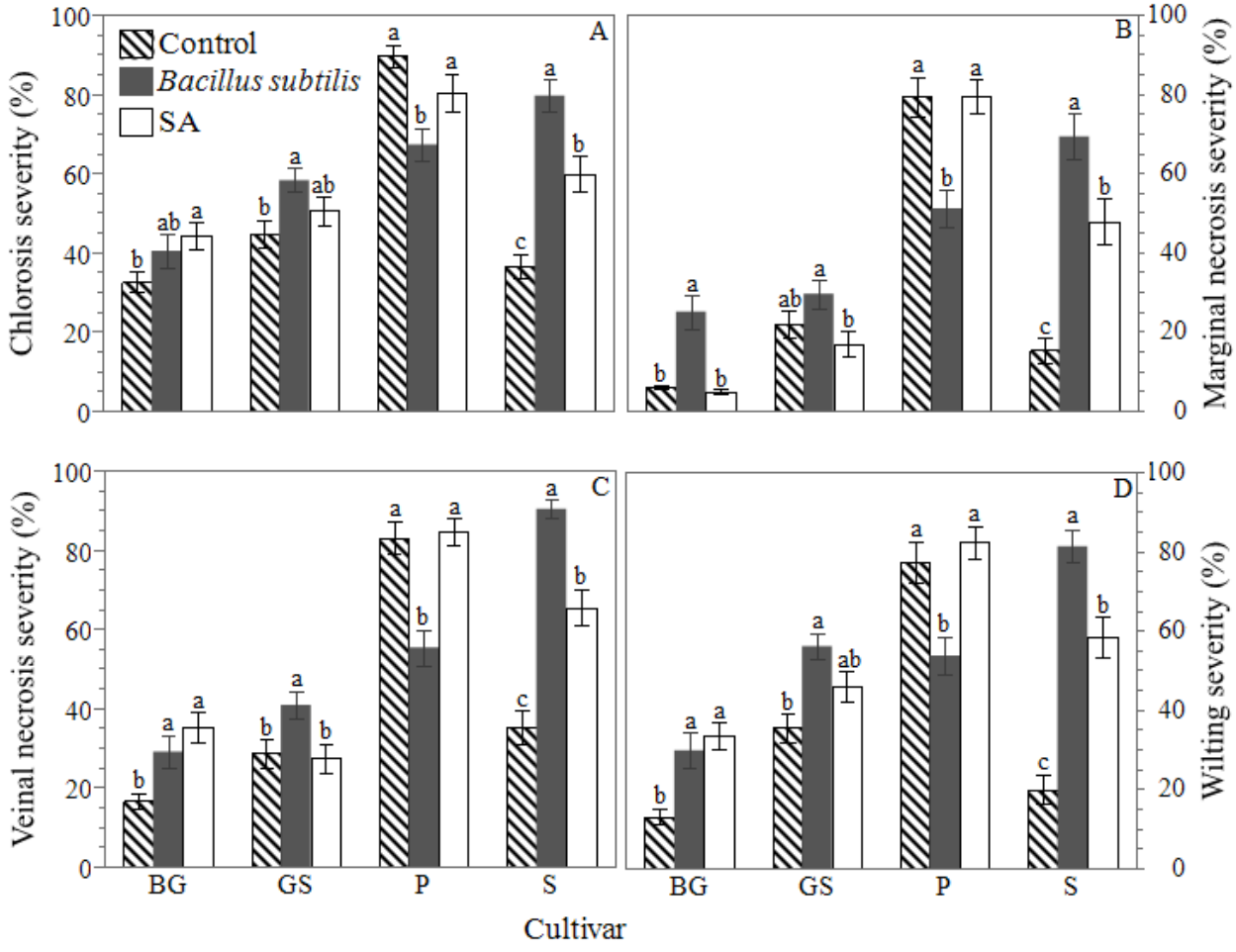

Figure 3.3. Effect of three weekly spray applications of biorational products (Bacillus subtilis and salicylic acid (SA)) made prior to harvest in the greenhouse on four leaf senescence severity symptoms (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) after 10 d of vase life (Expt. 2). The four chrysanthemum cultivars were (Bomber Green (BG), Green Screen (GS), Peridot (P), and Shrek (S). Leaf symptoms were recorded on day 10. Error bars = ±1SE. Different letters indicate significant

differences within cultivar and symptom using Fisher's least significant difference test at $P<0.05$.

Figure 3.4. Chrysanthemum ‘Peridot’ treated with A. control or B. three weekly spray applications of *Bacillus subtilis* made prior to harvest. Pictures were taken at 10 d vase life.

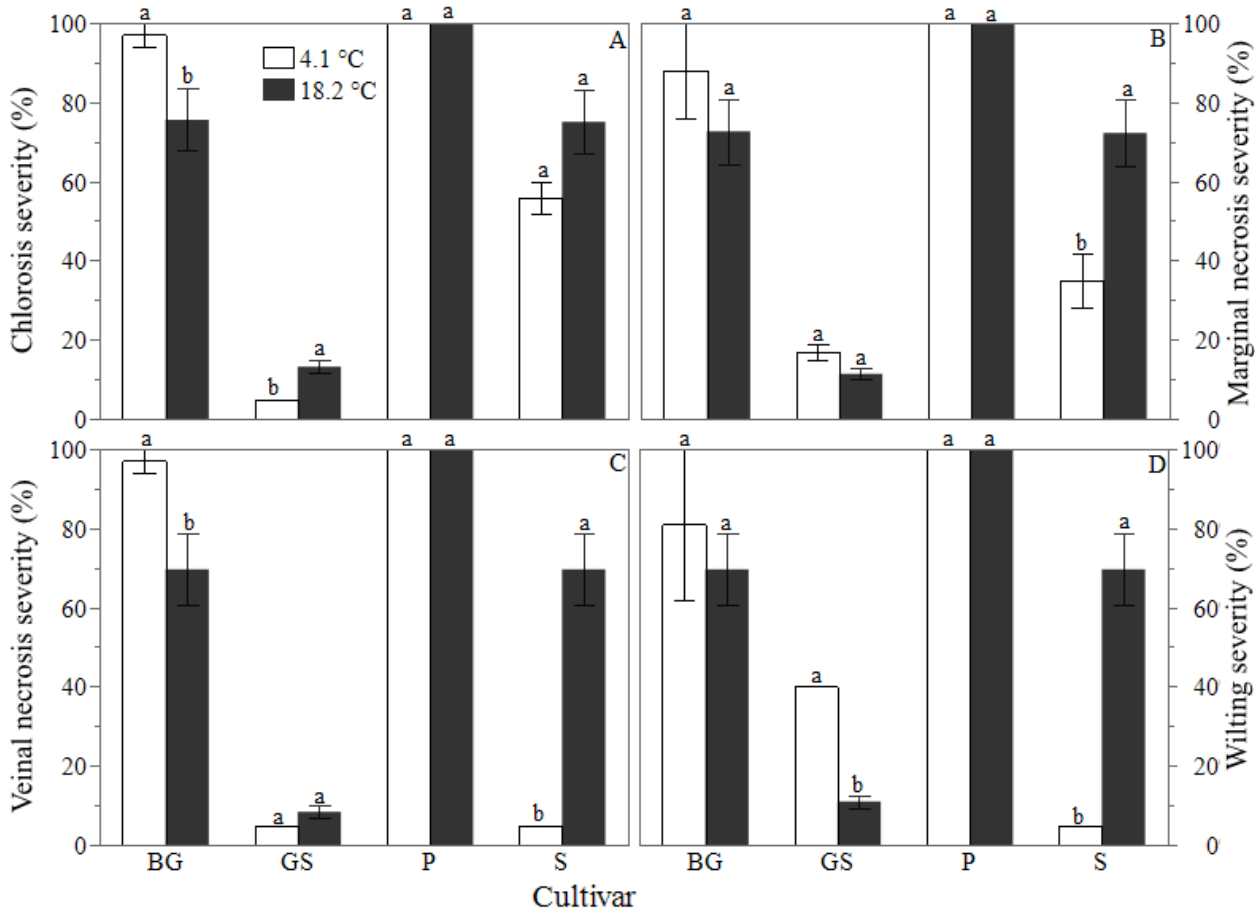

Figure 3.5. Effect of postharvest hydration temperature during a 12-h period. During this time, stems were held in hydration solutions inside 4.1 or 18.2 °C rooms. Then stems were packaged, stored for 10 d and shipped under the same environment conditions. Leaf senescence symptoms severity (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) were rated after 10 d of vase life. Chrysanthemum cultivars were Bomber Green (BG), Green Screen (GS), Peridot (P) and Shrek (S). Error bars = ±1SE. Different letters represent treatment differences within cultivar using Fisher's least significant difference test at $P<0.05$.

Figure 3.6. Chrysanthemum ‘Shrek’ hydrated for 12 h after harvest in a A. 4.1 °C room, and 4.9 °C hydration solution temperature or a B. 18.2 °C room, and 16.2 °C hydration solution temperature, and then packaged, stored, and shipped. Pictures were taken after 10 d in the vase.

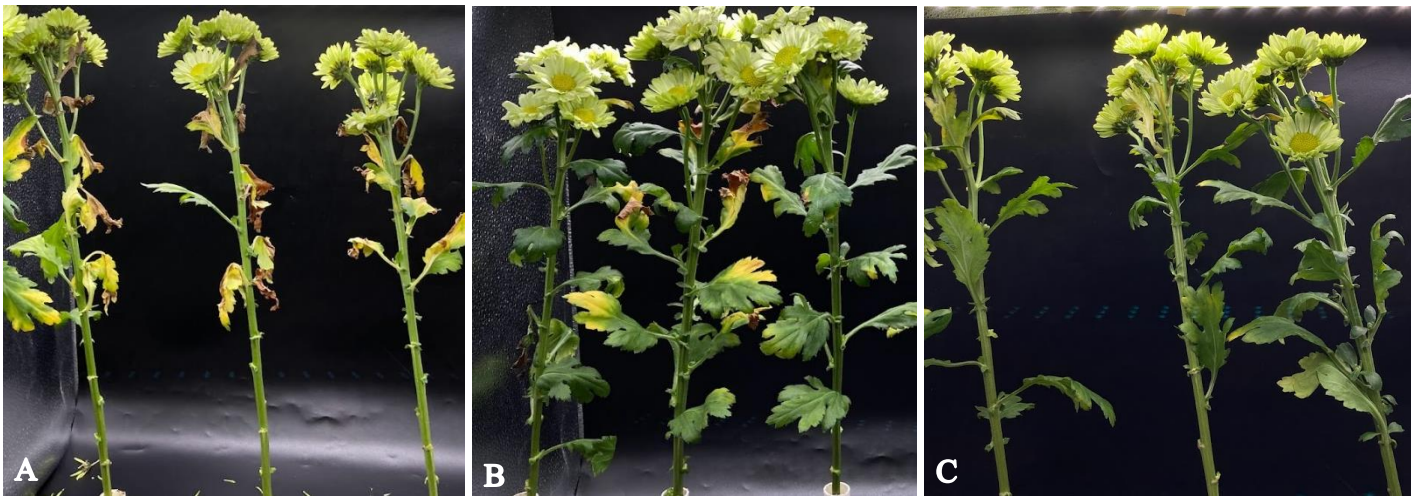

Figure 3.7. Chrysanthemum ‘Shrek’ hydrated for 4 h after harvest in the following solutions (3.8 pH): A. control, B. 40 ppm thidiazuron, or C. 20 ppm benzyladenine plus gibberellic acid in the postharvest area 18.2 °C room temperature, and then packaged, stored, and shipped. Pictures were taken after 10 d of vase life.

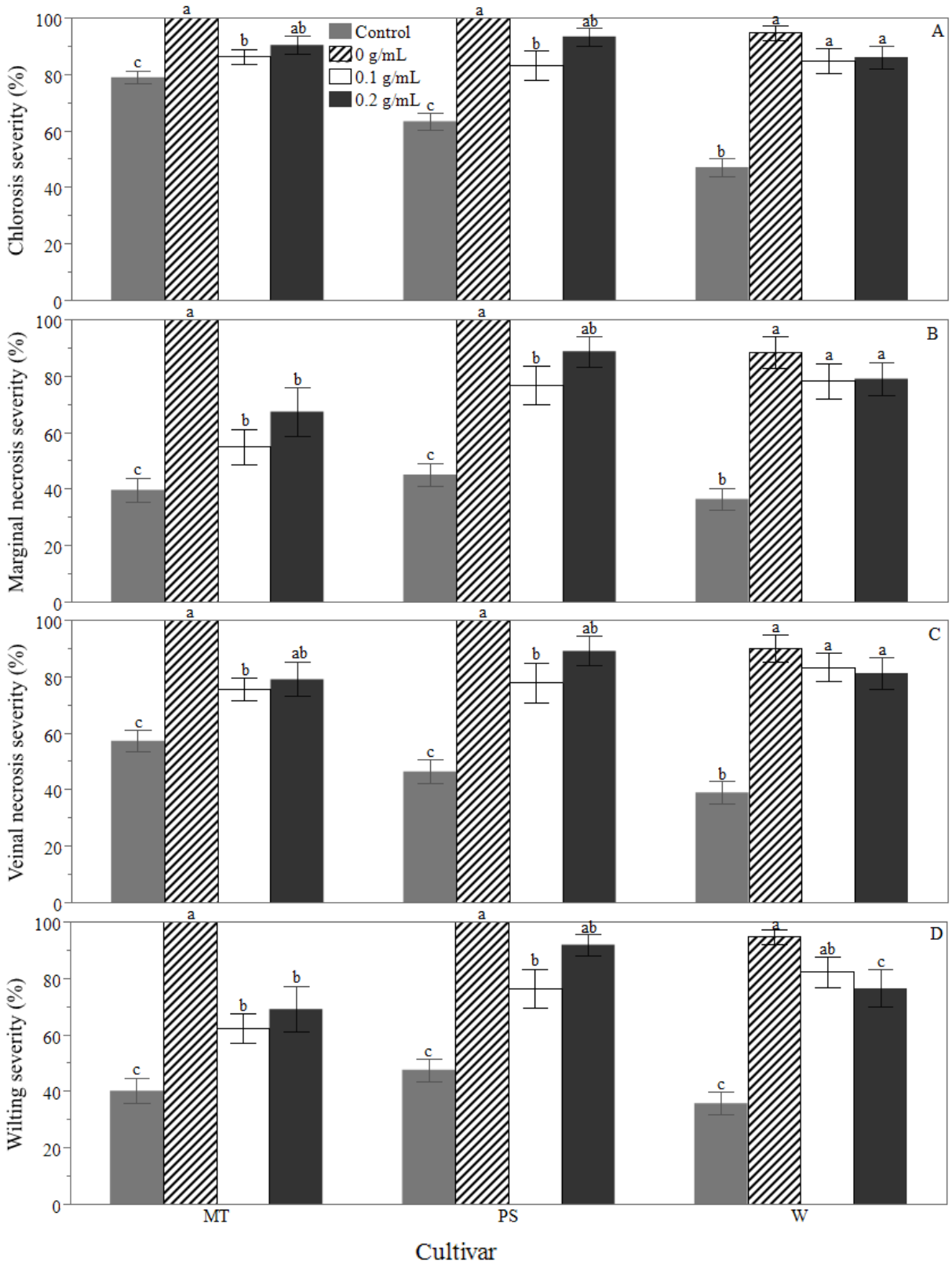
Control
0 g/mL
0.1 g/mL
0.2 g/mL
A
B
C
D
Chlorosis severity (%)
Marginal necrosis severity (%)
Veinal necrosis severity (%)
Wilting severity (%)
0
20
40
60
80
100
MT
PS
W
Cultivar

Figure 3.8. Effect of cut flower stems placed for 4 h (first replication) or 8 h (second replication) in a 0, 0.1, or 0.2 g/mL sucrose holding solution after shipment. Four leaf senescence severity symptoms were recorded (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) on chrysanthemum ‘Mark Twain’ (MT), ‘Paintball Sunny’ (PS), and ‘WhatsApp’ (W) after 10 d of vase life (Expt. 7). Error bars = ±1SE. Different letters represent treatment differences within cultivar using Fisher's least significant difference test $P<0.05$.

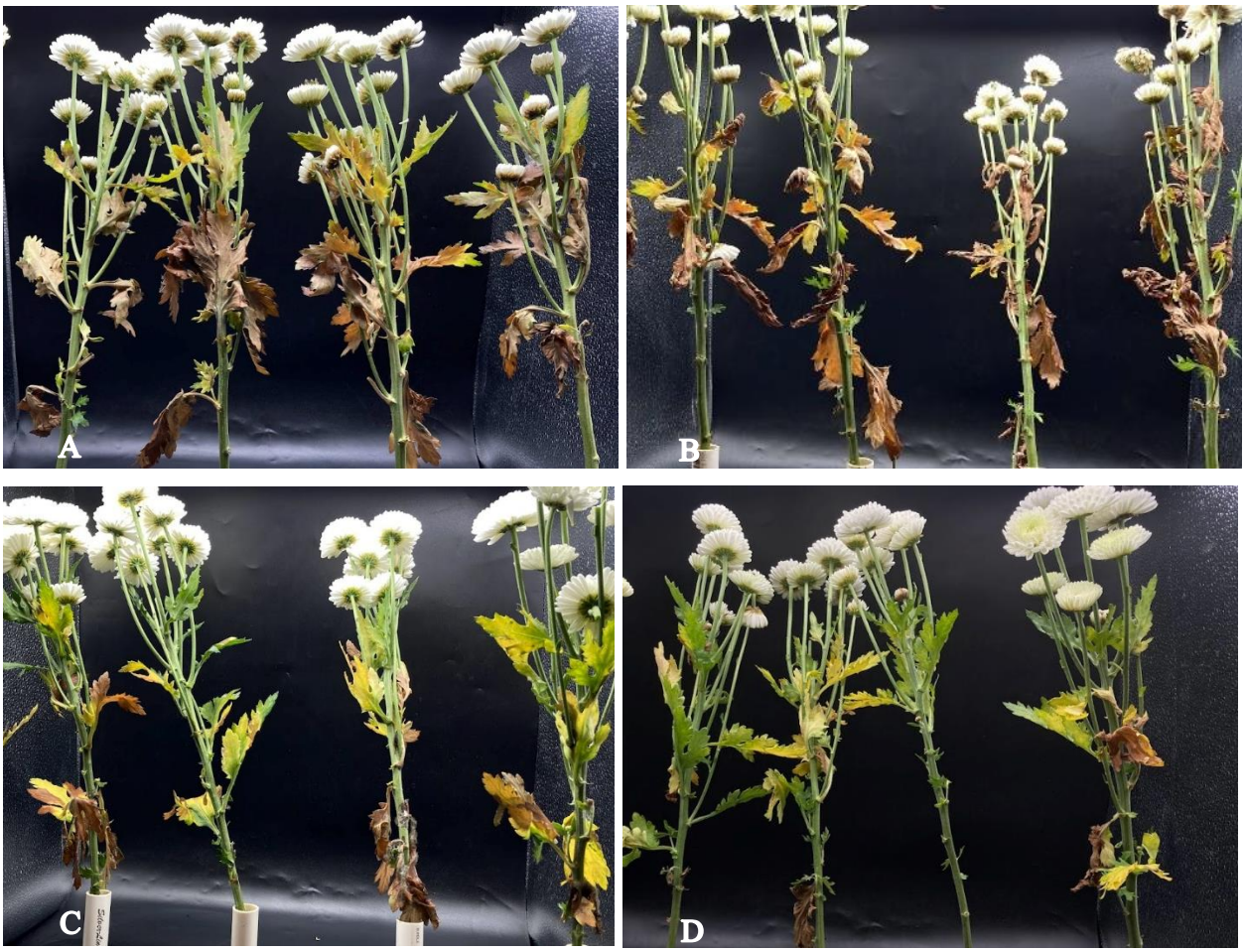

Figure 3.9. Chrysanthemum ‘Mark Twain’ A. with no holding solution application after shipment for 8 h in B. 0 g/mL, C. 0.1 g/mL, or D. 0.2 g/mL sucrose holding solutions (pH 3.8) in a vase life room. Pictures were taken after 10 d in the vase solution.

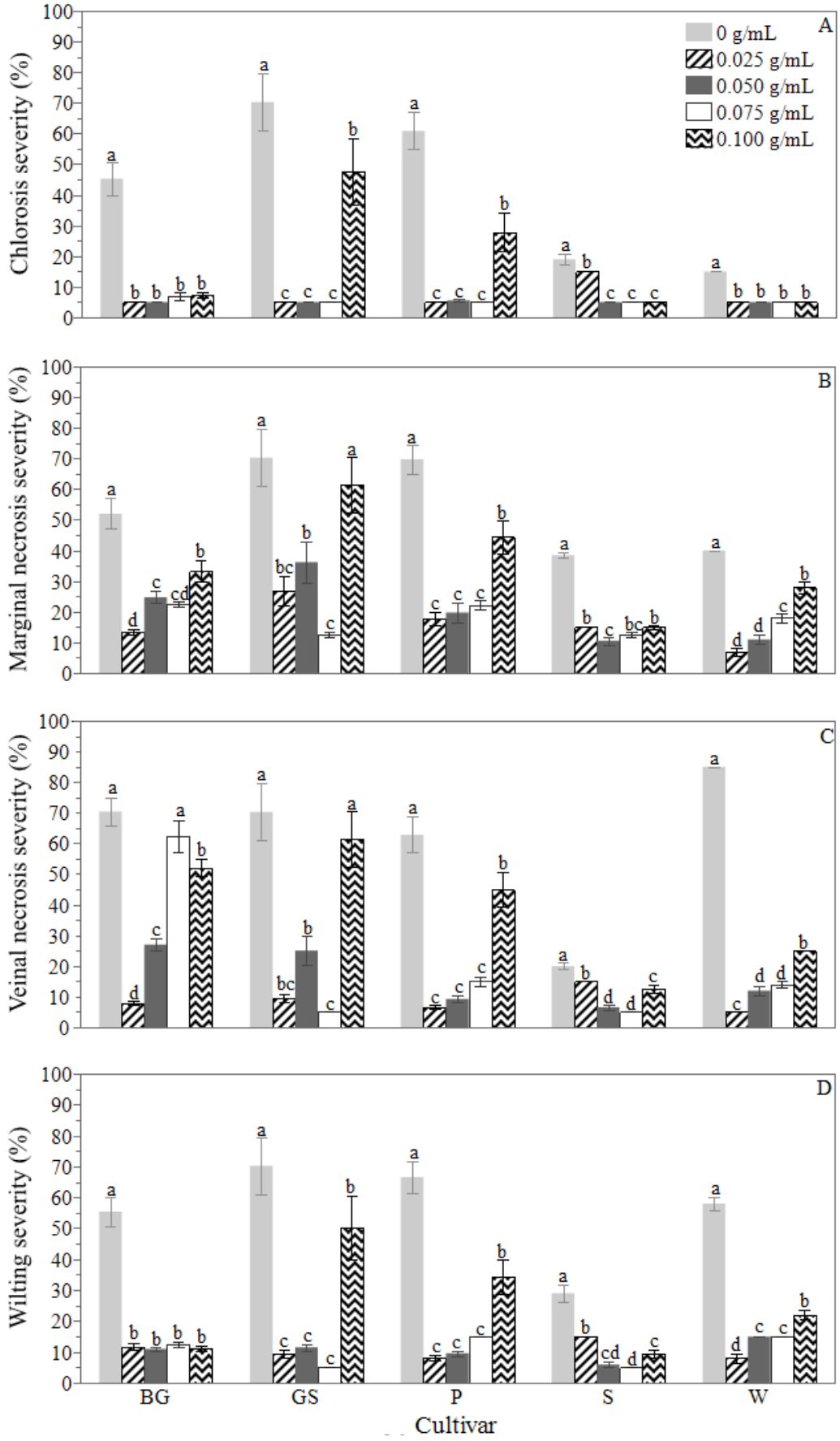
A
B
C
D
0 g/mL
0.025 g/mL
0.050 g/mL
0.075 g/mL
0.100 g/mL
Chlorosis severity (%)
Marginal necrosis severity (%)
Veinal necrosis severity (%)
Wilting severity (%)
BG
GS
P
S
W
Cultivar

Figure 3.10. Effect of cut flower stems placed in vase solutions after shipment containing 0, 0.025, 0.05, 0.075 or 0.1 g/mL sucrose. Four leaf senescence severity symptoms (A. chlorosis, B. marginal necrosis, C. veinal necrosis, and D. wilting) were recorded on five chrysanthemum cultivars: Bomber Green (BG), Green Screen (GS), Peridot (P), Shrek (S), and WhatsApp (W), after 10 d of vase life (Expt. 7). Error bars = ±1SE. Different letters represent treatment differences within cultivar using Fisher's least significant difference test (FLSD) $P<0.05$.

Figure 3.11. After shipment, chrysanthemum ‘WhatsApp’ stems were placed in a A. 0 g/mL or B. 0.025 g/mL sucrose vase solution. Pictures were taken after 10 d of vase life.