MBO4 **Basic Methods in Plant Physiology, Anatomy and Ecology UE 4h 5ECTS**
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Block course in Mai 2018 07.-11. and 14.-18., 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.

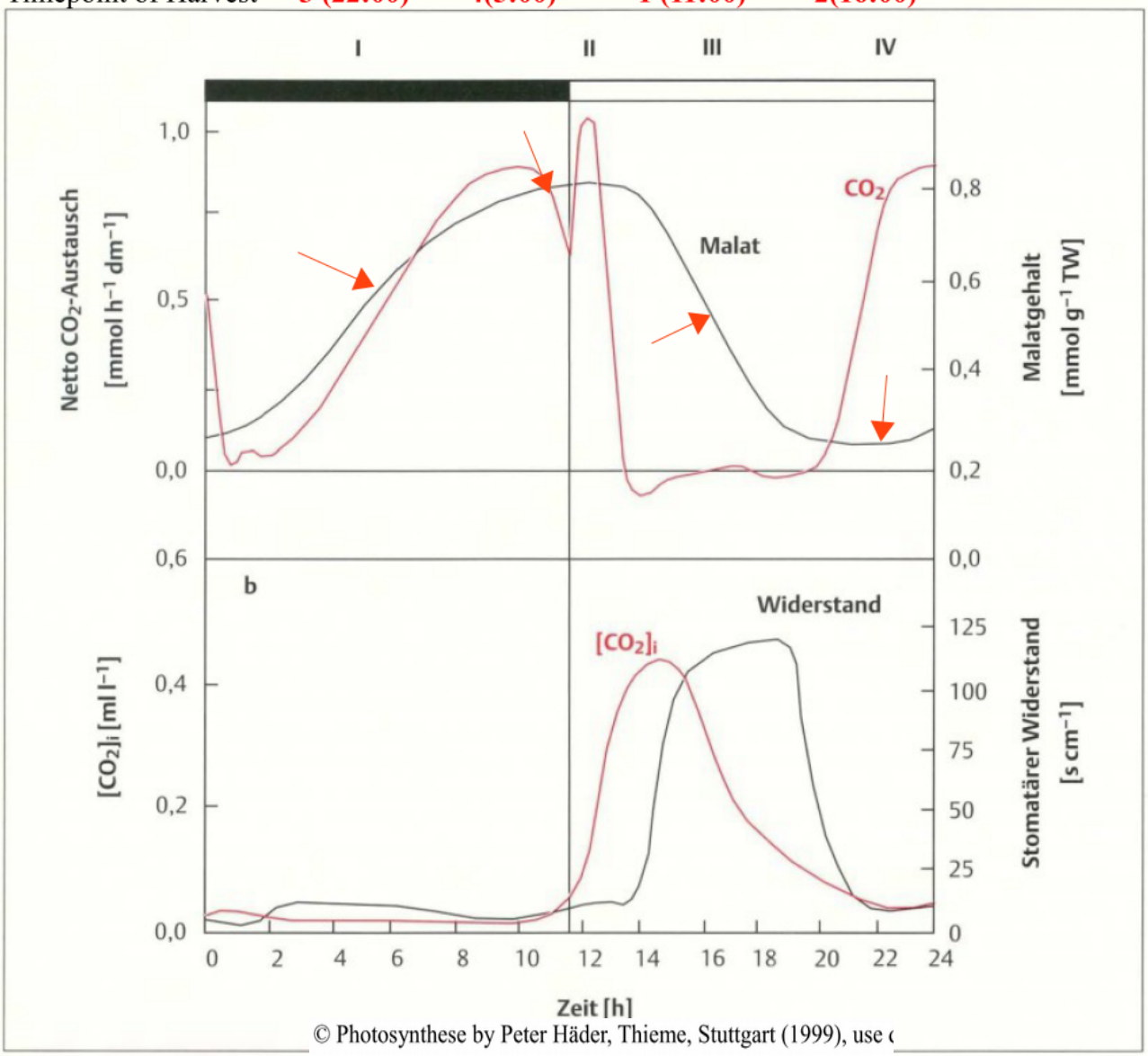
Experimental Design, Plants studied:
Main Experiment: C. rosea (CAM), C. minor (falcultative CAM), C. mexicana (C3)
These Plants are grown in outdoor cultivation conditions and subjected to two treatment conditions that may induce CAM or repress that metabolic option.
Demonstration: Sugar Caine, Field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

Program Summary:

Day	Start: 8:45 End: ~ 16:00
Mo 07	productivity tradeoff, design
Tue 08	Introduction in microclimate and photosynthesis measurement, measurements in the plant growth facilities
Wed 09	demonstration of analytical devices, day harvest
Th 10	Morning harvest of plant samples, sample preparation and processing
Fr 11	Sample Preparation and Analysis (GCMS)
Mo 14	plant anatomy, pH, KI-HPLC analysis
Tue 15	Photosynthesis pigment photometry, data processing (start)
Wed 16	Complete data processing, statistics
Th 17	Statistics, data evaluation, preparation of protocols
Fr 18	Presentation of Results

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

Timepoint of Harvest **3 (22:00) 4(5:00) 1 (11:00) 2(16:00)**

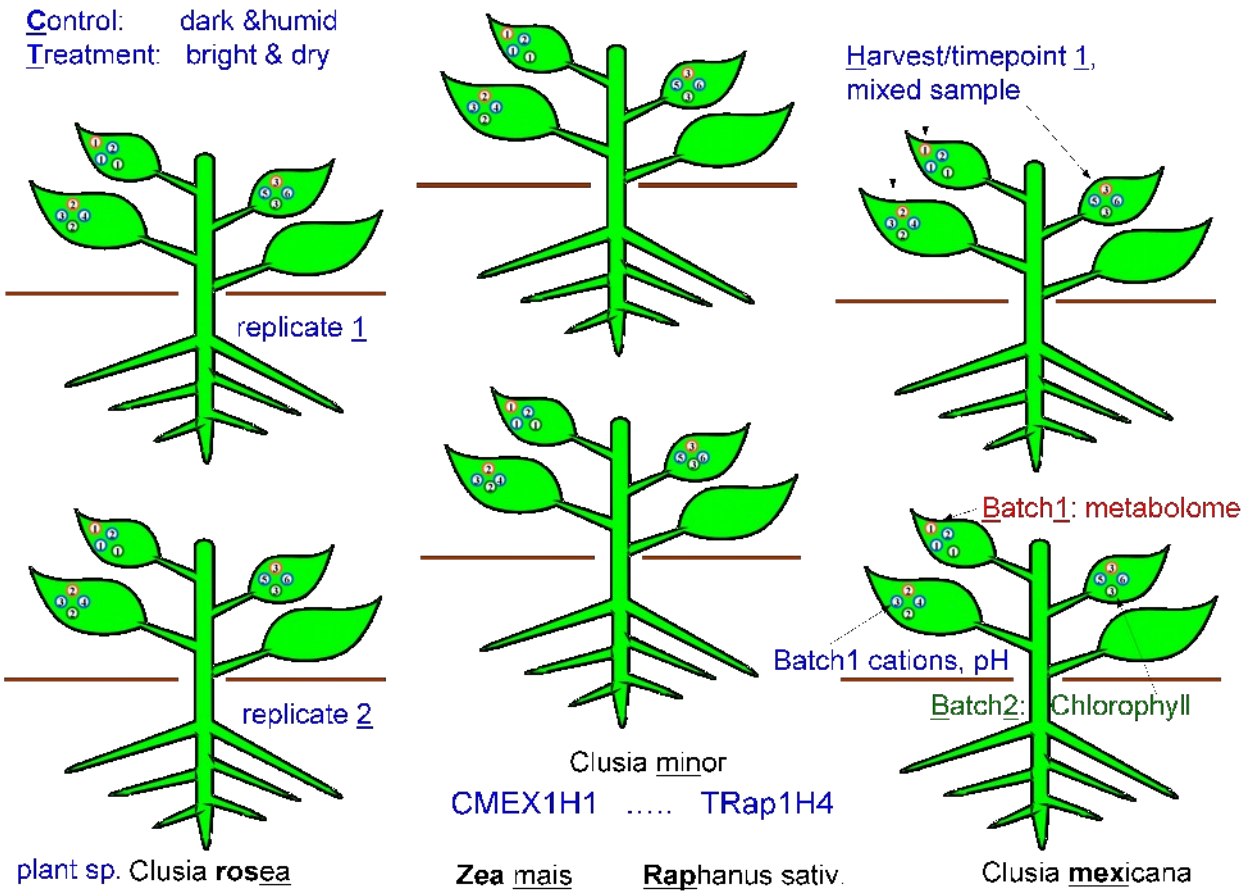


Detailed Programme:

MONDAY 07.05	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 08.05	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 09.05	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 10.05	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 11.05		Sample processing Analysis in Mosys Lab

MONDAY 14.05	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 15.05	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 16.05	morning	*Data evaluation:*	GCMS Cation HPLC Titration Chlorophyll photometer Statistics	
	afternoon	*Data evaluation:*	GCMS Cation HPLC Titration total acidity Chlorophyll photometer Statistics	
THURSDAY 17.05	morning	final statistics, graphics		
	afternoon	Final evaluation, prep. of protocols (3 presentations, each group)		
FRIDAY 18.05	Reserve	Final Presentation		

Harvest Method and Sample Labels: long Acronym on tubes, Number on Vials



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 4 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 mexicana		2	2	
clusia minor		2	2	
zea mais		2	2	
tropaeolum minus		2	2	
Sampling Roster:				
microclimate			recordings (24h)	
locations		2		
measuring days		2	4	
gas metabolism			Recordings (6)	
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^+	H1-4, 2 day, 2 night, 2 rep	8	48	
chlorophyll			Photometry_samples	
species		5		
treatment		2		
sampling	1 day H1, 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

Ecophysiology

Monitoring: at **every harvest** (H1-H4)

Air Temperature and Humidity	Thermo/Hygrometer, Laser Thermometer
Soil Humidity:	DeltaT ML3 Theta Probe
Microclimate and Quantum Yield	Photosynq MultispeQ,
Relative Chlorophyll Content:	Minolta SPAD,
Photoystem II Quantum Yield:	Hansatech PEA,
Porometry/Stomatal Conductance:	DeltaT AP4
Light response curve:	WALZ MiniPamII

Demo: Imaging PAM, Walz GFS3000 IRGA + leaf chamber, Delta T GP2 Datalogger

Sampling for Chlorophyll Photometry:

Only **on H1**(11:00):
cork driller, brown glass Flasks 5mL, 5 ml DMF, Photometer: UV glass cuvettes (10mm),
mixed sample of 3 discs from 3 leaves , 1 disc per leaf, **all** 24 Plants

Sampling for GCMS of Metabolome and HPLC of Kations :

Every Harvest 15 mL FalconTubes, see SOP
mixed sample of 4 discs, from 4 leaves (1 disc per plant leaf) **only Clusia** Sp.(12 Plants)

Plant Leaf Anatomy:

cross sections, stomatal imprints
one sample per Species

Further material:
PSE (personal safety equipment) Lab Gloves, Lab spectacles,
Lab coat to be used at all times in the Lab

A5 Lab protocol, fine black Permanent Markers, Laptops
Al monitoring results will be stored and left in the lab!

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