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The Role of Water Relations and Oxidative Stress in the Vase Life Response to Prolonged Storage: A Case Study in Chrysanthemum

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Abstract: Long storage periods have been associated with decreased vase life. In this study, the processes underlying the vase life response to prolonged storage were investigated, along with the potential of light reflectance profiles to estimate storage duration. Three cut chrysanthemum cultivars were exposed to four cold (5 °C) storage periods (0, 7, 14, and 21 d). Stomata were present on the leaves (either side) and stem, but not on petals. As compared to the leaves, stomata on the stem were non-functional, smaller, and less dense. Floral transpiration was a small portion of the whole-cut flower transpiration, with the major contributor being the leaves or stem depending on the cut flower hydration. Storage duration linearly decreased vase life, with the rate of decrease being cultivar specific. Storage duration (0–21 d) did not affect leaf stomatal functioning, non-leaf tissue transpiration, or the relative contribution of each organ to the whole-cut flower transpiration. Cut flower hydration was generally enhanced by storage, while water uptake restoration ability was not impaired. Membrane lipid oxidation increased in response to storage duration owing to enhanced H_2O_2 accumulation. A strong correlation between membrane lipid oxidation level and the vase life response to cold storage was apparent. By examining the light reflectance profiles (400–1050 nm) of leaves (either side) and flowers (top view), an indication of the storage period could not be deduced. In conclusion, cultivar differences in vase life response to cold storage were attributed to variation in oxidative state, whereas cut flower water relations are clearly not involved.

Keywords: keeping quality; long-term storage; multispectral imaging; oxidative stress; postharvest; rehydration; stomatal closing ability; transpiration

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1. Introduction

Unlike production, the market demand for cut flowers is very irregular. Major volumes are traded on specific days of the year [1]. To match supply and demand, cut flowers are often stored for long periods in the order of weeks [2–4]. In addition, a critical share of the cut flower trade is intercontinental, with the production and distribution sites being located overseas. Notably, there is an emerging trend of shipping cut flowers by sea containers at the expense of air freight [5]. Although in this way the transport time is considerably increased (from 2–4 d to 2–4 weeks), sea transport inherently has a lower environmental footprint, comes at a lower (≈50%) cost and offers a better control of environmental conditions [5]. However, long storage periods have been associated with decreased vase life [1,2,4], with the underlying processes being poorly understood.

Cut flower water balance depends on the rate upon which the water loss is replaced by the water uptake [6]. A negative water balance typically induces lethal wilting symptoms terminating vase life [7,8]. Recent work on rose indicates that long-term storage (28 d at 0.5 °C) de-regulated the control of water loss, and this effect was cultivar specific [5]. In this way, increased leaf transpiration partially underlays the negative effect of long-term storage on vase life [5]. However, whether or not stomatal functioning was gradually attenuated and the timing (within the 28 d period) of this response remain unknown. Since the environmental effects on stomatal functioning are species dependent [9,10], more cut flower species of importance ought to be evaluated. In addition, the effect of long-term storage on the transpiration of non-leaf tissues (stem, flowers) has not been previously addressed. Their contribution to whole-cut flower transpiration can be sizeable in some cut flower species (i.e., carnation and chrysanthemum) [8,11] and increases as stomata close [8,12]. During the supply chain, cut flowers often experience dry periods [7,8,13]. Whether or not long-term storage affects the cut flower ability to rehydrate following a dehydration event also remains elusive.

In cut flowers, dark refrigerated storage has been associated with oxidative stress [3,4,14], which elicits a range of adverse effects, including lipid peroxidation [15]. The associated symptomatology is often undetectable during storage, and expressed later in the marketing chain [16]. Oxidative stress is triggered by the imbalance between the accumulation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), and the antioxidant elements [4,14,15]. Polyphenolics and flavonoids are critical non-enzymatic antioxidant elements detoxifying ROS [17–19]. Therefore, monitoring ROS accumulation and antioxidant elements during storage may be potentially employed to control the risk of vase life deterioration.

Methods distinguishing cut flowers stored for different periods are not in existence. In this way, potential differences in vase life go unnoticed. The development of a non-invasive method to estimate the period where cut flowers had been stored could reduce postharvest losses, benefiting distributors and sellers [20]. More importantly, a continuous supply of cut flowers with long vase life would benefit the horticultural industry by stimulating the cut flower popularity to consumers [16,21]. Such a candidate is the light reflectance properties, which are affected by both biochemical properties, and physiological condition [22–26]. The changes in cut flower reflectance during storage have not previously been evaluated, and their potential in distinguishing batches stored for different periods has not been currently addressed.

The objectives of the current study were: (1) to investigate the effect of storage on the whole-cut flower (stem, flower, and leaf) transpiration response to water deficit, the rehydration ability following a dehydration event, and the magnitude of oxidative stress, as well as (2) to evaluate the potential of leaf (either side) and flower reflectance as an index of storage period. Chrysanthemum (*Chrysanthemum morifolium* L.) was employed as a model species since it is globally one of the most popular cut flowers [27,28], and the effect of long-term storage on keeping quality has not been previously addressed.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Cut chrysanthemum flowers were obtained from a commercial grower (35°0′31″ N 25°44′23″ E; Ierapetra, Crete, Greece). Single-stem plants were grown in a multispan plastic greenhouse. A single harvest was conducted (16 May 2021). Three 'spray' type cultivars ('Pina Colada', 'Code Green', 'Euro White') were tested. Since plants were grown under identical conditions, the noted phenotypic differences were related to the genotype.

Plants of similar height (1 m) were sampled. In this way, the percentage of stem length that was excised remained constant both within and among cultivars [27]. Harvested shoots had a length of approximately 0.9 m and at least three fully-open flowers. These were collected in the morning (07:00–08:00 h; 17–19 °C during harvest), and their cut ends (horizontal cut) were immediately placed in buckets with aqueous sodium hypochlorite

solution (1%, *v*/*v*) at a height of 0.12 m. The stems were transferred to the laboratory in these buckets at the day of harvest using refrigerated transport (7 °C). Cut flowers were stored overnight in buckets with water, at 5 °C and in darkness. Following this rehydration period, stem length was shortened to 0.75 m (horizontal cut) by submerging it under water (to prevent cavitation of xylem vessels that were exposed by cutting). Then the leaves on the lower 0.15 m of the shoot were stripped. Following rehydration and stem length shortening practices, that fresh weight was regarded as the initial one. To prevent bacterial growth, which would cause vascular blockage leading to low water uptake, sodium hypochlorite (1%, *v*/*v*) was added in the water, where the cut flowers were placed throughout the handling and evaluation processes [7,8].

Cut flower and organ (leaves, stems, or petals) level measurements were conducted. For leaf-level measurements, sampled leaves had grown under direct light and were fully expanded. For stem-level measurements, the lowermost part was employed. For petal-level measurements, five outermost petals were sampled. In all cases, the time between sampling and the start of the evaluation did not exceed 15 min. When this was not possible, samples were placed in vials, flash-frozen in liquid nitrogen, and transferred to a freezer (−80 °C) for storage. Replicate leaves, stems, or petals were sampled from separate cut flowers.

For the traits where the effect of storage period was assessed, different sets of cut flowers were evaluated following four periods (0, 7, 14, and 21 d) of dark refrigerated storage (5 °C). In 0 d storage, cut flowers were stored overnight (12 h), to ensure maximal turgidity [7,8].

2.2. Visual Quality Characteristics, Mass Partitioning and Stem Strength

Since previous studies on cut chrysanthemum are mostly limited to either morphological characteristics or postharvest longevity [15,28,29], combined examination (or selection) of these traits is currently not favored. To stimulate the overlap between these two trait groups and provide a more comprehensive data set [7], we here recorded the following important visual quality traits.

A morphometric analysis was conducted by analyzing leaf shape (n = 12). Leaf shape traits were derived from images acquired by a digital camera (Sony DSC-W830, Sony Corporation, Tokyo, Japan) under non-reflective glass from a distance of 0.5 m, employing a copy stand [30]. Using specialized software (ImageJ; Wayne Rasband/NIH, Bethesda, MD, USA), leaf lamina outlines were processed to calculate the following four (dimensionless) metrics of leaf form: (a) aspect ratio [(major axis)/(minor axis); axes of the best-fitted ellipse], (b) circularity [$(4\pi \times \text{area})/(\text{perimeter})^2$], (c) roundness [$(4 \times \text{area})/[4\pi \times (\text{major axis})^2]$], and (d) solidity [(area)/(convex area)] [28]. Each metric captures a distinct aspect of leaf shape. Aspect ratio and roundness are influenced by the length to width ratio, while circularity and solidity are sensitive to serration and lobing [31]. Aspect ratio ranges from 1 (circle) to value without upper bound (infinitely narrow). Roundness ranges from 0 (infinitely narrow) to 1 (circle). Circularity ranges from 0 (infinitely narrow) to 1 (circle). Solidity ranges from 0 to 1, being inversely related to boundary irregularities. Solidity is sensitive to leaves with deep lobes or a distinct petiole, and can be used to detect leaves lacking such structures [31]. Solidity, unlike circularity, is not greatly affected by serrations and minor lobings [31].

Number of flowers and flower buds (>5 mm) was determined (n = 40). Individual flower diameter was assessed in one fully-open flower per stem (n = 12). The flower position was also evaluated based on the distance between the highest and the lowest flower on the stem (the so-called flower distance; n = 12).

To evaluate cultivar differences in fresh mass allocation, the stem, leaf, and flower masses of each cut flower were recorded (±0.01 g; MXX-412; Denver Instruments, Bohemia, NY, USA) (n = 12). The strength (mass per unit length) [8] of the main stem (thus excluding axillary shoots) was also determined (n = 12).

2.3. Effect of Storage Period on Leaf and Flower Reflectance

Following the respective storage period (0, 7, 14, and 21 d), leaf (either side) and flower (top view) light reflection properties were assessed (n = 6). Samples were fixed horizontally on a frame by using wires. To reduce reflectance, frame and wires were black. Samples were illuminated with tungsten-halogen bulbs, covering the whole spectral acquisition range. Light was homogenously distributed across the field of view (<10% difference between center and edges). Illumination conditions remained constant across all acquisitions.

Multispectral images were collected with an XpeCAM X01 system (XpectralTEK, Braga, Portugal) coupled with a 25 mm F1.4 objective lens (Electrophysics Corp., Fairfield, NJ, USA). The distance between the sensor system and the samples was 1.5 m with 0° angle of incidence. Spectral reflectance data were obtained at 25 nm intervals over the range 400–700 nm (thus 13 bands), and at 50 nm intervals in the 700–1050 nm range (thus 7 bands). Before image acquisition, a white diffuse reflectance target (DRT) was used for calibration. DRT was placed next to the samples and in parallel direction. Following acquisition, each image was normalized with the corresponding DRT one [32]. Normalized images were then processed with a co-registration algorithm to correct for any image movements between the wavelength bands, resulting in a 3D dataset (the so-called spectral cube). Based on the spectral cube, the average reflectance spectrum of each image was calculated [33]. Eleven indices were further selected (Table 1), encompassing vegetation, moisture, and compositional characteristics [22–24]. In case a specific wavelength was not available in the obtained data set, the band with the center wavelength closest to the required one was employed instead. All processing and analysis was performed with custom made software developed in Python. The code was written in version 3.9, while the openCV library employed to process and analyze the images was of version 4.5.2 [33]. In addition, the PyElastix library (version 1.2), containing a collection of algorithms, was used for image registration [33]. To export the results in excel file, the library XlsxWriter was used (version 3.0.1).

Table 1. Indices used in this study.

Index Name (Abbreviation)	Equation	Reference
Photochemical reflectance index (PRI)	$\frac{\rho_{531}-\rho_{570}}{\rho_{531}+\rho_{570}}$	[24]
Renormalized difference vegetation index (RDVI)	$\frac{\rho_{800}-\rho_{670}}{\sqrt{\rho_{800}+\rho_{670}}}$	[23]
Water index (WI)	$\frac{\rho_{900}}{\rho_{970}}$	[22]
Normalized difference vegetation index (NDVI)	$\frac{\rho_{800}-\rho_{680}}{\rho_{800}+\rho_{680}}$	[22]
Ratio of WI with NDVI (WI:NDVI)	$\frac{\rho_{900}}{\rho_{970}} / \frac{\rho_{800}-\rho_{680}}{\rho_{800}+\rho_{680}}$	[22]
Normalized water index (NWI)	$\frac{\rho_{970}-\rho_{880}}{\rho_{970}+\rho_{880}}$	[24]
Optimized soil-adjusted vegetation index (OSAVI)	$\frac{(1+0.16)\times(\rho_{800}-\rho_{670})}{(\rho_{800}+\rho_{670}+0.16)}$	[23]
Structure independent pigment index (SIPI)	$\frac{\rho_{800}-\rho_{445}}{\rho_{800}+\rho_{680}}$	[23]
Blue/green index 2 (BGI2)	$\frac{\rho_{450}}{\rho_{550}}$	[23]
Triangular vegetation index (TVI)	$0.5\times[120\times(\rho_{750}-\rho_{550}) - 200\times(\rho_{670}-\rho_{550})]$	[23]
Modified chlorophyll absorption in reflectance index (MCARI)	$[(\rho_{700}-\rho_{670}) - 0.2\times(\rho_{700}-\rho_{550})]\times(\frac{\rho_{700}}{\rho_{670}})$	[23]

2.4. Effect of Storage Period on Vase Life

The effect of storage period on vase life was determined. Following the respective storage period (0, 7, 14, and 21 d), vase life was evaluated by placing cut flowers in the vase (one flower per flask). The vases contained 400 mL sodium hypochlorite solution (1%, v/v), employed to inhibit bacterial growth, with their top covered with Parafilm, to

ensure that water loss could only occur via the flower stalks. The flasks were placed in a climate-controlled room at 19.6 °C, 62% relative air humidity, and a light intensity of 15 µmol m^{-2} s^{-1} (fluorescent tubes; TLD 58 W/84, Philips, Eindhoven, The Netherlands) under a daily light period of 12 h. The height of the vase solution column was held constant at 0.06–0.08 m over the evaluation period to avoid hydrostatic pressure differences between flowers with different transpiration rates [7,8]. The termination of vase life was determined based on the occurrence of at least one of the following criteria: (i) visible wilting (i.e., loss of turgor) of more than 50% of the open flowers or their pedicels; (ii) discoloration of either ray or disc florets of more than 50% of the open flowers; (iii) discoloration or withering of more than 50% of the leaves; (iv) discoloration of the stem [34]. To reduce subjectivity, three observers independently evaluated the end of vase life (without being aware of treatment assignment), and decision was based on majority vote [12]. In this study, no *Botrytis cinerea* infections were observed. The flower and flask weights were recorded separately at the onset and end of vase life. The transpiration rate was calculated per unit fresh weight and per unit time [12]. At the end of the experiment, the number of leaves on the main stem, along with (leaf, stem, and flower) area (by using ImageJ, Wayne Rasband/NIH, Bethesda, MD, USA) [8] were recorded. Ten cut flowers were assessed per cultivar and storage period.

2.5. Investigation of Cut Flower Traits Potentially Related to Vase Life Response to Storage Period

2.5.1. Effect of Storage Period on Cut Flower Transpiration Partitioning between Organs during Water Deficit

The effect of storage period on the contribution of each individual organ involved in whole-cut flower transpirational water loss was investigated in the course of desiccation. This was achieved by both evaluating transpiration response to desiccation at the tissue scale (leaves, stem, flowers), and considering associated allometric parameters to scale up tissue-level properties to the whole-cut flower (obtained as described above) [8]. Following the respective storage period (0, 7, 14, and 21 d), the cut flowers were placed into the test room, where they were kept for 2 h prior to measurements. This period served the dual aim of inducing stomatal opening and bringing cut flowers to test room temperature [7]. Subsequently, cut flowers were separated into leaves, stem, and flowers. Since preliminary work comparing transpiration rates between cut flower organs with either intact or sealed (by using paraffin wax) cut surfaces (i.e., petiole, two stem cut ends, and peduncle, respectively) indicated that water loss through the cut surfaces was negligible (below the detection limit), these were not sealed during measurements [35,36]. The detached leaves, stem, and flowers were placed on a bench, and the rate of water loss over time was gravimetrically recorded for 8, 144, and 144 h, respectively (±0.001 g; Mettler ME303TE, Giessen, Germany). For leaves and flowers, the abaxial surface and the calyx were facing downwards, respectively. Test room conditions were air temperature of 21 °C, relative air humidity equal to 55%, and continuous light with an intensity of 50 µmol m^{-2} s^{-1}, which was provided by fluorescent lamps (TLD 58 W/84, Philips, Eindhoven, The Netherlands). As an indication of air velocity, the rate of evaporation from two glass beakers was recorded in the test room during measurements. The evaporation rate of distilled water was 0.72 ± 0.01 mmol H_2O m^{-2} s^{-1}, which indicated adequate air circulation [35,37]. The employed environmental conditions are typical for dehydration experiments [38,39]. Different cut flower organs were always assessed simultaneously. At the end of the measurement, dry weight was determined after drying the tissue for 48 h at 80 °C in a drying cabinet. Tissue relative water content (RWC; also referred as relative turgidity) was calculated using the following equation

$$\mathrm{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated fresh weight} - \text{dry weight}} \times 100 \quad (1)$$

Stabilization RWC was defined as the RWC at which the transpiration rate stabilized [8]. In brief, the stable transpiration rate was defined as the average of the three consecutive points that do not significantly differ, while stabilization RWC was taken as

the RWC where the first of these three points was noted. Twelve replicates were assessed per cultivar and storage period.

2.5.2. Stomatal Density and Dimensions on Cut Flower Organs

The transpiration rate of an organ largely depends on the presence of stomata, as well as on their density and size [10,40]. On this basis, measurements were carried out on the adaxial and abaxial sides of leaves and floral petals, as well as on the stem. Images were acquired using a confocal laser scanning microscope (LabRAM HR-800, HORIBA, Kyoto, Japan). The laser pulses were focused using an objective (LMPLanFL; Olympus Corp., Tokyo, Japan) of either 10× (0.25 numerical aperture and 21.0 mm working distance), or 50× (0.50 numerical aperture and 10.6 mm working distance). Images were acquired by a 2 million pixel CMOS sensor (IDS Imaging Development Systems GmbH, Obersulm, Germany). In leaves, the fields of view were located in interveinal areas since veins lack stomata [41]. Stomatal dimensions (length, width) were determined on four randomly selected stomata, while stomatal density was counted on four non-overlapping fields of view. Stomatal size was defined as stomatal length multiplied by stomatal width [42]. Image processing was performed with ImageJ software (Wayne Rasband/NIH, Bethesda, MD, USA) [30]. Eight (stomatal density) or four (stomatal dimensions) replicates were assessed per cultivar.

2.5.3. Effect of Storage Period on Hydration Status and Rehydration Ability following a Desiccation Event

Cut flowers with decreased hydration status often express reduced vase life depending on the cultivar [7,8]. The effect of storage period on cut flower hydration status was thus assessed. Following the respective storage period (0, 7, 14, and 21 d), cut flower fresh weight was recorded, and then dry weight was determined, as described above. Cut flower water content (WC) was calculated using the following equation

$$\mathrm{WC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100 \quad (2)$$

Dry handling is commonly employed in the supply chain of cut flowers [7,8,13]. Following dry handling, enhanced ability to regain weight is associated with improved hydration status. The effect of storage period on the rehydration ability following a dehydration event, taken as an indication of water transport restoration, was therefore determined. Following the respective storage period (0, 7, 14, and 21 d), cut flowers were imposed to desiccation at 21 °C, 55% relative air humidity, and 50 µmol m^{-2} s^{-1} light intensity. Cut flowers were allowed to dehydrate to 90% of the initial weight [8]. Then, the cut ends were immediately placed in buckets filled with water at a height of 0.12 m. Afterwards, the cut flowers were incubated for 12 h in the refrigerated storage (5 °C and darkness). Subsequently, cut flower weight was measured. The rehydration method used was independent of the water loss through the stomatal component [7]. Cut flower weight was established by the balance between water uptake through the stem cut end and water loss through transpiration. The latter was minimized by applying a very low vapor pressure deficit and darkening.

Measurements were conducted on twelve replicates per cultivar and storage period.

2.5.4. Effect of Storage Period on Leaf Hydrogen Peroxide Content

H_2O_2 is a critical ROS, which accumulates under adverse conditions [15,43]. Following the respective storage period (0, 7, 14, and 21 d), leaf H_2O_2 content was assessed. The reaction mixture consisted of tissue extract supernatant, 0.5 mL 0.1% trichloroacetic acid, 0.5 mL of 0.1 M potassium-phosphate buffer (pH 7.0), and 1 mL of 1 M KI (w/v). Color developed for 45 min in darkness, and absorbance was then spectrophotometrically assessed at 390 nm (UV-1800, Shimadzu, Kyoto, Japan). H_2O_2 content was calculated by using a calibration curve prepared with eight known H_2O_2 concentrations. Measurements

were conducted on three replicates per cultivar and storage period. For each replicate, four samples (collected by using separate cut flowers) were pooled, and the assay was performed twice.

2.5.5. Effect of Storage Period on Lipid Peroxidation

Leaf malondialdehyde (MDA) content, taken as an indication of lipid peroxidation level [15], was evaluated by employing the thiobarbituric acid reactive substance assay following the respective storage period (0, 7, 14, and 21 d). Freshly cut leaf discs (0.5 g) were homogenized, and then added in 5 mL of 20% (w/v) trichloroacetic acid and 0.5% (w/v) thiobarbituric acid. The suspension was subsequently centrifuged (6000× g for 15 min). The obtained solution was heated (100 °C for 25 min). After equilibration at 25 °C, the precipitate was removed by centrifugation (6000× g for 5 min). The amount of MDA was calculated from the absorbance at 535 nm after subtracting the non-specific absorption at 600 nm. The extinction coefficient 156 mmol MDA L^{-1} cm^{-1} was used [15]. Measurements were conducted on three replicates per cultivar and storage period. Four discs were sampled per replicate leaf, and the assay was performed twice.

2.5.6. Effect of Storage Period on Leaf Total Phenolic and Total Flavonoid Contents

As phenolics and flavonoids exhibit strong antioxidant properties, they can be beneficial for plant antioxidant defense [15,17,18]. Following the respective storage period (0, 7, 14, and 21 d), leaf total phenolic and total flavonoid contents were therefore assessed. Leaf samples (0.1 g) were ground in mortar and pestle with liquid nitrogen, and were then extracted with 1 mL of 80% aqueous methanol in an ultrasonic bath for 10 min, and were then centrifuged (15,000× g for 10 min). The contents of total phenolics and total flavonoids were determined by using the Folin–Ciocalteu assay and aluminum chloride colorimetric assay, respectively, following Yang et al. (2021) [19]. The absorbance against prepared reagent blank was determined using a microplate reader (Infinite 200 PRO, TECAN, Männedorf, Switzerland). For total phenolic content, gallic acid was used as the standard reference and gallic acid equivalent (GAE) was expressed as mg per g dry mass. For total flavonoid content, rutin was used as the standard reference and rutin equivalent (RUE) was expressed as mg per g dry mass. Measurements were conducted on three replicates per cultivar and storage period. For each replicate, four samples (collected by using separate cut flowers) were pooled, and the assay was performed twice.

2.5.7. Leaf and Floral Mineral Analysis

Ca content has been related to postharvest longevity of gerbera [44] and rose [45,46]. To assess its potential role in the noted vase life response to prolonged storage, leaf and floral mineral analysis was conducted. Samples were washed with distilled water and then dried. Afterward, they were grounded into fine powder, assessed by using a 30-mesh screen [47]. A fine powder portion of 1 g was dry-ashed in a muffle furnace (515 °C for 6 h). Then, the ash was dissolved in 5 mL of 6 N HCl and diluted with double distilled water up to 50 mL [47]. N was determined by the Kjeldahl method [48]. P was measured colorimetrically with the vanado-molybdate method [49], while the concentrations of K and Na were assessed by using flame photometer [50]. The contents of Ca, Mg, Fe, and Zn were evaluated by atomic absorption spectrometry [51]. Mineral content was expressed per dry weight basis. Six replicates were evaluated per cultivar. For each replicate, four samples (collected by using separate cut flowers) were pooled, and the assay was performed twice.

2.6. Statistical Design and Analysis

Data analysis was performed using the R software (version 3.6.2; R Development Core Team, Vienna, Austria). For trait comparison among cultivars, an one-way analysis of variance (ANOVA) was performed, and statistically significant differences were based on Tukey's HSD (honestly significant difference) test. For vase life and transpiration during

this period, a split-plot design was employed, with storage duration prior to vase life evaluation (0, 7, 14, 21 d) as the main factor, and cultivar as the split factor.

3. Results

3.1. Visual Quality Characteristics, Mass Partitioning and Stem Strength

Distinct differences in leaf shape (aspect ratio and circularity) and serration (circularity) were apparent among the cultivars under study (Table 2). These differences were independent of leaf size (data not shown).

In flower characteristics, a great variation was also noted among the studied cultivars (Table 2). Number of flowers (12.6–35.9), flower distance (10.9–21.8 cm), and flower diameter (3.6–8.4 cm) varied by at least 100%.

As a fraction of cut flower weight, the cultivar range of leaf weight was 30.1–34.6%, of flower weight was 26.1–29.9%, and of stem weight was 38.2–40.0% (Table 2). Among cultivars, differences in fresh mass allocation were most prominent in leaf and flower weight (i.e., up to 15% difference). Stem strength varied between 20 and 30 g m^{-1}.

3.2. Effect of Storage Period on Leaf and Flower Reflectance

Multispectral images of leaf (either side) and flower (top view) were obtained following different periods of storage (0, 7, 14, and 21 d) in the three cultivars under study. Based on these data, eleven indices (PRI, RDVI, WI, NDVI, WI:NDVI, NWI, OSAVI, SIPI, BGI2, TVI, and MCARI), encompassing vegetation, moisture, and compositional characteristics [22–24], were calculated (Table 1). No trend was apparent either in the whole reflectance range (400–1050 nm) or any of the eleven indices in relation to the storage period (Tables S1–S3).

3.3. Effect of Storage Period on Vase Life

When cut flowers were not subjected to storage (0 d), the three cultivars under study had the similar vase life (25.5–26.4 d; Figure 1A). Storage period linearly decreased vase life in all cultivars. The rate of this decrease was the highest in cultivar 'Euro White', and the lowest in cultivar 'Pina Colada'. Following 21 d of storage, cultivar 'Euro White' had a vase life of 2.3 d, while cultivar 'Pina Colada' of 6.8 d (Figure 1A).

The cut flower water loss during vase life was not affected by storage period in all three cultivars (Figure 1B).

3.4. Investigation of Cut Flower Traits Potentially Related to Vase Life Response to Storage Period

3.4.1. Effect of Storage Period on Cut Flower Transpiration Partitioning between Organs during Water Deficit

In all three cultivars, whole-cut flower transpiration decreased in the course of desiccation (8 h; Figure 2).

As desiccation progressed, transpiration also decreased in the leaves, stem, and flowers (8, 144, and 144 h, respectively; Figures S1–S3). Following 8 h of desiccation, leaves had the highest cumulative water loss (0.222–0.378 g g^{-1}), whereas flowers had the least (0.076–0.122 g g^{-1}; Table 4). Within 8 h of desiccation, the decrease in transpiration was more pronounced in leaves as compared to the stem (31.2–57.0% versus 10.1–26.4%, respectively; Table 4). With two exceptions, the decrease in stem transpiration was more prominent as compared to the respective decrease in floral transpiration following 8 h of desiccation (10.1–26.4% versus 2.0–15.7%, respectively; Table 4).

By plotting transpiration versus hydration status (RWC), it was noted that leaf transpiration actively decreased to a stable level, suggesting active (stomatal) regulation (8 h of desiccation; Figure 3). The RWC, where leaf transpiration stabilized, varied depending on the cultivar (Table 4). By contrast, relevant (active) regulation was noted in neither the stem nor flower, where transpiration continuously decreased without achieving a stable value after 144 h of desiccation (Figures 4 and 5).

Table 2. Leaf shape traits (aspect ratio, circularity, roundness, and solidity), as well as flower characteristics (number per cut flower, distance, and diameter), fresh weight distribution (to leaves, flowers, or stem), and main stem strength (mass per unit length) in three cut chrysanthemum cultivars. Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$.

Cultivar	Leaf				Flower			Leaf Weight (%)	Flower Weight (%)	Stem Weight (%)	Stem Strength (g m^{-1})
	Aspect Ratio	Circularity	Roundness	Solidity	Number	Distance (cm)	Diameter (cm)				
'Pina Colada'	1.85 ± 0.03 ^{b}	0.15 ± 0.00 ^{b}	0.54 ± 0.01 ^{b}	0.67 ± 0.00 ^{b}	35.9 ± 0.8 ^{a}	21.8 ± 0.9 ^{a}	8.4 ± 0.2 ^{a}	30.1 ± 0.7 ^{b}	29.9 ± 0.5 ^{a}	40.0 ± 0.5	20 ± 0 ^{b}
'Code Green'	1.63 ± 0.04 ^{c}	0.19 ± 0.00 ^{a}	0.62 ± 0.02 ^{a}	0.72 ± 0.00 ^{a}	16.9 ± 0.8 ^{b}	16.4 ± 1.1 ^{b}	3.6 ± 0.1 ^{c}	33.5 ± 1.4 ^{ab}	28.4 ± 1.0 ^{ab}	38.2 ± 0.8	27 ± 1 ^{a}
'Euro White'	2.21 ± 0.03 ^{a}	0.12 ± 0.00 ^{c}	0.46 ± 0.01 ^{c}	0.62 ± 0.01 ^{c}	12.6 ± 0.5 ^{c}	10.9 ± 0.7 ^{c}	7.6 ± 0.1 ^{b}	34.6 ± 0.9 ^{a}	26.1 ± 1.0 ^{b}	39.4 ± 0.9	30 ± 1 ^{a}
n	60	60	60	60	40	12	12	12	12	12	12
p	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	0.011487	0.013213	0.220553	<0.00001

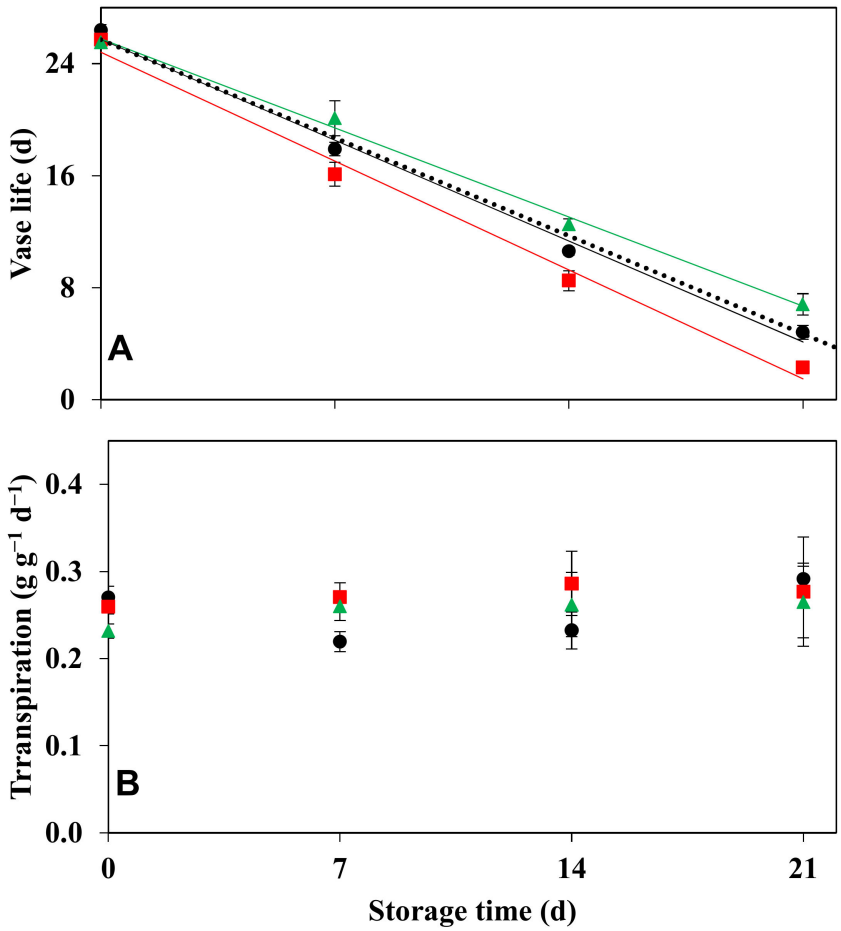

Figure 1. Vase life (**A**), and transpiration during vase life (**B**) as function of storage time (5 °C and darkness) prior to evaluation in three cut chrysanthemum cultivars (**triangle**, 'Pina Colada'; **circle**, 'Code Green'; **square**, 'Euro White'). In panel A, the dashed line depicts the 1:1 relationship [i.e., vase life at storage time t = (vase life at storage time 0)—(storage time t)]. Several morphological parameters of the employed cut flowers are provided in Table 3. Values are the means of ten cut flowers ± SE. Statistics are provided in Table S4.

Table 3. Cut flower fresh weight, number of leaves on the main stem, and (leaf, stem, and flower) area of three cut chrysanthemum cultivars, employed for vase life evaluation. Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$ (n = 40).

Cultivar	Fresh Weight (g)	Number of Leaves	Leaf Area (cm^2)	Stem Area (cm^2)	Flower Area (cm^2)
'Pina Colada'	98.2 ± 1.7 ^{a}	38.4 ± 1.2 ^{a}	637 ± 24 ^{a}	199 ± 5 ^{a}	387 ± 17 ^{a}
'Code Green'	84.8 ± 1.9 ^{b}	15.0 ± 0.5 ^{b}	543 ± 20 ^{b}	149 ± 6 ^{b}	111 ± 5 ^{c}
'Euro White'	94.6 ± 1.6 ^{a}	10.4 ± 0.4 ^{c}	610 ± 20 ^{ab}	149 ± 4 ^{b}	162 ± 7 ^{b}
p	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001

The relative input of each individual organ (leaves, stem, flowers) to the whole-cut flower transpiration was investigated (Figure 6). During 8 h of desiccation, the relative input of leaves decreased, whereas the respective input of the stem and flowers generally increased (Figure 6). Flowers acquired the lowest fraction of whole-cut flower transpiration (Figure 6). In the first hour of desiccation, the leaf relative input was higher than the stem relative input (Figure 6). As desiccation progressed, the major relative input was either the leaves or the stem (Figure 6).

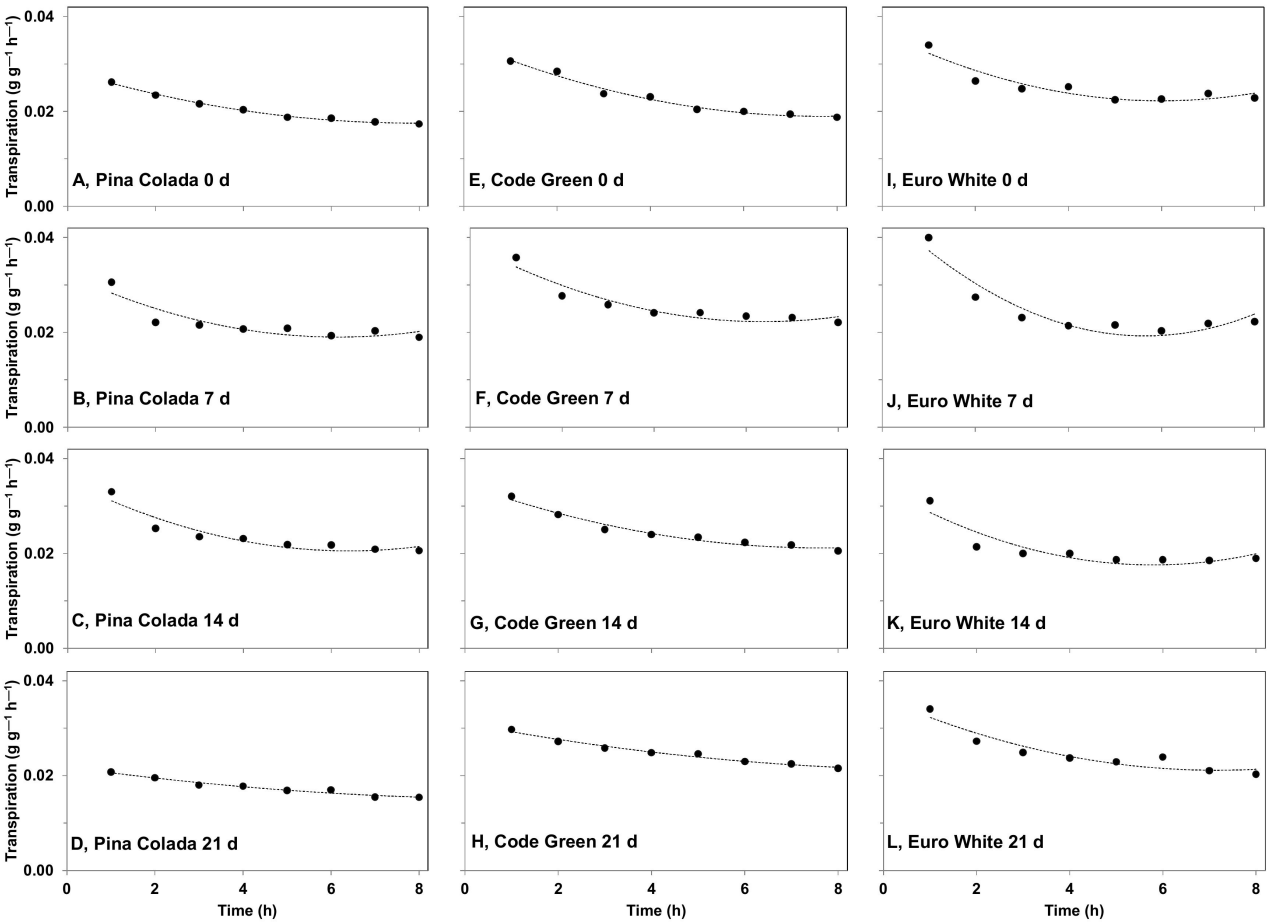

Figure 2. Cut flower transpirational water loss as a function of dehydration time in three cut chrysanthemum cultivars ['Pina Colada' (**A**–**D**); 'Code Green' (**E**–**H**); 'Euro White' (**I**–**L**)] stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). The transpiration (data in Figures S1–S3) in combination with the cultivar fresh mass fraction of each component (data in Table 2) were considered for the computation.

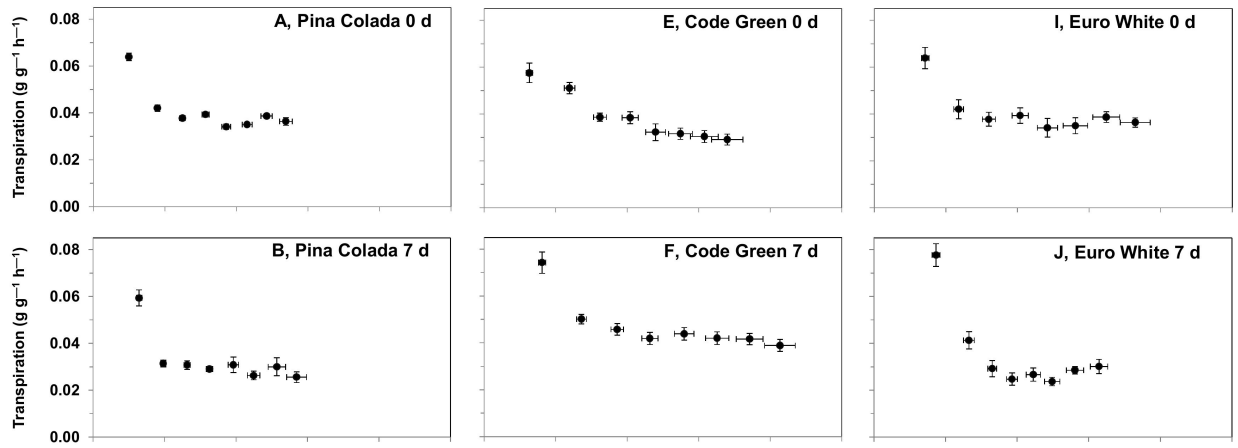

Figure 3. *Cont*.

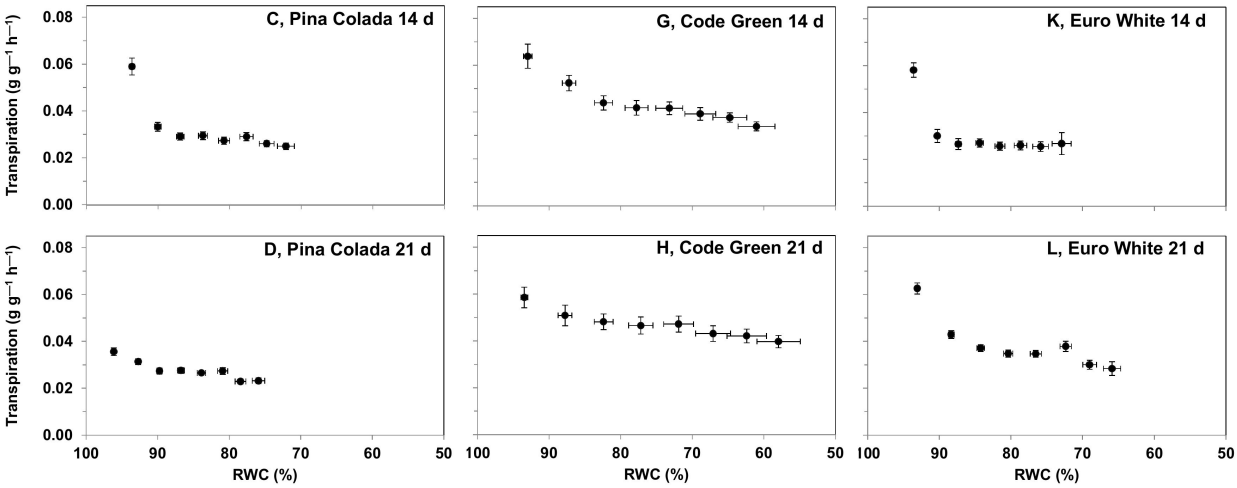

Figure 3. Leaf transpirational water loss, per initial weight, as a function of relative water content (RWC) in three cut chrysanthemum cultivars ['Pina Colada' (**A**–**D**); 'Code Green' (**E**–**H**); 'Euro White' (**I**–**L**)] stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). Leaves were left to desiccate for 8 h (data in Figure S1). When the SE bars are not visible, the SE is smaller than the symbol.

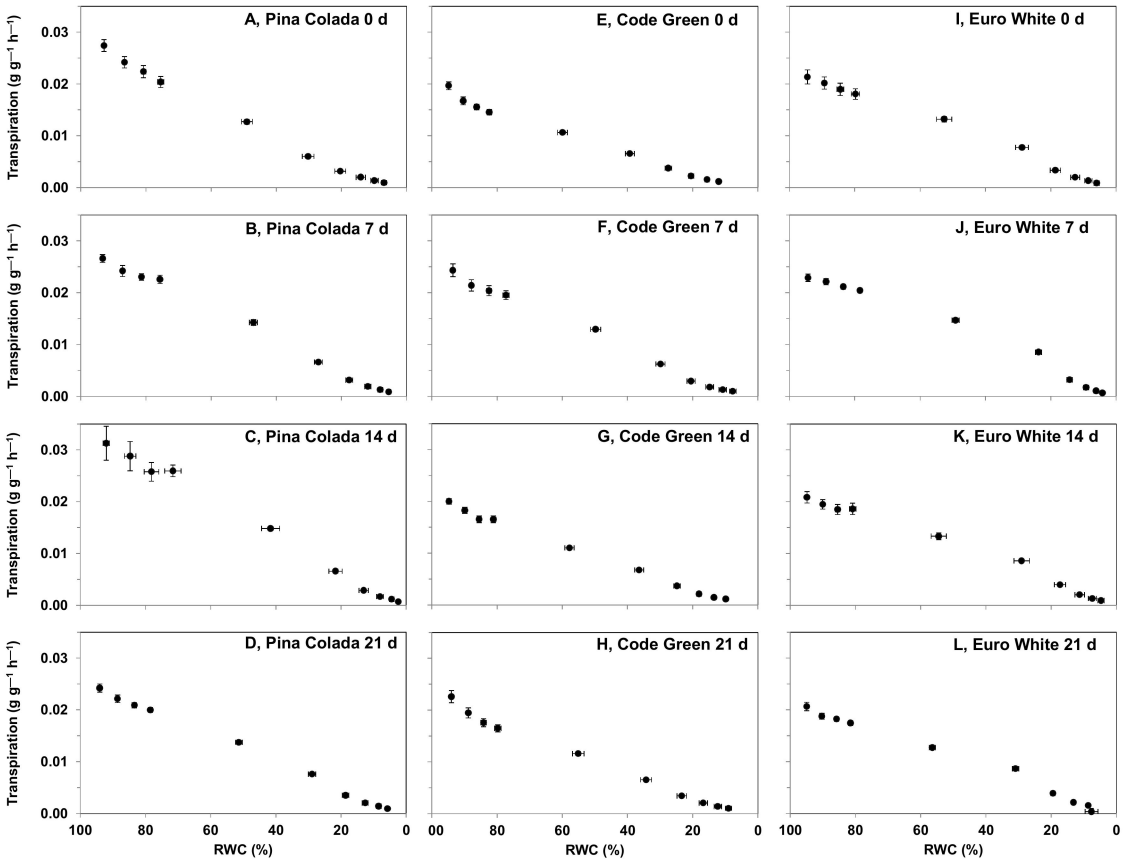

Figure 4. Stem transpirational water loss, per initial weight, as a function of relative water content (RWC) in three cut chrysanthemum cultivars ['Pina Colada' (**A**–**D**); 'Code Green' (**E**–**H**); 'Euro White' (**I**–**L**)] stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). Stems were left to desiccate for 144 h (data in Figure S2). When the SE bars are not visible, the SE is smaller than the symbol.

Table 4. Cumulative transpiration and transpiration rate decrease over 8 h of desiccation of cut flower (data in Figure 2), leaf (data in Figure S1), stem (data in Figure S2), and flower (data in Figure S3), as well as leaf relative water content (RWC) at which transpiration rate stabilizes (data in Figure 3) in three cut chrysanthemum cultivars. Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$ ($n = 12$).

Cultivar	Storage Period (d)	Cumulative Transpiration (g g^{-1})				Decrease in Transpiration (%)				RWC (%)
		Cut Flower	Leaf	Stem	Flower	Cut Flower	Leaf	Stem	Flower	Leaf
'Pina Colada'	0	0.164	0.248 ± 0.008 ^{de}	0.189 ± 0.009 ^{ab}	0.079 ± 0.004 ^{d}	33.7	45.7 ± 3.2 ^{ab}	25.8 ± 1.3	9.1 ± 1.5 ^{ab}	75.3 ± 0.9
'Code Green'		0.184	0.309 ± 0.019 ^{acd}	0.133 ± 0.005 ^{abc}	0.103 ± 0.007 ^{abc}	38.7	49.4 ± 1.7 ^{ab}	25.6 ± 1.9	8.7 ± 1.5 ^{ab}	69.6 ± 1.9
'Euro White'		0.202	0.327 ± 0.018 ^{ab}	0.157 ± 0.009 ^{bcd}	0.121 ± 0.002 ^{a}	32.8	40.8 ± 4.2 ^{ab}	15.0 ± 1.7	12.2 ± 1.0 ^{ab}	70.0 ± 3.1
'Pina Colada'	7	0.174	0.263 ± 0.013 ^{cd}	0.193 ± 0.005 ^{ab}	0.092 ± 0.008 ^{bc}	38.0	53.8 ± 6.3 ^{bc}	14.7 ± 3.0	3.8 ± 1.6 ^{ab}	76.9 ± 1.2
'Code Green'		0.206	0.378 ± 0.019 ^{a}	0.171 ± 0.008 ^{a}	0.079 ± 0.002 ^{d}	38.3	46.0 ± 4.2 ^{ab}	19.3 ± 1.5	10.8 ± 3.1 ^{ab}	68.9 ± 3.2
'Euro White'		0.198	0.282 ± 0.011 ^{bcd}	0.173 ± 0.004 ^{bcd}	0.122 ± 0.003 ^{a}	44.4	57.0 ± 7.3 ^{c}	10.1 ± 2.1	8.2 ± 1.1 ^{ab}	76.3 ± 1.8
'Pina Colada'	14	0.190	0.258 ± 0.011 ^{cd}	0.224 ± 0.018 ^{a}	0.073 ± 0.004 ^{de}	37.6	56.2 ± 3.0 ^{c}	13.4 ± 4.0	2.0 ± 1.2 ^{a}	77.2 ± 1.5
'Code Green'		0.197	0.354 ± 0.023 ^{ab}	0.143 ± 0.005 ^{ab}	0.083 ± 0.004 ^{cd}	36.0	45.0 ± 2.8 ^{ab}	17.3 ± 2.3	5.4 ± 1.5 ^{ab}	66.0 ± 3.7
'Euro White'		0.167	0.242 ± 0.011 ^{de}	0.155 ± 0.008 ^{bcd}	0.106 ± 0.004 ^{ab}	39.0	57.0 ± 5.5 ^{c}	10.8 ± 1.8	12.1 ± 1.0 ^{ab}	77.5 ± 1.3
'Pina Colada'	21	0.141	0.222 ± 0.008 ^{e}	0.174 ± 0.005 ^{bcd}	0.054 ± 0.004 ^{e}	25.8	33.9 ± 3.5 ^{ab}	17.1 ± 1.5	13.8 ± 5.4 ^{bc}	79.4 ± 0.8
'Code Green'		0.199	0.376 ± 0.028 ^{a}	0.152 ± 0.007 ^{a}	0.076 ± 0.003 ^{d}	27.6	31.2 ± 2.2 ^{a}	26.4 ± 1.5	4.8 ± 1.0 ^{ab}	63.1 ± 3.4
'Euro White'		0.198	0.308 ± 0.010 ^{abc}	0.150 ± 0.004 ^{de}	0.121 ± 0.007 ^{a}	40.4	55.2 ± 4.3 ^{c}	15.0 ± 1.9	15.7 ± 2.0 ^{c}	72.7 ± 1.5
p										
Cultivar			<0.001	<0.001	<0.001		0.009	<0.001	0.003	<0.001
Storage period			0.341	0.004	<0.001		0.001	<0.001	0.029	0.444
Cultivar × Storage period			<0.001	0.002	<0.001		0.007	0.272	0.005	0.093

Table 5. Leaf stomatal traits in three cut chrysanthemum cultivars. Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$.

Cultivar	Adaxial Stomatal					Abaxial Stomatal					Leaf Stomatal Density (mm^{-2})	Stomata on the Abaxial Side (%)
	Density (mm^{-2})	Length (μm)	Width (μm)	Size ($μm^2$)	Length to Width	Density (mm^{-2})	Length (μm)	Width (μm)	Size ($μm^2$)	Length to Width		
'Pina Colada'	10.0 ± 0.6 ^{a}	58.8 ± 2.1 ^{b}	32.2 ± 1.0 ^{b}	1903 ± 125 ^{b}	1.83 ± 0.04 ^{a}	32.9 ± 1.1 ^{b}	56.9 ± 2.8 ^{b}	38.4 ± 1.2 ^{b}	2192 ± 166 ^{b}	1.49 ± 0.04	42.9 ± 1.2	76.8 ± 1.3 ^{b}
'Code Green'	10.4 ± 0.5 ^{a}	63.6 ± 0.8 ^{a}	37.4 ± 0.8 ^{a}	2388 ± 79 ^{a}	1.72 ± 0.03 ^{ab}	28.8 ± 0.4 ^{b}	57.1 ± 1.1 ^{b}	37.9 ± 0.8 ^{b}	2167 ± 55 ^{b}	1.51 ± 0.05	39.3 ± 0.7	73.4 ± 1.5 ^{b}
'Euro White'	5.9 ± 0.4 ^{b}	57.3 ± 1.2 ^{b}	34.7 ± 0.8 ^{ab}	1990 ± 77 ^{b}	1.66 ± 0.03 ^{b}	37.1 ± 0.8 ^{a}	63.8 ± 1.1 ^{a}	42.7 ± 1.2 ^{a}	2731 ± 91 ^{a}	1.50 ± 0.05	43.0 ± 0.8	86.0 ± 0.9 ^{a}
n	8	4	4	4	4	8	4	4	4	4	8	8
p	<0.00001	0.003543	0.000482	0.000579	0.020969	0.000071	0.001509	0.000626	0.000035	0.886066	0.086436	<0.00001

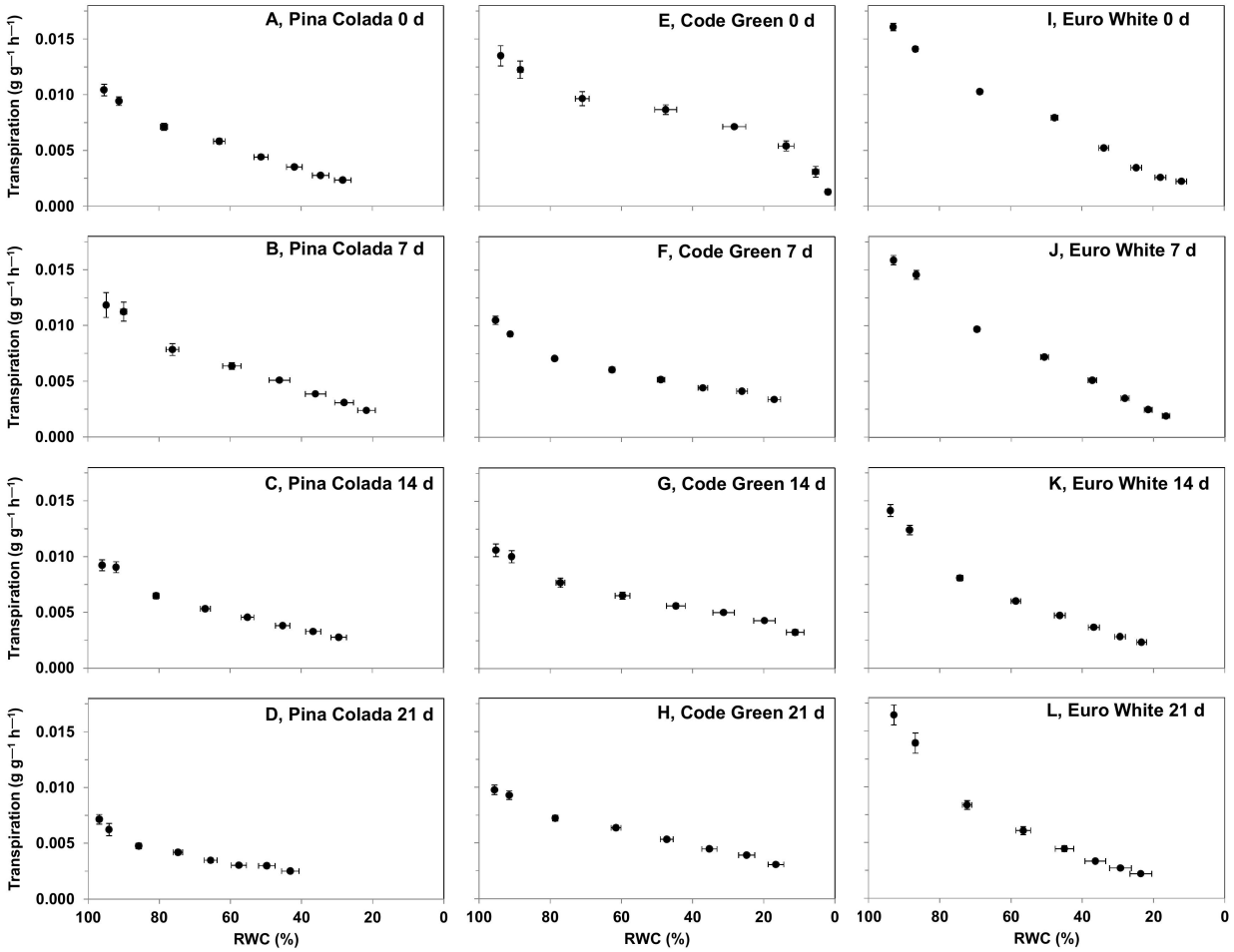

Figure 5. Floral transpirational water loss, per initial weight, as a function of relative water content (RWC) in three cut chrysanthemum cultivars ['Pina Colada' (**A**–**D**); 'Code Green' (**E**–**H**); 'Euro White' (**I**–**L**)] stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). Flowers were left to desiccate for 144 h (data in Figure S3). When the SE bars are not visible, the SE is smaller than the symbol.

No consistent effect of storage period on either the whole-cut flower or individual organ (leaves, stem, flowers) transpiration response to desiccation was apparent (Figures 2–5 and Table 4). Consequently, storage period did also not alter the relative input of each individual organ to the whole-cut flower transpiration (Figure 6).

3.4.2. Stomatal Density and Dimensions on Cut Flower Organs

Leaf stomatal density (39.3–43.0 stomata mm^{-2}) did not differ among the three cultivars under study (Table 5). In all cultivars, most stomata (73.4–86.0%) were situated on the abaxial leaf side, and were more rounded (i.e., lower length to width ratio) as compared to the adaxial one (Table 5).

Stem stomatal density (1.6–3.7 stomata mm^{-2}) did also not differ among the three cultivars (Table 6). Stomata on the stem were smaller and in a much lower density as compared to leaves (Tables 5 and 6). No stomata were observed on either side of petals (data not shown).

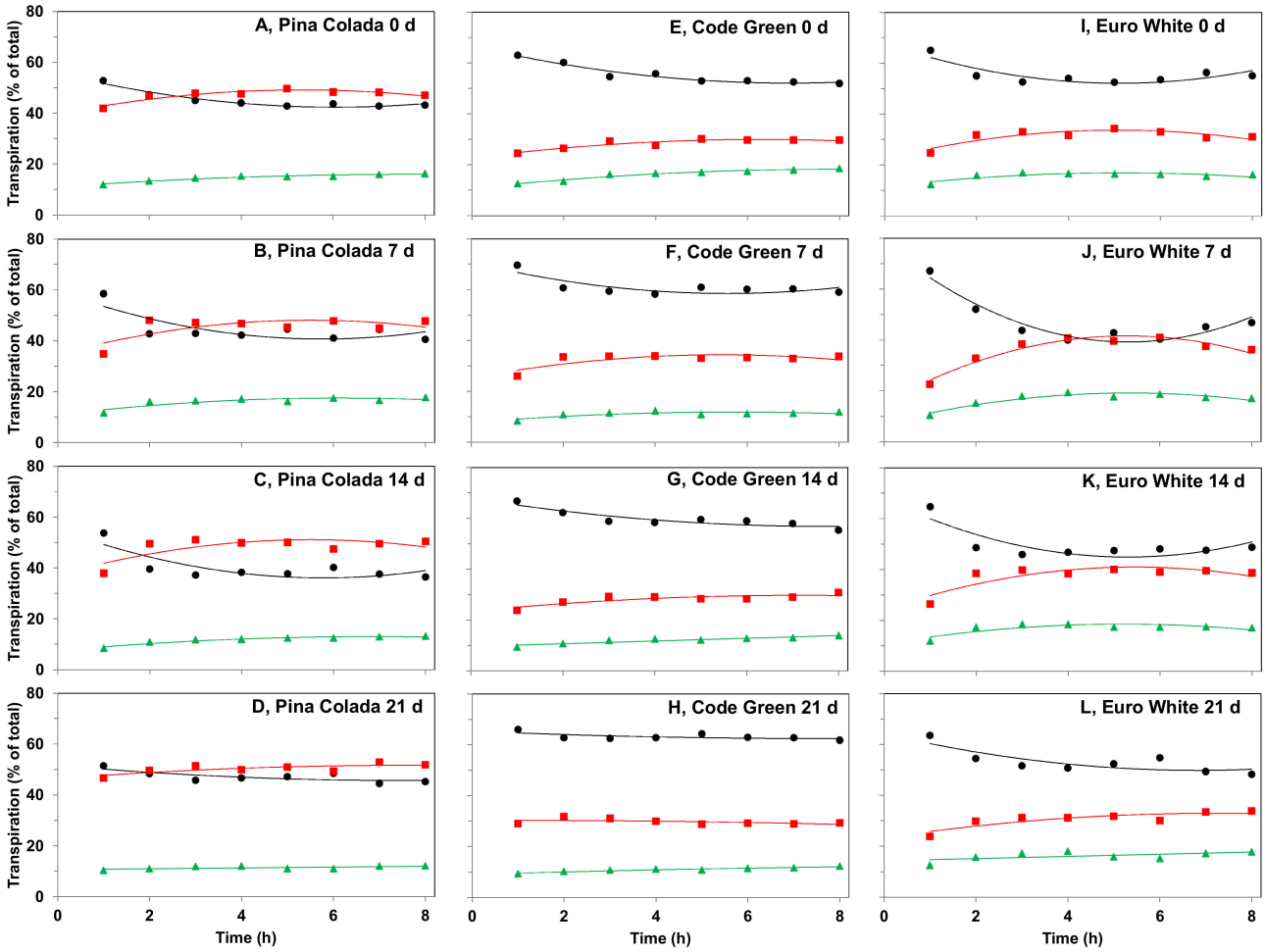

Figure 6. Transpirational water loss, as a percentage of total cut flower water loss (data in Figure 2), of leaves (**circle**), stem (**square**), and flower (**triangle**) as a function of dehydration time in three cut chrysanthemum cultivars ['Pina Colada' (**A**–**D**); 'Code Green' (**E**–**H**); 'Euro White' (**I**–**L**)] stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). The transpiration (data in Figures S1–S3) in combination with the cultivar fresh mass fraction of each component (data in Table 2) were considered for the computation.

Table 6. Stem stomatal traits in three cut chrysanthemum cultivars. Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$.

Cultivar	Stomatal				
	Density (mm^{-2})	Length (µm)	Width (µm)	Size ($µm^2$)	Length to Width
'Pina Colada'	2.2 ± 0.7	49.6 ± 2.6	24.0 ± 1.1	1194 ± 101	2.08 ± 0.12 ^{a}
'Code Green'	3.7 ± 0.8	47.0 ± 3.0	30.8 ± 2.5	1476 ± 220	1.55 ± 0.08 ^{b}
'Euro White'	1.6 ± 0.7	50.4 ± 3.9	28.9 ± 2.2	1484 ± 205	1.75 ± 0.10 ^{ab}
n	8	4	4	4	4
p	0.123582	0.742849	0.081331	0.80645	0.007452

3.4.3. Effect of Storage Period on Hydration Status and Rehydration Ability following a Desiccation Event

The effect of storage period on cut flower hydration status was investigated. Seven d of storage slightly enhanced cut flower hydration in all three cultivars, while an extension of the storage period to 14 or 21 d did not have any additional effect (Figure 7A).

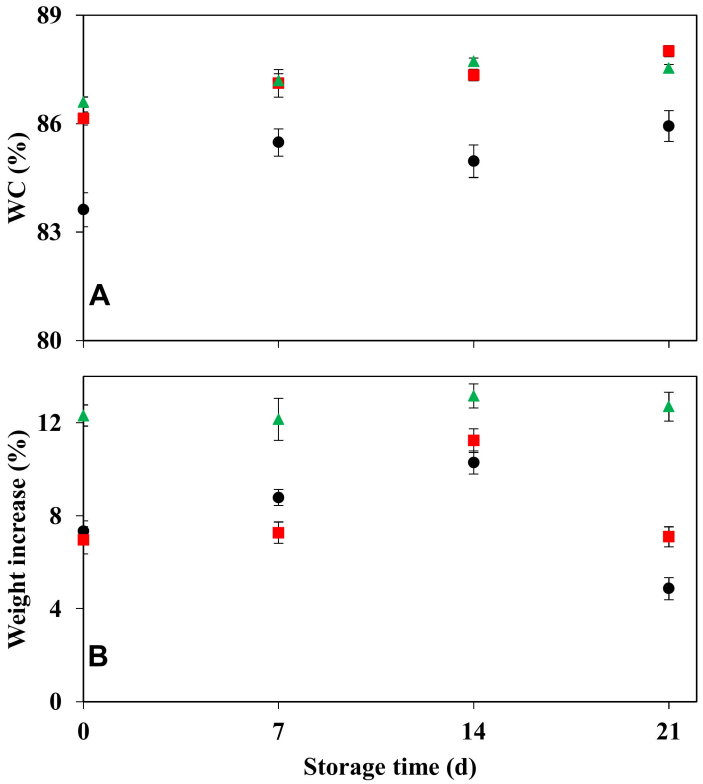

Figure 7. Cut flower water content (WC; **A**), and rehydration-induced cut flower fresh weight increase following 10% weight loss (**B**) as function of storage time (5 °C and darkness) prior to evaluation in three cut chrysanthemum cultivars (**triangle**, 'Pina Colada'; **circle**, 'Code Green'; **square**, 'Euro White'). Values are the means of twelve cut flowers ± SE. Statistics are provided in Table S4.

The effect of storage period on the rehydration ability following a dehydration event was also assessed. In cultivar 'Pina Colada', the storage period did not affect rehydration ability (Figure 7B). In cultivar 'Euro White', a higher rehydration ability was noted at 14 d of storage, with the remaining periods (0, 7, 21 d) showing similar values (Figure 7B). In cultivar 'Code Green', an increase in the rehydration ability was noted at 7 and 14 d of storage as compared to 0 d, followed by a decrease at 21 d (Figure 7B).

3.4.4. Effect of Storage Period on Leaf Hydrogen Peroxide Content and Lipid Peroxidation

H_2O_2 is a critical ROS. Leaf H_2O_2 content linearly increased as the storage period was extended (Figure 8A). The rate of this increase was the highest in cultivar 'Euro White', and the lowest in cultivar 'Pina Colada' (Figure 8A). The same trends were noted for leaf MDA content (Figure 8B), which was determined as a measure of lipid peroxidation.

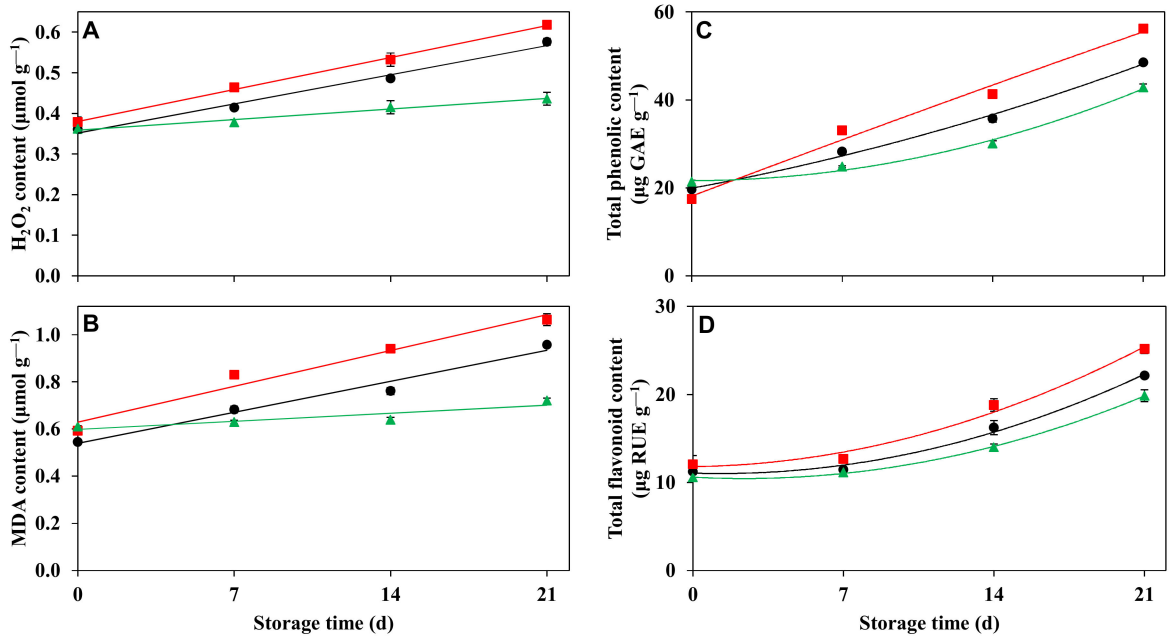

Figure 8. Leaf hydrogen peroxide (H_2O_2; **A**), malondialdehyde (MDA; **B**), total phenolic (**C**), and total flavonoid (**D**) contents as function of storage time (5 °C and darkness) in three cut chrysanthemum cultivars (**triangle**, 'Pina Colada'; **circle**, 'Code Green'; **square**, 'Euro White'). Values are the means of three leaves ± SE. GAE, gallic acid equivalent; RUE, rutin equivalent. Statistics are provided in Table S4.

3.4.5. Effect of Storage Period on Leaf Total Phenolic and Total Flavonoid Contents

Phenolics and flavonoids are key antioxidant elements. Leaf total phenolic and total flavonoid content increased with storage period (Figure 8C,D). This increase was consistently more prominent in cultivar 'Euro White', and weaker in cultivar 'Pina Colada' (Figure 8C,D).

3.4.6. Leaf and Floral Mineral Analysis

Cultivar differences in vase life response to cold storage were associated with variation among the minerals under study (P, K, Na, Ca, Zn, Fe, and Mg) in neither the leaves (Table S5) nor flowers (Table S6). An exception to this trend was N, which was noted at a lower leaf content as cultivar sensitivity to cold storage increased (Table S5).

4. Discussion

This study examines the processes underlying the long-term storage-induced reduction in vase life, and the potential of recording reflectance as a measure of storage duration.

4.1. The Contribution of Distinct Organs to the Whole-Cut Flower Transpiration in Relation to the Presence and Functionality of Stomata

A dissimilar transpiration rate and response to desiccation was noted among cut flower organs (Figures 3–5). During desiccation, the decrease in leaf transpiration followed a two-phase response (Figure 3), which is typical for stomatal closure [36]. Instead, no specific pattern in stem and floral transpiration was apparent (Figures 4 and 5), suggesting the absence of active control [36]. These findings are in agreement with recent work [8]. In this study, the distribution of stomata on different organs of cut chrysanthemum was additionally investigated. Stomata were not observed on either side of petals. This explains the low rate and the lack of active control in floral transpiration (Figure 5). Instead, early work reports that stomata were present on the abaxial petal side of two chrysanthemum cultivars, though at an extremely low density (0.2 stomata mm^{-2}) [52]. This

study for the first time indicates that stomata were also present on the cut chrysanthemum stem (Table 6). Stomatal anatomy, however, was distinctly different on the stem as compared to the leaves. On the stem, stomatal density was lower by at least a factor of 10 (1.6–3.7 versus 39.3–43.0 stomata mm^{-2}), and stomatal size was smaller by at least 28% (1194–1484 versus 1903–2731 μm^{-2}; Tables 5 and 6). The presence of these non-functional stomata, as manifested by their response to desiccation, at least partly underlies the high rates of stem transpiration (Figure 4).

Organ transpiration dynamics were also scaled up to the whole-cut flower level (Figure 6). Independently of the cut flower hydration status, flowers acquired a small fraction of whole-cut flower transpiration (Figure 6). Under enhanced cut flower hydration (i.e., at the onset of desiccation), leaves were the major component of the whole-cut flower transpiration (Figure 6). Under a decreased cut flower hydration level (i.e., in the progress of desiccation), instead, the major component of the whole-cut flower transpiration was either the leaves or the stem (Figure 6). Similar findings were recently reported [8]. Breeding for better cut flower water relations, as an avenue to improve vase life, has been mainly directed towards the leaf water loss trait [20,21,53]. Although not supported by the obtained data so far, Körner et al. (2021) [29] have pointed out that there is an inherent limit in decreasing leaf stomatal conductance without a yield penalty. The present results in combination with data on carnation [11] suggest that stem transpiration is undoubtedly an additional trait of high interest towards improved cut flower water relations, at least in some cut flower species. Including it in breeding programs will, thus, partly alleviate the pressure for selection of the low leaf weight loss trait, which may eventually limit productivity.

4.2. Vase Life Response to Long-Term Storage Is Related to Oxidative Stress

Storage duration exerted a negative effect on vase life (Figure 1A). Long-term storage has been earlier associated with decreased vase life in other cut flower species [2–5]. This study additionally indicates that vase life is linearly decreased in response to storage duration (Figure 1A). For the first time, it is also shown that the rate of the vase life decrease is strongly cultivar specific (Figure 1A). Differences in vase life among cultivars were magnified as storage duration increased. Several processes potentially underlying the adverse effect of storage on vase life were also evaluated.

Factors determining cut flower water balance exert a strong influence on vase life [5]. Water balance is set by the difference between water loss and uptake [6]. In this study, the effects of storage period (0, 7, 14, and 21 d) on both organ (leaves, stems, flowers) and whole-cut flower transpiration dynamics in response to a stomatal closing stimulus (desiccation) were evaluated (Figures 2–5 and Table 4). Storage duration (0–21 d) did not consistently affect stomatal functioning on leaves (Figure 3 and Table 4), non-leaf tissue transpiration pattern (Figures 4 and 5, and Table 4) or the relative contribution of each organ to the whole-cut flower transpiration (Figure 6). In addition, the cut flower water loss during vase life was also not altered by the storage period (Figure 1B). These data collectively indicate that the storage-induced reduction in vase life is not related to the water loss rate or regulation. Instead, recent work on rose indicates that long-term storage (28 d at 0.5 °C) attenuated stomatal functioning, which, in turn, was a factor contributing to the reduced vase life of these cut flowers [5]. Therefore, the effect of storage on stomatal functioning is species dependent. Species differences in the attenuation of stomatal functioning following post-cultivation exposure to high relative air humidity ($\geq$85%) have also been reported [9,10,40].

Impaired hydration prior to placement in the vase has been associated with lower cut flower longevity [7,8,13]. Under this background, cut flower hydration was also determined following storage. Cut flower hydration was enhanced by 7 d of storage, while further storage duration did not have any additional effect (Figure 7A). Therefore, the noted storage-induced vase life decrease is certainly not related to cut flower hydration.

In real-world situations, cut flowers are often held dry [7,8,13]. Following dry handling, an efficient water transport restoration will promote cut flower hydration. On this basis,

the effect of storage period on the rehydration ability following a dehydration event was assessed. With a single exception (cultivar 'Code Green' at 21 d of storage), the rehydration ability was either not affected or even increased by storage (Figure 7B). These findings clearly illustrate that water uptake is not impaired by cold storage, and in this way it does not contribute to the storage-induced vase life decrease.

Considering, collectively, data on water loss (Figures 1B and 2, Figures 3–5, and Table 4), hydration state (Figure 7A), and water uptake restoration ability (Figure 7B), it becomes apparent that the storage-induced progressive decrease in vase life is not associated with cut flower water relations in chrysanthemum.

As a by-product of membrane lipid oxidation, MDA content commonly increases in response to oxidative stress [15]. In this study, leaf MDA content increased with the duration of storage (Figure 8B), and this increase was associated with leaf H_2O_2 content (Figure 8A). An increase in membrane lipid oxidation owing to cold storage-induced ROS elevation has been earlier reported [3,4,14]. This study for the first time shows that the membrane lipid oxidation level was associated with cultivar sensitivity to cold storage (Figures 1A and 8B). These results might be taken to indicate that membrane stability was a major factor in determining cut flower longevity following cold storage.

The cultivars undergoing increased H_2O_2 accumulation (Figure 8A) also showed higher content of phenolics and flavonoids (Figure 8C,D). These antioxidant elements are accumulated to scavenge and detoxify ROS [17–19]. However, the increased activation of the oxidative protection networks was not sufficient to alleviate the cold storage-induced oxidative damage, as expressed by MDA content (Figure 8B). Contrary to our findings, increased stimulation of antioxidant defense has been earlier associated with a more pronounced alleviation of oxidative stress [4,14,15].

Enhanced leaf or floral Ca contents have been earlier associated with long vase life owing to advanced cell wall rigidity, postponement of senescence, and improved *B. cinerea* tolerance [44–46]. In this study, neither leaf nor floral Ca contents underlay cultivar differences in vase life response to storage duration. In conclusion, the processes controlled by Ca content are not primary in shaping genetic differences in the vase life of cut flowers that had been subjected to long-term storage.

4.3. Leaf and Flower Reflectance Were Not Indicative of Storage Period

Since light reflectance profiles are indicative of a range of biochemical and physiological aspects [22–26], it was hypothesized that they may be employed to provide an indication of storage period. This hypothesis was tested by analyzing either leaf side, as well as the flower (top view). However, the results of this study did not validate this hypothesis. By examining the whole reflectance range (400–1050 nm) or eleven commonly employed indices (Table 1) [22–24], no indication of storage period could be deduced (Tables S1–S3). Therefore, storage period-induced changes in biochemical and physiological features captured by reflectance profiles were either not present, or were so minor, being lower than the among batch variation. Our results indicate that an indication of storage period cannot be deduced by using cut flower reflectance profiles.

5. Conclusions

This study dealt with the processes underlying the vase life response to long-term storage (up to 21 d) and the possibility of using light reflectance profiles (400–1050 nm) as an indication of storage duration. Stomata were present on both the leaves (either side) and stem but not on petals (either side). As compared to leaves, stomata on the stem were non-functional, smaller, and at a lower density. Floral transpiration was only a minor portion of the whole-cut flower transpiration, with the main fraction accounted by either the leaves or stem depending on the cut flower hydration state. Vase life linearly decreased in response to storage duration, with the rate of this decrease being cultivar dependent. Storage duration (0–21 d) did not affect leaf stomatal functioning, non-leaf tissue (flower, stem) transpiration, or the organ contribution to the whole-cut flower transpiration. Storage

generally improved cut flower hydration and did not attenuate water uptake restoration ability. Storage duration triggered membrane lipid oxidation owing to H_2O_2 accumulation. The increase in membrane lipid oxidation level was associated with the cold storage-induced decrease in vase life. The light reflectance profiles of leaves (either side) and flowers (top view) were not consistently affected by the duration of storage. In conclusion, genetic variation in cold storage-induced vase life decrease was associated with cultivar differences in oxidative state, whereas cut flower water relations were clearly not implicated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12020185/s1, Figure S1: Leaf transpirational water loss, per initial weight, as a function of desiccation time in three cut chrysanthemum cultivars stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). When the SE bars are not visible, the SE is smaller than the symbol; Figure S2: Stem transpirational water loss, per initial weight, as a function of desiccation time in three cut chrysanthemum cultivars stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). When the SE bars are not visible, the SE is smaller than the symbol; Figure S3: Floral transpirational water loss, per initial weight, as a function of desiccation time in three cut chrysanthemum cultivars stored (5 °C and darkness) for different periods (0, 7, 14, and 21 d) prior to evaluation (n = 12). When the SE bars are not visible, the SE is smaller than the symbol; Table S1: Effect of storage period (5 °C and darkness) on the photochemical reflectance index (PRI), renormalized difference vegetation index (RDVI), water index (WI), normalized difference vegetation index (NDVI), ratio of WI with NDVI (WI–NDVI), normalized water index (NWI), optimized soil-adjusted vegetation index (OSAVI), structure independent pigment index (SIPI), blue/green index 2 (BGI2), triangular vegetation index (TVI), and modified chlorophyll absorption in reflectance index (MCARI) of the adaxial leaf surface in three cut chrysanthemum cultivars. The indices equation is provided in Table 1. Different letters within each line indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$ (n = 6); Table S2: Effect of storage period (5 °C and darkness) on the photochemical reflectance index (PRI), renormalized difference vegetation index (RDVI), water index (WI), normalized difference vegetation index (NDVI), ratio of WI with NDVI (WI–NDVI), normalized water index (NWI), optimized soil-adjusted vegetation index (OSAVI), structure independent pigment index (SIPI), blue/green index 2 (BGI2), triangular vegetation index (TVI), and modified chlorophyll absorption in reflectance index (MCARI) of the abaxial leaf surface in three cut chrysanthemum cultivars. The indices equation is provided in Table 1. Different letters within each line indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$ (n = 6); Table S3: Effect of storage period (5 °C and darkness) on the photochemical reflectance index (PRI), renormalized difference vegetation index (RDVI), water index (WI), normalized difference vegetation index (NDVI), ratio of WI with NDVI (WI–NDVI), normalized water index (NWI), optimized soil-adjusted vegetation index (OSAVI), structure independent pigment index (SIPI), blue/green index 2 (BGI2), triangular vegetation index (TVI), and modified chlorophyll absorption in reflectance index (MCARI) of the flower (top view) in three cut chrysanthemum cultivars. The indices equation is provided in Table 1. Different letters within each line indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$ (n = 6); Table S4: Vase life, transpiration during vase life (data in Figure 1), cut flower water content, rehydration-induced cut flower fresh weight increase following 10% weight loss (data in Figure 7), leaf hydrogen peroxide (H_2O_2) content, leaf malondialdehyde (MDA) content, leaf total phenolic content, and leaf total flavonoid content (data in Figure 8) as function of storage time (5 °C and darkness) in three cut chrysanthemum cultivars. Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$; Table S5: Leaf mineral analysis of three cut chrysanthemum cultivars, employed for vase life evaluation (n = 6). Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$; Table S6: Floral mineral analysis of three cut chrysanthemum cultivars, employed for vase life evaluation (n = 6). Means ± SE followed by different letters within each column indicate significant differences based on Tukey's Honest significant difference test at $p \leq 0.05$.

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Abbreviations

BGI2: blue/green index 2; DRT, diffuse reflectance target; GAE, gallic acid equivalent; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; n, number of replicates; MCARI, modified chlorophyll absorption in reflectance index; NDVI, normalized difference vegetation index; NWI, normalized water index; OSAVI, optimized soil-adjusted vegetation index; PRI, photochemical reflectance index; RDVI, renormalized difference vegetation index; ROS, reactive oxygen species; RUE, rutin equivalent; RWC, relative water content; SIPI, structure independent pigment index; TVI, triangular vegetation index; WC, water content; WI, water index; WI–NDVI, ratio of WI with NDVI; ρ_x, reflectance at wavelength x.

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