

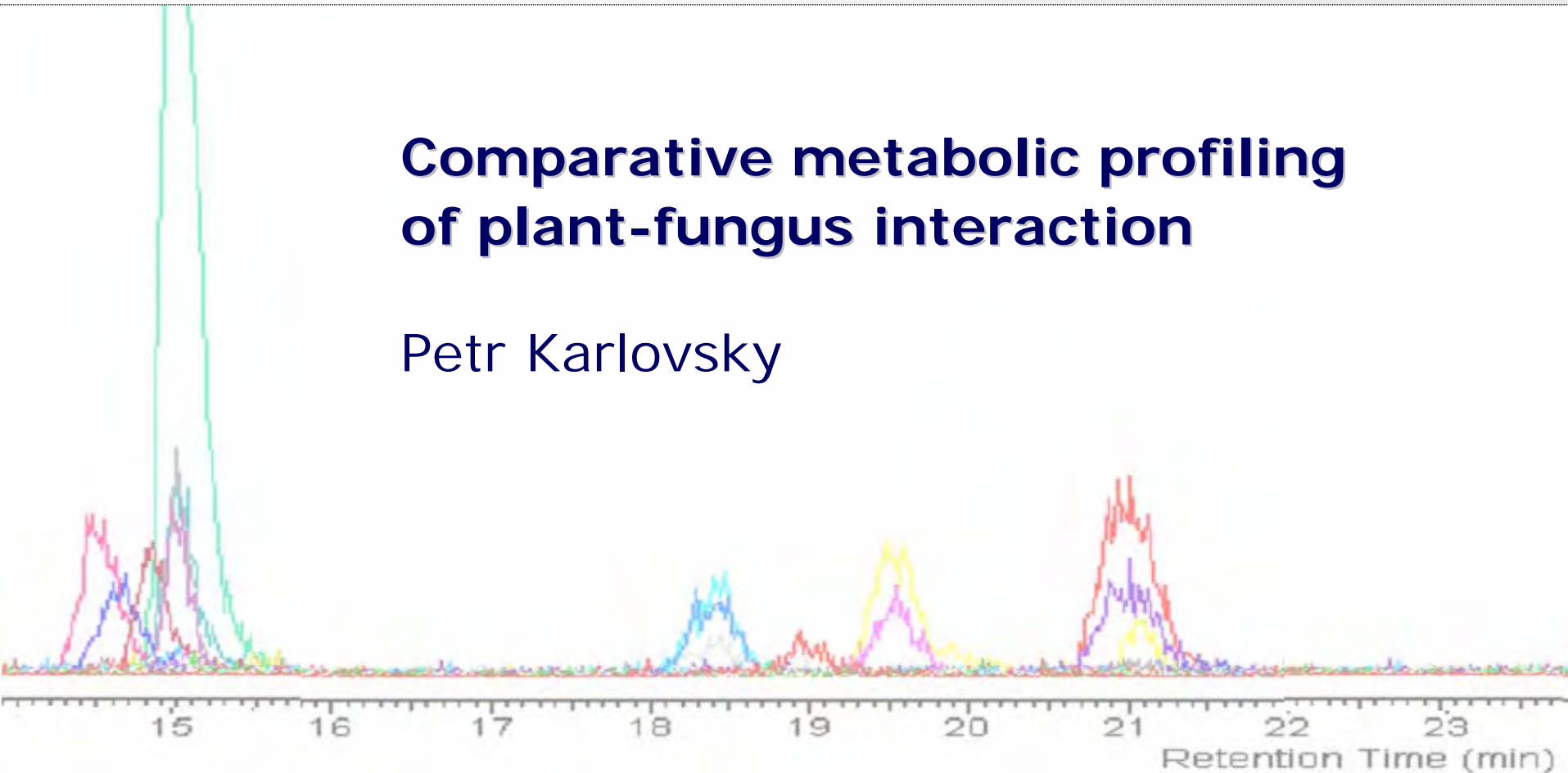


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Comparative metabolic profiling of plant-fungus interaction

Petr Karlovsky







www.gwdg.de/~instphyt/karlovsky
(go to Content -> 500-MS)

Ion trap 500-MS (Varian) as HPLC detector

Petr Karlovsky, Astrid Ratzinger, Uli Hettwer

Here we report about our experience with our new ion trap detector 500-MS in our work on plant and fungal metabolites. The date in the following list of content indicates when the notes were written.

- ♦ Mai 2006: [Installation](#)
- ♦ Juli 2006: [Gained/lost features as compared with triple quadrupole 1200LC](#)
- ♦ August 2006: [Fast "unidirectional triple resonance scanning"](#)
- ♦ September 2006: [Sensitivity and signal/noise issues](#)
- ♦ September 2006: [About settings](#)

Installation

In May 2006 we acquired a [new HPLC-MS system](#) from Varian, furnished with their brand new ion trap 500-MS. Our trap was the second 500-MS installed world-wide. Because of numerous inquiries concerning the system, we decided to share our experiences, performance data, solutions to problems and technical tips on this website.

We are continuously extending this website. Other users are welcome to





Topics

- Non-targeted metabolic profiling
 - ▶ Goals, data format, software
- Met. profiling projects in our laboratory
- *Brassica-Verticillium* pathosystem
 - ▶ Xylem sap analysis
 - ▶ Data processing
 - ▶ Interpretation



Non-targeted analysis: a new paradigm in analytical chemistry

	Analyte	Demands on determination
Traditional analytical chemistry	known	absolute accurate
Comparative metabolic profiling	unknown	relative approximate



Metabolite analysis

- Metabolomics
- Metabolic fingerprinting
- Differential/comparative profiling
- Metabonomics



Basic technology: full-scan MS

- Fundamental requirement: Detect many chemically diverse, uncharacterized substances
- Suitability of MS detectors:
 - ▶ Compare QQQ, IT, TOF, FT-ICRMS
- Varian 500-MS and triple resonance scanning



Data format

- Open standard is a must!
- XML: Universal - open - extensible
 - ▶ BUT biased towards proteomics,
+ not widely implemented yet
- ANDI/NetCDF: open and compatible
 - ▶ BUT unsuitable for parsing (binary)
+ not good for higher-order MS data



Software

- Open Source dominant in metab. profiling
 - ▶ XCMS
 - ▶ MzMINE
- ACD/MS Manager (ScienceServe)
- Custom-written Perl scripts

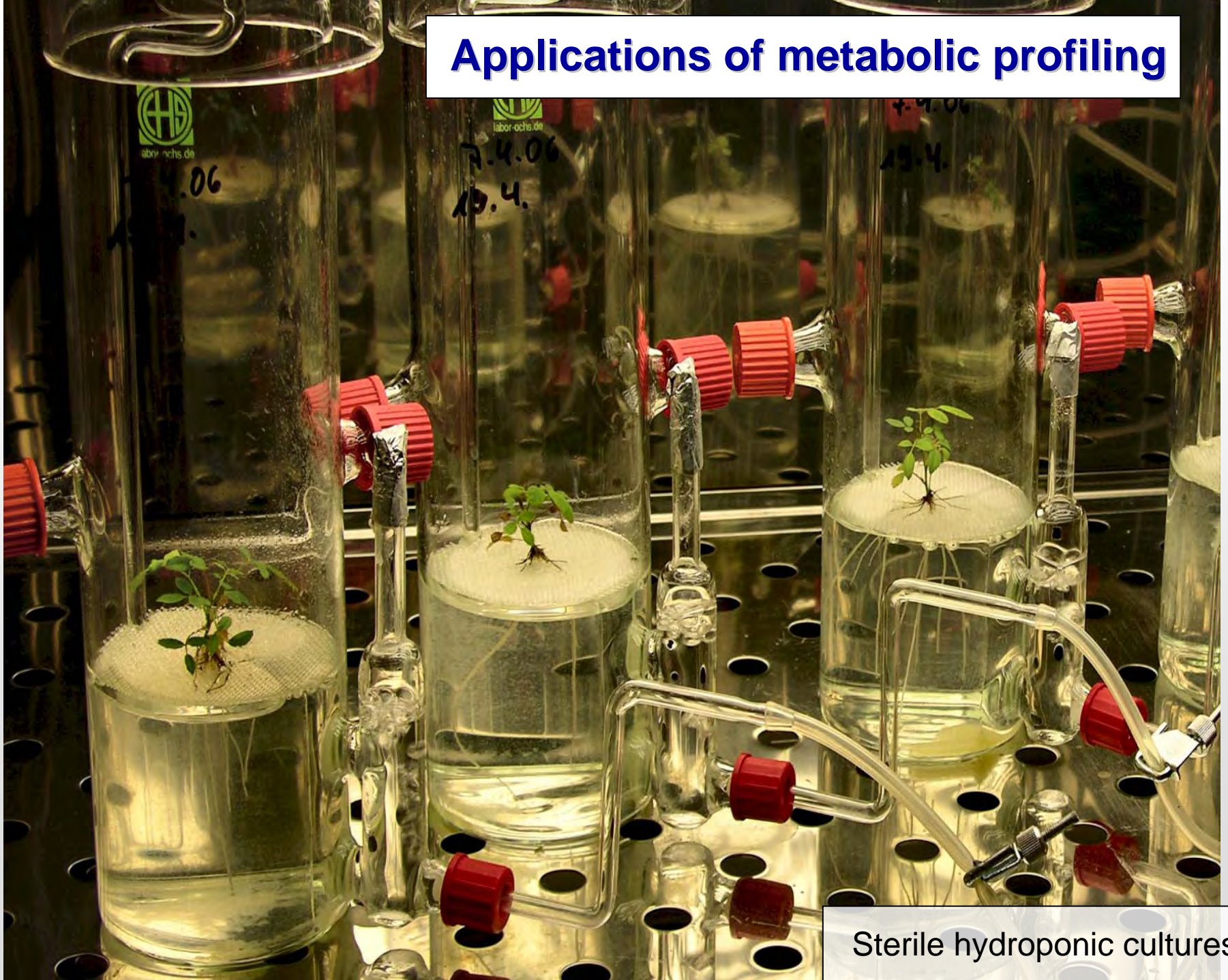
Applications of metabolic profiling



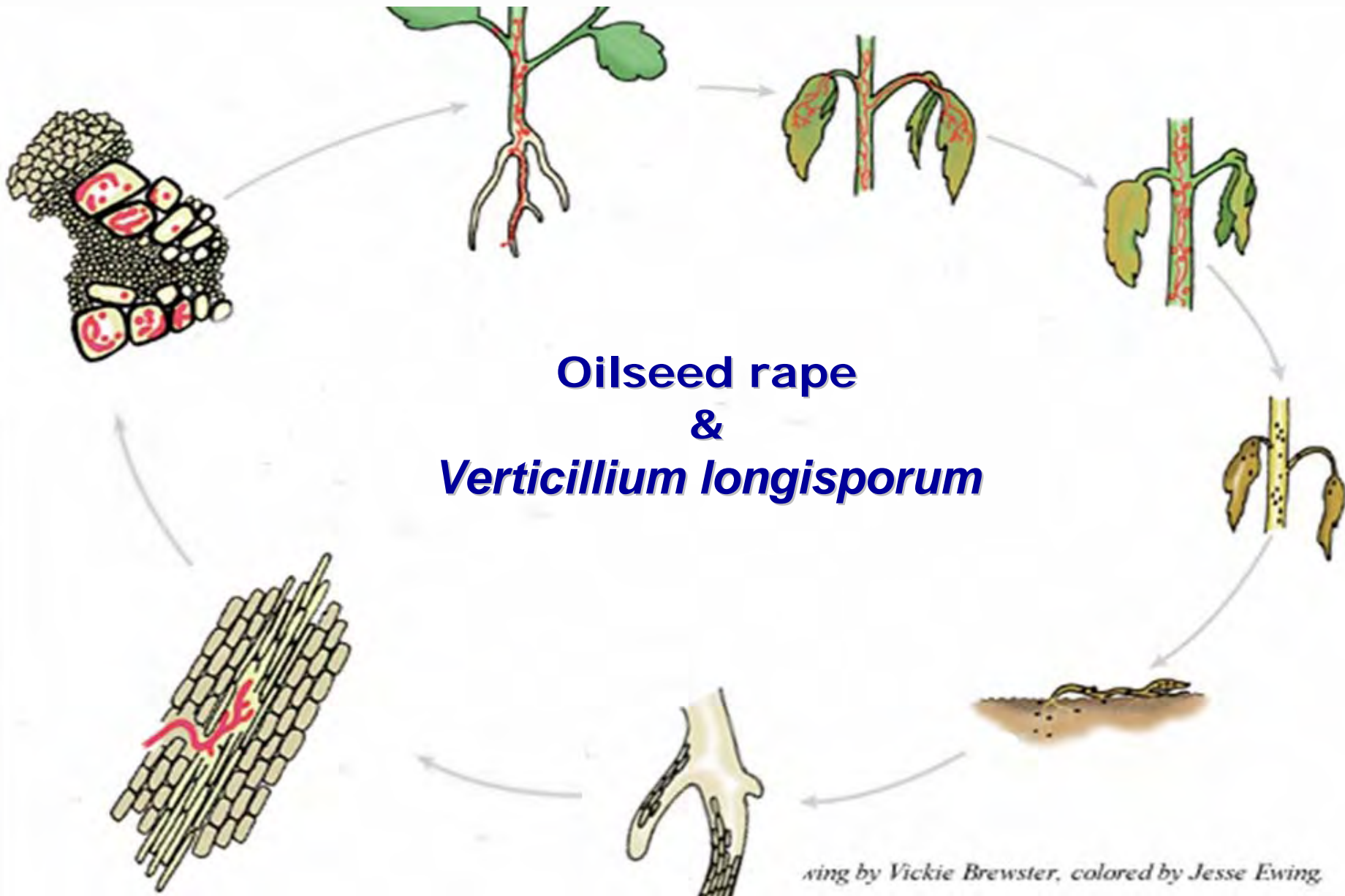
Sesame
(*Sesamum indicum* L.)



Applications of metabolic profiling

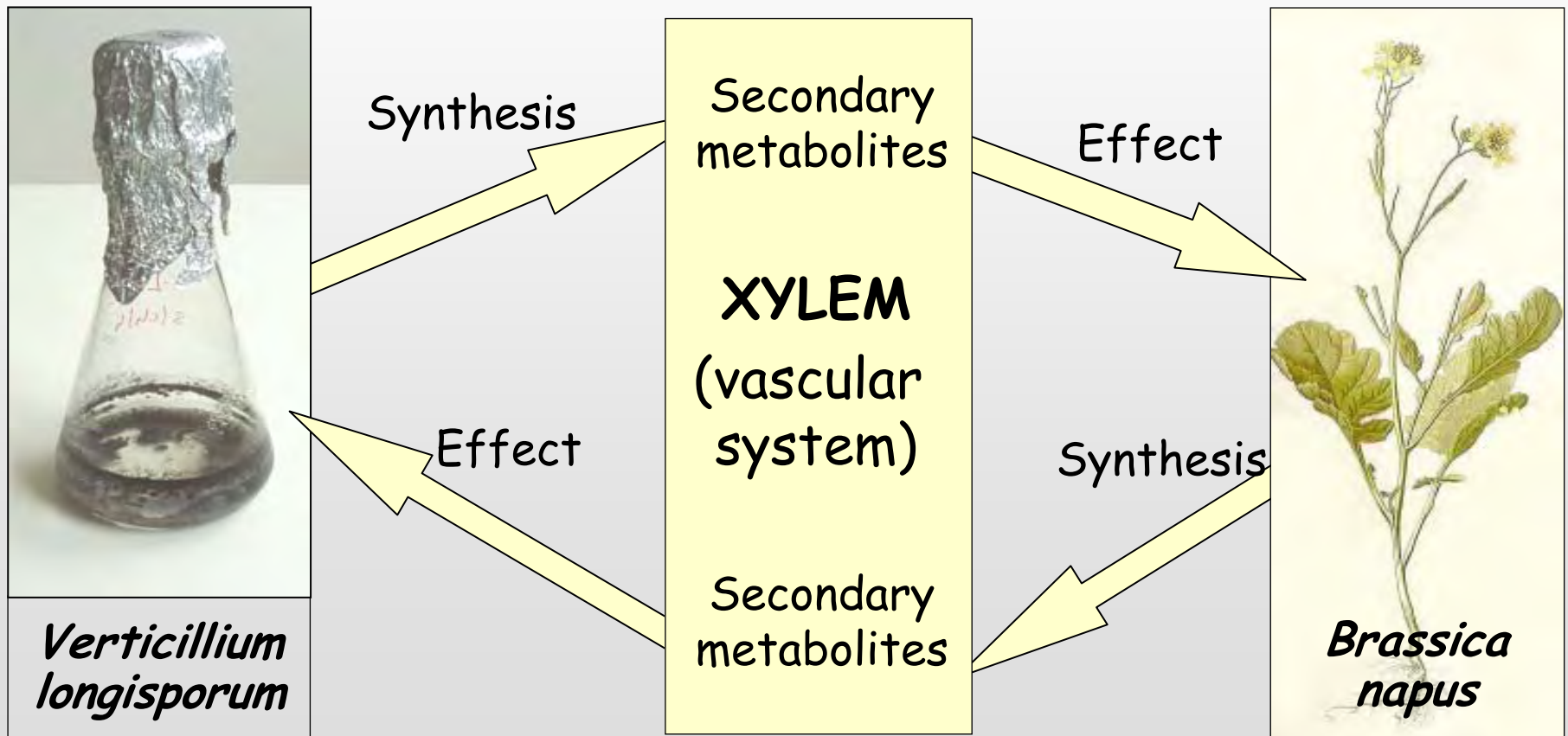


Sterile hydroponic cultures





Brassica napus – *Verticillium* pathosystem



Chemical interactions in xylem



*Verticillium
longisporum*

XYLEM

Metabolites
affected by
infection:

- Origin
- Identity
- Function



*Brassica
napus*

Extraction of xylem sap

Control plants



Infected plants



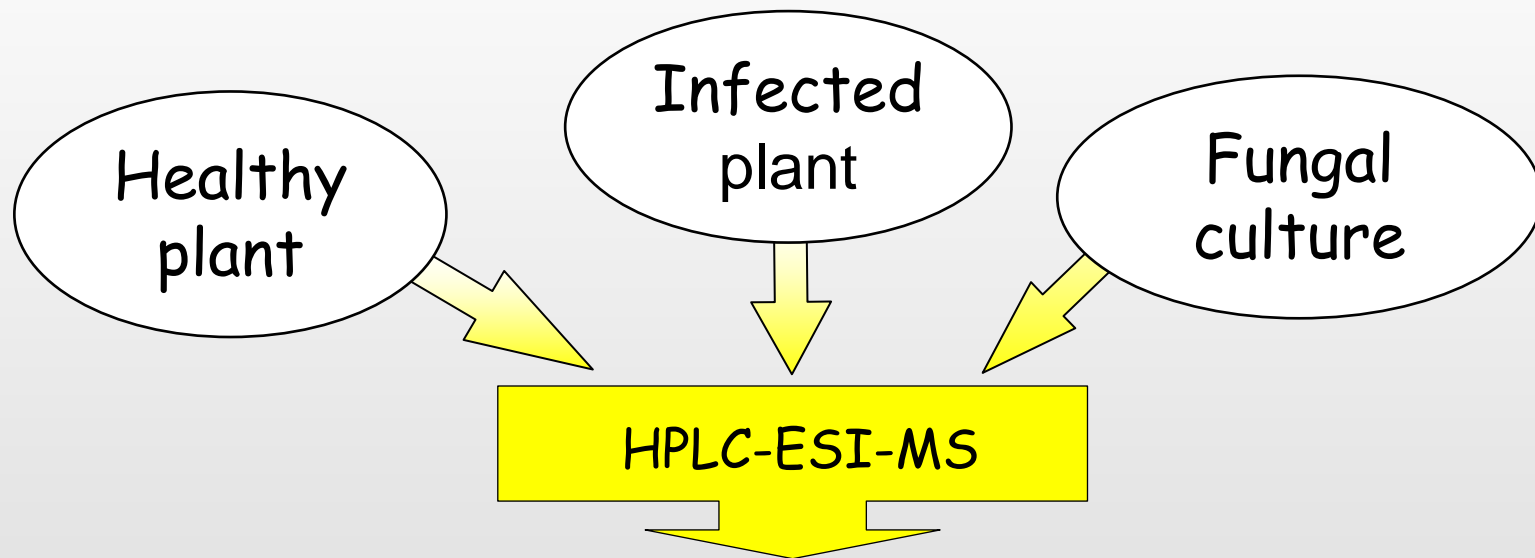
Pressure chamber



Xylem sap
20 - 60 μ l/plant

- Rapid cycling rape
- 7 days old seedlings infected
- 14 to 35 dpi harvest

Comparative profiling



1. Find metabolites specific for or affected by infection
2. Elucidate their origin
3. Identify metabolic transformations



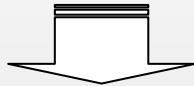
HPLC-MS

HPLC	Polar-modified C18, 3 μ m, 2x100 mm, MeOH/water 10 - 90%, 40°C, 0.2 ml/min
Ionization	ESI
Mass selection	m/z from 50 to 1000
Sampling	Once per second
Full-scan on IT	Fast triple-resonance scanning
Data reduction	CODA
Data processing	ACD/MS Manager XCMS (+R) Custom Perl scripts

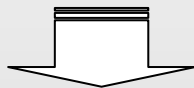


Metabolic profiles of xylem sap

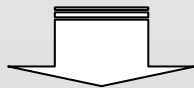
HPLC-ESI-MS:
500-MS full scan



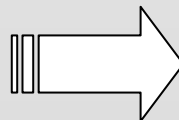
Data processing



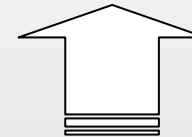
Normalization



Comparative analysis



Confirmation
on single plants



Candidate metabolites

m/z	C1 (4 pl.)	C 2 (4 pl.)	C 3 (4 pl.)	Mixed sample (50 pl.)	strong symptoms (4 pl.)	weak symptom A (4 pl.)	weak symptom B (4 pl.)	Mixed sample (50 pl.)
187	1.1	1.3	2.0	0.6	4.0	1.2	4.1	26.1
299	2.1	2.0	3.3	2.5	0.0	0.2	0.0	0.9
327	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
329	31.7	35.2	36.5	19.6	34.9	39.8	47.9	35.2
351	3.2	3.1	2.7	1.0	0.6	1.3	1.5	0.5
396	0.0	0.0	0.1	0.0	19.2	6.9	13.7	0.0
449	50.9	80.6	47.0	58.4	19.8	44.1	22.7	21.2
554	0.3	0.7	0.3	0.5	16.6	6.1	11.5	7.1
558	0.1	0.1	0.1	0.1	11.0	3.2	7.3	2.6
576	0.1	0.2	0.2	0.1	8.4	2.2	5.9	0.2
595	4.7	3.1	3.2	11.9	14.0	9.8	8.5	23.1
602	0.1	0.0	0.1	0.0	7.3	3.0	6.7	2.5
611	0.2	0.1	0.3	1.2	28.5	12.4	16.4	7.2
617	28.0	24.8	1.6	1.2	2.0	11.2	4.3	1.0
655	4.4	5.5	18.8	34.6	0.3	2.6	2.2	1.9
791	4.7	4.2	0.4	0.6	0.5	0.2	0.3	0.6



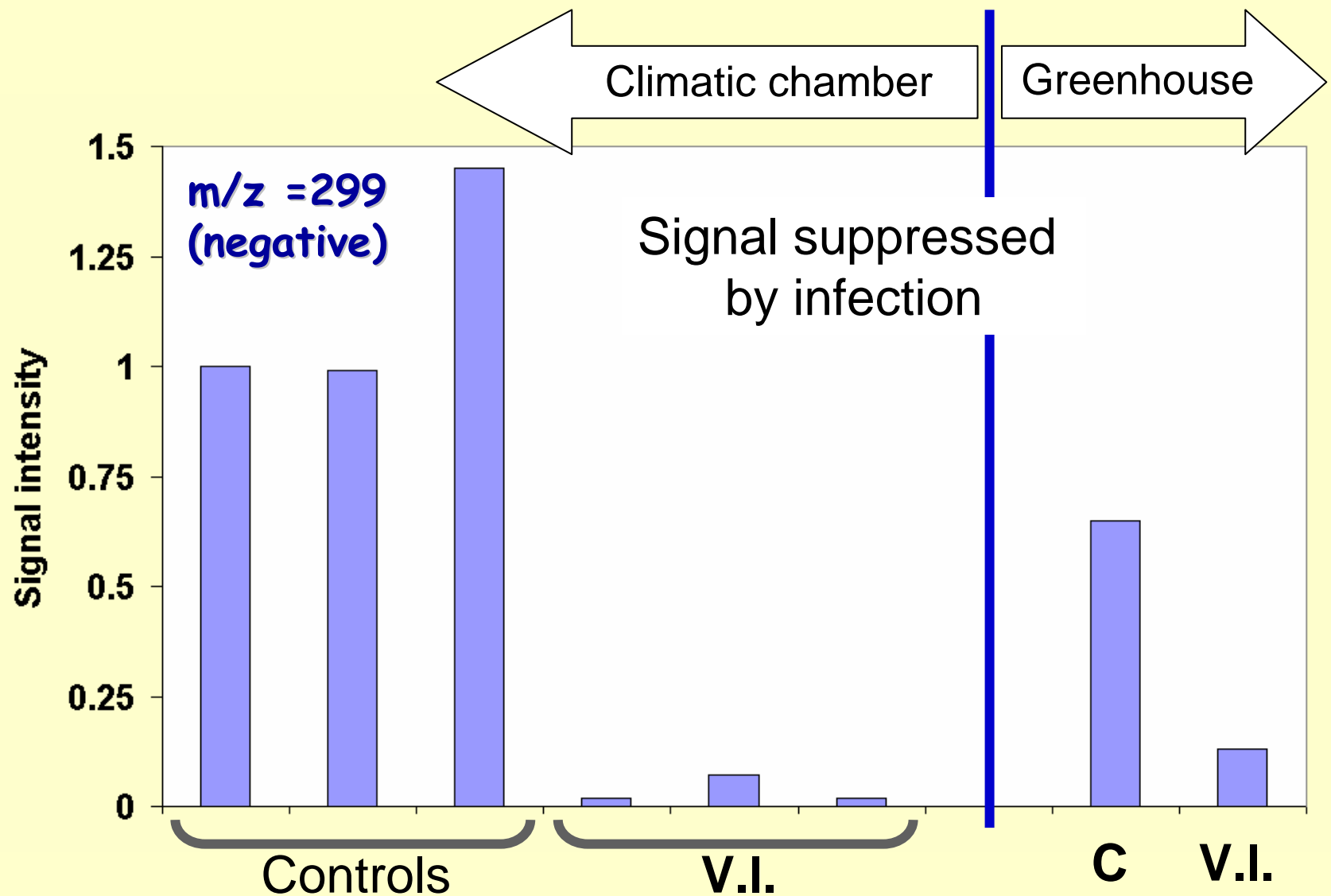
Relative intensities before normalizatio

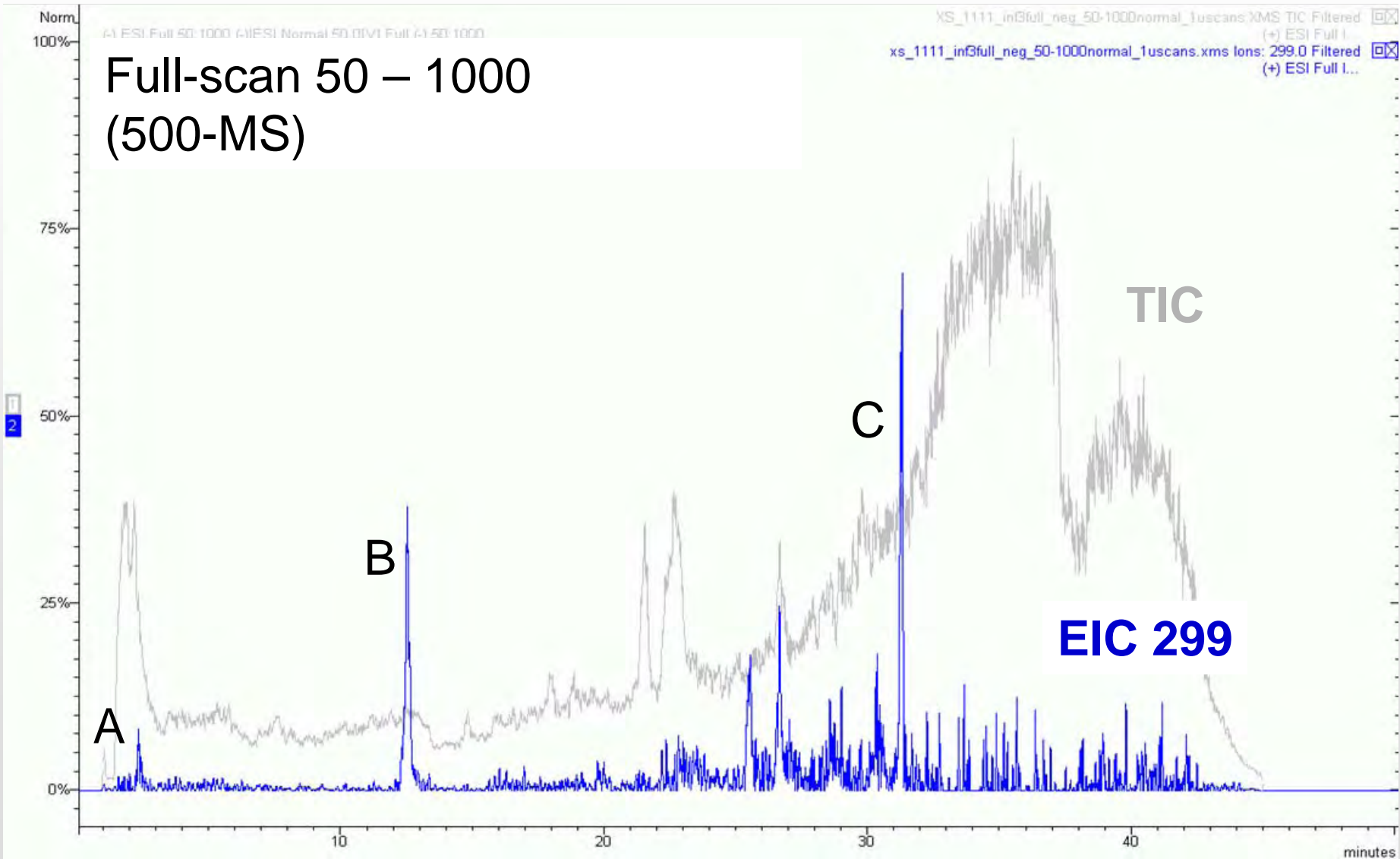
Peak ID	1	2	3	4	5	6
M299T1198	1	1.04	1.08	0.23	0.02	0.07
M223T1370	1	0.97	0.81	0.93	0.33	0.82
M655T1118	1	1.34	2.88	1.51	0.11	0.5
M293T1569	1	1.11	0.92	0.38	0.75	0.52
M294T1570	1	1.4	1.05	0.47	1.03	0.46
M253T1659	1	0.59	0.89	0.79	0.31	0.61

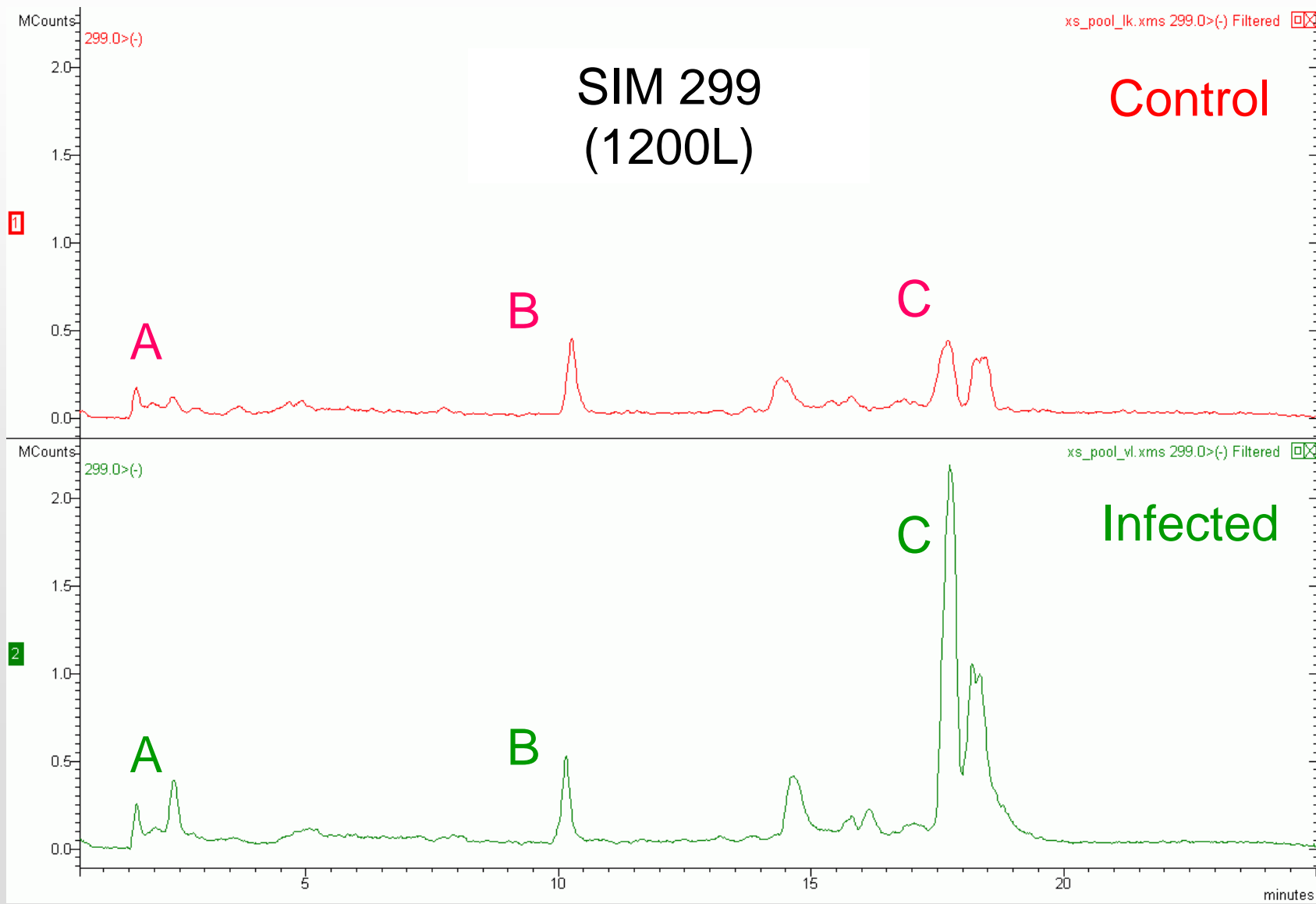
Normalization
is crucial

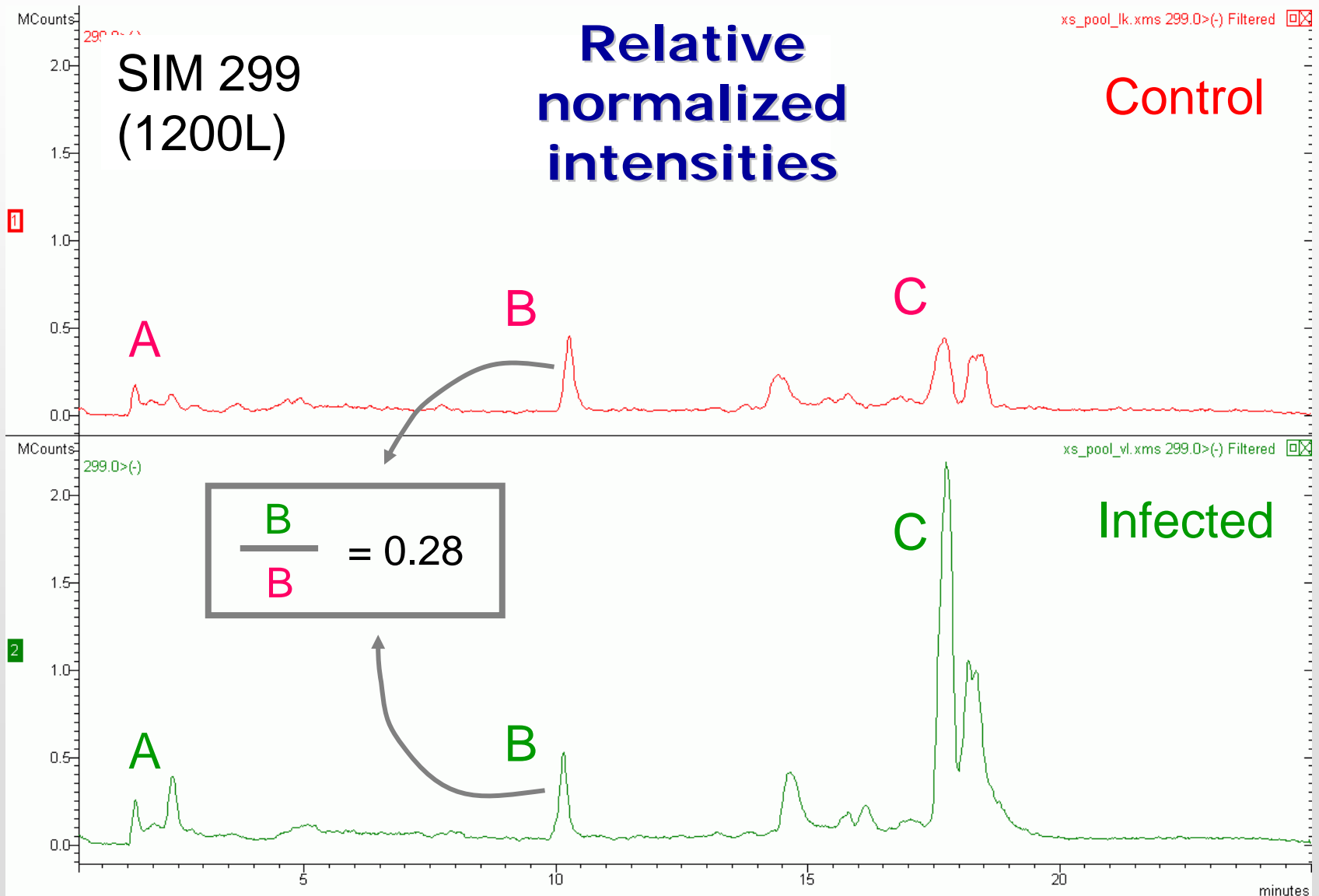
Relative normalized intensities

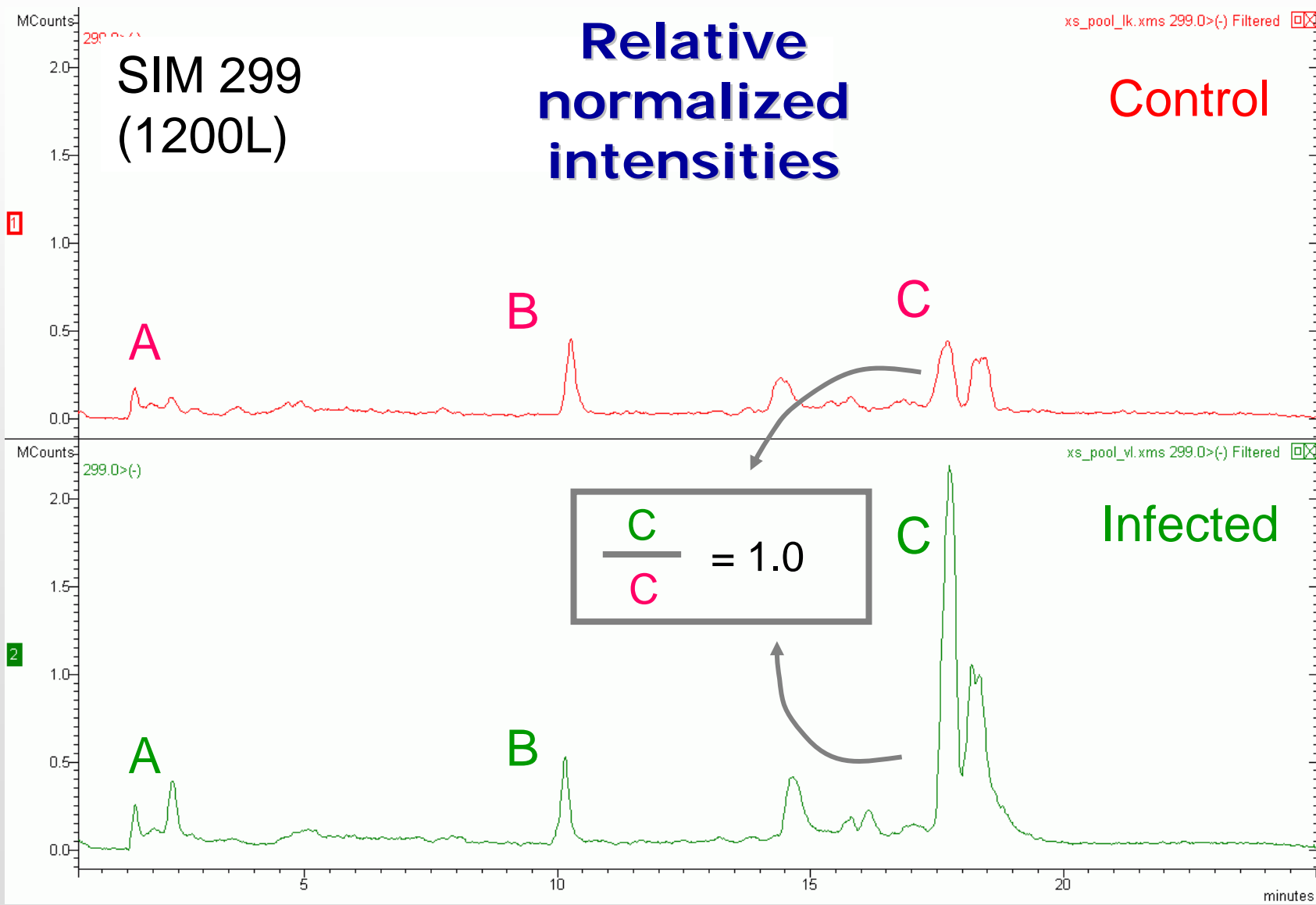
Peak ID	1	2	3	4	5	6
M299T1198	1	0.99	1.45	0.65	0.02	0.07
M223T1370	1	0.93	1.09	2.63	0.33	0.89
M655T1118	1	1.28	3.87	4.26	0.11	0.55
M293T1569	1	1.06	1.23	1.08	0.76	0.56
M294T1570	1	1.33	1.4	1.34	1.05	0.5
M253T1659	1	0.56	1.2	2.23	0.31	0.66

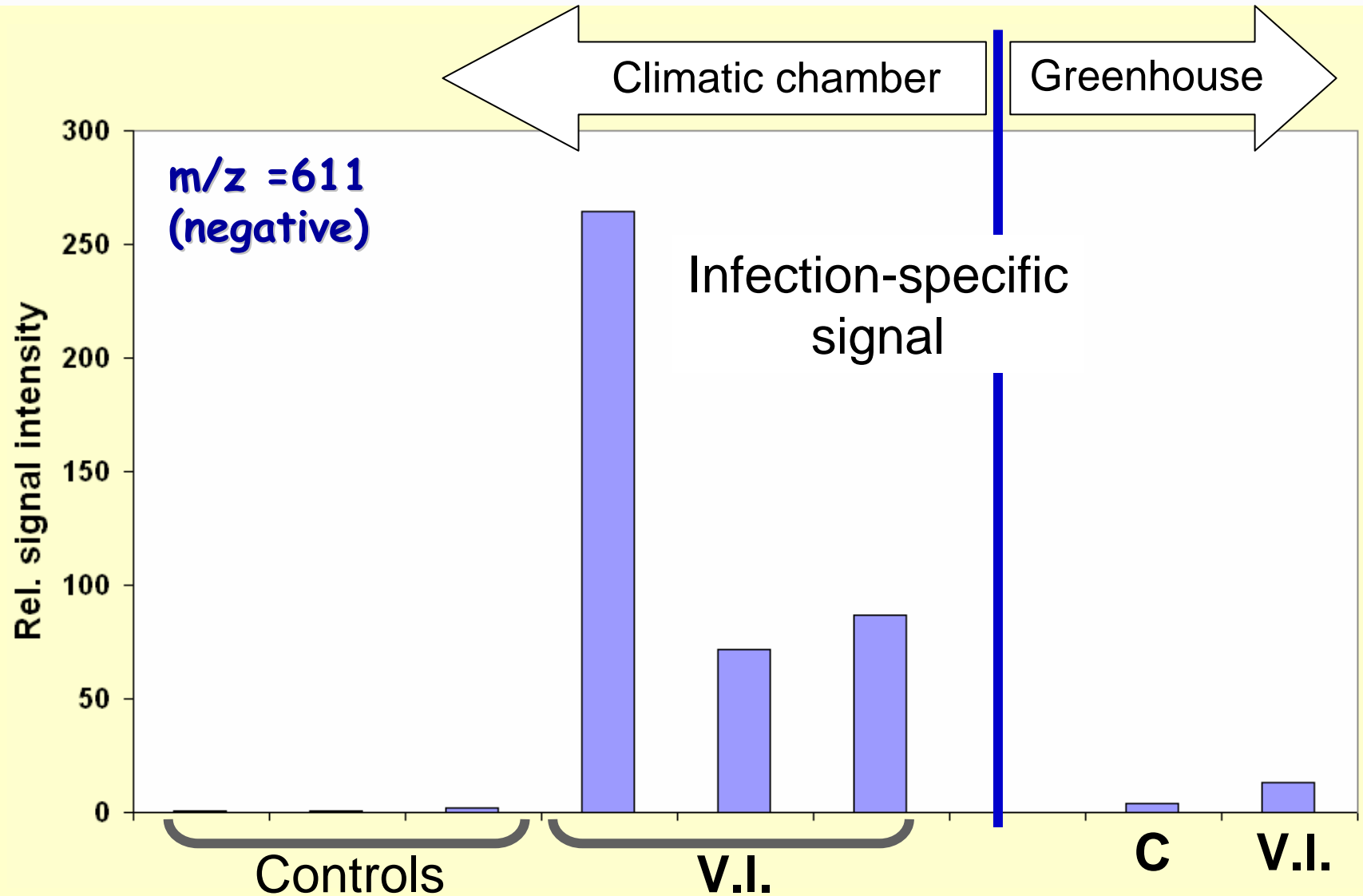


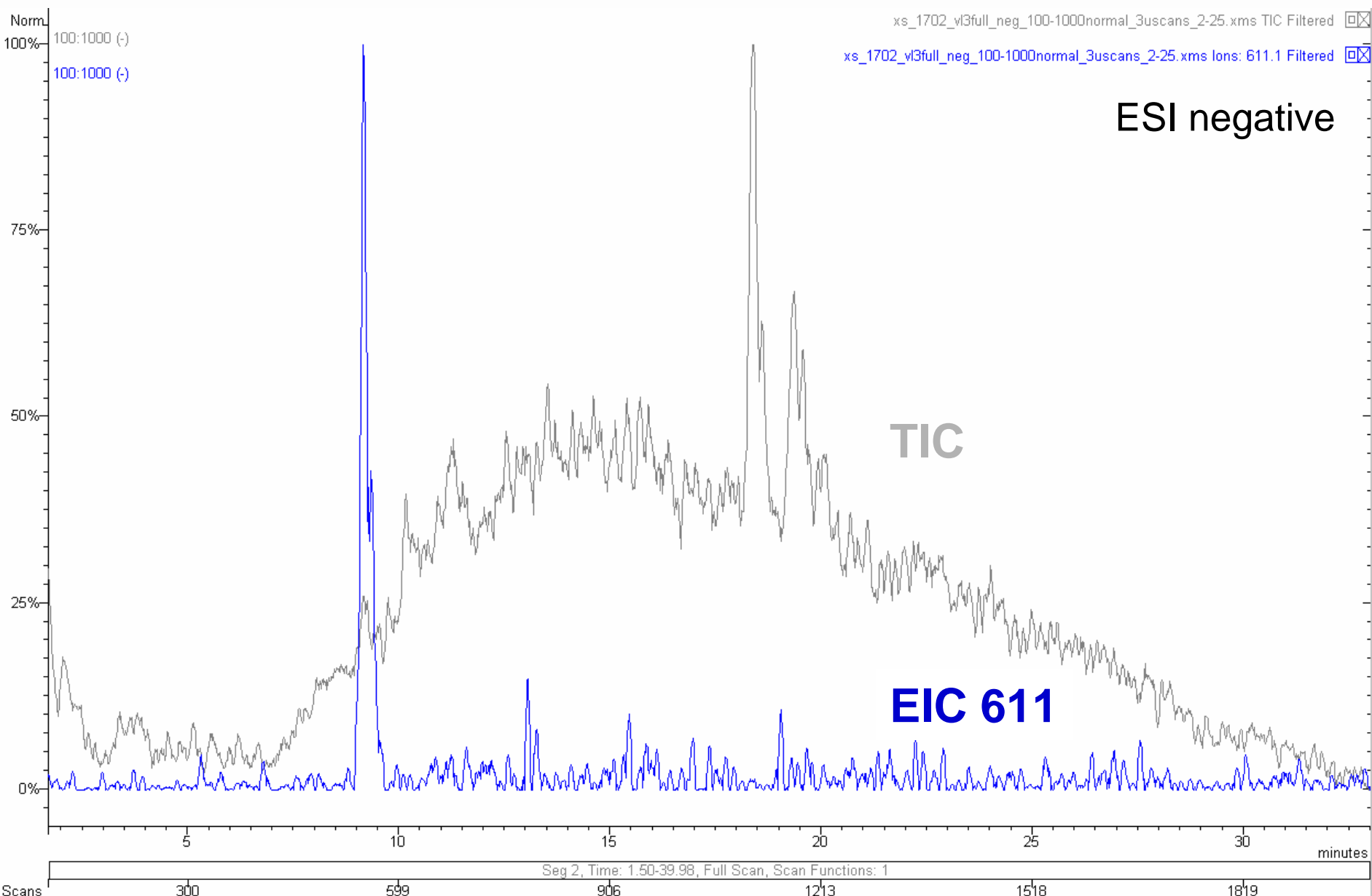




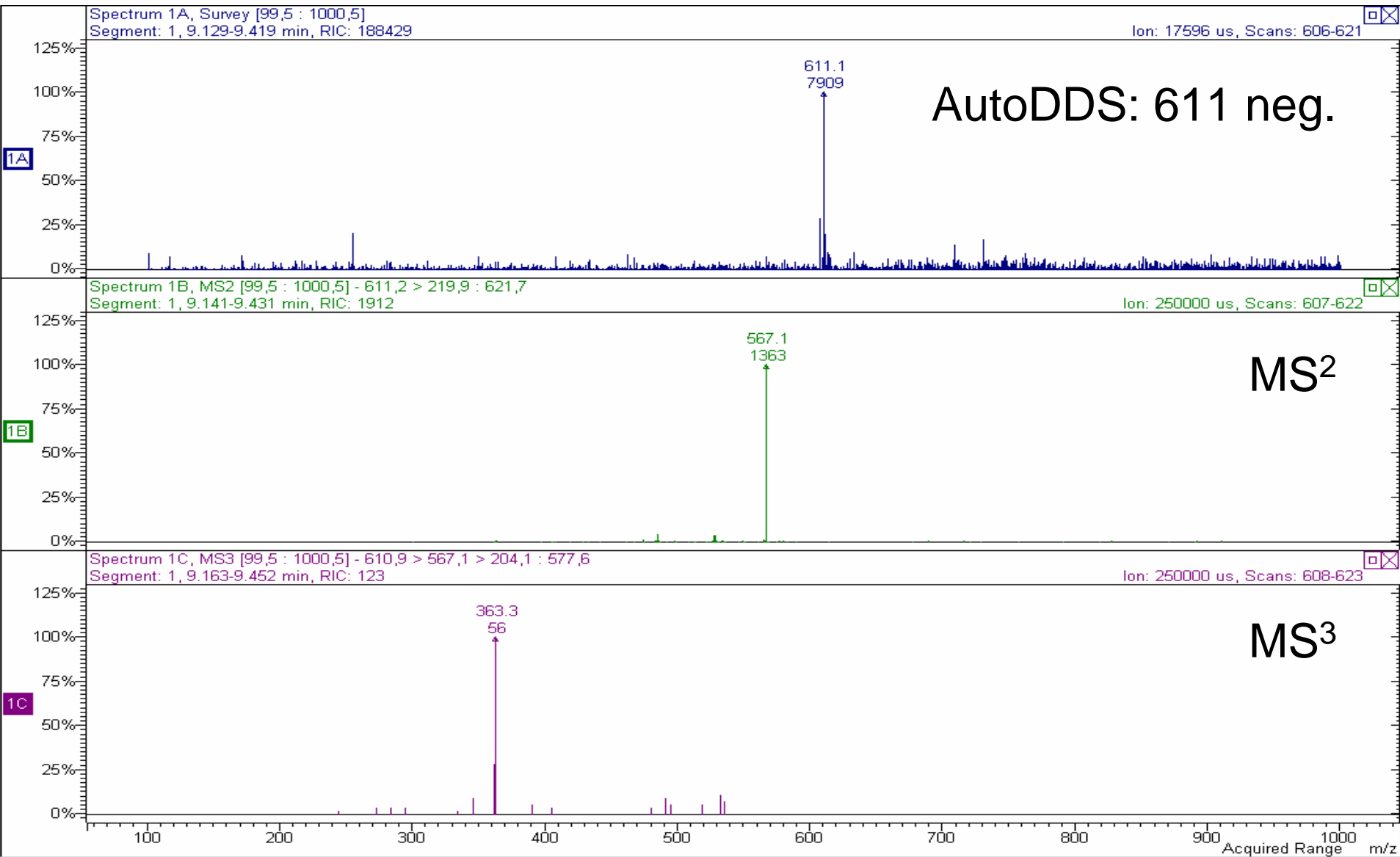


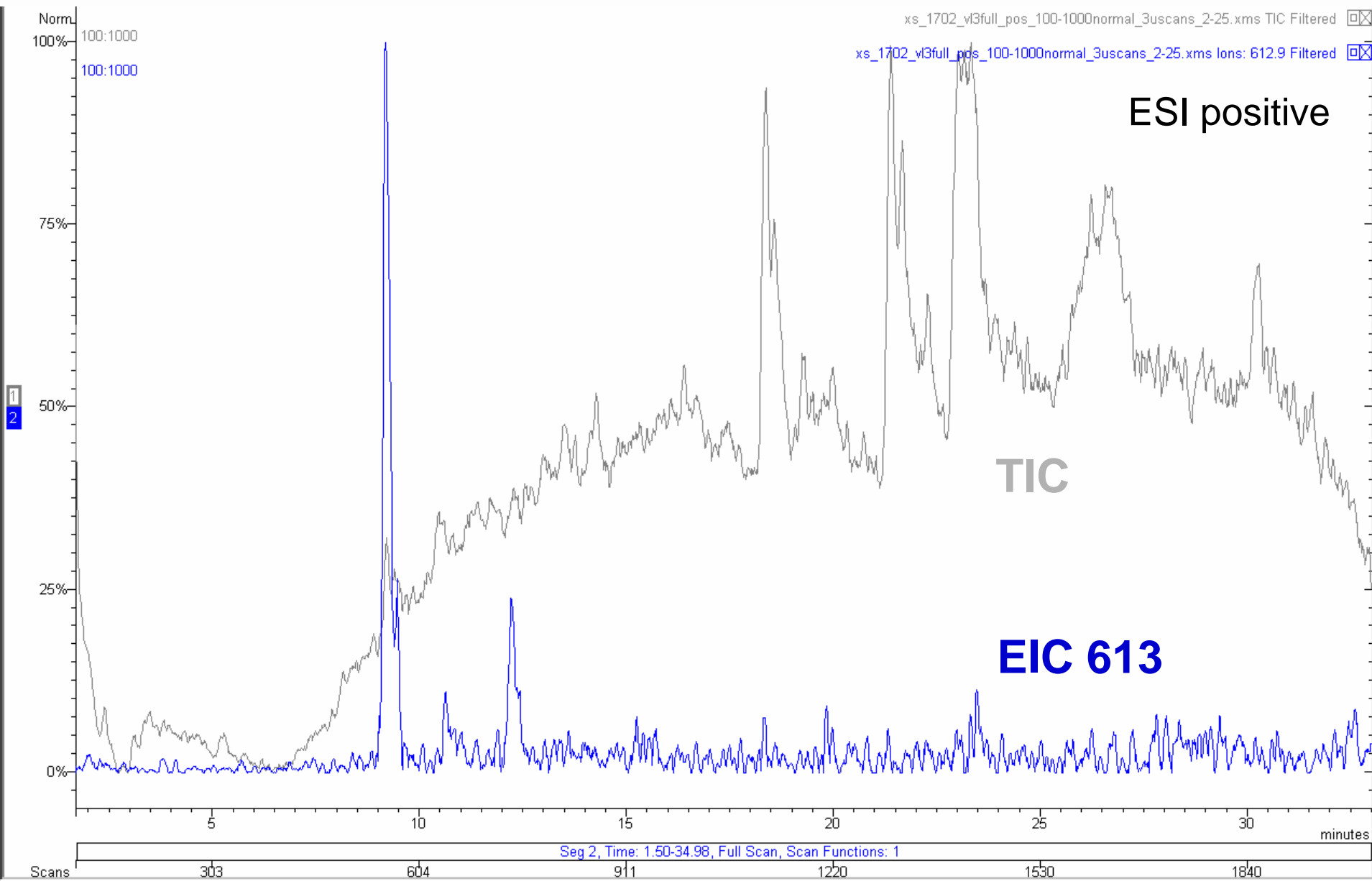




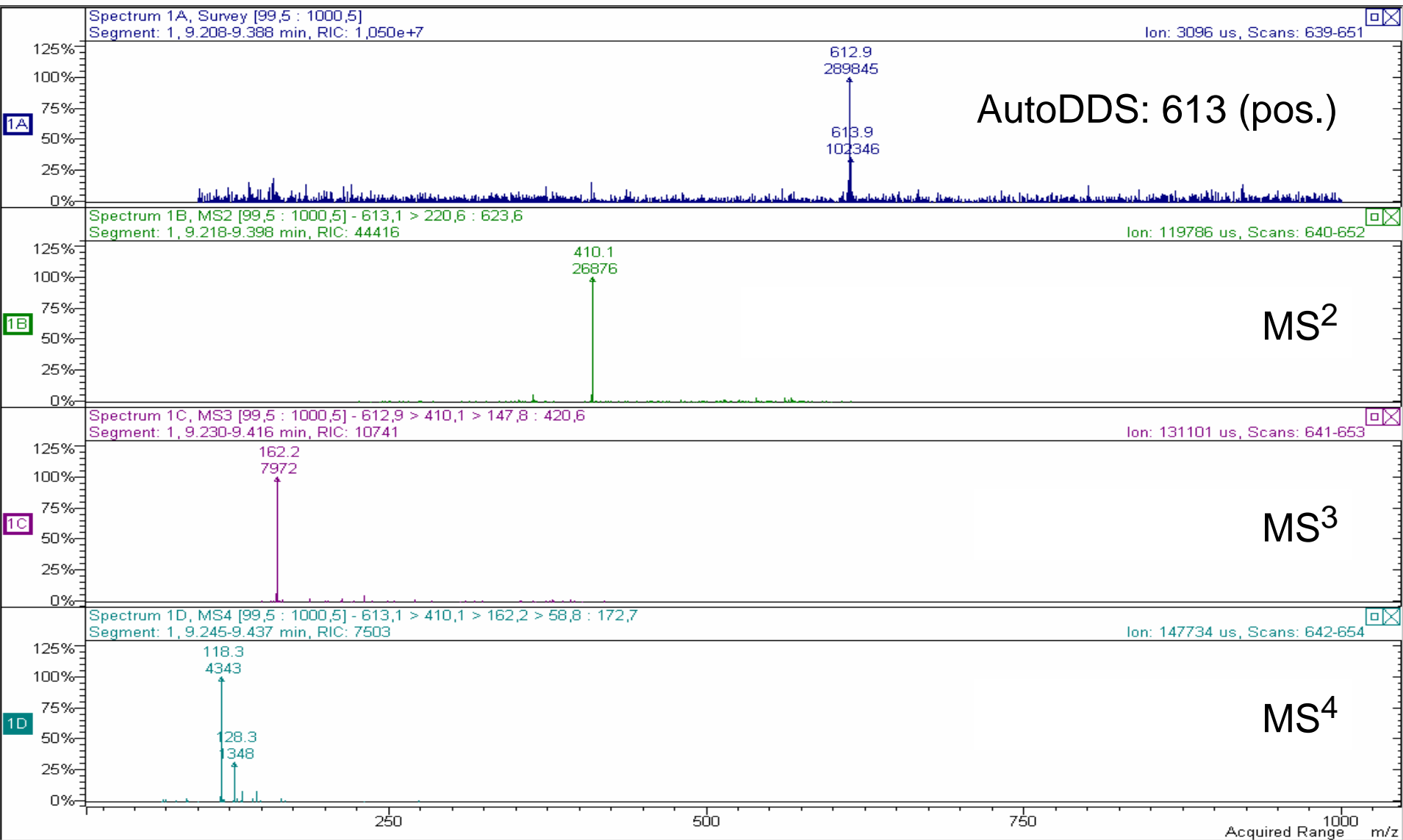


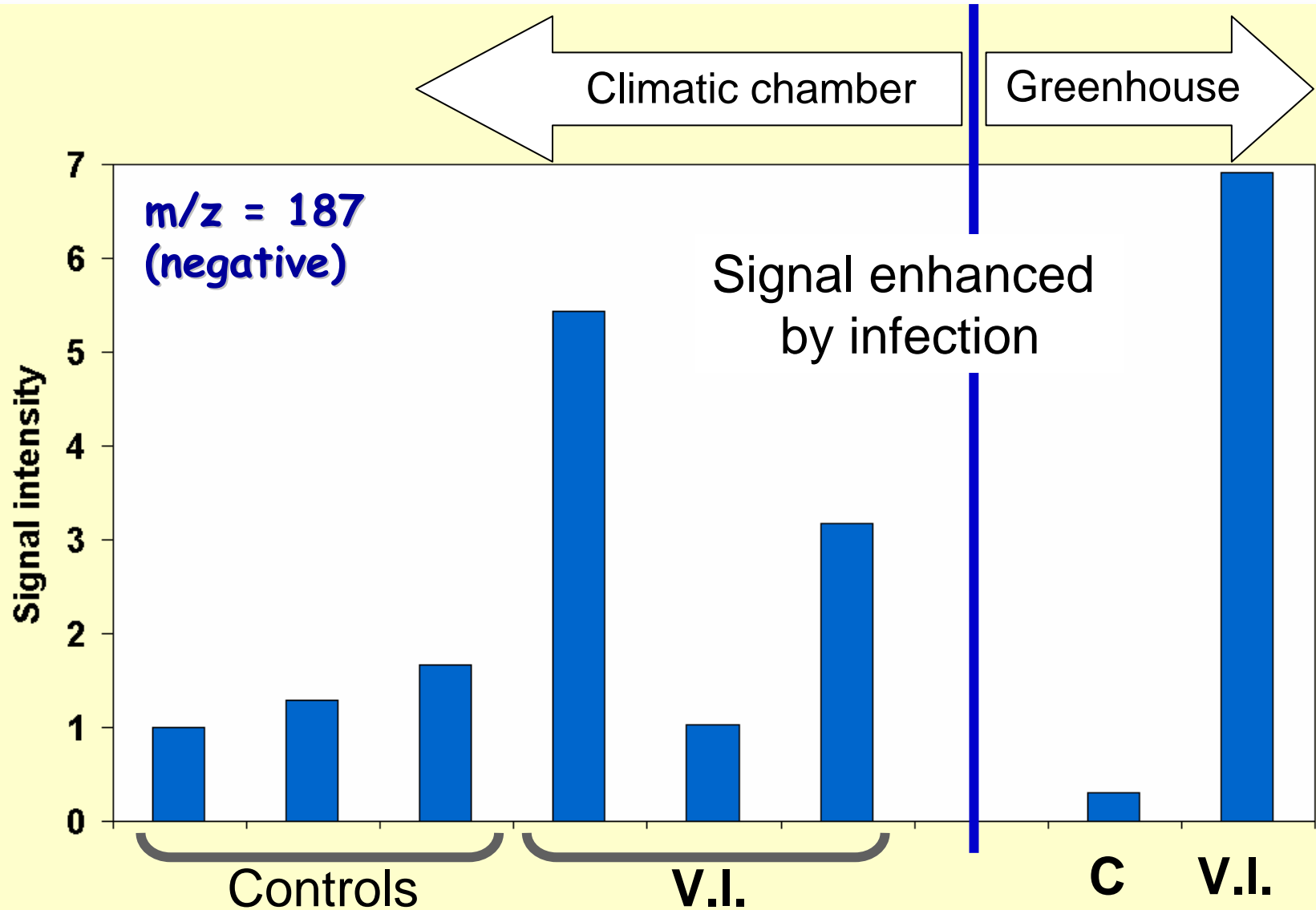
TurboDDS

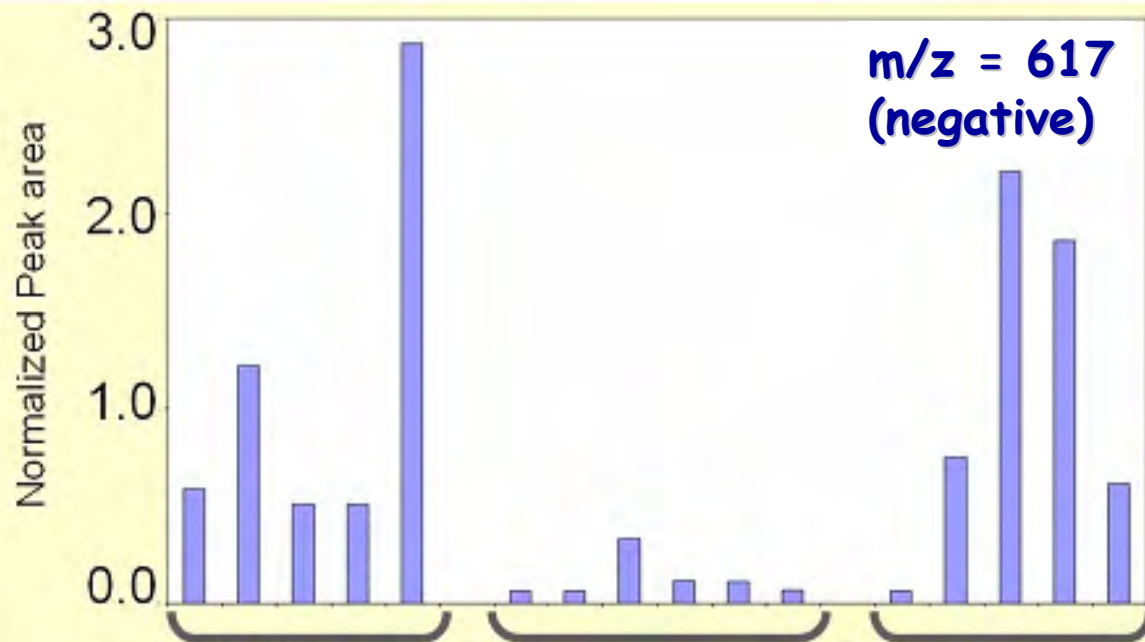




TurboDDS







Confirmation:
Single plant
analysis



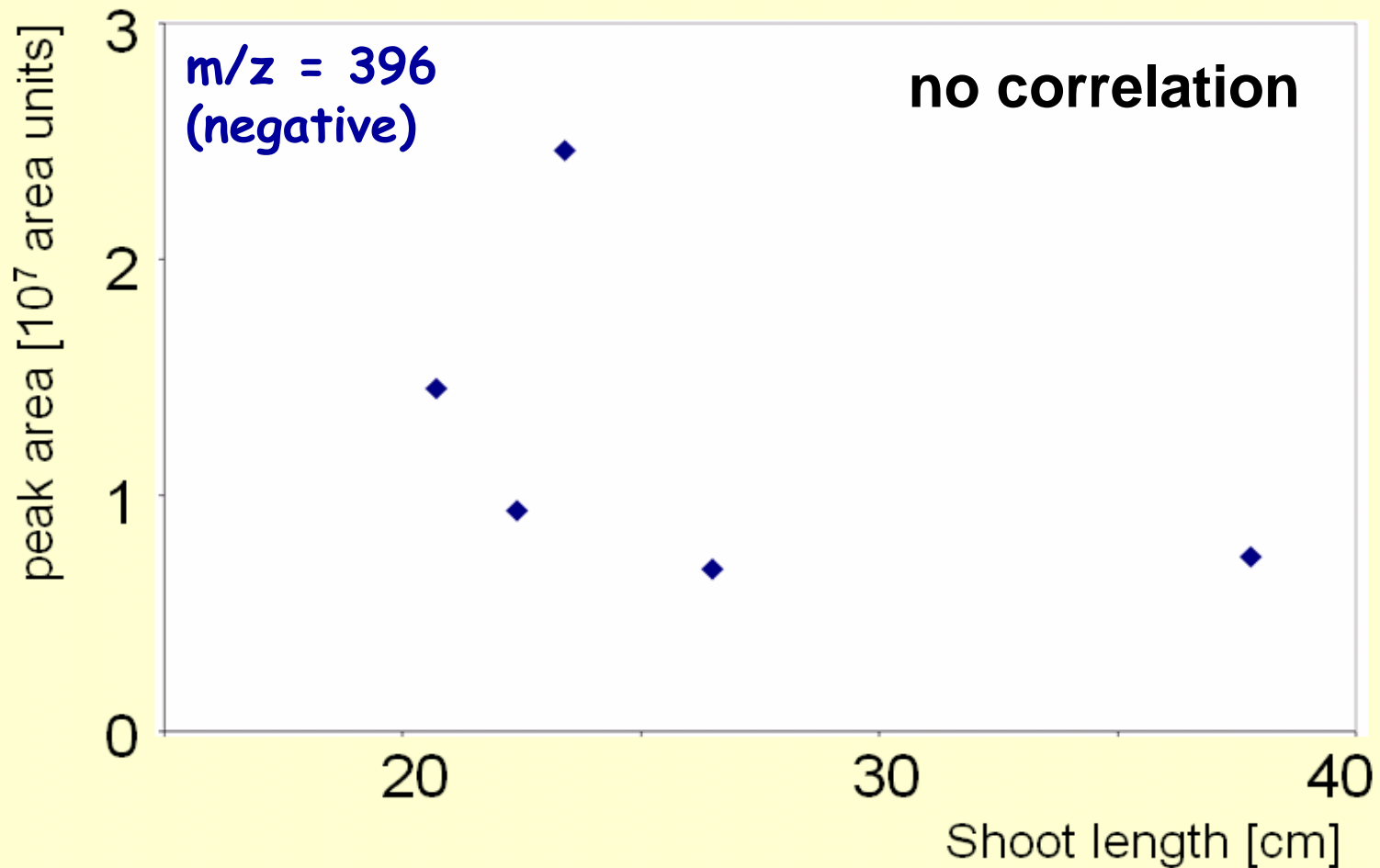
Control

V. longisporum

V. dahliae

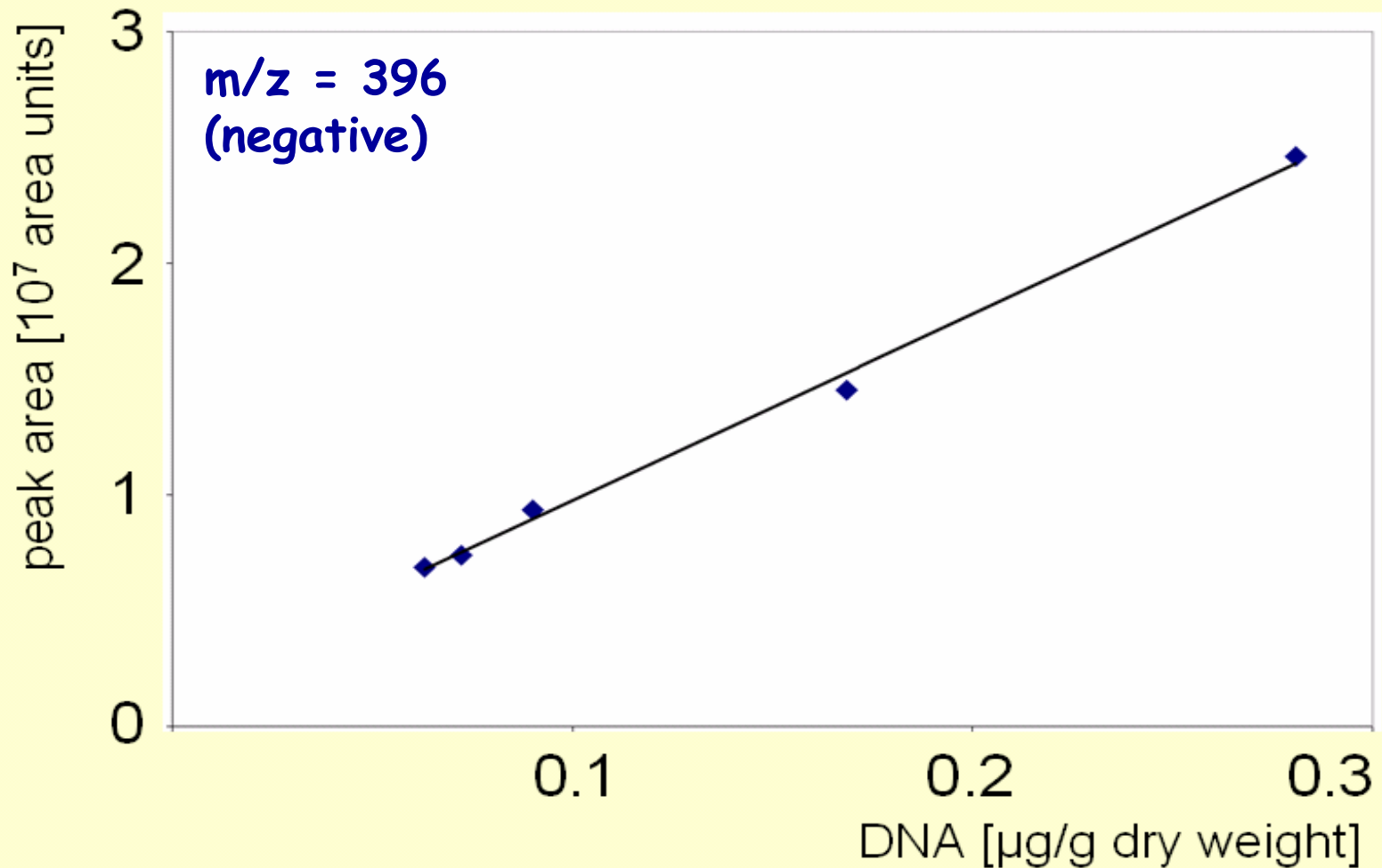


Metabolite signal vs. disease symptoms





Metabolite signal vs. fungal DNA





Xylem sap metabolites affected by infection

- Suppressed metabolites
 - ▶ Degradation of phytoanticipins

- Intensified metabolites
 - ▶ Phytoalexins, signal transduction

- Infection-specific metabolites
 - ▶ Virulence factors?



Acknowledgment

PhD students

Astrid Ratzinger
Arne Weiberg
Nadine Riediger

Funding

DFG (Research Group
FOR546 *Verticillium*)

Technical assistance

Monika Boßmann
Nadine Reimann
Ines Kunze

Special thanks

Dr. Susanne Sölter
(Varian Germany)