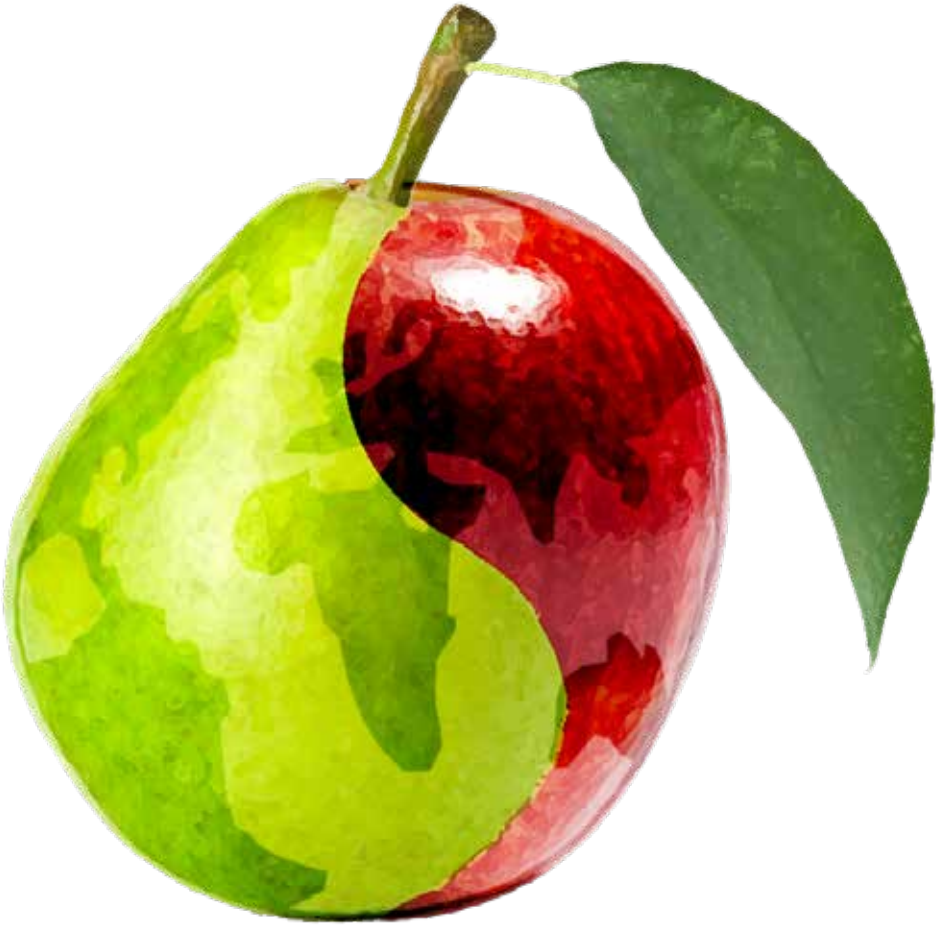
ISFB 2019

2nd International Symposium on Fire Blight of Rosaceous Plants



June 17-21, 2019

Hotel Indigo, Traverse City

Welcome and Table of Contents

WELCOME

Welcome to the 2nd International Symposium on Fire Blight in Rosaceous Plants in beautiful Traverse City, Michigan!

Glance through this program and find a detailed overview of the conference including the schedule, abstracts and participant list.

We graciously thank all of the sponsors that helped make this conference possible. Please refer to the complete listing on the back of the program.

We are excited to host this year's conference at the Hotel Indigo in Traverse City, Mich. Traverse City has been voted Best Small Town in the U.S. (Livability), one of the 10 Most Beautiful Towns in the World (Select City Magazine), one of the 21 Best Beaches in the World (National Geographic), one of America's Top 10 Foodie Towns (Livability), and best beer town (CNN Money). We encourage you to visit the outstanding restaurants, wineries, and breweries that Traverse City has to offer. If you have any questions or suggestions, please don't hesitate to let us know.

Throughout the week we hope you remain engaged, ask questions, and network with other attendees. We thank each of you for attending the 2nd International Symposium on Fire Blight in Rosaceous Plants!

Local organizing committee
Michigan State University,
Michigan State University Extension

George Sundin
Betsy Braid
Megghan Honke Seidel
Janette Jacobs
Nikki Rothwell
Jennifer Zielinski

Scientific committee
Awais Khan – Cornell University, USA
Emilio Montesinos – Universitat de Girona, Spain
Andreas Peil – Julius Kühn Institute, Germany
Joanna Pulawska – Research Institute of Horticulture, Poland
Fabio Rezzonico – ZHAW Wädenswil, Switzerland
Virginia Stockwell – Oregon State University, USA
George Sundin – Michigan State University, USA
Joel Vanneste – New Zealand Institute for Plant & Food Research Limited, New Zealand
Youfu (Frank) Zhao – University of Illinois, USA

TABLE OF CONTENTS

Conference Schedule

Monday, June 17, 2019

4:00-7:00 p.m. Registration, Hotel Indigo lobby

5:30 to 7:00 p.m. Welcome social, Hotel Indigo

Tuesday, June 18, 2019

7:30-9:00 a.m. Breakfast, Hotel Indigo

9:00-11:45 a.m. **OVERVIEW ON FIRE BLIGHT AND *ERWINIA AMYLOVORA***
Chair: George Sundin

9:00 a.m. Welcome, dedication of the meeting to the memory of Dr. Eve Billing
George Sundin, Michigan State University, East Lansing, MI USA

9:20 a.m. *Malus* species: Valuable Genetic Resource for Pest and Disease Resistance in Apple
Awais Khan, Cornell University, Geneva, NY USA

9:50 a.m. Preservation of Central Asian fruit tree forests from the fire blight pathogen *Erwinia amylovora*
Fabio Rezzonico, ZHAW Wädenswil, Switzerland

10:20 a.m. BREAK

10:45 a.m. Comparative genomics of Spiraeoideae-infecting *Erwinia amylovora* strains revealed higher genetic diversity than previously understood and identified the genetic basis of a low virulence strain
Quan Zeng, Connecticut Agricultural Experiment Station, New Haven, CT USA

11:15 a.m. Studying *Erwinia amylovora* biology by protein crystallography
Stefano Benini, Free University of Bolzano, Italy

11:45 a.m. LUNCH, Hotel Indigo

1:15-5:25 PM **DISEASE MANAGEMENT I**
Chairs: S. Tianna Dupont and Kari Peter

1:15 p.m. KEYNOTE: Open questions in fire blight management
Ken Johnson, Oregon State University, Corvallis, OR USA

Conference Schedule

2:00 p.m.	Fire blight control in New Zealand intensive orchards *Mary Horner, New Zealand Institute for Plant & Food Research Limited*
2:20 p.m.	Fire blight outbreaks and controls in Washington state, United States *S. Tianna Dupont, Washington State University, Wenatchee, WA*
2:40 p.m.	Fire blight management with copper – the effects on pollen germination, fruit size, and Starner resistance *Mary Dafny-Yelin, Northern Research & Development, 12900 Kiriat Shmona, Israel*
3:00 p.m.	Shifts in antibiotic sensitivity in *Erwinia amylovora* and evaluation of new antimicrobials for managing fire blight *Jim Adaskaveg, University of California, Riverside, CA USA*
3:20 p.m.	BREAK
3:45 p.m.	Fire blight efficacy field studies in Switzerland *Vanessa Reininger, Agroscope, Wädenswil, Switzerland*
4:05 p.m.	Vetting plant defense elicitor products for fire blight management: snake oils or silver bullets? *Kari Peter, Penn State University, Biglerville, PA*
4:25 p.m.	Floral colonization dynamics and specificity of Blossom Protect strains of *Aureobasidium pullulans* for fire blight suppression *Todd Temple, Oregon State University, Corvallis, OR USA*
4:45 p.m.	Anti-virulence approaches to combat bacterial diseases *Ching-Hong Yang, University of Wisconsin-Milwaukee, Milwaukee, WI USA*
5:05 p.m.	Quantifying the reduction effect of dormant copper in mix with bark penetrants on overwintering populations of *Erwinia amylovora* in cankers on apple wood using viability-digital PCR *Srdan Acimovic, Cornell University, Highland, NY USA*
5:25-7:00 p.m.	POSTER SESSION

Conference Schedule

Wednesday, June 19, 2019

7:30-9:00 a.m. Breakfast, Hotel Indigo

9:00 a.m. - 12:30 p.m. **PHAGE SYMPOSIUM – SPONSORED BY** CERTIS USA

Chairs: Kerik Cox and Michael Parcey

9:00 a.m. KEYNOTE: Control of fire blight with bacteriophages
Antonet Svircev, Agriculture and AgriFood Canada, Vineland, Ontario Canada

9:45 a.m. Fire Quencher – a microbiome replacement therapy for apple trees
Julianne Grose, Brigham Young University, Provo, UT USA

10:15 a.m. Quantitative host range of bacteriophages against a world-wide collection of *Erwinia amylovora*
Stephen Gayder, Brock University, St. Catharines, Ontario Canada

10:35 a.m. BREAK

11:00 a.m. The application of depolymerase DpoL1 and bacteriophage Y2 causes a strong synergistic inhibitory effect on *Erwinia amylovora*
Lars Fieseler, Zurich University of Applied Sciences, Wädenswil, Switzerland

11:30 a.m. Bacteriophages effective against both *Erwinia amylovora* and *Erwinia pyrifoliae* causing fire blight and black shoot blight in apple and pear
Chang-Sik Oh, Kyung Hee University, Republic of Korea

12:00 p.m. The toolkit utilized by bacteriophages to infect and lyse bacteria
D. William Thompson, Brigham Young University, Provo, UT USA

12:15 p.m. End of session

12:30 p.m. Embark on field trip to the Old Mission peninsula, box lunch on buses

7:30 p.m. Conference dinner

Conference Schedule

Thursday, June 20, 2019

8:30-10:00 a.m. Breakfast, Hotel Indigo

10:00 a.m. - Noon **MOLECULAR BIOLOGY AND GENOME ANALYSES**
Chairs: Tim McNellis and Quan Zeng

10:00 a.m. Insights into *Erwinia amylovora* parasitism and biocontrol via auxotrophic mutants
Tim McNellis, Penn State University, State College, PA USA

10:20 a.m. Microbiome associated with apple stigmas and its impact on fire blight infection
Quan Zeng, Connecticut Agricultural Experiment Station, New Haven, CT USA

10:40 a.m. Diversity of the CRISPR/CAS SYSTEM in *Erwinia amylovora*
Michael Parcey, Brock University, St. Catharines, Ontario Canada

11:00 a.m. PhytoTrakr: Creating a real time strain tracking tool for *Erwinia amylovora* and other plant pathogens
Anna Wallis, Cornell University, Geneva, NY USA

11:20 a.m. Molecular analysis of the distribution of two ancestral populations of *Erwinia amylovora* in Europe using CRISPR data
Fabio Rezzonico, ZHAW Wädenswil, Switzerland

11:40 a.m. Patterns of genetic variation and selection across the *Erwinia amylovora* genome
Jugpreet Singh, Cornell University, Geneva, NY USA

12:00-1:30 p.m. LUNCH, Hotel Indigo

1:30-5:20 p.m. **PLANT BREEDING, HOST-PATHOGEN INTERACTIONS**
Chairs: Roshni Kharadi and Jeff Schachterle

1:30 p.m. KEYNOTE: Fire blight resistance breeding
Andreas Peil, Julius Kuhn Institute, Dresden, Germany

2:00 p.m. Variation of fire blight susceptibility in a pedigree-connected apple germplasm set
Sarah Kostick, Washington State University, Wenatchee, WA USA

2:20 p.m. Rootstock genotypes influence response to fire blight in grafted apple scion cultivars
Ricky Tegtmeier, Cornell University, Geneva, NY USA

Conference Schedule

2:40 p.m.	The difference in gene expression between lowly and highly virulent *Erwinia amylovora* strains on two apple cultivars of different susceptibility to fire blight *Joanna Pulawska, Research Institute of Horticulture, Skierniewice, Poland*
3:00 p.m.	Role of type III secretion system during the infection of apple flowers by *Erwinia amylovora* and the influence of relative humidity to its expression *Zhouqi Cui, Connecticut Agricultural Experiment Station, New Haven, CT USA*
3:20 p.m.	BREAK
4:00 p.m.	Cyclic-di-GMP is a critical regulator of biofilm formation and virulence factors in *Erwinia amylovora* *Roshni Kharadi, Michigan State University, East Lansing, MI USA*
4:20 p.m.	Small RNA ArcZ regulates catalase and peroxide susceptibility in *Erwinia amylovora* *Jeff Schachterle, Michigan State University, East Lansing, MI USA*
4:40 p.m.	Small RNA rprA modulates biofilm dispersal in *Erwinia amylovora* *Jingyu Peng, Michigan State University, East Lansing, MI USA*
5:00 p.m.	Hfq controls bacterial virulence through linking c-di-GMP and two mechanistically distinct sRNAs *Xiaochen Yuan, University of Wisconsin-Milwaukee, Milwaukee, WI USA*
5:20-7:00 p.m.	POSTER SESSION

Conference Schedule

Friday, June 21, 2019

7:00-8:30 a.m.	Breakfast, Hotel Indigo
8:30 a.m. - Noon	**DISEASE MANAGEMENT II** *Chairs: Jingyu Peng and Suzanne Slack*
8:30 a.m.	In orchard population dynamics of *Erwinia amylovora* on apple flower stigmas *Suzanne Slack, Michigan State University, East Lansing, MI USA*
8:50 a.m.	Management of fire blight in young apple orchards *Darlene Nesbitt, Agriculture and AgriFood Canada, Vineland, Ontario Canada*
9:10 a.m	Prohexadione-Ca growth regulator and pruning as post symptom rescue treatments following fire blight infection during bloom *Vincent Philion, Research and Development Institute for the Agri-Environment, Saint-Bruno-de-Montarville, Quebec Canada*
9:30 a.m.	Post-infection applications of prohexadione-calcium can reduce/prevent shoot blight initiation of fire blight cankers on perennial apple wood *Srdan Acimovic, Cornell University, Highland, NY USA*
9:50 a.m.	Factors affecting the selective detection and quantification of *Erwinia amylovora* live cells in natural cankers by viability digital PCR using propidium monoazide *Ricardo Delgado Santander, Cornell University, Highland, NY USA*
10:10 a.m.	BREAK
10:40 a.m.	Evaluation of rapid and cost-effective pathogen detection assays for fire blight management in apple orchards *Jugpreet Singh, Cornell University, Geneva, NY USA*
11:00 a.m.	Summary (chairs of sessions) and Discussion
11:45 a.m.	Next meeting location
12:00 p.m.	Meeting closure

Abstracts - Oral Presentations

Malus species: Valuable genetic resource for pest and disease resistance in apple

Awais Khan

Cornell University, Geneva, NY USA

Apple productivity is constantly threatened by existing pathogens, emergence of new pathogens, and the changing populations of pathogen strains. New apple cultivars are needed for optimal performance under changing biotic and abiotic stresses and to meet market demands. Identification of novel genes and their functional alleles is a prerequisite to breed cultivars with enhanced disease resistance. Wild *malus* species are a vital resource for apple breeders/geneticists for their allelic diversity and novel resistance alleles. The potential contribution of the genetic diversity present in wild malus species for developing cultivars with improved disease and pest resistance has been demonstrated. With recent advances in sequence-based genotyping platforms and disease phenotyping, these wild malus species can be more rapidly characterized for specific diseases. We have evaluated a large collection of malus germplasm from 45 *Malus* species including *Malus sieversii*, the progenitor of domesticated apples, for fire blight and apple scab resistance/susceptibility in the greenhouse and field. Our results showed different levels of fire blight and apple scab susceptibility/resistance responses within these species, from highly susceptible to highly resistant. These accessions are promising for use in future genetic studies to identify novel sources of resistant alleles and to breed disease resistant apples. However, a major challenge is how to efficiently deploy this useful variation into elite apple cultivars and develop new disease-resistant and consumer-preferred cultivars. We will briefly discuss some new approaches for developing disease resistant cultivars, including rapid cycle breeding, genetic transformation and genome-editing.

Preservation of Central Asian fruit tree forests from the fire blight pathogen *Erwinia amylovora*

Fabio Rezzonico[1], Theo H.M. Smits[1], Jarkyn Samanchina[2], Galiya Zharmukhamedova[3]

[1]ZHAW Wädenswil, Switzerland; [2]Flora and Fauna International, Kyrgyzsan; [3]Kazakh Inst. of Horticulture & Viticulture, Almaty, Kazakhstan

Domesticated apple and pear species and their wild ancestors are native of Central Asia, where they represent the dominant species in mid-altitude forests located in mountainous regions. Together, they constitute a critical foundation for entire ecosystems of plants, insects and animals. Starting from 2008, the first fire blight cases were detected in orchards of Kyrgyzstan and Kazakhstan, with sporadic cases reported also in the natural forests. The arrival of fire blight in the center of origin of pome fruit species is a major threat to the whole forest ecosystem, the endangered flora and fauna species therein contained and local rural economies that subsist on the natural resources of the forests. Within a project funded by the Swiss Programme for Research on Global Issues for Development (r4d programme) we aim to understand and minimize the impact of fire blight in Central Asia by monitoring the presence and movements of the disease in natural forests and the influence of adjacent fruit-growing areas, where international apple cultivars are grown. The objectives of the project are (1) the determination of the dissemination routes of fire blight to and within Central Asia by implementing targeted surveys in forests and nearby orchards in combination with molecular source tracking; (2) to use the acquired data to understand the disease dynamics in natural forest ecosystems and to assess the level of damage therein inflicted; (3) to develop locally-adapted phytosanitary strategies to enhance ecological conservation efforts and crop protection; (4) to add a further biodiversity protection layer through networking and consolidation of the existing germplasm preservation facilities; (5) to monitor local apple varieties for relative tolerance to fire blight for use in sustainable production and breeding programs; (6) to identify biocontrol agents adapted to the local conditions that can be used to combat fire blight in the orchards and in the forests. In this keynote an overview of the project, including the first data of the current sampling season and technical advancements in molecular source tracking, will be presented.

Abstracts - Oral Presentations

Comparative genomics of Spiraeoideae-infecting *Erwinia amylovora* strains revealed higher genetic diversity than previously understood and identified the genetic basis of a low virulence strain

Quan Zeng[1], Zhouqi Cui[1], Jie Wang[2], Kevin L. Childs[2], George W. Sundin[2], Daniel R. Cooley[3], Ching-Hong Yang[4]

[1]Connecticut Agricultural Experiment Station, New Haven, CT USA; [2]Michigan State University, East Lansing, MI USA; [3]University of Massachussetts, Amherst, MA USA; [4]University of Wisconsin-Milwaukee, Milwaukee, WI USA

Erwinia amylovora is the causal agent of fire blight, one of the most devastating diseases of apple and pear. *Erwinia amylovora* is thought to have originated in North America and has now spread to at least 50 countries worldwide. An understanding of the diversity of the pathogen population and the transmission to different geographical regions is important for the future mitigation of this disease. In this research, we performed an expanded comparative genomic study of the Spiraeoideae-infecting (SI) *E. amylovora* population in North America and Europe. We discovered that, although still highly homogeneous, the genetic diversity of 30 *E. amylovora* genomes examined was about 30 times higher than previously determined. These isolates belong to four distinct clades, three of which display geographical clustering and one of which contains strains from various geographical locations ('Widely Prevalent' clade). Furthermore, we revealed that strains from the Widely Prevalent clade displayed a higher level of recombination with strains from a clade strictly from the eastern USA, which suggests that the Widely Prevalent clade probably originated from the eastern USA before it spread to other locations. Finally, we detected variations in virulence in the SI *E. amylovora* strains on immature pear, and identified the genetic basis of one of the low?virulence strains as being caused by a single nucleotide polymorphism in hfq, a gene encoding an important virulence regulator. Our results provide insights into the population structure, distribution and evolution of SI *E. amylovora* in North America and Europe.

Studying *Erwinia amylovora* biology by protein crystallography

Stefano Benini

Free University of Bolzano, Italy

Protein crystallography is a valuable tool to complement research data, either coming from knock out mutants, biochemistry or bioinformatics, providing valuable information towards the understanding of the structure and function of proteins and enzymes at the molecular level.

In the last few years, several crystal structures of proteins and enzymes, from the causal agent of fire blight, have been elucidated providing valuable information on the biology of *Erwinia amylovora* and therefore the molecular basis of the disease.

The structures of the negative regulator of amylovoran production AmyR1 and of the cysteine protease effector AvrRpt22 are examples of how to combine information from different sources to increase our knowledge on their mechanisms of action.

Started as a structural genomics project, to study proteins and enzymes important for the pathogenicity and the survival of *E. amylovora*, our line of research is now moving to focus on the study of proteins involved in its iron metabolism3, and of the enzymes of the amylovoran biosynthetic pathway.

1. Bartho, J. D., Bellini, D., Wuerges, J., Demitri, N., Toccafondi, M., Schmitt, A. O., Zhao, Y., Walsh, M. A., and Benini, S. (2017) The crystal structure of Erwinia amylovora AmyR, a member of the YbjN protein family, shows similarity to type III secretion chaperones but suggests different cellular functions. PLoS One. 12, e0176049.
2. Bartho, J. D., Demitri, N., Bellini, D., Flachowsky, H., Peil, A., Walsh, M. A., and Benini, S. (2019) The structure of Erwinia amylovora AvrRpt2 provides insight into protein maturation and induced resistance to fire blight by Malus x Robusta 5. J. Struct. Biol. 206, 233-242.
3. Polsinelli, I., Borruso, L., Caliandro, R., Triboli, L., Esposito, A., and Benini, S. (2019) A genome-wide analysis of desferrioxamine mediated iron uptake in Erwinia spp. reveals genes exclusive of the Rosaceae infecting strains. Scientific Reports. 9, 2818.

Abstracts - Oral Presentations

Open questions in fire blight management

Ken Johnson

Oregon State University, Corvallis, OR USA

Most of the world's pear and apple orchards are planted to fire blight susceptible cultivars that can require protective sprays for this disease before, during and after bloom to maintain tree health. This need to spray trees for protection has led to the registration and marketing of dozens of materials for fire blight control, particularly for organic production where antibiotics are not allowed. Under this scenario, we have been attempting to understand fire blight management through the impact of sprayed materials on epiphytic pathogen populations in flowers, which, in inoculated orchard trials, we now measure routinely through the bloom period. In natural epidemics, measurements of floral pathogen populations show that they are typically low at full bloom and grow to a peak during petal fall. Moreover, even in inoculated trials, epiphytic pathogen populations tend to peak during petal fall. Questions that arise from these observations include: how much infection occurs during petal fall when orchardists are generally less concerned about disease risk?, does management of late-building populations affect subsequent risk of shoot blight?, what is the expected population reduction after treatment with a specific material?, does an early, effective reduction of a pathogen population lessen the size of the later occurring peak population?, and do predictive models perform a disservice by focusing too much on the concept of 'infection period'? A second set of questions concerns the degree to which phyllosphere pH influences epiphytic populations of *Erwinia amylovora*, which are sensitive to mildly acidic conditions. Blossom Protect, a very effective biological product consisting of strains of *Aureobasidium pullulans*, is applied with a pH 3.3 buffer that contributes partially to control. Related questions arise with other materials. For example, is the effectiveness of alum (potassium aluminum sulfate) due to its effect on phyllosphere pH?, does acidification of other materials – e.g. oxytetracycline or kasugamycin – enhance their impact on epiphytic populations?, and is acidification of the phyllosphere detrimental to fruit quality? A third set of questions concerns materials that have little effect on epiphytic pathogen populations yet they contribute significantly to infection suppression. For example, 'how does Blossom Protect (A. pullulans) control fire blight given that it shows only small effects on epiphytic *E. amylovora* populations?' Competitive exclusion appears to be an unlikely mechanism for this biocontrol agent, which suggests the mechanism involves a potential interaction mediated through the host. Similarly, at least two materials, acibenzolar-S-methyl and prohexidione calcium, have been shown to enhance host resistance to floral infection without a direct effect on pathogen populations. What magnitude of contribution can be expected from routine applications of resistance-inducing materials?, are there potential interactions among resistance inducers?, do resistance inducers exist that can be relied on in organic production?, and if inducers are utilized before bloom, should the strategy for use of other materials change? A goal of this presentation is to encourage discussion as answers to many of these questions are unclear.

Fire blight control in New Zealand intensive orchards

Mary Horner[1], Rachel Kilmeister[2], Anna Lambourne[3], Peter Wood[1], David Manketlow4

[1]New Zealand Institute for Plant & Food Research Limited, Havelock North, New Zealand; [2]New Zealand Apples and Pears Limited, Hastings, New Zealand; [3] GWADA Group, Hawke's Bay, New Zealand; [4]Applied Research and Technologies Ltd, Napier, New Zealand

Major outbreaks of the disease fire blight, caused by *Erwinia amylovora*, occur sporadically in New Zealand. This has resulted in a loss of grower focus and management understanding when an outbreak of the disease occurs. Globally, there is increasing pressure from fire blight as new highly susceptible premium cultivars and susceptible rootstocks grown in high density planting systems become more common. Higher temperatures from climate change are also increasing the occurrence of infection events and greater disease pressure. The current and future economic consequences from increased disease pressure through lost tree productivity and tree death has the potential to be significant. To address this risk, the New Zealand apple industry has implemented a new research programme to develop and document a disease management strategy that will ensure a sustainable low risk status for fire blight.

An initial survey was conducted to ascertain the current industry practices, knowledge, and gaps in fire blight management. Results of this survey have been incorporated into the development of a research programme to create education packages, address knowledge gaps, and develop an online fire blight management information toolbox that can be easily updated in the future. This talk outlines current control practices, uncertainties and research knowledge gaps as identified by the survey, and how these are being addressed. Research includes the use of grower case study blocks to trial various combinations of registered agri-chemicals and cultural practices; glasshouse and field trials investigating new chemistries; pruning strategies to control fire blight strikes on susceptible new premium cultivars and dwarfing rootstocks in nursery and orchard trees with or without growth regulator application; development of an improved disease prediction model adapted to the NZ climate and growing practices; better ways to control fire blight without antibiotic use or in the presence of streptomycin resistance.

Abstracts - Oral Presentations

Fire blight outbreaks and controls in Washington state, United States

S. Tianna Dupont

Washington State University, Wenatchee, WA USA

With 157,000 bearing acres of apples and 20,000 acres of pears, Washington State has had minor fire blight outbreaks annually since 1991 and serious damage in 1993, 1997, 1998, 2005, 2009, 2012, 2015, 2016, 2017 and 2018. In 2017 and 2018 infections were severe due to multiple wetting events as well as high temperatures during bloom. For example, in a survey representing 3,229 acres of pears and 14,146 acres of apples (aprox. 10% of Washington apple and pear acreage), respondents documented that 88% of their pear acres and 17% of apple acres were impacted by fire blight in 2018. Costs included an average of $194 per acre for protective sprays and $168 per acre to cut out infected material. Survey respondents removed more than 230,000 trees or about 377 acres due to fire blight infections. Total direct losses from spray, cutting and tree replacement costs totaled $3.7 million on the 17,375 acres surveyed. Assuming surveyed acres represented industry averages, direct costs for managing fire blight in Washington were more than $37 million in 2018. When asked to estimate the percentage of their bearing acreage lost to fire blight in 2018 respondents on average responded nine percent. Assuming $2.5 billion in Washington apple and pear fresh market production, production losses due to fire blight in 2018 may have totaled $222 million.

Current grower conventional management is based on University trials. It includes a combination of antibiotics, systemic acquired resistance products, and some biologicals. Following the Cougar Blight Model, oxytetracycline (Fireline, Mycoshield) or kasugamycin (Kasumin) is ideally applied in the 24-hour window before a wetting event if temperatures have been high. Some streptomycin is also used in areas where growers have lower resistance, no more than once per season. Some conventional growers also apply biologicals (Blossom Protect) during early bloom. Increasingly in high risk blocks the systemic acquired resistance product Actigard is used both in combination with antibiotics in bloom sprays and as a trunk spray when cutting blight out of blocks.

More than fourteen thousand acres of apples and two thousand acres of pears in Washington are managed organically. Pear growers often use two applications of biologicals (Blossom Protect) during early bloom followed by Bacillus subtillis (Serenade Opti) or copper octanoate (Cueva) every 2-5 days dependent on risk as predicted by the Cougar Blight model. Apple growers in Washington use two to five lime sulfur applications for thinning which makes it difficult to apply biologicals during early bloom. Following lime sulfur thinning sprays, which are also considered antimicrobial, soluble coppers (e.g. Cueva, Previsto) or biologicals (Serenade Opti) are generally used. Recent research has shown that the percentage of metallic copper in the various coppers available is important to optimize efficacy.

The Washington industry is also extremely interested in the many new biologicals, plant extracts and systemic acquired resistance products coming to the market. We will discuss new product efficacy of a number of products trialed in Washington.

Kirby, E., and D. Granatstein. “Certified Organic Acreage and Sales in Washington State: 2007-2015.” WSU Extension: Washington State University, 2017.

Fire blight management with copper – The effects on pollen germination, fruit size, and Starner resistance

Mery-Dafny Yelin, Judith Moy, Orly Mairesse, Miriam Zilberstaine, and Gal Sapir

Northern Research & Development, 12900 Kiriat Shmona, Israel

For many years management of Fire blight (caused by the bacterium *Erwinia amylovora*) in Israel was based on the bactericide Starner (active ingredient - Oxolinic Acid, OA). Over the years, isolates with resistance to Starner at 50 ppm (a.i) developed. Bacteria with this resistance appeared after frequent use of Starner but, because of low fitness, they would disappear by the following year. However, in addition to this resistance, during 1998-2014 the percentage of isolates that developed on Starner at 5 ppm (a.i) slowly grew, reaching 100% in 2014. The slow decrease in OA efficacy led to use of increased concentrations of Starner – from 0.15 to 0.20%. In 2014 copper was added to the control system as a protective fungicide and was used at the high frequency of twice weekly during the flowering periods. However, previous reports showed that the use of copper could impair pollen germination. The aims of the present study were to monitor the effects of treatment with copper during flowering on: 1) *E. amylovora* resistance to Starner, and 2) seed count, and fruit size and weight. Results: 1) Infected blossoms were collected from commercial apple and pear orchards that were treated twice weekly with copper compounds during flowering (approximately 4-6 weeks). The blossoms were ground and sown on *E. amylovora*-selective media (CCT) containing OA at various concentrations. In pears, 46.2 and 63.6% of the samples developed on OA at 5 ppm or more, in 2017 and 2018, respectively. In addition, in 2017 and 2018, respectively, 23.08 and 0.0% developed on OA at 50 ppm or more. 2) Germination of apple and pear pollen in vitro was significantly reduced after 2 days of application of 0.1% Nechoshtan (copper tribasic sulfate at 190 g/l), but not after only one day. In a field trial in an apple orchard during 2017 we found that the use of five applications of Nechoshtan twice weekly during flowering significantly reduced fruit weight by 19.6%, from 168.56 g in the control to 136.52 g, and fruit diameter from 71.98 mm in the control to 68.82 mm in the Nechoshtan treatment. The seed count per fruit also was significantly reduced from 7.0 to 4.3. This experiment was performed four times during 2017-2018, but we saw the effect of Nechoshtan in reducing seed count, fruit weight and size, only once as described above. In this experiment significant changes in seed numbers have been seen with increasing distance from the pollinizer cultivar, indicating a problem with pollination quality. In the other three experiments, no significant effect of Nechoshtan on fruit size was seen, probably because there was no pollination problem. In two experiments, the copper did not reduce fruit size even though there was a problem with fertilization. In the pear orchard, six experiments were performed in three years (2016-2017) in the varieties Spadona and Costia. In three experiments there was a pollination problem; however, we saw a decrease in seed count only once – in variety Spadona – but it had no effect on fruit size. In one experiment, we saw an increase in fruit size – in Costia fruit. However, this was not related to a pollination problem (there was no reduction in seed count). In summary: The approach to Fire blight management in Israel changed in recent years; from application of Starner alone, in accordance with a Decision Supporting System, to use of copper twice weekly throughout the flowering period, supplemented with Starner application once or twice during the season. The present study shows that: 1) resistance to Starner at 5 ppm or more was dramatically reduced after adding copper to the *E. amylovora* control system; the resistance to OA at 50 ppm was reduced relative to the years when only Starner was used; 2) in apple, in cases of poor fertilization, the fruit weight, size, and seed count might be harmed by twice-weekly use of Nechoshtan during the flowering period. However, in pears we did not see reductions in fruit weight, size, or seed count, even under poor pollination.

Abstracts - Oral Presentations

Shifts in antibiotic sensitivity in *Erwinia amylovora* and evaluation of new antimicrobials for managing fire blight in California.

James E. Adaskaveg and Helga Förster

University of California, Riverside, CA USA

In three commercial pear orchards in California, strains of *Erwinia amylovora* with reduced sensitivity to oxytetracycline were detected with minimum inhibitory concentration (MIC) values of 1.8 to 3.5 µg/ml as compared to sensitive isolates with MICs of 0.2 to 0.4 µg/ml. Strains from two of these orchards were also highly resistant to streptomycin (MIC >100 µg/ml), and commercial applications with the two antibiotics resulted in poor fire blight management. Strains with reduced sensitivity to oxytetracycline from the third orchard were either sensitive (MICs <1 µg/ml) or moderately resistant (MICs 20 to 30 µg/ml) to streptomycin. High resistance to streptomycin was associated with a mutation in codon 43 of the chromosomal *rpsL* gene. In strains with moderate resistance, the *strA-strB* resistance genes were located on transposon *Tn5393a* that is lacking the insertion sequence *IS1133* that provides a promoter for efficient expression of *strA-strB*. Moreover, *Tn5393a* was located on plasmid pEU30, in contrast to previously described plasmids pEa34 or pEA29 harboring *strA-strB* on *Tn5393*::*IS1133*. Reduced sensitivity to oxytetracycline to date has not been molecularly characterized but the low levels of resistance indicate that a general detoxification mechanism may be involved. In small-scale trials where branches of pear apple (Asian pear) with flowers were treated with bactericides and then inoculated with strains of *E. amylovora* either sensitive to streptomycin and oxytetracycline or highly resistant to streptomycin and intermediately resistant to oxytetracycline, fire blight caused by the sensitive strain was effectively reduced to low levels by streptomycin, oxytetracycline, and kasugamycin, whereas disease caused by the resistant strain was most effectively reduced by kasugamycin or kasugamycin-mancozeb. The high incidence of streptomycin resistance in *E. amylovora* in many growing areas and the emergence of strains with reduced sensitivity to oxytetracycline mandates the development of new fire blight management treatments. The highly effective kasugamycin was federally registered in 2014 and in California in 2018. To date, no resistance to this antimicrobial has been found, and its efficacy needs to be protected. Among alternative treatments evaluated, the biocontrols Serenade, Serenade-copper mixtures, and Blossom Protect performed well against fire blight of pear and apple and were generally better than copper by itself, but only under low to moderate disease pressure. A new focus of our research are antimicrobials that are approved as food preservatives. Nisin and Ɛ-poly-L-lysine, especially when mixed with EDTA, were highly toxic to *E. amylovora* (a gram-negative bacterium) in vitro using direct contact assays. Under field conditions, these two compounds showed promising results with up to 70% reduction in disease as compared with the control. These treatments were slightly less efficacious than a standard kasugamycin-streptomycin treatment with 85% to 90% reduction in fire blight incidence. Using mixtures of Ɛ-poly-L-lysine and kasugamycin, the efficacy of the treatment was sometimes improved as compared to the bactericides by themselves, and this may provide an anti-resistance strategy. Current efforts are to improve the efficacy of nisin and Ɛ-poly-L-lysine with the addition of UV stabilizers.

Fire blight efficacy field studies in Switzerland

Vanessa Reininger, Anita Schöneberg, Perrine Gravalon, Eduard Holliger

Agroscope, Wädenswil, Switzerland

In Switzerland, plant protection trials against fire blight are conducted every year at Agroscope in an entirely covered field site. As antibiotics have not been allowed from 2016 on, alternative plant protection strategies became even more important as before. A national plan of action to reduce the potential risk of pesticides by 50% was launched by the Swiss government in 2017. This plan of action is already implemented in the Swiss agriculture and is meant to lead to a more sustainable system. Additionally, from 2020 on, the legal status of *Erwinia amylovora* will be changed in Switzerland, and in the EU, from a quarantine organism into a regulated non-quarantine organism. Nevertheless, fire blight will still be an economic important disease. In this context it is very important to create a comprehensive toolbox including alternative and sustainable plant protection solutions to cope with fire blight.

Therefore, artificial fire blight inoculation experiments in our field-site containment using 3-year old potted apple trees and different treatment strategies against *Erwinia amylovora* have been providing efficacy values and cell counts per blossom for several seasons. From directly inoculated trees, distributed throughout the field site, bacteria are spread by bumblebees, which take care of natural infection on opening blossoms within the containment. Up to eight treatment strategies with three plant protection applications each, are investigated during blooming if the weather conditions are accordingly. Blossoms are collected additionally, to determine cell densities depending on the treatment strategies and to verify the Maryblyt model using the untreated control. Usually, the highly susceptible variety ‘Gala Galaxy’ on M9 rootstock is used to test different plant protection strategies against fire blight. In 2018 though, we could show for the first time that flower clusters of the tolerant variety ‘Ladina’ got much less infested by Erwinia amylovora in comparison to ‘Gala Galaxy’. This might be due to the effect of combining a fire blight tolerant variety with an appropriate plant protection treatment.

In 2019, alternative plant protection strategies were applied closely spaced to yield high efficacy values due to the treatments. The plant protection agents LMA, LMA-Squall, Mycosin, Mycosin-Squall and BlossomProtectTM were applied and an untreated control included as well. First results will be presented at the conference.

Abstracts - Oral Presentations

Vetting plant defense elicitor products for fire blight management: snake oils or silver bullets?

Kari Peter, Brian Lehman

Pennsylvania State University, Biglerville, PA USA

Fire blight is a constant headache for Pennsylvania apple growers, with the last three out of five years being exceptionally challenging. Diverse growing systems existing throughout PA apple growing regions compound the fire blight problem: high density apple plantings next to older semi-dwarf apple plantings. Although blossom blight can be effectively managed during bloom since streptomycin is still effective, unpredictable weather events, protracted bloom periods, and yearly canker blight contribute to persistent fire blight threats. This is most critical for newly planted and young trees on dwarfing rootstocks. An alternative strategy for season-long fire blight management in vulnerable apple plantings is through controlled activation of the plant immune system by the application of materials containing plant defense activating compounds. Over the last several years, we have been vetting the different "plant defense elicitor" products on the market, asking the question: Do they live up to the hype? We have evaluated Actigard (*Acibenzolar-S-methyl*), Regalia (*Reynoutria sachalinensis*), Vacciplant (laminarin), and the different Bacillus subtilis formulations (Serenade, Double Nickel, LifeGard, and Stargus). Through potted tree greenhouse trials and field trials on different aged trees, we will discuss our results to date focusing on the following questions: what works and what doesn't?; is there an ideal application timing?; how long is the duration of the defense signal for disease control?; does tree age influence efficacy?; and can products be combined for a greater synergistic effect?

Floral colonization dynamics and specificity of Blossom Protect strains of *Aureobasidium pullulans* for fire blight suppression

Todd Temple[1], Evan C. Thompson[1], Sai-Sree Uppala[1], David Granatstein[2], and Ken B. Johnson[1]

[1]Oregon State University, Corvallis, OR USA; and [2]Washington State University, Wenatchee, WA USA

Aureobasidium pullulans is used as a biocontrol agent for fire blight protection in organic apple and pear production. We assessed colonization of pome flowers by A. pullulans in orchards located near Corvallis, OR and Wenatchee, WA. Blossom Protect, a mix of *A. pullulans* strains CF10 and CF40, and its citrate-based companion, Buffer Protect, were sprayed at 70% bloom. Later in bloom, the population size of putative A. pullulans on flowers was estimated by dilution plating; plate scrapings of putative *A. pullulans* were then sampled and subjected to PCR analysis. Sequenced PCR-amplicons of the internal transcribed spacer region and the elongase gene confirmed the presence of *A. pullulans*, while a multiplex-PCR with primers specific to CF10 and CF40 were used to determine the presence of the introduced strains. At Corvallis, a wet spring environment, *A. pullulans*, was recovered from most (> 90%) Bartlett pear and Golden Delicious apple flowers sampled from experimental trees regardless if the trees were treated with Blossom Protect. Nevertheless, population size estimates of *A. pullulans* on the flowers were correlated with the number of times Blossom Protect was sprayed onto the trees. At Wenatchee, an arid spring environment, *A. pullulans* was detected on most flowers from trees treated with Blossom Protect, but on only a minority of flowers from non-treated controls. Over both locations, the combined incidence of strains CF10 and CF40 on flowers averaged 89% on Blossom Protect-treated trees but only 27% on adjacent, non-treated trees. In subsequent trials, efficacy of Blossom Protect for fire blight control was compared to three alternative yeast isolates with each applied with Buffer Protect: a local isolate of *A. pullulans*, *Cystofilobasidium infirmominiatum,* and *Cryptococcus neoformans*. All yeasts suppressed fire blight to a degree but control with Blossom Protect was significantly superior ($P < 0.05$) to other yeast isolates in one of two trials. Because secondary flower-to-flower dispersal of strains CF10 and CF40 was limited, we recommend treating every tree row with Blossom Protect at least once for fire blight suppression.

Abstracts - Oral Presentations

Anti-virulence approaches to combat bacterial diseases

Ching-Hong Yang[1], Xiaochen Yuan[1], Liwei Fang[1], George W. Sundin[2]

[1]University of Wisconsin-Milwaukee, Milwaukee, WI USA; [2]Michigan State University, East Lansing, MI USA.

Fire blight, caused by *Erwinia amylovora*, is a destructive bacterial disease of apples and pears. Although this disease has been known for decades, control options remain limited. Antibiotic sprays have suppressed blossom infection in commercial orchards. However, the development of streptomycin-resistant populations of *E. amylovora* has limited the utility of this control method. In *E. amylovora*, the type III secretion system (T3SS)—a needle-like pilus that detects, then pierces and infects its target—is a primary pathogenicity factor. Specifically disabling a component of the T3SS significantly decreases the virulence of the pathogen: thus, T3SS is a promising therapeutic target for antimicrobial interventions. In our results, we identified small phenolic compounds that can potently inhibit the expression of key T3SS genes in the fire blight pathogen without affecting its growth. We proved that the virulence suppression could lead to effective control of fire blight in our pilot field study. Unlike many current antibiotics, which indiscriminately kill a broad range of bacteria by targeting metabolic processes needed for survival, a T3SS-specific inhibitor would disarm the pathogen's virulence without interfering with its survival, thus likely reducing selection pressure on the bacteria to develop metabolically expensive drug-resistant mutations. Because of its specificity, we also expect that the T3SS inhibitors will have little impact on indigenous microflora in the host and natural environment.

Quantifying the reduction effect of dormant copper in mix with bark penetrants on overwintering populations of *Erwinia amylovora* in cankers on apple wood using viability-digital PCR

Srdan Acimovic, Ricardo D. Santander, Christopher L. Meredith, Fatemeh Khodadadi

Cornell University, Highland, NY USA

The delayed dormant application of copper compounds during bud break in spring is one of the standard practices in all fire blight management programs. With every wetting event, the metallic copper ions on plant surfaces are gradually released from the deposits of copper compounds. This provides residual protection against fire blight through reduction of *Erwinia amylovora* populations on plant surfaces emerging from overwintered cankers and thus lowering the primary inoculum of this pathogen. However, during very rainy springs it can be challenging or impossible to secure enough copper deposits to remain on tree surfaces until the bacteria emerge from cankers with warm weather. Usually, three inches of rain are enough to wash almost all copper residues from plant surfaces and in most years, the pathogen emerges from cankers at the end of bloom and start of shoot growth. In addition, if applied after the ¼ to ½ inch green bud stages, copper can cause phytotoxicity on the developing green tissues, especially at the base of the flower buds that will develop into fruit. Under these circumstances, quite common question among practitioners is whether dormant copper application can be conducted in fall and have the same benefit as the delayed dormant application. In addition to this, other questions on delayed dormant copper use for fire blight management we were interested addressing were: (a) Can dormant spray applications of copper reduce the overwintering *E. amylovora* populations in cankers? (b) Do bark penetrating surfactants increase the efficacy of dormant copper sprays in reduction of *E. amylovora* populations in cankers? and (c) for how much copper reduces the emerging populations of *E. amylovora*, justifying the benefit of their spray application? To address some of these questions, we initiated a spray trial during the overwintering phase of *E. amylovora* life cycle on naturally developed fire blight cankers on wood of 'Cortland' apple trees. We hypothesized that surfactants in mix with copper sulfate or copper might enable penetration of copper ions into the apple bark and reduce the number of live overwintering *E. amylovora* cells in cankers. Accordingly, the treatments performed included 1. Bordeaux Mix (BM) + Loveland Bark Oil; 2. BM + Bark Oil Blue; 3. BM + Bark Oil Clear; 4. BM + Basal Oil; 5. BM alone; 6. Copper Hydroxide (CH; metallic copper equivalent 50%) + Loveland Bark Oil; 7 CH + Bark Oil Blue; 8. CH + Bark Oil Clear; 9. CH + Basal Oil; 10. CH alone. An additional set of three trees served as untreated control. Treatments were sprayed dilute to drip (300 gal/A) using a tractor-carried handgun sprayer on the same day: All the BM treatments delivered 12.7 lb/A, and the CH treatments delivered 8 lb/A of metallic copper. Each treatment was replicated on three trees. Fifteen days after the spray applications, we collected fire blight cankers by pruning removal from the treated trees, froze them in liquid nitrogen and stored at -80°C. We collected three cankers per tree at time 0 and 15 days after treatment (9 cankers per treatment and time point). The experiment was performed in mid-November 2016, before the onset of low winter temperatures which probably also contribute to E. amylovora population reduction in cankers. After canker tissue harvesting and processing, the selective detection and quantification of E. amylovora live cell populations in cankers was achieved by a viability-digital PCR (v-dPCR) protocol involving the use of propidium monoazide. The v-dPCR analysis showed that only 15 days after the application a significant reduction of overwintering pathogen cells was achieved by treatment with BM + Loveland Bark Oil, BM alone and CH + Bark Oil Blue. Depending on the treatment, the reduction of E. amylovora live cells ranged from 71.1 to 92.2%, while in the untreated control the reduction was of a 3.9% but not statistically significant. This trial was repeated in a second year and tissue analyzed with v-dPCR, however the fire blight cankers developed after shoot tip inoculations (1 × 10^9 CFU *E. amylovora*) did not develop into high-quality cankers on perennial wood, necessary for *E. amylovora* reduction detection by v-dPCR. The same experiment is being repeated again to determine the consistency of copper treatments in reduction of *E. amylovora* in cankers. Our results offer first quantitative evidence for capacity of copper compounds to reduce overwintering pathogen populations in fire blight cankers and the capacity of surfactants in improving this population reduction effect.

Control of fire blight with bacteriophages

Antonet Svircev[1], Alan Castle[2]

[1]Agriculture and Agri-Food Canada, Vineland, Ontario Canada; [2]Brock University, St. Catharines, Ontario Canada

In the1960s and 1970s, bacteriophages were considered as potential candidates for the control of *Erwinia amylovora* in the orchard. This agricultural application of phages was largely abandoned due to the integration in fruit production of the highly effective streptomycin for the control of blossom blight. Development of resistance of *E. amylovora* to streptomycin along with public concern about long term antibiotic use has made phage control an attractive option yet again. Our presentation will provide a brief overview on the development of the phage-mediated biological control program. Phage isolation, enrichment, characterization and the rationale behind the decision on which bacteriophages to incorporate into our field-based field trials are discussed. This program was developed using *Pantoea agglomerans* as phage-carrier or delivery system. The bacteriophages, initially grown in the pathogen isolation host, are isolated, purified and directly used to infect *P. agglomerans*. The phage infected *P. agglomerans* was designated as the phage-carrier. The phage-carrier is applied to the open blossoms where the bacteria and phage populations are established prior to the arrival of the pathogen. Quantitative PCR (qPCR) technologies were developed and standardized to quantify phage, pathogen and carrier populations on the blossom. The qPCR method provides numerical data rather than the time consuming and highly variable spot and double agar plaque assays. These data indicated that under field conditions both the carrier and phage populations increase on the stigma and hypanthium. Field-based trials have indicated that efficacy can range from 50-62% when compared to the control. Information was lacking on the field-based trials where phage-carrier failed. Current work on phage mixtures-carrier combinations and preliminary formulation work will be discussed. The presence of bacterial lysogens in wild type isolates of *E. amylovora* and *P. agglomerans* were studied since prophages in wild type populations may affect phage therapy and possibly act as agents of horizontal gene transfer. In addition, to fully optimize the phage-host-carrier interaction we have examined the role of exopolysaccharides on the ability of the phages to infect the bacterial pathogen. Integrated information from blossom bioassays, qPCR based host range data, qPCR based population detection of carrier-phage-pathogen in situ and field trials have ultimately provided direction on which phage mixtures/combinations will provide the highest efficacies under field conditions.

Fire Quencher – a microbiome replacement therapy for apple trees

Julianne Grose

Brigham Young University, Provo, UT USA

Widespread alterations in the microbiome have been shown to co-inside and often contribute to many disease states, including obesity, diabetes, Crohn's disease and certain cancers. In these and other disease states, a healthy microbiome contributes to disease prevention due to the ability of bacteria to outcompete invading pathogens. Disease therapies based solely on probiotic approaches are marginally successful treatments in part because the probiotic bacteria must compete with residing microbes. Several studies have shown enhanced microbiome replacement when the residing microbiome is displaced along with administration of a probiotic. Herein we present a microbiome replacement therapy for apple trees that utilizes bacteriophages to target bacterial pathogens combined with a probiotic to provide healthy competing bacteria. Bacteriophages were chosen from over 30 that were isolated and sequenced, and replacement bacteria were isolated from healthy apple trees. Data on successful treatment of fire blight with bacteriophage alone versus this combined therapy will be discussed.

Abstracts - Oral Presentations

Quantitative Host Range of Bacteriophages Against a World-Wide Collection of Erwinia amylovora

Stephen Gayder[1], Alan Castle[2], Antonet Svircev[1]

[1]Agriculture and Agri-Food Canada, Vineland, Ontario Canada; [2]Brock University, St. Catharines, Ontario Canada

The consumer demand for organic, antibiotic-free produce is growing while antibiotic resistance in the orchard has become an issue in certain growing regions. Bacteriophages of *Erwinia amylovora* have become a promising avenue for biological control. When selecting phages for mixtures it is critical to determine their host range on a wide selection of bacterial hosts. This is commonly carried out using spot tests. While quick and simple, these tests are only qualitative. The appearance of a clearing, or plaque, on a bacterial lawn is assumed to indicate successful cell lysis. However, spot tests can exhibit false positives, and plaque appearance on *E. amylovora* is affected by exopolysaccharides. This can lead to an erroneous determination of host range. A quantitative host range can provide greater insight into phage-host interactions, comparing relative phage progeny produced as an indicator of successful infection. We have developed a technique using plasmid standardized quantitative real-time PCR to measure the production of 10 phages on 106 global isolates of *E. amylovora*. A quantitative host range study of this scale would not be feasible using plaque-based methodologies. Final phage production differed by up to 8 logs and almost all hosts which were unable to produce certain phages were isolated in western North America. This effect was largely correlated with the amount of the exopolysaccharide amylovoran produced by the host cells. Collectively however, these phages have a very broad host range as every host is able to enrich at least one phage. This suggests that a mixture of these phages would make an effective biological control treatment.

The application of depolymerase DpoL1 and bacteriophage Y2 causes a strong synergistic inhibitory effect on *Erwinia amylovora*

Yannick Born[1], Leandra E. Knecht[1], 2, Cosima Pelludat[3], Martin J. Loessner[2], and **Lars Fieseler**[1]

[1]Zurich University of Applied Sciences, Wädenswil, Switzerland; [2]ETH Zurich, Zürich, Switzerland; [3]Institute for Plant Production Sciences, Agroscope, Wädenswil, Switzerland

Erwinia amylovora is the causative agent of fire blight, a severe disease of Rosaceae plants. To prevent infection, streptomycin is usually applied during the flowering period. However, in some countries, use of streptomycin was banned recently. As an alternative to antibiotics, the application of bacteriophages for fire blight control seems to be a promising alternative. E. amylovora usually infects host blossoms via the stigma and invades the ovary. Later it spreads through the xylem vessels of an infected plant. In the xylem vessels *E. amylovora* produces high amounts of exopolysaccharides (EPS), e.g. a capsule, which leads to ooze formation and canker development. Transposon mutagenesis of E. amylovora revealed that adsorption of the T7-like phage L1 and the SP6-like phage S2 is dependent on the amylovoran synthesis (ams) operon. Accordingly, both phages exhibit a Depolymerase (Dpo) with 59 % amino acid identity. The enzyme is a structural component of the virion. DpoL1 binds specifically to amylovoran and cuts the galactose backbone. In vitro treatment of a growing *E. amylovora* culture with DpoL1 did not inhibit growth. However, application of the enzyme together with the amylovoran-independent phage Y2 revealed a strong synergistic inhibitory effect and caused a 4 log reduction of viable cell counts. In addition, we also identified a dpo gene in phage Bue1, a novel member of the Vil-like phages, which is clearly different from the podoviral depolymerases (24 % amino acid identity only). However, Bue1 does also infect non-capsulated strains of *E. amylovora* and transposon mutagenesis indicated that Bue1 adsorbs to LPS. Here we discuss the impact of capsule removal on adsorption and infectivity of different Erwiniaphages.

Abstracts - Oral Presentations

Bacteriophages effective against both *Erwinia amylovora* and *Erwinia pyrifoliae* causing fire blight and black shoot blight in apple and pear

Chang-Sik Oh

Kyung Hee University, Republic of Korea

Bacteriophages are the viruses that infect only target bacteria very specifically. Recently, they have been used as phage therapy, which is a biological control tool for preventing bacterial pathogens. In this study, 21 bacteriophages effective against *Erwinia amylovora* and *Erwinia pyrifoliae* causing fire blight and black shoot blight in apple and pear, respectively, from total 18 soil samples collected from apple and pear orchards in Korea, were isolated. Based on restriction enzyme digestion patterns of their genomic DNAs with EcoRI and BamHI, either separately or together, isolated bacteriophages could divide into three groups. The host ranges of three representative bacteriophages, phiEaP-7, phiEaP-8, and phiEaP-21, were determined with 33 bacterial strains, including twenty-three *E. amylovora*, five *E. pyrifoliae* and five other related bacteria. All three bacteriophages were very effective against *E. amylovora* and *E. pyrifoliae* strains, while none of them showed positive effect on *Pectobacterium*, *Dickeya*, and *Pantoea* species. Morphology of three bacteriophages examined with transmission electron microscope showed that phiEaP-7 and phiEaP-21 belong to Myoviridae, but phiEaP-8 belongs to Podoviridae. Genome analysis also showed the same results. Lytic activity of bacteriophages was stable up to 40-50oC, within pH 5-10, and under 365nm UV light. These results suggest that selected bacteriophages may have the potential as antibacterial agents infecting target bacterial pathogens for phage therapy. Currently, their survival length in soil, minimum MOI (multiplicity of infection) value, and stability with diverse adjuvants for commercial products are being examined. These results will also be presented.

The toolkit utilized by bacteriophages to infect and lyse bacteria

D. William Thompson and Julianne H. Grose

Brigham Young University, Provo UT USA

Bacteriophages are the most abundant living entity on the planet. Through their ability to infect and kill their hosts or to incorporate into the host genome, they contribute highly to the evolution of their hosts by controlling their ecological concentrations and providing novel functions through the transfer of DNA. Phages utilize specific proteins which target and degrade bacterial cell walls (lysins) and disrupt the membrane (holins). These proteins, together with EPS depolymerase, play an important role in the effectiveness of phage infection. We have isolated and sequenced 30 phages that infect the host *Erwinia amylovora* which fall into eight families of related phages. A close inspection of the phage genomes revealed that each phage family had a unique method of lysing the cell. We were able to identify 5 different EPS depolymerases, 7 lysins and 1 holin. Interestingly, a majority of our 30 phages also infect a closely related genus, *Pantoea vegans.* This co-infectivity rate is rare, prompting us to study the specificity of the phages lysins and holins for each of these hosts. We cloned and expressed 5 different EPS depolymerases and 7 lysins encoded by these *Erwinia* phages and tested their ability to break down the *Erwinia* and *Panteoa* biofilm and cell well. Several of these proteins showed specificity for one bacterium over the other. The diversity of and characterization of lysins, holins, and EPS depolymerase provide an understanding into how these phages have evolved in order to infect such closely related hosts, one (*Erwinia*) a phytopathogen and the other (*Pantoea*) thought to be a commensal host.

Abstracts - Oral Presentations

Insights into *Erwinia amylovora* parasitism and biocontrol via auxotrophic mutants

Tim W. McNellis[1], Sara Klee[1], Judith Sinn[1], Melissa Finley[1], Erik Allman[1], Philip Smith[1], Osaretin Aimufua[2], Viji Sitter[2], Brian Lehman[1], Teresa Krawczyk[1], Kari Peter[1]

[1]Pennsylvania State University, State College, PA USA; [2]Morgan State University, Baltimore, MD USA

We have isolated a series of auxotrophic mutants of *Erwinia amylovora* and tested them for virulence on apples, avirulence on tobacco, metabolic activity, and ability to interfere with wild-type *E. amylovora* growth in apple flowers. This resulted in the identification of a number of metabolites, including amino acids, which are sufficiently available from the host to render their synthesis by the bacterium unnecessary for full virulence. It was also determined that certain other metabolites must be synthesized by the bacterium growing in plant tissues for fire blight disease to develop. There was generally a correlation between avirulence activity on tobacco and virulence activity in apples, with the major exception of a arginine biosynthesis mutants, which failed to cause disease, but caused a robust hypersensitive reaction in tobacco. Metabolic profiling analyses showed that *E. amylovora* auxotrophic strains actively utilize extracellular metabolites. Application of an avirulent auxotrophic strain to apple flowers substantially reduced the growth of virulent *E. amylovora*, possibly though a competitive mechanism. Implications for fire blight parasitic interactions with the host and potential practical applications of *E. amylovora* auxotrophs will be discussed.

Microbiome associated with apple stigmas and its impact on fire blight infection

Quan Zeng, Zhouqi Cui

Connecticut Agricultural Experiment Station, New Haven, CT USA

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a devastating disease of rosaceous plants such as apples and pears. *E. amylovora* enters hosts through natural openings on flowers. Stigma, the receptive tip of a carpel in the gynoecium of a flower, produces nutrient rich exudate that not only attracts pollen, but also supports growth of many microbes, including *E. amylovora*. Successful colonization by *E. amylovora* on the apple stigmas is essential for the subsequent occurrence of the disease. In our past field experiments, we observed an interesting phenomenon that even though all apple flowers were evenly inoculated with *E. amylovora*, only a proportion of those flowers would later develop disease symptoms. We hypothesize that each single flower may harbor a unique microbiome and that the microbiome differences in these flowers may affect the pathogen colonization and further determine whether a flower would develop disease symptoms. To test this hypothesis, we performed a controlled inoculation experiment on 100 single apple flower clusters, in which efforts were made to ensure all flowers were under the same developmental stage and received an equal amount of pathogen cells. Two days post inoculation, the amount of *E. amylovora* on each individual flower was quantified by qPCR. The microbiome composition on each individual flower was also determined by using a culture dependent method and by deep sequencing of the 16S rRNA. We observed a negative correlation between the pathogen amount and microbiome complexity: flowers with more E. amylovora tended to harbor a less complex microbiome, with Pseudomonas and Erwinia being the most dominate genera; flowers with less E. amylovora tend to harbor a more complex microbiome, which includes *Pseudomonas*, *Erwinia, Non-Erwinia Enterobacteriaceae, Bacillus*, and *Curtobacterium*. Findings from this study improved our understanding of fire blight infection in the natural environment. It also suggests the possibility of controlling fire blight through artificially increasing the complexity of the microbiome on apple flowers.

Abstracts - Oral Presentations

Diversity of the CRISPR/CAS system in *Erwinia amylovora*

Michael Parcey[1], Shawkat Ali[2], Guus Bakkeren[2], Alan J. Castle[1], Antonet M. Svircev[2]

[1]Brock University, St. Catharines, Ontario Canada; [2]Agriculture and Agri-Food Canada, Vineland, Ontario Canada

The CRISPR/Cas system in *Erwinia amylovora* acts as a record of the foreign genetic material which a strain has historically encountered. *E. amylovora* has three CRISPR regions called cassettes. Each cassette is composed of CRISPR repeats which flank non-repetitive sequences known as spacers. When *E. amylovora* encounters plasmids and phages, spacers are acquired from the antagonist's genetic sequence and are added sequentially to the CRISPR cassettes. The presence and pattern of these spacers within a cassette can be used for phylogenetic analysis as well as the identification of common antagonists. We have completed genomic sequencing of 94 *E. amylovora* isolates from Canada and around the world. These new genomes, in combination with the 33 published *E. amylovora* genomes, were analyzed to determine their relation and variability via the CRISPR/Cas system. In the total 127 genomes, 111 were isolated from hosts within the Amygdaloideae (previously Spiroideae) subfamily where the other 16 were isolated from *Rubus* hosts. Approximately 11 000 CRISPR spacers were extracted from all the CRISPR regions of these *E. amylovora* sequences. Phylogenetically, the Amygdaloideae-infecting *E. amylovora* can be distinguished by three primary clades: a high prevalence clade found globally and two low prevalence clades found in western and eastern North America. Based on the currently available genetic sequences, spacers of the CRISPR/Cas system in Amygdaloideae-infecting *E. amylovora* preferentially target plasmid antagonists such as pEU30, where as the CRISPR/Cas system of Rubus-infecting *E. amylovora* preferentially target phage.

PhytoTrakr: Creating a real time strain tracking tool for *Erwinia amylovora* and other plant pathogens

Anna E. Wallis[1], Andrea Ottesen[2], Lance Cadle-Davidson[1], and Kerik D. Cox[1]

[1]Cornell University, Geneva, NY USA; [2]Food and Drug Administration, College Park, MD USA

Understanding the distribution of *Erwinia amylovora* strains across geographic regions is integral to sustainable management of fire blight in commercial orchards. Investigations into phenotypic and genetic diversity of *E. amylovora* strains have identified differences in host range, aggressiveness, and virulence genes, with potential implication for their ability and extent to cause disease outbreaks (Pulawska and Sobiczewski 2012; Khan et al. 2018; Desnoues et al. 2018). In order to conduct tracebacks and epidemiological research efficiently and in real time, it is necessary to have a rapid, high-throughput strain identification protocol. Clustered regularly interspaced short palindromic repeats (CRISPR) pattern characterization is a reliable, inexpensive way to identify strains, but is currently a time-consuming operation requiring multiple Sanger sequencing runs and manual annotation (McGhee and Sundin 2012; Rezzonico et al. 2011). We are using next-generation amplicon sequencing technology to develop a high-throughput, multiplexed pipeline to identify CRISPR patterns efficiently (Yang et al. 2016). Additionally, whole genome sequencing may generate a more comprehensive approach for genome-wide *E. amylovora* strain characterization, as sequencing costs continue to decrease. A whole genome pipeline and database were recently created for human pathogens by the FDA and NIH, called the Genome Trakr Network: a publicly available searchable database of whole genomes of over 25 of the most important human pathogens, complied by public health and university laboratories and used regularly for scientific investigations and real-time surveillance of microbial foodborne pathogens (Allard et al. 2016). GenomeTrakr may serve as a suitable model and potential host for *E. amylovora* and other plant pathogens in the future, and we propose to name this 'Phytotrakr.' Suitable genomic information for populating such a database include whole genome sequences (WGS) or hypervariable regions of the genome such as CRISPR patterns and variable number tandem repeats (VNTRs) (McGhee and Sundin 2012; Rezzonico et al. 2011; Zeng et al. 2018). This presentation aims to coalesce a group of committed collaborators for creating a PhytoTrakr tool as a comprehensive strain resource.

Abstracts - Oral Presentations

Molecular analysis of the distribution of two ancestral populations of *Erwinia amylovora* in Europe using CRISPR data

Fabio Rezzonico, Mirjam Kurz

ZHAW Wädenswil, Switzerland

Erwinia amylovora isolates affecting pomaceous trees in Europe are genetically very homogenous and it is difficult to distinguish them with the aid of simple conventional molecular tools. Epidemiological statements are thus only possible on the basis of the analysis of hypervariable sections of the genome such as Variable Number Tandem Repeats (VNTR) or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) regions. Previous analysis of European isolates has shown that two ancestral CRISPR genotypes of the pathogen, denominated A and D, initially colonized Europe in the second half of the last century and that all other CRISPR genotypes were derived from them, mainly by loss of internal CRISPR spacers. In this work a total of about two hundred *E. amylovora* isolates from 13 European countries, collected between 1973 and 2017, were analyzed using a simple PCR assay designed to determine their ancestral CRISPR genotype. The strains belonging to the ancestral genotype D lack a duplication of spacer 1029 in the CRISPR Repeat Region 1 (CRR1), which can be found in the strains belonging to the ancestral genotype A. Following statements about temporal and spatial distribution of the two ancestral genotypes in Europe could be made based on the data obtained in this work: there were likely two major introduction events of *E. amylovora* with the ancestral genotype A appearing foremost on the Old Continent and within many single countries. Only starting from 1979 the ancestral genotype D could also be detected, even if it is now predominant in many Northern European countries such as Germany, Switzerland, Poland, the Czech Republic, Lithuania and Latvia. All the while the ancestral genotype A stayed prevalent in other Western European countries (Spain, Netherlands) and in the Mediterranean Area. The genetic diversity of E. amylovora in Europe has increased around the turn of the century with the diversification of A and D genotypes into A-derived and D-derived genotypes, respectively. This further discrimination of the isolates requires however complete sequencing of the CRISPR regions to be performed.

Patterns of genetic variation and selection across the *Erwinia amylovora* genome

Jugpreet Singh and Awais Khan

Cornell University, Geneva, NY USA

The fire blight disease caused by the bacterial pathogen *Erwinia amylovora* (EA) is a substantial threat to apple and pear production worldwide. Genome sequencing efforts have led to the annotation of several virulence factors and identification of variations across clustered regularly interspaced short palindromic repeats (CRISPR) loci and streptomycin resistance genes in EA. In addition, some genome resequencing studies have highlighted the extent of genetic variation and population structure in EA strains, but genomic signatures of selection due to geographical isolation, host range, and management practices remains unexplored. We used skim sequencing to analyze whole genomes of 41 strains to study the patterns of nucleotide diversity and selection affecting population structure in EA. A total of 72,741 single nucleotide polymorphisms (SNPs) and 2,500 insertions/deletions (Indels) were identified, representing approximately 6-fold more diversity than previously reported. We further identified nonsynonymous variants in the effectors, suggesting virulence differences between the EA strains. The extent of nucleotide diversity was slightly higher in EA plasmids than chromosome. EA strains formed three distinct sub-groups; North American strains had highest nucleotide diversity. A genome window scan for nucleotide diversity (π), fixation index (Fst), and TajimaD underlined several genomic regions associated with selection signatures and EA population differentiation into three distinct sub-groups. A detailed analysis of genome-wide selection effects suggested that both purifying and balancing selection played a role in shaping the genome of *Erwinia amylovora*.

Abstracts - Oral Presentations

Variation of fire blight susceptibility in a pedigree-connected apple germplasm set

Sarah Kostick[1], John (Jay) Norelli[2], Soon Li Teh[1], Kate Evans[1]

[1]Washington StateUniversity Tree Fruit Research & Extension Center, Wenatchee, WA USA; USDA-ARS
[2]Appalachian Fruit Research, Kearneysville, WV USA

Most commercial apple cultivars are susceptible to fire blight, caused by *Erwinia amylovora*, and management practices in the United States are not sustainable and/or not effective against all disease stages. Breeding for resistance offers a potentially sustainable, long-term solution; however most sources of fire blight resistance in apple have been identified in wild genetic backgrounds with poor fruit quality. Also, phenotyping fire blight resistance/susceptibility is challenging due the erratic nature of the disease, significant impacts of environmental conditions and tree vigor on susceptibility, and quantitative resistance. This study's objective was to characterize phenotypic variation of fire blight resistance/susceptibility levels in a pedigree-connected apple reference germplasm set.

Developed during the USDA-SCRI RosBREED project, this pedigree-connected apple reference germplasm set provides efficient representation of important breeding parents (IBPs) in three public US apple scion breeding programs. In this study, 27 IBPs were represented in a field planting of 556 individuals (i.e. seedlings, available IBPs and ancestors) planted in triplicate in a randomized complete block design.

Using the cut-leaf method, multiple actively growing shoots per tree were inoculated with *E. amylovora* 153n in 2016 (5×10^8 CFU mL^{-1}) and 2017 (1×10^9 CFU mL^{-1}). For each inoculated shoot, response was quantified as proportion of current season's shoot length that was blighted (SLB), calculated from shoot and lesion length measurements. Incidence and maximum age of wood that a fire blight lesion extended into were also recorded for each inoculated shoot. To estimate seedling effects within and across years, best linear unbiased predictions (BLUPs), adjusted by overall SLB mean, were used. K-means clustering was used to classify seedlings into resistance/susceptibility groups.

In both years, wide variation in fire blight susceptibility was observed among individuals with responses ranging from highly susceptible to highly resistant. Adjusted BLUPs for seedlings ranged from 0.04 to 0.97 SLB. Responses were relatively consistent across years with a correlation of $\rho = 0.75$ (p-value $<1 \times 10^{-4}$) between years for adjusted BLUPs. Phenotypic information from this study 1) enables increased understanding of variation of fire blight susceptibility in germplasm relevant to U.S. apple breeding programs, and 2) is being used in pedigree-informed QTL analysis to identify loci associated with fire blight resistance/susceptibility.

Rootstock genotypes influence response to fire blight in grafted apple scion cultivars

Ricky Tegtmeier and Awais Khan

Cornell University, Geneva, NY USA

Several studies have highlighted the use of fire blight resistant rootstocks to provide disease tolerance/resistance in grafted scion cultivars, but the mechanisms that account for this interaction are unknown. This study aims to better define the factors that contribute to a rootstock's ability to increase the resistance/tolerance of a susceptible scion cultivar. The tests utilized highly susceptible cultivar 'Gala' scions grafted on 21 different commercially valuable rootstocks. These rootstocks were also self-grafted as controls. The leaf tissues of scion cultivars were infected and monitored for differential phenotypic expression following artificial fire blight infections. Phenotypic data was collected for leaf length, leaf necrosis, and percent leaf lesion. The results indicated a significant ($p < 0.05$) rootstock genotype effect on fire blight tolerance in 'Gala' scions. The percent leaf lesion length ranged from 3.3 to 100% when 'Gala' was grafted on B118 and M9 rootstocks, respectively. Moreover, leaf length showed negative correlation with percent leaf lesion length, and the latter was significantly ($p < 0.05$) affected by leaf length when analyzed as a covariate in the statistical model. This observation suggests that developmental age of infected leaves can substantially influence the level of genotypic susceptibility against fire blight infection. We further explored the role of rootstock root traits to understand the cause of rootstock-derived tolerance in susceptible scions. It was observed that vigorous rootstocks had better tolerance against fire blight infection in susceptible scions. Further studies can elucidate the exact genetic and molecular mechanisms related to developmental-age and root-specific fire blight tolerance in susceptible scion cultivars.

Abstracts - Oral Presentations

Difference in gene expression between lowly and highly virulent *Erwinia amylovora* strains on two apple cultivars of different susceptibility to fire blight

Joanna Puławska, Monika Kałużna, Wojciech Warabieda, Artur Mikiciński

Research Institute of Horticulture, Skierniewice, Poland

Erwinia amylovora strains, although homogenic in terms of phenotypic and genetic features, show variations in their virulence appearing in differences in their host range and the intensity of caused symptoms. The aim of our study was to find differences on the level of transcription between lowly and highly virulent strains: 650 and B62. For this purpose we applied RNA-seq technique to compare transcriptomes of *E. amylovora* strains, during the growth on microbiological medium, and 24 h and 6 days after infection of shoots of two apple cultivars – susceptible (Idared) and resistant (Free Redstar) to fire blight. The analysis of differences in gene expression of 650 and B62 strains grown on TY medium showed already differences in expression of 37 genes. Out of them, 31 were up-regulated in B62 including genes known as playing a role in pathogenicity: *amsF* and *hrcQ*. The clear difference between strains was observed in transcriptome changes in planta. The large gene pool of strain 650 was commonly up- (698) and down-regulated (640) on both apple cultivars in two time points after shoot inoculation compared to TY substrate. However, in the case of strain B62, only a small number, 33 and 37 genes, were commonly up- and down-regulated, respectively in all in *planta* combinations. Generally, a small number of B62 genes were differently expressed on Idared and large changes were observed in the expression on Free Redstar. For strain 650, the largest differences between bacterial transcripts in *planta* were observed 24 h after inoculation - 150 genes with an increased and 142 genes with reduced expression on cv. Idared compared to Free Redstar. For B62 strain, 24 h after inoculation 245 genes had a decreased and 79 increased expression on Idared compared to Free Redstar. Six days after inoculation, in the case of strain 650, differences in expression of 10 genes were observed between apple cultivars, while for strain B62 - 25 genes. In *planta*, 24 h after inoculation, among the genes of known role in bacterial virulence, the strain with higher virulence - B62, showed a higher expression of part of the genes responsible for the synthesis of flagellin, two genes belonging to the phosphorelay system, higher expression of genes responsible for desferoxamine synthesis, while lower expression of type III secretion genes (T3SS).

The results obtained from transcriptome analyzes indicate a potential role in the pathogenicity of many genes for which such a role has never been confirmed. Among the genes with the highest level of transcription in planta and among the genes with the greatest difference in the level of transcription between experimental combinations, many genes coding for proteins of unknown function were found - they can constitute new, previously undescribed virulence factors of bacteria.

The project was financed by National Science Centre, Poland, grant no. DEC-2012/05/B/NZ9/03455

Role of type III secretion system during the infection of apple flowers by *Erwinia amylovora* and the influence of relative humidity to its expression

Zhouqi Cui, Neil Schultes, Quan Zeng

The Connecticut Agricultural Experiment Station, New Haven, CT USA

Erwinia amylovora is the causal agent of fire blight, a devastating disease of rosaceous plants. To successfully cause infection, *E. amylovora* utilizes the type III secretion system (T3SS) to suppress host immunity. Flowers are a major entry point of *E. amylovora*. During the flower infection, *E. amylovora* first colonizes on the stigma and builds up a large population before migrating down to the hypanthium and causing infection. This process is heavily influenced by environmental factors. Although flower infection by *E. amylovora* is well studied, the role of T3SS during the flower infection and the influence of environmental factors to its expression remain unclear. In this study, we developed a dual fluorescence promoter reporter and used it to monitor the expression of a T3SS gene *hrpA* in wild type *E. amylovora* and a T3SS mutant *ΔhrpL*. The two strains were inoculated at stigma and hypanthium of detached apple flowers, and the fluorescence and bacterial growth were monitored over a period of four days under different relative humidity (RH; 60%, 80%, and 100%). On stigma, *hrpA* is constantly expressed at a high level in the wild type during the four-day period (about 90% of the total cells expressed *hrpA*). A strong positive correlation between RH, T3SS expression, and bacterial population was also observed. Compared to the wild type, the *ΔhrpL* not only did not expressed *hrpA*, but also displayed reduced growth. These observations suggest *E. amylovora* modulates its virulence according to the environment. It also suggests that the T3SS expression is necessary for the epiphytic growth of *E. amylovora* on stigma. Compared to the stigma, expression level of *hrpA* at the hypanthium is much lower, even under high RH (100%). *hrpA* was not expressed in the first two days, and was only expressed in about 30% of the total cells at day 3 and day 4. No difference in growth between wild type and *ΔhrpL* was observed, suggesting that T3SS is not necessary for the growth of *E. amylovora* at the hypanthium. However, flowers inoculated with *ΔhrpL* at the hypanthium developed no symptoms while 100% of flowers inoculated with wild type were infected by day 4, with high level of *hrpA* expression (>95% of total cells) observed in the infected internal ovary tissues. This suggests that although T3SS is not necessary for the epiphytic growth of *E. amylovora* at hypanthium, it is however critical for the infection. Our results identified hypanthium as a T3SS-repressing environment. By growing on stigma initially, *E. amylovora* not only builds up a large population, but also primes its virulence, both of which are critical for the subsequent infection at the hypanthium.

Abstracts - Oral Presentations

Cyclic-di-GMP is a critical regulator of biofilm formation and virulence factors in *Erwinia amylovora*

Roshni R. Kharadi and George W. Sundin

Michigan State University, East Lansing, MI USA

Erwinia amylovora is the causal agent of fire blight, an economically significant disease affecting pome fruit. Following entry into the host through shoot tips, *E. amylovora* uses type III secretion to translocate effector proteins into host cells, thereby suppressing host defenses and facilitating pathogenesis. As leaf infection progresses, cell ultimately colonize xylem vessels through the formation of extensive biofilms, which impede the migration of water and nutrients through the xylem, eventually leading to tissue necrosis and shoot blight symptoms. Cyclic-di-GMP (c-di-GMP) is a bacterial messenger compound used by *E. amylovora* to regulate the transition into and out of an attached lifestyle within biofilms. C-di-GMP formation is catalyzed by the enzymatic action of five diguanylate cyclase enzymes (DGCs) in *E. amylovora*, and hydrolysis of c-di-GMP into 5'-phosphoguanylyl-(3'→5')-guanosine (pGpG) is regulated by three phosphodiesterase enzymes (PDEs). To study the effects of modulating intracellular levels of c-di-GMP, we interrupted c-di-GMP hydrolysis through the mutagenesis of the three PDE encoding genes, *pdeA, pdeB* and *pdeC* both singly and in combinations of two or three genes. Amylovoran production was found to be positively regulated by c-di-GMP at a transcriptional level in a concentration dependent manner. Virulence mediated by type III secretion was found to be negatively regulated by c-di-GMP, both through the transcriptional control of the alternate sigma factor encoding gene *hrpL*, and through the reduction of DspE translocation into the host. During the process of biofilm formation, *E. amylovora* cells encounter shear forces within the xylem vessels arising from host transpiration. We used a flow-cell based system, GFP-tagged *E. amylovora*, and confocal laser scanning microscopy to study the impact of varying c-di-GMP levels on biofilm formation. We found that the shear force arising from flow promotes the formation of robust biofilms when compared to conditions of static incubation. In addition, increasing levels of c-di-GMP positively regulate biofilm formation under flow. Further, the complete deletion of all eight *edc* and *pde* genes involved in c-di-GMP dimerization and hydrolysis led to an absence of intracellular c-di-GMP, and as a result, a total lack of attachment and biofilm formation in the flow cell-based system. Thus, c-di-GMP is a critical factor involved in the regulation of attachment and biofilm formation in *E. amylovora*.

Small RNA ArcZ regulates catalase and peroxide susceptibility in *Erwinia amylovora*

Jeff K. Schachterle and George W. Sundin

Michigan State University, East Lansing, MI USA

Precise control over virulence trait expression is critical for successful infection by E*rwinia amylovora*, the gram-negative bacterial causative agent of fire blight disease. Transcriptional control plays a major role in mediating *E. amylovora* adaptations to different environments including responding to different host cell and tissue types and host defenses. In addition to transcriptional regulation, post-transcriptional regulation plays a major role in contributing to expression of critical virulence traits, and is often mediated by small non-coding RNAs (sRNAs). Previous work identified 42 sRNAs regulated by the chaperone protein Hfq. Phenotypic testing indicated that the *E. amylovora* Hfq-dependent small RNA ArcZ plays a critical role for expression of enzymes involved in mitigating the threat of reactive oxygen species. *E. amylovora* wild-type strain Ea1189 elicits an oxidative response of up to ~4 mM H_2O_2 in apple leaves during disease progression, and this strain grows uninhibited *in vitro* in culture medium supplemented with 5 mM exogenous hydrogen peroxide. Expression of the catalase-encoding gene *katA* had the strongest effect on Δ*arcZ* phenotypes related to susceptibility to hydrogen peroxide. Further testing revealed that ArcZ regulates *katA* at the transcriptional level, suggesting that ArcZ likely regulates a transcriptional regulator of *katA*. Through transcriptomic analysis of the ArcZ regulon, we observed significant overlap with the regulons of transcription factors involved in oxidative sensing and response including the ArcBA two-component system, Fnr, and Fur. We determined that ArcZ is post-transcriptionally regulating *arcA*, providing a hypothetical mechanism for the ArcZ regulation of KatA through ArcA. Together this places *arcZ* in a regulatory cascade in which it can both contribute to oxidative sensing as well as mediating the response. Together, these findings suggest that oxidative sensing and response, including sRNA signaling, are critical bacterial behaviors for successful fire blight infection.

Abstracts - Oral Presentations

Small RNA *rprA* modulates biofilm dispersal in *Erwinia amylovora*

Jingyu Peng and George W. Sundin

Michigan State University, East Lansing, MI USA

Fire blight, caused by *Erwinia amylovora*, is a devastating bacterial disease threatening the worldwide production of many rosaceous fruits, such as apple and pear. During the primary infection through flowers, a high population of *E. amylovora* cells is established on the stigma that further disseminates down to nectarthodes, where infection occurs. Within xylem vessels of the plants, biofilms, adherent cells embedded in a matrix of exopolysaccharides (EPSs), are formed that block water transport and cause wilting symptoms. *E. amylovora* cells that dislodge from biofilms are relocated and cause new infections. Previous screening of RNA chaperone protein Hfq-dependent small RNAs in *E. amylovora* yielded the identification of *rprA*, which also contributes to the pathogenesis of *E. amylovora*. In this study, we demonstrate that *rprA* positively regulates amylovoran, the main exopolysaccharide of *E. amylovora,* and the type III secretion system, that both contribute to the primary infection in this bacterium. Interestingly, *rprA* also acted as a negative modulator of flagellum-dependent motility, and of levansucrase activity and cellulose production, two other EPSs that function synergistically with amylovoran to form the biofilm matrix of *E. amylovora*. An *in vitro* experiment modeling the dispersal of cells from biofilms indicated that RprA plays a substantial role in this stage of biofilm development in *E. amylovora*. Through confocal fluorescence microscopy, we also found that the expression of rprA, at single-cell resolution, was activated in *E. amylovora* cells actively infecting immature pears. We currently are leveraging this tool to examine if *rprA* is fine-tuned in *E. amylovora* during different infection stages within apple trees. Taken together, our results shed light on a better understanding of the roles of RprA in orchestrating multiple virulence factors of *E. amylovora* to facilitate the transition from sessile to planktonic modes of infection of this bacterium within a host.

Hfq controls bacterial virulence through linking c-di-GMP and two mechanistically distinct sRNAs

Xiaochen Yuan[1], Quan Zeng[2], Christopher M. Waters[3], George W. Sundin[3], Ching-Hong Yang[1]

[1]University of Wisconsin-Milwaukee, Milwaukee, WI, USA; [2]The Connecticut Agricultural Experiment Station, New Haven, CT, USA; [3]Michigan State University, East Lansing, MI, USA

Hfq and Hfq-dependent small RNAs (sRNA) are important post-transcriptional regulators in multiple bacterial species on different phenotypes, yet the detailed regulatory mechanism is often unelucidated. *Dickeya dadantii* is an important plant pathogen that causes soft rot disease on vegetable and potato crops. This pathogen utilizes two sets of virulence factors, plant-cell-wall degrading enzymes (PCWDE) and the type III secretion system (T3SS), to cause disease. Flagella motility is also important for the bacteria to migrate from infected plants to nearby uninfected plants. Here, we elucidated the regulatory mechanism of Hfq and an Hfq-dependent sRNA, ArcZ, on the two important virulence factors and flagella motility in *D. dadantii*. ArcZ represses *pecT* translation through the 5' untranslated region of the *pecT* transcript in an Hfq-dependent manner. PecT, a LysR-type transcriptional regulator, down-regulates the expression of PCWDE and T3SS by repressing the transcription of *rsmB*, encoding an Hfq-independent sRNA. Besides the Hfq-ArcZ-PecT-RsmB pathway, Hfq also controls the expression of PCWDE, T3SS and flagella motility through another regulatory pathway that is dependent on the bacterial secondary messenger, c-di-GMP. Hfq negatively controls the cellular level of c-di-GMP by down-regulating two diguanylate cyclases (DGCs), GcpA and GcpL. As cellular c-di-GMP levels in *D. dadantii* was demonstrated to be in negative correlation with PCWDE, T3SS and flagella motility, the down-regulation of c-di-GMP by Hfq results in activation of PCWDE, T3SS and flagella motility. Together, our results suggest that there is an interplay between the three signal transduction systems, Hfq and Hfq-dependent sRNA, Hfq-independent sRNA RsmB, and c-di-GMP.

Abstracts - Oral Presentations

In orchard population dynamics of *Erwinia amylovora* on apple flower stigmas

Suzanne M. Slack, Jeffery K. Schachterle, Emma M. Sweeney, Megan Botti-Marino, Roshni R. Kharadi, Jingyu Peng, Emily A. Pochubay, George W. Sundin

Michigan State University, East Lansing, MI USA

The fire blight disease cycle is typically initiated each spring with the disease stage known as blossom blight. For blossom blight to occur, the fire blight pathogen *Erwinia amylovora* establishes and builds populations on the stigma and subsequently moves into the hypanthium, ultimately gaining entry into the flower through natural openings in the nectaries. Epiphytic growth on the flower stigma is known to be pivotal to the ability of *E. amylovora* to cause disease. Flower stigmas are sensitive to temperature, and as they age are thought to be less conducive to supporting large bacterial populations. However, most studies assessing *E. amylovora* growth on stigmas have been done under environmentally-controlled conditions. In this study, we examined the population dynamics of a rifampicin-resistant *E. amylovora* strain over the course of bloom for three field seasons using 1, 3, and 5 day old flowers of five apple cultivars of varying disease susceptibility. Weather conditions including temperature, windspeed, relative humidity, and precipitation were monitored hourly during each experimental replicate. The ability for *E. amylovora* to colonize older flowers was diminished, as both population count and subsequent disease incidence decreased drastically. In contrast, on one-day old flowers, *E. amylovora* populations on stigmas were able to reach 10^7 colony forming units (CFU) in as little as 72 hrs after inoculation with 10^4 cells per flower. The first substantial population jumps occurred between the 24 and 48 hr time points, with as much as a 100-fold increase in population occurring in that time. These jumps occurred in conditions with atmospheric temperatures as low as 2.9oC, highs ranging from 9.4°C to 22°C, with averages around 12°C. In many cases, these temperatures were below the average mean daily temperature required for infection for the disease forecasting system MaryBlyt (15.6°C). Disease incidence observed was between 35-88% of flowers inoculated in these below threshold conditions. After this finding, more intensive population sampling revealed that the large increases between 24-48 hrs after inoculation were occurring in the evenings between 6 pm and 6 am. Our findings indicate that *E. amylovora* populations can grow and infect flowers at daily temperature averages below the forecasting minimums, with significant growth occurring at lower night temperatures.

Management of Fire Blight in Young Apple Orchards

Darlene Nesbitt and Antonet Svircev

Agriculture and AgriFood Canada, Vineland, Ontario Canada

Fire blight management practices for newly planted apple orchards are not well defined as current recommendations favour established orchards. These recommendations include cultural, chemical and biological control, varietal resistance, and the use of prediction models to forecast blossom infection and recommend spray timing. In 2015, this study was initiated to provide data to formulate the best practices for each apple growing region in Canada. The experiment was established on the AAFC Jordan Experimental Farm situated in Jordan, Ontario, Canada. The test site consisted of 320 one-year-old Brookfield Gala trees in a split-split-split plot design. The experiment, split between M9 and G41 rootstocks, studied the combined effects of three treatments: rates of nitrogen, application of prohexadione-calcium (Apogee), and application of Auerobasidium pullulans (Blossom Protect) with streptomycin. In 2016, only nitrogen applications at standard and half rate were implemented for vigour control. The experimental plan originally included artificial inoculation with Erwinia amylovora after the first year. In year two however, this proved unnecessary as approximately 50% of the young trees showed signs of infection in the spring of 2016. Experimental data collected in 2017 and 2018 included monthly measurement of shoot length and fire blight incidence. At the end of the season, an evaluation of total yield for each tree was also performed. We report preliminary results that were subjected to statistical analyses.

Abstracts - Oral Presentations

Prohexadione-Ca growth regulator and pruning as post symptom rescue treatments following fire blight infection during bloom

Vincent Philion and Valentin Joubert

Research and Development Insitute for the Agri-Environment, Saint-Bruno-de-Montarville, Quebec Canada

In many areas where apples are grown, fire blight (*Erwinia amylovora*) is a sporadic but potentially devastating disease. Infections occur primarily during bloom when warm weather conducive to bacteria multiplication on the stigma is followed by a wetting event which facilitates transfer of bacteria to the hypanthium where plant entry occurs. Most spray recommendations against this disease aim to prevent blossom infections. However, because fire blight is infrequent in any given orchard many growers opt not to spray during bloom, especially on old mature blocks or tolerant cultivars which can withstand some disease pressure. This practice can result in widespread disease which can then spread during the summer to neighboring younger blocks of higher economical value. Although Prohexadione-Ca (ProCa) treatments star=ted at bloom are effective at reducing disease spread in the summer, past studies have concluded this growth regulator isn't useful when treatments are initiated post-symptomatically. Although late ProCa applications may not be sufficient as the sole strategy against fire blight, we hypothesized that the cell thickening effect of ProCa which prevents infection could also reduce ooze production of infected shoots, thus providing some benefit by limiting inoculum spread. Our goal was to evaluate the benefits of both late ProCa treatments and summer pruning for fire blight management. The study was carried out in 2018 at the IRDA research orchard in Saint-Bruno-de-Montarville, Québec. Plots of 2 consecutive mature trees (cv Cortland) were randomly allocated to 6 different treatments and replicated in 4 blocks (CRBD). Trees were either not sprayed, sprayed three times with Apogee (BASF, 27.5% ProCa) (546g/ha) starting at full bloom (May 23rd) or twice with a higher dose (819g/ha) starting when blossom symptoms started to appear in the control (June 6th). The last spray was applied on June 21st. Thus, the same quantity of ProCa was applied to trees of the different sprayed treatments. All sprays were made with a custom tunnel airblast sprayer operated at 460L/ha. Two hours after the bloom ProCa spray, all trees were misted with 460L/ha of a 1E+6 CFU/ml suspension of a 1:1 mix of local strains of *Erwinia amylovora*. Approximately at weekly intervals, symptoms were pruned out from half the trees whereas symptoms from the three unpruned treatments were cut out after harvest. Individual tree cuttings were air dried and weighed. On June 15th, growing shoots (25 per tree) were inoculated by transversely bisecting the two youngest leaves with a pair of scissors dipped in a suspension of 10^9 CFU/ml of the pathogen. On June 27th, each of these shoots was rated for disease severity (0 = absence; necrosis on: 1 = inoculated leaves, 2 = extending to petiole, 3 = reaching shoot, 4 = reaching other leaves) and the length of the necrotic area was recorded. Shoots were also assessed for presence of exudate, and if it was alive, still actively growing. At harvest, yield was recorded per tree.

Trees were severely affected with blossom blight. We observed that significantly less wood was removed from trees pruned during the season (2.3 kg/tree) than from trees cleaned of symptoms only after harvest (3.8 kg/tree) (GLM: F = 8.8; P = 0.007). ProCa had no effect on the amount of wood affected (GLM: F = 1.04; P = 0.32). In contrast, ProCa reduced the severity score on inoculated shoots (CLM: LR = 62(2 df); P < 0.001) and the effect was influenced by pruning (CLM: LR = 6(2 df); P = 0.06). The effect of ProCa was similar (odds ratio (OR) approx. 5) for both spray initiation dates but was reduced on pruned trees when sprays were initiated at bloom (OR = 2.5). Furthermore, ProCa reduced the probability of observing exudate (OR =5, GLM: LR = 105(2 df), P < 0.001), whereas pruning did not (GLM: LR = 0.1(1 df), P = 0.75). ProCa also increased the probability of survival of inoculated shoots (GLM: LR = 38(2 df), P < 0.001). The OR of survival was similar for the two spray starting dates (approx. 4.5), but the effect was lower (OR =1.7, interaction GLM: LR = 7.2(2 df), P = 0.03) for trees sprayed with ProCa starting at bloom and pruned. Lastly, pruning (GLM: LR = 23(1 df), P < 0.001), ProCa (GLM: LR = 11(2 df), P = 0.004) and their interaction (GLM: LR = 10(2 df), P = 0.005) influenced growth. Live inoculated shoots were more likely to be still growing when ProCa treatments were initiated after bloom on pruned trees (OR=15). However, ProCa had no discernable effect on growth of inoculated shoots on unpruned trees, and pruning had no effect on growth in absence of ProCa. At harvest, yield was 5kg/tree higher from the ProCa treatments when trees were left unpruned during the season. Trees pruned during the season and/or not sprayed with ProCa had similar yields. If these observations are confirmed, use of ProCa soon after blossom blight symptoms appears could become a useful tool to reduce summer spread of fire blight.

Post-infection applications of prohexadione-calcium can reduce/prevent shoot blight initiation of fire blight cankers on perennial apple wood

Srdan Acimovic, Ricardo D. Santander, Christopher L. Meredith, Fatemeh Khodadadi
Cornell University, Highland, NY USA

Erwinia amylovora infections of flowers and shoots can lead to initiation of fire blight cankers on apple wood. Development of cankers frequently leads to death of dwarf trees in high density apple orchards and any cankers remaining in the orchard after pruning can serve as overwintering inoculum sources for infection renewal in the spring. With the implementation of high-density apple plantings with narrow spindle-shaped training systems that allow production of higher fruit yields, fruiting limbs of adjacent trees comprise a tree wall with flower clusters often overlapping and in physical contact thus contributing to an easier spread of fire blight bacterium epiphytically from tree to tree. Under adequate weather conditions favoring pathogen population growth, it takes few fire blight infections of flowers to rapidly reach a level of an outbreak and then a regional fire blight epidemic. Blossom or shoot blight infections on dwarf trees can quickly progress internally from branches into the trunk because the fruiting limbs are younger, shorter and thinner in comparison to the older, longer, and thicker limbs of the classic training systems (e.g. freestanding central leader). In addition, one to three limbs on the trunk thicker than 20 mm are removed and renewed every year on these spindle trees, constantly producing young i.e. blight-susceptible shoot growth allowing direct pathogen access to the trunk. The resulting fire blight cankers on limbs, trunks, and rootstocks of young trees can lead to significant tree losses ranging from 500 to 2000 dead trees per acre in just one year. We initiated experiments to develop chemical program/s with plant growth regulator prohexadione-calcium (PCA) and a systemic acquired resistance inducer acibenzolar-S-methyl (ASM) for post-infection management of shoot blight with the goal to reduce or prevent the formation of devastating cankers in cases of poorly managed fire blight outbreaks. We focused on the destructive effects of fire blight cankers formed on apple wood from shoot blight symptoms, which have been problematic even in orchards with recently successful blossom blight control programs. To reduce or prevent shoot blight severity and the resulting canker initiation on wood from the infected shoots, we evaluated twelve post-infection spray programs on mature trees of 'Royal Cort', including treatments with PCA and PCA plus ASM, combined or not with a surfactant, and applied at different concentrations 1-3 times in 14-day intervals. Treatments with copper were also tested for comparative purposes. The tested spray programs were: 1 application of PCA 123.6 mg/L (1 x PCA 123.6 mg/L); 2 x PCA 123.6 mg/L + surfactant; 1 x PCA 247.1 mg/L; 2 x PCA 247.1 mg/L; 1 x PCA 20.6, 1 x 41.2, 1 x 20.6 mg/L; 1 x PCA 41.2, 1 x 20.6, 1 x 41.2 mg/L; 1 x PCA 123.6 mg/L + ASM 25 mg/L; 2 x PCA 123.6 mg/L + ASM 25 mg/L; 2 x PCA 123.6 mg/L + ASM 25 mg/L + surfactant; 3 x PCA 123.6 mg/L + ASM 25 mg/L; 3 x 224.2 g/ha metallic copper + lime; 3 x 224.2 g/ha metallic copper. Sprays were applied 1.5 to 2 days after the manual inoculation of shoots (30 per treatment) with *E. amylovora*. All treatments except 1 x PCA 123.6 mg/L, which was applied at pink bud growth stage, were started at 2.5 to 7.5 cm shoot length. The parameters measured were the reduction of shoot blight severity and the control of canker initiation. The experiment was repeated over two years and the treatments were sprayed dilute to drip (300 gal/acre). Each spray program was applied to three trees. In comparison to the untreated control, we achieved the greatest reduction of shoot blight severity of 72.5 and 78.8% in years one and two, respectively, with two applications (2 x) of PCA 247.1 mg/L, 14 days apart. A single application of the same rate (1 x PCA 247.1 mg/L), reduced shoot blight severity by 53.5% the first year and 89.5% the second. The latter treatment also provided 71.5% and 100% control in canker initiation on perennial wood in two consecutive years, respectively. Three applications (3 x) of 123.6 mg/L PCA + 25 mg/L ASM, each 14 days apart, reduced canker initiation by 78.6 and 83.5% in years one and two, respectively. Therefore, our results indicate that the most effective post-infection programs of PCA and PCA plus ASM can be valuable tools for fire blight management in reduction or prevention of destructive canker development on apple wood, thus minimizing tree losses after un-prevented fire blight infections.

Abstracts - Oral Presentations

Factors affecting the selective detection and quantification of *Erwinia amylovora* live cells in natural cankers by viability digital PCR using propidium monoazide

Ricardo D. Santander, Christopher L. Meredith, Srdjan G. Acimovic
Cornell University

Selective detection of live cells can be considered an ideal goal for most microbial diagnosis applications. Bacterial viability has classically been linked to growth on solid media. However, there are plant pathogens unable to form colonies on media plates (e.g. Candidatus Liberibacter spp.), bacteria isolation from plant material is not always successful, and the adoption of the viable but nonculturable state by many phytopathogenic bacterial species has been reported since the discovery of this survival strategy in the 20th century (e.g. Erwinia amylovora, Ralstonia solanacearum, Rhizobium radiobacter, etc.). Nowadays, there are a variety of culture-independent methodologies allowing the determination of bacterial viability. These usually involve the detection of certain metabolic activities, RNA transcripts, cell multiplication, membrane potential, membrane integrity, etc. In this sense, one of the most recent approaches for the selective detection of viable bacteria in natural samples consists of staining with propidium monoazide (PMA), a DNA intercalating dye similar to propidium iodide that is able to penetrate only into dead bacterial cells due to their compromised membrane integrity. After photo-activation, PMA binds irreversibly to DNA making it unsuitable for PCR. Accordingly, only live cells' DNA, not exposed to PMA, can be detected by DNA amplification techniques. Despite the abundance of studies using this method, live cell discrimination involving PMA treatments may represent a significant challenge depending on plethora of factors, with several works showing protocol variations required even in a bacterial strain-dependent manner. Other elements determining success in PMA treatments are the type and concentration of analyzed organisms, sample properties (composition, texture, opacity), the target DNA sequence, etc. A poorly designed PMA treatment may lead to live cell number overestimation, i.e. detection of dead cells as live ones, due to underlying factors related, among other things, to i) the apparent difficulty of PMA to penetrate even damaged membranes, ii) an insufficient dye concentration to stain all the target DNA in the sample, or iii) sample matrix characteristics hampering the process of PMA photo-activation. In this work we conducted an optimization of a PMA-based method for the selective detection and quantification of *Erwinia amylovora* live cells in natural cankers, by combining PMA staining with the chip-based Quantstudio 3D digital PCR (dPCR) System. Some advantages of dPCR with respect to quantitative PCR are the reduced inhibition of PCR reactions by compounds in plant material, and the quantification of target DNA in a sample based on limiting dilution and Poisson distribution statistics, without the need for a calibration curve. We determined that some of the key factors affecting the viability dPCR (v-dPCR) performance were the composition of the plant tissue maceration buffer, the target DNA length, the thermal cycle number and the use of sodium dodecyl sulfate or a commercial PMA Enhancer for Gram negative bacteria, to improve the effect of PMA. The newly developed v-dPCR protocol was robust enough to determine *E. amylovora* live cell concentrations in both artificially inoculated tissues and natural canker samples, contaminated with different E. amylovora strains. This method also allowed the analysis of *E. amylovora* population dynamics in apple and pear cankers of different cultivars in different time periods, and it is being tested to assess the effect of commercial pesticides and bark penetrants on *E. amylovora* survival in cankers. In the future, PMA staining could also be optimized in combination with regular PCR and quantitative real-time PCR to improve fire blight diagnostics. Combined with plate counts, this methodology also allows the detection of viable but nonculturable cells. The v-dPCR protocol optimized in this work may shed light on important aspects of the *E. amylovora* biology which remain poorly studied, such as the pathogen population dynamics in plant tissues during infections and within less studied reservoirs such as mummified fruits or necrosed tissues, as well as accurate analysis of *E. amylovora* survival on plant surfaces, insects, soil and other environments.

Evaluation of rapid and cost-effective pathogen detection assays for fire blight management in apple orchards

Alejandra Rodriguez Jamarillo, **Jugpreet Singh**, and Awais Khan

Cornell University, Geneva, NY USA

Fire blight susceptibility of commercial scion and rootstock cultivars remains a significant risk to the profitability of apple orchards. Fire blight leads to necrotic plant tissues, some of which can be confused with spray damage, nectria twig blight, and similar symptoms from other abiotic and biotic factors. For instance, rootstock blight in tree suckers and water-sprouts can be confused with spray damage. Thus, an accurate and timely detection of fire blight pathogen, *Erwinia amylovora* (EA), can help reduce the disease risk in commercial orchards. In addition, detecting the level of pathogen invasion inside plant tissues can help design pruning measures for necessary and timely management of fire blight. We tested two lateral flow immunoassays (AgriStrip®, and Pocket Diagnostics kit), and two PCR-based methods, (LAMP; Loop mediated isothermal amplification, and quantitative PCR), to detect EA from direct lab grown cultures and apple tissues infected with fire blight. The sensitivity of detection assays mainly depended on the bacterial concentration in the solution. The AgriStrip® and Pocket Diagnostics kits were able to detect actively growing bacteria up to 10^7 and 10^6 concentrations, respectively. In addition, Pocket Diagnostics kit also detected other Erwinia species, hence less suitable to detect only *Erwinia amylovora*. In contrast, LAMP and qPCR assay can detect very low bacterial content diluted up to 10^{-2} concentrations. Moreover, LAMP assay sometimes provide unreliable results, probably due to formation of primer dimers in the reaction. Overall, we outline the potential factors restricting the adoption of these assays for onsite fire blight detection in commercial orchards, which still require more research to improve the accuracy of pathogen detection at lower concentrations.

Abstracts - Poster Presentations

Progress in biopesticides for fire blight management

Brad Fritz, **Greg Rogers**, and Mike Dimock

Certis USA, Columbia, MD USA

For the past decade, Certis USA has been developing biopesticides with demonstrated efficacy against the fire blight pathogen, Erwinia amylovora. Our portfolio of products to combat this devastating disease has grown to meet the demand for rotational partners and alternatives to antibiotics. In addition to our copper and microbial products for fire blight management, Certis USA has most recently launched AgriPhage™-Fireblight, a product based on a bacteriophage that specifically targets the fire blight pathogen. Here, we summarize the modes of action for AgriPhage™-Fireblight and our recently launched LifeGard® (*Bacillus mycoides* isolate J), a microbial inducer of natural plant defenses. Also highlighted are recent field trial data in pome fruits for Kocide® 3000 (copper hydroxide), Cueva® (copper octanoate), and Double Nickel® (*Bacillus amyloliquefaciens* D747).

Loss-of-function mutations in the Dpp and Opp permeases render *Erwinia amylovora* resistant to kasugamycin and blasticidin S

Yixin Ge[1], Jae-Hoon Lee[2], Baishi Hu[1], and **Youfu Zhao**[2]

[1]Nanjing University, Nanjing, China; and [2]University of Illinois, Urbana, IL

Extensive use of antibiotic streptomycin to control fire blight disease of apples and pears, caused by *Erwinia amylovora*, leads to the development of streptomycin-resistant strains in the United States and elsewhere. Kasugamycin (Ksg) has been considered as an alternative to control this disease. In this study, we investigated the role of two major peptide ABC-transporter systems in E. amylovora, the dipeptide permease (Dpp) and oligopeptide permease (Opp), in conferring sensitivity to Ksg and blasticidin S (BcS). Minimum inhibitory concentration and spot dilution assays showed that the dpp and opp deletion mutants exhibited enhanced resistance to Ksg and BcS. Deletion of both dpp and opp conferred higher level of resistance to Ksg as compared to the single mutant. In addition, bioinformatic analysis combined with qRT-PCR showed that the Rcs system negatively regulates opp expression and the rcsB mutant was more sensitive to both Ksg and BcS as compared to the wild-type. Electrophoresis motility shift assay further confirmed the direct binding of the RcsA/RcsB proteins to the promoter region of the opp operon. However, neither the Dpp nor the Opp permeases contributed to disease progress on immature pears, hypersensitive response on tobacco leaves, motility, and amylovoran production. These results suggested that Ksg and BcS hijack the Dpp and Opp permeases to enter *E. amylovora* cells, and the Dpp and Opp permeases act synergistically for illicit transport of antibiotics.

Abstracts - Poster Presentations

Current status of fire blight by *Erwinia amylovora* in Korea

Sung-Jun Hong, Kyong-Jae Lee, Tae-Geun Kim, Yeong Mok Baek, Hyeong-Il Roh, Jong-Ho Lee, Jun-yong Chung

Fire blight, caused by E*rwinia amylovora*, is a devastating disease and threat for apple and pear production worldwide. *E. amylovora* causes wilting and blight on most of the above-ground parts of host plants, including flowers, leaves, fruits and branches. In early May 2015, disease symptoms similar to typical blossom and shoot blight of fire blight disease were observed in young blossoms and shoots of Asian pear in the region of Anseong and Cheonan. Based on several microbiological characteristics including genetic and biological assays, the disease was finally confirmed as fire blight disease caused by *E. amylovora*. Consequentially, the fire blight occurred in 42.9ha of 43 orchards(3 regions) in 2015. In 2016, 17 orchards (15.1ha, 2 regions) were confirmed to be infected by this pathogen, and 33 orchards (22.7ha, 2 regions) were confirmed in 2017. Two years(2016 year and 2017 year) tended to decrease compared to 2015 year. However, in 2018, the disease spread to 67 orchards (48.2ha) in 6 regions. In 2018, a favorable environment was created for the occurrence of this disease in April, May and June, which is assumed to have caused a major outbreak. The South Korea government set up a management strategy for this disease in 2015, and recommended removing all host trees within 100m radius range from an infected plant. It was also banned from growing of host plants for five years. Moreover, 3 times intensive chemical treatment and 4 times monitoring of fire blight have been performed from 2015 in the occurrence regions. A total of 290 orchards (191.5ha) were removed from 2015 to 2018 in accordance with national management strategy. In a detailed analysis by year, 68 orchards (59.9ha) were removed in 2015, 32 orchards (19.7ha) in 2016, 55 orchards (31.7ha) in 2017 and 135 orchards (80.2ha) in 2018. From in 2019, the management strategy was changed to remove only occurrence orchard from 100m radius range from an infected plant. It was also changed the period of growing prohibition from five years to three years.

Comparative genomics of bacteria: Understanding host specificity of *Pseudomonas* on *Prunus*

Michelle T. Hulin[1], John W Mansfield[2], Samantha Lynn[1], Kieran Housley[1], Felicidad Fernandez[1], Marzena Lipska[1], Helen Neale[3], Dawn L Arnold[3], Robert W Jackson[4], Richard J Harrison[1]

[1]NIAB EMR, East Malling, United Kingdom; [2]Imperial College London, London, United Kingdom; [3]University of West of England, Bristol, United Kingdom; [4]University of Reading, Reading, United Kingdom

Population genomics of bacterial phytopathogens is now allowing us to address questions about the evolution of complex traits. To explore this, we studied *Pseudomonas* syringae pathovars, which are important pathogens globally. Individual strains are believed to be host specialised to a greater or lesser extent, which may be linked to their repertoire of type III effector proteins involved in both virulence and avirulence in planta. It is hypothesised that effectors may form functionally redundant groups that allow *P. syringae* to suppress the immune response of different hosts whilst utilising contrasting effector sets. Multiple clades of *P. syringae* have independently converged to cause canker disease of cherry (*Prunus avium*). They include *P.s* pv. morsprunorum races 1 and 2 and *P.s* pv. syringae. Genomic analysis of the Prunus strains revealed highly divergent effector and toxin repertoires between the different clades, indicating they use distinct mechanisms to cause disease. Despite these differences, the presence/absence of several effectors was associated with the disease and phylogenetics revealed effectors have been frequently swapped between cherry pathogens via horizontal gene transfer, on both plasmid and phage sequences. By contrast, the HopAB effector family has been lost or truncated in cherry-pathogenic clades likely due to an avirulence reaction in cherry. Utilising a Bayesian co-occurrence analysis of effector gene evolution, we now aim to dissect which effectors act redundantly in cherry pathogens, determine the set required for virulence and complement this with a polymutant approach in the laboratory.

Abstracts - Poster Presentations

Visual symptoms of fire blight infection on ornamental plants in Western Canada

Mario Lanthier

Crop Health Advising and Research, Kelowna, British Columbia Canada

This poster will be a pictorial presentation of flower, leaf and canker symptoms following fire blight infection in ornamental plant nurseries. Genus covered will be Amelanchier, Cotoneaster, Crataegus, Malus (crabapple and apple), Pyrus and Sorbus, with comments on the most susceptible cultivars. The pictures will be based on samples where pathogen confirmation was made with standard laboratory methods including Biolog and PCR.

Fire blight (caused by *Erwinia amylovora*) is frequently seen in commercial nurseries of British Columbia and Alberta. The pathogen is present at the nursery or on near-by infected landscape plants and is spread on infected nursery stock not presenting symptoms at the time of sale. The region is known for summer conditions of hot and dry weather, with long periods of temperatures around 30oC, which favors the growth and multiplication of the bacteria. New infections usually occur during bloom in spring but new infections are frequent on late bloom in early summer for plants planted in late spring. Epidemic disease outbreaks are seen after summer thunderstorms and hail storms.

Two-component systems and Lon protease regulate *hrpS* expression in *Erwinia amylovora*

Jae-Hoon Lee, and **Youfu Zhao**

University of Illinois, Urbana, IL USA

The bacterial enhancer binding protein HrpS is essential in activating *hrpL* and the type III secretion system (T3SS) in *Erwinia amylovora*. However, how the *hrpS* gene is regulated remains unknown in *E. amylovora*. In this study, we investigated the role of various two-component systems (TCSs) and Lon in regulating HrpS. Promoter deletion analyses and 5' RACE showed that the hrpS gene contains two promoters driven by HrpX/HrpY and the Rcs system, respectively. EMSA and qRT-PCR assays demonstrated that integration host factor IHF positively regulates *hrpS* expression through directly binding the *hrpX* promoter and positively regulating *hrpX/hrpY* expression. Western blot analyses showed that mutation in lon directly affected the accumulation and stability of HrpS. Mutation in lon indirectly influenced the expression of hrpS through accumulation of the RcsA/RcsB proteins. Furthermore, the *csrA* mutant showed significantly reduced transcripts of the *hrpX/hrpY, rcsA* and *rcsB* genes, indicating that CsrA is required for full hrpS expression. On the other hand, the csrB mutant exhibited up-regulation of the *rcsA* and *rcsB* genes, and *hrpS* expression was largely diminished in the *csrB/rcsB* mutant, indicating that the Rcs system is mainly responsible for the increased *hrpS* expression in the *csrB* mutant. These findings suggest that *E. amylovora* recruits multiple TCSs to regulate *hrpS* at the transcriptional level and HrpS is also regulated at posttranslational level by Lon.

Abstracts - Poster Presentations

Selection and characterization of plant-associated bacteria with biocontrol activities against *Erwinia amylovora*

Ester Marco-Noales[1], Silvia Barbé[1], Àngela Figàs-Segura[2], Inmaculada Navarro[1], Mohamed Benada[1], Telma M. Sampaio[1], **Ricardo D. Santander[2]**, Elena G. Biosca[2]

[1]Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investi
Cornell University, Highland, NY USA

Erwinia amylovora is as a very destructive and difficult to control pathogen worldwide. Agrochemicals, including antibiotics, which are banned in the European Union (EU), are not sufficiently effective against this highly adaptable pathogen, and also pose a negative environmental impact. This has led to the search for alternative strategies for biological and sustainable management, such as the use of bacteria with biocontrol activities. At present, there are only two registered antagonists against *E. amylovora* in the EU, so it is necessary to search for new biocontrol agents. From a collection of bacterial strains obtained from different plant sources, ten strains were initially selected for their potential antagonistic activity against *E. amylovora* reference strain CFBP 1430 both in normal (nutrient rich) and one ten diluted (nutrient poor) King's B medium. The biocontrol potential of these ten strains was also evaluated by means of several ex vivo tests on detached immature loquats, individually pre-treated with the candidate strains. A second round of selection was conducted with additional ex vivo tests on immature loquats (of two different cultivars) and pears, including other *E. amylovora* strains from different sources and origins. Subsequently, biocontrol candidates were also characterized by their ability to trigger or not a hypersensitive response (HR) on tobacco leaves (*Nicotiana tabacum* L. cv Xanthi) as an approach to discard potential phytopathogens, as well as by their ability to produce different hydrolytic enzymes (amylases, proteases, lipases and DNases) that may be related to biocontrol capacity. Some other biocontrol and/or plant growth promoting activities, such as nitrogen fixation, phosphate solubilization and siderophore and auxin production are also being investigated. Molecular identification of selected biocontrol candidates was carried out by amplification and sequencing of the 16S rRNA gene.
Interestingly, in vitro antagonistic activity of selected strains was best detected in diluted KB medium. Five out of ten selected candidates consistently inhibited the growth of all assayed E. amylovora strains. Since this activity can be influenced by different growth conditions, strains were also tested ex vivo. On immature loquat and pear fruits, most of the biocontrol candidates were able to delay the onset of symptoms, and some of them also effectively reduced the incidence and severity of the disease. In some cases, and depending on the loquat cultivar, some biocontrol candidates were able to suppress all fire blight symptoms. None of the five further selected candidates elicited an HR in tobacco leaves. All of them produced one or more hydrolytic enzymes, and it was found that the two best biocontrol candidates also produce proteases, DNases and lipases, which could contribute to their biocontrol potential. In addition, most of them also present other biocontrol and/or plant growth-promoting activities, such as siderophore production and phosphate solubilization. These five biocontrol candidates were identified as: *Enterobacter cancerogenus, Curtobacterium flaccumfaciens, Pseudomonas sp.*, and *Serratia plymuthica*. Overall, the results obtained in this study suggest the potential of these biocontrol candidates for fire blight ecofriendly management.

New *Pantoea ananatis* strains as biocontrol agents of fire blight

Artur Mikiciński[1], Anna Daniel Wójcik[2], Monika Kałużna[1], Katarzyna Góralska[3], Małgorzata Hałat-Łaś[3], Marcin Oleszczak[3], Piotr Sobiczewski[1], **Joanna Puławska**[1]

[1] Research Institute of Horticulture (InHort), Skierniewice, Poland,
[2] Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna
[3] Intermag Sp. z o.o., Olkusz, Poland

The main objective of the BioSafeFood project implemented by the Research Institute of Horticulture, Łukasiewicz Research Network - Institute of Industrial Organic Chemistry, Branch Pszczyna and biotech company Intermag in Poland is to develop safe biopreparations to protect fruit and vegetable crops against the most important infectious diseases. One of the diseases the work is concentrated on is fire blight. Screening of 358 isolates originating from leaves of different horticultural plants and soil using pear fruitlet test showed that 45 of them significantly protected pear fruitlets against the disease. Physiological and biochemical tests, as well as 16S rRNA sequence analyses, revealed the most effective were three isolates classified to *Pantoea ananatis* AM 3/18, AM 14/18 and AM 20/18. *P. ananatis* is known mostly as a plant pathogen, however recent studies show that it is the species inclusive a versatile group of strains occurring in diverse ecological niches and possessing also positive for plant features.

In our studies, protective spraying of 'Idared' apple trees growing in the greenhouse with water suspension of P. ananatis strains as well as their three prototype formulations containing bacteria at the concentration 107 cfu/ml (the concentration realistically achievable in practice) showed that its efficacy against fire blight ranged from 71.2% at 5th day after inoculation with *E. amylovora* (strain Ea 659) to 62.3% at 7th day. Efficacy of tested P. ananatis in protection of apple shoots (Idared) determined 12 and 26 days after inoculation ranged from 52.2 to 100%, respectively.

It was found that bacteria of *P. ananatis* survived well on apple blossoms cv. McIntosh and 'Ligol' and blueberry in an orchard. Ten days from their introduction by spraying with water suspension at 10^7 cfu/ml $2.0x10^4$ - $6.7x10^6$ cfu per blossom were detected.

Additionally to fire blight, the selected *P. ananatis* strains showed high protective activity against grey mold on apples (70-80%) and grapes (od 61 do 86%), anthracnosis on blueberry (80-82%) and grey mold (50-80%) and white mold (40-60%) on cabbage (test on fruits and cabbage leaves).

The strains were not pathogenic and did not show any phytotoxic effect on apple trees, blueberry, strawberry, grapes, cabbage, carrot, lettuce and celery. The selected strains were subjected to screening to eliminate microorganisms with potential toxic properties on mammalian cells and conducting toxicological and ecotoxicological studies on the formulations developed to assess their toxicity. The neutral red uptake (NRU) cytotoxicity test performed on the 3T3 clone A31 cell line of mouse BALB/c showed that the post-culture mediums of *P. ananatis* strains have no cytotoxic effect.

The studies were performed in the frame of project BioSafeFood "Development of a technology for producing high-quality, consumer-safe fruit and vegetables using new biopreparations to protect crops against diseases" no. POIR.04.01.02-00-0100/17-00 co-financed by UE European Regional Development Fund, POIR 2014-2010, and NCBiR.

Abstracts - Poster Presentations

Survival of *Erwinia amylovora* on surfaces of materials used in orchards and insect vector

Duck Hwan Park, and Hyun ju Choi

Kangwon National University, Republic of Korea

Fire blight disease caused by the bacterium *Erwinia amylovora*, was observed in apple and pear orchards in Korea in 2015. Since then, it has spread, sometimes over long distances to other orchards. Therefore, we examined the ability of *E. amylovora* to survive in soils and on the surfaces of common materials such as T-shirts, wrist bands, pruning shears, and rubber boots. *E. amylovora* was detected in all materials tested in this study and survived for sufficiently long periods to cause fire blight disease in new sites. Thus, based on the results of this study, sanitation protocols must be applied to equipment during orchard work. In addition, for the honeybees known as a vector, the survival period of bacteria in the surface of insect examined by various methods such as population test, conventional PCR and Real-time PCR. This is a result of future research that can support the role of insect vector as an important factor in disease transmission.

VNTR and genome-wide SNP analysis of genetic variation in *Erwinia amylovora*

Girim Park[1], Bomi Lee[1], Seong-Jin Lee[2], Ju Yeon Song[3], Jihyun F.Kim[3], Sung-Hwan Jo[1], Seong Hwan Kim[3] and **Moon Nam**[1]

[1]Xenotype Incorporated, Daejeon, Republic of Korea; [2]Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea; [3]Yonsei University, Seoul, Republic of Korea; and [4]Cheonan University, Republic of Korea

Since the recent report of *Erwinia amylovora* (fire blight) in South Korea in 2015, it continues to occur in various regions. Fire blight can have an adverse effect on agriculture and economy. So, it is regulated as a quarantine organism in many countries. To investigate the genetic diversity of fire blight isolates, we were carried out epidemiological survey using Variable Number Tendem Repeat (VNTR) and genome-wide SNP analysis. VNTR analysis is applied to fire blight isolates from bacterial collections representing global and regional distribution of the pathogen. To develop a more resolution analysis technology, fire blight isolates were examined and compared by genome-wide SNP analysis. Whole genome sequencing and analysis were carried out on 82 E. amylovora strains isolated from various regions. Based on these results, genome-wide SNP was selected, and representative SNPs for analysis were selected by filtering algorism. We conducted the Principal Component Analysis (PCA), Genetic Structure, and Phylogenetic tree using selected SNP markers for the analysis of the genetic relationships among 82 strains. When VNTR and Genome-wide SNP analysis are compared, gw-SNP analysis was confirmed more highly resolution. It is expected to be applicable to the field of epidemiological survey for the analysis of a genetic differentiation among the fire blight subgroups. The newly developed molecular epidemiology method, genome-wide SNP analysis will provide more reliability and rapidity in understanding on how fire blight spread over a given geographical region and genetic differentiation.

Malus fusca fire blight resistance: identification of a candidate gene on chromosome 10 and a novel minor locus on chromosome 16

Andreas Peil[1], O. Francis Emeriewen[1], K. Richter[1], and M. Malnoy[2]

[1]Julius Kuhn Institute, Dresden, Germany; [2]IASMA Research and Innovation Centre, Italy

Fire blight resistance of the wild apple species *Malus fusca* (accession MAL0045) has been previously reported. This accession, crossed with the domesticated apple cultivar 'Idared', allowed for studies on the genetics of the resistance of this crabapple with the resultant F1 population. A major fire blight locus (Mfu10), found on chromosome 10, explained up to 66% of the phenotypic variance amongst the *M. fusca* × 'Idared' progenies. Although fire blight resistance is strain specific for some Malus accessions, leading to the breakdown of resistance in few resistance donors by highly aggressive strains of *Erwinia amylovora*; no strain able to breakdown the resistance of *M. fusca* itself or Mfu10 has been found. This makes this wild apple an interesting model for resistance studies with different wild-type and mutant strains of *E. amylovora*. A candidate gene (FB_Mfu10), underlying the major locus, was recently proposed. FB_Mfu10 was predicted on the sequence of a bacterial artificial chromosome (BAC) clone, spanning the fire blight locus on chromosome 10 and encodes B-lectin and serine/threonine kinase domains. Preliminary functional analyses showed, that the open reading frame (ORF), together with its border sequences upstream of the start codon and downstream of the stop codon (~ 6000 bp), is present only in resistant F1 genotypes with 8bp distinguishing between susceptibility and resistance. Furthermore, with a dense genetic map of *M. fusca* and studies with a mutant of an aggressive strain of *E. amylovora*, a minor fire blight locus has been identified.

Direct molecular discrimination of *rpsL* variants leading to streptomycin resistance in *Erwinia amylovora* and detection of in vitro-generated streptomycin-dependent mutants

Fabio Rezzonico and Alexia Roschi

ZHAW Wädenswil, Zurich, Switzerland

Erwinia amylovora can quickly become highly resistant to streptomycin through a single mutation in the *rpsL* gene encoding the S12 protein of the 30S small ribosomal subunit. This causes an amino acid substitution that prevents inhibitory binding of streptomycin while preserving the functionality of the ribosome. Screening of streptomycin resistant (Sm^R) bacteria in the orchards mostly yields the same mutation at codon 43 of the *rpsL* gene, which leads to the substitution of a lysine through arginine (K43R) in the S12 protein. It has previously been demonstrated that, of all possible mutations in the RpsL protein, K43R is the one that retains the highest environmental fitness even in absence of antibiotic pressure. However, alternative mutations involving the replacement of lysine, either at the same codon (K43T, K43N) or at another position in the protein (K88R), have also been found to result in SmR isolates in natural or laboratory conditions. To improve direct detection of these rarer variants, we developed a molecular assay based on the SNaPshot™ Multiplex Kit from Thermo Fisher Scientific. This approach allows interrogating one or multiple SNP positions via a single-base extension reaction to label DNA fragments followed by analysis in the ABI3500 Genetic Analyzer or similar sequencing devices. Single discrete genotypes or mixtures of two genotypes down to a 1:10 ratio can be detected with this method. Using the developed protocol, the relative frequency of spontaneous appearance of the different rpsL mutations was assessed without antibiotic pressure in Luria Bertani medium. Next to the expected mutations resulting in the K43R and K88R genotypes, which were detected at an approximately 3:1 ratio, several smaller colonies were isolated that did not display any of the known base substitutions leading to streptomycin resistance in *E. amylovora*, but were nevertheless able to grow in presence of high concentrations of the antibiotic. Sequencing analysis of the *rpsL* gene in these isolates revealed the presence of two new RpsL variants, i.e. P91L (proline to leucine) and G92D (glycine to aspartic acid) resulting in conditional-lethal streptomycin-dependent (SmD) phenotypes, which are unable to grow in absence of the antibiotic.

Abstracts - Poster Presentations

Microbiological characterization of copper sensitive *Erwinia amylovora* strains: potential links between copper tolerance and exopolysaccharide secretion, oxidative stress resistance, and cadmium sensitivity

Ricardo Delgado Santander and Srdan Acimovic

Cornell University, Highland, NY USA

Copper is an essential trace element forming part of oxygenases, the cytochrome c oxidase and related proteins. Bacteria possess unspecific uptake systems to incorporate environmental copper ions into the cell. However, high copper concentrations are toxic and homeostasis systems ensure the maintenance of cytoplasmic copper ion concentrations below harmful levels. A variety of compounds containing different forms of copper are commonly used in agriculture as treatments to manage, prevent and/or control different phytopathogens. Hence, the survival of many phytopathogenic bacteria in the orchard will depend on their ability to cope with toxic copper concentrations. Some of the described mechanisms for achieving copper homeostasis are: i) exopolysaccharides (EPS) that bind copper ions and trap them outside the cell; ii) antioxidant enzymes like superoxide dismutases, which detoxify the reactive oxygen species that are produced in the presence of copper; and iii) extrusion of copper ions to the periplasmic space catalyzed by specific ATPases, usually also contributing to the removal of other toxic metal ions such as cadmium. During the analysis of fire blight samples from affected farms in New York (USA), some of the isolated *Erwinia amylovora* strains did not form colonies or showed a clear reduction in the colony numbers on RESC (King's B ,KB, agar containing 1.5 mM $CuSO_4$), a semi-selective and differential medium for this pathogen containing copper. A more detailed analysis revealed that 63.2% of the strains had similar growth on KB plus 0 to 3.5 mM $CuSO_4$, but a significant reduction in colony numbers on KB plus 5 mM $CuSO_4$. A 21.1% of the strains only tolerated copper concentrations equal or lower than 2.5 mM. A 5.2% of the strains were also inhibited by 2.5 mM $CuSO_4$, and the remaining 10.5% of the isolates experienced a strong growth inhibition even on RESC medium with the standard copper concentration of 1.5 mM. Knowledge on the basis of the E. amylovora copper sensitivity could help in developing strategies to increase efficacy of copper treatments in controlling fire blight, which could be particularly important in European countries where the use of antibiotics against fire blight is forbidden or restricted. As the first step in determining factors involved in E. amylovora copper sensitivity, a collection of 7 strains showing different degrees of copper tolerance were further characterized to determine possible links between copper sensitivity and other phenotypic traits. The copper tolerant strains were Ea273, Ea16, Ea20 and EaK. The three analyzed copper sensitive strains were Ea17 and EaR2 (highly sensitive), and Ea19 (intermediate sensitivity). Strains Ea17 and EaR2 were more virulent on 'Bartlett' pear leaves than most of the copper tolerant strains, except Ea16 which showed similar necrosis extents as Ea17 and EaR2. Strains Ea17, EaR2 and Ea16 also showed lower growth rates in minimal medium, produced 2.6-3.4 times more levan in LB, and were more sensitive to cadmium than the remaining assayed strains. Levan production is a well-known virulence factor in E. amylovora, which correlates well with our virulence assay results. The exposure of liquid cultures to 0.1 and/or 0.01 mM $CuSO_4$ in LB had no apparent effect on levan production. However, the assay of amylovoran production in minimal medium revealed a very significant induction of amylovoran secretion by 0.01 mM $CuSO_4$ occurring only in the copper sensitive strains Ea17 and EaR2, which produced about 30-35 times more amylovoran than in control medium. There are examples of microorganisms where EPS protect against copper, and other cases where EPS seem to increase copper sensitivity. More studies are needed to establish a real correlation between levan/amylovoran and copper tolerance/sensitivity in E. amylovora. The fact that two out of the three analyzed copper sensitive strains were also more sensitive to cadmium might indicate convergent mechanisms for cadmium and copper detoxification (e.g. common efflux transport systems). Finally, the copper sensitive strains Ea17, Ea19 and EaR2, together with the copper tolerant strain EaK were also more sensitive to the hydroxyl radical-inducer paraquat than the other strains. This indicates the potential role of superoxide dismutases as one of the mechanisms for copper detoxification in *E. amylovora*. These results together show common characteristics in the most copper sensitive strains, shared with some of the copper tolerant strains, and reveal the multifactorial nature of copper tolerance in *E. amylovora*. A potential connection between copper sensitivity, levan secretion, amylovoran induction and paraquat and cadmium sensitivity will be deeply investigated in future studies.

Systematic study of the roles of Hfq-dependent sRNAs in regulation of virulence-associated traits in *Erwinia amylovora*

Jeffrey K. Schachterle[1], Leire Bardaji Goiketxea[2], Justin Bi[1], Ishani Pandya[1], and George W. Sundin[1]

[1]Michigan State University, East Lansing, MI USA; and [2]Universidad Publica de Navarra, Pamplona, Spain

Erwinia amylovora, causative agent of fire blight disease of apple and pear trees, coordinates gene expression as it passes through several host environments, overcomes host defenses and emerges to disseminate to new hosts. *E. amylovora* has evolved to precisely regulate distinct virulence processes to be expressed during critical points in infection. Here we report a systematic study of the roles of Hfq-dependent small RNAs as post-transcriptional regulators of virulence-associated traits that play important roles in fine-tuning the regulation of critical virulence factors. We screened each identified sRNA by generating single-sRNA deletion mutants and overexpressing each sRNA singly in the wild-type genetic background. Several virulence-associated phenotypes were assessed in our library of sRNA mutants and overexpression strains, and we identified novel virulence functions for several sRNAs. Of note, we found that deletion of the sRNA *hrs1* led to a reduction in virulence, and we found that the sRNA Hrs21, previously associated with virulence by an unknown mechanism, is linked to multiple virulence-associated phenotypes. This work increases understanding of the essential roles that individual sRNAs are playing during disease development in *E. amylovora* and highlights the importance of post-transcriptional regulation in the evolution of this pathogen.

Abstracts - Poster Presentations

The effector protein AvrRpt2$_{EA}$ from *Erwinia amylovora* induces salicylic acid dependent response in fire blight susceptible apples

Susan Schröpfer[1], Christoph Böttcher[1], Thomas Wöhner[1], Klaus Richter[1], John Norelli[2], Erik Rikkerink[3], Magda-Viola Hanke[1], and **Andreas Peil**[1]

[1]Julius Kuhn Institute, Dresden, Germany; [2]USDA-ARS Appalachian Fruit Research Station, Kerneysville, WV USA; [3]The New Zealand Institute for Plant & Food Research

Fire blight disease, caused by the bacterium *Erwinia amylovora*, has developed to an economical important disease in cultivation of pome fruits in many regions of the world. The bacterial cysteine protease AvrRpt2$_{EA}$ was identified as a central molecule in the host-pathogen interaction and it is important for pathogen recognition in the fire blight resistant crabapple *Malus* × robusta 5. However, little is known about its role as virulence factor in susceptible apples. To investigate its function in planta, transgenic lines of the fire blight-susceptible cultivar 'Pinova' were generated, which contain an plant-optimized version of AvrRpt2$_{EA}$ driven by a heat shock-inducible promoter. After induced expression of AvrRpt2$_{EA}$, the transgenic lines showed symptoms similar to natural fire blight infections, such as shoot necrosis and browning of older leaves. Furthermore, an increase of the expression of the PR-1 gene was shown, which was used as molecular marker for salicylic acid (SA) dependent systemic acquired resistance (SAR). Additional analysis reveal that the levels of SA and its derivatives were increased after AvrRpt2$_{EA}$ expression, too, with diverse kinetics in leaves of different ages. In contrast, no induction of the expression level of VSP2 paralogs was found, which were used as marker genes for the activation of the jasmonic acid (JA)-dependent defense pathway. This was also confirmed by metabolic profiling of JA and its derivatives. In conclusion, the results of this study show that AvrRpt2EA alone acts as virulence factor causing fire blight disease symptoms in susceptible apple plants and induces the formation of SA and SA-dependent SAR.

Nucleobase transport in Fire Blight pathogen and host

Neil Schultes[1], Amanda J. Stoffer-Bittner[2], Candace R. Alexander[2], Ian T. Gatchell[2], Andrea M. Funk[2], Adrita Iman[2], and George S. Mourad[2]

[1]The Connecticut Agricultural Experiment Station, New Javen, CT USA; and [2]Purdue University Fort Wayne, Ft. Wayne, IN USA

Erwinia amylovora, a phytopathogenic bacterium, causes fire blight on apples and pears. It derives nutrients from the host during disease progression. Our research 1) identifies *E. amylovora* nucleobase transporter loci, 2) biochemically characterizes the encoded transporters and 3) determines if the loci are necessary for disease. Previous work revealed that *E. amylovora* nucleobase biosynthetic mutants differ in their ability to cause disease, suggesting either varied uptake or importance of nucleobases in disease establishment. Here the transport function and biochemical properties for three nucleobase cation symporter 2 transporters in *E. amylovora* are determined through heterologous expression in nucleobase transport deficient Escherichia coli Keio strains coupled with radiolabeled nucleobase uptake studies and toxic analog growth studies. Under in vitro culture conditions, the *E. amylovora* uracil transporter (EaUraA), is able to transport uracil and 5-fluorouracil into *E. coli* cells. Similarly, the guanine/hypoxanthine transporter (EaGhxP) moves guanine, hypoxanthine and 8-azaguanine and the adenine transporter (EaAdeP) transports adenine, guanine and 8-azaadenine. Gene disruption mutations, EauraA::Camr, EaghxP::Camr , EaadeP::Camr were generated in *E. amylovora* and the resulting strains were able to grow and show disease symptoms as determined by the immature pear growth tests. Our results confirm that nucleobase transporter genes are present in *E. amylovora* yet they are not required for disease progression. Many *E. amylovora* strains contain the Ycf operon which confers the ability to synthesize and excrete the toxic nucleobase analog 6-thioguanine (6TG). 6TG is believed to be involved in the infection process by inhibiting growth of competing microbes on host tissue or is toxic to host cells or both. Heterologous biochemical and growth tests reveal that EaUraA and EaAdeP do not transport 6TG, but EaGhxP does transport 6TG and has a high affinity (Ki 3.7 uM) for 6TG. Similar functional analysis was performed on *Malus domestica* nucleobase cation symporter 1 (MdNCS1), one of several plant transporters known to transport guanine. MdNCS1 has high affinities for and the ability to transport both guanine (Km 0.42 uM) and 6-thioguanine (Ki 0.015 uM).

Abstracts - Poster Presentations

Impact of antibiotics and specific weather parameters on population dynamics of *Erwinia amylovora* on apple flower stigmas

Suzanne M. Slack, Cory A. Outwater, and George W. Sundin

Michigan State University, East Lansing, MI USA

Fire blight, caused by the gram negative bacterium *Erwinia amylovora*, is typically initiated each season via flower infection. To reach population levels high enough to start the infection process, *E. amylovora* must grow on flower stigmas before moving down to the hypanthium and nectaries. Because of the surface association of epiphytic *E. amylovora* cells on flower stigmas, and since the flowering window for pome fruit trees is generally short, the bloom period has historically been considered the best time for antibiotic intervention. Specifically, targeting the bacteria population on the stigma with antibiotics could prevent the population build up required for infection. In the United States, there are three antibiotics currently registered for fire blight management at bloom: kasugamycin, oxytetracycline, and streptomycin. Our study aimed to identify factors that impact antibiotic effectiveness including timing of antibiotic application and weather factors. During three field seasons, the rifampicin-resistant *E. amylovora* strain Ea110 was inoculated directly onto apple cv. 'Gala' stigmas either 4 hr prior to antibiotic application or 4 hr post antibiotic application; stigma populations of strain Ea110 were then tracked for five days after inoculation. We observed steep declines in *E. amylovora* populations on flowers over a 48 hr time period after treatment with kasugamycin or streptomycin. In contrast, populations of *E. amylovora* were only minimally affected on flowers treated with oxytetracycline. Disease incidence in all treatments was lower when the antibiotics were applied prior to inoculation. During the five day experimental periods, weather factors including precipitation, relative humidity, temperature, windspeed, and solar irradiance were tracked hourly. When the weather trends were compared over the three years, it was found that solar irradiance negatively impacted the efficacy of kasugamycin and oxytetracycline. Further work into the solar sensitivity of kasugamycin is currently underway.

The effectiveness of copper-containing fungicides against the pathogen of bacterial fire blight *Erwinia amylovora*

Zhansaya Umiralijeva, Aliya Dzhajmurzina, Bakyt Kopzhasarov

Kazakh Research Institute of Plant Protection, Almaty, Kazakhstan

The article describes the effectiveness of copper-containing fungicides against the pathogen of bacterial fire blight *Erwinia amylovora* in the laboratory. Zones of inhibition of bacterial growth are noted. The most effective fungicides that showed good bactericidal properties were identified.

Causes and consequences of decreased streptomycin use for fire blight control in New Zealand

Joel Vanneste[1], Mary Horner[1], Peter Wood[1], David Manktelow[2], Nicola Park[3]

[1]New Zealand Institute for Plant & Food Research Limited, Havelock North, New Zealand; [2]Applied Research and Technologies Ltd, Napier, New Zealand; [3]The New Zealand Institute for Plant & Food Research

Streptomycin is the only antibiotic available for control of fire blight, caused by *Erwinia amylovora*, in New Zealand. Its use is strongly regulated: it is legally allowed on pipfruit only for control of fire blight and on a few other crops (stonefruit, tomato and kiwifruit) for specific diseases. Spraying streptomycin onto apple or pear flowers just before an infection period has proven to be very effective in reducing fire blight incidence. However, as early as 2005 an expert panel on antibiotic resistance recommended that New Zealand horticulture industries seek alternative strategies for control of bacterial diseases, so that the use of streptomycin for control of plant pathogenic bacteria could be phased out. The search for alternative treatments for fire blight had already started, and the sales of streptomycin in 2003 were less than half of those in 1999. The continuation of this trend is well illustrated by the average numbers of streptomycin applications per block of 'Braeburn' across the whole country, which went from 0.25 in 2000 to 0.01 in 2010. It stayed at that extremely low level until at least 2015. This dramatic reduction in streptomycin use can be attributed to several factors, including the introduction of alternatives to antibiotics, in particular the biological control agent *Pantoea agglomerans* P10c, sold as Blossom Bless™, commercially available since 2000; signals from the European markets against antibiotic use for crop protection; and climatic conditions during spring time which might have led to low levels of infection.

In the last five years the New Zealand pipfruit industry has moved to intensive production systems relying on novel cultivars, most of them very susceptible to fire blight, which are grafted on susceptible dwarfing rootstocks ('Malling 9'). These changes, combined with recent fire blight events, has seen some increase in streptomycin use, which is concerning for disease management and market acceptability. The main risk associated with this practice would be the selection of streptomycin-resistant strains of the pathogen, especially as streptomycin-resistant strains of *E. amylovora* were isolated from some apple orchards in the early 1990s.

Transcriptomic analysis of stringent response regulator (p)ppGpp in *Erwinia amylovora*

Ho-wen Yang, Menghao Yu, Jun Liu, and Youfu Zhao

University of Illinois, Urbana, IL USA

Stringent response, regulated by nucleotide second messengers (p)ppGpp, could be triggered by tremendous stress, such as nutrient starvation and acidity stress, in bacteria.Previous study showed that ppGpp is required for *Erwinia amylovora* virulence. However, global transcription patterns under the regulation of ppGpp in E. amylovora have not been analyzed comprehensively. In this study, we used RNA-Seq to analyze differential gene expression patterns in WT and (p)ppGpp0 mutant after 3 hours incubation in HMM medium. Whole gene expressions were subjected to principle component analysis, and the result showed that deletion of (p)ppGpp synthesis genes, spoT and relA, caused drastical changes in gene expression. After PCA, differentially expressed genes (DEGs) analysis was performed, and 1314 DEGs were detected (p-value < 0.05; logFC > 1 or < -1). Among them, 612 DEGs were up-regulated in (p)ppGpp0 mutant, including gene cluster related to amino acid biosynthesis gene (hut, met, trp, ilv), translation (rps, rpl, rpm), as well as nucleotide metabolism (pyr, pur, gua, deo). On the other hand, 702 DEGs were down-regulated in (p)ppGpp0 mutant, including genes related to T3SS (hrc, hrp), motility (flg, fli), as well as opp-dpp. We also verified the RNA-seq data by qPCR. Our finding suggested that (p)ppGpp inhibits genes related to biosynthesis of amino acid, translation, as well as nucleotide metabolism. On the other hand, (p)ppGpp triggers the expression of genes related to T3SS, motility, as well as opp-dpp operons.

Abstracts - Poster Presentations

Lactobacillus biocontrol strains active against fire blight in Kazakhstan

Elvira Ismailova[1], Amankeldy Sadanov[1], Mereke Alimzhanova[1,2], Olga Shemshura[1], Asel Molzhigitova[1], Karlygash Kebekbaeva[1], Zhanarbekova Alma[3]

[1] LLP "Scientific Production Center of Microbiology and Virology", Almaty, Kazakhstan; [2]Al-Farabi Kazakh National University, Almaty, Kazakhstan; and [3]Kazakh Research Institute of Plant Protection and Quarantine, Almaty, Kazakhstan

Lactic acid bacteria (LAB) may be biological control agents against fire blight of fruit crops in Kazakhstan. They are considered the most environmentally friendly for the environment. For this purpose, screening of strains of lactic acid bacteria from the SPC collection of microbiology and virology showing antagonistic activity against the pathogen of fire blight of fruit crops *Erwinia amylovora* was carried out. Also, the newly isolated strains from the Apple phyllosphere (flowers, fruits and branches) in Karasai district of Almaty region of Kazakhstan were studied. The test culture was a bacterium of the genus *Erwinia amylovora*, which was isolated from the affected organs of the Apple tree.

Species belonging of microorganisms was established by classical methods of identification and confirmed by molecular genetic analysis by determining the direct nucleotide sequences of the fragment 16S rRNA gene, followed by their comparison with the sequences of genes 16S rRNA reference strains deposited in the international database GenBank (NCBI, USA), as well as the construction of phylogenetic trees. Studies of antagonistic activity of lactic acid bacteria in relation to the causative agent of fire blight was carried out by diffusion in agar. The results of the studies showed that lactic acid bacteria had different antagonistic activity against *E. amylovora.* The pathogen suppression zones of the selected strains of lactic acid bacteria ranged from 22.7 mm to 36.6 mm. The maximum inhibitory activity was (36, 6 mm) in the strain *L. plantarum* 17 M. For further research, this strain of bacteria was selected as the most promising agent of biocontrol against the causative agent of fire blight. As a result of the research, 3 strains of bacteria of the genus Lactobacillus with inhibitory activity were selected: two of them was collection strains- *L. plantarum* 173, strain - *L. casei* 139 and one newly isolated from the surface of the fruit Apple - strain *L. plantarum* 17M. The study of the effect of different nutrient media on the antagonistic activity of *L. plantarum* 17 M, showed that the most suitable media are MRS and milk whey. The zone of suppression of the pathogen was made on the RS (36.6 mm) and milk whey (30 mm). These media can be used for the development of a biological product.

Analysis of the component composition of *L. plantarum* culture broth by GC-MS methods. As a result of the chromatographic analysis it was found that one of the active components of the inhibitory activity of lactic acid bacteria is acetic acid. In addition to the results of chromatographic analysis, a study on the effect of acetic acid on the suppression of pathogen growth was conducted. Study of different concentrations of acetic acid (70%,50%, 30%, 25%, 10%, 5%, 1%, 0,5%,0,25% and 0.1%) showed that high concentrations of acetic acid completely inhibit the growth of the pathogen. At a concentration of acetic acid equal to 1%, there are zones of inhibition of pathogen growth similar to the suppression zones obtained by spiking into the wells of lactic acid bacteria grown in a liquid medium. The study of the dynamics of the accumulation of acetic acid in the culture fluid of the main component of suppressing the growth of *E. amylovora* showed. This study confirms the potential use of certain strains of *L. plantarum* as an active agent of microbial biopesticides to combat bacterial burn of fruit crops in Kazakhstan.

Conference Attendees

Srdan Acimovic
Cornell University
3357 US Highway 9W
Highland, NY 12528 USA
acimovic@cornell.edu

James Adaskaveg
University of California
5970 Claridge Dr
Riverside, CA 92506 USA
jim.adaskaveg@ucr.edu

Stefano Benini
Free University of Bolzano
Piazza universita 5
Bolzano, NA 39100 IT
stefano.benini@unibz.it

Hyun Ju Choi
Kangwon National University
Chuncheon 24341, Korea
Chuncheon, NA KSXX0035 KR
godrn1017@gmail.com

Kerik Cox
Cornell University
630 West North Street
Geneva, NY 14456 USA
kdc33@cornell.edu

Zhouqi Cui
The Connecticut Agricultural
Experiment Station
123 Huntington Street
New Haven, CT 6511 USA
zhouqi.cui@ct.gov

Mery Dafny Yelin
Galilee Research Institute
Southern Industrial Zone
Kiryat Shmona, NA 12100 IL
merydy@gmail.com

Ricardo Delgado Santander
Cornell University
3357 US Highway 9W
Highland, NY 12528 USA
rd379@cornell.edu

Michael Dimock
Certis USA
9145 Guilford Rd., Suite 175
Columbia, MD 21045 USA
mdimock@certisusa.com

Madison Dobbins
Michigan State University
Dept. of Plant, Soil & Microbial Sciences
578 Wilson Rd.
East Lansing, MI 48824 USA
dobbin16@msu.edu

S. Tianna DuPont
Washington State University
1100 N Western Ave
Wenatchee, WA 98801 USA
tianna.dupont@wsu.edu

Lars Fieseler
Zurich University of Applied Sciences
Einsiedlerstrasse 31
Wädenswil, NA 8820 CH
lars.fieseler@zhaw.ch

Steven Gayder
Brock University
1812 Sir Isaac Brock Way
St. Catharines, ON L2S 3A1 CA
sg10yl@brocku.ca

Julianne Grose
College of Life Sciences,
Brigham Young University
4007 LSB 701 East University Parkway
Provo, UT 84602 USA
julianne_grose@byu.edu

Rebecca Harkness
Michigan State University
Dept. of Plant, Soil & Microbial Sciences
578 Wilson Road.
East Lansing, MI 48824 USA
harkne26@msu.edu

Mary Horner
Plant and Food Research
Private Bag 1401
Havelock North, NA 4157 NZ
mary.horner@plantandfood.co.nz

Drew Hubbard
Oregon State University
4415 NE 56th Ave.
Portland, OR 97218 USA
drewh@fine-americas.com

Michelle Hulin
NIAB EMR
West Malling, NA ME19 6BJ UK
michelle.hulin@emr.ac.uk

Amy Irish-Brown
Michigan State University Extension
775 Ball Ave NE
Grand Rapids, MI 49503 USA
irisha@msu.edu

Elvira Ismailova
SPC of Microbiology and Virology
str. Bogenbay batyr 105
Almaty, NA 50010 KZ
mereke.84@mail.ru

Kenneth Johnson
Oregon State University
2082 Cordley Hall
Corvallis, OR 97331 USA
johnsonk@science.oregonstate.edu

Conference Attendees

Achala KC
Southern Oregon Research andExtension Center, Ore
569 Hanley Rd
Central Point, OR 97502 USA
achala.kc@oregonstate.edu

Awais Khan
Cornell University
112 Barton lab, 15 Castle Creek Dr.
Geneva, NY 14456 USA
awais.khan@cornell.edu

Roshni Kharadi
Michigan State University
Dept. of Plant, Soil & Microbial Sciences
578 Wilson Rd.
East Lansing, MI 48824 USA
kharadir@msu.edu

Seong Hwan Kim
Dankook University
Cheonandaero 119, Dept. of Microbiology, Dankook University
Cheonan, NA 311169 KR
piceae@dankook.ac.kr

Sarah Kostick
Washington State University
1100 N Western Ave
Wenatchee, WA 98801 USA
sarah.kostick@wsu.edu

Mario Lanthier
CropHealth Advising & Research
PO Box 28098
Kelowna, BC V1W4A6 CA
office@crophealth.com

Jun Liu
University of Illinois at Urbana–Champaign
288E R Madigan Laboratory
1201 W. Gregory Dr.
urbana, IL 61801 USA
junliu25@illinois.edu

Xiangyang Liu
T3 Biocience
10120 N. Sheridan Dr.
Mequon, WI 53092 USA
xliu@t3biosci.com

Tim McNellis
Pennslyvania State University
318 Buckhout Lab
University Park, PA 16802 USA
twm4@psu.edu

Moon Nam
XENTYPE CO.,LTD.
N219, 11-3, Techno 1-ro, Yuseong-gu, Daejeon, Republic of Korea
Daejeon, NA 34015 KR
moonlit51@xenotype.co.kr

Darlene Nesbitt
Agriculture and Agri-Food Canada
4902 Victoria Avenue North
Vineland, ON L0R 2E0 CA
darlene.nesbitt@canada.ca

Chang-Sik Oh
Kyung Hee University
1732 Deogyoung-daero
Yongin, NA 17104 KR
co35@khu.ac.kr

Cory Outwater
Michigan State University
Department of Plant, Soil and Microbial Sciences
578 Wilson Rd.
East Lansing, MI 48824 USA
outwate3@msu.edu

Michael Parcey
Brock University
1812 Sir Isaac Brock Way
St. Catharines, ON L2S 3A1 CA
mp17ll@brocku.ca

Duck Hwan Park
Kangwon National University
Chuncheon 24341, Korea
Chuncheon, NA KSXX0035 KR
dhp@kangwon.ac.kr

Andreas Peil
Julius Kühn-Institut
Pillnitzer Platz 3a
Dresden, NA 1326 DE
andreas.peil@julius-kuehn.de

Jingyu Peng
Michigan State University
Dept. of Plant, Soil & Microbial Sciences
578 Wilson Rd.
East Lansing, MI 48840 USA
pengjin2@msu.edu

Kari Peter
Penn State University
290 University Dr., P.O. Box 330
Biglerville, PA 17307 USA
kap22@psu.edu

Vincent Philion
IRDA
335, Rang des Vingt-Cinq Est
Saint-Bruno-de-Montarville, QC J3V 0G7 CA
Vincent.philion@irda.qc.ca

Joanna Pulawska
Research Institute of Horticulture
ul. Konstytucji 3 Maja
Skierniewice, NA 96-100 PL
joanna.pulawska@inhort.pl

Vanessa Reininger
Agroscope Wädenswil
Müller-Thurgau Strasse 29
Wädenswil, NA 8820 CH
vanessa.reininger@agroscope.admin.ch

Fabio Rezzonico
ZHAW Wädenswil
Einsiedlerstrasse 31
Wädenswil, NA 8820 USA
rezz@zhaw.ch

Conference Attendees

Gregory Rogers
Certis USA
58 Middlecroft Rd
Elkton, MD 21921 USA
rogersgs_98@yahoo.com

Brian Rund
Nufarm
51 W 42nd St
Indianapolis, IN 46208 USA
brian.rund@nufarm.com

Jeffrey Schachterle
Michigan State University
Dept. of Plant, Soil & Microbial Sciences
578 Wilson Rd.
East Lansing, MI 48824 USA
schacht6@msu.edu

Neil Schultes
The Connecticut Agricultural Experiment Station
123 Huntington St
New Haven, CT 6511 USA
Neil.Schultes@ct.gov

Jugpreet Singh
Cornell University
A122 Barton Lab
Geneva, NY 14456 USA
js3444@cornell.edu

Suzanne Slack
Michigan State University
Dept. of Plant, Soil & Microbial Sciences
578 Wilson Rd.,
East Lansing, MI 48824 USA
slacksuz@msu.edu

George Sundin
Michigan State University
578 Wilson Rd.
East Lansing, MI 48824 USA
sundin@msu.edu

Antonet Svircev
Agriculture and Agri-Food Canada
4902 Victoria Avenue North
Vineland, ON L0R 2E0 CA
antonet.svircev@canada.ca

Todd Temple
Oregon State University
3335 sw chintimini ave
corvallis, OR 97333 USA
templet@science.oregonstate.edu

Daniel William Thompson
Brigham Young University
4007 LSB 701 East University Parkway
Provo, UT 84602 USA
d.william.thompson@gmail.com

Mark Trimmer
AgroSource, Inc.
23335 Red Robin Place
Bradenton, FL 34202 USA
Mark.Trimmer@Agrosource.onmicrosoft.com

Joel Vanneste
New Zealand Institute for Plant & Food Research
Ruakura Research Centre
Bisley Road
Hamitlon, NA 3214 NZ
Joel.Vanneste@plantandfood.co.nz

Sara Villani
North Carolina State University
Department of Entomology and Plant Pathology
Mills River, NC 28759 USA
sara_villani@ncsu.edu

Anna Wallis
Cornell University
630 West North Street
Geneva, NY 14456 USA
aew232@cornell.edu

EricaRose Warwick
AgroSource, Inc.
2063 NE Christopher Ct
Jensen Beach, FL 34957 USA
ericarose.warwick@agrosource.onmicrosoft.com

Ching-Hong Yang
University of Wisconsin - Madison
Lapham Hall 181, 3209 N. Maryland Ave.
Milwaukee, WI 53211 USA
CHYANG@UWM.EDU

Ho-Wen Yang
288 E R Madigan Laboratory 1201 W. Gregory Dr.
Urbana, IL 61810 USA
howenwy2@illinois.edu

Menghao Yu
University of Illinois Urbana-Champaign
288 E R Madigan Laboratory
1201 W. Gregory Dr.
Urbana, IL 61801 USA
myu37@illinois.edu

Xiaochen Yuan
3209 N Maryland ave
Milwaukee, WI 53211 USA
yxc881112@gmail.com

Quan Zeng
Connecticut Agricultural Experiment Station
123 Huntington Street
New Haven, CT 6511 USA
quan.zeng@ct.gov

Youfu Zhao
University of Illinois
288A ERML
1201 W Gregory Dr
Urbana, IL 61801 USA
zhao888@illinois.edu

Notes

Notes

Thank You Sponsors

Gold

MICHIGAN APPLES

Michigan Tree Fruit Commission

CERTIS USA
The Biopesticide Company

Platinum

AGROSOURCE™ Fruitful Thinking™

Arysta LifeScience
Innovation. Agility. Results.

Michigan State HORTICULTURAL Society

Nufarm

Westbridge Agricultural Products

Silver

Syngenta

Bronze

Gowan USA

Plant Food Systems