IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS AS BIOMARKERS FOR EARLY DETECTION OF STORAGE ROTS IN SUGAR BEETS

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Introduction

- Sugarbeet (SB)- Crop with high sucrose content

- Contributes to 40% of world's sugar (Dohm et al. 2014)

- US produces > **30 million tons annually** (USDA, 2023)

Introduction

- Tons harvested annually > sugar processing industries = long storage periods (up to 270d) $\longrightarrow$ sucrose loss

- **Sucrose loss** in SB- crucial challenge faced by the sugar industry in the US

- Sucrose loss ~ **$30 million loss** in North America (Strausbaugh 2018).

Introduction

- Sucrose loss: respiration or **microbial colonization** (rot development)

- Multiple bacterial and fungal strains are reported as causal agents

- **Bacteria** (*L. mesenteroides* & *Lactobacillus*), **Yeast** (*Pichia* & *Candida* spp) and **Fungi** (*Penicillium* spp, & *Fusarium* spp) (Straubaugh, 2018)

Introduction

- **Millions of dollars** in storage losses = need for **<u>early detection</u>** of SB storage rots

- PCR assay, ELISA assay: **drawbacks/cons**

- Development of **sensors to detecting VOCs** associated with SB rot development (**Unexplored**)

Objective

- Identify & profile potential pathogenic **VOC biomarkers** for the development of smart sensor platform- **rapid & early detection** of storage rots in SB piles.

Experimental design

Complete Randomized Design with 4 reps per treatment

- Day 3 : 1 var x 3 treatments x 4 bio reps = 12 samples
- Day 7: 1 var x 3 treatments x 4 bio reps = 12 samples
- Day 14: 1 var x 3 treatments x 4 bio reps = 12 samples
- Day 21: 1 var x 3 treatments x 4 bio reps = 12 samples
- Control : 1 var x 3 times interval x 4 bio reps = 12 samples
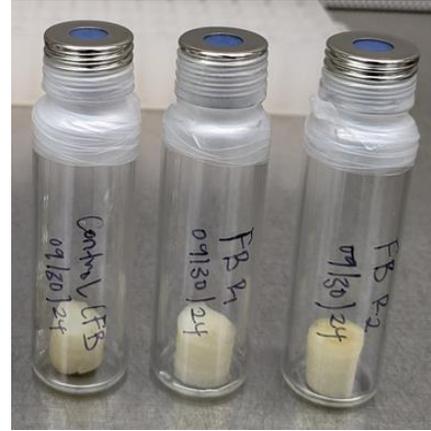
Total = 60 samples

Assessing VOCs profile from pathogens: *In vitro*

SB cultivation (1 variety, 17 wks)

Washed: 0.6% bleach & sliced

Inoculated plugs : 100µl of microbial suspension

Incubation: 3, 7,14 & 21d @ 30 °C, 95% RH

Bacteria: *L. mesenteroides*
Fungi: *P. expansum* & *P. paneum*

Bacteria: OD_{600}: 0.5)
Fungi: 10^6 spores/mL)

VOC analysis: GC-MS

Data analysis

- Data were expressed as relative percentage of the total peak area.

- Normalized by median and auto-scaled using Metaboanalyst version 6.0.

- Sparse Partial least square-discriminant analysis

- ANOVA and Post-hoc tests -discriminant compounds considering p-value inferior or equal to 0.05.

Total Occurrence of VOCs across samples

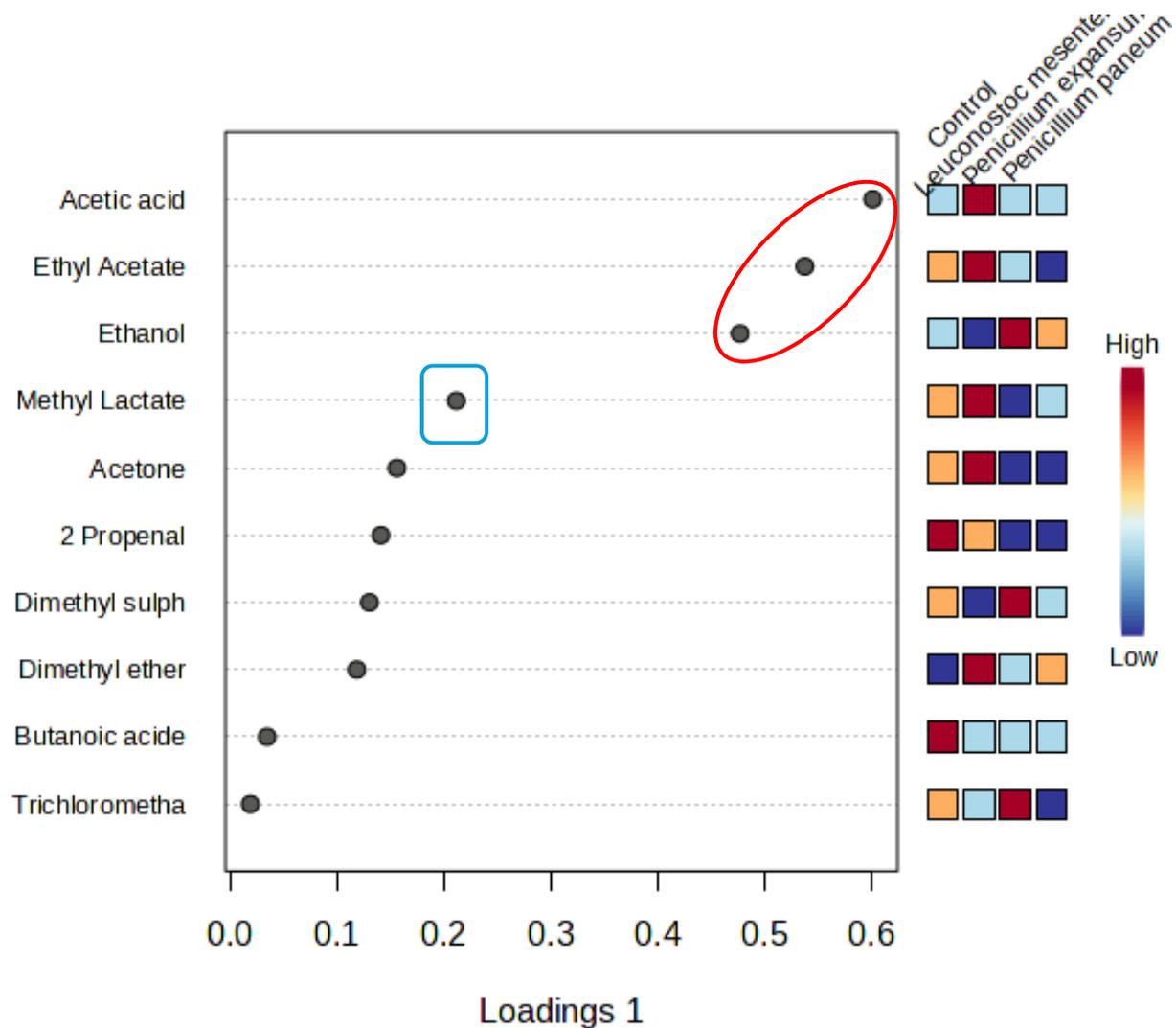| No	Volatile Organic Compounds	RT, (min)	Non-inoculated samples				Inoculated samples				Total Occurrence (%)
			Occurrence at sampling period (%)				Occurrence at sampling period (%)				
			Day 3	Day 7	Day 14	Day 21	Day 3	Day 7	Day 14	Day 21	
			n=4	n=4	n=4	n=4	n=12	n=12	n=12	n=12	
1	Ethanol	1.53	100	100	100	100	100	100	100	100	100
2	Dimethyl Sulphide	0.87	100	75	50	50	66.67	58.33	41.67	50.00	61.46
3	Methyl lactate	0.71	50	100	25	25	50	41.67	33.33	25	43.75
4	Ethyl acetate	1.25	0	0	50	75	0.00	33.33	33.33	66.67	32.29
5	Trichloromethane	2.15	100	0	0	0	100	8.33	0.00	0.00	26.04
6	Dimethyl ether	0.72	50	0	0	25	25	41.67	41.67	16.67	25
7	2 propenal	8.35	100	0	0	25	50	8.33	0.00	8.33	23.96
8	Acetone	0.98	50	50	25	25	0.00	25	0.00	0.00	21.88
9	Butanoic acidethyl ester	1.49	0	0	50	50	0.00	0.00	0.00	8.33	13.54
10	Acetic acid	9.29	0	0	0	0	0.00	33.33	33.33	33.33	12.50
11	Acetaldehyde hydroxy	1.6	0	0	25	0	0.00	0.00	0.00	8.33	4.17
12	Acetoin	6.45	0	0	0	25	0.00	0.00	0.00	16.67	5.21
13	Borane methyl sulphide	0.85	0	0	25	0	16.67	0.00	16.67	0.00	7.29
14	Methyl formate	2.45	0	0	0	25	0.00	0.00	0.00	0.00	3.13
15	Methyl nitrate	1.5	0	0	25	0	0.00	0.00	8.33	0.00	4.17
16	1 H tetrazole	1.52	0	0	25	0	0.00	0.00	0.00	0.00	3.13
17	Oxalic acid	1.55	25	0	0	0	0.00	0.00	0.00	0.00	3.13
18	Butanoic acid	10.79	0	0	25	0	0.00	0.00	0.00	0.00	3.13
19	Hydrogen isocyanate	0.79	0	0	0	0	0.00	0.00	8.33	16.67	3.13

Abundance of volatiles across sample groups
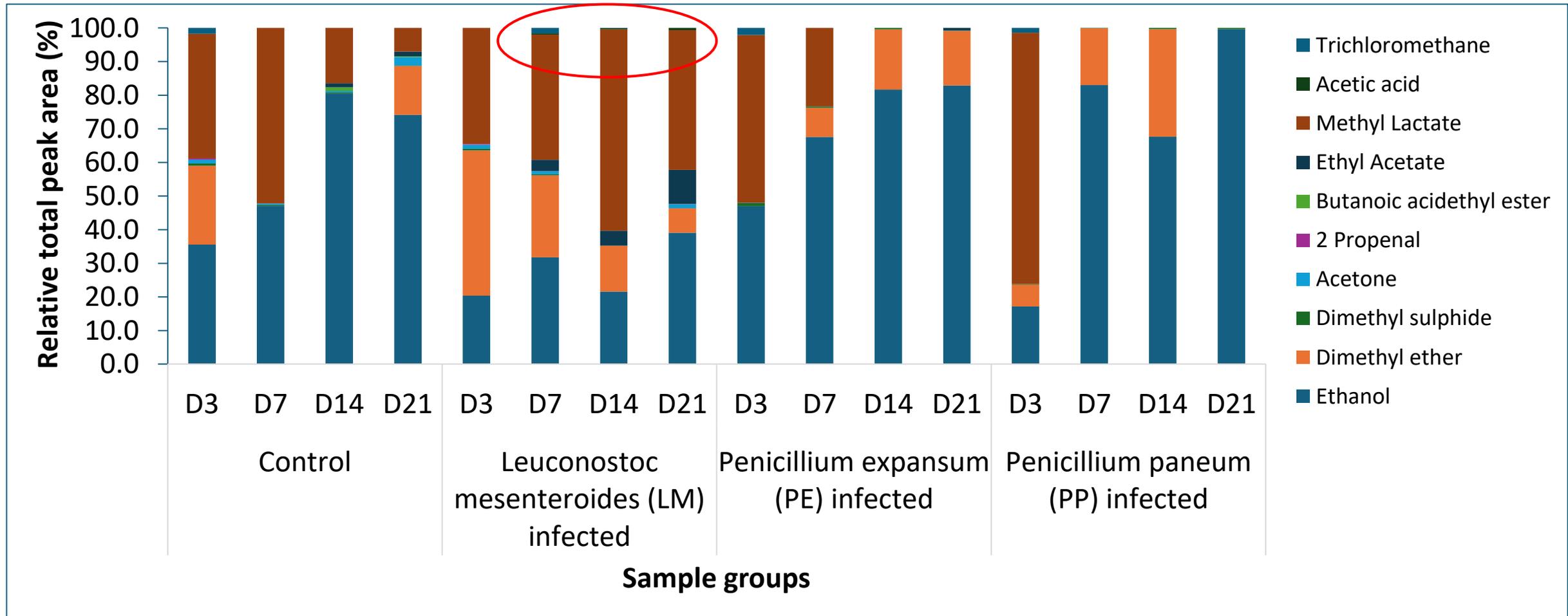
- Out of the 10 volatiles, ethanol was the most abundant across samples over time.

- Lower conc. of ethanol observed in LM- infected samples

Multivariate Discriminant analysis of VOCs using sPLS-DA

- Significant metabolic shifts as control group clusters separately from inoculated samples

- LM forms a separate cluster – distinct metabolites.

- PE & PP overlaps – shared VOC signatures
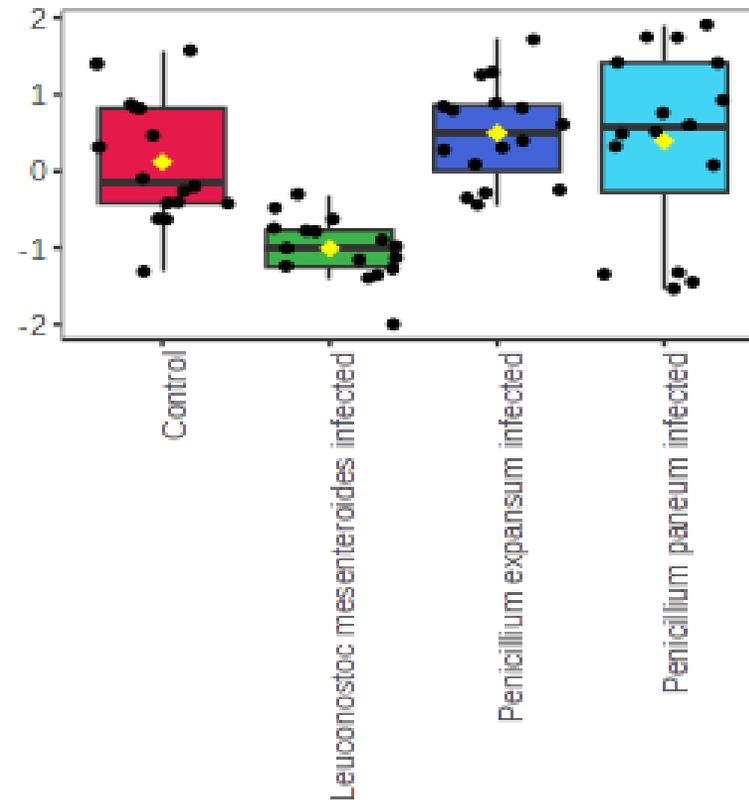
Loading score-key VOC discriminants across samples

- Significant – score close to 1

- Key discriminants – **Acetic acid, Ethyl acetate and Ethanol**

- LM associated with high acetic acid and ethyl acetate

- While PE & PP are associated with ethanol

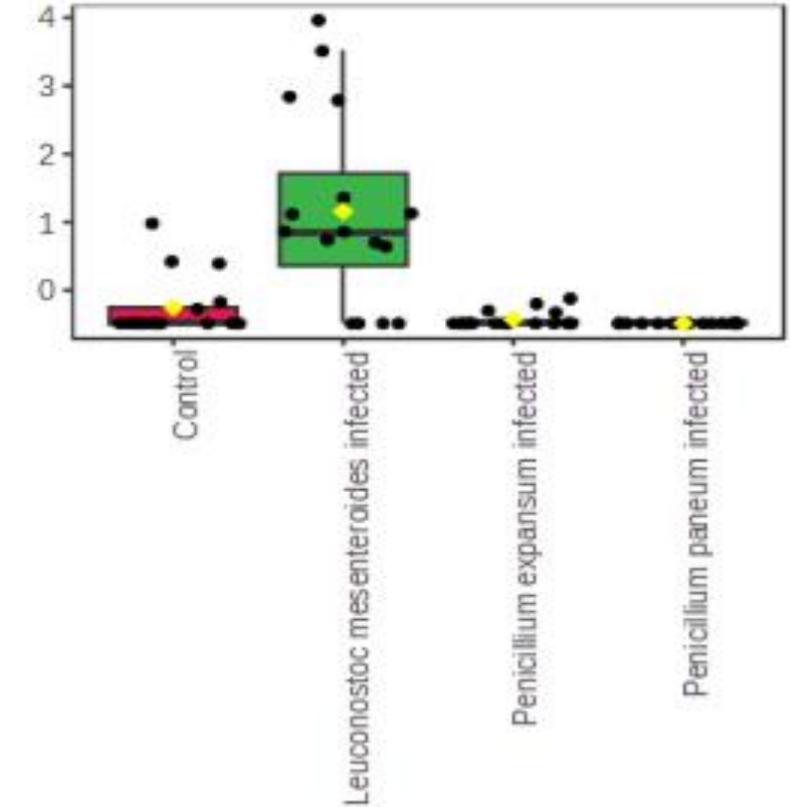# Potential markers for early detection of sugar beet rots using ANOVA and Post hoc tests
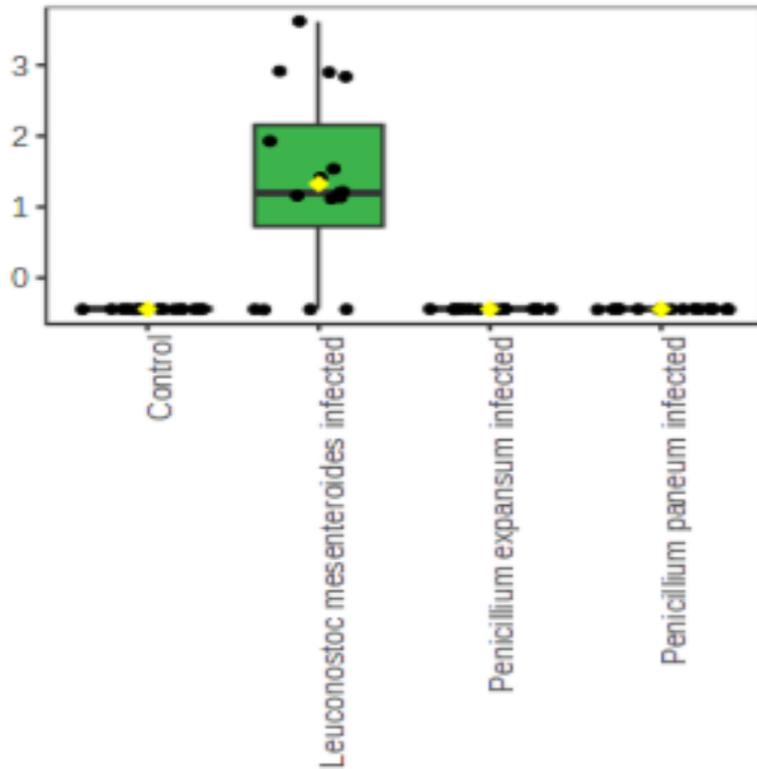
- 3 out of 10 volatiles showed (p>0.001) higher abundance in the presence of rot infection

Key findings

- **Ethanol** - most abundant and frequently

- Control groups clustered differently from inoculated groups

- Key discriminants – **Acetic acid**, **Ethyl acetate** and **Ethanol**

- LM - acetic acid and ethyl acetate instead of ethanol

- Significant increase of ethanol concentration with *Penicillium* spp

Conclusion and Future prospectives

Conclusion
- Acetic acid, Ethyl acetate and Ethanol - **Potential biomarkers**

Future prospective
- Studies to verify biomarker concentration critical limit as well as sucrose consumption levels in defining SB rots

- Effects of different SB varieties and storage temperatures on VOC profile

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THANK YOU

Questions?