

BRIEF COMMUNICATION

Crassulacean acid metabolism and survival of asexual propagules of *Sedum wrightii*

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Abstract

In *Sedum wrightii* grown in a growth chamber, detached leaves could survive for at least 120 d with a high rate of success for propagule formation. The pattern of gas exchange, associated with CAM, may be important in extending the period during which the detached leaf remains physiologically active. The added benefit for the developing propagule, still attached to the "parent" leaf, is an additional source of water and saccharide reserves over an extended period necessary for rooting. Drought survival of propagules may be determined by the amount of water-storing tissue in the detached leaf.

Additional key words: asexual reproduction; dry mass; malic acid; plantlet; stonecrop; water content.

Survival of seeds and seedlings in shallow soils of rock outcrops (Sharitz and McCormick 1973) and on the desert soil surface (Jordan and Nobel 1981) is low, resulting in low sexual reproductive success and limiting the establishment of many desert perennials to certain favorable years. The development of plantlets from leaves is an efficient mechanism of vegetative reproduction in many plant families. These vegetative plantlets may be more successful than seedlings because of several factors, including greater water and carbon reserves (Holthe and Szarek 1985).

Plants of *Sedum wrightii* produce numerous tiny seeds. In addition, the leaves fall from the stem with only slight pressure and take root from basal callous tissue, forming a new plant. Clausen (1975) casually noted that after 11 weeks, 82 % of detached leaves developed plantlets, each with a small rosette of leaves and well-developed roots.

In previous work, mature plants of *S. wrightii* under well-watered and drought-stressed conditions exhibited this "classic" CAM gas exchange pattern (Gurevitch *et al.* 1986, Kalisz and Teeri 1986, Gravatt and Martin 1992; for last list of CAM plants see Sayed 2001). Far less work has been done on seedlings or young propagules of

succulent species (Steenbergh and Lowe 1969, Jordan and Nobel 1981), which may be the most critical stage for establishment in the desert. The pattern of gas exchange, associated with CAM, may be important in extending the period during which the detached leaf remains physiologically active. The added benefit for the developing plantlet, still attached to the "parent" leaf, is an additional source of water and saccharide reserves over an extended period.

The purpose of this investigation was: (1) to determine the extent of vegetative propagule formation and survival using detached leaves of *S. wrightii*; (2) to monitor water content and CAM photosynthesis over time in order to document the effect of physiological activity in detached leaves on plantlet success.

S. wrightii is a leaf-succulent perennial occurring in the semi-deserts of the southwestern USA and Mexico. Plants were collected on the limestone cliffs overlooking the Devils Arm of the Amistad Reservoir, Val Verde County, Texas at an elevation of 360 m in August 2001. Plants were potted in soil sand : perlite : topsoil (3 : 3 : 3) using plastic pots (10×10 cm) and placed in a plant growth chamber. Environmental conditions were as follows: photosynthetic photon flux density (PPFD) of

Received 24 March 2003, accepted 17 September 2003.

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Acknowledgements: This research was partially supported by Stephen F. Austin State University, Research and Sponsored Programs. The author gratefully acknowledges the cooperation of the National Park Service, which administers the Amistad Reservoir, TX.

Table 1. Mean survival, water content, percent of leaves with propagules, tissue malic acid content of detached leaves, and fresh mass of propagules of *Sedum wrightii* during the 120 d growth chamber experiment. Means \pm SE of leaves placed on moist vermiculite, $n = 20$, except for tissue malate content where $n = 5$. Means with different letters are statistically different at $p \leq 0.05$. For the water content data, statistical significance of the difference between the means of each day and day 0 are indicated as follows: ns = not significantly different, ** and *** = significant at $p = 0.01$ and 0.001 levels, respectively, using Dunnett's multiple range test.

Day	Survival [%]	Water content [%]	Leaves with propagules [%]	Malic acid content [mol kg ⁻¹ (DM)]			Propagule mass [mg]
				Morning	Evening	Δ Malate	
0	100 \pm 0 ^a	98 \pm 0.7	0 ^a	2.320 \pm 0.058 ^a	0.330 \pm 0.176 ^a	2.519 \pm 0.120 ^a	0 ^a
5	100 \pm 0 ^a	98 \pm 0.6 ^{ns}	0 ^a	2.377 \pm 0.167 ^a	0.295 \pm 0.089 ^a	2.482 \pm 0.089 ^a	0 ^a
15	98 \pm 4.4 ^a	97 \pm 0.7 ^{ns}	26 \pm 1.4 ^b	2.359 \pm 0.102 ^a	0.305 \pm 0.103 ^a	2.294 \pm 0.127 ^b	0 ^a
30	87 \pm 4.8 ^{ab}	97 \pm 1.6 ^{ns}	63 \pm 3.4 ^{cd}	2.060 \pm 0.064 ^b	0.249 \pm 0.108 ^a	1.811 \pm 0.105 ^c	9.5 \pm 0.86 ^b
60	82 \pm 4.1 ^{bc}	96 \pm 1.8 ^{**}	79 \pm 3.9 ^d	1.627 \pm 0.184 ^c	0.242 \pm 0.130 ^a	1.385 \pm 0.097 ^c	24.9 \pm 1.82 ^c
90	76 \pm 4.3 ^c	93 \pm 1.6 ^{***}	76 \pm 4.3 ^d	1.555 \pm 0.098 ^c	0.240 \pm 0.194 ^a	1.315 \pm 0.129 ^c	35.2 \pm 1.97 ^d
120	78 \pm 4.1 ^c	89 \pm 1.5 ^{***}	89 \pm 4.2 ^d	1.145 \pm 0.154 ^d	0.233 \pm 0.185 ^a	0.912 \pm 0.107 ^d	54.9 \pm 2.64 ^e

900 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 25(\pm 1) °C air temperature, and 1.9(\pm 0.2) kPa vapor pressure deficit (vpd) during the day, and 20(\pm 1) °C air temperature and 1.5(\pm 0.2) kPa vpd at night. Environmental conditions were measured using an ADC (Hertfordshire, England) LCA4 CO₂ and H₂O analyzer. After transplanting, plants were kept well watered and fertilized biweekly with 0.5-strength *Peters Professional* fertilizer (*W.R. Grace and Co.*, Fogelsville, PA, USA) with trace elements, 15 % each of total N, K₂PO₅, and K₂O.

Plants were allowed to grow until they reached maturity, indicated by the presence of floral buds. Leaves were detached from whole plants ("parent leaves"), their fresh masses determined, and then they were randomly placed on moist vermiculite in plastic pots (10 \times 15 cm). Experimental units, pots, consisted of 20 leaves placed randomly on the surface of the moist vermiculite and sampled on days 0, 5, 15, 30, 60, 90, and 120, in a randomized complete block design with five replicates. The vermiculite was kept moist in the growth chamber for the duration of the experiment. Environmental conditions inside the chamber were the same as that described for the whole plants.

On each of the seven sampling days half of the leaves were removed from the pots on their respective sampling days just before lights-on. Later, just before lights-out, the remaining leaves for that sampling were removed. For each leaf sampled the condition of the "parent leaf" was recorded (alive or dead) and whether a propagule had formed at the base of the leaf. Leaf survival was determined visually by noting the color and the turgidity of the leaf. Following sampling, the leaves were weighed for fresh mass and immediately frozen at -65 °C until analysis.

Tissue malic acid content was determined according to Gutmann and Wahlefeld (1974) following extraction of leaf cell sap by centrifugation (Smith and Lüttge 1985). Leaf material was weighed prior to leaf sap extraction (FM) and after oven drying for 48 h at 65 °C (DM). Previous work indicated that malic acid accumulation

accounted for all fluctuations of organic acid in the leaves (Gravatt and Martin 1992). Individual leaves were too small to assay for malate separately. Therefore, all leaves from either the AM or PM sampling period, within a replicate, for each sampling day were pooled and analyzed as one sample.

The experimental design was a randomized complete block design with five replicates. Individual pots, containing 20 detached leaves, served as the experimental unit. Analysis of variance was used to test the differences between days for percent survival, leaf water content, weights, and tissue malic acid contents using *PC-SAS* statistical software (*SAS Institute*, Cary, NC, USA). Multiple comparison of means were conducted using Tukey-Kramer multiple range test between the sampling days for leaf survival, propagule number, and malate content, when the ANOVA indicated statistical significance ($p \leq 0.05$). Dunnett's multiple comparison, with leaves from day 0, was used to test leaf water content. Differences between means were considered to be significant when $p \leq 0.05$.

Detached *S. wrightii* leaves remained alive throughout the 120 d of the experiment (Table 1). A significant decline in survival was seen by day 60, but survival remained high at approximately 80 %. Detached leaves remained turgid and retained their characteristic yellow-green color throughout the experiment. Detached leaf water content did not show a significant decline until day 60 of the experiment (Table 1), after which the water content declined to 89 % by day 120.

Detached leaves of *S. wrightii* readily developed propagules at the base of the leaves. Each propagule developed 2 to 3 roots, with a small rosette of 3 to 5 leaves. Roots of propagules made contact with the soil surface at approximately day 45 to 50 of the experiment. Casual observations revealed that the orientation of the leaf with respect to the soil surface did not appear to have any relationship with the development of the propagule. Propagules began to appear as early as day 7, with 26 % of the detached leaves developing propagules by day 15

of the experiment (Table 1). Propagule fresh mass increased in a linear fashion throughout the experiment (Table 1). By the end of the experiment nearly 80 % (98 % of surviving leaves) of the detached leaves had developed propagules.

Nighttime malate accumulation did not significantly decline until days 30 of the experiment (Table 1) and further declined on days 60 and 90 to 52 % of day 0 content. By day 120, nighttime malate accumulation of detached leaves declined to 36 % of day 0 content. Tissue malate contents of detached leaves showed no significant change in PM content during the 120 d of the experiment (Table 1). However, malate content did significantly decline for AM values starting at day 30 and continuing to decline to day 120. Due to the small amount of tissue from the propagules on each sampling day, an insufficient amount of cell sap was collected. Therefore, propagule tissue malic acid contents were not possible.

CAM plants conserve water during periods of drought (Kluge and Ting 1978). Holthe and Szarek (1985) reported that detached cladodes of *Optunia* spp., placed outside on exposed soil, exhibited CAM activity for the 160 d period, with a predicted survival of the detached cladodes 2 or 3 months longer. In this study, four months (120 d) after detachment from the plant, 80 % of the leaves were still alive, with 98 % of those leaves developing propagules.

Propagule data from this study confirms the observations made by Clausen (1975) that in 7 weeks, 78 % of detached *S. wrightii* leaves had taken root. By 11 weeks, 82 % had developed into plantlets and taken root. Formation of propagules by detached leaves of *S. wrightii* may be an effective means of reproduction.

Throughout the 120-d experiment, detached leaves of *S. wrightii* remained visually alive. Leaf water content for day 0 was 98 % and remained high at 89 % on day 120. Malic acid contents are indicative of full-CAM gas exchange patterns (Osmond 1978) based on previous work

with *S. wrightii* (Gravatt and Martin 1992). This was found also in a study on *Marrubium frivaldszkyanum*, in which plant species the CAM pattern occurred during leaf ageing and was connected with malate accumulation (Markowska and Dimitrov 2001). In this study, leaves remained physiologically active throughout the 120 d of the experiment. Malic acid accumulation on day 0 [2.52 mol kg⁻¹(DM)] was consistent with those found for well-watered plants with attached leaves (Gravatt and Martin 1992). By day 120, malic acid accumulation had declined to approximately 36 % of those measured at the start of the experiment. Malic acid accumulation at day 120 indicates that carbon uptake, through the CAM pathway, was reduced by one-third. This indicates that the detached leaf remained physiologically active, and presumably could provide photosynthates necessary for new growth of the propagule.

In the field, *S. wrightii* matures and flowers from August to November (Clausen 1975). This coincides with the fall increase in precipitation characteristic of the area in which this species is found. Clausen (1975) suggested that moisture is important in breaking dormancy and stimulating growth and flowering. Late in the summer, field-grown plants have lost some of their turgidity; the leaves are more appressed against the stem and are not easily detached. However, within a couple of weeks of re-watering the leaves regain their turgidity and are easily detached with the slightest pressure. Greenhouse grown plants behave similarly to plants in nature (personal observation). Additional microscopic studies will be needed to determine if an abscission layer forms at the base of the leaf, leading to easy separation of the leaf from the stem. The timing of this phenomenon may insure that leaves that do not easily detach until moisture conditions are conducive for propagule formation, for example, the higher moisture content of the associated moss mats during the wetter periods of the growing season, from October through March.

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