PRO-GRACE
PROMOTING A PLANT GENETIC RESOURCE COMMUNITY FOR EUROPE

EMPHASIS

Euroseeds
Embracing Nature

CSIC
Consejo Superior de Investigaciones Científicas

Joint PRO-GRACE/EMPHASIS policy symposium and workshop about plant genetic resources and phenotyping

VOLATILOME PROFILING AS A NEW TOOL FOR SEED PHENOTYPING

Phen Italy
THE ITALIAN PLANT PHENOTYPING NETWORK

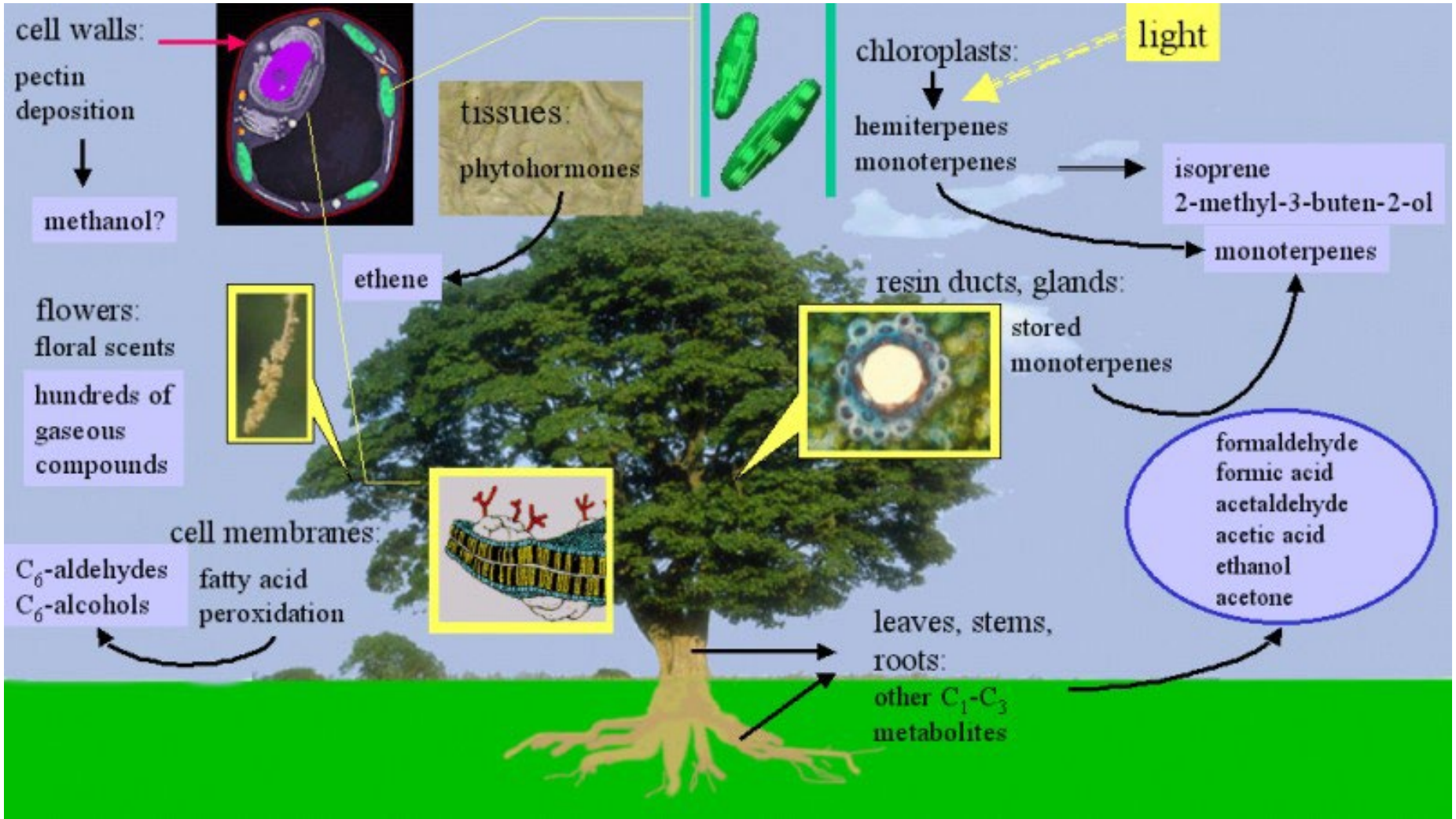
Dr. Michelina Ruocco

EMPHASIS

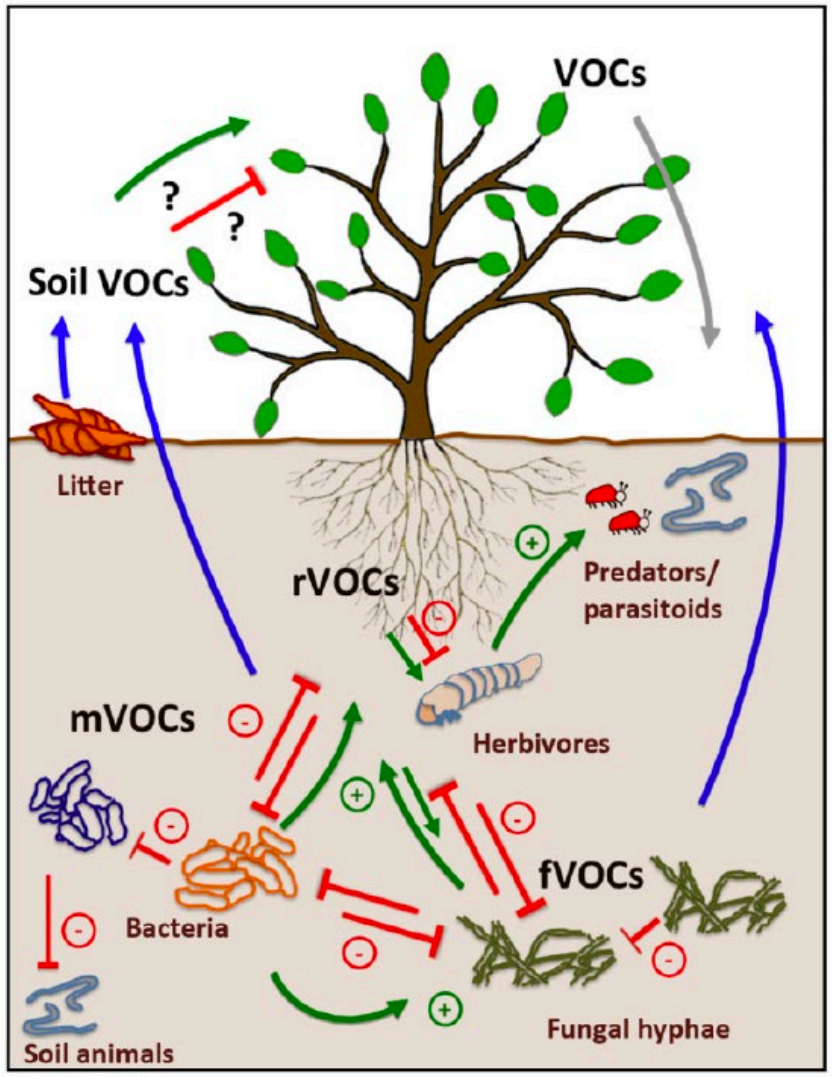
Consiglio Nazionale delle Ricerche | Istituto per la Protezione Sostenibile delle Piante
Sede Secondaria Portici

IPSP

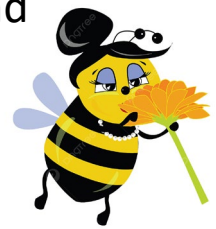
bVOCs, OR BIOGENIC VOLATILE ORGANIC COMPOUNDS, ARE A SUBSET OF VOCs EMITTED BY LIVING ORGANISMS, PRIMARILY PLANTS, BUT ALSO INCLUDING FUNGI, BACTERIA, AND ANIMALS. THESE COMPOUNDS ARE NATURALLY OCCURRING AND ARE RELEASED INTO THE ATMOSPHERE THROUGH VARIOUS BIOLOGICAL PROCESSES



Sources: The primary sources of bVOCs are plants, which emit these compounds through their leaves, flowers, and other parts. Other sources include soil microorganisms, fungi, and marine organisms.



Types of Compounds: Common bVOCs include isoprene, monoterpenes, sesquiterpenes, methanol, acetone, and various alkanes, alkenes, and alcohols.



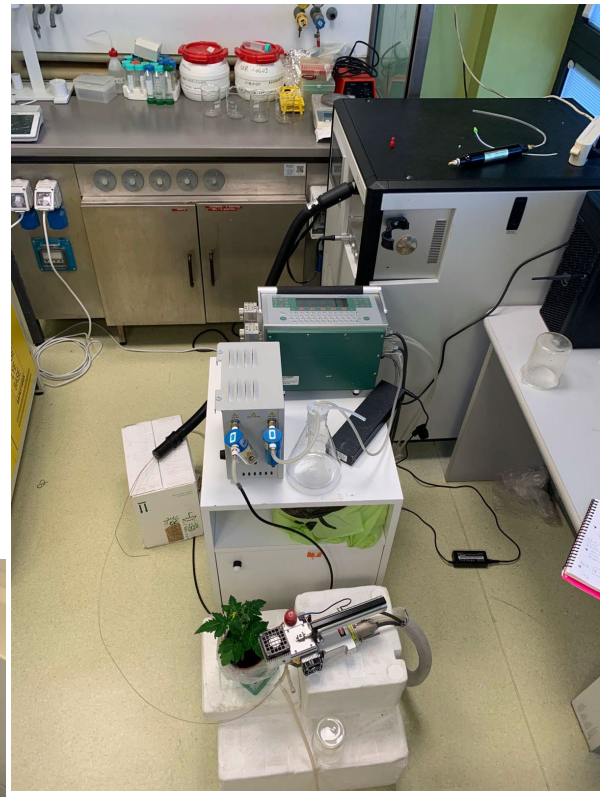
Ecological Roles: bVOCs serve multiple ecological functions, such as:

- **Attracting Pollinators**: Many plants emit specific bVOCs to attract pollinators to their flowers.
- **Defense Mechanisms**: bVOCs can help protect plants from herbivores and pathogens by deterring pests or by attracting predators of the herbivores.
- **Plant Communication**: Plants can release bVOCs to communicate with each other, warning nearby plants of potential threats and triggering defensive responses.

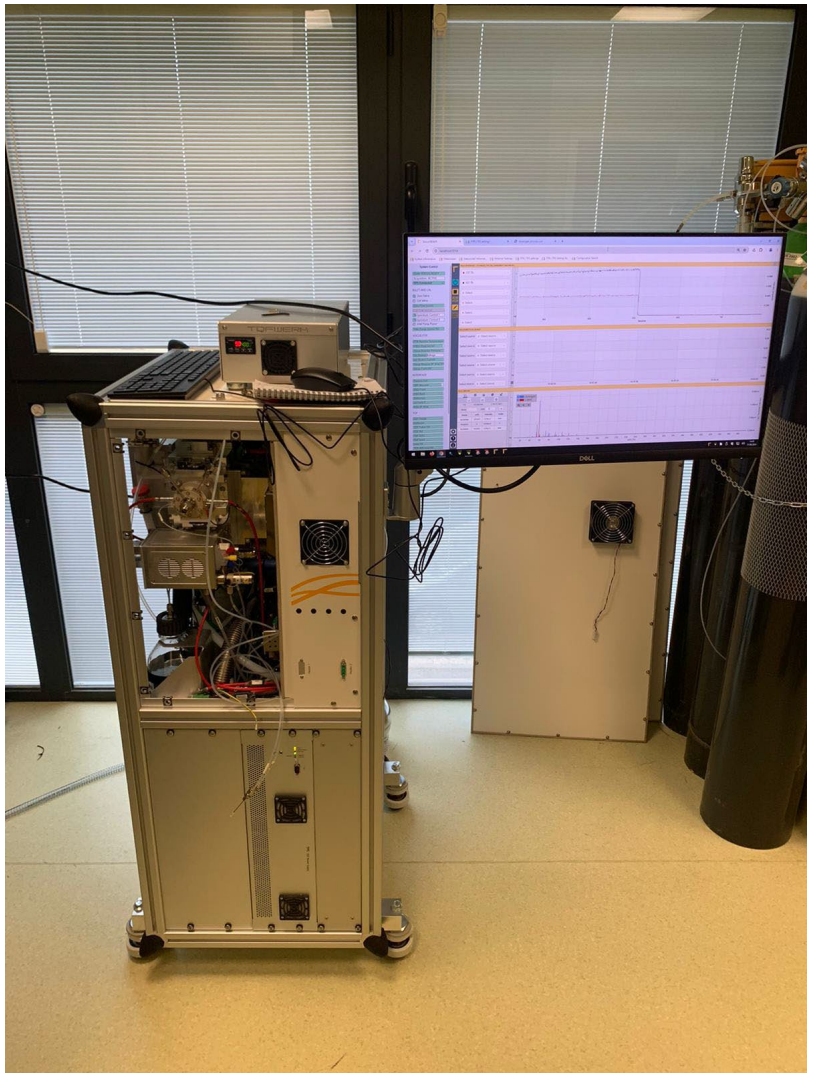
Proton Transfer Reaction Time-of-Flight Mass Spectrometry

- This modern technique is used for real-time monitoring of VOCs
- It is highly sensitive and can detect low concentrations of VOCs (parts per trillion volume [pptv])
- In air and gas samples providing a rapid, non-invasive fingerprinting of VOCs concentrations

INFRAVOL INFRASTRUCTURE

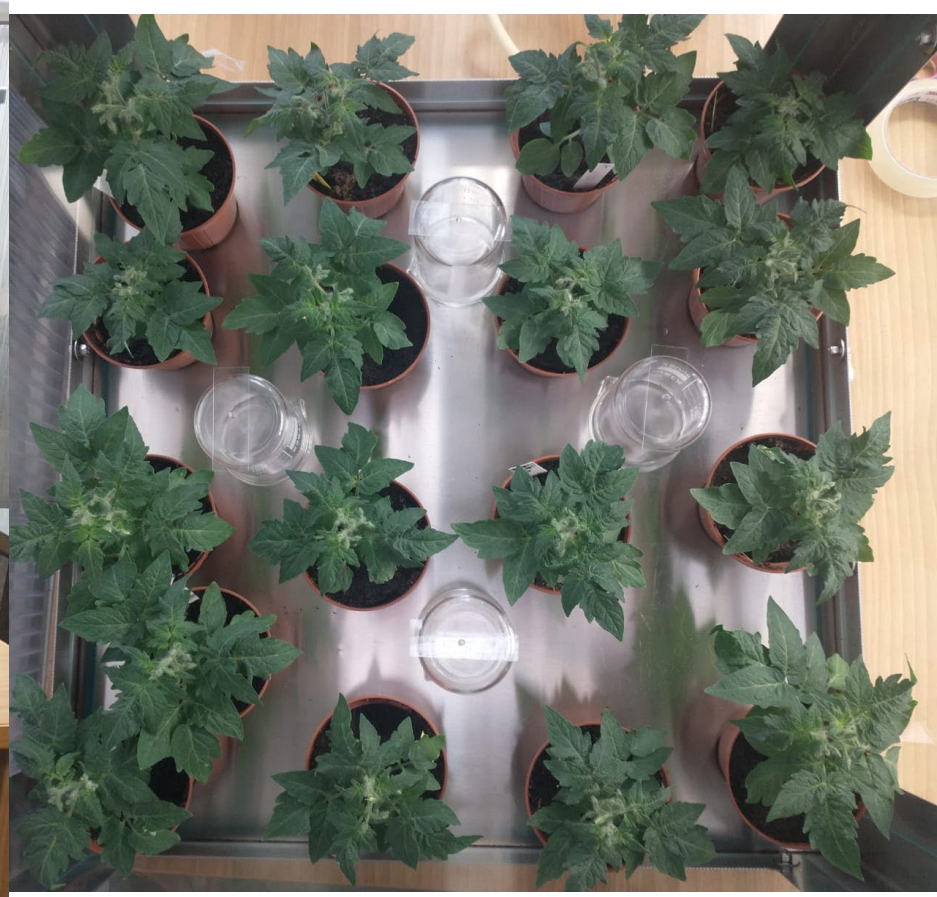


PTR-TOF-MS



Vocus-Ci-TOF

CHACKING FOR PLANT-PLANT PRIMING EFFECT WITH A SINGLE SPECIFIC VOC



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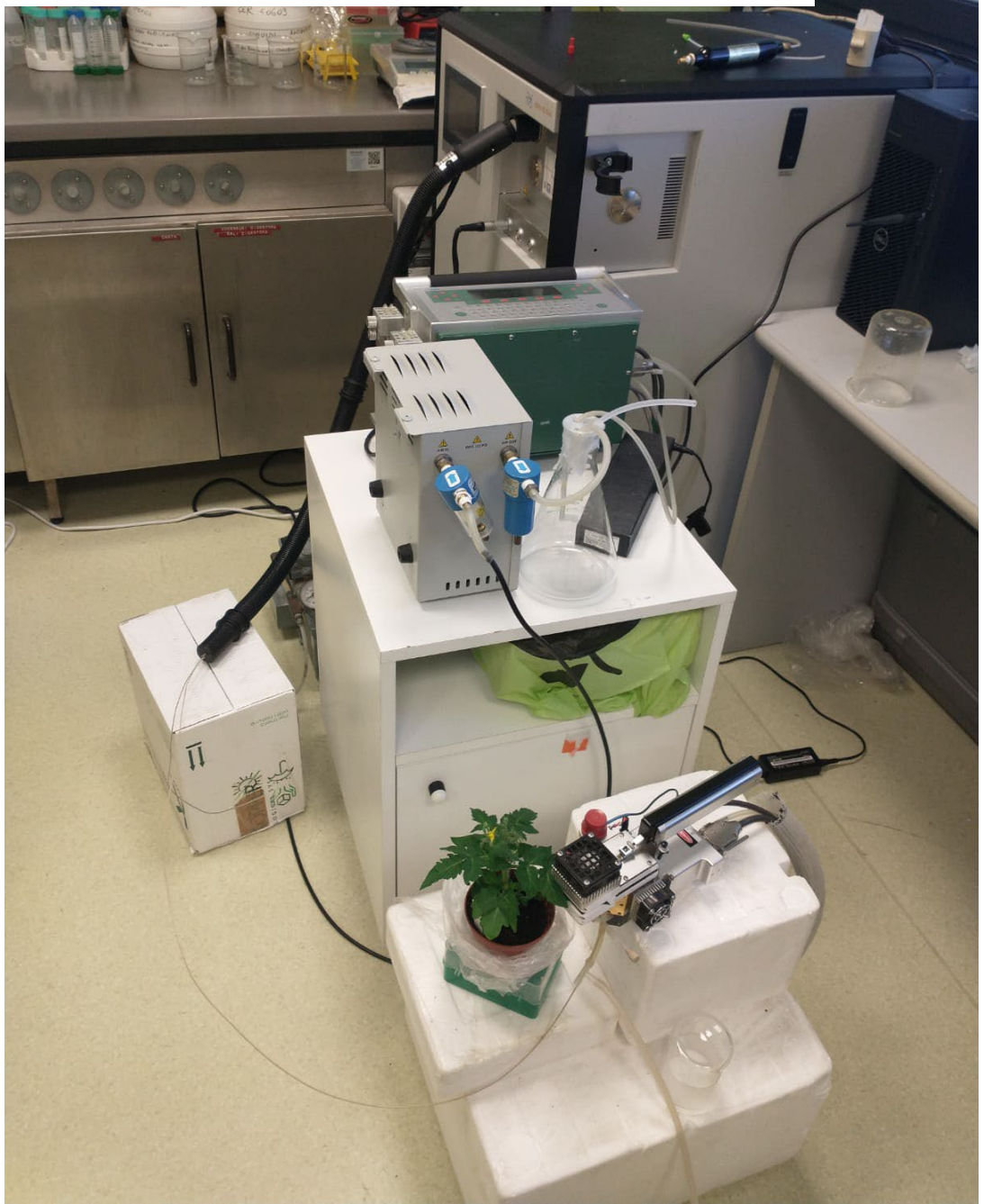
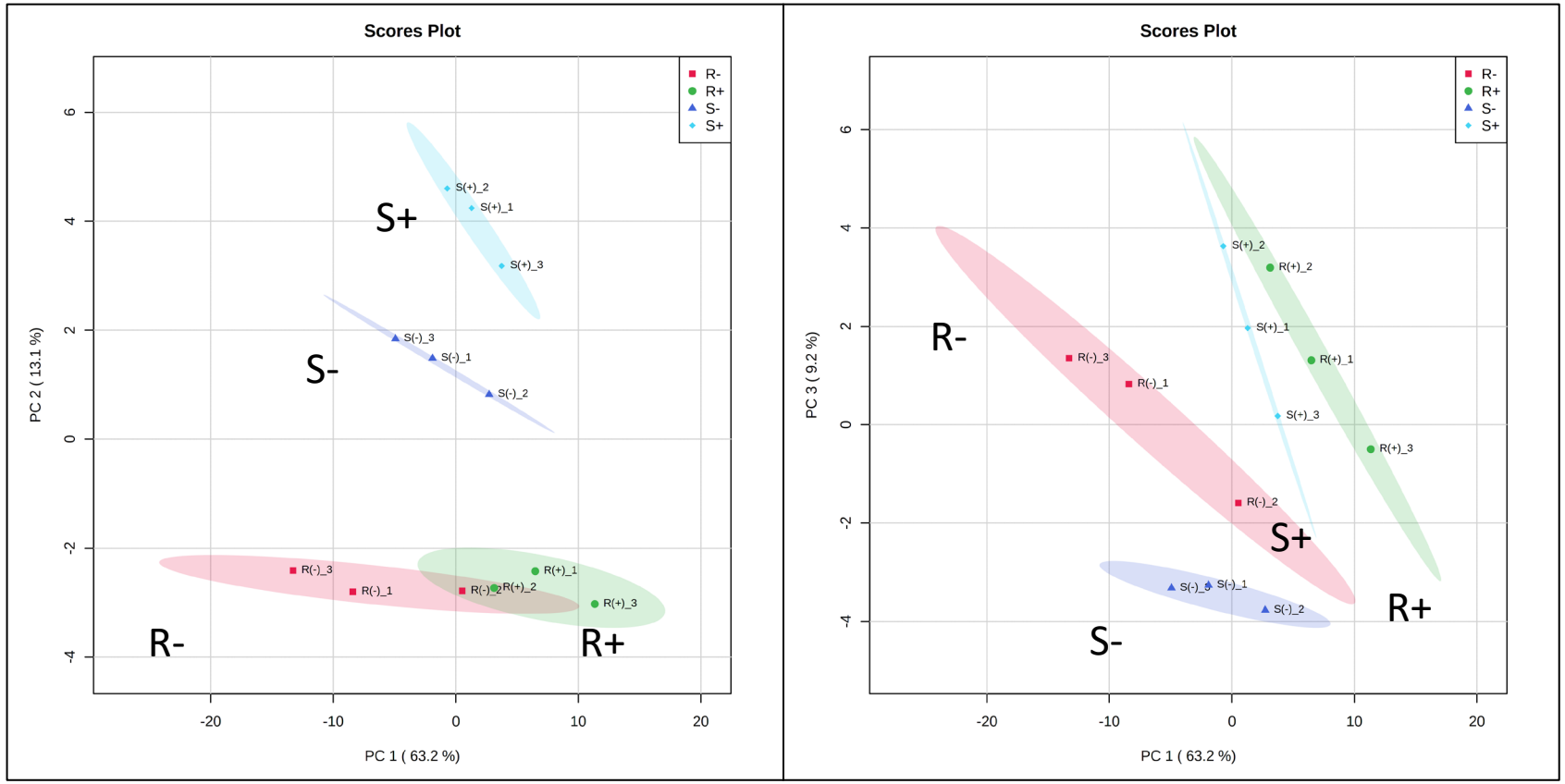
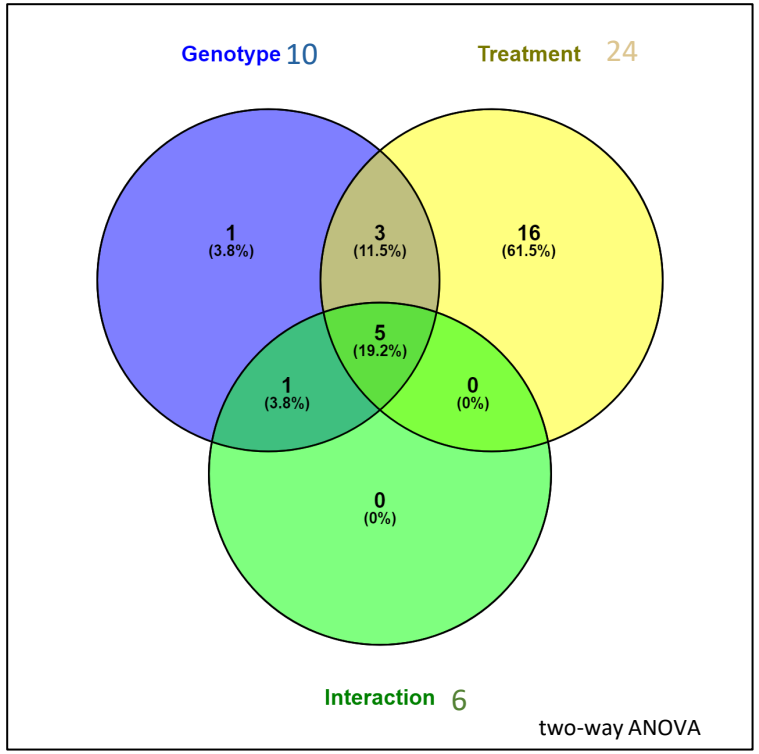


VOCs BLEND DATA ANALYSIS

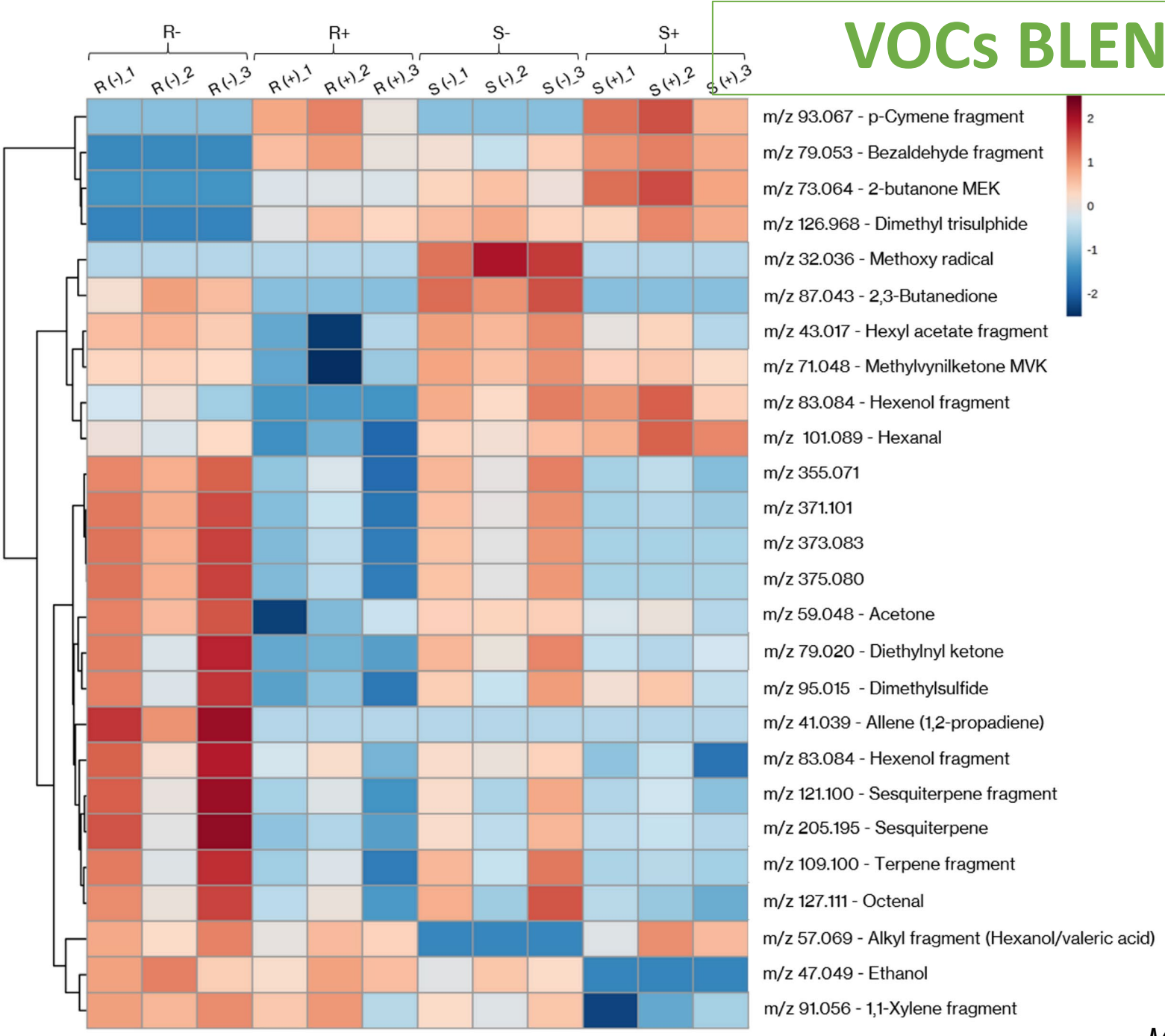


Identified VOCs, in genotype, treatment (*Colletotrichum* infection), or interaction-dependent groups



Principal component analysis (PCA; Metaboanalyst V5.0)

VOCs BLEND DATA ANALYSIS



Total emitted VOCs:

R- > R+ = S- = S+

5.13 > 3,65 = 3,97= 3,98 (ppb g^{-1}fw)

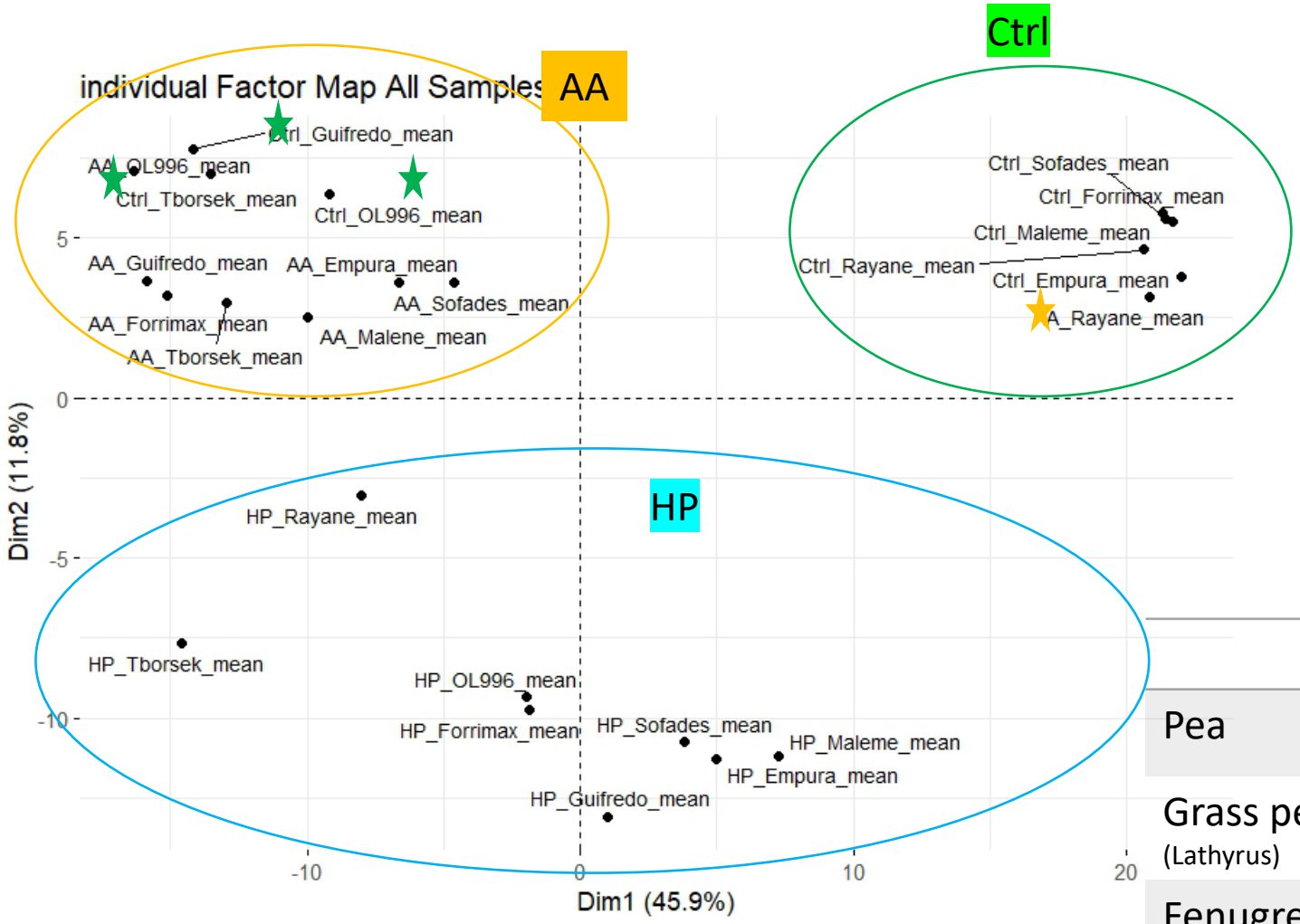
lower emission of volatiles in R+ could be due to **a stomatal closure in response to pathogen invasion**, reducing diffusion of VOCs outside leaves

two proteins (Munc-13-like and phototropin 1) which play fundamental roles in stomatal opening, **accumulated more in S+ than in R+ plants**:

Monti et al., 2023 Physiologia Plantarum. 2023;175:e14044.

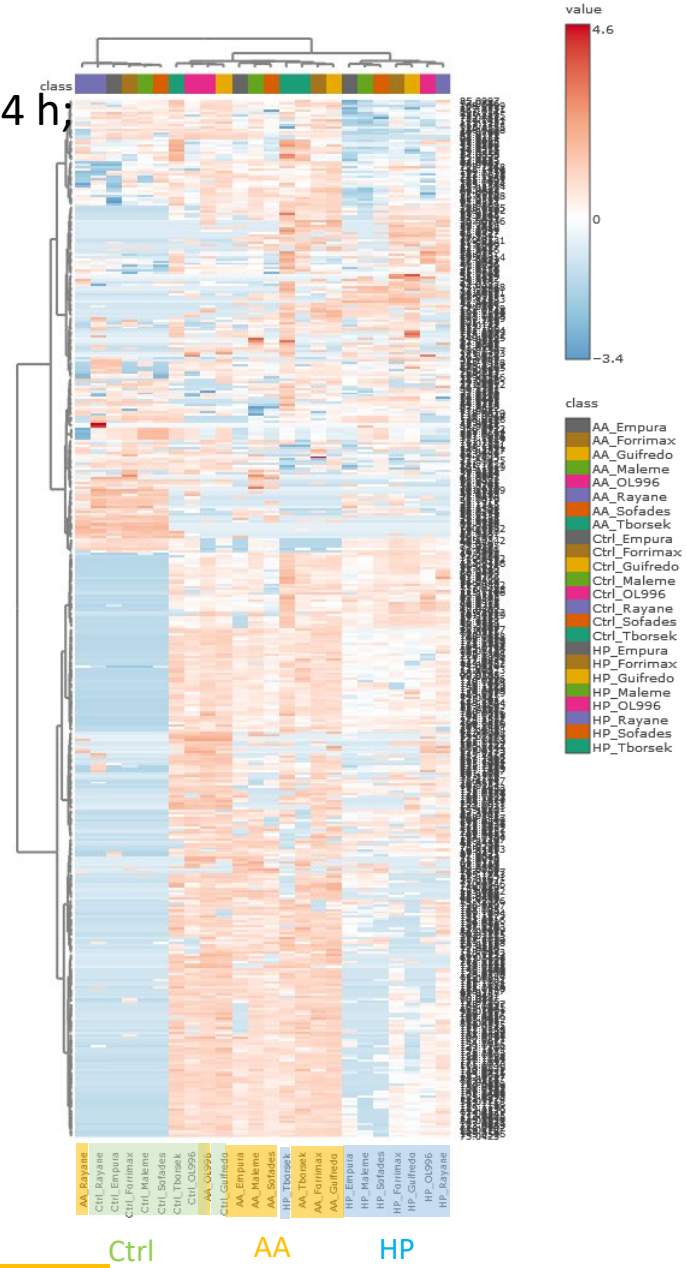
SEEDS VOLATILOME PROFILES

Ctrl= control; AA = (Artificial Aging) 55-60° 8 h; -HP = (Hydro Priming) In water 8 h; dryed 24 h;



PCA, mean values of 4 replicates

	accessions
Pea	Forrimax Guifredo
Grass pea (Lathyrus)	Maleme 107 Sofades
Fenugreek (Trigonella)	Rayane Tborsek
Soybean	Empura OL996



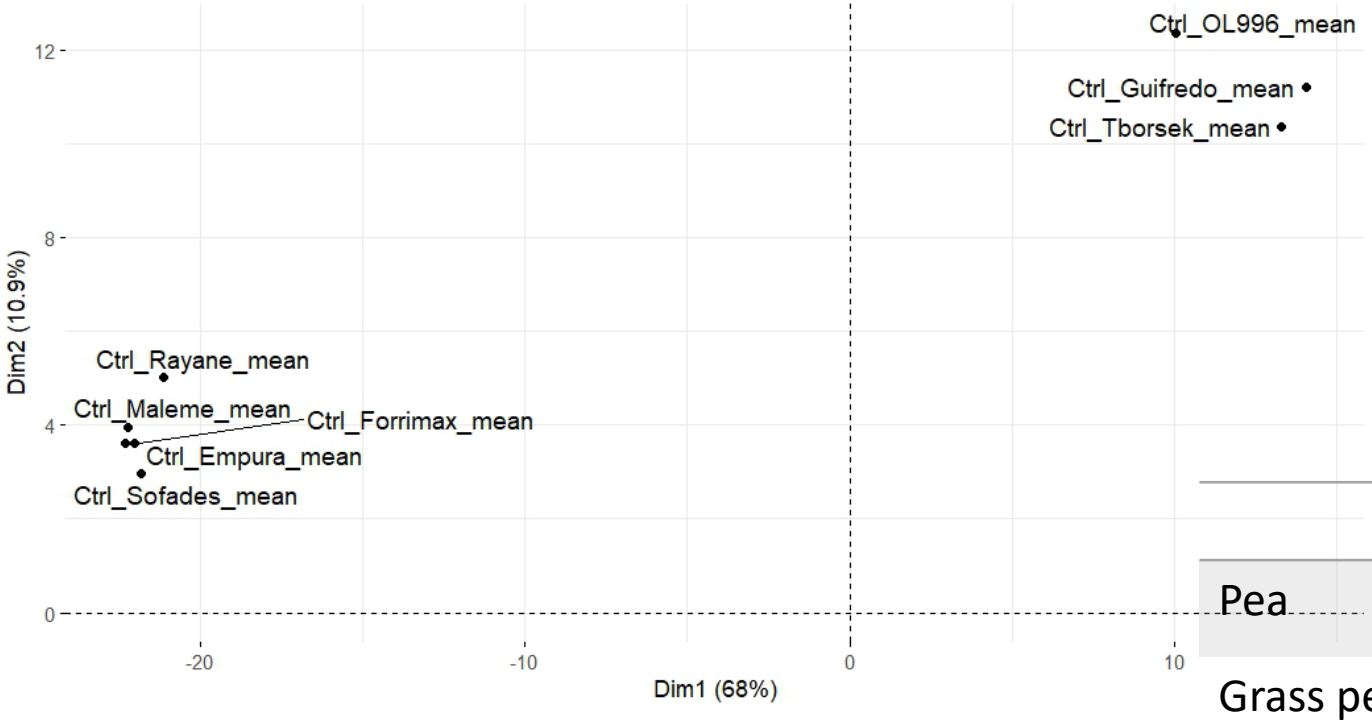
AA release more Volatiles

SEED ACCESSIONS CHARACTERIZATION

Volatilome profiles

Sample separation

PCA, mean values of 4 replicates



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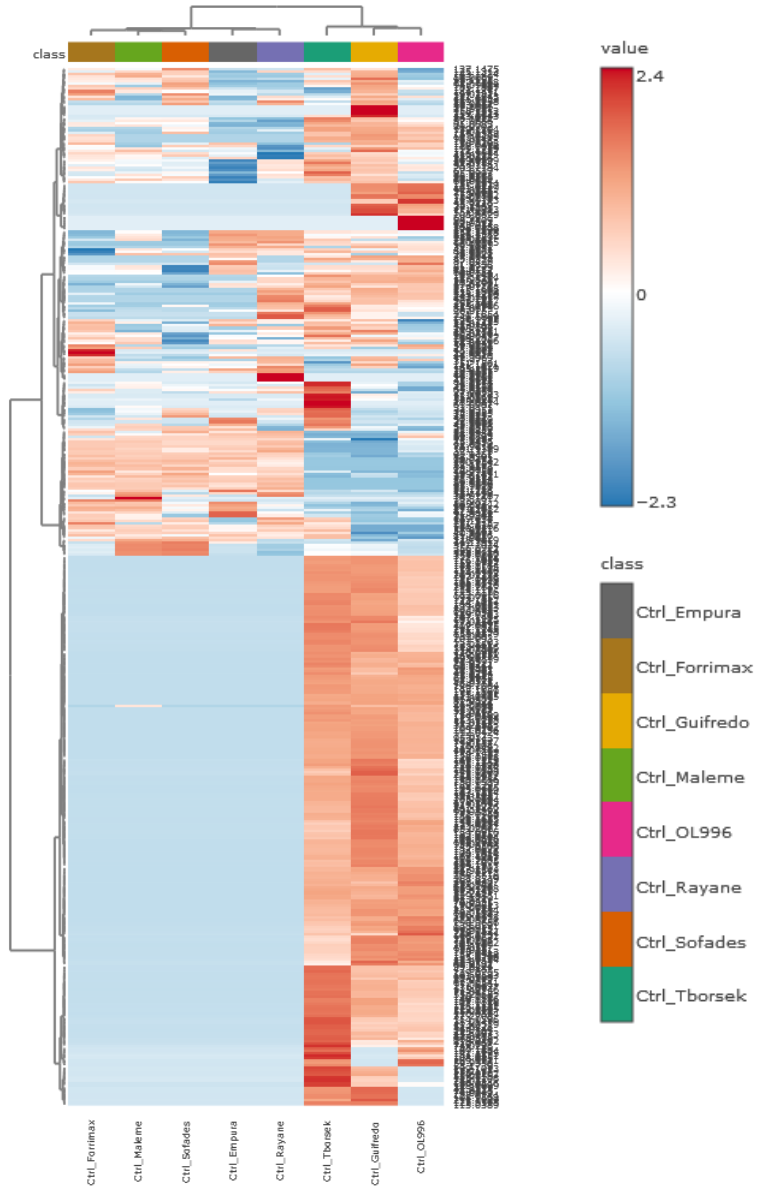

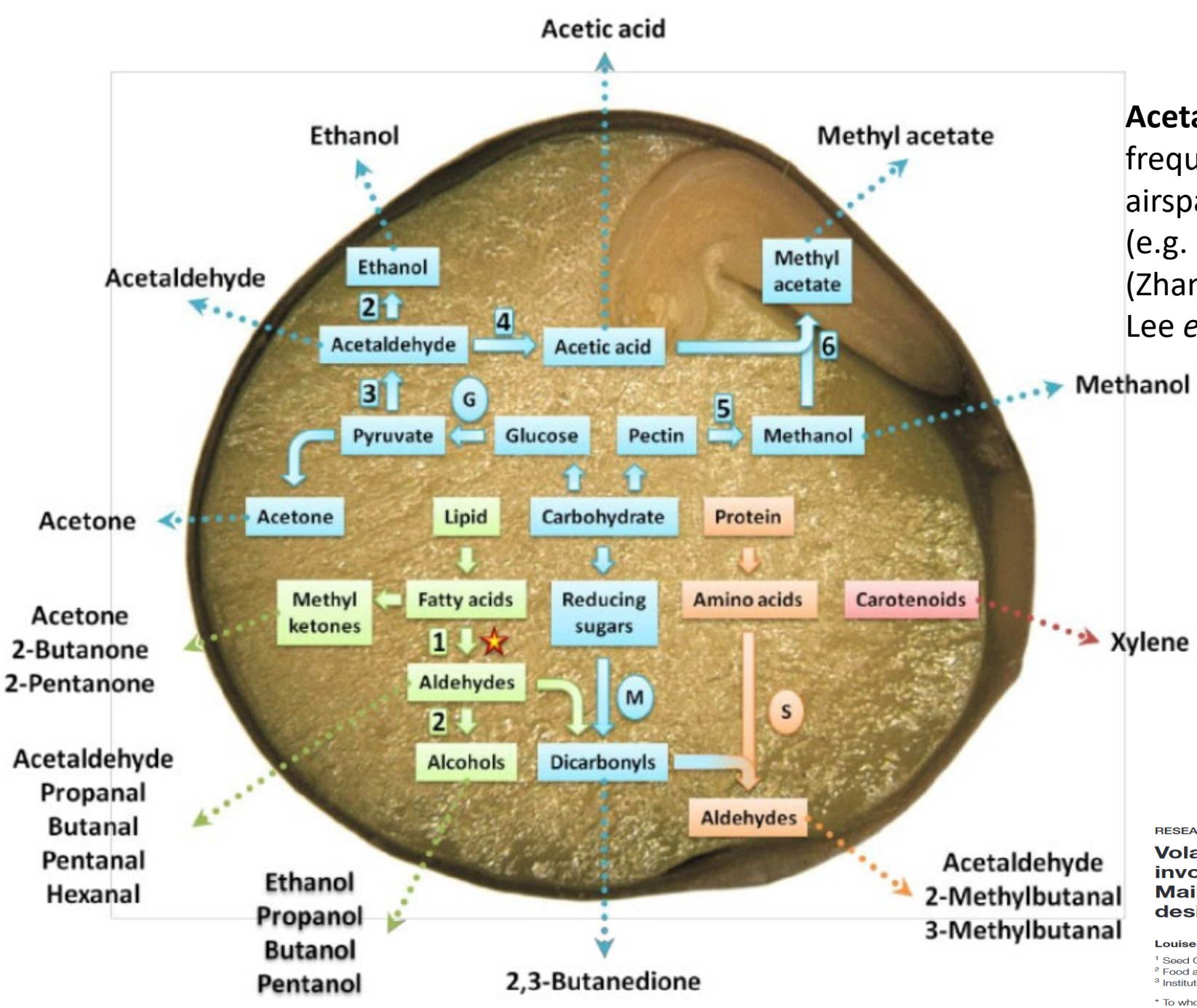

Acetaldehyde, **ethanol**, and **methanol** are frequently described as major constituents of airspace above seeds, with longer chain aldehydes (e.g. butanal, pentanal, or hexanal) also reported (Zhang *et al.*, 1993, 1995*a*; Trawatha *et al.*, 1995; Lee *et al.*, 2000*a*; Akimoto *et al.*, 2004).

Non-enzymatic oxidation of macromolecules produces a plethora of other by-products with diverse carbonyl groups (i.e. **alkanes, aldehydes, esters, ketones, and alcohols**).

For example, by-products of linoleic auto-oxidation include three to six carbon alkanes (propane, butane, pentane), aldehydes (propanal, butanal, pentanal, hexanal, etc), ketones (2-heptanone, etc), alcohols (propanol, butanol, pentanol, etc), acids (pentanoic acid, etc), and esters (methyl formate, etc) (Frankel, 1983; Grosch, 1987; Knutson *et al.*, 2000).

RESEARCH PAPER
www.jxb.oxfordjournals.org

Volatile fingerprints of seeds of four species indicate the involvement of alcoholic fermentation, lipid peroxidation, and Maillard reactions in seed deterioration during ageing and desiccation stress

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Future Prospectives

Agriculture: Understanding VOC profiles can aid in breeding programs for disease resistance or improve seed storage and viability.

Ecology: Insights into seed VOCs can elucidate plant-plant and plant-insect interactions, aiding in ecosystem management.

Pharmacology: Some seed VOCs have potential medicinal properties and can be explored for drug development.

Food Industry: Characterizing seed VOCs can improve the quality control of seeds and seed-derived products.

BIOTA TNA AGROSERV PROJECT

Seeds volatilome will be analysed as non-destructive markers for **seed aging, vigour, and health,** at InfraVOL (EMPHASIS RI). Seed volatilomes profiles will be associated to classical physiological seed quality parameters, determining seed germination, vigour and mass (physiological seed quality) and seed composition