Postharvest water relations in cut rose cultivars with contrasting sensitivity to high relative air humidity during growth

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\section*{A B S T R A C T}
A constant high relative air humidity (RH) during cultivation can strongly reduce the vase life in some cut rose cultivars. We studied three contrasting cultivars in their tolerance to high RH in order to analyse in detail the water relations during postharvest and better understand this genotypic variation. Plants were grown at moderate (60%) and high (95%) RH, and cut flowers were placed in water immediately after cutting. Flowers of cv. Pink Prophyla grown at high RH did not open throughout vase life, while flower opening of cvs. Frisco and Dream was not affected by preharvest RH. Cultivation at high RH resulted in about 80\% shorter vase life in Pink Prophyla, whereas in Dream and Frisco the negative effect was considerably smaller (15\% and 5\% shorter vase life, respectively). The shorter vase life and reduced flower opening of cut roses grown at high RH was due to a higher rate of transpiration both in the light and dark periods. It was found that the leaves of Pink Prophyla grown at high RH could partly close their stomata upon lowering of the water potential or when flower stalks were fed with abscisic acid, but stomata remained far more open than in leaves grown at moderate RH. The RH during cultivation did not affect stem hydraulic conductivity and its recovery after air emboli induction. Preventing vascular occlusion largely alleviated the high-cultivation-RH effect on vase life and flower opening, showing that the effect of high-cultivation-RH becomes only important if water uptake is limited.

\section*{1. Introduction}
The water relations of cut flowers are dependent on a number of physiological and anatomical traits that regulate water loss and water uptake rates (reviewed by van Doorn, 1997). These traits are established during the preharvest period, being the result of the complex interaction between genotype and environment during cultivation, and will subsequently determine potential vase life (i.e. maximum vase life) of a given cut flower. For instance, although the relative air humidity (RH) level during cultivation has no significant effect on crop growth and visual quality (Torre and Fjeld, 2001), cut roses grown at high RH (>85\%) often have a very short vase life (Mortensen and Fjeld, 1998). Nevertheless, the decrease in the life span after cultivation at high RH is strongly dependent on the genotype, varying between as little as 15\% (cv. Dream, Frisco, and Kardinal) to as much as 75\% (cv. Amadeus) (Mortensen and Gislerød, 1999). Sensitive cultivars grown under elevated RH show precocious postharvest senescence symptoms, which are typically related to water stress, including premature flower and leaf wilting as well as pedicel bending (Torre et al., 2001; Mortensen and Gislerød, 2005). The described phenotypic variation in the sensitivity to high RH during cultivation is not yet fully understood.

A limited capacity to reduce water loss, due to stomatal malfunctioning, is thought to be the main reason for the vase life reduction in plants grown under long-term high RH (Rezaei Nejad and van Meeteren, 2007; Fanourakis et al., 2011). The regulation of water loss is mostly under physiological control (i.e. stomata), whereas water uptake is basically a physical process in cut flowers. An adequate stomatal responsiveness to different closing stimuli (e.g. darkness and low water potential) will limit the net loss of water from the cut flower, and will consequently delay early wilting symptoms (van Doorn et al., 1989; Bleeksma and van Doorn, 2003). The flow rate (water uptake) is proportional to the driving force (water potential), and to the conductance (inverse of resistance) of the transport path (van Doorn, 1997). It has been shown that drought stress results in a reduction of xylem vessel

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van Doorn, 1997. *
diameter (Lovisolo and Schubert, 1998), which in turn leads not only to a lower stem hydraulic conductivity but also to a higher resistance to cavitation (Nijssse et al., 2001; McElrone et al., 2004). Since low water potentials during drought stress can change the xylem anatomy, some opposite changes might be expected when plants are subjected to long-term high water potentials, as a result of elevated RH levels. The effect of RH during growth on stem hydraulic conductivity has not been previously investigated.

The main objectives of the present work were: (i) to analyse the postharvest water relations of cultivars with contrasting sensitivity to high RH during preharvest, (ii) to assess the cultivation RH effect on the stem hydraulic conductivity and its recovery after artificial induction of air emboli, and (iii) to test if improvement of water uptake, by preventing vascular blockage, can compensate for the higher water loss found after cultivation at high RH. We hypothesized that a shorter vase life, after growth at high RH, results from the combined effect of a higher water loss and a changed xylem anatomy (leading to a higher sensitivity to air emboli). Moreover, we expect that factors which improve water uptake will alleviate this negative effect of high RH on cut flower longevity.

2. Materials and methods

2.1. Plant material and growth conditions

Rooted cuttings of three cut rose cultivars (Rosa hybrida L., cvs. Pink Prophyla, Frisco, and Dream) known to have different decreases in vase life after cultivation at high RH (Mortensen and Gisléröd, 1999), were obtained from a commercial propagator (Kordes, De Kwakel, The Netherlands). The cuttings were planted in 3.6 L pots containing a mixture of cocopeat (Jongkind Grond BV, Aalsmeer, The Netherlands) and perlite (Agapervilite nr. 3, Pull, Rhenen, The Netherlands), 3:1 (v/v). Cultivar Pink Prophyla (registered cultivar name RJ1kuiros) will be called Prophyla in the rest of this paper.

Five experiments were conducted. In each experiment, plants were grown in four growth chambers as a single shoot (one plant per pot) at a density of 30 plants m⁻². In two growth chambers the RH was 60 ± 3% (moderate RH) and in the other it was 95 ± 1% (high RH). The four chambers had a constant day and night temperature (19 ± 1°C), resulting in vapour pressure deficits (VPDs) of 0.88 ± 0.12 kPa (moderate RH) and 0.11 ± 0.03 kPa (high RH). Climate parameters were recorded automatically every 5 min by data loggers (Fourier Microlog EC650, MicroDAQ.com Ltd., Contocoook, NH, USA).

Fluorescent tubes (TLD 58W/84, Philips, Eindhoven, The Netherlands) provided an 18 h photoperiod and 300 ± 20 μmol m⁻² s⁻¹ of photosynthetically active radiation (PAR, determined with a Model LI-250, LI-COR, Lincoln, NE, USA). Radiation levels were measured at 70 cm from the root-shoot interface, which corresponds to the top of fully grown plants. The CO₂ concentration during the light period was 370 ± 50 μmol m⁻³ (determined using Indoor Air Quality Meter, Model 8760, TSI Incorporated, Shoreview, MN, USA). Plants were watered automatically with a nutrient solution as described by Fanourakis et al. (2009).

Experiments on postharvest characteristics used second-order shoots, each originating from an individual plant, at the commercial harvest stage (stage 2 according to the Association of Dutch Flower Auctions (VBN, 2005); described for experiment 1). The harvested shoots had a length of approximately 50–60 cm, measured from the primary shoot/secondary shoot junction to the top of the shoot. The night before the experiment, the plants were well irrigated and placed in darkness for 12 h, to ensure maximal turgidity and minimize the presence of natural air emboli (van Doorn and Siero, 1996).

2.2. Vase life under non-optimum water uptake conditions (experiment 1)

The flowering stems of the three cultivars were cut in air, left in air for 2–3 min, and their cut ends were then washed with sodium hypochlorite solution (2%, v/v); the concentration of commercial bleach solution. All flowers were cut to the same length (49 ± 2 cm), and the same number of five-leaflet leaves per cultivar was left (cvs. Frisco and Prophyla, four leaves; cv. Dream, five leaves). Subsequently, the cut flowering stems were put in flasks (one flower per flask) containing 300 mL of an artificial vase solution (0.7 mM CaCl₂, 2H₂O, 1.5 mM NaHCO₃, 5 μM CuSO₄, 5H₂O; van Meeteren et al., 2000). The presence of copper sulphate in the vase solution leads to a moderate inhibition of bacterial growth. The top of the flasks was covered with Parafilm, to ensure that water loss could only occur via the flower stalks. These flasks were placed in a climate-controlled room at 20°C, 50% RH and 10–12 μmol m⁻² s⁻¹ PAR at a 12h on-off cycle, provided by fluorescent tubes (TLD 58W/84, Philips, Eindhoven, The Netherlands). The height of the vase solution column was held constant over the evaluation period to avoid hydrostatic pressure differences between flowers with different transpiration rates (Mensink and van Doorn, 2001).

The termination of vase life was determined based on the occurrence of at least one of the following criteria: (i) bending of the pedicel (bent-neck; i.e. pedicel bends and flower angle becomes larger than 90° from the vertical position); (ii) abscission of more than two petals; (iii) visible wilting of the flower, i.e. loss of petal turgor; and (iv) more than 50% of the number of leaves had abscised, turned yellow, or had desiccated (VBN, 2005). Total leaf area was determined at the end of vase life, using a leaf area meter (model 3100 Area Meter, LI-COR, Lincoln, NE, USA).

During the postharvest phase, the flower and flask weights were recorded separately two times a day (time 0 and 12 h after the onset of the light period). The transpiration rate was calculated per unit leaf area. Treatments were compared based on the average transpiration rate over the complete postharvest period. The fresh weight (FW) of each flowering stem was expressed relative to its initial weight. The flower diameter and opening stage were recorded during daily life of vase life. The flower diameter was measured by assessing the maximum diameter and the diameter perpendicularly to that one. These two values were averaged. Flower stages were determined using the scale of VBN (2005) (i.e. stage 2: loose pointed bud with cylindrical shape; stage 3: half-open flower; stage 4: open flower; stage 5: maximally opened flower with visible anthers). In this experiment twelve flowers per treatment were assessed.

2.3. Vase life under optimum water uptake conditions (experiment 2)

In order to test if improving the water uptake conditions during vase life could compensate for the negative effect of high RH during cultivation on keeping quality, vascular blockage (caused by air emboli at the cut surface and bacterial growth in the vase water) was prevented. Flowering shoots of the three cultivars were cut under water to prevent air entrance into the xylem conduits that were opened by cutting. Thus, just prior to cutting, pots were placed in buckets containing degassed sterilized water, whereby the water level was about 5 cm above the primary shoot/secondary shoot junction. Each cut was made using shears that had been sterilized in ethanol and through an internode that had been surface-sterilized by rubbing with a cloth drenched in the same solution (sterile treatment; van Doorn et al., 1991). The flowering stems were cut to the same length, and the same number of five-leaflet leaves per cultivar as described earlier (experiment 1). Subsequently, to further reduce the effect of bacteria on xylem occlusion, the stems were placed in sterilized flasks containing 300 mL of an artificial vase solution.
(details in experiment 1), which was sterilized (autoclaved at 121 °C for 15 min) and its pH was reduced to 3 with addition of citric acid. This approach was used in place of a vase solution biocide, common in vase life work, to avoid possible effects of chemicals other than on microbes (van Doorn et al., 1990). The flasks were placed under test room conditions and cut flowers were submitted to the same procedures and measurements as described for experiment 1. Additionally, in the cvs. Frisco and Prophylta, the flower with the flask was weighed at regular intervals during the light period (time 2, 4, 8, and 10 h after the onset of the light period). In experiment 2, the measurements were carried out in eight flowers per treatment.

2.4. Recovery from the decrease in stem hydraulic conductivity ($K_s$) due to air aspirated at the stem cut surface, in cv. Prophylta (experiment 3)

In cv. Prophylta (sensitive) we investigated the effect of high RH on stem hydraulic conductivity ($K_s$). We also artificially induced the presence of air emboli at the cut surface. All manipulations with plants and stem segments in the laboratory were done under water to prevent the entrance of air into the xylem vessels at the cut surface. Stem segments of 35 cm length were cut from the plants at 5 cm above the primary shoot/secondary shoot junction with sharp shears. The stems were recut, removing 5 cm from both ends, with a new razor blade. Stem length was then approximately 25 cm. The number of xylem vessels exceeding 20 cm length is very low in rose (less than or equal to 5; van Doorn and Reid, 1995). Leaves were removed from the stem segment with a razor blade, leaving 0.2 cm of the petioles on the stem (van leperen et al., 2001). Each stem segment contained the same number of nodes, since a nodal structure offers higher resistance to water flow (Salleo et al., 1984), and had similar diameters at both cut ends compared to the other replications. The time between collection from the plant and the start of the measurement was approximately 20 min.

A silicone tube was pushed over the upper cut end of the stem segment (cut end at largest distance from the roots), while the lower end of the stem segment was placed in a container filled with degassed aqueous solution of sodium bicarbonate (1.5 mM), calcium chloride (0.7 mM) and copper sulphate (5 μM) at room temperature (20 ± 2 °C) (van Meeteren et al., 2000). The tube was then connected to a pump (7550-62, Barnant, Barrington, IL, USA) creating a pulling pressure difference of 40 kPa. Actual pressure was measured using a pressure transducer (DVR 5, Vacuubrand, Wertheim, Germany). During these measurements solution flow was always in the natural direction, from the lower (closest to the roots) to the upper cut end. Flow through the stem segments was calculated from weight changes of the solution, corrected for evaporation. Flow rates stabilized typically after 5 ± 2 min. Subsequently, the $K_s$ was calculated according to van Meeteren et al. (2000) (Eq. (1)), by using the stem segment length (l), the applied pressure difference ($\Delta P$) and the flow rate (q).

$$K_s = \frac{q}{\Delta P / l}$$

After the $K_s$ measurement without initial air emboli, and while keeping the stem segments under tension (40 kPa), the segments were lifted out of the solution to allow air entrance for approximately 3 min. The pressure exerted was far lower than the one needed to move the air–water interface (1.5 MPa) through the pores of the pit membranes (van Doorn and Suiro, 1996), but higher than the one needed to fill most xylem conduits at the cut surface with air (van leperen et al., 2001). After this period of exposure to air, the stem segments were lowered back into the solution and $K_s$ was followed for 2.4 h. In this experiment seven stem segments (one segment per plant) per treatment were used.

2.5. Stomatal response to a decrease in leaf water potential ($\Psi_{\text{leaf}}$), in cv. Prophylta (experiment 4)

The effect of ambient humidity during cultivation on the stomatal responsiveness to a decrease in leaf water potential ($\Psi_{\text{leaf}}$) was investigated in cv. Prophylta (sensitive). The transpiration rate as a function of leaf relative water content (RWC) was evaluated in one set of measurements. Terminal leaflets of the first five-leaflet leaves counting from the apex were detached. Their petioles were immediately recut under degassed water (to prevent cavitation-induced embolism), placed in flasks filled with water, and further incubated for 1 h at about 100% RH (21 °C, VPD close to 0) to establish their saturated FW. Since the leaflets were detached during the light period in the growth chamber, the rehydration process was therefore conducted in the light (15 μmol m$^{-2}$ s$^{-1}$ PAR; following darkness the light-induced stomatal opening requires several min; Mott et al., 1999). Subsequently, the leaflets were removed from water and placed on a table (abaxial surface down) at 21 °C, 50 ± 3% RH, and 50 μmol m$^{-2}$ s$^{-1}$ PAR. Transpiration rate was gravimetrically recorded during 4 h. The leaflets were then dried at 80 °C for 24 h. The RWC was calculated according to Slavik (1974).

In another set of measurements, the $\Psi_{\text{leaf}}$ as a function of leaf RWC was determined. Terminal leaflets of the first five-leaflet leaves counting from the apex were detached, their petioles were recut under degassed water and placed in vials filled with water. Subsequently, the leaflets were rehydrated in darkness (to promote stomatal closure). This was done in place of overnight rehydration, common in pressure–volume work, to prevent changes in osmotic potential which can occur within several hours (Auge et al., 1986). Afterwards, the leaflets were covered with a polyethylene sheet of a known mass and weighed to determine their saturated FW. Then the leaflet with the polyethylene sheet still around, was placed in a Scholander–type pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) and the balance pressure ($-\Psi_{\text{leaf}}$) was determined. The pressure in the chamber was increased at a rate not higher than 0.02 MPa s$^{-1}$ to avoid cell injury (Kikuta et al., 1985). Leaves were allowed to dry on a table at 21 °C, 50 ± 3% RH, and 50 μmol m$^{-2}$ s$^{-1}$ PAR. Incremental weight loss was gravimetrically determined. The dry weight of the leaflets was obtained as described above. All measurements included 14 leaves (one leaf per plant) per treatment.

2.6. Stomatal response to abscisic acid (ABA) feeding during postharvest, in cv. Prophylta (experiment 5)

The efficiency of an antitranspirant compound (abscisic acid, ABA) in decreasing the transpiration rate of cv. Prophylta (sensitive) grown at different moisture ambient conditions was assessed. Care was taken that the vascular blockage was prevented in the tested cut flowers (as described for experiment 2). Flowering stalks were placed in artificial vase solution with 0 (control) or 100 μM (±) ABA (Sigma, St. Louis, MO, USA), and kept under test room conditions (as described for experiment 1). The top of the flask was covered with Parafilm, while its sides were wrapped in aluminum foil (to reflect light), since ABA is light sensitive (Davies and Jones, 1991). The experiment was stopped when leaf abscission was observed in ABA-fed flowers, and total leaf area was then measured. The transpiration rate was determined as described earlier (experiment 1). In this experiment eight flowers per treatment were used.

2.7. Statistical design and analysis

Data were subjected to analysis of variance using Genstat software (10th edition, VSN International Ltd., Hemel Hempstead, Herts, UK). Experiments 1, 2 and 5 had a split-plot design, with RH
level as the main factor, and cultivar (experiments 1 and 2) or duration of ABA feeding (experiment 5) as the split factors, respectively. Experiments 3 and 4 were analysed by one-way ANOVA. Treatment effects were tested at 5% probability level and mean separation was carried out using least significant differences based on Student’s t-test ($P \leq 0.05$).

3. Results

3.1. Vase life, flower opening stage and flower diameter

The life span of flowering stems cut in air and placed in vase solution with moderate inhibition of bacterial growth (i.e. non-optimum water uptake conditions; experiment 1) was reduced in stalks from plants grown at high RH (95%), compared with those from plants grown at moderate RH (60%). The effect was largest in cv. Prophyta. At moderate RH its vase life was about 19 days, whereas the vase life of flowering stems grown at high RH was only 4 days (Table 1; experiment 1). A small negative effect of high RH during cultivation was found on the vase life of cv. Dream, while no significant effect was observed in cv. Frisco (Table 1; experiment 1). Moreover, flower diameter was only significantly inhibited in cv. Prophyta when grown under high RH, resulting in about 30% reduction at the end of vase life (Table 1; experiment 1). A similar trend was observed for the flower opening stage, but since these data were not normally distributed, no analysis of variance was performed (Table 1; experiment 1). Symptoms that resulted in early termination of vase life after cultivation at high RH were pedicel bending and leaf desiccation in cv. Prophyta, and pedicel bending in cv. Dream. Vase life termination was thus due to early water stress symptoms (Table 2; experiment 1). Such precocious senescence symptoms were not observed in cv. Frisco grown at high RH, which ended its vase life as a consequence of a natural flower wilting as observed in nearly all flowers of the studied cultivars grown at moderate RH (Table 2; experiment 1).

In experiment 2 the flowers were surface-sterilized, cut from the plant under degassed water, and placed in sterilized vase water. Uptake of air into the cut stems was thereby prevented and micro-bial effects were drastically reduced (i.e. optimum water uptake conditions). These conditions largely alleviated the negative effect of high RH during cultivation on the length of vase life and on the flower opening stage and diameter in cv. Prophyta (Table 1; experiment 2). In cv. Dream these conditions completely prevented the effect of high RH during cultivation (Table 1; experiment 2). Contrary to the expectation there was still a negative effect of cultivation RH on the vase life of cv. Frisco (Table 1; experiment 2). In this experiment no water stress symptoms were noted, except leaf desiccation in cv. Dream grown at high RH (Table 2; experiment 2).

3.2. Transpiration rate and its diurnal rhythm in the light period

A two day vase life trial, in which the leaves were totally removed on the second day, showed that leaves in cv. Frisco accounted for about 80% of the total transpiration during the light period in flowers grown both at moderate and high RH (data not shown).

Cut roses grown at high RH had higher transpiration rates in the light period, compared with roses grown at moderate RH (Fig. 1A, C and E). In the cvs. Dream and Frisco the effect was only found during the first days of vase life (Fig. 1C and E). Cultivar Prophyta had an average transpiration rate in the light period that was three times higher when grown at high RH, compared to stalks grown at moderate RH (Fig. 1A). The effect was smaller in the cvs. Frisco and Dream (32 and 22% higher transpiration in flowers grown at high RH compared to roses grown at moderate RH, respectively; Fig. 1C and E). These data refer to normal cutting and a

### Table 1

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>RH (%)</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tr>
<td></td>
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<td>Vase life (days) Flower stage</td>
<td>Flower diameter (mm)</td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Prophyta</td>
<td>60</td>
<td>19.4$^{a}$</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>4.0$^{b}$</td>
<td>3.0</td>
</tr>
<tr>
<td>Frisco</td>
<td>60</td>
<td>18.0$^{a}$</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>16.4$^{a}$</td>
<td>5.0</td>
</tr>
<tr>
<td>Dream</td>
<td>60</td>
<td>13.6$^{a}$</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>11.5$^{a}$</td>
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<td></td>
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<td>RH</td>
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Means in each column followed by different letters indicate significant differences according to LSD-test.

*Flower stage* did not show a normal distribution, therefore no F probability is given.

### Table 2

<table>
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<th>Experiment 2</th>
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<tr>
<td></td>
<td></td>
<td>Pedicel bending</td>
<td>Desiccated leaves</td>
<td>Desiccated petals</td>
<td>Wilted flower</td>
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<tr>
<td>Prophyta</td>
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<td>100</td>
<td>-</td>
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<tr>
<td></td>
<td>95</td>
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<td>17</td>
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<td>Frisco</td>
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<td></td>
<td>95</td>
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<td>Dream</td>
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moderately large vase water microbial population. When flower stems were surface-sterilized, cut under degassed water, and placed in sterile vase solution, the transpiration was also higher in flowers cultivated at high RH compared to those grown at moderate RH (Fig. 1B, D and F; see also Fig. 7A). There was no clear difference between this experiment (Fig. 1B, D and F) and the one in which the stems had aspirated air and the vase water microbial population was much higher (Fig. 1A, C and E).

The diurnal course of transpiration rate was recorded during the vase life of the cvs. Prophyta and Frisco, under water uptake conditions that were close to optimum (stem surface-sterilization followed by cutting stems from the plant under degassed water and placement in sterilized vase solution). A relatively large diurnal oscillation in the transpiration rate was observed in flowers grown at moderate RH, and a much smaller oscillation in flowers grown at high RH (Fig. 2A and B). The transpiration rate during the light period showed a peak during the first hours, while the lowest value was always observed at the end of the light period (Fig. 2A and B). The amplitudes between the highest and the lowest transpiration rates within the light period are shown in Fig. 2C and D. Roses grown under high RH exhibited a considerably smaller amplitude of transpiration during the light period, compared to those grown at moderate RH.

3.3. Transpiration rate in darkness

In all three cultivars tested, a high RH during cultivation significantly increased the transpiration rate in the dark period during vase life (Fig. 3A, C and E). Cultivar Prophyta grown at high RH had on average a five times higher transpiration rate in the dark compared to roses grown at moderate RH. The cvs. Frisco and Dream showed an increase of approximately a factor of two. Compared to these data on roses placed under suboptimal water uptake conditions, the effect of cultivation RH on the transpiration rate in darkness was similar in roses that were subjected to optimized water uptake conditions during vase life (Fig. 3B, D and F; see also Fig. 7B).

The difference between the transpiration rate during the light period and the rate of nocturnal transpiration is shown in Fig. 4. This difference was large at the beginning of vase life in roses grown under moderate RH, but became lower later on, in roses placed under suboptimal water uptake conditions (Fig. 4A, C and E). This decrease in the difference between transpiration during the light and in darkness, during the course of vase life, was not found (cv. Prophyta; Fig. 4B) or was less pronounced ( cvs. Frisco and Dream; Fig. 4D and F) in roses that were subjected to more optimal water uptake conditions.

3.4. Fresh weight (FW)

A large initial increase in FW was observed in roses that were held under optimum water uptake conditions during vase life (Fig. 5B, D and F) while a smaller FW increase was found in flowers that were exposed to non-optimum conditions (Fig. 5A, C and E). The RH during cultivation had little effect on the FW in the cvs. Dream (Fig. 5C and D) and Frisco (Fig. 5E and F). Only in cv. Prophyta the FW remained lower, almost throughout vase life, in flowers grown under high RH compared to those grown under moderate RH (Fig. 5A and B).

3.5. Recovery from the decrease in stem hydraulic conductivity ($K_h$) due to air aspirated at the stem cut surface, in cv. Prophyta

Stem segments of cv. Prophyta were cut under water. A suction force of 40 kPa was applied to the upper end while the lower end of the stem segment was maintained under water. The absolute $K_h$ values were not affected by the preharvest RH level ($P = 0.458$; data not shown). The initial $K_h$ was set to 100% (Fig. 6A). After some initial measurements, air was allowed to be aspirated at the basal cut surface for 3 min, by lifting the basal end of the segment above
Fig. 2. Transpiration rate (2, 4, 8, 10, 12 h after the onset of the light period, and 12 h after the onset of the dark period) during the first 10 days of vase life under optimum water uptake conditions (experiment 2) of two cut rose cultivars grown at moderate (60%, open symbols) or high (95%, closed symbols) relative air humidity. The light and dark periods were 12 h each. Details of the experiment are given in Table 1. (C) and (D) depict the relative decrease between the maximum ($T_{Lmax}$) and minimum ($T_{Lmin}$) values of transpiration rate during the light period [i.e. $((T_{Lmax} - T_{Lmin})/T_{Lmax}) \times 100$]. Values are the means of 8 replications ± S.E.

Fig. 3. Transpiration rate in the dark period during the vase life under non-optimum (A, C and E; experiment 1) and optimum (B, D and F; experiment 2) water uptake conditions of three cut rose cultivars, grown at moderate (60%, open symbols) or high (95%, closed symbols) relative air humidity. Details of the experiments are given in Table 1. Values are the means of 12 (experiment 1) or 8 (experiment 2) replications ± S.E.
the solution surface. This was followed by lowering the segment back into the solution. \( K_h \) then showed an initial fast recovery (Fig. 6A). Previous research found that this recovery is due to the partial refilling with solution of xylem conduits in which air had been taken up, resulting in a reconnection between the vase water and the xylem conduits that had not been opened by cutting (van Ieperen et al., 2002). Later on (from about \( t = 30 \text{ min} \)), a slower increase in \( K_h \) was found (Fig. 6A). This has been related to the

![Diagram](image)

**Fig. 4.** The relative decrease between the transpiration rate during the light \( (T_L) \) and dark \( (T_D) \) periods [i.e. \( (T_D - T_L) \times 100 \)], during vase life under non-optimum (A, C and E; experiment 1) and optimum (B, D and F; experiment 2) water uptake conditions of three cut rose cultivars, grown at moderate (60%, open symbols) or high (95%, closed symbols) relative air humidity. Details of the experiments are given in Table 1. The transpiration rate during the light and dark periods is shown in Figs. 1 and 3, respectively. Values are the means of 12 (experiment 1) or 8 (experiment 2) replications ± S.E.

![Diagram](image)

**Fig. 5.** Relative fresh weight during the vase life under non-optimum (A, C and E; experiment 1) and optimum (B, D and F; experiment 2) water uptake conditions of three cut rose cultivars, grown at moderate (60%, open symbols) or high (95%, closed symbols) relative air humidity. The fresh weight of each flowering stem was expressed relative to its initial weight. Details of the experiments are given in Table 1. Values are the means of 12 (experiment 1) or 8 (experiment 2) replications ± S.E.
relatively slow dissolution of the remaining trapped air at the top of the xylem conduits (van Ieperen et al., 2002). After 2.4 h of measurement, the $K_s$ tended to stabilize at 63% of the initial value (before air entrance at the lower stem end). No effects of RH during cultivation were found in the response to air aspiration for 3 min (Fig. 6A).

3.6. Stomatal response to a decrease in leaf water potential ($\Psi_{\text{leaf}}$), in cv. Prophyta

Transpiration rates and $\Psi_{\text{leaf}}$ were measured in detached leaves of cv. Prophyta. The transpiration rate was taken as a measure of stomatal opening. In leaves from plants that had been cultivated at moderate RH, the stomata showed a rapid closure reaction, starting when the $\Psi_{\text{leaf}}$ had dropped to $-2.0$ MPa. The stomata were almost fully closed when the $\Psi_{\text{leaf}}$ was $-2.5$ MPa (Fig. 6B). The reaction was quite different in leaves taken from plants that had been cultivated at high RH. The stomata showed a small closing reaction also starting at about $-2.0$ MPa, but they closed only slightly further at a lower $\Psi_{\text{leaf}}$. When the $\Psi_{\text{leaf}}$ had reached $-3.0$ MPa the stomata were still about half open (Fig. 6B).

3.7. Stomatal response to abscisic acid (ABA) feeding during postharvest, in cv. Prophyta

The efficacy of adding ABA into the vase solution (antitranspirant compound) on decreasing postharvest water loss was evaluated in cv. Prophyta roses grown at high RH. Long-term ABA feeding (100 $\mu$M) through the stem base resulted in lower transpiration rates during both the light and dark postharvest periods (Fig. 7). However, the long-term ABA feeding via the vase solution was only partly able to counteract the effect of high RH during cultivation on the increased water loss. This is particularly evident during the nocturnal period, where the transpiration rate in ABA-fed stalks from plants grown at high RH is five times higher, as compared to unfed stalks from plants grown at moderate RH (Fig. 7B).

4. Discussion

Cut rose flowers often show postharvest water stress symptoms. In previous studies it has been found that in some cultivars these water stress symptoms become aggravated by long-term high RH during cultivation (Mortensen and Gislérud, 1999, 2005). The present results confirm those findings (Table 1). Moreover, we have studied in detail the water relations of cut flowers grown at moderate and high RH and we addressed the question of how stomatal opening changes in time and reacts to darkness and to a decrease in water potential. In this study we also initiated work to explain the cultivar differences in their sensitivity to long-term high RH.

Fig. 6. Stem hydraulic conductivity recovery upon air emboli induction, and transpiration rate as a function of leaf water potential in cv. Prophyta grown at moderate (60%, open symbols) or high (95%, closed symbols) relative air humidity. (A) Hydraulic conductivity ($K_s$) changes following artificial induction of air emboli at the cut surface of 25 cm stem segments (experiment 3). The arrow depicts the time where the stem segment was allowed to aspirate air at the basal cut surface (air aspiration duration was 3 min). Thereafter the end of the stem segment was again placed into the solution. Values are the means of 7 replications ± S.E. (B) Transpiration rate as a function of leaf water potential ($\Psi_{\text{leaf}}$) during desiccation of detached leaves (experiment 4). Vertical bars indicate S.E (n = 14).

Fig. 7. Transpiration rates in the light (A) and darkness (B) of cv. Prophyta roses placed in a vase solution containing 0 (solid lines) or 100 $\mu$M ABA (dashed lines) (experiment 5). Flowers were cultivated at moderate (60%, open symbols) or high (95%, closed symbols) relative air humidity. Xylem occlusion was largely prevented as described for experiment 2 in Table 1. The experiment was terminated when leaf abscission was observed in ABA-fed flowers. Values are the means of 8 replications ± S.E.
When the problems with water uptake were largely prevented (stem surface-sterilization, cutting under degassed water, and placement in sterilized vase solution; experiment 2) the shorter vase life and the inhibition of flower opening in cv. Prophyta were strongly alleviated in high RH-grown plants (Tables 1 and 2). Similarly, it has been shown that treatment with silver nitrate, an antibacterial compound, increased the vase life in cut roses cultivated at high RH (Torre and Ejlid, 2001). These data show that the water uptake problems, which often occur in most rose cultivars, are the initial cause of the water stress symptoms. However, the poor control of water loss in flowers cultivated at high RH aggravates this problem, whereas flowers grown at moderate RH react to the low Ψleaf by rapidly closing their stomata (Fig. 6B), which enables a positive water balance during a longer period. Nonetheless, in flowers grown at moderate RH the water uptake still eventually becomes so low that a net water loss occurs, even though the stomata are largely closed. This net water loss is due to the ongoing residual stomatal and cuticular transpiration (Kerstiens, 1995). So wilting symptoms will eventually ensue.

The water loss of cv. Prophyta roses that were grown at high RH, and placed in a vase solution, was considerably larger than that in the other two cultivars studied (Figs. 1 and 3). These results are consistent with an earlier study, where stomatal responsiveness to leaf desiccation was significantly lower in cv. Prophyta as compared to cv. Frisco in high RH-grown plants (Fanourakis et al., 2009). Additionally, it was found that elevated RH during cultivation resulted in a weakened diurnal rhythm during the light period, as expressed by the amplitude between the highest and the lowest transpiration rate (Fig. 2). Prophyta roses grown at high RH and placed in a vase solution containing ABA had a lower water loss, compared to unfed high RH-grown flower stalks, but still their transpiration rate especially during the darkness was higher than in moderate RH-grown plants (Fig. 7). Recently it has been demonstrated that the role of ABA, in alleviating the negative effects of high RH on stomatal functioning, is restricted to the period of leaf expansion (Fanourakis et al., 2011). Thus, the limited capacity of a long-term ABA feeding to induce stomatal closure when applied via the vase solution (Fig. 7) can be explained by the fact that flower stalks at harvest are totally composed of fully developed leaves. Moreover, in the current study the 100 μM ABA feeding solution was continuously reaching the leaf via the transpiration stream, whereas in Fanourakis et al. (2011) ABA was brushed daily on fully developed leaves at a lower concentration (30 μM). These differences can explain the total absence of stomatal response to ABA in their study and a partial (though limited) response in the current one.

Although stomatal density in cv. Prophyta is not affected by ambient humidity during leaf development, stomata formed under elevated RH levels have longer pore length (Fanourakis et al., 2011), which results in a higher water loss rate at the same pore aperture values (Parlange and Waggoner, 1970). Higher cuticular water loss, as a result of poor cuticular development, might also contribute to the high rates of water loss in high RH-grown leaves (Karbulkova et al., 2008), though this conclusion has been questioned by other authors (Torre et al., 2001). Thus, part of the higher water loss observed in cv. Prophyta can be possibly related to anatomical features (e.g. bigger stomata and higher cuticular permeability), which might contribute to the increased sensitivity of this cultivar to long-term high RH during growth. Even in the cvs. Frisco and Dream there was a higher water loss as a result of high RH during cultivation, but this did not lead to early visible water stress symptoms (Table 1). Apparently, the increase in water loss in these cultivars was not high enough to reduce the water potential to a level that induced earlier visible symptoms of water stress. Future research is needed to evaluate the relative importance of the physiological and anatomical components in the enhanced water loss among contrasting genotypes grown at elevated RH.

A decrease of the water potential in cut flowers leads to cavitation events (Dixon et al., 1988; Spinarova et al., 2007). When a high number of conduits becomes inoperative, as a result of cavitation, water uptake will become additionally inhibited (van Doorn, 1997). Both air emboli at the cut surface (van Doorn and Jones, 1994) and bacterial occlusion (Bleekema and van Doorn, 2003) have been shown to induce cavitation. We have no data on sensitivity to cavitation in the cvs. Prophyta and Dream, but cv. Frisco was highly resistant to cavitation (which started at a considerably lower water potential than in cv. Sonia roses; van Doorn and Suiro, 1996). It is therefore possible that the genotypic variation in the vase life decrease, as a result of more humid air during cultivation, arises partially from an effect on cavitation, but the role of cavitation in the present differences between cultivars is not yet known.

Unlike our initial hypothesis, no effect of cultivation at high RH was found on the initial values of stem hydraulic conductivity. The absence of such an effect indicates that RH during cultivation had no effect on stem xylem anatomy to an extent that it affected hydraulic conductivity. When the hydraulic conductivity of cv. Prophyta stem segments was reduced because of aspiration of air into the xylem conduits opened by cutting, there was no effect of the RH level during cultivation (Fig. 6A). This suggests that factors such as the wetting angle of the xylem conduits (van Ieperen et al., 2002) were also not considerably affected.

This study clearly demonstrates that the main effect of ambient humidity during preharvest on the water relations during postharvest is closely related to the regulation of water loss, since the stem hydraulic conductivity and its recovery by air emboli were not affected by long-term high RH. Therefore, it is concluded that xylem anatomy does not explain the differential cultivar sensitivity to high RH. Instead, differences between the cultivars could be largely explained by their contrasting capacity to control water loss. For instance, the early water stress symptoms and the inhibition of flower opening in cv. Prophyta (sensitive) grown at high RH were due to a higher rate of water loss compared to those grown at moderate RH, while there was apparently a similar blockage in water uptake. This higher water loss seems to be closely related to stomatal malfunctioning. Prophyta roses that had been cultivated at high RH were found not to close their stomata to the same degree as those cultivated at moderate RH (1) in darkness and (2) when the Ψleaf decreased. The increase of cut flower water loss, as a result of plant growth at high RH, was much less pronounced in two other cultivars (cvs. Frisco and Dream), compared to cv. Prophyta. It is likely that the higher water loss in these two cultivars grown at high RH results in a water potential, lower than the one needed to induce significant cavitation events. Preventing vascular occlusion, caused by air emboli at the cut surface and bacterial physical blockage, considerably extended the time to wilting and enhanced flower opening in cv. Prophyta roses grown at high RH. This indicates that the high rate of water loss, as a result of plant growth at elevated atmospheric humidity, can only be detrimental for cut flower longevity under limiting water uptake conditions.

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