



„Perspectives and challenges for VOC phenotyping in plants“

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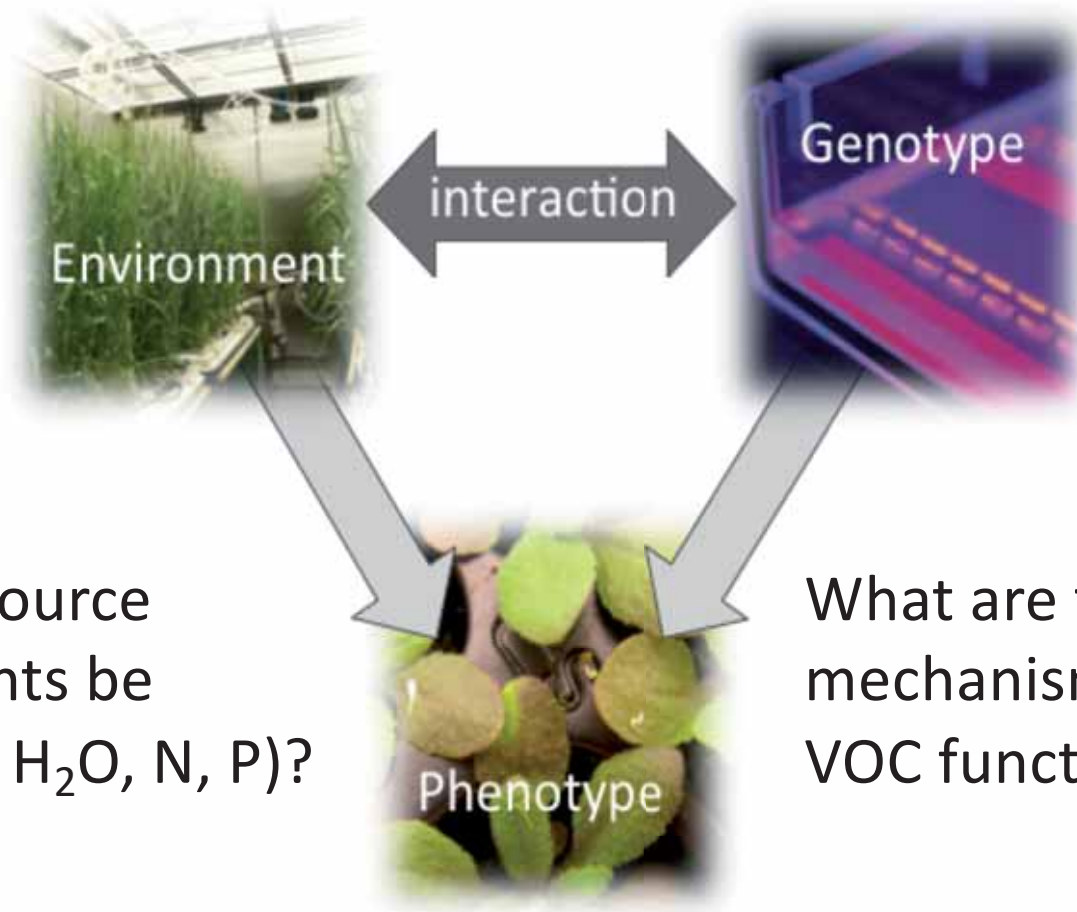
05.-.06. 09. 2018 | The Italian plant phenotyping landscape and the other international initiatives

The Munich phytotron facilities



Our research interests.....

How does biotic (microbe/fungus/herbivore -plant) interactions change with changing climate?



How can the resource efficiency of plants be improved (use of H₂O, N, P)?

What are the underlying mechanisms of biogenic VOC functioning?

GM plants

Natural phenotypes

VOC-based phenotyping in plants, fungi, & humans



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Chapter (Book)

Volatile Organic Compounds (VOCs) for Noninvasive Plant Diagnostics

Alexander A. Aksenov¹, Ana V. Guaman Novillo², Sindhuja Sankaran³, Alexander G. Fung¹, Alberto Pasamontes¹, Frederico Martinelli⁴, William H. K. Cheung¹, Reza Ehsani³, Abhaya M. Dandekar⁵, Cristina E. Davis^{*1}

PDF (489 KB) | PDF w/ Links (452 KB) | Full Text HTML

Fungal Genetics and Biology 54 (2013) 25–33

Contents lists available at SciVerse ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Basanta et al. *Respiratory Research* 2012, **13**:72
<http://respiratory-research.com/content/13/1/72>

RESPIRATORY RESEARCH

RESEARCH Open Access

Exhaled volatile organic compounds for phenotyping chronic obstructive pulmonary disease: a cross-sectional study

María Basanta¹, Baharudin Ibrahim¹, Rachel Dockry¹, David Douce², Mike Morris², Dave Singh¹, Ashley Woodcock¹ and Stephen J Fowler^{1,3*}

OPEN ACCESS Freely available online

Profiling of Volatile Organic Compounds in Exhaled Breath As a Strategy to Find Early Predictive Signatures of Asthma in Children

Agnieszka Smolinska^{1,2*}, Ester M. M. Klaassen³, Jan W. Dallinga¹, Kim D. G. van de Kant³, Quirijn Jobsis³, Edwin J. C. Moonen¹, Onno C. P. van Schayck⁴, Edward Dompeling³, Frederik J. van Schooten¹

¹Department of Toxicology, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, Maastricht, The Netherlands. ²Top Institute Food and Nutrition, Wageningen, The Netherlands. ³Department of Pediatric Pulmonology, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC), Maastricht, The Netherlands. ⁴Department of General Practice, School for Public Health and Primary Care (CAPHRI), Maastricht University Medical Center (MUMC), Maastricht, The Netherlands.

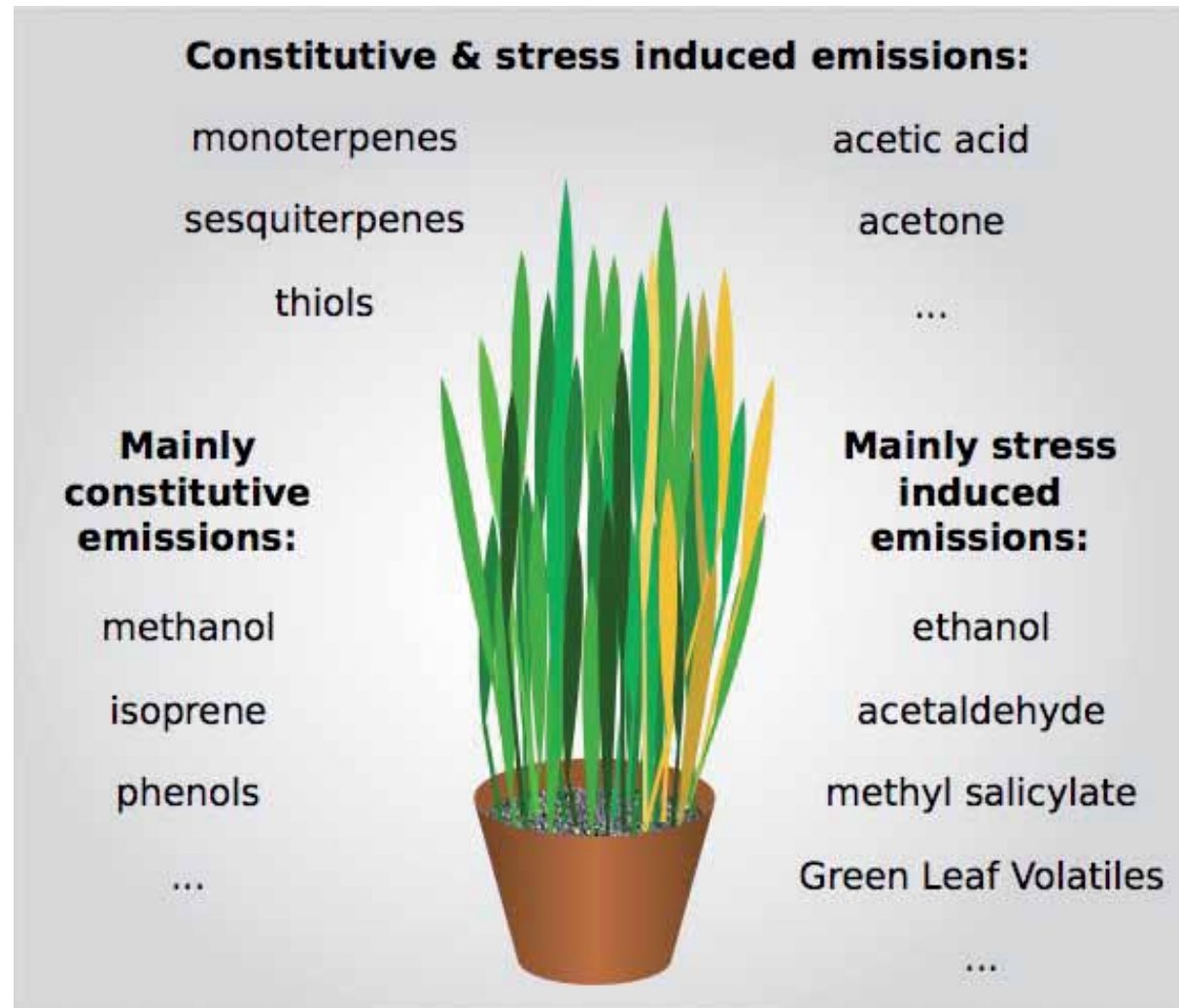
Volatile profiles of fungi - Chemotyping of species and ecological functions

Anna Müller^{a,c}, Patrick Fauber^{b,1,2}, Michael Hagen^c, Wolfgang zu Castell^c, Andrea Polle^a, Jörg-Peter Schnitzler^b, Maaria Rosenkranz^{b,*}

^aBüsgen Institute, Forest Botany and Tree Physiology, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
^bResearch Unit Environmental Simulation, Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Ingolstädter Landstraße 1, 85764 Neuherberg, Germany
^cResearch Unit Scientific Computing, Institute of Biomathematics and Biometry, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany



Typical VOCs released under stress



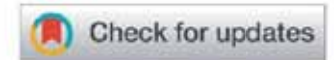
Jud et al, under revision



Importance of VOCs in plant-pathogen/pest-interaction

- involved in direct (e.g. ROS quenching) and indirect defense (repellent/ attractant) against insect pests
- messengers of plant-plant communication
- priming agents to activate plant defense responses
- variable profiles from

The Plant Cell, Vol. 29: 1440–1459, June 2017, www.plantcell.org © 2017 ASPB.



Monoterpenes Support Systemic Acquired Resistance within and between Plants

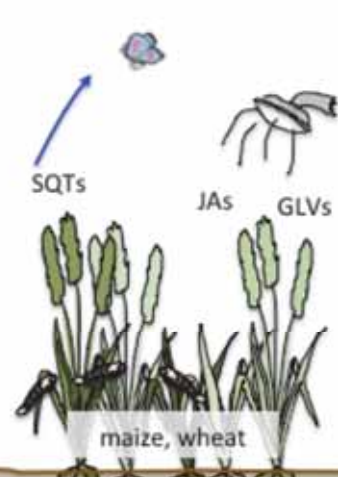
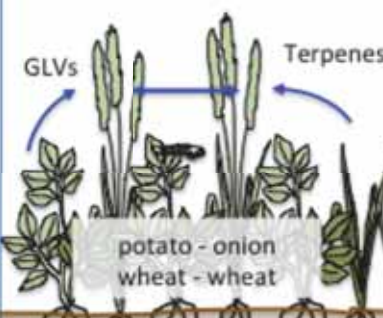
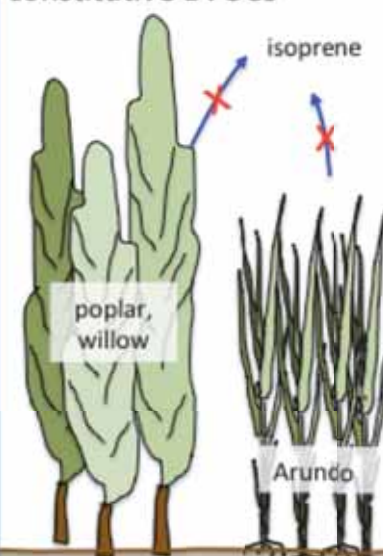
Marlies Riedlmeier,^{a,1} Andrea Ghirardo,^{b,1} Marion Wenig,^{a,1} Claudia Knappe,^a Kerstin Koch,^b Elisabeth Georgii,^a Sanjukta Dey,^a Jane E. Parker,^c Jörg-Peter Schnitzler,^b and A. Corina Vlot^{a,2}

Ideal non-invasive

- Online monitoring of pathogenesis (susceptibility/resistance)
- Identification of chemotypes
- Monitoring of priming effects
- Analysis of cross-talk between microbes, fungi, insect pest
- Determination of C-fluxes under pathogenesis (¹³C labelling)



Potential applications of VOCs.....

Applications of BVOCs in smart agriculture			
	Monocultures	Co-cultivation systems	Bioenergy plantations
Aim of treatment	Direct priming; Plant-to-plant signalling; attraction of beneficials	Plant-to-plant signalling; attraction of beneficials;	Removal/ downregulation of constitutive BVOCs
Examples	 <p>maize, wheat</p>	 <p>potato - onion wheat - wheat</p>	 <p>poplar, willow</p> <p>Arundo</p>
Methods	<ul style="list-style-type: none"> • VOC elicitor spraying (e.g. MeJA, GLVs) • GM crops (e.g. SQTs over-expression) 	<ul style="list-style-type: none"> • Selection of emitter types (e.g. volatile terpenoids) 	<ul style="list-style-type: none"> • Phenotyping for low emitters (e.g. isoprene) • GM plants (e.g. isoprene knockouts)

Rosenkranz et al. (PC&E 2015)



Comparison of various types of plant disease detection

Challenges

Characteristics	Molecular techniques	Imaging and spectroscopic techniques	VOCs profiling-based techniques
Accuracy of the method	Presently the most accurate method	Accuracy is plant and disease specific	Unknown accuracy since actual under development
Costs	Moderately expensive.	Expensive (i.e. hyperspectral imaging).	Cost depends on the desired accuracy for VOC profiling.
Applicability for rapid detection	Not fast for a huge amount of samples.	Allows rapid detection of disease symptoms	Shows the potential for rapid plant disease detection.
Applicability for field work/Ruggedness	Field kits are being developed	Moderately ruggedness	Moderately rugged, depending on VOCs detection
Speed of detection	May require 24 - 48 h for reliable results	May require minutes for disease detection	May require less time, if proven as an effective method for a particular disease

Computers and Electronics in Agriculture 72 (2019) 1–13

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Computers and Electronics in Agriculture

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Review

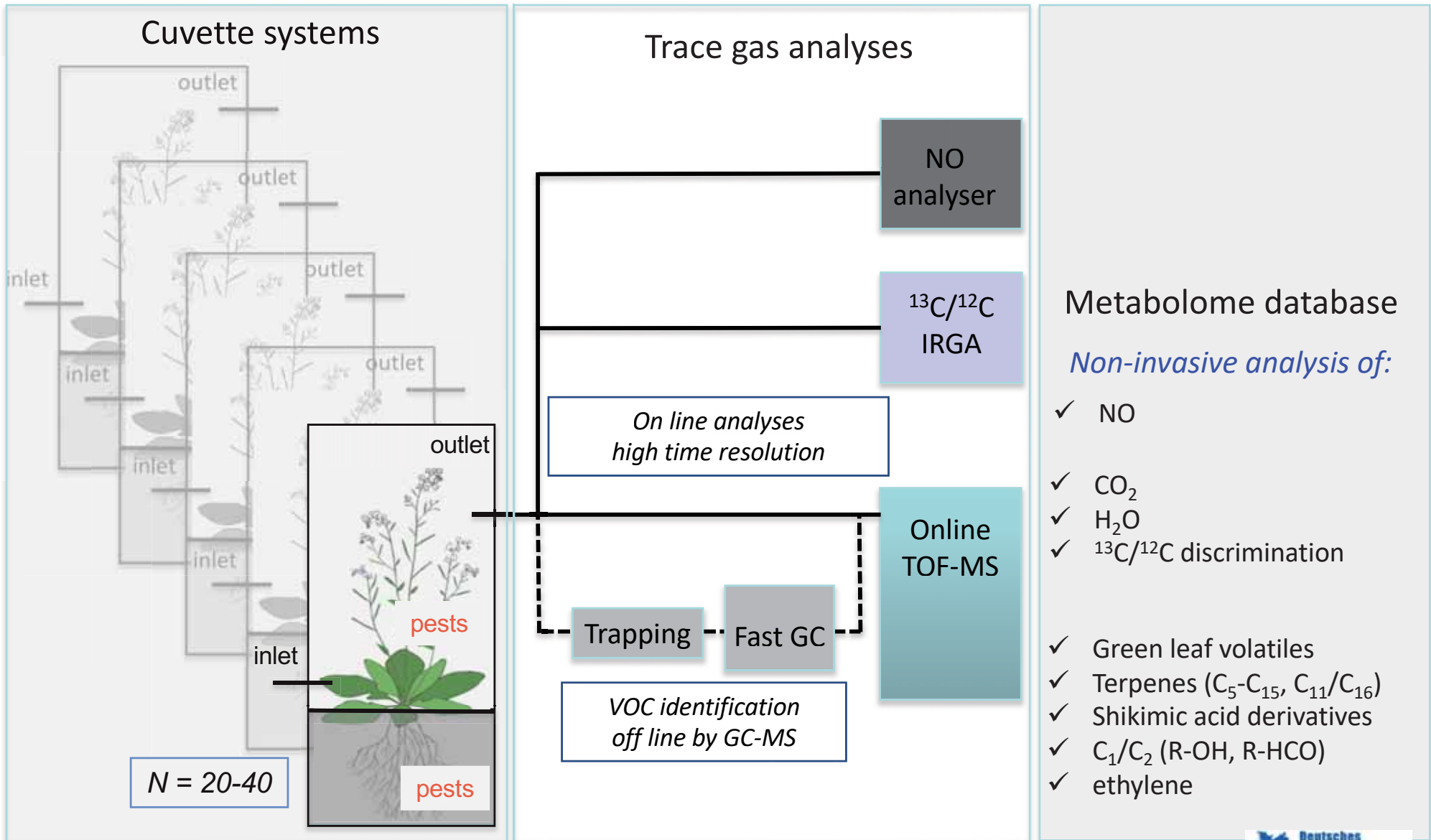
A review of advanced techniques for detecting plant diseases

Sindhuja Sankaran^a, Ashish Mishra^a, Reza Ehsani^{a,*}, Cristina Davis^b

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^b Department of Mechanical and Aerospace Engineering, University of California, One Shields Avenue, Davis, CA 95616, United States



Concept of a VOC and gas exchange phenotyping platform



Challenge of cuvette design



Problems

Homogeneous air flow
Gas tightness
Rapid gas turnover
No H₂O condensation
Inertness of materials
High light permeability
Minimizing VOC absorption
VOC-free inlet air
No overheating

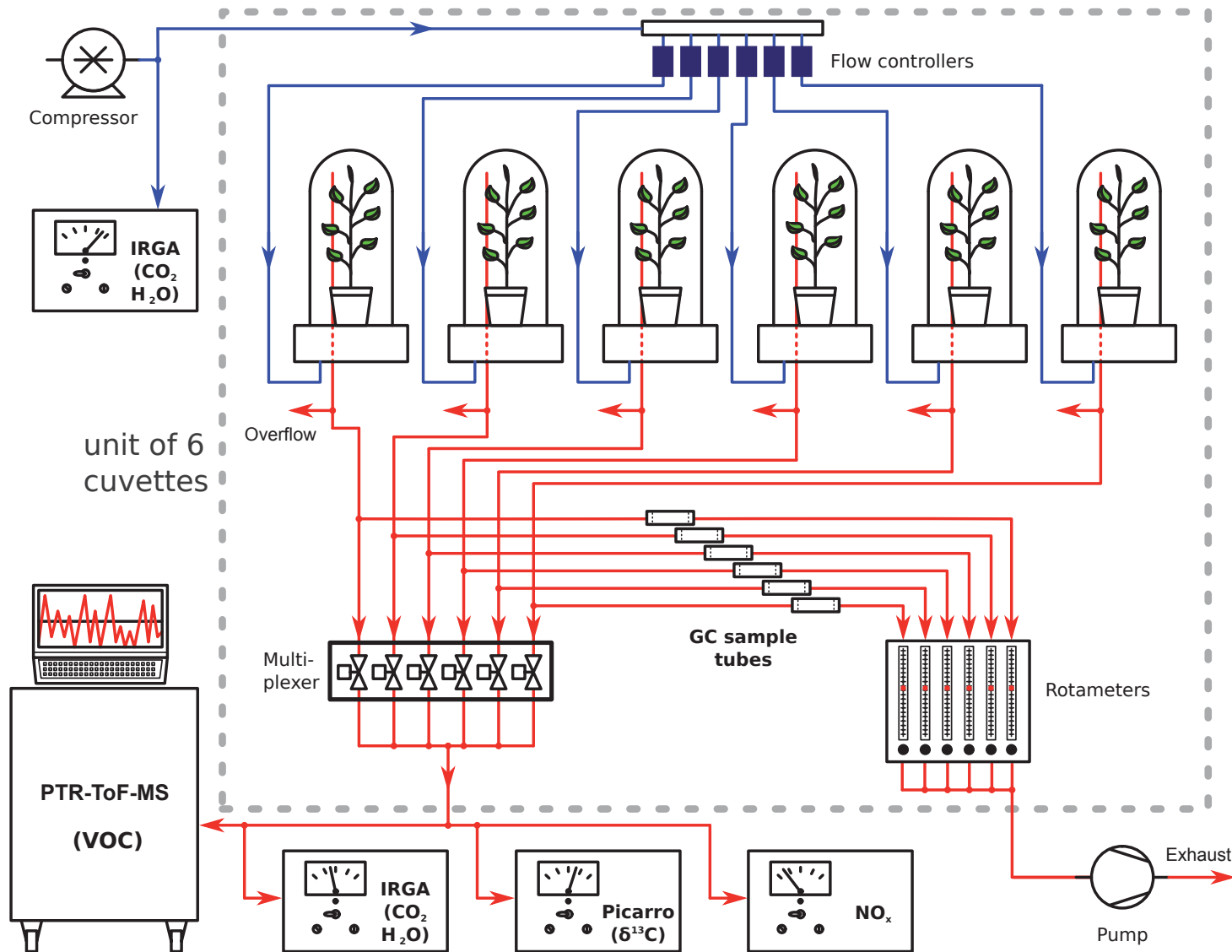
Solutions

- ✓ Circular gas inlet
- ✓ VOC-free sealing
- ✓ High air flow (20L/min)
- ✓ Line heating
- ✓ Stainless steel / teflon
- ✓ Glass
- ✓ Steel / PEEK tubings

- ✓ Catalyst
- ✓ Cooling of chamber



Modular system of cuvette blocks



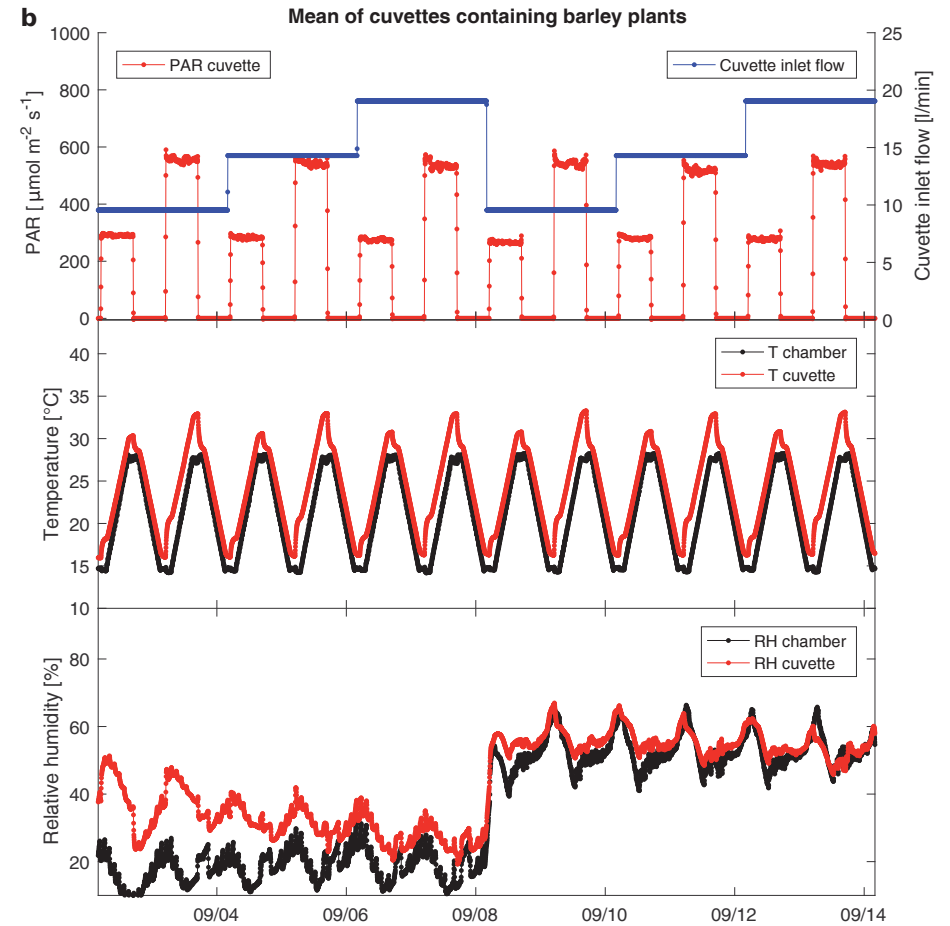
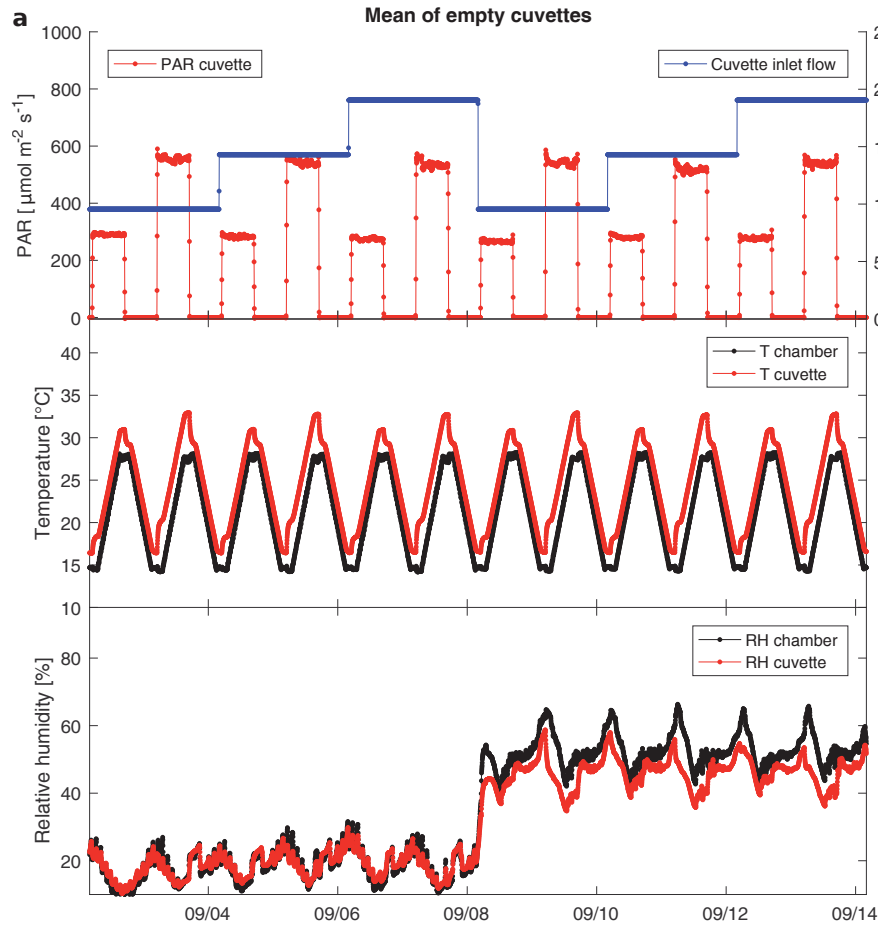
Construction of a 24 cuvette platform



Jud et al, under revision



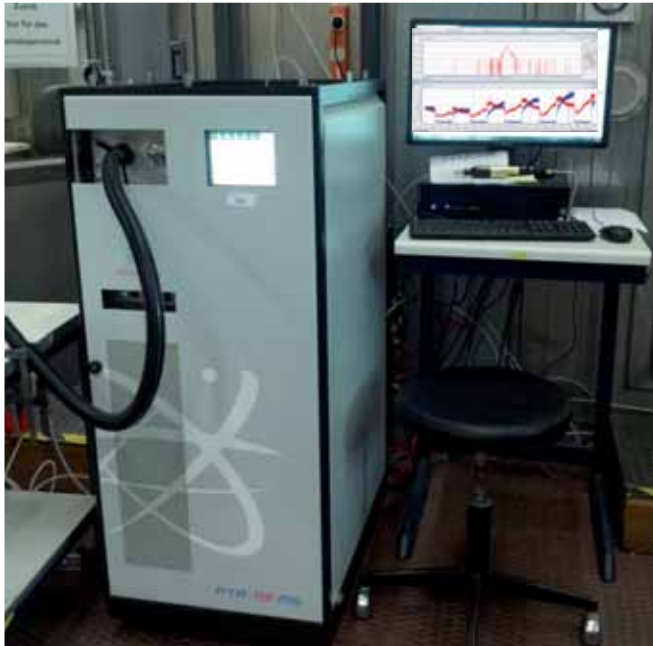
Characterization of cuvette dynamics



Jud et al, under revision



Non-targeted volatilomics by online Proton Transfer Reaction Mass Spectrometry (PTR-TOF MS)

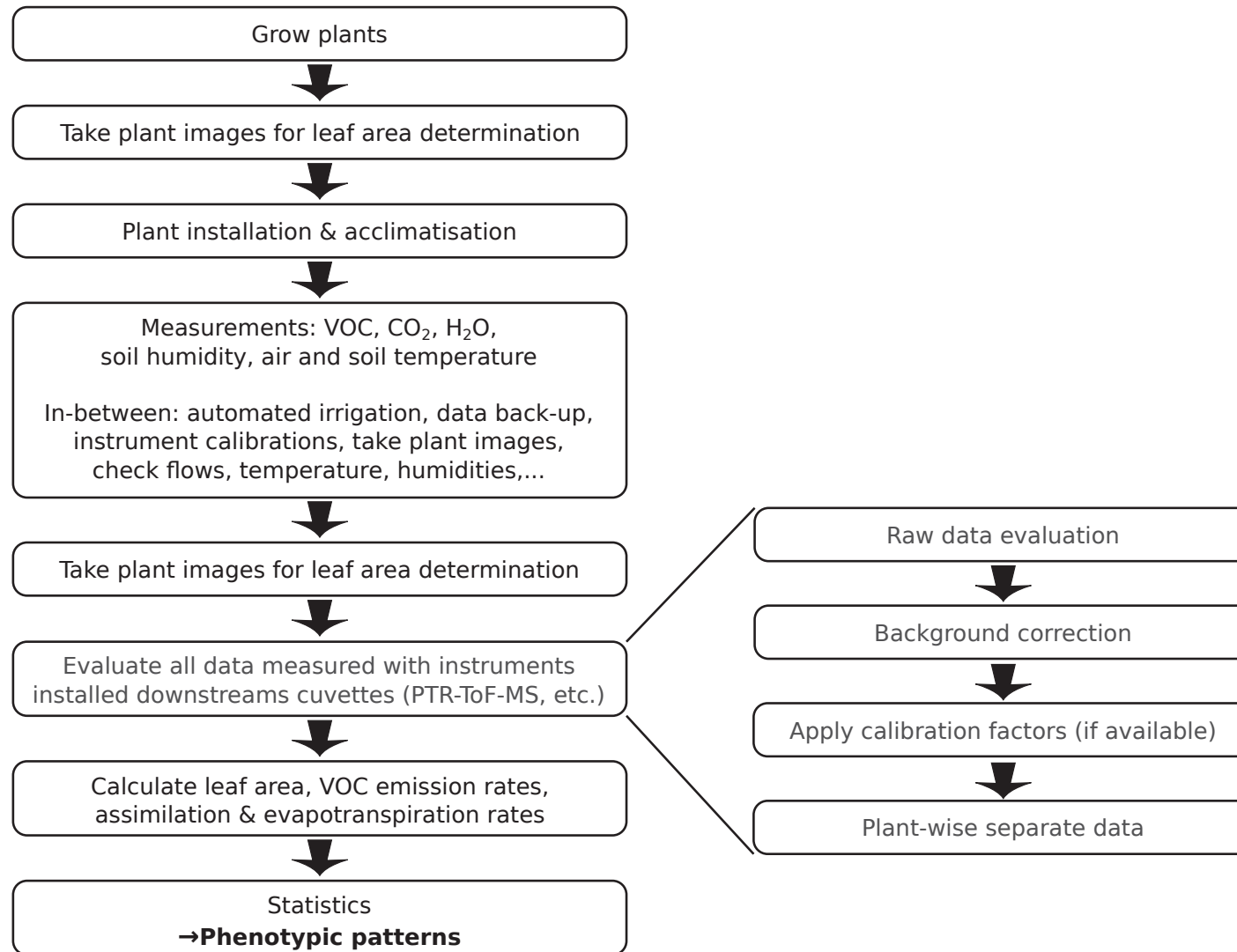


- 1) allows for online measurement of VOCs at concentrations as low as a few ppt
no need for pre-concentration
- 2) soft ionization (little fragmentation) of virtually all VOCs via proton transfer from H_3O^+ ions:

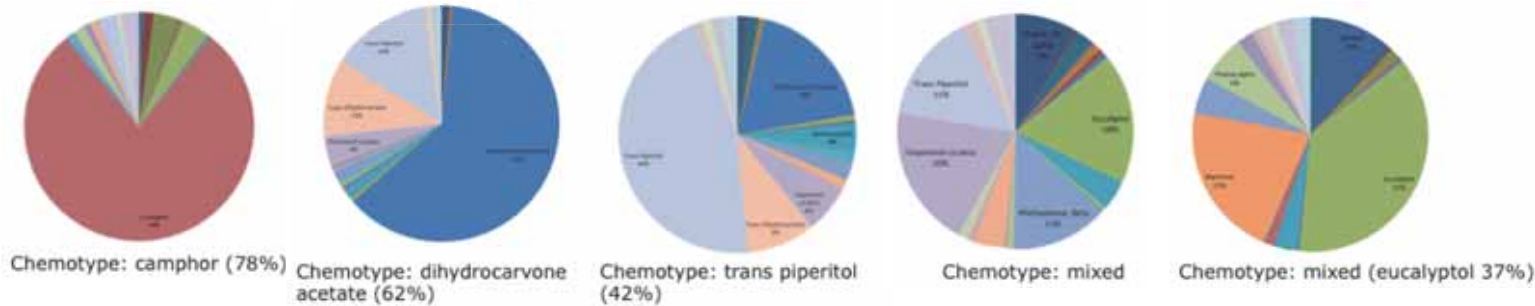
Proton Transfer Reaction



Workflow for plant volatilome analyses



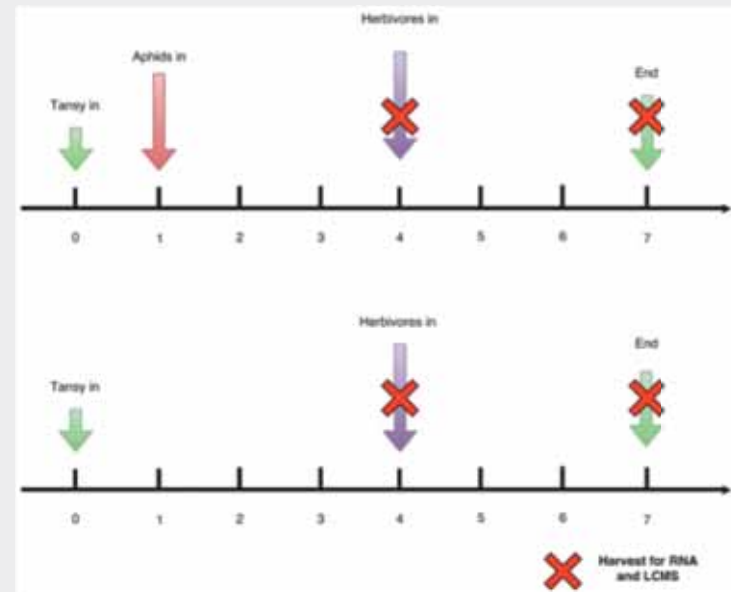
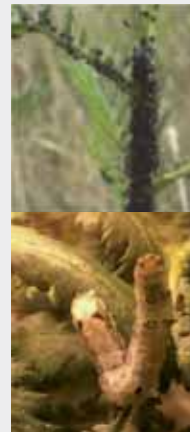
1st Proof of principle experiment with Tansy terpenoid chemotypes



Testing the VOC profiles of 5 chemotypes following subsequent treatments by

Aphids: *Metopeurum fuscoviride*
 &
 Herbivore: *Spodoptora littoralis*

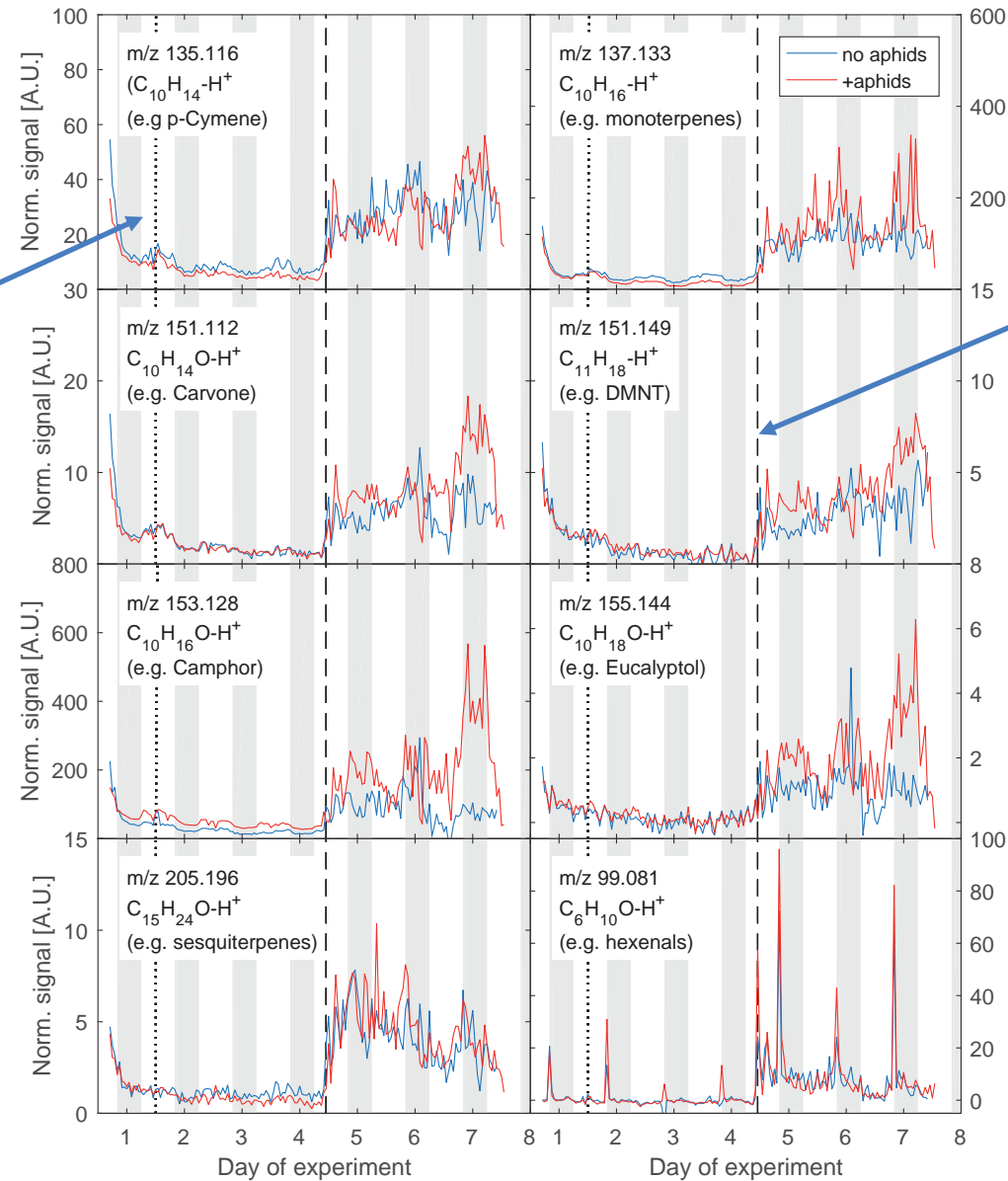
Clancy, Jud et al, in preparation



Profiling of VOC emissions in tansy following herbivory

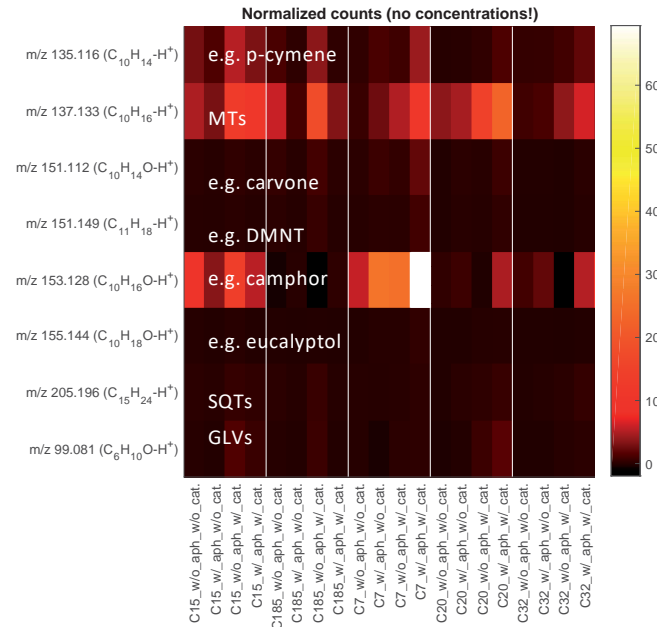
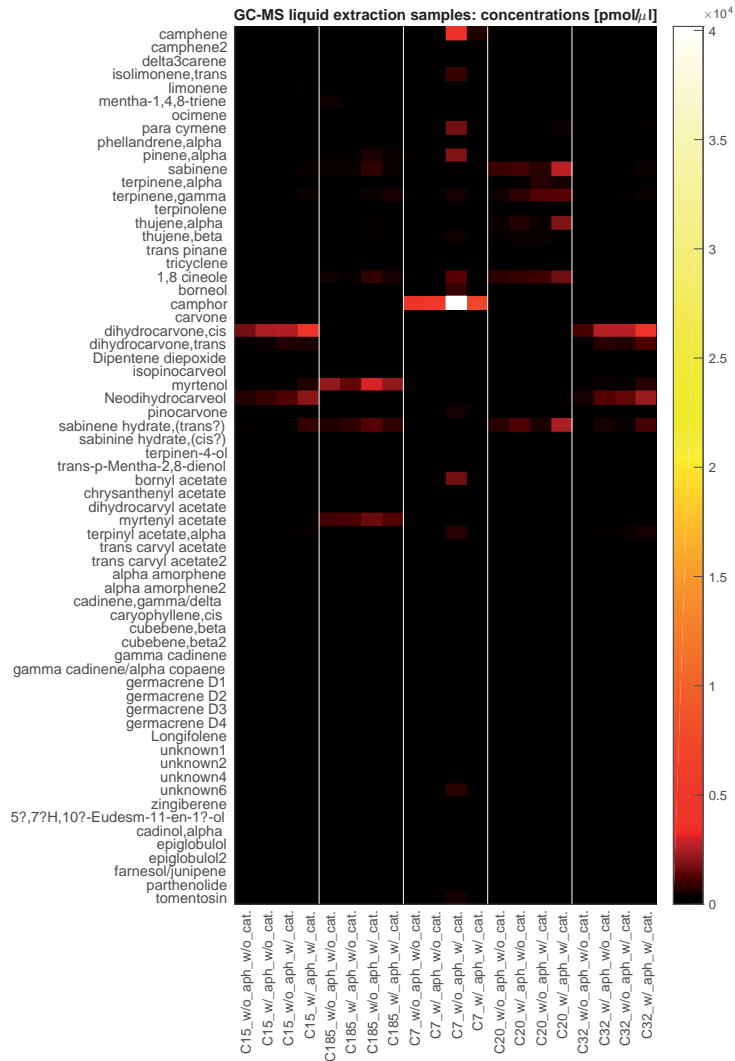
Addition of aphids

Addition of caterpillar larvae

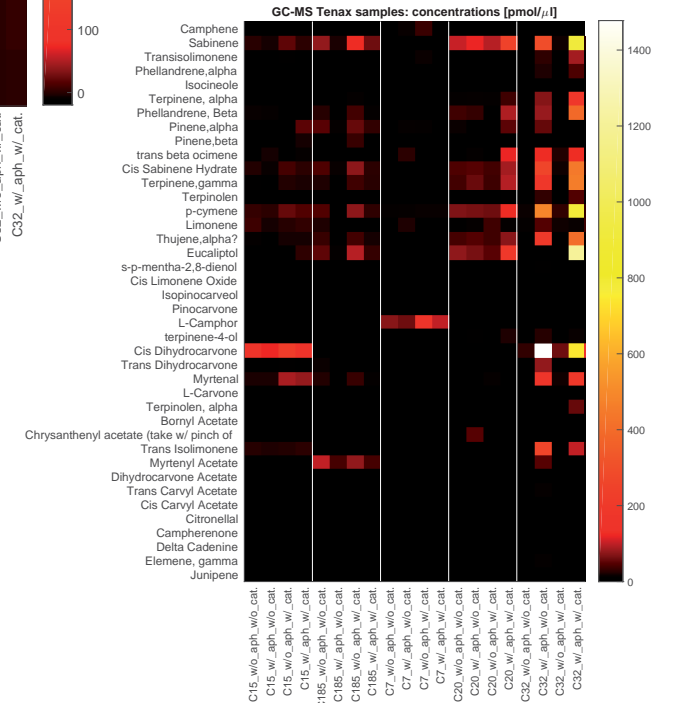


Profiling of volatilomes in tansy following herbivory

Stored terpenoids



Offline GC-MS VOC analysis



Online VOC analysis by PTR-TOF MS

Challenges.....

- Cuvette design and platform construction require indepth experience in gas exchange analysis and analytics
- Platform software for automatisisation and analysis tools must be developed in your own
- Physical properties (air mixing, tightness, exchange time, line effects, etc...) of the cuvettes must be characterized precisely
- Data analysis of online mass spectrometry (also offline GC-MS) requires automatisisation including quality checks
- High maintenance of the system is required

BUT finally it works nicely for low emitting crops 😊

24-cuvette system is operational (for interest please contact)

<https://www.helmholtz-muenchen.de/eus/index.html>



Thanks to.....



Werner Jud

Bishu Niederbacher



Jana B. Winkler

