

**2nd INTERNATIONAL SYMPOSIUM ON BIOLOGICAL CONTROL OF BACTERIAL PLANT DISEASES**



**4-7 November 2008**  
**Orlando, Florida, USA**

**2<sup>nd</sup> International Symposium on Biological Control of  
Bacterial Plant Diseases**

**November 4-7, 2008, Orlando, FL, USA**

<b>Organizing Committee</b>	<b>Scientific Committee</b>
<p>Jeffrey Jones, <i>Coordinator</i>, USA Timur Momol, <i>Co-coordinator</i>, USA Botond Balogh, USA Aleksa Obradovic, Serbia Pamela Roberts, USA Wolfgang Zeller, Germany</p>	<p>Dr. Herb Aldwinckle, USA Dr. Brion Duffy, Switzerland Dr. M'barek Fatmi, Morocco Dr. Nicola Sante Iacobellis, Italy Dr. Jeffrey Jones, USA (<i>Co-Chair</i>) Dr. Steven Lindow, USA Dr. Rosa Mariano, Brazil Dr. Timur Momol, USA (<i>Co-Chair</i>) Dr. Maria Lopez, Spain Dr. Aleksa Obradovic, Serbia Dr. Peter Ott, Hungary Dr. Reginaldo Romeiro, Brazil Dr. Virginia Stockwell, USA Dr. Antonet Svircev, Canada Dr. Mark Wilson, USA Dr. Sandra Wright, Sweden Dr. Kerstin Wydra, Germany Dr. Wolfgang Zeller, Germany</p>

## Daily Schedule

Start Time	End Time	Function	Room
<b>Monday, November 3<sup>rd</sup>, 2008</b>			
5:00 PM	9:00 PM	Registration	Solar
5:00 PM	9:00 PM	Poster Setup	Horizons Salons 7-8
<b>Tuesday, November 4<sup>th</sup>, 2008</b>			
7:00 AM	8:00 AM	Continental Breakfast	Horizons Foyer 2
7:00 AM	5:00 PM	Registration	Solar
7:00 AM	8:00 AM	Poster Setup	Horizons Salons 7-8
8:00 AM	5:30 PM	General Session	Horizons Salons 9-11
5:30 PM	6:00 PM	Poster Session	Horizons Salons 7-8
6:00 PM	7:00 PM	Reception	Horizons Foyer 3
<b>Wednesday, November 5<sup>th</sup>, 2008</b>			
7:00 AM	9:00 AM	Continental Breakfast	Horizons Foyer 2
7:45 AM	5:00 PM	Registration	Solar
9:00 AM	4:00 PM	General Session	Horizons Salons 9-11
4:00 PM	6:00 PM	Poster Session	Horizons Salons 7-8
6:00 PM	7:00 PM	Reception	Horizons Foyer 3
7:00 PM	10:00 PM	Dinner	Horizons Salons 1-4
<b>Thursday, November 6<sup>th</sup>, 2008</b>			
7:00 AM	9:00 AM	Continental Breakfast	Horizons Foyer 2
9:00 AM	12:10 PM	General Session	Horizons Salons 9-11
2:00 PM	3:30 PM	Poster Session	Horizons Salons 7-8
4:00 PM	5:00 PM	Summary Session	Horizons Salons 9-11
6:00 PM	7:00 PM	Reception	Horizons Foyer 3
<b>Friday, November 7<sup>th</sup>, 2008</b>			
7:00 AM	9:00 AM	Continental Breakfast	Horizons Foyer 2

# Program

**Tuesday, November 4<sup>th</sup>**

(Horizons Salons 9-11)

- 8:00 **WELCOME: Raghavan Charudattan**, Chair, Plant Pathology Department, University of Florida, Gainesville, FL, USA.
- 8:15 **Wolfgang Zeller**, Organizer of 1<sup>st</sup> International Symposium on Biological Control of Bacterial Plant Diseases. Julius-Kühn-Institut, Darmstadt, Germany.
- 8:25 **Jeff Jones**, Co-Organizer of 2<sup>nd</sup> International Symposium on Biological Control of Bacterial Plant Diseases. Plant Pathology Department, University of Florida, Gainesville, FL, USA.

## **Session: *Mechanisms of Biological Control***

**Moderator: Kerstin Wydra**

- 8:30 **Induced systemic resistance by root colonizing fluorescent *Pseudomonas* spp.** Peter Bakker, Institute of Biology, Section of Phytopathology, Utrecht University, PO Box 80084, 3508 TB Utrecht, The Netherlands.
- 9:10 **Quorum sensing in rice associated bacteria; possible interplay between pathogenic and beneficial bacteria.** Vittorio Venturi, Iris Bertani, Laura Cabrio, Giuliano Degrassi, Giulia Devescovi, Maura Mattiuzzo, Sara Ferluga, Laura Steindler, Zulma Rocio Suarez Moreno and Sujatha Subramoni. Bacteriology Group, International Centre for Genetic Engineering & Biotechnology (I.C.G.E.B.) Padriciano 99, 34012 Trieste Italy and Plant Bacteriology Group, I.C.G.E.B., Biosafety Outstation, Via Piovega 23, 31052, Ca' Tron di Roncade, Treviso, Italy.
- 9:50 **A model of silicon-induced basal resistance in tomato against *Ralstonia solanacearum* based on transcriptomic and biochemical analyses,** Kerstin Wydra, Leibniz Universität Hannover, Hannover, Germany.
- 10:20 **Break**

## **Session: *Safety and Regulation of Biocontrol Agents***

**Moderators: Aleksa Obradovic and M'Barek Fatmi**

- 10:45 **Safety, regulation and commercialization of bacterial biocontrol agents in the European Union** Emilio Montesinos<sup>1</sup> and María M. López<sup>2</sup>. <sup>1</sup>Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, Spain. Email: [emonte@intea.udg.edu](mailto:emonte@intea.udg.edu); <sup>2</sup>Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Valencia, Spain.
- 11:20 **Biopesticide Research - Will it end with Publication or EPA Registration?** Michael Braverman, Biopesticide Program, IR-4 Project, Rutgers University, Princeton, New Jersey 08540, USA.
- 11:40 **Commercial Perspective on the Regulatory and Development Process for Biopesticides.** Pam Marrone, CEO/Founder, Marrone Organic Innovations, Inc. (MOI), Davis, CA 95618.
- 12:00 **Biopesticides regulation in the United States.** Gail S. Tomimatsu, Biopesticides and Pollution Prevention Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C., USA.
- 12:20 **Genotypic comparison of *Pantoea agglomerans* biocontrol and clinical isolates to address taxonomic and biosafety questions.** F. Rezzonico<sup>1</sup>, T.H.M. Smits<sup>1</sup>, C. Pelludat<sup>1</sup>, E. Montesinos<sup>2</sup>, J.E. Frey<sup>1</sup>, B. Duffy<sup>1</sup>. <sup>1</sup>Agroscope Changins-Wädenswil ACW, Plant Protection Division, CH-8820 Wädenswil, Switzerland; <sup>2</sup>University of Girona, Girona, Spain.
- 12:40 **Lunch**

## **Afternoon Session: *Genomics***

**Moderator: Brion Duffy**

- 2:30 **Genomics illuminates mechanisms involved in biological control of crown gall disease by *Agrobacterium radiobacter* K84.** Stephen K. Farrand, Department of Microbiology, University of Illinois at Urbana-Champaign Urbana, Illinois USA.
- 3:10 **Genomics of secondary metabolite production by *Pseudomonas fluorescens* Pf-5.** Joyce E. Loper<sup>1</sup>, Ian Paulsen<sup>2</sup>, Denny Bruck<sup>1</sup>,

Maria Pechy-Tarr<sup>3</sup>, Monika Maurhofer<sup>4</sup>, Christoph Keel<sup>3</sup>; and Harald Gross<sup>5</sup>. <sup>1</sup>U.S.Department of Agriculture, Agricultural Research Service, Corvallis, Oregon, USA; <sup>2</sup>The Institute for Genomic Research, Rockville, Maryland, USA; <sup>3</sup>Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; <sup>4</sup>Phytopathology/Institute of Integrative Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland; and <sup>5</sup>Institute for Pharmaceutical Biology, University of Bonn, Germany.

3:30 **Complete genome sequencing of *Pantoea agglomerans* C9-1.** T.H.M. Smits<sup>1\*</sup>, F. Rezzonico<sup>1</sup>, C. Pelludat<sup>1</sup>, V.O. Stockwell<sup>2</sup>, A. Goesmann<sup>3</sup>, J.E. Frey<sup>1</sup>, B. Duffy<sup>1</sup>. <sup>1</sup>Agroscope Changins-Wädenswil ACW, Plant Protection Division, CH-8820 Wädenswil, Switzerland; <sup>2</sup>Oregon State University, Corvallis, USA; <sup>3</sup>CeBiTec, University of Bielefeld, Germany.

3:50 **Break**

4:15 **How beta-aminobutyric acid (BABA) primes the Arabidopsis defense response against the bacterial pathogen *Pseudomonas syringae*.** CH Tsai<sup>a</sup>, I Fiatte<sup>b</sup>, CW Chen<sup>a</sup>, B Boachon<sup>b</sup>, J Thomas<sup>c</sup>, H Weber<sup>c</sup>, B Mauch-Mani<sup>b</sup>, and L Zimmerli<sup>a</sup>. <sup>a</sup>Institute of Plant Biology, The National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 106, Taiwan. <sup>b</sup>Laboratory of Cell and Molecular Biology, University of Neuchatel, Rue Emile-Argand 11, 2009 Neuchatel, Switzerland. <sup>c</sup>DNA Array Facility, Center for Integrative Genomics, University of Lausanne, Genopode Building, 1015 Lausanne, Switzerland.

4:35 **The creation of attenuated mutants in *X. perforans* for use as a control method toward *X. euvesicatoria*.** A.P. Hert<sup>a</sup>, M. Marutani-Hert<sup>b</sup>, J.B. Jones<sup>a</sup>, M.T. Momol<sup>c</sup>, and P.D. Roberts<sup>d</sup>. <sup>a</sup>Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA; <sup>b</sup>United States Horticultural Research Laboratory, Fort Pierce, FL, USA <sup>c</sup>North Florida Research and Education Center, University of Florida, Quincy, FL 32060, USA; <sup>d</sup>Southwest Florida Research and Education Center, University of Florida, Immokalee, FL 34142, USA.

4:55 **Genome comparison of the bacterial fire blight antagonists *Erwinia billingiae* and *E. tasmaniensis*.** M. Kube<sup>1</sup>, R. Reinhardt<sup>1</sup>, I. Müller<sup>2</sup>, and K. Geider<sup>2</sup>. <sup>1</sup>MPI for molecular Genetics, Berlin; <sup>2</sup>JKI for Plant Protection, Dossenheim, Germany.

- 5:15     **Discussion**
- 5:30     **Visit posters** (Horizons Salons 7-8)
- 6:00     **Reception** (Horizons Foyer 3)
- 7:00     **Dinner on own**

**Wednesday, November 5<sup>th</sup>**  
(Horizons Salons 9-11)

**Session: *Biocontrol of Bacterial Diseases of Agronomic and Horticulture Crops***

**Moderators: Nicola Iacobellis and Antonet Svircev**

- 9:00     **Bacteriophages and phage therapy.** Stephen Abedon, The Ohio State University, Mansfield, OH USA.
- 9:40     **Challenges involved in deploying bacteriophages for control of bacterial plant diseases.** J. B. Jones<sup>1</sup>, Fanny Iriarte<sup>2</sup>, Botond Balogh<sup>3</sup>, Aleksa Obradovic<sup>4</sup>, and M. Timur Momol<sup>1</sup> <sup>1</sup>Department of Plant Pathology, University of Florida, Gainesville, FL USA; <sup>2</sup>Department of Plant Pathology, Iowa State University, Ames, IA USA; <sup>3</sup>Department of Plant Pathology & Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT USA; and <sup>4</sup>Plant Pathology Department, Faculty of Agriculture, University of Belgrade, Belgrade-Zemun, Serbia.
- 10:00    **Selecting bacteriophages for biological control.** B. Balogh<sup>1</sup> and J. B. Jones<sup>2</sup>. <sup>1</sup>Connecticut Agricultural Experiment Station, New Haven, CT, USA; <sup>2</sup>University of Florida, Plant Pathology Dept., Gainesville, USA.
- 10:40    **Break**
- 11:10    **Management of bacterial blight on cotton and rice with bacteriophages.** E. Kamilova<sup>1</sup>, S. Monakov<sup>1</sup>, T. Gabisonia<sup>2</sup>, M. Loladze<sup>2</sup>, and K. Severinov<sup>3</sup>. <sup>1</sup>Institute of Genetics and Plant Experimental Biology, AS RUz, Tashkent Region, 102151, Kibrai

District, P/o Yukori-Yuz, Uzbekistan; <sup>2</sup>G.Eliava Institute of Bacteriophage, Microbiology and Virology, AS G, Gotua Street 3, Tbilisi, 380060, Georgia; <sup>3</sup>Rutgers University, Waksman Institute, 190 Frelinghuysen Road, Piscataway, New Jersey 08854-8020, USA.

- 11:30 **Bacteriophage translocation in tomato plants and prospects for control of tomato bacterial wilt.** A. Obradovic, F. B. Iriarte, G. V. Minsavage, J. C. Hong, T. M. Momol<sup>3</sup>, and J. B. Jones, University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia; Iowa State University, Plant Pathology Dept., Ames, IA; University of Florida, Plant Pathology Dept., Gainesville, FL, USA.
- 11:50 **Involvement of *pcoR-pcoI* quorum-sensing system in biocontrol of plant diseases by *Pseudomonas fluorescens* 2P24.** Q. Yan, H.L. Wei, Q.X. Zhang, X.G. Wu, and L.Q. Zhang. Department of Plant Pathology, China Agricultural University, Beijing 100193, China.
- 12:10 **Lunch**

### ***Bacterial Diseases of Agronomic and Horticulture Crops (Cont.)***

- 2:00 **Evaluation of PGPR and acibenzolar-S-methyl for control of bacterial spot of tomato.** S. Zhang and T. L. White. Tropical Research and Education Center, University of Florida, IFAS, Homestead, FL 33031.
- 2:20 **Biological control of Pierce's disease in the greenhouse and field with a benign strains of *Xylella fastidiosa*.** D. Hopkins, University of Florida, MREC, 2725 Binion Road, Apopka, FL 32703, U.S.A.
- 2:40 **Understanding the mechanism of grape crown gall biological control by *Agrobacterium vitis* strain F2/5.** T. J. Burr, J. E. Creasap, C. L. Reid and D. Zheng Department of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456.
- 3:00 **Characterization of mutants of *Agrobacterium* strain K84 affected in production of exopolysaccharides, surface motility and biofilm formation: their role in the biocontrol of crown gall.** R. Penyalver<sup>1</sup>, L.P. Burbank<sup>2</sup>, S.B. von Bodman<sup>2</sup> and M. M. López<sup>1</sup>. <sup>1</sup>IVIA, Ctra. Moncada-Naquera Km 5, 46113 Moncada, Valencia, Spain. <sup>2</sup>Department of Plant Science, University of Connecticut, 302 B Agricultural Biotechnology Laboratory, Storrs, CT 06269, USA.



3:20 **Effectiveness of *Punica granatum* L. extracts on kiwifruit and tomato bacterial pathogens.** G.M. Balestra, A. Quattrucci and A. Rossetti. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, Italy.

3:40 **Break**

4:00 **Poster session** (Horizons Salons 7-8)

**Short presentations** (3-5 minutes)

***Pseudomonas fluorescens* for management of bacterial blight in cotton.** R. M. Gade\*, O.V.Ingole<sup>1</sup> and B.R. Patil<sup>1</sup>. \*Department of Plant Pathology, 1. AICCIP, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M. S.) India, 444 104.

**Antagonistic and plant growth promotion activities of soil actinomycetes isolated from potato fields in Mongolia.** B. Tsetseg, G. Nyamsuren and T. Gantuya. Laboratory of Microbiology, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia.

**Serenade MAX<sup>®</sup>, a biopesticide for use against bacterial pathogens in specialty food crops.** H. Brett Highland PhD., 1069 Eisenhower Dr., Nokomis, FL, USA, and T. Smith PhD., AgraQuest, Inc., 1540 Drew Ave., Davis, California, USA.

**Characterization of bacterial antagonists and their resistance inducing effect against bacterial wilt caused by *Ralstonia solanacearum* in tomato.** H. Kurabachew, and K. Wydra. Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany.

**An efficient method in genetical discrimination of biological control agents by molecular ISSR and RAPD markers.** Ö. Baysal<sup>1</sup>, H. İkten<sup>1</sup>, M. Çalışkan<sup>2</sup> and W. Zeller<sup>3</sup>. <sup>1</sup>West Med. Agr. Research Inst. (BATEM) Plant Pathology and Molecular Biology Department P:B. 35 07100 Antalya, Turkey; <sup>2</sup>Turkish Ministry of Agriculture and Rural Affairs Central Research Institute for Field Crops, Ankara, Turkey; and <sup>3</sup>Julius – Kühn - Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany.

**Antibacterial activity of certain plant extracts against bacterial wilt of tomato.** K. Abo-Elyousr<sup>1</sup>, A. M. Asran<sup>2</sup> and W. Zeller<sup>3</sup>. <sup>1</sup> Plant Pathology Dept. Faculty of Agriculture, Assiut University - 71526, Assiut, Egypt; <sup>2</sup>Dept. of Plant Pathology, Faculty of Agriculture, Sohag Univ., Sohag, Egypt; and <sup>3</sup>Julius-Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen

Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt.

6:00 **Reception** (Horizons Foyer 3)

7:00 **Dinner** (Horizons Salons 1-4)

## **Thursday, November 6<sup>th</sup>**

(Horizons Salons 9-11)

### **Session: *Fire Blight***

**Moderators: Maria Lopez and Virginia Stockwell**

9:00 **The discovery and development of *Pseudomonas fluorescens* A506 for suppression of *Erwinia amylovora*.** Steven Lindow, University of California, Berkeley, CA USA.

9:40 **Potential for biological control of fire blight in the eastern United States.** Herb Aldwinckle<sup>1</sup>, George Sundin<sup>2</sup>, Nicole Werner<sup>1</sup> and Keith Yoder<sup>3</sup>. <sup>1</sup>Cornell University, Geneva, NY 14456, USA; <sup>2</sup>Michigan State University, East Lansing, MI 48824, USA; <sup>3</sup> Virginia Tech Ag. Research & Extension Center, Winchester, VA 22602, USA.

10:00 **Antibiosis and acidification may contribute to control of fire blight by *Pantoea agglomerans* E325.** P.L. PUSEY (1), V.O. Stockwell (2), and D.R. Rudell (1). (1) USDA-ARS, Wenatchee, WA, USA; (2) Oregon State University, Corvallis, OR, USA.

10:20 **Break**

11:00 **Implications of pathogenesis on stigmas to biological control of fire blight.** Kenneth B. Johnson, Teresa L. Sawyer, Virginia O. Stockwell and Todd N. Temple. Department of Botany and Plant Pathology, Oregon State University Corvallis OR 97331-2902. USA.

11:30 **Use of *Pantoea agglomerans* as a phage delivery system for control of fire blight.** A.M Svircev<sup>1</sup>, S.M.Lehman<sup>2</sup> and A.J. Castle<sup>3</sup>. <sup>1</sup> Agriculture and Agri-Food Canada, Vineland Station, ON Canada L0R 2E0; <sup>2</sup> Centers for Disease Control and Prevention, Atlanta, Georgia USA 30333; <sup>3</sup> Department of Biological Science, Brock University, St. Catharines, ON Canada L2S 3A1.

- 11:50 **Detection and differentiation of antagonistic *Erwinia* species by PCR and MALDI TOF analyses.** K. Geider<sup>1</sup>; I. Gehring<sup>1</sup>, A. Wensing<sup>1</sup>, A. Freiwald<sup>2</sup>, and S. Sauer<sup>2</sup>. <sup>1</sup>JKI for Plant Protection, Dossenheim,<sup>2</sup>MPI for molecular Genetics, Berlin, Germany.
- 12:10 **Lunch**
- 2:00 **Poster session (brief oral presentations on selected posters as determined by moderators)** (Horizons Salons 7-8)
- 3:30 **Break**
- 4:00 **General meeting summary session/discussion** (Horizons Salons 9-11)
- 5:00 **End of symposium**
- 6:00 **Reception**
- 7:00 **Dinner on own**

### **Posters - Mechanisms of Biological Control**

- P1 BABA-induced resistance against *Pseudomonas syringae* is based on the inhibition of the hijacking of plant hormone signaling by the bacteria.** B. Boachon<sup>1</sup>, X. Daniel<sup>1</sup>, V. Flor<sup>2</sup>, and B. Mauch-Mani<sup>1</sup>. <sup>1</sup>Laboratory of Molecular and Cellular Biology, Institute of Botany, University of Neuchâtel, Rue Emile-Argand 11, Case Postale 158, 2009 Neuchâtel, Switzerland. <sup>2</sup>Departamento de Ciencias Agrarias y del Medio Natural, Area de Fisiologia Vegetal, Universitat Jaume I, Borriol s/n, 12071 Castellon, Spain.
- P2 Absolute quantification of specific plant defense pathways and *Pseudomonas syringae* growth in *Arabidopsis thaliana* using real-time PCR.** B. Boachon, J. Robert, and B. Mauch-Mani. Laboratory of Molecular and Cellular Biology, Institute of Botany, University of Neuchâtel, Rue Emile-Argand 11, Case Postale 158, 2009 Neuchâtel, Switzerland.
- P3 Resistance induction by silicon against bacterial diseases of tomato, eggplant, cucumber and geranium.** T. Hartmann and K. Wydra. Leibniz

Universität Hannover, Institute of Plant Diseases and Plant Protection.  
Herrenhäuser Str. 2. 30149 Hannover, Germany.

- P4 Phenotypic and biochemical analyses of silicon – induced resistance against *Ralstonia solanacearum* in potato genotypes.** Dritan Sadikaj and Kerstin Wydra. Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany.
- P5 Histochemical analyses of silicon-induced resistance in the interaction of tomato (*Solanum lycopersicum* L.) and *Ralstonia solanacearum*.** T. Schacht and K. Wydra. Leibniz Universität Hannover, Institute of Plant Diseases and Plant Protection, Herrenhäuser Str. 2. 30149 Hannover, Germany.

### **Posters - Agronomic and Horticulture Crops**

- P6 Enhancement of Induced systemic Resistance on Cucumber by *Bacillus vallismortis* EXTN-1 with L-Alanine.** Kyungseok Park, Diby Paul<sup>a</sup>, Srinivasan Bharathkumar, Young Ki Lee and Sang Yeb Lee, Sung Sook Han. \*Plant Pathology Division, National Institute of Agricultural Science and Technology, RDA, Suwon. 441-707, South Korea, <sup>a</sup>Dept. of Environmental Engineering, Konkuk University, Seoul, 143-701 South Korea.
- P7 Suppression of bacterial palea browning of rice by bacteriophages and non-pathogenic bacterium.** K. Azegami and Y. Inoue, National Agricultural Research Center, Tsukuba, Ibaraki, 305-8666, Japan.
- P8 Biological control of citrus bacterial canker disease using *Pseudomonas fluorescens* and *P. putida* strains.** Gh. Khodakaramian<sup>1</sup>, G.M. Balestra<sup>2</sup> and A. Heydari<sup>3</sup>. <sup>1</sup>Department of Plant Protection, Hamedan University, Hamedan, Iran. <sup>2</sup>Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università della Tuscia, Viterbo, Italy. <sup>3</sup>Iranian Research Institute of Plant Protection, Tehran-Iran.
- P9 Antibacterial activity of grape (*Vitis vinifera*) waste extracts.** G.M. Balestra, A. Rossetti and A. Quattrucci. Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy.
- P10 Eradication of *Pseudomonas syringae* pv. *syringae* from micropropagated kiwifruit plants (*Actinidia deliciosa* cv. Hayward).** L. Fratrarangeli, M. Muganu and G.M. Balestra, Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy.

- P11 A natural antagonist against phyto-bacteria of kiwifruit plants.** A. Rossetti and G.M. Balestra, Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy.
- P12 Preliminary results of in vitro antagonist bacteria on the development of fungi isolated from *Brachiaria brizantha* (Poaceae) seeds.** M. del S. Balcázar, Á. L. Rivera and B. Pineda L. Genetic Resources Unit, International Center of Tropical Agriculture (CIAT, Colombia).
- P13 Antagonistic and plant growth promotion activities of soil actinomycetes isolated from potato fields in Mongolia.** B. Tsetseg, G. Nyamsuren and T. Gantuya. Laboratory of Microbiology, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia.
- P14 An efficient method in genetical discrimination of biological control agents by molecular ISSR and RAPD markers.** Ö. Baysal<sup>1</sup>, H. İkten<sup>1</sup>, M. Çalışkan<sup>2</sup> and W. Zeller<sup>3</sup>. <sup>1</sup>West Med. Agr. Research Inst. (BATEM) Plant Pathology and Molecular Biology Department P:B. 35 07100 Antalya, Turkey; <sup>2</sup>Turkish Ministry of Agriculture and Rural Affairs Central Research Institute for Field Crops, Ankara – Turkey; and <sup>3</sup>Julius-Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany.
- P15 Searching for new antibiotics in endophytic microorganisms.** Erik Saenz; Charlotte Borda; Ligia Renata; Márcia Da Silva; Gabriel Padilla. University of São Paulo, São Paulo, Brazil.
- P16 Eco-friendly management of bacterial black spot disease of mango using bacterial antagonists.** Choudhary Sharfuddin, C.Kumar, R.Mohanka and G.Tabassum. Plant pathology and Microbiology lab, Department of Botany, Patna University, Bihar, India.
- P17 Preliminary results on the biocontrol of bacterial diseases of cultivated mushrooms by using potential antagonistic bacteria.** S. Prashanth, P. Lo Cantore, and N.S. Iacobellis. Dipartimento di Biologia Difesa e Biotecnologie Agro Forestali, Università degli Studi della Basilicata, viale Ateneo Lucano, 10, 85100 Potenza, Italy.
- P18 Preliminary results on the antagonistic activity of bean rhizosphere bacteria toward common bean pathogenic bacteria and fungi.** V. Shanmugaiah, P. Lo Cantore and N.S. Iacobellis. Dipartimento di Biologia Difesa e Biotecnologie Agro Forestali, Università degli Studi della Basilicata, viale Ateneo Lucano, 10, 85100 Potenza, Italy.
- P19 *Pseudomonas fluorescens* for management of bacterial blight in cotton.** R. M. Gade\*, O.V.Ingole<sup>1</sup> and B.R. Patil<sup>1</sup>. \*Department of Plant

Pathology, 1.AICCIP, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M. S.) India, 444 104.

- P20 Biological control of Verticillium wilt of tomato using antagonistic and root colonizing bacteria.** M. Fatmi<sup>1</sup>, Y.A.A. Alkhalayali<sup>2</sup>, and M. Achouri<sup>1</sup>.  
<sup>1</sup> Institut Agronomique et Vétérinaire Hassan II, Complexe d'Agadir, B.P. 18/S, Agadir, Morocco and <sup>2</sup> University of Sebha, Sebha, Lybia.
- P21 Transmission of *Ralstonia solanacearum* in tomato seed and true potato seed (TPS).** Charlotte Borda<sup>1</sup>; Erik Saenz<sup>1</sup>; Pedro Aley; Lilian Gutarra<sup>2</sup> and Sylvie Priou<sup>2</sup>. <sup>1</sup>University of São Paulo, São Paulo, Brazil, <sup>2</sup>International Potato Center, Lima, Peru.
- P22 Antibacterial activity of certain plant extracts against bacterial wilt of tomato.** K. Abo-Elyousr<sup>1</sup>, A. M. Asran<sup>2</sup> and W. Zeller<sup>3</sup>. <sup>1</sup> Plant Pathology Dept. Faculty of Agriculture, Assiut University - 71526, Assiut, Egypt; <sup>2</sup>Dept. of Plant Pathology, Faculty of Agriculture, Sohag Univ., Sohag, Egypt; and <sup>3</sup>Julius-Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt.
- P23 Characterization of bacterial antagonists and their resistance inducing effect against bacterial wilt caused by *Ralstonia solanacearum* in tomato.** H. Kurabachew, and K. Wydra. Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany.
- P24 Serenade MAX<sup>®</sup>, a biopesticide for use against bacterial pathogens in specialty food crops.** H. Brett Highland PhD., 1069 Eisenhower Dr., Nokomis, FL, USA, T. Smith PhD., AgraQuest, Inc., 1540 Drew Ave., Davis, California, and D. Long, 125 Willow Wisp Hill, Demorest GA 30535, USA.
- P25 Effects of soil amendment with silicon fertilizer and sugarcane bagasse on tomato bacterial wilt under field conditions in Ethiopia.** G. Hordofa<sup>1</sup>, C. Fininsa<sup>2</sup> and K. Wydra<sup>3</sup>. <sup>1</sup>Ethiopian Institute of Agricultural Research, Melkassa Research Center P.O. BOX 436, Adama, Ethiopia, <sup>2</sup>Haramaya University, Department of Plant Sciences P.O. BOX 138 Dire Dawa, Ethiopia, <sup>3</sup>Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Str.2., 30419, Hannover, Germany.
- P26 A new antibiotic of 21<sup>st</sup> century from livestock excrements.** K. Rajamohan, V. Kurucheve and S. Usharani. Department of Plant Pathology, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India.
- P27 Biological control of black rot of cabbage by non-pathogenic *Xanthomonas campestris* and bacteriophage.** Y. Inoue and K. Azegami, National Agricultural Research Center, Tsukuba, Ibaraki, 305-8666, Japan.

- P28 Biocontrol of soft rot bacteria of the genus *Erwinia* in potato – Secondary metabolites of rhizobacteria affecting transcription of the pectinase genes of *Erwinia chrysanthemi*.** Barbara Żurek<sup>1</sup>, Mieczysław Żurek<sup>2</sup>. <sup>1</sup>Department of Microbiology, <sup>2</sup>Department of Plant Protection University of Podlasie, Prusa 12 Street, 08-110 Siedlce, Poland.
- P29 Recombinant protein for biocontrol of *Ralstonia solanacearum*.** S.S.Kabeil, Elsayed E. Hafez, Ayman S. Daba and A. El-Saadani. Mubarak City for Scientific Research and Technology, Borg-Elarab, Alexandria, Egypt. Corresponding author: Sanaa Soliman Ahmed, [sanaa5769@yahoo.com](mailto:sanaa5769@yahoo.com).
- P30 Potato brown rot disease in Egypt: current status and prospects.** S. S. Kabeil\*, S. M. Lashin\*\*, M. A. El-Saadani\*, M. M. Abd-Elgawad\*\* and A. M. Aboul-Einean\*\*\*. \*Mubarak City for Scientific Research and Technology, Borg-Elarab, Alexandria; \*\*Plant Pathology Department, National Research Center, El-Tahrir St, Dokki, 12622, Giza;\*\*\*Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt.

### **Posters – Fire Blight**

- P31 Reduction of *Erwinia amylovora* (fire blight) infection of apple shoots by the biofungicide Taegro.** H.S. Aldwinckle<sup>1</sup>, C. Mpoy<sup>1</sup>, K. Moktan<sup>1</sup>, S.S. Gnanamanickam<sup>2</sup>, and S. Semones<sup>2</sup>. <sup>1</sup>Department of Plant Pathology and Plant Microbe Biology, Cornell University, Geneva, NY 14456 and <sup>2</sup>Novozymes Biologicals, 5400 Corporate Circle, Salem, VA 24153.
- P32 Effect of exopolysaccharides on the infection of *Erwinia amylovora* by bacteriophages.** D. R. Roach<sup>1</sup>, A. J. Castle<sup>1</sup>, and, A. M. Svircev<sup>2</sup>. <sup>1</sup>Department of Biological Sciences, Brock University, St. Catharines ON, Canada L2S 3A1, <sup>2</sup>Agriculture and Agri-Food Canada, Vineland Station ON, Canada L0R 2E0.
- P33 Integrated control of fire blight with bacterial antagonists and oxytetracycline.** Virginia O. Stockwell<sup>1</sup>, Todd N. Temple<sup>1</sup>, Kenneth B. Johnson<sup>1</sup>, and Joyce E Loper<sup>1,2</sup>. <sup>1</sup>Dept. of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331 and <sup>2</sup> USDA ARS, Horticultural Crops Research Lab, Corvallis, OR 97330, USA.
- P34 Recent studies on the biocontrol of Fireblight (*Erwinia amylovora*) with BioZell-2000 B, an etheric oil of *Thymbra spicata*.** W. Zeller, K. Abo-Elyousr and O. Yegen. Julius-Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany.

- P35 Nicotinic acid degradation: a novel method for selection of a biocontrol agent against *Erwinia amylovora*.** Thomas Paternoster<sup>1,2\*</sup>, Brion Duffy<sup>3</sup>, Geneviève Défago<sup>1</sup>. <sup>1</sup> Phytopathology group, Swiss Federal Institute of Technology, ETH-Z, CH-8092 Zürich, Switzerland; <sup>2</sup> SafeCrop Institute, San Michele all'Adige, Trentino, Italy; <sup>3</sup> Agroscope Changins-Wädenswil, Swiss National Competence Center for Fire Blight, CH-8820 Wädenswil, Switzerland.
- P36 Field evaluation of biological control agents for fire blight control in Michigan.** George W. Sundin, Gayle C. McGhee, and Gail R. Ehret. Michigan State University, Department of Plant Pathology, 103 CIPS, East Lansing, MI 48824 USA.
- P37 Isolation and characterization of *Erwinia amylovora* specific bacteriophages.** L. Fieseler, Y. Born, B. Duffy, and M.J. Loessner. ETH Zurich, Institute of Food Science and Nutrition, Schmelzbergstrasse 7, 8092 Zurich, Switzerland. (2) Agroscope Changins-Wädenswil, 8820 Wädenswil, Switzerland.
- P38 Evaluating the effect of some chemical and plant-based compounds against fire blight disease (*Erwinia amylovora*) in Lorestan province of Iran.** M. Haji<sup>1</sup>, N. Hasanzadeh<sup>2</sup>, S. H. Vafaei<sup>1</sup>, and S. Rezaei<sup>2</sup>. <sup>1</sup>Dept. of Plant Protection, Agriculture Collage, Islamic Azad University, Khorramabad branch, Lorestan, Iran and <sup>2</sup>Dept. of Plant Protection, Agriculture Collage, Islamic Azad University, Research & Science branch, Tehran, Iran.



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## **ABSTRACTS FOR ORAL PRESENTATIONS AND POSTERS**

**BACTERIOPHAGES AND PHAGE THERAPY.** Stephen T. Abedon.  
Department of Microbiology, The Ohio State University, Mansfield, OH USA.

Bacteriophages, or phages for short, are the viruses of bacteria. They can be classified in various ways according to, for example, their host range, virion morphology, genome characteristics, infection aspects, etc. Among phages, the majority have a tailed morphology, dsDNA genomes, and release their progeny from infected bacteria via a lytic process. The resulting lysis not only liberates phage progeny but also kills the bacterial cell and terminates the phage infection. Also in terms of infection characteristics, tailed phages may be characterized into two basic types, so-called temperate phages and phages that may be variously described as virulent, obligately lytic, professionally lytic, etc. The latter, under favorable environmental conditions (e.g., such that bacteria are replicating), will, upon successful infection, destroy their bacterial host. Given this obligately lytic potential, bacteriophages can be harnessed as highly effective anti-bacterials. Unlike other bacteriocidal agents, including antibiotics as well as disinfectants, phages not only kill target bacteria but self-amplify their bacterial-killing activity in the process. They are also easily isolated in forms to which bacteria are not able to evolve cross resistance, and in the course of evolving resistance bacteria may incur significant metabolic costs and/or reduced virulence. Finally, and perhaps of greatest relevance regarding their anti-bacterial utility, most phages are relatively benign, resulting in a high therapeutic index (ratio of toxic dosage to effective dosage) as well as insignificant environmental toxicity, plus can narrowly target bacteria rather than broadly impacting otherwise beneficial normal flora. For all of these reasons, phage therapy could serve as a highly efficacious anti-bacterial strategy against pathogenic or nuisance bacteria, via treatment of individuals or of environments, plus, depending on circumstances, can be used either prophylactically or as specific cures.

**ANTIBACTERIAL ACTIVITY OF CERTAIN PLANT EXTRACTS AGAINST BACTERIAL WILT OF TOMATO.** K. Abo-Elyousr<sup>1</sup>, A. M. Asran<sup>2</sup> and W. Zeller<sup>3</sup>. <sup>1</sup>Plant Pathology Dept. Faculty of Agriculture, Assiut University - 71526, Assiut, Egypt; <sup>2</sup> Dept. of Plant Pathology, Faculty of Agriculture, Sohag Univ., Sohag, Egypt ; and <sup>3</sup>Julius-Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany.

Five isolates of *Ralstonia solanacearum* were isolated from a naturally wilted root of tomato plants grown in Assiut governorate of Egypt. The antibacterial activity of plant leaf extracts of Datura, Garlic and Nerium were tested in controlling *R. solanacearum* in vitro and in vivo. Garlic extract exhibited the strongest antibacterial activity against bacterial wilt in vitro and in vivo followed by Datura and then Nerium. Cold water extract of these plant species were more effective than hot water extract in the development of the disease in vivo. In greenhouse experiments, the application of the tested plant extract to soil at the time of inoculation, two days before inoculation and two days after inoculation with the pathogen, significantly reduced the disease index of wilt on tomato cultivar Super Marmande. The application of plant extracts at the same time of inoculation resulted in the highest reduction of disease index.

**REDUCTION OF *ERWINIA AMYLOVORA* (FIRE BLIGHT) INFECTION OF APPLE SHOOTS BY THE BIOFUNGICIDE TAEGRO.** H.S. Aldwinckle<sup>1</sup> C. Mpoy<sup>1</sup>, K. Moktan<sup>1</sup>, S.S. Gnanamanickam<sup>2</sup> and S. Semones<sup>2</sup>. <sup>1</sup>Department of Plant Pathology and Plant Microbe Biology, Cornell University, Geneva, NY 14456 and <sup>2</sup>Novozymes Biologicals, 5400 Corporate Circle, Salem, VA 24153, USA.

Taegro is a biofungicide that contains *Bacillus amyloliquefaciens* strain FZB24 at  $5.0 \times 10^{10}$  cfu/g of the powdered formulation. It has been shown to have a broad-spectrum of activity against both fungal and bacterial plant pathogens. In laboratory dual plate assays Taegro inhibited the growth of *Erwinia amylovora* strains Ea273 and Ea276. A field trial was carried out in Geneva, NY during June-August, 2008, applying Taegro at three rates, 0.21, 0.29 and 0.58 g/L as a foliar spray to apple shoots. Applications were made with a Solo backpack sprayer to twenty randomly selected and tagged, growing shoots on six trees each of apple cv. Empire (moderately susceptible to fire blight) and cv. Idared (susceptible), 24 h before inoculation and 24 h after inoculation. Besides using Taegro alone, there was a treatment with an alternation of 0.21g/L Taegro in the first application and 0.05 g/L streptomycin plus Regulaid surfactant in the second. Non-treated shoots served as checks. All treated and non-treated shoots were inoculated by laterally bisecting the two youngest leaves with scissors dipped in a suspension of *E. amylovora* strain Ea273 at  $10^7$  cfu/ml. The development of fire blight infection in the shoots was recorded at intervals following inoculation using a severity scale from 1 (no or mild infection) to 4 (very highly infected). In the untreated check, there were 36% and 66% severely infected shoots on Empire and Idared, respectively, 20 days after inoculation. The streptomycin treatment resulted in 18% and 44% severely infected shoots of Empire and Idared, respectively. Among the three Taegro treatments, the 0.29 and 0.58 g/L rates appeared to reduce the development of shoot infection in both cultivars. Taegro at 0.58 g/L resulted in 21% and 27% severely infected shoots of Empire and Idared, respectively. The alternation of 0.21 g/L Taegro with streptomycin resulted in 21% and 32% severely infected shoots of Empire and Idared, respectively. The reductions in severity of shoot infection by the higher rates of Taegro, and by Taegro alternated with streptomycin are sufficient to encourage further trials of Taegro to reduce the incidence and severity of fire blight infections in apple orchards.

**POTENTIAL FOR BIOLOGICAL CONTROL OF FIRE BLIGHT IN THE EASTERN UNITED STATES.** Herb Aldwinckle<sup>1</sup>, George Sundin<sup>2</sup>, Nicole Werner<sup>1</sup> and Keith Yoder<sup>3</sup>. <sup>1</sup>Cornell University, Geneva, NY 14456, USA; <sup>2</sup>Michigan State University, East Lansing, MI 48824, USA; <sup>3</sup> Virginia Tech Ag. Research & Extension Center, Winchester, VA 22602, USA.

Research on biological control of fire blight has been carried out for several years in various apple and pear growing regions of the United States. Although effective control has been reported from Pacific Coast growing regions, results in the eastern United States have generally shown less effectiveness. This paper will report research results over six years from three eastern states. Several biological materials have EPA labels for use on apple to manage fire blight infection of flowers (blossom blight). The commercial products, Blightban A506 and C9-1 (containing the bacterial antagonists *Pseudomonas fluorescens* A506 and *Pantoea agglomerans* C9-1, respectively) and Bloomtime Biological (*Pantoea agglomerans* E325), as well as Serenade (a lyophilized culture filtrate of *Bacillus subtilis* QST 713 ) were evaluated for efficacy in controlling fire blight in Michigan, New York, and Virginia. When examined individually, none of these biological control materials was consistently effective in reducing blossom infection. Average reductions in blossom infection observed in experiments conducted between 2002 and 2007 ranged from 9-36%, whereas control with the antibiotic streptomycin was consistent and ranged from 60-67% (in orchards where streptomycin resistance had not developed in *E. amylovora*). Blossom colonization by the bacterial antagonists was variable and <60% in 12 of 25 experiments. Consistent control of blossom infection was observed when the biological control materials were integrated into programs with streptomycin resulting in a reduction in the number of streptomycin applications needed to yield similar levels of control. These results indicate that the prospects for effective biological control of fire blight in conventionally managed orchards in the eastern United States are currently poor due to the variability and inadequacy in performance of existing biological control options.

**SUPPRESSION OF BACTERIAL PALEA BROWNING OF RICE BY BACTERIOPHAGES AND NON-PATHOGENIC BACTERIUM.** K. Azegami and Y. Inoue. National Agricultural Research Center, Tsukuba, Ibaraki, 305-8666, Japan.

Bacterial palea browning of rice, caused by *Erwinia ananas* (*Pantoea ananatis*), occurs in Japan when temperature and humidity are high. It characteristically frequently discolors only paleae to brown, although it sometimes does both paleae and lemmata. The discolored grains sometimes amount to 10% or more on a panicle. It makes hulled rice brown and lighter, and degrades (Yoshida et al. 1983). To search for potential biological control agents, the effects of bacteriophages and non-pathogenic *E. ananas* on the disease occurrence were examined. *E. ananas* is ubiquitously distributed on rice panicles, and the virulence varies with individual isolate (Azegami et al. 1983). So, one of such non-pathogenic *E. ananas* isolates was used. Phages lytic for both pathogenic and non-pathogenic *E. ananas* were isolated from an inflorescence of eulalia (*Miscanthus sinensis*). The results showed that single spray application of the phage(s) or non-pathogenic *E. ananas* at the flowering time, as well as coapplication of them, suppressed the disease. As for bacterial leaf blight of rice, caused by *Xanthomonas oryzae*, only co-application of its phage and non-pathogenic *X. campestris* suppresses the disease occurrence from wounded sites, but single application does not (Azegami et al. 2003, 2004). The possible reasons for the efficacy of *E. ananas* phage(s) are as follows. 1. *E. ananas* proliferates rapidly on anthers (Hasegawa et al. 2003), and the phage(s) was/were sprayed at the flowering stage which is the prime period for infection (Yoshida 1986). 2. Non-pathogenic *E. ananas* inhabiting rice panicles acts as “reservoir”, “medium”, “shelter” from UV light, and “delivery vector” for the multi-host phage.

**INDUCED SYSTEMIC RESISTANCE BY ROOT COLONIZING FLUORESCENT *PSEUDOMONAS* SPP.** Peter A.H.M. Bakker, Plant-Microbe Interactions, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Selected strains of plant root colonizing fluorescent *Pseudomonas* spp. can effectively suppress plant diseases caused by fungal and bacterial pathogens. Direct interactions with the plant pathogen can play an important role in disease suppression, for instance siderophore-mediated competition for iron or the production of antimicrobial metabolites. However, particular strains are effective even when they are applied, and remain, spatially separated from the pathogen on the plant surface. Typically the biocontrol bacteria are applied on the roots of the plant and the pathogen is challenge inoculated on the above ground plant parts, or, in case of soil borne diseases, split root systems are used. When appropriately stimulated, the plant develops a state of enhanced defensive capacity and this mode of action is termed induced systemic resistance (ISR). ISR has been described for several strains of fluorescent pseudomonads in a variety of crop plants and against a wide range of pathogens, including bacterial pathogens. Using the model plant *Arabidopsis thaliana* progress has been made in understanding signal transduction pathways involved in induced resistance. It was established that perception of ethylene and jasmonic acid is important in ISR. Our current understanding of ISR signaling will be reviewed. The traits of the *Pseudomonas* bacteria that can elicit ISR appear to be very diverse. Siderophores, antibiotics, lipopolysaccharides, cyclic lipopeptides, salicylic acid and flagella have all been implicated as bacterial elicitors of ISR. Moreover there appears to be redundancy of ISR elicitors. Future directions of research on both the plant and the bacterial side of ISR will be discussed.

**PRELIMINARY RESULTS OF *In Vitro* ANTAGONIST BACTERIA ON THE DEVELOPMENT OF FUNGI ISOLATED FROM *Brachiaria brizantha* (Poaceae) SEEDS.** M. del S. Balcázar, Á. L. Rivera and B. Pineda L. [m.balcazar@cgiar.org](mailto:m.balcazar@cgiar.org) . Genetic Resources Unit, International Center of Tropical Agriculture (CIAT), Colombia.

Regeneration and multiplication of *Brachiaria* spp. grass germplasm are normally carried out under field conditions in Popayán, Colombia. Under such conditions fungi, such as *Drechslera* spp., *Phoma* spp., *Curvularia* spp., *Sphacelia* spp., *Cerebella* spp., *Curvularia* spp and *Fusarium* spp. can either infest or infect seeds and significantly reduce the quality of the seed produced and stored. Trials with different fungicides proved to be inadequate for disease control. We used an antagonistic bacteria as a preliminary biological control approach for control these fungi. Naturally occurring bacterial isolates were obtained from *Brachiaria* spp. seeds. An *in vitro* assay with three groups (morphology and Gram stain separation) of bacterial isolates was performed. Isolates of bacteria (G1, G2, G3) and fungi (*Drechslera* spp, *Fusarium* spp., *Epicoccum* spp., *Curvularia* spp., and *Phoma* spp. were plated on two artificial media (NA and PDA) and incubated for a week. The growth inhibition zone of the fungi was measured. Results showed that bacterial isolates from group G3 (Gram-negative Bacillus) were most effective. Trials where G3 bacterial filtrates were used resulted in 98.5%, 98.4%, 97.4 %, 96.5% and 90.0% fungal growth inhibition of *Drechslera* spp., *Fusarium* spp., *Epicoccum* spp., *Curvularia* spp, and *Phoma* spp., respectively.



## **EFFECTIVENESS OF *PUNICA GRANATUM* L. EXTRACTS ON KIWIFRUIT AND TOMATO BACTERIAL PATHOGENS.**

G.M. Balestra, A. Quattrucci and A. Rossetti. Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy. Email: [balestra@unitus.it](mailto:balestra@unitus.it).

The control of kiwifruit and tomato bacterial pathogens it is based on appropriate cultural practices (irrigation, fertilization, seed certification) and phytosanitary treatments (preventive cupric treatments). Due to the increase of these bacterioses during the last decade and the EU restriction to use copper in organic agriculture, new control strategies are requested. Peel extracts of *Punica granatum* L. (pomegranate) were tested. The extracts were obtained by ethanol (EtOH) and *in vitro* they were utilised to inhibit the growth of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*, Psa, *Pseudomonas syringae* pv. *syringae*, Pss, *Pseudomonas viridiflava*, Pv) and tomato (*Clavibacter michiganensis* subsp. *michiganensis*, Cmm, *Pseudomonas syringae* pv. *tomato*, Pst and *Xanthomonas vesicatoria*, Xv) bacterial pathogens. Minimal Inhibitory Concentration (MIC) and broth tests were carried out. In MIC tests the *P. granatum* extract was used at 0.05%, 0.5% and 5 % and the bacterial pathogens at  $10^6$  and  $10^8$  cfu ml<sup>-1</sup> concentration, respectively. In broth tests the pomegranate peel extract was utilised at 5% and the bacterial strains at  $10^4$  cfu ml<sup>-1</sup> concentration. In MIC and broth tests all phyto bacteria strains resulted completely inhibited by *P. granatum* extract at 5%. MIC tests gave the most evident results on Cmm and Xv strains. By broth tests, among the Pseudomonads the best growth inhibition was recorded on Psa and Pst strains. Further studies are in progress in the field and to characterize the active principles of *P. granatum* peel extracts to develop a new biocontrol strategy against dangerous bacterial pathogens for kiwifruit and tomato plants.

Research supported by MIPAAF n° 893/2006 project

**ANTIBACTERIAL ACTIVITY OF GRAPE (*VITIS VINIFERA*) WASTE EXTRACTS.** G.M. Balestra, A. Rossetti and A. Quattrucci. Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy. Email: [balestra@unitus.it](mailto:balestra@unitus.it).

In nature there are a large number of antimicrobial compounds that play an important role in the biocontrol of different living organisms. Extracts of herbs, spices and their derivatives are the most common plant materials used for this purpose. Due to the recent EU restriction on copper use in organic agriculture (Reg. EU n° 473/2002) as an alternative to copper compounds, different natural extracts have been recently studied. Here, are reported the results obtained using grape (*Vitis vinifera*) waste extracts obtained from different cv. (Cabernet, Merlot and Sangiovese), to inhibit the growth of different phytopathogenic bacterial pathogens (*Agrobacterium tumefaciens*, *Pseudomonas savastanoi* pv. *savastanoi*, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas vesicatoria*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *actinidiae*, *Pseudomonas syringae* pv. *syringae* and *Pseudomonas viridiflava*). Grape (*Vitis vinifera*) is considered the main fruit crop cultivated in the world and its waste extracts, rich in polyphenols, are potentially utilised in biocontrol of bacterial pathogens. *In vitro* tests were carried out. Spot agar tests shown that grape waste extracts obtained from different grape cv. had an antibacterial activity against all bacterial strains ( $10^6$  cfu ml<sup>-1</sup>) tested. Results obtained, pointed out that these extracts tested could be tested in field experiments for an effective biocontrol of different bacterial pathogens. Further studies are necessary to evaluate greenhouse and field-doses of grape waste extracts and on the possibility to spread them in combination with copper compounds, to reduce the use of cupric ion to control bacterial pathogens.

Research supported by MIPAAF n° 893/2006 project.

**SELECTING BACTERIOPHAGES FOR BIOLOGICAL CONTROL.** B. Balogh<sup>1</sup> and J. B. Jones<sup>2</sup>. <sup>1</sup>Connecticut Agricultural Experiment Station, New Haven, CT, USA; <sup>2</sup>University of Florida, Plant Pathology Dept., Gainesville, USA.

Bacteriophages have been used for control of bacterial plant diseases in a number of pathosystems. In most studies phages were chosen as biocontrol agents solely based on the ability to lyse the target bacterium *in vitro* (i.e., produce plaques). However, our results show that plaque forming ability does not necessarily translate to disease control capability. We found that two of three phages capable of lysing *Xanthomonas axonopodis* pv. *citri* *in vitro*, failed to reduce citrus canker disease severity on grapefruits in greenhouse trials. The two phages that did not reduce disease were not even able to multiply in the phyllosphere. Later we investigated whether it is possible to forecast phage disease control ability based on preliminary tests. Seven phages, which caused different levels of reduction in tomato bacterial spot disease severity, were chosen for the study. We found that *in vitro* characteristics, such as efficiency of bacterial kill or efficiency of multiplication in liquid culture medium, did not correlate with disease control efficacy. Similarly, there was no clear correlation between disease control efficacy and ability to persist or multiply in the phyllosphere.

**AN EFFICIENT METHOD IN GENETICAL DISCRIMINATION OF BIOLOGICAL CONTROL AGENTS BY MOLECULAR ISSR AND RAPD MARKERS.** Ö. Baysal<sup>1</sup>, H. İkten<sup>1</sup>, M. Çalışkan<sup>2</sup> and W. Zeller<sup>3</sup>.

<sup>1</sup>West Med. Agr. Research Inst. (BATEM) Plant Pathology and Molecular Biology Department P:B. 35 07100 Antalya, Turkey; <sup>2</sup>Turkish Ministry of Agriculture and Rural Affairs Central Research Institute for Field Crops, Ankara – Turkey; and <sup>3</sup>Julius-Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany.

Some *Bacillus subtilis* strains are known as prominent biological control agents that show very efficient control when applied against bacterial canker disease caused by *Clavibacter michiganensis* subs. *michiganensis* (*C.m*). In the present study we have determined that although bacteria has no microsatellite in its genome, ISSR markers, which have been selected in previous screening studies, can also efficiently be used in order to discrimination of its genetic variability besides RAPD primers. The efficiency of each strain that has been detected on tests carried out in climatic conditions has been correlated well with their genetic differences. A *Bacillus subtilis* isolate called BS2008 has showed very effective results and resulted in disease control and suppressing on bacterial growth by 74% on tomato plants. Furthermore while we were investigating the reason for this inhibitory effect of BS2008 some regions in its genome have showed significant polymorphism ratio that can be related to suppression effect of S2008 on bacterial growth compared to QST713 and FZB24 which are known as commercial strains. In accordance with the results obtained by molecular tools the effect of BS2008 showed dramatically higher suppression on pathogen *C.m*. than QST713 and FZB24, suggesting a postulated induction of avirulence gene of pathogen by S2008 treatment.

**ABSOLUTE QUANTIFICATION OF SPECIFIC PLANT DEFENCE PATHWAYS AND PSEUDOMONAS SYRINGAE GROWTH IN ARABIDOPSIS THALIANA USING REAL-TIME PCR.** B. Boachon, J.

Robert, and B. Mauch-Mani. <sup>1</sup>Laboratory of Molecular and Cellular Biology, Institute of Botany, University of Neuchâtel, Rue Emile-Argand 11, Case Postale 158, 2009 Neuchâtel, Switzerland.

Monitoring specific plant defence pathways during pathogen infection by measuring the expression of marker genes has been widely used to understand plant-microbe interactions. Different methods are used despite the lack of sensitivity and the difficult task one is faced with the normalising of data based on the expression of housekeeping genes which does not remain constant, especially during plant-microbe interactions. Moreover, quantification of disease development is usually based on visual observations that are subjective or based on time-consuming methods that require many replicates. Here, we present a novel method to measure the implication of SA, ET, JA, ABA and IAA specific plant defence pathways during infection coupled to the quantification of *Pseudomonas syringae* growth. Absolute quantification by real-time PCR for each marker or bacterial growth was done using a standard curve of a well defined cloned gene product. The expression of marker genes and the quantification of the bacterial infection were normalised between samples by the sensitive quantification of cDNA and DNA, respectively. This method could be applied to other plant species and pathogens. The advantages and disadvantage of the method will be discussed.

**BABA-INDUCED RESISTANCE AGAINST *Pseudomonas syringae* IS BASED ON THE INHIBITION OF THE HIJACKING OF PLANT HORMONE SIGNALING BY THE BACTERIA.** B. Boachon<sup>1</sup>, X. Daniel<sup>1</sup>, V. Flor<sup>2</sup>, and B. Mauch-Mani<sup>1</sup>. <sup>1</sup>Laboratory of Molecular and Cellular Biology, Institute of Botany, University of Neuchâtel, Rue Emile-Argand 11, Case Postale 158, 2009 Neuchâtel, Switzerland. <sup>2</sup>Departamento de Ciencias Agrarias y del Medio Natural, Area de Fisiología Vegetal, Universitat Jaume I, Borriol s/n, 12071 Castellon, Spain.

In *Arabidopsis*, BABA-induced resistance against *Pseudomonas syringae* is assumed to be based on the potentiation of defence responses mediated by the SA signalling pathway. In order to further investigate this phenomenon, we monitored this plant-pathogen interaction during well-defined kinetics using sensitive methods. We analysed different levels of response during signalisation and infection establishment including whole transcriptomic changes, real-time PCR quantifications of specific gene expression, and hormone balances. The investigation of the plant hormone homeostasis during infection, as well as the expression of different genes encoding enzymes implicated in the conjugation/deconjugation of SA and JA, suggest that BABA is responsible for a dramatic change in the hormone unbalance that is usually controlled by the bacteria. BABA treatment induces the biosynthesis of SA but does not induce any significant accumulation of the free active form but rather of the glycosylated conjugates of SA. Consequently, *PR1* transient expression also remains low. BABA induces the accumulation of a pool of SA conjugates prior to infection that could be responsible for the BABA-dependent earlier and stronger plant defence activation called priming. On the other hand, BABA inhibits the *Pseudomonas*-dependent reduction of free SA due to its conversion in glycosylated and methylated forms during the infection. Our results demonstrate that priming plants with BABA inhibits the bacterial control of plant defence responses and hormone homeostasis, including JA, ET and ABA pathways. Thus, BABA is responsible for the observed plant defences priming that could be explained by an accumulation of inactive forms of specific hormones. On the other hand, BABA counteracts the bacterial-dependent control of plant hormone and defences. Our results point to an interesting possibility of inhibiting bacterial-dependent plant control that generally leads to efficient disease spread.

**TRANSMISSION OF *RALSTONIA SOLANACEARUM* IN TOMATO SEED AND TRUE POTATO SEED (TPS).** Charlotte Borda<sup>1</sup>; Erik Saenz<sup>1</sup>; Pedro Aley; Lilian Gutarra<sup>2</sup> and Sylvie Priou<sup>2</sup>. <sup>1</sup>University of São Paulo, São Paulo, Brazil, <sup>2</sup>International Potato Center, Lima, Peru.

Bacterial wilt caused by the soilborne bacterium *R. solanacearum*, is one of the most destructive bacterial diseases of economically important crops. Tomato seed and mesocarp extracts were inoculated with different concentrations of *R. solanacearum* race 3/biovar II (10 to 10<sup>8</sup> ufc/ml in tenfold serial dilutions) and incubated at room temperature for 6, 12, 24 and 48 hours with constant agitation (150 r.p.m.) previously incubated at 4<sup>0</sup>C for 2 hours. After enrichment, the extracts were analyzed in plated on medium SMSA, ELISA and double-PCR to repeat the methodology with TPS and potato mesocarp. The method developed in tomato seed inoculated was applied to samples of tomato seed and mesocarp obtained from plants with bacterial wilt symptoms from field infested and plants inoculated in greenhouse with *R. solanacearum*, were analyzed to check the effectiveness of the detection method. Different infected plant parts (roots, stem and fruit) were examined to confirm the presence of the pathogen. The analyses demonstrated the method effectiveness and that *R. solanacearum* is a bacterial that infects tomato seed. Finally, it was examined samples of TPS and mesocarp of two resistant varieties and two susceptible varieties of potato, obtained from plants with bacterial wilt symptoms from fields infected. The analyzes demonstrated that neither TPS nor mesocarp from wild plants of 4 varieties harbor *R. Solanacearum*. Support: CIP

**BIOPESTICIDE RESEARCH - WILL IT END WITH PUBLICATION OR EPA REGISTRATION?** M. P. Braverman, J.J. Baron , D. L. Kunkel and V. S. Starnier. IR-4 Project, Rutgers University, 500 College Road East, Suite 201 W, Princeton , New Jersey 08540, USA.

The regulatory considerations in transitioning from a basic research project into a marketable product will be described including the registration process and requirements, networking to enhance data and waiver development, and information on funding and organizational infrastructure related to product development. This presentation is a primer for scientists involved in university and governmental research programs on how they can integrate regulatory research to satisfy regulatory requirements as a tandem to their research projects. Biopesticide research strategies and waiver construction are important considerations that can greatly assist in the process of bringing products to market.



**UNDERSTANDING THE MECHANISM OF GRAPE CROWN GALL BIOLOGICAL CONTROL BY *A. vitis* STRAIN F2/5.** T. J. Burr, J. E. Creasap, C. L. Reid and D. Zheng Department of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456, USA.

Crown gall disease of grapevines is a limiting factor in grape production worldwide. It is caused by *Agrobacterium vitis*, a host specific species of *Agrobacterium*. In addition to inciting tumors on grapevines, *A. vitis* also causes a necrosis on grape roots and a hypersensitive-like response on certain other plants like tobacco. *A. vitis* survives systemically in vines and therefore is spread in propagation material. Control of grape crown gall is limited to the selection of cultivars and rootstocks that are relatively resistant and by using vineyard management practices that limit the possibility of freeze injuries to vine trunks. Biological control of the disease is also being studied in different labs; *A. rhizogenes* strain K84 is not effective against grape crown gall. We previously demonstrated that non-tumorigenic *A. vitis* strain F2/5 is effective for preventing grape crown gall if applied to wounds on vines prior to inoculation with tumorigenic *A. vitis*. Variable responses are seen when testing F2/5 for control of crown gall by *A. vitis* and *A. tumefaciens* strains on other plant species. We also reported the mechanism of control was not associated with antibiotic production. To study the control further transposon mutants of F2/5 were evaluated for biological control and one mutant with an insertion in a *clpA* homolog was found to be biological control negative. The mutant was also negative for hypersensitive response and had a reduced grape necrosis phenotype. Upstream of the *clpA* gene is a *clpS* homolog, a putative adapter protein that modulates a ClpA-ClpP complex. The Clp protease system functions in protein folding or degradation. A site-directed mutation in *clpA* revealed the same phenotypes as the transposon mutant. The *clpS* mutant was not affected in biological control or necrosis but was hypersensitive response-negative. Interestingly, expression of a truncated *clpA* clone (lacking a putative *clpS* binding domain) in the *clpA* mutant, was biological control and grape necrosis positive but hypersensitive response negative. These results suggest that the *clp* protease system of *A. vitis* plays roles in biological control, grape necrosis and hypersensitive response. ClpS is not essential for biological control but required for the other plant responses. This research provides new insights into mechanisms of biological control and will help to identify how the mechanisms of these responses are related.

**GENOMICS ILLUMINATES MECHANISMS INVOLVED IN BIOLOGICAL CONTROL OF CROWN GALL DISEASE BY *AGROBACTERIUM RADIOBACTER* K84.** Stephen K. Farrand. Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA, and the *Agrobacterium* Sequencing Consortium.

*Agrobacterium radiobacter* K84 is used commercially world-wide as an effective biocontrol agent for crown gall disease caused by *Agrobacterium tumefaciens*. Its derivative, K1026, modified to insure genetic containment, is the only recombinantly engineered microorganism certified for commercial use in the field in the United States. Strain K1026 also is approved for and commercially used in Australia, and in select countries in Africa and Asia. Strain K84 is unique in its ability to control crown gall disease; while often times effective in laboratory or greenhouse tests no other natural isolate tested to date has shown the degree of biocontrol effectiveness in the field exhibited by strain K84. Moreover, attempts to engineer efficient *Agrobacterium*-based biocontrol agents based on other strains have not been successful. Properties thought to contribute to biocontrol by strain K84 include its ability to efficiently colonize roots, its survival in the rhizosphere, and the production of three diffusible agents, an active siderophore and two novel antiagrobacterial antibiotics, agrocin 84 and agrocin 434. We report the complete nucleotide sequence of strain K84. The genome of this bacterium is composed of a 4 Mb circular chromosome, a 2.65 Mb megaplasmid, and three additional plasmids of 388 kb, 185 kb and 44 kb. Gene order on the chromosome is strongly syntenic over its entire length with that of the chromosomes of the nitrogen-fixing symbionts *Rhizobium leguminosarum* 3841 and *R. etli* CFN42. The megaplasmid, while unique in its gene complement compared to such replicons in other sequenced members of the family Rhizobiaceae, contains five genes that are essential for successful life in the wild. Three of these genes, *minCDE*, generally are found on secondary chromosomes in members of the  $\alpha$ -proteobacteria. Although closely related to nitrogen fixing symbionts, the genome of strain K84 does not contain significant homologs of any *nif*, *nod*, or *fix* genes associated with nodulation and nitrogen fixation. Analysis of the sequence predicts gene systems that could or are known to contribute to biocontrol including synthesis of the siderophore and the two agrocin antibiotics, and synthesis of extracellular polysaccharides that may contribute to root colonization. The genome of strain K84 also contains three Non-Ribosomal Peptide Synthetase-like genes, two of which are not found in other members

of the Rhizobiaceae, and two very large genes that could code for RTX-like bacteriocins. The genome of strain K84 codes for one type III and three type IV macromolecular secretion systems. While the function of the type III system is not known, bioinformatics analysis has identified genes coding for several possible substrates for this transporter. The genome sequence indicates that strain K84 is genetically active; there are two intact conjugative transfer systems, one on the chromosome, the other on the 185 kb plasmid which is known to be transmissible. In addition, the 388 kb plasmid codes for an orphan type IV secretion system and the 44 kb plasmid, which also is transmissible, codes for the DNA metabolism portion of a conjugative transfer system. The 185 kb plasmid is of special interest; this element shares significant sequence relatedness with nopaline-agrocinopine-type Ti plasmids such as pTiC58, but lacks any sequence relatedness to T-regions or components of the *vir* regulon found on all Ti plasmids sequenced to date. The analyses presented in this study speak to the unique lifestyle of this organism. Although best known as a biocontrol agent, the sequence predicts that strain K84 has evolved to invade and occupy the niche provided by crown gall tumors induced by nopaline-agrocinopine-type pathogenic agrobacteria.

**BIOLOGICAL CONTROL OF VERTICILLIUM WILT OF TOMATO USING ANTAGONISTIC AND ROOT COLONIZING BACTERIA.** M. Fatmi<sup>1</sup>, Y.A.A. Alkhalayali<sup>2</sup>, and M. Achouri<sup>1</sup>. <sup>1</sup> Institut Agronomique et Vétérinaire Hassan II, Complexe d'Agadir, B.P. 18/S, Agadir, Morocco and <sup>2</sup> University of Sebha, Sebha, Lybia. Corresponding author: [fatmi@iavcha.ac.ma](mailto:fatmi@iavcha.ac.ma).

A procedure of biological control of tomato *Verticillium* wilt based on the use of wild unaltered rhizobacterial strains with high antagonistic activity toward *Verticillium dahliae* Kleb. (*V. dahliae*) and tomato root colonization capacity, was developed. Methods for researching, screening and selecting potential antagonistic bacterial strains were described. *In vitro* evaluation of the collected rhizobacteria showed that 50% of these strains inhibit growth of *V. dahliae*. Six strains were selected for their antagonistic activity toward *V. dahliae* and their capacity for colonization of seeds and roots. Trials for the evaluation of the potential of these strains for biological control of *Verticillium* wilt, were carried out under greenhouse and open field conditions. The *V. dahliae* inoculum was added to the substrate at the point of transplantation at a level of 100 propagules/g of substrate. The tested strains were applied separately in two different ways, as seed bacterization alone or both seed and seedling root bacterization. These strains reduced significantly *Verticillium* wilt incidence as compared to the control (susceptible cultivar) and "Daniela" (a tomato hybrid bearing the *Ve* gene of resistance to *V. dahliae*). The seed and root bacterization method was significantly better than seed bacterization alone. Using seed and root bacterization, the recorded data showed the high performance of the strains YF184 and YF195 as compared to the control and the genetic resistance. Under greenhouse conditions, the percentage of disease reduction was evaluated to 75 % and 69 %, respectively for YF184 and YF195. While, in the case of Daniela, this percentage didn't exceed 15.3 %. The results from open field studies, confirmed the effectiveness of these selected strains. The strains YF184 and YF195 reduced the disease, respectively by 75.2 % and 79.7 % against 42.6 % for "Daniela".

**ISOLATION AND CHARACTERIZATION OF *ERWINIA AMYLOVORA* SPECIFIC BACTERIOPHAGES.** L. Fieseler (1), Y. Born (1), B. Duffy (2), and M.J. Loessner (1). (1) ETH Zurich, Institute of Food Science and Nutrition, Schmelzbergstrasse 7, 8092 Zurich, Switzerland. (2) Agroscope Changins-Wädenswil, 8820 Wädenswil, Switzerland.

*Erwinia amylovora* is the causative agent of fire blight, a severe infectious disease of apple and pear fruit trees. As an alternative to antibiotic treatment we aim to use bacteriophages as a tool for specific detection and eradication of *E. amylovora*. This study further explores diversity of *E. amylovora* specific bacteriophages. We report on phages isolated from soil samples of infected orchards during summer periods of 2007 and 2008 in Switzerland, Europe. Isolated phages belong to the *Podoviridea* and *Myoviridea*. They exhibit clearly different host ranges and genome sizes ranging from 33 kb – 165 kb. Additionally, the phage life cycle was determined and phages were tested for their ability to infect and kill *E. amylovora* in *in vitro* assays. Determination of phage genome sequences will further provide deeper insights into *Erwinia* phage biology.

**ERADICATION OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* FROM MICROPROPAGATED KIWIFRUIT PLANTS (*ACTINIDIA DELICIOSA* CV. HAYWARD).** L. Fratracangeli<sup>1</sup>, M. Muganu<sup>2</sup> and G.M. Balestra<sup>1</sup>.

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Bacterial disease caused by *Pseudomonas viridiflava* and *Pseudomonas syringae* pv. *syringae* are responsible of heavy damage on kiwifruit plants worldwide. This paper refers the results of the *in vitro* culture of shoot tips, meristem tips and thermotherapy applied on kiwifruit of cv. Hayward shoots to obtain the eradication of *P. s. pv. syringae*. Micropropagation of shoot tips was obtained on Murashige and Skoog medium (MS) added with benzylaminopurine. Initially, the value of *P. s. pv. syringae* population detected on individual micropropagated shoots was of  $9,8 \times 10^3$  cfu/cm<sup>2</sup>. Afterwards thermotherapy was applied during 52 days on individual shoots growing in test tube and kept with a photoperiod of 16 h at 36° C and 8 h at 31 °C. At the end of the experiment, meristem tips were excised from elongated buds and placed on MS medium at 24±1° C with a photoperiod of 16 h at 40 µmol m<sup>-2</sup>sec<sup>-1</sup>. *P. s. pv. syringae* population was not detected on elongated shoots submitted to heat treatment. Possibilities to develop specific protocols and also to eradicate vascular phyto bacteria from micropropagated kiwifruit plants are discussed.

Research supported by Regione Lazio, PRAL n° 118/2003 project.

**PSEUDOMONAS FLUORESCENS FOR MANAGEMENT OF BACTERIAL BLIGHT IN COTTON.** R. M. Gade\*, O. V. Ingole<sup>1</sup> and B. R. Patil<sup>1</sup>.

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*Pseudomonas fluorescens* was selected for intensive study on the basis of its *in vitro* antagonistic activity against the most predominant and virulent race of *Xanthomonas auxanopodis* pv *malvacearum*, causing bacterial blight of cotton. Biochemical analysis was carried out for the isolates of *Pseudomonas fluorescens*. The isolates showed positive results for starch hydrolysis, gelatin liquefaction, and H<sub>2</sub>S production. All were found positive for HCN, IAA production. All had capacity to produce siderophore but the level of production was different. Activity of defense related enzymes like esterase, peroxidase, and polyphenol oxidase banding pattern was also detected through Polyacrylamide Gel Electrophoresis (PAGE). Consistent appearances of esterase, peroxidase and polyphenol oxidase isozyme bands were visualized. Pf I was the strongest antagonist of *Xam* which gave an average 1.4 cm zone of inhibition. These isolates were selected for management of bacterial blight in field. Carrier based formulation of *Pseudomonas fluorescens* was tested against *Xanthomonas auxanopodis* pv *malvacearum* causing bacterial blight of cotton. Copper oxychloride @ 0.2% + streptomycin @ 100 ppm foliar application were found effective to minimize bacterial blight incidence (37.41%), intensity (13.08%) with maximum yield (1287 kg/ha). However, seed treatment with *Pseudomonas fluorescens* Pfl @ 10 g/kg seed with three foliar sprays @ 0.2% at 30, 60 and 90 DAG was also found equally effective in reducing incidence (38.34%) and intensity (14.54%) with 1252 Kg/ha seed cotton yield. These two treatments were statistically at par with each other. The incremental cost benefit ratio (ICBR) was maximum (1:3.48) in this treatment as compared to foliar spray of Copper oxychloride @ 0.2% + streptomycin @ 100 ppm (1:2.08). Maximum disease incidence (57.59%) with intensity (32.84%) and minimum seed cotton yield was recorded in untreated control.

**DETECTION AND DIFFERENTIATION OF ANTAGONISTIC *ERWINIA* SPECIES BY PCR AND MALDI TOF ANALYSES.** K. Geider<sup>1</sup>; I. Gehring<sup>1</sup>, A. Wensing<sup>1</sup>, A. Freiwald<sup>2</sup>, and S. Sauer<sup>2</sup>. <sup>1</sup>JKI for Plant Protection, Dossenheim,<sup>2</sup>MPI for molecular Genetics, Berlin, Germany.

The genus *Erwinia* comprises phytopathogenic and non-pathogenic species. The latter can be applied for competition of *E. amylovora* to control fire blight. The species *Erwinia tasmaniensis* and *E. billingiae* were isolated from the apple and pear flora. *E. persicina* and *E. rhapontici* have apparently no or low virulence, lack a hypersensitive response on tobacco and have been detected on many plant surfaces. Both were also isolated from lesions with fire blight. By applying single nucleotide polymorphisms (SNPs) in house keeping genes, we designed specific PCR primers for differentiation of the *Erwinia* species and used PCR applying two annealing temperatures. An SNP can also differentiate strains isolated in Europe/the Mediterranean region and in New Zealand from North American isolates of *E. amylovora*. Other primers specifically detected *E. billingiae* and *E. tasmaniensis*. TaqMan probes identified simultaneously strains of both species and *E. amylovora* by real-time PCR in bacterial samples from flowers after treatment for control of fire blight. For fast detection and differentiation of strains of *Erwinia*-species, protein patterns from whole cells were analyzed by MALDI TOF profiles. The patterns were evaluated with a software program developed by Bruker Daltonik (Leipzig) and matched for differentiation of *Erwinia* species in a dendrogram. For identification of strains in the genus *Erwinia*, protein patterns were matched in a score scheme. MALDI TOF analysis is useful for rapid identification of *Erwinia* species including *E. amylovora*, *E. billingiae* and *E. tasmaniensis* from plant tissue, whereas real-time PCR allows quantitative determination of strains in the genus *Erwinia* even at a low bacterial level and of mixed cultures.



**EVALUATING THE EFFECT OF SOME CHEMICAL AND PLANT-BASED COMPOUNDS AGAINST FIRE BLIGHT DISEASE (*ERWINIA AMYLOVORA*) IN LORESTAN PROVINCE OF IRAN.** M. Haji<sup>1</sup>, N. Hasanzadeh<sup>2</sup>, S. H. Vafaei<sup>1</sup>, and S. Rezaei<sup>2</sup>. <sup>1</sup>Dept. of Plant Protection, Agriculture Collage, Islamic Azad University, Khorramabad branch, Lorestan, Iran and <sup>2</sup>Dept. of Plant Protection, Agriculture Collage, Islamic Azad University, Reaserch & Science branch, Tehran, Iran.

Fire blight (*Erwinia amylovora*) is one of the most important pome-fruits diseases. During 2006-2007 surveys were made on pome fruits orchards in Alashtar, Brojerd and Khorramabad regions. Many infected plant samples were collected from the above-mentioned regions and all isolates were characterized on the basis of routine bacteriological methods. To evaluate effect of some chemical and plant-based compounds on FB pathogen, the selected compounds were first tested in culture media. The appropriate compound/doses were prepared for field trails. The experiment was conducted in year 2006 using a completely randomized design with four treatments, each with three replication in Brojerd region under natural orchard infection. Sprays were done twice at 50% and 100% flowering stages. Treatments were included streptomycin 100 ppm, biozell 0.5:1000, Thymus essential oil (EO) 1:1000 and negative control (water spray). The experiment was repeated in the spring of 2007 with four treatments including streptomycin 100 ppm, Thymus EO 1:1000, starner 300 ppm and negative control treatment (water spray). *In vitro* bioassay results indicated the positive antibacterial effects of chemical compounds as well as thymus EO. On the other hand, statistical field analyses showed significant differences among the treatments at level of 1%. Streptomycin (100 ppm ) with 75 percent blossom infection reduction, gave the most promising result. The efficacy of Thymus, biozell and starner with blossom blight reduction was recorded 38.5%, 56% & 53% respectively. This is worth to mention the field experiments were done in two most severe disease years according to Maryblyt forecasting system and this may support the pronounced effects of the treated compounds.

## **RESISTANCE INDUCTION BY SILICON AGAINST BACTERIAL DISEASES OF TOMATO, EGGPLANT, CUCUMBER AND GERANIUM.**

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To contribute to the development of new integrated practices for the control of bacterial diseases, root and/or foliar application of silicon to eggplant, geranium, tomato and cucumber were investigated. In eggplant and geranium, wilt incidence caused by *Ralstonia solanacearum* was reduced in silicon-treated plants, and initially delayed by two days. Bacterial numbers were significantly reduced in stems of eggplant and geranium. Immunohistochemical studies of possible molecular mechanisms of silicon-mediated resistance on cell wall level in eggplant and geranium showed a strong yellow autofluorescence of the xylem parenchyma and vessel walls, indicating the production of phenolic substances in inoculated plants without silicon application, but not in plants treated with silicon. In geranium, inoculation resulted in an increased staining for (1→5)- $\alpha$ -L-arabinan side chains of rhamnogalacturonan-I in cell walls, which was less in inoculated plants without silicon application. Foliar- and/or root-application with silicon to tomato genotypes with different levels of resistance, and to cucumber, inoculated with *Pseudomonas syringae* pv. *tomato* and *Pseudomonas syringae* pv. *lachrymans* causing bacterial speck in tomato and angular leaf spot in cucumber, respectively, resulted in slightly reduced bacterial speck in a moderately resistant tomato genotype. In cucumber, a weak retardation in the initial development of angular leaf spot was observed. A reduction in bacterial populations was observed in tomato, but not in cucumber. In tomato, the enzyme activity of guaiacol peroxidase was increased after inoculation, but no effect due to silicon treatment was observed, while in cucumber, the enzyme activity was also increased after inoculation, and was higher in silicon-treated plants. A major role of the genetically determined resistance of a genotype was decisive and, thus, silicon-induced resistance can most effectively be triggered in genotypes exhibiting a moderate resistance against a pathogen.

**THE CREATION OF ATTENUATED MUTANTS IN *XANTHOMONAS PERFORANS* FOR USE AS A CONTROL METHOD TOWARD *X. EUVESICATORIA*.** A.P. Hert<sup>a</sup>, M. Marutani-Hert<sup>b</sup>, J.B. Jones<sup>a</sup>, M.T. Momol<sup>c</sup>, and P.D. Roberts<sup>d</sup>. <sup>a</sup>Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA; <sup>b</sup>United States Horticultural Research Laboratory, Fort Pierce, FL, USA <sup>c</sup>North Florida Research and Education Center, University of Florida, Quincy, FL 32060, USA; <sup>d</sup>Southwest Florida Research and Education Center, University of Florida, Immokalee, FL 34142, USA.

Bacterial spot of tomato is caused by several *Xanthomonas* species: *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri*. *Xanthomonas perforans* has been previously shown to produce at least three bacteriocins (BcnA, BcnB, and BcnC) that are antagonistic toward *Xanthomonas euvesicatoria* in the greenhouse and in the field and that deletion of *bcnB* produced the highest level of antagonism toward sensitive *X. euvesicatoria* strains. In this study we looked at several *Xanthomonas perforans* strain 91-118 mutants (*opgH*, *hpaA*, *hpaB*, *hpaC*, *avrBs2*, *xopA*, and *xopD*) for their ability to attenuate *X. perforans* to create a weakly pathogenic strain for use as a delivery system for these bacteriocins in the field. In greenhouse studies, mutants were evaluated for growth rate, disease severity, and antagonism toward *X. euvesicatoria* populations epiphytically and within the plant. Three mutants, in *xopA*, *opgH*, and *gumD*, were significantly less pathogenic than the wild-type strain. The *opgH*- mutant was the most severely attenuated in growth rate while still inhibiting *X. euvesicatoria* populations. The *opgH* mutant strain was further evaluated in the field as a biological control agent toward *X. euvesicatoria*. Seven and fourteen day application intervals of 91-118:: $\Delta$ *opgH* $\Delta$ *bcnB* were evaluated for control of *X. euvesicatoria* populations compared to the grower's standard control (copper hydroxide + mancozeb applied weekly and acibenzolar-S-methyl applied every 2 weeks). The *opgHbcnB* mutant applied weekly significantly reduced *X. euvesicatoria* populations compared to the grower's standard control and plants did not exhibit bacterial spot lesions. *X. perforans* *opgH*- mutant was an effective mutant to reduce pathogenicity while maintaining bacteriocin activity.

**SERENADE MAX<sup>®</sup>, A BIOPESTICIDE FOR USE AGAINST BACTERIAL PATHOGENS IN SPECIALTY FOOD CROPS.** H. Brett Highland PhD., 1069 Eisenhower Dr., Nokomis, FL, USA, and T. Smith PhD., AgraQuest, Inc., 1540 Drew Ave., Davis, California, USA.

Serenade MAX<sup>®</sup>, (Rhapsody ASO<sup>®</sup>, Serenade ASO<sup>®</sup>), active ingredient *Bacillus subtilis* QST 713, a naturally occurring bacterial strain, discovered and commercialized by AgraQuest, Inc., USA, has been shown to possess significant efficacy against a broad spectrum of economically important diseases of fresh market fruits, vegetables, and field crops. In addition to fungal pathogens such as early blight (*Alternaria solani*), botrytis blight (*Botrytis cinerea*), Sclerotinia white mold (*Sclerotinia spp.*), and powdery mildews of various crops (*Uncinula necator*, *Podosphaera leucotricha*, *Leveillula taurica*), Serenade MAX also provides activity of key bacterial diseases, such as bacterial spot (*Xanthomonas campestris*) of tomato and pepper, and fire blight (*Erwinia amylovora*) of pome fruit. Serenade MAX works through novel, multiple modes of action that involve the biological action of *B. subtilis* competing for nutrients on the host surface, in addition to the antimicrobial activity of lipopeptide metabolites produced by the bacteria, causing permeability changes of the cytoplasmic membrane and subsequent disintegration of the pathogen cells. Given the novel, multiple, modes of action, Serenade MAX is utilized as a resistance management tool in rotation and tank mix programs with chemical fungicides, which are susceptible to resistance development due to a specific metabolic site modes of action. *Bacillus subtilis* QST 713 is currently registered in more than 20 countries under the trade name Serenade<sup>®</sup>. As determined by global regulatory authorities, *B. subtilis* QST 713 is exempt from the requirement of a tolerance because there are no synthetic chemical residues, and it is safe to workers and the environment. Serenade MAX has been shown in field trials to be very effective in controlling many bacterial pathogens in agricultural systems, including apples, peaches, tomatoes, peppers, and cucurbits. Use of Serenade MAX is especially useful for export markets which demand reductions in conventional pesticide use and require no-detectable pesticide residues on the harvested commodity.

**BIOLOGICAL CONTROL OF PIERCE'S DISEASE IN THE GREENHOUSE AND FIELD WITH A BENIGN STRAIN OF *XYLELLA FASTIDIOSA*.** D. Hopkins, University of Florida, MREC, 2725 Binion Road, Apopka, FL 32703, U.S.A.

There is no control for Pierce's disease (PD), caused by *Xylella fastidiosa*, in susceptible grapevines. ED92-1, a benign strain of *X. fastidiosa*, provided cross protection against virulent PD strains in susceptible *Vitis vinifera* cultivars in greenhouse tests. In a vineyard test, this benign strain has provided control of PD in Cabernet Sauvignon for 11 years in Central Florida. In current tests in our vineyard and in commercial vineyards in Central Florida, strain EB92-1 is providing control of PD in various genotypes, *V. vinifera*, *V. aestivalis*, and *Vitis* spp. (French/American hybrids). Contrary to many systems, the biological control of PD has been more effective and consistent in the vineyard than in the controlled greenhouse environment. Our hypothesis was that this inconsistency in greenhouse tests was due to very high pathogen inoculation levels. In our needle-pricking inoculations in the greenhouse, each plant was being inoculated with approximately  $10^7$  bacterial cells; whereas, vectors containing as few as  $10^2$  bacterial cells per insect head could transmit *X. fastidiosa*. The effect of the inoculum level of the pathogenic strain of *X. fastidiosa* on control was evaluated on plants treated with biocontrol strain, EB92-1. When the pathogen was inoculated at  $10^4$  or  $10^5$  bacteria per plant, the control was effective and consistent, as occurs in the vineyard. This benign strain of *X. fastidiosa* (EB92-1) has the potential to provide biological control of Pierce's disease in vineyards in Florida and other areas where PD occurs.

**EFFECTS OF SOIL AMENDMENT WITH SILICON FERTILIZER AND SUGARCANE BAGASSE ON TOMATO BACTERIAL WILT UNDER FIELD CONDITIONS IN ETHIOPIA.** G. Hordofa<sup>1</sup>, C. Fininsa<sup>2</sup> and K. Wydra<sup>3</sup>.

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Silicon fertilizer in form of silicon dioxide (Agrosil LR ®) and sugarcane bagasse were tested for their bacterial wilt reducing effect in tomato under field conditions in Ethiopia. In tomato genotype King Kong 2, moderately resistant, bacterial wilt incidence and severity expressed as area under the disease progress curve were reduced by 36.5 % and 40.3%, with a reduction in final incidence and severity of 51.6 % and 57.8 %, respectively, while amendment with sugarcane bagasse reduced the AUDPCs of incidence and severity by 6.1 and 2.9%, respectively. Both amendments also caused a reduction of bacterial numbers in stems. Fruit yield was increased in the infected plants by both amendments, but not in non-infected tomato, with a higher effect of the silicon fertilizer than of the organic material. No significant reduction was observed in the susceptible genotype Marglobe with either treatment. The silicon concentration at the collar region of tomato genotypes was significantly higher in silicon fertilizer amended plants, followed by sugarcane bagasse amended plants than in the control plants. Our data confirm trials under controlled conditions, where a significant silicon effect on bacterial wilt was observed mainly in moderately resistant genotypes.

**BIOLOGICAL CONTROL OF BLACK ROT OF CABBAGE BY NON-PATHOGENIC *XANTHOMONAS CAMPESTRIS* AND BACTERIOPHAGE.**

Y. Inoue and K. Azegami. National Agricultural Research Center, 3-1-1, Kannondai, Tsukuba, Ibaraki, 305-8666 Japan.

The strain of non-pathogenic *Xanthomonas campestris* 98106 isolated from the culm of *Panicum bisulcatum* suppressed the occurrence of black rot of cabbage when sprayed on the leaf, but the effect was not stable. The bacteriophage Xocp1 and Xop20S lyse the strain 98106 and most of the strains of *X. c. pv. campestris*. The mixture of the phage(s) and the strain 98106 suppressed the occurrence of the disease stably. But the effect decreased when the population of the phage(s) was too high. The bacteriophage XcpSFC211 and XcpGTB311 lyse a part of strains of *X. c. pv. campestris* but does not the strain 98106. A high population of the latter two phages reinforced the effect of the strain 98106.

**IMPLICATIONS OF PATHOGENESIS ON STIGMAS TO BIOLOGICAL CONTROL OF FIRE BLIGHT.** Kenneth B. Johnson, Teresa L. Sawyer, Virginia O. Stockwell and Todd N. Temple. Department of Botany and Plant Pathology, Oregon State University Corvallis OR 97331-2902, USA.

Prior to floral infection, *Erwinia amylovora* grows epiphytically on stigmas, which provide a conducive but nonselective habitat for bacterial growth. This nonselectivity allows for biocontrol of fire blight; although, in practice, it is very difficult to exclude *E. amylovora* completely from this habitat. We investigated the dynamics of growth suppression of *E. amylovora* by comparing the ability of virulent and avirulent strains of *E. amylovora* to compete with each other on stigmas of pear and apple, and to compete with a co-inoculated mixture of effective bacterial antagonists. When strains were inoculated individually, virulent *E. amylovora* strain Ea153 attained the highest population size on stigmas with 'epiphytic yields' that were approximately double those of the avirulent derivative or the bacterial antagonists. In competition experiments, growth of the avirulent *hrpL* mutant of Ea153 was suppressed by the antagonist mixture to a greater extent than the virulent strain. Unexpectedly, the virulent strain enhanced the epiphytic yield of the antagonist mixture. Similarly, a small dose of virulent Ea153 added to inoculum of Ea153 HrpL<sup>-</sup> significantly increased the epiphytic yield of the avirulent strain. Virulent gene reporter strain, Ea153 *dspE::gfp*, expressed the green fluorescent protein while growing epiphytically on stigmas of apple. These results are consistent with the hypothesis that virulent *E. amylovora* modifies the epiphytic habitat presented by the stigma through the expression of pathogenesis-related genes, which increases resources available to itself and coincidentally to nonpathogenic competitors. In nine orchard trials, the avirulent Ea153 suppressed incidence of fire blight by an average of 37%, which was less than a 44 % reduction achieved by the antagonist mixture. The degree of biological control achievable with an avirulent strain of *E. amylovora* likely is limited by its inability to utilize the stigmatic habitat to the same degree as a virulent strain.



## **CHALLENGES INVOLVED IN DEPLOYING BACTERIOPHAGES FOR CONTROL OF BACTERIAL PLANT DISEASES. J. B. Jones<sup>1</sup>, Fanny Iriarte<sup>2</sup>, Botond Balogh<sup>3</sup>, Aleksa Obradovic<sup>4</sup>, and M. Timur Momol<sup>1</sup>**

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<sup>3</sup>Department of Plant Pathology & Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT USA; and <sup>4</sup>Plant Pathology Department, Faculty of Agriculture, University of Belgrade, Belgrade-Zemun, Serbia.

Biological control using bacteriophages has proven to be an excellent alternative to conventional chemical control strategies for controlling bacterial spot of tomato. In several studies which we conducted, bacteriophages outperformed standard chemical control practices (principally copper-based compounds) and other biological control strategies. One of the major challenges in deploying bacteriophages relates to the ability of phages to persist at levels necessary for being effective in limiting bacterial populations. Persistence of phages in the phyllosphere is influenced by various factors including the presence or absence of the phage host, sunlight irradiation (especially in the UV zone), temperature, desiccation, and copper-based bactericides. These factors were evaluated in two studies (Balogh 2007, Ph.D. dissertation, University of Florida; Iriarte et al., 2007, Appl. & Env. Microbiol. 73:1704-1711) for persistence of non-formulated phage (nothing added to enhance longevity) and formulated phage (phage mixed with skim milk). Field studies were conducted to address the effects of a copper bactericide and sunlight on phage survival. Copper was shown to adversely affect phage if applied the same day as phage applications but not if applied at least 4 days prior to phage application. Sunlight UV was evaluated for detrimental effects on formulated and non-formulated phage survival on tomato foliage in which phage was applied at four time points during the day (early morning, midmorning, early afternoon, and late evening). In that study in which UV (UVA and UVB) irradiation and phage populations were monitored, populations declined most precipitously in the early afternoon when intensity of UV irradiation was highest. Application of phage in the evening resulted in minimal reduction in phage populations on leaf surfaces when assayed the following morning. There was clearly a negative correlation between UV intensity and phage populations with the protective formulation consistently reducing the deleterious effects of UV. In growth room experiments, ambient temperature was shown to have a pronounced effect on non-formulated phage but not on formulated phages. We also

determined the effects of desiccation and fluorescent light on phage survival. Desiccation without exposure to light caused a slight decline in phage populations after 60 days, whereas desiccation combined with fluorescent light was much more destructive. The formulation protected phage from adverse effects of both factors. Phage persistence was most dramatically affected by UV, while the other factors had much less pronounced effects. The skim milk formulated phage reduced deleterious effects of the studied environmental factors. Combining the use of formulated phage with evening applications potentially should optimize efficacy. Future research should focus on developing other strategies to optimize stability of phages on the leaf surface.

**MANAGEMENT OF BACTERIAL BLIGHT ON COTTON AND RICE WITH BACTERIOPHAGES.** E. Kamilova<sup>1</sup>, S. Monakov<sup>1</sup>, T. Gabisonia<sup>2</sup>, M. Loladze<sup>2</sup>, and K. Severinov<sup>3</sup>. <sup>1</sup>Institute of Genetics and Plant Experimental Biology, AS RUz, Tashkent Region, 102151, Kibrai District, P/o Yukori-Yuz, Uzbekistan; <sup>2</sup>G. Eliava Institute of Bacteriophage, Microbiology and Virology, AS G, Gotua Street 3, Tbilisi, 380060, Georgia; <sup>3</sup>Rutgers University, Waksman Institute, 190 Frelinghuysen Road, Piscataway, New Jersey 08854-8020, USA.

Gram-negative bacteria of the genus *Xanthomonas* are plant pathogens that infect many economically significant crops. Infection spreads by seeds. A recent survey showed that cotton as well as rice were affected by *X. oryzae* and *X. malvacearum*, leading to extremely large yield losses (e.g. 15 - 60% (Bilay, Gvozdyak, Scripal, 1988) of cotton in Tashkent region, 20% of rice in Karakalpakia region). Lytic bacteriophages, bacterial viruses, have narrow "specialization" in damaging and killing phytopathogenic bacteria but do not endanger the environment otherwise. Our collection of bacteriophages has been isolated using well-known techniques (F.B. Iriarte et al, 2007, A. Sulakvelidze, 2005) from environmental substrates which contain phytopathogenic bacteria: soil, water – both irrigating and draining, herbarium of diseased plants, seeds of infected plants, etc. The main advantage of the invention is development of a preparation to treat and prevent bacterial plant diseases by the method which is an alternative to chemical bacteriocides that can have negative effects on the environment. Prevention and fight against bacterial diseases of agricultural plants are carried out using a mixture of bacteriophages specific to strains of the bacteria. Bacteriophages are used to treat the following objects: infected seeds, diseased plants or plant materials, soil, etc. where the infected plants are growing, irrigation water, soil in other sites, soil supplements. The set of bacteriophages and the method of its application have been described. A polyvalent concentrate of bacteriophage reduces infection of plants with bacterial diseases by 50-90% contributing to increased yield.

**BIOLOGICAL CONTROL OF CITRUS BACTERIAL CANKER DISEASE USING *PSEUDOMONAS FLUORESCENS* AND *P. PUTIDA* STRAINS.** Gh. Khodakaramian<sup>1</sup>, G. M. Balestra<sup>2</sup> and A. Heydari<sup>3</sup>. <sup>1</sup>Department of Plant Protection, Hamedan University, Hamedan, Iran. <sup>2</sup>Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università della Tuscia, Viterbo, Italy. <sup>3</sup>Iranian Research Institute of Plant Protection, Tehran-Iran. Email: [asheydari@hotmail.com](mailto:asheydari@hotmail.com).

Bacterial citrus canker (*Xanthomonas axonopodis* pv. *citri*) is one of the most important and serious diseases of citrus trees in Iran. Selected Pseudomonads (*Pseudomonas fluorescens* and *P. putida*) bacterial strains, isolated from citrus orchards in southern Iran, were utilized *in vitro* and in greenhouse experiments against known *X. a. pv. citri* strains. All biocontrol agents utilised *in vitro* experiments were effectiveness with various antagonistic activities on *X. a. pv. citri*. The most active strains were selected and then were evaluated in the greenhouse experiments against the agent of citrus canker disease. Two *P. fluorescens* strains were particularly effective in reducing citrus canker leaf spot symptoms. Results in greenhouse experiments were promising and pointed out selected strains able to reduce the number of citrus disease spots between 23.8 to 64.0%. Based on the results, biocontrol strategies seem to be potentially effective if applied as component of different and integrated approaches to control and to manage citrus canker disease.

**GENOME COMPARISON OF THE BACTERIAL FIRE BLIGHT ANTAGONISTS *ERWINIA BILLINGIAE* AND *E. TASMANIENSIS*.** M. Kube<sup>1</sup>, R. Reinhardt<sup>1</sup>, I. Müller<sup>2</sup>, and K. Geider<sup>2</sup>. <sup>1</sup>MPI for molecular Genetics, Berlin; <sup>2</sup>JKI for Plant Protection, Dossenheim, Germany.

The genomes of the fire blight antagonists *Erwinia tasmaniensis*, strain Et1/99, and *Erwinia billingiae*, strain Eb661, have a size of 3.8 and 5.1 Mb, respectively. A plasmid of *E. tasmaniensis* encoding a bacteriocine is typical for strains from Australia. Its absence in strains from other origins renders these susceptible to tasmancin. Both epiphytic species share a substantial amount of genes, but differ in some important aspects. *E. tasmaniensis* cannot utilize sorbitol. *E. billingiae* cannot metabolize sucrose and is also deficient in levan formation lacking the responsible *Isc* gene. The inability of *E. tasmaniensis* to synthesize capsular exopolysaccharide (EPS) despite of the presence of an EPS synthesis encoding region similar to *E. amylovora* or *Pantoea stewartii* can be overcome by increasing expression of EPS activator proteins. *E. tasmaniensis* strains are able to cause a hypersensitive response in tobacco leaves in contrast to *E. billingiae*. An *hrp*-gene cluster is present in the genome of strain Et1/99 but absent in the genome of Eb661. *E. billingiae* synthesizes large quantities of EPS, which is related in the sugar composition to EPS of other *Erwinias*. It can be degraded by a viral EPS depolymerase indicating similar sugars in the backbone of the repeating unit as found for amylovoran. Judged from sequence comparison of house keeping genes, *E. billingiae* strains are more divergent than *E. amylovora* and *E. tasmaniensis*. A population of *E. tasmaniensis* is apparently more stable in apple flowers of orchards than cells of *E. billingiae* surpassing this species for control of fire blight.

**CHARACTERIZATION OF BACTERIAL ANTAGONISTS AND THEIR RESISTANCE INDUCING EFFECT AGAINST BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM* IN TOMATO.** H. Kurabachew, K. Wydra. Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany. [wydra@ipp.uni-hannover.de](mailto:wydra@ipp.uni-hannover.de).

Among 150 bacterial strains isolated from tomato and potato rhizosphere soil from Ethiopia, 15 showed antagonistic activity against *R. solanacearum* in *in vitro* tests. Strains were so far identified by fatty acid analysis as *Bacillus cereus* and *Pseudomonas putida* biotypes A and B, and characterized by colony morphology, oxidase and catalase test, gelatine liquefaction, levan formation, starch hydrolysis, carbohydrate utilization and growth at different salt concentrations. Key compounds of plant growth promoting (PGP) activity were tested by determination of the production of siderophores, indole-acetic acid and hydrogen cyanide and the ability to solubilize phosphate. The strains were variable in acyl-homoserine lactone (AHLs) production, the common quorum sensing signal, using cross-feeding assays with selected antagonists and the mutant biosensor strain *Chromobacterium violaceum* CV026. In *ad planta* tests with 5 antagonists on the moderately resistant tomato genotype King Kong 2 and the susceptible L390, disease severities and incidences were reduced in both genotypes, with a reduction of bacterial multiplication in stems by 16.0%-24.7% and 27.0%-33.6%, respectively, depending on antagonist applied. Split root tests applying the antagonists to one pot apart from the pot with *R. solanacearum* inoculation confirmed the reduction of disease development and bacterial numbers in antagonist treated plants indicating the induction of a rhizobacteria-induced systemic resistance (ISR). Increases in plant biomass in antagonist-treated plants indicated the plant growth promoting activity of the strains. Enzymatic assays and q-RT-PCR for quantification of key enzymes of signaling pathways are ongoing.

**DISCOVERY AND DEVELOPMENT OF *PSEUDOMONAS FLUORESCENS* STRAIN A506 FOR SUPPRESSION OF *ERWINIA AMYLOVORA* AND OTHER PLANT COLONIZING BACTERIA.** Steven Lindow. Dept. Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102 USA. Email: [icelab@berkeley.edu](mailto:icelab@berkeley.edu).

*Pseudomonas fluorescens* strain A506, identified in an *in planta* screen as successful in preemptive competitive exclusion of ice nucleation active *Pseudomonas syringae* strains leading to a lower incidence of frost injury, was subsequently found to be highly effective in reducing growth of *Erwinia amylovora* on pear flowers. Application of this strain to pear and apple flowers shortly after opening has led to substantial reductions in the incidence of fire blight disease, frost injury, and fruit russet (incited by a variety of 3-indole-acetic acid-producing bacteria) in field studies. As strain A506 is naturally resistant to both streptomycin and oxytetracycline it has been successfully integrated into pear and apple management schemes in which antibiotics and other pesticides are applied when incompatibilities have been identified and accounted for. Strain A506 migrates efficiently to untreated flowers if they open within several days of the application to early-opening flowers enabling fire blight control to trees with only 3 or fewer spray applications. Application of strain A506 with organosilicon surfactants having a low surface tension to unopened flower buds enable its subsequent colonization of flowers as they open without the need for direct application to flowers. Since this strain is an excellent colonist of many plant surfaces it has emerged as a model organism for use in microbial ecology studies in which the plant environment has been interrogated at small spatial scales. The movement and hence invasion/virulence of behavior of *P. syringae* is strongly suppressed by production of n-acyl homoserine lactones (AHLs) produced by neighboring species on plants. Such naturally-occurring strains or transgenic AHL-producing variants of antagonists such as strain A506 are attractive as agents of a new process of biological control.

**GENOMICS OF SECONDARY METABOLITE PRODUCTION BY *PSEUDOMONAS FLUORESCENS* Pf-5.** Joyce E. Loper<sup>1</sup>, Ian Paulsen<sup>2</sup>, Denny Bruck<sup>1</sup>, Maria Pechy-Tarr<sup>3</sup>, Monika Maurhofer<sup>4</sup>, Christoph Keel<sup>3</sup>; and Harald Gross<sup>5</sup>. <sup>1</sup>U.S.Department of Agriculture, Agricultural Research Service, Corvallis, Oregon, USA; <sup>2</sup>The Institute for Genomic Research, Rockville, Maryland, USA; <sup>3</sup>Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; <sup>4</sup>Phytopathology/Institute of Integrative Biology, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland; and <sup>5</sup>Institute for Pharmaceutical Biology, University of Bonn, Germany.

The rhizosphere bacterium *Pseudomonas fluorescens* Pf-5 is known to produce six secondary metabolites, and the genomic sequence of Pf-5 revealed three additional gene clusters, which encode for the biosynthesis of unknown natural products but contain conserved sequences of genes encoding for non-ribosomal peptide synthetases or polyketide synthases. Natural products synthesized from two of these orphan gene clusters have since been identified. Orfamide A, a novel cyclic lipopeptide produced by Pf-5, contributes to swarming motility of Pf-5 and has the capacity to lyse zoospores produced by phytopathogenic *Phytophthora* spp. (Gross et al. 2007. Chem. Biol. 14: 53-64). Several analogs of rhizoxin, a macrocyclic lactone with antifungal activity and phytotoxicity, are synthesized from a large biosynthetic gene cluster in the Pf-5 genome (Loper et al. 2008. Appl. Environ. Microbiol. 74:3085-93). Linked to the rhizoxin biosynthetic locus is a cluster of genes encoding for an insect toxin termed FitD, which is related to Mcf (for “makes caterpillars floppy”) produced by *Photorhabdus luminescens*, an inhabitant of the gut of entomopathogenic nematodes in the genus *Heterorhabditis*. If injected into the hemocoel, Pf-5 kills caterpillars of tobacco hornworms (*Manduca sexta*) whereas a *fitD* mutant of Pf-5 is less virulent (Péchy-Tarr et al. 2008. Environ. Microbiol. PMID: 18484997). The genomic sequence of *P. fluorescens* Pf-5 provides a variety of insights and into this organism’s interactions with plants and other organisms in the environment.



**COMMERCIAL PERSPECTIVE ON THE REGULATORY AND DEVELOPMENT PROCESS FOR BIOPESTICIDES.** Pam Marrone, CEO and Founder of Marrone Organic Innovations, Inc., Davis, CA, USA. [pmarrone@marroneorganics.com](mailto:pmarrone@marroneorganics.com).

The global chemical pesticide market is approximately \$30 billion and has had no real growth in ten years. The market for biological pesticides is growing at 20% per year and is expected to reach \$1 billion in sales by 2010. While biopesticides often get pigeonholed into products only for organic production, the facts are that more than 75% of biopesticides are used in conventional programs. Today's biopesticides offer a combination of benefits increasingly being recognized and valued in the market. Some of the reasons they are being used more and more in conventional programs are, 1) equal or higher yields and quality, 2) resistance management, 3) short pre-harvest intervals - residue management for exported crops, 4) worker safety and short re-entry intervals, 5) environmentally friendly. Getting a pesticide registration can have its challenges, but most of the challenges are after the registration is granted. Most small companies underestimate the requirements and costs for market adoption. This talk will provide an overview of the market for biopesticides, briefly describe biopesticide discovery screening and development, and provide a commercial perspective on the regulatory process.

**SAFETY, REGULATION AND COMMERCIALIZATION OF BACTERIAL BIOCONTROL AGENTS IN THE EUROPEAN UNION.** Emilio Montesinos<sup>1</sup>

and María M. López<sup>2</sup>. <sup>1</sup>Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, Spain. Email: [emonte@intea.udg.edu](mailto:emonte@intea.udg.edu);

<sup>2</sup>Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología, Valencia, Spain.

An important issue in microbial pesticides is biosafety which is necessary to avoid nontarget effects on plants, animals, or on the environment, because the isolation of a given microorganism from plants obviously is not a proof of safety. However, risk evaluation is a very complex task, because the risk of nontarget effects caused by a strain of a given microorganism is estimated from the intrinsic toxicity–pathogenicity, the degree of exposure, and the susceptibility of the possible receiver. Most strains of microbial pesticides meet biosafety rules. However, in a few cases, there are uncertainties regarding the potential risk, because human isolates reported in clinical opportunistic infections cannot be distinguished from environmental isolates, which may be part of the normal microbiota found in plants and could be present in several plant-derived fresh products. These uncertainties are the reason for specific and differing regulations on risk classification of microorganisms among countries. In the European Union, the registration and authorization of microbial pesticides as plant protection products (PPP) depends on the Health and Consumer Protection Directorate (SANCO), and is regulated by the Directives 2001/36/EC and 2005/25/EC. Under these regulations only a few products have been authorized to be commercialized in the last ten years. Recently, the European Food Safety Agency (EFSA) has been involved in the registration and the establishment of a Qualified Presumption of Safety (QPS) for every candidate microorganism under study, may facilitate the registration process. A second possibility is open at specific country level, consisting on a simple notification and a reduced dossier, in cases where the microorganism can be considered as non-biocide or non-fertilizer, or as a plant enhancer. A few examples will be provided and discussed on successful and unsuccessful registration of microbial biocontrol agents in the European context.

## **BACTERIOPHAGE TRANSLOCATION IN TOMATO PLANTS AND PROSPECTS FOR CONTROL OF TOMATO BACTERIAL WILT. A. Obradovic<sup>1</sup>, F. B. Iriarte<sup>2</sup>, G. V. Minsavage<sup>3</sup>, J. C. Hong<sup>3</sup>, T. M. Momol<sup>3</sup>, and J. B. Jones<sup>3</sup>.**

<sup>1</sup>University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia; <sup>2</sup>Iowa State University, Plant Pathology Dept., Ames, IA; <sup>3</sup>University of Florida, Plant Pathology Dept., Gainesville, FL, USA.

Bacterial wilt caused by *Ralstonia solanacearum* is a serious soilborne disease of many economically important Florida crops, such as tomato and potato, and is caused predominantly by race 1, biovar 1. Since it is caused by a soilborne pathogen with a wide host range, bacterial wilt is very difficult to control once it becomes established in the field. Although considerable effort has focused on identifying effective chemical treatments, extensive research has focused on identifying biological control agents for use in plant protection. Bacteriophages have been utilized for controlling plant pathogens either in the rhizosphere or phyllosphere. In order to use phages for control of this soilborne vascular tomato pathogen, it was necessary to determine the ability of phages to survive in the rhizosphere and the possibility to be absorbed by the plant root system and translocated to above-ground parts. Therefore, we tested the systemic nature of bacteriophages by using tomato plants and specific phage strain as a model system. In the first experiment four-week old tomato plants with either damaged or undamaged roots were drenched with phage suspension (approx.  $10^8$  PFU/ml). In three other experiments only undamaged roots were used. Tissue samples were cut from several locations on the plants and analyzed for phage concentration. During a two-week period of observation, the phage strain was constantly detected in the rhizosphere of drenched tomato plants, proving ability to maintain its population without the presence of compatible host. Detection of phages in roots and above-ground plant parts provided evidence for phage uptake through the tomato root system and translocation within the plant tissue. In order to study effectiveness of the host specific phage treatment for control of tomato bacterial wilt in controlled conditions, three-week old tomato plants were inoculated with bacterial wilt pathogen and drenched with a suspension of lytic phages (approx.  $10^8$  PFU/ml) applied either three days before, following inoculation, and/or three days after inoculation. The most effective wilt control was achieved in treatments based on phage application following inoculation. This indicates that phage application can contribute to control of the disease even in conditions of artificial inoculation favoring infection. Consequently, such treatment could potentially play an important role in the integrated disease management strategy.

**ENHANCEMENT OF INDUCE SYSTEMIC RESISTANCE ON CUCUMBER BY *BACILLUS VALLISMORTIS* EXTN-1 WITH L-ALANINE.** Kyungseok Park, Diby Paul<sup>a</sup>, Srinivasan Bharathkumar, Young Ki Lee and Sang Yeb Lee, Sung Sook Han. \*Plant Pathology Division, National Institute of Agricultural Science and Technology, RDA, Suwon. 441-707, South Korea., [kspark@rda.go.kr](mailto:kspark@rda.go.kr). <sup>a</sup>Dept. of Environmental Engineering, Konkuk University, Seoul, 143-701 South Korea.

*Bacillus vallismortis* strain EXTN-1 is a potential elicitor agent that induces systemic resistance in many crops against various pathogens. In an effort to boost this bacterial ISR with a chemical inducer of ISR, L-Alanine was experimented in cucumber. Both L-Alanine and EXTN-1 brought about significant levels of disease suppression in cucumber against Anthracnose disease. When cucumber plants were treated with EXTN-1 and L-Alanine together there was augmentative disease suppression. Treatment with different concentrations of L-Alanine (10 - 250ppm) on PR-1a::GUS transgenic tobacco plant showed strong GUS activity. In addition to that the treatment also induced the expression of resistance genes PR-1a and PDF 1.2 in transgenic (PR-1a or PDF 1.2 over expressing) *Arabidopsis* plant, as confirmed by RT-PCR analysis. The defense gene activation was higher with EXTN-1 than L-Alanine and even higher with EXTN-1 and L-Alanine together.. Inclusion, simultaneous application of L-Alanine on EXTN-1 treated plants showed augmentative induction of systemic resistance against cucumber anthracnose. This throws light on the fact that an SA mediated and JA mediated systemic resistance mechanisms act in an additive mode to bring about a cumulative effect. This cumulative ISR brings about higher disease suppression in plants. To our knowledge, this is the first report of use of L-Alanine as an ISR-elicitor. This could be an effective strategy in a practical scale to boost rhizobacteria mediated ISR in crops.

**CHARACTERIZATION OF MUTANTS OF *AGROBACTERIUM* STRAIN K84 AFFECTED IN PRODUCTION OF EXOPOLYSACCHARIDES, SURFACE MOTILITY AND BIOFILM FORMATION: THEIR ROLE IN THE BIOCONTROL OF CROWN GALL.** R. Penyalver<sup>1</sup>, L.P. Burbank<sup>2</sup>, S.B. von Bodman<sup>2</sup> and M. M. López<sup>1</sup>. <sup>1</sup>IVIA, Ctra. Moncada-Naquera Km 5, 46113 Moncada, Valencia, Spain. <sup>2</sup>Department of Plant Science, University of Connecticut, 302 B Agricultural Biotechnology Laboratory, Storrs, CT 06269, USA.

*Agrobacterium rhizogenes* strain K84 is a commercial biocontrol agent utilised worldwide to control crown gall disease. Strain K84 produces several exopolysaccharides (EPS), display surface motility in soft agar medium and form biofilms on polypropylene wells. In order to establish whether or not these bacterial traits are important for biocontrol of crown gall, we have begun a detailed analysis of them, and 1700 transposon-induced mutants were independently analyzed for these phenotypes. We identified 26 mutants defective or displaying abnormal patterns and they were grouped into four categories based on their EPS, surface motility and biofilm formation patterns. In the first one, 16 mutants moved less than the wild-type, were generally less mucoid and showed normal or stronger calcofluor binding reaction but displayed normal for biofilm formation. Among the mutated genes of this mutant category were two genes involved in the nucleoside metabolism (*udp* and a permease ABC transporter gene), a GGDEF family gene (*yhe00032*), a putative transcriptional regulator, a peptide ABC transporter gene, the two component regulatory system *feuPQ*, the C-terminal processing peptidase *ctpA*, and two genes involved in EPS synthesis: the *ndvB* gene required for the synthesis of cyclic beta 1-2 glucan and a glycosyl transferase involved in the synthesis of succinoglycan. In the third category, seven mutants moved further than the wild-type, were normal or more mucoid, displayed generally a normal calcofluor binding reaction and biofilm formation. Among the mutated genes were *etfAch*, an electron transport flavoprotein and a polysaccharide deacetylase gene involved in EPS metabolism. In the third group, one mutant did not form biofilms on polypropylene plates, showed a slightly weaker calcofluor reaction but displayed normal for surface motility. The mutated gene was *wcbD*, an LPS export protein likely involved in the synthesis of a capsular polysaccharide. The last category was formed by two mutants that displayed enhanced ability to form biofilms but showed normal mucosity, calcofluor binding and surface motility. One of the mutated genes was *rkpK*, a UDP-glucose 6-dehydrogenase involved in LPS biosynthesis. Preliminary results on the efficacy of these 26 mutants on the biocontrol of crown gall caused by tumorigenic *Agrobacterium* strains sensitive and resistant to agrocin 84 will be presented.

**NICOTINIC ACID DEGRADATION: A NOVEL METHOD FOR SELECTION OF A BIOCONTROL AGENT AGAINST *ERWINIA AMYLOVORA*.** Thomas Paternoster<sup>1,2\*</sup>, Brion Duffy<sup>3</sup>, Geneviève Défago<sup>1</sup>. <sup>1</sup> Phytopathology group, Swiss Federal Institute of Technology, ETH-Z, CH-8092 Zürich, Switzerland; <sup>2</sup> SafeCrop Institute, San Michele all'Adige, Trentino, Italy; <sup>3</sup> Agroscope Changins-Wädenswil, Swiss National Competence Center for Fire Blight, CH-8820 Wädenswil, Switzerland.

*Erwinia amylovora* is the causal agent of fire blight, often devastating disease of apple, pear, and other rosaceous plants. The disease is usually initiated by epiphytic populations of *E. amylovora* developing on blossoms. The pathogen requires nicotinic acid and thiamine as growth factors in laboratory culture media. Unlike thiamine, which is required by a few wild-type strains, nicotinic acid is a specific requirement among species of the genera *Erwinia*. Starting from these evidences, the aim of the work was to select a new biocontrol agent against fire blight making use of this specific pathogen's peculiarity. For this purpose, a collection of 735 bacteria and 1237 epiphytic yeast, respectively, was created from apple and pear flowers collected in orchards situated in different locations of Switzerland and Trentino. The whole collection was screened for the ability to degrade nicotinic acid in a system based on the use of nicotinic acid as sole N-source. Ten percent of the isolates showed this capacity. Among several isolates tested, JAN strain displayed the best growth performance and the strongest biocontrol effect against *E. amylovora* in pear slice bioassay. JAN1 F12 strain was characterized as *Pseudomonas rhizosphaerae* species by 16S rDNA gene sequence analysis. The strain proved to be a competent colonizer of apple blossoms, and strongly suppressed pathogen growth in detached flower assays and in greenhouse flowering apple tree trials with reduced development of blossom blight.

**PRELIMINARY RESULTS ON THE BIOCONTROL OF BACTERIAL DISEASES OF CULTIVATED MUSHROOMS BY USING POTENTIAL ANTAGONISTIC BACTERIA.** S. Prashanth, P. Lo Cantore, and N. S. Iacobellis. Dipartimento di Biologia Difesa e Biotecnologie Agro Forestali, Università degli Studi della Basilicata, viale Ateneo Lucano, 10, 85100 Potenza, Italy. [iacobellis@unibas.it](mailto:iacobellis@unibas.it).

Mushrooms are becoming increasingly popular among consumers and there is increased market interest and demand for mushrooms all over the world. The market for mushrooms continues to grow due to interest in their culinary, nutritional, and health benefits. Mushrooms contain many essential amino acids, unsaturated fatty acids, vitamin D and several of the vitamin B group. Some of them even contain significant levels of vitamin C as well as the minerals potassium, phosphorus, calcium, and magnesium. Among various diseases on cultivated mushrooms, bacterial ones may be limiting factors on these crops. Bacterial brown blotch disease on *Agaricus bisporus* and *Pleurotus ostreatus*, caused by several bacteria including *Pseudomonas tolaasii* and *P. reactans*, are endemic in mushroom farms and are the cause of significant yield and mushroom quality losses. *P. reactans* is also the cause of the yellowing of *P. eryngii*. Chemical control by using chlorine are still used for the bacterial diseases control but the limited efficacy of this measure, the concomitant possible toxicity of the above substance and the increasing concern over the use of chemicals for the disease management suggest the need for alternative methods for the mushroom diseases management. Hence, in the present study efforts were taken to control disease by the use of potential antagonistic bacteria. Among 234 bacterial isolates, obtained from the niches of *Agaricus bisporus*, *Pleurotus ostreatus* and *P. eryngii*, 48 and 30 ones showed *in vitro* antagonistic activity against *P. tolaasii* and/or *P. reactans*, respectively. Fifteen of them were active against both the pathogens. Among the latter six isolates were shown to produce hydrolytic enzymes (i.e. pectinase, cellulase, protease, glucanase, chitinase and amylase). Twenty-four isolates showing in *in vitro* assay zone of inhibition against both *P. tolaasii* and *P. reactans* above 15 mm were selected and further tested in *A. bisporus* tissue block assay for their efficacy to reduce symptoms. Among the selected isolates two significantly reduced symptoms caused by  $10^7$  and  $10^6$  cfu/ml population of *P. tolaasii* whereas three others reduced symptoms when  $10^6$  cfu/ml population of *P. tolaasii* were used. Three and six isolates reduced symptoms caused by  $10^8$ ,  $10^7$  and  $10^6$  cfu/ml population of *P. reactans* and by  $10^7$  and  $10^6$  cfu/ml

population of *P. reactants*, respectively. The above bacteria, which reduced symptoms severity of blotch disease in *Agaricus* tissue blocks against both pathogens are potential antagonists to be used in biological control of brown blotch disease in mushrooms cultivation. Further studies are in progress involving the bacterial isolates in brown blotch disease management.



**ANTIBIOSIS AND ACIDIFICATION MAY CONTRIBUTE TO SUPPRESSION OF *ERWINIA AMYLOVORA* BY *PANTOEA AGGLOMERANS* STRAIN E325.** P.L. PUSEY (1), V. O. Stockwell (2), and D. R. Rudell (1). (1) USDA-ARS, Wenatchee, WA, USA; (2) Oregon State University, Corvallis, OR, USA.

*Pantoea agglomerans* strain E325, a commercially-available antagonist for fire blight of apple and pear, was originally selected through screening based on suppression of *Erwinia amylovora* on flower stigmas, but specific mechanisms of antagonism were unknown. Bacterial modification of pH was evaluated as a possible mechanism by analyzing stigma exudates extracted from 'Gala' apple stigmas. The pH values for field samples were only slightly lower than controls, but indicated a range (pH 5-6) conducive for antibiotic activity according to subsequent assays. Under low-phosphate and low-pH conditions, an antibacterial product of E325 with high specificity to *E. amylovora* was effective at low concentrations. A minimum of 20 to 40 ng of a ninhydrin-reactive compound purified using RP-HPLC caused visible inhibition in assays. Activity was heat stable and unaffected by amino acids, iron, or enzymes known to affect antibiotics of *P. agglomerans*. Antibiosis was diminished, however, under basic conditions, and with increasing phosphate concentrations at pH 6 and 7. Inhibition was not observed in media containing phosphate concentrations commonly used in antibiosis assays. We propose that E325 suppresses the fire blight pathogen not only by competing for nutrients on the stigma, but by producing an antibiotic specific to *E. amylovora*. Further work is necessary to substantiate that the compound is produced and active on flower stigmas.

**A NEW ANTIBIOTIC OF 21<sup>ST</sup> CENTURY FROM LIVESTOCK EXCREMENTS.** K. Rajamohan, **V. Kurucheve** and S. Usharani. Department of Plant Pathology, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India. E-mail: [vkurucheve@yahoo.co.in](mailto:vkurucheve@yahoo.co.in)

India has the largest area under rice, however, the productivity is far below (2 tonnes/ha) than that of China (6-7 tonnes/ha), which has the second largest area. In spite of all available means of plant protection about one-third of agricultural production is lost by pests. Natural products like livestock excrements were reported to possess antimicrobial and sanitizing properties as mentioned in the Vedhas books but so far, there was only scanty literature available. Hence, a study was initiated using various excreta (urines and dungs of cow, buffalo, sheep; manures of poultry and turkey) and garlic bulbs against bacterial blight pathogen of rice, *Xanthomonas oryzae* pv. *oryzae*. All the aqueous extracts (60%) significantly inhibited the growth of the bacteria and found superior than streptomycin sulphate. Combinations of buffalo and sheep excrements; sheep dung plus poultry manure and turkey plus poultry manure exhibited total inhibition even at 40% concentration thus, synergism was observed. There was no rejuvenation of the growth upon re-inoculation thereby bactericidal nature of toxicity was documented. The toxicity was retained as such for three months of storage under air tight containers. Maximum inhibition zone, further merger of such zones and surrounding area also free from the bacterial colony was observed in sheep excrements alone. All the natural products reduced the disease incidence significantly under two pot trials conducted. They also reduced the sugar content and influenced more on the growth and yield parameters; phenols, chitinase and  $\beta$ 1-3 glucanase activity. Among them, sheep excrements were found supremacy over others. Thus, livestock wastes particularly sheep excreta can be recommended as an alternative to streptomycin sulphate because of the above said advantages and thereby the biodiversity can be maintained.

**GENOTYPIC COMPARISON OF PANTOEA AGGLOMERANS BIOCONTROL AND CLINICAL ISOLATES TO ADDRESS TAXONOMIC AND BIOSAFETY QUESTIONS.**

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*Pantoea agglomerans* strains have recently been registered for agricultural application in the US, Canada and New Zealand for biocontrol of fire blight. This destructive bacterial disease of apples and pears raged in Switzerland in 2007. However, registration in Europe is hindered because *P. agglomerans* is currently listed as a biosafety level 2 (BL2) organism, due to clinical isolates reported as opportunistic human pathogens. The aim of this work was to understand if clinical isolates of *P. agglomerans* have undergone a discrete evolution implying specialization toward human pathogenicity and to look for molecular markers enabling the selection of safe biocontrol strains. The taxonomy of a compilation of strains gathered from culture collections designated as “*Pantoea agglomerans*” (or with the old species name “*Enterobacter agglomerans*”) was assessed by sequencing of two housekeeping genes and by PCR amplification of biocontrol-relevant and *P. agglomerans* specific genes. Surprisingly, only about one third of the strains investigated belonged to *P. agglomerans* (*sensu stricto*), while other could be identified as *Enterobacter* spp., suggesting that the countless taxonomical rearrangements that this genus has undergone in the last decades resulted in the mis-assignment of many isolates into *P. agglomerans*. In *P. agglomerans* (*sensu stricto*) both sequencing and fAFLP data show that no discrete evolution occurred between biocontrol and clinical strains. It is possible that clinical isolates acquired pathogenicity factors on plasmids or other mobile elements, but markers potentially pointing to pathogenicity remains to be found. Furthermore, only one of four Koch’s postulates (isolation from a diseased host) has been plainly demonstrated for *P. agglomerans* (*sensu stricto*). If no more evidence of the pathogenicity of *P. agglomerans* can be collected, it might be necessary to reconsider the classification of this species as a BL 2 organism.

**EFFECT OF EXOPOLYSACCHARIDES ON THE INFECTION OF *ERWINIA AMYLOVORA* BY BACTERIOPHAGES.** D.R. Roach<sup>1</sup>, A.J. Castle<sup>1</sup>, and, A.M. Svircev<sup>2</sup>. <sup>1</sup>Department of Biological Sciences, Brock University, St. Catharines ON, Canada L2S 3A1, <sup>2</sup>Agriculture and Agri-Food Canada, Vineland Station ON, Canada L0R 2E0.

Like many plant-pathogenic bacteria, *Erwinia amylovora*, the causative agent of fire blight on apple and pear, produces extracellular polysaccharides (EPS). These capsular polysaccharides are believed to play a protective function against bacteriophage attack. Six *E. amylovora* isolates were selected to examine the effects of EPS on bacteriophage induced cell lysis. Plaque morphology and efficiency of plating (EOP) of 54 *Erwinia spp.* phages was used to study the phage-host interaction. Phages showed a high EOP and clear, confluent lysed plaques on primary hosts. These same phages showed a reduced EOP and overgrown or turbid plaques their secondary hosts. EPS production was measured grouping the hosts as being either a high (isolates EaD-7, Ea29-7, Ea110R) or low EPS producer (isolates EaG-5, Ea6-4, Ea17-1-1). Primary hosts were influenced by the amount of EPS produced and specific to bacteriophage family. Primary hosts for *Podoviridae* were high EPS producers whereas *Myoviridae* were low EPS producers. Secondary hosts were the opposite EPS group. Overexpressing EPS in primary hosts had no effect on EOP. Overexpressing EPS in secondary hosts decreased EOP for *Myoviridae* phages only. These results suggest that *E. amylovora* isolates are susceptible to a wide range of *Erwinia spp.* phages. Differences in susceptibility are caused, at least partially, by amount of EPS produced by the cells. Members within the *Erwinia spp.* phage family had a similar efficiency at lysing cells that produce a specific amount of EPS.

**A NATURAL ANTAGONIST AGAINST PHYTOBACTERIA OF KIWIFRUIT PLANTS.** A. Rossetti and G.M. Balestra. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy. E-mail: balestra@unitus.it.

The bacterial blight and floral buds necroses diseases on kiwifruit plants caused by *Pseudomonas syringae* pv. *syringae* (Pss) and *Pseudomonas viridiflava* (Pv), have become during last 15 years a serious problem in Italy. During 2007, three kiwifruit orchards cv. Hayward characterised by a relevant presence of Pss and Pv situated in the provinces of Verona (Veneto region), Viterbo and Latina (Latium region), were submitted (in April, July and December) to *Bacillus subtilis* (strain QST 713, Serenade<sup>®</sup>, Intrachem) treatments to control these bacterial pathogens. To evaluate the effect of *B. subtilis* on Pss and Pv bacterial populations on kiwifruit organs, monthly samplings were carried on and processed in lab. Pss and Pv populations were studied concerning their epiphytic survival and about their morphological, nutritional, physiological, biochemical characteristics and ice nucleation activity (INA<sup>+</sup>). The populations of Pss and Pv on not treated (control) kiwifruit organs (twigs, leaves, buds and fruits) ranged from  $1 \times 10^3$  to  $2 \times 10^4$  cfu/cm<sup>2</sup>; the highest value was recorded on buds in spring for Pv ( $4 \times 10^4$  cfu/gr). Kiwifruit vines treated by *B. subtilis* pointed out values of Pss and Pv ranging on buds from  $6 \times 10^2$  cfu/gr to  $6 \times 10^3$  cfu/gr, on leaves from  $3 \times 10^2$  cfu/cm<sup>2</sup> to  $2 \times 10^3$  cfu/cm<sup>2</sup> and on twigs from  $4 \times 10^2$  cfu/cm<sup>2</sup> to  $3 \times 10^3$  cfu/cm<sup>2</sup>. *B. subtilis* strain reduced Pss and Pv values from 6 to 30% respect to the untreated plots. During the kiwifruit vegetative season, the *B. subtilis* effect resulted equivalent to copper treatments to reduce Pss and Pv populations on kiwifruit plants.

Research supported by Regione Lazio, PRAL n° 118/20063 project.

**PHENOTYPIC AND BIOCHEMICAL ANALYSES OF SILICON – INDUCED RESISTANCE AGAINST *RALSTONIA SOLANACEARUM* IN POTATO GENOTYPES.** Dritan Sadikaj and Kerstin Wydra. Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany. [wydra@ipp.uni-hannover.de](mailto:wydra@ipp.uni-hannover.de)

Treatment of potato genotypes Desirée, moderately resistant, Saxon, susceptible, and Estima, highly susceptible to bacterial wilt, with silicon identified potato as Si-non-accumulator (less than 0.13% Si in roots). Silicon treatment in form of monosilicic acid and silicon dioxide reduced disease severity and incidence in genotype Desirée by 35% and 25%, respectively, while for genotype Saxon the influence of silicon was variable, and no effect was observed in Estima. Silicon amendment reduced the colonisation of roots and stems in the three genotypes. No significant differences in PAL activity and soluble phenols were found across treatments in genotypes Desirée and Saxon. In genotype Saxon PAL activity was tendenciously suppressed in the *Ralstonia solanacearum* infected plants, regardless of the Si amendment and plant organ tested, root or stem. No significant differences were found between treatments and genotypes in PAL activity and total soluble phenols. Probing cell walls of mid-stem xylem vessels with the anti-arabinogalactan protein (AGP) antibody LM2 revealed higher accumulation of AGPs due to *R. solanacearum* infection in genotype Desirée. The induction was stronger in the *R. solanacearum*-infected, silicon-amended treatment, whereas un-inoculated treatments showed no increased fluorescence. No differences were found in genotypes Saxon and Estima. The results of this study indicate that Si nutrition can induce resistance mechanism in a non-accumulator plant species, in a genotype-dependent interaction, as we similarly observed in here not presented studies with eggplant and geranium.

**SEARCHING FOR NEW ANTIBIOTICS IN ENDOPHYTIC MICROORGANISMS.** Erik Saenz; Charlotte Borda; Ligia Renata; Márcia Da Silva; Gabriel Padilla. University of São Paulo, São Paulo, Brazil.

All microorganisms that inhabit in the interior of a plant, at least for one period of their life cycle, may be considered as an endophytic. These organisms may contribute to their host plant by producing one or many substances that provide protection and survival value to the plant. These compounds may also have potential for use in, agriculture, industry and medicine. In this study, we used genomic DNAs from endophytic Actinomycetes microorganisms collection isolated from sugarcane and citrus plants, as templates in amplification by degenerated PCR approaches, which were able to amplify a conserved portion of the ketosynthase gene present in the PKS type II gene cluster, in order to screen potential producers of polyketide antibiotic compounds. The sequence analysis of amplified products of about 600pb showed a high identity within the known sequences in GenBank the ketosynthase genes related with the production of polyketides type II compounds. 16S rRNA gene sequence analysis of the selected isolates revealed that the most predominant among the endophytic bacteria isolates belonging to the genus *Streptomyces* and *Nocardiopsis*. The bioactive metabolites were assayed against phytopathogenic bacteria and human enterobacteria, identified inhibition of pathogenic bacterial. A phylogenetic tree constructed from the amino acid sequences of the amplified fragments and the known KS sequences in public databases showed a distribution the endophytic sequences within the antibiotics group separate the spore group. This study, demonstrates the existence of novel sequences KS genes in the endophytic bacterial suggest their potential to synthesize new aromatic polyketides.

Support: CAPES

**HISTOCHEMICAL ANALYSES OF SILICON-INDUCED RESISTANCE IN THE INTERACTION OF TOMATO (*SOLANUM LYCOPERSICUM* L.) AND *RALSTONIA SOLANACEARUM*.** T. Schacht and K. Wydra. Leibniz Universität Hannover, Institute of Plant Diseases and Plant Protection, Herrenhäuser Str. 2. 30149 Hannover, Germany. wydra@ipp.uni-hannover.de

Silicon-induced resistance has been studied on molecular genetic and immuno-histochemical level and revealed an increase in expression of genes involved in the basal resistance reaction and of signalling genes belonging mainly to the jasmonic acid pathway. To further characterize and localize the reaction on histochemical level, various biochemical parameters linked to resistance in different pathosystems, such as tylosis formation, hydrogen peroxide accumulation, lignification and callose deposition were studied microscopically at different times after inoculation. Tomato genotype King Kong 2 showed significantly increased tylosis formation in vessels of the midstem in silicon-treated and *R. solanacearum*-inoculated plants compared to non-inoculated plants of the same treatment at eight days post inoculation (dpi), and an elevated tylosis formation compared to inoculated and non-inoculated plants of the non-silicon treatment. At 12 dpi an increased tylosis formation was additionally observed in silicon-treated inoculated plants compared to all other treatments. Further investigations for differences in lignification, hydrogenperoxide accumulation and callose deposition revealed that these mechanisms are apparently not involved in the silicon-induced resistance of tomato to *R. solanacearum*.



**PRELIMINARY RESULTS ON THE ANTAGONISTIC ACTIVITY OF BEAN RHIZOSPHERE BACTERIA TOWARD COMMON BEAN PATHOGENIC BACTERIA AND FUNGI.** V. Shanmugaiah, P. Lo Cantore and N. S. Iacobellis. Dipartimento di Biologia Difesa e Biotecnologie Agro Forestali, Università degli Studi della Basilicata, viale Ateneo Lucano, 10, 85100 Potenza, Italy. [iacobellis@unibas.it](mailto:iacobellis@unibas.it).

Sustainable and environmentally acceptable control measures of bacterial diseases of plants may be achieved by using microbial biocontrol agents. The aim of this study was to assess the potential of bacteria isolated from bean rhizosphere to control the common bean blight caused by the varieties *fuscans* and non *fuscans* of *Xanthomonas campestris* pv. *phaseoli* by using different screening methods. A total of 162 bacteria isolates were obtained from the bean rhizosphere soils and then evaluated in the agar plate assays for the ability to inhibit the growth of strains of the varieties of *X. c.* pv. *phaseoli*. Moreover, the same assays were performed on common bean bacterial and fungal pathogens such as *Pseudomonas syringae* pv. *phaseolicola*, *P.s.* pv. *syringae*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotinia sclerotiorum*. 60 and 57 out of 162 isolates suppressed the growth of above bacteria and fungi, respectively, and most of them were shown to produce amylase, protease, pectinase, cellulase, glucanase and chitinase. Furthermore, the above bacteria were evaluated for their possible interference on the pathogenicity and /or virulence of strains of the varieties of *X. c.* pv. *phaseoli*. For the purpose seeds of a susceptible bean variety were bacterized with the potential antagonistic bacteria and then put to germinate in sterile Petri dishes and sown in pot in the greenhouse. Cotyledon and trifoliate leaves were then inoculated with the representative strains of the varieties of *X. c.* pv. *phaseoli* and scored for the symptom appearance. Plants either *in vitro* or in greenhouse treated with seven out 44 isolates so far analysed showed reduced lesions when compared with the plant control not bacterised and challenged with the pathogens. It is not excluded that induced systemic resistance is involved in that feature.

**ECOFRIENDLY MANAGEMENT OF BACTERIAL BLACK SPOT OF MANGO USING BACTERIAL ANTAGONISTS.** C. Sharfuddin, C.Kumar, R.Mohanka and G.Tabassum. Plant pathology and Microbiology lab, Department of Botany, Patna University, Patna 800005, Bihar, India.

Mango is the most important fruit in India, occupying 33% of the total area under fruit crops. The northeastern states of India viz. Bihar and West Bengal are the chief producers of mango, the prominent cultivars of which are known as Maldai, Bamba, Gulabkhas and Zardalu. In the present investigation Bacterial Black Spot disease of mango was studied with special reference to its biological control. Mango leaves, stems and fruits are all susceptible to infection. It was observed that small, irregular, water soaked speck symptoms appear on fruits, which soon become raised and usually cracks open, from which a bead of bacterial ooze developed. On nutrient agar medium, colonies were developed by pour-plate method which were smooth circular and cream white in color. The slides were prepared and the pathogen was identified as *Xanthomonas campestris* pv. *mangiferae* (*Xcm*). Epiphytic bacteria on mango leaves from the different orchards of Darbhanga, Samastipur, Begusarai and Khagaria districts of Bihar were investigated for two years (March 2006 to Feb 2008) to select strains, antagonistic to *Xcm*. The two major groups of the isolated bacteria were identified as *Bacillus* and *Erwinia*. Experiments were then performed, both as *in-vitro* and *in-vivo* conditions. The best results were obtained with the two *Bacillus* strains. For each experiment three replicates were taken and inhibition zones were measured for statistical analysis. Similarly for *in-vivo* experiment, young mango plants were grown in controlled conditions and after treatment a significant reduction in disease incidence was observed, which was analyzed in percentage.

**COMPLETE GENOME SEQUENCING OF *PANTOEA AGGLOMERANS* STRAIN C9-1.** T.H.M. Smits<sup>1\*</sup>, F. Rezzonico<sup>1</sup>, C. Pelludat<sup>1</sup>, V.O. Stockwell<sup>2</sup>, A. Goesmann<sup>3</sup>, J.E. Frey<sup>1</sup>, B. Duffy<sup>1</sup>. <sup>1</sup>Agroscope Changins-Wädenswil ACW, Plant Protection Division, CH-8820 Wädenswil, Switzerland; <sup>2</sup>Oregon State University, Corvallis, USA; <sup>3</sup>CeBiTec, University of Bielefeld, Germany.

*Pantoea agglomerans* strains are among the most effective biocontrol agents for fire blight and other bacterial and fungal plant diseases. Commercialization efforts are hindered however because the species biodiversity includes some strains reported as opportunistic human pathogens. Nonetheless, fire blight biocontrol formulations based on *P. agglomerans* strains C9-1 and Eh325 were registered in the US in 2007, and strain P10c is sold in New Zealand. We sequenced the complete genome of the commercialized *P. agglomerans* strain C9-1. The sequencing revealed a 4.88 Mb size, divided over the chromosome (4.025 Mb, currently one gap remaining) and three non-self-transmissible plasmids (0.168, 0.166 and 0.530 Mb, respectively). The preliminary annotation gave a total of 4618 ORFs, and showed the presence of 4 regions containing bacteriophage-related genes and a small genomic island with lower G+C content as the genome backbone. Genes for sugar metabolic pathways found in the genome largely reflect the lifestyle of the organism in a sugar-rich environment. Their presence corresponds to the metabolic spectrum of C9-1 and of the type strain *P. agglomerans* ATCC27155T, which in fact is a clinical isolate. Currently, we are assembling the genome sequence of the clinical isolate *P. agglomerans* ATCC27155T, for comparison with the genome of *P. agglomerans* C9-1. This comparison is expected to reveal the presence of regions important for the biocontrol ability of the latter strain, and for pathogenicity in the clinical isolate. These genotypic features will be tested with other isolates of *P. agglomerans* strains from biocontrol or clinical origin.

**INTEGRATED CONTROL OF FIRE BLIGHT WITH BACTERIAL ANTAGONISTS AND OXYTETRACYCLINE.** Virginia O. Stockwell<sup>1</sup>, Todd N. Temple<sup>1</sup>, Kenneth B. Johnson<sup>1</sup>, and Joyce E Loper<sup>1,2</sup>. <sup>1</sup>Dept. of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331 and <sup>2</sup> USDA ARS, Horticultural Crops Research Lab, Corvallis, OR 97330, USA.

In the Pacific Northwest of the United States, the antibiotic streptomycin provided excellent control of fire blight until resistant isolates of *Erwinia amylovora* were prevalent. Oxytetracycline (Mycoshield) is now sprayed as an alternative antibiotic. We found that the duration of inhibitory activity of oxytetracycline is similar to that of streptomycin, but oxytetracycline is considerably less effective than streptomycin when the antibiotics are targeted toward sensitive strains. In an effort to improve disease control, we evaluated combinations of biological control agents (*Pseudomonas fluorescens* A506 or *Pantoea agglomerans* C9-1) and oxytetracycline in 11 orchard trials inoculated with an antibiotic-sensitive strain of *E. amylovora*. Two bloom sprays of streptomycin or oxytetracycline reduced the incidence of fire blight of blossom clusters by an average of 73% and 44%, respectively compared to water-treated controls. A combination of C9-1 and a protease-deficient A506 provided 41% disease control. An integrated treatment, i.e., one application of biological control agents followed by one application of oxytetracycline, provided 59% control. Biological and chemical methods of fire blight suppression were complementary, and consequently, an integrated strategy consisting of a biological control agent sprayed in early and near full-bloom, followed by an oxytetracycline treatment at late bloom, improved disease control with a reduced number of antibiotic applications.

**FIELD EVALUATION OF BIOLOGICAL CONTROL AGENTS FOR FIRE BLIGHT CONTROL IN MICHIGAN.** George W. Sundin, Gayle C. McGhee, and Gail R. Ehret. Michigan State University, Department of Plant Pathology, 103 CIPS, East Lansing, MI 48824 USA.

The bacterial antagonists *Pseudomonas fluorescens* A506, *Pantoea agglomerans* C9-1, and *Pantoea agglomerans* E325, preparations of *Bacillus subtilis* QST 713 (Serenade MAX) containing bacterial endospores and lipopeptide metabolites, and the algal extract laminarin were evaluated for efficacy in controlling fire blight in Michigan from 2005-2008. Control of blossom blight in inoculated trials was variable with the biologicals ranging from good-excellent disease reductions to complete failures. For example, reductions in blossom blight following applications of *Pseudomonas fluorescens* A506 and *Pantoea agglomerans* C9-1 or a combination of the two bacteria ranged from -43% to 83% in 10 experiments. Blossom blight control with laminarin ranged from 14-88% in six experiments. In these trials, the best disease control was always achieved under the lowest disease pressure. Serenade MAX was the most consistent material averaging about 70% disease control; control was increased with the addition of Phostrol, although Phostrol alone provided a similar level of disease control in 2008. The occurrence of shoot blight was consistently higher in trees treated with biological control agents than those treated with streptomycin, indicating a consequence of not killing bacterial inoculum on blossoms. Treatments incorporating biologicals in programs with a reduced number of antibiotic applications typically performed equally to antibiotic treatments. These treatments resulted in a net reduction of antibiotic applications which is effective for resistance management and represents the most promising application regimen for biological control agents in Michigan.

**USE OF *PANTOEA AGGLOMERANS* AS A PHAGE DELIVERY SYSTEM FOR CONTROL OF FIRE BLIGHT.** A. M. Svircev<sup>1</sup>, S. M. Lehman<sup>2</sup> and A. J. Castle<sup>3</sup>.

<sup>1</sup> Agriculture and Agri-Food Canada, Vineland Station, ON Canada L0R 2E0; <sup>2</sup> Centers for Disease Control and Prevention, Atlanta, Georgia USA 30333; <sup>3</sup> Department of Biological Science, Brock University, St. Catharines, ON Canada L2S 3A1.

The success of a bacteriophage-based biopesticide for the control of *E. amylovora* in the orchard depends on a number of factors. One of the critical components is the population ratio of pathogen: phage. The phages need to be present in sufficiently high numbers once the pathogen appears on the surface of the flower. The carrier-based delivery system can be optimized to yield high phage concentrations during the open bloom period. The *in vitro* and *in planta* growth characteristics and interactions of *E. amylovora*, *P. agglomerans*, and *E. amylovora* phages were studied in order to establish parameters for treatment preparation and application during field trials. *In vitro* growth curves were constructed for *P. agglomerans* Eh21-5, *E. amylovora* Ea6-4 and Ea29-7, and two *Erwinia* phages. The *in planta* interactions among *E. amylovora*, the *P. agglomerans* carrier, and four *Erwinia* phages were also studied. Four phages were used to test the effects of treatment timing and multiplicity of infection on biocontrol efficacy using a pear blossom assay. Phage-carrier combinations were more effective at reducing fire blight symptom severity than the carrier alone if 3 h (vs. 0 h) was allowed to elapse between treatment application and pathogen application. In field trials, six of twelve treatments consisting of *E. amylovora* phages and a *P. agglomerans* significantly reduced the incidence of blossom blight. The control afforded by the phage-carrier treatments was as effective as streptomycin.

**BIOPESTICIDES REGULATION IN THE UNITED STATES.** Gail S. Tomimatsu. Biopesticides and Pollution Prevention Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460, USA.

Biopesticides are manufactured from naturally occurring substances and include pheromones, microorganisms and genetically engineered organisms. Because of their unique modes of action, low volume use, target species specificity, and advances in formulation technology, these pesticides offer additional approaches to plant pest control. Before a pesticide is made, sold, commercially distributed, and used in the United States, manufacturers must register their products with the U.S. Environmental Protection Agency (US EPA) to ensure that their uses will not pose unreasonable risks to human health or the environment. The pre-registration review process encompasses risk assessments from estimates of hazard and exposure under “worst-case” scenarios (Tier I). These estimates are developed from a synthesis of test results, intended pesticidal uses, and publically available scientific literature to fulfill data requirements addressing the primary disciplines of product analysis and manufacturing, mammalian toxicity and ecological/environmental effects. The US EPA has over 30 years of regulatory experience in performing risk assessments for the registration of biopesticides. Since 1984, registration requirements have been modified periodically to refine the risk assessment and eliminate unnecessary testing. The biopesticide data requirements were updated, clarified and revised in December 2007. The presentation will provide an overview of the biopesticide registration process, relevant definitions, and the current data requirements.

**HOW BETA-AMINOBUTYRIC ACID (BABA) PRIMES THE ARABIDOPSIS DEFENSE RESPONSE AGAINST THE BACTERIAL PATHOGEN *PSEUDOMONAS SYRINGAE*.** CH Tsai<sup>a</sup>, I Fiatte<sup>b</sup>, CW Chen<sup>a</sup>, B Boachon<sup>b</sup>, J Thomas<sup>c</sup>, H Weber<sup>c</sup>, B Mauch-Mani<sup>b</sup>, and L Zimmerli<sup>a</sup>. <sup>a</sup>Institute of Plant Biology, The National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 106, Taiwan. <sup>b</sup>Laboratory of Cell and Molecular Biology, University of Neuchatel, Rue Emile-Argand 11, 2009 Neuchatel, Switzerland. <sup>c</sup>DNA Array Facility, Center for Integrative Genomics, University of Lausanne, Genopode Building, 1015 Lausanne, Switzerland.

The xenobiotic beta-aminobutyric acid (BABA) enhances Arabidopsis resistance to the virulent bacterial pathogen *Pseudomonas syringae* pv *tomato* DC3000 (*Pst* DC3000) through priming of the salicylic acid (SA) defense response. The molecular mechanisms underlying BABA-priming remain elusive. In this presentation, I will demonstrate that BABA inhibits the Arabidopsis response to the bacterial effector coronatine (COR). COR promotes bacterial virulence by inducing the jasmonate (JA) response to antagonize SA signaling activation. BABA was found to specifically inhibit the JA response induced by COR, without affecting other Arabidopsis JA responses. BABA also inhibited the Arabidopsis response to purified COR. BABA inhibition was SA-independent. Moreover, I will describe a direct connection between BABA-priming and COR. Indeed, treatment with a high concentration of purified COR was found to counteract BABA inhibition. In these conditions, BABA did not protect Arabidopsis plants and PR1 priming was strongly reduced. These data suggest that BABA primes the SA response through inhibition of the COR response. A high concentration of purified COR also inhibited BABA-priming of PAMP-triggered PR1. We propose that BABA primes Arabidopsis resistance to *Pst* DC3000 by interfering with the bacterial manipulation of the Arabidopsis PAMP-triggered immune response. These data also point to the existence of a signaling node that can distinguish COR from other JA responses.



**ANTAGONISTIC AND PLANT GROWTH PROMOTION ACTIVITIES OF SOIL ACTINOMYCETES ISOLATED FROM POTATO FIELDS IN MONGOLIA.** B. Tsetseg, G. Nyamsuren and T. Gantuya. Laboratory of Microbiology, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar-51, Mongolia.

It has been shown that actinomycetes - streptomycetes and non-streptomycete actinomycetes - can promote plant growth and be used as biological control agents of soil-borne plant pathogens. Furthermore, non-pathogenic streptomycetes can act as controlling agents of potato scab caused by the pathogenic *Streptomyces scabies*. Potato scab is widely occurred in Mongolia. Its dry climate is favorable for spread of this disease. Therefore, screening of biological control agents for pathogens causing potato scab is important issue of microbiology in Mongolia. Due to this, our study was aimed at isolation of soil actinomycetes and testing them as biological control agents against streptomycetes isolated from potato scab lesions (*Streptomyces canus*, *S. achromogenes* and *S. turgidiscabies*). The ability of actinomycetes isolated to promote the plant growth was also evaluated using wheat seeds. Based on assumption that indigenous microflora can survive easier being introduced back, isolation was carried out from potato fields. Three different media were used for isolation of actinomycetes: starch-ammonium agar (ISP4), humic acid-vitamin agar (HVA) and Czapek agar. As a result, 78 strains of actinomycetes in total were isolated from soils of two potato and one control (without potato) fields. Just 5.1% of isolates did not show antimicrobial activity against tested microorganisms and 62.8% were active against *S. canus*, 71.8% - against *S. achromogenes* and 84.6% - against *S. turgidiscabies*. Up to 15 isolates produced the clear zones of growth inhibition of *S. turgidiscabies* with diameter more than 25 mm. Three isolates promoted the root growth by 37.7, 29.7 and 20.2% respectively in comparison with control. Among them strain PTX-32 belonged to the non-streptomycete genus and had a high antagonistic activity against *S. turgidiscabies* (the clear zone diameter=31 mm).

## **QUORUM SENSING IN RICE ASSOCIATED BACTERIA: POSSIBLE INTERPLAY BETWEEN PATHOGENIC AND BENEFICIAL BACTERIA.**

Vittorio Venturi, Iris Bertani, Laura Cabrio, Giuliano Degrassi, Giulia Devescovi, Maura Mattiuzzo, Sara Ferluga, Laura Steindler, Zulma Rocio Suarez Moreno and Sujatha Subramoni. Bacteriology Group, International Centre for Genetic Engineering & Biotechnology (I.C.G.E.B.) Padriciano 99, 34012 Trieste Italy and Plant Bacteriology Group, I.C.G.E.B., Biosafety Outstation, Via Piovega 23, 31052, Ca' Tron di Roncade, Treviso, Italy.

It is now recognized that bacteria behave/synchronize as a community employing a level of gene regulation involving intercellular communication (quorum sensing, QS) organized by the production and detection of small signal molecules. QS has been studied in many bacterial species and shown to provide a significant advantage to a community of bacteria by adapting to environmental conditions and often enhancing its defense capabilities against other microorganisms or eukaryotic resistance mechanisms. In Gram-negative bacteria, *N*-acyl homoserine lactones (AHLs) are to date the most commonly used signal molecules being produced by a synthase enzyme belonging to the LuxI protein family; a transcriptional regulator, belonging to the LuxR family, then forms a complex with the cognate AHL at threshold levels altering the transcriptional activity of target genes. Research interests on are focused on AHL QS in important Gram-negative bacteria associated rice; more precisely with (i) rice-rhizosphere beneficial *Pseudomonas* spp., (ii) in beneficial rice endophytic *Burkholderia* spp. and (iii) in rice pathogenic *Xanthomonas oryzae*, *Burkholderia glumae* and *Pseudomonas fuscovaginae*. How these bacteria employ similar QS mechanisms to either establish a beneficial association with the plant in the rhizosphere or to colonize and cause disease are currently being studied. The overall picture of AHL QS of rice-associated bacteria is beginning to emerge as well as possible interplay between beneficial and pathogenic bacteria which could lead to the design of possible biocontrol and biofertilizer strategies.

**A MODEL OF SILICON-INDUCED BASAL RESISTANCE IN TOMATO AGAINST *RALSTONIA SOLANACEARUM* BASED ON TRANSCRIPTOMIC AND BIOCHEMICAL ANALYSES.** K. Wydra. Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Str. 2, 30419 Hannover, Germany. [wydra@ipp.uni-hannover.de](mailto:wydra@ipp.uni-hannover.de).

Application of silicon (Si) in form of Si dioxide and monosilicic acid induced resistance in tomato against bacterial wilt caused by *Ralstonia solanacearum*. Analyzing the gene expression of defense-related genes by quantitative real time PCR (qRT-PCR), up-regulation of *JERF3*, *TSRF1*, *ACCO*, *FD-I*, *POD* and *AGP-1g* in Si-amended, but not in untreated plants was observed after challenging with the pathogen. These genes play a role in ethylene (ET) and jasmonic acid (JA) signaling pathways and/or are involved in expression of basal resistance. Former immunohistochemical analysis of plant cell wall reactions to infection were partly confirmed by expression of *AGP-1g*, playing a role in defense related structural changes of the cell wall. Gene expression was generally highest at 72 hours post inoculation. At this time point gene expression analysed by the TOM2 microarray revealed sixteen genes significantly up- or down-regulated in plants treated with Si challenged with *R. solanacearum* compared to plants without Si application. The microarray results were validated by qRT-PCR, and the genes were annotated and functionally classified. At least twelve genes involved in defense, signal transduction, response to stresses, transcription, ubiquitinylation and metabolism were up-regulated. Highest up-regulation of 11.0 log<sub>2</sub> fold was found with the tify (ZIM)-protein JAZ1, involved in regulation of the JA-signaling pathway. A role of Si in priming the defense capacity of the plant involving reactive oxygen species (ROS), ET and JA signaling pathways and thereby alleviating biotic stress imposed by the pathogen is suggested and a hypothetical model of silicon-induced resistance (SiIR) presented.

**INVOLVEMENT OF *PCOR-PCOI* QUORUM-SENSING SYSTEM IN BIOCONTROL OF PLANT DISEASES BY *PSEUDOMONAS FLUORESCENS* 2P24.** Q. Yan, H.L. Wei, Q.X. Zhang, X.G. Wu, and L.Q. Zhang. Department of Plant Pathology, China Agricultural University, Beijing 100193, China. Email: [zhanqlq@cau.edu.cn](mailto:zhanqlq@cau.edu.cn).

*Pseudomonas fluorescens* 2P24 is a biocontrol agent isolated from a wheat take-all decline soil in China. Antibiotic 2,4-diacetylphloroglucinol produced by strain 2P24 is a principal factor enabling this bacterium to suppress plant diseases. Our recent work revealed that strain 2P24 employed a LuxR/I family quorum-sensing (QS) system, PcoR/I, to regulate its biocontrol activity. Mutation on signal biosynthesis gene *pcol* did not detectably affect the antibiotic production, but significantly influenced biofilm formation, colonization on wheat/tomato rhizosphere and biocontrol ability against wheat take-all and tomato bacterial wilt. The complementation of *pcol* restored the colonization and biocontrol activity to the wild-type level. We further investigated the upstream regulators that influenced the transcription of the *pcol* gene using a chromosomal *pcol::lacZ* fusion reporter strain. Stationary sigma factor RpoS and the two-component regulatory system PhoP/PhoQ, which responds to environmental Mg<sup>2+</sup> starvation, were identified as negative regulators of QS system using a random mini-Tn5 mutant procedure. Furthermore, deletion mutagenesis and complementation experiments revealed that the two-component system GacS/GacA positively regulated the QS system by upregulating *pcol* transcription. Our results demonstrate genetically the involvement of QS in the regulation of biocontrol activity in *P. fluorescens* 2P24, and indicate that the QS in strain 2P24 is under the pleiotropic regulation of a number of upstream genes.

**Acknowledgements:** This work was funded by the Chinese 863 project (2006AA10A211), NNSF(30671403) and MOST-DEST Project (2007DFA31570).

**RECENT STUDIES ON THE BIOCONTROL OF FIREBLIGHT (*ERWINIA AMAYLOVORA*) WITH BIOZELL-2000 B, AN ETHERIC OIL OF *THYMBRA SPICATA*.** W. Zeller, K. Abo-Elyousr and O. Yegen. Julius-Kühn-Institut, Bundesforschungsinstitut für Kulturpflanzen, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, 64287 Darmstadt, Germany.

Latest results with the natural product based on an etheric oil of thyme, BIOZELL 2000B, which was developed with Turkish colleagues (Yegen et al. 2002) will be presented. The natural compound was tested on the efficacy against fireblight (*Erwinia amylovora*) and on its resistance induction activity. For the studies M26 apple rootstock was used as host plant. Moreover as marker of resistance in physiological studies enzyme activities of  $\beta$ -glucosidase and PR-protein (chitinase) were estimated. The treatment with BioZell-2000B resulted in a marked reduction of the disease index. This was correlated with a decreasing effect on the growth of bacteria during the course of infection. In the physiological studies significant changes in the activities of  $\beta$ -glucosidase and chitinase were found after treatment. Thus the biocontrol effect was correlated with a resistance induction in the host plant of the apple rootstock M26.

**EVALUATION OF PGPR AND ACIBENZOLAR-S-METHYL FOR CONTROL OF BACTERIAL SPOT OF TOMATO.** S. Zhang and T. L. White. Tropical Research and Education Center, University of Florida, IFAS, Homestead, FL 33031.

Greenhouse and field trials have been conducted to evaluate plant growth-promoting rhizobacteria (PGPR) and acibenzolar-S-methyl (ASM) for the potential to control bacterial spot of tomato. In greenhouse assays, tomato seeds were sown in potting mix in Styrofoam flats. PGPR ( $1 \times 10^7$  CFU/ml) and ASM (Actigard 50WG) were applied three or four times as weekly soil drenches beginning 1 week after planting. Tomato plants were transplanted into 4-inch pots 1 week after the last treatment. One week after transplanting (WAT), plants were sprayed to run-off on both leaf surfaces with suspensions of *Xanthomonas campestris* pv. *vesicatoria* ( $1 \times 10^8$  CFU/ml) and incubated for 7 days on a greenhouse bench when disease was rated on a 0-6 scale. ASM at 30 mg/l and PGPR strains SE76 and INR7 significantly reduced disease severity on tomato cultivars 'FL 47' and 'Tygress', compared to the nontreated control ( $P = 0.05$ ). Two field trials were conducted with the same cultivars in 2008. Plants were treated with PGPR or ASM in the same manner as described above for greenhouse assays and transplanted into field plots 1-2 weeks after the last treatment where they were naturally infected with the pathogen. ASM at 30 mg/l consistently reduced bacterial spot on both cultivars compared to the nontreated control when disease was rated weekly from 8 to 11 WAT for the first trial ('FL 47'), and 3 WAT for second trial ('Tygress'). Plants of tomato ('Tygress') treated with PGPR strain IN 937a also had significantly less disease than the nontreated plants. Interestingly, treatments with silicon nutrient, silicic acid applied at 1.5 and 0.15 mM in the first trial, significantly suppressed bacterial spot on tomato ('FL 47') at 8 WAT.

**BIOCONTROL OF SOFT ROT BACTERIA OF THE GENUS ERWINIA IN POTATO – SECONDARY METABOLITES OF RHIZOBACTERIA AFFECT TRANSCRIPTION OF THE PECTINASE GENES OF *ERWINIA CHRYSANTHEMI*.** Barbara Żurek<sup>1</sup>, Mieczysław Żurek<sup>2</sup>. <sup>1</sup>Department of Microbiology, <sup>2</sup>Department of Plant Protection University of Podlasie, Prusa 12 Street, 08-110 Siedlce, Poland.

The pectinolytic bacteria of the genus *Erwinia* are known as causal agents of soft rot diseases among many plants, including potato. The major factors responsible for development of soft rot diseases are: the ability of bacteria to multiply and the release of cell-wall degrading enzymes. In our previous studies it was shown that metabolites of rhizobacteria, *Pseudomonas putida* PS-14/3, selected as potential biocontrol agents against fungal disease in carrot, and *P. fluorescens* PS-2N/3 affect both multiplication and enzymatic activity of tested *Erwinia* strains *in vitro*. In this work we studied the effect of metabolites of these bacteria on expression of pectinase genes of *E. chrysanthemi* 3937, six *pels* (*pelA*, *pelB*, *pelC*, *pelD*, *pelE* and *pelL*) encoded for pectate lyases and one *pem* encoded for pectin methylesterase, by application of derivative mutants of *E. chrysanthemi* possessing transcriptional fusion in one of the pectinase genes, with the reporter gene encoding glucuronidase activity. The studies were conducted *in vitro* and *in vivo* in potato tubers. The received results indicate that metabolites of rhizobacteria can affect the transcription of pectinase genes of *E. chrysanthemi*. The expression of almost all of studied genes (beside *pelA*) was significantly lower in the presence of metabolites of *P. putida* PS-14/3, however some variability in genes responses to action of these antimicrobial compounds, was observed. By contrary, the metabolites of *P. fluoresces* PS-2N/3 induced expression of pectinase genes and especially *pem*. The effect of culture filtrates of *P. putida* PS-14/3 against wild strains of both *E. chrysanthemi* and *E. carotovora* was studied on potato tubers in humid chamber. After incubation time it was observed the reduction in disease severity for both erwinias but *E. chrysanthemi* was more sensitive to this biocontrol agent than *E. carotovora*.

**RECOMBINANT PROTEIN FOR BIOCONTROL OF *RALSTONIA SOLANACEARUM*.** S.S. Kabeil, Elsayed E. Hafez, Ayman S. Daba and A. El-Saadani. Mubarak City for Scientific Research and Technology, Borg-Elarab, Alexandria, Egypt.

In Egypt, potato has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato. Recently, potato crop is infected with the brown rot disease producing a major problem which caused by *Ralstonia. Solanacearum*. Soil samples were collected from Gharbia governorate, bacterial isolation was carried out. Many bacterial isolates were obtained and used for bioassay against the *R. solanacearum*. One isolate showed high activity against the pathogenic bacteria *R. solanacearum*, subjected to identification using the 16S rRNA gene and the sequence analysis revealed that the strain is *Pantoea agglomerance*. The bacteria was cultivated on PBG medium and the culture filtrate was fractionated and the fraction were used to control such bacteria. One of these fractions showed high ability to control the pathogenic bacteria (named *Biocine*). The Molecular weight of the purified *Biocine* was determined by SDS polyacrylamide gel electrophoresis revealing one band with a molecular weight of 29kDa. Differential display was used to study the gene regulating the production of *Biocine* in the isolated strain using arbitrary primer. Band in molecular weight 800bp were obtained, cloned using TOPO TA cloning kit. The fragment was released using the *EcoR1* restriction enzyme and subcloned into prokaryotic expression vector (PtracA). The recombinant bacteria was induced using the IPTG and the purified protein was obtained based on the His-tag technology. Bioassay was carried out for the purified recombinant protein compared with the wild one, the same activity was shown. Now we can concluded that we are able to produce a high amount of *biocine* on semi-industrial scale and we will try to transfer the experiment from lab to the field.



**POTATO BROWN ROT DISEASE IN EGYPT: CURRENT STATUS AND PROSPECTS.** S. S. Kabeil\*, S. M. Lashin\*\*, M. A. El-Saadani\*, M. M. Abd-Elgawad\*\* and A. M. Aboul-Einean\*\*\*. \*Mubarak City for Scientific Research and Technology, Borg-Elarab, Alexandria; \*\*Plant Pathology Department, National Research Center, El-Tahrir St, Dokki, 12622, Giza;\*\*\*Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt.

Potatoes are Egypt's largest horticultural export. Yet, the total value of Egyptian potato exports fell from a peak value of US\$ 102.12 million in 1995 to \$US 7.7 million in 2000 mainly due to quarantine restrictions on the potato brown rot imposed by the European Union (EU) which used to account for about 70% - 90% of Egyptian potato exports. Therefore, The Central Administration for Plant Quarantine (CAPQ) of the Egyptian Ministry of Agriculture has recently set up a new Directorate for Internal Potato Quarantine in order to delimit pest free areas (PFAs), i.e. areas in which *Ralstonia solanacearum* which causes the brown rot was known not to have occurred. In order for PFAs to be approved, extensive documentation including detailed maps, cropping pattern, irrigation sources and other relevant information must be submitted to the EU Standing Committee on Plant Health. Integrated management of such a disease with special emphasis on the biological control approach is presented herein. Bacteriocin has proved to be effective against the disease. On the other hand, recognizing that zero tolerance requires sampling every tuber in the potato lot, the default strategy is to define an acceptable tolerance limit. The binomial probability distribution is presented as a base for determining probabilities of detecting various infestation levels as increasing numbers of samples are collected. Such a method saves time, labor and money in the detection and ensures the relative proportional certainty emanating from the inspected sample to the actual matter. However, a zero tolerance is required for complete eradication of the disease. Neither Poisson nor the negative binomial distributions can be applied to such a zero tolerance. Eventually, the tolerance limit must be backed up by sound technical information and consequent judgments for each specific case.

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