ISFB 2022

Third International Symposium on Fire Blight of Rosaceous Plants

06 – 09 September 2022

Dresden-Pillnitz, Germany

JKI
Julius Kühn-Institut
Federal Research Centre for Cultivated Plants

The 3rd ISFB is dedicated to the memory of Dr. Brion Duffy (1967–2021), who devoted most his research career to Erwinia amylovora and was a driving force within the fire blight community. With his work, that included the publication of the first complete genome of the pathogen, he made major contributions to the understanding of the molecular mechanisms that make this bacterium the source of the most dangerous pome fruit disease.

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Welcome to Dresden-Pillnitz. We, at the Institute for Breeding Research on Fruit Crops, of the Julius Kühn Institutes, Germany, have the honour of hosting the third ISFB. We are grateful to our partners – Landesanstalt für Umwelt, Landwirtschaft und Geologie (LfULG) and Hochschule für Technik und Wirtschaft Dresden (HTW Dresden) for supporting to ensure the successful hosting of this conference. We are grateful to all the participants for attending and for presenting scientific contents, which are required for a successful conference. This program provides a detailed overview of the conference, abstracts and participant list.

We encourage you to take time during this week to enjoy the beautiful city of Dresden. We will be organizing a tour of the city for those interested.

Finally, we hope that you have a great time in Dresden and enjoy the conference. We encourage you to be active in making the conference a memorable one by staying engaged and networking. We hope future collaboration will emerge from this meeting. We thank you for attending.

Organizing committee

Institute for Breeding Research on Fruit Crops, JKI
Ofere Francis Emeriewen
Henryk Flachowsky
Monika Höfer
Janne Lempe
Andreas Peil
Jens Pflug
Stefanie Reim
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Mirko Schuster
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Joanna Puławska (Research Institute of Horticulture, Poland)
Joel Vanneste (New Zealand Institute for Plant and Food Research Limited, New Zealand)
Fabio Rezzonico (Zurich University of Applied Science, Switzerland)
Virgina Stockwell (Oregon State University, USA)
Youfu (Frank) Zhao (Washington State University, USA)

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- **Keynote: Fire Blight - issues, challenges and future prospects** (45 mins)
- Joanna Pulawska
- **An app for apples: citizen-led mapping of fire blight in Central Asia** (20 mins)
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- **Annual *Erwinia amylovora* population dynamics in fire blight cankers and effect of pome fruit host and environmental factors on pathogen survival** (20 mins)
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  Kenneth B. Johnson
- **Control of pear shoot blight and fire blight cankers with Regalia and antibiotics** (20 mins)
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  S. Tianna DuPont, Kerik Cox, Ken Johnson, Kari Peter, Misbakhul Munir, Aina Baro

12:40 – 13:45
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Posters will also be available for viewing during coffee breaks

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Chair: Joanna Pulawska

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Posters will also be available for viewing during coffee breaks

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10:50 – 12:30
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- **Improvement of the citric acid buffer used for fire blight control with Blossom Protect™** (20 mins)
  Timothy Jenkins, Stefan Kunz, Armin Weiß, Jan Wunderle
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12:30 – 13:30
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13:30 – 15:15
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Chair: Fabio Rezzonico

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  Ho-wen Yang, Awais Khan, Ken Johnson, Tianna Dupont, Youfu Zhao

15:15 – 15:40
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Posters will also be available for viewing during coffee breaks

15:40 – 17:00
Session 4 - Molecular biology and pathogen genome analyses
Chair: Fabio Rezzonico

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- **Genome editing in Erwinia amylovora by rpsL counter-selection** (20 mins)
  Laura Binmöller, Ofere Francis Emeriewen, Andreas Peil, Annette Wensing, Wilhelm Jelkmann
- **RpoN regulon in *Erwinia amylovora* revealed by transcriptional profiling and *in silico* binding site analysis** (20 mins)
  Ho-wen Yang, Youfu Zhao

17:30 – 19:30
Dresden City Tour with the Red Double Decker Bus

08.09.2022 (THUR)

9:30 – 10:35
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Chairs: Theo Smits, Youfu Zhao

- **Keynote: Host-*Erwinia amylovora* interaction** (45 mins)
  George Sundin
- **The Ams proteins and the amylovoran biosynthetic pathway in the phytopathogen *Erwinia amylovora*** (20 mins)
  Lavinia Carlini, Alfonso Esposito, Luca Mauro Invernizzi, Luca Ambrosino, Silvano Piazza and Stefano Benini

10:35 – 11:00
Coffee Break

11:00 – 12:20
Session 5 - Host-*Erwinia amylovora* interaction
Chairs: Theo Smits, Youfu Zhao

- **The complex and compartmentalized cyclic di-GMP signaling network is a global regulator of phase-transition and host colonization in *Erwinia amylovora*** (20 mins)
  Roshni R. Kharadi, George W. Sundin
- **Colonization of yeast-like fungi on apple flowers induces host immunity and prevents fire blight infection** (20 mins)
  Zeng Q.
- **Probing metabolite requirements for *Erwinia amylovora* disease establishment** (20 mins)
  Neil P. Schultes, Judith P. Sinn, Timothy W. McNellis

Posters will also be available for viewing during coffee breaks

- **Glandular and non-glandular trichomes are colonization sites and host entry points of the fire blight pathogen on apple leaves** (20 mins)
  Millett F., Cui Z., Miller, K., Zeng Q**.**

12:20 – 13:30
Lunch

14:30 – 16:00 Excursion Pillnitz Palace Park

16:00 – 17:00 Apple tasting

17:00 – 18:00 JKI orchard visit

Posters will be on display at the venue (Schurichtbau)

18:00 –
Conference Barbecue including wine tasting

09.09.2022 (FRI)

9:30 – 10:35
Session 6 - Plant breeding and breeding research
Chairs: Andreas Peil, Ofere Francis Emeriewen

- **Keynote: The dichotomy of polygenic and monogenic resistance to fire blight: the case of wild and domesticated apple genotypes** (45 mins)
  Ofere Francis Emeriewen, Thomas Wöhner, Annette Wensing, Henryk Flachowsky, Andreas Peil
- **Advances in breeding fire blight resistant apple cultivars at Agroscope** (20 mins)
  Simone Buehlmann-Schuetz, Marius Hodel, Luzia Lussi, Giovanni AL Broggini, Andrea Patocchi, Markus Kellerhals

10:35 – 11:00
Coffee Break

11:00 – 11:40

- **Mapping novel QTL for fire blight resistance in the primary progenitor species of domesticated apples (*M. × domestica*)** (20 mins)
  Richard Tegtmeier, Awais Khan
- **Fire Blight resistance breeding in Dresden-Pillnitz** (20 mins)
  Andreas Peil, Ofere Francis Emeriewen, Klaus Richter, Monika Höfer, Henryk Flachowsky

11:40 – 12:30
Conclusion, Final Remarks, next meeting

12:30 – 13:30
Lunch

Posters will also be available for viewing during coffee breaks

Abstract - Oral presentation

Keynote: Fire Blight - issues, challenges and future prospects.

Joanna Puławska

The National Institute of Horticultural Research (InHort), Skierniewice, Poland

Keywords: resistance inducers, biocontrol, remote sensing, transcriptomics

Fire blight, caused by the bacterium *Erwinia amylovora*, is the oldest known plant bacterial disease causing big losses in apple and pear production worldwide. In Poland, it was detected for the first time in 1966 and since that time, it has been the subject of intensive research at InHort. Although several research results were introduced into practice, new challenges emerge that require new solutions. The general tendency in EU to limit pesticide usage, a ban on the use of antibiotics, and plans to withdraw copper preparations from use in plant protection requires new solutions. An alternative to chemical protection measures may be the introduction of plant resistance inducers and biocontrol with microorganisms. In our research we worked on the new compounds - inducers, eg. derivatives of salicylic acid as well as we selected a new promising strain *Pantoea agglomerans* T16/8, which application allowed for satisfactory protection of apple and pear trees against fire blight.

A dynamically developing trend in agriculture is the use of remote sensing and photogrammetric technologies, inter alia, drones. As part of cooperation with the Polish phytosanitary service, we developed a system facilitating the work on field inspectors. The use of dedicated sensors and flying platforms allows for obtaining reliable data in the orchard with great potential in assessing the degree of plant infestation by pathogens, e.g. in fire blight detection.

Using the RNA-seq analysis we generated a dataset describing the differences in the transcriptional response of three *E. amylovora* strains of different virulence in susceptible and resistant apple cultivars. The strains, although homogenous in terms of phenotypic and genetic features, differed in gene expression levels. 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.

Abstract - Oral presentation

An app for apples: citizen-led mapping of fire blight in Central Asia

Mirjam Kurz[1], Werner Tischhauser[2], Tinatin Doolotkeldieva[3], Mariia Cherniavskaia[4], Bolot Tagaev Bolo[4], Ormon Sultangaziev[4], Jarkyn Samanchina [4], Theo HM Smits[1], Fabio Rezzonico[1]

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[2]A&K Strategy GmbH, Chastelstrasse 14, 8732 Neuhaus, Switzerland

[3]Kyrgyz-Turkish Manas University, Plant Protection Department, Bishkek 720044, Kyrgyzstan

[4]Fauna & Flora International, The David Attenborough Building, Pembroke Street, Cambridge, CB2

Keywords: Wild apples, Walnut-fruit Forest, germplasm, IUCN Red List

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a severe bacterial disease of apple and pear that can quickly destroy whole plants. In the last decade, it arrived also in Central Asia, where pomaceous fruit plants represent the dominant species in mid-altitude forests and constitute a critical foundation for the entire ecosystem. Efficiently informing farmers, forestry services and private persons about the instances and dangers of fire blight, the correct way to recognize the symptoms, and the methods of disease control is thus of paramount importance in a vast and fragmented natural landscape like the one characterizing countries like Kyrgyzstan, Kazakhstan, and Tajikistan. For that purpose, beside the direct contact to the local population, we have developed an app for smartphones [1] that can inform stakeholders about fire blight, simultaneously allowing a citizen science approach for mapping the spread of this dangerous pome fruit disease in Central Asia. Utilizing the gathered data, a more precise field monitoring approach can be implemented, thus improving the quality of the existing data. Altogether, this should give a clearer picture of the risk for fire blight around the endangered apple and pear species in the forests of Central Asia, while allowing the app users to better protect their orchards and gardens. The app will be made available for mobile Android devices in Russian, English and in the respective local languages and can easily be adapted to new countries, languages or even diseases.

Abstract - Oral presentation

Annual *Erwinia amylovora* population dynamics in fire blight cankers and effect of pome fruit host and environmental factors on pathogen survival

Ricardo Delgado Santander[1], Fatemeh Khodadadi[2], Željko Rađenovic[1], Christopher L. Meredith[1], Jon Clements[2], Srđan G. Aćimović[3]

[1]Hudson Valley Research Laboratory, Cornell University, Highland, NY, USA
[2]UMass Cold Spring Orchard Research and Education Center, University of Massachusetts Amherst, Belchertown, MA
[3]Alson H. Smith Jr. Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Winchester, VA, USA

Keywords: Fire blight, irrigation, drought, logistic regression analysis

Fire blight cankers are one of the main reservoirs and sources of inoculum of *Erwinia amylovora*. However, little is known about *E. amylovora* cell concentrations in canker tissues, its population dynamics over time, and the effect of environmental conditions on the pathogen survival in cankers. To obtain cankers, we inoculated tree shoots of different apple (irrigated and non-irrigated), pear and Asian pear cultivars in May/June with *E. amylovora* in Highland, NY, and Belchertown, MA, in an assay repeated in two different growing seasons. The quantification of live *E. amylovora* cells in cankers collected in July, October, January and April was assessed using a sample processing and viability digital PCR protocol optimized in our previous work. The analysis showed a decrease in the percent of positive *E. amylovora* detections and cell numbers in cankers sampled during fall and winter compared to summer and spring. Additionally, a logistic regression analysis of the results revealed lower chances for detecting the pathogen in cankers in samples collected from non-irrigated trees and/or trees in the first experiment repeat, performed during one of the most severe droughts recorded in New York state. *E. amylovora* detection in cankers after winter (April) was inversely linked to fire blight symptom severity, meaning that the success of the pathogen overwintering increased significantly in the more resistant host species and cultivars in comparison to the most susceptible ones. Our results reveal novel information about *E. amylovora* life cycle and provide important data that will help improve fire blight management strategies.

Abstract - Oral presentation

Keynote: Insights into fire blight management

Kenneth B. Johnson

Dept. Botany and Plant Pathology, Oregon State University, Corvallis, OR, 97331

Keywords: fire blight, *Erwinia amylovora*, disease management, floral infection

For the last 30 years, we have studied suppression of floral infection by *Erwinia amylovora* in experimental and commercial orchards of susceptible pear and apple cultivars. Major strategies to reduce floral infection include sanitation of overwintering inoculum, biological control, chemical protection, and induced host resistance. Sanitation slows the rate of epiphytic colonization of flowers by the pathogen. Biological antagonists sprayed in early bloom colonize floral surfaces, which can limit the pathogen's potential epiphytic population size. Chemical materials, applied from full bloom to petal fall, primarily protect the hypanthial surface of flowers where the pathogen gains entry to the host. Induction of systemic acquired resistance in the host reduces incidence of infection. Used by themselves these strategies generally result in meaningful but partial infection suppression, which is frequently unsatisfactory at commercial scale. Spray programs that integrate strategies, e.g., sanitation then biocontrol then chemical control or chemical control combined with resistance induction, increase the likelihood of achieving commercially-satisfactory disease suppression. This concept of 'integrated control' will be illustrated with examples from both antibiotic-allowed and antibiotic-prohibited production systems.

Abstract - Oral presentation

Control of pear shoot blight and fire blight cankers with Regalia and antibiotics

Srđan G. Aćimović[1], Christopher Meredith[2]

[1]Virginia Polytechnic Institute and State University, Winchester, VA, USA
[2]Cornell University, Highland, NY, USA

Keywords: fire blight cankers, plant extract, antibiotics, management

Cankers are infected zones of bark on branches, trunk, or rootstock that develop after *Erwinia amylovora* invades wood from diseased flowers, shoots and rootstock suckers, or by residual transfer through tree xylem. On pear trees cankers cause tree death within one year. On 3-yr-old trees of cultivar 'Bartlett', we evaluated efficacy of spray and trunk injection applications of giant knotweed extract (Regalia) for shoot blight and canker control and compared it to antibiotics. We applied spray treatments to drip using a handgun sprayer at 100 gal/A. We delivered injection treatments with Quik-jet injector. Untreated control developed 80.9% shoot blight severity and 46.7% canker incidence. Five spray applications of Regalia delivered at bud burst, green cluster, white bud, petal fall, and fruit set, gave 100% control of both shoot blight severity and canker incidence. Trunk injected Regalia did not control the disease allowing 75% shoot blight severity and 38.5% canker incidence. Spray applied oxytetracycline (FireLine + Regulaid adjuvant) at bloom and fruit set completely prevented shoot blight severity and canker incidence. A single trunk injection of oxytetracycline (Arbor-OTC) gave 94.8% control of shoot blight severity and 91% control of canker incidence in comparison to untreated control. Two spray applications of streptomycin (Agri-Mycin + Regulaid) at bloom and fruit set gave 86.3% control of shoot blight severity and 84.2% control of canker incidence. A multi-year experiments are ongoing on apple and pear to determine the consistency of these results which could allow developing treatments for shoot blight and fire blight cankers prevention.

Abstract - Oral presentation

New sustainable treatment against fire blight disease in pears using quorum-sensing disruption

Mery Dafny-Yelin[1], David Gurevich[2], Shlomit Dor[2], Mayan Erov[2], Yoav Dan[3,4], Jehudith Clara Moy[1], Orly Mairesse[1], Lihi Adler-Abramovich[3,4,5], Livnat Afriat-Jurnou[2,6]
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[6]The Faculty of Sciences and Technology, Tel-Hai Academic College, Upper Galilee, Israel

Keywords: Directed enzyme evolution, Enzyme's encapsulation, Fire blight, Quorum-sensing.

Under suitable weather conditions on blossoming pear trees, the bacterium *Erwinia amylovora* can reproduce on the flower stigma, enter the receptacle and cause fire blight disease. In Israel, the antibiotic Starner (a.i. oxolinic acid) was the main product used to fight fire blight, applied according to a decision support system. Copper can also serve as a protective compound when applied before infection occurs. In 2021, world production of Starner was stopped, leaving the Israeli farmers with only one active ingredient against fire blight. Additional compounds with different modes of action are needed. Many Gram-negative bacterial pathogens use quorum-sensing, a population-density-dependent regulatory mechanism, to monitor the secretion of N-acyl-homoserine lactones (AHLs) and regulate pathogenicity. AHL lactonases hydrolyze AHLs, thus exhibiting antibacterial activity; however, most AHL lactonases are thermally unstable and short-lived. Using directed enzyme evolution, we increased a bacterial AHL lactonase's temperature resistance by 8◦C. Then, by encapsulating the enzyme in nanospherical structures composed of tert-butoxycarbonyl-Phe-Phe-OH peptide, its shelf life was extended by more than 5 weeks. Use of the encapsulated and free mutant significantly inhibited (up to 70%) blossom infection in the field, achieving the same efficacy as Starner. We conclude that directed enzyme evolution combined with the enzyme's encapsulation can inhibit fire blight symptoms in the field, constituting a sustainable approach for bacterial disease management. These findings have been published in ACS Applied Materials & Interfaces (https://doi.org/10.1021/acsami.0c15808).

Abstract - Oral presentation

Evaluation of Fire Blight Removal Strategies

S. Tianna DuPont[1], Kerik Cox[2], Ken Johnson[3], Kari Peter[4], Misbakhul Munir[1], Aina Baro[1]

[1]Washington State University, Wenatchee, United States
[2]Cornell University, Geneva, United States
[3]Oregon State University, Corvallis, United States
[4]Penn State University, Biglerville, United States

Keywords: Fire blight, cutting, sanitation

Effective in season management of fire blight infections is critical to reduce tree loss, cutting costs, and the risk of new infections. Nine experiments were conducted to evaluate the success of fire blight removal strategies in orchards with different scion, rootstocks, age, vigor and training system combinations. Experiments were arranged in a randomized complete block with 6-15 replications. Seven fire blight cutting treatments were compared including Best Management Practice (cuts 30 cm from the edge of noticeably infected tissue into 2-year or older wood with sanitized loppers), BMP No-sanitize (no sanitization between cuts), Aggressive (cuts 76 cm from noticeably infected tissue with sanitized loppers), Short and Long Stub (cuts leaving a 5 cm or 13 cm stub from structural wood) and Breaking (infected shoots broken by hand). Timely summer pruning of fire blight infections significantly ($P < 0.05$) reduced the number of trees which developed rootstock blight and died from fire blight infections. The standard best management practice where cuts are made 30 cm from the edge of the noticeably infected tissue into 2-year or older wood with sanitized loppers reduced the number of new systemically caused infections compared to no-treatment controls in most experiments. While Breaking provided a fast fire blight removal method it left many cankers in the orchard which can provide a source for infection in subsequent years. Pruning which left a 13 cm Long Stub from structural wood reduced the number of cankers on structural wood.

Abstract - Oral presentation

Evaluation of biopesticides for the control of *Erwinia amylovora* in Apple and Pear

S. Tianna DuPont[1], Kerik Cox[2], Ken Johnson[3], Kari Peter[4], Misbakhul Munir[1], Aina Baro[1], Tim Smith[1]

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[4]Penn State University, Biglerville, United States

Keywords: Fire Blight, essential oils, peracetic acid-peroxide, copper

With increasing acreage of organic and increased scrutiny of the use of antibiotics in agriculture, alternatives to antibiotics for the control of Erwinia amylovora are of interest to stakeholders. At the same time failures of minimally tested, newly marketed products have resulted in severe infections leading to costly tree and orchard removal. We evaluated antibiotic alternatives in 8 Washington, 3 Oregon, 3 New York and 2 Pennsylvania field experiments conducted between 2013 and 2022. Antibiotic alternatives included essential oils (thyme and cinnamon extracts), mineral compounds (potassium aluminum sulfate), oxidizers (peracetic acid-peroxide), soluble coppers (copper octanoate, copper hydroxide, copper suflate pentahydrate), and biological controls (Bacillus subtilis, bacteriophage). Orchard studies were conducted in 'Bartlett' and 'dAnjou' pear, 'Gala', 'Cameo,' and 'Red Delicious' apple with 4 to 6 single tree replicates arranged in a randomized complete block. Trees were inoculated with a suspension of E. amylovora (1x106 cfu ml-1) at 80-100% bloom. In summary analysis of 8 Washington trials Alum (potassium aluminum sulfate), several copper products (Previsto, Mastercop, Instill), and Blossom Protect averaged 70-72% control not significantly different than antibiotic checks. Several essential oil, copper, and peracetic acid-peroxide and biological products (Serenade Opti, Cueva, Oxidate 5.0, Jet Ag, Thymegard and Thymox) provided intermediate control between 45-56% significantly better than the water-treated control. In multistate trials, Alum with 2-3 applications also provided good control (2019: NY 77%; PA 57%; 2020: OR 86%, NY 65%; 2021: NY 87%) and essential oils with 3-4 applications good to intermediate control (2021: NY 81% control with 23% thyme oil; 2020, 2021: NY 70-85% control with 60% cinnamon oil).

Abstract - Oral presentation

Field testing of fire blight control strategies in Switzerland

Perrine Gravalon[1], Sandrine Kammerecker[2], Vanessa Reininger[3], Sarah Perren[1], Eduard Holliger[4]

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[4]Swiss fruit union, Zug, Switzerland

Keywords: Fire blight, field treatment, pesticide efficacy test, sustainable strategy

Since 2020, the bacteria *Erwinia amylovora* is no longer considered a quarantine pest in the European Union and Switzerland, even though fire blight is still responsible for damaging losses for producers. In addition to a low range of products registered in Switzerland against this disease and a ban of antibiotics since 2016, there is also a government incentive to reduce the use of plant protection products and the withdrawal of certain products in neighbouring countries. In this context, it is essential to gain experience and create a reliable knowledge base in fire blight control using alternative and sustainable strategies to support Swiss pome fruit production.

For this reason, the Swiss research institute Agroscope, as part of the HERAKLES Plus project, continues to conduct orchard trials every year, where different products (registered or in pending registration) and combinations of products are tested. The trials are conducted under biosafety guidelines in an orchard completely enclosed by insect netting on three-year-old potted apple trees. Trees are directly inoculated with the *E. amylovora* bacteria during flowering and bumblebees spread the disease to neighbouring trees, which are treated using different strategies. The efficacy of the variants is compared to untreated trees and trees treated with Blossom Protect™ (*Aureobasidium pullulans*).

The results from previous years will be presented at the conference.

Abstract - Oral presentation

Monitoring of *Erwinia amylovora* abundance in blossoms to support timing of control measures

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Keywords: fire blight monitoring, qPCR, abundance, blossom infection

Erwinia amylovora, the causal agent of fire blight is well established in the pome fruit growing areas around the Lake of Constance. Applications of control agents like Blossom Protect or LMA are timed using forecast models, which calculate the infection risk using weather data. Since 2014 blossom samples from pear, apple or quince orchards were analyzed using a qPCR assay quantifying *E. amylovora*. Orchards, from which blossom samples were analyzed, were also controlled for symptom development. Since 2014 more than 1100 cases (orchard-year combinations) were studied. This fire blight monitoring was financed by German, Suisse and Austrian cooperatives and advisory institutions.

In some cases, *E. amylovora* was detected in blossoms in high abundance, although forecast models did not indicate a high risk for infections. Monitoring proofed to be suitable to detect infection risk due to the real abundance of the pathogen and to time application of control agents.

In a lot of cases, no symptoms occurred, although the forecast models indicated a high risk for infections. If so, control agents timed according to the forecast models were applied without a real need. Quantitative monitoring of *E. amylovora* in blossoms allowed the definition of minimum levels of bacteria needed for high levels of symptom development in the orchard under different weather conditions. Without detectable bacteria in the specific orchard or as long as the abundance stayed below the defined minimum levels, the risk for infections was low.

Abstract - Oral presentation

Computer Vision-based Deep Learning for Fire Blight Recognition

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Keywords: Deep Learning, Computer Vision, Recognition, GAN

Fire Blight is a bacterially caused disease occurring from the pathogen *Erwinia amylovora.* This disease is particularly ruinous to apple and pear cultivation. When it occurs, all flora within a certain radius must be buried given the absence of proper treatment to date. Therefore, periodic forecasting for early prevention is currently the best possible method for minimizing damages from Fire Blight. This study utilized smartphone filming to determine pest infestation for quick and accurate Fire Blight forecasting from the cultivation site. Additionally, artificial intelligence training data was generated, and a deep learning-based recognition model was developed. First, similar diseases to Fire Blight such as Scab, Black Necrotic Leaf Spot, Valsa Mali, and White Rot 5 were accounted for, and 1,546 labeled images were created from 4,709 original images. For Fire Blight recognition, two types of deep learning models are proposed. The first model FireBlight GAN considers the manifesting location and intensity of the symptom to augment relatively low quantities of Fire Blight images. In FireBlight GAN, the manifesting location of Fire Blight is automatically detected. Further, damages are caused on the spot of occurrence to receive a healthy leaf image and generate a Fire Blight leaf image. The second model as the Fire Blight recognition model recognized five types of crop damages similar to those caused by Fire Blight. The performance results showed 97.77% precision and 96% recall from the proposed deep learning-based recognition model.

Abstract - Oral presentation

Fire blight management system in Korea

Yong Hwan Lee[1], Dong Suk Park[1], Hyeonheui Ham[1], Eunjung Roh, Se-Weon Lee[1], Hyeonseok Oh[1], Mi-Hyun Lee[1], Yeon-Jeong Lim[1] , Mihyung Kang[2], Uiseok Chae[2]

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Keywords: Fire blight, Management, diagnosis, monitoring, and control

Fire blight in apple and pear, caused by *Erwinia amylovora*, was first reported in three cities of Korea in 2015. Because *E. amylovora* is a prohibited quarantine pathogen in Korea, the government eradicated all hosts within a 100m radius range from an infected plant until 2018. However, the disease fast spread from five cities in 2018 to twenty-six cities and counties in 2021. The control system has been operated with greatly improved methods of diagnosis, monitoring, and control from 2022. After diagnosing on-site using the strip kit and removing the infected tree, the detection of amylovora and pyrifoliae kit (DAP kit) is used to distinguish between *E. amylovora* and *E. Pyrifoliae.* Branches with canker symptoms were inspected between March and April using the DAP kit. It was divided into the contaminated area and the disease-free area. In the contaminated area, if more than 5% of trees in the orchard are infected, all hosts are removed, and if less than 5%, only infected trees are removed. In the disease-free area, all host plants of an infected orchard are immediately removed. Administrative duties were imposed on farmers to conduct self-monitoring of the disease. Using the K-Maryblyt model improved from Maryblyt, 382 forecasting stations were installed in 36 cities and counties and the chemicals such as streptomycin were sprayed twice or more according to the prediction information during the flowering period.

Acknowledgements:
This study was carried out with the support of Cooperative Research Programs (Project No. PJ015059012022) from Rural Development Administration, Republic of Korea.

Abstract - Oral presentation

Fire blight control in Korea: the status of burial control

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Keywords: fire blight**,** burial control**,** bacterial diversity, fungal diversity

Fire blight disease caused by *Erwinia amylovora* has been occurred in apple and pear orchards in Korea since 2015. Because its risk for spreading is very high, burial control of all pear and apples trees in the disease detected orchards has been strictly performed by the Law of Plant Protection Act of the Korean Ministry of Agriculture, Food, and Rural Affairs. The removed host trees were buried to a depth of 2 meters below from the surface of the orchard soils. Replanting of apple and pear trees in the burial sites was prohibited for five years until two years ago. Now, the prohibition period has been reduced for three years. However, there have been concern about the justification on the burial control period among some apple and pear growers. Thus, to get some data for the justification, we investigated the survival of the pathogen and the microbial diversity from the buried host tissues and surrounding soils. The media-based solation and sequencing methods and PCR assay using specific primers were used to detect *E. amylovora* in both the plant and soil samples. *E. amylovora* was not detected in any samples investigated. Diverse bacterial and fungal groups were found in the soil samples using a microbial taxonomic profiling analysis. Our results suggest that burial control is effective and safe for fire blight in Korea.

Abstract - Oral presentation

Possible mechanisms that reduce sensitivity of *Erwinia amylovora* against oxytetracycline

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Keywords: Control, Effector, LPS, Oxytetracycline

Fire blight, which was first reported in Korea in 2015, is still a devastating disease in apple and pear orchard. Antibiotics such as streptomycin and tetracycline have been used as main agent within a three-time treatment strategy during blooming seasons. To date, no resistance to these antibiotics has been recorded in Korea. However, *in vitro* efficacy test of antibiotics and anecdotic evidence by farmers showed that there is reduce sensitivity by oxytetracycline compared to that by streptomycin. Thus, we obtained tetracycline-reduced sensitivity (TcR) strain of *E. amylovora* after exposures to light under laboratory conditions and compared its characteristics with those of wild type (WT). TEM analysis showed that TcR strain has thicker biofilm layers than WT. Quantitatively, levan and cellulose were reduced in TcR strain compared to those in WT; however, both strains had a similar amount of amylovoran, indicating that the thick layers were likely more of LPS than EPS. Regarding the patterns of gene expression, *waaL* gene was upregulated, and *ams* and *lsc* genes were downregulated, corroborating the increased LPS. In addition, TcR strain experienced more reductions in growth rate and motility than the WT. In pathogenicity tests, TcR strain showed lower necrotic symptoms than WT on apple seedlings; the strain could not produce any symptoms on immature apple fruits. However, hypersensitive response (HR) was not different between the two strains. Experiment of patterns of proteins secreted from WT and TcR strains is currently being performed to elucidate whether effector protein is connected to reduced pathogenicity.

Abstract - Oral presentation

Novel biocontrol agent RejuAgro to control fire blight, citrus greening, and citrus canker

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Keywords: *Erwinia amylovora*, Huanglongbing, *Candidatus* Liberibacter asiaticus, *Xanthomonas citri* subsp. *citri*

Fire blight caused by *Erwinia amylovora* is ranked as the most concerned pome fruit disease in the U.S. Due to the lack of consistent control efficacy from biocontrol agents, fire blight management heavily relies on using human antibiotics streptomycin and oxytetracycline. Since the initial report in 1971, the pathogen's resistance to streptomycin has been constantly observed in almost all apple-producing regions in the U.S. The continued use of the antibiotic streptomycin has not only resulted in high losses of crops and trees due to failures in controlling the disease but is also threatening the environment and human health.

We have built the first extensive collection of microbes from different orchards and natural environments in Wisconsin and other states of the U.S. A total of ~44,000+ microbial isolates were screened over five years to identify biocontrol agents for plant disease control. The biocontrol bacterium *Pseudomonas soli* T3-07 was found to be the most capable of suppressing fire blight. *P. soli* T3-07 and its metabolites have since been named to RejuAgro. We performed greenhouse and field experiments on RejuAgro and demonstrated that it effectively reduces fire blight disease incidences with an efficacy comparable to the streptomycin treatment. RejuAgro also suppresses citrus Huanglongbing and citrus canker when sprayed on the surface of citrus leaves. The formulated biocontrol agent RejuAgro was developed for long-term storage of two years at room temperature and orchard applications.

Abstract - Oral presentation

Development of a digital monitoring system for fire blight in fruit orchards

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Keywords: *Erwinia amylovora*, UAV, Image annotation, Georeferencing, Photogrammetry

Changing climatic conditions promote the increasing immigration and spread of quarantine phytopathogens, which is a major challenge for European commercial fruit growing and fruit breeding. Most control measures are aimed at detecting an infestation at an early stage, containing it and preventing further spreading. Currently, there are no standardized methods for detection and map pathogens on a small scale. In the MONIQUA project, a digital monitoring system will be developed for detecting and localizing pathogens in fruit orchards. As model-pathogens were used fire blight and pear rust. Classified as a quarantine disease in Europe until January 1, 2020, fire blight (*Erwinia amylovora*) remains one of the most dangerous diseases in fruit growing. It can spread epidemically, which is why regular controls are mandatory.

Low-altitude UAV flights have enabled large datasets of high spatio-temporal RGB images of species-specific disease symptoms in apple orchards. The focus is initially on the detection of leaf and shoot fire blight symptoms. The annotation of each symptom by using the Computer Vision Annotation Tool (CVAT) creates a training data set for the machine-learning model. Using a photogrammetric approach on georeferenced images, the infested plants of the orchard are precisely located. Later the spread of diseases will linked to the geographic information from weather services. A monitoring model will developed, based on this data.

Establishing a high-throughput control system for fruit production and breeding is the long-term goal of the project to enable continuous spatial detection and documentation of fruit diseases.

Abstract - Oral presentation

Keynote: Status of research on phage-mediated control of *Erwinia amylovora*

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Keywords: Bacteriophage, Biocontrol, Molecular Quantification, Genomics, CRISPR-Cas resistance

Bacteriophages can shape the microbiome and control phytopathogenic populations through their lytic activity. This presentation will provide an overview of the recent advances in the development of phage-mediated biological control of *E. amylovora*. Alternative pest management strategies are important to organic growers and regions where antibiotics are restricted or antibiotic resistance is present. In our system, *Pantoea agglomerans* acts to deliver and propagate *Erwinia* phages on the stigma. Comparative genomic analysis of 127 *E. amylovora* isolates defined three primary clades of the pathogen in North America. Furthermore, a host range study identified 3 families of phages capable of infecting all 95 *Amygdaloideae*-infecting *E. amylovora* isolates tested.

We have developed multiplex qPCR assays to quantify the carrier, pathogen, and multiple phages within solution simultaneously. The lytic lifecycle of individual phages was established through qPCR from which the burst size was determined. Recently, we've shown that *E. amylovora* is capable of surviving exposure to individual phages at a high MOI under ideal growth conditions. The continued study of phage resistance mechanisms through comparative genomic analysis revealed that each primary clade of *E. amylovora* has a unique CRISPR-Cas system. These systems provided protection against invasive plasmids but offer an incomplete degree of resistance against *Erwinia* phage φEa21-4. However, *P. agglomerans* works synergistically with the phage cocktail to overcome the observed phage resistance. Thus, the future of phage-mediated biological control for *E. amylovora* will involve the identification of resistance genes and the optimization of the phage-carrier system to better mitigate these mechanisms.

Abstract - Oral presentation

A qPCR-based, population dynamics approach for the development of a bacteriophage-based biopesticide

Steven Gayder[1], Sandrine Kammerecker[1], Kellen KG Gervásio[2], André Henriques[2], Gloria Torres-Cortés[2], Szilvia Kőrösiné Papp[3], Tamás Kovács[3]; Krisztina Raffai[3], Sixto Cabezón Largas[4]; Borja de Santo Prietos[4], Lars Fieseler[1]

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Keywords: Bacteriophages, qPCR, Population Dynamics, Biopesticide

Bacteriophages are viruses that target specific bacteria and kill them through their own replicative life cycle. As more countries ban the use of antibiotics for the control of fire blight and antibiotic resistant strains of *Erwinia amylovora* become more widespread, the use of phages as biological control agents is rapidly gaining interest. In the multinational Horizon 2020 Project PhageFire, several academic and industry partners have teamed up to develop a phage-based product for the control of fire blight.

To design an effective and affordable phage formulation, the choice of phages in the cocktail is critical. The ultimate goal is to maximize synergistic interactions and minimize antagonistic interactions between the different phages while using the fewest number of phages possible to reduce production costs. To achieve this goal, we used a quantitative real-time PCR (qPCR) approach to study different combinations of a collection of phages against a combined library of *E. amylovora* strains representative of the pathogen diversity in Europe. With qPCR, the populations of the phages and *E. amylovora* can be measured individually over time in liquid cultures. This allows us to determine which phages synergize best together, while eliminating those that get outcompeted and add little overall value to the cocktail. With this work, in conjunction with additional product formulation, regulatory efforts, and field trials we aim to develop an effective, affordable, phage-based biopesticide for the control of fire blight.

Abstract - Oral presentation

The effects of *Aureobasidium pullulans* formulations and acibenzolar-S-methyl on the incidence and severity of fire blight floral infections

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Keywords: fire blight, *Aureobasidium pullulans*, Actigard, control

The New Zealand pome fruit industry currently uses antibiotics to control fire blight. Concerns regarding the transfer of streptomycin resistance from plant production into human clinical bacteria or the environment has prompted a proposed antibiotic phase out.

This study looked at the effect of biological control agents (BCAs) containing *Aureobasidium pullulans* formulations (Botector®, Aureo® Gold, Blossom Protect™, OPA), with or without the plant defence elicitor acibenzolar-S-methyl (Actigard™), on incidence of fire blight in *Malus domestica* 'Scilate' potted trees and *M*. *domestica* 'Royal Gala' flowers in the field.

Actigard was applied to potted 'Scilate' trees 3 days before *Erwinia amylovora* (1 x 10^6 cfu/mL) inoculation and the BCAs (Botector, Aureo Gold or OPA) were applied 1 day before inoculation. Actigard alone and all of the tested BCAs with or without Actigard significantly reduced infection numbers compared with untreated control trees. The best performing treatments were Actigard + OPA and OPA alone.

In the field trial, Actigard was applied 7 days and the BCAs (Botector, Blossom Protect, Aureo Gold or OPA) 24 h before inoculation with *E. amylovora* (1 x 10^6 cfu/mL). All of the BCAs with or without Actigard provided good fire blight control, similar to that of KeyStrepto™. The treatment combinations of Actigard + OPA and Actigard + Botector gave better disease control than the treatments Aureo Gold, Blossom Protect, or Actigard + Aureo Gold. Treatments containing Blossom Protect, Botector or OPA resulted in significantly more side russet of fruit, whereas this was not observed in treatments containing Aureo Gold.

Abstract - Oral presentation

Formulation of a bacteriophage-based biopesticide against *Erwinia amylovora*

Sandrine Kammerecker[1], Steven Gayder[1], Kellen Gervásio[2], André Henriques[2], Gloria Torres-Cortés[2], Szilvia Kőrösiné Papp[3], Tamás Kovács[3], Krisztina Raffai[3], Sixto Cabezón Largas[4], Borja de Santos Prieto[4], Lars Fieseler[1]

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Keywords: Bacteriophages, Biopesticide, Formulation, UV-protection

Many plant protection agents traditionally used to combat fire blight are subjected to restrictions in an increasing number of countries. The emergence of streptomycin-resistant bacteria has called for antibiotic-free alternatives, while the use of aluminum- and copper-based products has raised environmental concerns. In this scope, there is a high demand for alternative products with lower risk profiles, for example antagonistic bacteria, yeasts, or bacteriophages (phages). Phages are considered safe for both the environment and human health, and they are highly specific for their target bacterium. Some phage-based agents have already been approved for use in plant protection and food safety. However, diverse environmental factors can impair the phages' stability. UV-light, for example, which is damaging to DNA, can inactivate phages in the field.

In the European Horizon 2020 project PhageFire, the goal of all involved partners is to develop such a phage-based biopesticide against fire blight. Here, we tested different UV-B absorbing substances and surfactants to develop an effective formulation to protect the bacteriophages from UV-B light and to ensure an even foliage coverage and adherence of the phages to the plant surface. Under artificial UV-B illumination, the phages' survival could be increased by the addition of UV-B absorbents. We could also show that many commercially available surfactants are compatible with phages and do not affect their stability.

With these contributions, complemented by phage characterization, field trials, and regulatory aspects, we aim to develop an effective and safe biopesticide against fire blight.

Abstract - Oral presentation

Improvement of the citric acid buffer used for fire blight control with Blossom Protect™

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Keywords: Buffer Protect NT™, *Aureobasidium pullulans*, pH buffer, *Erwinia amylovora*

Blossom Protect™ contains *Aureobasidium pullulans*, a yeast-like biocontrol fungus that blocks the fire blight pathogen, *Erwinia amylovora*, from colonizing pome fruit blossoms. Buffer Protect NT™, buffers the spray suspension to around pH 3.5 and lowers blossom pH. The low pH impedes the growth of *E. amylovora*, while *A. pullulans* applied with the buffer tolerates the low pH well and proliferates on stigmas and in the hypanthium.

Buffer Protect NT™ has been developed with improved solubility and storage stability compared to an old formulation (Buffer Protect™). This user-friendly SG formulation (soluble granules) significantly reduces the application rate from 10.5 kg/ha for the old buffer to 6 kg/ha and is certified for use in organic agriculture.

The previous formulation could sometimes contribute to fruit russet in sensitive pome fruit varieties dependent on weather conditions. The two formulations were compared in Blossom Protect™ field trials with 3 to 4 applications per growing season. In 14 bridging trials the Buffer Protect NT™ showed significantly less russeting than the old formulation ($P<0.01$). In ten tests on russet sensitive apple cultivars, the fruit russet after treatment with Buffer Protect NT™ showed no significant difference from the untreated control.

Field trials in pears and apples over 4 years in 6 countries (Austria, France, Germany, Italy, Spain, USA) confirmed that Buffer Protect NT™ could replace the old formulation, significantly enhancing efficacy of *A. pullulans* biocontrol ($P<0.05$). Blossom Protect™ + Buffer Protect NT™ reduced fire blight incidence in these trials by up to 93% in the best case.

Abstract - Oral presentation

Improving tissue processing, sensitivity and dynamic range of viability droplet digital PCR (v-ddPCR) for detection and quantification of *Erwinia amylovora* in fire blight cankers

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Fire blight bacterium *Erwinia amylovora* (*Ea*) continues to threaten commercial apple and pear production globally. The development of fire blight cankers is caused by *Ea* invasion of wood from infected flowers and shoots. The importance of cankers is growing due to spindle-shaped apple training systems in high density orchards suffering an increase in tree death from cankers on the central leader, trunk and/or rootstock. In contrast, there are few reliable and sensitive detection techniques for studying fire blight cankers which are the main sites for *Ea* overwintering. Using culture-dependent approaches to determine *Ea* cell viability in cankers is complicated by faster growing saprophytic microorganisms in culture and the possibility that *Ea* cells are stressed and thus nonculturable. Traditional PCR diagnostic methods cannot quantify and distinguish live and dead *Ea* cells. To detect and quantify live *Ea* cells in cankers, we previously developed a v-dPCR assay using the chip-based QuantStudio 3D platform. To date, we have improved fire blight canker maceration using Geno/Grinder homogenizer, increased DNA extraction efficiency from bark tissues, and increased the assay's dynamic range and sensitivity by optimizing ddPCR assay on the BioRad QX200 platform. QX200 can run 15,000 to 17,000 reactions per well in a 96 well plate, allowing accurate quantification of target copies using the same Taq-Man probe and primers for *Ea* we used previously. Our v-ddPCR assay will enable new ways to evaluate resistant pome fruit tree germplasm, provide further dissection of *Ea* life cycle, and elucidate *Ea* epidemiology and new options for canker management.

Abstract - Oral presentation

Keynote: Evolution of *Erwinia amylovora* genome: what, how and why?

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Keywords: Genome structure, inversion, virulence, RNA operons

The first two genome sequences of *Erwinia amylovora* strains CFBP1430 and Ea273, isolated from France and New York, respectively, shared 99.99% identity. Thus, *E. amylovora* is generally believed to be genetically homogeneous at the nucleotide level. However, large chromosome inversions (LCIs) have been reported in *E. amylovora* and are believed to be driven by homologous recombination. Syntenic analyses discovered two LCIs between the chromosome of the CFBP1430 and Ea273 strains. When we sequenced the Ea1189 genome, we revealed a new type of chromosome arrangement, which differs from those previously published. MAUVE alignment of complete *E. amylovora* genome sequences available in NCBI reveals four LCI types (Ea1189, CFBP1430, Ea273, and MAGFLF-2). More recently, we combined genome sequencing and PCR analysis using a set of molecular markers to explore novel LCIs in *E. amylovora* and established what LCI type exists in nature, how LCI happens and why. We further predicted potential LCIs based on estimated replichore balance, determined their geographical distribution, and proposed the evolutionary history of *E. amylovora* genome based on known and newly discovered LCI types. These findings provide strong evidence about the prevalence of certain LCI types and potential evolutionary history, which might help determine the spread of the fire blight pathogen/disease. In addition, the methods we developed will provide essential tools to further discover novel LCI types, which will expand our knowledge of genomic diversification in *E. amylovora*.

Abstract - Oral presentation

Comparative genomics provides new insights into host specificity and evolutionary history of *Erwinia amylovora*

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Keywords: Fire blight, phytopathogen, intraspecies diversity, *Malus* x *domestica*, *Rubus idaeus*

The bacterial pathogen *Erwinia amylovora* can be divided by host-specificity into a group of strains infecting members of the Amygdaloideae subfamily and a group infecting the *Rubus* genus. We have completely sequenced eleven *E. amylovora* genomes from North America, the geographical origin of the organism, in which we identified unique features of these strains. Comparative genomics was used to reveal differences between Amygdaloideae-infecting (AI) and *Rubus*-infecting (RI) strains.

The phylogenetic clades (AI and RI) do not entirely reflect the host-specificity. Four deletions in clade-specific gene clusters were identified from this comparison in the RI clade. The function of one cluster is unknown, while the other three encode for L-arabinose metabolization, degradation of sulfur compounds and degradation of phenolic compounds. Based on the absence thereof in RI strains, novel information on the interaction of AI *E. amylovora* with its host plant may have been obtained. Furthermore, two host-specific differences were identified: the lipopolysaccharide biosynthetic gene cluster and the deletion of non-ribosomal peptide and polyketide synthase genes in strains from Amygdaloideae hosts. Some of the genomes showed phage insertions, while anti-phage defense systems were identified in a number of strains.

The results of this study implicate an instance of convergent evolution for two distinct clades of *E. amylovora* with *Rubus* hosts, resulting in complex host-specificity mechanisms which are not reflected by the phylogeny. Additionally, it was shown that intraspecies diversity of *E. amylovora* is much higher than previously thought.

Abstract - Oral presentation

Increasing genetic diversity suggests multiple independent introductions of fire blight in Central Asia

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Keywords: source tracking, epidemiology, *Malus sieversii*, *Pyrus korshinskyi*

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. In 2017, isolates were also obtained from neighboring Uzbekistan and Tajikistan. PCR and sequence analysis revealed an initial discrete geographical distribution of different CRISPR genotypes exclusively belonging to the archetypal genotype A, which is typically prevalent in the Mediterranean Area and the Caucasus region. Genetic investigation of newly acquired isolates now reveals that archetypal genotype D, which is predominant in Northern Europe, was already present in South-Kazakhstan in 2014 and was recently detected also in the Chui Valley in Kyrgyzstan, as well as near Samarkand in Uzbekistan. Taken together this data show that multiple (and partially geographically overlapping) independent introductions of fire blight have occurred in Central Asia in the last fourteen years. Genome sequencing of selected isolates is now underway to establish the possible origin of these invasions.

Abstract - Oral presentation

Genome editing in *Erwinia amylovora* by *rpsL* counter-selection.

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Keywords: Lambda-red, genome editing, *avrRpt2*, *Malus* ×*robusta* 5 resistance

Spontaneous streptomycin resistant mutants have long been a useful tool for functional screenings via random transposon mutagenesis in various phytopathogenic bacteria. With the recent methodic advances in targeted genome editing, *rpsL* selection has found a new use as an effective counter-selectable marker. This application relies on the fact, that introduction of a wt copy *rpsL* into a streptomycin-resistant *rpsL* mutant will render the resulting strain once again sensitive towards the antibiotic. In combination with lambda-red engineering this has been used to generate markerless gene-deletions or gene modifications in a number of bacteria. Here we demonstrate the usefulness of this genome editing strategy in *E. amylovora*.

The AvrRpt2 effector of the fire blight pathogen has been shown to trigger a crucial function in the defense response of *Malus* x *robusta* 5 (Mr5). A mutation found in *avrRpt2* of the Canadian isolate 3049 (Ea3049) allows this strain to cause typical fire blight symptoms in the otherwise resistant wild apple. In contrast, virulence towards cultivars of *Malus domestica* does not differ between *E. amylovora* Ea222 carrying the common cysteine-allele (C-allele) of the effector or Ea3049 with the deviating serine-allele (S-allele). In order to investigate if additional genomic features of Ea3049 contribute toward its symptomatic infection of Mr5, the *avrRpt2* gene of this isolate and of C-type isolate Ea222 were edited in their native chromosomal location. In two subsequent rounds of lambda-red assisted marker exchange the *avrRpt2* of each strain was substituted for its counterpart. The resulting phenotypes are discussed.

Abstract - Oral presentation

RpoN regulon in *Erwinia amylovora* revealed by transcriptional profiling and *in silico* binding site analysis

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Keywords: Type III secretion, virulence, sigma factors, transcriptome

Erwinia amylovora causes a devastating fire blight disease in apples and pears. One of the main virulence determinants in *E. amylovora* to cause disease is the hypersensitive response (HR) and pathogenicity (*hrp*)-type III secretion system (T3SS), which is activated by the RpoN-HrpL sigma factor cascade. However, the RpoN regulon in *E. amylovora* has not been investigated. In this study, we determined the regulon of RpoN in *E. amylovora* by combining RNA-seq transcriptomic analysis with *in silico* binding site analysis. RNA-seq revealed that 262 genes, approximately 7.5% genes in the genome of *E. amylovora*, were differentially transcribed in the *rpoN* mutant as compared to the wild type. Specifically, genes associated with virulence, motility, nitrogen assimilation, the PspF system, stress response, and arginine biosynthesis are positively regulated by RpoN, whereas genes associated with biosynthesis of amino acids and sorbitol transport are negatively regulated by RpoN. *In silico* binding site analysis identified 46 potential target genes with putative RpoN binding site, and the upstream sequences of 6, 3, and 3 genes also contain putative GlnG, PspF, and YfhA binding site, respectively. Overall, RpoN directly regulates genes associated with virulence, nitrogen assimilation, the PspF system, motility and the YfhA/YfhK two component regulatory system.

Abstract - Oral presentation

Keynote: Host-*Erwinia amylovora* interaction

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Fire blight, caused by the bacterial phytopathogen *Erwinia amylovora,* is an economically critical and a mechanistically complex disease that affects apple and pear production in most geographic production hubs worldwide. *E. amylovora* utilizes distinct virulence strategies during host infection including type III secretion and biofilm formation. An understanding of *E. amylovora* host-pathogen interactions is critical to direct management strategies including gene-for-gene based host resistance, use of SAR inducers and growth inhibitors, and even impacts blossom blight tactics. Research from the fire blight community spanning 50+ years has shown that the type III effector DspE and the amylovoran exopolysaccharide are essential for disease, and that a wide variety of other factors influence virulence and systemic infection. Strict genetic regulation of these pathogenicity and virulence factors is also critical for the orchestration of pathogenesis; the major regulatory systems known to control virulence factor expression include two-component regulators, small RNAs, and the second messenger compound cyclic di-GMP. I will present an overall outlook of our knowledge of *E. amylovora* host-pathogen interactions and discuss some key directions to address critical unanswered questions.

Abstract - Oral presentation

The Ams proteins and the amylovoran biosynthetic pathway in the phytopathogen *Erwinia amylovora*

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Keywords: Ams, amylovoran, *Erwinia*, glycosyltransferase, pyruvyl transferase

Amylovoran is the most important pathogenicity factor of *Erwinia amylovora*, the etiological agent of “Fire blight” disease. It is an exopolysaccharide essential in the formation of a protective biofilm used by the bacterium to escape the plant protection mechanisms. The amylovoran biosynthetic pathway involves 12 genes, clustered in the 16kb *ams* region and corresponding to different Ams proteins. Among them, the glycosyltransferases AmsB, AmsD, AmsE, AmsG, AmsK and the pyruvyl transferase AmsJ are presumed to play a part in annealing the sugar subunits of the branched polysaccharide. Since the inability to synthesize amylovoran results in non-pathogenicity, to achieve the bio-molecular characterization of these carbohydrate-active enzymes will be the base for the development of efficient and sustainable control methods to Fire blight threat. To understand the chemo-enzymatic reactions, a functional and structural analysis of each target is then required, mainly considering spectroscopic methods and X-ray crystallography.

A genome wide analysis was carried out between species belonging to the *Erwinia* family to identify what makes the pathway unique. By a proteome comparative approach, we identified the functional domains considered to be fundamental for each enzyme. After several purification attempts, the Ams proteins proved to be difficult to express and solubilize. Computational approaches were used to identify several hydrophobic patches on their surface. Biophysical techniques confirmed the instability problem, which lead to aggregation and incorrect folding. A modified plasmid is being designed to remove the hydrophobic fractions and a co-expression experiment will attempt to check for possible protein-protein interaction to stabilize the targets.

Abstract - Oral presentation

The complex and compartmentalized cyclic di-GMP signaling network is a global regulator of phase-transition and host colonization in *Erwinia amylovora*

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Keywords: biofilm, cyclic-di-GMP, global regulation, phase transition

The bacterial second messenger cyclic-di-GMP (c-di-GMP) is a regulator of several virulence factors in the fire blight pathogen, *Erwinia amylovora*. The presence of multiple (12) formative (diguanylate cyclases encoded by *edc* genes) and degradative (phosphodiesterases encoded by *pde* genes) enzymes that control the intracellular levels of c-di-GMP in *E. amylovora* elevates the complexity of this signaling system. In order to study regulatory contribution of each individual Edc and Pde enzyme, we constructed a c-di-GMP null strain by deleting all 12 *dgc* and *pde* genes in *E. amylovora* strain Ea1189Δ12. Ea1189Δ12 was unable to colonize xylem vessels in apple shoots due to an impairment in surface anchoring and biofilm initiation dependent on both the flagellar filament and the type IV pilus. An RNA-Seq study comparing the transcriptomic profiles of Ea1189Δ8 (lacking the 5 Edcs and 3 Pdes that are enzymatically active) and Ea1189Δ8 overexpressing each of the 5 *edc* genes highlighted that a small subset of genes involved in metabolism and transcriptional regulation were regulated by all 5 Edcs. However, a majority of the regulatory targets of each of the Edcs were unique. Thus, our study highlights that c-di-GMP is critical for host colonization and that this process is mediated by a dual modality of compartmentalized and global regulatory frameworks in *E. amylovora.*

Abstract - Oral presentation

Colonization of yeast-like fungi on apple flowers induces host immunity and prevents fire blight infection

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Keywords: Microbiome, Systemic Acquired Resistance (SAR), *Aureobasidium pullulans*

Microbiome on plants is well recognized for its potential to influence plant disease occurrence through impacting the pathogen-host interactions. Fire blight, caused by a bacterial pathogen *Erwinia amylovora*, is a devastating disease of apple and pears. Blossom blight stage of fire blight infection, in which *E. amylovora* mutiplies eiphytically on flower surfaces such as stigma and stamen, prior to entering host through the hypanthium, is a critical step of the disease cycle. Previous research investigating the function of microbiome on fire blight mostly focused on the microbiome-pathogen interactions, however, to what extent the microbiome interacts with the host, and whether/how such interactions influence disease outcome is less understood. In this study, we characterized the composition and dynamics of the mycobiome on hypanthium of apple flowers. We showed that some members of the mycobiome, namely the yeast-like fungi belonging to the *Aureobasidium* genus, induced the expression of pathogenicity related (PR) genes in the salicilic acid (SA) pathway in apple hypanthium. Some of the fungal isolates that induce host immunity genes also can colonize under cuticles of immature fruits and cause russeting of fruits. Spray-inoculating an *Aureobasidium pullulans* suspension to apple flowers significantly repressed fire blight infection. Our study indicates certain yeast-like fungi, through causing a minor russeting on apple cuticle, induced host immunity and thus primed the host to resist the infection of fire blight, a much more devastating disease that can cause tree mortality.

Abstract - Oral presentation

Probing metabolite requirements for *Erwinia amylovora* disease establishment

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Keywords: aspartate, tyrosine, asparagine, mutant, stigma, fruitlets

Erwinia amylovora must proliferate in different tissues of the host, including the stigma, developing fruit, vasculature and over winter in cankers during the disease process. Each host locale presents a unique nutritional landscape, necessitating *E. amylovora* to be biochemically flexible to meet its needs. We are utilizing mutants in biochemical pathways to probe the metabolic requirements of *E. amylovora* particularly for growth on stigma and in developing fruit. During apple flowering, the major nitrogen transport molecules (aspartate, asparagine and glutamine) peak in xylem sap as they are transported from roots to developing flowers and leaves. This period coincides with the main infection time for fire blight. We have engineered aspartate prototrophs by deleting both the complementary aspartate aminotransferase (AspC) and tyrosine aminotransferase (TyrB) loci. Heterologous complementation experiments in an analogous *E. coli aspC tyrB* mutant using EaAspC and EaTyrB expression constructs and growth experiments in *E. amylovora DaspC::Cam*^{R} *DtyrB::Kan*^{R} strains verify the function of the genes. Despite the amino acid synthesis deficiencies the *E. amylovora DaspC::Cam*^{R} *DtyrB::Kan*^{R} strain can still grow on stigma surfaces. Apple fruitlet growth experiments are in progress. Similar experiments using asparagine synthase deficient *E. amylovora* strains are also under way. These investigations are part of a larger research program exploiting metabolic mutations to better understand nutritional interactions and limitations for *E. amylovora* growing in the host environment.

Abstract - Oral presentation

Glandular and non-glandular trichomes are colonization sites and host entry points of the fire blight pathogen on apple leaves

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Keywords: Glandular and non-glandular trichomes, Pathogen entry points, Shoot blight

Unlike fungal pathogens, bacterial plant pathogens do not have penetration structures thus rely on natural openings or wounds to enter host and cause disease. Characterized natural openings as host entry points by bacterial pathogens include stomata (*Pseudomonas syringae*) and hydathodes (*Xanthomonas campestris*). Fire blight, caused by a bacterial pathogen *Erwinia amylovora*, is a devastating disease of apple and pears. On leaves and shoots, *E. amylovora* has been long thought to enter host through injuries caused by insects, wind, and hailstorm, however, the level of infection observed in the field suggests there could be additional host entry points other than artificial wounds. In this study, we demonstrated that *E. amylovora* can infect apple leaves without artifiicial injuries (grown in a plant growth chamber). Epiphytic colonization of *E. amylovora* was observed on glandular and non-glandular trichomes. *E. amylovora* was later found in interceluar space of leaf tissue adacent to the glandular trichomes and the veins. Additionally, we observed the glandular and non-glandular trichomes gradulally rupture and fall off during leaf development, which provide naturally occurred wounds for *E. amylovora* to enter. Although the type III secretion system is not required for colonization of *E. amylovora* on the glandular trichomes, it is however essential for *E. amylovora* to establish initial colonziation in messophil tissue adjacent to the glandular trichomes. Finally the host entry and infection of shoots is heavily impacted by the shoot water content. When shoot water potential is below -18 bar, pathogen entry and shoot blight infection would not occur.

Abstract - Oral presentation

Keynote: The dichotomy of polygenic and monogenic resistance to fire blight: the case of wild and domesticated apple genotypes

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Keywords: *Erwinia amylovora*, polygenic resistance, monogenic resistance, broad-spectrum resistance

It has long been thought that resistance to fire blight is polygenic in nature, which is further strengthened by the discovery of several quantitative trait loci mapped in the genome of some apple cultivars, clones and wild species accessions. However, resistance to this disastrous disease of *Malus*, which is caused by *Erwinia amylovora*, is also known to be strain-dependent, with the most notable breakdown of resistance being the result of a single nucleotide polymorphism in *E. amylovora* effector avrRpt2$_{EA}$. This exchange of cysteine amino acid to serine amino acid led to the breakdown of the resistance of *Malus* ×*robusta* 5 (Mr5) and the gene *FB_MR5*, which underlies the resistance locus on linkage group (LG) 3. In principle, strain-specificity of disease resistance does not conform to polygenic resistance, which is touted as more durable; but rather to monogenic resistance, which is less durable and often overcome due to pathogen selective pressure. It is unclear which resistance model fits *Malus* fire blight resistance. What is clear however is that not all resistances in *Malus* conform to the clear distinction between monogenic and polygenic resistance. The fire blight resistance loci of *Malus fusca* accession MAL0045 and *M*. ×*arnoldiana* accession MAL0004 on LGs 10 and 12, respectively, are the only ones known to not be broken down by the single nucleotide polymorphism found in strains of the avrRpt2$_{EA}$ effector of *E*. *amylovora*. Nevertheless, their progenies are severely affected by the aggressiveness resulting from this nucleotide exchange. Even so, their resistance appear to fit both models. We will also present results of phenotypic evaluation and QTL mapping using avrRpt2$_{EA}$ mutant strains on populations derived from MAL0045 × 'Idared' and 'Idared' × MAL0004, and show evidence of previously unmapped strain-specific QTLs in MAL0045.

Abstract - Oral presentation

Advances in breeding fire blight resistant apple cultivars at Agroscope

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Keywords: Apple, resistance breeding, *Erwinia amylovora*

Fire blight remains one of the world's most damaging diseases in apple (*Malus* x *domestica*) fruit production. Although the bacterium *Erwinia amylovora* is no longer classified as quarantine organism in Switzerland, breeding for fire blight resistance remains an important breeding objective at Agroscope. The apple breeding program develops new varieties with high fruit quality and excellent agronomic characteristics suitable for sustainable fruit production, including the combination and stacking of different resistance genes against various diseases.

For more than a decade, different methods and strategies (e.g. marker assisted selection and phenotyping) have been implemented in fire blight resistance breeding.

As a result, knowledge about the susceptibility or resistance of different apple varieties and Agroscope breeding material has increased considerably. Breeding material, varieties and heirloom accessions were tested by disease screening with artificial inoculation under greenhouse and field conditions. Genotypes that were found to be less susceptible or resistant to *E. amylovora* were used in new cross combinations to obtain new varieties with a high tolerance against *E. amylovora* and other diseases combined with good fruit quality and productivity. A “Fast Track” breeding approach for the accelerated introgression of fire blight resistances from wild or exotic donors (*Malus x robusta* 5, ‘Evereste’ and *Malus fusca*), combining marker assisted breeding with a controlled and optimized cultivation in the greenhouse and an artificial winter simulation, has been continuously optimized. Genotypes of the fifth pseudo-backcross generation combining the resistance genes *FB_MR5* and *Fb_E* with the quantitative trait locus *FB_F7* from ‘Fiesta’ were obtained. Recent results, progress and new approaches in various Agroscope apple breeding projects will be presented.

Abstract - Oral presentation

Mapping novel QTL for fire blight resistance in the primary progenitor species of domesticated apples (*M. × domestica*)

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Keywords: QTL mapping, Fire blight, *M. sieversii*

Fire blight, caused by *Erwinia amylovora*, is a devastating bacterial disease that significantly impacts apple production worldwide with few exceptions. Most commercial apple cultivars are moderately to highly susceptible to fire blight. The use of known major effect fire blight resistance QTL from wild *Malus* species is inhibited by high heterozygosity, long juvenile periods, sexual incompatibility, and linkage drag of undesirable fruit quality alleles. To overcome these obstacles, there is great interest in identifying resistance QTL in *M. sieversii*, the primary progenitor of domesticated apples. *M. sieversii* has broad sexual compatibility with *M. × domestica*, moderate fruit size, and several accessions identified with major fire blight resistance. To further map in this background, we performed artificial inoculations with E. amylovora for 298 F1 progeny across two families from a biparental cross of 'Royal Gala' and two *M. sieversii* accessions. Fire blight inoculation was repeated for three years (2018-2020) with 5 clonal replicates of each F1 genotype, parents, and controls. Utilizing approximately 20K GBS SNPs, single-family parental linkage maps were constructed from pseudo-test cross markers for both families. Interval QTL mapping and compositive interval mapping were performed to identify novel QTL for fire blight resistance. Across two years (2019-2020), a major effect QTL for the percent shoot lesion length and area under the disease progress curve was identified on the distal end of LG07. The percent variance explained for this QTL range from 45-55% with a confidence interval of 6-8cM. Identifying a major fire blight resistance QTL in *M. sieversii* has the potential to accelerate the development of fire blight resistant apple cultivars.

Abstract - Oral presentation

Fire Blight resistance breeding in Dresden-Pillnitz

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Keywords: apple breeding, fire blight, resistance, genetic resources, introgression, rapid cycle breeding

Resistance to fire blight caused by *Erwinia amylovora* has become an important topic in pome fruit breeding in Dresden-Pillnitz. Since most apple and pear cultivars are more or less susceptible to this dangerous disease, the demand for fire blight resistant cultivars has gained more importance. The first step in breeding is the evaluation of genetic resources to identify resistant sources. Therefore, both apple and pear cultivars and wild species collections have been phenotyped for resistance/susceptibility. Whereas in apple breeding for fire blight resistance relies mainly on wild species accessions, in pear donors have been selected from the cultivar collection. An important consideration in pome fruit breeding is the long juvenile phase especially if wild species are used as donors requiring several backcrosses to eliminate genetic drag. Thus, rapid cycle breeding, which could shorten the long-lasting breeding process, is tested for its usefulness in apple breeding. We will present the state of the art with respect to fire blight resistance breeding in apple and pear in Dresden-Pillnitz including results obtained from rapid cycle breeding.

Abstract - Poster presentation

Characterization of oxytetracycline resistance in *Erwinia amylovora* from commercial pear orchards in California

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Fire blight caused by *Erwinia amylovora* is a major disease of pome fruit crops in California. With low chilling conditions during tree dormancy, flowering is often protracted, resulting in a prolonged period of susceptibility to infection. Thus, multiple bactericide applications are needed during the spring season to manage the disease. Streptomycin (STR) and oxytetracycline (OXY) have been widely and very effectively used by California growers since the 1950s and 1970s, respectively. Resistance to STR was first reported in 1971 and has been an ongoing problem with high and moderate resistance levels among strains. We have been surveying orchards since 2005 and examining the sensitivity of *E. amylovora* isolates to OXY and other bactericides (e.g., STR and later kasugamycin). Resistance to OXY with minimum inhibitory concentrations of >40 µg/ml was found for the first time in *E. amylovora* isolates from two commercial pear orchards in 2018, and again in 2019 and 2020. At the location with the highest incidence of OXY resistance, nine applications of the antibiotic were done between 2017 and 2018. High dependency on one antibiotic in a two-year period may be responsible for the selection of the resistance detected. All strains highly resistant to OXY were also highly resistant to STR, were similar in virulence as a sensitive wildtype strain, and their fitness was confirmed in co-inoculations of pear flowers with sensitive and resistant strains. In small-scale studies, applications at labeled rates with OXY or STR showed little or no efficacy in reducing blight caused by strains highly resistant to both antibiotics. In whole genome sequencing of resistant strains, the *tet*A gene was found to be located on a 43.6-kb IncX plasmid that also contained the *tnp*A, *tnp*R, and IS*1133* sequences from Tn*5393*, as well as *str*A and *str*B that are responsible for STR resistance. This plasmid likely originated from a member of the Enterobacteriacae such as a *Pantoea* sp.

Abstract - Poster presentation

Proteins Involved in Siderophore Mediated Iron uptake in Erwinia amylovora

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Keywords: *Erwinia amylovora*, FhuD, Iron uptake, Siderophore, ViuB

Despite being the fourth most abundant element in the earth's crust, Iron is a limiting factor for survival of microorganisms due to its low bioavailability. In iron-deficient conditions, many microbes secrete low molecular weight metal-chelating compounds called siderophores. *Erwinia amylovora*, a causal agent of fire blight of rosaceous plants, also secretes hydroxamate type siderophore (Desferrioxamines), which facilitates indirect iron uptake and plays a significant role in pathogenesis. Siderophore-assisted iron uptake requires outer membrane receptors (FhuA and FoxR), periplasmic binding protein (FhuD), ABC cassette type receptor components (FhuB and FhuC), and siderophore utilization protein (ViuB). Among these, ViuB and FhuD are exclusively present in rosaceous infecting *Erwinia spp* and are the primary targets for the current project. These proteins have been mainly studied at the genetic level by gene mutations followed by analysis of the resulting phenotypes. However, we are the first research group using structural biology tools to study these proteins which could be the potential drug targets. ViuB was found soluble and purified successfully. The preliminary conditions for crystal growth were found. However, further optimization is ongoing to obtain high-quality crystals. FhuD being a periplasmic protein, remains insoluble when expressed in cytoplasm. A strategy to solubilize FhuD has been designed, using mutagenesis to insert signal peptides and subcloning in the vector used for periplasmic expression. Conclusively, solving 3D structures of these proteins could contribute to the discovery of sustainable chemical control against fire blight disease.

Abstract - Poster presentation

Exploring bacteria from Mediterranean settings as a source of potential biocontrol agents against *Erwinia amylovora*.

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Keywords: Fire blight, environmental bacteria, antagonistic activity, biocontrol

Erwinia amylovora, responsible for fire blight, causes major economic losses in pome fruit crops worldwide. Chemical control is not always effective and poses a serious threat to the environment and health. Social demands for eco-sustainable and safe control methods make it necessary to search for new biocontrol agents as those based on antagonists. A bacterial collection from different Mediterranean environmental sources free of fire blight was tested for antagonistic activity against Spanish and reference strains of *E. amylovora*. *In vitro* antagonistic assays were carried out on culture medium plates and *ex vivo* tests in immature loquat and pear fruits. Results revealed that 12% of 82 bacterial isolates tested were able to inhibit the growth of several strains of the pathogen. Some of the isolates also maintained their antagonistic activity even after chloroform inactivation. Selected isolates were further tested *ex vivo*, with several of them being able to delay and/or reduce fire blight symptom severity in both loquats and pears, and against some *E. amylovora* strains. The isolates showing the best antagonistic activity also produced different hydrolases linked to biocontrol (protease, lipase, amilase or DNAse) and were able to fix molecular nitrogen. Based on this additional characterization, four biocontrol strain candidates were further selected and identified by MALDI-TOF MS. Three of them were Gram-positive bacteria belonging to *Bacillus* and *Paenarthrobacter* genera, and the fourth one was a *Pseudomonas*.

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Abstract - Poster presentation

Isolation and characterization of *Erwinia amylovora* bacteriophages from Mediterranean environments with biocontrol potential

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Keywords: Fire blight, phages, lytic activity, biocontrol

Fire blight, caused by *Erwinia amylovora*, is a difficult disease to control due to the high dissemination and survival capacity of this bacterium and the lack of effective control methods. Bacteriophages (phages) may be an environmentally friendly and sustainable alternative, being necessary to search for phages adapted to Mediterranean environmental conditions. In this study, phages active against *E. amylovora* were isolated from plots in Mediterranean orchards and characterized. Phages were isolated from plant material, soil and water samples from plots where the pathogen was present. A collection of phages with lytic activity against Spanish and reference strains of *E. amylovora* was generated. Phage characterization was initiated by determining their host range, specificity and ability to control the pathogen *in vitro* and *ex vivo*. A selection of phages was able to specifically infect and lyse *E. amylovora* cells, significantly reducing the pathogen populations *in vitro*. *Ex vivo* tests on immature fruits revealed that applying some of the phages or their combinations delayed the onset of fire blight symptoms and reduced the disease severity, suggesting their biocontrol potential. Morphological and molecular characterization of the selected *E. amylovora* phages classified them as members of the family *Myoviridae*. These results open new perspectives for the biological control of fire blight in Mediterranean settings.

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Abstract - Poster presentation

A novel transcription factor CdeR regulates type III secretion system in a c-di-GMP-dependent manner

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Keywords: *Dickeya dadantii*, T3SS, diguanylate cyclase, c-di-GMP

The enterobacterium, *Dickeya dadantii,* is an opportunistic bacterial pathogen that causes disease in many plants. C-di-GMP is a ubiquitous bacterial second messenger, regulating multiple cellular behaviors through several c-di- GMP-associated components. Here, we identified a novel transcriptional regulator named CdeR that regulates T3SS in a c-di-GMP-dependent manner. GcpD is a diguanylate cyclase responsible for the synthesis of c-di-GMP. Compared to a *gcpD* mutant, the *gcpD* and *cdeR* double mutant exhibited a reduced T3SS expression. In addition, we found that, under the *gcpD* mutant background, CdeR regulates T3SS by manipulating intracellular c-di-GMP levels, involving an additional diguanylate cyclase GcpL upregulated by CdeR. This is the first report that uncovers CdeR as a transcriptional regulator involved in the regulation of T3SS. A model is proposed on how CdeR regulates T3SS expression by manipulating the c-di-GMP network.

Abstract - Poster presentation

Novel detection and quantification of *Erwinia amylovora* and *Erwinia pyrifoliae in planta* by Real-time PCR

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Keywords: *Erwinia amylovora*, *Erwinia pyrifoliae*, Detection, Real-time PCR

Fire blight, caused by *Erwinia amylovora*, is a destructive disease that attacks apple and pear trees worldwide. Black shoot blight, caused by *Erwinia pyrifoliae*, is less dangerous than the fire blight and usually emerges in South Korea. The government thoroughly controls the disease caused by these two pathogens to prevent their dispersion by swiftly removing the diseased plants. Therefore, rapid and precise detection of these pathogens is an essential factor for controlling fire blight and black shoot blight in South Korea. This study developed novel detection and quantification primers targeted for *E. amylovora* and *E. pyrifoliae*. The bacterial genome sequences were downloaded from NCBI GenBank and compared by real-time PCR data-mining algorithms to identify the pathogens' unique genes and primers. Regarding specificity tests of the primers, we performed with various *Erwinia* spp. and other closely related species. Sensitivity confirmation was performed under different concentrations of plasmid DNA, genomic DNA, and bacterial cell suspensions. Furthermore, a Bio-PCR assay, using a template from the plant extracts, was performed to detect pathogens directly from the diseased samples. These results demonstrated that these novel primers identify and quantify *E. amylovora* and *E. pyrifoliae*, even directly from plant samples, without DNA extraction. Our newly improved detection methods would be helpful for the quick removal of diseased plants, preventing the spread of fire blight and black shoot blight in Korea.

Abstract - Poster presentation

Effect of sterilant on *Erwinia amylovora* viability on secateurs

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Keywords: fire blight, sterilant, Dettol, *Erwinia amylovora*

Fire blight is caused by the bacteria *Erwinia amylovora.* The disease can spread through orchards and nurseries as a result of poor sanitation practices such as contaminated secateurs. This research investigated the efficacy of various commercial sterilants to kill *E. amylovora* on secateurs. To screen a wide range of sterilants for kill efficacy, secateurs were dipped into an *E. amylovora* inoculum and then sprayed with a test sterilant. After 10 s secateurs were swabbed and plated onto a CCT Agar plate, incubated at 26°C for 48 h and bacterial colonies counted. Sterilants were also assessed for cotton bleaching and metal corrosion. The best performing sterilants were then tested as described above using infected plant material containing sticky bacterial ooze as inoculum. Each of the sterilants tested (methylated spirits (95% and 70%), NaOCl (1%, 0.5%, 0.135%), Bac-stop/benzalkonium chloride (2%, 1%), Virkon® (label rate), Dettol™ (50%, 10%, 1%, 0.1%), and HarvestCide® gel (0.1%, 0.5%, 1%)) were found to be effective in killing *E. amylovora* on inoculum-coated secateurs. The best performing sterilants (methylated spirits, Dettol (active ingredient 4.8% w/v), HarvestCide gel, NaOCl) were also effective in killing *E. amylovora* on infected plant material when compared with the untreated control. Many of the best performing sterilants were likely to damage tools over time and cause bleaching on clothing. However, Dettol and methylated spirits did not cause metal corrosion or bleaching.

Abstract - Poster presentation

Fire blight resistance in flowers of pear accessions in New Zealand.

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Keywords: fire blight, disease resistance, *Pyrus*, control

The Plant & Food Research pear breeding programme in New Zealand is developing interspecific pear hybrids to suit pear industries globally. The major aim is to produce a new type of pear that is productive and has attractive, crisp, juicy, flavoursome fruit with long storage life. Since 2003, breeding lines were initiated to actively introgress multiple fire blight resistances from nine original progenitors. In 2021, twenty advanced selections and 18 commercial pear cultivars were assessed in the field for fire blight resistance through artificial floral inoculation with *Erwinia amylovora*.

The mean shoot lesion lengths of the commercial cultivars and advanced selections ranged from 0 to nearly 3m. Flowers of the pear cultivars ‘Moonglow’ and ‘Harovin Sundown’ (HW614) were infected multiple times, yet the infections did not travel beyond the peduncle into the bourse. In fact, floral infections on these cultivars appeared to abscise from the bourse and no cankers would develop. As a result, the mean lesion lengths of ‘Moonglow’ and ‘Harovin Sundown’ were 0 and 0.9 mm respectively.

There was a range of responses to infection in the advanced selections. Thirty percent of advanced selections showed evidence of floral abscission yet some still had large mean lesion lengths. Forty-five percent of advanced selections had a mean lesion length less than 3 cm whilst the remainder had lesion lengths between 6 and 300 cm long. One advanced selection in particular abscised many infected flowers and had a very small mean canker lesion length, which indicates it could be grown under high fire blight pressure. Results to date suggest that the resistance breeding strategy is making advances towards a fire blight resistant interspecific pear.

Abstract - Poster presentation

Zinc-dependent virulence regulation occurs through cyclic-di-GMP signaling in *Erwinia amylovora*

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Keywords: zinc, cyclic-di-GMP, type III secretion, transcriptional regulation

The bacterial second messenger cyclic-di-GMP (c-di-GMP) is a critical regulator of the phase transition from type III secretion system (T3SS)-dependent to biofilm-dependent disease progression in *Erwinia amylovora*. Changing extracellular zinc levels have been associated with c-di-GMP dependent transcriptional regulation of the *znuABC* operon involved in zinc uptake in *E. amylovora*. In this study, we examined if the levels of extracellular zinc could alter the intracellular levels of c-di-GMP in *E. amylovora*, and if these changes in c-di-GMP levels could be involved in downstream virulence regulation. Our results indicated that elevation of extracellular levels of zinc (Zn^{2+}) could trigger an increase in c-di-GMP generation within *E. amylovora* Ea1189 cells. Of the virulence factors examined, the major impact of this zinc-mediated increase in c-di-GMP levels was on the downregulation of the T3SS via a reduction in *hrpL* promoter activity in a zinc dose dependent manner. Through a transposon mutant screen, we identified that the zinc-dependent increase in c-di-GMP production was able to affect *hrpL* promoter activity through EAM_0627 (CdrZ), which encodes for a LysR-family transcriptional regulator. Our results indicate that CdrZ along with YajQ, its accessory protein, positively regulate the transcription of *hrpL* through a putative CdrZ binding box in the promoter region of *hrpL*. The elevated levels of c-di-GMP can alter the stability of the CdrZ/YajQ complex, thus impacting *hrpL* transcription. Our results highlight a new mode of transcriptional regulation of *hrpL* occurring in *E. amylovora* channeled through zinc sensing and c-di-GMP signaling.

Abstract - Poster presentation

Investigation on molecular mechanism of fire blight resistance protein FB_MR5 ortholog originated from a wild apple species *Malus baccata*

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Keywords: NLR, effector, AvrRpt2, FB_MR5

Erwinia amylovora is a bacterial pathogen infecting Rosaceae plants and causes fire blight. During infection, *E. amylovora* secretes effector proteins to dampen plant resistance response. AvrRpt2 is one of the effector proteins secreted by *E. amylovora* and cleaves apple RPM1-INTERACTING PROTEIN 4 (RIN4) into three AvrRpt2-cleavage products (ACP1~3). Previously, we showed that ACP3 activates apple nucleotide-binding leucine-rich repeat receptor (NLR), FB_MR5, and triggers immune responses. In this study, we identified a novel FB_MR5 homolog (MbMR5-Kor) by screening Korean wild apple species *Malus baccata*. Sequence alignment of FB_MR5 and MbMR5-Kor showed only eight amino acid substitutions between two NLRs. However, unlike FB_MR5, which requires ACP3 for its activation, MbMR5-Kor showed auto-activity when transiently expressed in *Nicotiana benthamiana*. Substitution of each amino acid on FB_MR5 showed that one amino acid change in N-terminal coiled-coil domain enables auto-activation of FB_MR5, while another amino acid substitution in nucleotide-binding domain has a minor effect on the gain of auto-activity. Interestingly, both apple RIN4 and ACP3 failed to suppress auto-activity of MbMR5-Kor. We are aiming to further investigate on a molecular basis for auto-activity of MbMR5-Kor and identify natural suppressor of MbMR5-Kor to understand evolution of apple NLR in wild apple species.

Abstract - Poster presentation

Investigation on apple resistance gene activation by *Erwinia amylovora* effector *avrRpt2*

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Keywords: FB_MR5, AvrRpt2, *Erwinia amylovora*, Fire blight

Fire blight is a bacterial disease caused by *Erwinia amylovora,* and it affects *Rosaceae* family. It causes severe economic loss. Therefore, studying about interaction of apple and *E. amylovora* is important to breed fire blight resistant apples.

E. amylovora secretes specialized proteins called effectors into the plant cells to suppress plant immune response. In response, resistant hosts have a surveillance mechanism to recognize the effectors. In apple, RPM1-INTERACTING PROTEIN4 (RIN4) is cleaved by AvrRpt2, an effector secreted by *E. amylovora.* AvrRpt2 cleaves apple RIN4 into three products which are ACP1, ACP2 and ACP3, and ACP3 is sufficient to activate a nucleotide-binding leucine-rich repeat receptor (NLR) called FB_MR5 from a wild apple, *Malus x robusta 5*.

To study the mechanism of AvrRpt2-triggered immunity by FB_MR5, we screened a wild apple from Korea called *Malus baccata* KW-JB-4 accession and found FB_MR5 homolog (FB_MR5^{KW}). FB_MR5^{KW} carries only eight amino acid variations compared to FB_MR5. Similar to FB_MR5, FB_MR5^{KW} triggered defense response when co-expressed with AvrRpt2 in *Nicotiana benthamiana*. However, further transient expression assay showed that ACP3 was not sufficient to activate FB_MR5^{KW}. Also, the transient expression of ACP1, ACP2, and ACP3 with FB_MR5 ^{KW} did not activate immune response. These results suggest that unlike FB_MR5, apple RIN4 cleavage products are not sufficient for activating FB_MR5^{KW}. In the future, we will study additional interaction of AvrRpt2 and host targets required to activate FB_MR5^{KW}.

Abstract - Poster presentation

Transformation of Korean apple cultivar 'Kamhong' using the *MR5* resistance gene of *Malus* × *robusta* 5

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Keywords: Fire blight, *MR*5, Transgenic plant

Fire blight disease, caused by *Erwinia amylovora*, firstly broke out at Korea in 2015, it is necessary to investigate potential spread of the invasive pathogen. The fire blight susceptible apple cultivar, Korean bred 'Kamhong' was transformed with the candidate fire blight resistance gene MR5 originating from the crab apple accession *Malus* × *robusta* 5. The *MR5* gene cloned into *Agrobacterium tumefaciens* strain LBA4404 harboring the vector pICH86988 carrying with CaMV 35S promoter and resistance to kanamycin as selective agent to obtain its transgenic plants. Five putative transgenic lines were positively tested on the presence of the *npt*Ⅱ marker gene and tested for transgene integration by Southern hybridization. A total of two different transgenic lines were obtained. Efficient regeneration and transformation system is a priority for successful application of genetic engineering to vegetative propagated plants such as apple. Phenotyping experiments of transgenic plants will be performed with virulent strains of *Erwinia amylovora*, the causal agent of fire blight.

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Abstract - Poster presentation

Pear fire blight resistance breeding research

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Keywords: pear, fire blight, resistance, cultivar screening, QTL-mapping

In Germany, demand for both conventional and organic pears cannot currently be met from domestic production. Pear sales are dominated by the varieties 'Alexander Lucas', 'Conference' ahead of 'Williams Christ', the 'Delicious of Charneux' and 'Clapps Favourite'. These cultivars, as well as most other pear cultivars, are highly susceptible to the regulated non-quarantine pest (RNQP) *Erwinia amylovora*, which is the causal agent of fire blight, the most important disease in pear production. Since no suitable control measures are available in pear cultivation, the cultivation of resistant varieties and the close and regular monitoring of pear plants and neighboring host plants are regarded as the best control options. Whereas in apple, intensive work is being done in various breeding programs to investigate fire blight resistance mechanisms and to breed resistant cultivars, efforts in pear are limited and available data are sparse. The project aims to contribute to improved effectiveness in breeding pear cultivars with resistance to fire blight, and to evaluate genetic resources in pear and *Pyrus* wild species to identify potential sources of resistance and make them available for breeding. The goals are to analyze possible additive effects of resistance QTL as well as to detect and test the efficacy of additional resistance loci, to use them in pear breeding using molecular markers, and to select pear genotypes with good resistance to fire blight. The development of molecular markers for effective resistance loci are the prerequisite for early selection in pear breeding for resistance to fire blight and accelerate the development of new resistant varieties.

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Project manager Federal Office for Agriculture and Food

Abstract - Poster presentation

Detection of fire blight in Almaty region adjacent to the distribution area of wild apples

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Keywords: fire blight, wild apple trees, *Malus sieversii,* epidemiology

Fire blight caused by *Erwinia amylovora* reached Kazakhstan in 2010. Here, the disease not only poses a threat to agricultural production of domesticated apples, but also to the wild forests of *Malus sieversii*, which is the progenitor of most apple varieties worldwide. In the previous three years, the spread of fire blight in the growth area of wild apples was limited by the weather conditions. In 2022, late spring and early summer were characterized by increased rainfall and moderate temperatures favorable for the disease. The goal of this study was to monitor the distribution of fire blight in private households and small orchards in the zones adjacent to wild apple distribution zones in Zailiyskiy Alatau. A total of 55 samples with fire blight–compatible symptoms was collected from cultural apples (51), pear (3) and quince (1) in the Almaty region, resulting in 21 isolates (one from pear and the rest from apple) of *E. amylovora* found in private gardens and small orchards. All isolates belonged to the archetypal CRISPR genotype A. Taking into account the relative proximity of the wild apple trees, additional measures for fire blight control and prevention in the growth areas of wild apples will have to be implemented, including state monitoring of the wild apple forests for fire blight symptoms and awareness campaigns for specially protected natural territories that safeguard *M. sieversii*, as well as for local pomaceous-fruit growing communities.

Abstract - Poster presentation

Priority effects in the apple flower determine if the siderophore desferrioxamine is a virulence factor for *Erwinia amylovora* CFBP1430

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Keywords: apple flowers, siderophore, secondary colonization, replication

Erwinia amylovora (EA), the causative agent of fire blight, produces desferroxamine (DFO) siderophores under iron-limiting conditions. The impact of DFO as a virulence factor during the epiphytic phase of *E. amylovora* CFBP1430 on apple flowers was reassessed. To this end, siderophore synthesis (DfoA) and uptake (FoxR receptor) mutants were constructed and tested in various flower assays.

In semi-sterile Golden Delicious (GD) flowers, the parental and mutant strains did not differ in replication, induction of calyx necrosis and weak induction of a foxR promoter-*gfp*mut2 reporter construct. In contrast, when inoculated onto GD flowers with an established microbiome, the FoxR mutant showed significantly lower replication than the parental strain.

The results suggest that apple flowers are not *per se* an iron limited environment for EA and that DFO is an important factor for the fire blight pathogen when apple flowers are pre-colonized.

Abstract - Poster presentation

Quantitative analysis of *Erwinia amylovora* population dynamics during apple shoot infection

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Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is one of the most devastating diseases of apple. The introduction of high-density orchard planting systems has heightened the need for active shoot blight management due to the deployment of susceptible apple cultivars and younger fruit-bearing trees. Young trees are particularly susceptible to the shoot blight phase of the disease, and rapid spread of *E. amylovora* from infected shoot tips throughout trees will necessitate tree removal. The aim of this research is to quantify and track *E. amylovora* migration through infected shoot tissues to gain insight into the systemic movement of the pathogen. Bacterial populations were monitored over 20-day periods in 2021 and 2022 in 'Gala' trees (4 yrs old in 2021) in the field. Each shoot was sampled at the shoot tip, end of the new growth, beginning of the 2nd year wood, and at the branch/trunk junction to assess *E. amylovora* population density. *E. amylovora* colonized the entire new growth of shoots sampled by 5 days post inoculation (dpi) and cells were consistently detected in the wood by 7 dpi. *E. amylovora* was able to maintain populations up to 10^9 CFU/g in the new growth over the sampling period. This research will aid in development of treatments for shoot blight and give us further insight of fire blight disease progression.

Abstract - Poster presentation

Isolation and identification of bacterial strains from apple flowers in Trentino and their evaluation as biocontrol agents of Erwinia amylovora

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Keywords: Fire blight, Microbiota, Biocontrol agents, Apple flowers

Fire blight caused by *Erwinia amylovora* (Ea) represents a great threat to apple and pear production worldwide. For instance, the outbreak of fire blight occurred in Trentino caused a relevant reduction of crop yield in 2020. Since Ea can spread rapidly in the environment, it is difficult to manage this devastating phytopathogenic bacterium. It is now widely accepted that apple flowers may harbor bacterial taxa that might hinder the ability of Ea to colonize apple flower. Based on this body of knowledge, we aimed at investigating the microbiota of apple flowers to select new potential biocontrol agents active against Ea. Flowers of *Malus domestica* cv. Golden Delicious from Trentino apple orchards were sampled at the 'Baloon stage' and surface sterilised to isolate only bacteria residing within the flowers. Bacterial isolates were initially selected on R2A dishes according to their colony morphology and subsequently identified through 16S rRNA gene sequencing. The phylogenetic analysis showed the bacterial isolates mainly belonged to the *Enterobacteriaceae*, *Pseudomonadaceae*, and *Microbacteriaceae* families. One member of each bacterial family was selected and tested against Ea both on newly open apple flowers and on pear slices. Preliminary results showed some of these strains might have a significant effect on the control of Ea. In particular, *Pantoea agglomerans* and *Curtobacterium flaccumfaciens* strains showed the highest efficacy. In the future, we will carry out further experiments to investigate and understand the modes of action of these bacterial strains.

Abstract - Poster presentation

Pathogenicity Related gene expression in the bark surrounding fire blight cankers on apple and Asian pear

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Keywords: *Erwinia amylovora*, PR genes, RT-PCR, fire blight resistance, irrigation

Erwinia amylovora infection of apple leaves and shoots induces the expression of pathogenicity-related *(PR*) genes. However, there is limited information about *PR* gene expression in other host species, perennial tissues, and the effect of tree irrigation on *PR* gene expression patterns. In our study, we assessed the expression levels of the genes *PR-1* (anti-fungal activity), *PR-2* (□-1,3-glucanase), *PR-5* (thaumatin-like protein) and *PR-8* (class III chitinase) genes in perennial bark adjoining cankers of apple and Asian pear cultivars with differing susceptibilities to fire blight. In both species, *PR-1, PR-2, PR-5* and *PR-8* showed an enhanced expression compared to the controls. In apple, the highest relative expression values were observed in *PR-5* and *PR-8* genes, but in Asian pear, the most expressed genes were *PR-1* and *PR-2*. Although these results were consistent among samples, expression level differences between the assayed *PR* genes were non-significant. In apple, we observed higher *PR* gene expression levels in a more resistant cultivar to fire blight. However, this trend was not observed in Asian pear. The expression of *PR* genes in the bark surrounding cankers was not affected by irrigation treatments, as irrigated and non-irrigated apple trees showed similar expression patterns. Our work shows for the first time the *E. amylovora*-triggered induction of *PR* genes in apple and Asian pear perennial tissues during canker formation/maturation.

Abstract - Poster presentation

Phenotypic and transcriptomic differences between copper sensitive and copper tolerant *Erwinia amylovora* strains

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Keywords: Fire blight, copper responses, RNA-Seq, virulence, exopolysaccharides, paraquat

Most *Erwinia amylovora* strains can grow on selective/differential media amended with copper, but a small proportion of strains show high sensitivity to the same copper concentrations. In this work, we compared different phenotypic traits and the transcriptomic responses to copper-shock and copper adaptation in a copper sensitive (EaR2) and a copper tolerant (Ea273) *E. amylovora* strains. Strain EaR2 showed lower virulence and higher sensitivity to paraquat than the type strain Ea273. Strain EaR2 additionally developed a quicker and more abundant exopolysaccharide secretion in the presence of low copper concentrations compared to Ea273. Further, RNA-seq analysis revealed a significant downregulation of genes linked to cell motility, chemotaxis and secretion systems in EaR2 in control conditions without copper, including virulence genes and/or genes related to the type three secretion system. The transcriptomic responses to copper shock in both strains involved upregulation of genes linked to the resistance to copper (*copA, cueO*), other heavy metals (*zntA*), and reactive oxygen species (ROS) (*aHP, katG, soxS, ybaK*). However, Ea273 overexpressed additional genes linked to ROS detoxification (*trxC, grxA*) which were not induced in EaR2. The upregulation of *copA* and *cueO* was also the primary response during copper adaptation in both strains. However, EaR2 also upregulated amylovoran biosynthesis genes and modulated the expression of hundreds of genes unrelated to copper resistance. Our findings shed light on the molecular mechanisms of copper tolerance in *E. amylovora,* which might contribute to improving the application of copper for fire blight management in the field.

Abstract - Poster presentation

Detection of novel pear genetic resources resistant to *Erwinia amylovora* strain TS3125 and YKB14808

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Keywords: Asian pear, CenSall, Magness, Moonglow

Outbreak of the fire blight on Asian pear in Korea was reported in 2015. Though antibiotics provide effective control for fire blight, they are not allowed increasingly due to ecological considerations and emergence of antibiotic resistant strains. Planting resistant cultivar is regarded as the promising strategy for fire blight control. We had screened 93 cultivars following artificial inoculation of leaf-cutting method with mixed inoculum of Korean strain TS3125 and YKB14808 to find resistant resources. According to the results, 90 cultivars in our germplasm collection displayed susceptible phenotype, while 3 cultivars displayed resistant phenotype. These findings were inconsistent with previous studies. Known resistant cultivars, including 'Harrow delight', 'Old home', 'Hosui', 'Shinko', 'Jing Bai Li' and 'Xue Hua Li', did not display resistance to *E. amylovora* strain TS3125 and YKB14808. However, 'Magness', 'Moonglow' and 'CenSall' remained as the resistant. These support the pathogenic differences among European and Korean *E. amylovora* strains. Specific breeding programs for each countries or regions should be operated by considering their own environments.

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Characterization of oxytetracycline resistance in *Erwinia amylovora* from commercial pear orchards in California

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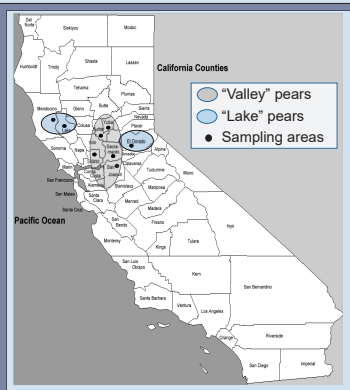
Introduction

Blighted young fruit after flower infection | Shoot blight | Blighted flower on rattail bloom | Branch canker

Fire blight caused by *Erwinia amylovora* is a major disease of pome fruit crops worldwide. With low chilling conditions during tree dormancy in California, flowering is often protracted (i.e., 'rattail bloom'). This results in a prolonged period of susceptibility to infection, and multiple bactericide applications need to be done to manage the disease.

Oxytetracycline (OXY) and streptomycin (STR) have been widely and effectively used in the United States for the management of fire blight since the 1950s and 1970s, respectively. Resistance to STR was first reported in California in 1971 and has been an ongoing problem. Resistance to OXY has not been reported previously in *E. amylovora*.

Surveys on antibiotic resistance in *E. amylovora* in California



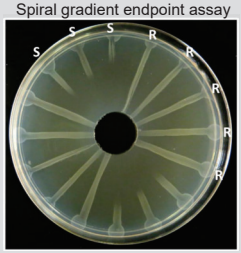
- Strains of *E. amylovora* were obtained from pear orchards in two major California growing areas.
- Surveys were done annually since 2005.
- Sensitivities to OXA and STR were determined using the spiral gradient endpoint and direct dilution assays.

E. amylovora strains highly resistant to OXY detected

Surveys in 2018-2020:

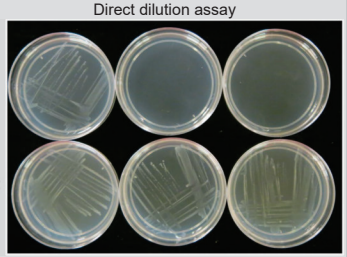
- In 2018, **resistance to OXY with MICs of >40 µg/ml** was detected in two orchards (6 of 7 strains or 1 of 8 strains, respectively). Resistance in these and in an additional orchard was also present in 2019. **Thus, these resistant strains persisted**. Resistance also detected in 2020.
- All strains highly resistant to OXY were **also highly resistant to STR**.

Spiral gradient endpoint assay



OXY concentration gradient from 0.02 (edge of plate) to 4 µg/ml (center of plate). Resistant (R) strains are not inhibited at 4 µg/ml).

Direct dilution assay

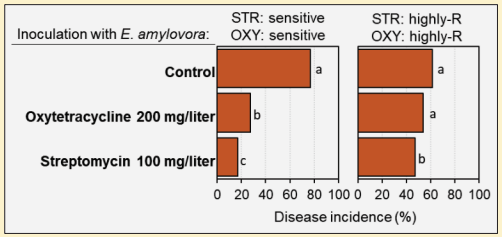


Growth of resistant strain not inhibited at 40 µg/ml OXY.

Efficacy of OXY and STR against fire blight caused by strains highly resistant to OXY and STR

Ornamental pear flowers were treated in the laboratory, allowed to air-dry, and inoculated with *E. amylovora*.

- **OXY and STR showed little or no efficacy in reducing blight caused by *E. amylovora* strains highly resistant to both antibiotics.**



Virulence and competitiveness of OXY-resistant strains

- Pear flowers were inoculated with single strains or 1:1 mixtures of sensitive and resistant strains.
- The incidence of infected flowers was determined after 7 days.
- *E. amylovora* was re-isolated from diseased flowers, and sensitivity to OXY was determined.

Inoculation with	Incidence of flower infections (%)
STR^S/OXY^S	56.1 a
STR^R/OXY^R -1	62.1 a
STR^R/OXY^R -2	47.1 a

Two representative STR^R/OXY^R strains are similar in virulence as a sensitive strain.

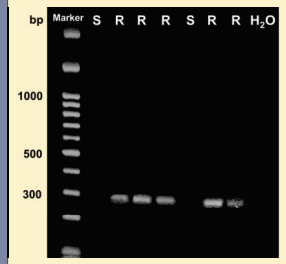
Inoculation with		Recovery of resistant phenotype (%)
Single strains	STR^S/OXY^S	0
	STR^R/OXY^R -1	94.5
	STR^R/OXY^R -2	100
Mixtures	STR^S/OXY^S + STR^R/OXY^R -1	61.5
	STR^S/OXY^S + STR^R/OXY^R -2	63.8

STR^R/OXY^R strains are competitive in the presence of sensitive strains with >60% recovery.

*-We acknowledge the California pear industry for financially supporting this research.

Molecular mechanism of OXY resistance

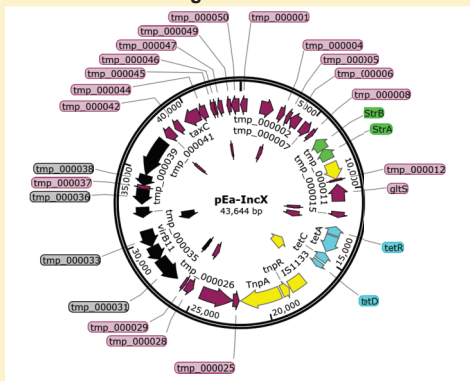
Identification of OXY resistance genes



Amplification using degenerate primers Tet1 and Tet2 with a 55ºC annealing temperature (Jones et al. 1999)

- A fragment of tetracycline resistance genes *tetA*, *tetB*, *tetC* with the expected length of 293 bp was amplified for OXY-resistant strains.
- **Sequencing of the amplicon resulted in 98% homology with a *tet* gene from *Pantoea agglomerans* and other members of the Enterobacteriacae.**

Location of the *tet*A gene



*Tet*A was found to be located on a 43.6-kb IncX plasmid that also contains the *tnp*A, *tnp*R, and IS*1133* sequences from Tn*5393*, as well as *str*A and *str*B that are responsible for streptomycin resistance.

Summary

- **High resistance to OXY in *E. amylovora* was detected for the first time.**
- **All OXY-resistant strains were also highly resistant to STR.**
- **Labeled rates of OXY and STR had little or no effect in reducing disease caused by resistant strains.**
- **STR^R/OXY^R strains had similar virulence as a sensitive strain, and they were competitive in the presence of sensitive strains**
- **At the location with the highest incidence of OXY resistance, nine applications of the antibiotic were done between 2017 and 2018.**
- **High dependency on one antibiotic in a two-year period may be responsible for the selection of the resistance detected.**
- **OXY resistance in *E. amylovora* was found to be due to the presence of a *tet*A gene that is located on an IncX plasmid.**

Proteins involved in siderophore mediated iron uptake in *Erwinia amylovora*

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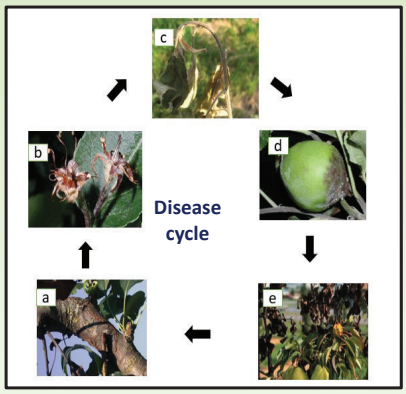
1. Background

Erwinia amylovora,

- A causal agent of fire blight disease in rosaceous plants including apple and pear.
- Caused outbreaks in apple orchards in South Tyrol, a region contributes up to 10 % of total apple production in Europe.
- No specific-sustainable curative control measures available so far, so is a great concern for commercial apple cultivation.
- Understanding of virulence factors at structural level could contribute to the discovery of sustainable chemical control against fire blight.
- The main virulence factors are Exopolysaccharides, T3SS and Siderophore mediated iron uptake.
- Siderophore are low molecular weight compounds secreted by microorganism in iron deficient conditions.

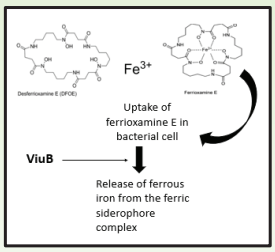


Fire blight disease cycle (a) perennial canker (b) symptoms on flowers (c) shoot tip with typical curvature (d)bacterial ooze on fruits (d) symptoms on trees

***E. amylovora* secretes hydroxamate type siderophore.**

Uptake of iron loaded siderophore requires

- Outer membrane receptors (FhuA and FoxR)
- periplasmic binding protein (FhuD)
- ABC cassette type receptor components (FhuB and FhuC)
- Siderophore utilizing protein ViuB (an oxidoreductase)



2. Research gap

- *fhuD* and *sidE* are exclusively present in Rosaceae-infecting strains (polsinelli et al., 2019).
- These targets are important due to their potential druggability.
- 3D structure have not been experimentally solved yet.

3. Objective

- **Structural and functional characterization of FhuD and ViuB using x-ray crystallography and enzymatic assays.**

4. Methods

Cloning, transformation and expression

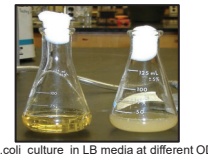
- LIC in vector pMCSG49
- Transformation in E.coli Shuffle, E.coli BL21 (DE3), and BL21 Star™ (DE3)pLysS
- Overexpression

Purification

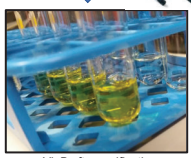
- Immobilized metal affinity Chromatography (IMAC) using Ni column
- Size Exclusion Chromatography with Superdex 75 10/300 GL

Crystallization

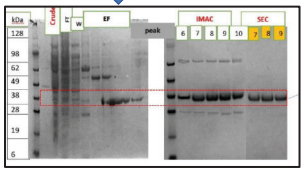
- Microbatch underoil crystallization trials were performed keeping homologues proteins from PDB as references.
- Further optimization is ongoing



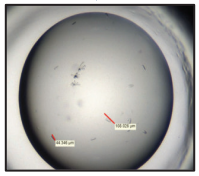
E.coli culture in LB media at different OD



ViuB after purification



SDS PAGE depicting proteins before and after IMAC and SEC

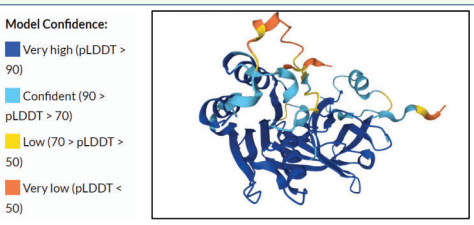


5. Preliminary Results

- *sid E* which encodes protein ViuB overexpressed successfully in all the E. coli strains used.
- However, E. coli Shuffle yields the best
- ViuB was found soluble while FhuD remains in pellet fraction.

- Protein was eluted at 190mM Imidazole in IMAC and at 11.5 ml in SEC.
- FAD as a cofactor gives protein a yellow coloration.

- Small crystal growth has been observed with the following conditions
- 0.005 M Na Acet (additive), 0.1M MES pH 6.5 (buffer), 10%w/v PEG 8K (precipitant), 1mM TEW
- 0.01 M MgCL2 (Additives), 0.1 M NaCl (Salt), 1mM TEW (Additive)
- These conditions may need further optimization to get high quality crystals.



Predicted structure of ViuB, Source: Alphafold

6. Conclusion and future perspectives

- 3D structure of target proteins will provide clear insights about molecular mechanism of siderophore mediated iron uptake and release of iron from the siderophore iron complex.
- This information could eventually be useful in rational drug design against the fire blight disease.

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EXPLORING BACTERIA FROM MEDITERRANEAN SETTINGS AS A SOURCE OF POTENTIAL BIOCONTROL AGENTS AGAINST *Erwinia amylovora*

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INTRODUCTION

Erwinia amylovora, responsible for fire blight, causes major economic losses in pome fruit crops worldwide. Chemical control is not always effective and poses a serious threat to the environment and health. Social demands for eco-sustainable and safe control methods make it necessary to search for new biocontrol agents such as those based on antagonists.

Aim:

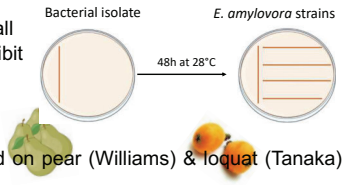
To select and characterize bacteria from Mediterranean environments as a source of potential biocontrol agents against *E. amylovora*

MATERIALS & METHODS

- **82 environmental bacterial isolates** (soil, water, rhizosphere)
- ***E. amylovora*** (CFBP 1430, IVIA 1554, IVIA 1614.2, IVIA 1892.1)

1. *In vitro* antagonist activity

Bacterial isolates were tested against all *E. amylovora* strains. Those ableto inhibit pathogen growth were also assayed after chloroform inactivation.



2. *Ex vivo* antagonist activity

The selected isolates were also tested on pear (Williams) & loquat (Tanaka) inmature fruits.

3. Activities related to biocontrol or plant growth promotion:

a. Exoenzymatic activities (amylase, cellulase, Dnase, lipase protease).
b. Nitrogen-fixing activity.

4. Molecular identification of best candidates

Bacterial isolates with the best antagonistic potencial where identified through MALDI-TOF/MS.

RESULTS & DISCUSSION

1. *In vitro* antagonist activity

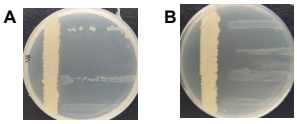


Fig. 1. Representative images of an active (A) and inactivated (B) antagonist against different *E. amylovora* strains.

Table 1. Results of *in vitro* antagonistic activity against *E. amylovora*. Candidates were tested before and after chloroform inactivation.

Bacterial isolate	Status	*E. amylovora* strains			
		CFBP 1430	IVIA 1554	IVIA 1614.2	IVIA 1892.1
9	Active	-	+	+	++
	Inactive	-	+	+	++
12	Active	-	+++	++	+
	Inactive	-	-	-	-
17	Active	+	+++	++	+
	Inactive	-	+++	+	-
18	Active	++	+++	+++	+++
	Inactive	++	+++	+++	+++
19	Active	++	+++	+	+++
	Inactive	+	+++	+	++
20	Active	+	+++	++	++
	Inactive	-	++	+	+
30	Active	-	+++	++	++
	Inactive	-	-	-	-
41	Active	++	++	++	++
	Inactive	++	++	++	++
59	Active	-	-	++	+
	Inactive	-	-	++	+
60	Active	-	-	++	-
	Inactive	-	-	++	-

(-): without antagonist activity; (+): low antagonistic activity; (++): medium antagonist activity; (+++): strong antagonistic activity

- 12% of 82 bacterial isolates tested were able to inhibit the growth of at least one strain of the pathogen.
- Some of the isolates also maintained their antagonistic activity even after chloroform inactivation.

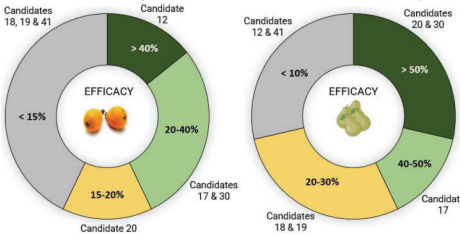
Seven candidates (12, 17, 18, 19, 20, 30, 41) were selected based on their ability to strongly inhibit two or more *E. amylovora* strains.

2. *Ex vivo* antagonist activity

Fig. 2. Efficacy of inhibition of pathogen infection on inmature fruits (loquat & pear).

On loquats:
12 > 17 > 30 > 20

On pears:
20 > 30 > 17 > 18 > 19



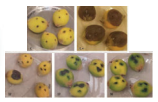


Fig. 3. Symptomatology in loquat at end time.

Fig. 4. Severity of pathogen infection on inmature fruits (loquat & pear).

On loquats:
30 > 12 > 17 > 20

On pears:
30 > 17 > 20 > 18 > 19

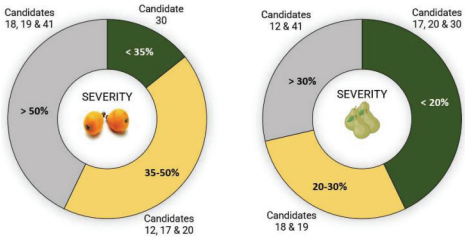


Fig. 5. Symptomatology in pear at end time.

Several of selected isolates were able to delay and/or reduce fire blight symptoms severity in both loquats and pears, and against some *E. amylovora* strains.

3. Activities related to biocontrol or plant growth promotion:

Fig. 6. Representative images of exoenzymatic and growth-promoting activities tested. **A**. Amylase; **B**. Cellulase; **C**. DNase; **D**. Lipase; **E**. Protease; **F**. Nitrogen-fixing activity.

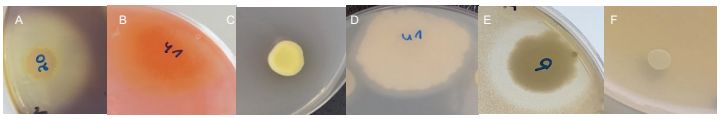


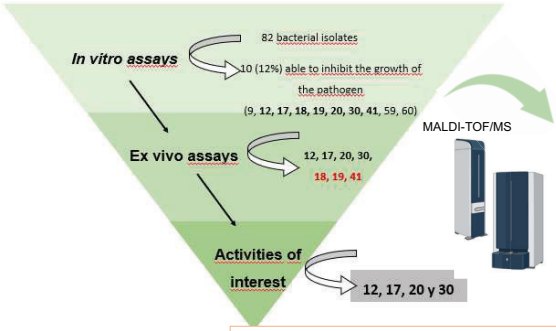
Table 2. Results of exoenzymatic and nitrogen-fixing activities in selected candidates.

Bacterial isolate	Exoenzymatic activity							N_2-fixing activity	
	Protease		Lipase		Amylase	Cellulase	DNase		
	48 h	96 h	48 h	96 h	96 h	96 h	96 h	48 h	96 h
12	++ [a]	++	-	++	-	-	+	+ [b]	+
17	-	++	-	-	+++	-	+	+	+
18	++	+++	-	-	+++	-	+	-	+
19	++	+++	-	-	+++	-	+	-	+
20	-	++	-	-	+++	-	-	+	+
30	-	-	-	++	-	-	-	+	+
41	-	++	+	+++	+++	+++	-	-	-

a (-): without activity; (+): weak activity; (++): moderate activity; (+++): strong activity. b (+): bacterial growth indicative of nitrogen-fixing activity; (-): absence of growth and activity.

- All candidates show at least one activity of interest.
- Candidates 18 & 19 fix N_2 more slowly than the rest.
- Candidate 41 is cellulase positive and unable to fix N_2

4. Molecular identification of best candidates.



- Candidates 12, 17 & 20 were initially identified as gram-positive bacteria belonging to *Bacillus simplex*, *Paenarthrobacter aurescens and P. sp., respectively*.
- Candidate 30 was initially identified as *Pseudomonas moraviensis*.
- **All three genera include bacteria previously described in environmental samples - such as soil - and with biocontrol potential**

CONCLUSIONS

- ✓ *In vitro* antagonistic activity against *E. amylovora* was demonstrated in selected bacterial isolates through nutrient competition and, in some cases, also by the production of antimicrobial compounds.
- ✓ The isolates showing the best *ex vivo* antagonism to *E. amylovora* also produced different hydrolases linked to biocontrol (protease, lipase, amilase or DNAse) and were able to fix molecular nitrogen.
- ✓ Selected antagonistis could provide or complement new environmentally friendly biocontrol methods against fire blight.

FUNDING

Project RTA2015-00087-C02 funded by MCIN/AEI/10.13039/501100011033, INIA and ERDF "A way of doing Europe".

GOBIERNO DE ESPAÑA — MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

ISOLATION AND CHARACTERIZATION OF *Erwinia amylovora* BACTERIOPHAGES FROM MEDITERRANEAN ENVIRONMENTS WITH BIOCONTROL POTENTIAL

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INTRODUCTION

Fire blight, caused by *Erwinia amylovora*, is a difficult disease to control due to the high dissemination and survival capacity of this bacterium and the lack of effective control methods. Bacteriophages (phages) may be an environmentally friendly and sustainable alternative, being necessary to search for phages adapted to Mediterranean environmental conditions.

Aim:
To isolate and characterize phages active against *E. amylovora* from plots in Mediterranean orchards.

MATERIALS & METHODS

Samples (plant material, soil and water from plots with *E. amylovora*)
Phages Isolation (direct/enriched using *E. amylovora*)
Biological characterization:
- Host range (15 Spanish and 4 international strains from different host plants and geographical origins) & specificity
- Ability to control the pathogen by *in vitro* and *ex vivo* (on immature loquats).

Morphological characterization: TEM
Molecular characterization:
- Phage genome sequencing (Illumina)
- Genomic annotation (RASTtk)
- Phylogenetic analysis (VICTOR)



RESULTS & DISCUSSION

1. Phage Isolation

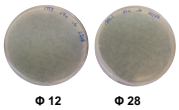


Fig. 1.1. Plaques of lytic phages 12 & 28 on lawns of *E. amylovora* on NA.

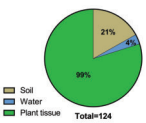
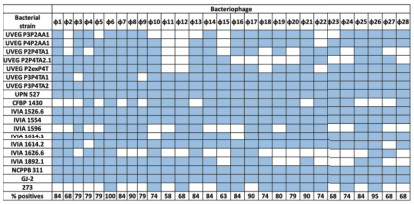


Fig. 1.2. Graph showing the ratio of phages isolated from different Mediterranean environmental samples

2. Phage Biological Characterization

2.1. Host range

Fig. 2.1. Heat-map showing the host range of each isolated phage.



White, no sensitive *E. amylovora* strain; blue, sensitive *E. amylovora* strain.

2.2. Specificity: Lytic activity was negative to strains of other bacterial species, the main genera tested being *Agrobacterium, Bacillus, Escherichia, Micrococcus, Pseudomonas, Ralstonia and Serratia.*

Most of the selected phages were isolated from plant material, showing lytic activity, a wide host range and specificity for *E. amylovora*.

2.2. *In vitro E. amylovora* biocontrol assays

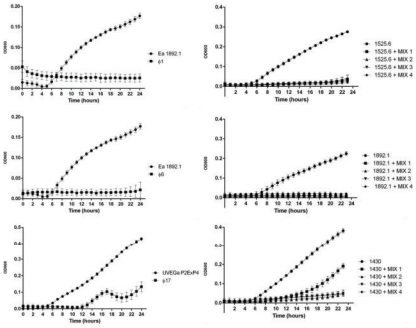


Fig. 2.2. Dynamics of representative single phages (left) or phage cocktails (1-4) (right) on *E. amylovora*. Mean values of triplicate assays plus Standard error.

Some individual phages or phage cocktails were able to significantly reduce pathogen populations *in vitro*.

2.3. *Ex vivo E. amylovora* biocontrol assays

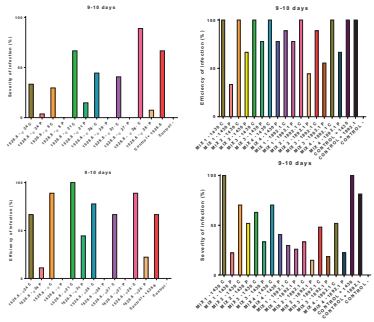


Fig. 2.3. Efficiency (top) and severity (bottom) of infection produced by *E. amylovora* on immature loquat fruits treated with individual phages (left) or phage cocktails (1-4) (right) by co-inoculation (C) or prevention (P) assays. Mean data from triplicate experiments with 3 fruits per condition.

Preventive application and phage cocktails are more effective than co-inoculation and use of single phages.

3. Phage Morphological & Molecular Characterization

3.1. TEM of selected *E. amylovora* phages

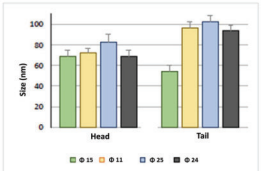


Fig. 3.1. A. Mean size (nm) of representative phage capsids and tails. Lines represent the SD.

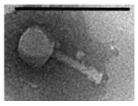


Fig. 3.1. B. Virion morphology of phage 24. Scale bar 200 nm.

3.2. Annotated genome map of one representative *E. amylovora* phage

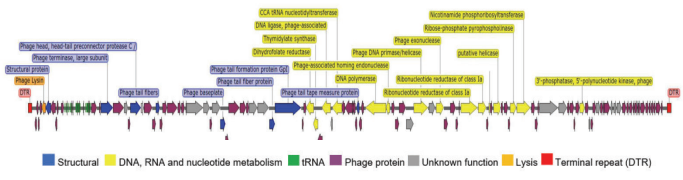
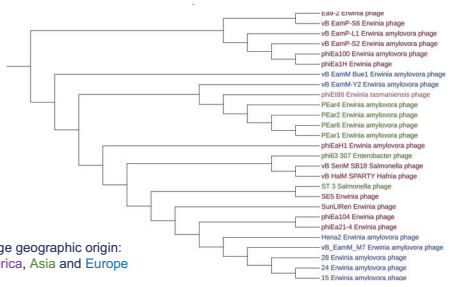


Fig. 3.2. Gene organization of phage 24. Arrows represent ORFs identified in the genome and their length, the gene size. Colors indicate the functional module to which they belong.

Morphological characterization of the selected phages classified them as myovirus.

The genome of phage 24 is organised into distinct functional modules with necessary genes for the development of lytic cycle and its replication.

3.3. Phylogenetic tree of selected *E. amylovora* Mediterranean phages



Phage geographic origin:
America, Asia and Europe

Fig. 3.2. Genomic phylogenetic tree with VICTOR of selected Mediterranean *E. amylovora* phages and the nearest phages.

Phylogenetic analysis indicates that the four *E. amylovora* phages belong to the same species as the phage vB_Eam_MM7, within genus *Kolesnikvirus*

CONCLUSIONS

- ✓ **Most of the selected Mediterranean phages showed lytic activity against *E. amylovora*, being specific and having broad host range. Some of them or their combinations were able to control the pathogen *in vitro*.**
- ✓ **Biocontrol assays on immature loquats fruits with single selected phages or their combinations delayed the onset of fire blight symptoms and reduced disease severity, with preventive application and phage cocktails being more effective than co-inoculation and single phages.**
- ✓ **Morphological characterization of the selected *E. amylovora* phages classified them as members of the family *Myoviridae* .**
- ✓ **Genomic and phylogenetic analyses revealed that the selected *E. amylovora* phages are highly related to the reference phage vB_Eam_MM7, forming a monophyletic group within the genus *Kolesnikvirus*.**

FUNDING

Project RTA2015-00087-C02 funded by MCIN/AEI/10.13039/501100011033, INIA and ERDF "A way of doing Europe".

A novel transcription factor CdeR regulates type III secretion system in a c-di-GMP-dependent manner

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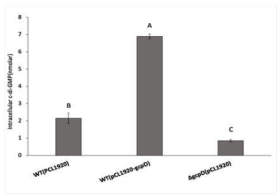
ABSTRACTS

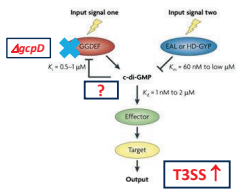
The enterobacterium, *Dickeya dadantii,* is an opportunistic bacterial pathogen that causes disease in many plants. c-di-GMP is a ubiquitous bacterial second messenger, regulating multiple cellular behaviors through several c-di- GMP-associated components. Here, we identified a novel transcriptional regulator named CdeR that regulates T3SS in a c-di-GMP-dependent manner. GcpD is a diguanylate cyclase responsible for the synthesis of c-di-GMP. Compared to a *gcpD* mutant, the *gcpD* and *cdeR* double mutant exhibited a reduced T3SS expression. In addition, we found that, under the *gcpD* mutant background, CdeR regulates T3SS by manipulating intracellular c-di-GMP levels, involving an additional diguanylate cyclase GcpL upregulated by CdeR. This is the first report that uncovers CdeR as a transcriptional regulator involved in the regulation of T3SS. A model is proposed on how CdeR regulates T3SS expression by manipulating the c-di-GMP network.

RESULTS

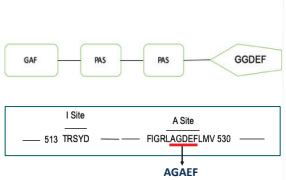
I. Regulatory role of GGDEF domain protein (GcpD) in *Dickeya dadantii*

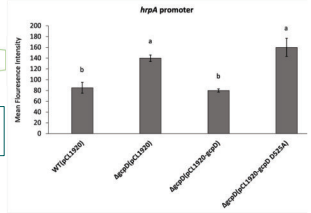
GcpD synthesizes bis-(3',5')-cyclic dimeric guanosine monophosphate (c-di-GMP)



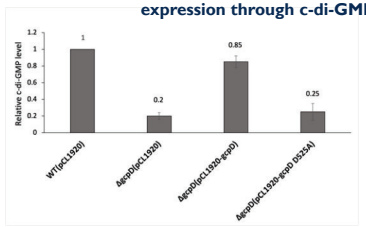


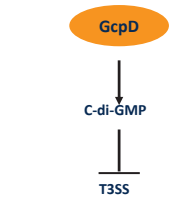
The DGC activity of GcpD is essential for the T3SS expression





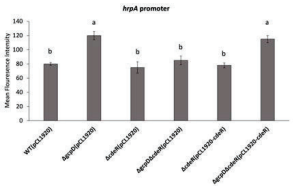
A-site motif in GcpD is essential for its DGC activity to regulate T3SS expression through c-di-GMP signaling

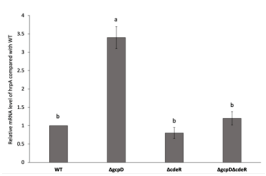




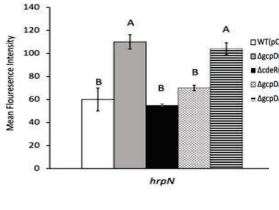
II. Identify the role of the transcriptional regulator CdeR on T3SS expression

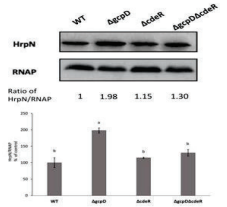
Knock out of *cdeR* under *ΔgcpD*, decreased promoter activity and mRNA level of *hrpA*. However, deletion of *cdeR* in wild type showed no regulatory effect on T3SS expression



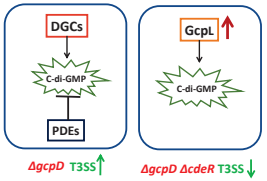


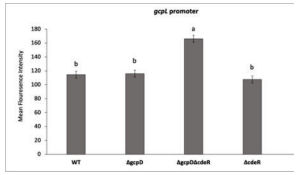
Knock out of *cdeR* under low c-di-GMP background (*ΔgcpD*) decreased the promoter activity and protein expression of *hrpN*



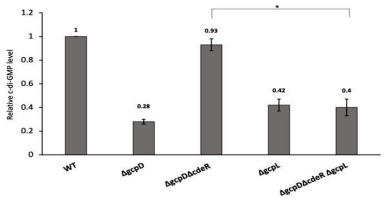


Deletion of *cdeR* in *gcpD* mutant background upregulated *gcpL*

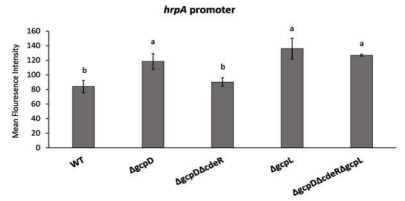




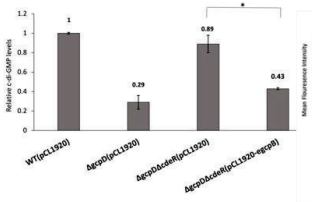
c-di-GMP level in *ΔgcpDΔcdeRΔgcpL* restored to the *ΔgcpL* level

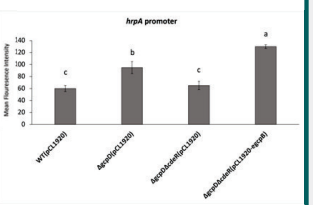


Deletion of *gcpL* under *ΔgcpDΔcdeR* recovered the *hrpA* promoter activity to *ΔgcpL* level

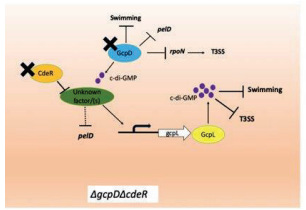


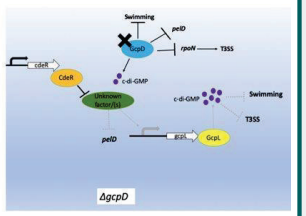
The impact of overexpression of PDE in c-di-GMP level and T3SS expression





Proposed mechanism of how CdeR regulates T3SS in *D. dadantii*.





SUMMARY

- We have shown GcpD, which contains a conserved A- site, is a genuine DGC. Moreover, our findings demonstrated that GcpD relies on its DGC activity to regulate T3SS expression.
- We found that under low c-di-GMP levels(*ΔgcpD*) , CdeR controls T3SS expression through GcpL.
- Upregulation of *gcpL* overrides the lower c-di-GMP level caused by the deletion of *gcpD* in the *cdeR* mutant and results in the phenotypes of *hrpA* expression

ACKNOWLEDGEMENTS

This work is dedicated to Prof. Noel T. Keen. We thank Vy Huyen Ton Nu Bao for making the poster and insightful discussion.

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National Institute of Agricultural Sciences

60th Anniversary Rural Development Administration

Novel detection and quantification of *Erwinia amylovora* and *Erwinia pyrifoliae* in planta by Real-time PCR

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Abstracts

Fire blight, caused by *Erwinia amylovora*, is a destructive disease that attacks apple and pear trees worldwide. Black shoot blight, caused by *Erwinia pyrifoliae*, is less dangerous than fire blight and usually emerges in South Korea. The government thoroughly controls the disease caused by these two pathogens to prevent their dispersion by swiftly removing the diseased plants. Therefore, rapid and precise detection of these pathogens is essential for controlling fire blight and black shoot blight in South Korea. This study developed novel detection and quantification primers for *E. amylovora* and *E. pyrifoliae*. The bacterial genome sequences were downloaded from NCBI GenBank and compared by real-time PCR data-mining algorithms to identify the pathogens' unique genes and primers. Regarding specificity tests of the primers, we performed with various *Erwinia* spp. and other closely related species. Sensitivity confirmation was performed under different concentrations of plasmid DNA, genomic DNA, and bacterial cell suspensions. Furthermore, a Bio-PCR assay, using a template from the plant extracts, was performed to detect pathogens directly from the diseased samples. These results demonstrated that these novel primers identify and quantify *E. amylovora* and *E. pyrifoliae*, even directly from plant samples, without DNA extraction. Our newly improved detection methods would be helpful for the quick removal of diseased plants, preventing the spread of fire blight and black shoot blight in Korea.

Material & Methods

In silico-based species-specific gene selection and primer design

Primers specific for *E. amylovora* and *E. pyrifoliae* were designed by pipeline of Lang et al. (2010) with slight modifications.

Specificity tests via conventional PCR

Target bacterial species and other closely related species were used as a template for the primer specificity tests.
PCR was performed in a final reaction volume of 25 µL containing 0.2 mM dNTPs, 0.1 µM of each primers, 25 ng/µL of template DNA, and 1.25 U of GoTaq® Flexi Polymerase (Promega, Madison, WI, USA). PCR conditions: denaturation at 95℃ for 5 min, followed by 35 cycles of denaturation at 95℃ for 60 s, annealing at 65℃ for 60 s, and extension at 72℃ for 60 s, and a final extension at 72℃ for 10 min.

Sensitivity tests via real-time PCR

Genomic DNA, cloned DNA, and bacterial suspension were serially diluted 10-fold to use for the template.
Lesions of diseased apple and pear leaves were surface sterilized, cut by 5 × 5 mm size, extracted at 500 µl of distilled water, diluted 10-fold and used for the template of Bio-PCR assay.
Each real-time PCR reaction contained 12.5 µl of 2X Direct qRCR premix (Nanohelix, Korea), 5 pmol of each primer, and 1 µl of purified genomic DNA (5 ng/µl), cloned DNA (5 ng/µl), or bacterial suspension (9.0 × 10^6 CFU/ml) from each sample. Real-time PCR conditions: 95°C for 3 min; 40 cycles of 95°C for 10 s and 60°C for 20 s; and the melting curve was assessed from 65°C to 95°C in increments of 0.5°C. Standard curves were generated by plotting the cycle threshold (Ct) values.

PCR primers and condition

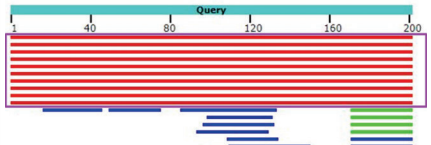
Target	Primer	Oligonucleotide sequence	Annealing
E. amylovora	RS19160-201F	5′-CTG TTT CAA ATT TGG CGA TGT A-3′	60℃
	RS19160-201R	5′-AAG GTG TTT TGT GCT GTT TGT AT-3′	
E. pyrifoliae	RS14185-320F	5′-GGG GCT ACA AGT CGC AAA GAT A-3′	
	RS14185-320R	5′-TAA GCC CAT TCG AAC CCA GAC-3′	

Commercial Detection Kit

Results & Conclusion

In silico assay evaluation

(A) *Erwinia amylovora*



(B) *Erwinia pyrifoliae*

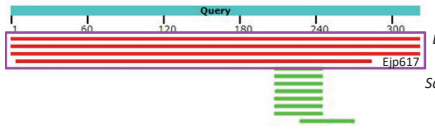
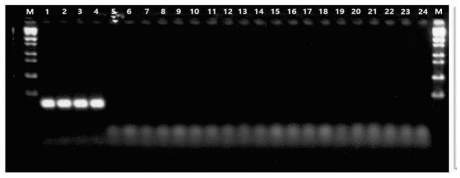


Fig. 1. Distribution of the BLASTn hits of target genes from NCBI. A, *Erwinia amylovora*. B, *Erwinia pyrifoliae*.

Specificity tests via conventional PCR

(A) *Erwinia amylovora*

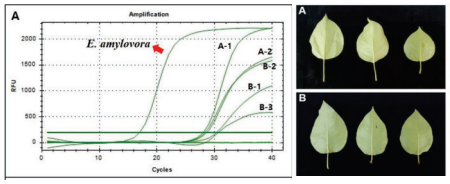


(B) *Erwinia pyrifoliae*

Fig. 2. Specificity test of developed specific primers. Lane M, size marker (1 kb ladder). A, Lanes 1–4, *E. amylovora* strains; lanes 5–23, *Erwinia pyrifoliae*, *Pectobacterium*, *Dickeya*, and *Pantoea* strains; lane 24, negative control (distilled water). B, Lanes 1 and 6, *Erwinia pyrifoliae* strains; lanes 7 to 23, *Erwinia*, *Pectobacterium*, *Dickeya*, and *Pantoea* species; lane 24, negative control.

Bio-PCR assay via real-time PCR

(A) *Erwinia amylovora*



(B) *Erwinia pyrifoliae*

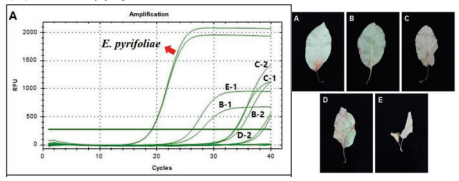


Fig. 3. Real-time PCR assay of diseased leaves. A, *Erwinia amylovora*. B, *Erwinia pyrifoliae*.

Sensitivity tests via real-time PCR

Table 1. *Erwinia amylovora* TS3128 threshold cycle (Ct) of 10-fold dilution series by real-time PCR with RS19160-201 primer set

Cloned DNA		Genomic DNA		Cell suspension	
Weight/µl reaction mix	Ct ± SD (n=3)	Weight/µl reaction mix	Ct ± SD (n=3)	CFU/ml reaction mix	Ct ± SD (n=3)
5ng	10.94 ± 0.12	5ng	23.98 ± 0.06	1.9×10^8	25.99 ± 0.14
500pg	13.13 ± 0.10	500pg	27.86 ± 0.03	1.9×10^7	29.24 ± 0.02
50pg	16.33 ± 0.10	50pg	31.44 ± 0.07	1.9×10^6	32.44 ± 0.05
5pg	19.62 ± 0.11	5pg	35.10 ± 0.08*	1.9×10^5	35.73 ± 0.07*
500fg	23.18 ± 0.08	500fg	38.38 ± 0.15*	1.9×10^4	38.58 ± 0.31*
50fg	26.61 ± 0.10	50fg	N.D.	1.9×10^3	N.D.
5fg	30.04 ± 0.07	5fg	N.D.	1.9×10^2	N.D.
500ag	33.39 ± 0.09	500ag	N.D.	1.9×10^1	N.D.

N.D.: Not Determined; * Not approved

Table 2. *Erwinia pyrifoliae* YKB12327 threshold cycle (Ct) of 10-fold dilution series by real-time PCR with RS14185-320 primer set

Cloned DNA		Genomic DNA		Cell suspension	
Weight/µl reaction mix	Ct ± SD (n=3)	Weight/µl reaction mix	Ct ± SD (n=3)	CFU/ml reaction mix	Ct ± SD (n=3)
5ng	13.90 ± 0.09	5ng	25.11 ± 0.16	1.6×10^8	24.79 ± 0.10
500pg	16.76 ± 0.18	500pg	29.30 ± 0.16	1.6×10^7	28.14 ± 0.08
50pg	19.60 ± 0.11	50pg	33.18 ± 0.14	1.6×10^6	31.53 ± 0.12
5pg	23.18 ± 0.31	5pg	37.11 ± 0.21*	1.6×10^5	35.17 ± 0.22*
500fg	26.93 ± 0.13	500fg	N.D.	1.6×10^4	38.41 ± 0.08*
50fg	30.69 ± 0.37	50fg	N.D.	1.6×10^3	N.D.
5fg	34.33 ± 0.11	5fg	N.D.	1.6×10^2	N.D.
500ag	38.12 ± 0.21*	500ag	N.D.	1.6×10^1	N.D.

N.D.: Not Determined; * Not approved

Conclusion

- RS19160-201 and RS14185-320 primers can specifically amplify the target bacteria. None of the other closely related bacteria that tested here were amplified.
- RS19160-201 and RS14185-320 primers were sensitive enough to detect target bacteria, even from plant extracts without DNA extraction.

Reference

Lang, J. M. *et al*. 2010. Genomics-based diagnostic marker development for *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. Plant Dis. 94:311-319.

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Effect of sterilant on *Erwinia amylovora* viability on secateurs

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Introduction

Fire blight, caused by *Erwinia amylovora*, is difficult to control and once established can only be removed by pruning out infections. Pruning tools used to cut out fire blight strikes potentially transfer bacteria between trees. Use of a suitable sterilant to kill the bacteria would reduce transmission through pruning. The efficacy of various commercial products to kill *E. amylovora* on secateurs and the potential for clothing bleaching and metal corrosion were tested in a series of trials over 3 years.

Methods

Sterilant efficacy

Sterile secateurs were dipped into a beaker containing *E. amylovora* inoculum (1 x 10^6 cfu/mL in phosphate buffered saline solution, pH 7.2) for 4 s, or cut through bacterial ooze on a fire blight infected pear shoot (Figure 1A). The secateurs were removed from the inoculum, shaken to remove excess, then one of the sterilant treatments (Table 1) was applied using a spray bottle until the secateur blades were coated. After 10 s, the secateur's blade edge and anvil were swabbed with a sterile cotton swab (Figure 1B), and placed into 1.160 mL sterile distilled water. A dilution series was made from the washings. A 0.1 mL aliquot of each dilution was spread onto King's B agar, incubated at 26°C for 2–3 days and bacterial colonies counted. Each sterilant treatment was replicated six times.

Bleaching properties

Colour bleaching of fabric was determined by applying 0.1 mL of each sterilant onto a black woven square of cotton fabric. Bleaching rankings were: 0 = none, 1 = mild, 2 = moderate, 3= major, 4 = extreme.

Metal corrosion

Corrosivity of each sterilant was assessed using stainless steel blades. Six blades were placed in each of the trial sterilant baths for 15 min to facilitate enough corrosion to compare between treatments. The blades were then drained, blotted dry and left exposed to the atmosphere for 4 days. Blades were scored on amount of corrosion. Corrosiveness rankings were: 0 = nil corrosion, 1 = mild, 2 = moderate, 3 = high, 4 = extreme.

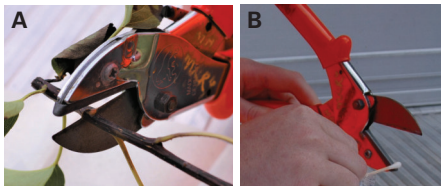


Figure 1: (A) Secateurs cutting through bacterial ooze on a fire blight infected pear shoot. (B) swabbing secateur blade to sample for live bacteria after sterilant treatment.

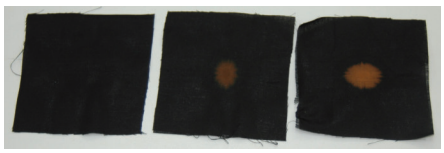


Figure 2: Bleaching of cotton cloth from sodium hypochlorite (NaOCl): untreated control (left), 0.5% NaOCl (middle), 1% NaOCl (right).

Acknowledgements

This work was funded by New Zealand Apples and Pears Inc. and the New Zealand Sustainable Food and Fibre Futures Fund.

Results

Sterilant efficacy using *E. amylovora* suspension as inoculum

Most of the trialled sterilants appeared effective in Years 1 and 2 (Table 1).

Bleaching properties

Sodium hypochlorite was found to visibly bleach fabric within 30 min of exposure (Table 1). The 1% solution exhibited higher bleaching than the 0.5% solution (Figure 2). HarvestCide® gel caused a very slight almost negligible bleaching. No other sterilant treatments caused damage to cloth fabric, suggesting that there would not be any issue of orchard worker clothing damage.

Corrosion of tools by sterilants

Bac-Stop, HarvestCide gel (Figure 3) and sodium hypochlorite caused corrosion of metal blades. No other products tested caused significant degradation of metal.

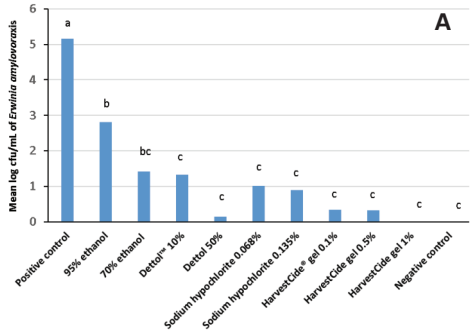
Sterilant efficacy using infected plant material as an inoculum

The sterilants that performed well in the kill test, corrosivity test, and bleaching test were re-evaluated using bacterial ooze from an infected plant as an inoculum. All re-evaluated sterilants significantly reduced the number of bacteria in swabs ($p<0.001$) compared with the untreated control (Figures 4A and B), although some treatments were far better than others.

Table 1: Effect of various test sterilants on *Erwinia amylovora* population on secateurs, metal corrosiveness and bleaching of cotton. Corrosive rankings are 0–4, where 0 = nil corrosion, 1 = mild, 2 = moderate, 3 = high, 4 = extreme. Cotton bleaching rankings are 0–4, where 0 = nil, 1= mild, 2 = moderate, 3 = high, 4 = extreme bleaching. '-' = not tested

Sterilant	Concentration of sterilant's active ingredient	Mean log cfu of Erwinia amylovora	Mean corrosive ranking	Mean cotton bleach ranking
No washing with sterilant	-	6.23	-	-
Sterile distilled water	-	5.45	0	0
Methylated spirits	95%	0.67	0	0
	80%	0	-	-
	70%	1.4	0.66	0
Sodium hypochlorite	1%	0.09	4	4
	0.5%	0	3	1.67
	0.135%	0	3	0
	0.068%	0	2	0
Bac-Stop (4° amine; benzalkonium chloride)	2%	0	3	0
	1%	0	3	0
Virkon™	1%	0	4	0
Dettol™ (Chloroxylenol 48mg/mL)	50%	0	0	0
	10%	0	0	0
	5%	1.6	-	-
	1%	3.3	-	-
	0.5%	3.33	-	-
	0.1%	4.4	-	-
HarvestCide® gel (1-Bromo-3-chloro-5,5-dimethylhydantoin 30-40% w/w)	1%	0	4	2.6
	0.5%	0.333	3	1
	0.1%	0.099	1.7	0
Lemon oil	10%	3.5	-	-
	5%	6.6	-	-
	2%	7.53	-	-
	1%	6.88	-	-
Mānuka oil	10%	6	-	-
	1%	8.3	-	-
	0.1%	7	-	-
Pine oil	10%	7.6	-	-
	1%	8	-	-
	0.1%	7.8	-	-
Aussan	0.3%	4	-	-
Cetrimide	2%	0	-	-
	1%	1.06	-	-
	0.5%	1.7	-	-
Colgate® Total	100%	2	-	-

Figure 3: Corrosion caused by 1% HarvestCide® gel.



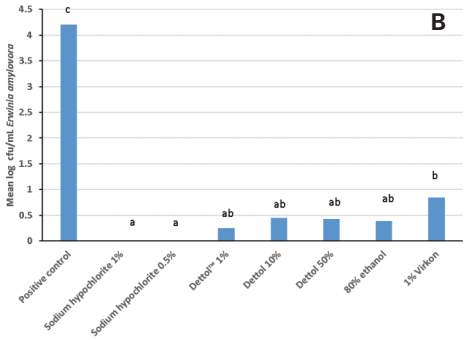


Figure 4: Mean *Erwinia amylovora* colony forming units (cfu)/mL that grew from a 100 µL washing off secateurs used to cut through fresh fire blight cankers on pear shoots and subsequently sprayed with one of the test sterilants. Different groupings of letters indicate significant differences between treatments based on the Fisher's least significant difference (LSD) method and 95% confidence. A = Year 1, B = Year 2.

Conclusions

- 1% sodium hypochlorite gave good bacterial kill. However, it is very damaging to pruning tools and causes bleaching of fabric.
- HarvestCide gel (1%) gave 100% bacterial kill when pruning sticky fire blight infected shoots and caused little or no bleaching to clothing. However, it caused corrosion to metal. Additionally, it would not be an acceptable sterilant for use in organic orchards.
- 80% methylated spirits proved a good sterilant. However, it is highly flammable posing a health and safety risk, especially where workers smoke. Also, it evaporates easily, potentially leading to concentration decline during use.
- Dettol™ substantially reduced bacterial populations though it did not quite reach a 100% kill with sticky bacterial plant material. It was easy to work with, did not bleach fabric, was not flammable and didn't corrode metal tools.

ESA_09/2022_CBO-1283

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Fire blight resistance in flowers of pear accessions in New Zealand

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Introduction

The New Zealand-based Plant & Food Research pear breeding programme is developing interspecific pear hybrids for the global pear industry. The major aim is to produce a new type of pear that is productive and has attractive, crisp, juicy, flavoursome fruit with long storage life. Since 2003, we have been actively introgressing multiple fire blight resistances from nine original progenitors into breeding lines. In 2021, 20 advanced selections and 18 commercial pear cultivars were assessed in the field for fire blight resistance through artificial floral inoculation with *Erwinia amylovora*.

Methods

Ten floral clusters per tree containing freshly opened flowers were tagged, inoculated with *Erwinia amylovora* (ICMP 236) [International Collection of Micro-organisms from Plants (ICMP), Manaaki Whenua – Landcare Research New Zealand Ltd] at a concentration of 1 x 10^6 cfu/mL in phosphate buffer, pH 7.2 and covered in foil bags (Figure 1) for 5 days.

After bag removal, the floral clusters were assessed for fire blight infection 5, 7, 10, 15, 20, 25, 30, 35 days post inoculation and also monitored for abscission of infected flowers.

Infection was recorded on a 0–4 scale where 0 = no infection, 1 = infected flower, 2 = infected flower and peduncle, 3 = infected flower, peduncle and bourse leaves, and 4 = entire floral cluster infected, including the bourse shoot.

Branch lesion lengths were recorded after 14–18 weeks.

Figure 1: Floral clusters on pear tree artificially inoculated with *Erwinia amylovora* and bagged for 2–3 days in foil bags.

Figure 2A: Fire blight-infected 'Harovin Sundown' fruitlet showing delimitation of infection prior to abscission. B. Fruiting bourse showing where infected flowers have abscised from the bourse, preventing infection travelling through to other fruit and into the branch.

Figure 3A: Fire blight-infected 'Moonglow' pear flowers showing delimitation of infection prior to floral abscission. B. Infected flowers abscised from bourse, hence preventing infection travelling through to other fruit.

Results

The mean shoot lesion lengths of all genotypes ranged from 0 to nearly 3 m.

Flowers of the pear cultivars 'Moonglow' and 'Harovin Sundown' (HW614) were infected multiple times, yet the infections did not travel beyond the peduncle into the bourse (Figures 2 and 3). In fact, infected flowers on these cultivars appeared to abscise from the bourse and no cankers developed. As a result, the mean lesion lengths of 'Moonglow' and 'Harovin Sundown' were 0 and 0.9 mm, respectively.

In contrast, once flowers from other commercial cultivars, such as 'Doyenné du Comice', became infected, this infection rapidly spread from the infected bourse, forming large lesions and sometimes killing entire branches.

A range of responses to infection were observed in the advanced selections. Thirty percent of genotypes showed evidence of floral abscission (Figures 4 and 5), yet some still occasionally had large mean lesion lengths. Forty-five percent had a mean lesion length less than 3 cm, the remainder had total shoot lesion lengths between 6 and 300 cm long.

One genotype in particular abscised many infected flowers and had a very small mean canker lesion length, indicating it could tolerate high fire blight disease pressure.

Figure 4: Advanced selection showing inhibited floral infection, confined to the ovary and upper peduncle. B. Bourse from an advanced selection showing peduncle scars where the infected flower abscised from the bourse, ensuring fire blight infection would not travel into the bourse.

Figure 5: Pear advanced selection showing a scar from a floral abscission.

Conclusion

Results to date suggest that the resistance breeding strategy is making advances in developing a fire blight-resistant, interspecific pear.

Investigation on molecular mechanism of fire blight resistance protein FB_MR5 ortholog originated from a wild apple species *Malus baccata*

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Summary

- We newly identified MbMR5-Kor by screening FB_MR5 orthologs in Korean wild apple species, *Malus baccata*.
- We observed that MbMR5-Kor induces cell death without apple RIN4 when transiently expressed in *Nicotiana benthamiana*.
- We found that A54V substitution on the CC domain is responsible for MbMR5-Kor auto-activity.
- We showed that apple RIN4 cleavage product 3 activates FB_MR5 but not V54A mutant MbMR5-Kor.

Results

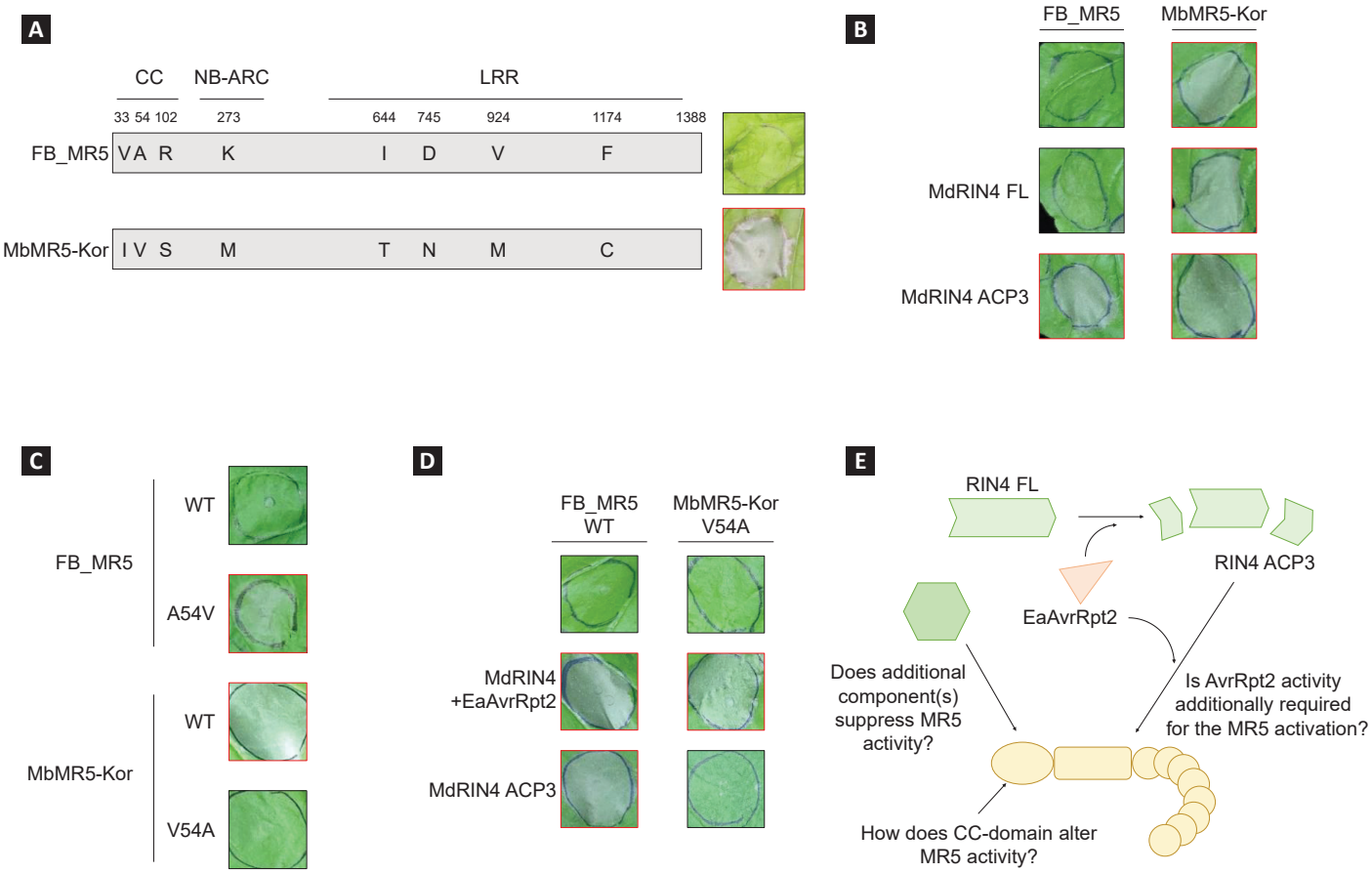

Figure.

A. MbMR5-Kor carries eight polymorphisms compared to FB_MR5 and induces cell death without apple RIN4 cleavage products.

B. MbMR5-Kor-induced cell death cannot be suppressed by apple RIN4.

C. Substitution of single amino acid residue on CC domain is sufficient for gain or loss of MbMR5-Kor-induced cell death.

D. Apple RIN4 cleavage product 3 activates FB_MR5 but not V54A substituted MbMR5-Kor.

E. Schematic representation of current questions for further research.

Indicated genes were transiently expressed by *Agrobacterium* infiltration in *ptr1*-silenced *N. benthamiana* to avoid AvrRpt2-triggered cell death. A black or red border indicates the absence or presence of cell death, respectively. MdRIN4 refers to apple RIN4-1 (*Vogt et al.*, 2013). ACP3 indicates AvrRpt2-cleavage product 3 and corresponds to apple RIN4 amino acid 181-239.

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Acknowledgements

This research was carried out with the support of Cooperative Research Program for Agricultural Science and Technology Development (PJ0153012021) Rural Development Administration, Republic of Korea. Also, this research was supported by the BK21 funded by the Ministry of Education, Republic of Korea (4120200313623)

POSTECH POHANG UNIVERSITY OF SCIENCE AND TECHNOLOGY

농촌진흥청 Rural Development Administration

Investigation on apple resistance gene activation by *Erwinia amylovora* effector *avrRpt2*

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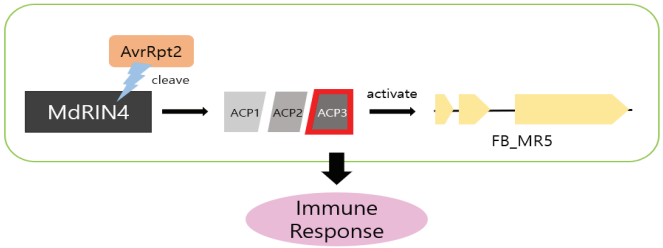
Abstract

Fire blight is a bacterial disease caused by *Erwinia amylovora,* and it affects *Rosaceae* family. It causes severe economic loss. Therefore, studying about interaction of apple and *E. amylovora* is important to breed fire blight resistant apples. *E. amylovora* secretes specialized proteins called effectors into the plant cells to suppress plant immune response. In response, resistant hosts have a surveillance mechanism to recognize the effectors. In apple, RPM1-INTERACTING PROTEIN4 (RIN4) is cleaved by AvrRpt2, an effector secreted by *E. amylovora.* AvrRpt2 cleaves apple RIN4 into three products which are ACP1, ACP2 and ACP3, and ACP3 is sufficient to activate a nucleotide-binding leucine-rich repeat receptor (NLR) called FB_MR5 from a wild apple, *Malus x robusta 5*.

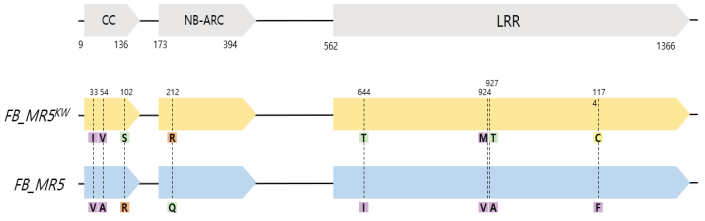
To study the mechanism of AvrRpt2-triggered immunity by FB_MR5, we screened a wild apple from Korea called *Malus baccata* KW-JB-4 accession and found FB_MR5 homolog (FB_MR5^{KW}). FB_MR5^{KW} carries only eight amino acid variations compared to FB_MR5. Similar to FB_MR5, FB_MR5^{KW} triggered defense response when co-expressed with AvrRpt2 in *Nicotiana benthamiana*. However, further transient expression assay showed that ACP3 was not sufficient to activate FB_MR5^{KW}. Also, the transient expression of ACP1, ACP2, and ACP3 with FB_MR5 ^{KW} did not activate immune response. These results suggest that unlike FB_MR5, apple RIN4 cleavage products are not sufficient for activating FB_MR5^{KW}. In the future, we will study additional interaction of AvrRpt2 and host targets required to activate FB_MR5^{KW}.

Background



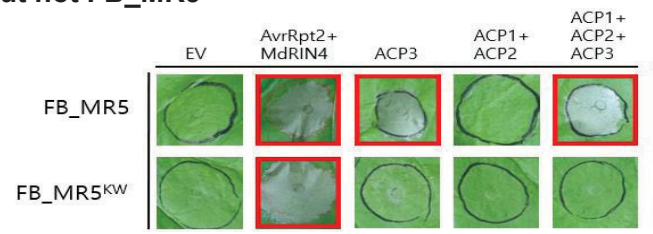
Ea effector AvrRpt2 is a cysteine protease and it can cleave apple RIN4 protein into three products, ACP1, ACP2 and ACP3. NLR protein, FB_ MR5 can be activated by ACP3, leading to immune responses.

FB_MR5^{KW} carries eight amino acid variations compared to FB_MR5



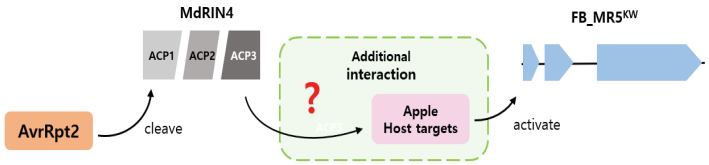
There are only eight amino acid variations between *Malus baccata* KW-JB-4 accession from Korea and *Malus robusta* 5. Different amino acids between two FB_MR5 sequences are denoted.

RIN4 cleavage product, ACP3 can activate FB_MR5 but not FB_MR5^{KW}



FB_MR5 and FB_MR5^{KW} triggered immune response when co-expressed with AvrRpt2 and apple RIN4 in *Nicotiana benthamiana (Nb)*. Agrobacterium-mediated transient expression assay showed that apple RIN4 cleavage product, ACP3 can trigger defense response when co-expressed with FB_MR5. However ACP3 cannot activate FB_MR5^{KW}. Also, ACP1, ACP2 and ACP3 with FB_MR5 can trigger immune response but three RIN4 cleavage products cannot activate FB_MR5. Red border indicates the programmed cell death.

Further study



We will study additional reaction of AvrRpt2 and its host target to activate FB_MR5^{KW}.

Conclusion

- Korean wild *Malus baccata* has full length FB_MR5 gene, FB_MR5^{KW} and it shows sequence polymorphism.
- Apple RIN4 cleavage product, ACP3 is not sufficient to activate FB_MR5^{KW}.

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- Cooperative Research Program for Agricultural Science and Technology Development (PJ0153012021) Rural Development Administration Republic of Korea

Transformation of Korean apple cultivar 'Kamhong' using the *MR5* resistance gene of *Malus* × *robusta* 5

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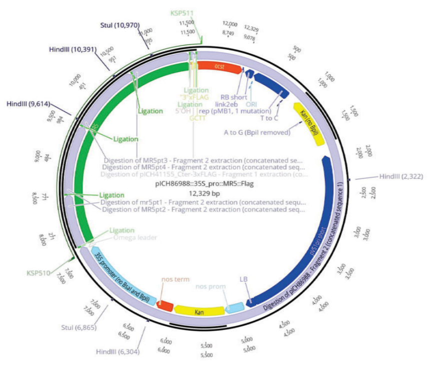
Abstract

Fire blight disease, caused by *Erwinia amylovora*, firstly broke out at Korea in 2015, it is necessary to investigate potential spread of the invasive pathogen. The fire blight susceptible apple cultivar, Korean bred 'Kamhong' was transformed with the candidate fire blight resistance gene *MR5* originating from the crab apple accession *Malus* × *robusta* 5. The *MR5* gene cloned into *Agrobacterium tumefaciens* strain LBA4404 harboring the vector pICH86988 carrying with CaMV 35S promoter and resistance to kanamycin as selective agent to obtain its transgenic plants. Five putative transgenic lines were positively tested on the presence of the *npt*Ⅱ marker gene and tested for transgene integration by Southern hybridization. A total of two different transgenic lines were obtained. Efficient regeneration and transformation system is a priority for successful application of genetic engineering to vegetative propagated plants such as apple. Phenotyping experiments of transgenic plants will be performed with virulent strains of *Erwinia amylovora*, the causal agent of fire blight.

Material and Methods

Plant material
Leaf material used for all transformation and regeneration studies was from *in vitro* cultures of the apple cultivar 'Fuji', 'Hongro', 'Kamhong'.

***Agrobacterium* strain and plasmids**
- LBA4404
- pICH86988 vector



***Agrobacterium* transformation**
- *Agrobacterium* cultures were grown overnight at 28 ℃ and used for infection at an optical density reading of approximately 0.8 at 600nm.
- Leaf discs were placed adaxial side up, *Agrobacterium* co-cultured for 3days in the dark at 25℃ on MS medium supplemented with IBA 0.1mg/L, TDZ 5g/L, sorbitol 30g/L, Daishin agar 7g/L.
- Transferred to dishes containing appropriate selection and regeneration media, cefotaxime inhibit bacterial growth and enhance shoot regeneration.
- All selection and regeneration took place at 25℃ in the dark for 4 weeks.

Acknowledgments

This work was carried out with the support of the National Institute of Horticultural & Herbal Science (PJ01530103) Rural Development Administration.

Results

Table 1. Formation of callus rate and shoot regeneration rate from leaf explants of the apple cultivar 'Fuji', 'Hongro', 'Kamhong'.

Apple cultivar	Formation of callus(%)	Formation of shoots(%)
Fuji	71.7±1.2	0.18
Hongro	41.7±0.6	0.21
Kamhong	91.7±1.1	0.62

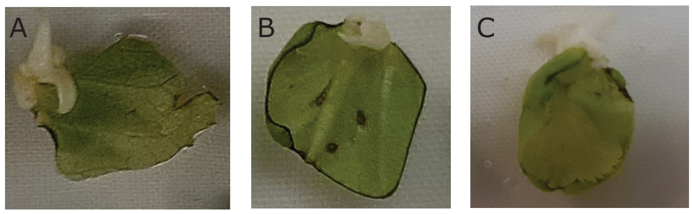


Fig. 1. Shoot regeneration from leaf explants of the apple cultivar on petri dishes. Adventitious shoot on MS medium supplemented with IBA 0.1mg/L, TDZ 5mg/L, sorbitol 30g/L, Daishin agar 7g/L. (A) 'Fuji', (B) 'Hongro', (C) 'Kamhong'.

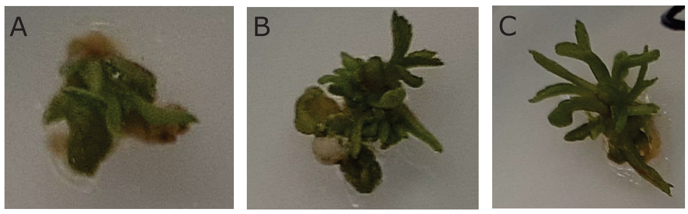


Fig. 2. Induction of adventitious shoots from leaf explants of the apple cultivar. Adventitious shoot on MS medium supplemented with BA 1mg/L, IBA 0.3mg/L, GA_3 0.5mg/L, sucrose 30g/L, plant agar 8g/L. (A) 'Fuji', (B) 'Hongro', (C) 'Kamhong'.

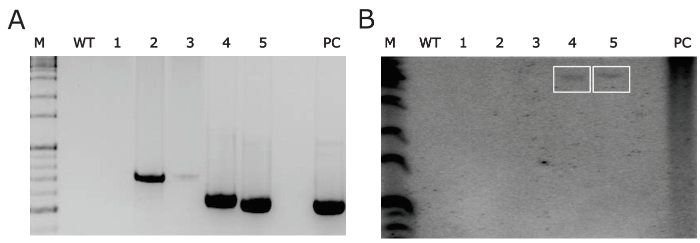


Figure 3. Molecular analysis of transgenic plants. (A) Genomic DNA from *Malus domestica* cv. 'Kamhong' wild type (WT) and transgenic apple 1, 2, 3, 4 and 5 plants, PCR amplified using 5' -forward and 3' -reverse *NPT*II gene primers. PC: positive control. (B) Southern-blot analysis of genomic DNA digested with *Hind*Ⅲ.

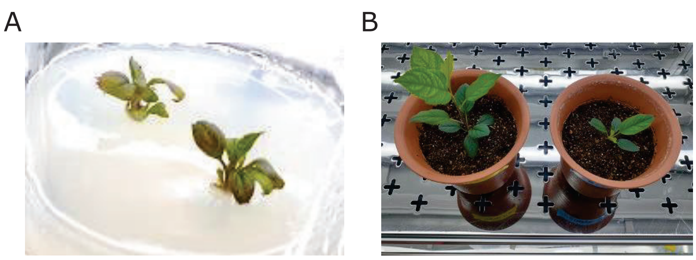


Figure 4. Seedling of transgenic apple 'Kamhong' plants in which gene introduction was confirmed as a result of Southern analysis. (A) Transgenic apple 'Kamhong' plants incubated on medium. (B) Transgenic apple 'Kamhong' plants being acclimatized in soil.

Pear fire blight resistance breeding research

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Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen
Federal Research Centre for Cultivated Plants

The project aims to contribute to improved effectiveness in breeding pear cultivars with resistance to fire blight, and to evaluate genetic resources in pear and *Pyrus* wild species to identify potential sources of resistance and make them available for breeding.

Aims:

- Evaluation of pear genetic resources - Identification of donors for fire blight resistance
- Create new segregating populations
- Establishment of dense genetic maps for 'Harrow Sweet' and 'Conference'
- Mapping of fire blight resistance
- Analysis of epistatic effects
- Narrowing QTL intervals by identifying recombinants
- Development of tightly linked molecular markers

Phenotyping

- 131 pear cultivars of the pear collection of JKI have been phenotyped (Fig. 1)
- 'Diels Butterbirne', 'Römische Schmalzbirne' and 'Petersbirne' have been identified as robust or less susceptible to fire blight and used for crosses
- 186 progenies from a cross of 'Conference' × 'Harrow Sweet' have been phenotyped (Fig. 2). The mean and median PLL are 37.6 % and 30.3 %.

class	PLL
resistant	0
robust	>0 - < 10
less susceptible	10 - < 25
mid susceptible	20 - < 50
suceptible	50 - < 75
highly susceptible	>= 75

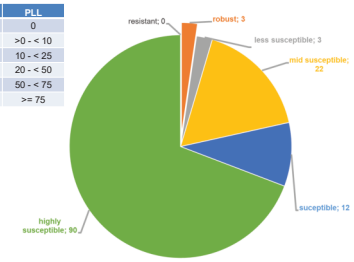

Fig. 1: distribution of pear cultivars to different classes of resistance/susceptility to fire blight

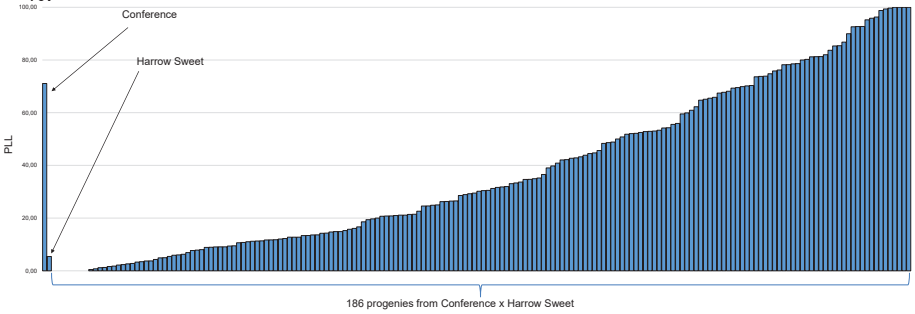

Fig. 2: percentage lesion length (PLL) of 186 progenies of the cross 'Conference' × 'Harrow Sweet' after inoculation with *E. amylovora* strain Ea222

Genotyping and QTL mapping

- 186 progenies of the 'Conference' × 'Harrow Sweet' population have been genotyped with the 70k Axiom pear SNP array (Montanari et al. 2019)
- Mapping with JoinMap5 (van Ooijen 2018) resulted in 17 linkage groups for 'Harrow Sweet' built from 1441 markers, a total length of 1273 cM, with a mean density of one SNP per 0.9 cM
- QTL mapping using MapQTL5 (van Ooijen 2004) with 'Harrow Sweet' linkage maps and PLL values of 186 progenies yielded four linkage groups showing QTLs, HS02, HS05, HS11, and HS17, linked to fire blight resistance, explaining around 56 % of the phenotypic variance
- LeRoux et al. 2012 identified fire blight QTLs for 'Harrow Sweet' on LG2 and LG4 and Dondini et al. (2005) on LG2, LG4 and LG9, only the QTL on LG2 (HS02) could be confirmed from our analysis

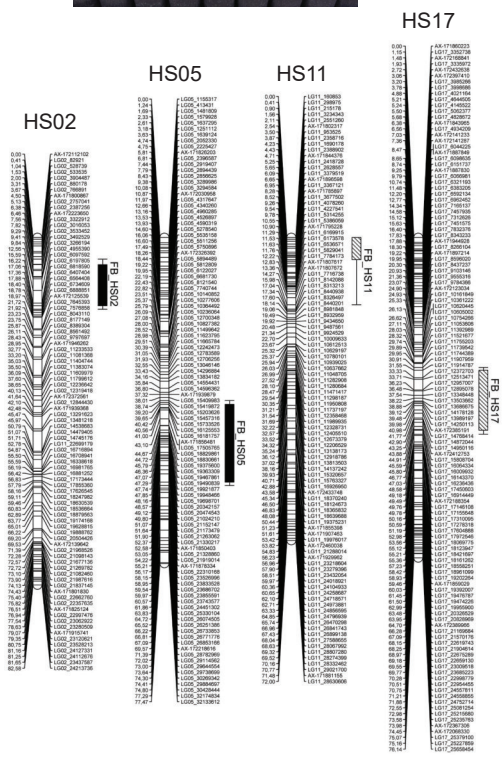

Fig.3: Linkage groups of 'Harrow Sweet' and the position of detected fire blight QTLs
black bars indicate QTLs above the genome wide threshold
bars with stripes indicate QTLs above the chromosome wide threshold

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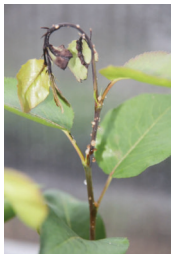

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Project manager
Federal Office for Agriculture and Food

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Isolation and identification of bacterial strains from apple flowers in Trentino and their evaluation as biocontrol agents of *Erwinia amylovora*

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Introduction

- *Erwinia amylovora* (*Ea*), the causal agent of fire blight, represents a **great threat** to the apple and pear production of many regions worldwide [1].
- **Flowers** are considered the *main sites of infection* from which *Ea* enters the host plant [2].
- Increasing evidence indicates that the **microbial communities** residing in apple flowers might hinder *Ea* host colonization [3].
- Studying the **interaction** between these microorganisms and *Ea* might lead to the devolpment of new sustainable strategies for the management of fire blight.

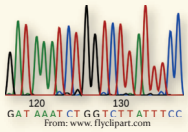
Aim of the study

To select new potential biocontrol agents active against fire blight by investigating the interaction between *Ea* and bacteria isolated from apple flowers from Trentino

Methods

SAMPLING
apple flowers at "Baloon stage"

IDENTIFICATION
through 16S rRNA gene sequencing

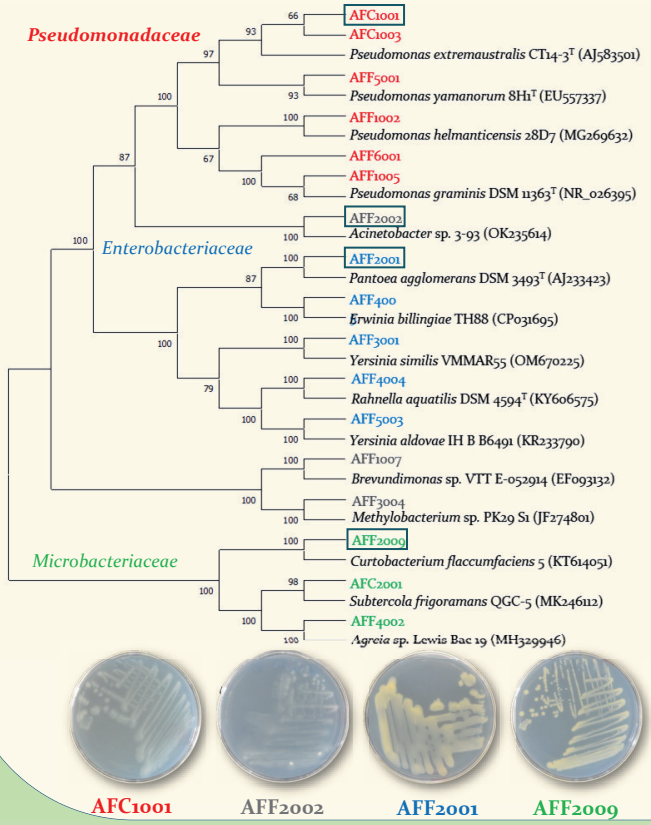


PHYLOGENETIC ANALYSIS
with MEGA-X [4]

TESTS
on detached apple flowers and immature pears

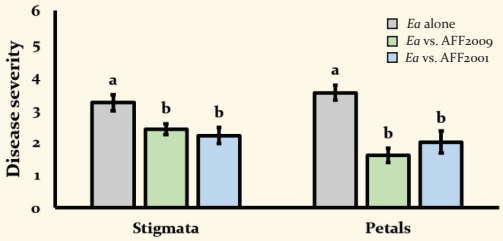
Results

- **Bacterial strains isolated from healthy apple flowers belonged to *Enterobacteriaceae*, *Microbacteriaceae* and *Pseudomonadaceae*. One member of each bacterial family was evaluated against *Erwinia amylovora*.**

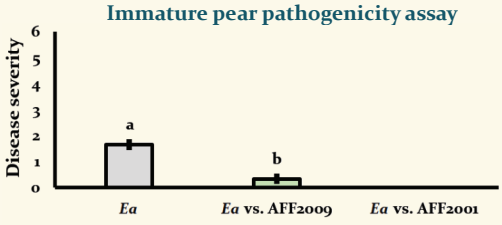


- ***Curtobacterium flaccumfaciens* AFF2009 and *Pantoea agglomerans* AFF2001 were able to effectively control *Erwinia amylovora* both on apple flowers and immature pear slices.**

Test on detached apple flowers



Immature pear pathogenicity assay



***Ea* alone** | ***Ea* vs. AFF2009** | ***Ea* vs. AFF2001**

Future perspectives

Since *Curtobacterium flaccumfaciens* **AFF2009** and *Pantoea agglomerans* **AFF2001** might have a significant effect on the control of *Ea,* further experiments will be carried out to investigate and understand the mode of action of these potential biocontrol agents.

References:
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[2] CABI *Erwinia amylovora* (fireblight)
[3] Cui et al, (2021) Temporal and spatial dynamics in the apple flower microbiome in the presence of the phytopathogen *Erwinia amylovora*. ISME Journal 15, 318–329
[4] MEGA11: Molecular Evolutionary Genetics Analysis version 11 (Tamura, Stecher, and Kumar 2021)

Pathogenicity related gene expression in the bark surrounding fire blight cankers on apple and Asian pear

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1. Cornell University, Hudson Valley Research Laboratory, Highland NY;
2. Virginia Polytechnic Institute and State University, Alson H. Smith Jr Agricultural Research and Extension Center, Winchester VA;
3. University of Massachusetts Amherst, Cold Spring Orchard Research and Education Center, Belchertown MA.

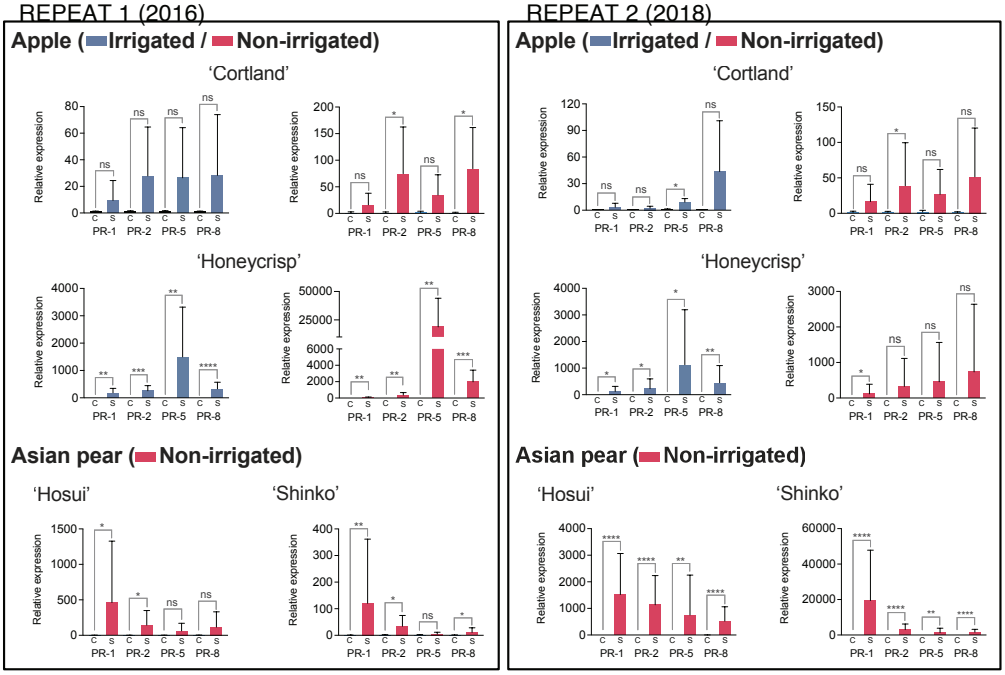
Introduction

Erwinia amylovora infections of apple leaves and shoots induces the expression of pathogenicity-related (PR) genes. However, there is limited information about PR gene expression in other host species, perennial tissues, and the effect of tree irrigation on PR gene expression patterns. In our study, we assessed the expression levels of the genes PR-1 (anti-fungal activity), PR-2 (b-1,3-glucanase), PR-5 (thaumatin-like protein) and PR-8 (class III chitinase) genes in perennial bark adjoining cankers of apple and Asian pear cultivars with differing susceptibilities to fire blight. This is the first report of induction of PR genes by *Erwinia amylovora* in the bark surrounding fire blight cankers in apple and Asian pear.

Materials & Methods

1. **Induction of canker formation** → Shoot inoculation with *Ea*273 during May-June (apple 'Cortland' and 'Honeycrisp'; Asian pear 'Hosui' and 'Shinko')
2. **Canker harvesting and flash freezing with liquid N_2** (July)
3. **RNA extraction and relative expression analysis of genes *PR-1, PR-2, PR-5* and *PR-8* by RT-PCR in bark tissues surrounding cankers using qRT-PCR**

Results & Conclusions



Relative expression levels of PR genes in cankers of *E. amylovora* host plants with different degrees of resistance to fire blight. We analyzed cankers from the apple cultivars 'Cortland' (susceptible) and 'Honeycrisp' (resistant) and the Asian pear cultivars 'Hosui' (highly susceptible) and 'Shinko' (susceptible). The blue and red columns show results obtained with cankers from irrigated and non-irrigated trees, respectively. Columns represent average relative expression values calculated by the 2-DDCt method, normalizing data to the reference actin gene and using average values of the expression of *PR* and actin genes in control samples as calibrator.

- The genes *PR-1, PR-2, PR-5*, and *PR-8* were overexpressed in the bark surrounding apple and Asian pear fire blight cankers.
- **In apple**, the highest average expression levels were observed in *PR-5, PR-8* and/or *PR-2*, compared to *PR-1*, although these differences were not supported statistically (one-way ANOVA, $P \geq 0.1307$).
- The resistant cultivar 'Honeycrisp' showed higher relative expression values for all the analyzed PR genes when compared to the more susceptible cultivar 'Cortland' regardless of the experimental repeat, these differences being especially significant when comparing to irrigated trees.
- Comparisons between the average relative expression values for each gene in cankers from irrigated and non-irrigated trees indicated no significant differences linked to the irrigation treatment, regardless of the assayed apple cultivar or the experimental repeat.
- **In Asian pear**, we detected a significant induction of all the analyzed PR genes compared to control samples. In this case, *PR-5* and *PR-8* showed the lowest relative expression levels, while *PR-1* and *PR-2* were the most expressed PR genes. Although this trend was evident in all the assays, the statistical analysis indicated no significant differences between PR gene expression levels (one-way ANOVA, $P \geq 0.0699$).
- Comparisons between relative expression levels of the more susceptible and less susceptible Asian pear cultivars to fire blight did not reveal any clear relationship between the susceptibility to fire blight and the relative expression values. Depending on the experimental repeat and the analyzed PR gene, the highly susceptible Asian pear cultivar 'Hosui' showed higher or lower relative expression values than the more resistant cultivar 'Shinko'.

Acknowledgements

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Phenotypic and transcriptomic differences between copper sensitive and copper tolerant *Erwinia amylovora* strains

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2. Cornell University, Cornell AgriTech - New York State Agricultural Experiment Station, Geneva, NY;
3. Virginia Polytechnic Institute and State University, Alson H. Smith Jr Agricultural Research and Extension Center, Winchester VA.

Introduction

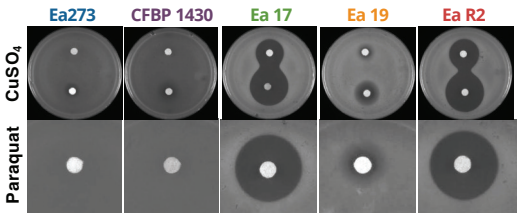
Most *Erwinia amylovora* strains can grow on selective/differential media amended with copper (Cu), but a small proportion of strains show high sensitivity to the same copper concentrations. In this work, we compared different phenotypic traits and the transcriptomic responses to Cu-shock and Cu adaptation in a Cu sensitive (EaR2) and a Cu tolerant (Ea273) *E. amylovora* strains. Our findings shed light on the molecular mechanisms of copper tolerance in *E. amylovora*, which might contribute to improving the application of copper for fire blight management in the field.

Materials & Methods

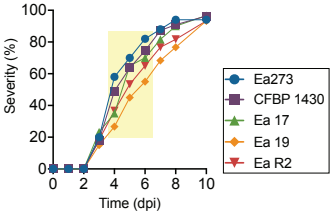
1. **Strain characterization** (Cu sensitivity, virulence, EPS production with/without copper, ROS sensitivity)
2. **Transcriptional responses to Cu-shock** (5 min exposure to 1 mM $CuSO_4$ in LB) **and Cu adaptation** (4.5h growth in LB + 1 mM $CuSO_4$)

Results & Conclusions

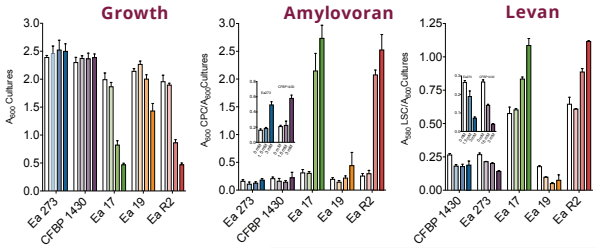
A) Strain characterization



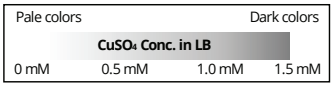
Growth inhibition disk assay. $CuSO_4$: 1.5 mM (upper disk) and 5 mM (bottom disk); Paraquat, 15 mM.



Virulence on 'Bartlett' fruitlets.



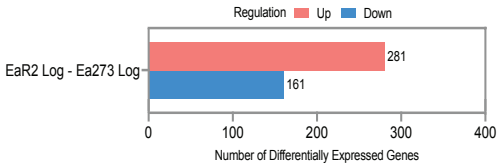
Effect of copper in **EPS secretion.**



1. Compared to the type strains, the Cu-sensitive strains Ea17, Ea19 and EaR2 showed increased sensitivity to paraquat, lower virulence and atypical patterns of EPS production in the presence of low copper concentrations.

B) RNA-Seq Analysis

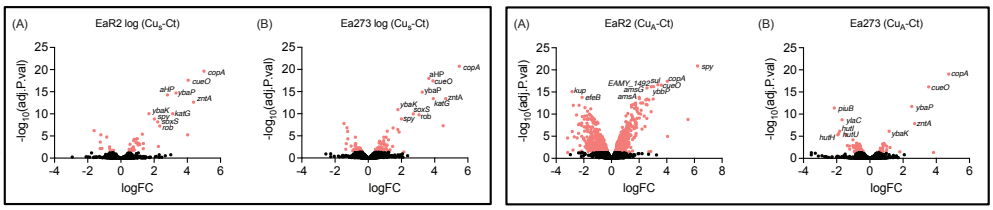
Differentially Expressed Genes (DEGs) in control samples



DEGs in EaR2 with respect to Ea273 in log-phase cells (limma+voom; FDR cutoff = 0.05; log2-fold change ≥ 1.5).

Cu-Shock response in EaR2 (A) and Ea273 (B)

Cu adaptation response in EaR2 (A) and Ea273 (B)



Volcano plots highlighting the 10 most differentially expressed genes (lowest adj. *P* values and highest log2 fold expression values).

2. EaR2 **control cells** → 442 DEGs compared to Ea273. A gene ontology (GO) enrichment and STRING Network analysis showed an upregulation of genes linked to Arg catabolism and downregulation of genes linked to the T3SS, motility / chemotaxis and ribosomal structure and regulation.

3. Almost identical **Cu-Shock response** in Ea273 and EaR2, with small differences consisting of upregulation in Ea273 of some genes linked to ROS responses (*trxC, grxA*) and the downregulation in EaR2 of *ylaC,* also linked to protection against ROS.

4. **Cu adaptation response:** Both strains upregulate genes addressing Cu and ROS detoxification (*copA, cueO, ybaP*), but, overall, they show widely different transcriptomic responses that match the observed phenotypical differences between the two strains:

Ea273: Downregulates GO terms linked to His metabolism.

EaR2: Upregulates GO terms linked to EPS biosynthesis and pathogenicity.

Downregulates GO terms associated to motility and flagellum structure.

5. Possible causes of a higher Cu sensitivity in EaR2 might be:

1. Biased energy resources towards genes not helping protect against Cu.
2. Different translational/post-translational regulation of proteins linked to Cu detoxification.
3. Mutations of global regulators participating in responses to Cu and other phenotypic traits.

Acknowledgements

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National Institute of Horticultural and Herbal Science

Detection of Novel Pear Genetic Resources Resistant to *Erwinia amylovora* strain TS3125 and YKB14808

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ABSTRACT

The incidence and spread of fire blight throughout the world is reporting. Outbreak of the fire blight on Asian pear in Korea was first reported in 2015. Antibiotics such as streptomycin provide effective control for fire blight, however these are not allowed increasingly due to ecological considerations and possibility for emergence of antibiotic resistant strains in *Erwinia amylovora*. Therefore, planting resistant cultivar is potentially regarded as the most promising fire blight control strategy. To select fire blight resistant cultivar, we had screened 93 cultivars by artificial inoculation of leaf-cutting method. Each plants with 5 replications was inoculated with the mixed inoculum of Korean strain TS3125 and YKB14808. Plants were kept under controlled conditions of relative humidity of 100% and temperature of 25±2℃ for 48hours after the inoculation in the clear vinyl house, and the relative humidity was adjusted from 85 to 90% for 4 weeks without other changes. We inoculated new leaves from the first inoculation position to upper sides and evaluated phenotypes every week. According to the results, 90 cultivars, including 'Harrow delight', 'Bartlett', 'Niitaka' and *et al*., in our germplasm collection displayed susceptible phenotype, while 3 cultivars, i.e. 'Magness', 'Moonglow' and 'CenSall', displayed resistant phenotype. These findings were inconsistent with previous studies. Interestingly, known resistant cultivars, including 'Harrow delight', 'Old home', 'Hosui', 'Shinko', 'Jing Bai Li' and 'Xue Hua Li', did not display resistance to *E. amylovora* strain TS3125 and YKB14808. However, 'Magness' and 'Moonglow' remained as the resistant. Based on these findings, we will try to find fire blight resistance genes through plant-pathogen interaction studies, and select elite seedlings to develop resistant cultivars.

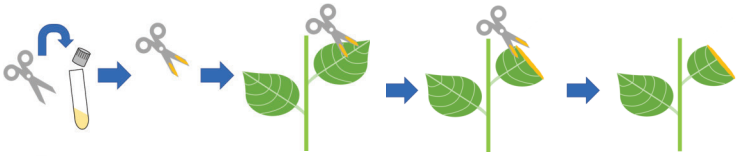
MATERIALS & METHODS

1 Materials

- ✓Plants : 93 kinds of pear cultivars(Oriental pears 'Niitaka', 'Hosui', 'Shinko', 'CensSall', 'Jing Bai Li', 'Xue Hua Li' and *et al*., European pears : 'Bartlett', 'Harrow Delight', 'Magness', 'Moonglow', 'Old Home' and *et al*.)
- ✓Pathogens : Mixed isolates of *Erwinia amylovora* strain TS3125 and YKB14809(collected from Ansung city in Korea)

2 Methods

- ✓Artificial inoculation of leaf-cutting method



3 Phenotype evaluation

- ✓Scored according to visual symptoms(1: No symptoms or Hypersensitive response(HR), 2: Weak vein necrosis, 3: Strong vein necrosis, 4: Vein and branch necrosis, 5: Vein, branch necrosis and ooze)

Score 1 (Resistant) | Score 2~3 (2: R / 3: S) | Score 4-5 (Suseptible) | Ooze Leaking

RESULTS & DISCUSSION

1 Results

- ✓Cultivar Distribution

Score 1: Magness
Score 2: Moonglow, CenSall
Score 3: Ames, Chein pali, Honey sweet, Manning-Miller, Old home, Danbae, Wonhwang, Heukseong-1
Score 4: Bartlett, Ducheese d'Angouleme, Farmingdale, Harrow delight, Oliam OPR-113, 114, P-12, W-1,Gamcheonbae,Gwansangdream, Gwansang-bae3, Geumchonjosaeng, Imamuraaki, Dudream, Okusankichi, Mansoo, Manpungbae, Seonhwang, Supergold, Shinko, Josaenghwangeum, Jinhwang, Sowon, Wongyo Na-79, 82, 84, 86, Waseaka, Hosui, Heukseong-2
Score 5: Abate fetel, California, Harbin, Idaho, NY10262, 10355, Okolo, Gwansangbae 1, 2, Greensis, Noksoo, Manhwang, Baeyeon 3, Jing Bai Li, Satangbae, Seolwon, Soojinjosaeng, Sweet skin, Niitaka, Xue Hua Li, Yali, Yeongsanbae, Yescool, Changjo, Gihooilho, Shinhwa, Solmi, Sodam, Aramchan, Soohwangbae, Seolrem, Jinhyang, Jeoksaek1, Wongyo Na-76,77,78,80,81,83,85,87, 88,89,Nijiseiki,Joyskin,Chuhwangbae,Dangshansuli, Hanareum, Kosui, Hongli, Hwasan, Heukseong 3

2 Discussion

- ✓Pathogenic differences among European and Korean *E. amylovora* strains supports that breeding programs for each countries or regions should be done by considering their own environments and pathogen strains

REFERENCE & ACKNOWLEDGEMENT

❖Peil, A., Bus, V. G., Geider, K., Richter, K., Flachowsky, H., & Hanke, M. V. (2009). Improvement of fire blight resistance in apple and pear. *Int J Plant Breed*, *3*(1), 1-27.

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