EUPHRESCO Kick-off Meeting, PHYTFIRE WP3

Population analysis via MALDI-TOF ID

What goes on during fire blight treatment?

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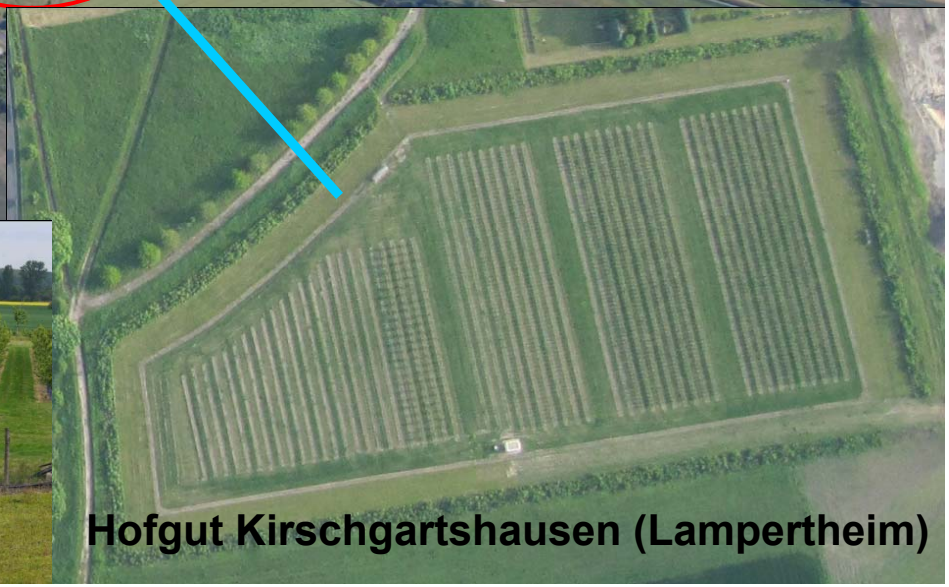
PHYTFIRE

Euphresco Phytosanitary ERA-NET

Example: Field trials for fire blight control



Hofgut Kirschgartshausen (Lampertheim)

Field trials

Setup (EPPO PP 1/166(3))

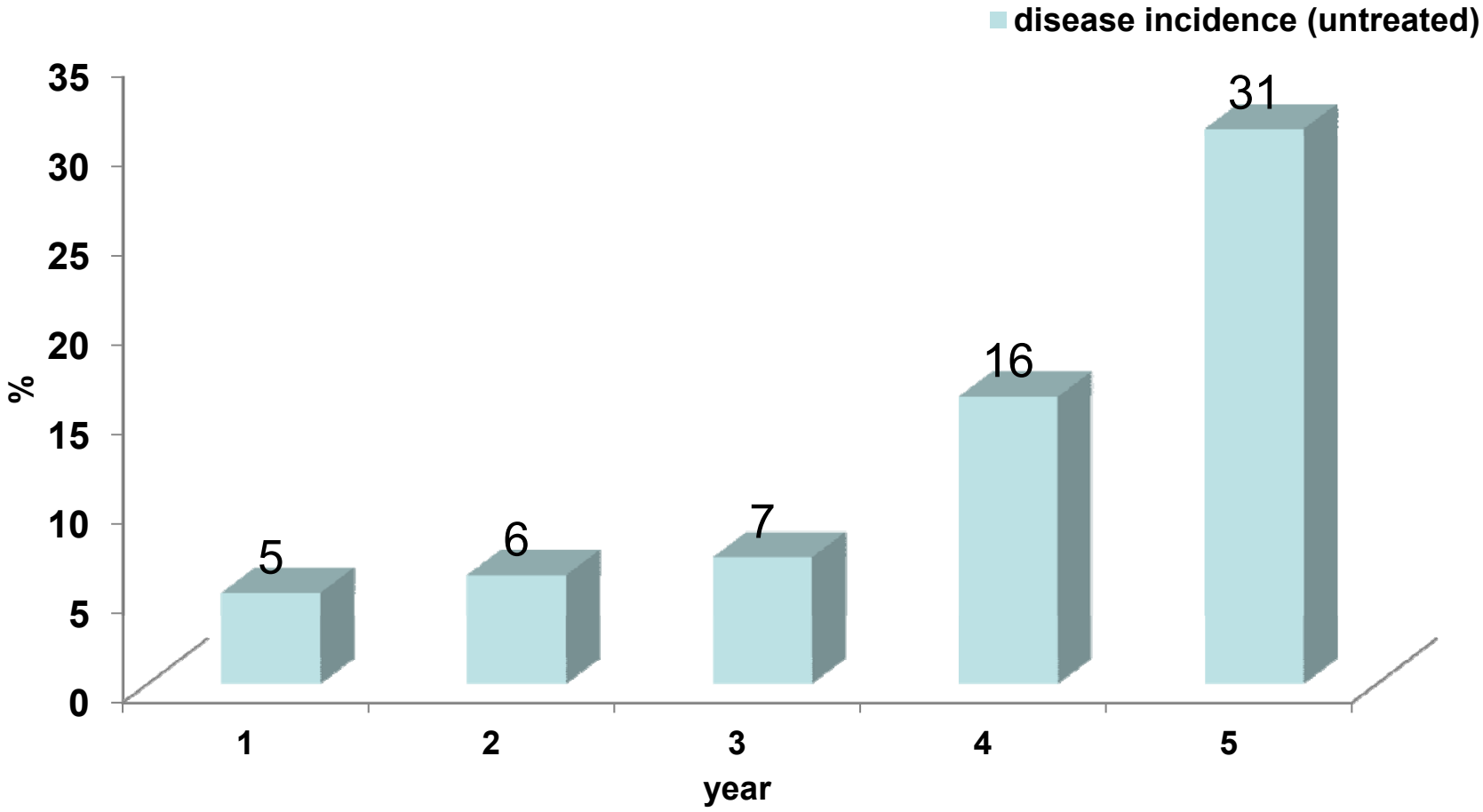
4 blocks with 10 trees each per variant

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

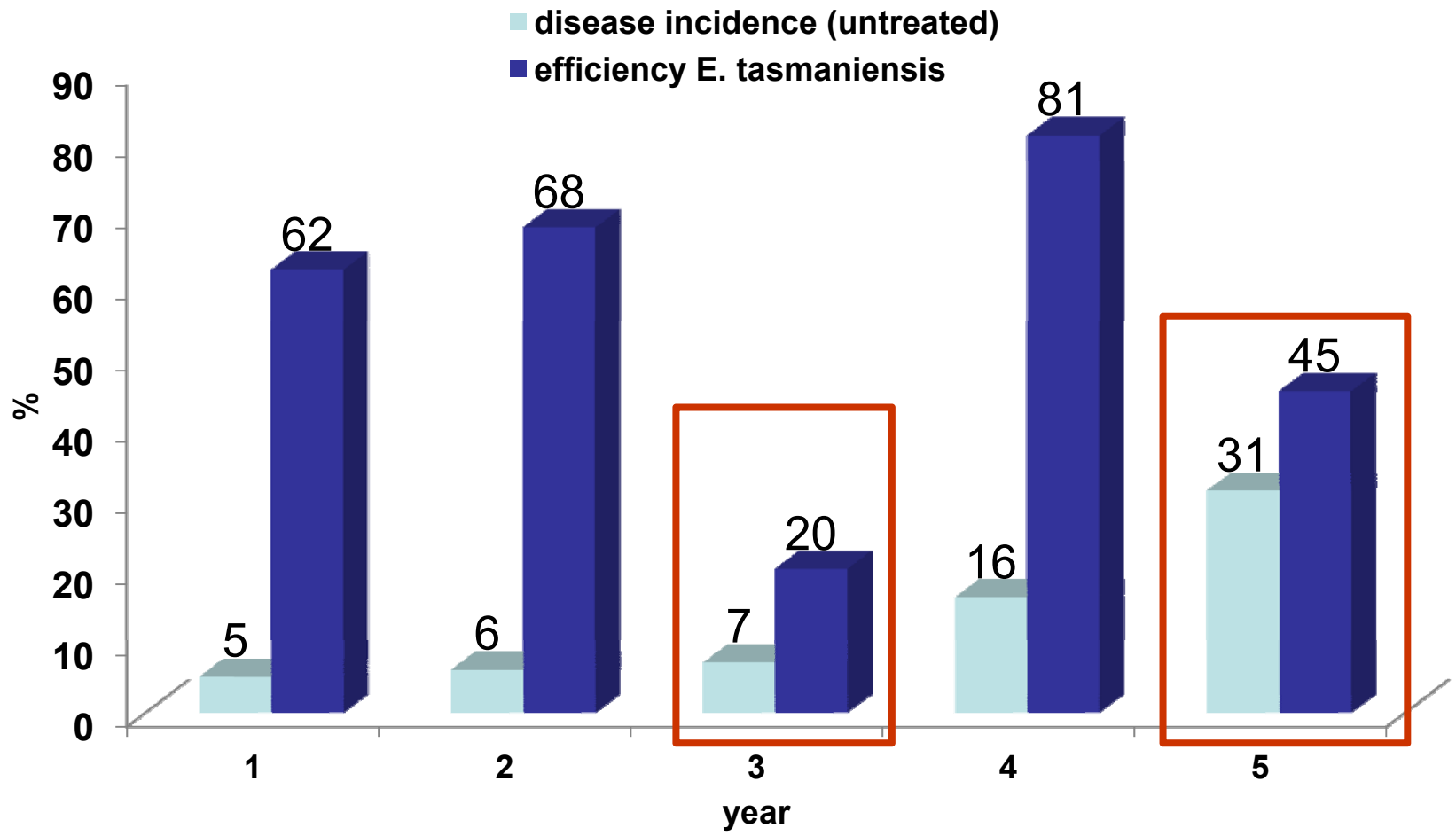
secondary infection of neighboring trees via natural vectors

→ defined conditions

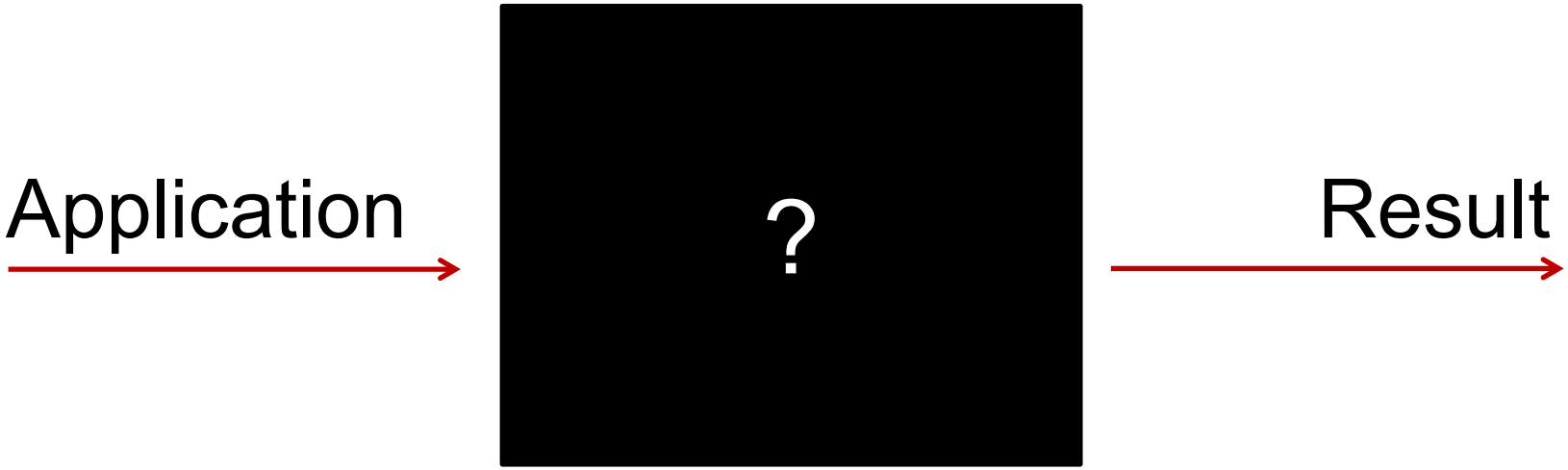
Differences in disease incidence



Differences in efficiency



Objective:
What happens on the flower?

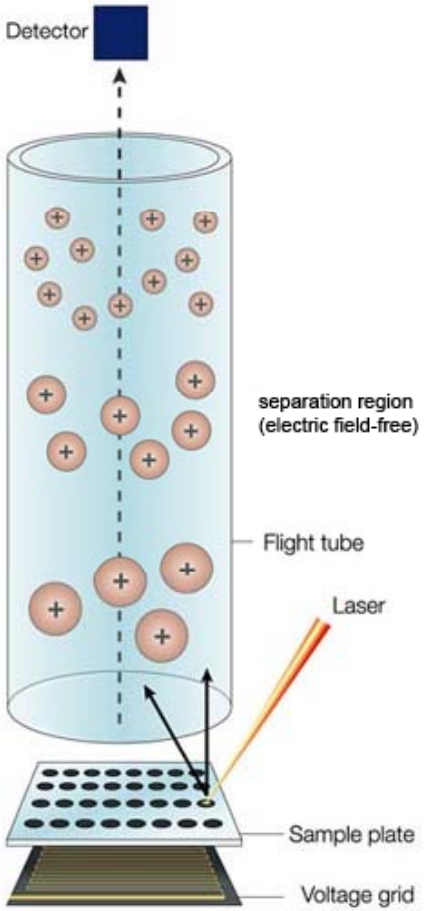


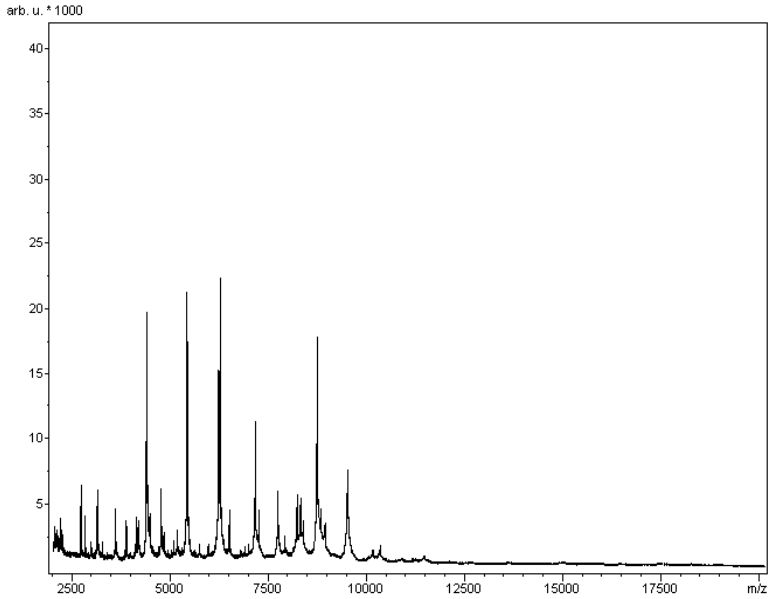
→ screening for specific organisms

→ evaluation of population composition

MALDI-TOF

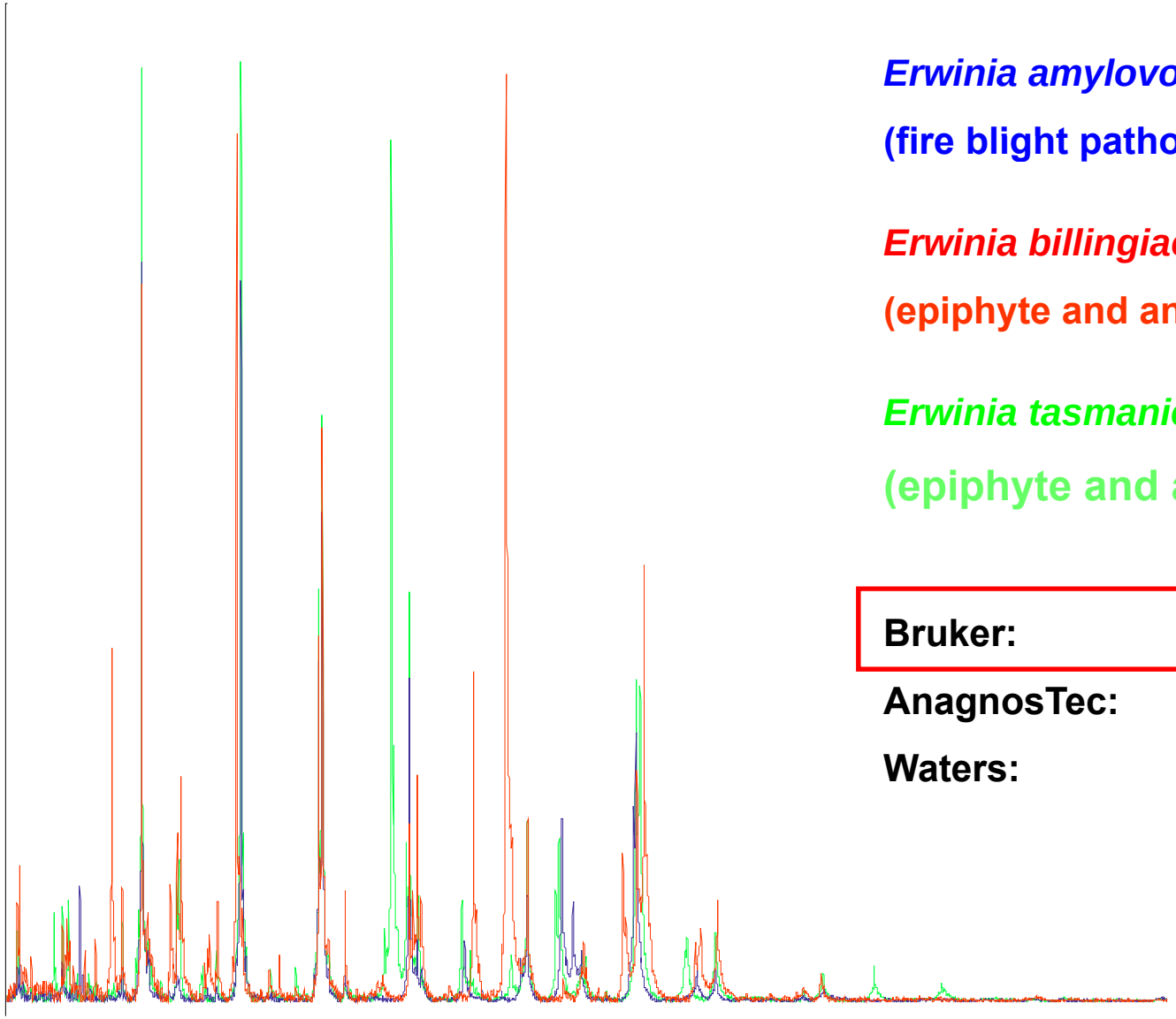
Matrix assisted laser dissorption/ionisation time of flight analysis





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

Protein fingerprinting



m/z

***Erwinia amylovora* CFBP1232**

(fire blight pathogen)

***Erwinia billingiae* Eb661**

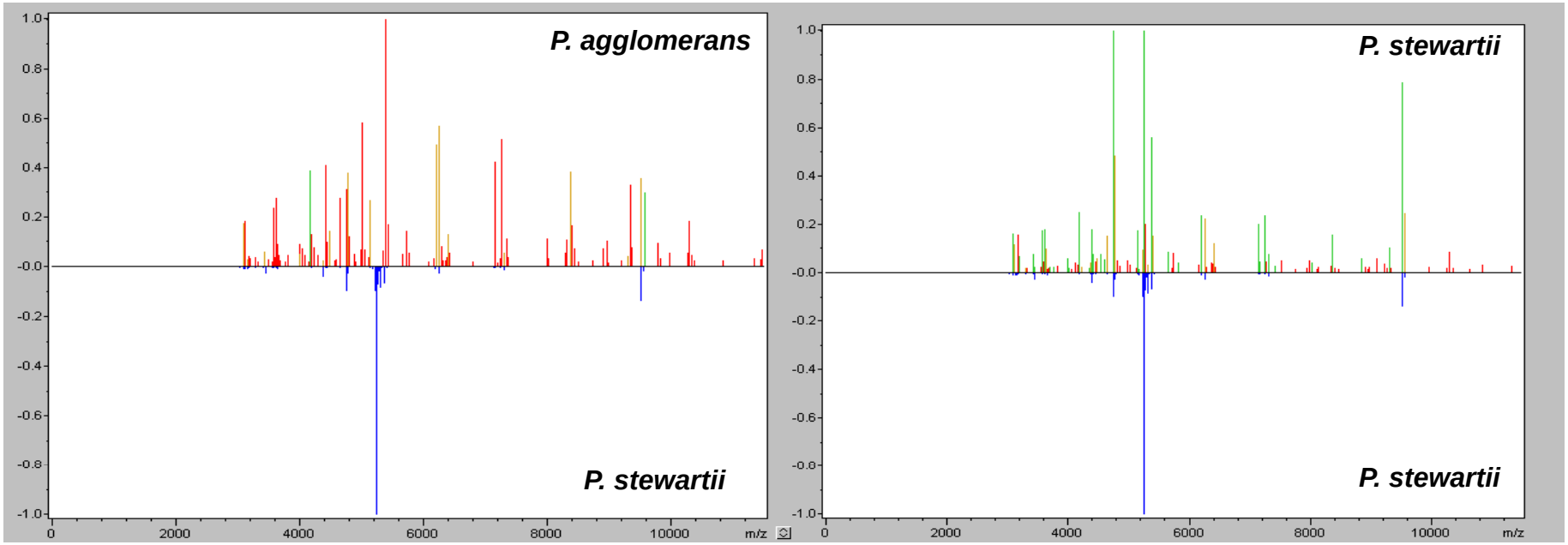
(epiphyte and antagonist)

***Erwinia tasmaniensis* Et1/99**

(epiphyte and antagonist)

Bruker:	**Biotyper**
AnagnosTec:	**Saramis**
Waters:	**MicrobeLynx**

Reference spectra matching:

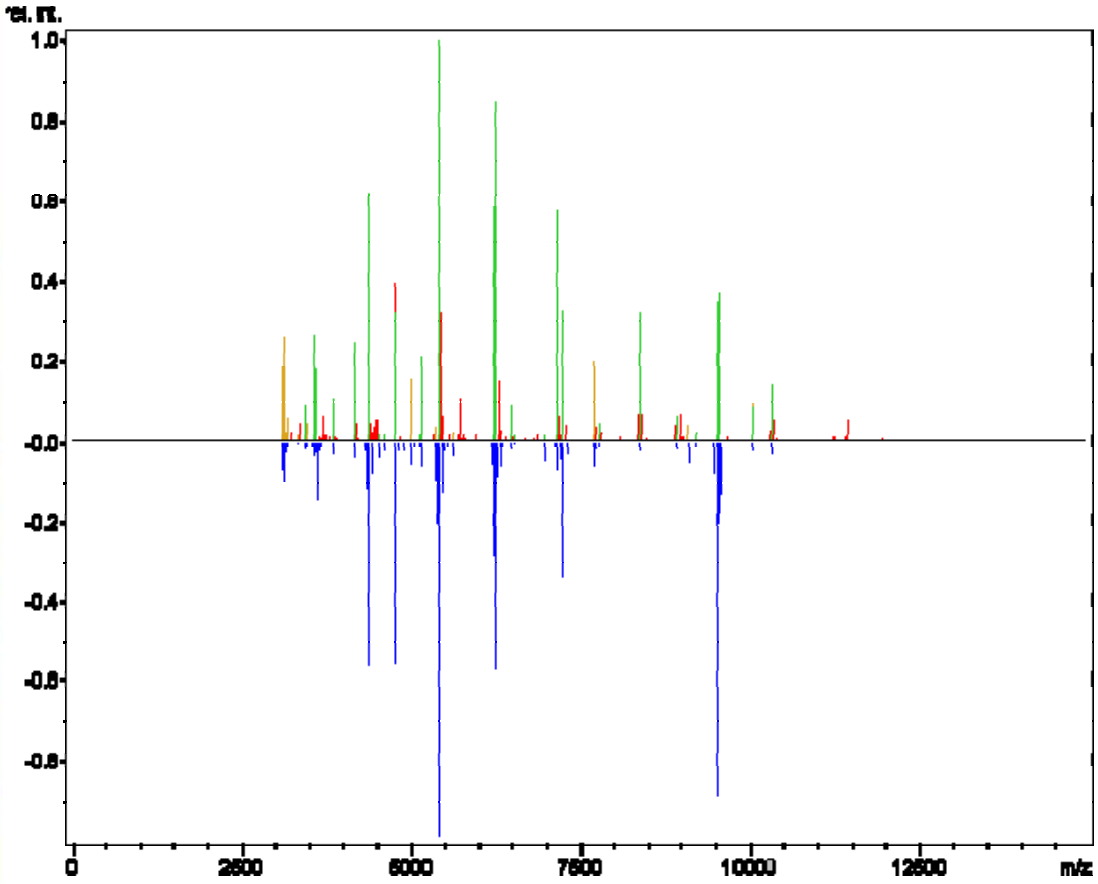


full match = green
partial match = yellow
non-matching = red

Wensing A, Zimmermann S, Geider K (2010)
Appl Environ Microbiol 76: 6248-6256

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

Isolate identification:

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Table 1: Strain identification by Biotyper analysis:

	strain	log score ID
E. amylovora	CFBP1430	Ea 2.1
	Ea273	Ea 2.0
	Ea1/79	Ea 2.2
	Ea7/74	Ea 2.0
	Tp3	Ea 2.2
	EaRW1/06	Ea 2.2
	Ea775	Ea 2.0
	OR6	Ea 2.1
	Ea4/82	Ea 2.2
	EaSp1767-3	Ea 2.2
	EaSp1951-5	Ea 2.3
E. pyrifoliae	Ep1/96	Ep 2.0
	Ep2/96	Ep 2.2
	Ejp557	Ep 2.3
E. tasmaniensis	Et1/99	Et 2.3
	Et2/99	Et 2.2
	Et4/99	Et 2.4
	Et09-1	Et 2.4
	SM88	Et 2.3
	SM882	Et 2.3
	Esa13	Et 2.3
	Esa41	Et 2.3
	FLA03	Et 2.2
E. billingiae	Eb660	Eb 2.2
	Eb661	Eb 2.0
	Eb1261	Eb 2.0
	Kae2a	Eb 2.0
	Kae9a	Eb 2.3
	RIPFgo2	Eb 2.3

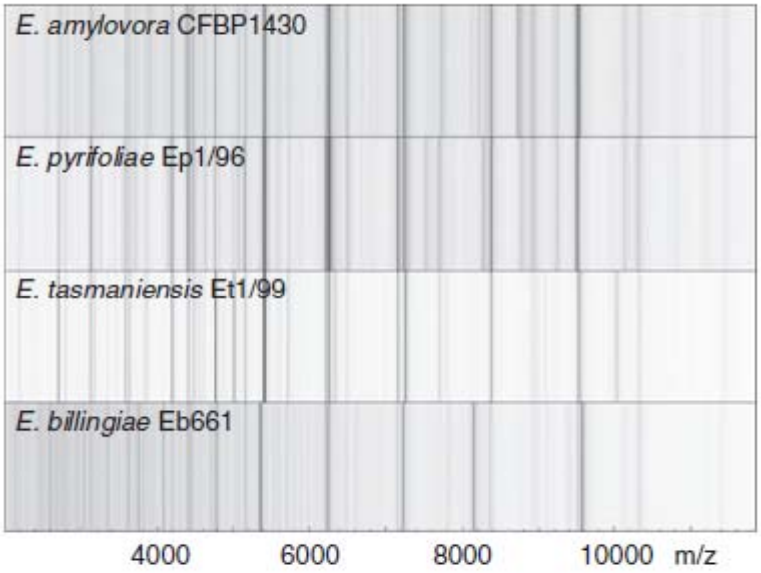


Figure 4 Bar code representation of protein profiles of MALDI–TOF MS spectra. The protein spectra of various *Erwinia* species were visualized in a pseudo-gel representation with peak heights converted to colour intensity.

Wensing A, Gernold M, Geider K (2012)
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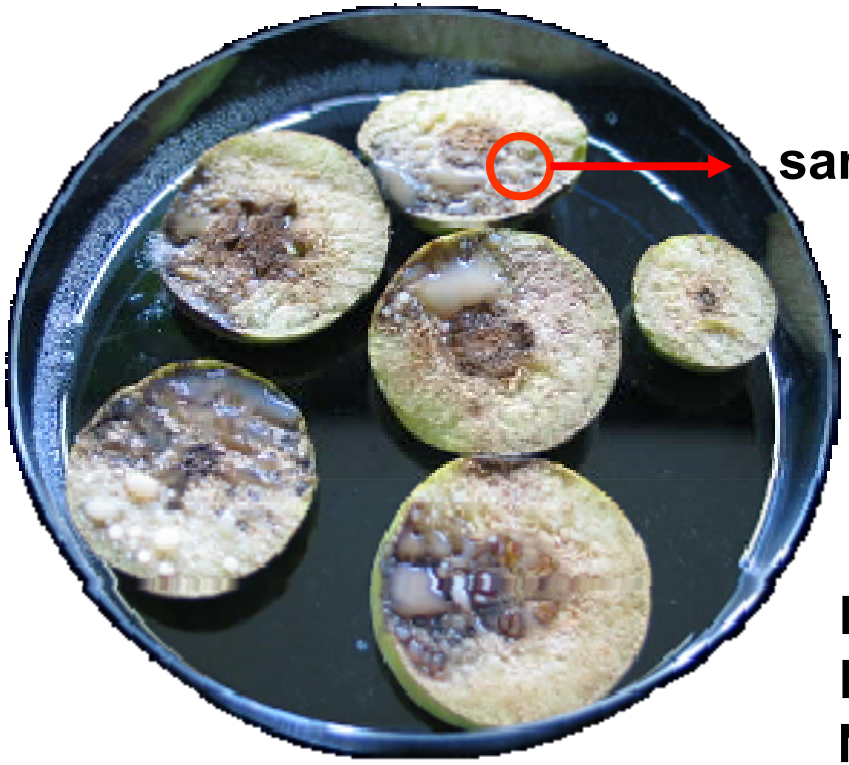
Method comparison:

Example fire blight

artificial infected tissue (immature pear):



sample with starting symptom formation

Detected Species	Lo...
Erwinia amylovora CFBP1232 ...	2.020
Erwinia amylovora 1-79 MMG	1.822
Erwinia pyrifoliae Ep 16_96_PAH	1.570
Erwinia tasmaniensis Et 2_99_...	1.512

BUT:
For most samples only mixed spectra,
NOT reliable yet!

Wensing A, Gernold M, Geider K (2012)
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sample preparation:

1ml overnight culture / colony

↓

harvest cells

↓

wash in 70% EtOH

↓

lyse in 35% formic acid with 50% acetonitrile

↓

apply to target

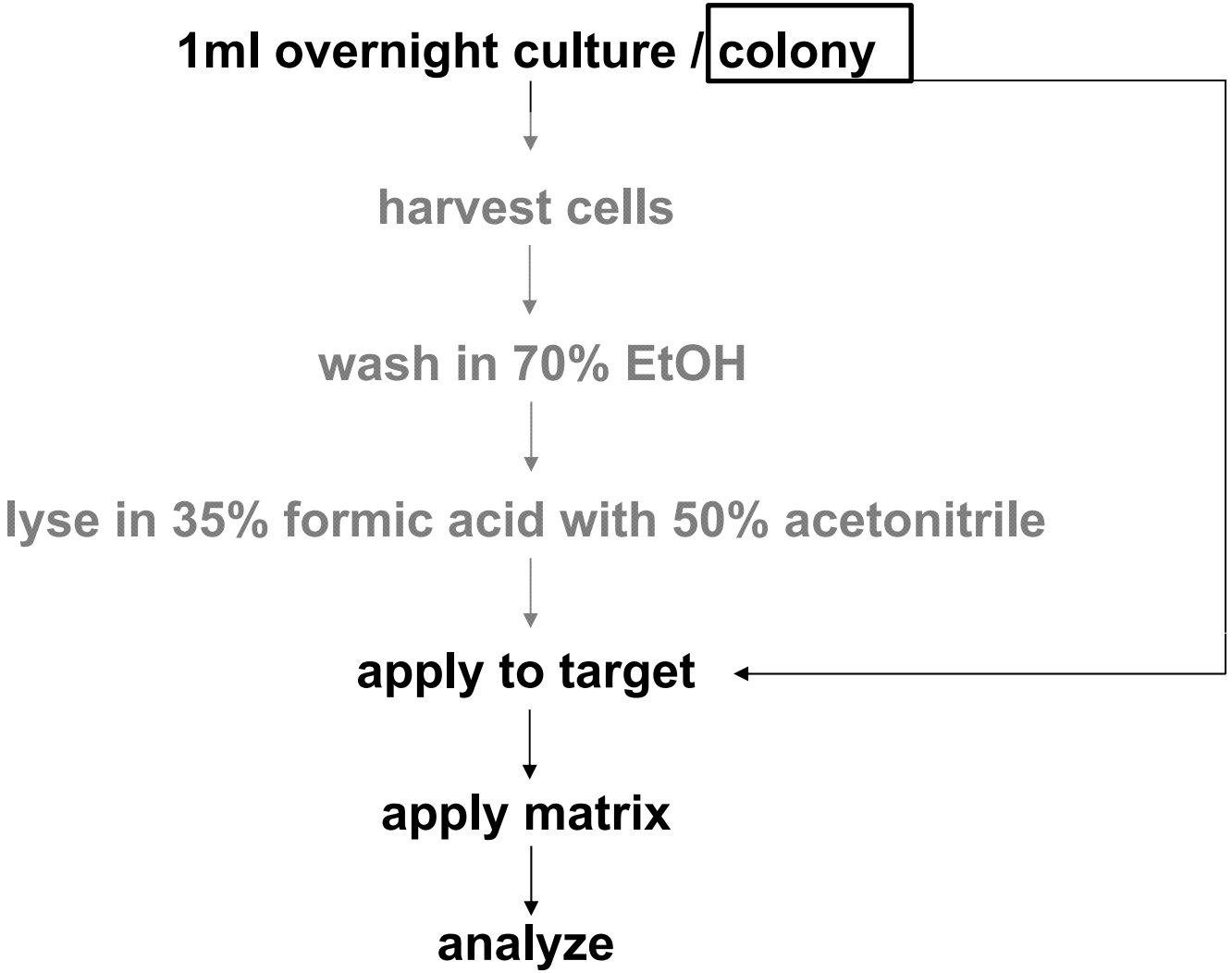
↓

apply matrix

↓

analyze

sample preparation:



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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]*

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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

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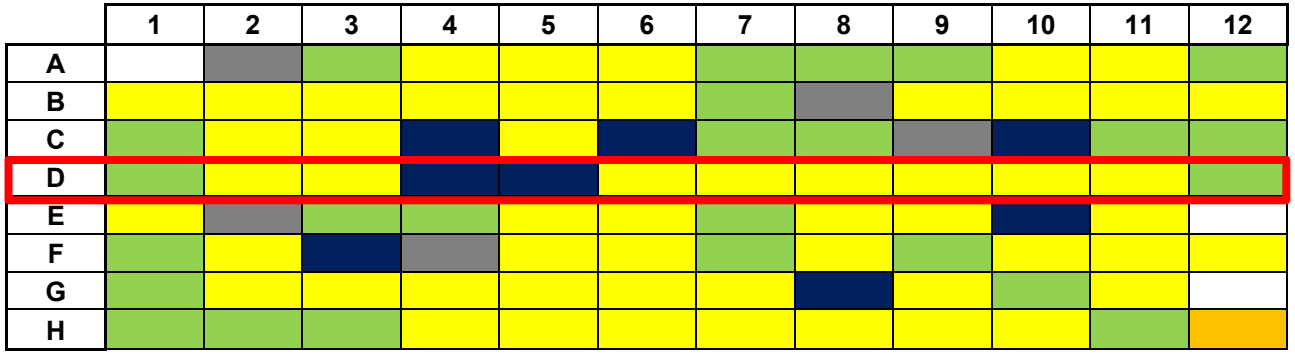
Applied and Environmental Microbiology, Oct. 2011, p. 6858–6866 Vol. 77, No. 19
0099-2240/11/$12.00 doi:10.1128/AEM.05465-11

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▽

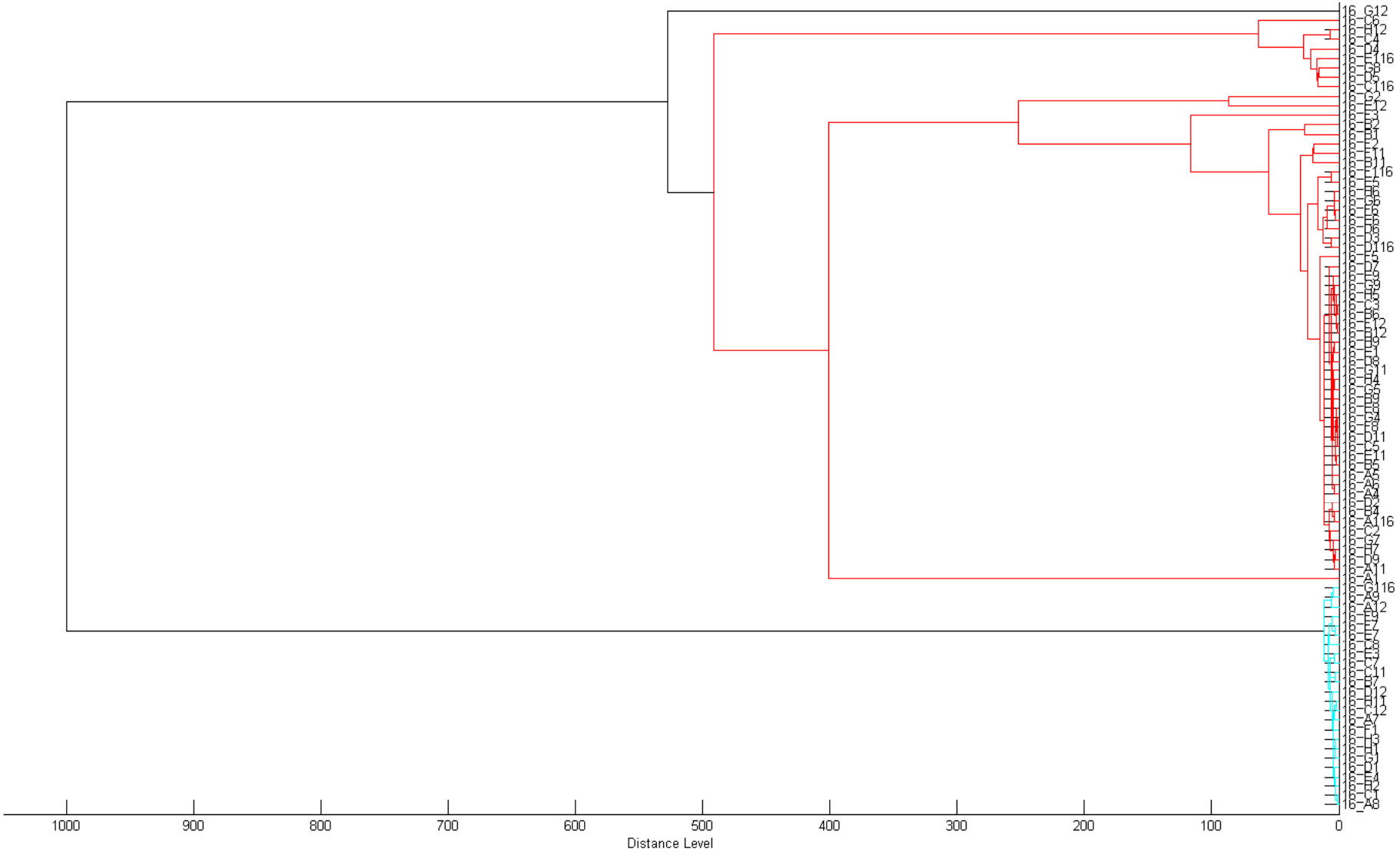
Ondrej Uhlik,[1,2]§ Michal Strejcek,[1]§ Petra Junkova,[1] Miloslav Sanda,[2] Miluse Hroudova,[3] Cestmir Vlcek,[2] Martina Mackova,[1,2] and Tomas Macek[1,2]*

comparison of samples:



Matched Pattern	D1 Score Value	D2 Score Value	D3 Score Value	D4 Score Value	D5 Score Value	D6 Score Value	D7 Score Value	D8 Score Value	D9 Score Value	D10 Score Value	D11 Score Value	D12 Score Value
D1	2.845	0.127	0.828	1.3	1.234	0.098	0.884	0.53	0.825	0.44	0.825	2.606
D2	0.451	2.845	2.13	1.015	1.162	2.17	2.206	2.199	2.435	2.051	2.18	0.123
D3	0.608	2.227	2.845	0.269	0.431	2.601	2.586	2.478	2.192	2.623	2.464	0.485
D4	1.364	1.388	0.733	2.845	2.354	0.838	1.078	0.902	1.19	0.868	0.856	1.436
D5	1.224	1.089	0.712	2.427	2.845	0.395	0.791	0.172	1.205	0.696	0.691	0.97
D6	0.519	2.161	2.498	0.817	0.524	3	2.465	2.444	2.26	2.485	2.392	0.513
D7	0.868	2.31	2.579	0.659	0.79	2.472	2.845	2.566	2.323	2.584	2.486	0.46
D8	0.791	2.264	2.521	0.884	0.637	2.465	2.553	2.877	2.246	2.538	2.497	0.783
D9	0.793	2.334	2.067	1.045	0.917	2.217	2.22	2.147	3	2.196	2.214	0.732
D10	0.7	2.16	2.666	0.534	0.638	2.589	2.627	2.593	2.211	2.845	2.461	0.698
D11	0.781	2.341	2.523	0.616	0.509	2.399	2.56	2.553	2.336	2.443	2.845	0.076
D12	2.66	0.463	0.716	1.426	1.209	0	0.488	0.324	0.501	0	0.893	2.845

comparison of samples:



Sampling technique:

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

Sample Size?

vs.

Sampling technique:

Timeframe?

Protocol development:

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

Comparison of systems

Bruker:	**Biotyper**
AnagnosTec:	**Saramis**
Waters:	**MicrobeLynx**

Evaluation with established methods

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

Output:

- Protocol development for application of a new high-throughput technique for microbial identification to fire blight settings

- Evaluation of systems, sampling and culturing conditions

- Information on development of *E. amylovora* in the context of a competing microflora

- Information on effect of fire blight treatment on the natural microflora

- Information on development of microbial biocontrol organisms (biosafety)


Thank you!