

# Using Thermography to Estimate Leaf Transpiration Rates in Cut Roses for the Development of Vase Life Prediction Models

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**Abstract.** While the flower market has traditionally placed emphasis on the external quality (appearance) of cut flowers, the internal quality (longevity) has become increasingly important for retail marketing and consumers. In this study, we investigated key factors affecting the vase life of cut roses via multiple regression analysis (MRA) and examined the use of thermography for estimating the internal quality of flowers. The MRA results show that the vase life of cut roses depends primarily on, and is affected negatively by, transpiration in the dark, as well as high humidity growing conditions. A strong correlation ( $r^2 = 0.86$  in the dark,  $r^2 = 0.82$  in the light) was observed between leaf transpiration rate and leaf temperature differences (estimated with thermography). Finally, we developed vase life prediction models using environmental parameters and phenotypic parameters using either transpiration in the dark (VL-model 1) or leaf temperature difference (VL-model 2). The MRA results indicate that no significant difference exists between the predictive ability of VL-model 1 and VL-model 2. Thus, thermography is an effective technique for estimating leaf transpiration rate and is a practical approach for developing vase life prediction techniques.

**Additional key words:** grading, leaf temperature, longevity, multiple regression analysis, postharvest, potential vase life

## Introduction

Grading based on cut flower quality standards is an essential postharvest process for commercial cut flower growers. In the cut flower industry, quality evaluation is commonly performed based on external characteristics, such as flower color and shape, as well as stem form and length. However, the internal quality of cut flowers (potential vase life, longevity) is not currently incorporated into these grading standards. Vase life is one of the most important factors in consumer flower choice (Ichimura, 2000; Reid, 2005; Rihn et al., 2011; Rihn et al., 2014). The brief, unpredictable nature of vase life has resulted in decreased customer satisfaction. This is particularly the case with flowers for home use, which comprise a considerable portion of sales in countries with high per capita consumption of cut flowers (e.g., the EU and Japan). Therefore, cut flower retailers worldwide are increasingly demanding vase life guarantees

on behalf of their customers (van Kootena and Kuiper, 2009). The development of new techniques to predict vase life is a high priority, as it will provide an effective means to assess cut flowers with a view to guaranteeing their vase life for consumers. Researchers are beginning to devise effective vase life prediction methods that can be incorporated into existing quality grading systems for cut flowers (Elibox and Umaharan, 2008; In et al., 2009; Tromp et al., 2012). To develop vase life prediction techniques, variables that influence vase life must first be quantified.

Substantial research exists to show that an interaction between flower variety (genotype) and preharvest environment determines the morphological and physiological factors (phenotype) that affect vase life (Fanourakis et al., 2012a; Ichimura et al., 2002; Kim and Seo, 2013; Mortensen and Gislørød, 1999; van Meeteren et al., 2005). These phenotypic traits determine the water status of flowers (van Doorn, 1997; van Doorn, 2012). Specifically, transpiration rate at

harvest is the strongest determinant of the potential vase life of cut roses and is a significant parameter in vase life prediction models (In et al., 2009; In et al., 2007). However, detecting the transpiration rate at harvest is difficult due to technical limitations. For instance, while porometers are accurate and widely used to measure leaf transpiration in the laboratory, they are not practical for commercial use because of their inability to measure multiple plants simultaneously and the potential for damage to the plants during measurement. Thus, using transpiration as a predictor of vase life requires the development of rapid, non-destructive measurement techniques. Infrared thermography is a relatively fast and non-contact technique used to detect plant stress, including water deprivation and viral infection (Nilsson, 1995; Raskin and Ladyman, 1988). This technique detects infrared radiation (heat) emitted by plants and converts it to thermal images that indicate surface temperature (Chaerle and Van Der Straeten, 2000; Lee and Kim, 2015).

This study was conducted to assess the use of thermography as a method for assessing cut flower vase life in roses. We aimed to determine key environmental and phenotypic factors that influence transpiration rate, and thus vase life, in three rose cultivars. We then investigated how to effectively estimate transpiration via thermography and examined the utility of thermography for developing vase life prediction models.

## Materials and Methods

### Plant Materials and Growing Environment

*Rosa hybrida* L. ‘Rote Rose,’ ‘Bridal Pink,’ and ‘Sonia’ plants were grown via an “arching” technique (Ohkawa and Suematsu, 1999) on Rockwool slabs at 10-cm intervals in a greenhouse at Osaka Prefecture University, Japan. The plants were grown in natural light and drip-irrigated hourly with half-strength Enshi standard nutrient solution containing 8.0 me·L<sup>-1</sup> NO<sub>3</sub>-N, 0.65 me·L<sup>-1</sup> NH<sub>4</sub>-N, 2.0 me·L<sup>-1</sup> PO<sub>4</sub>-P, 4.0 me·L<sup>-1</sup> K, 4.0 me·L<sup>-1</sup> Ca, 2.0 me·L<sup>-1</sup> Mg, 2.0 me·L<sup>-1</sup> S, and trace amounts of micronutrient elements. Heating and ventilation were provided when the temperature in the greenhouse was lower than 16°C and higher than 25°C, respectively.

### Harvest Conditions and Vase Life Evaluation

Between March 2004 and March 2006, 212 rose flowers were cut from ‘Rote Rose’ (140 stems), ‘Bridal Pink’ (36 stems), and ‘Sonia’ (36 stems) at commercial maturity (outer petals beginning to unfold). Flowers were harvested at 17:00 and immediately carried to the laboratory in a bucket containing tap water. The flower stems were trimmed to 50, 60, or 70 cm with five upper leaves (two with three-leaflets and three with five-leaflets). Each cut stem was

placed in a glass jar containing 500 mL distilled water through a hole (1 cm diameter) in the center of a plastic cap. The cut flowers were placed in the dark for approximately 15 h (18:00 to 09:00 in the next morning) at 25°C and 50% relative humidity (RH). Their morphological and physiological characteristics were then measured (see next section, “Measurement of parameters”). The cut flowers were then transferred to an environment-controlled room (25°C, 50% RH, and 10 μmol·m<sup>-2</sup>·s<sup>-1</sup> of fluorescent lighting) for 12 h.

The following senescence cues were used to determine if a flower had reached the end of its vase life: wilting (i.e., loss of petal turgor), petal “bluing,” and bent neck. The vase life was considered terminated when one of these symptoms appeared.

### Measurement of Parameters

Based on the results of our previous quantitative study (In et al., 2007), 25 parameters affecting vase life were selected and measured, including 11 preharvest environmental parameters and 14 phenotypic parameters (Table 1).

Preharvest environmental parameters. Environmental factors in the greenhouse were measured at 2-m intervals along the Rockwool slabs, with all roses in the same 2-m section categorized under identical conditions. The local temperatures and RH in the greenhouse were recorded every 10 min using thermo recorders (RS-11, Espec Mic, Aichi, Japan). The local photosynthetic photon flux (PPF) was measured with an OPTLEAF sensor system (R-2D film and THS-470 T-METER, Taisei E&L, Tokyo, Japan). PPF data were standardized based on the values from a PPF radiation sensor (IKS-27, Koito, Yokohama, Japan) connected to a data logger (HR-1300, Yokogawa, Tokyo, Japan). The vapor pressure-deficit variables (Table 1) were calculated from temperature and RH data. Daily values of all parameters for 15 d before harvest were averaged and used in subsequent analyses.

Morphological parameters at harvest. The morphological parameters were as follows: fresh weight, stem length, neck stem diameter, cut end stem diameter, leaf area, stomatal size (length and width), and stomatal density. To measure stomatal size and density, Suzuki’s Universal Micro-Printing (SUMP) method was performed on the abaxial side of the lowermost five-leaflet leaf on the first morning (9:00) in the dark (see previous section, “Harvest and vase life evaluation”). Images of the leaf surface resulting from SUMP were taken with a digital camera (Coolpix 4500, Nikon, Tokyo, Japan) connected to an optical microscope (B202, Olympus, Tokyo, Japan). Stomatal size and density were then calculated with Scion Image (Version 4.02, Scion, Frederick, MD, USA). After flowers reached the end of their vase life, all leaves were removed from the cut stems and scanned to measure leaf area.

**Table 1.** Parameters at preharvest and harvest stages used for multiple regression analysis

Stage	Parameter	Abbreviation	Unit	Note
Preharvest	Supplemental lighting time	SLT	h·day <sup>-1</sup>	Daily average hours
	Integrated PPF	PPF	mol·m <sup>-2</sup> ·day <sup>-1</sup>	Average of daily total value
	Maximum temperature	Tem-Max	°C	Average of daily maximum value
	Average temperature	Tem-Avg	°C	Average of daily average value
	Minimum temperature	Tem-Min	°C	Average of daily minimum value
	Maximum relative humidity	RH-Max	%	Average of daily maximum value
	Average relative humidity	RH-Avg	%	Average of daily average value
	Minimum relative humidity	RH-Min	%	Average of daily minimum value
	Maximum vapor pressure deficit	VPD-Max	kPa	Average of daily maximum value
	Average vapor pressure deficit	VPD-Avg	kPa	Average of daily average value
	Minimum vapor pressure deficit	VPD-Min	kPa	Average of daily minimum value
Harvest	Fresh weight	FW	g	Total weight after trimming
	Stem length	Stem-Len	cm	Stem length including flowers after trimming
	Stem diameter of neck	SD-Ne	mm	1 cm below the flowers
	Stem diameter of cut end	SD-En	mm	1 cm above cut end
	Leaf area	Area-Leaf	cm <sup>2</sup>	Leaf area after trimming
	Stomatal length	Stom-Len	μm	Length measured in the dark
	Stomatal width	Stom-Wid	μm	Width measured in the dark
	Stomatal density	Stom-Dens	mm <sup>-2</sup>	Number of stomata per 1 mm <sup>2</sup>
	Transpiration in the dark	Trans-Dark	μg·cm <sup>-2</sup> ·s <sup>-1</sup>	Transpiration rate in the dark
	Transpiration in the light	Trans-Light	μg·cm <sup>-2</sup> ·s <sup>-1</sup>	Transpiration rate in the light
	Water potential in the dark	WP-Dark	MPa	Water potential of leaf in the dark
	Water potential in the light	WP-Light	MPa	Water potential of leaf in the light
	Brix of leaf	Brix	%	Soluble solids content of leaf
	Temperature difference in the dark	Temp-Diff	°C	Leaf temperature – reference temperature (T <sub>L</sub> - T <sub>R</sub> )

Preharvest parameters are average values at 15 days before harvest.

Physiological parameters at harvest. The transpiration rate, soluble solids content (SSC), and leaf water potential were measured simultaneously with stomatal characteristics (09:00 in the dark) at 25°C and 50% RH. The flowers were then transferred to light conditions (three-band fluorescent lamps at 80 μmol·m<sup>-2</sup>·s<sup>-1</sup>), and 90 min later, the transpiration rate and leaf water potential were measured again to evaluate stomatal opening and closing (In et al., 2007). A steady state porometer (LI-1600, LI-COR, Lincoln, NE, USA) was used on the uppermost three-leaflet terminal leaves to obtain transpiration rates. Total daily transpiration on day-1 was calculated using the following equation:

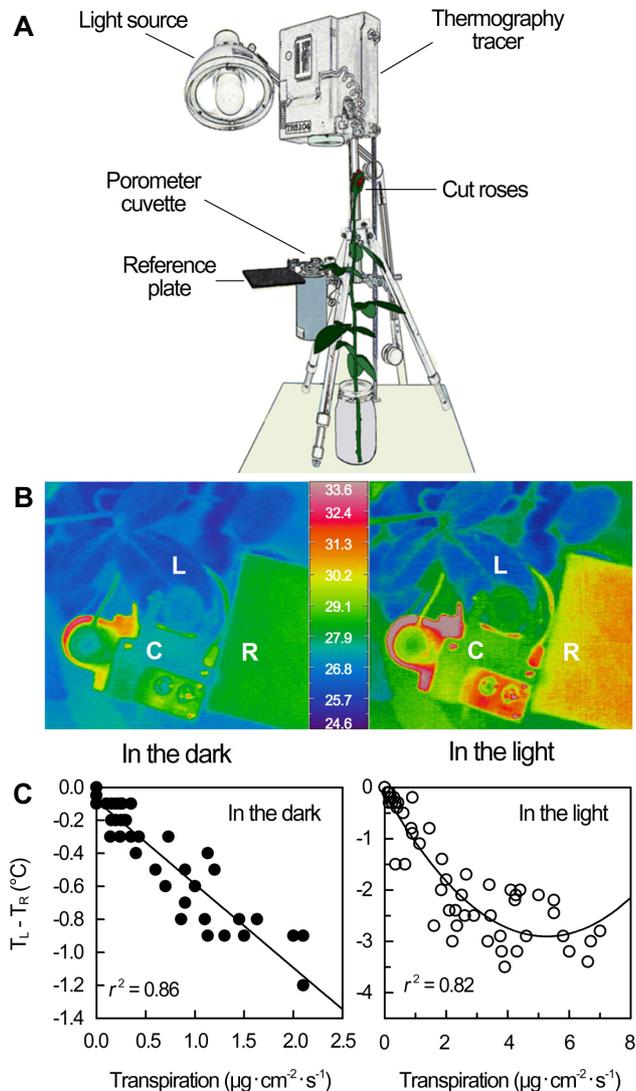
$(WA - FWC)/\text{leaf area}$ , where WA is the amount of water absorption and FWC is fresh weight change.

Next, to measure water potential, a fresh leaf disk (5 mm in diameter) was sampled from a second uppermost three-leaflet leaf. The disk was placed in a sample chamber (C-52, Wescor, Logan, UT) connected to a microvolt meter (HR-33T, Wescor, Logan, UT) and allowed to equilibrate for 2 h. The

dew point of the headspace was then measured following the instruction manual for the microvolt meter. Finally, to measure SSC, a tissue sample (0.1 g) of the lowermost leaf was ground in a mortar with 0.9 mL distilled water. SSC was obtained from the resulting mixture using a digital refractometer (PR-101, Atago, Tokyo, Japan).

### Thermography Measurement

Thermal images of 50 cut roses (20 'Rote Rose,' 15 'Bridal Pink,' and 15 'Sonia' flowers) were taken under the same conditions and time points as the transpiration measurements (see "Physiological parameters at harvest" above). To obtain thermal images and transpiration data simultaneously on the same leaves, thermography equipment (TH51-701, NEC, Japan) was installed at the top of the porometer (Fig. 1A). The distance between the leaves and the thermography camera lens was set to 40 cm for all samples to avoid the effect of variation in distance. A reference plate (10 cm × 10 cm × 3 mm) was installed at the same height (Fig. 1A). The



**Fig. 1.** Estimation of cut rose transpiration using thermography. (A) Schematic diagram of the setup for measuring leaf temperature and transpiration of cut roses. (B) Infrared thermal images of rose leaves. The images were taken with a thermography tracer in a controlled environment (25°C, relative humidity 50%). The left panel shows an image obtained in the dark. The right panel shows an image obtained in the light. The middle panel shows the temperature scale. L, leaf; R, reference plate; C, cuvette of the porometer. (C) The relationship between leaf temperature difference ( $T_L - T_R$ ), obtained via thermography, and leaf transpiration under dark (left panel) and light (right panel) conditions. The relationship was linear ( $y = -0.08 - 0.51x$ ) in the dark and quadratic ( $y = -0.11 - 1.06x + 0.10x^2$ ) in the light.

average temperature per unit area was measured using the image processing software included with the thermography equipment (Fig 1B). The temperature difference (Temp-Diff) between the leaf ( $T_L$ ) and the reference plate ( $T_R$ ) was calculated as  $T_L - T_R$ . Linear and quadratic regression analyses were then performed between the Temp-Diff and the transpiration data.

To examine whether thermography could be used to

accurately measure transpiration rates under water stress conditions, the method described above was also used to obtain data from cut roses that were held out of water for 0, 2, and 4 h at 25°C and 50% RH.

### Statistical Analysis

All data were analyzed with SPSS 18.0 (IBM, Somers, NY, USA). One-way analysis of variance (ANOVA) was performed to determine seasonal variations in transpiration rate, stomatal characteristics, and vase life. Only the data from ‘Rote Rose’ flowers (140 stems) were used for the seasonal variation comparison due to unequal sample sizes across seasons in the other two cultivars. Means between groups were compared using Duncan’s multiple range test ( $p = 0.05$ ).

To determine the major determinant of vase life and to develop vase life prediction models, stepwise multiple regression analysis (MRA) and simultaneous MRA were performed, respectively. Independent variables that exhibited a high correlation with vase life from the stepwise MRA were used in the simultaneous MRA for model prediction. For stepwise MRA and simultaneous MRA, data from 212 cut roses (see previous section, “Harvest and vase life evaluation”) and 50 cut roses (see previous section, “Thermography measurement”) were used, respectively. For both MRAs, vase life was set as the dependent variable, while the environmental and phenotypic parameters were set as the independent variables. Two models were developed with the simultaneous MRA: VL-model 1, which included Trans-Dark as a phenotypic parameter, and VL-model 2, which included Temp-Diff.

To avoid multicollinearity among the independent variables, parameters were removed based on their tolerance and variance inflation factor (VIF) values.

## Results

### Relationship between Stomatal Characteristics, Transpiration Rate, and Vase Life

Significant seasonal variation occurred in ‘Rote Rose’ stomatal size, transpiration rate, and vase life (Table 2). In the dark, winter stomatal length was shortest across all seasons, whereas winter stomatal width was significantly wider, indicating that during the winter, the cut roses failed to sufficiently close their stomata in the dark (Table 2).

Seasonal variation also occurred in transpiration rate. During spring and summer, the transpiration rate of cut flowers significantly decreased after transfer from light to dark conditions. By contrast, cut flowers in autumn and winter exhibited markedly higher transpiration rates under both light and dark conditions compared with cut flowers in

**Table 2.** Average values of stomatal characteristics, transpiration rates, and vase life of cut 'Rote Rose' roses grown year-round in a greenhouse

Season <sup>z</sup>	Stomatal length ( $\mu\text{m}$ ) <sup>y</sup>	Stomatal width ( $\mu\text{m}$ ) <sup>x</sup>	Stomatal density (number-mm <sup>-2</sup> )	Transpiration in the dark ( $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ )	Transpiration in the light ( $\mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ )	Transpiration on day 1 ( $\text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}$ ) <sup>w</sup>	Vase life (days)
Winter	35.7±0.4b	13.9±0.5a	94.7±2.6b	1.94±0.26a	5.10±0.35a	0.11±0.01a	8.6±0.5d
Spring	37.5±0.9ab	13.1±0.4ab	94.5±4.0b	0.16±0.03c	3.86±0.45b	0.06±0.01c	13.0±0.4a
Summer	38.3±0.6a	12.7±0.3b	104.2±2.5a	0.95±0.16b	3.86±0.29b	0.08±0.00bc	10.9±0.2b
Autumn	38.1±0.4a	13.1±0.3ab	97.2±2.8ab	1.62±0.32a	5.48±0.47a	0.09±0.01ab	9.9±0.3c

<sup>z</sup>Winter, December–February; Spring, March–May; Summer, June–September, Autumn, October–November.

<sup>y</sup>Stomatal length was measured in the dark during the first morning of the experiment (d 1).

<sup>x</sup>Stomatal width was measured in the dark during the first morning of the experiment (d 1).

<sup>w</sup>Total daily transpiration on day 1 was divided by the leaf area of cut flowers.

Values are means with standard errors. Different letters (a-d) in each column indicate statistically significant differences (Duncan's multiple range test;  $p < 0.05$ ;  $n = 35$ ).

**Table 3.** Results of multiple regression analysis of the relationship between preharvest/harvest parameters and the vase life of cut 'Rote Rose', 'Bridal Pink', and 'Sonia' flowers. Data from 212 cut roses of three cultivars (140 'Rote Rose', 36 'Bridal Pink', and 36 'Sonia') were used for the analysis. Preharvest environmental parameters are averages of daily values for 15 days before harvest. Regression statistics:  $r^2 = 0.61$  ( $n = 212$ )

Independent variable	Partial regression coefficient	Standard error	Standard partial regression coefficient
Intercept	2.74*	2.40	
Tem-Avg	0.23***	0.06	0.42
RH-Min	-0.08**	0.03	-0.28
VPD-Max	-1.00*	0.47	-0.30
Trans-Dark	-1.30***	0.13	-0.64
Trans-Light	0.29***	0.06	0.30
Stom-Wid	0.13***	0.03	0.23
SD-Ne	0.56**	0.19	0.18
WP-Dark	-1.77 <sup>NS</sup>	0.80	-0.10

\*, \*\*, and \*\*\* indicate significant difference at  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively.

<sup>NS</sup> indicates a non-significant difference ( $p > 0.05$ ).

spring and summer. In particular, transpiration rates for cut flowers in the dark in the autumn and winter were 12- and 10-times higher than those in the spring, respectively. Together, these results indicate that cut flowers grown in autumn and winter have less functional stomata, which reduces their ability to regulate stomatal closing during the transition from light to dark. Consequently, daily total transpiration rates were significantly higher for autumn and winter flowers than for spring flowers. The resulting high water loss shortened the vase life of winter flowers by 4.4 and 2.3 days compared with the vase life of spring and summer roses, respectively (Table 2). By contrast, the stomata of spring flowers were the most functional, resulting in the lowest transpiration rates and the longest vase life across all seasons.

### Multiple Regression Indicates the Importance of Transpiration in the Dark

Eight environmental and phenotypic parameters were

selected by stepwise MRA. The results reveal a significant relationship between vase life and these parameters ( $r^2 = 0.61$ ,  $p < 0.05$ ; Table 3). The partial regression coefficients indicate that vase life was significantly associated with all independent variables, except water potential in the dark. Of all parameters, however, transpiration in the dark had the highest standard partial regression coefficient, indicating that it contributes the most to predicting vase life.

### Effectiveness of Thermography at Measuring Leaf Transpiration

Fig. 1B shows thermal images of leaves that were taken under dark and light conditions. Under dark conditions, the average temperature of the leaf and the reference plate was 26.6°C and 28.8°C, respectively, resulting in a temperature difference (Temp-Diff) of -1.2°C. Under light conditions, the average temperature of the leaf and the reference plate was 26°C and 30.5°C, respectively, resulting in a higher Temp-Diff (-3.5°C) than in the dark. The relationship between

transpiration and Temp-Diff ( $T_L - T_R$ ) was highly significant both in the dark and in the light, although the nature of the relationship differed (Fig. 1C). In the dark, the relationship was linear ( $y = -0.08 - 0.51x$ ,  $r^2 = 0.86$ ), whereas in the light, the relationship was quadratic ( $y = -0.11 - 1.06x + 0.10x^2$ ,  $r^2 = 0.82$ ). The quadratic relationship in the light indicates that once the transpiration rate reaches a particular level (approximately  $4 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ ), leaf temperature no longer drops.

While thermography appears to be effective at estimating transpiration under regular conditions, it proved to be less effective under water stress conditions. We found that while leaf transpiration in both the dark and light significantly decreased after 2 h of water stress (Fig. 1D), linear regression analyses revealed no significant relationship between Temp-Diff and transpiration (Fig. 1E), even after 4 h of water stress. However, when the flowers experienced 0 h of water stress, a strong relationship existed between transpiration and Temp-Diff under both dark ( $r^2 = 0.92$ ) and light ( $r^2 = 0.90$ ) conditions.

### Vase Life Prediction Models

In addition to Trans-Dark (in VL-model 1) and Temp-Diff (in VL-model 2), six variables from the preharvest environmental and phenotypic parameters were highly correlated with vase life and were therefore included in both vase life prediction models (Fig. 2). In VL-model 1 (Fig. 2A), the independent variables explained approximately 60% of the variance in vase life. The regression equation is as follows:

$$y = 0.121 \cdot X_1 + 0.065 \cdot X_2 + 1.082 \cdot X_3 + 0.239 \cdot X_4 - 0.858 \cdot X_5 + 0.182 \cdot X_6 - 7.530 \quad (r^2 = 0.604, p < 0.001),$$

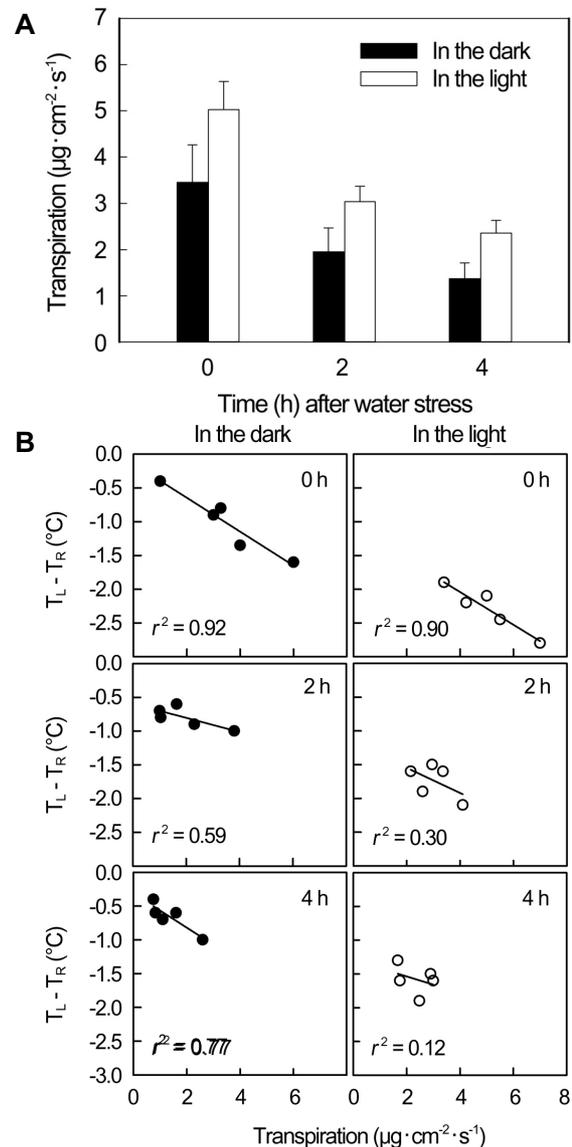
where  $y$  is VL;  $X_1$ – $X_6$  are Tem-Avg, RH-Min, SD-Ne, Stom-Wid, Trans-Dark, and Brix, respectively (see Table 1 for abbreviations). In VL-model 2 (Fig. 2B), the independent variables explained approximately 62.5% of the variance in vase life. The regression equation is as follows:

$$y = 0.096 \cdot X_1 + 0.066 \cdot X_2 + 1.021 \cdot X_3 + 0.216 \cdot X_4 - 1.615 \cdot X_5 + 0.162 \cdot X_6 - 6.194 \quad (r^2 = 0.625, p < 0.001),$$

where  $y$  is VL;  $X_1$  –  $X_6$  are Tem-Avg, RH-Min, SD-Ne, Stom-Wid, Temp-Diff, and Brix, respectively. We found no significant difference between the predictive ability of VL-model 1 and VL-model 2.

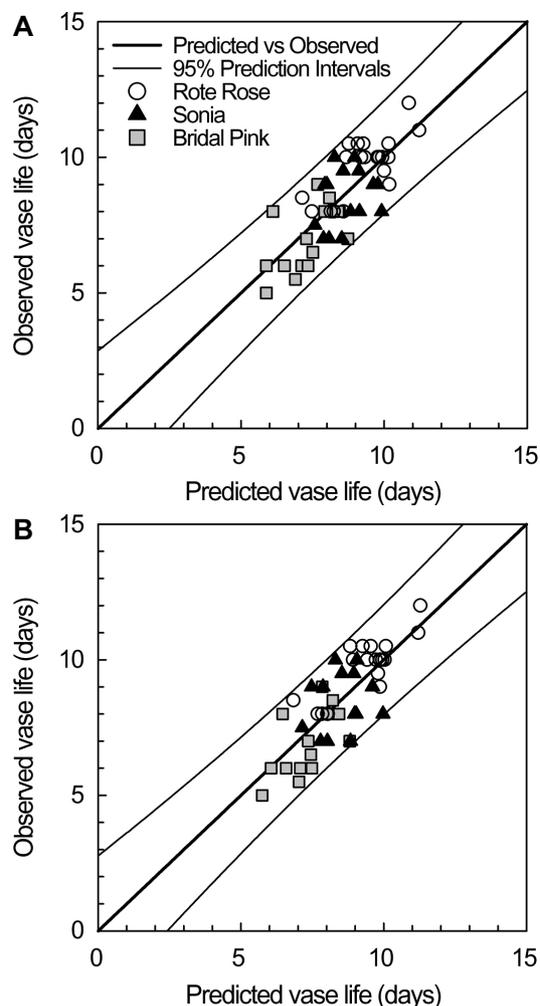
## Discussion

The present study revealed that the ability of cut roses to control water loss varies seasonally, which is similar to the findings of previous studies (In et al., 2006; Slootweg et al., 2001; Torre and Fjeld, 2001). Cut roses grown in the winter could not close their stomata completely in the dark and transpired excessively during postharvest periods, resulting



**Fig. 2.** Estimation of cut rose transpiration under water stress conditions using thermography. (A) Leaf transpiration under dark and light conditions after water stress treatment for 0 h, 2 h, and 4 h at 25°C and 50% RH. (B) The relationship between leaf temperature difference ( $T_L - T_R$ ) and leaf transpiration under dark (left panels) and light (right panels) conditions after water stress treatment for 0 h, 2 h, and 4 h. Linear regression equations were as follows:  $y = -0.14 - 0.25x$  (0 h),  $y = -0.59 - 0.10x$  (2 h), and  $y = -0.31 - 0.25x$  (4 h) in the dark and  $y = -1.07 - 0.24x$  (0 h),  $y = -1.18 - 0.18x$  (2 h), and  $y = -1.29 - 0.12x$  (4 h) in the light.

in a shortened vase life. These results are consistent with previous work showing that water stress is the major cause of short vase life in cut roses (Ferreira and De Swardt, 1981; Mayak and Halevy, 1980; van Doorn, 1997; Zieslin et al., 1978) and that leaf transpiration is responsible for postharvest water loss (Carpenter and Rasmussen, 1974; Mayak et al., 1974). Furthermore, rose plants grown under winter greenhouse conditions, with high RH and low VPD (similar to the



**Fig. 3.** The relationship between observed and predicted vase life (number of days), via multiple regression. The vase life prediction models were developed using environmental parameters and phenotypic parameters at harvest, obtained from 50 cut roses of three cultivars (20 'Rote Rose,' 15 'Bridal Pink,' and 15 'Sonia'). For variable abbreviations, see Table 1. (A) VL-model 1 (dark transpiration used as a predictor).  $y = 0.121 \cdot X_1 + 0.065 \cdot X_2 + 1.082 \cdot X_3 + 0.239 \cdot X_4 - 0.858 \cdot X_5 + 0.182 \cdot X_6 - 7.530$  ( $r^2 = 0.604$ ,  $p < 0.001$ ), where  $y$  is vase life;  $X_1 - X_6$  are Tem-Avg, RH-Min, SD-Ne, Stom-Wid, Trans-Dark, and Brix, respectively. The thin lines are 95% prediction intervals ( $\pm 2.9$ ). (B) VL-model 2 (temperature difference between leaves and reference plate used as a predictor).  $y = 0.096 \cdot X_1 + 0.066 \cdot X_2 + 1.021 \cdot X_3 + 0.216 \cdot X_4 - 1.615 \cdot X_5 + 0.162 \cdot X_6 - 6.194$  ( $r^2 = 0.625$ ,  $p < 0.001$ ), where  $y$  is vase life;  $X_1 - X_6$  are Tem-Avg, RH-Min, SD-Ne, Stom-Wid, Temp-Diff, and Brix, respectively. The thin lines are 95% prediction intervals ( $\pm 2.8$ ).

winter conditions employed in this study) produce cut flowers with failed stomatal closure (Fanourakis et al., 2015; Mortensen and Gislerød, 1999; Torre and Fjeld, 2001). Flowers grown under these conditions experience no water stress during growth and consequently lose the ability to close their stomata during postharvest periods, resulting in water stress when transferred to a different environment.

Next, supporting our observations, multiple regression ana-

lyses indicated that vase life was negatively correlated with high humidity preharvest conditions and attenuated stomatal function (high transpiration in the dark), but positively correlated with temperature: as temperature increased, VPD also increased. These results support the previous finding that cut roses grown under dry conditions (high temperature and low RH) have a longer vase life, whereas high RH conditions result in increased transpiration rates in the dark and a shorter vase life (Fanourakis et al., 2012b; In et al., 2007). Overall, our data and previous reports strongly indicate that the vase life of cut roses depends primarily on transpiration in the dark at the harvest stage. In other words, dark transpiration may be an important parameter for predicting the longevity of cut rose flowers.

Consistent with research demonstrating a high correlation between leaf temperature and transpiration (Garbe et al., 2002; Jones, 1999), our thermography analysis revealed that the temperature difference between leaves and the reference plate accurately represented leaf transpiration. Additionally, a predictive model using this temperature difference was just as effective in predicting vase life as a model using dark transpiration. Our results suggest that using thermography to measure leaf temperature may be effective for estimating the internal quality of cut flowers at pre-marketing stage. However, while our thermography model performed well, we did not find its predictive ability ( $r^2 = 0.625$ , prediction interval:  $\pm 2.8$  days) high enough for practical use as a guarantee of the vase life of cut flowers. Additionally, we found that thermography was less effective at estimating transpiration after flowers were water deprived, likely because the leaf temperature was affected by water stress. This finding suggests that thermography may not be a preferred method for measuring the vase life of cut flowers that were previously exposed to water deprivation during storage or transport. To improve the performance of vase life prediction models, further studies will be necessary both to better understand the circumstances under which various estimation methods may be ineffective and to investigate more comprehensive data processing methods that can be used with thermography, such as neural networks.

In conclusion, the current study tested the utility of thermography to estimate dark transpiration, the major determinant of cut flower longevity. Our results clearly demonstrate that thermography is a rapid and effective method for detecting leaf transpiration rates at harvest. With some modifications, the current approach should prove extremely useful for developing a practical vase life prediction technique for use in the flower industry.

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