https://doi.org/10.17221/144/2017-HORTSCI

Stomatal anatomy and closing ability is affected by supplementary light intensity in rose (*Rosa hybrida* L.)

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Citation: Fanourakis D., Hyldgaard B., Giday H., Aulik I., Bouranis D., Körner O., Ottosen C.O. (2019): Stomatal anatomy and closing ability is affected by supplementary light intensity in rose (*Rosa hybrida* L.). Hort. Sci. (Prague): 46: 81–89.

Abstract: Increasing the light level in protected cultivation of ornamental crops via supplementary lighting is critical to enhance both production and external quality especially during the periods of low light availability. Despite wide applications the effects of light intensities were not previously addressed on water loss pathways. In this study rose plants were cultivated at 100, 200 or 400 μmol/(m^2·s) photosynthetic photon flux density (PPFD). The stomatal responsiveness to desiccation, stomatal anatomical features and cuticular transpiration were determined. Plant biomass as well as photosynthesis response to light and CO_2 were also assessed. Increasing growth PPFD led to a considerable increase in plant biomass (85 and 57% for 100 to 200 and 200 to 400 μmol/(m^2·s) respectively). Photosynthesis was marginally affected by increasing growth PPFD from 100 to 200 μmol/(m^2·s) while a further rise to 400 μmol/(m^2·s) considerably increased photosynthetic rate at high light intensities. Higher PPFD during cultivation generally led to larger stomata with bigger pores. A PPFD increase from 100 to 200 μmol/(m^2·s) had a small negative effect on stomatal closing ability whereas a further rise to 400 μmol/(m^2·s) had a substantial stimulatory effect. Cultivation at a PPFD higher than 100 μmol/(m^2·s) led to lower rates of cuticular transpiration. In conclusion, high growth PPFD (> 200 μmol/(m^2·s)) enchanced both photosynthetic and stomatal anatomical traits. High light intensity (> 200 μmol/(m^2·s)) also led to a better control of water loss due to more responsive stomata and decreased cuticular permeability.

Keywords: cuticular water loss; photosynthesis; stomatal size; transpiration

Supplementary light is frequently employed in protected cultivation of northern Europe to enhance both photoperiod and light intensity (Heuvelink et al. 2006). The number of crops where supplementary light is applied is continuously increasing and the applied light levels are reportedly higher compared to five years ago (Heuvelink et al. 2006). Additionally, higher light intensities are used in modern greenhouses through newly introduced designs (less constructional elements resulting in less shading), cover materials with higher light permeability and materials increasing light transmission inside the greenhouse (e.g. white ground cover; Heuvelink et al. 2006; Fanourakis et al. 2013b). Higher light levels during cultivation not only enhance productivity, but also reduce the visual variation in flower quality throughout

Supported by the Aarhus University and was linked to the projects REDHUM (funded by GUDP) and GreenGrowing (Interreg ERDF).
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https://doi.org/10.17221/144/2017-HORTSCI

the year (Fanourakis et al. 2013b). Although these effects on external quality of cut flowers are well-documented limited attention has been devoted on the respective effects on cut flower longevity following harvest with the undelying processes, such as the water loss regulation via both stomata and cuticle being unexplored. Studying the light environment effects on these processes is essential for the horticultural industry because of both increased competition for production of high quality plants and rise in number of growers working with a label for postharvest quality and shelf life guarantee.

Contradictory findings are reported on the role of light levels during cultivation on post-harvest longevity of cut rose flower (Fanourakis et al. 2013b). Enchanced cut flower carbohydrate content under high light intensities did not impart any effect on post-harvest life of cut rose flowers (Fanourakis et al. 2013b). There is a possiblity of light mediated regulation of water loss pathways in rose and its subsquent effects on cut flower longevity. However, the effects of light level on stomatal closing ability have not been addressed. Only a single study showed faster stomatal closure in response to light/ dark transition in rose plants grown under the highest light intensity (150 μmol/(m^2·s); Blom-Zandstra et al. 1995).

Water loss takes place not only through stomatal openings, but also via the cuticular pathway. Similarly to stomatal characters the cuticular properties are affected by the growth environment (Domínguez et al. 2011; Fanourakis et al. 2013a; Fanourakis et al. 2016; Xue et al. 2017). The effect of light intensity during growth on cuticular transpiration remains largely unexplored in ornamnetal crops. Research on tree species reported increased cuticle thickness under high light intensities (James, Bell 2000), which is expected to reduce water loss. To the best of our knowledge, the role of light intensity on water loss pathways in rose has not previously been studied.

The present investigation was done (1) to investigate the biomass and biomass allocation responses to different light environments during growth, (2) to analyse the photosynthesis response to light and carbon dioxide concentration of leaves expanded at different light levels, and (3) to evaluate the effect of light intensity during cultivation on both stomatal and cuticular water loss pathways. We hypothesize that leaves expanded at high light intensity are not only more efficient in driving photosynthesis, but also more able to regulate water loss upon water severity.

MATERIAL AND METHODS

Plant material and growth conditions. One month old plants of pot rose cv. 'Pasadena' (*Rosa hybrida* L.) were obtained from a commercial nursery (Rosa Danica, Marslev, Denmark). Pots (0.55 l) were filled with a mixture of peat and perlite (9 : 1, v/v; Meegaa substrates BV, Rotterdam, Netherlands). Upon arrival plants were prunned leaving two nodes on the main shoot, each bearing a leaf and its axillary bud. From those two buds, the most vigorous one was kept (formed the shoot that was later on assessed) whereas the other one was discarded. Plants were transferred into three growth chambers (MB-teknik, Brøndby, Denmark), which were previously set at three different light levels (i.e. 100 (low), 200 (moderate) or 400 (high) μmol/(m^2·s) PPFD). Light level was determined by LI-250A (LI-COR, Lincoln, USA) and was supplied by white LED lamps (HQI-BT 400W/D pro, Philips, Eindhoven, Netherlands) for 18 h per day. The remaining environmental factors were identical among three chambers. Air temperature was maintained at 20.5 ± 1.4°C and relative air humidity (RH) at 60 ± 3%. Carbon dioxide concentration was set to 400 μmol/mol. Climate data was automatically logged (Datataker, Thermo Fisher Scientifid Australia Pty Ltd., Scoresby, Australia) throughout the experiment by sensors placed at 60 cm (corresponding to the top of fully grown plants). Plants were daily watered with a nutrient solution optimised for rose (Giday et al. 2013b). Potting-media moisture was maintained at or near max. water holding capacity. Electrical conductivity and pH were monitored daily and adjusted to 2 mS/cm and 5.5 respectively (Giday et al. 2013b).

After four weeks in the climate chambers measurements were performed on fully-grown plants (defined as bearing at least two flower buds with cylindrical shape and pointed tip). Sampled leaves were young, fully-expanded, and grown under direct light. Replicate leaves were collected using separate plants. In all cases the time between sampling and the start of the evaluation did not exceed 15 minutes.

Leaf morphological components. To evaluate the effect of light intensity on leaf morphological components, leaf area together with leaf, stem and flower bud dry masses were recorded. Leaf area was determined using a leaf area meter (LI-COR Model Li-3100, LI-COR, Lincoln, USA) and dry weight was assessed after drying the tissue for 48 h at 80°C.

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Specific leaf area (SLA; leaf area/leaf mass) and leaf mass ratio (LMR; leaf mass/plant mass) were calculated (Poorter et al. 2010). All the measurements were conducted on six plants per treatment.

Chlorophyll content. The effect of growth irradiance on leaf chlorophyll content was assessed by using a SPAD chlorophyll meter (SPAD-502 meter, Konica Minolta, Tokyo, Japan). Lateral leaflets of the first and second penta-foliate leaf (counting from the apex) were used for measurements. Thirty leaflets were analysed from each treatment.

Plant water loss. The effect of different levels of light intensity on whole plant transpiration was evaluated at growth conditions. During a five day period the pot water loss was daily determined by weighing (± 0.1 g; MXX-2001; Denver Instruments, Bohemia, USA) the pots two times a day (time 0 and 18 h after the onset of the light period) as well as the supplied nutrient and drainage solutions. Growth-media evaporation was assessed daily in four pots without plants, but with identical media water content to the pots containing plants. Direct evaporation from the media was subtracted from pot water loss to determine plant water loss. Plant transpiration rate was calculated per unit leaf area. Treatments were compared using the average transpiration rate over the period of five days. Plant transpiration was assessed in six intact plants per treatment.

Photosynthetic light-response and CO_2-response curves. The effect of growing irradiance on photosynthetic light–response and CO_2-response curves was investigated. Lateral leaflets of the first and second five-leaflet leaves (counting from the apex) were assessed. A portable gas analysis system (CIRAS-2, PP systems, Amesbury, USA) was used. The response of photosynthetic rate (A_n) to light was determined by gradual increase in irradiance from 0 to 1500 μmol/(m^2·s) while keeping CO_2 at ambient concentration (400 μmol/mol). At each irradiance level, steady-state was achieved within 15 minutes. A_n was taken as the 50 s mean value following the establishment of a stable rate. The response of A_n to internal CO_2 concentration (C_i) was measured immediately after reaching the saturating irradiance level (1,500 μmol/(m^2·s)). CO_2 was gradually decreased from 400 to 75 μmol/mol before returning to the initial concentration. This was followed by a stepwise increase to 1,500 μmol/ mol. During measurements the leaf temperature was set at 20°C and air flow at 200 μmol/s. Measurements were recorded when A_n stabilized to the new condition (≥ 5 min). Measurements were performed on attached leaves of six intact plants per treatment.

Stomatal size, density and pore dimensions. The effects of light intensity during leaf expansion on stomatal length (i.e. longest diameter), width (i.e. shortest diameter) and density (i.e. number per unit leaf area) together with pore length (i.e. longest diameter), aperture (i.e. shortest diameter) and (projected) area were determined. The silicon rubber impression technique was employed (elite HD+, Zhermack, Badia Polesine, Italy; Giday et al. 2013b) using a lateral leaflet of the first penta-foliate leaf (counting from the apex). The sampling area (1 × 1 cm) was located midway the leaflet base and tip as well as between the midrib and lateral margin (Fanourakis et al. 2015a). Images were acquired using an optical microscope (LeitzAristoplan; Ernst LeitzWetzlar GmbH, Wetzlar, Germany) connected to a digital camera (Nikon DXM-1200; Nikon Corp., Tokyo, Japan). As *Rosa hybrida* L. is a hypostomatous species (Fanourakis et al. 2013a), only the abaxial (lower) leaflet surface was assessed. Sampling took place 2 h following the onset of the light period, since this time is required for plants experiencing the dark period to open stomata and reach a steady-state stomatal conductance (Drake et al., 2012). Stomatal (length and width) and pore (length and aperture) anatomical features were determined on 25 randomly selected stomata (magnification ×200) while stomatal density was counted on five non-overlapping interveinal fields of view per leaflet (magnification ×100). Stomatal size was defined as stomatal length multiplied by stomatal width (Drake et al. 2012). Pore area per leaf area was taken as the product of pore area per stoma (π × 0.5 (pore length × pore aperture)) and stomatal density (Fanourakis et al. 2015a). Images were processed by using the UTHSCSA ImageTool program (University of Texas Health Science Centre, San Antonio, USA). Ten leaflets were assessed for each treatment.

Stomatal responsiveness to desiccation. Stomatal responsiveness was evaluated by exposing detached leaflets to dehydrating conditions. First and second five-leaflet leaves (counting from the apex) were sampled. Terminal leaflets with long petioles (> 2 cm) were selected to facilitate both rehydration (see below) and handling (i.e. preventing contact with the leaflet lamina) procedures. Leaflets were detached at the same time of the day (2 h following the onset of the light period) and re-cut by submerging their petiole under water (to prevent cavitation of xylem vessels that were opened by cut-

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ting) and finally placed in flasks filled with degassed water. The leaflets were incubated at 21°C, ~100% RH (i.e. VPD close to 0) and 15 μmol/(m^2·s) PPFD for 1 h to establish their maximum fresh weight. The leaflets were then placed on a bench (down-facing abaxial surface) in the climate-controled test room and the transpiration rate was recorded for 4 h by gravimetry. Preliminary work comparing transpiration rates between leaves with either intact or sealed (by using paraffin wax) petioles indicated that water loss through the petiole cut end was negligible and thus petioles were not sealed during measurements. Test room conditions were air temperature 21°C, RH equal to 50% and 50 μmol/(m^2·s) PPFD provided by fluorescent lamps (T5 fluorescent lamp; GE lighting, Cleveland, USA). 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), which indicated adequate air circulation. Different treatments were always assessed simultaneously. At the end of the measurement, leaflet area and dry weight were determined. Leaflet relative water content (RWC) was calculated using the following equation:

$$RWC = \frac{fresh\,weight - dry\,weight}{saturated\ fresh\,weight - dry\,weight} \times 100$$

Stabilization RWC was defined as the RWC at which the transpiration rate stabilized and was calculated as explained by Giday et al. (2013b). The stable transpiration rate was defined as the average of the three consecutive points that did not differ significantly while stabilization RWC was taken as the RWC where the first of these three points was noted. Measurements were carried out on seven leaflets per treatment.

Cuticular transpiration rate. The effect of growth conditions on the transpiration rate through the leaf epidermis was assessed. Astomatous cuticular water loss was estimated via gravimetric measurements of detached leaflets. Sampling and rehydration procedures were as described above. Following rehydration, leaflets were sealed on the abaxial surface by coating with silicone vacuum grease to which a polyethylene sheet was then attached (Fanourakis et al. 2013a). The sealed leaflets were further left to desiccate from the adaxial (upper) cuticle (stomata free) at 21°C, 50% RH, and darkness. Darkness was employed to prevent leaf heating as light *per se* has no direct effect on cuticular water loss (Boyer et al. 1997). The rate of water loss over time was gravimetrically recorded for 84 hours. Preliminary work showed that leaves sealed from both sides exhibited negligible weight loss (below the detection limit), which confirmed the effectiveness of the sealing method. At the end of the measurement, leaflet area and dry weight were determined. Leaflet RWC was also calculated. Eight leaflets were assessed per treatment.

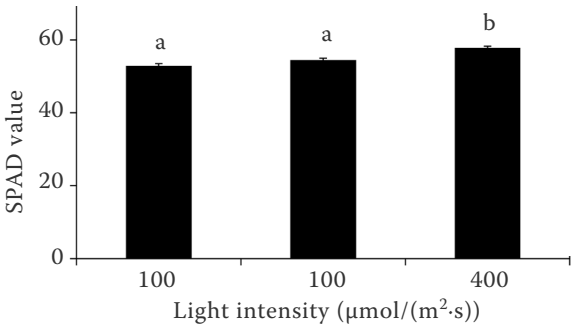

Fig. 1. Effect of light intensity during cultivation on chlorophyll index (SPAD value) of *Rosa hybrida* L. Data refer to 30 replications (± SEM (standard error of mean)). Different letters indicate significant differences based on Tukey's Honest Significant difference at $P < 0.05$

Data analysis. Data were subjected to two-way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows, Version 24.0. (IBM Corp., Armonk, USA). Variance homogeneity was tested using Levene's test and data was log-transformed if necessary. Tukey's multiple range tests were used to identify significant differences between mean values at the 5% significance level.

RESULTS

Leaf morphological components

Doubling light intensity during plant growth resulted in an increase in plant above-ground biomass by at least 57% (Table 1). More plant mass was allocated to the leaves in low (100 μmol/(m^2·s)) light (i.e. higher LMR) in comparison to moderate (200 μmol/(m^2·s)) and high (400 μmol/(m^2·s)) light intensity (Table 1). Leaves were thinner (i.e. higher SLA) at low and moderate light intensity in comparison to plants grown under high light intensity (Table 1). The highest light intensity (400 μmol/(m^2·s)) positively affected leaf chlorophyll content (Fig. 1) and led to greener leaves.

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Table 1. Effect of light intensity during cultivation on biomass accumulation and biomass allocation of *Rosa hybrida* L. Data refer to six replications. Standard error of mean is indicated in brackets

Light intensity (μmol/(m^2·s))	Leaf area (cm^2)	Leaf mass (g)	Stem mass (g)	Bud mass (g)	Above ground biomass (g)	SLA (cm^2/g)	LMR
100	893^a (51)	3.4^a (0.9)	1.1^a (0.1)	0.7^a (0.1)	5.2^a (0.3)	262.2^b (4.4)	0.66^b (0.03)
200	1,415^b (104)	5.5^b (0.5)	2.3^b (0.3)	1.7^b (0.2)	9.6^b (0.6)	258.7^b (8.5)	0.57^a (0.01)
400	1,716^b (141)	8.2^c (0.3)	3.8^c (0.3)	3.1^c (0.3)	15.1^c (0.5)	209.9^a (16.0)	0.54^a (0.02)
P-value	0.000	0.000	0.000	0.000	0.000	0.006	0.001

means followed by different letters indicate significant differences based on Tukey's Honest Significant difference at $P < 0.05$ (comparisons per column); SLA – specific leaf area (leaf area/leaf mass); LMA – leaf mass ratio; LMR – leaf mass ratio (leaf mass/plant mass)

Plant water loss

Water loss per plant was greatly enhanced by an increase in light intensity during plant growth (Suppl. Fig. S1). However, no significant effect of growth light intensity was noted in plant water loss per leaf area basis (Fig. 2).

Photosynthesis light and CO_2 response curves

Higher light intensity under growth environment caused an increase in A_n, when light intensity exceeded 400 μmol/(m^2·s) at ambient CO_2 concentration (Fig. 3a). No difference in CO_2 response curves was noted between 100 and 200 μmol/(m^2·s) light intensities during cultivation (Fig. 3b). A further increase in growth light intensity to 400 μmol/(m^2·s) resulted in higher A_n when C_i exceeded 360 μmol/mol under strong illumination (1,500 μmol μmol/(m^2·s); Fig. 3b).

Stomatal size, density and pore dimensions

Stomatal density was not affected by light intensity during leaf expansion (Table 2). Stomata were generally larger and had enhanced pore areas when light intensity increased during growth (Table 2). Pore area per leaf (i.e. cross-sectional area available for gas exchange) also increased by higher growth light intensities (Table 2).

Stomatal responsiveness to dessication

Transpiration rate declined upon leaf dessication (i.e. RWC decreased) in all cases (Fig. 4). Leaves expanded at 200 μmol/(m^2·s) showed higher transpiration rates compared to leaves developed at 100 μmol/(m^2·s) when RWC was higher than 50%. Contrary to this the leaves detached from plants grown at 400 μmol/(m^2·s) had lower transpiration rates compared to leaves developed at 100 μmol/(m^2·s) throughout the

Table 2. Effect of light intensity during cultivation on stomatal and pore anatomical features of *Rosa hybrida* L. Values are the means of 10 leaflets. Per replicate leaflet, five fields of view (stomatal density) and 25 stomata (stomatal and pore anatomy) were analysed (SEM (standard error of mean) is indicated in brackets). Measurements took place 2 h following the onset of the light period

Light intensity (μmol/(m^2·s))	Stomatal					Pore			
	density (mm^{-2})	length (μm)	width (μm)	size (μm^2)	length to width[1]	length (μm)	aperture (μm)	area (μm^2)	area/ leaf area (μm^2/mm^2)
100	78.8^{ab} (2.1)	13.9^a (0.1)	10.7^a (0.2)	149^a (4)	1.31^a (0.02)	9.9^a (0.2)	5.1^a (0.2)	40.1^a (1.8)	3,158^a (160)
200	73.9^a (2.3)	15.1^b (0.2)	11.5^{ab} (0.3)	174^b (6)	1.32^a (0.02)	11.3^b (0.2)	6.3^b (0.2)	56.5^b (2.3)	4,129^b (162)
400	81.8^b (2.5)	15.7^c (0.1)	11.8^b (0.2)	186^b (5)	1.33^a (0.02)	11.6^b (0.2)	6.7^b (0.2)	61.6^b (2.5)	4,998^c (164)
P-value	0.053	0.000	0.008	0.000	0.727	0.000	0.000	0.000	0.000

[1]calculated as an indication of shape; means followed by different letters indicate significant differences based on Tukey's Honest Significant difference at $P < 0.05$ (comparisons per column)

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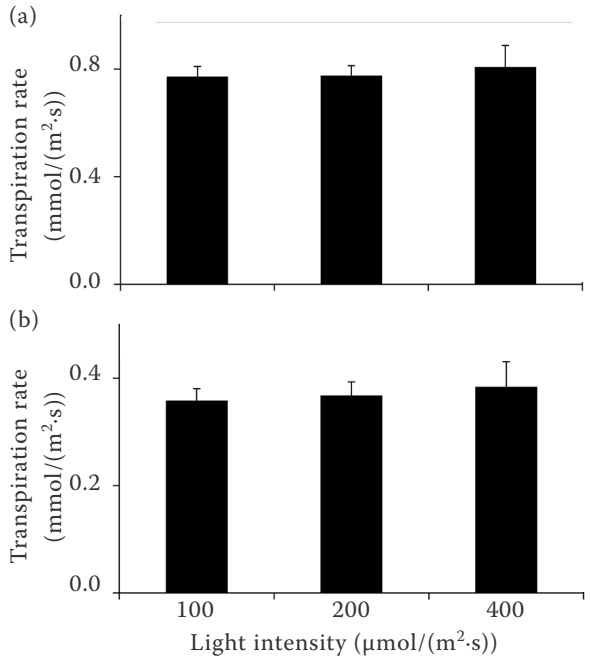

Fig. 2. Day-time (a) and night-time (b) plant transpiration rate (per leaf area basis) of *Rosa hybrida* L. grown at different light intensities. Measurements were conducted in the growth environment and using fully-grown intact plants (at least two flower buds with cylindrical shape and pointed tip) (n = 6). Error bars indicate standard error of mean. The difference in the y-axis scale should be noted. Transpiration rate per plant is provided in Fig. S1, and leaf area is given in Table 1

RWC range. Besides these differences in transpiration rate, the leaf RWC at which transpiration stabilized also varied between treatments. The RWC of stabilization was the highest at 400 μmol/(m^2·s) growth light intensity and the lowest at 200 μmol/(m^2·s). Less severe leaf drying was needed to close the stomata when leaves expanded at 400 μmol/(m^2·s) in comparison to leaves that developed at low (100 μmol/(m^2·s)) or moderate (200 μmol/(m^2·s)) light intensities.

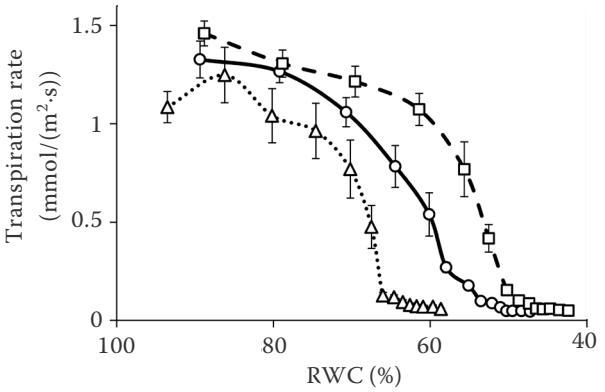

Fig. 4. Transpiration as a function of relative water content (RWC) during leaflet desiccation in *Rosa hybrida* L. grown at different light intensities (100 (circles, continuous line), 200 (squares, dashed line), 400 (triangles, dotted line) μmol/(m^2·s)). Leaflets were left to desiccate for 4 hours. Values are the mean of 7 leaves (± standard error of mean)

Cuticular transpiration rate

Cuticular water loss decreased following leaf dessication (Fig. 5). Leaves that expanded at a light intensity of 200 μmol/(m^2·s) or more had significantly lower cuticular water loss compared to leaves that were developed at 100 μmol/(m^2·s).

At the noted RWC range (≥ 80%), transpiration rate of unsealed leaves (thus including stomata;

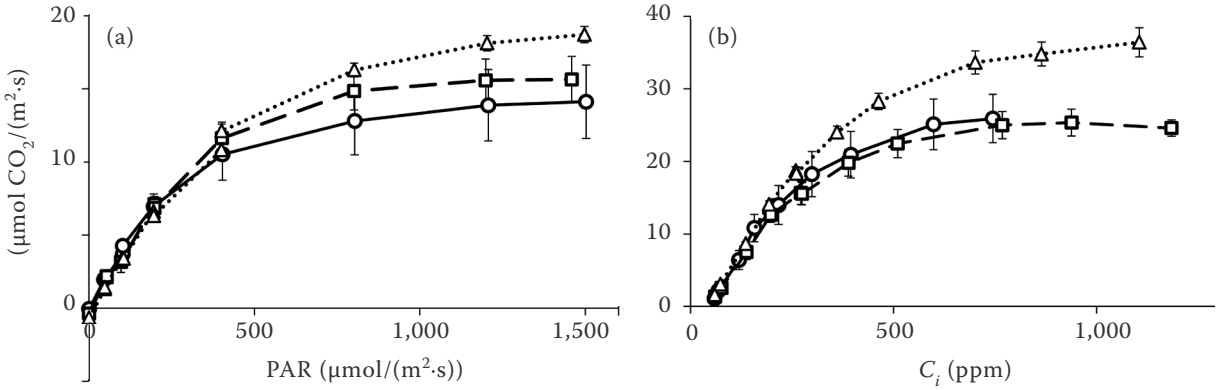

Fig. 3. Net CO_2 assimilation rate in response to photosynthetic active radiation (PAR) (a) as well as in response to internal CO_2 concentration (C_i) (b) of *Rosa hybrida* L. grown at different light intensities (100 (circle, continuous line), 200 (square, dashed line), 400 (triangle, dotted line) μmol/(m^2·s)). Measurements were conducted in attached leaves of intact plants (n = 6). Error bars indicate SEM (standard error of mean). The difference in the y-axis scale should be noted

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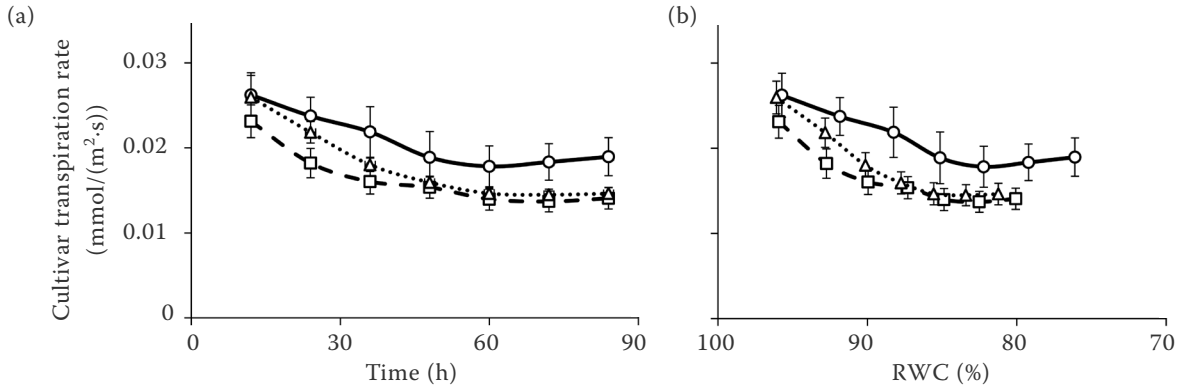

Fig. 5. Cuticular transpiration as a function of time (A) and relative water content (RWC, B) during leaflet desiccation in *Rosa hybrida* L. grown at different light intensities (100 (circle, continuous line), 200 (square, dashed line), 400 (triangle, dotted line) μmol/(m^2·s)). Leaflets were left to desiccate for 84 hours. Values are the mean of 8 leaves (± standard error of mean)

Fig. 4) was around 1.00 mmol/(m^2·s) while the respective rate of sealed ones (thus excluding stomata; Fig. 5) was less than 0.03 mmol/(m$^{/2}$·s). In other words, a 30-min water loss through the stomatal pores was equivalent to 84 h of desiccation through the cuticle.

DISCUSSION

Supplementary light is often applied to ornamental crops to promote both productivity and external quality of cut flowers and potted plants under protected cultivation (Fanourakis et al. 2013b). Despite wide application, it has not been previously assessed whether or not the light level during cultivation affects the regulation of water loss which is a factor of outmost importance for a long keeping quality (Fanourakis et al. 2012, 2015b; Carvalho et al. 2016). This is the first study, where we explored the role of light levels in mediating differences in leaf water loss pathways.

Higher growth light intensity strongly stimulates plant biomass

An increase in light intensity during growth led to a considerable enhancement of plant biomass (Table 1). The applied light levels (100 to 400 μmol/(m^2·s) PPFD) were thus well below the light saturation point of the crop. Given these effects on growth, increasing the light level by means of supplementary lighting is critical to enhance productivity in protected cultivation during periods of low light availability (i.e. autumn and winter; Heuvelink et al. 2006; Ieperen 2012).

More plant mass was partiotioned to the leaves at low (100 μmol/(m^2·s)) light intensity environment (i.e. higher LMR) in comparison to moderate (200 μmol/(m^2·s)) or high (400 μmol/(m^2·s)) light intensity growth regimes (Table 1). Therefore plants allocated more biomass to light-capturing tissue to maximize carbon gain in the low light environment whereas this allocation was shifted to organs related to the acquisition of other resources (e.g. water and nutrients) in the moderate and high light environments. Adjustment of leaf morphology (SLA variation) by adjusting their surface of light capture also appeared to be an important component of adaptation to light availability in rose. Leaves were thinner at low and moderate light intensity environments (i.e. higher SLA) as compared to the high (400 μmol/(m^2·s)) light intensity growth condition (Table 1). An increase in SLA enhances the amount of light interception which can maximize carbon gain. This morphological adaptation is typical at low light environments and has been documented in a wide range of species (Poorter et al. 2010).

Photosynthesis is driven more efficiently in high light intensity-expanded leaves

Light response curves revealed that high light intensity (400 μmol/(m^2 s)) during growth period significantly enhanced the rate of photosynthesis under ambient CO_2 concentration (400 μmol/(m^2·s); Fig. 3a). The A_n/C_i curves indicated that the highest growth light intensity increased A_n when C_i was high-

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er than 360 μmol/mol (Fig. 3b). These results demonstrated that leaves that developed under high light conditions had improved capacity for driving photosynthesis. This improvement has been attributed to differences in the amounts of ribulose bisphosphate carboxylase/oxygenase, cytochromes, and photosystem I and II core complexes (Yano, Terashima 2004). Additionally, leaves expanded under high light environments have thicker laminae, thicker palisade tissue, and larger cumulative mesophyll surface area per unit leaf area than leaves developed at low light environments (Yano, Terashima 2004; Terashima et al. 2005). The larger mesophyll surface area of these leaves also decreased the resistance to CO_2 diffusion from the intercellular spaces to the chloroplast stroma (Yano, Terashima 2004).

Light intensity promotes both stomatal size and pore dimensions

The pore area per leaf area was greatly enhanced as growth light intensity increased (Table 2). This increase was driven by individual pore area (per stoma), since stomatal density was not significantly affected by light intensity during cultivation (Table 2). The increase in the individual pore area (per stoma) was at least partly driven by the larger stomatal size (Table 2) because larger stomata have bigger pores (Fanourakis et al. 2014). An increase in stomatal size with increasing irradiance has also been previously noted in other species (James, Bell 2000). The larger stomata in high light intensity provide both higher CO_2 intake and enhanced leaf cooling through transpirational water loss. Therefore, leaves expanded at a higher light intensity are advantageous in driving gas exchange when water supply is not limiting.

Stomata formed at high light intensity are more responsive to desiccation

Increasing light intensity during growth from 100 to 200 μmol/(m^2·s) PPFD led to small though significant attenuation of stomatal closing ability (Fig. 4). However, a higher light intensity of 400 μmol/(m^2·s) PPFD resulted in considerable improvement (Fig. 4). Leaves expanded under the highest light intensity (400 μmol/(m^2·s)) were able to decrease transpiration at a much smaller RWC loss (Fig. 4). Thus leaves grown under high light intensity also hold the advantage under water limting conditions where water conservation takes priority over carbon gain and stomata need to close fast to manage the available resources prior to the onset of damaging water potentials.

Leaves expanded at moderate or high light intensity lose less water through the epidermis

Stomata are rarely completely closed (i.e. sealed) and continuously leak, thus contributing to total water loss (Kerstiens 1996). Cuticular transpiration also plays an important role in water loss (Boyer et al. 1997).

Higher light level beyond 100 μmol/(m^2·s) PPFD led to a significant decrease in cuticular transpiration (Fig. 5). Therefore, light intensity not only regulated the stomatal transpiration (Fig. 4), but also controlled the cuticular water loss (Fig. 5). However, an uncoupling between these two processes was evident. High growth light intensities (> 200 μmol/(m^2·s)) were required to promote stomatal closing ability while a moderate growth light intensity (> 100 μmol/(m^2·s)) was sufficient to enhance the control of water loss through the cuticle. Decreased cuticular water loss under high light intensity is probably related to increased cuticular thickness under high light intensities (James, Bell 2000).

The cuticular water loss declined during dehydration, which indicates that the diffusion resistance of the cuticle depends on the condition of the underlying cells (Fig. 5). Similar observations have also been previously documented in other species (Boyer et al. 1997).

The cuticular contribution was a small fraction of the total water loss in *Rosa hybrida* L. (Fig. 4 vs. Fig. 5) and this fraction increased following leaf dessication. During leaf dessication residual stomatal transpiration decreased more as compared to cuticular water loss (Fig. 4 vs. Fig. 5). This indicates that gas exchange through the cuticle becomes increasingly important under (stress) conditions promoting stomatal closure.

CONCLUSION

The stomatal and cuticular water loss pathways were assessed in *Rosa hybrida* L. cultivated at 100, 200 or 400 μmol/(m^2·s) PPFD. An increase in light intensity led to a considerable enchancement in plant biomass. Photosynthetis rate was higher in plants grown at 400 μmol/(m^2·s) light level. The effects of growth light intensity on plant transpiration (per leaf area) were trivial. Increasing growth PPFD generally led to

https://doi.org/10.17221/144/2017-HORTSCI

larger stomata with bigger pores. High light intensity (> 200 μmol/(m^2·s)) led to more responsive stomata while moderate light (> 100 μmol μmol/(m^2·s)) was sufficient to decrease the cuticular permeability. Residual stomatal transpiration was a much larger fraction of the total leaf water loss in compariosn to cuticular contribution. Leaves expanded at high light (> 200 μmol/(m^2·s)) are more efficient in regulating water loss via both stomata and cuticle.

Acknowledgments

We would like to thank Helle K. Sørensen and Ruth Nielsen for their help in conducting the measurements. The valuable comments of two anonymous reviewers are greatly acknowledged.

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Received for publication August 18, 2017
Accepted after corrections August 17, 2018