ISFB2016

Girona

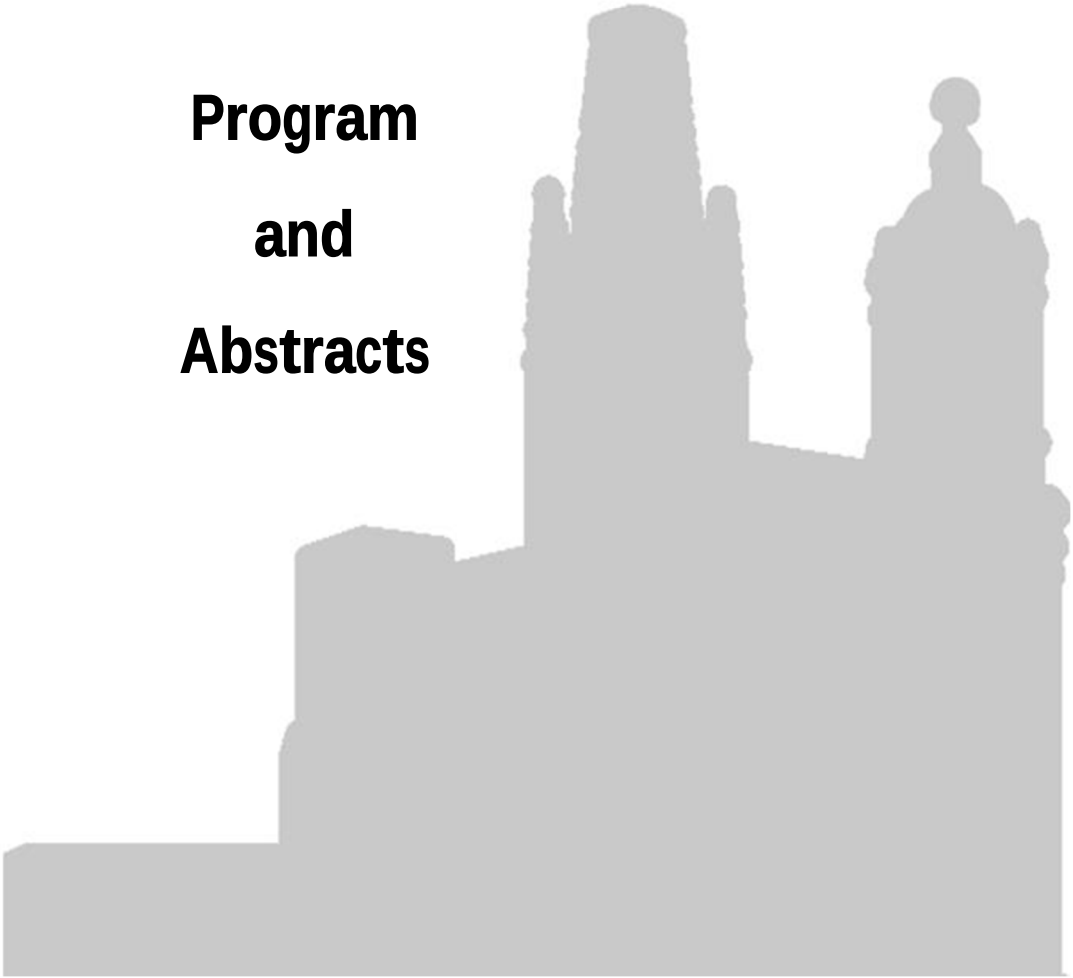
1st International Symposium on Fire Blight of rosaceous plants

Girona (Spain), 5-8 July, 2016

Program

and

Abstracts



Universitat de Girona

Local organizing committee

Institut de Tecnologia Agroalimentària (INTEA). Universitat de Girona.

Emilio Montesinos
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Maria M. López (Instituto Valenciano de Investigaciones Agrarias-IVIA, Spain)
Fabio Rezzonico (Zurich University for Applied Sciences, Switzerland)
Virginia Stockwell (Oregon State University, USA)
George Sundin (Michigan State University, USA)
Andreas Peil (Julius Kühn Institute, Germany)
Joanna Pulawska (Research Institute Horticulture, Poland)
Joel Vanneste (Plant and Food, New Zealand)
Youfu (Frank) Zhao (University of Illinois, USA)

ISFB 2016 Venue

Science and Technology Park (Parc Científic i Tecnològic)
Pic de Peguera 15 (La Creueta)
17003 Girona
http://intea.udg.edu/ISFB2016/

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LOCATION OF BUILDINGS & SERVICES

Buildings

A - Jaume Casademont Building
B - Centre of Innovation in subacuatic robotics
C - Center for New Food Technologies
D - Giroemprèn Business Center
E - Narcís Monturiol Building
F - Greenhouse

Services

1 - Auditorium (Narcís Monturiol building)
2 - Lunch room (Giroemprèn building, ground floor)

ISFB2016 Venue map

Parc Científic i Tecnològic
Universitat de Girona

Program overview

	Tuesday, July 5th	Wednesday, July 6th	Thursday, July 7th	Friday, July 8th
9:00				Departure from Girona (8:15)
10:00		**SESSION 2- Genetics and genomics**	**SESSION 4-Epidemiology & DSS**	**VISIT TO MAS BADIA** **Agricultural Experimental Station**
11:00	**Registration** *Hall Monturiol building*	Coffee/break	Coffee/break	Coffee/break
12:00		**SESSION 2- Genetics and genomics** *Poster viewing*	**SESSION 4-Epidemiology & DSS** *Poster viewing*	**VISIT TO COOPERATIVE**
13:00	**Official Opening**	**ROUND TABLE. The genome of *E. amylovora*. An update.**		
14:00	Lunch *Giroemprèn building*	Lunch *Giroemprèn building*	Lunch *Giroemprèn building*	Lunch Coming back to Girona
15:00	**SESSION 1-Overview. *Ea* & Fire Blight**	**SESSION 3-Interactions and plant breeding**	**SESSION 5- Control Strategies**	
16:00	Coffee/break	Coffee/break	Coffee/break	
17:00	**SESSION 1-Overview. *Ea* & Fire Blight**	**SESSION 3-Interactions and plant breeding**	**SESSION 5- Control Strategies**	
18:00	**Beer tasting** ***Monturiol building***	*Poster viewing*	**Overview & Next Meeting**	
19:00		**Visit to Girona**	**Human towers**	
20:00				
21:00				
22:00	**Social Dinner**		**BBQ&Show**	
23:00				

Program of the 1st International Symposium on Fire Blight of Rosaceous Plants

July 5th to 8th, 2016

Auditorium, Narcís Monturiol building
Science and Technology Park (STP)
University of Girona
Girona (Spain)

Tuesday, July 5th

10:45 Bus from Girona to the Park
(meeting point in front of the Correus building)

11:00-12:30 **Registration** (Hall Monturiol building)

12:30-13:00 **Official opening** (Auditorium)

13:00-15:00 **Lunch** (Giroemprèn building, ground floor)

15:00-17:30 **Overview on fire blight and *Erwinia amylovora***
(Auditorium, Narcís Monturiol building) Chair: Emilio Montesinos.

15:00-15:30 **Comparative genomics of *Erwinia* species.**
Theo Smits. Zurich University of Applied Sciences, Switzerland.

15:30-16:00 **Breeding for host resistance to fire blight.**
Markus Kellerhals. Agroscope, Institute for Plant Production Sciences, Wädenswil, Switzerland.

16:00-16:30 Coffee break

16:30-17:00 **Use of decision support systems for fire blight management**.
Dani Shtienberg. ARO theVolcani Center, Israel.

17:00-17:30 **Improving fire blight control by knowing *Erwinia amylovora* epidemiology in the European Union countries.**
María M. López. Instituto Valenciano de Investigacions Agrarias-IVIA, Spain.

17:30-18:30 **Beer tasting (Narcís Monturiol building entrance)**

18:30 **Bus from STP to Girona**

20:45 **Bus from Girona to Social dinner**

21.00-24:00 **Social Dinner (Siloc restaurant, La Creueta)**

24:00 **Bus from restaurant to Girona**

Wednesday, July 6th

8:30 **Bus from Girona to STP**

9:00-11:30 **Genetics and Genomics**
Chairs: Brion Duffy and George W. Sundin

9:00-9:30 **Keynote: Transcriptional and post-transcriptional regulation of the T3SS in *Erwinia amylovora*.** Youfu (Frank) Zhao. University of Illinois, USA.

9:30-10:30 Oral presentations

Towards the understanding of iron uptake and siderophore biosynthesis in *Erwinia amylovora*. Ivan Polsinelli, Marco Salomone-Stagni, Joseph Dale Bartho, and Stefano Benini.

The structural biology of *Erwinia amylovora* desferoxamine biosynthetic pathway at a glance. Marco Salomone-Stagni, Joseph Dale Bartho, and Stefano Benini.

Polymorphism of the VNTR F region of *Erwinia amylovora* and comparison between some VNTR F and CRISPR-groups. Nataliya Drenova, Galina Matiashova, Denis Belkin, and Maxim Kondratyev.

10:30-11:00 Coffee break

11:00-11:30 Oral presentations

Regulation of flagellar motility by the small RNA ArcZ in *Erwinia amylovora*. Jeffrey Schachterle, Quan Zeng, and George W. Sundin.

11:30-12:00 **Poster viewing**

12:00-13:00 **Round table:** The genome of *E. amylovora*. An update.

13:00-15:00 **Lunch** (Giroemprèn building, ground floor)

15:00-18:00 **Session-Pathogen/Microbe/Plant Interactions and plant breeding** Chairs: Joanna Pulawska and Andreas Peil

15:00-15:30 **Keynote: Role of cyclic di-GMP in *Erwinia amylovora*-apple interactions.** George W. Sundin. Michigan State University, USA.

15:30-16:30 Oral presentations

The *Malus fusca* fire blight resistance locus: validation of *Mfu10* and genetic resolution of the region containing the locus. Ofere Francis Emeriewen, Klaus Richter, Mickael Malnoy, Magda-Viola Hanke, and Andreas Peil.

Functional roles of *Erwinia amylovora* catalases during plant-pathogen interactions and exposure to starvation. Ricardo D. Santander, Àngela Figàs-Segura, and Elena G. Biosca.

Comparative analysis of transcriptomes of *Erwinia amylovora in planta*, on two apple cultivars of different susceptibility to fire blight. Joanna Pulawska, Monika Kalużna, Wojciech Warabieda, and Artur Mikiciński.

16:30-17:00 Coffee break

17:00-18:00 Oral presentations

Testing of resistance of pome fruit cultivars after artificial inoculation with *Erwinia amylovora* in field conditions. Jana Sillerova, Josef Korba, Jiri Sedlak, Frantisek Paprstein, Ales Matejicek, and Tomas Necas.

Modulating the plant-pathogen interaction using antimicrobial peptides to control fire blight. Esther Badosa, Laura Montesinos, Lidia Ruz, Jordi Cabrefiga, Jesús Francés, Beatriz Gascón, Cristina Camó, Marta Planas, Lidia Feliu, and Emilio Montesinos.

Functional analysis of the fire blight effector protein AvrRpt2$_{EA}$ in apple. Susan Schröpfer, Thomas Wöhner, Dieter Treutter, Magda-Viola Hanke, Andreas Peil, and Henryk Flachowsky.

18:00-18:30 **Poster viewing**

18:30 **Bus to Girona**

19:30-21:00 **Girona Downtown Visit** (meeting point at Vicens Vives square)

Thursday, July 7th

8:30 **Bus from Girona to the STP**

9:00-12:30 **Epidemiology and Decision Support Systems**
(Chairs: Fabio Rezzonico and Maria M. López)

9:00-9:30 **Keynote: Perspectives on streptomycin-resistant *Erwinia amylovora* in New York.** Kerik Cox. Cornell University, USA.

9:30-10:30 Oral presentations

Tracking of *Erwinia amylovora* dissemination in Eastern Europe and central Asia using CRISPRs. Fabio Rezzonico, Nataliya Drenova, Inga Morocko-Bicevska, Laima Baranauskaite, Anita Végh, Aboubakr Moradi, Hatice Ozaktan, Zhansaya Umiralieva, and Galiya Zharmuhamedova.

Epidemiology of fire blight (caused by *Erwinia amylovora*) in the Pink Lady® apple cultivar in Israel. Mery Dafny Yelin, Orly Mairesse, Judit Moy, Miriam Silberstein, Shulamit Manulis-Sasson, and Dani Shtienberg.

New host for *Erwinia amylovora* in wild plants: first detection in *Pyrus bourgaeana* (Iberian wild pear). E. Marco-Noales, J. Peñalver, I. Navarro, M.T. Gorris, C. Morente, C. Balguerías, J.A. Ramírez, C. Recio, T. Ruiz de la Hermosa, R. Sancho, C. Aedo, and M.M. López.

Monitoring *Erwinia amylovora* in Southern Germany, Switzerland and Austria during the blossoming periods since 2010. Stefan Kunz, David Szalatnay, Klaus Altherr, Eduard Holliger, Béatrice Schoch, Dennis Mernke, Urs Müller-Widmer, Richard Hollenstein, Nina Thomas, Markus Hunkeler, Daniel Schnegg, Louis Suter, Hans-Jakob Schärer, Ulrich Höfert, Klemens Böck, Karin Wudler, Christian Scheer, Sonja Weißhaupt, Malin Hinze, and Peter Triloff.

10:30-11:00 Coffee break

11:00-12:30 Oral presentations

Ocurrence of fire blight disease on Asian pear caused by *Erwinia amylovora* in Korea. Duck Hwan Park , Ji-GangYu, Kyu-Suk Han, Eom-Ji Oh, Mi Chi Yea, Seong Jin Lee, and Chang-Sik Oh.

The incidence and prevalence of fire blight in apple trees in Lake Van Basin, Turkey. Ahmet Akköprü and Cevdet Kipçak.

Effect of low temperatures on *Erwinia amylovora* virulence and starvation responses. Ricardo D. Santander, and Elena G. Biosca.

A microbiological examination of *Erwinia amylovora* exopolysaccharide ooze. Suzanne M. Slack, Quan Zeng, Cory A. Outwater, and George W. Sundin.

Differential fitness of *Erwinia amylovora* isolates carrying different variants of the *rpsL* gene leading to high level of streptomycin resistance. Mireia Marcé Escursell, Virginia O. Stockwell, Theo H.M. Smits, and Fabio Rezzonico.

Shifts in the prevalence of streptomycin-resistant *Erwinia amylovora* on pear in Oregon, USA. Virginia O. Stockwell

12:30-13:00 **Poster viewing**

13:00-15:00 **Lunch** (Giroemprèn building, ground floor)

15:00-18:00 **Control Strategies**
Chairs: Virginia Stockwell and Stefan Kunz

15:00-15:30 **Keynote: Non-antibiotic strategies for fire blight control in the USA.** Kenneth Johnson. Oregon State University, USA.

15:30-16:30 Oral presentations

Integration of biological control agents and plant defense activators against fire blight in Morocco. Ait Bahadou Smail, Ouijja Abderrahmane, Boukhari Mohammed Amine, and Tahiri Abdessalem.

Field trials for fire blight control in the experimental orchard "Kirschgartshausen". Arno Fried, Annette Wensing, Dennis Mernke, and Wilhelm Jelkmann.

Experiences with Kasumin for fire blight control in Michigan. Cory A. Outwater and George W. Sundin.

Contribution of native plasmids of *Pantoea vagans* strain C9-1 to fitness and biocontrol efficacy in apple and pear orchard trials. Jeannie M. Klein, Joyce E. Loper, Kenneth B. Johnson, and Virginia O. Stockwell.

16:30-17:00 Coffee break

17:00-18:00 Oral presentations

Further results on SAR-inducing tree paints as a therapy to aid restoration of tree health after fire blight infection. Todd N. Temple and Kenneth B. Johnson.

Osmoadaptation increases the performance of *Bacillus amyloliquefaciens* EPS2017 for biological control of fire blight. Jordi Cabrefiga, Isabel Mora, and Emilio Montesinos.

Lactobacillus plantarum strains as efficient biocontrol agents of fire blight. Núria Daranas, Gemma Roselló, Esther Badosa, Jordi Cabrefiga, Jesús Francés, Emilio Montesinos, and Anna Bonaterra.

Control of fire blight in Norway 1986-2015. A. Sletten, V. Talgø, T. Rafoss, and N.S. Melbøe.

18:15-19:00 **Overview** (chairs of the sessions)

19:00-19:15 **Next meeting and official closure**

19:15-20:15 **Human towers exhibition**
(green area between Giropemprèn and Casademont buildings)

20:15 **Bus from STP to the restaurant BBQ**

21:00-24:00 **BBQ and Show** (Mas Bes restaurant and farmers house)

24:00 **Bus from restaurant to Girona**

Friday, July 8th

8:15 Bus departure from Girona

9:00-11:20 **Visit to Mas Badia Agricultural Experimental Station (IRTA),** La Tallada d'Empordà (Girona)

11:20-11:40 Coffee break

11:40-12:45 **Visit to a Fruit-tree Cooperative**, Torroella de Montgri (Girona)

13:00-15:00 **Lunch** (back to Girona)

15:00 Return to Girona (estimated arrival at 15:45)

Abstracts

Oral presentations

Comparative genomics of *Erwinia* species

Theo H.M. Smits

Research Group for Environmental Genomics and Systems Biology, Institute of Environmental Resource Sciences, Zurich University of Applied Sciences (ZHAW), CH-8820 Wädenswil, Switzerland

The current availability of large amounts of genome sequences of the genus *Erwinia* allow to examine in more detail how *Erwinia amylovora* became the successful pathogen. Common features observed between *Erwinia* and the closely related genus *Pantoea* show that there was a common ancestor. From there, it can be observed that within the both genera, a convergent evolutionary pathway has been followed in the shaping of multiple current-day pathogens, as based on the reduction of genome size followed by uptake of similar virulence factors.

This presentation will also go into depth on the evolution within the species *E. amylovora*. We have sequenced a large number of strains from different origin and different times, and are in the progress of analyzing the data. Plasmids were found in nearly all strains, with pEA29 as most common plasmid. Other plasmids were identified only in few strains. These include largely novel families of plasmids that rather would reflect the general plasmid pool in nature, as some of these plasmids were also found in clinical settings as backbone plasmid for antibiotic resistance cassettes. Analysis of singular nucleotide polymorphisms (SNPs) indicate that there is only a low genetic diversity in CRISPR-type I strains of *E. amylovora*, which is the most commonly found around the world. Nevertheless, the SNP pattern gives indications that multiple introductions have taken place within Europe and the Middle East with a subsequent spread within Europe and Asia.

Breeding for host resistance to fire blight

Markus Kellerhals, Simone Schütz, and Andrea Patocchi

Agroscope, Schloss 1, 8820 Wädenswil, Switzerland

Developing fire blight resistant apple cultivars is a promising approach to manage the disease. Efforts to breed fire blight resistant apple and pear cultivars started in the USA at the end of the 19th century. In the USA, the disease was first observed around 1793. In 1880, Burrill discovered that the causal agents might be bacteria and Waite examined the way the pathogens enter the host and started a pear breeding program in 1908. Many pear and apple cultivars from Europe were introduced to the US at that time. They showed differential susceptibility to the disease. Fire blight resistant material was also introduced from China and Japan. Therefore, a range of resistance sources was available for breeding. As the disease spread to Europe and other parts of the world, breeding activities for host resistance were intensified. A combination of phenotypic testing and molecular selection is currently underpinning the development of fire blight resistant cultivars carrying commercial fruit quality in several breeding programs worldwide. For phenotypic resistance testing, artificial syringe-inoculation of shoot tips or infection by cutting leaves with scissors dipped in a bacterial solution are common practices in glasshouse tests. Additionally, artificial flower inoculation tests in protected orchards or quarantine greenhouses are performed. Towards the end of the last century a range of apple cultivars with resistance to scab and some with additional resistance or tolerance to fire blight were released especially from breeding programs in the USA, Germany and France. However, only few reached commercial impact.

Transgenic pear and apple lines expressing attacin genes and therefore enhanced fire blight resistance were developed in Geneva, USA at the end of the last century.

In order to enlarge the genetic base of resistance for breeding, the resistances of wild apple species accessions including *M. x robusta* 5, *M. fusca*, *M. floribunda* 821 and the ornamental cultivar.

Use of decision support systems for fire blight management

Dani Shtienberg

Department of Plant Pathology and Weed Research, ARO, the Volcani Center, P.O.B. 15159 Rishon Lezion 7588809 Israel

Fire blight, caused by *Erwinia amylovora* (*Ea*), is the most destructive pathogen of pears worldwide. The effects of this disease are devastating and severely infected trees may eventually die. The erratic nature of fire blight, coupled with its destructive potential, make management of this disease a difficult task. Prevention of blossom infection by *Ea* is the key to fire blight management. Bactericide sprays may protect the blossoms provided that they are applied within a narrow window: 1 day before or up to 2 days after an infection episode. Inadequate spray timing may result in insufficient disease suppression. As the number of infection episodes per year in individual orchards in many places of the world is limited, it is expected that a few properly timed bactericide sprays would adequately protect the blossoms. For predicting when infection episodes are most likely to occur, numerous warning and decision support systems (DSS) were developed over the years in various places. A DSS named Fire Blight Control Advisory (FBCA) was developed in Israel in 1997. The system was evaluated between 1997 and 2000 in simulation experiments and 35 replicated experiments carried out in commercial orchards in which there were natural infections. For comparison, the performance of the forecast system `Maryblyt', which was developed in Maryland, USA, was evaluated as well. The simulation experiments included 193 orchard-plots in which the time of disease onset enabled us to determine the date of infection. In 10 of the experiments fire blight developed naturally. Analysis of the data revealed that FBCA predictions were accurate in 98% of the case; Maryblyt' was significantly less accurate. Pear managers in Israel readily accepted FBCA and since 2001 most pear orchards in Israel have been managed according to its recommendations. The performed of the system since its commercialization, and the improvements that were carried out over the year will be presented and discussed.

Improving fire blight control by knowing *Erwinia amylovora* epidemiology in the European Union countries

María M. López[1], Ester Marco-Noales[1], and Emilio Montesinos[2]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), CV-315, Km 10,7, 46113 - Moncada (Valencia), Spain; [2]Institute of Food and Agricultural Technology-INTEA, University of Girona, 17071 Girona, Spain.

Erwinia amylovora outbreaks are still feared by pome fruit growers in most European Union (EU) countries, although information about its integrated control in their local conditions is generally available. However, the situation is quite different in Spain because the efforts since 1995 were concentrated on active and rapid eradication actions taken after discovering new foci. Indeed, the Spanish experience has been for many years the unique example of a long-term eradication program of the pathogen. Unfortunately, in the last five years, eradication has been neglected in some areas and new foci have appeared. The implementation of integrated management programs in the orchards of a given area requires the knowledge of the local critical factors determining fire blight development in each season: inoculum potential, amount of susceptible plant material and favorable weather conditions. Information on pathogen sources, life cycle of *E. amylovora* and cultivar susceptibility should also be known. Moreover, the number of currently authorized products is very limited, and studies on efficacy and phytotoxicity are also required. Therefore, innovative research is still a cornerstone in fire blight management strategies. At local level, it is strictly necessary to know the type of symptoms, reduce the inoculum by appropriate pruning in winter, apply risk assessment systems, acquire information on patterns of disease in space and time, and select the more efficient chemical and biological preventative treatments. The objective is to reduce the pathogen populations and damage levels to an economically acceptable threshold. Nurseries are key players in fire blight management, and in the EU the intensity and quality of the inspections and laboratory controls of the plant material should be increased in order to guarantee its sanitary status and *E. amylovora* absence. Comparisons of strategies among several countries will be presented.

Transcriptional and post-transcriptional regulation of the T3SS in *Erwinia amylovora*

Youfu (Frank) Zhao

Department of Crop Sciences, University of Illinois at Urbana-Champaign, USA

It is well understood that the T3SS in *Erwinia amylovora* is transcriptionally regulated by the master regulator HrpL; however, how *hrpL* is regulated remains elusive. Here we presented genetic and biochemical evidences to demonstrate that the *hrpL* transcription is positively regulated by alternative sigma factor 54 (RpoN), its modulation protein (YhbH), an enhancer binding protein (HrpS) and an integration host factor (IHF). Bioinformatic analysis and electrophoretic mobility shift assay further determined the binding sites of HrpS and IHF on the *hrpL* promoter. In addition, we showed that the linear nucleotide second messengers (p)ppGpp-mediated stringent response is the internal molecular "switch", which activates RpoN-dependent regulation of the *hrpL* gene when bacteria are under nutrient stress. Furthermore, we revealed that the global regulator GacS/GacA and the small non-coding regulatory RNA *rsmB* negatively regulate the T3SS through neutralizing the positive effect on T3SS by the RNA-binding protein RsmA at the post-transcriptional level. A comprehensive model for the T3SS regulatory pathway in *E. amylovora* will be presented and future research directions will be discussed.

Towards the understanding of iron uptake and siderophore biosynthesis in *Erwinia amylovora*

Ivan Polsinelli[1], Marco Salomone-Stagni[1], Joseph Dale Bartho[1,2], and Stefano Benini[1]

[1]Free University of Bozen-Bolzano, Faculty of Science and Technology, Piazza Università 5, 39100 - Bolzano, Italy; [2]Ludwig-Maximilians Universität München, Professor-Huber-Platz 2, 80539 - München, Germany

A better understanding of *Erwinia amylovora* biology is of paramount importance to develop alternative strategies to control fire blight. Iron is an indispensable micronutrient used as cofactor for numerous proteins in almost all living organism. In conditions of poor availability of iron, *E. amylovora* produces, for its uptake, cyclic hydroxamate-type siderophores, called desferrioxamines (DFOs). The structural and functional characterization of the enzymes of the biosynthetic pathway of DFOs (DfoJ, DfoC and DfoA), of the proteins involved in ferrioxamine uptake (FhuD, FoxR, FhuA) and in ferrioxamine utilization (ViuB) will lead to an increased knowledge of *Erwinia amylovora* iron metabolism and will provide the basis for the development of sustainable strategies to prevent fire blight infection. Here we show the structures of the DfoJ/C/A enzymes and our current research plan in which our efforts are focused on the elucidation of the structures of the other proteins involved in ferrioxamine uptake and utilization.

The structural biology of *Erwinia amylovora* desferoxamine biosynthetic pathway at a glance

Marco Salomone-Stagni[1], Joseph Dale Bartho[1,2], and Stefano Benini[1]

[1]Free University of Bozen-Bolzano, Faculty of Science and Technology, Piazza Università 5, 39100 - Bolzano, Italy; [2] Ludwig-Maximilians Universität München, Professor-Huber-Platz 2, 80539 - München, Germany

Iron is an essential nutrient for almost every living organism. Although iron is a very abundant element on earth, its bioavailability in aerobic condition at neutral to alkaline pH is poor. Hence, to satisfy their iron requirement bacteria utilize various strategies. The most widespread and successful tool for high-affinity iron acquisition is the use of siderophores, which play roles also in iron homeostasis, various metals transport and sequestration, signaling and oxidative stress. Thus, the exploitation of the structural knowledge on siderophores biosynthetic enzymes may result in the design of next generation antimicrobials. As principal iron scavenger, *Erwinia amylovora* uses desferoxamine E (nocardamine), which is among the strongest hydroxamate siderophores known. Herein, we present the structural biology of the all three *Erwinia amylovora* proteins involved in dfo biosynthesis. The three dimensional structures of DfoJ, DfoA and DfoC are the starting point for the creation of rational inhibitors that would lead to the impairment of iron availability in *E. amylovora*. In particular, DfoA and DfoC have no human homologues, making them very attractive targets.

Polymorphism of the VNTR F region of *Erwinia amylovora* and comparison between some VNTR F and CRISPR-groups

Nataliya Drenova, Galina Matiashova, Denis Belkin, and Maxim Kondratyev

All-Russian Plant Quarantine Centre

Erwinia amylovora, causal agent of fire blight, is the most dangerous bacterial disease of *Rosaceae*. Source tracking of the pathogen is important for disease control but genomic polymorphism of strains is relatively low. During EUFRESCO II PHYTFIRE project several regions containing Various Number of Tandem Repeats (VNTR) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have been found and six VNTR (A, B, C, D, F, H) and CRISPR region 1 (CRR1) have been selected for strain typing. In additional VNTR F region has polymorphic sequences of tandem repeats. At this stage polymorphism in the VNTR F region (so structure of individual repeats as repeats' number and composition) has been studied for over 100 original strains from Russian Federation and neighboring countries and for 46 world collection strains. Sequences of individual repeats consist 18 nucleotides including common part from 8 nucleotides (excl. 2 strains) and various tails. We were found 6 types of tails in individual repeats with 1 to 7 differences. Whole VNTR F regions consist 8 repeats (1 composition type), 5 and 7 repeats (2 types for each one) and 6 repeats (3 types). The highest variability was found among strains from USA. Comparison of repeats compositions for strains with 5, 6 and 7 repeats from Europe was detected possibility of generation of 6-repeats strains from 7-repeats ones by deletion of its 6th repeat. Additional argument for this version is correlation of groups VNTR F consisting 6 or 7 repeats and CCR1 groups A ('native') and D (which has deletion of 2 repeats). We were found A7, D7 and D6 groups of strains among Old World collection. Thus polymorphism of VNTR region F could be additional instrument for strain differentiation. In the other hand, possibility of generation of monomorphic VNTR regions with equal length by different ways (insertions, deletions of whole repeats) should be taken into account for detection of strains identity and source tracking.

Regulation of flagellar motility by the small RNA ArcZ in *Erwinia amylovora*

Jeffrey Schachterle[1], Quan Zeng [1,2], and George W. Sundin[1]

[1]Department of Plant, Soil and Microbial Sciences and Center for Microbial Pathogenesis Michigan State University, East Lansing, MI, USA, 48824; and [2]Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, New Haven, CT 06504, USA

Erwinia amylovora, causal agent of fire blight disease of apple and pear trees, requires flagellar motility for efficient primary infection of flowers. We have shown that the Hfq-dependent sRNA ArcZ positively regulates flagellar motility in *E. amylovora*. However, the mechanism of this regulation is unknown. This study explores the mechanism of ArcZ regulation of flagellar motility and identifies the first known direct target of ArcZ in *E. amylovora*. RNA was isolated from wildtype, *hfq*, and *arcZ* mutant cells, reverse transcribed to cDNA and used in qPCR reactions to determine relative abundances of *fliC*, *motA*, *motB*, *fliA*, *flhD*, and *flhC* mRNA. Translational fusions were developed by cloning of *flhD* 5' UTR in-frame to a GFP reporter. The *arcZ* and *flhD* translational fusion were additionally subjected to site-directed mutagenesis at sites identified using the RNAHybrid online server. Reporter mean fluorescence intensity was measured by flow cytometry. Analysis of mutants by qPCR showed that for all genes tested, mRNA levels were significantly lower in *hfq* and *arcZ* mutants relative to wildtype, including the global regulator *flhDC*. RNAHybrid search of ArcZ and *flhD* 5' UTR identified a candidate interaction region. Testing of an *flhD* translational fusion in revealed that translation of FlhD is upregulated 3-fold in *hfq* and *arcZ* mutants. Wildtype *arcZ* complemented the effect, but *arcZ* mutated in the interaction region failed to complement the effect. Introduction of compensatory mutations in the *flhD* translational fusion enabled complementation by the mutated ArcZ. These results demonstrate that ArcZ regulates flagellar motility via regulation of *flhDC*. Mutation and compensatory mutation analysis suggest that the 5' UTR of *flhD* acts as a direct target of ArcZ in *E. amylovora*. However, because mutations of *hfq* and *arcZ* led to opposing effects at the mRNA and translational level, there are likely additional ArcZ targets involved in regulation of flagellar motility.

The *Malus fusca* fire blight resistance locus: validation of *Mfu10* and genetic resolution of the region containing the locus

Ofere Francis Emeriewen[1], Klaus Richter[2], Mickael Malnoy[3], Magda-Viola Hanke[1], and Andreas Peil[1]

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The apple wild species accession *Malus fusca* MAL0045 was found to be resistant in artificial inoculation trials with fire blight. *Erwinia amylovora* strain Ea222_JKI, usually used for mapping purposes in Dresden, as well as the highly virulent strain Ea3049 did not affect MAL0045. The corresponding fire blight resistance locus of *M. fusca* (*Mfu10*) was previously mapped on chromosome 10. This quantitative trait locus explaining up to 66 % of phenotypic variation at a logarithm of the odd (LOD) ratio of 31.0 was based on 134 individuals derived from a cross of *M. fusca* × Idared and phenotyped with *Erwinia amylovora* strain Ea222_JKI. To gain more insight into the resistance mechanism of *M. fusca*, we phenotyped the original mapping population of 134 individuals with the highly virulent *E. amylovora* resistance-breaking strain of *M.* ×*robusta* 5 - Ea3049, and fine mapped the region containing *Mfu10*. Ea3049 could not break down the resistance of *Mfu10* but significantly affected it. Furthermore, the closest tightly linked SSR markers to *Mfu10* were used to genotype an increased population of 1,202 individuals, leading to the identification of interesting recombinants in the region of interest. The increase in population, addition of more tightly linked SSR markers to linkage group 10 as well as the phenotypic evaluation of recombinants ensured the genetic resolution of the resistance locus.

Functional roles of *Erwinia amylovora* catalases during plant-pathogen interactions and exposure to starvation

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Non-obligated bacterial phytopathogens such as *E. amylovora* need antioxidant enzymes to cope with the hydrogen peroxide (H_2O_2) produced by plant defenses, and metabolism during exposure to environmental starvation. Catalases decompose H_2O_2 into innocuous compounds for the cell. *E. amylovora* possesses two catalases, KatA and KatG, but their roles during plant-pathogen interactions and survival under natural starvation had not yet been explored. In this work, we characterized the functional roles of *E. amylovora* catalases by expression analyses, activity assays as well as virulence and survival experiments, using single and double catalase mutants. Our study revealed: i) different regulation patterns for each catalase; ii) a higher catalase activity of KatA, but a greater contribution of KatG to virulence and survival in non-hosts; iii) that exopolysaccharides apparently do not participate in H_2O_2 protection *in vitro*; iv) the contribution of catalase activity to the maintenance of culturability during starvation, delaying the viable but nonculturable response. Accordingly, we describe for the first time catalases as important virulence and survival factors in *E. amylovora.*

Comparative analysis of transcriptomes of *Erwinia amylovora in planta,* on two apple cultivars of different susceptibility to fire blight

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Erwinia amylovora is generally considered as a homogeneous species in terms of phenotypic and genetic features. However, strains show variations in their virulence appearing in differences in their host range and the intensity of caused symptoms. The aim of our study was to find differences on the level of transcription during bacterial interaction with host plants of different susceptibility to fire blight. For this purpose we applied RNA-seq technique to compare transcriptomes of *E. amylovora* strain 650, 24h and 6 days after infection of shoots of two apple cultivars – susceptible and resistant to fire blight. The analysis of differences in gene expression revealed that approximately 50% of the *E. amylovora* 650 genes were differentially expressed *in planta,* both 24 h and 6 days after inoculation of apple shoots, comparing to the transcriptome of bacteria in pure culture in liquid TY medium. A total of 640 down-regulated genes and a set of another 698 up-regulated genes were common for both apple cultivars in both time points after inoculation. The 640 down- and 698 upregulated genes were classified into the same 19 eggNOG/COG categories but genes belonging to different categories were over-represented in groups of up- and down-regulated genes. The highest number of differentially expressed *E. amylovora* genes between two apple genotypes was observed 24 h after inoculation time point. Six days after inoculation only a few bacterial genes had different expression in susceptible and resistant apple cultivar. Among genes of different expression several hypothetical proteins of unknown function were found.

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Testing of resistance of pome fruit cultivars after artificial inoculation with *Erwinia amylovora* in field conditions

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Fifteen pear and eighteen apple genotypes comparing with commercially grown varieties as standards were tested for their relative susceptibility level to fire blight after artificial inoculation in the climatic conditions of the Czech Republic. Tests were carried out in research station of Crop Research Institute in Slany in technical isolators covered insect proof net in suitable climatic conditions (temperature > 15°C, relative humidity > 75%). Artificial inoculations were carried out in the period of strong extending growth by decapitation of shoot tips by scissors dipped in the bacterial suspension (concentration 10^6 cfu/ ml) of mix virulent strains of *Erwinia amylovora* (Czech isolates). The level of resistance was determined by percentage of necrotic lesion development to total length of shoots (in cm) after 40 days. Calculated intensity of infection was transferred to 6 point evaluation scale. From pear tested genotypes, only ´Bohemica´ was evaluated as high resistant (4.74% infection intensity). From old cultivars only ´Avranzska´ was evaluated as resistant (9.83% infection intensity). Resistant level of ´Avranzska´ and ´Bohemica´ was comparable to newly bred US-62-537-48 or US 625-63-4. The most of testing genotypes were categorized as moderately susceptible, susceptible or highly susceptible. From apple tested genotypes, only ´Selena´ was evaluated as high resistant (4.01% infection intensity). Resistant level of ´Selena´ was comparable to ´Remo´ (4.77% infection intensity). The most of testing genotypes were categorized as moderately susceptible, susceptible or highly susceptible.

Modulating the plant-pathogen interaction using antimicrobial peptides to control fire blight

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Synthetic antimicrobial peptides (AMPs) are reliable compounds to develop strategies for integrated pest management. AMPs can act directly against the pathogens and/or induce plant defense responses. In this work, 50 peptides from the CECMEL11 (linear undecapeptides, cecropin A-melittin hybrids) and the CYCLO10 (cyclic decapeptides) libraries and some derivatives were tested for induction of plant defense genes. These peptides showed different antimicrobial, hemolytic and phytotoxic activities as well as susceptibility to proteolytic degradation. A first screening for plant defense elicitation was carried out with BY2 tobacco cells using assays to determine the alkalinization and the production of hydrogen peroxide. Peptides BP13, BP100, BP143 and BPC200W were slightly effective in these assays and were tested for expression of defense-related genes in tomato by RT-qPCR. Within a set of 11 genes representative of the jasmonic, salycilic acid and ethylene pathways, the peptides BP13 and BPC200W induced overexpression of several genes that are also overexpressed by reference plant defense peptide elicitors. The results obtained with the two platforms (tobacco cells and tomato) were confirmed in plant assays with pear potted plants for control of *E. amylovora* infections. Plants were treated with peptides BP13, BP100, BP143 and BPC200W at different treatment schedules related to the pathogen inoculation (7, 2, 0 days bpi). All the peptides were effective in controlling infections in the full schedule, but peptides BP13 and BP200W showed efficacy in the preventative application, 7 and 2 days bpi. Interestingly, peptide BP200W did not have antibacterial activity against *E. amylovora*. It was concluded that these peptides or their combination can be used to modulate the plant-pathogen interaction by triggering defense mechanisms in the host plant, as well as to directly attack the pathogen.

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Functional analysis of the fire blight effector protein AvrRpt2$_{EA}$ in apple

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During the last decades the fire blight disease, caused by the bacterium *Erwinia amylovora*, has become an economical important disease in pome fruit cultivation in many regions of the world. Beside the characteristic necrosis of shoots, an infection with the bacterium triggers also a modification of the phenolic profile in apple leaves. The bacterial cysteine protease AvrRpt2$_{EA}$ was identified as a central molecule in the apple-*Erwinia amylovora* host-pathogen interaction, playing an important role in the resistance induction as well as infection development in resistant or susceptible plants. To analyze the function of the type III effector AvrRpt2$_{EA}$ in apple, a fire blight susceptible apple cultivar was transformed with a T-DNA construct containing the bacterial *AvrRpt2$_{EA}$* gene under the control of the heat-shock inducible promoter *Gmhsp17.5-E* from soya bean *Glycine max*. Three *AvrRpt2$_{EA}$*-transgenic plant lines have been obtained, which were characterized in detail. These lines were transferred to the greenhouse and used for experiments. The expression of *AvrRpt2$_{EA}$* was induced by repeated heat-shock treatments. To analyze the effect of AvrRpt2$_{EA}$ on the flavonoid metabolism, we analyzed the content of different phenolic substances in the plant tissue. The results of these experiments are discussed.

Perspectives on streptomycin-resistant *Erwinia amylovora* in New York

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Resistance to streptomycin in *Erwinia amylovora* (SmR Ea) was first observed in New York in 2002. From 2011 to 2015, 1,280 isolates of *E. amylovora* were collected from 80 commercial apple orchards in New York. Thirty-four SmR Ea isolates were found in 19 orchards. Thirty-two of the 34 resistant isolates contained the streptomycin resistance gene pair *strA/strB* in the transposon Tn*5393* on the non-conjugative plasmid pEA29, while two had the K43R mutation in the *rpsL* gene. CRISPR spacer sequencing was use to explore the diversity and origins of SmR Ea in New York. The spacer array regions CR1, CR2, and CR3 were sequenced for both SmR and streptomycin sensitive (SmS) *E. amylovora* isolates revealing nineteen CRISPR spacer profiles. The majority of SmR Ea isolates had the same CRISPR profile as the strains from 2002, suggesting that they may have spread throughout the region. Several CRISPR profiles were identical between SmR Ea and of SmS Ea strains collected from the same orchards, suggesting that SmR Ea may have originated on site. Some SmR Ea isolates had profiles identical to those from other regions in the United States suggesting movement of infested materials.

Tracking of *Erwinia amylovora* dissemination in Eastern Europe and Central Asia using CRISPRs

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The genetic variability of *Erwinia amylovora* in Europe is very narrow due to a bottleneck effect following the first introduction of the disease in the late 1950's. This makes standard methodologies for assessing population diversity ineffective. CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) are DNA loci containing short repetitions of base sequences interspersed with variable spacer sequences derived from phages or plasmids. Together with the *cas* (CRISPR-associated) genes they protect bacteria and archaea against invasion by mobile DNA elements via an RNA-interference-like mechanism. CRISPR Repeat Regions (CRR) display higher-than-average variability than the rest of the genome and can thus be used for strain typing. Using this approach we investigated the recent spread of fire blight to Eastern Europe and Central Asia, the latter being the geographic origin of domestic apple and pear species. The deletion of a spacer duplication in CRR1 was determined to have appeared in Europe about twenty years after the introduction of *E. amylovora*, either by modification of the existing genotype or, more likely, by a second independent introduction from North America. All European isolates so far analyzed are either identical to or can traced back to one of these two genotypes, which thus represent the two ancestral populations in the Old World. An increase in genetic diversity among European strains was apparent only in the last decade. A PCR assay was designed to swiftly identify to which of the two European ancestral populations an isolate can be traced back and allowed to build, together with data obtained from whole genome sequencing, a streamlined model of the dissemination routes of fire blight toward Central Asia. According to our data, the strains responsible for the recent outbreaks in Kazakhstan and Kyrgyzstan share a common ancestry with the *E. amylovora* population that is present in the Caucasus region and around the Black Sea.

Epidemiology of fire blight (caused by *Erwinia amylovora*) in the Pink Lady® apple cultivar in Israel

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Local decision supporting systems developed in Israel (Fire Blight [FB] control advisory [FBCA]) and can successfully predict the occurrence of FB (Caused by *Erwinia amylovora*) infection in pears. FB is not considered a serious pathogen in Israeli apple orchards, due to early flowering in the 'Ana' cultivar, when temperatures are too low for infection; or late flowering beyond the common period of rain events. However, growers of Pink Lady® (PL) apple cultivars have reported severe FB infections in their orchards due to early flowers, when significant rainfall events occur. The long-term objective of the present project was to develop a strategy for FB management in the PL apple cultivar. The specific objectives of this report are: to evaluate the accuracy of FBCA in predicting infections in PL and to record pathogen survival in the trees. FB infections were observed in 87.5% of the orchards with severe infections only in 2 orchards which were located in close proximity to infected pear orchards. In 2015 FBCA predicted 83% of the infection events with no visible cankers with ooze. During April to August, 100% of the infected inflorescences contained viable *E. amylovora* cells but none in December to June of the following year. In this research we found that FBCA can successfully predict FB in Israeli PL apple orchards, and that the initial inoculum probably originates from nearby infected pear orchards.

New host for *Erwinia amylovora* in wild plants: first detection in *Pyrus bourgaeana* (Iberian wild pear)

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The Iberian wild pear (*Pyrus bourgaeana* Decne) is a deciduous tree species typical of the Mediterranean forest and evergreen open woodland (dehesas) of central and southern Spain. This tree can reach 10 m in height, and it has an irregular crown with an average diameter of approximately 5 m. It plays an important trophic role in the context of ecological balance, since it produces palatable leaves as well as a good quantity of fleshy fruits throughout the summer, very attractive for animals, at a time when other resources are scarce. However, it is not an abundant species, and has been included in the indexes of threatened species. In the Iberian Peninsula, *P. bourgaeana* populations are dispersed in the southern Portugal and western Spain, becoming rarefied towards the eastern areas. From 2010 to 2012, typical fire blight symptoms in some trees located near a National Park (Cabañeros) in the Southern Central area of Spain were observed. *Erwinia amylovora* was isolated in pure culture in a 70.5% of the analyzed samples. The isolates of the pathogen were phenotypically homogeneous, with characteristic biochemical profile and pattern of carbon source utilization, with only some minor discrepancies. Genotypically, differences in the pEA29 and pEI70 plasmid content have been shown, corresponding to two different VNTRs profiles. Pathogenicity of all isolates was assayed in immature pears or apples, and also in pear and Iberian wild pear shoots, developing typical fire blight symptoms. This is the first report of *P. bourgaeana* as host of *E. amylovora*. Given the significance of Iberian wild pear for fauna and landscape, the increasing interest of the dehesas of southern of Europe, and the biodiversity harbored in the Mediterranean region, it is of critical importance to protect the populations of *P. bourgaeana* from fire blight.

Monitoring *Erwinia amylovora* in Southern Germany, Switzerland and Austria during the blossoming periods since 2010

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Forecasting blossom infection periods for fire blight is important to allow for countermeasures that reduce economic damage in pome fruit production. Current forecasting models are based on physical factors such as temperature and moisture, but not on the actual presence of *Erwinia amylovora*. As qPCR allows for early and fast detection and quantification of *E. amylovora* in pome fruit blossoms, measuring the abundance of the pathogen accurately timed for setting countermeasures is possible. Bio-Protect, located in the apple growing region around Lake of Constance, in cooperation with cooperatives and consultants from southern Germany, Switzerland and Austria established a monitoring for *E. amylovora* during the blossoming period. Up to 160 samples per day originated from distances up to 200 km from the laboratory were processed in 24 hrs. So actual information on the abundance of *E. amylovora* in the specific orchards and an overview on the disease pressure in the whole region was generated. In some selected orchards samples were taken daily. This data showed that implementing the real abundance of *E. amylovora* into the fire blight forecast could improve its reliability.

Occurrence of fire blight disease on Asian pear caused by *Erwinia amylovora* in Korea

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Fire blight, a bacterial disease in Rosaceous plants, has been reported worldwide, but not in Korea. In early May 2015, disease symptoms similar to typical blossom and shoot blight of fire blight disease were observed in young blossoms and shoots of Asian pear (*Pyrus pyrifolia* cv. `Shingo') in Korea. Disease incidence ((infected tree numbers/total tree numbers) x 100) varied from 0.2 to 93.2%. The casual pathogen, *Erwinia amylovora*, was isolated from disease shoots and identified by a PCR method with several *E. amylovora*-specific primers, DNA sequencing and also MLST (Multi Locus Sequence Typing) analysis. Moreover, all Korean isolates of *E. amylovora* produced levan. To confirm pathogenicity of Korean *E. amylovora* isolates, immature Asian pear and apple fruits were inoculated with bacterial suspension and typical necrosis and bacterial ooze appeared at the inoculated sites in both apple and Asian pear fruits. Bacteria were re-isolated from the inoculated pear, and PCR with *Ea*-specific primer sets confirmed *E. amylovora*. To our knowledge, this is the first report on the occurrence of fire blight disease caused by *E. amylovora* on Asian pear in Korea.

The incidence and prevalence of fire blight in apple trees in Lake Van Basin, Turkey

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Apple is an economically important product in Lake Van Basin. The aim of the study is to determine Erwinia amylovora, and incidence and prevalence of fire blight on apple trees in Lake Van basin. Observations and samplings were carried out in 42 apple orchards, which generally consist of Golden delicious, Starking and local varieties of apple, from 9 district (Van, Bitlis, Edremit, Gevaş, Tatvan, Adilcevaz, Ahlati, Erciş, Muradiye) in Lake Van basin in June 2015. The samples from apple trees having the fire blight symptoms were collected for identification of E. amylovora. Identification of obtained isolates was done by the phenotypic tests, molecular detection by G1-F/G2-R primer pair and pathogenicity on pear sapling. The incidence of fire blight on apple orchards was detected in the range of 0,0086 and 6,63 in Ahlat and Edremit districts, respectively, and the incidence of disease in all over the basin was detected as 0,32 percent. The disease prevalence in the basin was 36,6% and maximum prevalence was in Erciş district with 66,6%.

Effect of low temperatures on *Erwinia amylovora* virulence and starvation responses

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Erwinia amylovora is a psychrotrophic bacterium able to grow from 4ºC to 37ºC. Under field conditions blossom blight epidemics are produced at temperatures over 18ºC. Consequently, most studies on *E. amylovora* have been performed at 18ºC or at its optimal growth temperature (28ºC). However, the significance of *E. amylovora* psychrotrophy on its life cycle is still poorly understood. In this work, we investigated the effects of three temperatures (28ºC, 14ºC and 4ºC) on different aspects related to *E. amylovora* virulence and/or starvation survival. *E. amylovora* caused fruit blight at all the temperatures assayed, and different pathogenicity/virulence factors (exopolysaccharides, biofilms, etc) were more efficiently induced at 4ºC and/or 14ºC than at 28ºC. Besides, while starvation at 14ºC favored the maintenance of culturability together with changes in cell size and shape, 28ºC and 4ºC induced a faster entry into the viable but nonculturable state, with less apparent morphological changes than at 14ºC. These results unveil unknown information on the *E. amylovora* pathogenicity and survival at temperatures below 18ºC, possibly influencing the seasonal development of the disease.

A microbiological examination of *Erwinia amylovora* exopolysaccharide ooze

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Fire blight, caused by the pathogen *Erwinia amylovora*, is the most devastating bacterial disease of pome fruits in North America and around the world. The primary dispersal method of *E. amylovora* is through ooze, a mass of exopolysaccharides and bacterial cells that is exuded from infected host tissue. Over the 2013 and 2014 seasons, the virulent strain *E. amylovora* Ea110 was used to inoculate actively-growing apple shoots (cv. Jonathan) in field experiments, and 631 ooze droplets (201 in 2013 and 435 in 2014) were collected from blighted shoots. Mean populations of *E. amylovora* in ooze droplets ranged from 10^6 to 10^8 colony forming units per microliter (cfu/µl) depending on droplet color. The droplets that had higher populations were typically smaller in total volume and had darker coloring, such as orange, red, or dark red hues. These darker colors may be more attractive to insects that disperse *E. amylovora* cells from ooze. Examination of host tissue at the emergence site of ooze droplets using scanning electron microscopy revealed that ooze was not exuding through natural openings; instead, we only observed erumpent mounds and small (10 µm) tears in tissue. These observations suggested that *E. amylovora*-induced wounds in tissue provided the exit holes for ooze extrusion from the host. Analyses of internal *E. amylovora* populations and populations in ooze droplets indicated that approximately 33% of the total bacterial population from infected stems is diverted to ooze. Genetic expression analysis indicated that *E. amylovora* cells in stem sections located above ooze droplets and in ooze droplets were actively expressing virulence genes such as *hrpL, dspE, lsc,* and *amsK*. Thus, our study identified ooze as a source of very large, concentrated populations of *E. amylovora* that emerge from the host in a physiologically-ready mode to be dispersed and initiate disease.

Differential fitness of *Erwinia amylovora* isolates carrying different variants of the *rpsL* gene leading to high level of streptomycin resistance

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Erwinia amylovora can quickly become highly resistant to streptomycin through a single mutation in the *rpsL* gene, encoding the S12 protein of the 30S small ribosomal subunit. This causes an amino acid substitution that prevents inhibitory binding of streptomycin while preserving the functionality of the ribosome. Screening of streptomycin resistant (Sm^R) bacteria in the orchards consistently yielded the same mutation at codon 43, which leads to the substitution of a lysine through arginine (K43R). However, alternative mutations involving the replacement of a lysine, either at the same codon (K43T, K43N) or at another position in the protein (K88R), have also been found to result in Sm^R isolates under lab conditions. We address here the question whether the prevalence of the K43R variant in the wild is due to a higher probability of emergence of this particular mutation, to an increased tolerance to the antibiotic or is the result of enhanced fitness. For this purpose the resistance level of the different Sm^R genotypes was tested by assessing their respective minimal inhibitory concentrations (MICs). Moreover, the fitness of the various mutants was tested in different media with or without selective pressure by measuring their growth profiles, both separately and in competitive assays, using optical densitometry and plate counts. In rich medium, all variants have been found to display MICs exceeding 16'000 mg/l with only minor differences in their relative growth rates at most Sm concentrations. However, in minimal medium simulating the nutrient composition of apple flowers containing streptomycin, variant K43R showed a faster growth rate with respect to the isolates carrying the other mutations and was the sole strain that could compete with the wild-type when no antibiotic was present. We thus propose that the prevalence of Sm^R variant K43R in the field is due to an enhanced fitness that allows it to persist even in absence of selective pressure.

Shifts in the prevalence of streptomycin-resistant *Erwinia amylovora* on pear in Oregon, USA

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Streptomycin has been used for control of fire blight in the US since 1958. Streptomycin provided excellent disease control in the western region of the US and did not mar fruit finish. Oxytetracycline was registered for pear in 1972 after streptomycin resistance was detected in California. Streptomycin was the primary antibiotic used in Oregon; oxytetracycline was used sparingly, in part because it was considered less effective than streptomycin. Streptomycin-resistant *E. amylovora* emerged in northern Oregon in the 1990's. In an epidemic in 1998, nearly every isolate tested was resistant to streptomycin. In the 1990's in southern Oregon, streptomycin resistance was present in nearly every orchard sampled, but only a third of the isolates from each orchard was resistant. In 2002, a major epidemic occurred in southern Oregon, but nearly every isolate tested was sensitive to the antibiotic. Since then, the prevalence of streptomycin-resistant *E. amylovora* has increased in southern Oregon; about 75% of isolates in orchards were resistant to streptomycin by 2013. Resistance to streptomycin in Oregon was due to spontaneous mutation in *rpsL*; acquired resistance was not detected. Resistance to oxytetracycline or copper has not been detected in Oregon. Resistance status of *E. amylovora* in Oregon to kasugamycin, registered in 2014, is unknown. Sustainable antibiotic-based disease management will depend on use of resistance mitigation strategies and continuous monitoring the emergence of resistance.

Non-antibiotic strategies for fire blight control in the USA

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Fire blight-susceptible, organic pome fruit orchards occupy 9000 ha in the Pacific Northwest region of the United States. In 2014, the U. S. National Organic Program phased-out antibiotics as allowable materials for fire blight suppression in these orchards. In response, we have developed non-antibiotic programs for fire blight control that integrate cultural and copper-based sanitation, crop load thinning, biological control and non-antibiotic chemical protection. Parsing the pathogen's biology into stages - prebloom activity, epiphytic increase on floral stigmata, and infection via the floral cup - has been key in timing and maximizing the effectiveness of individual materials. Non-antibiotic programs have now been used commercially for two seasons with a high level success. To date, important materials for non-antibiotic fire blight control have included lime sulfur, a filamentous yeast (*Aureobasidium pullulans*), and soluble copper bactericides, with limited contributions from biological products comprised of gram-positive (*Bacillus* spp.) or gram-negative (*Pseudomonas* and *Pantoea* spp.) bacteria. Ongoing research is investigating i) the mechanism(s) by which the *A. pullulans*-based product, Blossom Protect, suppresses fire blight, ii) the environmental conditions that trigger fruit russeting by non-antibiotic materials, and iii) the relative ability of individual materials to impact/eradicate epiphytic pathogen populations. In 2016, replicated orchard trials focused on the evaluation additional alternative materials including potassium aluminum sulfate and pathogen-specific phage for their potential to eradicate the fire blight pathogen from pear and apple flowers.

Integration of biological control agents and plant defense activators against fire blight in Morocco

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The bacterial antagonists (*Pantoea agglomerans P10c, Bacillus subtilis GB03, B. subtilis QST713 and B. subtilis Y1336*) and plant defense activators (Acibenzolar-S-methyl (ASM), Fosetyl aluminium (F-Al), Potassium phosphites (PH) and Prohexadione-Ca (ProCa)) were evaluated individually and in combination for efficacy in controlling fire blight in Meknes. In laboratory on detached blossoms, two biological control treatments have shown remarkable efficiencies compared to others on apple and pear, it is the treatment based *P. agglomerans P10c*, and the mixture of the two strains *P. agglomerans P10c* and *B. subtilis QST713*. Under field, this mixture of biological control agents and all others strains were tested alone and in combined with plant defense activators using split-split-plot design. Result, showed that under field when applied alone *P. agglomerans P10c, B. subtilis QST713,* their mixture (½ + ½), *B. subtilis GB03* and *B. subtilis Y1336* reduce blossom infection by 70.1%, 67.7%, 66.8%, 65.4%, and 48.5% respectively. For plant defense activators this reduction was 73.5%, 69.9%, 60.6% and 57.3% for ASM, ProCa, F-Al and PH respectively. The combination of plant defense activators and biological control agents gave the greatest protection against blossom blight, which ranged from 76% to 95.7%. The most combinations were the mixture of *P10c* and *QST713* combined with ASM or ProCa.

Keys words: Fire Blight, *Pantoea agglomerans, Bacillus subtilis,* Acibenzolar-S-methyl, Fosetyl aluminium, Potassium phosphites, Prohexadione-Ca and mixture of biological control agents.

Field trials for fire blight control in the experimental orchard "Kirschgartshausen"

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Field experiments are the critical bottleneck for evaluation of fire blight control strategies. High fluctuation in natural infection pressure necessitates artificial inoculation for reproducible results but deliberate infection with fire blight is often restricted. The experimental orchard in Mannheim Kirschgartshausen is approved for artificial *E. amylovora* infection and field experiments for fire blight control have been conducted since 1994. One feature of the experimental setup in Kirschgartshausen is a three-year rotation between plots with clearing and replanting of each infected plot after evaluation. This minimizes age related resistance effects and yields trees of uniform size with approximately one hundred evaluated flower clusters per tree and one thousand flower clusters per replicate per variant for each experiment in addition to the requirements of EPPO guideline PP1/166 (3).

The presentation will summarize results of the field experiments from 2014-2016 with special focus on two non-antibiotic control strategies, application of the aluminum containing product "LMA" and of formulations of the antagonistic bacterium *Erwinia tasmaniensis*.

After consistent good results in field trials from 2010-2012 LMA has been available for fire blight control in commercial orchards in Germany since 2013 (plant protection act, Art.53). Due to the low level of natural infection during those years, there is only limited practical experience for this product till now. Application of *E. tasmaniensis* is still at a much more experimental stage. It has been tested in field experiments with good efficacy but with high fluctuation margins. The influence of application time point for chemical control products compared to the biological control agent *E. tasmaniensis* is discussed.

Experiences with Kasumin for fire blight control in Michigan

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The antibiotic Kasumin 2L (Arysta Corp.; Cary, NC) was registered for use on pome fruit for fire blight control in the United States in 2015, and has been used under a Section 18 Specific Exemption in Michigan in apple orchards with streptomycin-resistant *Erwinia amylovora* since 2010. We evaluated Kasumin for control of the blossom blight phase of fire blight in field experiments conducted between 2006 and 2015. Orchard studies were mostly conducted on the highly-susceptible varieties 'Gala' and 'Jonathan', and sometimes conducted on 'McIntosh'. Trees were treated with Kasumin at 70-80% bloom, inoculated with a concentrated suspension (1×10^6 cfu/ml) of the virulent strain *E. amylovora* Ea110 the next evening (full bloom), and sprayed with Kasumin again the next day. In other experiments, Kasumin was only applied once after inoculation. In many of the experiments, control achieved with Kasumin was compared to that with the other antibiotics streptomycin or oxytetracycline. Incidence of fire blight disease, as measured by the occurrence of blossom blight symptoms in inoculated plots, was moderate to high in nontreated control trees in 13 of 14 experiments conducted between 2006 and 2015, ranging from 24.5% to 80.5% infection. Application of Kasumin to trees at least 24 h prior to and at least 24 h following inoculation with strain Ea110 resulted in high levels of disease control with a mean of 76.8%. These levels of control were not significantly different from the standard streptomycin treatment in any experiment that included both antibiotics. In three experiments when Kasumin was only applied after inoculation, the level of control was very good, with a mean of 84.3% control. Our results with Kasumin and growers' experiences using Kasumin in commercial orchards have been very positive, and we feel that Kasumin is a highly effective blossom blight control option especially for highly-susceptible apple varieties under high disease pressure.

Contribution of native plasmids of *Pantoea vagans* strain C9-1 to fitness and biocontrol efficacy in apple and pear orchard trials

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Pantoea vagans strain C9-1 is a registered biocontrol agent for fire blight. We cured C9-1 of two of its three plasmids: pPag2, pPag3, and both pPag2 and pPag3, and evaluated flower colonization and survival through to mature fruit in the field. We conducted disease efficacy trials with C9-1 and a derivative lacking pPag3. pPag3 is a member of the plasmid group called Large *Pantoea* Plasmid (LPP-1) and carries genes that are thought play a critical role in environmental fitness. In trials in experimental orchards, loss of pPag2 and/or pPag3 did not affect establishment, growth or survival on apple and pear flowers through petal fall. Loss of pPag2 did not affect survival on fruit. However, population sizes of C9-1 lacking pPag3 decreased on apple fruit, and on pear fruit, the incidence of detectable populations decreased over time. In fire blight control trials, C9-1 cured of pPag3 reduced the incidence of disease to levels similar to the wild-type C9-1. Loss of pPag3 (LPP-1) did not reduce epiphytic fitness on apple and pear flowers or affect C9-1's ability to reduce disease incidence; however, loss of pPag3 did reduce long-term survival during fruit maturation in our trials.

Further results on SAR-inducing tree paints as a therapy to aid restoration of tree health after fire blight infection

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Induction of systemic acquired resistance as a therapeutic aid to the restoration of tree health was evaluated in 3- to 8-year old pear and apple trees diseased with fire blight. Acibenzolar-S-methyl (ASM) was applied to diseased trees in spring near the time of removal of primary fire blight cankers, which had originated from floral infections. Expanding cankers in young, susceptible pear or apple trees can be severely damaging in spite of pruning owing to residual pathogen inoculum that resides internally below the pruning cut, which can `re-ignite' disease symptoms. In the greenhouse, ASM applied as a pot drench or foliar spray was shown to suppress fire blight canker expansion in young trees, but similar rates of ASM by drench or spray in the orchard have proven ineffective. In contrast, in six seasons of orchard experiments, branch paints of ASM applied to the symptomless branch stub after pruning or sprayed onto an 80- to 100-cm length of the central trunk have reduced by 40 to 60% the end of season `yield' of blighted branches that developed as a result of disease re-ignition compared to pruning alone. Moreover, branch and trunk paints of ASM have suppressed canker expansion in other pear cultivars and apple rootstocks, and compared to sprays and drenches, ASM paints have shown a five- to ten-fold increase in induction of pathogenesis related protein genes (PR-1 and PR-2), an indicator of SAR expression in the host. An U. S. EPA registration for therapeutic applications of ASM was obtained in 2015 with the 2016 season representing first use in commercial orchards. The pome fruit growing community of the western U.S. will provide evaluations on the practical significance of this methodology.

Osmoadaptation increases the performance of *Bacillus amyloliquefaciens* EPS2017 for biological control of fire blight

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Biological control is a reliable method for the management of fire blight caused by *Erwinia amylovora*, but the experience in field evaluations have shown limitations due to variability in efficacy and consistency from trial to trial. The main objective have been focused to reduce or suppress *E. amylovora* on flowers, the main entry sites. Pathogen control may be due to competition for growth-limiting nutrients or antibiosis, or by excluding the pathogen from infection sites. Efficient biological control requires the establishment of the antagonistic bacterium on the surface of plant host organs, prior to the arrival of the pathogen. However, the success of its colonization/survival is often limited by adverse environmental conditions. Physiological adaptation during growth of the antagonist in the laboratory has been reported to improve its fitness in the field conditions. In our laboratory, *Bacillus amyloliquefaciens* EPS2017, an efficient fire blight biocontrol agent, was subjected to osmoadaptation to increase cell survival in the phyllosphere. Osmoadaptation consisted of saline stress and osmolyte amendment to the growth medium that induced the synthesis and accumulation of osmolytes, thus improving cell survival under desiccation and low relative humidity conditions. This procedure increased the survival of EPS2017 under low relative humidity conditions and showed a highly consistent efficacy in the reduction of infections caused by *E. amylovora* under semi-field experimental conditions.

Lactobacillus plantarum strains as efficient biocontrol agents of fire blight

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Several strains of *Lactobacillus plantarum* are suitable candidates for development of biocontrol agents of fire blight because they are potent antagonists of *Erwinia amylovora*, efficient colonizers of host plant and benefit of the lack of biosafety concerns of lactic acid bacteria. A set of 45 isolates of *L. plantarum* obtained from a wide range of plant sources were characterized in order to select lead candidates. The characterization by multilocus sequence type analysis (MLST) of *pgm*, *ddl*, *gyrB*, *purK1*, *gdh* and *mutS* genes revealed that strains collection showed eight sequence types (ST). Besides, almost all strains shared the same plantaritype that harbour 20 out of 29 genes present in the *pln* locus. The strains PM411 and TC92 have been selected as leads because of their wide spectrum of *in vitro* antagonism against five bacterial plant pathogens, including *E. amylovora*. They showed differential MLST profiles and shared the same plantaritype. More in detail, these strains showed a strong inhibitory activity against *E. amylovora* (*in vitro* antagonism of cells and culture supernatants, and inhibition of infections on flowers, leaves and immature fruits), and their efficacy in preventing infections of fire blight was confirmed in pear plant assays. A specific viability qPCR has been developed as a tool to study the colonization and survival of *L. plantarum* on plant surfaces in field conditions. Moreover, different strategies were studied to preadapt the cells to stress conditions and increase its survival in plant surfaces, consisting of saline and acid amendment to the growth medium during inoculum preparation. The expression of several stress-related genes using RT-qPCR (*groEL*, *dnaK*, *ftsH*, *ctsR*, *clpB*, *clpC*, *hsp1*, *hsp2*, *hsp3*, *cspP*, *cspL*, *efTU*, *trxB1*) was studied in preadapted cells, and during cell survival under low relative humidity conditions. Particularly, the physiological adaptation of strain PM411 increased its performance under stress and field conditions.

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Control of fire blight in Norway 1986-2015

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Fire blight was detected for the first time in Norway in 1986. It was a limited outbreak on the West Coast, only on ornamentals, particularly on *Cotoneaster*. An organization for the eradication and containment of fire blight was quickly established, and given comprehensive statutory powers and government resources to do surveys and eradicate diseased plants and highly susceptible plants from contaminated areas. The work has managed to restrict fire blight to the West Coast. Eastern and Northern parts of the country are considered pest free areas. The disease has not moved into important fruit-growing areas. Spread of fire blight to new areas has mainly been due to uncontrolled movement of beehives. From 1969 to 2016 import of all host plants from countries with fire blight has been prohibited. Systematic yearly surveys by foot and car in all parts of the country, using digital maps, internet connected tablets with GPS, and software for registrations made in the field have proved to be an efficient tool to spot new outbreaks at an early stage and start eradication, thus limiting further spread.

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P01-01

RNAi mutants for determination of candidate gene function in resistance of apple to *Erwinia amylovora* (Fire blight)

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To determine the function of candidate apple genes that confer resistance to fire blight, RNAi mutants of M.26 apple rootstock were produced using an efficient multiplex transformation system. Five RNAi EST-silencing vectors were used in each transformation experiment to allow selection of up to five types of mutants from a single experiment. RNAi-silencing constructs were created using ESTs associated with response of apple to *Erwinia amylovora* that were selected based upon transcript profiling data and bioinformatics. Candidate genes in six functional categories were evaluated by using the pHELLSGATE8 RNAi-mediated gene silencing vector to silence a specific EST derived sequence and then observing the resulting resistance phenotype. Silencing of candidate genes was confirmed by RT-qPCR. Transgenic lines were phenotyped following inoculation of young plants with *E. amylovora* by determining the area under the disease progress curve (AUDPC), the cumulative percentage of shoot length that was blighted and the population of *E. amylovora* in young plants as determined by qPCR. Some of transgenic lines with the p8L01 and pfbox silencing construct had a significantly lower population of *E. amylovora* than non-transformed M.26. The disease severity in the lines with the p4H09 silencing construct was significantly different from that of non-transformed M.26. Also some lines with the pfbox, p8L01, pPO53 and pSGT1 silencing constructs had significantly lower disease severity than non-transformed M.26. The genes associated with reduced disease-severity and/or lower *E. amylovora* population are potentially useful in maker assisted breeding and GE for cultivars and rootstocks with increased resistance to fire blight.

P01-03

Erwinia gerundensis sp. nov., a cosmopolitan epiphyte originally isolated from pome fruit trees in Catalonia

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A survey performed in the province of Girona to discover potential antagonists of pome fruit tree diseases yielded two yellow epiphytic bacterial isolates morphologically similar to *Pantoea agglomerans*, but showing no biocontrol activity. Whole-cell MALDI-TOF MS and analysis of 16S rRNA gene and *gyrB* sequences suggested the possibility of a novel species with a phylogenetic position in either the genus *Pantoea* or *Erwinia*. Multi-locus sequence analysis placed the two strains within *Erwinia* and supported their classification as a novel species. The strains showed general phenotypic characteristics typical of this genus and results of DNA-DNA hybridizations confirmed that they represent a single novel species. Both strains showed a G+C content of 54.5mol% and could be discriminated from phylogenetically related *Erwinia* species by their ability to utilize potassium gluconate, potassium 2-ketogluconate, maltose, melibiose and raffinose. Whole-genome sequencing of strain EM595^T, isolated from pear leaves in La Tallada d'Empordà, revealed the presence of a chromosomal carotenoid biosynthesis gene cluster similar to those found in species of the genera *Cronobacter* and *Pantoea* that explains its atypical pigmentation. Additional strains belonging to the same species were later recovered from other hosts in three different continents, revealing the cosmopolitan nature of this epiphyte. Although the majority of the strains in our work were detected among epiphytic bacteria living on plants of the *Amygdaloideae* subfamily, this may not reflect the preferred habitat of the species, but be the outcome of a sampling bias. Indications that this species may also be associated to cereals are suggested by a number of 16S rRNA sequences retrieved in GenBank that are closely related to that of EM595^T. To honor of the city of Girona, the name *Erwinia gerundensis* is proposed, with EM595^T (= LMG 28990^T = CCOS 903^T) as the designated type strain.

P01-04

Cellulose production, activated by cyclic di-GMP through BcsA and BcsZ, is a virulence factor and an essential determinant of the three-dimensional architecture of biofilms formed by *Erwinia amylovora*

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Multicellular aggregates in bacterial biofilms are encased in an extracellular matrix mainly composed of exopolysaccharides (EPSs), protein, and nucleic acids, which determines the architecture of the biofilm. *Erwinia amylovora* forms a biofilm inside the xylem of its host, which results in vessel plugging and water transport impairment. Production of the EPSs amylovoran and levan are critical for the formation of a mature biofilm. Additionally, cyclic dimeric GMP (c-di-GMP) was reported to positively regulate amylovoran biosynthesis and biofilm formation in *E. amylovora*. In this study, we demonstrate that cellulose is synthesized by *E. amylovora,* is a major modulator of the three-dimensional characteristics of biofilms formed by this bacterium, and contributes to virulence during systemic host invasion. Additionally, we demonstrate that cellulose biosynthesis activation in *E. amylovora* is a c-di-GMP dependent process, through allosteric binding to the cellulose catalytic subunit BcsA. We report that the endoglucanase BcsZ is a key player in c-di-GMP activation of cellulose biosynthesis. Our results provide evidence of the complex composition of the extracellular matrix produced by *E. amylovora* and the implications of cellulose biosynthesis in shaping the architecture of the biofilm and in the expression of one of the main virulence phenotypes of this pathogen.

P02-01

Proteomics of resistance of B.9 apple genotype to *Erwinia amylovora* (Fire blight)

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Fire blight (*Erwinia amylovora)* infection of the rootstock of apple trees is an increasing threat to productivity and longevity in high-density orchards of susceptible high-quality cultivars. Resistant rootstocks are the only reliable option to prevent development of rootstock blight. Budagovsky 9 (B.9) dwarfing rootstock displays varying resistance to fire blight at different stages of plant development. It is very susceptible to *E. amylovora* as a young shoot, but becomes increasingly resistant as it ages. Understanding the basis of this unusual resistant phenotype would potentially facilitate further exploitation of this form of disease resistance. Technologies for studying protein expression provide one method to increase our understanding of this phenomenon. Using the TMT technology, protein profiles of young and older B.9 tissue were identified. By blasting an apple database with the TMT-identified proteins, 3,733 proteins showing differential expression were identified. Proteins were characterized by up-regulation or down-regulation at 95% confidence in samples of first-year and second-year tissue at intervals after *E. amylovora* inoculation: 0h, 154 proteins; 12h, 156 proteins; 24h, 146 proteins. Several of these proteins have been shown to be correlated with increased disease resistance, and may have a role in developing more resistant apple cultivars and rootstocks by marker assisted breeding an/or GE.

P02-02

Occurrence of SCAR markers to the major QTL LG7 “Fiesta” for fire blight resistance among apple trees in Russia and close countries

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Apple tree, one of the host plants of fire blight, is the most important fruit and one of the most widespread ornamental and forest shelter belt’s culture in the Russian Federation. Due the climatic conditions and the people preferences, domestic and traditional frost resistant foreign varieties are the most prevalent excluding south regions of country. We was tested more than 150 varieties, rootstocks and wild forms of apple trees from different regions for the presence of F7 major QTL-markers for fire blight resistance as the first step of investigation of potential host plant resistance background and breeding possibilities against fire blight in Russia. During this assay high occurrence of varieties with one of QTL F7 markers has been found. Several varieties including Severniy Sinap, Spartan, Stroevskoe, Zaslavskoe, and some wild forms from different regions have both markers.

P02-03

Development of a novel selection scheme to detect *Erwinia amylovora* antagonists

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For *Erwinia amylovora* entry through host blossoms is the predominant infection pathway. Thus, the detached (apple) flower assay mimics natural conditions under which an antagonistic activity of a microorganism against *E. amylovora* can be tested in small scale laboratory settings. This assay is time-consuming and also dependent on the availability of blossoms, a temporally limited resource. Therefore, we developed a stepwise *in vitro* assay that preselects bacteria likely to show antagonistic activity in the detached flowers. Bacterial isolates mainly collected from apple and pear leaves in autumn were classified using MALDI-Biotyping. Stains were tested for growth inhibition of *E. amylovora* under iron limited conditions as well as growth rate and competition against *E. amylovora* in stigma based medium. Correlations of the acquired *in vitro* data and antagonistic activity of the strains in the detached flower assay are presented.

P03-01

Detection of *Erwinia amylovora* in asymptomatic plants: evaluation of bulk samples

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The ability of *E. amylovora* to survive in planta and produce latent infections can lead to false negative results in routine diagnostic protocols, since the pathogen can be present at very low numbers under the detection limit of some techniques. An experimental design to evaluate detection limits in asymptomatic plant material from different hosts was made in order to determine the minimum amount of plant material necessary for detecting *E. amylovora* with several techniques. To this end, samples from pear, apple and loquat trees were inoculated with different concentrations of an *E. amylovora* strain (spiked samples) and analyzed individually by isolation, ELISA-DASI and PCR. Thereafter, using the spiked samples that were positive for any of the techniques used, bulk samples were prepared by adding to spiked samples more amount of healthy plant material. Thus, bulk samples simulated 3, 5, 10 or 20 plants with *E. amylovora* concentrations ranging from 10^1 to 10^8 cfu/ml of plant extract. Interestingly, results showed that all the tested techniques allowed reaching a detection limit of 10^1-10^2 cfu/ml of extract after the enrichment, even analyzing samples from 20 plants. The enrichment was confirmed as a necessary step in the analysis protocol of bulk samples to achieve a greater sensitivity, since it favors the multiplication of the pathogen when it is found in very low numbers. Whole results show that asymptomatic samples can be processed as a pool in extensive screening surveys, facilitating the detection of latent infections.

P03-02

Fire blight epidemiology in the Republic of Kazakhstan

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The disease spread by regions of the republic, symptoms and host plants of the Rosaceae family: Map of the spread, total damaged area, photos of symptoms on various plants of the *Rosaceae* family.

Identification methods and morphological characteristics of the pathogen colonies on nutrient media: Identification methods: isolation of a pure culture, check of the pathogenic properties of hypersensitivity reactions by the Clement and White (photos), PCR methods. Morphology of the colonies, the pathogen of fire blight (photos).

The strategy of Fire blight control: Scheme of control strategies in the focus of infection.
Decreasing of infectious load: -autumn pruning of the infected burn wood organs, trimming cankers and whitewash of lime with addition of 10% copper sulfate, spraying by 4% solution of Bordeaux mixture; -early spring preventive spraying on the green cone by 4% solution of Bordeaux mixture and three times spraying by copper-bearing fungicides to flowering.
Repression of the fire blight pathogen breeding intensity: During flowering three times treatment by biologics (saturation of agrocenosis by antagonists and competitors)
Activation of the immune System: - After flowering three times treatments by immunomodulators; - autumn pruning, whitewash, spraying; Carrying out of protective measures in the fire blight focus for 3-5 consecutive years to eradicate the disease.

P03-03

Fire blight risk assessment in Girona (Catalonia). Comparison of Maryblyt and CougarBlight models

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Fire blight, caused by *Erwinia amylovora*, is a serious disease of apple and pear in some areas of Catalonia (North-East Spain). However, fire blight has only been detected in a few trees of two orchards in Girona region in the last 10 years. The first detection took place in 2007 on *cv* Conference pear in Bordils and the second one occurred in 2013 on apple *cv* Pink Lady in Torroella de Montgrí. The entirely trees of the affected orchards were eradicated according to the legal measures. Fire blight can quickly become an epidemic, for this reason an intensive surveillance has been done in the last years through rigorous disease monitoring. In order to have a better guidance for inspection, two fire blight predictive models were used: Maryblyt and CougarBlight. The fire blight risk was established during 2013, 2014, 2015 and 2016 on pear (*cv* Conference) and apple (*cvs* Fuji, Gala and Golden) using meteorological data of three weather stations (La Tallada d'Empordà, Torroella de Fluvià and Vilobí d'Onyar). The levels of infection risk were established using Mariblyt 7.0 and 7.1 and CougarBlight 2010 with the blight history scenario 2 (fire blight occurred in neighborhood orchard last year). A detailed analysis was also performed in Torroella de Montgrí, in an orchard placed near to the location where the last outbreak had been detected in 2013. Meteorological data from 2012 to 2016 were used and the fire blight risk was obtained for apple *cv* Pink Lady, the same variety of eradicated orchard. Models indicate that the infection observed in this orchard was probably due to the secondary bloom of apple trees.

P04-01

Control of fire blight (*Erwinia amylovora*) on apple trees with trunk-injected plant resistance inducers and antibiotics and assessment of induction of pathogenesis-related protein genes

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Trunk injection is a target-precise pesticide delivery method, that utilizes tree xylem to distribute injected compounds. Trunk injection could decrease antibiotic usage in the open environment and increase the effectiveness of compounds in fire blight control. In field experiments, after 1-2 apple tree injections of either streptomycin, potassium phosphites (PH) or acibenzolar-S-methyl (ASM), significant reduction of blossom and shoot blight symptoms was observed compared to water- or non-injected control trees. Overall disease suppression with streptomycin was lower than typically observed following spray applications to flowers. Trunk injection of oxytetracycline resulted in excellent control of shoot blight, suggesting that injection is a superior delivery method for this antibiotic. Injection of both ASM and PH resulted in the significant induction of PR-1, -2 and -8 protein genes in apple leaves, and ASM and PH suppressed fire blight even after cessation of induced gene expression. With the development of injectable formulations and optimization of doses and injection schedules, the injection of protective compounds could serve as an effective option for fire blight control.

P04-02

Biological control of fire blight (*Erwinia amylovora*) on quince by beneficial bacteria

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Fire blight caused by *Erwinia amylovora* has threatened quince cultivation in Turkey. Chemical control of fire blight is difficult because there are few effective bactericides registered and streptomycin, which is effective, and other antibiotics are not registered worldwide. Additionally the pathogen, *E. amylovora*, has developed resistance to streptomycin in several important production areas. Alternative control strategies are urgently needed for fire blight. Biological control with beneficial bacteria against *E. amylovora* has been considered as a potential method for controlling the disease. In this work, bacterial strains belonging to the species *Pantoea agglomeras, P. vagans* and *Pseudomonas fluorescens* were extensively studied as potential biological control agents of fire blight on immature pear fruits, detached blossoms and transplants of quince. Bacterial antagonists were found as effective as streptomycine treatment (100 µg/ml) to inhibit the development of *E. amylovora* on immature pear fruit assay. *P. agglomerans* strains Eh24 and Eh325 and *P. fluorescens* strain PfA506 significantly reduced the percentage of blighted blossoms in the detached quince blossom assay by 33 % to 55 % compared to the untreated control. Then, bacterial antagonists and streptomycine were applied onto one year old quince transplants (*cv.* Ege1) as spray treatment. *E. amylovora* inoculation was performed by injecting 10 µl of the bacterial suspension (10^8 cfu/ml) into the apical bud of quince transplants 24h after beneficial bacteria application. Disease severity was determined by measuring the length of shoot necrosis divided by the total shoot length and expressed as a percent 7 days after pathogen inoculation in seedling assays. *P. vagans* strain C9/1 and *P. fluorescens* strain PfA506 inhibited the development of shoot blight at the rate of 80 % and 76 % compared to pathogen alone treatment, respectively. These bacterial strains were found more promising than streptomycine tratment (100 µg/ml) for controlling the shoot blight symptoms of *E. amylovora* on quince transplants.

P04-03

Formulations for the microbial pesticides of *Lactobacillus plantarum* for the biological control of fire blight

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Lactic acid bacteria (LAB) which have been used in food technology including fermented vegetables or fruit juices as biopreservatives are considered with the status of GRAS (Generally recognized as safe) is a novel approach for biological control of fire blight. LAB strains *Lactobacillus plantarum* str. TC92 and *Lactobacillus plantarum* str. PM411 that showed suppressive activity against *E. amylovora* in detached plant organs in a previous research were selected in order to develop bioformulations. The production of the LAB were carried out in bioreactors using a modified media with cheap carbon and nitrogen sources as an alternative to MRS media. Formulations of the LAB by the methods of freeze drying, spray drying, microencapsulation and fluidized bed drying are the main objectives of the research followed by the observation of the shelf life viability performance in several periods of time and the biocontrol activity tests with detached plant organs. With the freeze dried formulations of *L. plantarum* TC92 using several lyoprotectants viability results observed after two months of storage and the preliminary results of the spray dried formulations are promising.

P04-04

Selection of potential fire blight biological control agents among loquat microbiota

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Spain is the world second producer and first exporter of loquat in global market. In eastern Spain, Protected Designation of Origin (PDO) "Loquats from Callosa d'En Sarria" comprises 19 villages, whose main economic activity is the loquat production. Although there have been outbreaks of fire blight in nearby locations, the disease has not been detected in the PDO, so prevention efforts are essential to maintain this important loquat producing area free of fire blight. Among the preventive activities, one of our research lines is the search and selection of potential biological control agents adapted to this crop. In a first approach, isolation of bacterial microbiota from loquat leaves and flowers was made in several culture media, and all colonial morphotypes from the culturable diversity were selected. The 173 representative isolates were tested in immature loquats for their ability to inhibit or slow down the infection caused by *E. amylovora*. Those that delayed the onset of symptoms and that did not produce hypersensitivity reaction in tobacco plants were selected. Four isolates were challenged for antagonistic activity against *E. amylovora*, and also for their ability to inhibit or slow down infection in pear blossoms. Then, phenotypic and molecular characterization has been initiated and they have been identified by complete sequencing of the 16S gene rRNA as *Enterobacter cancerogenus*, *Curtobacterium* sp., *Pseudomonas rhizospherere* and *Rosenbergiella epipactidis*.

P04-05

Influence of *Pantoea vagans* strain C9-1 plasmids on phenotypes associated with epiphytic fitness

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Pantoea vagans strain C9-1 is an effective biological control agent for fire blight. We cured C9-1 of two of its three plasmids: pPag2 (166 kb), pPag3 (530 kb), and both pPag2 and pPag3, and tested phenotypes of derivatives in laboratory assays. pPag3 carries genes for carotenoid pigment, thiamine biosynthesis, and quorum sensing; expression of phenotypes associated with these genes was confirmed previously. UV-C tolerance did not differ between yellow-pigmented C9-1 and white derivatives lacking pPag3. Swarming on nutrient broth-glucose solidified with 0.5% agar was influenced by pPag2 and pPag3. C9-1 exhibited wide dendritic swarms that coalesced, whereas C9-1 lacking pPag3 formed thin dendritic swarms. Swarms of C9-1 lacking pPag2 exhibited less radial distribution. Exopolysaccharide production and biofilm formation was greater with a derivative lacking both pPag2 and pPag3 compared to the wild-type and single plasmid mutants. pPag2 and pPag3, both alone and together, influenced expression of phenotypes of C9-1 associated with epiphytic fitness.

P04-06

Nutritional environment influences transcription of the Pantocin A promoter in *Pantoea vagans* strain C9-1.

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Pantoea vagans strain C9-1 is an effective biological control agent for suppression of *Erwinia amylovora*. C9-1 is thought to control *E. amylovora* on stigmas via resource competition and production of two antibiotics: pantocin A (herbicolin O) and dapdiamide E (herbicolin I). We focus on the expression of pantocin A. In antibiosis assays, pantocin A was detected on MOPS, Gluconate, Asparagine (MGA) medium, but not on Luria Bertani (LB). We used a green fluorescent protein (GFP) promoter probe to monitor the activity of the pantocin A (operon *paaPABC*) promotor. We found that yeast extract and tryptone in LB each inhibit expression of pantocin A, and then tested effects of amino acids that may be present in these two ingredients. The amino acids histidine, tryptophan, and arginine, when added to MGA medium, down-regulated transcription of the pantocin A promoter, whereas proline, aspartic acid, and asparagine increased transcription. The amino acids that induced *paaPABC* promoter activity, including proline and asparagine, are in high abundance on apple and pear stigmas, while those that down-regulate expression are in low abundance, indicating that pantocin A may be produced on stigmas.

P04-07

Comparison of test performance criteria for detection and identification *Erwinia amylovora* in plant extract

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In recent years there have been designed a sufficient amount of molecular tests for detection and identification of bacteria *Erwinia amylovora*. However only a small number of them undergo to the full validation process with recognition of such performance criteria as analytical specificity and selectivity, analytical sensitivity, repeatability and reproducibility (EPPO PM 7/97 (2)). Four molecular tests for validation were chosen by us: two of them for Conventional PCR (according to Stoger et al., 2006; Gottsberger adapted from Obradovic et al., 2007) was designed for plasmid and chromosomal region, third one was a Real-time PCR test (according to Gottsberger, 2010) and the fourth was FLASH-PCR (designed by OAO «AgroDiagnostika» company). Commercial kits for DNA extractions, primers and enzymes for PCR reactions were produced by Russian companies.

Obtained results shows that conventional PCR test (according to Stoger et al., 2006) demonstrates even better analytical sensitivity then it is stated in EPPO PM 7/20 (2), from 10^3 to 10^6 cfu mL^{-1}. Specificity of other test was comparable to published data as well as other test performance criteria.

The comparison of the obtained performance criteria with known data allows us to make the conclusion that tests with used commercial kits and enzymes could be use successfully for detection and identification of bacteria *Erwinia amylovora* in the laboratory analysis.

P04-08

Plant protection field trials against fire blight in Switzerland

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In Switzerland an entirely covered field trial has been established to investigate alternative strategies against fire blight as a replacement for the antibiotic Streptomycin, which was allowed in this context from 2008-2015 in Switzerland but not in 2016. In two consecutive experimental runs, open flowers on potted apple trees (var. `Gala Galaxy') were inoculated with *Erwinia amylovora* ($5x10^6$ and $1x10^6$ cfu/ml, respectively). Honeybees spread bacteria to newly opened blossoms. Five strategies using the following products were applied: Streptomycinsulphate (21,8%), LMA (80% potassium aluminum sulphate), Myco-Sin (65% aluminium sulphate, 0,2% horsetail extract), lactic acid (90%), Vacciplant (4,3% laminarin) and BlossomProtect (*Aureobasidium pullulans* $5x10^9$ cfu/g, citric acid). Infected flower clusters were counted and infestation was calculated in comparison with untreated control trees. Additionally, bacterial cells in blossoms were quantified using real-time PCR and counting colony forming units. Infestation rates in untreated trees were high, with 31 and 36%, respectively. The efficacy of the reference treatment with Streptomycin and LMA was highest, whereas Myco-Sin, used as a single substance, showed the lowest efficacy. However, most of the treatments showed infestation rates significantly different compared with the untreated control, except for lactic acid, Myco-Sin and the treatment including BlossomProtect in one of the two runs. Cell quantification data were in accordance with our infestation data, i.e. most cells were detected in the control and least cells in the reference treatment containing Streptomycin. Due to high infestation rates, efficacy values were rather low. Further experiments in 2016 and the following years are needed to adjust the timing for application of alternative strategies depending on weather conditions and the products in order to attain satisfactory efficacy values.

P04-09

Fire blight monitoring by UAV system carrying spectral sensors

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Today, regular visual inspections of fruit orchards, nurseries and other host plants of *E. amylovora* remain an important measure to control fire blight and to define fire blight free production area for fruit export. However, visual monitoring is very labor intensive and time consuming. Hence, in the framework of a joint research project between Pcfruit and Vito, spectral sensors were mounted on an Unmanned Airborne Vehicle (UAV) or drone to test their potential for the monitoring of fire blight infections on pear. Remote sensing with drones allows to monitor larger areas than the current field inspections. Drones can offer, unlike the more traditional remote sensing platforms as manned aircraft and satellites, a higher flexibility and extremely detailed images. An UAV platform carrying a RGB, Red Edge, Multispec and hyperspectral cosicam camera was used to fly over a heavily infected pear orchard. The hyperspectral data were used to examine which wavebands or combination of wavebands could be used to distinguish between healthy and infected trees.

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