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Physiological responses of garden roses to hot and humid conditions

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Abstract: Garden roses do not grow well under hot and humid conditions. The objective of this study was to investigate the physiological responses of 'Marie Curie' and 'Lapjau' to high temperatures and relative humidity. The study included temperatures of 25/18°C (day/night) and 35/28°C (day/night), and relative humidity of 70% and 100%. 'Marie Curie' was more tolerant to heat stress than 'Lapjau' based on relative electrolyte leakage (REL), malondialdehyde (MDA), and activities of superoxide dismutase (SOD). The heat tolerance of cultivars also was confirmed by the levels of chlorophyll content and the net photosynthesis rate. Both cultivars were more stressed under more water vapour deficit than saturated vapour at 35/28°C (day/night), while at 25/18°C (day/night) the cultivars were more stressed under saturated humidity condition than at 70% relative humidity. In conclusion, combined hot and saturated humidity does not necessarily result in increased stress over separated heat or humidity elevations to the garden roses. Rose growers can use this information in regions where hot and humid conditions concur.

Keywords: abiotic stress; heat; Rosa; water vapour

Roses are very popular garden plants worldwide. However, poor adaptation under conditions of temperature and humidity extremes has been a practical problem in garden rose application (Manners 1999; Mackay et al. 2008). Besides of lowering the flower quality and growth rate, high temperature and high partial vapour pressure favour *Botrytis* flower blight, black spot, and powdery mildew diseases on roses (Marois et al. 1988; Wenefrida et al. 1993; Host 1995; Hagan et al. 2005). Ongoing breeding efforts to improve the adaptability to such environmental conditions shall provide the most economical solution to the problems in the future (Byrne et al. 2010; Liang et al. 2017). Meanwhile, adaptation of garden roses in a hot and humid region relies heavily on selective use of the best suited cultivars and modifications of management practices, such as pruning, fertilization and irrigation (Manners 1999).

High temperature conditions adversely affect the growth and development of roses. Cut roses produce flowers of less dry weight and reduced concentration of anthocyanin under heat in greenhouses (Shin et al. 2001; Dela et al. 2003). Field grown roses showed increased flower abortion and reduced flower sizes (Greyvenstein et al. 2012) as well as decreased growth and flowering of the bushes (Nadeem et al. 2011) under hot climate as compared to more favourable growing conditions. Greyvenstein et al. (2014) also reported that garden rose bud stage is important to differentiate cultivar sensitivity to heat stress under controlled conditions. Heat conditions also favour certain insect pests as shown by the strong positive correlation between ambient temperature and thrips population on leaves and flowers of roses (Kumar et al. 2006).

Performance of roses under humid conditions has mostly been investigated in greenhouses with stomata ontogenesis as the primary objective. Grown under saturated vapour pressure, roses showed increased number of stomata and wider stomatal apertures (Torre et al. 2003). Elevated relative

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humidity combined with continuous lighting also caused rose stomata failure to close post-harvest or in dark conditions (Pettersen et al. 2007).

Research on the effects of high relative humidity combined with high temperatures have focused mainly on cut flower production instead of landscape cultivars. Therefore, understanding the influence of cultural practices on plant health and the physiological responses under hot and humid stresses for garden roses shall provide information regarding the selection of site-specific management practices. Research has shown that from the ecological point of view, different woody plants have different adaptation strategies with respect to water use efficiency, assimilation rate, and other morphological and physiological traits (Tomlinson et al. 2013).

There are over 200 rose cultivars in the collection of Shenzhen Rose Center (22.708688 N, 114.253768 E), which is an associate member of the World Federation of Rose Societies. The most recent introduction of 72 cultivars was from France (Wang et al. 2013). The annual average temperature is 22.4°C with an average mean minimum of 11.7°C and an average mean maximum of 32.2°C. Annual precipitation is 1948.4 mm with 80% in March to October. Many cultivars stop growing during July to October. The stresses also have negative impact on the growth and flower quality after the plants come out of the stress period. A preliminary study (Luo et al. 2013; Wang et al. 2013) based on flower quality and vegetative growth revealed that with regular fungicide application, 'Hi-Ohgi', 'Ice Berg', 'Blue Ribbon', 'Perfume Delight', 'Double Delight', 'Perfume Yellow', and 'Chicago Peace' were ranked as adaptive among old cultivars. The newly introduced 72 cultivars were classified into nine groups conforming to the traditional rose classification (American Rose Society 1995), among which 'Marie Curie' (registration name MEIlomit) and 'ORAgofe' were the most adaptive, while 'Lapjau' was one of the susceptible cultivars (Luo et al. 2013; Wang et al. 2013).

The objective of this study was to further investigate the physiological responses to high temperatures and high relative humidity using some of the previously evaluated garden roses that showed different levels of adaptation in Shenzhen.

MATERIALS AND METHODS

Material establishment. Two rose cultivars, 'Marie Curie' and 'Lapjau', were propagated by cuttings. Eight weeks after propagation, plants with a healthy growth vigour and root system were transferred to pots (20 cm diameter and 20 cm deep) that were filled with a media of sand and peat mixture in 1 : 1 (v/v) ratio. The plants were grown in a greenhouse with temperatures about 25/15°C (day/night), relative humidity around 70%, and a 12-h photoperiod at natural sunlight.

Treatment and experimental design. After the generation of at least one new fully expanded leaf, the plants were transferred to growth chambers (BIC-300, Boxun Industry Cooperation Ltd., Shanghai) for temperature and humidity treatments. Temperature treatment levels were 25/18°C (day/night) and 35/28°C (day/night). Relative humidity treatment levels were 70% and 100%. Four growth chambers were assigned to the four temperature and relative humidity factorial combinations all set to have a 12-h light photoperiod with light intensity of 30,000 lx from LED light at 430–500 nm and 600–680 nm. Each chamber contained 12 plants placed on three shelves within the chamber, each shelf had 2 plants of each cultivar. Three runs were conducted to constitute three replications. Plants were watered every other day with water and weekly with half-strength Hoagland solution to pot capacity.

Sampling and measurement of physiological parameters. The plants were sampled at 0, 1, 2, 3, and 4 weeks after the start of treatments (WAT) from the oldest leaflets of the mature leaves.

For the measurement of relative electrolyte leakage (REL), leaves were cut into 1 cm^2 pieces, and 100 mg of the samples were then placed in test tubes containing 15 ml deionized water. Initial electric conductivity ($EC1$) was measured after the test tubes were shaken for 24 h on a gyratory bench shaker at 200 rpm. Thereafter, the test tubes with samples were placed in a boiling water bath for 10 min and cooled down to room temperature before another electric conductivity ($EC2$) was measured. The REL of samples was calculated as:

$$REL(\%) = \frac{EC1}{EC2}$$

For chlorophyll (Chl) content measurement, about 250 mg of fresh leaf samples from each plant was homogenized in 80% acetone with a mortar and pestle, and rinsed to a final volume of 25 ml in flasks. After extraction for 24 h in dark, the absorbance was measured at a wavelength of 645 nm, and 663 nm using a 2802S spectrophotometer (Unico,

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New Jersey, USA). The content of Chl (mg/g) was calculated based on the equations developed by Hiscox and Israelstam (1979).

Malondialdehyde (MDA) content in the leaf samples was measured using the thiobarbituric acid (TBA) reaction following the method describe by Dhindsa et al. (1981). The 1-g leaf samples were ground with liquid nitrogen before adding 10 ml of 5% trichloroacetic acid. After homogenization, the mixture was centrifuged at 3,000 g_n for 10 minutes. Aliquot of 2 ml supernatant was transferred to a new centrifuge tube and mixed with an equal volume of 0.67% TBA. The mixture was incubated in a water bath at 100°C for 30 min before the reaction was stopped in an ice bath. The light absorbance of the mixtures at the wavelengths of 450, 532 and 600 nm was read using a 2802S spectrophotometer. The MDA content was calculated using an extinction coefficient of 155 mM/l·cm (Heath, Packer 1968).

Tetrazolium method was used to measure activities of superoxide dismutase (SOD) (Chowdhury, Choudhuri 1985). Each 0.5-g leaf sample was ground thoroughly with an ice cold mortar and pestle in 50 mM potassium phosphate buffer (pH 7.8) containing 0.7% of $NaH_2PO_4 \cdot 2H_2O$ and 1.64% $Na_2HPO_4 \cdot 12H_2O$. The homogenate was centrifuged at 10,000 g_n for 15 min at 4°C. To the supernatant was reacted with the reaction solution under 4,000 lx fluorescent light for 15 min before measuring the absorbance at 560 nm wave length using a 2802S spectrophotometer. The reaction solution contained 0.05 M Na_2CO_3, 0.1 m*M* EDTA, 63 μM nitroblue tetrazolium, 13μ*M* methionine, 20 μL enzyme extract and 1.3 μM riboflavin (added last).

The net photosynthesis rate was measured using a LI-6400 Portable Photosynthesis System (Li-Cor, Nebraska, USA) under the same conditions where the plants were grown.

Data processing and statistical analysis. Data were subjected to ANOVA using the GLM procedure in SAS (SAS Institute, North Carolina, USA). Means were separated using Tukey's least significant difference when *F*-test was significant. Two-way and three-way interactions were presented graphically with standard deviations labelled on data points.

RESULTS AND DISCUSSION

There were differences in chlorophyll content between the two cultivars both before and after being subjected to hot and humid conditions (Table 1). 'Marie Curie' had higher chlorophyll content and greener genetic colour than 'Lapjau', which may translate to higher photosynthesis rate. The treatment effects between two temperature levels were significant but not between the two humidity levels. Hot temperature decreased chlorophyll content in the leaves starting at 1 WAT and continued to the end of the study. Two cultivars responded

Table 1. Chlorophyll content (mg/g) of two cultivars tested under combination of temperature, 25/18°C (day/night) and 35/28°C (day/night), and relative humidity (70% and 100%) using growth chambers

		0 WAT	1 WAT	2 WAT	3 WAT	4 WAT
Cultivar	Marie Curie	55.92	54.49	52.60	51.66	50.23
	Lapjau	45.93	45.46	42.16	39.83	36.36
Temperature (day/night)	25/18°C		51.57	50.31	49.97	49.57
	35/28°C		48.38	44.45	41.52	37.03
Relative humidity	70%		50.40	47.95	46.33	43.70
	100%		50.56	47.81	46.16	43.89
ANOVA						
Source of variation	*Df*			*Pr > F*		
Cultivar (C)	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Temperature (T)	1	n/a	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Humidity (H)	1	n/a	0.2202	< 0.4521	< 0.4261	0.3022
C × T	1	n/a	0.8352	< 0.0001	< 0.0001	< 0.0001
C × H	1	n/a	< 0.0001	0.0297	0.0120	0.0893
T × H	1	n/a	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C × T × H	1	n/a	0.8503	0.4692	0.5267	0.6248

WAT – week after treatment

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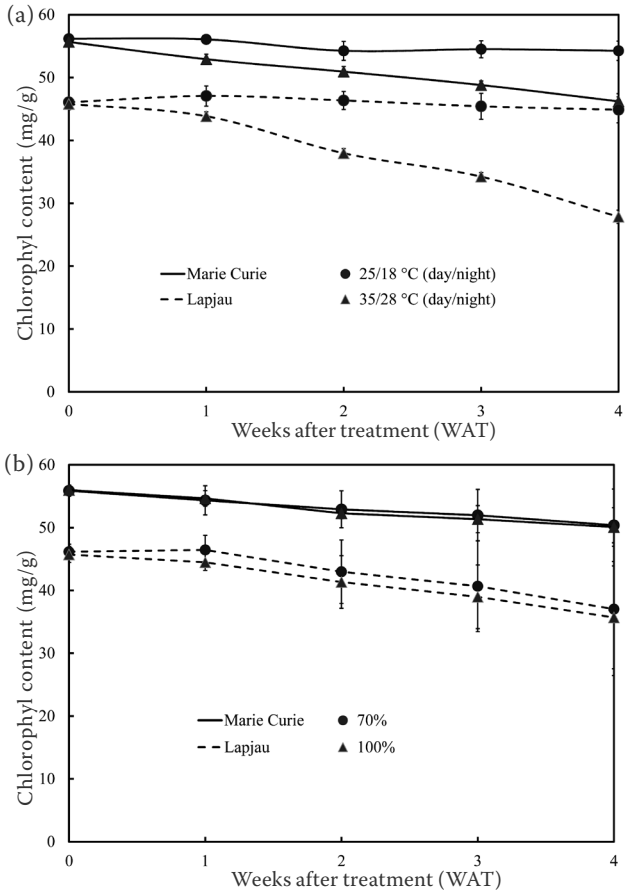

Fig. 1. Chlorophyll content of garden rose cultivars 'Marie Curie' and 'Lapjau' under (a) temperatures of 25/18°C (day/night) and 35/28°C (day/night), and (b) relative humidity of 70% and 100% grown in growth chambers

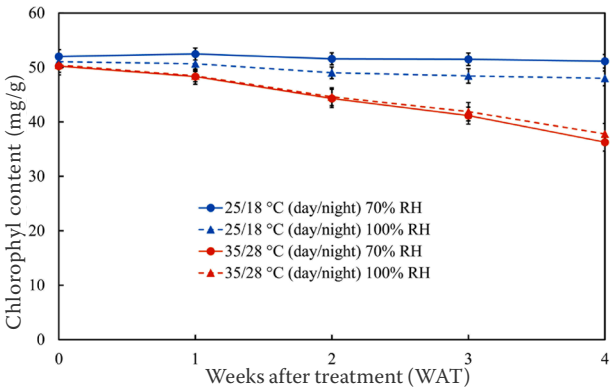

Fig. 2. Chlorophyll content of garden rose cultivars under temperatures of 25/18°C (day/night) and 35/28°C (day/night), and relative humidity of 70% and 100% grown in growth chambers

differently to both heat and humidity with 'Lapjau' more sensitive than 'Marie Curie', especially to heat (Fig. 1). Interactions between temperature and humidity were observed at all weekly measurements (Fig. 2). Specifically, under 25/18°C (day/night) saturated humidity reduced chlorophyll content compared to 70% relative humidity, while under 35/28°C (day/night) the saturated humidity treatment did not result in large changes in chlorophyll content compared to 70% relative humidity.

Malondialdehyde (MDA) is an indicator of the lipid oxidation in plants due to stresses and may be responsible for the cell membrane integrity. There

Table 2. Malondialdehyde content (μmol/g) of two cultivars tested under combination of temperature, 25/18°C (day/night) and 35/28°C (day/night), and relative humidity (70% and 100%) using growth chambers

		0 WAT	1 WAT	2 WAT	3 WAT	4 WAT
Cultivar	Marie Curie	2.68	3.16	4.65	6.13	6.71
	Lapjau	3.28	5.01	6.28	8.37	9.57
Temperature (day/night)	25/18°C		3.24	3.35	3.71	3.88
	35/28°C		4.94	7.58	10.79	12.40
Relative humidity	70%		4.19	5.73	7.26	8.11
	100%		4.00	5.20	6.54	7.42
ANOVA						
Source of variation	*Df*			*Pr > F*		
Cultivar (C)	1	0.0008	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Temperature (T)	1	n/a	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Humidity (H)	1	n/a	0.0208	0.0032	< 0.0041	< 0.0051
C × T	1	n/a	< 0.0001	< 0.0001	< 0.0001	< 0.0001
C × H	1	n/a	0.0484	0.0072	< 0.0001	< 0.0001
T × H	1	n/a	< 0.0001	< 0.0001	0.1223	< 0.0001
C × T × H	1	n/a	0.3403	0.3314	0.2234	0.0223

WAT – week after treatment

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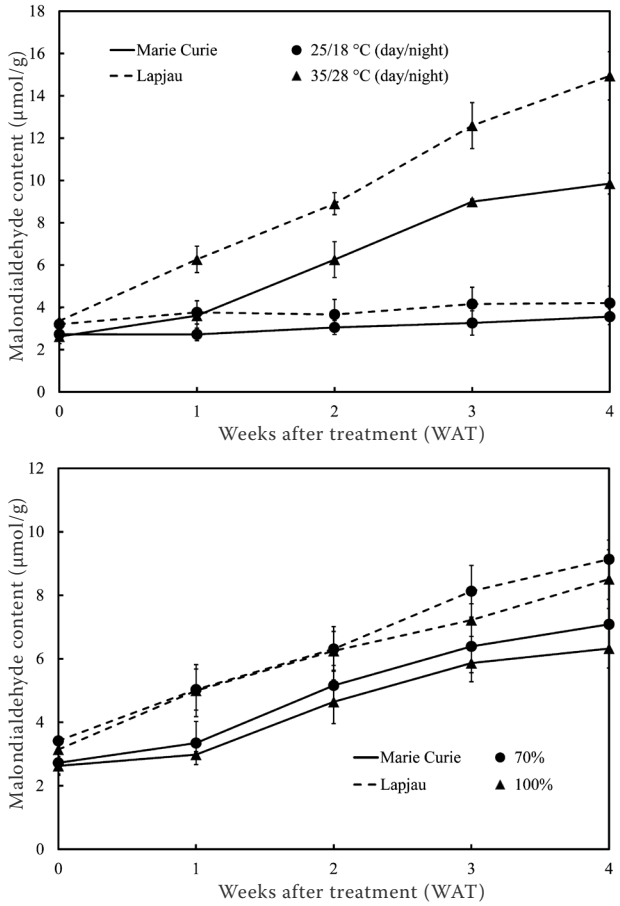

Fig. 3. Malondiadehyde content of garden rose cultivars 'Marie Curie' and 'Lapjau' under temperatures of 25/18°C (day/night) and 35/28°C (day/night), and relative humidity of 70% and 100% grown in growth chambers

were differences between two cultivars in MDA content before as well as after subjecting to hot and humid conditions (Table 2). Both cultivars were more stressed under 35/28°C (day/night) as compared to 25/18°C (day/night) (Table 2). However, the cultivars were less stressed under saturated relative humidity than at 70% (Table 2). Interaction between cultivar and temperature showed that 'Lapjau' was more sensitive to high temperature than 'Marie Curie' (Fig. 3). 'Marie Curie' was more responsive to water vapour deficiency than 'Lapjau' (Fig. 3). An interaction between temperature and humidity was detected. Both cultivars were more stressed under more water vapour deficit than saturated vapour at 35/28°C (day/night), while at 25/18°C (day/night) the cultivars were more stressed under saturated humidity condition than at 70% relative humidity (Fig. 4).

Relative electrolyte leakage (REL) is an indicator of cell membrane integrity and stress levels in plants. The results of REL in two cultivars showed a trend similar to the MDA data (Table 3). 'Lapjau' showed more leakage than 'Marie Curie'. High temperatures increased the REL levels at all weekly measurements (Table 3). Two cultivars showed different response to heat, 'Lapjau' was more stressed under heat than 'Marie Curie' (Fig. 5) at third and fourth week after treatment (Table 3). Under saturated humidity, the cultivars had lower REL as compared to the more deficit vapour condition at 70% (Table 3). There were no interactions between cultivar and humidity, or between temperature and humidity.

Table 3. Relative electrolyte leakage content (%) of two cultivars tested under combination of temperature, 25/18°C (day/night) and 35/28°C (day/night), and relative humidity (70% and 100%) using growth chambers

		0 WAT	1 WAT	2 WAT	3 WAT	4 WAT
Cultivar	Marie Curie	25.77	29.22	37.22	38.15	41.06
	Lapjau	29.68	34.06	39.50	45.70	43.91
Temperature (day/night)	25/18°C		28.37	31.62	31.69	31.94
	35/28°C		34.91	45.09	52.16	55.07
Relative humidity	70%		32.89	40.18	43.47	43.31
	100%		30.39	36.53	40.38	39.91
ANOVA						
Source of variation	*Df*			*Pr > F*		
Cultivar (C)	1	0.0010	< 0.0001	0.0173	< 0.0001	< 0.0001
Temperature (T)	1	n/a	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Humidity (H)	1	n/a	0.0021	0.0006	0.0031	0.0042
C × T	1	n/a	0.1297	0.0828	0.0129	0.0004
C × H	1	n/a	0.0601	0.6456	0.1754	0.2746
T × H	1	n/a	0.4368	0.7769	0.0847	0.4728
C × T × H	1	n/a	0.0849	0.6353	0.4561	0.7396

WAT – week after treatment

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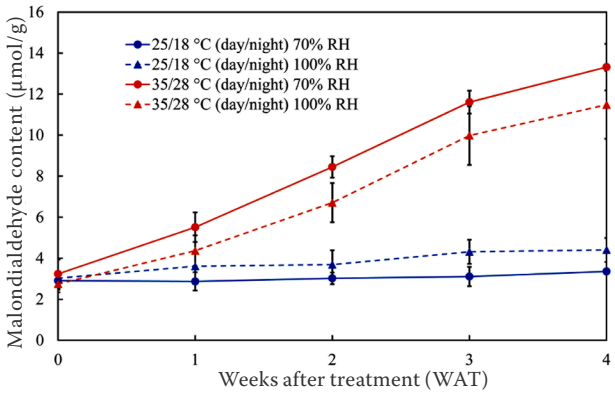

Fig. 4. Malondiadehyde content of garden rose cultivars under temperatures of 25/18°C and 35/28°C (both day/night), and relative humidity of 70% and 100% grown in growth chambers

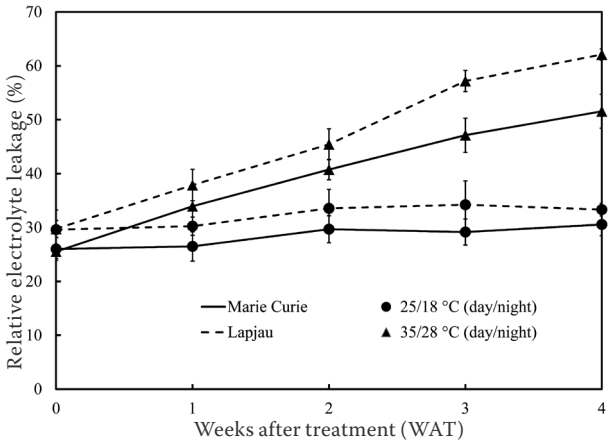

Fig. 5. Relative electrolyte leakage of garden rose cultivars 'Marie Curie' and 'Lapjau' under temperatures of 25/18°C and 35/28°C (both day/night) grown in growth chambers

Superoxide dismutase (SOD) activity may increase in plants under stress which often causes elevated levels of oxidants (Sahin et al. 2017). 'Marie Curie' showed increased levels of SOD content more than 'Lapjau' (Table 4). Both cultivars showed elevated SOD at 35/28°C (day/night) than at 25/18°C (day/night). There were no differences in SOD between two humidity levels.

Ultimately, the net photosynthesis rate was higher for 'Marie Curie' than 'Lapjau' before and after subjecting the plants to hot and humid conditions. The photosynthesis rate was lower when the cultivars were grown under 35/28°C (day/night) than under 25/18°C (day/night) (Table 5). Two relative humidity levels did not result in different photosynthesis rate in the two cultivars. Under elevated temperature conditions, 'Lapjau' showed more decrease in photosynthesis than 'Marie Curie' at one, three, and four weeks after treatment (Fig. 6).

In summary, two garden roses grown at 35/28°C (day/night) were more stressed than at 25/18°C (day/night) based on all the physiological parameters such as MDA content, REL, and SOD levels. The end results were decreased chlorophyll content and net photosynthesis rate. Saturated vapour did not change the chlorophyll and SOD content as com-

Table 4. SOD content (µg/g) of two cultivars tested under combination of temperature, 25/18°C (day/night) and 35/28°C (day/night), and relative humidity (70% and 100%) using growth chambers

		0 WAT	1 WAT	2 WAT	3 WAT	4 WAT
Cultivar	Marie Curie	12.58	12.17	13.37	13.37	11.61
	Lapjau	11.12	10.52	11.33	11.25	9.52
Temperature (day/night)	25/18°C		11.84	11.08	11.89	9.08
	35/28°C		11.86	11.61	12.72	11.39
Relative humidity	70%		11.59	12.43	12.41	11.10
	100%		11.19	12.27	12.21	10.86
ANOVA						
Source of variation	*Df*			*Pr > F*		
Cultivar (C)	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Temperature (T)	1	n/a	0.0070	< 0.0001	< 0.0001	< 0.0001
Humidity (H)	1	n/a	0.0602	0.1850	0.2309	0.8040
C × T	1	n/a	0.5751	0.3924	0.5422	< 0.0601
C × H	1	n/a	0.7126	0.0803	0.0540	0.0901
T × H	1	n/a	0.1802	0.1398	0.0573	0.0517
C × T × H	1	n/a	0.6216	0.2006	0.6468	0.3367

WAT – week after treatment

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Table 5. Net photosynthesis rate (μmol $CO_2/m^2 \cdot s$) of two cultivars tested under combination of temperature, 25/18°C (day/night) and 35/28°C (day/night), and relative humidity (70% and 100%) using growth chambers

		0 WAT	1 WAT	2 WAT	3 WAT	4 WAT
Cultivar	Marie Curie	2.27	2.23	2.03	1.98	1.69
	Lapjau	1.67	1.59	1.34	1.13	1.08
Temperature (day/night)	25/18°C		2.00	1.88	1.81	1.76
	35/28°C		1.82	1.49	1.30	0.99
Relative humidity	70%		1.94	1.69	1.55	1.51
	100%		1.88	1.68	1.56	1.46
ANOVA						
Source of variation	*Df*			*Pr > F*		
Cultivar (C)	1	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Temperature (T)	1	n/a	0.0371	< 0.0001	< 0.0001	< 0.0001
Humidity (H)	1	n/a	0.3921	0.4826	0.5402	0.1913
C × T	1	n/a	0.0476	0.6181	0.0002	0.0008
C × H	1	n/a	0.3700	0.7902	0.3875	0.4037
T × H	1	n/a	0.6001	0.6794	0.6534	0.6626
C × T × H	1	n/a	0.1760	0.9266	0.2012	0.4309

WAT – week after treatment

pared to 70% relative humidity. On the contrary, two cultivars showed increased MDA and REL under 70% relative humidity compared to saturated humidity. Therefore, saturated humidity did not necessarily result in more physiological stress for the two rose cultivars. Combined hot and saturated humidity may actually result in less stress than hot condition alone to the garden roses. Therefore, high relative humidity may be a favourable condition for diseases instead of as an abiotic stress. The heat stress may accelerate the disease incidence because of the weakening of plant health (Marois et al. 1988, Byrne et al. 2010). Combined heat and high relative humidity may contribute to physiological stress and disease pressure, respectively. Therefore, breeding for cultivars with better adaptation in hot and humid regions should be focused on tolerance to heat as an abiotic stress, and disease tolerance or resistance as a biotic stress. From management point of view, practice should be taken to increase the plant health and carbohydrate reservation prior to the onset of hot and humid conditions.

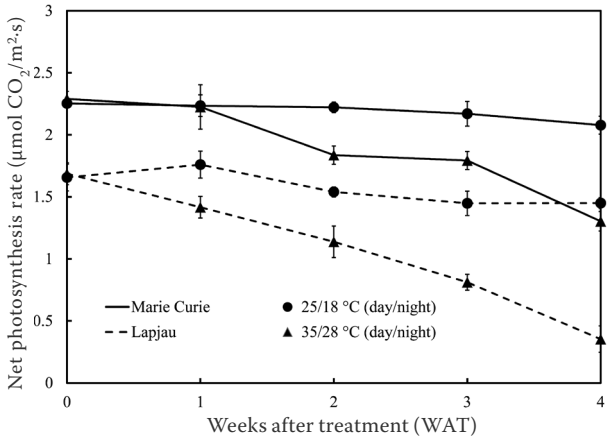

Fig. 6. Net photosynthesis rate of garden rose cultivars 'Marie Curie' and 'Lapjau' under temperatures of 25/18°C (day/night) and 35/28°C (day/night) grown in growth chambers

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