13th International Fire Blight Workshop
2.-5. July 2013, Zürich, Switzerland
ETH Zürich Main Building, HG E7

Local Organizing Committee
Fabio Rezzonico (Agroscope Changins-Wädenswil, Wädenswil)
Cesare Gessler (ETH, Institute of Integrative Biology, Zürich)
Brion Duffy (Agroscope Changins-Wädenswil, Wädenswil)
Katja Gruber (Agroscope Changins-Wädenswil, Wädenswil)
Eduard Holliger (Agroscope Changins-Wädenswil, Wädenswil)
Markus Kellerhals (Agroscope Changins-Wädenswil, Wädenswil)
Cosima Pelludat (Agroscope Changins-Wädenswil, Wädenswil)
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Sponsors Listing

Swiss Federal Office for Agriculture - www.blw.admin.ch
Swiss Expert Committee for Biosafety - www.efbs.admin.ch
Swiss Federal Research Station - www.agroscope.admin.ch

www.snf.ch

www.swissfruit.ch

www.swisscofel.ch

www.chevita.com

www.lubera.com

www.gatc-biotech.com
1. Local Information

1.1. WLAN access

Choose the WLAN “public” as connection. Start your usual browser and an ETH website will open. In order to get access, provide the following information:

Login: fireblight
Password: ishs

1.2. ETH Zürich – Campus Zentrum
Hotels
a  Hotel Sunnehus
b  Hotel Arc Royal Comfort Inn
c  Hotel St. Josef
d  Hotel St. Georges
e  Hotel du Theatre
f  The Astor Hotel (Apartments)
g  Leonardo Boutique Hotel Rigihof
h  Hotel Bristol
i  Swiss Q-Hotel Rex

1  ETHZ – Main building
2  Zürich Hauptbahnhof
3  Bauschänzli
1.3. Zürich public transport network
### 1.4. Quick timetable Zürich Main Station – Zürich Airport

<table>
<thead>
<tr>
<th>Route</th>
<th>Time</th>
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<tbody>
<tr>
<td>Zürich HB – Zürich Flughafen</td>
<td>07:16</td>
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Note: Check the official website for the latest updates and changes in the timetable.
1.5. Tram 10 timetable

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<th>Montag-Freitag</th>
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*a bis Zürich, Bahnhof Oerlikon*
2. Programm

Monday, July 1

13.00 - 18.00
PhytFire meeting
LFW D12

13.00 - 18.00
PhytFire meeting
LFW D12

Tuesday, July 2

8.30 - 12.30
Registration, poster setup & meet-and-greet coffee

9.30 - 12.15
IP ProfiCrops Satellite Meeting
Chair: Anna Crole-Rees
HG G60

IP ProfiCrops Satellite Meeting
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HG G60

Wednesday, July 3

13.00 - 18.00
Phytosanitary Control Strategies
Chair: U. Persen & H. Özaktan

Biological and Chemical Control 1
Chair: C. Wend & A. Surcev

Biological and Chemical Control 1
Chair: C. Wend & A. Surcev

Thursday, July 4

13.00 - 18.00
Pathogen Genetics and Genomics 1
Chair: F. Rezzonico & V. Stockwell

Pathogen Genetics and Genomics 2
Chair: J. Pulawaska & B. Rodoni

Pathogen Genetics and Genomics 2
Chair: J. Pulawaska & B. Rodoni

Friday, July 5

13.00 - 18.00
Germplasm Resources and Host Resistance Breeding
Chair: A. Peil & P. Sobiczewski

Host Genetics and Genomics
Chair: M. Malnoy & R. Contreras

ISHS Business Session

Closing remarks
End of ISHS Conference

16.00 - 22.00
Excursion to Agroscope in Wädenswil and BBQ

17.30 - 19.30
Poster session & Wine-Cheese-Chocolate social

17.30 - 19.30
Poster session & Wine-Cheese-Chocolate social
### Tuesday, July 2nd

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>09.00-12.30</td>
<td>Registration, poster set-up &amp; meet-and-greet coffee</td>
</tr>
<tr>
<td>12.15-13.15</td>
<td>Stand-up lunch (together with ProfiCrops)</td>
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<tr>
<td>13.30</td>
<td>Welcome addresses</td>
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<tr>
<td>14.00-15.40</td>
<td><strong>Keynote Session 1</strong> (Chair: B. Duffy)</td>
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<tr>
<td>14.00</td>
<td>Key01 Fire blight: barriers to control in the past and present; future control strategies Tim Smith</td>
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<tr>
<td>15.00</td>
<td>Key02 Addressing concerns surrounding antibiotic use for control of fire blight Virginia Stockwell</td>
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<tr>
<td>15.40-16.20</td>
<td><strong>Coffee break</strong> (opportunity to view posters)</td>
</tr>
<tr>
<td>16.20-17.40</td>
<td><strong>Keynote Session 2</strong> (Chair: B. Duffy)</td>
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<tr>
<td>16.20</td>
<td>Key03 The biopesticide innovation chain: template for development of microbial agents from discovery to registration Antonet Švircev</td>
</tr>
<tr>
<td>17.00</td>
<td>Key04 Host resistance to <em>Erwinia amylovora</em> – germplasm, breeding, genetics Andreas Peil</td>
</tr>
<tr>
<td>19.30-22.00</td>
<td><strong>Social dinner</strong> – Bauschänzli Beer Garden, Zürich on the lake.</td>
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</table>

### Wednesday, July 3rd

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.00-10.40</td>
<td><strong>Phytosanitary Control Strategies</strong> (Chair: U. Persen &amp; H. Özaktan)</td>
</tr>
<tr>
<td>9.00</td>
<td>T01-01 Control of fire blight in Baden-Württemberg at the end of the streptomycin era A. Fried, E. Schell, E. Moltmann, A. Wensing</td>
</tr>
<tr>
<td>9.20</td>
<td>T01-02 Fire blight control strategy in Belgium H. Schoofs, T. Deckers, W. Verjans, K. Vrancken, R. Valcke</td>
</tr>
<tr>
<td>9.40</td>
<td>T01-03 Phytosanitary strategies against fire blight in Switzerland H. Dreyer</td>
</tr>
<tr>
<td>10.20</td>
<td>T01-05 Necrotrophic survival of <em>Erwinia amylovora</em> in apple leaf tissue P. Sobiczewski, A. Mikiciński, A. Stępowska</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
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</tr>
<tr>
<td>10.40-11.10</td>
<td>Coffee break (opportunity to view posters)</td>
</tr>
<tr>
<td>11.10-12.40</td>
<td>Biological and Chemical Control 1</td>
</tr>
<tr>
<td>11.40</td>
<td>T02-02</td>
</tr>
<tr>
<td>12.00</td>
<td>T02-03</td>
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<tr>
<td>12.20</td>
<td>T02-04</td>
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<tr>
<td>12.40-14.00</td>
<td>Lunch (opportunity to view posters)</td>
</tr>
<tr>
<td>14.00-15.30</td>
<td>Biological and Chemical Control 2</td>
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<tr>
<td>14.00</td>
<td>T03-01</td>
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<td>14.30</td>
<td>T03-02</td>
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<td>T03-03</td>
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<td>15.10</td>
<td>T03-04</td>
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<tr>
<td>15.30-16.00</td>
<td>Coffee break (opportunity to view posters)</td>
</tr>
<tr>
<td>16.00-17.30</td>
<td>Epidemiology and Decision Support Systems</td>
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<tr>
<td>16.00</td>
<td>T04-01</td>
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<td>16.30</td>
<td>T04-02</td>
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<td>16.50</td>
<td>T04-03</td>
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<tr>
<td>17.10</td>
<td>T04-04</td>
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<tr>
<td>17.30-19.30</td>
<td>Poster Session &amp; wine-cheese-chocolate social</td>
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</table>
# Thursday, July 4th

## 9.00-10.30 Pathogen Genetics and Genomics 1 (Chair: F. Rezzonico & V. Stockwell)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.00</td>
<td>T05-01</td>
<td>Small RNAs play big: role of bacterial small RNAs in regulating <em>Erwinia amylovora</em> pathogenesis</td>
<td>Q. Zeng, G.W. Sundin</td>
</tr>
<tr>
<td>9.30</td>
<td>T05-02</td>
<td>t.b.d. (Sponsor talk: GATC)</td>
<td>B. Busch</td>
</tr>
<tr>
<td>9.50</td>
<td>T05-03</td>
<td>Multidrug efflux in <em>Erwinia amylovora</em></td>
<td>D. Pletzer, H. Weingart</td>
</tr>
<tr>
<td>10.10</td>
<td>T05-04</td>
<td>The role of Erwinia amylovora CRISPR/Cas system in resistance to phage infection</td>
<td>A. Yagubi, A.J. Castle, A.M. Svircev</td>
</tr>
</tbody>
</table>

## 10.30-11.00 Coffee break (opportunity to view posters)

## 11.00-12.40 Pathogen Genetics and Genomics 2 (Chair: J. Puławska & B. Rodoni)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<th>Authors</th>
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<tbody>
<tr>
<td>11.00</td>
<td>T06-01</td>
<td>An alternative sigma factor cascade regulates expression of the type III secretion system in <em>Erwinia amylovora</em></td>
<td>Y. Zhao, V. Ancona, W. Li, J.-H. Lee</td>
</tr>
<tr>
<td>11.20</td>
<td>T06-02</td>
<td>Investigating the molecular basis of fire blight by structural and functional genomics of <em>Erwinia amylovora</em></td>
<td>S. Benini, J.D. Bartho, M. Salomone-Stagni, M. Toccafondi</td>
</tr>
<tr>
<td>11.40</td>
<td>T06-03</td>
<td>MALDI-TOF as tool to distinguish species in <em>Erwinia</em> and related genera</td>
<td>Pflüger V., Rezzonico F., Pothier J., Smits T.H.M., Duffy B.</td>
</tr>
<tr>
<td>12.00</td>
<td>T06-04</td>
<td>6-thioguanine biosynthesis of <em>Erwinia</em> species</td>
<td>A. Wensing, A. Beck, M. Gernold, S. Epple, R. Jansen, W. Jelkmann, K. Geider</td>
</tr>
</tbody>
</table>

## 12.30-13.10 ISHS Business Session – election of next meeting venue & discussion of the future framework for our group

## 13.10-14.10 Lunch (opportunity to view posters)

## 14.10-15.50 Plant-Microbe Interactions (Chair: J. Vanneste & R. Vögele)

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<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
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</thead>
<tbody>
<tr>
<td>14.10</td>
<td>T07-01</td>
<td>Biphenyls and dibenzofurans – fire blight-induced phytoalexins of apple and pear</td>
<td>C. Chizzalli, B. Liu, K. Richter, H. Flachowsky, A. Peil, M.-V. Hanke, L. Beerhues</td>
</tr>
<tr>
<td>14.30</td>
<td>T07-02</td>
<td>Proteome investigation of the plant pathogen <em>Erwinia amylovora</em></td>
<td>M. Holtappels, R. Valcke</td>
</tr>
<tr>
<td>14.50</td>
<td>T07-03</td>
<td>Differential transcriptome analysis of <em>Malus × robusta</em> 5 after inoculation with the virulent <em>Erwinia amylovora</em> avrRpt2Ea deletion strain ZYRKD3-1 and the non-virulent wild type strain Ea1189</td>
<td>L. Vogt, K. Richter, A. Dahl, M.-V. Hanke, H. Flachowsky, A. Peil</td>
</tr>
<tr>
<td>15.10</td>
<td>T07-04</td>
<td>Pathogenicity and infection strategies of the fire-blight pathogen <em>Erwinia amylovora</em> in <em>Rosaceae</em>: state of the art</td>
<td>K. Vrancken, M. Holtappels, H. Schoofs, T. Deckers, R. Valcke</td>
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<tr>
<td>15.30</td>
<td>T07-05</td>
<td>Cyclic di-GMP regulates the expression of virulence factors in <em>Erwinia amylovora</em></td>
<td>L.F. Castiblanco, A.C. Edmunds, C.M. Waters, G.W. Sundin</td>
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</tbody>
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**16.00-22.00 Excursion to Agroscope in Wädenswil and BBQ**

**16.00 h** Departure from ETH (Vorfahrt Main building) by coaches (www.heggli.com)

**16.30 h** Group visits at Agroscope Wädenswil (coaches will directly go to Sandhof, Center and Gottshalde)

<table>
<thead>
<tr>
<th>Time</th>
<th>Group A (Sandhof)</th>
<th>Group B (Center)</th>
<th>Group C (Gottshalde)</th>
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<tr>
<td>16.45 - 18.00</td>
<td>Low input trial (A. Naef)</td>
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<td>Variety testing (S. Egger)</td>
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<td>Plant protection (phytopathology) (A. Naef)</td>
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<td>Fire blight biosafety glasshouse from outside, research (B. Duffy, F. Walsh)</td>
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<td>Breeding (M. Kellerhals) incl. ZUEFOS fast track, early flowering (I.O. Baumgartner)</td>
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<td>Post-harvest and sensory research (Chr. Brugger)</td>
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<td>‘Herakles’ in Gottshalde (S. Perren)</td>
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<td>BEVOG II scab and mildew testing plot (J. Gassmann)</td>
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<td>Entomology total netting (G. Brand)</td>
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<td>Vinquest: trap orchard (A. Patocchi)</td>
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**18.15 h** Wine tasting (Daniel Pulver)

**19.00 h** Barbecue Dinner (Glasshouse 7 with rainy weather or Schlosshof with good weather)

With short welcoming speeches by Director M. Gysi, Agroscope and J.P. Mayor, Head ACW and probably representative of the city Wädenswil

Music: Alphorngruppe Albisblick and Drumlin (www.drumlin.ch)

Stands of sponsors and Agroscope

**22.30 h** transport back to ETH Zürich with coaches

**23.00 h** arrival at ETH Zürich
Friday, July 5th

9.00-10.50 Germlasm Resources and Host Resistance Breeding (Chair A. Peil & P. Sobiczewski)

9.00 T08-01 Breeding for fire blight resistance and sterility in Cotoneaster R. Contreras, J. Rothleutner, V.O. Stockwell

9.30 T08-02 Breeding high quality apples with fire blight resistance M. Kellerhals, I.O. Baumgartner, L. Leumann, L. Lussi, A. Patocchi

9.50 T08-03 Overview of cultivar testing and pathogenesis of apple with respect to a tolerance against Erwinia amylovora M. Joos, R. T. Vögele

10.10 T08-04 Fire blight resistance breeding for real customers M. Kobelt (Sponsor talk: Lubera AG)

10.40-11.20 Coffee break (opportunity to view posters)

11.20-12.40 Host Genetics and Genomics (Chair: M. Malnoy & R. Contreras)


11.40 T09-02 Investigation on fire blight resistance in the cross population of ‘Idared’ x Malus × robusta 5 with different Erwinia amylovora strains Th. Wöhner, K. Richter, M.-V. Hanke, G.A.L. Brogini, H. Flachowsky, A. Peil

12.00 T09-03 Accelerated introgression of fire blight resistance from Malus x robusta 5 and other wild germplasm into elite apple germplasm I.O. Baumgartner, A. Patocchi, L. Lussi, A. Peil, M. Kellerhals

12.20 T09-04 Evidence of a major QTL for fire blight resistance in the apple wild species Malus fusca O. Emeriewen, A. Kilian, M.-V. Hanke, M. Malnoy, A. Peil

12.40-13.10 Closing remarks & announcement of the host for the next International Fire Blight Meeting in 2016

13.10 End of meeting
Fire blight: barriers to control in the past and present; future control strategies

Tim J. Smith

Washington State University, Wenatchee, WA 98801, USA - smithtj@wsu.edu

There is a great body of information about fire blight and its management based on research and field experience. The current state of knowledge and technology is not complete, but excellent control can be attained most years utilizing an integrated program of careful sanitation of the orchard and its neighborhood, weather monitoring, modeling of infection conditions, orchard surveillance, and properly timed appropriate rates of currently available effective spray materials. However, when all the conditions are conducive for fire blight infection, some orchards suffer great losses due to fire blight, many others do not. Why do we continue to see thousands of hectares damaged almost every year? This is the most-feared and dangerous orchard disease that many affected orchardists face, why are they not more careful to prevent it? During the past 30 years in the Pacific Northwest USA, fire blight has increased acreage affected, intensity of outbreaks, and value of crop loss. There are biological, weather and human aspects of this disease that complicate matters and lead to imperfect control.

The biological factors are dominated by the fact that apples, pears and other susceptible hosts evolved for millennia in the absence of Erwinia amylovora. These bacteria are native to eastern North America and cause minor damage when attacking Crataegus sp., its’ natural host. Apples and pears are native to an area from the temperate mountain region stretching from Eastern Europe to Western Asia, and did not have a chance to develop resistance under pressure from this organism. All apples and pears are susceptible to fire blight with a wide variation of resistance. The potential and severity of damage from fire blight outbreaks depends on many circumstances that may exist at the time of infection. The biological factors include: relative resistance of the cultivar, age of the tree, stage of tree growth, relative vigor of the tree, flower numbers present, temperatures during the days leading up to the wetting of flowers, the number of days that flowers have been open, duration and intensity of flower wetting, and especially, the number and proximity of carryover or current season active fire blight cankers serving as a rapid, high volume source of bacterial contamination.

The human factors that influence the severity of a fire blight outbreak include the status of the managers in regards to knowledge, attitude, situational awareness, state of mind, and ability to pay attention to multiple tasks that change from day-to-day. We plant pathologists and horticultural advisors often recommend conflicting or incompatible spray timings that are nearly impossible to apply at the recommended time due to the limited number of hectares (7-9) that may be covered by a employee/tractor/sprayer combination during a normal work day. Rain that may lead to infection of flowers is often followed by cool, windy conditions unsuitable for spray application. Amount of damage that occurs after a fire blight infection event also depends on how the orchard manager reacts to an infection event. Asking most growers to spray every three days actually results in almost daily spray applications, and it is easy to understand why they will not continue this during the very busy two or three weeks leading up to and after blossom time.
Some of these biological and weather related factors can be monitored; others are difficult or almost impossible for the manager to know. An orchardist in an area that experiences fire blight on a regular basis is usually much more knowledgeable and vigilant than a grower who has never had a problem with this disease. Many growers have never had significant fire blight in the orchards, so gain a false sense of security, even when a few fire blight strikes appear. They may not realize that the following year, due to the increased bacterial presence that follows those few strikes, relatively normal weather conditions could lead to much worse than usual infection. The presence of active cankers is the key factor in the conditions that lead to the most spectacular fire blight outbreaks.

Why, if we already know so much about the disease and its management, is this more than a minor sporadic problem? Are we addressing all the issues and carrying out research in order of a priority that would achieve the most reduction in crop loss and control expense? Have we in the world fire blight research community ever formally discussed the barriers to fire blight control, the relative impact of each barrier, and used this consensus to decide research and education priorities? Which important barriers might be quickly overcome with new technology, which important barriers must be addressed with long-term solutions, and which are not likely to be resolved using current methods? Would we make more progress if fire blight research was carried out by teams made up of a mixture of disciplines such as plant pathology, horticulture, economists, social scientists, with advisory groups which included expertise from the tree fruit growing community and people involved in the storage, packing and marketing of fruit? The author will further discuss these and other practical fire blight management problems that currently face orchardists, including application timing issues, machinery issues, spray weather conditions, and the difficulties facing the fruit industry and researchers in the necessary development of numerous fire blight resistant pear and apple cultivars that have both high-quality fruit and enthusiastic market demand.
Addressing concerns surrounding antibiotic use for control of fire blight

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Antibiotics were first used to control fire blight in the 1950’s and quickly became an important tool for disease management. Since the inception of antibiotic use on plants, questions were raised over the potential for unintended detrimental effects on the environment and human health. The list of antibiotics used on plants is short; streptomycin, oxytetracycline, oxolinic acid, kasugamycin, and gentamicin. Streptomycin is used in several countries, whereas oxytetracycline, oxolinic acid, kasugamycin, and gentamicin are permitted in only a few countries. The sustainability of antibiotics for disease prevention has been threatened by emergence of antibiotic-resistant populations of *Erwinia amylovora*, which has reduced the efficacy of some of the antibiotics in certain locations. To reduce selection of resistant populations, antibiotics are applied primarily when disease risk is high, and consequently the majority of orchards in the USA are not treated annually. In addition to resistance in the targeted pathogen, questions about unintended consequences have challenged the registration of antibiotics for fire blight control. Some of the persistent questions posed relate to the potential for antibiotics to persist in the environment, the potential for significant antibiotic residues on fruit, and the potential for antibiotic applications in orchards to contribute to the antibiotic-resistance crisis in human medicine. These concerns have not been supported by studies using improved chemical detection methods and molecular methods to characterize environmental bacteria residing in orchards. Antibiotics are active on plant surfaces for less than a week, are rapidly inactivated in soils, and residues exceeding permissible levels set by regulatory agencies have not been found on harvested fruit. It is evident that antibiotic applications in orchards can have an immediate impact on bacterial populations on flowers and leaves, but within a month after application bacterial communities in orchards treated with antibiotics were not significantly different from those from non-treated orchards. Clinical bacteria have not been reported as resident microbes in the orchard phyllosphere and a direct link between antibiotic use in orchards and antibiotic resistance in human pathogens has not been demonstrated. Overall, antibiotics have been indispensable for fire blight management for more than 60 years without reports of adverse effects on human health or persistent impacts on the environment.
The biopesticide innovation chain: template for development of microbial agents from discovery to registration

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Historical and present-day discoveries of biological control agents, or biopesticides, for the control of Erwinia amylovora depended on the initial ‘eureka’ moment from a single research laboratory. Through perseverance and hard work, the prospective biopesticide made it through initial in vitro, in vivo and field trials. To date in Canada, there are five biopesticides that have made it through registration process to commercial production.

The quest for the high efficacy biopesticide(s) for the control of the fire blight pathogen continues to this day. In this presentation the biopesticide innovation chain, developed at Agriculture and Agri-Food Canada, is described. The model uses multiple-laboratories that focus on different aspects of biopesticide development. Promising biologicals are passed down the chain, where efficacy trials and formulation processes often occur concurrently. The process may start with 300-500 potential candidates that are weaned down to 4-5 most promising candidates. The presentation will use the development of bacteriophages for the control of fire blight to describe this model. The goal of the presentation is to achieve an open discussion among workshop participants on the development, commercialization and grower adoption of new biopesticides for the control of E. amylovora. Have we reached our goals in the development of the present day products? What should be the focus of future research on biologicals?
Host resistance to *Erwinia amylovora* – germplasm, breeding, genetics

Andreas Peil¹, Klaus Richter², Annette Wensing³, Ofere Emeriewen¹⁴, Pierre-Marie LeRoux⁵, Thomas Wöhner¹, Andrzej Kilian⁶, Andrea Patocchi⁵, Magda-Viola Hanke¹, Henryk Flachowsky¹, Mickael Malnoy⁴

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The most important bacterial disease affecting pome fruit is fire blight caused by *Erwinia amylovora*. It can cause devastating economic losses and is reliably controlled only by the application of antibiotics, which are banned in many European countries due to environmental, sustainable and consumer friendly issues. One solution could be the utilization of fire blight resistant cultivars in apple production.

In 2003, we started an approach at Dresden-Pillnitz to detect different mechanisms conferring resistance to fire blight aimed at their combination in new cultivars. Four segregating populations were established to map QTLs for fire blight resistance. The donors used were three wild species accessions *Malus baccata* (MALD0004), *M. fusca* (MALD0045), *M. × robusta* 5 and the Pillnitz cultivar Rewena. The susceptible parent in each case was Idared. Grafted scions of each progeny were inoculated with *E. amylovora* strain Ea 222 JKI in at least for two years. Average percent lesion length (PLL) of all progenies was determined. Genetic linkage maps were established using DArT-, SCAR-, SNP-, and SSR-markers. Whereas in Rewena no QTL could be determined, major QTLs were detected in *M. baccata* on linkage group 12, in *M. fusca* on linkage group 10, and in *M. × robusta* 5 on linkage group 3 explaining up to around 50, 85 and 85% of the phenotypic variance, respectively. The case that all resistance QTLs are located on different linkage groups enhances the chance that different mechanisms are acting in the donors.

Additionally, trees of the Idared by *M. × robusta* 5 population were planted in an orchard and flowers were inoculated in two consecutive years. The QTL on linkage group 3 could be confirmed after mapping.
4. Oral Presentations – Wednesday, July 3rd

Control of fire blight in Baden-Württemberg at the end of the streptomycin era

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Streptomycin application for fire blight control has been allowed in Baden-Württemberg since 1994 following the first heavy outbreak in orchards in South-West Germany. As antibiotics in general are not allowed in plant production in Germany the use of streptomycin was strictly regulated and made possible only in emergency situations:

- purchase and application of streptomycin required a qualification certificate issued by the plant protection service
- maximum number of applications was limited to three including one possible application after hail, since 2009 maximum number of applications was reduced to two and strictly limited to blooming time
- official warning by the local plant protection service based on prediction systems had to precede any application.

The demand of the European and German politics to reduce the use of antibiotics in agriculture and the findings of streptomycin residues in some batches of honey lead to a stepwise exit. Problems with strains resistant to streptomycin did not occur and no resistance built-up was detected in thorough screenings over the years. In 2013, streptomycin is restricted to a single application in highly endangered young plantings (up to five years old). In other orchards only alternative products are allowed. Since 1994, the plant protection service Baden-Württemberg together with the Julius Kühn-Institute, Dossenheim, performed field trials in the experimental plot in Kirschgartshausen in order to find alternatives to streptomycin application. A special experimental design has been developed which allowed to test compounds in the field under nearly natural conditions. It has been adopted in EPPO Guideline PP1/166(3). About 55 different compounds which gave promising results in lab tests or were claimed to control fire blight, were tested over the years. The products "Blossom Protect" (Aureobasidium pullulans) and "LMA" (potassium alum) were the only ones which showed consistently high efficacies, close to streptomycin. Blossom Protect has the drawbacks to increase russetting in sensitive varieties and to require timely distance to the application of certain fungicides, whereas LMA is effective only at high concentrations and requires time and effort to get solved in water. Both products have to pass the registration procedure which is ongoing. For 2013 a restricted amount of Blossom Protect and LMA for an emergency situation has been authorized. The advice of the plant protection service is to use Blossom Protect preferably in the beginning of bloom, LMA during full bloom and end of the bloom depending on infection risk and sensitivity of the varieties to russetting.
Fire blight control strategy in Belgium

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¹pcfruit- Research Station, Pomology department, Sint-Truiden, Belgium; ²University of Hasselt, Laboratory of Molecular and Physical Plant Physiology, Diepenbeek, Belgium.

Fire blight, caused by the bacteria *Erwinia amylovora* (Burill, Winslow et al.), is already more than 30 years present in the Belgian fruit growing and is still remaining an important threat leading each year to economic losses in fruit orchards as well as in fruit tree nurseries. Under the Belgian weather conditions, the fire blight infection risk during primary bloom period on pear is rather limited. On apple the late blooming cultivars have an increased risk for primary blossom infection. Later in the season, secondary blossoms on apple and pear and primary blossoms on other host plants like *Cotoneaster sp.* and *Crataegus sp.* can become infected. In the summer period, the infection risk can increase extremely after thunderstorms with hail damage on immature fruitlets and young shoots. The typical ooze droplets on the infected tissues lead to a severe spread and outbreak of fire blight in the orchard. Since 2002, the use of the antibiotic Streptomycin is forbidden in Belgium and new strategies to control fire blight have been developed. The early season copper applications are considered to be important: they can bring the bacteria in the VNBC status and help to reduce the inoculum in the orchards in the beginning of the season. During bloom it is possible to apply Blossom protect, a compound based on the antagonist *Aureobasidium pullulans* when the infection risk is high. With this antagonist, the colonization of blossoms with *E. amylovora* can be blocked. In the postfloral period a decrease in the susceptibility of the host plants towards fire blight infections using PDE molecules (Plant Defense Enhancing molecules) becomes important. Two PDE molecules Vacciplant (laminarin) and Aliette (fosetyl-Al) have been registered against fire blight. PDE molecules should be positioned preventively, prior to the infection, so that the defense mechanisms of the plants can be switched on in time. Vacciplant (laminarin) is more considered for the protection of the blossoms while fosetyl-Al shows an effect against different types of infections on blossoms, shoot and fruits. A reduction in the disease progression and a clear reduction in the ooze formation on the infected tissues were observed on plants treated 3 times with fosetyl-Al. This reduction in ooze production on fruits and shoots is considered to be a very interesting factor in the fire blight epidemiology. The induction of plant defense mechanism with fosetyl-Al in the host plants is linked with the internal phosphonate content in the tree and this level can be high for some weeks after repeated treatments. Fosetyl-Al can also have an inhibitory effect on the bacteria through its low pH and the Al cation itself can act as a heavy metal, inducing abiotic stress in the host plants. This abiotic stress inducing effect was also observed with other heavy metals like Cu and Mn. Therefore Mn applications during season for fruit quality improvement can be useful for fire blight control. During season applications of Cu are not considered due to the cumulative phytotoxic effect.
Phytosanitary strategies against fire blight in Switzerland

H. Dreyer

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Distribution, characteristics and diagnostic methods for fire blight (*Erwinia amylovora*) in the Russian Federation

Angela Kharchenko, Anna Kuznetsova, Marina Balandina, Maria Erohova, Juliana Kulakova, Natalia Kvashnina, Elena Shneider, Nataliya Drenova

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Fire blight was first detected in the Russian Federation (RF) in 2003. Since 2007, during annual NPPO monitoring, *Erwinia amylovora* has been detected in 11 regions located in the European part of the RF. By areas of their detection, the outbreaks may be grouped as follows: outbreaks in the Kaliningrad Region (the western enclave of RF), the Central Black Earth Area (Voronezh, Tambov, Belgorod and Lipetsk regions), Stavropol territory and the North Caucasus (Kabardino-Balkaria and Karachaevo-Cherkessia), as well as the Lower Volga area (Samara, Saratov and Volgograd regions). These areas vary in terms of their climatic conditions and practices utilized for agricultural production. This accounts for the significant differences in the severity of damage caused by fire blight in these areas. The first study of five *E. amylovora* strains from Kaliningrad, Voronezh, Tambov, Volgograd regions and Kabardino-Balkaria, indicated that all of these strains were related (except for the strain from Kaliningrad). Currently, the study on the biology, origin, distribution and potential damage caused by the pest is being conducted. The bacteriological laboratories of the All-Russian Plant Quarantine Centre (the main scientific institution of the Russian NPPO) located in eight regions of the RF. Detection and identification of fire blight perform according to Standard of Organization based on the EPPO Standard PM 20(1). Furthermore, a FLASH-PCR commercial kit for detection of *E. amylovora* developed in Russia has been validated and recommended as simple and reliable screening and confirming test.
Necrotrophic survival of *Erwinia amylovora* in apple leaf tissue

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To elucidate the possibility of *Erwinia amylovora* survival in dead host plant tissue a study was conducted during 2 seasons (2011/12 and 2012/13) using apple trees cv. Idared/M26 growing in pots in the greenhouse and in insect cage with mesh walls and a glass roof. Tips of actively growing terminal shoots were inoculated by cutting just below the first undeveloped leaf, using scissors previously immersed in a water suspension of the highly virulent wild strain Ea 659 of *E. amylovora* containing $10^7$ cfu/ml. After 4 weeks fire blight affected all shoots and leaves, and spread on to the woody shoots. Five, 6, 7, and 8 months after inoculation samples of entirely necrotized leaf tissue were collected for a study of the presence of bacteria in the midrib, lateral veins, and parenchyma.

In 2011/12, using conventional methods, live *E. amylovora* cells were detected in the midrib and lateral veins during the whole period of study. In the period between the 5th and 6th months the number of bacteria in the midrib increased almost 3-fold, and then steadily decreased. However, in the lateral veins a continuous, gradual decline in the number of bacteria was noted. In necrotized parenchyma live bacteria were never detected but their presence was confirmed using a nested-PCR method that detected both live and dead bacterial cells.

Similarly in 2012/2013 the pathogen was present during the whole period in vascular tissue of the necrotized leaves but never in the parenchyma. An analysis of the status of the studied leaves, using Evans blue staining and toluidine blue dyes and observations with a light microscope performed 5 months after inoculation, showed that some of the tissue cells were still alive. However, a similar analysis conducted 7 and 8 months after inoculation confirmed that the cells were dead.

Our study showed that *E. amylovora* can survive not only as a biotroph or epiphyte but also as a necrotroph or semi-necrotroph.
Strategy for non-antibiotic fire blight control in U.S.-grown organic pome fruit

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Pome fruit produced organically under the United States Department of Agriculture National Organic Program (NOP) standard can be treated with streptomycin or oxytetracycline for fire blight suppression, but a recent NOP regulatory review set a 2014 sunset (phase out) date for these materials. In response, we have employed a systems approach to research and develop non-antibiotic programs for fire blight control in the western United States. Objectives have been to: 1) understand the effect of sanitation (e.g., a copper spray at the delayed dormant stage of growth) on pathogen presence in flowers; 2) quantify the impact of fruit load thinning materials on pathogen and biocontrol agent populations in flowers, and 3) develop integrated biocontrol programs where registered products are utilized at specific stages of flowering based their relative ability to suppress the pathogen on the floral stigmata or in the floral cup. Under Objective 1 (presented in detail in another paper at this meeting), the effect of sanitation on pathogen presence in flowers is being evaluated with a molecular scouting technique termed ‘loop mediated isothermal DNA amplification’ (LAMP), which in the future could be available as an on-site decision aid. Experiments under Objective 2 demonstrated that the fruit load thinning material, lime sulfur, reduced flowers potentially infected by E. amylovora and also suppressed bacterial growth in flowers, and therefore compressed the time in bloom when other materials are needed for fire blight control. Under Objective 3, biocontrol programs beginning with stigmata colonizers (e.g., the gram negative bacteria in Bloomtime Biological® or BlightBan A506®, or the yeast Aureobasidium pullulans in Blossom Protect®) followed by floral cup protectors (gram positive bacterium in Serenade Max® or Blossom Protect®) have provided significant and consistent fire blight control. For example, over four trials, treatment with Blossom Protect® after lime sulfur reduced the incidence of fire blight by an average of 92% compared with water only; this level of control was similar to treatment with the antibiotic streptomycin. Finally, within integrated control programs, a ‘fruit safe’ formulation of a copper bactericide, as yet unregistered, is being evaluated as direct substitute for antibiotics; this copper bactericide has provided outstanding fire blight control in replicated orchard trials.
Field results for the efficacy of fire blight control agents in the last 15 years in Germany

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Fire blight caused by *Erwinia amylovora* is the most serious bacterial disease in apple and pear. During the last four decades it has spread throughout Europe. Sanitation methods like pruning of infected shoots and uprooting of infected trees are necessary to reduce infection pressure in the orchards. Under favorable weather conditions *E. amylovora* multiplies on blossom surfaces (e.g. stigma) and invades the plant tissue by the nectarthodes in the hypanthium routed by chemotaxis. Each blossom is a potential infection site and therefore efficient control agents are needed to prevent blossom infections. Streptomycin was banned in the EU but is further used with exceptional permission in some countries. In USA and Israel *E. amylovora* developed resistance against this antibiotic. Since 1997 field trials were conducted in Germany to find alternative control agents or strategies to replace streptomycin. Up to 6 trial sites in Southern Germany with allowance for artificial inoculation were used per year. A summary of the published results from this trials will be given. More than 70 different preparations were tested during the last 15 years. Some of them are now registered for use for fire blight control in Germany. From the products available in 2013, Blossom Protect showed the highest efficacy of all alternatives tested, followed by LMA and calcium formate, Myco-Sin and Serenade. Blossom Protect or spray strategies of different products can replace streptomycin in fire blight control. Its efficacy was comparable to that of streptomycin in trials in Germany as well as in the USA. Meanwhile Blossom Protect composed of two strains of *Aureobasidium pullulans* and an acidic buffer component is registered in many European countries, Morocco, the USA and Canada for use in organic orchards as well as in orchards managed according to Integrated Pest Management (IPM).
Development of antagonistic bacteria for field control of fire blight

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Application of antagonists is considered a possible alternative towards use of antibiotics like streptomycin in fire blight control. Mechanisms of control are divers and can base on competition or include more direct means like toxin production. The Gram-negative bacterium Erwinia tasmaniensis is closely related to the fire blight pathogen E. amylovora. It shares not only many physiological traits, but is also well adapted to the fire blight habitat. Whereas E. tasmaniensis does not produce any toxins against E. amylovora, an inhibition due to competition between both bacteria is conceivable. Another strategy of fire blight control by antagonists can be application of natural inhibitors. The Gram-positive Bacillus amyloliquefaciens is considered a classical soil inhabitant. While it is not well adapted to survival on aerial plant surfaces, it possesses interesting antagonistic features due to the production of a broad spectrum of secondary metabolites. In this current work we compared performance of both antagonists in a number of laboratory setups and in field trials. Co-cultivation of E. tasmaniensis and a luminescent reporter strain of E. amylovora as indicator strain revealed reduced pathogen growth, but no inhibition after application of supernatant. We also investigated various Bacillus isolates for their effect on E. amylovora. Application of supernatants revealed promising results in growth inhibition of the pathogen. However, efficiency of secondary metabolites depends on medium and growth phase. The effects were visible in dual-culture assays as well as in agar diffusion analysis. In detached-flower assays reduction in symptom development could be observed after application of antagonists, but the results still revealed considerable fluctuations. We also compared population development on inoculated flowers. Nevertheless, for field operation, any antagonist has to be conserved for long term storage and formulations have to become active within a short time frame after application. A combination of both metabolite formulations and antagonists might help to cover part of this problem and should be tested in further examinations.
Strange case (reports) of Dr. \textit{P. agglomerans} and Mr. \textit{Enterobacter} sp.

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\textit{Pantoea agglomerans} is an ecologically versatile species that has important biocontrol applications in agriculture as alternative to antibiotic use for orchard protection against fire blight, an invasive threat to global apple and pear production. \textit{P. agglomerans} based products are registered for biocontrol in the USA, Canada and New Zealand, but clinical reports have proven a regulatory obstacle in Europe. The persistent reports of clinical outbreaks attributed to \textit{P. agglomerans} are alarming and would have significant socio-economic impact, if the conclusion of clinical pathogenicity was indeed correct. Yet, there is mounting evidence that many, if not most, \textit{P. agglomerans} clinical reports are based upon inaccurate isolate recognition, resulting from investigators’ reliance on inadequate identification methods and/or obsolete nomenclature. Inaccurate identification has been shown for 96\% of ‘\textit{P. agglomerans}’ clinical strains deposited in ATCC. The primal \textit{P. agglomerans}-\textit{Enterobacter agglomerans}-\textit{Erwinia herbicola} complex has experienced several taxonomic revisions, and both biochemical profiling and 16S rRNA gene sequencing lack the resolution needed to discriminate \textit{P. agglomerans sensu stricto} isolates. Databases for metabolic tests performed in routine clinical diagnostic such as those included in the Vitek-2, API or Phoenix systems are unable to discriminate \textit{P. agglomerans} from the other 13 \textit{Pantoea} spp. and even the best-available match can deliver false positives. Moreover, only 57\% of 16S rRNA sequences retrieved from NCBI as \textit{P. agglomerans} cluster indeed with type strain LMG1286. The presence of such rogue data constitutes a dangerous pitfall for anyone trying to identify \textit{P. agglomerans} on the basis of 16S rRNA gene sequences.

Regrettably, these errors result in alarming misrepresentations of \textit{P. agglomerans} as a serial killer. The potential for serious, lasting consequences on regulatory evaluation of plant beneficial \textit{P. agglomerans}, but also for the choice of the appropriate medical treatment, calls for proactive action in raising the awareness of physicians and clinical diagnostic laboratories upon the use of appropriate methods for unambiguous identification of \textit{P. agglomerans} and closely related species. Here we show that Intact Cell MALDI-TOF Mass Spectrometry can provide faster and more reliable identification of \textit{P. agglomerans} with respect to routine biochemical methods and compare our approach with the current gold standard \textit{gyrB} gene sequencing.
Prospects and limitations of synthetic antimicrobial peptides for fire blight control

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Chemical control of fire blight of rosaceous plants is strongly limited by the scarce number of bactericides available as active ingredients. In the EU only copper is authorized, whereas in the USA and other countries antibiotics are permitted, but often fail due to the selection of plasmid-borne resistance in the pathogen. There are also other compounds that act through the host as plant strengtheners but generally have moderate to low efficacy. Thus, there is a need for novel compounds against Erwinia amylovora, with new mechanisms of action. Antimicrobial peptides (AMPs) are potential candidates because they are natural compounds produced by animals and plants as a first defence barrier, and by microorganisms in antibiosis as a competitive factor. Synthetic AMPs can be designed and produced by peptide chemistry approaches with optimized activity, toxicity and biodegradability. Our laboratories have prepared libraries of small AMPs with linear, cyclic, 5-arylhistidine-containing peptides, cyclolipopeptides, peptidotriazole derivatives, and multivalent display structures. Several leads from linear undecapeptides (BP100 and its D-amino acid homologue BP143), cyclic decapetides (BPC194, BP500 a cyclolipopeptide derivative) and multivalent display peptides (linked to cyclodithioerythritol or α-D-galactopyranoside) have been developed and optimized against E. amylovora, and minimizing fitotoxicity and protease susceptibility. A few selected candidates have been tested for fire blight control in the greenhouse, semi-field and field tests in apple and pear, and the efficacy is comparable to other commercially available compounds. However, the main limiting factor for its development is mass production that can be achieved by chemical synthesis, or using microorganisms or plants as biofactories (only for some linear versions). BP100 derivatives have been produced in the endosperm and embryo of seeds of GM transgenic rice plants and the expression products are under evaluation for structure, activity and feasibility of the yield obtained.
Gram-positive bacteria producing antimicrobial peptides as efficient biocontrol agents of fire blight

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Currently available biocontrol agents of fire blight are strains of Gram-negative (*Pseudomonas fluorescens*, *Pantoea agglomerans*, *Pantoea vagans*) or Gram-positive (*Bacillus subtilis/amyloliquefaciens*) bacteria. Many of the efficient strains produce antimicrobial peptides (AMPs) like cyclolipopeptides (CLPs) including surfactins, iturins, and fengycins, or pseudopeptides (e.g. pantocins). In several cases AMPs have been implicated in the mechanism of action of fire blight biological control. Also, the genomes of several BCA strains have been sequenced and information on AMP genes is already available. Taking advantage of this knowledge, we have designed PCR tools targeted to several AMP genes that have been used for molecular marker assisted screening of a large collection of Gram-positive bacteria (mainly *Bacillus* spp. and *Lactobacillus* spp.) isolated from plant environments in the Mediterranean area. Several strains of *B. subtilis/amyloliquefaciens* that are multiple producers of CLPs and of *L. plantarum* producing the bacteriocin plantaricin have been obtained. A set of selected strains were good colonizers of flowers as well as other plant organs, and revealed as highly efficient in fireblight biocontrol in greenhouse and semi-field tests, comparable to the existing BCAs and reference antibiotics. These strains have the additional advantage that *Bacillus subtilis/amyloliquefaciens* and *L. plantarum* are considered as biosafe by the EPA (USA) and EFSA (Europe), and will be the active ingredients of future microbial pesticides for fire blight control.
Sensitivity of *Erwinia amylovora* in Illinois apple orchards to antibiotics and copper

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Fire blight (*Erwinia amylovora*) is the most serious disease of apple in Illinois. Copper and streptomycin is widely used to control fire blight in commercial apple orchards. Statewide surveys were conducted in 2010, 2011, and 2012, and 117, 129, and 170, *E. amylovora* isolates were collected, respectively, from 20 counties, to determine sensitivity of *E. amylovora* to streptomycin, other antibiotics, and copper. None of the 416 *E. amylovora* isolates tested were resistant to streptomycin (Agrimycin 17WP) at 50 mg/L. Seven non-*E. amylovora* bacterial isolates were collected from blossoms and *E. amylovora*-infected shoots that contained both a *strA-strB* streptomycin-resistance gene and IS1133 on transposon Tn5393. Colony development of all 84 *E. amylovora* isolates tested were inhibited on Luria-Bertani medium amended with oxytetracycline at 50 mg/L and kasugamycin (Kasumin 2L) at 100 mg/L. Similarly, colony development of the 84 *E. amylovora* isolates was inhibited on the casitone-yeast extract medium amended with copper sulfate (Cuprofix Ultra 40DF) at 0.16 mM. In 2011 and 2012, field trials were conducted to evaluate efficacy of oxytetracycline (Mycoshield), kasugamycin (Kasumin 2L and ARY-0416-06), copper hydroxide (Kocide-3000 41.6DF), *Bacillus subtilis* (Serenade Max, QST713), and *Pseudomonas fluorescens* (Blight Ban A506) for management of fire blight in an apple orchard. Only kasugamycin (Kasumin 2L and ARY-0416-06) reduced blossom infection significantly.
How does *Erwinia amylovora* face up to stress by copper?

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Copper compounds are widely used for control of bacterial diseases. Copper acts as a co-factor in essential cellular functions but over a threshold it becomes toxic, at least in part due to the reactive oxygen species (ROS) released. Bacteria have developed mechanisms to control copper levels and guarantee their survival when this metal is present. *Erwinia amylovora* enters into the viable but nonculturable state induced by this element as a survival strategy. The aim of this work was to examine the differential expression of *E. amylovora* genes in the presence or absence of copper, using microarray hybridization transcriptomic technology. Source RNA was isolated from exponential grown cells after CuSO₄ 0.5mM shock exposure for five minutes. Under these experimental conditions, 44 genes were differentially expressed. The induced genes were distributed in the functional categories of transport, stress, movement, and metabolism, besides a conserved protein and some genes of unknown function. To validate gene expression patterns observed in the microarray analysis, selected genes were analyzed for expression profile by qRT-PCR, and 23 up-regulated genes were confirmed. Then, the following genes were selected for mutational analysis: copA, soxS, arcB, yjcE, ygcF, yhhQ, EAM_3469, and galF. Mutants and wild type strain were challenged to six different copper concentrations (0.5mM, 1mM, 5mM, 10mM, 20mM and 35mM) to test their sensitivity to copper. The mutants exhibiting the most severe decrease in growth by copper effect were complemented and then assayed to prove restoration of normal phenotype. A model for shock response of *E. amylovora* under copper stress and the ongoing genetic analysis is presented.
Use of real-time PCR for *Erwinia amylovora* detection during bloom and potential for integration in fire blight disease forecast in Québec.

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In Québec, fire blight outbreaks are infrequent but can be devastating. Disease forecast systems such as RIMpro are used to identify weather conditions favorable for disease outbreaks and time antibiotic sprays during bloom. Because bacteria are not always present, the models are prone to false positive prognosis (cry wolf). The objective of our study was to evaluate the potential of bacterial population monitoring in conjunction with disease forecast to improve spray recommendations. In 2012, samples of flower clusters were frozen upon collection at early-mid bloom, mid-late bloom and petal fall from 112 individual unsprayed orchard plots deriving from 31 orchards scattered in 5 growing regions. Sample size was adjusted to about 100 clusters for 0.1 ha. Disease incidence was evaluated in each plot in early June. Samples were sonicated with water for 15mn, filtered, resuspended in TPEB and directly amplified with qPCR using chromosomal probes. Our detection threshold was approximately 100 CFU/cluster. In 2012, we found that 31% of plots sampled had at least 5% diseased trees, whereas models predicted disease everywhere (sensitivity =1, specificity =0). On the first sampling date, 33% of samples were positive. The rate of positive samples increased to 40% after the second sampling, and 66% after bloom. Because the detected bacterial population was initially low, sensitivity and specificity of detection was 32% and 67% respectively after one sample. After the second sampling, combined sensitivity climbed to 75%, while specificity dropped to 46% because bacteria was detected in sites without disease. After bloom, detection sensitivity was 100% and specificity 28%. Although bacterial detection increased the overall accuracy of disease prediction to 62%, the consequence of lower sensitivity (tree loss) are much greater than the benefit of higher specificity (less spray). So unless the detection threshold during bloom is improved to lower the false negative rate, grower adoption is unlikely. Furthermore, the complicated logistics involved for processing samples ahead of spraying decisions limits the potential of this technology.
Use of loop-mediated isothermal amplification of *Erwinia amylovora* DNA to monitor efficacy of inoculum sanitation for fire blight suppression

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Copper-based bactericides applied at green tip (i.e., delayed dormant timing) to reduce populations of *Erwinia amylovora* associated with overwintering (‘holdover’) cankers has been a historical component of some fire blight management programs, but in the western United States the practice became little used as treatment with effective antibiotic materials became common. The epiphytic inoculum of *E. amylovora* that originates from holdover cankers can be detected rapidly and efficiently in samples of pear or apple flowers with a molecular DNA detection assay termed ‘loop-mediated isothermal amplification’ (LAMP). From 2010 to 2013, LAMP-based molecular scouting was used to evaluate the impact of delayed dormant copper treatments on the detectability of epiphytic populations of the fire blight pathogen during the bloom period. 6 to 13 commercial pear orchards in California, each approximately 5 ha in size, were split into two plots with the orchardist applying a fixed copper bactericide (6.7 kg/ha of 25% CuOCL and 23% CuOH (Badge X2), Isagro, Morrisville, NC) to one of the plots 3-4 weeks before first bloom. During the bloom period, the orchardists also applied their usual antibiotic program to the entire orchard. Flowers from each plot were sampled at mid-bloom, at full bloom, and once or twice at petal fall. On each sampling date, three samples of 100-flower clusters (~600 flowers) were made in each plot following a standard W-shaped scouting pattern with the LAMP protocol performed on the floral wash of each sample. Overall, *E. amylovora* was detected rarely at mid-bloom (10% of samples) but detected commonly at petal fall and rat tail (25 and 53% of samples, respectively). In three of four seasons, positive pathogen detection in flower samples as a function of bloom stage increased more rapidly on non-treated plots compared to copper-treated plots. Among orchards, development of fire blight was sporadic but was decreased significantly by the copper treatment at a few locations. Among seasons, differences in detection of epiphytic *E. amylovora* by LAMP-based scouting indicated that orchardists could potentially benefit from ‘point-of-care’ LAMP testing in individual orchards each season. The data depicting epiphytic detection of the fire blight pathogen over time as influenced by a delayed-dormant timing of copper suggest that additional copper-based sanitation during the bloom period (e.g., with a non-phytotoxic formulation of a copper bactericide) could further suppress *E. amylovora* inoculum over that achieved with similarly timed antibiotic treatments.
Bacteria commonly isolated from the phyllosphere of fire blight-susceptible hosts in Australia

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The microbial diversity of the phyllosphere provides a challenge to plant pathogen diagnostic design; a plethora of organisms that can produce false positive amplicons with diagnostic tests. In order to further scrutinize Erwinia amylovora-specific diagnostic tests (both molecular and culture based) and to ascertain what bacteria colonise fire blight-susceptible-hosts in Australia, a sub-set of culturable bacteria from the phyllosphere of a variety of Rosaceous plants were identified. The composition of bacterial families that colonise fire blight-susceptible hosts in Australia is consistent with those identified in studies of culturable phyllosphere populations on apple and pear in other parts of the world. A number of species closely related to E. amylovora were also present in these populations, such as E. tasmaniensis, that do not appear to be as prevalent in other parts of the world. The number of bacteria isolated from the phyllosphere of rosaceous plants in this study that are closely related to E. amylovora emphasises the importance of validating diagnostic tests in planta (on E. amylovora-free plant samples) so that tests can be reliably used directly on plant material to screen for a target pathogen.
Real-time and conventional PCR allow detection of *Erwinia piriflorinigrans*, a new pathogenic species causing necrosis of pear blossoms and its distinction from the fire blight pathogen, *Erwinia amylovora*

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*Erwinia piriflorinigrans* is a new pathogenic species of the genus *Erwinia* recently isolated in Spain. Its accurate detection and identification can be difficult because the symptoms produced on pear blossoms are similar to those caused by *E. amylovora* and other *Erwinia* species affecting pear trees and they also share phenotypic, biochemical, serological and molecular characteristics. It has been observed that all analyzed strains of *E. piriflorinigrans* harbor a stable plasmid named pEPIR37. Based in the sequence of this ubiquitous plasmid we have developed novel real-time and conventional PCR protocols to detect, identify and differentiate this new species from *E. amylovora*. Detection can be completed in short time with high sensitivity from plant material without DNA extraction. When the specificity was evaluated, all *E. piriflorinigrans* strains tested were amplified, whereas closely related pathogenic and non-pathogenic *Erwinia* species or epiphytic pear tree bacteria were negative. Sensitivity assays detected $10^{-1}$-$10^2$ cells/ml by real time PCR and $10^2$-$10^3$ cells/ml by conventional PCR. These new protocols have been employed in epidemiological studies and for fast detection of *E. piriflorinigrans* in symptomatic and asymptomatic plant material as well as in naturally infected samples of mixed infections with *E. amylovora*. 
5. Oral Presentations – Thursday, July 4th

Small RNAs play big: role of bacterial small RNAs in regulating *Erwinia amylovora* pathogenesis

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*Erwinia amylovora* is a gram negative phytopathogenic bacterium that causes fire blight, a devastating disease of apple and pear trees world-wide. To successfully develop disease symptoms, *E. amylovora* utilizes a complex regulatory system to coordinately control the expression of various pathogenicity and virulence determinants (type III secretion system (T3SS), exopolysaccharide amylovoran, biofilm formation, and motility) at the early, mid, and late stage of infection. Bacterial small RNAs (sRNAs) are a group of non-translated regulatory RNAs that bind to their mRNA targets and control their expression at the post-transcriptional level. Many of these sRNAs require the participation of the global RNA chaperone Hfq. Recently, Hfq-regulated sRNAs were identified as critical virulence regulators in many animal pathogens. However, the presence and virulence regulation of these sRNAs in plant pathogens were poorly understood. In this work, we identified Hfq-regulated bacterial sRNAs in *E. amylovora* and evaluated their virulence regulation. Using bioinformatic predictions as well as RNA-seq analysis, 45 sRNAs were identified in the genome of *E. amylovora*, and the expression of 15 of them was confirmed by Northern blot. Deletion of two sRNA-encoding genes, *rprA* and *arcZ*, significantly reduced virulence of *E. amylovora*. The regulatory effect of ArcZ on biofilm formation, amylovoran, motility, and the T3SS was also characterized. Our results showed that post-transcriptional regulation by bacterial sRNAs is an important regulatory strategy in controlling the pathogenesis of *E. amylovora*. 
Multidrug efflux in *Erwinia amylovora*

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*Erwinia amylovora* is the causative agent of the disease fire blight on members of the plant family *Rosaceae*, with economic importance on apple and pear. During pathogenesis, the bacterium is exposed to a variety of plant antimicrobials. Bacteria have developed various ways to resist the toxic effects of such antimicrobial compounds. Extrusion of toxic agents from cells by multidrug efflux is one of these mechanisms. In Gram-negative bacteria, members of the RND family of efflux transporters are the most relevant in respect of resistance to antimicrobial compounds. We identified the RND-type pump AcrAB as one of the major efflux system which confers resistance to a broad range of structurally unrelated compounds, including phytoalexins. Moreover, the ability of an *acrB*-deficient mutant to multiply *in planta* was severely reduced.

The availability of the genome sequence of *E. amylovora* has allowed functional genomics approaches to identify three additional operons encoding RND-type transporter homologous to *acrD*, *yegMNO*, and *mdtABC* of *E. coli*. Knockout mutants were generated to study the role of these RND-type transporters in multidrug resistance and virulence of *E. amylovora*. Determination of MICs revealed that mutational inactivation of the efflux systems reduced the tolerance to a varying range of antimicrobials as well as heavy metals depending on the efflux system. To learn more about their role during pathogenesis, we analyzed the expression of these efflux pumps during infection of the host plant using transcriptional fusions of the respective promoter region to the *egfp* reporter gene. Fluorescence of bacteria re-isolated from infected apple tissue, and quantified by confocal laser scanning microscopy, revealed an induction of the *yegMNO* operon *in planta*.

A link between antibiotic resistance and pathogenicity was found in several enterobacteria, e.g. *Salmonella enterica*. An intact AcrAB-TolC system is required for the colonization, and persistence, of these bacteria in the host. Members of the AraC/XylS family of regulators, e.g. *marA*, *soxS*, *rob* and *ramA*, have been shown to activate expression of RND efflux systems. Furthermore, overexpression of *ramA* in *S. enterica* lead to decreased expression of virulence genes suggesting that the regulation of multidrug efflux systems and expression of virulence genes show considerable overlap. We could identify four regulators of the AraC/XylS family homologous to *soxS* and *rob* in the available genome sequences of *E. amylovora*. Overexpression of the homologous genes was used to investigate the influence of the regulators on multidrug resistance in *E. amylovora*. We found that overexpression of the global regulator *rob* increased the expression of the *acrAB* and *yegMNO* operons more than twofold.

Our results show that disabling of multidrug efflux in *E. amylovora* leads to a striking increase in activity of plant antimicrobials. The use of multidrug efflux inhibitors to control and treat fire blight will be discussed.
The role of *Erwinia amylovora* CRISPR/Cas system in resistance to phage infection

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Field based trials have demonstrated that bacteriophages can control *Erwinia amylovora*, at efficacies comparable to antibiotics. Further research is required to understand the biological relationship between phages and their bacterial hosts. The potential emergence of phage-resistant bacteria due to the use of phage-based biopesticide is a valid concern. The various mechanisms by which *E. amylovora* may gain resistance against phages may include adsorption block, restriction/modification, abortive infection, lysogeny, and CRISPR immunity. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) are novel aspects of the bacterial defense system. Bacterial CRISPRs are sites containing conserved short direct repeats found in the genomes of some bacteria. The repeats are separated by sequences termed "spacers". Spacers are utilized by the CRISPR system to recognize and silence exogenous genetic elements via a mechanism possibly similar to RNA-interference in eukaryotes. The spacers may match sequences in phage genomes and bacterial hosts may acquire resistance or immunity against invading phages following previous exposure to the same phage. The role of the CRISPR system in protecting *E. amylovora* against phages has not been investigated. In the present study, we have determined the DNA sequence of CRISPR arrays of selected *E. amylovora* isolates, and existence of phage sequences as spacers was explored. The efficiency of CRISPR/Cas systems of some bacterial isolates was investigated; the level of expression of Cas genes of different isolates was measured and compared. Furthermore, the ability of *E. amylovora* to gain new spacers in CRISPR arrays upon sequential phage infection was examined. As a part of this study, we are exploring the role of CRISPR/Cas system in protecting bacterial host from lysogeny by temperate phages and we are also examining the ability of *E. amylovora* phages to down regulate or inactivate the CRISPR system during phage infection.
An alternative sigma factor cascade regulates expression of the type III secretion system in *Erwinia amylovora*

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In bacteria, gene expression is mainly regulated at the transcription initiation level and its core RNA polymerase (RNAP) requires sigma factors for promoter recognition and initiation. In *Erwinia amylovora*, ECF alternative sigma factor HrpL-RNAP complex regulates the transcription of *hrp*-type III secretion (T3SS) genes by binding to a consensus sequence known as the *hrp* box in *hrp* gene promoters. In turn, expression of *hrpL* has been proposed to be positively controlled by sigma factor 54 (RpoN) and HrpS, a member of $\sigma^{54}$ enhancer-binding proteins (EBPs). However, the function of both HrpS and RpoN has not been genetically characterized in *E. amylovora*. Furthermore, T3SS genes are induced when *E. amylovora* is in contact with plant tissue or in minimal media thought to mimic conditions in plant apoplast. In culture, T3SS gene expression is stimulated at acidic minimal media and repressed in rich LB medium. However, the molecular mechanism as how T3SS is activated is not well understood. In this study, we examined an alternative sigma cascade with an emphasis on components of HrpS-RpoN-RNAP complex in regulating T3SS gene expression in *E. amylovora*. Results showed mutations in *hrpS*, *hrpL*, *rpoN* and *yhbH*, but not *rpoS*, resulted in non-pathogenic phenotype on apple and immature pear fruits, and no hypersensitive response on non-host tobacco, suggesting that HrpL, HrpS, RpoN, and YhbH, a novel ribosome-binding protein, are essential for virulence and absolutely required for T3SS gene expression in *E. amylovora*. We also determined the regulons of both HrpS and HrpL *in vivo* using oligo array. New perspectives on how T3SS is activated in *E. amylovora* will be discussed.
Investigating the molecular basis of fire blight by structural and functional genomics of *Erwinia amylovora*

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Virulence and pathogenicity are determined by specific interactions between host and guest. Such interactions are regulated by a complicated network of protein-protein interactions determining the ability of the pathogen to shut down the host defense and enable the pathogen to colonize the host by exploiting its metabolites. The availability of the sequenced genome of the phytopathogen *Erwinia amylovora* has allowed us to begin a structural and functional study of several known pathogenicity factors and metabolic pathways. We are concentrating our efforts on the study, in solution and by X-ray crystallography, of *E. amylovora* proteins involved in sugars metabolism (e.g., GalE, Lsc) and amylovoran biosynthesis (the Ams cluster), desferrioxamine biosynthesis (DfoJ, DfoA, DfoC) as well as proteins such as the disease specific proteins DspA/E and DspB/F. We collected X-ray diffraction data from *E. amylovora* levansucrase crystals, the structure of has been solved to a resolution of 2.77 Å and refinement is underway. The enzyme has also been characterized *in vitro* showing that the main product of polymerization is not the long chain levan but mainly short chain fructo-oligosaccharides. The results of our research will lead to a better understanding of the molecular basis of fire blight.
MALDI-TOF as tool to distinguish species in Erwinia and related genera

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Rapid and reliable identification of plant pathogenic bacteria is critical for effective implementation of phytosanitary measures. The genus *Erwinia* include a number of economically important plant pathogens such as *Erwinia amylovora* or *Erwinia pyrifoliae*, together with closely related plant epiphytes of no known pathogenicity or even with a potential use for biological control. Current laboratory methods to achieve satisfactory discrimination are based on semi-selective isolation, serology, PCR and gene locus sequencing: these approaches are time-consuming and complicated. Here we present a streamlined approach based on whole-cell Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) demonstrating the potential of this technology for species identification in plant diagnostics within the genus *Erwinia*.
6-thioguanine biosynthesis of *Erwinia* species

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*Erwinia amylovora* has been described to release a compound absorbing at 340 nm and connected with the yellow color formation of colonies in the presence of Cu-ions. We confirmed that this substance is 6-thioguanine (6-TG) and purified the same compound from *E. tasmaniensis* culture supernatant. Structure was confirmed by GC/MS and NMR. In 1968 Feistner and Staub tested 6-TG as a possible phytotoxine of *E. amylovora* but could not find a clear link between 6-TG production and virulence. While 6-TG has been extensively studied as a chemotherapeutical compound, its biological function for *E. amylovora* remained unclear. We investigated distribution of 6-TG biosynthesis among *Erwinia* species and related genera. A five open-reading-frame gene cluster involved in 6-TG biosynthesis was identified and analyzed in heterologous expression. While transfer of the respective *E. amylovora* genes into *Escherichia coli* enabled the transformants to produce 6-TG, they still remained highly sensitive towards this compound. An independent resistance mechanism has to exist in *Erwinia*. Among other plant pathogenic bacteria, saprophytes and common epiphytes, we observed a varying degree of sensitivity towards 6-TG. While no strain from the genus *Pectobacterium* and *Dickeya* was inhibited by 6-TG, *Pantoea agglomerans* isolates responded to 6-TG to a greater or lesser extent. Considering the low minimal inhibitory concentration of 6-TG necessary to inhibit sensitive bacteria, 6-TG production might enable *E. amylovora* to compete with bacteria and other microorganisms in their host environment. Its incorporation in DNA of plants may also damage their host.
Biphenyls and dibenzofurans – fire blight-induced phytoalexins of apple and pear

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In response to fire blight inoculation, shoots of apple and pear formed biphenyls and dibenzofurans as defence compounds. The phytoalexins were only present in the transition zone of stems, whereas leaves were devoid of these compounds. Cell cultures of Sorbus aucuparia, which belongs to the same subtribe as apple and pear, were used to study the biosynthesis of the Pyrinae-specific phytoalexins. In shoots of the apple cultivar ‘Holsteiner Cox’, biphenyls and dibenzofurans started to accumulate in the downward advancing transition zone 28 days after Erwinia amylovora inoculation. The flanking stem segments, i.e. the necrotic and healthy zones, lacked detectable quantities of phytoalexins. The transition zone of apple stems contained four biphenyls and two dibenzofurans. In the pear cultivar ‘Conference’, three biphenyls and one dibenzofuran were detected. The total phytoalexin content in the transition zone of pear was 25 times lower than that in apple. A number of biphenyls and dibenzofurans were tested for their in vitro antibacterial activity against some E. amylovora strains. The most efficient compound was 3,5-dihydroxybiphenyl (MIC = 115 μg/ml), the immediate product of biphenyl synthase (BIS), which initiates phytoalexin biosynthesis. In apple, BIS is encoded by a gene family, members of which fall into four subfamilies. These subfamilies were differentially regulated after inoculation of shoots with E. amylovora. Stems contained only BIS3 transcripts, with maximum expression levels in the transition zone – approx. 4,000 times that in the respective stem segment of mock-inoculated control shoots. Leaves expressed the BIS2 gene, however, no enzyme protein was immunodetectable. The BIS3 protein was immunochemically localized to the parenchyma of the bark. Dot-shaped immunofluorescence was restricted to the junctions between neighbouring cortical parenchyma cells, which may point to an association with plasmodesmata in primary pit fields.
Proteome investigation of the plant pathogen *Erwinia amylovora*

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Former research has led to the conclusion that *E. amylovora* is a homogenous species although the particular strains show differences in their pathogenic ability. Because on genomic level no great differences were found, our research focuses on the proteomic level. For this investigation we considered two strains of *E. amylovora* with a difference in pathogenicity, a high pathogenic and a low pathogenic. We want to find out which proteins are responsible for the differences in virulence between these two strains and try to find out which mechanisms help the more virulent strains to be more effective at dispersion inside the host. Also we want to investigate the defense and repair mechanisms *E. amylovora* uses after being exposed to ROS produced by the host as a defense mechanism. In this part of the study, the proteome of the two strains grown *in vitro* is studied. The two strains were grown in a minimal medium and the complete proteome was extracted. A 2D Differential in-gel electrophoresis (DIGE) approach has been used. The differentially regulated protein spots were excised, trypsinized and identified.
Differential transcriptome analysis of *Malus × robusta* 5 after inoculation with the virulent *Erwinia amylovora* *avrRpt2EA* deletion strain ZYRKD3-1 and the non-virulent wild type strain Ea1189

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*Erwinia amylovora* causes the bacterial disease fire blight, one of the most important diseases in apple production worldwide. As the most gram-negative bacteria, *E. amylovora* possess a type three secretion system that enables it to deliver effector proteins into the host cell. Effectors, like *AvrRpt2EA* from *E. amylovora*, play an important role in the pathogenicity by manipulating key functions of the host cells. *Malus × robusta* 5 (Mr5) is a highly fire blight resistant apple wild species accession. Only few strains are able to overcome the resistance, including the *avrRpt2EA* deletion mutant ZYRKD3-1. The related wild type strain Ea1189, expressing the *avrRpt2EA* is not able to break down resistance of Mr5. The analysis of the gene expression of Mr5 after inoculation with these two strains gives the opportunity to analyze the host-pathogen interaction of *E. amylovora* and Mr5 in a system differing only in the *avrRpt2EA* gene. Therefore the transcriptome of Mr5, inoculated with a) the virulent mutant strain ZYRKD3-1 and b) the non-virulent wild type strain Ea1189, were sequenced 2 and 48 hours after inoculation. The comparison of the transcriptome of Mr5 inoculated with the two strains shows in total 211 significant differentially expressed genes for both time points. 140 of these genes were significantly higher expressed in Mr5 inoculated with the non-virulent wild type strain Ea1189, while only 71 genes had a higher expression level in Mr5 inoculated with the virulent *avrRpt2EA* deletion mutant. 38 percent of the 71 genes are involved in the stress response and the most of them are coding for heat shock proteins. Genes expressed after the inoculation with the wild type strain of *E. amylovora* are mainly involved in metabolism and regulation of transcription or belong to the miscellaneous enzyme families. To confirm the results received by sequencing and to verify further time points as well as an uninfected sample, quantitative real time analysis was performed using the Fluidigm Biomark system.
Pathogenicity and infection strategies of the fire-blight pathogen *Erwinia amylovora* in *Rosaceae*: state of the art

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Plants are host to an important amount of infectious diseases caused by a vast array of fungi, bacteria, viruses and nematodes. In great contrast to the mammalian immune system composed of specialized and mobile defense cells such as lymphocytes, plants however have to rely on the ability of each cell to recognize a pathogen and its signals emanating from the infection site in order to protect itself from the disease by using several defense mechanisms.

These mechanisms consist of physical barriers and the production of antimicrobial components, both in a preformed and an inducible manner. Inducible defense responses are activated upon the recognition by plant cell receptors of elicitor molecules, either derived from invading microorganisms or from pathogen-induced degradation of plant tissue. This recognition event triggers a signal transduction cascade, leading to a range of defense responses (reactive oxygen species (ROS), plant hormones, secondary metabolites, …) and redeployment of cellular energy in a fast, efficient and multiresponse manner, which prevents further pathogen ingress. This review highlights the research that has been performed during the last years regarding the *Erwinia-Rosaceae* plant-pathogen interaction, with special emphasis on the pathogenicity and the infection strategy of *E. amylovora* and the possible defense mechanisms of the plant against this disease.
Cyclic di-GMP regulates the expression of virulence factors in *Erwinia amylovora*

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Cyclic di-GMP (c di-GMP), a secondary intracellular messenger molecule, is involved in the regulation of many cellular processes in numerous bacterial species, including the transition from motile to sessile lifestyle, virulence, biosynthesis of exopolysaccharides and adhesion structures, and cell differentiation. The cellular levels of this messenger compound are controlled by enzymatic synthesis and degradation, catalyzed by diguanylate cyclases and phosphodiesterases, respectively. Signal transduction and phenotypic modulation is determined by binding of c di-GMP to specific downstream receptors. Although c di-GMP has been shown to be an important intracellular signal in several plant pathogenic bacteria, the role of this molecule in *Erwinia amylovora* has not been previously investigated. Using gene overexpression and site-directed mutagenesis analyses, we identified three active diguanylate cyclases (EdcA, EdcC and EdcE) in this bacterial pathogen. Phenotypic analyses demonstrated that c di-GMP positively regulates the biosynthesis of both cellulose and amylovoran, positively regulates biofilm formation, and represses motility. Disease assays on immature pears and apple tree shoots demonstrated that c di-GMP negatively regulates virulence in these infection models. In addition, using a bioinformatic approach, two candidate receptor proteins, EAM_3387 and EAM_1855 containing the PilZ domain, were predicted. Binding affinities of these proteins to c di-GMP were evaluated *in vitro*. These results demonstrated that c di-GMP is a key component of the regulatory networks that govern the expression of virulence traits in *E. amylovora*. 
Breeding for fire blight resistance and sterility in *Cotoneaster*

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*Cotoneaster* is a genus of over 400 species in the apple subfamily (Maloideae) commonly used as ornamental landscape plants. Many species are extremely drought and urban tolerant, making them desirable for modern sustainable landscapes. However, susceptibility to fire blight is common in the genus, which has reduced their use where there is significant disease pressure. In addition to fire blight susceptibility, some species have escaped cultivation to form substantial populations in native areas. We initiated a breeding program to develop disease resistant and sterile cotoneaster cultivars. To identify potential sources of resistance, we artificially inoculated 52 taxa of *Cotoneaster* with *Erwinia amylovora* strain Ea153 at $10^9$ CFU/mL. Percent shoot infection (lesion length/ total shoot length) was used to assess disease severity. Disease screenings were conducted over two years with 13 species repeated over years to validate results. Susceptibility was highly variable among species. Inoculation of several highly susceptible species resulted in whole plant mortality (*C. rhytidophyllus*, *C. rugosus*, *C. wardii*), while other taxa repeatedly showed no disease symptoms (*C. arbusculus*, *C. sikagensis*, *C. splendens*). We used results of our disease screening in combination with knowledge of the natural ploidy level variation in *Cotoneaster* to design crosses. In 2011, we used *C. splendens*, a resistant tetraploid ($2n = 4x = 68$), as a pollen parent in crosses with the susceptible diploid ($2n = 2x = 34$) *C. × suecicus* ‘Coral Beauty’ as the pistillate parent. The resulting hybrids were screened using flow cytometry and confirmed to be triploids ($2n = 3x = 52$). These triploid hybrids have been propagated by stem cuttings and will be screened for fire blight susceptibility to determine heritability of resistance. All hybrids will be evaluated for fertility, landscape performance, and aesthetics, as we have observed substantial phenotypic variability in leaf shape and habit among hybrids.
Breeding high quality apples with fire blight resistance

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Fire blight (Erwinia amylovora) is one of the most damaging diseases in apple (Malus x domestica) growing areas worldwide. Breeding of resistant cultivars is a promising approach to control this disease in combination with other disease prevention measures. Agroscope is developing high quality apples combined with excellent agronomic features suitable for sustainable production systems. This includes durable disease resistance against fire blight, scab (Venturia inaequalis) and powdery mildew (Podosphaera leucotricha). For fire blight resistance parents are being selected based on shoot infection tests with artificial inoculation of E. amylovora in the glasshouse. In addition to the glasshouse tests, flowering trees of advanced selections are also tested in protected field sites under artificial inoculation with E. amylovora. Related to the genetics of the parents, molecular markers are applied in a marker assisted breeding (MAB) approach to screen progeny plants for desired resistances. Examples of artificial shoot inoculation tests and selection of breeding parents will be shown and the use of molecular markers for the progeny selection illustrated. A new variety carrying moderate resistance to fire blight as well as scab resistance was developed and released for advanced testing under the name 'Ladina'.
Overview of cultivar testing and pathogenesis of apple with respect to a tolerance against *Erwinia amylovora*

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Due to the fact that there are still no vertical resistance genes known against *Erwinia amylovora*, the causative agent of fire blight, one main focus of research is the generation of tolerant cultivars. In the past three years we tested a total of 55 apple cultivars, mostly old and regional varieties, in greenhouse experiments by shoot inoculation and visual rating to assess their level of tolerance against *E. amylovora*. Some tested cultivars indeed performed better than the control variety Rewena.

Additionally, we carried out pathogenesis experiments with selected cultivars in order to test for a correlation of symptoms and actual pathogen spread. The background of these pathogenesis experiments is that the classification into tolerant and susceptible cultivars so far is only done based on visual ratings and field observations. Results from these experiments clearly proofed that there is no such correlation. Depending on the cultivar tested there could be huge differences between the distribution of bacteria and the actually visible symptoms. In a variation of the experimental setup, the plant growth regulator Prohexadion-Calcium (Regalis®) was found to reduce symptom development, but it did not alter pathogen load significantly. It is therefore not suitable for controlling the disease, but only obscuring infections.
Fire blight resistance breeding for real customers

M. Kobelt (Sponsor talk: Lubera AG)
FB_MR5 is an apple gene providing resistance to fire blight

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To date the use of natural resistances to manage fire blight epidemics in apple orchards has been limited by the reduced availability of such trait in commercial varieties as well as the very poor fruit quality of resistant wild apple genotypes. Such natural resistance offers great environmental advantages compared to any other disease control methods i.e. less treatments with chemicals and less tractor rides. We undertook the positional cloning of the fire blight resistance gene located on the linkage group 3 of *Malus x robusta* 5, a wild apple genotype immune to European strains of *Erwinia amylovora*. A single candidate gene (FB_MR5) was identified and validated by transgenic approach, transforming the fire blight susceptible cultivar ‘Gala’. This represents an unprecedented opportunity to deploy *Malus*-own resistance by cisgenics, similarly to what we recently reported for the scab resistance gene *Rvi6*, showing that such GM product may represent an effective and sustainable approach in fire blight management. Current state of the research will be presented.
Investigation on fire blight resistance in the cross population of ‘Idared’ x Malus × robusta 5 with different Erwinia amylovora strains

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Fire blight caused by the bacterial pathogen Erwinia amylovora is the most devastating disease in apple production worldwide. Breeding for fire blight resistance is therefore one of the major aims in nearly all apple breeding programs. Whereas common cultivars are all susceptible, several apple wild species accessions bear resistance against fire blight. First studies on mapping of fire blight resistance in the cross population Malus × domestica ‘Idared’ x Malus × robusta 5 (Mr5) using the E. amylovora strain Ea 222 resulted in the detection of a major QTL on linkage group 3 (LG3) of Mr5. Recent results suggest that the resistance of Mr5 seems to be based on a CC-NBS-LRR resistance gene (Fb_Mr5) responsible for a gene-for-gene relationship between Mr5 and the avrRpt2_Ea effector gene of E. amylovora. The resistance of Mr5 can be overcome by several Erwinia strains characterized by a substitution of the amino acid cysteine at position 156 by serine in the AvrRpt2_Ea effector protein sequence. In this study a QTL mapping of fire blight resistance in the cross population ‘Idared’ x Mr5 was performed after the inoculation with different E. amylovora strains which differ in their virulence to Mr5. Using strains containing cysteine at the position 156 in the AvrRpt2_Ea protein sequence, the QTL was stable over years whereas strains with the substitution to serine resulted in a breakdown of the QTL on LG3 of Mr5. The results gave further evidence for the existence of a gene-for-gene relationship and demonstrated clearly that the fire blight resistance of Mr5 is strain specific. Additionally, we screened all Malus wild species accessions of the German National Fruit Collection at the Julius Kühn-Institut in Dresden with two SSR markers flanking the fire blight resistance locus on LG3 of Mr5. First results demonstrated that the resistance locus seems to be also present in different accessions of M. × robusta and Malus baccata jackii. The accession number MAL0804 of Malus baccata jackii showed a similar allele combination of the flanking SSR markers Ch03e03 and Fem18 as Mr5. Subsequently, the CC-NBS-LRR candidate resistance gene was cloned from the Malus baccata jackii accession MAL0804. The predicted protein sequence of Fb_Mr5 in MAL0804 showed a number of amino acid substitutions compared to the FB_Mr5 protein sequence. When inoculated with the virulent and avirulent E. amylovora strains, both Mr5 and MAL0804 displayed similar disease severity, suggesting that these genotypes are undergoing the same gene-for-gene relationship.
Accelerated introgression of fire blight resistance from *Malus x robusta* 5 and other wild germplasm into elite apple germplasm

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Introgression of fire blight resistances from wild apples into high quality apple cultivars is an important breeding strategy of the Agroscope apple breeding programme. In order to achieve parental material suitable for cultivar breeding several pseudo-backcrosses with high quality parents are required. On F2 progeny plants of *Malus x robusta* 5 a low-input fast-track breeding approach was applied to accelerate the generation cycle. Seedlings were screened with molecular markers for *FB_MR5* fire blight resistance QTL and *Rvi6* scab resistance. Selected seedlings were grown on their own roots under optimal growing conditions, including non-limiting fertilisation, regular prohexadione-Ca and ethephon treatments followed by winter simulation, to enhance flowering. From flowering plants carrying the *FB_MR5* QTL pollen was collected for crosses with high quality *Malus x domestica* cultivars and flowers were pollinated with elite apple germplasm. Parents for new introgression cycles carrying the *FB_MR5* QTL were selected based on fruit evaluations and on results of artificial shoot inoculation tests with a Swiss strain of *Erwinia amylovora*.

In order to include new sources of fire blight resistance into the Agroscope breeding programme additional *Malus* crabapples, described as fire blight resistant, were inoculated by shoot injection. In this paper we report on the progress of the introgression of the fire blight resistance *FB_MR5* using a fast-track breeding approach and present results of fire blight shoot inoculation tests in wild and pre-breeding germplasm.
Evidence of a major QTL for fire blight resistance in the apple wild species *Malus fusca*

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Fire blight is a common and very destructive disease affecting apple (*Malus x domestica*) and pear (*Pyrus communis*) productions. Caused by the bacteria *Erwinia amylovora*, its devastating economic effects range from losses in yield to severe damage or death of trees in the orchard, and in extreme cases loss of a whole orchard. The only reliable and effective management measure available to producers is the use of antibiotics. However, dependence on antibiotics treatment is not sustainable given such risks as the rapid selection of antibiotic-resistant populations and environmental-associated risks, which have led to their application being forbidden in many countries. Therefore, the planting of fire blight-resistant cultivars seems to be the most probable strategy since it is eco-friendly. However, until now, there is no fire blight resistant apple variety produced for the global market. Host resistance to fire blight in *Malus* is thought to be quantitatively controlled. Thus, several quantitative trait loci for resistance have been identified in some accessions of *Malus* spp. In this study, we explored a segregating population derived from a cross between the apple wild species *Malus fusca* and the *Malus x domestica* cultivar ‘Idared’. F\textsubscript{1} progenies used for mapping were artificially inoculated with *E. amylovora* strain Ea 222 JKI at a concentration of 10\textsuperscript{9} cfu/ml in 3 different years. Highest value of average percent lesion length (PLL) of all progenies was 23.1\% in 2006 with 9.0\% as lowest in 2012. The averages of PLL of all replicates of each genotype were used as numerical traits for statistical analysis. A Kruskal-Wallis analysis, used to determine marker-phenotype association, revealed two DArT markers (971000 and 970840) with the highest significance. The blast results of the sequences of both markers on the ‘Golden Delicious’ reference genome revealed their localisation on LG10. SSR markers were designed from the ‘Golden Delicious’ genome to replace these DArT markers and to determine the QTL region. Interval mapping revealed a strong QTL on LG10 explaining about 65\% of the phenotypic variation. This is the first report on a fire blight resistance QTL of *M. fusca* and the only one located on LG10.
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**SESSIONS**

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- **P5** Pathogen genetics and genomics
- **P7** Plant-microbe interactions
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**Erwinia amylovora** loop-mediated isothermal amplification (LAMP) assay for rapid pathogen detection and on-site fire blight diagnosis

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Several molecular methods have been developed for detection of *Erwinia amylovora*, the causal agent of fire blight in pear and apple, but none are truly applicable for on-site use in the field. We developed a fast, reliable and field applicable detection method using a novel target on the *E. amylovora* chromosome that we identified by applying a comparative genomics pipeline. The target coding sequences (CDS) are both uniquely specific for and all-inclusive of *E. amylovora* genotypes. This avoids potential false negatives that can occur with most commonly used methods based on amplification of plasmid gene targets, which can vary among strains. Loop-mediated isothermal Amplication (LAMP) with Optigene Geniell chemistry and instrumentation proved to be an exceptionally rapid (<15 minutes) and robust method for detecting *E. amylovora* in orchards, as well as simple to use in the plant diagnostic laboratory. Comparative validation results using plant samples from inoculated greenhouse trials and from natural field infections (of regional and temporal diverse origin) showed that our LAMP had equivalent or greater performance regarding sensitivity, specificity, speed and simplicity than real-time PCR (TaqMan), other LAMP assays, immunoassays and plating, demonstrating its utility for routine testing.


**Validation of the FLASH-PCR method for detection *Erwinia amylovora* in a plant extract**

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PCR-based methods are widely applied for detection and identification of *Erwinia amylovora* and some other bacteria in quarantine laboratories of the Russian Federation. FLASH-PCR (Fluorescent Amplification-based Specific Hybridization) and Real-time PCR with commercial kits developed by a Russian company “Agrodiagnostics” (Moscow) are among the main screening methods. These kits are customized for equipment produced by “DNA-technology” company (Moscow, Russia) and RT-PCR machine “IQ-5” produced by “Bio-Rad” (USA). The FLASH-PCR is an end-point fluorescent PCR method enables the use of conventional thermocycler and relatively inexpensive fluorescence detector fitted with the specifically developed software. The key element of the FLASH-PCR technology is the use of destructible oligonucleotide hybridization probes (beacons) marked by fluorophore molecules and fluorescent quencher. A commercial kit consists of the following elements: ready-to-use reaction tubes sealed with paraffin with a PCR mix containing internal control, stable Taq-polymerase, buffer with no Taq-polymerase for background fluorescence, positive control. The FLASH-PCR method for detection of *E. amylovora* in a plant extract with the above mentioned commercial kit has been validated according to the EPPO Standard 7/98. The commercial kit for DNA purification based on silica sorbent principal by the same company has been used. The plant extracts from pear, apple, quince, raspberry, cotoneaster and hawthorn material were prepared according to the EPPO Standard 7/20 (1) for asymptomatic samples and spiked by reference *E. amylovora* strain CFBP 1430. 60 strains of *E. amylovora* and 20 strains of other phytopathogenic bacteria including *E. pyrifoliae, E. piriformingrans, E. tasmaniensis, E. billingiae* have been tested for specificity. Also, we checked the specificity of primers “in silico” using “Primer-BLAST” database. The following performance criteria have been obtained: analytical sensitivity - 10⁻³⁻¹⁰ cells/mL, analytical specificity – 100%, selectivity – not observed, repeatability – 75% for concentration of 10⁻³ cells/mL and 100% for 10⁻⁴ cells/mL, reproducibility – 80% for concentration of 10⁻³ cells/mL and 100% for 10⁻⁴ cells/mL. Thus, the FLASH-PCR method for detection of *E. amylovora* in a plant extract with the above mentioned commercial kits are recognized as quick, simple and reliable. It can also be recommended for identification of pure cultures.
Detection of fire blight infections in apple trees using photo-optical methods

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To prevent damage in pome fruit production caused by fire blight, early detection of *Erwinia amylovora* is essential due to limited options for disease control. The advantages of photo-optical methods are the non-invasiveness and speed of measurements - a quick survey of large areas and remote sensing is possible. The method uses the reflectance characteristics of plant tissue, which provides information about the health status of the plant. Changes in plant reflectance spectra can be used to make statements about the presence of pathogens. Measurements were done using different devices i.e. fluorescence and infrared spectrometers as well as an infrared camera on inoculated and healthy apple trees. Results show that a differentiation between healthy and infected trees is possible. The specificity of the photo-optical detection concerning the discrimination of fire blight and other diseases has to be further evaluated.

Evaluation of three extraction methods for detection of *Erwinia amylovora* from pear leaves by real time PCR

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Extraction of bacterial DNA of sufficient purity for PCR is often a limiting step in plant sample processing. In this study we compared three DNA extraction procedures prior to real time PCR reaction. Healthy pear leaves and twigs were crushed in antioxidant maceration buffer and spiked with *E. amylovora* to final concentrations from $2,1 \times 10^6$ to $2,1 \times 10^7$ cells ml$^{-1}$. DNA was extracted from aliquots of spiked crude extracts using (i) isopropanol, (ii) RedeExtract-N-Amp™ Plant PCR kit, and (iii) Taylor’s modified DNA purification procedure. The *ams* region of the chromosomal DNA was selected as target for the real time PCR reaction. This region of the *E. amylovora* chromosome is involved in synthesis of the capsular polysaccharide amylovoran, which is considered unique to *E. amylovora*. Specific primers Ams116F, Ams189R and TaqMan™ Ams141T probe were used in the reaction for 74 base pair fragment amplification. In this study, the RedeExtract-N-Amp™ and Taylor’s modified DNA extraction procedure were most successful in removing inhibitors, leading to detection of $2,1 \times 10^2$ *E. amylovora* cells ml$^{-1}$. This concentration can be efficiently detected in less than 5 hours in spite of inhibitors and plant DNA reducing sensitivity of the reaction. These two methods increased amplification efficiency in real time PCR compared to a simple isopropanol DNA extraction procedure from plant tissues where the lowest detected concentration of bacterial cells was $2,1 \times 10^4$ ml$^{-1}$. In our tests real time PCR has proven to be the most sensitive method for detection of *E. amylovora* in plant material. It was 10 times more sensitive than Nested PCR and 100 times more sensitive compared to other conventional PCR procedures.

This research was supported by the project III46008 financed by Ministry of Education, Science and Technological Development, Republic of Serbia.
Monitoring the presence of *Erwinia amylovora* in apple orchards using honey bees

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Temperature-based fire blight forecasting models provide an accurate prediction of conditions for blossom blight infections. However, additional information about the actual presence of *Erwinia amylovora* in apple orchards and surrounding host plants would provide valuable data to support the decision on treatments. A detection system has been developed, to monitor *E. amylovora* using tube collectors mounted at the flight entrances of beehives. This approach enables a less time consuming and more representative sampling within the flight range of honeybees compared to blossoms taken within the orchard. Thus qualitative and quantitative data about the occurrence of *E. amylovora* in the vicinity of apple or pear orchards could be gained. Prior to the field experiments a validation of different inlays for the tube collectors was performed by determining the recovery rate of *E. amylovora* after artificial spiking. The material from which the highest recovery rates of *E. amylovora* could be obtained was subsequently used for the orchard experiments. In 2012 and 2013 the combined monitoring was carried out in three commercial apple orchards in Austria and in an experimental station in Switzerland (Agroscope Wädenswil) to get information about the presence of *E. amylovora* during bloom. The experimental setup per orchard consisted of 2-5 beehives each equipped with 2 daily sampled tube collectors. DNA-extraction and confirmation of *E. amylovora* was carried out by qPCR. Using this combined monitoring system it was possible to confirm the presence of *E. amylovora* in the orchards (blossom samples) and/or in its vicinity. Qualitative and quantitative differences were found between individual beehives, sampling dates, collectors and blossom samples. Recorded *E. amylovora* occurrence was set in relation to the data obtained from the Maryblit™ prognosis model.

Sensitivity of fire blight detection in composite twig samples

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Testing of asymptomatic samples serves several purposes in the European Union as (i) checking for presence of the disease in uninfected areas and buffer zones around places of propagating material production and (ii) as a technical support to determination of areas with “zone protecta” status. Countries have different approaches to testing asymptomatic samples with sampling recognized as being the biggest source of uncertainty. From a survey, recently conducted by the European and Mediterranean Plant Protection Organization (EPPO), it was shown that laboratories test single twigs or composite samples of up to 30 twigs. Here we report on the analytical sensitivity of enrichment – real time PCR (ITS sequence; Pirc et al., 2009) and enrichment isolation on CCT plates when testing single and composite samples of 10 and 30 twigs with defined concentrations of *Erwinia amylovora* ranging from $10^6$ – 1 cfu/mL. Under our experimental conditions, no significant differences were observed in analytical sensitivity of enrichment - real time PCR or enrichment isolation on CCT media in different background matrix (single twigs, 10 or 30 twigs).
A new experimental design for testing control agents for fire blight following simulated hail injury

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Leaf damages after hail are points of entry for \textit{Erwinia amylovora} and can cause new fire blight infections. Control agents are able to reduce the degree of infection. Until now, there has been no method established for testing control agents after hail injury. The goal of this work was to develop an experimental design for testing control agents for fire blight following simulated hail injury. Saplings (Gala/M9), 10 plants per treatment, were cultivated in the climate chamber. Leaves were injured by a portable flower thinning machine with rotating plastic strings. Plants were spray inoculated with an \textit{E. amylovora} suspension (10\textsuperscript{6} cfu/ml). Application of the control agents took place 4 h after injury. After 3 weeks in the climate chamber plants were scored. Statistical analyses included the infection rate (binomial model) and length of necrosis in infested shoots (ANOVA). Streptomycin showed high efficiency. Of 13 agents tested, 3 showed an efficiency sufficient for practical use (Juglon, LMA und Myco-Sin). Infection rate depended on temperature and plant physiology. Infection risk in the field is likely to dependent on weather conditions, plant physiology, infection thread and degree of injuries.

Bacteriophage of \textit{Erwinia amylovora} – host range and fire blight control potential

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Fire blight, caused by \textit{Erwinia amylovora}, is the most destructive bacterial disease of pome fruits in Serbia. In search for an alternative to ineffective chemical disease control, we isolated bacteriophages specific to this bacterium and studied their host range and efficacy in disease control. Seven bacteriophage strains specific to \textit{E. amylovora} were collected from localities near Belgrade, during 2010. Three phage strains were isolated from water, three strains from symptomless pear leaves and one strain from apple leaves with characteristic fire blight symptoms. The phages’ host range study showed that they differed in the ability to lyse 40 strains of \textit{E. amylovora} isolated in Serbia. Based on its lytic activity, phage Φ\textit{Ea1} was chosen for further study of its biological control potential in pear and apple blossom bioassays. The ability of Φ\textit{Ea1} to control fire blight was tested by application of 10 µl of phage suspension (10\textsuperscript{8} PFU/ml) on blossoms either 2 h before, 2 h after or at the same time of application of bacteria. Inoculation was performed by pipetting 10 µl of bacterial suspension (10\textsuperscript{8} CFU/ml) on the each of five blossoms per treatment. Inoculated but untreated blossoms and blossoms without any treatment were used as controls. Treated flowers were incubated in a growth chamber under 70% relative humidity at 22-25°C. Disease severity was evaluated 3 and 5 days after inoculation, using a rating scale modified from Pusey (1997). Data were statistically analyzed using ANOVA and Duncan's multiple range test. Our results showed that bacteriophages specific to \textit{E. amylovora} can be isolated from various substrates. In three repeated bioassay experiments, application of host-specific phages 2 h before inoculation significantly reduced fire blight symptom development compared to untreated control. Potential of specific phages to efficiently lyse \textit{E. amylovora} strains originating from different hosts and localities, and to reduce fire blight symptoms, makes them a good candidate for future use in biological control of fire blight.

This research was supported by the project III46008 financed by Ministry of Education, Science and Technological Development, Republic of Serbia.
**Efficacy and phytotoxicity of Aliette 80 WG used for control of fire blight**

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The protective action of Aliette 80 WG was tested on blossoms and terminal shoots of potted apple trees growing in the greenhouse. Blossoms at full bloom were sprayed with a water suspension of Aliette at a concentration of 7500 ppm (3.75 kg/ha) once or twice (second application 3 days after first). After 48 h they were inoculated with a water suspension of the highly virulent strain Ea 659 of *Erwinia amylovora* (10⁶ cfu/ml). Observations performed on the cv. Szampion 5 days after inoculation showed that the efficacy was 70.0% with one treatment and 78.5% with two treatments, but they did not differ significantly. The efficacy evaluated 7 days after inoculation was lower in comparison to the 5 day observations with 60.7% and 66.2% efficacy, respectively. Similar treatments on cv. Idared blossoms showed 70.4% efficacy after one application and 75.9% for two treatments. The efficacy of a standard copper oxichloride preparation (Miedzian 50 WP at a dose of 1.5 kg/ha) was higher in comparison with Aliette 80 WG and reached c. 100%.

In two other separate experiments, Aliette 80 WG was applied on cv. Idared trees either once or twice at 7500 ppm (3.75 kg/ha) to protect terminal shoots against fire blight. Inoculation was done 48 or 72 h after the last treatment. Observations performed 5, 9, and 15 days after inoculation showed that this compound was not effective enough to control fire blight in terminal shoots.

A further experiment testing the phytotoxicity of Aliette 80 WG on pear fruitlets showed that the product was significantly less phytotoxic than the standard copper oxichloride preparation.

In conclusion, Aliette 80 WG can be recommended for the protection of apples against fire blight during flowering, especially when a low risk of disease is predicted.

**Biological control of fire blight: summary of recent study**

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The aim of our study was to select bacterial isolates, originating from apple phyllosheres and soil environments, showing high protective activity of apple and pear against fire blight. Screening of 299 isolates using the pear fruitlet test resulted in the selection of 13 isolates (3M, L16, 35M, B90, 43M, 48M, 49M, 55M, 56M, 59M, 91M, 19CK, and 141M) which completely or almost completely protected fruitlets against the disease. Of these isolates, 48M and 35M appeared to be the most effective in protection of both apple blossoms and shoots; 59M only protected blossoms and 43M only protected shoots. When used in comparison with strains A506 of *Pseudomonas fluorescens* and C9-1 of *Pantoea agglomerans*, which are active ingredients of commercial biopreparation BlightBan, our strains showed similar or slightly higher efficacy.

Studies on possible mechanisms of action of these isolates revealed that 3M possesses gene *prnD* encoding pyrrolnitrin biosynthesis, 59M – gene *phiD* encoding synthesis of 2-4 diacetylfluoroglucinol, and genes *pltB* and *pltC* encoding pyoluteorin. In the case of isolate 35M, the product of amplification with primers complementary to gene *pltB* was also obtained. Additionally, isolates 3M, 35M, 56M, 59M, 91M, 19CK, and 49M gave PCR product with primers complementary to gene *gacA*, the regulatory gene of secondary metabolite synthesis, including antibiotics. Biotic relationships between effective isolates and *Erwinia amylovora* studied on four agar media (NAS, King’s B and LB, whereas, on minimal medium 925, all isolates to a lesser or greater extent stimulated growth of *E. amylovora*. Isolates L16, 19CK, 3M, 35M, 49M, 56M, 59M, and 91M produced siderofores, whereas only 3M, 35M and 48M produced N-acyl homoserine lactones (AHL). All isolates produced biofilm, wherein the most intense were 43M, 48M 91M and L16.

Phenotypic characterization and sequence analysis of the 800 bp-length fragment of the 5’ end of 16S rRNA gene allowed identification of isolate 3M as *Pseudomonas chlororaphis*, 35M as *Pseudomonas syringae*, L16 as *Pseudomonas fluorescens*, 43M as *Citrobacter farmeri* or *Pantoea agglomerans*, 48M and B90 as *Pantoea agglomerans*, 55M as *Pseudomonas mosselli*, 56M as *Pseudomonas sp.*, 59M as *Pseudomonas fluorescens*, 91M as *Pseudomonas putida*, 19CK as *Pseudomonas sp.*, 49M as *Pseudomonas graminis*, and isolate 141M as *Erwinia bilingiae*. It is worth studying that our study showed for the first time the antagonistic properties against *E. amylovora* of isolates representing the following species *Pseudomonas chlororaphis*, *P. mosselli*, *P. putida*, and *P. graminis*, and also the ability of those bacteria to protect apple blossoms against fire blight.
Mechanism of different salicylic acid effects on fire blight control of apple and pear

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Previous reports demonstrated contrast response of apple and pear to systemic acquired resistance (SAR) induction by salicylic acid (SA) against fire blight disease. This research was conducted to reveal mechanisms of SA effects that cause these responses, considering bi-lateral role of SA on catalase inhibition and SAR activation. At first, effects of SA were studied on catalase activity of extracted enzyme, as well as, in the host tissues of 1 and 7 days pre-treated in vitro shootlets of MM-111 (resistant) and MM-106 (semi-susceptible) pears; and MM-107 (resistant) and Spadona (semi-susceptible) pears. SA had similar inhibitory effects on extracted catalase of both species, but increased this activity on pre-treated hosts at different rates in tissues. Considering similar catalase behavior of two species to SA induction, subsequently, response of hosts were evaluated in low (0.1 to 0.001 mg/l) and high (50 mg/l to 50 g/l) SA concentrations in pears and apples, respectively, at presence of Erwinia amylovora. By these, the disease susceptibilities were inversed in comparison to the intermediate range SA concentrations (1 to 50 mg/l), in both species. Also, catalase activity comparison in equalized total protein extracts showed higher catalase activity in apples. It was concluded that the contrast responses of two hosts to SA, is an interaction between catalase inhibition and SAR activation by SA.

Biocontrol studies of fire blight on pear and apple orchards in Turkey

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Fire blight disease, caused by Erwinia amylovora has threatened pear cultivation in Turkey since 1985. Since then the disease has spread out to apple and quince orchards and caused severe damages on all pome fruits in Turkey based on favorable weather conditions. Suppression of the blossom-blight phase of fire blight is a key point in the management of this destructive and increasingly important disease of pome fruits. Biological control with epiphytic bacteria against E. amylovora has been considered as a potential method for controlling the disease on pear and apple blossoms. In this research, Pantoea vagans strain C9-1 (formerly P. agglomerans C9-1), and P. agglomerans strain EH24, and Pseudomonas fluorescens strain A506 were produced as freeze-dried bioformulations. The efficacy of these bioformulations against blossom infections from natural populations of E. amylovora in commercial pear and apple orchards were evaluated. The freeze-dried formulations of antagonistic bacteria were applied as single strains and in combination at 30 % and 100 % bloom in pear and apple orchards. In the pear orchard trials conducted in 2008 and 2009, freeze-dried formulations of antagonistic bacteria, applied as single strains, reduced the percentage of blighted blossoms in the pear orchard by 20% to 70%, and strain C9-1 was more efficacious than PIA506 and EH24, as significant reductions in blossom blight was observed in the pear orchard experiment for consecutive two years. Three different combinations of bioformulations such as C9-1+PIA506, EH24+PIA506 and C9-1+EH24 which were applied on pear blossoms were found as effective as the alone treatment of C9-1 on pear orchard to protect the blossom infections. In the apple orchard trials conducted in 2008 and 2009, freeze-dried formulations of antagonistic bacteria, applied as single strains and in combination, reduced the percentage of blighted blossoms on pear orchards by 32% to 60% comparing with the pathogen alone treatment, and were found more efficacious than copper-oxchloride + maneb treatment. Antagonistic bacteria successfully colonized on pear and apple blossoms and resulted a 10 to 100 fold increase in surviving bacteria within 12 days after the last application.
Identification of novel biocontrol mechanisms for *Pantoea agglomerans* E325 (BloomTime) from *Pantoea* spp. pan-genome analysis

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*Pantoea* spp. have been isolated from diverse habitats and exhibit different lifestyles as opportunistic phytopathogens, beneficial plant epiphytes (biocontrol), insect-associates, and clinical co-isolates. Recent availability of several genomes of members of this genus enables us to apply comparative genomics and investigate similarities/dissimilarities that may reveal unique determinants for differential behavior. The genome of the fire blight antagonist *P. agglomerans* E325 was analyzed for features involved in biocontrol. Comparative genomics with other *P. agglomerans* genomes revealed that plasmid pPag4 yields most singletons within the genome of *P. agglomerans* E325. Mutants negative for the biosynthesis of the E325-specific antibiotic carried insertions in two genes within a low-G+C gene cluster that is located on pPag4. From the biosynthetic genes, no indications to the pathway and nature of the antibiotic could be deduced, but the mutants were unable to control fire blight on apple trees. The availability of the genome sequence for *P. agglomerans* E325 plus the extended knowledge from phylogeny within the genus *Pantoea* has facilitated the development of tools for strain- and species-specific environmental monitoring of *P. agglomerans* for the evaluation of its biosafety, which is a critical regulatory element for registration. The increased knowledge from the pan-genome analysis opens new doors to optimize biocontrol performance by the determination of environmental modulators of biocontrol gene expression (in orchards, during formulation).

Streptomycin use in apple orchards did not adversely alter the soil bacterial communities

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This study addresses the important topic of the influence of the use of antibiotics in agriculture on the total bacterial population within the soil ecosystem. We hypothesized that as streptomycin is produced naturally by soil bacteria from the Actinomycetales order, that additional streptomycin would not alter the bacterial composition of the soil. High throughput 16S rRNA gene amplicon sequencing was used to generate datasets from each soil. The application of streptomycin did not influence the abundance and diversities of major bacterial taxa of the soils. We also discovered that apple orchards under the same management practices, did not harbor the same bacterial communities. Our results show that soil pH did not influence the abundance of specific taxa, as reported in previous studies, but we identified strong differences in the abundances of the entire bacterial communities due to pH, indicating that small changes are noticeable at the entire population level when taken together. The application of streptomycin in the protection of apple orchards from the fire blight pathogen *Erwinia amylovora* did not alter either the bacterial diversity nor abundance within these soil ecosystems either in the short term or after three years of treatment.
Streptomycin use in apple orchards did not increase abundance of mobile resistance genes

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Streptomycin is used as a first-line defense, and tetracycline as a second-line defense, in the fight against fire blight disease in apple and pear orchards. We have performed the first study to quantitatively analyze the influence of streptomycin use in agriculture on the abundance of streptomycin and tetracycline resistance genes in apple orchards. Flowers, leaves and soil were collected from three orchard sites in 2010, 2011 and 2012. Gene abundance distribution was analyzed using two-way ANOVA to investigate relationships between gene abundance data over time and treatment. The antibiotic resistance genes were detected prior to streptomycin treatment in almost all samples, indicating the natural presence of these resistance genes in nature. The statistically significant increases in the resistance gene abundances were occasional, inconsistent and not reproducible from one year to the next. We conclude that the application of streptomycin in these orchards was not associated with sustained increases in streptomycin or tetracycline resistance gene abundances.

Is fire blight present in Saudi Arabia?

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Fire blight is one of the most destructive diseases of apple and pear trees. Outbreaks are sporadic in most parts of the Northeast, but can cause extensive tree damage when they do occur. Fire blight is caused by the bacterium Erwinia amylovora. During April 2013 unusual disease symptoms were observed on the new shoots, flowers and fruits on pear trees (Pyrus spp.) in the Aljouf Governorate, in the north of Saudi Arabia. The naturally infected shoot and flowers showed typical symptoms of fire blight, including terminal young shoots with brown necrotic lesions and both flowers and young fruits turned dry and brown. We plan to characterize the causative agent of these symptoms for first time in Saudi Arabia. The bacteria will be isolated on selective media, PCR using primers specific for E. amylovora will be performed and the resulting products sequenced.

The monitoring of Erwinia amylovora in Lithuania (1998-2012)

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Lithuania has been recognized as a protected zone with regards to Erwinia amylovora (Burr.) Wsln. et al. as a causal agent of fire blight for a period until 31 March 2014. State Plant Service under the Ministry of Agriculture of the Republic of Lithuania has implemented systematic monitoring, prevention, eradication and control measures for this bacterium in the territory of Lithuania. Fire blight has been monitored in Lithuania since 1998. For the first time the pathogen was discovered in 2005. There was a high outbreak of this pathogen in 17 localities in northern and northwestern parts of Lithuania. 27% of investigated samples were positive for E. amylovora using the immunofluorescence (IF), plating, nutritional, HR and enzymatic assays. Although in the following years of 2006 and 2007 the new outbreak sites were identified, the infection prevalence in tested samples decreased till 7,9% and 16,2%, respectively. The results of the tested samples in 2006 -2012 by PCR highly matched the E. amylovora detection by other methods (plating etc.). In 2008-2012 the monitoring results revealed the greatly reduced presence of bacteria in the tested samples (from 0% to 3,6%). Thus, the disease prevention and eradication programmes were very successful in Lithuania by reducing the spread of fire blight in some occasionally infested cases.
Etiology of causative agent fire blight - *Erwinia amylovora* (Burrill) Winston *et al.* - in the southeast of Kazakhstan

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Phytosanitary monitoring of apple and pear orchards were conducted in the farms of southeast of Kazakhstan. The samples of different parts of trees (branches, bark, fruits) with typical fire blight symptoms were selected for laboratory analysis. The causative agent was isolated in pure culture and the isolates were assayed for pathogenicity by the means of infectious-filtration as proposed by Clement as well as the method of Uetta on immature pear fruit. The study of morphological, cultural and pathogenic properties of the isolates revealed their identity *Erwinia amylovora*. The results were confirmed by experts of the All-Russia Quarantine Center (Moscow).

GM crops, antibiotics and pesticides: good or evil and for whom? A case study with apple and fire blight

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Fire blight (FB), caused by *Erwinia amylovora*, is probably the most destructive bacterial disease in apple and pear orchards worldwide. In Switzerland, the first observation of this pathogen dates 1989 on *Cotoneaster* sp. After this first observation, the disease spread in most of the north and central Swiss regions, reaching the peak in 2007. Due to the highly destructive nature of this pathogen, quarantine and eradication measures were promptly adopted. In 2008 the Federal Office for Agriculture permitted the use of streptomycin in pear and apple orchards to control the FB disease and in 2009 the Swiss Expert Committee for Biosafety (SECB) initiated a monitoring project for the period of 3 years to assess the evolution of antibiotic resistance upon streptomycin application. Besides streptomycin also other control measures (biocontrol agents and phytosanitary products) are used against this bacterial disease depending on the cultural praxis (Bio or IP production) adopted in the orchard. Another important control strategy would be the production of resistant cultivars. What is missing until today is a study comparing all these different control strategies for potential risks from the production to the consumption. Therefore we will perform a study based on literature researches and interviews with experts chosen depending their field of expertise (e.g. genetic transformation, breeding, antibiotic resistance, plant protection, human health and environmental security). This study aims at comparing different FB-control strategies (biological control, chemical control, control by antibiotics and by resistant cultivars obtained through classical or genetic engineering breeding) within the frame of most commonly used cultural practices (IP and Bio) in Swiss apple orchards. Special emphasis is given to biosafety aspects and to the protection goals: durability of FB-control measures, FB-free agricultural crops and environment, preservation of cultural diversity and diversity of cultural practices, marketable products, protection of workers, consumers and the environment.
Fire blight in Latvia: occurrence, management and problems

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Fire blight is one of the most destructive and invasive diseases in a wide range of commercial fruit species, ornamentals and wild Rosaceae host plants. Since its first detection in England more than 50 years ago it has spread almost in all European countries and established in most of the regions despite of the quarantine status, restrictions of planting material movement and eradication actions. State Plant Protection Service has carried out monitoring and tests for fire blight in Latvia since 1998. From 2004 the whole territory of Latvia has status of Zona Protecta. The first fire blight outbreak in Latvia was detected in 2007 and the disease was detected in 26 localities including commercial orchards, home gardens, ornamentals and wild stands in eight counties. The strict eradication actions were applied in infected zones in 2007, where more than 2500 trees in commercial and home gardens were eradicated. The eradication was continued and in 2012 no positive cases were detected by NPPO. However, taking into the consideration the movement and spread of fire blight across the Europe and other regions of the world, the question remains: the positive cases were not detected and disease was not observed due to the successful eradication or due to the its sporadic character and lack of favorable weather conditions for development and new outbreaks will occur in the coming years?

The detection of Erwinia amylovora (Burr.) Winslow et al. strains, causing fire blight in pome fruit trees in Bursa

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Fire blight caused by Erwinia amylovora (Burr.) Winslow et al., is the most serious and destructive bacterial disease of pome fruit trees. In 2011, in Bursa, bacterial isolations were done from different host organisms (apple, pear, quince) and their various cultivars belonging to Rosaceae family. A total of 311 E. amylovora strains which were characterized on the basis of biochemical and pathological tests isolated from pear, apple and quince in our study. All strains were gram negative, produced levans types colonies on SNA (saccharose nutrient agar) and MS (Miller and Schrot) also occurred orange colonies on MS, too. Milky ooze was determined on immature pear slices in pathogenicity test. In the diagnosis of the pathogen, growth in King B broth medium which was non-fluorescent. Bacteria were negative for oxidase, growth in 36°C, indole production, generation of H₂S from cystine; positive for catalase, gelatin hydrolyze. Oxidative/fermentative tests result oxidative. In the study, 311 plant samples were collected, as a result of biochemical and pathological tests, 120 bacterial strains isolated. Key words: Fire blight, Erwinia amylovora, apple, pear, quince

Assessment of the sanitary status of pome fruit crops in Kosovo, with particular emphasis to the bacterial disease the fire blight

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Pome fruits represent a very important fruit crops in Kosovo, covering around 50% of the total fruit production. Economic losses induced by Erwinia amylovora were reported in neighbouring countries. In order to understand the real situation of the bacterial diseases on pome fruits in the Kosovo, assessment was carried out for detecting 3 bacteria (E. amylovora, Pseudomonas syringae pv. syringae, Pseudomonas syringae pv. papulans) on apple and pear. Morphological, biochemical (LOPAT test) and molecular (rep-PCR) tests were performed. This survey showed that several bacteria were present and in some cases widely distributed. Furthermore, this current sanitary status could be worsened due to the wide distribution of E. amylovora, which seems to be widely distributed on apple and pear in the different cultivated areas of the country.
Role of *Erwinia amylovora* plasmids pEA29 and pEI70 on the expression of chromosomal genes during symptoms development

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*Erwinia amylovora* is considered a genetically homogeneous species based on physiological, biochemical, phylogenetic and genetic analysis. The major differences found in the genomes of several strains are based on extrachromosomal material and the plasmids seem to be the main cause of genetic diversity among *E. amylovora* strains. Plasmid pEA29, almost universal in this species, has been studied for years, several genes identified as important for the virulence of *E. amylovora* and the loss of the plasmid was related to a decrease in symptoms. Another plasmid recently discovered, pEI70, widespread in European isolates, provides a similar effect on the increase of fire blight symptoms when introduced in some strains, despite its different gene content compared to pEA29. Currently, it is still unknown how plasmids that play such important roles in *E. amylovora* are influencing the expression of the genes of this bacterium. Here, we describe the use of a microarray for studying the expression of chromosomal genes of strain CFBP 1430 in presence and absence of plasmids pEA29 and pEI70. It was designed with probes from strain ATCC99446 that includes genes from the genome and also from the most common plasmids found in this pathogen (pEA29, pEL60, pEU30 and pEI70). Several independent microarray experiments were performed using RNA from exudates obtained in immature pear fruits. The results show that in presence of pEA29, 38 genes from the chromosome were up regulated whereas 142 were down regulated, with fold-change expression ratios greater than 1.5. In the case of pEI70, its presence increased the expression of 60 genes and 60 other genes were down regulated. The functional analysis of these gene expression experiments will provide additional clues about pathogenicity factors related to plasmids, filling an important gap in the knowledge of the virulence of this species.

Structural studies of pathogenicity associated proteins from *Erwinia amylovora*

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The ability of phytopathogenic bacteria to successfully cause infection requires the subversion of its host’s defences and exploitation of its host’s resources. This involves a complicated network of interactions at the molecular scale. Many proteins from *Erwinia amylovora* have been associated with its ability to infect a wide host range, but the specific functions for many of these proteins remain unknown or poorly understood. Furthermore, many of these proteins do not have homologs of known function. Structural characterisation of *E. amylovora* proteins remains largely unstudied. This study seeks to characterise several pathogenicity associated proteins of *E. amylovora* through determination of their structures, primarily by x-ray crystallography. The mode of action of these proteins may be derived from these structures, to further our understanding of how *E. amylovora* causes the fire blight disease.

Biosynthesis of ovothiol A by *Erwinia amylovora* and *Erwinia tasmaniensis*

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Ovothiol A is a N-N-methyl-5-thiohistidine that we detected in both *Erwinia amylovora* and *Erwinia tasmaniensis*. Based on the antioxidant properties of this compound, it is conceivable that ovothiol A plays a considerable role to protect *Erwinia* spp. against oxidative species produced by plants in defense of microbial infection. We will present our progress on deciphering the biosynthetic pathway of ovothiol A and its physiological role in *Erwinia* spp.
Characterization of *Erwinia amylovora* strains isolated from quince trees in Serbia using REP-PCR method

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In Serbia, quince is traditionally grown using predominant native cultivars Leskovacka and Vranjska (as a polinator). However, quince proved to be very susceptible to *Erwinia amylovora*, resulting in destruction of whole orchards as well as individual trees in crofts. To support that fact, the first report of *E. amylovora* in Serbia was registered in quince as a host plant in 1989. A severe occurrence of fire blight in quince orchards was registered between 2010-2012 in Southeastern, Western and Central Serbia. At the same time, sporadic occurrence of this disease was observed in pear orchards, while apple trees remained symptomless. Therefore, the aim of this study was to investigate population of *E. amylovora* strains isolated from quince trees in Serbia and compare their characteristics with the bacterium reference strains NCPPB 595 and CFBP 1430, as well as with Serbian *E. amylovora* strains isolated from other hosts (pear, medlar etc.) in order to determine whether strains from quince trees represent a specific population of the bacterium. First symptoms of the disease were recorded after the blooming period, expressed as necrosis of immature fruits and shoot blight. The highest disease severity was observed in cultivars Leskovacka and Vranjska, while cultivar Triumph exhibited disease symptoms to a lesser degree. Characterization of isolated strains was conducted based on pathogenicity tests, biochemical characteristics and REP PCR. Colonies of the bacterium were levan forming on NSA medium and nonfluorescent on KB medium. Strains induced HR on tobacco leaves, and necrosis of immature pear fruit followed by occurrence of exudate as well as necrosis of inoculated pear seedlings. The results of biochemical tests of the bacterium were as follows: oxidase negative; catalase positive; gelatine hydrolysis positive, starch and aesculin hydrolysis negative; acid produced from glucose, fructose, galactose and sucrose. All strains were susceptible to copper-hydroxide in *in vitro* tests. REP PCR showed no difference between *E. amylovora* strains isolated from quince trees belonging to different cultivars and from different localities in Serbia. In addition, Serbian strains showed identical genetic profiles with the bacterium reference strains as well as strains obtained from other hosts of the bacterium. The results obtained in this study implied that severe occurrence of fire blight was caused by ecological conditions favorable for the disease development during the blooming period when the plants are most susceptible to infection.

**pEA68 – a novel plasmid of *Erwinia amylovora***

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A new plasmid called pEA68 was found in *Erwinia amylovora* strain 692, which was isolated from a diseased shoot of *Sorbus* in Poland in 1997. The plasmid was first detected with RFLP analysis of plasmid DNA of strain 692 digested with *Bam*HI. The sequence of the plasmid showed that it is circular and consists of 68,761 bp with a 60.37% G+C content. Annotation revealed that the plasmid contains 68 predicted CDS with a *repAB* system of replication and two mobile elements: insertion sequences IS26 and IS100. Among the CDSs, two operons associated with mobility were found: *tra* genes encoding for conjugal transfer and *pil* region encoding a putative type IV fimbrial system. The plasmid did not possess any genes associated with antibiotic resistance. Blast analysis of the sequence of pEA68 and predicted amino acid sequences of coding regions showed that it is similar to pEA72 isolated from *E. amylovora* strain ATCC 49946 (GenBank: FN666577). The majority of the genes of pEA68 with known homologies were related to genes found on plasmids of members of different genera of the γ-proteobacteria. Over 450 isolates of *E. amylovora* from five European countries (Belgium, The Netherlands, Serbia, Bulgaria, and Poland) and the Pacific Northwest of the USA were tested for the presence of pEA68 by PCR and/or *Bam*HI patterns. pEA68 was found only in two strains isolated from *Cydonia* in Belgium in 2002. The influence of pEA68 in pathogenicity and virulence of *E. amylovora* strain 692 will be tested.

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The HrpL regulon in *Erwinia amylovora* Ea1189 includes two virulence factors (YdcN and NlpI) involved in regulating biofilm formation and motility

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*Erwinia amylovora* is the causal agent of fire blight and is one of the most significant pathogens affecting apple and pear production world-wide. While *E. amylovora* exhibits an array of virulence mechanism, little is known about the deployment of virulence factors at spatial and temporal scales during *E. amylovora* infection. Our current model for fire blight disease development emphasizes the role of two pathogenicity factors: the type three secretion system (T3SS) and biofilm formation. The T3SS is hypothesized to facilitate interactions with herbaceous tissues near the site of infection early during pathogenesis. Biofilm formation contributes to the colonization of the host vasculature and to systemic fire blight and biofilm-related genes appear to be needed late during disease development. To dissect the temporal nature of fire blight disease development, *hrpL*, a master regulator of the T3SS, was subjected to *in vitro* microarray analysis. While transcript accumulation in Ea1189*ΔhrpL* relative to wild-type Ea1189 confirmed the role of HrpL in regulating the T3SS at early time points, HrpL failed to regulate the T3SS at later time points. In turn, multiple non-T3SS genes were found to exhibit HrpL-dependent transcriptional activity including *ydcN* (EAM_1248) and *nlpI* (EAM_3066). HrpL positively regulated *ydcN* while negatively impacting *nlpI* transcript abundance. As *ydcN* is a predicted transcription regulator, Ea1189*ΔydcN* was subjected to microarray and quantitative PCR analyses revealing that *ydcN* negatively regulates *nlpI* transcription. This suggests that HrpL-mediated regulation of *nlpI* is likely due to the intermediary regulator *ydcN*. To understand the role of *ydcN* and *nlpI* during infection, Ea1189*ΔydcN* and Ea1189*ΔydcN* were subjected to multiple phenotypic analyses. Results indicate that both *ydcN* and *nlpI* are virulence factors required for full disease development in immature pears. In addition, *ydcN* and *nlpI* promote swarming motility on soft agar plates while inhibiting biofilm production during flow cell analyses. Collectively these results suggest that HrpL may play a previously unknown role in regulating biofilm formation late in infection.

Towards triggering iron starvation in *Erwinia amylovora*: biophysical, biochemical and structural characterization of the desferoxamine metabolism gene products

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A deep knowledge about the molecular mechanisms behind the pathogenesis is thought to be essential for a specific and efficient treatment of the fire blight disease. Considering the antibiotic resistance acquisition of some *E.amylovora* strains and the worldwide fire blight distribution, it becomes easy to understand that the need for a proper treatment is urgent and economically relevant. A potentially very effective strategy to fight the fire blight would be to create specific inhibitors of the *E. amylovora* enzymes necessary for the infection. It is therefore essential to study the proteins involved in the pathogenesis, such as the proteins involved in the extracellular polysaccharide (EPS) formation (*ams* genes products); the hypersensitive response and pathogenicity proteins (*hrp* genes products). Besides, numerous other factors are necessary to overcome nutrient limitation and thereby considered good targets: desferrooxamine metabolism proteins (*dfo* cluster products); sorbitol metabolism proteins (*srl* gene products); sucrose metabolism proteins (*src* gene products). My project focuses on the biochemical, biophysical and structural characterization of the protein involved in the desferrooxamine (DFO) siderophore biosynthesis (*dfo* genes: DfoJ, DfoA, DfoC) and uptake (FoxR). Particularly, during flowers infection, DFO is essential for the acquisition of the iron ions necessary to the bacterium metabolism and protects the pathogen against lethal doses of hydrogen peroxide and other ROSs produced by the plant as defence. I created several plasmidic constructs starting from the wild type *dfo* genes amplified from *E. amylovora* and the relative products have been expressed with different fusion tags. For each construct a purification test has been carried out with promising results. Next steps will be: a) Optimization of the purification protocols for each target b) Large scale expressions and purification c) Biophysical, biophysical and structural characterization. The final aim of our research group, which is involved in studying numerous targets involved in *E. amylovora* pathogenesis, is the design of multiple specific molecular inhibitors in order to block the infection. The overall work might lead us towards an efficient control of the fire blight.
Molecular responses of *Erwinia amylovora* to starvation at environmental temperatures

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*Erwinia amylovora* persistence in nature depends on its ability to withstand and to overcome the characteristic oligotrophic conditions of most environments. Temperature is another abiotic factor affecting bacterial survivability under natural conditions. *E. amylovora* responds to nutrient scarcity by adopting both the starvation-survival and the viable but nonculturable (VBNC) states, but the ratio of bacterial cells sub-population adopting one or the other responses is strongly temperature dependent. With the aim to characterize the molecular responses of *E. amylovora* to starvation at environmental temperatures, natural water oligotrophic microcosms incubated at 20°C and 37°C were inoculated and the expression of starvation (rpoS, cstA, dps), oxidative stress (oxyR, katA, katG) and virulence/pathogenicity related (hrpL, rcsB, rlsA y dfoA) genes monitored for seven days by semi-quantitative RT-PCR using the 16S rRNA gene (rrs) as a control. In parallel, culturable, viable, and total cell population dynamics as well as pathogenicity in immature fruits were monitored during the same period. Most of the analyzed genes showed a modulation of their expression during the first 24 h of exposure to starvation at 20°C, reaching a steady state that continued until the end of the study. Culturable cell populations remained nearly constant during the assayed period at this temperature, and cells retained pathogenicity. Incubation at 37°C induced a pronounced down-regulation of all the assayed genes (with the exception of the control), which was correlated to the progressive entry of cells into the VBNC state and a decrease of virulence. Ultimately we observed a complete loss of pathogenicity when gene expression and culturability became undetectable (the entire culturable cell populations entered the VBNC state). These results contribute to a better understanding of the behavior of the fire blight pathogen under conditions resembling natural environments outside the host where starvation prevails.

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pEA27 in *Erwinia amylovora* from orchards in the Pacific Northwest, USA

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Genome sequences of isolates of *Erwinia amylovora* from the Pacific Northwest (PNW) region of the USA revealed the presence of a novel plasmid called pEA27. The plasmid has a ColE1-type replicon and is thus lacking repA. Analysis of the sequence of the plasmid relaxase gene indicates that pEA27 is a member of the MOB₉₁₁ family. The plasmid has a *trb-tra* system that likely is involved in mobilization. Genes associated with antibiotic resistance or tolerance to ultraviolet radiation were not detected on the plasmid. Previously, we confirmed the presence of pEU30 in numerous isolates of *E. amylovora* from the PNW and also reported that some isolates of the pathogen lack pEA29, the native plasmid of *E. amylovora*. pEA27 was detected in several isolates of the fire blight pathogen from Washington state, including some lacking pEA29, with a PCR assay. In addition, the novel plasmid pEA27 was detected in several isolates that also carried pEU30. It is not known if pEA27 and pEU30 were acquired simultaneously or sequentially over time. Currently, only 50 isolates of *E. amylovora* from the PNW have been tested for pEA27. Additional isolates will be tested to gain a better understanding of the distribution of pEA27 among isolates of *E. amylovora*. The results illustrate that *E. amylovora* has the capacity to acquire and maintain several plasmids in the orchard environment.
Genome sequences of *Erwinia amylovora* from Mexico

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In Mexico, apple production is centralized in the northern states of Chihuahua, Durango, and Coahuila. Fire blight in Mexico is managed routinely with three applications of oxytetracycline during bloom. In years with several disease risk periods, antibiotics may be applied up to seven times. In addition to oxytetracycline, about 30% of the orchards are treated with gentamicin. Streptomycin is not used routinely for fire blight control in Mexico because the antibiotic is considered ineffective due to widespread resistance. We isolated *Erwinia amylovora* from Golden Delicious apple tissues with symptoms of fire blight from two orchards near Cuauhtemoc and one near Creel in the state of Chihuahua, Mexico. Identity of the isolates as *E. amylovora* was confirmed with molecular assays and the Ea-AgriStrip of Bioreba. Antibiotic resistance was assayed in culture media. None of the isolates of the pathogen were resistant to oxytetracycline or gentamicin. The presence of streptomycin-resistant isolates of the pathogen varied among the orchards sampled. All isolates of the pathogen in one orchard in Cuauhtemoc were sensitive to streptomycin, whereas nearly every isolate of the pathogen was resistant to the antibiotic in another orchard located six km away. Another orchard near Creel, harboured a mixture of streptomycin-resistant and -sensitive isolates of the pathogen. Sequence of rpsL of selected isolates revealed the K43R mutation, which is associated with spontaneous mutation to resistance to streptomycin. One isolate from each orchard was sequenced on an Illumina HiSeq 2000 at the CGRB Core Lab at Oregon State University. Final assembly and annotation of the genomes are in progress.

Evolutionary genomics of *Erwinia* to elucidate virulence factors of the plant pathogen *Erwinia amylovora*

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Evolutionary genomics of the enterobacterial genus *Erwinia* is coming into focus with accumulating availability of complete genome sequences. *Erwinia amylovora* causes fire blight, a major disease threat to pome fruit production worldwide with further impact on a wide-range of *Rosaceae* species. Although important insights have been acquired regarding this important phytopathogenic bacterium, much remains uncertain about the evolutionary genetics of *E. amylovora*. The recent genome sequencing of six *Erwinia* species provides a solid genomics foundation to infer the evolution of these species within the genus *Erwinia*. A genealogy based on the core and assayory gene pools was generated explaining the origin and evolution of the *Erwinia* spp., which agrees with the phylogeny of these species, but introduces additional levels of differentiation. We propose *E. amylovora*, *E. pyrifoliae*, *E. piriflorinigrans* and *E. tasmaniensis* to be descendants of a pathoadapted *Erwinia* ancestor that has undergone genome reduction and acquired several traits involved in pathogenicity. Moreover, at the level of species delineation, some traits were introduced or lost, giving rise to species-specific phenotypes. Using comparative genomics, we can now start finding the answer to what gave *E. amylovora* the evolutionary edge to become such a successful pathogen based on species-specific virulence, host range, or fitness determinants. The ultimate aim is to identify novel targets for pathogen suppression that can be integrated into fire blight control strategies.
Structural and functional characterization of GalE, an epimerase involved in amylovoran biosynthesis

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One of the main pathogenicity factors of Erwinia amylovora is the exopolysaccharide amylovoran, composed of galactose, glucuronic acid and pyruvate. E. amylovora strains deficient in amylovoran biosynthesis were found to lose pathogenicity. The amylovoran biosynthesis gene cluster (ams) comprises 12 genes mainly encoding for glycosyl transferases. Besides the ams gene cluster proteins, GalE (UDP-glucose 4-epimerase) is of key importance in amylovoran biosynthesis as it catalyses the reversible epimerization of UDP-glucose to UDP-galactose one of the main amylovoran building blocks. On the other hand glucose is converted by UDP-glucose dehydrogenase to glucuronic acid, another essential molecule for amylovoran production. Therefore GalE with its reversible reaction exerts a pivotal role in E. amylovora pathogenicity.

Aim of this study is to compare structural and catalytic properties of the enzyme from E. amylovora with GalE isoforms from the non-phytopathogenic Erwinia tasmaniensis and the virulent phytopathogenic Erwinia pyrifoliae. Crystallization trials are currently ongoing as well as the characterization of the enzymes in solution by NMR spectroscopy.

The GrrSA-Csr global regulatory system plays a critical role in Erwinia amylovora virulence

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Extensive genetic studies in the past decade or so have demonstrated that a functional hypersensitive response and pathogenicity (hpr) - type III secretion system (T3SS) and its associated effectors, as well as production of the exopolysaccharide amylovoran are primary determinants in E. amylovora to cause fire blight disease. We have also reported that the GrrS/GrrA two-component phosphorelay system negatively regulates motility, amylovoran production and expression of T3SS genes in E. amylovora. To further understand the molecular mechanism as how GrrS/GrrA regulates various virulence traits, we generated and characterized csrB/rsmB and csrA/rsmA mutants. CsrA(carbon storage regulator) /RsmA(Regulator of secondary metabolites) is an RNA-binding protein, whereas csrB/rsmB is a small regulatory RNA. Results showed that csrB/rsmB mutant was hypermotile, produced higher amount of amylovoran than that of wild type strain, and had increased expression of T3SS genes in vitro, which are the same as reported for the grrA/grrS mutants. In contrast, csrA/rsmA mutant exhibited complete opposite phenotypes, including positive regulation of motility and expression of T3SS genes. Furthermore, csrA/rsmA mutant did not induce hypersensitive response on non-host tobacco plants or caused disease on immature pear fruit and apple shoots, indicating that CsrA/RsmA is a positive regulator of virulence factors. These findings demonstrated that negative regulation of virulence factors by GrrS/GrrA acts through csrB/rsmB small non-coding RNA, which likely binds to translational regulator CsrA/RsmA and neutralizes its positive effect on T3SS gene expression, flagellar formation and amylovoran production.
Contrast generation of hydrogen peroxide and superoxide radicals in pears following attack of *Erwinia amylovora*

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Responses of host cells that lead to the susceptibility level against *Erwinia amylovora* attack has not well studied. Resistance level of pear rootstocks OHF40, OHF87, OHF69, OHF333 and two control pear cultivars, Bartlett (susceptible) and Harrow Sweet (resistant), were studied by pathogen inoculation and evaluation of necrosis progress rate of in vitro shootlets. Also, generation of hydrogen peroxide (H₂O₂) and superoxide (O₂⁻) radicals were traced in response to the attack of pathogen. Pear shootlets were inoculated with *E. amylovora* type-strain Ea273 and the disease progress was followed for 144 h after inoculation. H₂O₂ and O₂⁻ generations were traced with use of DAB (Diaminobenzidine) and NBT (Nitro Blue Tetrazolium), respectively, at the same period of time. Results indicated a classification of resistance from OHF69>OHF87>Harrow Sweet>OHF333>Bartlett>OHF40, respectively. H₂O₂ production demonstrated inverse relation with necrosis appearance, so more tolerant cultivars and rootstocks had faster H₂O₂ generation and slower necrosis progress. O₂⁻ production expressed a contrast pattern from H₂O₂ in resistant and susceptible pears. Regarding to the pattern of ROS generation, it is likely that H₂O₂ play more inhibitory role than destructive against pathogen, while role of O₂⁻ are significantly more destructive than H₂O₂ for hosts tissues.

Tail-associated depolymerase of T7-like phage L1 is essential for adsorption to *Erwinia amylovora* and synergistically enhances Y2 phage efficacy

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The depolymerase encoded by T7-like *E. amylovora* phage L1 (DpoL1) was characterized in this study. It efficiently degrades extracellular polysaccharides (EPS) of *E. amylovora* cells and was found to be most active at pH 6 and 50°C. ESI-MS analysis demonstrated that DpoL1 cleaves the galactose backbone of amylovoran. The enzyme has a modular architecture indicating horizontal gene transfer. The N-terminal domain appears to mediate attachment to the phage-particle, as typical for T7-like phages, while the C-terminus exhibits enzymatic function. Deletion of the 180 N-terminal amino acids did not abolish enzyme activity. Electron microscopy showed that DpoL1 specific antibodies cross-link phage particles at their tails either lateral or frontal. Antibody labeling with gold-conjugated secondary antibodies confirmed that DpoL1 is located at the tail spikes of L1. Combination of DpoL1 with the virulent myovirus Y2 resulted in synergistic increased growth inhibition of a high EPS-producing *E. amylovora* strain CFBP1430. No such enhanced efficacy was observed against an EPS-mutant and a low EPS-producing *E. amylovora* strain 4/82. This suggests that the synergistic effect is due to enzymatic removal of the EPS-capsule as a physical barrier to Y2 infection and rapid multiplication. Since *E. amylovora* exopolysaccharides play an important role in pathogen fitness and virulence, DpoL1 and DpoL1/phage cocktails hold potential for suppression of fire blight development.
Type III secretion chaperones and HrpN from *Erwinia amylovora* interact with several effector proteins and modulate their translocation to the plant cell

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The delivery of effector proteins through the type III secretion system (TTSS) is a major pathogenicity determinant of pathogenic bacteria. Type III secretion (TTS) chaperones, are small, acidic cytoplasmic proteins often found to be associated with TTS effector proteins, stabilizing them in the bacterial cytoplasm and promoting their successful delivery to plant cells. Moreover, several TTS chaperones have been demonstrated to interact with more than one substrate including not only effectors but also regulatory proteins and components of the TTSS. *Erwinia amylovora* secretes several effector proteins including DspA/E, Eop1, Eop3 (HopX1$_{Ea}$) and Eop4 (AvrRpt2$_{Ea}$). In addition, TTS chaperone proteins DspF and Esc1 have been shown to interact with cognate effectors DspA/E and Esc1, respectively. Using yeast two hybrid analyses, we identified functional interactions between effector proteins DspA/E, Eop1, and Eop3 and the TTS chaperones DspF, Esc1 and Esc3, suggesting a multicargo role for these proteins. Moreover, the harpin protein HrpN, was also demonstrated to interact not only with DspA/E and Eop1, but also with the DspF chaperone. Using site-directed mutagenesis, secretome analyses and translocation assays we determined that these TTS chaperone proteins and HrpN play a functional role in regulating the translocation of DspA/E, Eop1, and Eop3 to the plant cell.

Survival of *Erwinia amylovora* in the digestive tract and faeces of honey bees

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Honey bees were fed with sucrose syrup (50%) contaminated with *E. amylovora* (5x10$^7$ cfu/ml). After 24 h bacteria were detected in all parts of the bee digestive tract. Addition of pollen grains (of 1.5x10$^4$ solid parts per ml of syrup) delayed the movement of bacteria in the tract. After 48 h the pathogen was detected in the midgut and hindgut of 3 and 27% of bees fed with syrup, with and without the addition of pollen grains, respectively. The authenticity of the isolated bacteria was confirmed on the basis of their colony morphology on NAS and King’s medium B, O/F reaction, HR on tobacco, and pathogenicity on pear fruitlets.

The introduction of water-diluted faeces filling the rectum, obtained from bees fed with contaminated syrup, on pear fruitlets caused fire blight symptoms on only 7% of treated fruitlet slices. However, symptoms were not observed when bee faeces was not diluted in water. On the other hand, the addition of bee faeces to a *E. amylovora* water suspension caused a 30% reduction of fire blight symptoms on pear fruitlet slices when compared with a pure suspension of bacteria.

The study on *E. amylovora* survival on leaves of apple cv. Szampion trees growing in the greenhouse showed that bacteria survived longer on their surface after deposition in water drops mixed with bee faeces (at least 96 h) than in water drops only (not detected after 72 h).
The epiphytic survival of *Erwinia amylovora* on the apple fruit surface

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Using molecular and conventional detection methods the presence of *Erwinia amylovora* was confirmed on the surface of 4% of tested apple fruits cv. Antonovka picked from moderately infected trees and on 23% apples collected from severely infected cv. James Grieve trees. In another experiment, the survival of *E. amylovora* was tested on the calyx of apple fruits cv. Gala collected from the orchard where fire blight symptoms were not observed. Two hundred µl of *E. amylovora* suspensions at the concentration of $10^3$ or $10^7$ cfu/ml were introduced on the calyx and afterwards apples were stored for 5 months at 3˚C and humidity of 85%. Every month, 10 fruits were tested for the presence of *E. amylovora*. On the fruits which were contaminated with suspension at lower concentration, the pathogen was not detected already after two months, but in case of apples treated with pathogen suspension at higher concentration, *E. amylovora* presence was confirmed even after 5 months of storage. Analysis of the influence of the humidity on the survival of pathogen on calyx showed that the humidity of about 100% was less favorable for bacteria survival than that of about 30%. The longevity of pathogen could be restricted by associated epiphytic microorganisms. We found out that some isolates of bacteria and fungi obtained from tested fruits inhibited the pathogen growth in vitro.

HrpW interacts with NADH dehydrogenase of apple

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During the infection, *Erwinia amylovora* secretes in the host cell at least twelve different effectors, which are involved in the establishment of the disease. Harpins are part of the proteins delivered in the plant cell via the type three secretion system. These proteins are known to be heat stable, glycine rich, without cysteine residues, and trigger a HR in non-host plant. In particular, the mechanism of action of harpin N has been well studied. HrpW, one of the other harpin members, is present in many phytopathogenic bacteria such as *E. amylovora* and *Pseudomonas syringae* pv tomato, and possesses a class III pectate lyase domain, but nobody so far has reported a pectate lyase activity. Also *hrpW* mutants induce symptoms as strong, or even stronger, than wild-type strains, suggesting that this protein, contrarily to HrpN, is not required for a full virulence of the bacteria. However, recently HrpW has been described as a HR negative modulator in non-host plants such as *Nicotiana tabacum* and *Arabidopsis thaliana*. On *Arabidopsis* cell cultures, depending on its concentration, it could trigger, like HrpN, a defense response, or act antagonistically to HrpN, inhibiting cell death and ROS production.

At present nobody has deeply investigated the effect of HrpW in host plants. We undertook this project to identify apple proteins interacting with this effector by screening a yeast two hybrid apple library. We identified several potential protein interactors that have been further tested by Bimolecular Fluorescent Complementation (BiFC). This test in tobacco confirmed that HrpW is able to interact with the N domain of the apple NADH dehydrogenase. Indeed when HrpW and the apple NADH dehydrogenase are co-infiltrated in tobacco an HR response can be observed after 2 days. The Interaction between HrpW and the apple NADH dehydrogenase located in the mitochondria can the ROS production and induce the HR reaction observed in non-host plant. The effect of this interaction in apple is under investigation.
Determination of fire blight disease reactions in loquat varieties in Turkey

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The aim of the study was to determine fire blight disease, caused by *Erwinia amylovora*, reactions. Five different loquat varieties (Akko XIII, Gold Nugget, Sayda, Hafif Cukurgobek and Champagne de Grasse) and 14 *E. amylovora* strains, isolated from different cities of Turkey, were used in the study. Shoot and leaves infections of fire blight disease were evaluated in 2011 and 2012 on these loquat varieties. According to evaluations of two year studies, all loquat varieties were highly susceptible for leaves infections of fire blight disease. Champagne de Grasse, Akko XIII and Hafif Cukurgobek varieties were found as moderately susceptible whereas, Sayda and Gold Nugget varieties were few susceptible for shoot infections of fire blight disease.

The presence of *Erwinia amylovora* in asymptomatic apple bud wood: a threat to newly established apple plantings

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The development of shoot blight in new apple plantings in NY occasionally seems to originate from infections at the site of budding. Budwood for new cultivars and those of limited availability is often collected from orchards where fire blight is established. An investigation was undertaken to examine the presence of *Erwinia amylovora* in the budwood from commercial apple trees used for nursery stock. Budwood was collected from two commercial nursery stock plantings of ‘Gala’ and ‘Topaz’ apples in western NY. Individual replicated collections of buds were made from budwood with differing proximities to shoot blight symptoms and evaluated for the presence of epiphytic and endophytic *E. amylovora*. On both cultivars, virulent *E. amylovora* was recovered from both the surface and the internal contents of buds from asymptomatic shoots. For ‘Topaz’ there were no significant (*P* > 0.05) differences in the frequency of *E. amylovora* recovery in regards to proximity to observed shoot blight symptoms. By contrast, the frequency of ‘Gala’ buds from which *E. amylovora* was recovered from the internal tissues was significantly (*P* = 0.034) higher for shoots within 1 m of a shoot blight strike (>80%) than for buds from shoots more than 20m from a tree with a shoot blight strike (<60%). Although it is unlikely that the majority of such cryptic infections will result in shoot blight, these observations may necessitate a reexamination of budwood collection practices.
Selections for resistance fire blight disease in young F1 hybrid pear seedlings in Turkey

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Fire blight caused by pathogenic bacterium Erwinia amylovora, is the serious disease of pear, and there is no effective disease management. Therefore it is very important to improve new resistant cultivars to fire blight. With this purpose, different crosses (resistant x resistant; resistant x susceptible) have been made between resistant Magness variety and resistant-susceptible cultivars and cultivgins (Akca, Ankara, Bursa, Conference, Guz, Kaiser Alexandre, Kieffer, Moonglow, Tas). The susceptibility levels of hybrids were determined by artificial inoculations by E. amylovora in greenhouse conditions. In pathogenicity tests, 10^8 cfu/ml populations of seven E. amylovora strains, isolated from different cities in Turkey, were used by shoot injections to hybrid plants. Eight weeks after inoculations, the percentage of the necrotic lesion to the total length of the shoot was calculated for each shoot. The experiments were repeated twice in August 2010 and May 2011. The main percentage of two experiments gave the disease severity. Index of the susceptibility was scored as A to E (A: very low susceptibility; B: low susceptibility; C: medium susceptibility; D: high susceptibility; E: very high susceptibility). Within 1242 young F1 hybrid seedlings inoculated, 31.64% of them were “very few susceptible” (A), 8.62% were “few susceptible” (B), 18.60% were “moderately susceptible” (C), 30.27% were “highly susceptible” (D), 10.87% were “very highly susceptible” (E), and 95 of hybrids were destroyed by the pathogen. The resistant 393 F1 hybrids were planted in Eskişehir, middle part of Turkey, for screening in agronomical and pomological characteristics.

Evaluation of traditional pip fruit genetic resources in Switzerland

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The project BEVOG II deals with the description and characterization of fruit varieties present in Swiss introduction collections for fruit genetic resources. The project is part of the program NAP-PGREL (National Action Plan for Plant Genetic Resources for Food and Agriculture). It is a project of the NGO Fructus (www.fructus.ch) and financed by the Federal Government (Federal Office of Agriculture). The project is divided into 4 work modules:

- Pomological characterization with fruit and tree descriptors
- Molecular fingerprints of the accessions to check for synonyms and homonyms
- Evaluation of disease tolerance and susceptibility towards fire blight (Erwinia amylovora), apple scab (Venturia inaequalis), powdery mildew (Podosphaera leucotricha) in selected accessions
- Public relations

Disease evaluation is especially important for possible use of the accessions in breeding and practical fruit-growing.

Search for fire blight tolerant accessions

During the last 6 years around 160 apple and pear accessions were evaluated in the glasshouse shoot tests for their susceptibility towards fire blight. The experiments were conducted in the security glasshouse of Agroscope. Each accession is represented by ten replicated plants grafted on rootstock M9 and BA29 for apple and pear respectively. A bacterial suspension was inoculated with a syringe containing an E. amylovora solution of 10^9 cfu/ml strain FAW610 at the top of a young shoot. Disease progress was measured three times in weekly intervals. The percent lesion length in relation to the total shoot length was calculated. This allows to draw conclusions as to the tolerance or susceptibility of the accession. However, it is not possible to fully conclude on the filed situation where flower infection usually is more relevant. Nevertheless, comparison of shoot tests and additional flower infection tests with the same accessions have resulted in a relatively good correlation. An especially fire blight tolerant Swiss apple accession with the name ‘Alant’ was identified.
Selecting effective indices for evaluation of fire blight resistance in quince germplasm in orchard condition

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Quinces rather have a divergent growth habit from apples and pears. Therefore, evaluation of fire blight susceptibility of quince germplasm by the same indices of other hosts will be uncertain and doubtful. This research was conducted to investigate the efficiency and compare the indices used for evaluation of fire blight susceptibility in orchard condition in quince germplasm. Thirty quince genotypes from Iranian National Quince Collection were selected and the disease severity were monitored by three indices including index of Beltsville, developed by USDA (I USDA), index of susceptibility of varieties (ISV) and index of frequency (IF) during two year of fire blight outbreak, 2011 and 2012. All indices were judged against total percentage of fire blight damage on trees, indexed as IT. Correlation analysis showed that IF had the highest affinity with IT, but each single index demonstrated various efficiency for evaluation the real disease damage and severity. Due to these variant results, the genotypes were classified differently in resistant classes. In order to obtain more reliable judgment, using cluster analysis on all indices demonstrated more effective ranking of fire blight susceptibility in quince.

Key Words: Cydonia oblonga Mill., Erwinia amylovora, Resistant Cultivars, Beltsville Index, Cluster Analysis.

Resistance against fire blight of Gala transformed with a gene from Malus x robusta 5

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The use of natural fire blight (FB) resistances from wild apples to increase the resistance level of new commercial cultivars is an environmental and consumer friendly approach. Little is known about the genetic determinants of the resistance and so far no resistance genes against this disease have been cloned in apple. A major quantitative trait locus (QTL) for resistance against Erwinia amylovora, the causal agent of FB, was identified on linkage group 3 of Malus x robusta 5. Recent results show that the resistant genotype M. x robusta 5 undergoes a gene-for-gene interaction with the pathogen, as a single amino acid mutation in the AvrRpt2$_{EA}$ effector protein of E. amylovora led to a change from avirulence to virulence of the pathogen. A positional cloning of the QTL region was successfully accomplished and a NBS-LRR gene (called FB_MR5) was proposed as candidate resistance gene. Genes belonging to this gene family were often shown to be involved in pathogen recognition. We complemented the FB susceptible cultivar Gala with the FB_MR5 gene via Agrobacterium-mediated transformation. Transgenic Gala lines carrying this gene were regenerated and subjected to artificial E. amylovora inoculation in the greenhouse. Results confirm the functionality of this gene and therefore FB_MR5 is the first FB resistance gene identified and cloned in apple. Currently we are investigating the mechanism of resistance defence initiated by FB_MR5. Using this FB resistance gene together with apple scab resistance genes we are planning to generate cisgenic lines with pyramided resistances. The current state of research is presented.
**Somaclonal variation - usefull tool to improve desease resistance of pear (Pyrus communis L.) rootstock Old Home x Farmingdale (OHF 333)**

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Somaclonal variation is a potentially useful source of genetic variability for plant improvement and could result in new valuable genotypes. Several examples of somaclonal variation resulted in disease resistant forms have been reported in temperate fruit species. In most of these cases, somaclonal variation was spontaneous and observed after regeneration from adventitious buds or somatic embryos. The aim of our study was to obtain somaclones of pear (Pyrus communis L.) rootstock Old Home x Farmingdale (OHF 333) with resistance/tolerance to the fire blight. The artificial inoculation of this rootstock with local strain of *Erwinia amylovora* showed that 50% of examined plants demonstrate low degree of visible symptoms of disease and 10 % - high degree of visible symptoms. In order to obtain genotypes resistant/tolerant to *E. amylovora* we developed an efficient system for shoot regeneration from in vitro leaf explants. All obtained regenerants were cloned, propagated, rooted and acclimatized to greenhouse conditions. Somaclones were tested for resistance to fire blight (*E. amylovora*). It was found out that ten of the studied clones demonstrated a low susceptibility/tolerance to the fire blight. The field tests of the selected genotypes are in progress.

**In vitro evaluation of cultivar resistance to fire blight**

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Pyrus communis L. is a globally important vegetatively propagated fruit species, which is widely cultivated in Central Europe. Unfortunately field grown trees and trees in nurseries are affected by many pathogens. Especially fire blight, which is highly destructive and increasingly important contagious disease caused by a bacterium *Erwinia amylovora*, is a serious threat for commercial production of pears. During the last four decades, pathogen has spread throughout Europe including the Czech Republic. The aim of the present study was to test the resistance of eight selected pear cultivars against *E. amylovora* in *in vitro* culture conditions. Pear *in vitro* shoots were grown on MS medium with 1 mg L⁻¹ IBA (indole-3-butyric acid) and 0.1 mg L⁻¹ BAP (6-benzylaminopurine). The isolate of *E. amylovora* (Ea 8/95) with stable virulence was used in this study. After careful removal of 3 mm of the shoot apex with scissors, the bacterial suspension in concentration 10⁶ colony forming units (cfu)/ml was applied to the cut area by pipette. The evaluation was done 3, 5 and 9 days after inoculation. On the basis of obtained results, cultivar ‘Margeurite Marillat’ was the most resistant and ‘Salisbury’ the most susceptible. Results of artificial inoculation of *in vitro* cultures by *E. amylovora* indicate the possibility of using *in vitro* plants for testing of pear resistance to fire blight.
Improvement of system for testing of pome fruit resistance to *Erwinia amylovora* using *in vitro* artificial inoculation

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Fire blight, a destructive bacterial disease presents potential threat to apple and pear industries throughout the whole Europe. Specific, safe and reliable methods for identifying the pathogen *Erwinia amylovora* and a consistent system for cultivar resistance testing can be very important factors in the management of the disease. Therefore a screening system for testing of pome fruit resistance to *E. amylovora* was developed based on *in vitro* artificial inoculation. Selected pome fruit genotypes were successfully established *in vitro* using mercuric chloride in a concentration of 0.15% as a sterilization solution. MS medium according to Murashige and Skoog (1962) with addition of cytokinins BAP (2 and 4 mg L⁻¹) or TDZ (0.5 and 1 mg L⁻¹) proved to be suitable for *in vitro* proliferation. We obtained multiplication coefficient higher than 1.5 for all tested pome fruit genotypes. After the phase of multiplication, three MS based media were tested for shoot elongation: full MS medium with 1 mg L⁻¹ IBA and 0.1 mg L⁻¹ BAP; 50 % MS with 1 mg L⁻¹ IBA and MS medium with macronutrients reduced to 40 % and micronutrients reduced to 35 % with 2.5 mg L⁻¹ IBA. The aim in this phase was to obtain actively growing in vitro shoots elongated to at least 2.0 cm for experiments with *in vitro* artificial inoculation of *E. amylovora*. Out of the tested media, only full MS medium with 1 mg L⁻¹ IBA and 0.1 mg L⁻¹ BAP produced actively growing shoots longer than 2 cm suitable for monitoring of the development of bacterial lesions. Obtained results indicate the possibility of using in vitro plants for testing of pome fruit for resistance to fire blight.

Testing of susceptibility level of apple cultivars to fire blight (*Erwinia amylovora*) in field conditions

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Ten older apple cultivars and Czech landraces and two commercially grown varieties (´Selena´, ´Golden Delicious´) as standards were tested for their relative susceptibility level to fire blight after artificial inoculation in the climatic conditions of the Czech Republic. The experiment was established in a screen-house covered with insect proof net. The artificial inoculation was carried out by cutting of the apical part of one year old shoot tips with scissors dipped in a bacterial suspension of mix isolates of *Erwinia amylovora* (concentration 10⁶ cfu/ml) in suitable climatic conditions (temperature > 15°C, relative humidity > 75%). Evaluation of susceptibility level was performed 40 days after inoculation according to the percentage of necrotic lesion development. Calculated intensity of infection was consequently transferred to 6 point evaluation scale: highly resistant 0.0 - 7.0%, resistant 7.1 - 13.0%, moderately resistant 13.1 – 26.0%, moderately susceptible 26.1 - 60.0%, susceptible 60.1 – 80.0% and highly susceptible 80.1 – 100.0%. From tested genotypes, only ´Selena´ was evaluated as resistant (11.2% infection intensity). The remaining genotypes were categorized as moderately susceptible, susceptible or highly susceptible.
Increased whole-tree fire blight tolerance through the right rootstock/scion combination

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Experiences made in the USA in commercial Royal Gala apple orchards infected with fire blight (Erwinia amylovora) suggested that trees grafted on fire-blight tolerant rootstocks were less affected by the infection compared to the standard M9-type rootstocks. As in organic fruit production streptomycin containing control agents are not permitted, and alternative natural products, so far, show only partial efficacy, all preventive measures avoiding E. amylovora infection and damage – primarily cultivar choice - are of high importance. The central hypothesis we tested in this study is: “Specific apple and pear rootstocks enhance the tolerance of the whole tree when the scion gets infected with E. amylovora; whereas the effect is more expressed when both plant parts, rootstock and scion have a certain E. amylovora tolerance”. If this is true, fruit growers wouldn’t entirely depend on highly tolerant cultivars but could also work with medium tolerant cultivars in combination with tolerant rootstocks. Our studies with over 40 full-factorial rootstock x cultivar combinations with apple and 30 with pear over 4 years with scion infections on freshly grafted, potted trees under greenhouse conditions repeatedly confirmed the hypothesis with apples but less with pears: i) tolerant rootstocks (e.g. CG 41, CG 11, A-296 and B.9) could reduce the fire blight infection length compared to susceptible rootstocks (e.g. M.9 or Supporter 2); and ii) this effect was more expressed in combination with tolerant cultivars (e.g. Ariane, Rewena, Galiwa) but less or not significant with susceptible cultivars (e.g. Gala, Topaz, Natyra).

Are chloroplasts key organelles for determining susceptibility of apple and pear genotypes to fire blight?

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Primary reports demonstrated role of electron transport chain (ETC) of chloroplasts in course of Erwinia amylovora/hosts interaction. This research was carried out to approve the role of chloroplast in this interaction and to determine the eventual site of pathogen effector protein(s) influence. Effects of several inhibitors of chloroplasts ETC including methylviologen, Glutaraldehyde and Diuron, with various sites of inhibition were evaluated on in vitro shootlets of apples, MM-111 (resistant) and MM-106 (semi-susceptible) and pears, Harrow Sweet (resistant) and Spadona (semi-susceptible). Distinctness of necrosis causes by inhibitory effects of ETC inhibitors from those of pathogen was accomplish in a series of primary experiments in absence of pathogen and removed sucrose from growth media for activation of ETCs in chloroplasts. These results presented at least 168 h free of necrosis of ETC inhibitors for study of host/pathogen interactions. Effects of ETC inhibitors were evaluated by necrosis of pathogen after inoculation of shootlets and showed that ETC inhibitors retarded symptom progress in all tested cultivars. Interestingly, responses of resistant and susceptible cultivars were divergent and varied after exposure to the inhibitors. These retardant effects were more considerable in susceptible than resistant cultivars. The results confirm previous results observed by uracil inhibition of chloroplasts and represent more evidence for role of this chain in host/E. amylovora interaction. In addition, according to the putative site of inhibitions, NADP reduction complex is the most probable site of effector proteins interaction in chloroplasts.

Key words: Fire Blight, Chloroplasts, Electron Transport Chain, Inhibitors.
Expression of some PR genes of apples in responses to attack of Erwinia amylovora

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Pathogenesis related genes play important role in defense strategies of plants against biotic stresses. In order to evaluate responses of apples to Erwinia amylovora, expression of some candidate PR genes from chitinase family including PR3-Ch2, PR3-Ch4, PR3-Ch5 and catalase-I was studied in a 72 h time course of host/pathogen interaction. In vitro grown shootlets of MM-111 (resistant) and MM-106 (semi susceptible) were inoculated by strain Ea273 of pathogen and sampling for RNA extraction followed at 0, 18, 31, 48 and 72 h after inoculation. RNA extraction and purification successfully achieved by using lithium chloride method and use of DNase I. Specific primers for real time expression studies were designed following isolation, sequencing and deposition of candidate genes, respecting maximum 150 to 200 bp length for each EST and using elongation factor1α (ef1α) as reference gene in all reactions. The results firstly showed that 31 h after inoculation is a threshold point of variation in expression of candidate defense genes in apples. Interestingly, in MM-106, expression of catalase-I gene was significantly higher than more resistant apple, MM-111. This expression pattern is likely subsidiary of reactive oxygen generation of host cells. Expression of PR genes showed more complicated expression pattern and seems to be under co-control of more defensive pathways of hosts.

Role of calcium dependent protein kinases (CDPKs) in resistant and susceptible cultivars of Malus x domestica in response to the pathogen Erwinia amylovora

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Plant calcium (Ca²⁺) signals are involved in a wide array of intracellular signaling pathways after pathogen invasion. Ca²⁺-binding sensory proteins such as Ca²⁺-dependent protein kinases (CDPKs) have been predicted to mediate the signaling following Ca²⁺ influx after pathogen infection. However, until now this prediction remains elusive. We conducted a genome-wide analysis of Malus x domestica CDPKs and identified 30 CDPK genes. Malus CDPKs were found to be similar to their counterparts in Arabidopsis thaliana in gene structure and subgroup classification. Furthermore, comparative quantitative real-time RT-PCR and intracellular cytosolic calcium analysis were conducted between a fire blight resistant and susceptible M. x domestica cultivar upon invasive pathogen (Erwinia amylovora) and/or mechanical damage. We found that there is striking difference between resistant and susceptible cultivars. Our genomic and bioinformatics analyses will provide important information about the M. x domestica CDPKs role in modulating the defense responses between the susceptible and resistant cultivars. It also sheds light for the further elucidation of early signaling and downstream signaling cascades for the pathogen and wound responses.
Localization of phytoalexin biosynthesis in Pyrinae

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In response to fire blight infection, Pyrinae produce biphenyls and dibenzofurans as phytoalexins. The antimicrobial activity of these inducible defense compounds was demonstrated. Thus, improving the production of biphenyls and dibenzofurans in apple and pear cultivars may be a promising strategy to obtain plants less sensitive to fire blight. In spite of the economic importance of the Pyrinae the biosynthesis of their phytoalexins is poorly understood. The key enzyme which forms the carbon skeleton is biphenyl synthase (BIS). In Malus, there are four subfamilies of BIS isoenzymes, which are differentially regulated. Recently, cDNAs encoding three downstream enzymes were cloned and characterized from elicitor-treated cell cultures of Sorbus aucuparia: two O-methyltransferases (OMT1 and OMT2) and biphenyl 4-hydroxylase (B4H), a cytochrome P450 enzyme. Cooperation of these four enzymes was found to be sufficient to produce aucuparin, the most widely distributed phytoalexin of the Pyrinae. In immunofluorescence studies, the BIS3 protein was detected in the parenchyma cells of the bark in the transition zone of fire blight-infected apple plants. The fluorescence was dot-shaped and only present at the junctions between neighboring cells, suggesting association with plasmodesmata. Dot-shaped fluorescence was also observed in immunolocalization studies with elicitor-treated cell cultures of Sorbus aucuparia. However, fusions of BIS3 to fluorescent proteins showed cytoplasmatic localization in transformed epidermal cells of Nicotiana benthamiana. Reporter studies with B4H demonstrated ER-localization. OMT1 and OMT2 are likely to be soluble. It is tempting to speculate that B4H may act as an anchor for a metabolon containing in addition BIS, OMT1 and OMT2. There may be an assembly of this protein cluster on lipid rafts moving to plasmodesmata following pathogen infection.
## 8. List of participants

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