PHYTFIRE WP3

Population analysis via MALDI-TOF ID

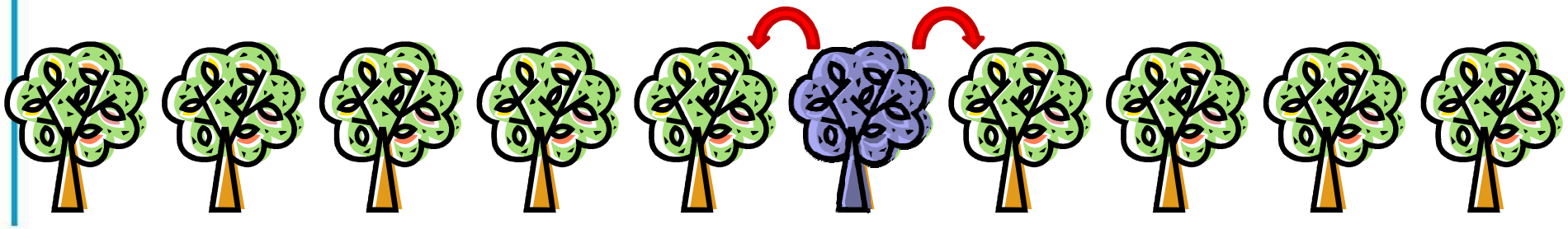
What goes on during fire blight treatment?

Example: Field trials for fire blight control

Hofgut Kirschgartshausen (Lampertheim)

Setup (EPPO PP 1/166(3))

4 blocks with 10 trees each per variant

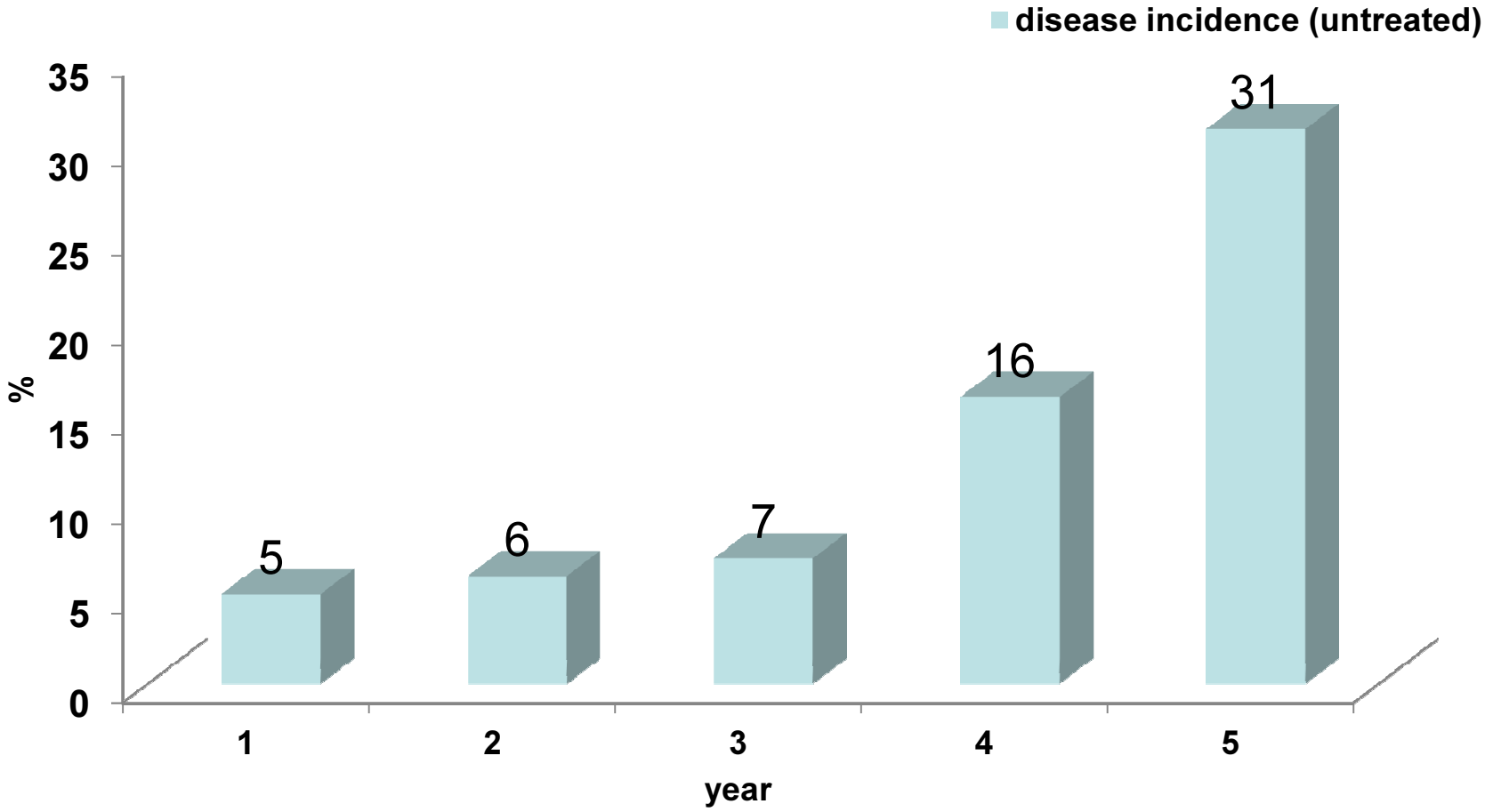


artificial inoculation of one tree/block (10^8 cfu/ml)

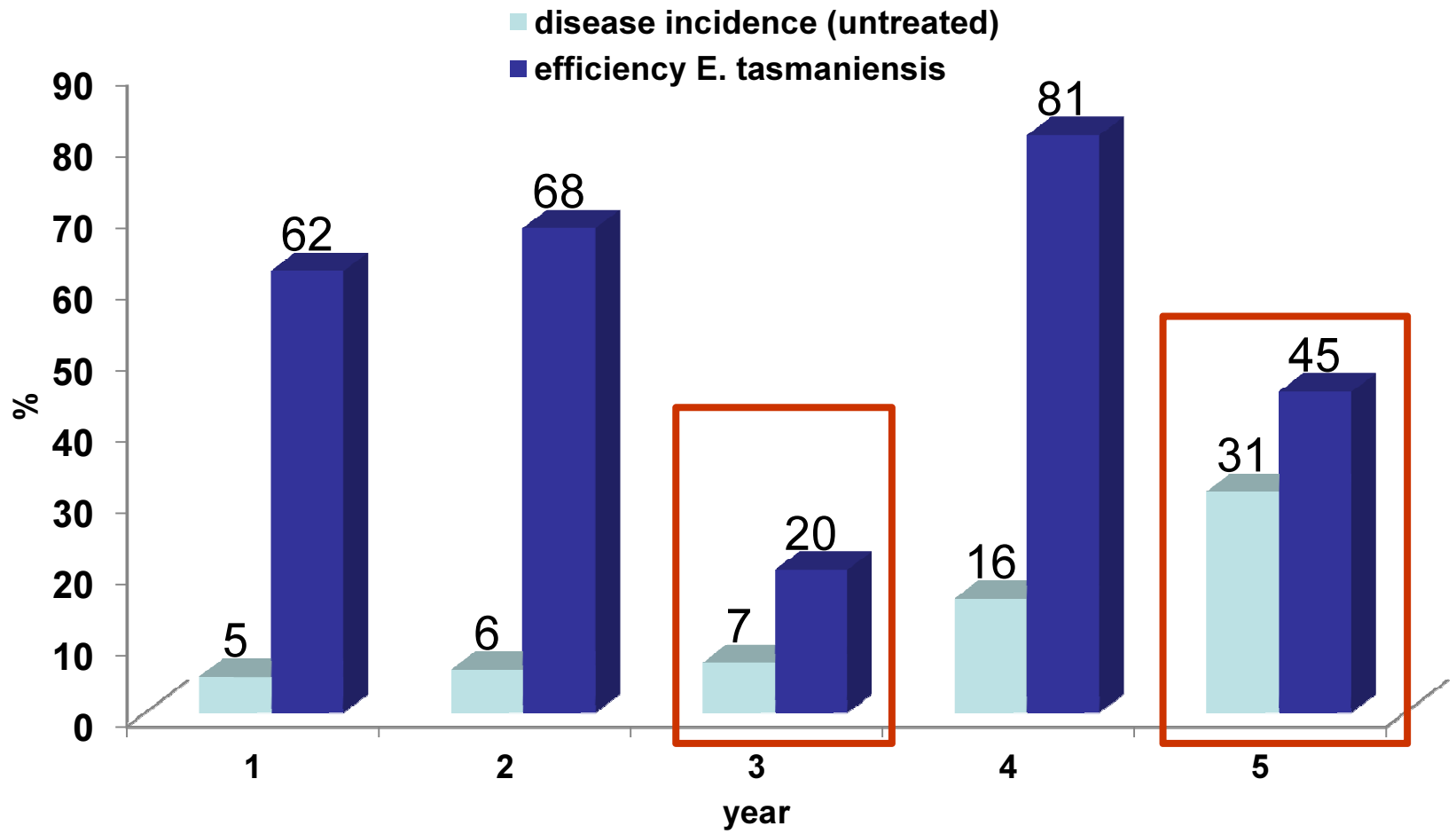
secondary infection of neighboring trees via natural vectors

→ defined conditions

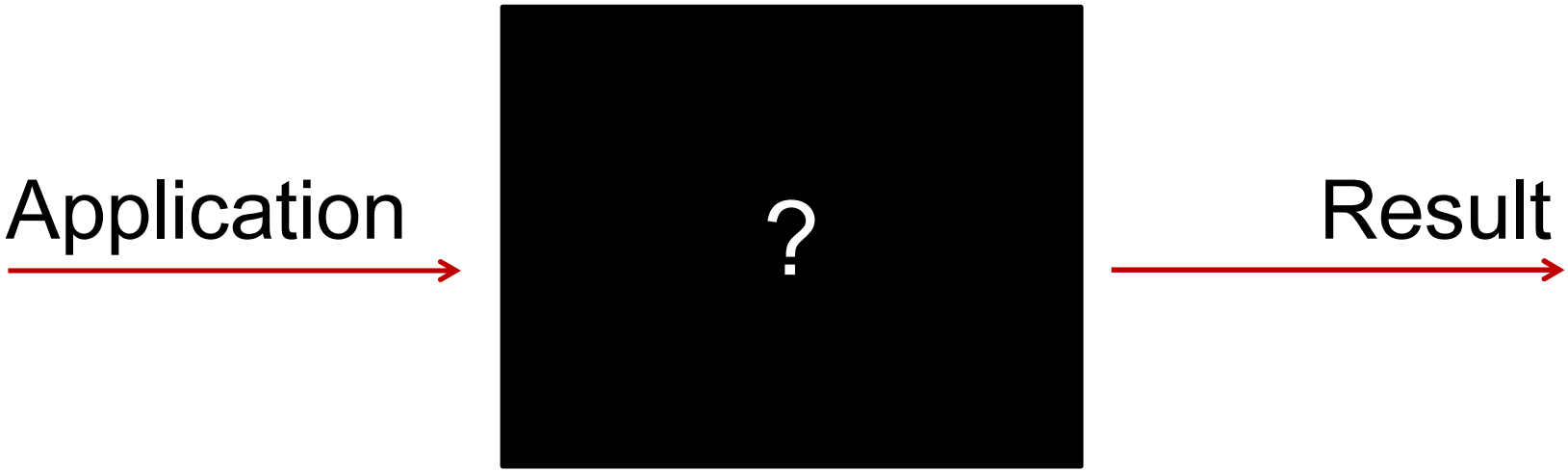
Differences in disease incidence



Differences in efficiency



Objective:
What happens on the flower?

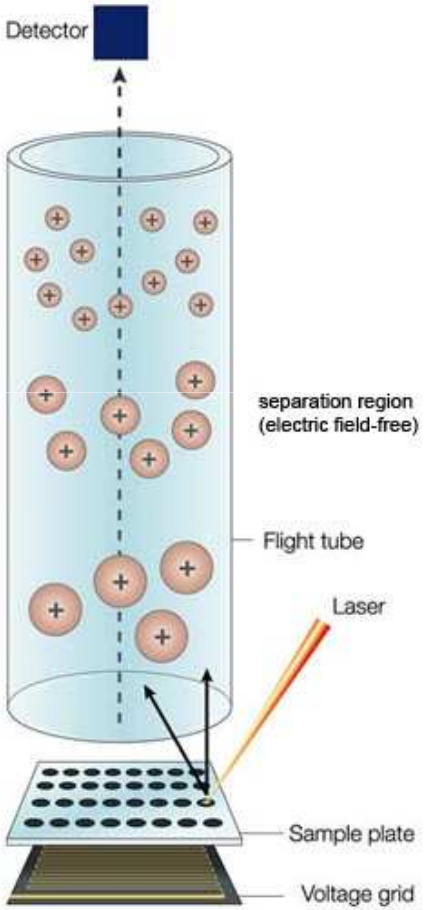


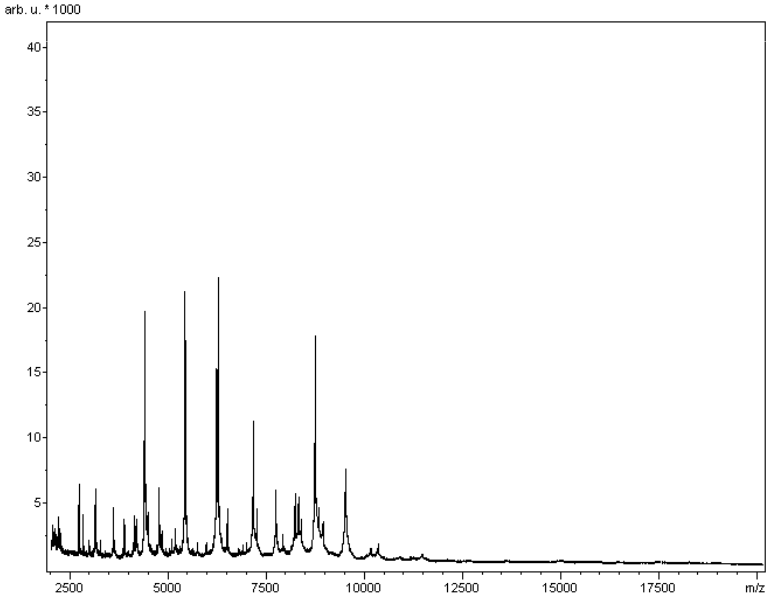
→ screening for specific organisms

→ evaluation of population composition

MALDI-TOF

Matrix assisted laser dissorption/ionisation time of flight analysis

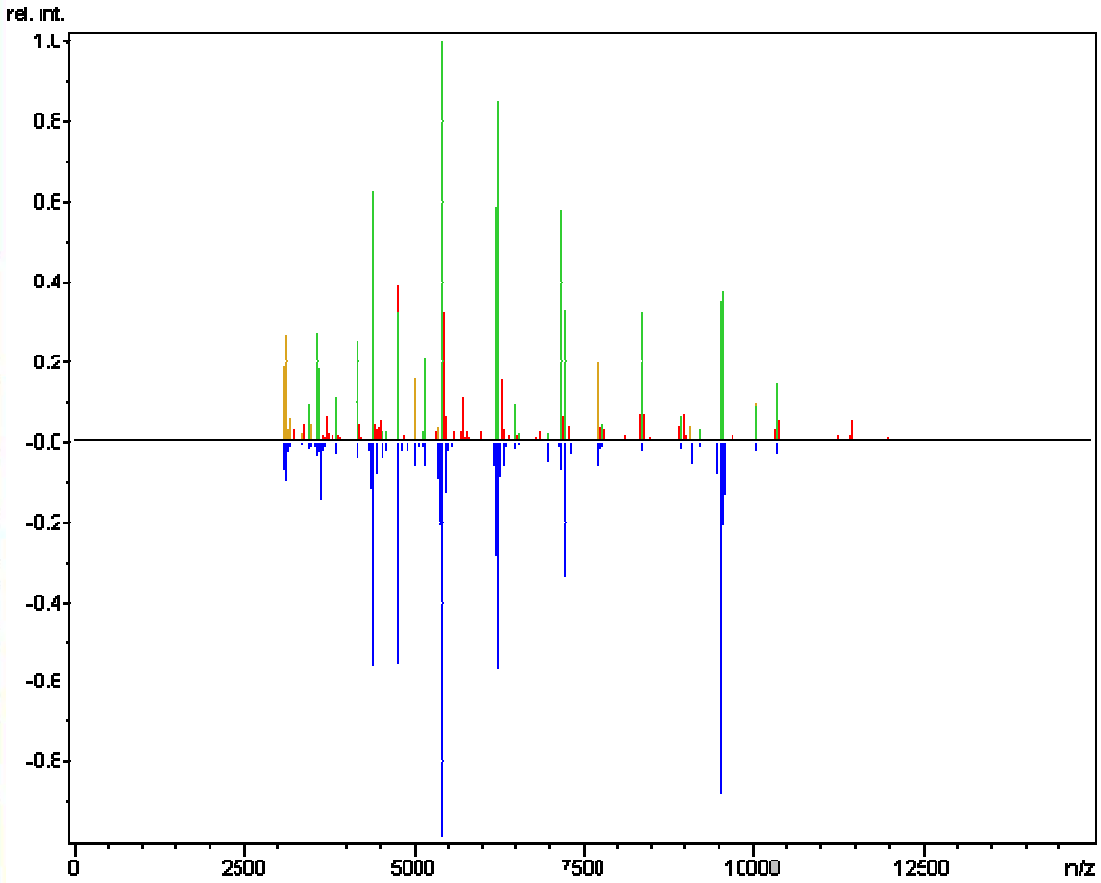




Claydon et al., 1996; Holland et al., 1996;
Krishnamurthy et al., 1996; Keys et al., 2004

Reference spectra matching:

Example: German *E. tasmaniensis* isolate



Detected Species	Log(Score)
Erwinia tasmaniensis Et 2_99_PAH	2.200
Erwinia tasmaniensis 1-99 MMG	2.088
Erwinia tasmaniensis Et 1_99_PAH	2.047
Erwinia pyrifoliae 16-96 MMG	1.807
Erwinia amylovora CFBP1232 MMG	1.729
Enterobacter asburiae DSM 17506_DSM	1.672
Erwinia pyrifoliae 1-96 MMG	1.607
Erwinia sp EJP 562_PAH	1.595
Erwinia pyrifoliae DSM 12163 HAM	1.571
Enterobacter cloacae 20105_2 CHB	1.570

Wensing A, Gernold M, Geider K (2012)
J Appl Microbiol **112:** 147-158

sample preparation:

1ml overnight culture / colony

↓

harvest cells

↓

wash in 70% EtOH

↓

lyse in 35% formic acid with 50% acetonitrile

↓

apply to target

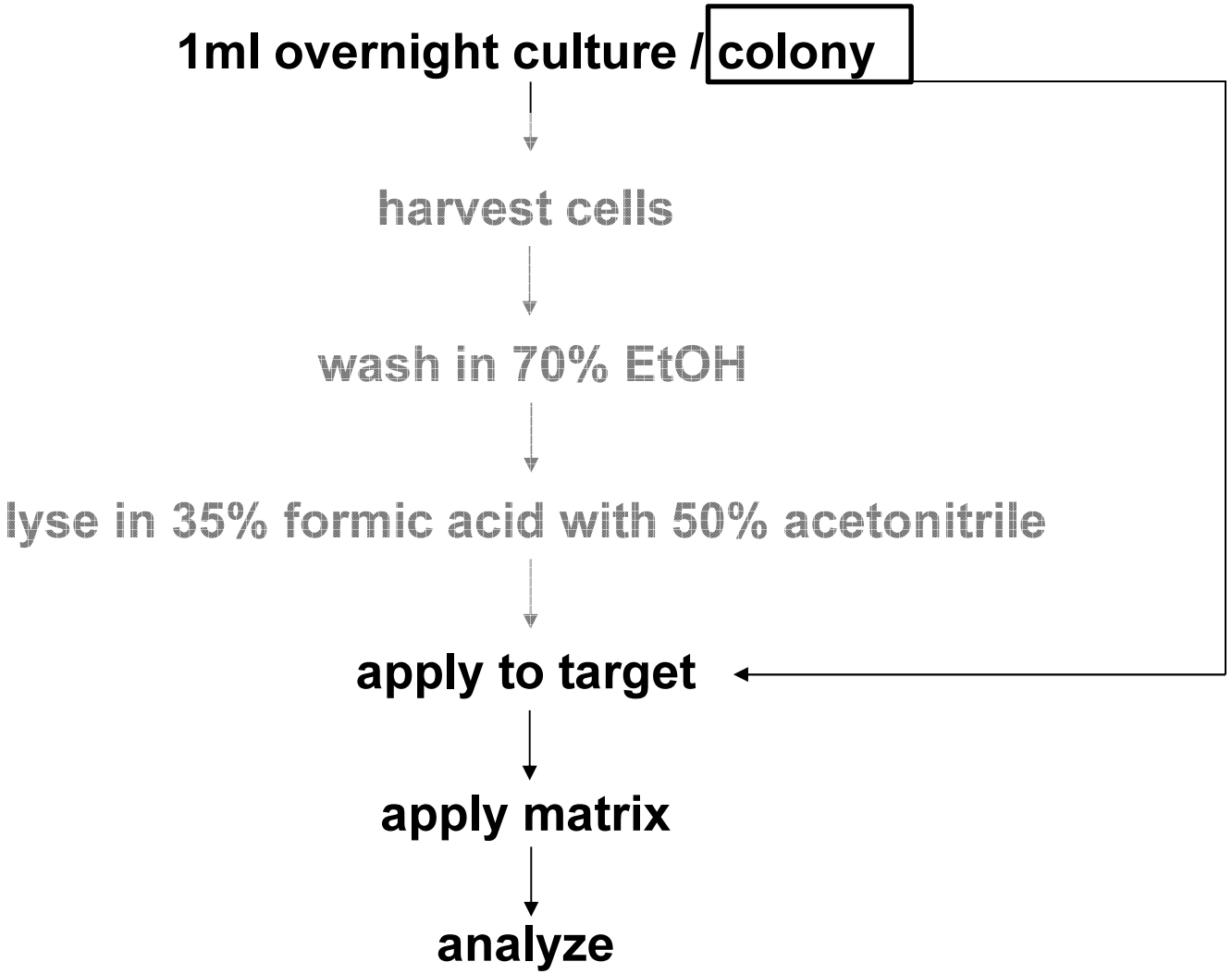
↓

apply matrix

↓

analyze

sample preparation:



OPEN ACCESS Freely available online

PLoS one

MALDI-TOF Mass Spectrometry Is a Fast and Reliable Platform for Identification and Ecological Studies of Species from Family *Rhizobiaceae*

Laura Ferreira[1], Fernando Sánchez-Juanes[1], Paula García-Fraile[2], Raúl Rivas[2], Pedro F. Mateos[2], Eustoquio Martínez-Molina[2], José Manuel González-Buitrago[1,3], Encarna Velázquez[2]*

1 Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain, 2 Departamento de Microbiología y Genética, Universidad de Salamanca, Salamanca, Spain, 3 Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca, Salamanca, Spain

International Biodeterioration & Biodegradation 69 (2012) 82–86

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

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

Whole-cell MALDI-TOF: Rapid screening method in environmental microbiology

Jiri Koubek[1], Ondrej Uhlik, Katerina Jecna, Petra Junkova, Jana Vrkoslavova, Jan Lipov, Veronika Kurzawova, Tomas Macek, Martina Mackova*

Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague, Technicka 3, 166 28 Prague 6, Czech Republic

Vol. 77, No. 19

Matrix-Assisted Laser Desorption Ionization (MALDI)–Time of Flight Mass Spectrometry- and MALDI Biotyper-Based Identification of Cultured Biphenyl-Metabolizing Bacteria from Contaminated Horseradish Rhizosphere Soil▽

Ondrej Uhlik,[1,2]§ Michal Strejcek,[1]§ Petra Junkova,[1] Miloslav Sanda,[2] Miluse Hroudova,[3] Cestmir Vlcek,[2] Martina Mackova,[1,2] and Tomas Macek[1,2]*

Sampling over time

Protocol development:

- **sampling procedure**
- **culture conditions for selection of isolates**

Comparison of systems

Bruker:	**Biotyper**
AnagnosTec:	**Saramis** → **no longer „send-in“ samples**
Waters:	**MicrobeLynx**

Evaluation with established methods

- **specific PCR +qPCR for selected species**
- **sequencing approaches**

Getting cell material to analyze:

Wash samples from flowers/young fruitlets

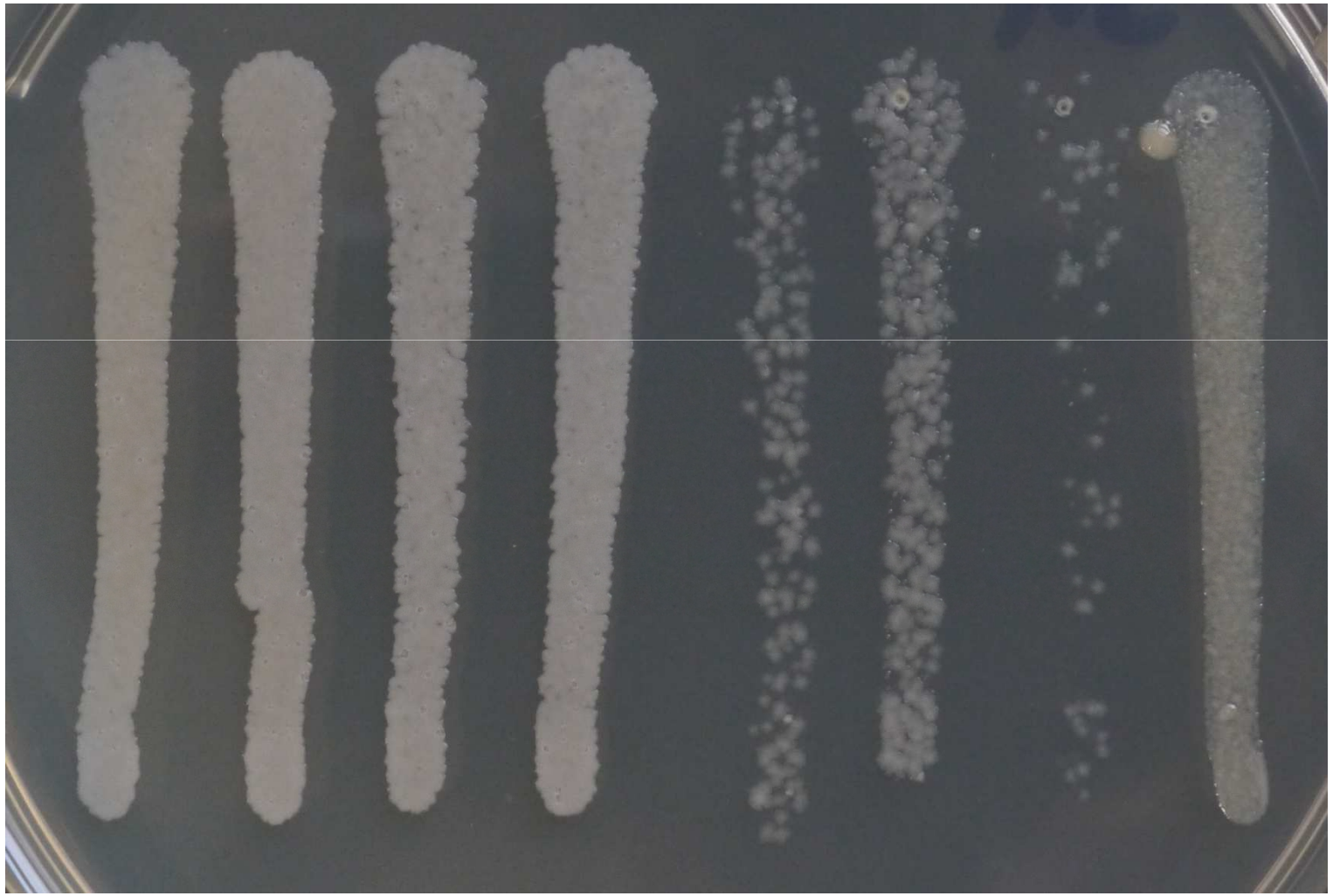
→ prepare glycerol stock + tween lysate

→ aliquot of wash sample in microwell plate

→ place 5µl droplets to medium

Getting cell material to analyze:

→ get dilution by gravity flow



sample preparation:

1ml overnight culture / colony

↓

harvest cells

↓

wash in 70% EtOH

↓

lyse in 70% formic acid on target

↓

apply matrix

↓

analyze

What happens in the field?

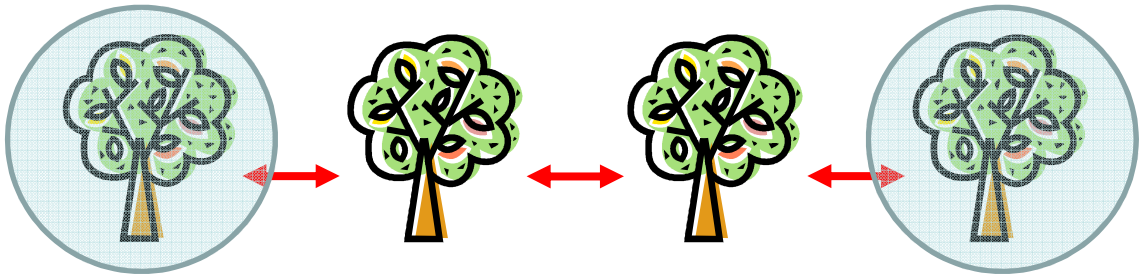
Inoculation with

- *Erwinia tasmaniensis*

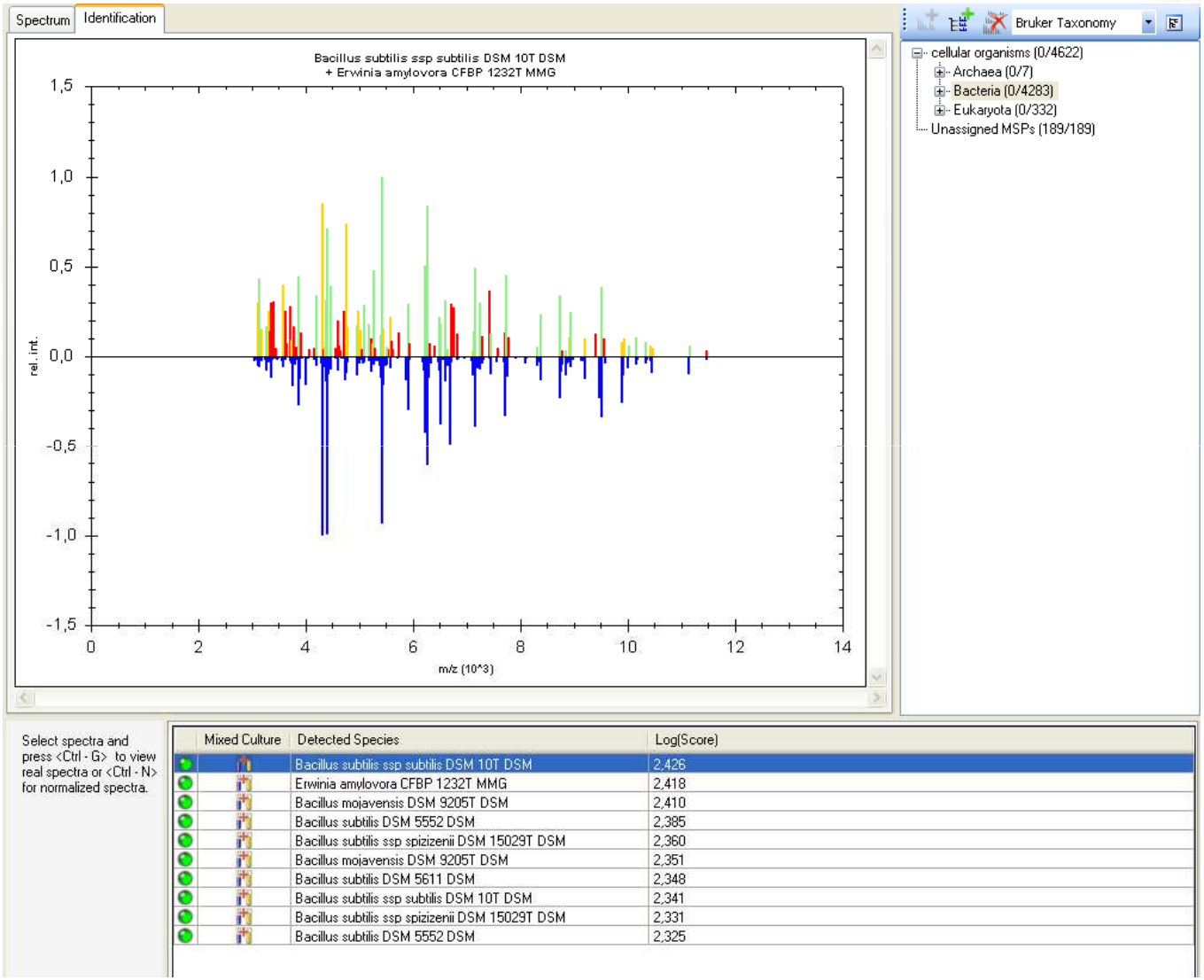
- *Bacillus amyloliquefaciens*

- for half the samples with *E. amylovora.*

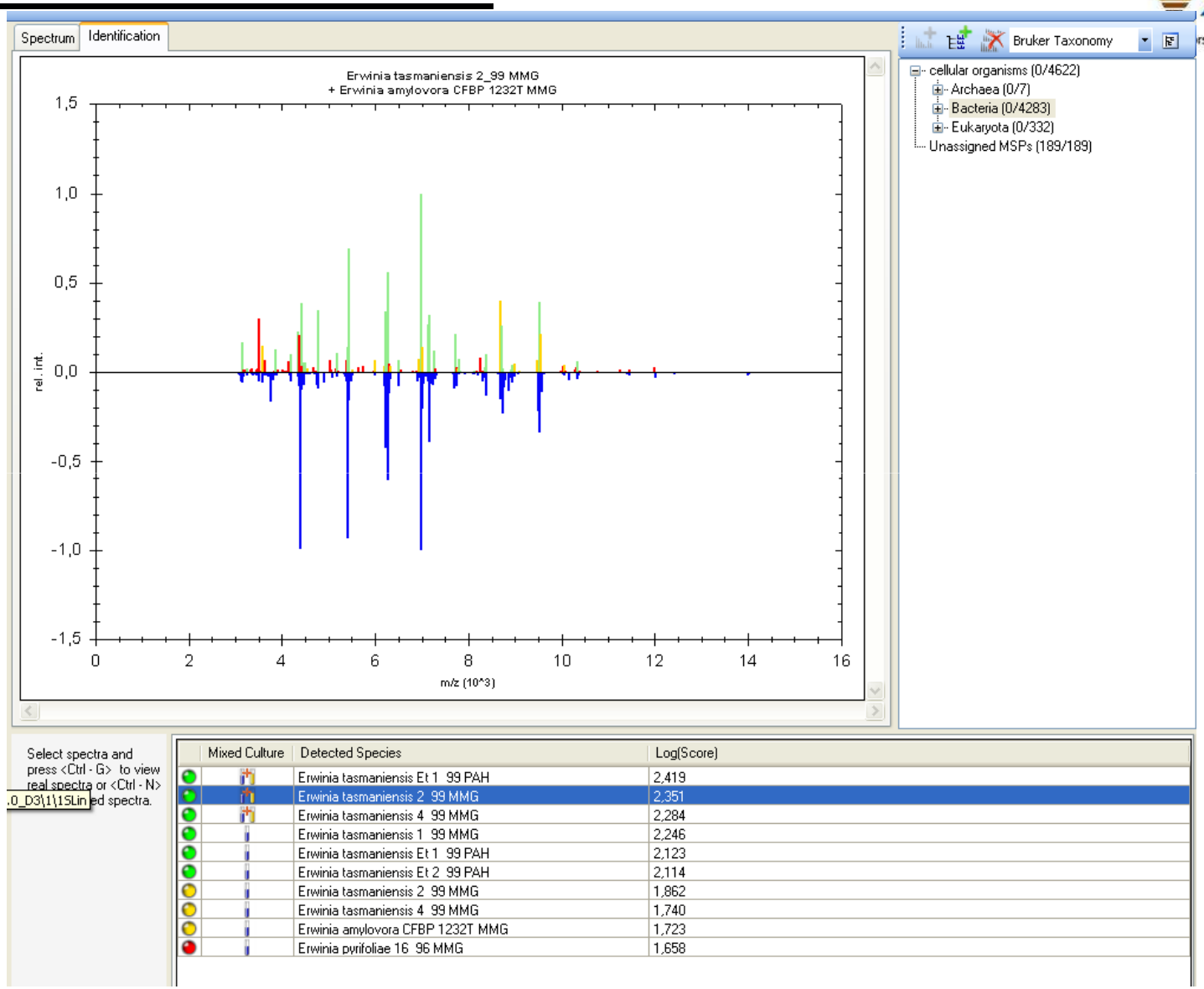
In addition samples without artifitial inoculation.



Mixed culture mode



Mixed culture mode



Dominant species in mixed culture?

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Inoculation with *Erwinia tasmaniensis*:

	T0	T1	T2	T3	T4	T5
a	Erwinia tasmaniensis	Bacillus mojavensis	Erwinia tasmaniensis	Bacillus vallismortis	Pantoea agglomerans	Pseudomonas chlororaphis
b	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	no identification	Erwinia tasmaniensis	Pseudomonas graminis
c	Bacillus subtilis	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	no identification	Pseudomonas congelans

Inoculation with *Erwinia tasmaniensis* + *E. amylovora*:

	T0	T1	T2	T3	T4	T5
a	no identification	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	Pseudomonas graminis	Pantoea agglomerans
b	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	Metschnikowia pulcherrima	Erwinia tasmaniensis	Erwinia amylovora
c	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	Bacillus subtilis	Pseudomonas congelans

Comparison to direct PCR?

Inoculation with *Erwinia tasmaniensis* -> *PCR Et* (qPCR $10^5/10^6$ cfu)

	T0	T1	T2	T3	T4	T5
a	Erwinia tasmaniensis	Bacillus mojavensis	Erwinia tasmaniensis	Bacillus vallismortis	Pantoea agglomerans	Pseudomonas chlororaphis
b	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	no identification	Erwinia tasmaniensis	Pseudomonas graminis
c	Bacillus subtilis	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	no identification	Pseudomonas congelans

Inoculation with *Erwinia tasmaniensis* + *E. amylovora* -> *PCR Et*:

	T0	T1	T2	T3	T4	T5
a	no identification	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	Pseudomonas graminis	Pantoea agglomerans
b	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	Metschnikowia pulcherrima	Erwinia tasmaniensis	Erwinia amylovora
c	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	Bacillus subtilis	Pseudomonas congelans

Comparison to direct PCR?

Inoculation with *Erwinia tasmaniensis* -> *PCR Ea*:

	T0	T1	T2	T3	T4	T5
a	Erwinia tasmaniensis	Bacillus mojavensis	Erwinia tasmaniensis	Bacillus vallismortis	Pantoea agglomerans	Pseudomonas chlororaphis
b	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	no identification	Erwinia tasmaniensis	Pseudomonas graminis
c	Bacillus subtilis	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	no identification	Pseudomonas congelans

Inoculation with *Erwinia tasmaniensis* + *E. amylovora* -> *PCR Ea*:

	T0	T1	T2	T3	T4	T5
a	no identification	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	Pseudomonas graminis	Pantoea agglomerans
b	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	Metschnikowia pulcherrima	Erwinia tasmaniensis	Erwinia amylovora
c	Erwinia tasmaniensis	Erwinia tasmaniensis	Erwinia tasmaniensis	Pantoea agglomerans	Bacillus subtilis	Pseudomonas congelans

More teamwork friendly options???

Proteomics & Bioinformatics

Raus and Šebela, J Proteomics Bioinform 2013, 6:12
http://dx.doi.org/10.4172/jpb.1000292

Short Communication Open Access

BIOSPEAN: A Freeware Tool for Processing Spectra from MALDI Intact Cell/Spore Mass Spectrometry

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Abstract

Here we introduce the software BIOSPEAN, which is applicable for processing of peptide/protein profiles obtained from MALDI intact cell/spore mass spectrometry. It has been developed as a web application using common technology. The BIOSPEAN automatically finds peaks in a mass spectrum. The peak detection principle involves a local scanning of intensity values around individual *m/z* positions. To cope with the level of noise, a threshold signal-to-noise ratio is adjusted for filtering relevant results. Based on the detected peak pattern, a similarity search in a custom spectral database can subsequently be performed for sample identification. When a spectrum is analyzed by comparison with a database entry, a percentage score value is calculated from the number of identical peak positions found (they are assigned with an adjustable mass tolerance) divided by the number of all detected peaks in the analyzed spectrum. Each two spectra may also be compared in the opposite way and both score values averaged. The database search results are finally sorted in a table.

```
About input file format - its possible load only TXT files (10MB
max. size), input values are pairs [m/z, intensity], each pair
is one row, values are separated by Space or Tab character.
example:
100.207 62
100.217 62
100.226 70
100.235 55
100.245 66
```

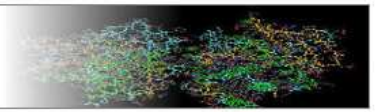
Online database and analysis

Online database and analysis

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

BIOSPEAN

MAIN | SPECTRA LIST | SPECTRA | ADD NEW | COMPARE ALL | COMPARE TWO | FINDER | GROUPS | PROFILE/FRIENDS | MANUAL | LOGOUT **WENSING.A**



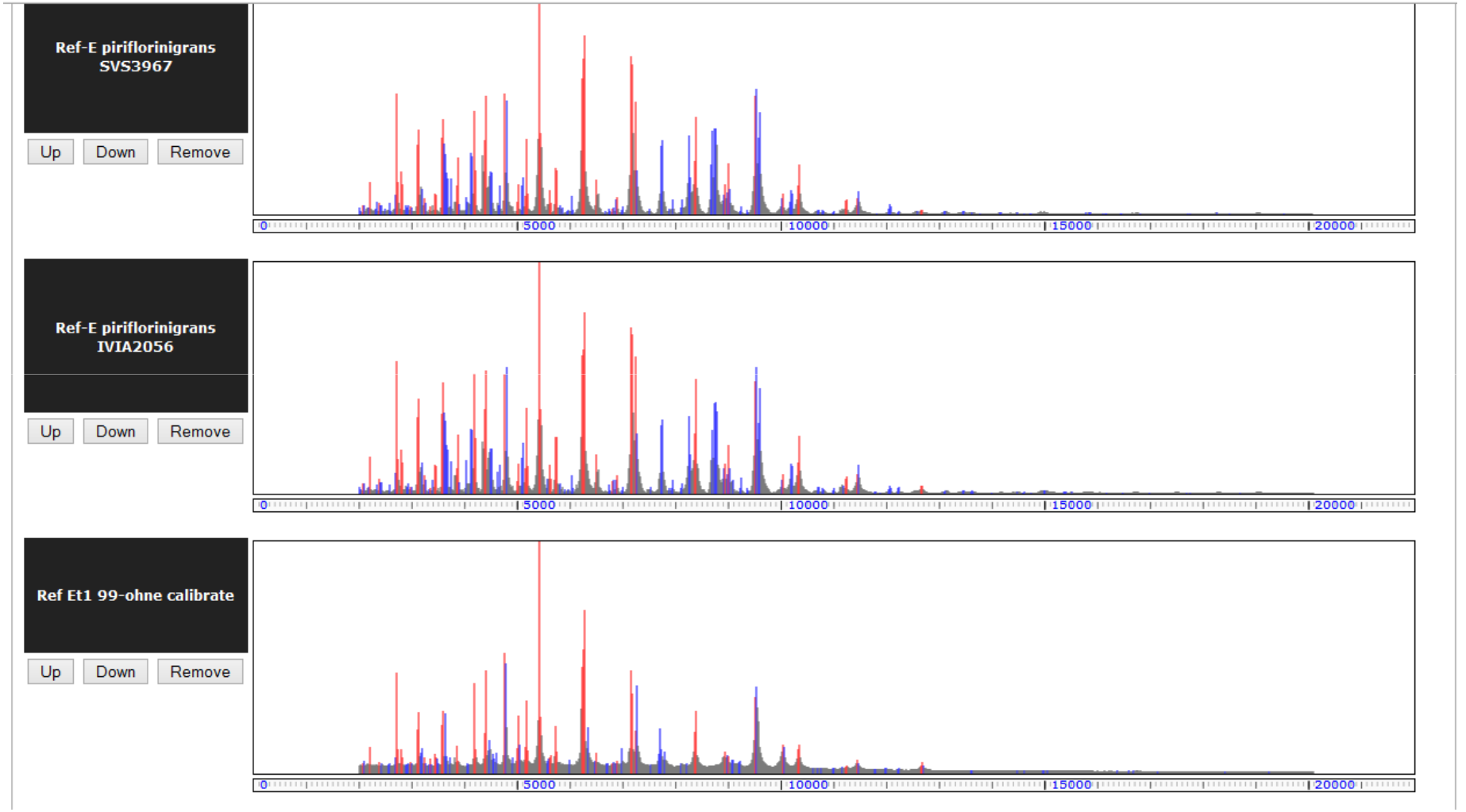
AVERAGE SIMILARITY

%		Et1_B5	Et1_B5_sqrt	Et1_B5_smooth	Et1_B5_baseline	Et2-99_B8	Ref_Et1_99-ohne calibrate	E. piri IVIA2055_2a_C5
Et1_B5	show	100.00	90.04	80.47	78.47	75.74	58.92	55.96
Et1_B5_sqrt	show	90.04	100.00	79.66	77.43	74.85	59.74	56.10
Et1_B5_smooth	show	80.47	79.66	100.00	93.22	72.15	57.89	52.65
Et1_B5_baseline	show	78.47	77.43	93.22	100.00	71.37	58.30	53.23
Et2-99_B8	show	75.74	74.85	72.15	71.37	100.00	65.75	57.20
Ref_Et1_99-ohne calibrate	show	58.92	59.74	57.89	58.30	65.75	100.00	64.50
E. piri IVIA2055_2a_C5	show	55.96	56.10	52.65	53.23	57.20	64.50	100.00

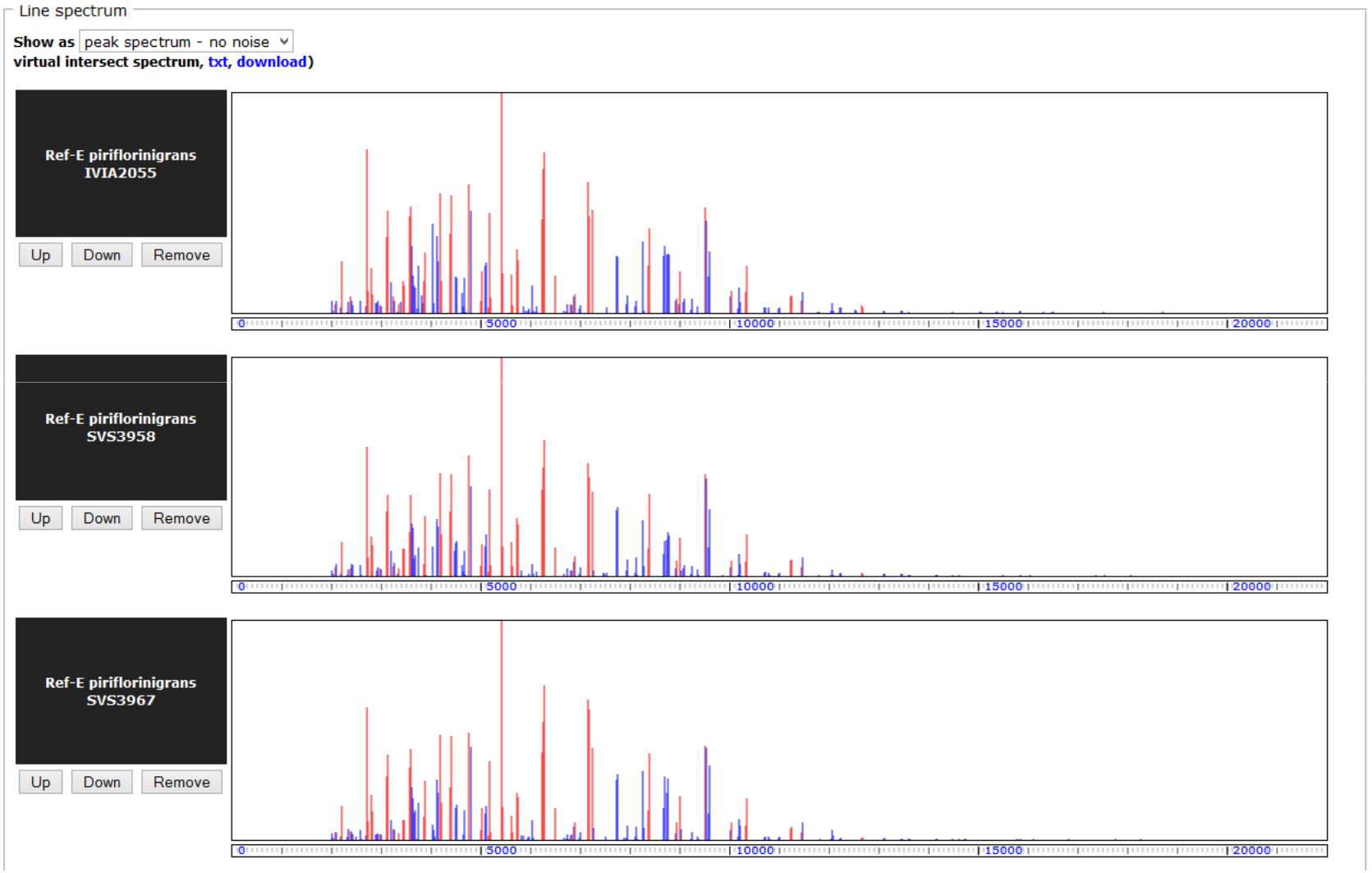
SIMILARITY ROW-COLUMN

%		Et1_B5	Et1_B5_sqrt	Et1_B5_smooth	Et1_B5_baseline	Et2-99_B8	Ref_Et1_99-ohne calibrate	E. piri IVIA2055_2a_C5
Et1_B5	show	100.00	100.00	71.83	67.30	72.37	59.36	64.15
Et1_B5_sqrt	show	80.07	100.00	62.49	58.14	63.29	53.57	58.14
Et1_B5_smooth	show	89.11	96.82	100.00	89.91	76.71	64.53	65.80
Et1_B5_baseline	show	89.64	96.71	96.53	100.00	78.39	67.02	68.28
Et2-99_B8	show	79.10	86.40	67.59	64.34	100.00	69.16	68.04
Ref_Et1_99-ohne calibrate	show	58.47	65.90	51.24	49.57	62.33	100.00	73.47
E. piri IVIA2055_2a_C5	show	47.76	54.06	39.49	38.17	46.35	55.53	100.00

Online database and analysis



Online database and analysis



How to “free” your spectra???

MALDIquant and MALDIquantForeign

-> open source in R

BIOINFORMATICS APPLICATIONS NOTE Vol. 28 no. 17 2012, pages 2270–2271
doi:10.1093/bioinformatics/bts447

Genome analysis

Advance Access publication July 12, 2012

MALDIquant: a versatile R package for the analysis of mass spectrometry data

Sebastian Gibb* and Korbinian Strimmer

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Associate Editor: Alex Bateman

ABSTRACT

Summary: `MALDIquant` is an R package providing a complete and modular analysis pipeline for quantitative analysis of mass spectrometry data. `MALDIquant` is specifically designed with application in clinical diagnostics in mind and implements sophisticated routines for importing raw data, preprocessing, non-linear peak alignment and calibration. It also handles technical replicates as well as spectra with unequal resolution.

Availability: `MALDIquant` and its associated R packages `readBrukerFlexData` and `readMzXmlData` are freely available from the R archive CRAN (http://cran.r-project.org). The software is distributed under the GNU General Public License (version 3 or later) and is accompanied by example files and data. Additional documentation is available from http://strimmerlab.org/software/maldiquant/.

Contact: mail@sebastiangibb.de

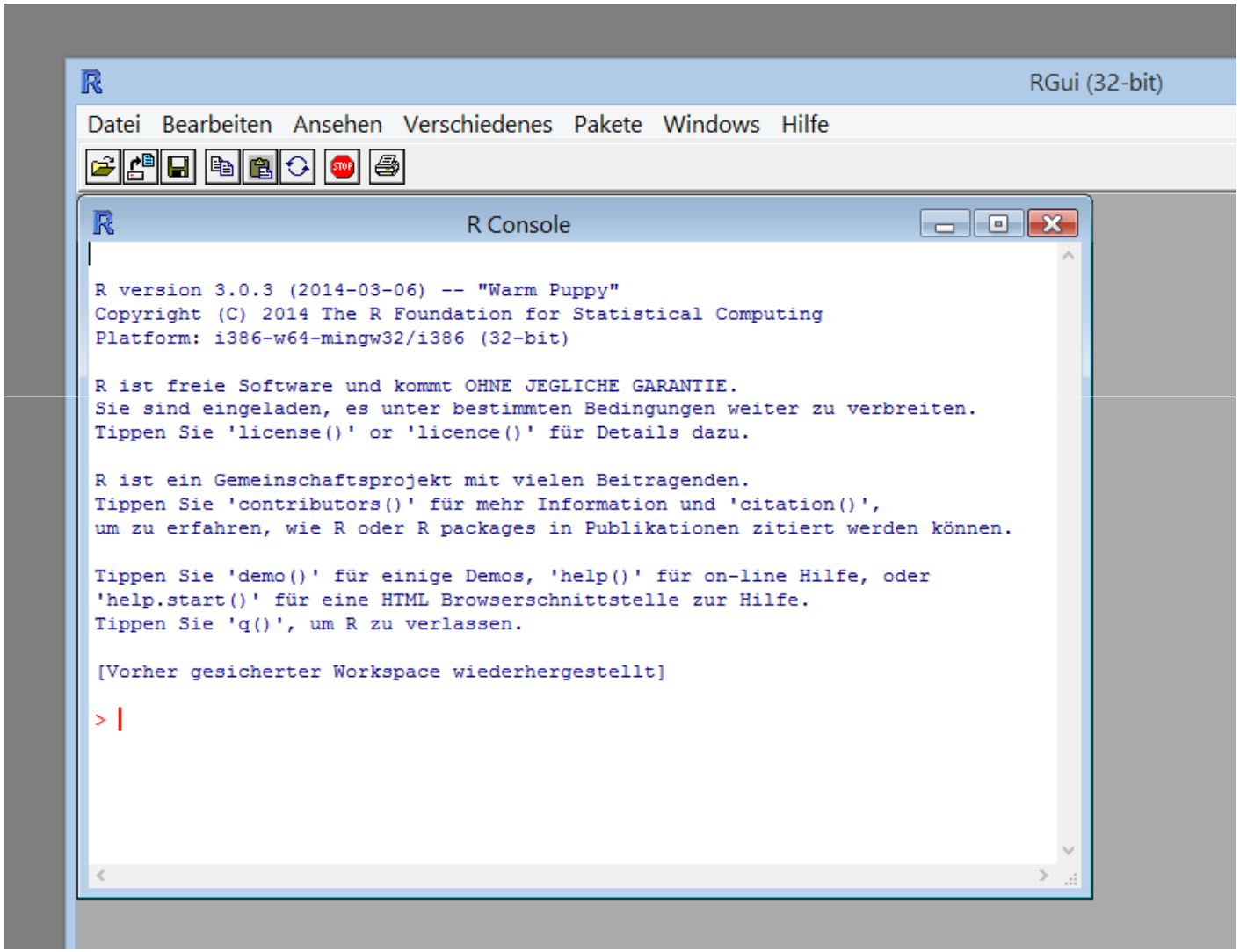
Received on June 6, 2012; revised on July 5, 2012; accepted on July 9, 2012

In particular, it implements a sophisticated non-linear peak alignment algorithm (He *et al.*, 2011; Wang *et al.*, 2010) as well as a calibration procedure for normalization of peak intensities across spectra that are modeled on a related method for sequence count data (Anders and Huber, 2010). In addition, `MALDIquant` allows to analyze technical replicates and spectra with unequal resolution, a crucial feature in clinical mass spectrometry where spectra from multiple sources need to be compared.

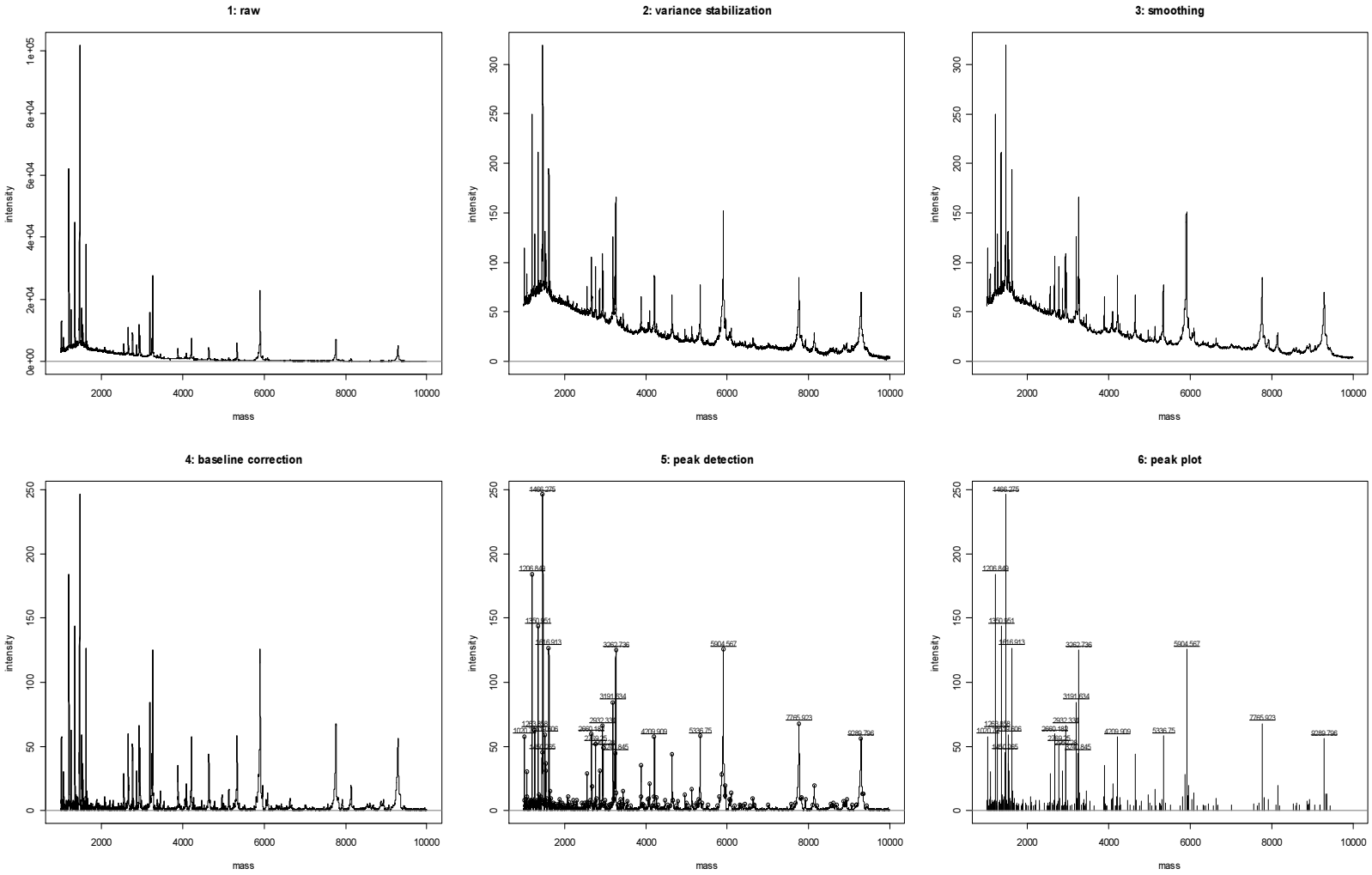
3 DETAILS ON ALGORITHMS

An example workflow for mass spectrometry analysis using `MALDIquant` is depicted in Fig. 1, starting with a raw unprocessed MALDI spectrum (A), followed by smoothing, baseline correction and peak detection (B), local alignment of peaks across spectra by warping (C–E) and merging and visualization (F). In the following, we briefly provide some background on the respective algorithms.

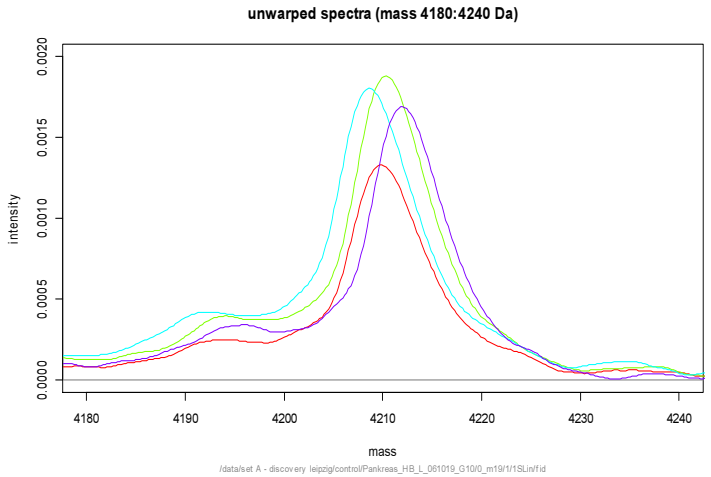
How to “free” your spectra???

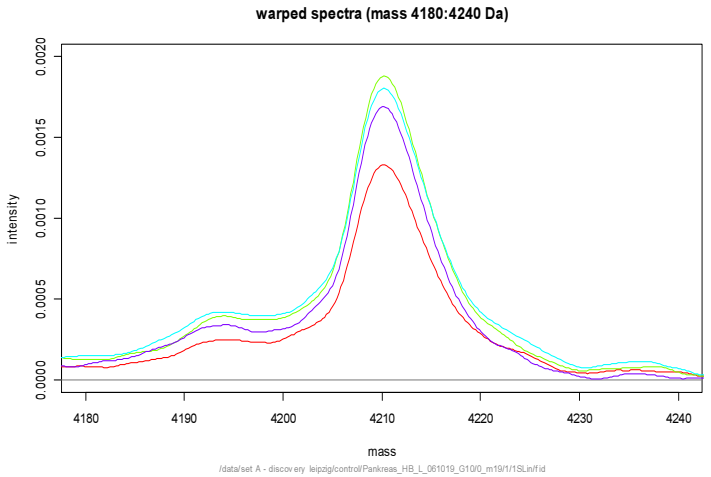


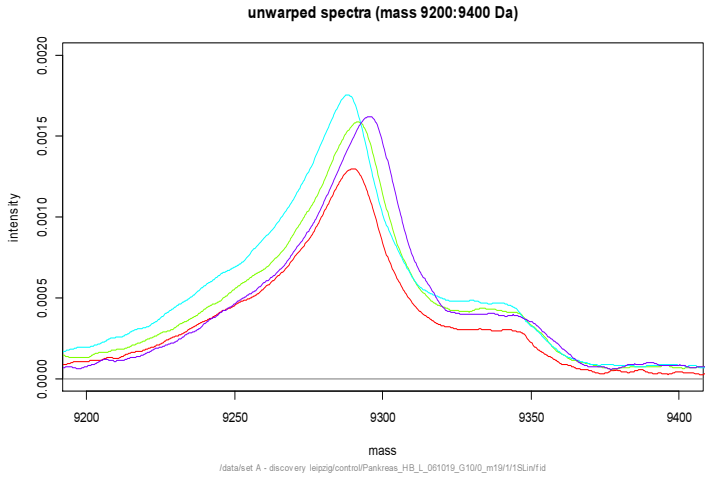
Freeware for analysis:

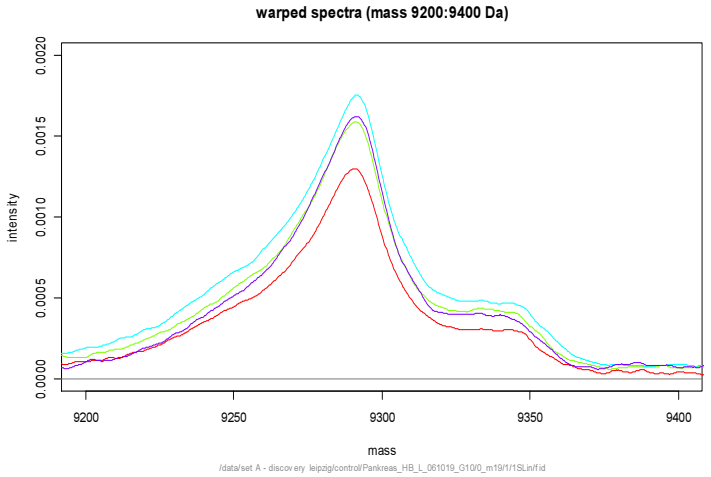


Freeware for analysis:

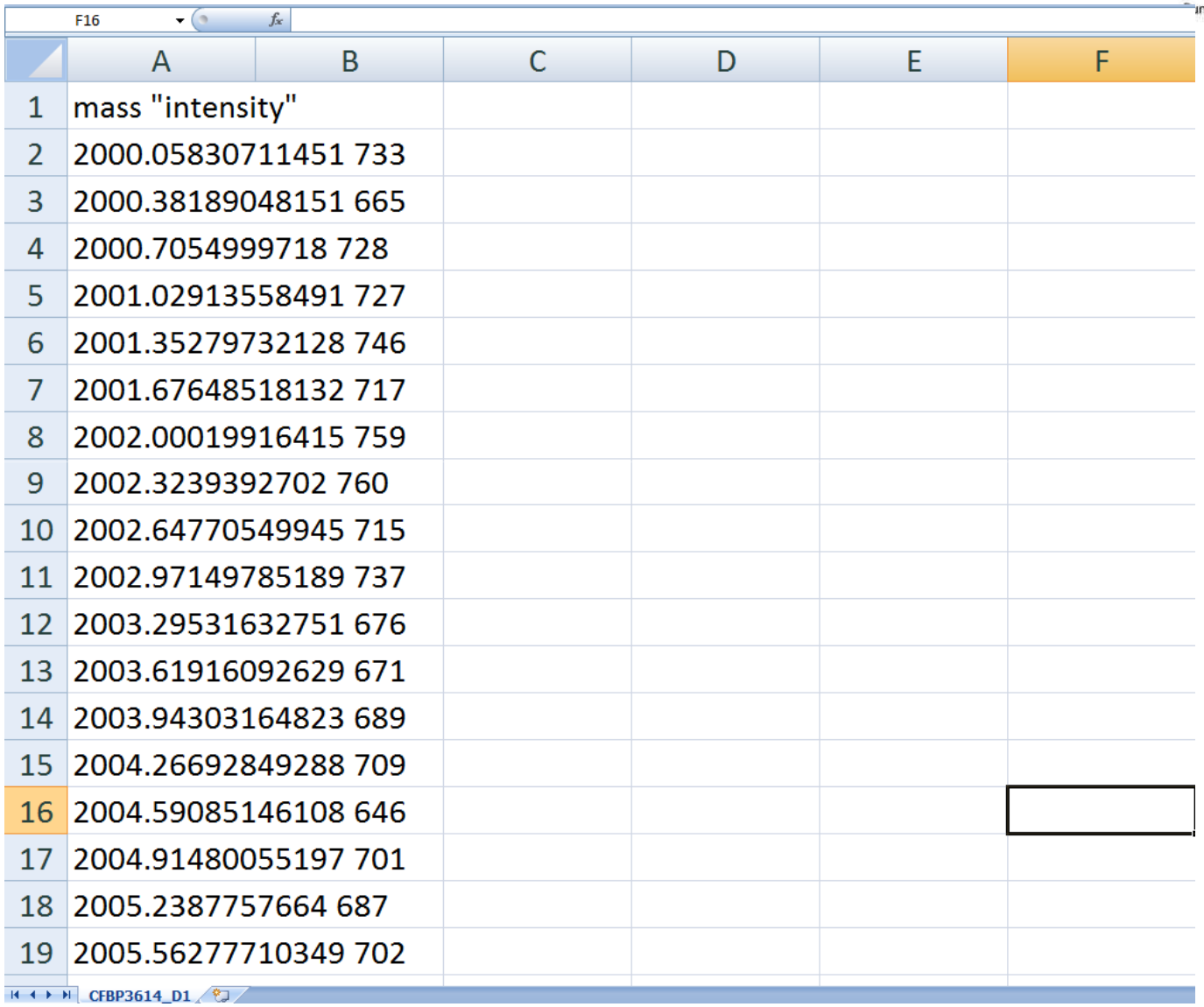








Export to *.csv:



F16

	A	B	C	D	E	F
1	mass "intensity"					
2	2000.05830711451 733					
3	2000.38189048151 665					
4	2000.7054999718 728					
5	2001.02913558491 727					
6	2001.35279732128 746					
7	2001.67648518132 717					
8	2002.00019916415 759					
9	2002.3239392702 760					
10	2002.64770549945 715					
11	2002.97149785189 737					
12	2003.29531632751 676					
13	2003.61916092629 671					
14	2003.94303164823 689					
15	2004.26692849288 709					
16	2004.59085146108 646					
17	2004.91480055197 701					
18	2005.2387757664 687					
19	2005.56277710349 702					

CFBP3614_D1

PHYTFIRE user group and database?


Thank you!