MBO4 **Basic Methods in Plant Physiology, Anatomy and Ecology UE 4h 5ECTS**
Wolfram Weckwerth, Wolfgang Postl, Gert Bachmann, Lena Fragner, Thomas Nägele
Block course in Mai 2016 09.-13. and 16.-20., 9:00-16:00

Aims, Topics:
The relationship between anatomical traits, metabolome and physiotype shall be presented and analyzed for model plants featuring C3, C4 and CAM (crassulacean acid metabolism) photosynthesis types.
The employed methods include microclimatic monitoring as well as metabolic profile analysis and diurnal acid metabolism (GCMS), cation content (HPLC), and a variety of photosynthesis activity parameters (Imaging PAM, SPAD ...) including the detection of photosynthesis pigments, stomata imprint and conductive vessel microscopy.

Experimental Design, Plants studied:
Main Experiment: C. rosea (CAM), C. minor (falcultative CAM), C. multiflora (C3)
These Plants are grown in outdoor cultivation conditions and subjected to two treatment conditions that may induce CAM or repress that metabolic option.
Demonstration: Sugar Caine, Field Bean, Tropeolum, Arabidopsis th.

Program Summary:

Day	Start: 8:45 End: ~ 16:00
Mo 09	Theory and introduction: photosynthesis types, water retention / productivity tradeoff, biochemistry of photosynthesis, experiment design
Tue 10	Introduction in microclimate and photosynthesis measurement, measurements in the plant growth facilities
Wed 11	Introduction in laboratory procedures (KI-HPLC, GCMS, Titration) demonstration of analytical devices, day harvest
Th 12	Night harvest of plant samples, sample preparation and processing
Fr 13	Sample Preparation and Analysis (GCMS)
Mo 16	plant anatomy, pH, KI-HPLC analysis
Tue 17	Photosynthesis pigment photometry, data processing (start)
Wed 18	Complete data processing, statistics
Th 19	Statistics, data evaluation, preparation of protocols
Fr 20	Presentation of Results

Sampling Schedule:

Sampling at 11:00, 16:00, 22:00, 06:00 for acidity and CO_2- Metabolism

Timepoint of Harvest 4 1 2 3

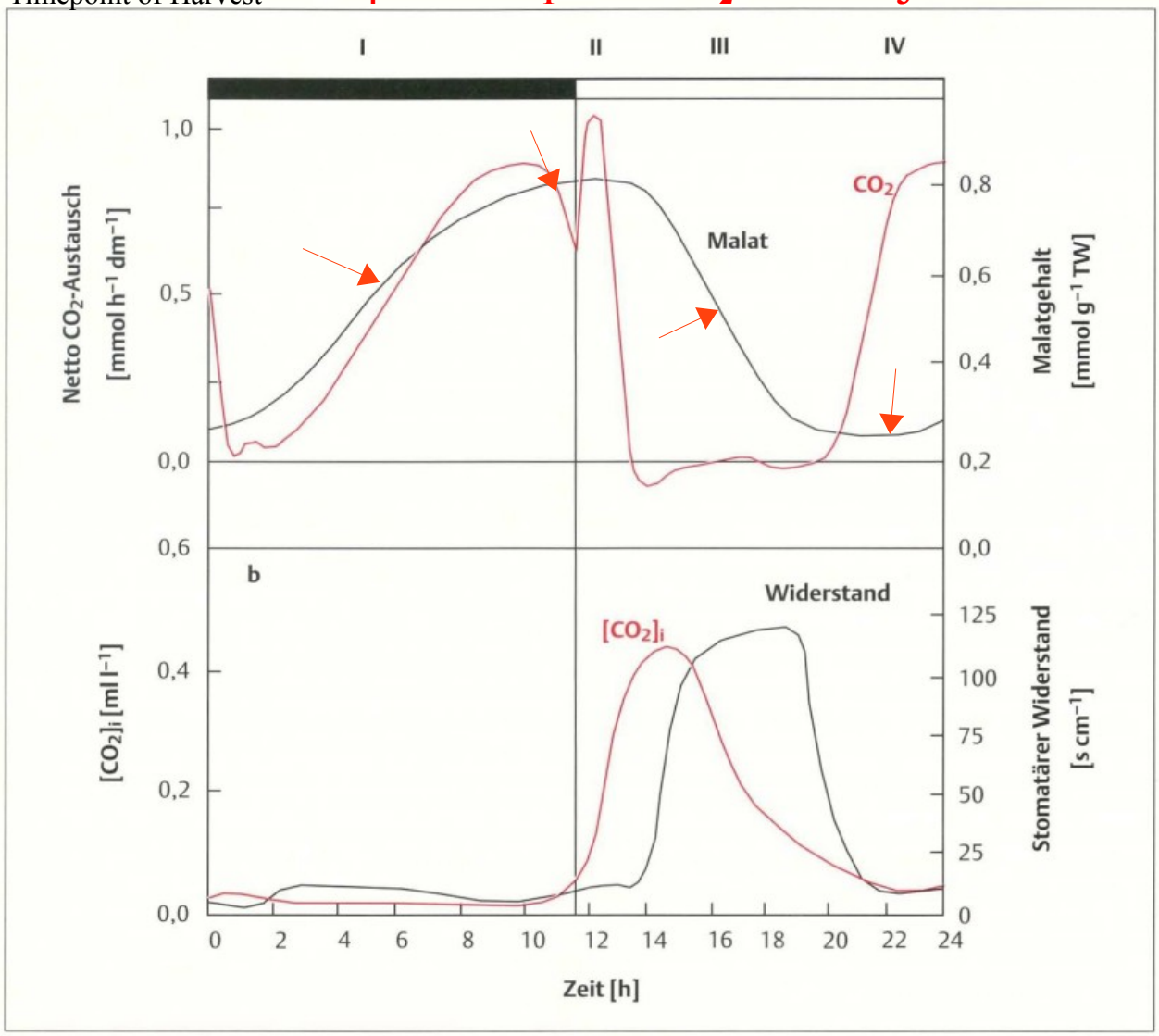


Abb. 12.2 Tagesgang einiger physiologischer Grundphänomene des CAM, wie sie von der obligaten CAM-Pflanze *Kalanchoë daigremontiana* (Crassulaceae) unter optimaler Bewässerung und deutlichem Temperaturunterschied zwischen Tag (25 °C) und Nacht (15 °C) gezeigt werden. (**a**) Netto-CO_2-Austausch (rote Linie; Werte oberhalb der 0-Linie bedeuten Netto-CO_2-Aufnahme) und Malatgehalt im Blattgewebe (schwarze Linie). (**b**) Stomatärer Widerstand (schwarze Linie) und mit Hilfe einer gaschromatographischen Methode ermittelte CO_2-Konzentration ($[CO_2]_i$) in den Interzellularen des Blattes (rote Linie). Der schwarze Querbalken zeigt die Dauer der Dunkelperiode und die römischen Zahlen die einzelnen Phasen (nach Osmond, 1978) des CAM-Gaswechsels an (nach Daten aus Kluge et al., 1981).

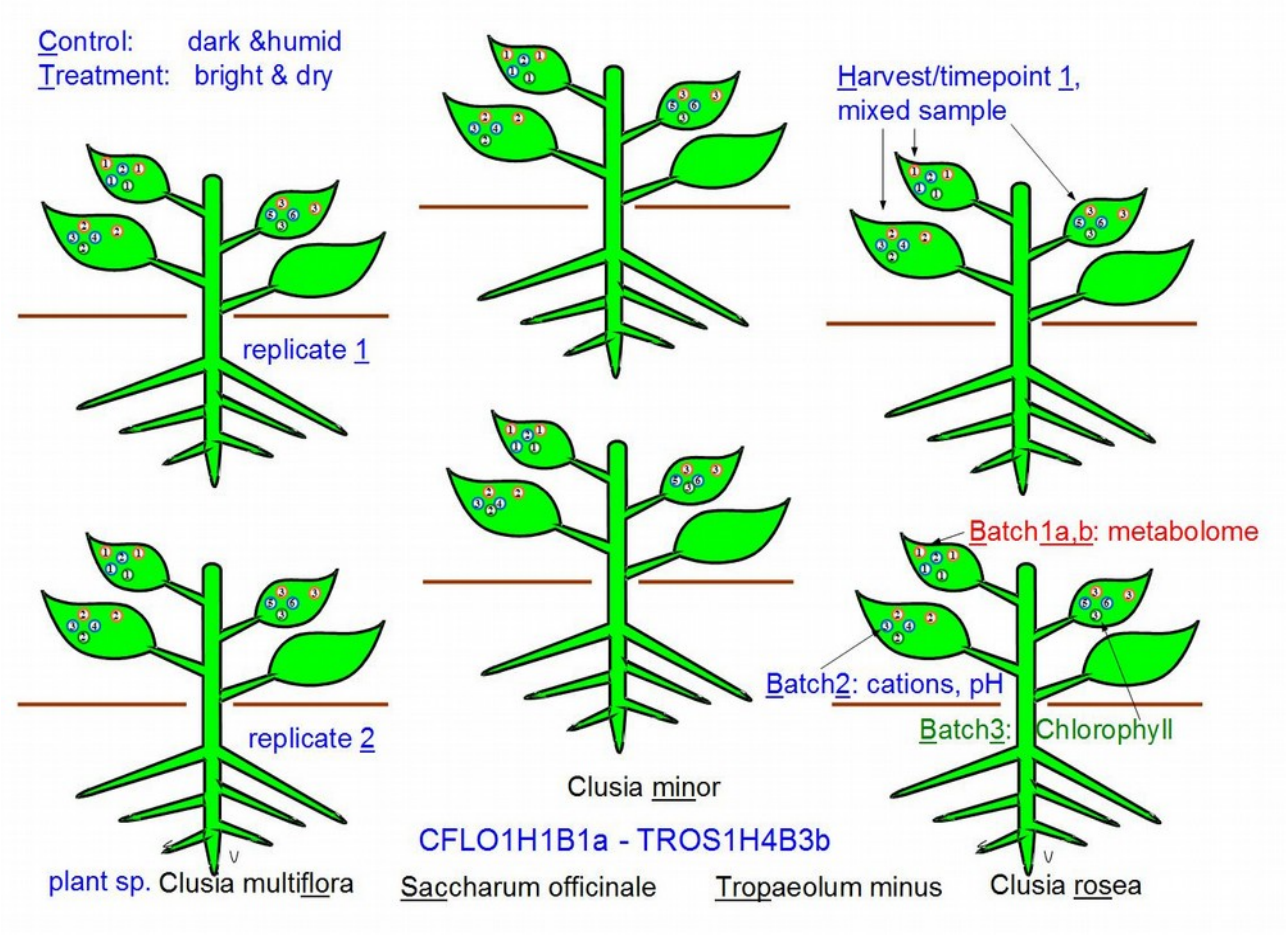
* © Photosynthese by Peter Häder, Thieme, Stuttgart (1999), use during course only

Detailed Programme:

MONDAY 09.05	morning	Introduction: Light, Water, Temperature, CO2: Photosynthesis Types as a flexible Response to Microclimate Theory: Biochemistry of photosynthesis
	afternoon	Theory: Photosynthesis Metabolome Experimental design of the experiment, Clusia species and varieties Visiting the experimental locations
TUESDAY 10.05	morning	greenhouse facilities: microclimate, Introduction to microclimate and photosynthesis measurement Fluorescense: PEA, Imaging PAM, SPAD, Soil moisture, Porometry
	afternoon	Theory: photosynthesis measurement Fluorescense: PEA, Imaging PAM, SPAD, Porometry
WEDNESDAY 11.05	morning	DEMO of analytical devices: GC-MS 1st harvest of Cusia – **10-11:00 = peak of CAM acidity**
	afternoon	DEMO of analytical devices: KI -HPLC, Titration 2nd harvest of Cusia – **15- 16:00 = medium of CAM acidity** 3rd harvest of Clusia – **21-22:00 = minimum of CAM acidity**
THURSDAY 12.05	morning	Predawn measurements: PEA 4th harvest of Clusia – **05:00- 6:00 = buildup of CAM acidity**
	afternoon	Sample processing in ÜR5 and Mosys Lab
Friday 13.05		Sample processing Analysis in Mosys Lab

MONDAY 16.05	morning	Plant anatomy Crosssections C3, C4, CAM	Plant anatomy Stomata Imprints C3, C4, CAM	
	afternoon	Plant anatomy Crosssections C3, C4, CAM	Chemistry pH	Chemistry Cation HPLC
TUESDAY 17.05	morning	Chlorophyll content Photometer	Chemistry pH, EC, RP	
	afternoon	Climatic Station Microclimate Readout of data Estimation of Specific Leaf Area SLA	*Data evaluation:*	Porometry PEA Imaging PAM Soil moisture Chlorophyll SPAD SLA Stomata imprints Leaf temperature
WEDNESDAY 18.05	morning	*Data evaluation:*	GCMS Cation HPLC Titration Chlorophyll photometer Statistics	
	afternoon	*Data evaluation:*	GCMS Cation HPLC Titration total acidity Chlorophyll photometer Statistics	
THURSDAY 19.05	morning	final statistics, graphics		
	afternoon	Final evaluation, prep. of protocols (3 presentations, each group)		
FRIDAY 20.05	Reserve	Final Presentation		

Harvest Method and Sample Labels:



Clusia minor Biometry / Harvest

disk	disk_r	area cm2	FW g	DW g	DW%FW	SLA dm2g-1
1	r 6mm	1.131	0.080	0.017		
3			0.240	0.052		
10			0.800	0.173	21.6	
1	r 5mm	0.785	0.056	0.012		
3			0.167	0.036		
10			0.556	0.120		
	small leaf	42.411	3.000	0.649		0.654

1 small leaf: ~ 3g ~ 30 disks

Leaf disks are cut from three leafs using a disk cutter, collected as a mixed sample in a plastik tube and immediately stored in a -80°C freezer. For analysis, the frozen leaf samples are grinded in liquid nitrogen or boiled in demineralized water as given in the respective SOP.

Table of Sampling and Analysis Frequency:

MBO4	photosynthesis variants, diurnal acid metabolism			
Exp. Design:				
locations	Rollhaus	Schattenhalle		
species	Treatment dry/bright	Control humid/shade		starts on:
clusia rosea		2	2	1 of Mai
clusia multiflora		2	2	
clusia minor		2	2	
saccharum officinale		2	2	
tropaeolum minus		2	2	
Sampling Roster:				
microclimate			recordings (24h)	
locations			2	
measuring days			2	4
gas metabolism			recordings (10')	
plant species			5	
days			2	
recordings	2 day, 2 night (2 rep)		4	40
experiment: diurnal acid metabolism			GCMS	
clusia sp.			3	
Treatment			2	
replicates, harvest GCMS	H1-4, 2 day, 2 night, 2 rep		8	48
			Kations_samples	
clusia sp.			3	
Treatment			2	
sampling ΔH^+	1 day H1, 1 night H3, 2 rep		4	24
chlorophyll			Photometry_samples	
species			5	
treatment			2	
sampling	1 day H2, 2 rep		2	20
anatomical properties	demo		Microscopy	
species			5	
sections	1treatment		2	10
stomata /inprint	1treatment		2	10

H1: 11:00, **H2**: 16:00, **H3**: 22:00, **H4**: 5:00

SOP, material/consumables:

Microclimatic Monitoring: Pyranometers, Hygrometer

Photosynthesis Monitoring: SPAD, Imaging PAM, IRGA + leaf chambers, Porometer

Chlorophyll Photometry: Brown glass 5mL Flasks Photometer, UV glass cuvettes (10mm), DMF, cork driller

GCMS of Metabolome: 5 mL Eppendorf Tubes, see SOP

HPLC-Kations: 15 mL sample tubes

Anatomy (cross sections)
PSE (personal safety equipment) Lab Gloves, Lab spectacles,
Lab coat to be used at all times in the Lab

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