UNIVERSITY OF CALIFORNIA
RIVERSIDE

Comparing Hydraulic Strategies Across Three Species of *Clusia* Differing in Expression of CAM Photosynthesis

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Evolution, Ecology and Organismal Biology

by

Jacob Edward Vogenberg

December 2015

Thesis Committee:
Dr. Louis Santiago, Chairperson
Dr. Michael Allen
Dr. Giles Waines

The Thesis of Jacob Edward Vogenberg is approved:

Committee Chairperson

University of California, Riverside

ABSTRACT OF THE THESIS

Comparing Hydraulic Strategies Across Three Species of *Clusia* Differing in Expression of CAM Photosynthesis

by

Jacob Edward Vogenberg

Master of Science, Graduate Program in Evolution, Ecology and Organismal Biology
University of California, Riverside, December 2015
Dr. Louis Santiago, Chairperson

Crassulacean acid metabolism (CAM), the night time fixation of CO_2 and daytime stomatal closure, may be linked to differences in hydraulic traits in the plants in which it is expressed. Three species of Panamanian *Clusia,* expressing differing levels of CAM strength (measured as a factor of their carbon isotope discrimination) were measured on variables of water relations. Leaves of the strong CAM species *Clusia rosea* showed a high modulus of elasticity (ε), high relative water content at turgor loss point (RWC_{TLP}), and a low absolute capacitance at full turgor (C_{FT*}), suggesting a strategy that retains water better than the intermediate CAM species *Clusia uvitana*, with a low ε, RWC_{TLP}, and a high C_{FT*}. With *C. rosea* expressing a high level of growth habit variability, and increased strength of CAM over *C. uvitana* in the same region, may

represent a development of different strategies allowing these two plants to persist in the same habitat – with CAM strength potentially contributing to an increase in ability to adapt to multiple growth habits. The C_3/facultative CAM *Clusia pratensis*, also persisting in the same geographic range, showed values intermediate of *C. rosea* and *C. uvitana*, and its persistence primarily as a tree, with limited CAM expression, may also be an adaptation to coexist in the same range as *C. rosea* and *C. uvitana*.

Contents

TABLES AND FIGURES

Introduction

Crassulacean Acid Metabolism (CAM) is a photosynthetic pathway whereby plants take up CO_2 at night, carboxylate it to malate, and store as malic acid in vacuoles. During the day the stomata are closed, the malic acid is decarboxylated, liberating the CO_2, which is then refixed by ribulose 1-5-bisphosphate carboxylase/oxygenase (RuBisCO) to enter the dark reactions of photosynthesis (Winter, Aranda & Holtum 2005, Ting 1985). Noted for their high water use efficiency, CAM plants are most frequently encountered in water limited habitats and have a d13C value typically higher than C_3 plants (Silvera et al. 2010, Ting 1985, Borland et al. 2011). Widely used in plant sciences, carbon isotope discrimination has been shown to be a powerful tool in estimating the proportional contribution of dark CO2 fixation to total carbon gain in CAM plants (Cernusak et al. 2013). Crassulacean acid metabolism exists over a spectrum, and the variability of CAM expression and induction has been a topic of debate for over a decade now (Borland et al. 2011, Silvera et al. 2010**,** Winter and Holtum 2007, Winter et al. 2008, 2009, 2011, Guralnick 2002). By examining the correlation between the degree of night time CO_2 uptake in a plant (as determined by carbon isotope discrimination δC^{13} (‰)) and various hydraulic parameters, the effect of the extent of night time CO_2 uptake on plant hydraulic properties can be determined and CAM specific hydraulic properties can be identified.

It is suspected that epiphytes, plants who grow on other plants, are particularly susceptible to changing climate conditions, and although most vascular epiphytes are well adapted to periodically low water availability, rising temperatures and increased light exposure may adversely affect epiphyte survival in the long term (Zotz and Bader 2009). As epiphytes are often prone to living in water-limited habitats, many epiphytes express CAM to some degree, and CAM has also been shown in many tropical hemi-epiphytes (plants who spend part of their life epiphytically) (Silvera et al. 2010, Holtum et al. 2004). Understanding the link between CAM and water relations is an integral part to predicting the response of vulnerable epiphytic and hemi-epiphytic species to changing climate conditions. It has been documented that species exhibiting night time CO_2 uptake have pronounced differences in hydraulic parameters; such as transpiration and water use efficiency (Winter et al. 2005). By creating models, analysing meta-data, and running large trait based analyses, important advances in ecological physiology have arisen that pin point large scale trends between different plant functional groups as well as biome types across plant groups (Santiago and Wright 2007, Swenson and Enquist 2007).

Examples of inducing a shift from C_3 photosynthesis to CAM has been well documented in species such as *Mesembranthymum crysallinum* (Winter and Holtum 2007), *Portulaca grandiflora* (Guralnick 2002) and *Portulaca oleraceae* (María et al. 2004), as well as a comprehensive summary by Winter and Holtum in 2014, but no group has been as well studied in photosynthetic variability and plasticity as the genus *Clusia.* A genus of tropical plants with roughly 300 representative species, *Clusia* plants can grow as self-supporting trees, epiphytes or hemi-epiphytes. Their range of growth form adaptations is about as wide as their range of expression of CAM, with species exhibiting strongly expressed CAM (*C. rosea*), to obligate C_3 species (*C. multiflora*) **(**Holtum et al. 2004).

A major prevailing idea is that plants that as plants continue on the continuum from C_3 to CAM photosynthesis, they will be accompanied by a suite of traits that assist in drought tolerance. With more and more species being found to be facultative CAM species, a formally major question “what physiological differences separate C_3, C_4 and CAM plants?”, has shifted to “What physiological differences exist along the continuum of CAM, as well as in species that can singlehandedly exhibit the continuum of CAM (i.e. Facultative CAM species). Using the well-studied genus *Clusia* as a study group, three CAM species C. rosea (a largely constitutively CAM epiphyte, hemi-epiphyte and tree), C. uvitana (weakly expressed constitutive CAM with some facultative CAM properties- grows mainly as a tree, but can grow as a hemi-epiphyte and epiphyte), and C. pratensis (a strong C_3/facultative CAM tree). Using pressure volume curves and basic measures of relative water content (RWC) and succulence, the differences between hydraulic traits in the three levels of CAM expression are examined. The results indicate schlerophylly in the leaves of C. rosea, with a high modulus of elasticity (ε), a high relative water content at the turgor loss point (RWC_{TLP}), and a low capacitance at full turgor (C_{FT}). This contrasted against the similar results of C. uvitana and C. pratensis – both expressing facultative CAM and C_3 photosynthetic properties, they exhibitied a higher C_{FT}, a lower ε and a lower RWC_{TLP} – suggesting leaves that are able to store large quantities of water, with cells that may be subject to larger volume changes, and stretching, due to changing in water content.

Selection of material and Site Conditions

Initial measurements on the water relations among photosynthetic type were sampled on 14 trees of three species (Family Clusiaceae) in the Santa Cruz Field Facility, Smithsonian Tropical Research Institute, Gamboa Panama [(9°07’13.48”N, 79°42’07.85”W), approximately 30km north-west of Panama City]. Trees were grown under optimal conditions for water, light and nutrient availability. Leaves of *Clusia rosea, Clusia uvitana and Clusia pratensis* were harvested midmorning, immediately sealed with parafilm, bagged, and returned to the laboratory in Ancon, Panama City for analysis. The three species were chosen based on tree health, tree availability and the variety CAM expression which was chosen based on prior research and δC^{13} values: *C. pratensis* (-25.6‰), *C. uvitana* (-22.1‰) and *C. rosea* (-16.7‰) (Holtum et al. 2004). The site experiences average temperatures of 26.4°C, with an average humidity of 87.7% and precipitation of 243.5mm/month (IWMI Online Climate Summary Model)

Hydraulic Measurements

Measurements of relative water content (RWC, %) were performed on leaves of similar size and branch location on all species. Leaves were allowed to soak in distilled water for one day to saturate leaves. Succulence was also measured, and the suggested format was saturated water content (SWC) as suggested by Ogburn and Edwards 2012. Pressure-volume curve parameters were determined by the procedure used by Loren Sacks as documented on PrometheusWiki.com (Sack et al. 2011). The procedure generates a set of data that, when graphed, can be used to determine ψ_{leaf}, leaf water potential (MPa), π_{ft}, osmotic pressure at full turgor pressure (MPa), π_{tlp}, osmotic potential (there is no osmotic potential; there is only osmotic pressure) at the turgor loss point (MPa), RWC_{tlp}, relative water content at turgor loss point (%), ε, modulus of elasticity at full turgor (MPa), C_{ft}, relative capacitance at full turgor (MPa^{-1}) C_{tlp}, relative capacitance at zero turgor (MPa^{-1}) C_{ft*}, absolute capacitance per leaf area, at full turgor ($g \cdot m^{-2} \cdot MPa^{-1}$). All analyses were performed using R statistical software. Data were analyzed using ANOVA analyses with Tukey HSD post-hoc testing to determine statistical significance at $p<0.05$ (Figures 1 -9). Analyses of correlation were also performed and a correlation matrix was generated using Pearson Correlation Coefficients (Table 4).

Results

Measures of RWC and succulence through hydration and oven drying, did not yield any statistical differences, with $p=0.05$. Average values of RWC and succulence, respectively, for the three species are: *C. rosea*: 98.06±1.80% , 770.05±152.9 $g*m^{-2}$, *C. uvitana*: 96.78±2.109%, 675.51±43.38 $g*m^{-2}$, *C. pratensis*: 98.36±4.03%, 605.55±43.31 $g*m^{-2}$. Measurements of pressure-volume curve derived parameters showed variables with significant differences. The modulus of elasticity was statistically higher for *C. rosea* then the other two species (66.44±32.70 MPa), as well as the relative water content at turgor loss point (97.01±2.04%), saturating water content (2.96±1.42). All three species showed statistically different absolute capacitance at full turgor: *C. rosea*: 0.46±0.28 $mol*m^{-2}MPa^{-1}$, *C. uvitana*: 4.33±1.00 $mol*m^{-2}MPa^{-1}$, *C. pratensis*: 2.03±0.79 $mol*m^{-2}MPa^{-1}$.

Measures of Pearson's correlation found the most significant correlation across the three species between the variables of C_{FT*} and ε with a strong negative correlation (-0.86), and C_{FT*} and SWC with a medium strength positive correlation (0.51). There was also a strong negative correlation between the RWC_{TLP} and the absolute capacitance (-0.85).

Discussion

Our results indicate that among three tree species that co-exist within a similar geographic range, differences in leaf hydraulic function correspond to their use of contrasting photosynthetic pathways. The low absolute capacitance at full turgor, high ε and high RWC_{TLP} in *C. rosea* indicate that its cells are not highly elastic and likely not able to hold large quantities of water. Due to the strong CAM nature of *C. rosea*, which reduces daytime transpiration loss, C. rosea would be unable to mechanically cope with the stresses of high water loss on its un-elastic cells. One of the lowest transpiration ratios of measured in Panamanian *Clusia* was in *C. rosea*, supporting the evidence that its high CAM usage may contribute to a high WUE which mechanically supports high water retention in cells (Winter et al. 2005). *C. rosea* is a strong CAM plant that frequently exhibits a hemi-epiphytic growth habit, and it has been previously shown in *Ficus* that tropical hemi-epiphytes exhibit a more conservative water use strategy, and CAM may potentially be one adaptation to developing a vining habit in the *Clusia* (Sack et al. 2010).

The higher absolute capacitance, lower ε and lower RWC_{TLP} of the leaves of *C. uvitana*, could be a different resource acquisition strategy that allows it to co-exist in similar geographic areas as *C. rosea*. With its elastic cell walls, high water capacitance at full turgor and low RWC_{TLP}, the cells can mechanically handle the strain of water loss to a lower RWC (82.42% vs. C. rosea: 97.01%). The lowered carbon isotope discrimination value of *C. uvitana* would suggest a less strong reliance on the CAM pathway (C. uvitana: -22.1‰ and C. rosea: -16.7‰) and more daytime CO_2 uptake, causing a higher rate of transpiration. Both *C. rosea* and *C. uvitana* are hemi-epiphytic in nature, therefore not only occupy a more unique niche, but also need to cope with the potential hydraulic strains of establishing in a variable micro-habitat like a tree branch (Holbrook and Putz 1996). Although literature is not highly supportive of a link between CAM and a climbing growth habit, further research may uncover whether CAM may be one of many potential adaptations vining plans can utilize to deal with the safety versus efficiency trade-off while growing in tropical forests.

C. pratensis is identified as a C_3-facultative CAM plant that almost only grows as a tree or shrub. It is possible that the difference in growth habit of *C. pratensis* from *C. rosea* and *C. uvitana* contributes to its non-significant values, and may be operating in a very similar fashion to *C. uvitana*, a hemi-epiphyte, whereby it is mechanically adapted to withstand high transpiration rates and water fluctuations within leaf tissue cells (Miraya and Correa 2004). The differences in water use strategies provided by the data between *C. rosea* and *C. uvitana* could suggest that *C. uvitana* maximizes its water storage capability and ability to cope with higher transpiration rates, whereas *C. rosea* maximizes its ability to retain water- and these strategies may have developed due to the two species overlapping niches in their native range.

The high biodiversity of tropical forests causes potential for a high degree of niche overlap, and to overcome mechanisms to help partition resources. A divergence in photosynthetic pathway is one such example, where the high WUE CAM plants will exploit water variable microhabitats, yet cannot compete with the growth rate of C_3 plants present in the high water availability microhabitats. By employing a spectrum of growth habits and photosynthetic pathway usage, as well as a high level of phenotypic plasticity, *Clusia* species have adapted to utilize their photosynthetic pathway, coupled with differences in water usage strategies, to persist over a range of growth habits and in a range of environments (Holbrook and Putz 1996).

The data presented suggests a trade-off in water usage mechanisms between two *Clusia* species with mainly C3 photosyntheic CO2 fixation and one species with mainly CAM photosynthetic CO2 fixation (of similar growth habit, geographic range, yet slightly different carbon isotope discrimination values.) The data alludes to a possible interaction between growth habit, usage of CAM, and hydraulic traits. A larger study examining hydraulic functional traits and growth habits of plants along the C_3 – CAM spectrum, using carbon discrimination values as a proxy for CAM strength, could show overarching trends relating divergences in photosynthetic pathway to resource partitioning in plant communities. This data comes as the community of Crassulacean Acid Metabolism is seeking to bio-engineer tree species to utilize CAM. The *Populus* tree genus is the primary target for bio-engineering, and as *Clusia* is one of the only genera to express strong CAM in a plant capable of growing as a tree, this research may be invaluable to assisting in the design and production of a new CAM tree.

References

Borland AM, Zambrano VAB, Ceusters J, Shorrock K. 2011. The photosynthetic plasticity of crassulacean acid metabolism: an evolutionary innovation for sustainable productivity in a changing world. *New Phytologist* **191**(3): 619-633.

Cernusak LA, Ubierna N, Winter K, Holtum JAM, Marshall JD, Farquhar GD. 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. *New Phytologist* **200**(4): 950-965.

Guralnick LJ, Edwards G, Ku MSB, Hockema B, Franceschi VR. 2002. Photosynthetic and anatomical characteristics in the C-4-crassulacean acid metabolism-cycling plant, Portulaca grandiflora. *Functional Plant Biology* **29**(6): 763-773.

Hao GY, Sack L, Wang AY, Cao KF, Goldstein G. 2010. Differentiation of leaf water flux and drought tolerance traits in hemiepiphytic and non-hemiepiphytic Ficus tree species. *Functional Ecology* **24**(4): 731-740.

Holbrook NM, Putz FE. 1996. From epiphyte to tree: Differences in leaf structure and leaf water relations associated with the transition in growth form in eight species of hemiepiphytes. *Plant Cell and Environment* **19**(6): 631-642.

Holtum JAM, Aranda J, Virgo A, Gehrig HH, Winter K. 2004. delta(13)C values and crassulacean acid metabolism in Clusia species from Panama. *Trees-Structure and Function* **18**(6): 658-668.

Mireya D, Correa A. 2004. *Catalogo de las Plantas Vasculares de Panama*: Universidad de Panama.

Ogburn RM, Edwards EJ. 2012. Quantifying succulence: a rapid, physiologically meaningful metric of plant water storage. *Plant Cell and Environment* **35**(9): 1533-1542.

Lawren Sack, Jessica Pasquet-Kok and PrometheusWiki contributors. "Leaf pressure-volume curve parameters." *PrometheusWiki.* , 05 Apr. 2011 Web. 02 Jul. 2015.

Santiago LS, Wright SJ. 2007. Leaf functional traits of tropical forest plants in relation to growth form. *Functional Ecology* **21**(1): 19-27.

Silvera K, Neubig KM, Whitten WM, Williams NH, Winter K, Cushman JC. 2010. Evolution along the crassulacean acid metabolism continuum. *Functional Plant Biology* **37**(11): 995-1010.

Swenson NG, Enquist BJ. 2007. Ecological and evolutionary determinants of a key plant functional trait: Wood density and its community-wide variation across latitude and elevation. *American Journal of Botany* **94**(3): 451-459.

Ting IP. 1985. CRASSULACEAN ACID METABOLISM. *Annual Review of Plant Physiology and Plant Molecular Biology* **36**: 595-622.

Winter K, Aranda J, Holtum JAM. 2005. Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. *Functional Plant Biology* **32**(5): 381-388.

Winter K, Garcia M, Holtum JAM. 2008. On the nature of facultative and constitutive CAM: environmental and developmental control of CAM expression during early growth of Clusia, Kalanchoe, and Opuntia. *Journal of Experimental Botany* **59**(7): 1829-1840.

Winter K, Garcia M, Holtum JAM. 2009. Canopy CO2 exchange of two neotropical tree species exhibiting constitutive and facultative CAM photosynthesis, Clusia rosea and Clusia cylindrica. *Journal of Experimental Botany* **60**(11): 3167-3177.

Winter K, Garcia M, Holtum JAM. 2011. Drought-stress-induced up-regulation of CAM in seedlings of a tropical cactus, Opuntia elatior, operating predominantly in the C-3 mode. *Journal of Experimental Botany* **62**(11): 4037-4042.

Winter K, Holtum JAM. 2007a. Environment or development? Lifetime net CO(2) exchange and control of the expression of crassulacean acid metabolism in Mesembryanthemum crystallinum. *Plant Physiology* **143**(1): 98-107.

Winter K, Holtum JAM. 2007b. Environment or development? Lifetime net CO(2) exchange and control of the expression of crassulacean acid metabolism in Mesembryanthemum crystallinum. *Plant Physiology* **143**(1): 98-107.

Zotz G, Winter K. 1993. SHORT-TERM REGULATION OF CRASSULACEAN ACID METABOLISM ACTIVITY IN A TROPICAL HEMIEPIPHYTE, CLUSIA-UVITANA. *Plant Physiology* **102**(3): 835-841.

Zotz G, Winter K. 1994. A ONE-YEAR STUDY ON CARBON, WATER AND NUTRIENT RELATIONSHIPS IN A TROPICAL C-3-CAM HEMI-EPIPHYTE, CLUSIA-UVITANA PITTIER. *New Phytologist* **127**(1): 45-60.

Zotz G, Winter K. 1996. *Seasonal changes in daytime versus nighttime CO2 fixation of Clusia uvitana in situ*. Berlin 33: Springer-Verlag Berlin.

Tables and Figures

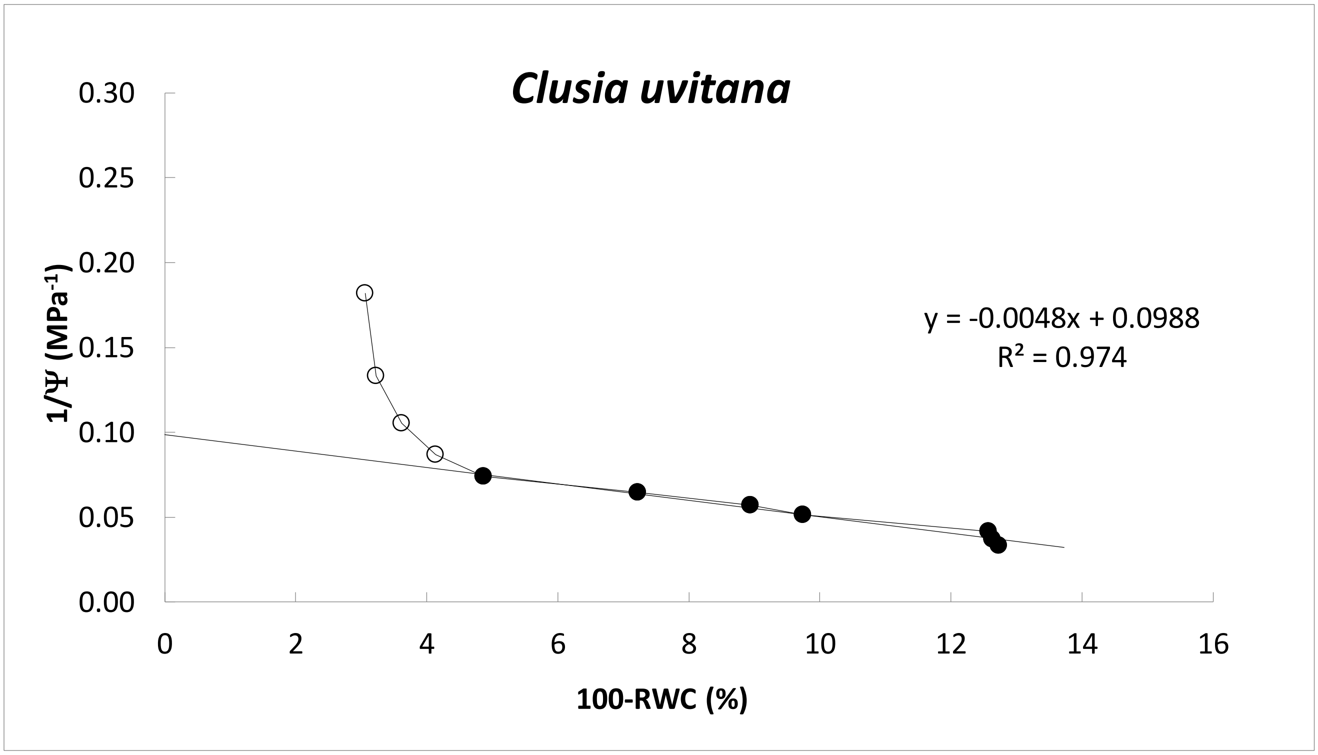

Table 1: Pressure-volume curve for one representative experiment with C. uvitana. The line of best fit that extends to the y-axis intersects the axis at the osmotic potential at full turgor of the leaf. Open circles represent measurements taken before the turgor loss point, and filled circles represent measurements after the leaf has passed its turgor loss point. All variables in figures 4 through 12 are taken from this curve or derived from values from this curve, except saturated water content and relative water content.

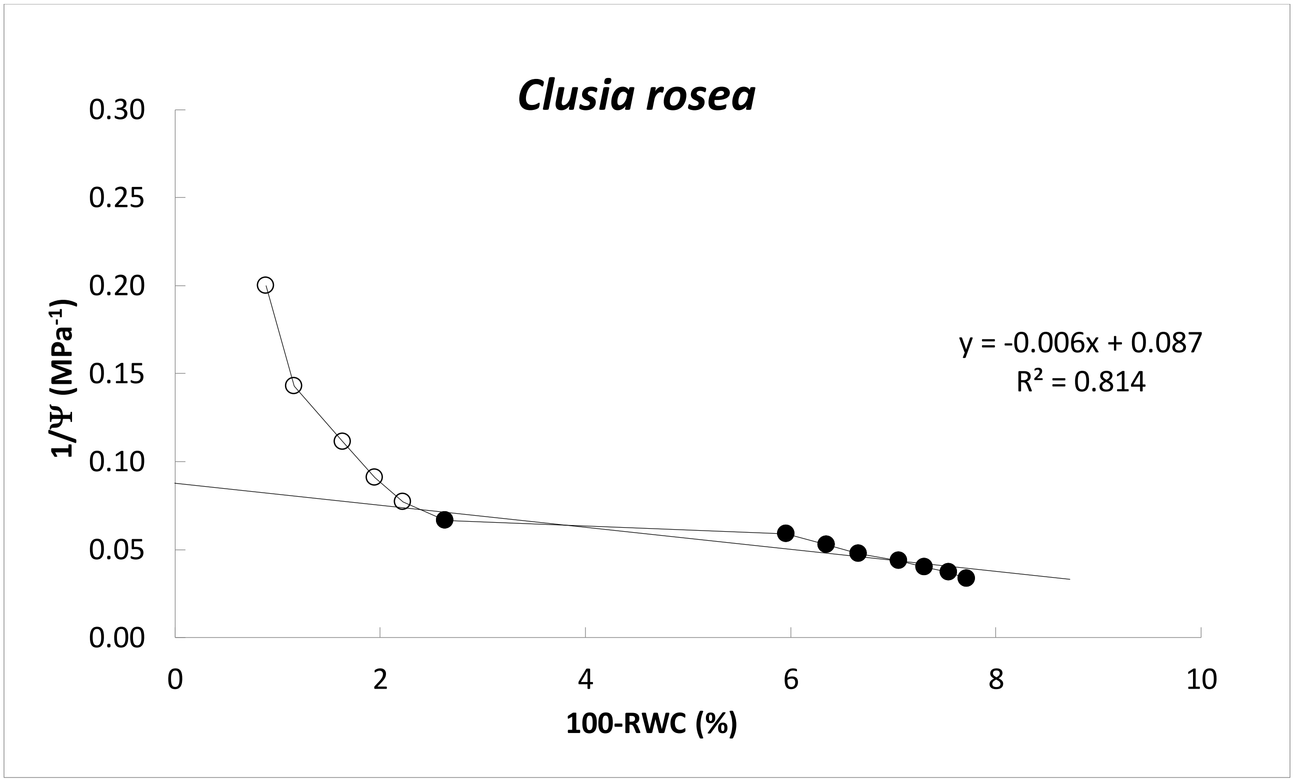

Table 2: Pressure-volume curve for one representative experiment with C. rosea. The line of best fit that extends to the y-axis intersects the axis at the osmotic potential at full turgor of the leaf. Open circles represent measurements taken before the turgor loss point, and filled circles represent measurements after the leaf has passed its turgor loss point. All variables in figures 4 through 12 are taken from this curve or derived from values from this curve, except saturated water content and relative water content.

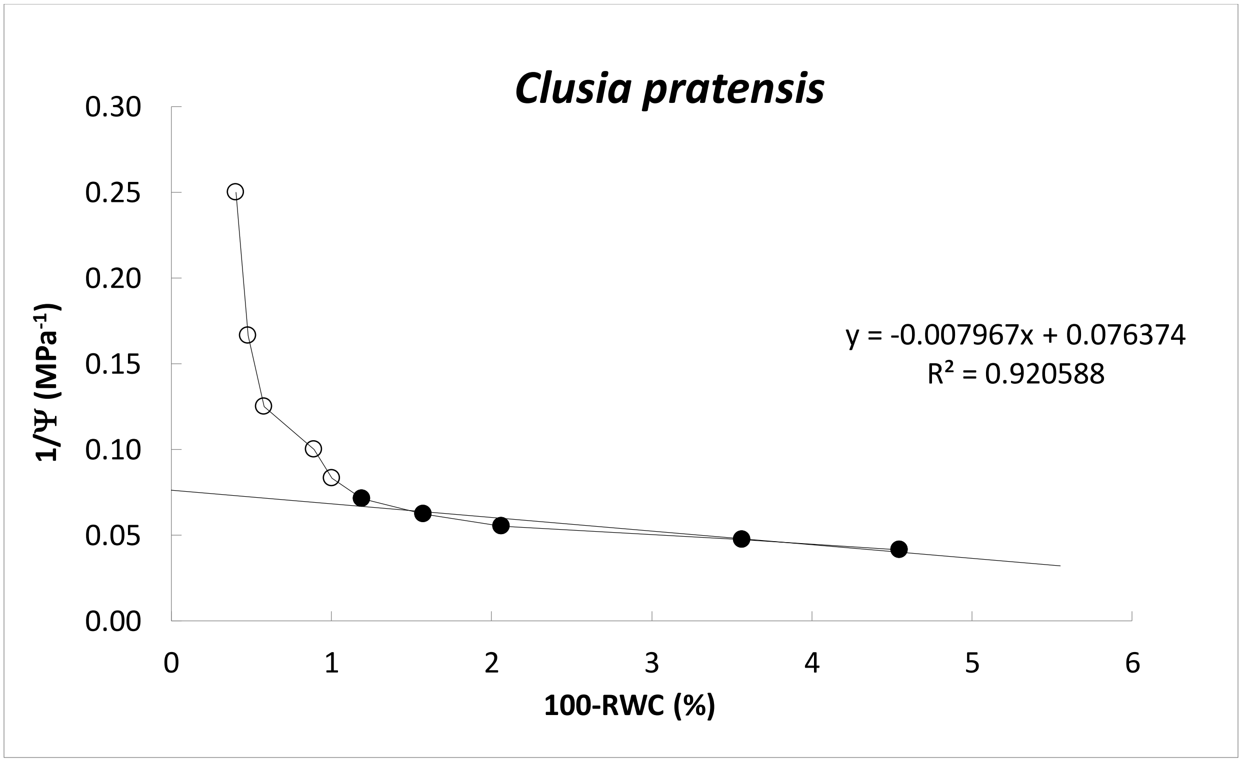

Table 3: Pressure-volume curve for one representative experiment with C. pratensis. The line of best fit that extends to the y-axis intersects the axis at the osmotic potential at full turgor of the leaf. Open circles represent measurements taken before the turgor loss point, and filled circles represent measurements after the leaf has passed its turgor loss point. All variables in figures 4 through 12 are taken from this curve or derived from values from this curve, except saturated water content and relative water content.

	SWC	**π_0**	**π_{TLP}**	**Ψ_{TLP}**	**RWC_{TLP}**	**ε**	**C_{FT}**	**C_{TLP}**
π_0	-0.30							
π_{TLP}	-0.45	**0.97**						
Ψ_{TLP}	0.47	0.09	-0.03					
RWC_{TLP}	**-0.82**	-0.11	0.10	-0.59				
ε	-0.25	-0.03	0.09	-0.09	0.49			
C_{FT}	0.58	0.38	0.16	0.60	-0.91	-0.48		
C_{TLP}	0.49	-0.69	-0.64	-0.01	-0.16	-0.07	-0.26	
C_{Ft}^*	0.54	0.55	0.36	0.59	**-0.85**	-0.46	0.95	-0.29

Table 4: Pearson's correlation matrix of all 9 values obtained or derived from a pressure-volume curve (Tyrell and Hammel 1972). Bolding indicates a significant correlations. Abbreviations are as follows: SWC (saturated water content), π_0 (osmotic potential at full turgor), π_{TLP} (osmotic potential at the turgor loss point), Ψ_{TLP} (water potential at the turgor loss point), RWC_{TLP} (relative water content at the turgor loss point), ε (elasticity), C_{FT} (capacitance at full turgor), C_{TLP} (capacitance at the turgor loss point), C_{Ft}* (area specific capacitance at full turgor).

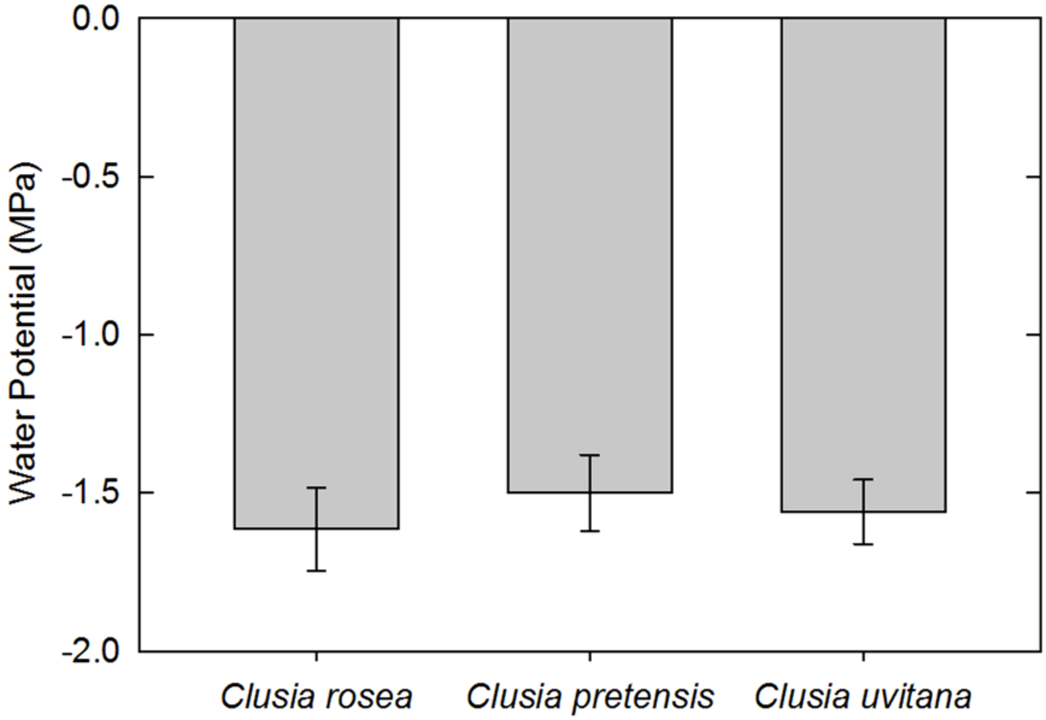

Figure 1: Water potential at the turgor loss point (ΨTLP) . Extrapolated from the pressure-volume curve as the intercept of the line of turgor loss, plus the slope of the line at turgor loss plus 100- the relative water content, all divided by negative one.

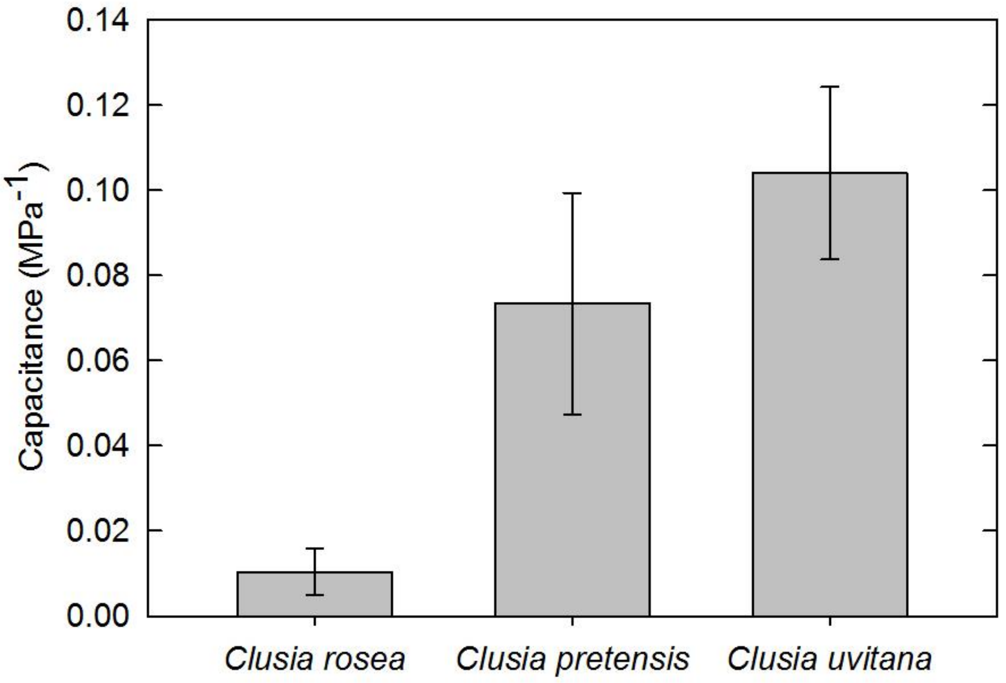

Figure 2: Leaf capacitance at full turgor (CFT). Measured as the standard deviation of the leaf's relative water content divided by the standard deviation of the leaf's water potential, all before turgor loss. Significant differences are seen between C. rosea, and C. pretensis and C. uvitana

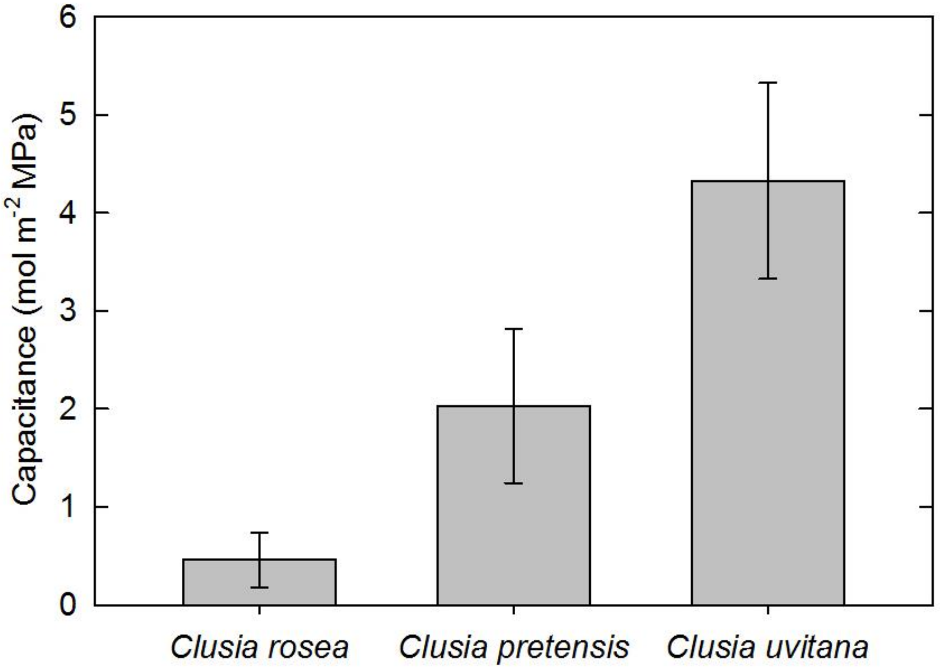

Figure 3: Capacitance of a leaf on an area scale at full turgor (CFT*). Measured as the standard deviation of the leaf's relative water content divided by the standard deviation of the leaf's water potential, all at full turgor. This value is divided by the leaf area to obtain a measure of the capacitance with area taken into account. All three species significantly different from one another, $p<0.05$, based on ANOVA testing with Tukey Post-Hoc testing with permutation to account for variation in normality.

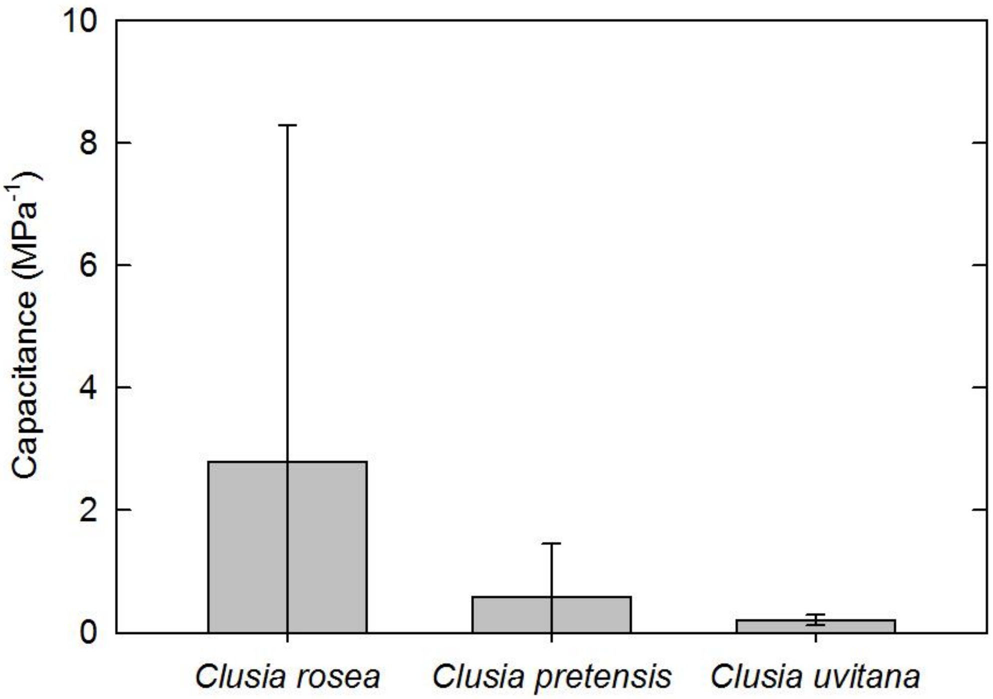

Figure 4: Leaf capacitance at the turgor loss point (CTLP). Measured as the standard deviation of the leaf's relative water content divided by the standard deviation of the leaf's water potential, all after turgor loss.

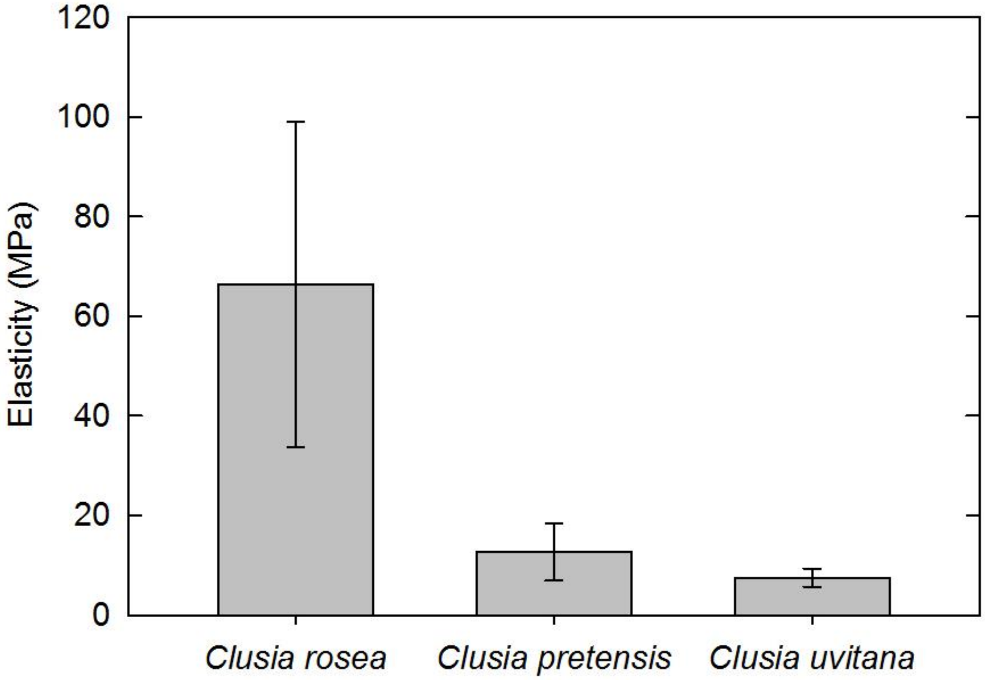

Figure 5: Elasticity (ε), calculated as the standard deviation of the water potentials of the leaf at turgor, divided by the standard deviation of the relative water contents of the leaf, in turgor. This measure represents the leaf cell's abilities to stretch and compress in response to changing water content (entering incipient plasmolysis). All three species significantly different from one another, $p<0.05$, based on ANOVA testing with Tukey Post-Hoc testing with permutation to account for variation in normality.

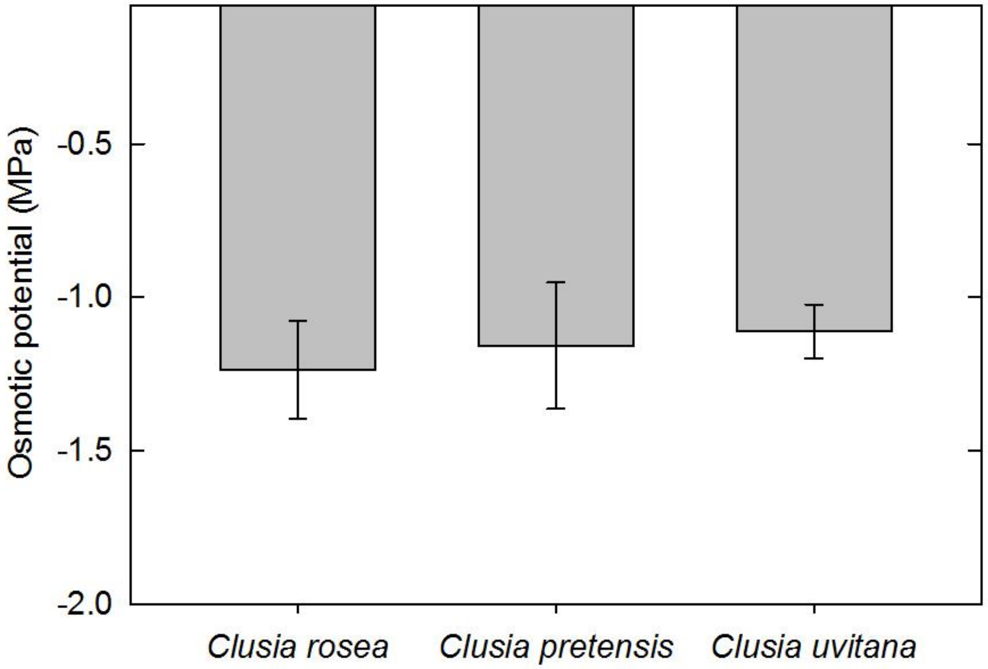

Figure 6: Osmotic potential at the turgor loss point (πTLP). Calculated by finding the y-intercept of the point on the graph where the leaf loses turgor.

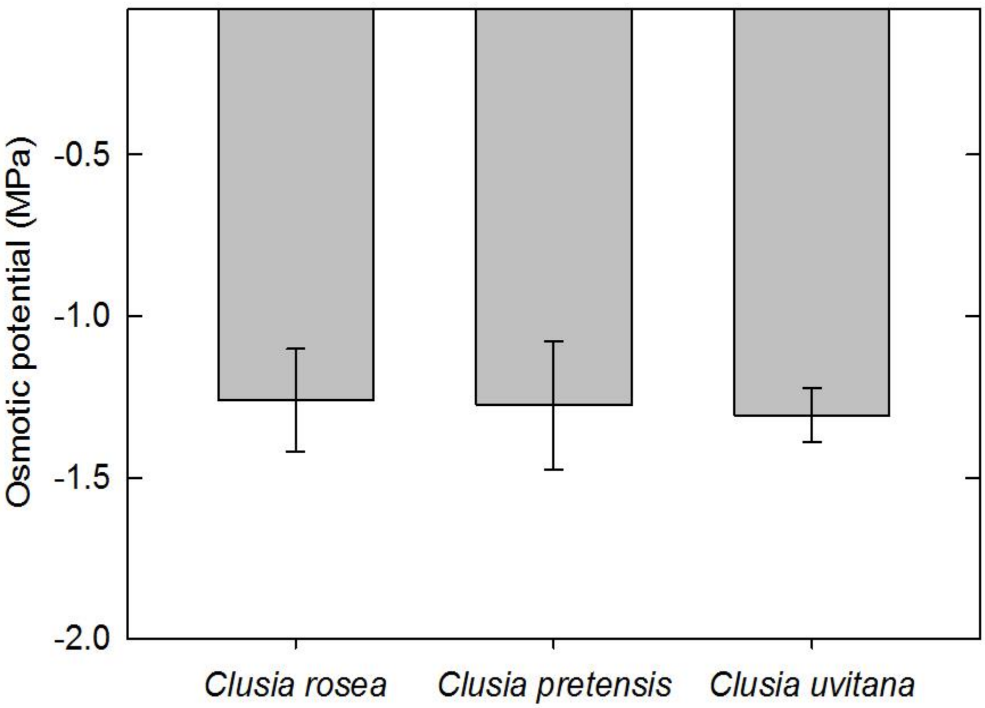

Figure 7: Osmotic potential at full turgor (π0). To determine, the slope of the line of the measurements after loss of turgor is determined, and extrapolated to the y-intercept.

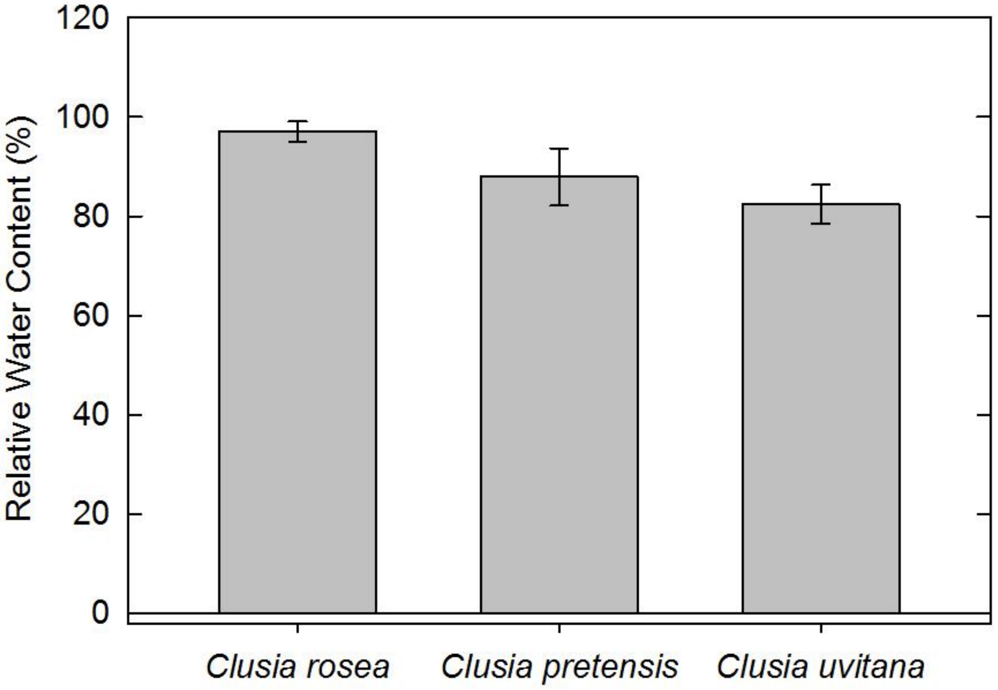

Figure 8: Relative water content at the turgor loss point (RWCTLP). Turgor loss point is determined as the point at which a pressure-volume curve begins to plateau (see tables 1-3). The RWC_{TLP} is measured as the dry (leaf weight/saturated leaf weight)*100. C. rosea and C. uvitana are statistically different from one another, $p<0.05$, based on ANOVA testing with Tukey Post-Hoc testing with permutation to account for variation in normality.

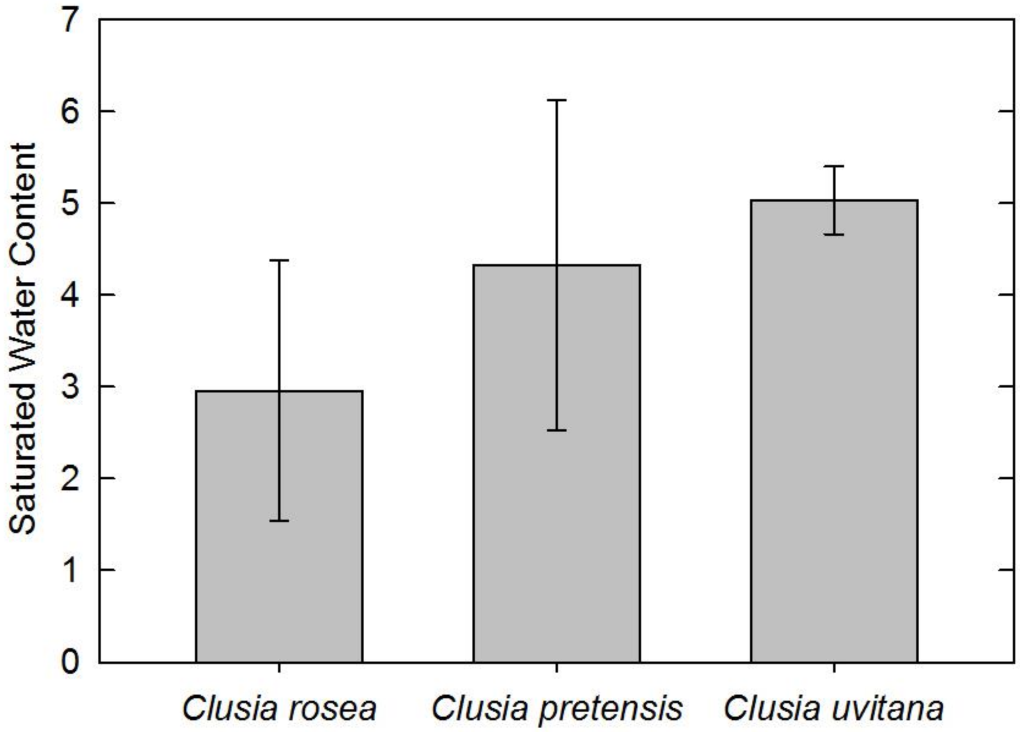

Figure 9: Saturated Water Content (SWC), measured as the theoretical saturating water leaf mass (g) over the dry mass (g), forming a unit-less measure of how saturated the leaf can become compared to its dry weight. Extrapolated from plotting the mass of the leaf (g) against the water potential of the leaf (MPa). C. rosea and C. uvitana are statistically different from one another, $p<0.05$, based on ANOVA testing with Tukey Post-Hoc testing with permutation to account for variation in normality.