Nocturnal Photosynthesis and How to Find it

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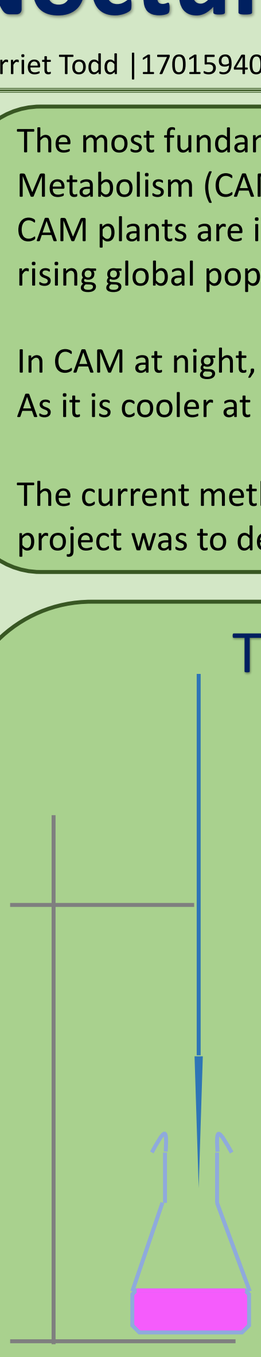
The most fundamental process in plants is photosynthesis, which is the use of sunlight to form sugars. Some 90% of plants are C3 whilst Crassulacean Acid Metabolism (CAM) is a special type of photosynthesis that gives capacity to take up carbon at night. CAM is more efficient as plants require less water to grow. CAM plants are incredibly valuable to research when improving crop species productivity given the rapidly growing concern for current climate change and the rising global population.

In CAM at night, carbon dioxide is converted into malic acid. At dawn the acid levels are high and decrease throughout the day as the plant metabolises the acid. As it is cooler at night, the plant can have the leaf pores (stomata) open for longer but lose less water compared to non-CAM plants.

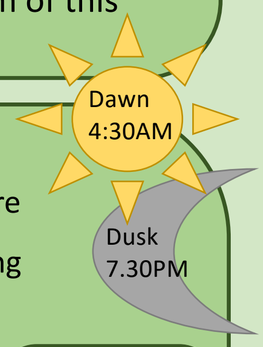
The current method for identifying CAM species is time consuming, which subsequently limits the number of species that can be investigated. The aim of this project was to develop a rapid and reliable method for identifying CAM plants.

1 Titration Assay Method



- Samples were collected from the National Sedum Collection in Bedlington Northumberland.
- Samples were taken at dawn and dusk, flash frozen in liquid nitrogen, weighed, ground into a powder and placed in a tube with methanol.
- Samples were heated, added to water and phenolphthalein (a colour changing pH indicator), creating a colourless solution.
- NaOH (an alkali) was titrated against it, until it turned pink. This gives us the titratable amount of acid in the leaf.

2 Titration Results



Plants were selected that had a wide array of morphologies and were from around the world. Five of these samples were tested were using the TA method, providing a baseline.

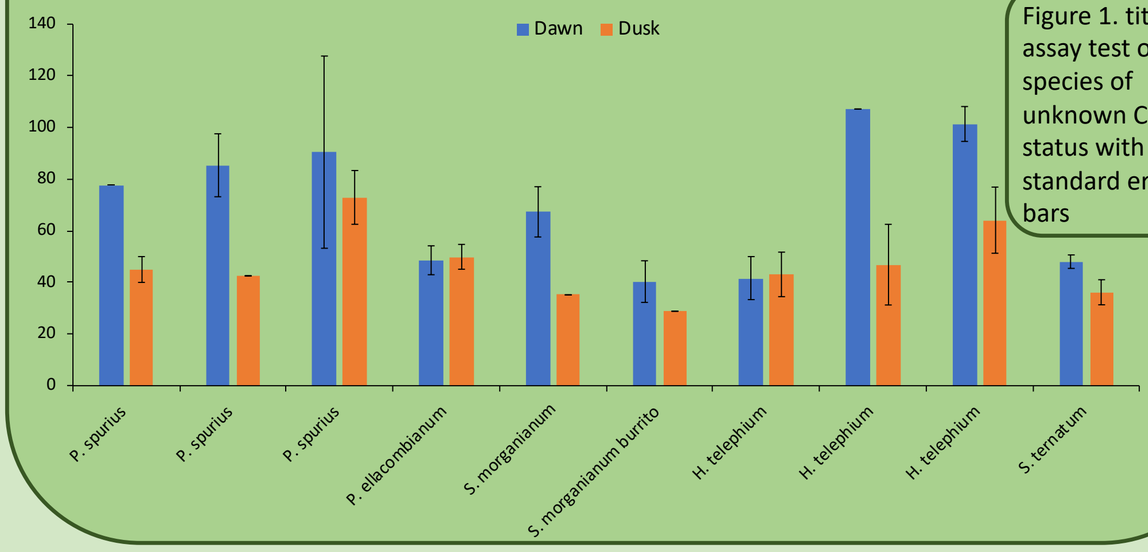


Figure 1. titration assay test of 5 species of unknown CAM status with standard error bars

3 Plate Reader

- All colours have known wavelength ranges, the reader identifies them.
- To make the method universal for all morphologies samples were freeze dried.
- A tissue lyser (shaking equipment) was used to powder samples.
- Powder was added to methanol, heated and then plated in 96 well plates.
- Chlorophenol (CPR), a pH indicator, was used. It has a change colour at pH4 (acidic) to red and at pH7 (alkali) it turns purple (Cushman et al 2008).

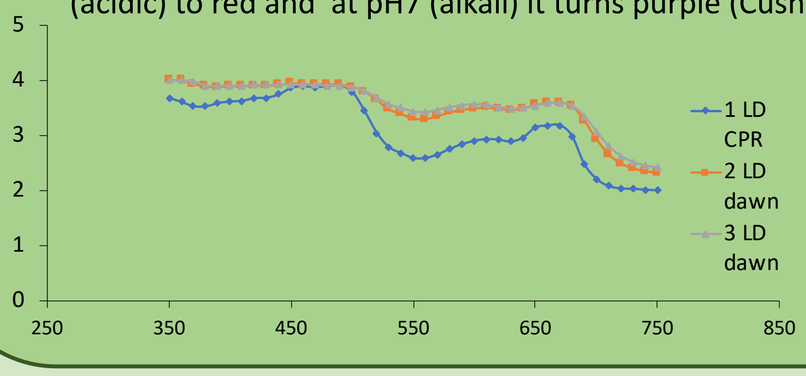


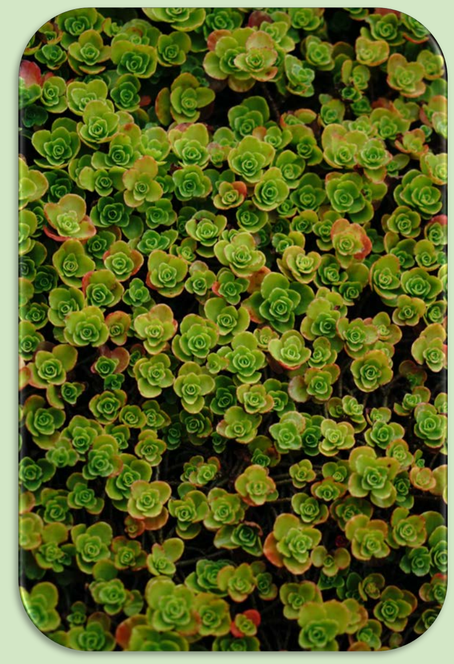
Figure 2.Spectrum test: shows 1 leaf disc and 595nm were found to be optimum. Any colour change was clearly read by the machine.

This is an example of one of the plates used in the experiment. Each plate has 96 wells which allows for a higher volume of species to be tested at once with repeats.

4 Final Results

Species	HT CAM status	TA CAM status
Phedimus spurius	CAM	CAM
Phedimus spurius (Red Cultivar)	C3	CAM
Phedimus spurius (Small leaf	CAM	CAM
Phedimus takesimense	C3	
Phedimus aizoon	C3	
Phedimus	CAM	
Sedum confusum	CAM	
Sedum confusum	CAM	
Phedimus ellacombianum	CAM	C3
Phedimus karntchaticus	CAM	
Rhodiola yuannaensis	CAM	
Rhodiola ishidae	CAM	
Sedum morganianum	CAM	CAM
Sedum morganianum burrito	CAM	CAM

Species	HT CAM status	TA CAM status
Hylotelephium populiform	C3	
Hylotelephium telephium (Northumberland)	CAM	C3
Hylotelephium telephium (Derbyshire)	C3	CAM
Hylotelephium telephium maxim (Europe)	C3	CAM
Hylotelephium viviparum	C3	
Sedum dassphyllum mesatlanticum (Spain)	C3	
Sedum dassphyllum mesatlanticum (Italy)	CAM	
Petrosedum montanum (France)	C3	
Petrosedum sediforme (France)	CAM	
Sedum ternatum	C3	CAM
Sedum spathuliform	CAM	
Sedum moranense	C3	



40% of the high throughput results were the same are the titration assay ones. This forms a solid and promising start point for the method. Some of the samples were lost during processing (e.g. didn't freeze dry properly) so had to be discounted. Further fine tuning of the method is needed to progress this method. However, it would seem that CAM is prevalent across the species in the National collection.

With special thanks to Ray Stephenson for giving us access to the National sedum collection. To Anne and Max for their guidance. To Jonathan Todd taking and providing the brilliant photos. To Amy Bell, Helen Martin and all the staff at the Devonshire building and Ridely 2 for their help. Finally and especially, Danny Cowan Turner for coming with me on a 4AM sampling trip!

JC Cushman S Agarie, RL. Albion, SM Elliot, T Taybi, AM Borland. Isolation and Characterization of Mutants of Common Ice Plant Deficient in Crassulacean Acid Metabolism. Plant physiology 2008 147(1): 228–238