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# The influence of the gas exchange and transpiration of C<sub>3</sub> and CAM-facultative species on the green roof's cooling performance

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## Abstract

Crassulacean acid metabolism (CAM) allows succulent species to survive under drought by reducing the daytime water loss and gas exchange. We compared gas exchange of two CAM-facultative species (*Sedum lydium* Boiss. and *Sedum kamtschaticum* Fisch.) and three C<sub>3</sub> species of semi-xeric habitats (*Lotus corniculatus* L., *Bromus erectus* Huds. and *Salvia officinalis* L.) during a gradual reduction of substrate moisture content (SMC). The aim was to describe how the C<sub>3</sub> and the CAM-facultative species modify their gas exchange rates when the substrate dries out gradually, as well as to identify the critical value of SMC that stops the transpiration. Additionally, we tested the cooling capacity of specie in a week-long glasshouse experiment, in vegetated containers with commercial green roof substrate. The net CO<sub>2</sub> assimilation (A) rates of C<sub>3</sub> species were significantly higher than those of CAM-facultative species. When SMC fell below 12% v/v, *Sedum* species showed a rapid decline of A due to the likely switch from C<sub>3</sub> to CAM metabolism. However, the high rates of gas exchange of C<sub>3</sub> species caused a rapid decline of RWC over the course of the experiment and death of some leaves. The lower substrate temperatures under C<sub>3</sub> plants confirmed the positive effect of high gas exchange rates and transpiration on the cooling capacity. We conclude that the use of C<sub>3</sub> plants, with sustainable irrigation (to maintain at least, on average, 10% v/v of SMC), has a potential to provide greater cooling to a green roof than the use of *Sedum* species.

**Keywords:** green infrastructures (GI), cooling effect, wild plants, net assimilation rate (A)

## INTRODUCTION

*Sedum* species (family *Crassulaceae*) are a popular plant choice on green roofs thanks to their ability to survive drought by activating the facultative crassulacean acid metabolism (CAM) (Herrera, 2009). Facultative CAM plants reduce daytime gas exchange and transpiration under drought conditions, which aids survival but decreases the transpirational cooling performance of green roofs. Many C<sub>3</sub> wild plant species in the Mediterranean area, however, are adapted to grow under harsh conditions without the use of CAM metabolism and could thus represent an important resource for increasing the pool of species for green roofs (Caneva et al., 2015; Vestrella et al., 2015). In this paper we compared the net CO<sub>2</sub> assimilation (A) rates of two *Sedum* facultative CAM species (*Sedum lydium* Boiss. and *Sedum kamtschaticum* Fisch.) and three C<sub>3</sub> species (*Bromus erectus* Huds., *Lotus corniculatus* L., *Salvia officinalis* L.) during a gradual reduction of moisture content in the growing medium. The aim was to observe if the evapo-transpiration (estimated via net assimilation rate) of C<sub>3</sub> species is higher than *Sedum*. Also to establish a substrate moisture level at which *Sedum* species change their metabolism from C<sub>3</sub> to CAM and, consequently, deduce if C<sub>3</sub> species could have better cooling capacity than facultative CAM species under both well-watered and drought conditions.

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## MATERIALS AND METHODS

This study was conducted in the ventilated glasshouses within the School of Agriculture, Policy and Development, University of Reading (UK). Twelve boxes ((l) 43.5 cm × (w) 35.0 cm × (d) 9.0 cm) were prepared, two for each plant species tested and two with bare substrate (control). All boxes were filled with the same weight of green roof substrate (Shire Extensive Substrate, Shire Minerals, UK) and they were planted to achieve full ground coverage. All boxes were well watered until the field capacity (on average 29% v/v) 24 h before the start and then, left to dry until reaching critical values of substrate moisture content (under 5% v/v). During the experimental period (from June 14 to 21), the mean daytime air temperature ranged 23-30°C.

Net CO<sub>2</sub> assimilation rate (A) was measured daily, between 12 pm and 4 pm, using portable infrared gas analyzer (LCi Portable Photosynthesis System, ADC BioScientific Ltd., Hoddesdon, UK), on five leaves or springs per box. Except for *S. officinalis*, it was impossible to fit individual leaves into the analyser's cuvette, so springs containing several leaves (and covering the entire cuvette) were chosen and repeatedly measured. At the end of the experiment, the leaf areas of each spring were determined using a leaf area meter (WinDias 3, Leaf Image Analysis System, Delta-T Devices Ltd., Cambridge, UK) and assimilation outputs adjusted where necessary, to relate to the area of the cuvette (6.25 cm<sup>2</sup>). The substrate moisture content (SMC) of each box was monitored daily using SM200 probe (Delta-T Devices Ltd., Cambridge, UK). The substrate temperatures were monitored continuously using two thermistors (type T fine PTFE insulated twin twisted wires) positioned at 1-2 cm depth in the middle of each box and connected to the DL2e data logger (Delta-T Devices Ltd., Cambridge, UK); data were collected every 1 min and averaged over 30 min. The leaf temperatures were measured daily (concurrently with A measurements) for each box using Infrared Thermal Camera NEC TH7700 (NEC San-ei Instruments Ltd., Japan). Leaf area index (LAI) of each species was determined at the end of the experiment by dividing the plants' leaf area with the 20×20 cm area of the substrate, from which plants were collected. The relative water content (RWC) was determined according to Živčák (2008) at the end of experiment. Data were statistically analysed using GenStat (15<sup>th</sup> Edition, Lawes Agricultural Trust, Rothamsted Experimental Station, UK) to compare the species for their A and final RWC.

## RESULTS

### Net assimilation rate

The C3 species showed higher assimilation rates than the two *Sedum* species; all species showed a rapid decline of net A (Table 1) from day 5, as SMC ranged between 13% v/v (*S. officinalis*) and 9% v/v (*B. erectus*). The net A rate decreased faster for *B. erectus* and *L. corniculatus* than *Sedum* species and *S. officinalis*, particularly between days 5 and 6.

### Leaf temperatures

All studied species had leaf temperatures that were 1.6-8.8°C lower than surface temperatures of bare substrate (Figure 1A). Between the species, except on day 3 and on day 5, *B. erectus*, *L. corniculatus* and *S. officinalis* showed lower leaf temperatures than *Sedum* species for the whole duration of the experiment. On average, the leaf temperatures of C3 species were 2.06°C lower than the leaf temperatures of two *Sedum* species. Also during the decline of net A (from day 5) due to a decrease of SMC, all C3 species continued to show the coolest leaf temperatures, in particular *S. officinalis*.

Table 1. Mean net assimilation rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and the corresponding substrate moisture content (SMC) value for all studies species over the course of the experiment. Net assimilation data are mean of 10 measurements per species. The percentages of SMC (v/v) are mean of six measurement per treatment.

Net assimilation rate and SMC (v/v)	Day 1 14/6	Day 2 15/6	Day 3 16/6	Day 4 17/6	Day 5 18/6	Day 6 19/6	Day 7 20/6	Day 8 21/6
<i>B. erectus</i>	13.79	12.87	12.83	13.97	12.17	3.85	1.00	-
SMC	29%	20%	17%	14%	9%	4%	2%	-
<i>L. corniculatus</i>	22.76	19.68	22.53	24.14	23.62	11.40	9.32	-
SMC	26%	20%	16%	15%	11%	5%	4%	-
<i>S. officinalis</i>	17.90	15.99	17.97	18.07	17.63	16.36	10.31	4.75
SMC	25%	21%	18%	15%	13%	10%	3%	2%
<i>S. lydium</i>	7.71	8.52	9.11	10.41	7.60	5.50	2.60	0.38
SMC	26%	19%	17%	16%	12%	10%	5%	3%
<i>S. kamtschaticum</i>	7.13	5.80	6.69	7.14	5.29	2,73	0.78	0.21
SMC	28%	21%	19%	15%	12%	6%	4%	2%
Net assimilation least significant difference (LSD)	2.88	3.37	3.60	3.37	2.31	2.73	2.63	1.78

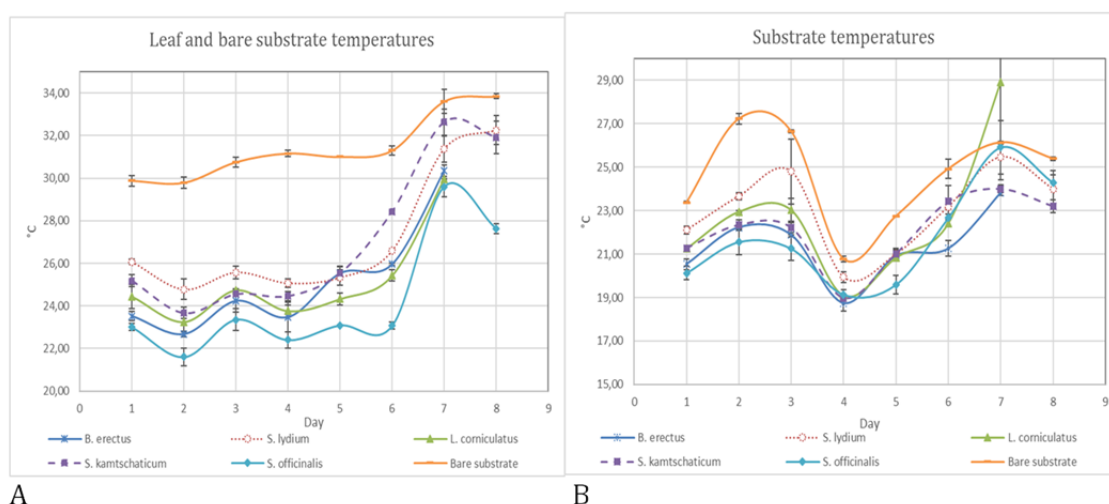


Figure 1. A) Leaf temperatures and bare substrate temperatures measured between 12 pm and 4 pm. Data are mean of 6 measurements per species and substrate; B) Mean substrate temperatures at 1 cm depth for all species and bare substrate. Data are means of two measurements per treatment.

### Substrate temperatures

As we expected, substrate temperatures (Figure 1B) were always cooler than leaf or surface temperatures, but we observed also that the substrate temperatures in all planted boxes with plants were lower than in the boxes without plants. In general, *L. corniculatus* had hottest substrate temperatures among the C3 species and *S. lydium* showed the hottest temperatures among all plant species considered. In particular, until the day 4 (under well-watered conditions), *S. officinalis*, *B. erectus* and *S. kamtschaticum* showed the lowest substrate temperatures (by 3.6°C lower than bare substrate on day 3 for example). From day 5, when SMC decreased under 13% v/v, all boxes increased their substrate temperatures, but the C3 species continued to show the coolest substrate temperatures or, in the worst cases, the same substrate temperatures of *Sedum* species (except for *L. corniculatus* on day 7). On day 6, for example, the C3 species and *Sedum* species showed, on average,

temperatures 2.8 and 1.6°C, respectively, lower than bare substrate.

On the other hands, *Sedum* species and *S. officinalis*, provided a small cooling effect for one more day than *L. corniculatus* and *B. erectus* for which the experiment ended one day earlier because of their precarious conditions due to water stress.

### **Final relative water content (RWC)**

The C3 species, especially *L. corniculatus* and *B. erectus*, showed a poor ability to maintain acceptable values of RWC until the end of the experiment; these two species reached critical values of RWC (40 and 46%, respectively, data not shown) by day 7. In fact, the leaves of some individuals of *L. corniculatus* and *B. erectus* began rolling already from day 6. *S. officinalis* showed, at the end of the experiment (day 8), the highest RWC values (72%) among the C3 species tested. However, also for this species, at the end of the experiment, a few leaves per plant were slightly wilted.

## **DISCUSSION**

### **Net assimilation rate, substrate moisture content and final RWC**

C3 species had higher net A (and thus likely, transpiration rates) than *Sedum*. In some cases, net assimilation values of *L. corniculatus* exceeded 3-fold those of *Sedum* (e.g., on day 5 when SMC was already below 12% v/v). There was a decline of the net assimilation when the substrate moisture reached 12% v/v in *Sedum* and from 11, 10 and 9% v/v in *L. corniculatus*, *S. officinalis* and *B. erectus*, respectively. CAM species are known to reduce the net assimilation and transpiration in the periods of water stress (Olivares et al., 1984; Borland and Griffiths, 1990). In addition, the results of our previous study (D'Arco et al., 2016) showed the malate accumulation during the dark period in the *S. lydium* and *S. kamtschaticum* leaves, at around 13-10% v/v of SMC. It is thus likely that the shift we are seeing in *Sedum* was due to the change from C3 to CAM metabolism. The final RWC values showed that the A decline in C3 species is followed by a rapid and significant drop in RWC. *Sedum* species demonstrated to have the capacity to maintain a high quantity of water in the leaves also under severe drought (>87% of RWC when the SMC values dropped below 6% v/v). *B. erectus* and *L. corniculatus* plants, which transpired more than *Sedum* species, reached at the end of experiment, values of SMC considered critical (Babu et al., 1999) and started to show the visible signs of water stress (rolling leaves and drying). *S. officinalis* is an exception; it transpired more than *Sedum*, but at the end of the experiment, the average RWC values were still higher than the critical limit and no part of plant material was lost.

We suggest that the use of C3 species (in particular *S. officinalis*) could increase significantly the cooling effect of the green roof through the transpiration at least until about 9% v/v of SMC.

### **Leaf and substrate temperatures**

Active gas exchange (CO<sub>2</sub> and water vapour) has an important role in the cooling effect; *B. erectus* and *S. officinalis* showed the lowest substrate and leaf temperatures and the highest A values until day 6 when the SMC dropped to 4% v/v and 10% v/v for these species, respectively. Other factors, including light leaf colour and presence of leaf hairs may also explain *Salvia*'s advantage (Vaz Monteiro et al., 2016). Despite high A, *L. corniculatus* showed, almost for the whole duration of the experiment, higher substrate temperatures than *S. kamtschaticum*. This may be explained by the lower LAI of *L. corniculatus* compared to *S. kamtschaticum* (almost four-fold). *S. kamtschaticum* could thus provide a positive contribution to the cooling effect by the shading effect of their leaves. Similarly, despite of lower gas exchange of *B. erectus* than *L. corniculatus*, the substrate of *B. erectus* was cooler and the LAI value was higher than *L. corniculatus*. The contribution of *B. erectus* to the cooling effect was likely the sum of a good transpiration activity, but also of shading. Otherwise, *S. officinalis* showed a lower LAI value (4.45) than *B. erectus* and *S. kamtschaticum*, but the substrate temperatures of boxes with *S. officinalis* were the coolest thanks to its high gas exchange activity.

## CONCLUSIONS

This study demonstrates that all C3 species tested showed greater gas exchange activity than *S. lydium* and *S. kamtschaticum* under well-watered conditions but, also, under drought (from about 10% v/v of SMC), when *Sedum* facultative CAM species begin to change their metabolism from C3 to CAM. The higher gas exchange capacity of C3 species reduces leaf temperatures and consequently increases the cooling effect of green roofs, particularly during the hot and dry weather. However, this study, also revealed that high LAI and ground coverage are required in addition to high transpiration activity, to maximise the green roof cooling effect.

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