18th International Reinhardshbrunn Symposium
www.reinhardshbrunn-symposium.de

Modern Fungicides and Antifungal Compounds

24th – 28th April 2016
Friedrichroda, Germany

Program and Abstracts
# Timetable

**Sunday, April 24, 2016**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>16:00-19:00</td>
<td>Arrival Registration</td>
</tr>
<tr>
<td>20:00-22:00</td>
<td>Welcome Reception with Evening Buffet</td>
</tr>
</tbody>
</table>
### 08:30  CONFERENCE OPENING  
Prof. Dr. Holger B. Deising

#### 1a  KEYNOTE LECTURES  Part I - Chair: Dr. Gerd Stammler

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:40</td>
<td>Gurr, S.</td>
<td>UK University of Exeter</td>
<td>Global movement of fungi</td>
</tr>
<tr>
<td>09:10</td>
<td>Lucas, J.</td>
<td>UK Rothamsted Research</td>
<td>Resistance management: we know why, but do we know how?</td>
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<tr>
<td>09:40</td>
<td>Norman, K.</td>
<td>UK Velcourt Ltd</td>
<td>The challenges of farming with resistance</td>
</tr>
<tr>
<td>10:10</td>
<td>Coffee break</td>
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</tr>
</tbody>
</table>

#### 10:30  KEYNOTE LECTURES  Part II - Chair: Prof. Dr. Holger B. Deising

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:30</td>
<td>Steinberg, G.</td>
<td>UK University of Exeter</td>
<td>Cell biology informs fungicide development</td>
</tr>
<tr>
<td>11:00</td>
<td>Kogel, K.-H.</td>
<td>D University of Gießen</td>
<td>The agronomic potential of gene silencing applications</td>
</tr>
<tr>
<td>11:30</td>
<td>Tietjen, K.</td>
<td>D Bayer CropScience</td>
<td>Contribution of plant responses to efficacy of fungicides</td>
</tr>
<tr>
<td>12:00</td>
<td>Zhou, M.</td>
<td>CN Nanjing Agricultural University</td>
<td>Myosin as a selective target for new fungicide phenamacril</td>
</tr>
<tr>
<td>12:30</td>
<td>Lunch break</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### NEW TECHNOLOGIES AND APPLICATIONS - Chair: Dr. Bart Fraaije

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Country</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:00</td>
<td>Calaora, V.</td>
<td>F</td>
<td>BIO-TRANSFER New methodology breakthrough in microscopy: Fluorescence Visualization of Living Fungal Pathogens inside Plant Tissues</td>
</tr>
<tr>
<td>14:20</td>
<td>Koch, A.</td>
<td>D</td>
<td>University of Gießen Towards an RNAi based control of plant diseases: Assessment of a potential spray application</td>
</tr>
<tr>
<td>14:40</td>
<td>Beattie, D.</td>
<td>CH</td>
<td>Syngenta Crop Protection Oxathiapiprolin: Low and Slow Mobility Still Provides Longlasting Protection, and Protection of New Growth</td>
</tr>
<tr>
<td>15:00</td>
<td>King, K.</td>
<td>UK</td>
<td>Rothamsted Research Development of rapid in-field diagnostic assays to detect CYP51 and/or MgMfs1 over-expressing strains of <em>Zymoseptoria tritici</em> in leaf samples</td>
</tr>
<tr>
<td>15:20</td>
<td>Schnabel, G.</td>
<td>USA</td>
<td>Clemson University Communication of FRAC Code Principles With Growers with Smartphones</td>
</tr>
<tr>
<td>15:40</td>
<td>Sierotzki, H.</td>
<td>CH</td>
<td>Syngenta Crop Protection ADEPIDYN (TM): A New Broad Spectrum Foliar Fungicide for Multiple Crops</td>
</tr>
<tr>
<td>16:00</td>
<td>Hernandez, C.</td>
<td>F</td>
<td>CONIPHY Resistance to SDHI fungicides: A new method for monitoring populations of <em>Pyrenophora teres.</em></td>
</tr>
<tr>
<td>16:20</td>
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<td></td>
<td>Coffee break</td>
</tr>
</tbody>
</table>

**16:30-18:00** **POSTER DEMONSTRATION**
### 3 FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECT I -
**Chair: Dr. Erich-C. Oerke**

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
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<th>Topic</th>
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</thead>
<tbody>
<tr>
<td>08:30</td>
<td>Jørgensen, L.</td>
<td>DK</td>
<td>Aarhus University</td>
<td>Azoles Have Different Strengths and Perform Diverely Across Europe</td>
</tr>
<tr>
<td>08:50</td>
<td>Piotrowska, M.</td>
<td>UK</td>
<td>SRUC</td>
<td>Fungicide Sensitivity Monitoring in Cereals, Forest and Minor Crop Pathogens in the UK</td>
</tr>
<tr>
<td>09:10</td>
<td>Nanni, I.</td>
<td>I</td>
<td>University of Bologna</td>
<td>Efficacy of Carboxylic Acid Amides (CAA) Fungicides towards CAA Sensitive and CAA Resistant <em>Plasmopara viticola</em> Populations: In Vivo Tests and Molecular Studies on PvCesA3 Gene</td>
</tr>
<tr>
<td>09:30</td>
<td>Scalliet, G.</td>
<td>CH</td>
<td>Syngenta Crop Protection</td>
<td>Fungicide Resistance: Learning from Botrytis Monitorings</td>
</tr>
<tr>
<td>09:50</td>
<td>Fraaije, B.</td>
<td>UK</td>
<td>Rothamsted Research</td>
<td>Azole and SDHI sensitivity status of <em>Zymoseptoria tritici</em> field populations sampled in France, Germany and the United Kingdom during 2015</td>
</tr>
<tr>
<td>10:10</td>
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<td>Coffee break</td>
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</tbody>
</table>

### 4 FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECTS II -
**Chair: Dr. Helge Sierotzki**

<table>
<thead>
<tr>
<th>Time</th>
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<th>Country</th>
<th>Institution</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:30</td>
<td>Mehl, A.</td>
<td>D</td>
<td>Bayer CropScience</td>
<td>SBI sensitivity status of major cereal pathogens in Europe</td>
</tr>
<tr>
<td>10:50</td>
<td>Kildea, S.</td>
<td>IRE</td>
<td>Teagasc</td>
<td>Developing Fungicide Control Programmes for <em>Septoria tritici</em> Blotch in Irish Winter Wheat Crops</td>
</tr>
<tr>
<td>11:10</td>
<td>Rehfus, A.</td>
<td>D</td>
<td>BASF SE</td>
<td>Sensitivity of <em>Pyrenophora teres</em> to Succinate Dehydrogenase Inhibitors in Europe</td>
</tr>
<tr>
<td>11:30</td>
<td>Hall, B.</td>
<td>AUS</td>
<td>South Austral. Research &amp; Development Institute</td>
<td>Fungicide Resistance in Australian Viticulture</td>
</tr>
<tr>
<td>11:50</td>
<td>Toffolatti, S.</td>
<td>I</td>
<td>University of Milan</td>
<td>Evaluation of a CAA-based Management Strategy for the Downy Mildew Control in a Vineyard with CAA Resistance</td>
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<tr>
<td>12:30</td>
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<td>Lunch break</td>
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**14:00**  SYMPOSIUM EXCURSION
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>14:00</td>
<td>Departure for the Conference Excursion by bus. Busses leave from the Ramada Hotel main entrance.</td>
</tr>
<tr>
<td>15:30</td>
<td>Visit to Heidecksburg baroque palace in Rudolstadt, guided tours through the The Banquet Rooms, and the Museum of Natural History; further open for exploration are the Rococo en miniature exhibit and the Painting Gallery</td>
</tr>
<tr>
<td>18:00</td>
<td>Leaving Rudolstadt for the Waldhotel Berghof in Luisenthal</td>
</tr>
<tr>
<td>19:30 - 22:00</td>
<td>Conference dinner with Thuringian specialties buffet at the Waldhotel Berghof</td>
</tr>
<tr>
<td>22:30</td>
<td>Returning to Ramada Hotel in Friedrichroda</td>
</tr>
</tbody>
</table>
### 5 MODES OF FUNGICIDE RESISTANCE: DIAGNOSTICS, MOLECULAR, AND GENETIC ASPECTS - Chair: Dr. Gerd Stammler

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<th>Topic</th>
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<tbody>
<tr>
<td>08:30</td>
<td>Torriani, S.</td>
<td>CH</td>
<td>Syngenta Crop Protection</td>
<td>Succinate-Dehydrogenase Inhibitor (Sdhi) Resistance Evolution in Plant Pathogens</td>
</tr>
<tr>
<td>08:50</td>
<td>Gazzetti, K.</td>
<td>I</td>
<td>University of Bologna</td>
<td>Role of Single Site-Specific Allele Replacement into SvHK1 Locus in the Study of Stemphylium vesicarium Dicarboximide and Phenylpyrrole Fungicides Resistance</td>
</tr>
<tr>
<td>09:10</td>
<td>Jung, G.</td>
<td>USA</td>
<td>U of Massachusetts</td>
<td>Mechanisms of fungicide detoxification in Sclerotinia homoeocarpa</td>
</tr>
<tr>
<td>09:30</td>
<td>Luo, C.</td>
<td>CN</td>
<td>Huazhong Agricul. University</td>
<td>Function and Detection of the Genetic Element ‘Mona’ Associated with Fungicide Resistance in Monilinia fructicola</td>
</tr>
<tr>
<td>09:50</td>
<td>Tietjen, K.</td>
<td>D</td>
<td>Bayer CropScience</td>
<td>Crystallographic studies of yeast CYP51 with fungicides</td>
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<tr>
<td>10:10</td>
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<td>Coffee break</td>
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### 6 FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECTS III - Chair: Dr. Andreas Mehl

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Country</th>
<th>Institution</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:30</td>
<td>Trkulja, N.</td>
<td>SRB</td>
<td>Inst. for Plant Protection &amp; Environment</td>
<td>Monitoring of Cercospora beticola Resistance to Fungicides in Serbia</td>
</tr>
<tr>
<td>10:50</td>
<td>Fernandez-Ortuño, D.</td>
<td>E</td>
<td>IHSM; La Mayora</td>
<td>Resistance to Multiple Fungicides in Botrytis cinerea Isolates from Commercial Strawberry Fields in Spain</td>
</tr>
<tr>
<td>11:10</td>
<td>Chong, P.</td>
<td>ECU/ NL</td>
<td>Wageningen University</td>
<td>Genetic Dynamics In Azole Fungicide Resistant In Pseudocercospora fijiensis</td>
</tr>
<tr>
<td>11:30</td>
<td>Adaskaveg, J.</td>
<td>USA</td>
<td>University of California</td>
<td>Potassium phosphate resistance and new modes of action for managing Phytophthora diseases of citrus in the United States</td>
</tr>
<tr>
<td>11:50</td>
<td>Cherrad, S.</td>
<td>F</td>
<td>CONIDIA</td>
<td>Resistance of Erysiphe necator to SDHI fungicides: first identification in french vineyards, biological and molecular characteristics</td>
</tr>
<tr>
<td>12:10</td>
<td>Hawkins, N.</td>
<td>UK</td>
<td>Rothamsted Research</td>
<td>Adaptive landscapes in fungicide resistance: fitness, epistasis, constraints and predictability</td>
</tr>
<tr>
<td>12:30</td>
<td></td>
<td></td>
<td></td>
<td>Lunch break</td>
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</tbody>
</table>
### FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECTS IV -
Chair: Dr. Bart Fraaije

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Country</th>
<th>Affiliation</th>
<th>Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:00</td>
<td>Kleemann, J.</td>
<td>D</td>
<td>Bayer Crop Science</td>
<td>SDHI resistance in monocot and dicot pathogens</td>
</tr>
<tr>
<td>14:20</td>
<td>Olaya, G.</td>
<td>USA</td>
<td>Syngenta Crop Protection</td>
<td>Detection of the G143A Mutation that Confers Resistance to QoI Fungicides in <em>Alternaria tomatophila</em> isolates from Tomatoes.</td>
</tr>
<tr>
<td>14:40</td>
<td>Klosowski, A.</td>
<td>BR</td>
<td>Uni. Fed. Paraná</td>
<td>Detection of Mutations in Cyp51 and Cytb Genes of <em>Phakopsora pachyrhizi</em> Isolates and Competitive Fitness of Mutated and Wild Type Isolates</td>
</tr>
<tr>
<td>15:00</td>
<td>Lin, D.</td>
<td>CN</td>
<td>China Agricult.. Universit.</td>
<td>Resistance to the Novel Fungicide Oxathiapiprolin in <em>Phytophthora capsici</em>: Risk Assessment and Molecular Mechanism</td>
</tr>
<tr>
<td>15:20</td>
<td>Lopez, F.</td>
<td>AUS</td>
<td>Curtin University</td>
<td>Exploring the Molecular Basis Underlying Fungicide Resistance in <em>Pyrenophora teres f. Sp. teres</em> Infecting Barley in Australia</td>
</tr>
<tr>
<td>15:40</td>
<td></td>
<td></td>
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<td>Coffee break</td>
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### BIORATIONAL FUNGICIDES - Chair: Dr. Erich-C. Oerke

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<tr>
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<tbody>
<tr>
<td>16:00</td>
<td>Schmitt, A.</td>
<td>D</td>
<td>Julius Kühn-Institut; Darmstadt</td>
<td>CO-FREE alternative test products for copper reduction in agriculture</td>
</tr>
<tr>
<td>16:20</td>
<td>Ramseyer, J.</td>
<td>CH</td>
<td>University of Basel</td>
<td>Search for Alternatives to Copper in Organic Farming: Fungicidal Activity of a <em>Juncus effusus</em> Medulla Extract and its Active Constituent Dehydroeffusol against Downy Mildew and Apple Scab</td>
</tr>
<tr>
<td>16:40</td>
<td>Glienke, C.</td>
<td>BR</td>
<td>Federal University of Paraná</td>
<td><em>Xylaria cubensis</em> Isolated from the Medicinal Plant <em>Maytenus ilicifolia</em> as a Biological Control of <em>Phyllosticta citricarpa</em></td>
</tr>
<tr>
<td>17:00</td>
<td>McDonald, M. R.</td>
<td>CA</td>
<td>University of Guelph</td>
<td>A food grade oil reduces foliar diseases of carrot</td>
</tr>
<tr>
<td>17:20</td>
<td>Young, D.</td>
<td>USA</td>
<td>Dow Agro Sciences</td>
<td>Enhancing the Efficacy of Copper Fungicides through Synergism with Salicylaldehyde Benzoylhydrazones</td>
</tr>
<tr>
<td>17:40</td>
<td>Ryabchenko, A.</td>
<td>RUS</td>
<td>Main Botanical Garden</td>
<td>The effectiveness of chemical and biological agents against the pear scab pathogen <em>Fusicladium pyrorum</em></td>
</tr>
</tbody>
</table>
### FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECTS V - Chair: Dr. Andreas Mehl

<table>
<thead>
<tr>
<th>Time</th>
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<th>Presentation Title</th>
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</thead>
<tbody>
<tr>
<td>08:30</td>
<td>Graf, S.</td>
<td>BASF SE</td>
<td>Status of <em>In Vivo</em> and Molecular Diagnosis of Fungicide Resistance in Powdery Mildews</td>
</tr>
<tr>
<td>08:50</td>
<td>Strobel, D.</td>
<td>BASF SE</td>
<td>Field Performance of DMI Fungicides against <em>Zymoseptoria tritici</em> across Europe – Compromized by Further Sensitivity Shift?</td>
</tr>
<tr>
<td>09:10</td>
<td>McDonald, M.R.</td>
<td>University of Guelph</td>
<td>Plant health effects and pathogen sensitivity of <em>Stobilurin</em> fungicides on lentil, chickpea and field pea.</td>
</tr>
<tr>
<td>09:30</td>
<td>Glienke, C.</td>
<td>Federal University of Paraná</td>
<td>Sensitivity of <em>Monilinia fructicola</em> from Brazil to Thiophanate-Methyl</td>
</tr>
<tr>
<td>09:50</td>
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<td></td>
<td>Coffee break</td>
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### RESISTANCE MANAGEMENT - Chair: Dr. Helge Sierotzki

<table>
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<tbody>
<tr>
<td>10:10</td>
<td>Walker, A.</td>
<td>INRA BIOGER</td>
<td>Durable strategies for fungicides use: lessons from the past and leads for improving the future</td>
</tr>
<tr>
<td>10:30</td>
<td>Oliver, R.</td>
<td>CCDM, Curtin University</td>
<td>Fungicide resistance management in practice; mixtures, alternations and cross resistance patterns</td>
</tr>
<tr>
<td>10:50</td>
<td>Carolan, K.</td>
<td>Rothamsted Research</td>
<td>Managing the evolution of fungicide resistance in potato blight</td>
</tr>
<tr>
<td>11:10</td>
<td>Khan, M.</td>
<td>N. Dakota State Univ.; U of Minnesota</td>
<td>Developing, Disseminating and Evaluating Management Strategies for Controlling Rhizoctonia solani on Sugar Beet in the United States</td>
</tr>
<tr>
<td>11:30</td>
<td>Wieczorek, T.</td>
<td>Aarhus Universitet</td>
<td>Spraying Strategies Avoiding Selection of <em>Zymoseptoria tritici</em> Triazole Resistance</td>
</tr>
<tr>
<td>11:50</td>
<td></td>
<td></td>
<td>CONFERENCE CLOSING - Prof. Dr. Holger B. Deising</td>
</tr>
</tbody>
</table>
Events like this are made possible through the financial support of donors, interested in promoting science.

Therefore, we would like to thank the *German Science Foundation (DFG)* for their generous financial support.

In addition we appreciate the donations to finance the proceedings volume of

![DFG](image)

![FRAC](image)

![BASF](image)

![Bayer CropScience](image)

![Syngenta](image)
Abstracts

Oral Presentations

(Listed in chronological order)
Monday, April 25, 2016 - Morning

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<td></td>
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<td>08:40</td>
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<td>Gurr, S.</td>
<td>UK</td>
<td>University of Exeter</td>
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<td>Global movement of fungi</td>
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<td>Lucas, J.</td>
<td>UK</td>
<td>Rothamsted Research</td>
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<td>Velcourt Ltd</td>
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<td>Coffee break</td>
</tr>
<tr>
<td>10:30</td>
<td>KEYNOTE LECTURES Part II</td>
<td>Steinberg, G.</td>
<td>UK</td>
<td>University of Exeter</td>
</tr>
<tr>
<td></td>
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<td>Cell biology informs fungicide development</td>
</tr>
<tr>
<td>11:00</td>
<td></td>
<td>Kogel, K.-H.</td>
<td>D</td>
<td>University of Gießen</td>
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<td>The agronomic potential of gene silencing applications</td>
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<td>Bayer CropScience</td>
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<td>Contribution of plant responses to efficacy of fungicides</td>
</tr>
<tr>
<td>12:00</td>
<td></td>
<td>Zhou, Ming-guo</td>
<td>CN</td>
<td>Nanjing Agricultural University</td>
</tr>
<tr>
<td></td>
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<td>Myosin as a selective target for new fungicide phenamacril</td>
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<td>Lunch break</td>
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</table>
Global movement of fungi

Abstract ID 105
Sarah Gurr

*Biosciences, University of Exeter, Exeter EX4 4QD, United Kingdom
S.J.Gurr@exeter.ac.uk

Over the past centuries, crop diseases have led to the starvation of the people, the ruination of economies and the downfall of governments. Of the various challenges, the threat to plants of fungal infection outstrips that posed by bacterial and viral diseases combined. Indeed, fungal diseases have been increasing in severity and scale since the mid. 20th Century and now pose a serious threat to global food security and ecosystem health.

We face a future blighted by known adversaries, by new variants of old foes and by new diseases. Modern agricultural intensification practices have heightened the challenge - the planting of vast swathes of genetically uniform crops, guarded by one or two inbred resistance genes, and use of single target site antifungals has hastened emergence of new virulent and fungicide-resistant strains. Climate change compounds the saga as we see altered disease demographics - pathogens are on the move poleward in a warming world.

This presentation will highlight some current notable and persistent fungal diseases. It will consider the evolutionary drivers underpinning emergence of new diseases and allude to the accelerators of spread. I will set these points in the context of recent disease modelling, which shows the global distributions of crop pathogens and their predicted movement and will discuss the concept of crop disease saturation. I shall conclude with some thoughts on future threats and challenges, on fungal disease mitigation and of ways of enhancing global food security.
Resistance management: we know why, but do we know how?

Abstract ID 109
John A Lucas

Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK
John.lucas@rothamsted.ac.uk

The targeted use of chemistry to control pests, diseases and weeds has played an important role in improving crop yield and quality, and ensuring stability of production. Growers have had access to a diversity of effective and affordable crop protection products that in most circumstances have given an economic return. This scenario is now changing due to an adverse regulatory regime and the emergence of resistance to many previously effective pesticides. In particular, this has compromised the efficacy of several classes of highly active single-site inhibitors that have dominated the recent crop protection market. Resistance has become a fact of life for both agrochemical producers and users, with resistance risk assessments and management strategies becoming an integral part of product stewardship. The good news is that technological advances have provided more rapid, sensitive, and accurate methods for the detection and quantification of resistance in pathogen populations. There have also been major advances in understanding the genetics and mechanisms of resistance to specific fungicide classes. The less good news is that there is still no fool-proof way to predict when and where resistance will occur, how quickly it might increase to affect field efficacy, and what management strategies will be most effective in preventing it. We still rely to a large extent on extrapolation from known cases, and intensive monitoring of field populations, to assess resistance development in practice. What can be done to change this? Mutation studies, along with experiments on the in vitro evolution of resistance, can provide clues to the potential for resistance development, and possible evolutionary pathways. Insights from comparative genomics are now shedding light on some previously unknown mechanisms affecting pathogen sensitivity. Improved understanding of the recent history of resistance development might inform future scenarios. Resistance risk assessments based on multiple biological, chemical and molecular parameters are helping to refine the process. Mathematical models evaluating the likely outcomes of different resistance management strategies are also being applied, and in addition to suggesting optimum tactics for contrasting situations, can identify current knowledge gaps. Despite all these advances, many uncertainties remain, and resistance management is still an inexact science. Delaying or reducing directional selection for resistance through use of mixtures and improved integration with cultivar resistance and agronomic measures, where available, is the main strategy, but depends on a diversity of effective modes of action. In the medium to longer term, new tools for crop protection such as RNAi and host-induced gene silencing should become available, but in many cases the disease control and resistance management options are currently limited.
The Challenges of Farming with Resistance

Abstract ID 107
Keith Norman, Technical Director
Velcourt Ltd Great North Road, StrettonOakham, Rutland, LE15 7QT, UK
knorman@velcourt.co.uk

The presentation will look at the progression of *Septoria tritici* resistance in the UK with the two key triazoles, epoxiconazole and prothioconazole and what this looks like in the field. Changes of insensitivity between seasons and within a crop season will be illustrated. In addition, evidence of the first signs of SDHI insensitivity will be discussed.

The implications of fungicide resistance on the growing of wheat in the UK will be discussed in more detail, together with various cultural and chemical options available to growers to mitigate the risks.

Regulatory issues will also be highlighted and the importance of EU regulatory authorities being kept up to date with field performance to key fungicides, to avoid vital actives being withdrawn.

The talk will also include resistance of Light Leaf Spot (*Pyrenopeziza brassicae*) in Oilseed rape to triazoles. The decreasing control of this disease with triazoles is becoming more apparent in the field and has been cited in the literature.

The talk will conclude looking at areas of development that are needed to manage the situation more effectively.
Cell biology informs fungicide development

Abstract ID 106
Gero Steinberg

*University of Exeter, UK, College of Life and Environmental Sciences, Ex4 4QD, Exeter, UK*

G.Steinberg@exeter.ac.uk

Fungi comprise the most devastating challenges to our crops. Their haploid genome and short life cycle enables them to rapidly develop resistance against anti-fungal drugs. New insight into the biology of fungi is required to better understand crucial processes that provide novel fungicide targets. Recently, the development of powerful live cell imaging techniques and tools has opened new avenues in studying fungal pathogens. In this talk, I will summarize recent advances in understanding the cell biology of the pathogen *Zymoseptoria tritici*, the causative agent of septoria tritici blotch of wheat. Furthermore, I shall provide insight into the use of cell biology to better define fungicide mode of action.
RNA interference (RNAi) has emerged as a powerful genetic tool for scientific research over the past several years. It has been utilized not only in fundamental research for the assessment of gene function, but also in various fields of applied research, such as human and veterinary medicine and agriculture. In plants, RNAi strategies have the potential to allow manipulation of various aspects of food quality and nutritional content. In addition, the demonstration that agricultural pests, such as insects and nematodes, can be killed by exogenously supplied RNAi targeting their essential genes has raised the possibility that plant predation can be controlled by lethal RNAi signals generated in planta. Indeed, recent evidence argues that this strategy, called host-induced gene silencing (HIGS), is effective against sucking insects and nematodes; it also has been shown to compromise the growth and development of pathogenic fungi, as well as bacteria and viruses, on their plant hosts. In this talk, I will review recent studies that reveal the enormous potential RNAi strategies hold not only for improving the nutritive value and safety of the food supply, but also for providing an environmentally friendly mechanism for plant protection.
Contribution of plant responses to efficacy of fungicides – A perspective

Abstract ID 100
Klaus Tietjen

*Bayer AG, Bayer Cropscience Research and Development, Disease Control Monheim, Building 6240, 40789 Monheim, Germany*

*Email: Klaus.Tietjen@bayer.com*

It is a frequent field observation, that fungicides exert beneficial effects in crop plants beyond their direct fungicidal action. Such crop strengthening effects, which are described e.g. as greening or as stress tolerance, apparently can increase yield. Although in the past years advanced phenotyping methods have been developed, molecular explanations are only fragmentary. Focussing on azoles and strobilurins some biochemical, molecular biological and physiological mechanisms are outlined. A comparison with host plant defence inducers sheds a light on plant-driven mechanisms, which successful fungicides also might acticate.
Myosin as a selective target for new fungicide phenamcaril

Abstract ID 79
Zhitian Zheng, Yiping Hou, Bin Li, Xiumei Liu, Yiqiang Cai, Yanjun Li and Mingguo Zhou
Coll. of Plant Protection, Nanjing Agricult. University, Nanjing, Jiangsu Province, China
mgzhou@njau.edu.cn

Phenamcaril (JS399-19) is a new selective fungicide specifically controlling *Fusarium* diseases. Lab resistance of phenamcaril was easily to obtain in the target fungi and resistance degree would decrease dramatically by disrupting fimbrin gene encoding actin-bundling protein. Meanwhile, we sequenced and annotated the genome of phenamcaril resistant strain YP-1 generated from *F. graminearum* reference strain PH-1. Compared with strain PH-1, 132 genes of YP-1 have nucleotide mutation leading amino acid shifts. Of those genes, 22 genes related to actin function in *Fusarium asiaticum*, a major causal agent of fusarium head blight in China, were sequenced and compared between phenamcaril resitants and their original sensitive strain 2021. Mutations of all resistant strains occurred in the same gene encoding myosin5. Mutations in myosin5 confer resistance to phenamcaril were confirmed by myosin5 locus homologous double exchange between sensitive and resistant strain. Phenamcaril resistant mutants were randomly selected for analyzing relationship of resistance level and mutation genotype. Of 82 resistant mutants, 25.6%, 7.3% and 67.1% showed low resistance (LR, 1.5-15μg/ml), moderate resistance (MR, 15.1-75μg/ml) and high resistance (HR, >75μg/ml), respectively, to phenamcaril determined by EC50 values. LR includes genotypes of A135T, V151M, P204S, I434M, A577T, R580G/H or I581F. MR includes of S418R, I424R or A577G and HR includes K216R /E, S217P/L or E420G/D. Interestingly, all of the mutations concentrated in myosin5 motor domain and most of mutations conferring to high resistance occurred at codon 217 and 420, which we called that ‘core region’. Homology modeling revealed that mutations far away from the ‘core region’ lead to lower resistance degree.

Homology of myosin5 motor domain in different pathogenic fungi was associated with their sensitivity to phenamcaril. *F. verticilloides* and *F. oxysporum* have higher homologous myosin5 sequence than *F. graminearum* and showed sensitive to phenamcaril. While *Botrytis cinerea*, *Magnaporthe oryzae* and *Blumeria graminis* have lower homologous myosin5 than *F. graminearum* and appeared insensitive. It revealed that the variety of myosin5 is selectivity basis of phenamcaril. Regulation role of phenamcaril sensitivity and resistance by fimbrin, myosin class V myosin 2B (FGSG_07469.1) and myosin passenger protein Smy1p as well as some ncRNAs was verified through these gene heterocaryotic disruption, deletion and over expression. Finally, mechanism of hypha growth inhibition by interfering myosin5 was discussed.
**Monday, April 25, 2016 - Afternoon**

### NEW TECHNOLOGIES AND APPLICATIONS - Chair: Dr. Bart Fraaije

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<td>14:20</td>
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<td>University of Gießen</td>
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<td>King, K.</td>
<td>UK</td>
<td>Rothamsted Research</td>
<td>Development of rapid in-field diagnostic assays to detect CYP51 and/or MgMfs1 over-expressing strains of <em>Zymoseptoria tritici</em> in leaf samples</td>
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<tr>
<td>15:20</td>
<td>Schnabel, G.</td>
<td>USA</td>
<td>Clemson University</td>
<td>Communication of FRAC Code Principles With Growers with Smartphones</td>
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<tr>
<td>15:40</td>
<td>Sierotzki, H.</td>
<td>CH</td>
<td>Syngenta Crop Protection</td>
<td>ADEPIDYN (TM): A New Broad Spectrum Foliar Fungicide for Multiple Crops</td>
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<td>16:00</td>
<td>Hernandez, C.</td>
<td>F</td>
<td>CONIPHYS</td>
<td>Resistance to SDHI fungicides: A new method for monitoring populations of <em>Pyrenophora teres</em>.</td>
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<td>16:20</td>
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### POSTER DEMONSTRATION

16:30-18:00
New methodology breakthrough in microscopy: Fluorescence Visualization of Living Fungal Pathogens inside Plant Tissues

Abstract ID 65
Viviane Calaora¹, Benjamin Perotin², Bernard Straebler², Jean-Marc Seng³, Sergej Buchet¹
¹ BIOtransfer Ltd., 41 rue Emile Zola, F-93100 Montreuil, France
² Du Pont de Nemours SAS, 24 rue du Moulin, F-68740 Nambsheim, France
³ Institut of Plant Sciences Paris-Saclay (IPS2), UMR 9213/UMR1403, CNRS, INRA, Université Paris-Sud, Université d'Evry, Université Paris-Diderot, Sorbonne Paris-Cité, Bâtiment 630, F-91405 Orsay, France
Viviane.Calaora@Biotransfer.fr

The development of the optical microscope in the seventeenth century opened up new areas of study in many fields of science. In particular, the observations of plant, animal tissues and micro-organisms gave rise to cell biology. In the past twenty years, the use of highly sensitive and specific fluorescence probes made optical studies of living cells possible as light is a relatively non-destructive method of probing cells. Two main applications of live cell imaging have emerged: first to follow the morphology and dynamics of different cell compartments and organelles (morphological staining) and secondly to infer physiological information (vital and death staining). BIOtransfer has been developing these techniques since 2000 to visualize the biological efficacy of fungicides towards a range of fungal plant pathogens. One important question was unsolved: managing to visualize in situ the viability of a fungal pathogen directly in the plant tissues (in a non destructive assay). Indeed, the limiting factor was to introduce the fluorescent probes into the plant tissues / cells in order to give a good signal-to-noise without causing toxic effects or significant disturbance to both plant and pathogen cell morphology and physiology.

The problem/difficulty was recently solved by the researchers of BIOtransfer, and has allowed visualizing the viability and development of some fungal pathogens of economical interest, in planta. As a case study, a collaboration project has been carried out with Du Pont de Nemours, on a new antioomycete fungicide. The innovative methodology has allowed showing curative effects towards oomycetes (Plasmopara viticola and Phytophthora infestans) in plant tissues (respectively vine leaves and potato leaflets). This new technique has allowed showing curative effect of a fungicide protection on each epidemiological and microbiological development stages of each pathogen as well within the different plant tissue layers (inside) and on the plant epidermis / cuticle (outside). As all the cellular and subcellular structures can be visualized and combined with the viability status of the fungal pathogen, a very accurate picture precise image of the biological fungicide activity can be obtained. The outcomes of this new methodology will be discussed.
Towards an RNAi based control of plant diseases: Assessment of a potential spray application

Abstract ID 43
Aline Koch; Karl-Heinz Kogel
Institute for Phytology, JLU Giessen, Institute for Phytology, JLU Giessen
Aline.Koch@agrari.uni-giessen.de

Meeting the increasing food and energy demands of a growing population will require developing ground-breaking strategies that promote sustainable plant production. RNA interference (RNAi) has emerged as a powerful genetic tool for scientific research over the past several years. It has been utilized not only in fundamental research for the assessment of gene function, but also in various fields of applied research, such as human and veterinary medicine and agriculture.

In plants, RNAi strategies have the potential to allow manipulation of various aspects of food quality and nutritional content. In addition, the demonstration that agricultural pests, such as insects and nematodes, can be killed by exogenously supplied RNAi targeting their essential genes has raised the possibility that plant predation can be controlled by lethal RNAi signals generated in planta. Indeed, recent evidence argues that this strategy, called host-induced gene silencing (HIGS), is effective against sucking insects (Abdellatef et al. 2015) and nematodes; it also has been shown to compromise the growth and development of pathogenic fungi (Koch et al. 2013), as well as bacteria and viruses, on their plant hosts (Koch and Kogel 2014).

However, while delivery of inhibitory noncoding double-stranded (ds)RNA by transgenic expression is a promising concept, it requires the generation of transgenic crop plants which may cause substantial delay for application strategies depending on the transformability and genetic stability of the crop plant species. Using the agronomically important barley - Fusarium graminearum pathosystem, we alternatively demonstrate that spraying a long noncoding dsRNA, which targets the biosynthesis of fungal ergosterol, silences the expression of these fungal genes and inhibits fungal growth.

Given the ease of design, high specificity, and applicability to diverse pathogens, the use of target-specific dsRNA as an anti-fungal agent offers unprecedented potential as a new plant protection strategy.
Oxathiapiprolin: Low and Slow Mobility Still Provides Longlasting Protection, and Protection of New Growth

Abstract ID 88
Helge Sierotzki¹, David Beattie², Karen Meade³, Sarah Allen³
¹Syngenta Crop Protection AG, Shaffhauserstrasse, CH-4332 Stein, Switzerland
²Syngenta Crop Protection AG, Schwarzwaldallee 215, CH-4058 Basel, Switzerland
³Syngenta Crop Protection Ltd, Jealott's Hill Research Station, Bracknell RG42 6EY, UK

Oxathiapiprolin is a new active ingredient for the control of downy mildews, blights, and other Oomycete pathogens, providing highly potent and long-lasting control in the field without cross-resistance to current standards, which will be marketed by Syngenta under the ORONDISTM brand name.

Oxathiapiprolin has a LogP of 3.66, PKA >1 and water solubility in the range of 0.5-13ppm. Based on this, the compound should show relatively low mobility in the plant. Studies using radiolabelled material in foliar and root applications of tomato and grapevine confirmed this hypothesis, with uptake and movement clearly lower than commercial standards.

Despite the low mobility of the compound, oxathiapiprolin offers protection of new growth. A single foliar application to young tomato plants at 15ga/ha provided long-lasting protection against Phytophthora infestans, with infection only appearing 28-30 days after application even under regularly repeated inoculation. LCMS analysis from this study showed that a small amount of oxathiapiprolin moved into the new growth as the plant developed, which supports the hypothesis that oxathaipiprolin shows weak tissue diffusion, no phloem mobility and limited xylem mobility, although the biology data shows that even this small amount of movement is enough to give activity against infection on new growth.

The protection on new growth is most likely provided by a.i. that landed on the stem, and translocated to new growth via the xylem. This hypothesis is still being investigated.

In the field, this translates into long-lasting control, with ORONDIS products outperforming competitors for disease control, yield and duration of control.
Development of rapid in-field diagnostic assays to detect CYP51 and/or MgMfs1 over-expressing strains of Zymoseptoria tritici in leaf samples

Abstract ID 78
Kevin King, Narin Kirikyali, Bart Fraaije
Fungicide Research Group, Biological Chemistry & Crop Protection Department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom

Septoria leaf blotch caused by the fungus Zymoseptoria tritici is currently controlled by programmed applications of two groups of systemic fungicides: the DeMethylation Inhibitors (DMIs) and a new generation of Succinate DeHydrogenase Inhibitors (SDHIs). Some strains of the fungus have adapted to the fungicides by evolving target and/or non-target site related resistance mechanisms. Resistance can be caused by point mutations in the target-encoding gene, which results in amino acid substitutions leading to a lower binding affinity of fungicides with the target protein. DNA inserts in gene promoter regions can change the expression of fungicide target proteins or efflux pumps. A 120 bp insert in the CYP51 promoter has been linked with CYP51 overexpression and reducedazole sensitivity while a 519 bp insert in the promoter of the Major Facilitator Superfamily transporter MgMfs1 has been linked with MgMfs1 overexpression and reduced sensitivity to several classes of fungicides, including DMIs and SDHIs. Rapid diagnostic tools able to measure the frequency of fungicide resistant alleles before spray applications can inform disease management strategies. Here we present the development and application of loop-mediated isothermal amplification (LAMP) assays for rapid in-field detection of CYP51 and MgMfs1 promoter inserts in Zymoseptoria tritici populations.
Communication of FRAC Code Principles With Growers with Smartphones

Abstract ID 68
Guido Schnabel, Mengjun Hu, Gregory Edison, and Roy Pargas

Clemson University, Department of Agricultural and Environmental Sciences and Department of Computer Sciences, Clemson, SC

schnabe@clemson.edu

Effective communication of resistance management strategies with growers is important for practical resistance management, but restrictions to crops and the many choices of active ingredients, trade names, and chemical classes make it difficult for growers to make informed decisions. We developed a smartphone application, MyIPM, to promote integrated disease and resistance management principles for fruit growers. The app features registered active ingredients and trade names for about a dozen of the most important diseases of blueberry, strawberry, and peach. Active ingredients are color-coded by FRAC code and an interactive table lets the user swiftly sort by FRAC code and efficacy ratings. The associated trade names can be requested by pushing on the active ingredient name. Pictures of signs and symptoms, descriptions of the causal agent, and a 2-min audio from the regional specialist provide additional diagnostic tools and communicate IPM principles. The app also features field EIQ values for formulated products as published by the Cornell IPM Program. MyIPM content is controlled by an external database that can be updated within seconds using an authoring tool allowing for up to date information. It is expandable to more crops and is currently being developed for apple, pear, cherry, and cranberry. A sister product ‘MyIPM-SEF-P’ was just published for blueberry pest management in the southeastern USA. The apps are available free of charge in Google Play and the Apple Store.
ADEPIDYN™ fungicide: A New Broad Spectrum Foliar Fungicide for Multiple Crops

Abstract ID 58
Helge Sierotzki¹, Hans-Ulrich Haas¹, Michael Oostendorp¹, Harald Walter², Daniel Stierli¹ and Cosima Nuninger²

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²Syngenta Crop Protection AG, Schwarzwaldallee 215, 4058 Basel, Switzerland
helge.sierotzki@syngenta.com

ADEPIDYN™ fungicide is the new carboxamide fungicide discovered by Syngenta, which is the first member of a new chemical subgroup among the succinate dehydrogenase inhibitor (SDHI) fungicides, the phenyl-ethyl pyrazole carboxamides. The ISO common name for ADEPIDYN™ fungicide is pydiflumetofen. The compound was selected based on its particular strength against Fusarium species, especially Fusarium Head Blight of cereal crops. It possesses high binding properties to the complex II enzyme. It also delivers a very high efficacy against many leaf spots (such as Cercospora spp., Alternaria solani and Venturia inaequalis) setting a new performance standard in various crops (such as apples, wheat and peanuts). Further, it provides excellent control of powdery mildews across multiple crops. In addition, ADEPIDYN™ fungicide is highly active on difficult to control diseases such as Botrytis cinerea, Sclerotinia sclerotiorum, and Corynespora cassicola, that cause severe damage on important crops. This spectrum makes it the ideal fungicide to complement the Syngenta fungicide portfolio and to introduce a new mode of action for Fusarium control. The observed movement of ADEPIDYN™ fungicide combined with excellent quantitative rainfastness provides long lasting activity. It can be safely mixed with various other active ingredients, which allow ADEPIDYN™ fungicide formulations to provide activity against a comprehensive spectrum of pathogens on a wide range of crops and also provide a tool for the management of fungicide resistance in the target populations.
Resistance to SDHI fungicides: A new method for monitoring populations of *Pyrenophora teres*.

Abstract ID 99
Hernandez C.¹, Vacher S.² and Steva H³
¹CONIPHY, Parc d’activités en Chuel, Route de Chasselay, 69650 Quincieux, France
²CONIDIA, Parc d’activités en Chuel, Route de Chasselay, 69650 Quincieux, France
³CJH SARL, 21 C Chemin de la Girotte, 33650 LA BREDE, France
c.hernandez@conidia.fr

Succinate Deshydogenase Inhibitor (SDHI) molecules are used in a wide variety of crops against various pathogens. These last two years, evolution of *Pyrenophora teres* to SDHI resistance in Europe is remarkable.

CONIPHY developed a new original method for studying the structure of population of *Pyrenophora teres* to SDHI. Collection and obtaining sporulation of spores of *Pyrenophora teres* spores from infected leaves was the first challenge.

The method developed is based on the study of hundred of spores from each location and allow to quantify the resistance of spores to different concentrations of SDHI. The evolution of structures of population of *Pyrenophora teres* can also be studied year by year to manage the crops treatments.

The presentation will focus on the results of structure of populations of *Pyrenophora teres* to SDHI on more than 40 sites in France in 2015.
### FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECT I -
Chair: Dr. Erich-C. Oerke

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<td>Aarhus University</td>
<td>Azoles Have Different Strengths and Perform Diversely Across Europe</td>
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<td>SRUC</td>
<td>Fungicide Sensitivity Monitoring in Cereals, Forest and Minor Crop Pathogens in the UK</td>
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<td>Nanni, I.</td>
<td>University of Bologna</td>
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<td>Fungicide Resistance: Learning from <em>Botrytis</em> Monitorings</td>
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<td>09:50</td>
<td>Fraaije, B.</td>
<td>Rothamsted Research</td>
<td>Azole and SDHI sensitivity status of <em>Zymoseptoria tritici</em> field populations sampled in France, Germany and the United Kingdom during 2015</td>
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### FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECTS II -
Chair: Dr. Helge Sierotzki

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<td>Developing Fungicide Control Programmes for <em>Septoria tritici</em> Blotch in Irish Winter Wheat Crops</td>
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<td>Sensitivity of <em>Pyrenophora teres</em> to Succinate Dehydrogenase Inhibitors in Europe</td>
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<td>South Austral. Research &amp; Development Institute</td>
<td>Fungicide Resistance in Australian Viticulture</td>
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<td>11:50</td>
<td>Toffolatti, S.</td>
<td>University of Milan</td>
<td>Evaluation of a CAA-based Management Strategy for the Downy Mildew Control in a Vineyard with CAA Resistance</td>
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**14:00** SYMPOSIUM EXCURSION
Azoles Have Different Strengths and Perform Diversely Across Europe

Abstract ID 50
Lise Nistrup Jørgensen, Niels Matzen¹, Roma Semaskiene², Marek Korbas³, Mariola Glazek⁴, Claude Maumene⁵, Bernd Rodemann⁶, Stephan Weigand⁷, Michael Hess⁸, Jonathan Blake⁹, Bill Clark¹⁰, Stephen Kildea¹¹, Charlotte Batailles¹², Rita Ban¹³.
Aarhus University, Flakkebjerg, 4200 Slagelse, Denmark¹.
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Institute of Plant Protection -National Research Institute, Poznań, Poland³.
Institutet of Plant Protection, Sosnicowice, Poland⁴.
Arvalis Institut du Végétal, 91720 Boigneville, France⁵.
JKI, 38104 Braunschweig, Germany⁶.
Institut für Pflanzenschutz Bayerische Landesanstalt für Landwirtschaft, Bavarian State Research. 85354 Freising-Weihenstephan, Germany⁷.
Phytopathology, TUM School of Life Sciences, 85354 Freising-Weihenstephan, Germany⁸.
ADAS Rosemaund, Preston Wynne, Hereford, HR1 3PG, UK⁹.
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Teagasc, Oak Park Crops Research Centre, Carlow, Ireland¹¹.
Gembloux, Protection des Plantes et ‘Eco-toxicologie, 5030 Gembloux, Belgium¹².
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Leaf diseases cause major yield losses in winter wheat every year across Europe. Septoria leaf blotch - STB (Zymoseptoria tritici) is the most serious leaf disease in Northern Europe but also yellow rust - YR (Puccinia striiformis) and brown rust - BR (Puccinia triticina) are known to cause major problems in some regions and seasons. In recent years increasing problems with fungicide resistance in the population of Z. tritici have given concerns for future options for control. Azoles have been used for more than 35 years but are still seen as the backbone of disease management and provide moderate to good control of STB depending on locality and the specific azole used. Due to varying disease pressures and fungicides available in the different countries the patterns of fungicide use vary greatly between different European countries. Because of this, fungicides efficacies also vary considerably. In 2015, 26 individual field trials were carried out in Europe representing different climate and cropping conditions. A common protocol was used in order to compare the efficacy profiles of 6 azole fungicides against STB, YR and BR in nine
European countries. Fungicide application was carried out once at BBCH 37-43 using full and half label doses of the specific fungicides. Diseases were assessed at regular intervals with focus on assessments carried out around GS 75. Apart from efficacy evaluation; leaf samples were also collected to investigate, the composition of CYP51 mutations of the *Z. tritici* populations and isolates were analyzed for EC$_{50}$ values to the main azoles. The presentation will focus on field performances. 17 trials had STB as the major disease. Major differences in the ranking of products against STB were seen depending on the location across Europe. However certain common patterns were detectable using comparable rates. Generally the products provided better preventive control than curative control. Overall the best preventive and curative control of STB was provided by Opus Max, Osiris and Prosaro. The co-formulations Osiris and Prosaro gave the most consistent control of STB across all sites. Treatments with Caramba gave in France, Belgium and Ireland a better control of STB than in other countries providing high levels of control (70-90 %) relative to other countries (40-70 %). The opposite was true of the curative control of STB by Proline, which in these countries stood out as being relatively low (40-50 % compared to 60-90 % in the other countries). Furthermore, Folicur performed very well in Ireland (89 %), whereas this product performed poorly in other countries (ca. 50%). The products were generally much more effective in their control of YR (ca. 80-90 %) compared to STB (ca. 60-70 %). This was especially the case for Opus Max and Folicur, which together with Prosaro provided the highest preventive control (ca. 90 % control). Against BR the two best products were Osiris and Opus Max (ca. 80 %), whereas the control from Proline was clearly inferior (ca. 50 %). The trials gave positive and significant yield increases. Higher yield increases were achieved by treatments in trials dominated by YR (rel. 122-142), than those dominated by STB (rel. 106-112) or brown rust (rel. 105-118). The activity is to continue in 2016.
Fungicide Sensitivity Monitoring in Cereals, Forest and Minor Crop Pathogens in the UK

Abstract ID 51
Marta Piotrowska, Fiona Burnett, Ashleigh Mackenzie, Carolyn Riddell, Jeanette Taylor, Kalina Gorniak, Neil Havis

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One of the biggest concerns in modern agriculture is the evolution of resistance to fungicides used to control fungal pathogens. Intensive monitoring programs can prove helpful in effective disease and resistance management; however for many fungal pathogens we still lack basic information on baseline sensitivity to which subsequent testing can be compared. In this paper we discuss this issue using examples from three different pathosystems: (i) increasingly well-studied barley pathogen, *Ramularia collo-cygni*; (ii) less-studied pine pathogen, *Dothistroma septosporum* and (iii) under-studied pathogen of minor crops, *Botrytis cinerea*. *Ramularia collo-cygni* and *Dothistroma septosporum* are genetically closely related ascomycete fungi but different management options have been used to minimise damage caused by the pathogens. Effective protection against *Ramularia* has been almost exclusively based on fungicide applications and both fungicide resistance status and sensitivity profiles to major fungicides groups have been well documented throughout the years. In contrast *Dothistroma* has been mainly managed by silvicultural methods in forest plantations but in addition, a limited number of fungicides have been applied to forest nursery stocks. The effectiveness of fungicide classes applied, however, as well as the possible declines in sensitivity to some of the high risk fungicides, remains largely unknown. Similarly to forestry, minor crops in the UK face a problem of limited numbers of approved actives and fewer management options available for the growers. One example is *Botrytis cinerea*, a fungal pathogen with particularly unstudied fungicide sensitivity profiles in the UK. In this paper we present current fungicide efficacy data for the barley pathogen, *Ramularia collo-cygni* and we discuss the results from sensitivity testing to major fungicide classes in *Dothistroma septosporum* and *Botrytis cinerea*. We also discuss how knowledge transfer between broad acre crops, forestry and minor crops can be used to manage resistance risk in the most effective way.
Efficacy of Carboxylic Acid Amides (CAA) Fungicides towards CAA Sensitive and CAA Resistant *Plasmopara viticola* Populations: *In Vivo* Tests and Molecular Studies on PvCesA3 Gene

Abstract ID 111

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*Plasmopara viticola* is controlled by fungicides with different modes of action, including carboxylic acid amides (CAAs). Dimethomorph was the first CAA introduced in 1988, followed by iprovalicarb, flumorph, benthiavalicarb, mandipropropamid, valifenalate and latest pyrimorph in 2010 (Gisi et al. 2012). The mode of action of CAA compounds is linked to the inhibition of cellulose synthesis in the Oomycete plant pathogens. The mutations conferring CAA resistance in *P. viticola* located on the CesA3 gene are G1105S and G1105V. The aim of this work was to evaluate the activity of CAAs on CAA sensitive and CAA resistant isolates of *Plasmopara viticola*. Bioassays on leaf discs, on detached leaf and on grape plants trials were carried out. In leaf discs assay, CAAs tested on Italian strains showed different level of activity and in particular dimethomorph showed lower levels of EC95. The detached leaf tests showed under preventative conditions that the CAA-sensitive strains were fully controlled by all CAAs, while the CAA-resistant strains were best controlled by dimethomorph (Nanni et al. 2015). Under curative conditions all CAAs showed good activity on the sensitive strains, while the resistant strains were not controlled by any CAA. This finding confirms the cross resistance between CAAs. The good activity of dimethomorph on CAA resistant isolates under preventive conditions was also confirmed in greenhouse tests where whole plants were inoculated. All CAA resistant strains carried the G1105S/V mutations, which were detected by molecular techniques such as pyrosequencing, CAPS-PCR or qPCR. In order to gain a better understanding of the different behaviour among the CAA fungicides, molecular modelling and docking studies are still ongoing.


Fungicide Resistance: Learning from Botrytis Monitorings

Abstract ID 59
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Botrytis cinerea, causing gray mold on a large variety of crops is a difficult to control disease and a high risk pathogen for fungicide resistance development. We will present European monitoring results gathered over the past 6-10 years and discuss the different situations across crops and spray regimes. The identification of novel resistance mechanisms was followed by the development of a range of molecular assays which inform us more precisely about the resistance situation. Our molecular characterization encompasses type of strain, target-related resistances for mode of actions such as the SDHIs, the QoIs and the APs and non-target-related resistance mechanisms such as multi-drug resistance (MDR). We will give an overview of the frequency of these genotypes and associated phenotypes, which sometimes reflects differential fitness penalty. Finally we will discuss the exceptional robustness of SWITCH® introduced more than 20 years ago but still delivering excellent control.
Azole and SDHI sensitivity status of *Zymoseptoria tritici* field populations sampled in France, Germany and the United Kingdom during 2015

Abstract ID 62
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Azole fungicides have been used for more than three decades to control Septoria leaf blotch caused by the fungus *Zymoseptoria tritici*. However, the fungus has adapted over time to resist azoles and studies have shown that combinations of three different resistance mechanisms can be present in field isolates. The most common mechanisms are CYP51 target alterations with up to 9 different amino acid residues simultaneously affected. More recently, overexpression of *CYP51* and/or *MgMfs1*, a Major Facilitator Superfamily transporter (efflux pump), have been shown to contribute to azole insensitivity. Due to resistance to Quinone outside Inhibitors (QoIs) and loss of azole efficacy, Succinate DeHydrogenase Inhibitors (SDHIs) and multi-site inhibitors have become key components in spray programmes. SDHIs are at risk of resistance development and continued monitoring of fungicide sensitivity in field populations is required to inform optimal disease management strategies. Here we present the latest genotype-to-phenotype relationships for azole and/or SDHI insensitive field strains of *Zymoseptoria tritici* sampled at different locations in 2015 and discuss the practical implications for Septoria leaf blotch control.
SBI sensitivity status of major cereal pathogens in Europe

Abstract ID 110
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Septoria leaf blotch, caused by the ascomycete *Mycosphaerella graminicola* (anamorph *Zymoseptoria tritici*), is the most important disease of wheat in North-Western European cereal growing areas. As field resistance of *M. graminicola* populations towards QoI fungicides is widely spread since many years, today’s control of Septoria leaf blotch depends mainly on the use of DMI- and SDHI fungicides to ensure high yield and quality of the crop. Consequently, resistance research during the past years focused mainly on *M. graminicola* and both fungicide classes.

Resistance of practical importance towards site-specific and systemic fungicides is mostly based on point mutations of the target. E.g., with QoIs, the widespread presence of field isolates of many pathogens carrying a single nucleotide polymorphism in the fungal cytochrome b gene, leading to disruptive resistance patterns, correlates generally well with a decreasing efficacy of solo applied QoI products on the field level. Although DMIs target as well a single protein, and presence of point mutations of the sterol-14α-demethylase (*cyp51*) target site has been reported particularly in *M. graminicola* since more than ten years, sensitivity changes during the last decade have developed much slower, still following a ‘shifting-type’ pattern. During this period of time, the relevance of *cyp51* alterations in the development of DMI sensitivity changes has been thoroughly described by different research groups as well as an increasing number of genotype and phenotype groups. Further on it was reported that, with regard to loss of *in vitro* efficacy, not all DMIs are equally affected.

The consequences of these findings for praxis are still controversially discussed, particularly in regard to different spray regimes and DMI products applied on the regional level. Besides *M. graminicola*, several other important pathogens also on other cereal crops like barley are on a regular basis controlled by fungicide programs containing DMI fungicides. The aim of this study is to discuss the current DMI sensitivity status of further major cereal diseases like powdery mildew, rust, net blotch, and Rhynchosporium leaf blotch.

Latest monitoring data based on standard *in vitro*- and *in vivo* bioassays generated with thousands of single field strains sampled in different European cereal growing regions are summarized and used to describe the DMI sensitivity of those pathogens’ populations exposed to this fungicide group in Europe for the last three decades. Molecular biological characterizations alone of target site mutations or other resistance mechanisms in a limited number of strains cannot sufficiently predict field performance of a DMI containing product in the regarded cereal growing area and the right resistance management strategy.
Developing Fungicide Control Programmes for *Septoria tritici* Blotch in Irish Winter Wheat Crops

Abstract ID 92
Steven Kildea, Hilda Dooley, Sinead Phelan, Liz Glynn, Jeanne Mehenni-Ciz and John Spink

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*Septoria tritici* blotch (STB) caused by the fungal pathogen *Zymoseptoria tritici* continues to be the most economically destructive disease of Irish winter wheat crops. With the potential to reduce yields by 50% control of STB is currently reliant on the routine application of fungicides belonging to the azole and SDHI groups of fungicides. With the number of available fungicide actives decreasing through changes in registration and/or the development of resistance it is imperative to ensure fungicide programmes both attain the desired control but also minimise the potential for resistance development and spread. To achieve these goals extensive efficacy, selection and population fungicide sensitivity monitoring programmes have been established. Since the late 2000s an erosion of sensitivity to the azole fungicides has been detected in Irish *Z. tritici* populations, whilst strains showing high levels of insensitivity to the SDHIs were first detected in 2015. These changes have resulted in reduced efficacy of the azole fungicides, and it is anticipated that any proliferation of the SDHI insensitive strains will have a similar impact upon their efficacy against STB. To maintain control it is essential to initially deploy strategies that minimise risk of serious infections, followed by ensuring fungicides are only applied in accordance with anti-resistance guidance; minimising where possible the number of individual fungicide applications and only applying fungicides in mixtures with an effective partner fungicide of a different mode of action.
Sensitivity of *Pyrenophora teres* to Succinate Dehydrogenase Inhibitors in Europe

Abstract ID 77
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One of the most important diseases of barley worldwide is net blotch caused by *Pyrenophora teres*. In addition to strobilurins (QoIs) and azoles, succinate dehydrogenase inhibitors (SDHIs) are very effective fungicides for net blotch control. Recently, isolates with reduced SDHI sensitivity have been found in the field. The first less sensitive isolates towards SDHIs registered in barley were found in Germany in 2012 and carried the H277Y amino acid exchange in the SDH-B subunit. Since 2013 there has been an increase of isolates with reduced SDHI sensitivity detected predominantly in France and Germany and with a broader range of target site mutations. The majority of such isolates contained the amino acid exchange G79R in the SDH-C subunit. Other substitutions were observed in a lower frequency. Microtiter tests indicated cross-resistance for all SDHIs tested. Subsequent glasshouse tests showed that all isolates with a reduced SDHI sensitivity were still effectively controlled by the SDHI fluxapyroxad when applied as preventative treatment. In contrast to the development of the QoI mutation G143A, the number and impact of the *sdh* mutations is significantly more complex and appears to be a highly dynamic process. Strict resistance management strategies are recommended to maintain SDHIs as effective tools for net blotch control.
Fungicide Resistance in Australian Viticulture

Abstract ID 38
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²Centre for Crop & Disease Management, Curtin University, Western Australia, Australia
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Fungicide resistance has previously been reported within Australian vineyards: in *Erysiphe necator* (powdery mildew) to the demethylation inhibiting (DMI) and QoI (strobilurin) group of fungicides, *Botrytis cinerea* to the dicarboximide and anilinopyrimidine fungicide groups and *Plasmopara viticola* (downy mildew) to metalaxyl.

To determine the incidence and severity of fungicide resistance, samples of all three diseases were collected from vineyards in the main viticultural regions of Australia and tested against a range of commonly used fungicides. The pathogens were tested phenotypically for resistance using leaf disc assays (*E. necator* and *P. viticola*) or mycelial growth assays (*B. cinerea*). Representative samples were genotyped for the presence of known mutations conferring resistance.

Phenotypic resistance of *E. necator* to QoI was found in 42% of 72 sites, with the G143A allele present in 87%. Phenotypic resistance of *E. necator* to DMIs was not observed, however the Y136F allele was present in ~60% of the isolates.

*B. cinerea* resistant populations to fenhexamid, iprodione, boscalid and pyrimethanil were detected in 7, 20, 21, and 27% respectively of the 72 sites tested. While 54% of sites had no resistance detected, two sites had populations resistant to all four fungicides. A number of mutations were found in the target genes; H272R or H272Y in the sdhB gene (boscalid target), I365S or Q369P/N373S in the bos-1 gene (iprodione target), F412S in the erg27 gene (fenhexamid target) and L8P or D416E in cgs gene (pyrimethanil target).

Populations of *P. viticola* resistant to metalaxyl now exist in Western Australia and Tasmania as well as Victoria and New South Wales.

The results of the testing have confirmed the presence of resistant populations of these three pathogens to many fungicides throughout Australia. However more work is needed to confirm how these laboratory results relate to the potential for field failure.
Evaluation of a CAA-based Management Strategy for the Downy Mildew Control in a Vineyard with CAA Resistance

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Plasmopara viticola (Berk. et Curt.) Berlese and De Toni, is an Oomycete causing grapevine downy mildew, one of the most devastating diseases of Vitis vinifera L. in regions characterized by high precipitation rate and mild temperatures. Chemical control is performed to obtain adequate yields at the quantitative and qualitative levels. Active ingredients belonging to CAA fungicide class (dimethomorph, iprovalicarb, benthiavalicarb, valifenalate and mandipropamid) are often used in spray programs to control P. viticola. All CAAs are cross resistant and considered by FRAC as medium risk to evolve fungicide resistance. Sensitivity monitoring activity is required as a consequence of the combined risk derived from the interaction of the single components: pathogen, fungicide and agronomic conditions. Anti-resistance strategies are usually recommended for the fungicide class.

In the present study, disease intensity and level of CAA’s sensitivity of P. viticola populations have been evaluated over a three-year period (2013-2015) in a commercial vineyard located in a region of Northern Italy with severe downy mildew epidemics. At the beginning of the project, P. viticola CAAs resistance were already present in the vineyard, due to the high utilization of the class in previous years. The vineyard was divided into three plots: the first (0.1 ha) was not treated against downy mildew, the others (1 ha each) were treated according to an identical strategy differing only for the application of cymoxanil or ametoctradin instead of CAAs at three/four phenological stages. All the active substances were applied in mixture with anti-resistance partner. Each year at berry touch, the disease intensity was assessed in the three plots and leaves showing downy mildew symptoms were sampled to perform fungicide sensitivity tests. The disease severity was evaluated by scoring four replicates of 100 leaves and bunches for the percentage of symptomatic area of each organ and calculating the percentage infection index (I%), that also encompasses the disease incidence. The sensitivity of the three P. viticola populations was evaluated by biological and molecular assays, aiming at calculating the EC₅₀ values and quantifying the point mutations leading to resistance (G1105S and G1105V) in sporangia bulks. The results showed that both the disease management strategies adequately protected leaves and bunches from the...
pathogen infection in comparison with the untreated plot, where the I%I were higher than 66 % on leaves and 95 % on bunches. Despite the populations were characterized by the presence of resistant strains (EC$_{50}$>10 mg/L) and both allelic variants leading to resistance at high rates (G1105V in particular), no significant differences in the efficacy were found between the two strategies.

As a conclusion, disease management strategy based on CAA applications in mixture effectively protects grapevine from downy mildew epidemics in high disease pressure area and in presence of resistant strains, contributing to avoid the occurrence of practical field resistance.
Sensitivity of Fungal Strains Isolated from Rice Sheath Blight Symptom to the Sdhi Fungicides Furametpyl and Benzovindiflupry

Abstract ID 80
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The three QoI fungicides metominostrobin, azoxystrobin, and orysastrobin have been used for the control of blast and sheath blight diseases, caused by *Magnaporthe oryzae* and *Rhizoctonia solani*, respectively, on rice in Japan. The isolates of *M. oryzae* resistant to QoI fungicides have been detected since 2012 and are widely distributed now in Japan (Miyagawa and Fuji, 2013). Under such circumstances, it is not unlikely that QoI-resistant strains also exist in the field populations of other fungal species. In the summer 2015, fungal strains were isolated from sheath blight symptom naturally developed on rice at Minami-awaji, Hyogo, Japan. The isolates were tested for their sensitivity to azoxystrobin on fungicide-amended potato dextrose agar plates and it was indicated that QoI-resistant strains were present in the sampling area (Kurosaki and Ishii, unpublished). In the same area, the SDHI fungicide furametpyl has been continuously applied for the control of sheath blight once a year for over the last ten years. The sensitivity of *R. solani* and related fungal strains isolated from sheath blight symptom was then examined on YBA agar plates supplemented with furametpyl and a novel SDHI fungicide benzovindiflupyr. Although the isolates of *R. solani* were sensitive to these two fungicides, some isolates belonging to the other species than *R. solani* were less sensitive to furametpyl. Interestingly, benzovindiflupyr showed high inhibitory activity to mycelial growth of less furametpyl-sensitive isolates. Species identification of these isolates and sensitivity tests for the other SDHI fungicides are under investigation currently. The differential inhibitory activity of benzovindiflupyr resembled with that against *Colletotrichum* species (Ishii et al. accepted) and recent progress in our research on benzovindiflupyr will also be discussed in this paper.

### 5 Modes of Fungicide Resistance: Diagnostics, Molecular, and Genetic Aspects - Chair: Dr. Gerd Stammler

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<td>08:30</td>
<td>Torriani, S.</td>
<td>CH Syngenta Crop Protection</td>
<td>Succinate-Dehydrogenase Inhibitor (Sdhi) Resistance Evolution in Plant Pathogens</td>
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<td>Gazzetti, K.</td>
<td>University of Bologna</td>
<td>Role of Single Site-Specific Allele Replacement into SvHK1 Locus in the Study of Stemphylium vesicarium Dicarboximide and Phenylpyrrole Fungicides Resistance</td>
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<td>Jung, G.</td>
<td>USA U of Massachusetts</td>
<td>Mechanisms of fungicide detoxification in Sclerotinia homoeocarpa</td>
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<td>Luo, C.</td>
<td>CN Huazhong Agricul. University</td>
<td>Function and Detection of the Genetic Element ‘Mona’ Associated with Fungicide Resistance in Monilinia fructicola</td>
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<td>Tietjen, K.</td>
<td>D Bayer CropScience</td>
<td>Crystallographic studies of yeast CYP51 with fungicides</td>
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### 6 Fungicide Resistance Monitoring: Regional and Global Aspects III - Chair: Dr. Andreas Mehl

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<td>Fernandez-Ortuño, D.</td>
<td>E IHSM; La Mayora</td>
<td>Resistance to Multiple Fungicides in Botrytis cinerea Isolates from Commercial Strawberry Fields in Spain</td>
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<td>Chong, P.</td>
<td>ECU/ NL Wageningen University</td>
<td>Genetic Dynamics In Azole Fungicide Resistant In Pseudocercospora fijiensis</td>
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<td>Adaskaveg, J.</td>
<td>USA University of California</td>
<td>Potassium phosphate resistance and new modes of action for managing Phytophthora diseases of citrus in the United States</td>
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<td>11:50</td>
<td>Cherrad, S.</td>
<td>F CONIDIA</td>
<td>Resistance of Erysiphe necator to SDHI fungicides: first identification in french vineyards, biological and molecular characteristics</td>
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<td>12:10</td>
<td>Hawkins, N.</td>
<td>UK Rothamsted Research</td>
<td>Adaptive landscapes in fungicide resistance: fitness, epistasis, constraints and predictability</td>
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Succinate-Dehydrogenase Inhibitor (Sdhi) 
Resistance Evolution in Plant Pathogens

Abstract ID 81
Stefano F. F. Torriani, Regula Frey, Carolina Buitrago, Jürg Wullschleger, Maya Waldner, Reto Kuehn, Fabiano Orelli, Gabriel Scalliet, Helge Sierotzki
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Different evolutionary forces shape fungicide resistance evolution with respect to the different fungicide classes and target fungal species. Disease control is critical to control most fungal pests threatening yield and quality of harvest in different crops. Succinate-DeHydrogenase Inhibitors (SDHIs) block the TCA cycle at the level of succinate to fumarate oxidation, leading to an inhibition of respiration. SDHIs display broad spectrum and are active to a variety of diseases in different crops. To date, resistance has been reported about 15 fungal pathogens. SDHI resistance is associated to various mutations in the target genes encoding for the mitochondrial succinate dehydrogenase (SDH) enzyme. SDH enzyme consists of four subunits: 2 hydrophilic subunits (sdhA, sdhB) forming the soluble part of the complex involved in the succinate dehydrogenase activity and 2 hydrophobic membrane spanning subunits (sdhC, sdhD). The binding cavity of ubiquinone is formed by the subunits sdhB, sdhC and sdhD. SDHI fungicides specifically interrupt fungal respiration by blocking the electron transport from the heme group to ubiquinone at regions overlapping with the ubiquinone sites. SDHI resistance is monogenic and several target site mutations have been described targeting sdhB, sdhC and sdhD subunits. Molecular modelling studies showed that most selected mutations are positioned within or close to ubiquinone-binding site and are expected to result in a decreased or loss in binding affinity for SDHI fungicides. Each mutation could lead to different sensitivity losses within the SDHI fungicides Distinct species can co-evolved a similar panel of mutations at sdh target genes associated to decreased SDHI sensitivity. For example mutations modulating SDHI sensitivity of Alternaria species and Pyrenophora teres largely overlap and differ from those monitored in Zymoseptoria tritici. Parallel genetic adaptation to SDHIs is influenced by selection imposed by fungicide application and genetic background at target sites. Today field performance reduction of SDHIs were not reported, however this depends on the resistance factors associated to the single mutations and the frequency in a particular fungal population. The impact and the possible evolution of resistance/adaptation in different species will be discussed in relation to fungicide resistance management.
Role of Single Site-Specific Allele Replacement into SvHK1 Locus in the Study of *Stemphylium Vesicarium* Dicarboximide and Phenylpyrrole Fungicides Resistance

Abstract ID 53
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*Stemphylium vesicarium* is the fungal agent of pear Brown Spot and its resistance to dicarboximide fungicides has been a known concerning phenomenon since the 1990s. Henceforward, pear orchards have been monitored and field strains have been tested by mycelial growth inhibition assays to understand the sensitivity to dicarboximide and phenylpyrrole fungicides. Four phenotype classes were recognized according to *in vitro* responses to procymidone and iprodione: S (sensitive), S+ (low resistance), R1 (moderate resistance), R2 (high resistance). Cross-resistance to fludioxonil was only detected in R2 phenotype. Previous molecular studies correlated dicarboximide resistance class with single aminoacid substitutions observed in a two-component histidine kinase (HK1), corresponding to single nucleotide polymorphism (SNPs) in the nucleotide sequence of *SvHK1* gene [1]. The goal of this ongoing study is to define the role of SNPs in *SvHK1* sequence on dicarboximide resistance by the replacement of the S allele with S+, R1 or R2 alleles. A reference sensitive strain was selected through biological and molecular assays and DNA was properly extracted and Fusion PCR technique was used to build the linear disruption vector (KOSvHK1). Fungal protoplast were obtained by enzymatic lysis of cell wall and transformed. KOSvHK1 replacement of *SvHK1* gene produced null mutants which were able to grow up on Hygromycin B. Transformants are currently screened for unique, complete and site-specific insertion of KOSvHK1 using PCR-based methods and Southern Blotting assays. Interesting mutants will be transformed with linear complementation vectors, and complemented strains will be tested for the expected acquired resistance level. Assessment of the role of SNP mutations in *SvHK1* sequence in *S. vesicarium* resistant phenotypes to dicarboximides will allows us to develop a RealTime PCR assay to quickly determine resistant allele-frequency in monitored populations. The results obtained so far will increase the possibility of quantify, prevent and manage the iprodione and fludioxonil resistance risk in the field.

Mechanisms of fungicide detoxification in
Sclerotinia homoeocarpa

Abstract ID 94
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Dollar spot is caused by the “sterile” ascomycete fungus Sclerotinia homoeocarpa and is the most economically significant turfgrass disease. Repeated fungicide applications are required throughout the growing season in order to provide high turf quality, however; fungicide resistance has developed to three fungicide classes (demethylation inhibitor, dicarboximide, and benzimidazole) in the United States. Cross-resistance to different active ingredients in the demethylation inhibitor class and resistance to multiple fungicide classes has been documented in S. homoeocarpa. Furthermore, multi-drug resistance is becoming increasingly problematic in both plant and human pathogenic fungi. Recent studies suggest overexpression of ATP-binding cassette efflux transporters and cytochrome P450 monooxygenases may confer multi-drug resistance through various detoxification pathways. In addition, a novel fungal specific transcription factor has been discovered for regulating these fungicide detoxification genes/proteins and this gain-of-function mutation gives constitutive overexpression of the genes/proteins. Current advances in understanding the genetic mechanisms of fungicide detoxification in S. homoeocarpa using functional genomics techniques such as RNA-seq, quantitative RT-PCR, and knockout mutants will be presented.
Function and Detection of the Genetic Element ‘Mona’ Associated with Fungicide Resistance in Monilinia Fructicola

Abstract ID 40
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In this study, the promoter activity of the genetic element ‘Mona’ was determined in Monilinia fructicola. Through a series of deletions, promoter activity of Mona was narrowed down to a 20-bp active region. Mona knockout transformants were generated from DMI-resistant isolate Bmpc7 and EC50 values as well as expression of the MfCYP51 gene were found to be reduced in transformants compared to the parental isolate. When the Mona element was inserted into the upstream region of the MfCYP51 gene of the DMI-sensitive isolate HG3, the EC50 values as well as the expression of the MfCYP51 gene increased in the transformants compared with the parental sensitive isolate. Both knockout and insertion transformants showed similar mycelial growth rate, sporulation, and ability to cause lesions on fruit compared with their parental isolates. A loop-mediated isothermal amplification (LAMP) method was developed to detect the Mona element located upstream MfCYP51. The assay consistently amplified the target DNA from as low as 0.1 fg of genomic DNA from DMI-resistant but not DMI-sensitive isolates. The assay also amplified the target DNA directly from mycelium, making it a useful tool for in-field detection of isolates resistant to DMI fungicides.
Crystallographic studies of yeast CYP51 with fungicides

Abstract ID 101
Joel D.A. Tyndall\textsuperscript{1}, Brian C. Monk\textsuperscript{2}, Manya Sabherwal\textsuperscript{2}, Rajni Wilson\textsuperscript{2}, Matthew Woods\textsuperscript{2}, Jacopo Negroni\textsuperscript{3}, Klaus Tietjen\textsuperscript{4}

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The crystal structure of \textit{Saccharomyces cerevisiae} lanosterol 14α-demethylase (CYP51) is the first complete structure of this important antifungal target and is the first full-length structure of a membrane-inserted cytochrome P450. The yeast structure includes an N-terminal domain comprising a membrane associated helix and a transmembrane helix that interacts with the cytosolic catalytic domain. The catalytic domain of the yeast enzyme has the standard cytochrome P450 fold but differs in primary sequence from other cyp51s for which crystal structures have been solved. Complexes of yeast CYP51 with several important fungicides will be described.
Monitoring of *Cercospora beticola* Resistance to Fungicides in Serbia

Abstract ID 86
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Over the last two years, sugar beet production in Serbia was faced with dramatic decrease in efficacy of fungicides intended for *Cercospora beticola* control. A cercospora leaf spot (CLS) management over the years included intensive use of three groups of fungicides with different modes of action i.e. benzimidazole (MBC), triazole (DMI) and strobilurin (QoI), consequently imposing *C. beticola* resistance selection pressure. The aim of this study was to verify possible decrease in sensitivity of *C. beticola* populations to MBC, DMI and QoI fungicides. In the 2014 we obtained isolates from two different locations, Vajska and Bezdan, while in 2015 from localities Ruma and Stari Tamiš. Sensitivity testing was set up as a micelial growth measurement for *C. beticola* isolates at discriminatory concentration (DC=1 mg/l) for MBC fungicides, carbendazim and thiophanate methyl, and DMIs, flutriafol and tetraconazole. Test of conidial germination was used to detect sensitivity of the isolates to QoI, trifloxystrobin and pyraclostrobin at DC=5 mg/l. In the 2014 frequencies of *C. beticola* isolates resistant to MBC, QoI and DMI fungicides at locality Vajska were 61 %, 82 % and 98% respectively, while at locality Bezdan were 50 %, 90 % and 100 %. Isolates colected during 2015 from the locality Stari Tamiš were resistant to MBC, QoI and DMI fungicides in frequencies of 38 %, 90 %, 96 %, while at locality Ruma were 54 %, 77 %, 96 %, respectively. Obtained results clearly indicate emergence of *C. beticola* populations resistant to all three groups of fungicides applied in control of the pathogen over the years. This study provides a new insight on development of highly frequent resistance of *C. beticola* to MBC, QoI and DMI fungicides which had a strong impact on decline of their efficacy in the sugar beet fields in Serbia.
Resistance to Multiple Fungicides in *Botrytis cinerea* Isolates from Commercial Strawberry Fields in Spain

Abstract ID 67
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*Botrytis cinerea* Pers., is one of the most economically important pre- and post-harvest pathogen of strawberry. The main strategy to control the disease involves the application of different classes of fungicides despite that *B. cinerea* is considered a high-risk pathogen for resistance development. We collected a total of 367 *B. cinerea* isolates from 14 strawberry fields in Huelva (Spain) during 2014 and 2015 and determined in vitro fungicide sensitivity to all classes of fungicides currently used for gray mold control in Spain. The overall resistance frequencies of 242 isolates collected in 2014 for pyraclostrobin, boscalid, cyprodinil, fenhexamid, iprodione, and fludioxonil were 79, 71, 40, 24, 18, and 0%, respectively. Frequencies of 125 isolates collected in 2015 were 66, 52, 32, 24, 9, and 3%, respectively. Resistant isolates were resistant to either one (9%), two (18%), three (40%), four (13%), five (3%), or six (0%) chemical classes of fungicides in 2014. In 2015, this distribution was 9, 22, 26, 10, 2, and 1%, respectively. Resistance to boscalid, fenhexamid, iprodione, and pyraclostrobin was correlated with point mutations in the corresponding target genes (*sdhB, erg27, bos1*, and *cytb*). The presence multifungicide resistance phenotypes in *B. cinerea* from strawberry fields in Spain must be considered in future resistance management practices for sustained gray mold control.
Genetic Dynamics in Azole Fungicide Resistant in *Pseudocercospora fijiensis*

Abstract ID 113

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*Pseudocercospora fijiensis* is the causal agent of Black Sigatoka disease in bananas and plantains. This economically most costly banana disease is mainly controlled by azoles fungicides that belong to the sterol demethylation-inhibiting (DMIs) class, that target the sterol 14α demethylase enzyme *CYP51*. We examined the variation in sensitivity to azole fungicides in 589 field isolates of *P. fijiensis* collected from Colombia, Costa Rica, Dominican Republic, Ecuador, Philippines, Guadalupe, Martinique and Cameroon. A large variation in sensitivity was observed among isolates towards the DMIs fungicides difenoconazole, epoxiconazole and propiconazole, with a clear pattern of cross-sensitivity. Sequence analysis of the *PfCyp51* gene revealed genetic dynamics within the different populations. We identified point mutations that resulted in amino acid substitutions. Most prominent were Y136F, A313G, H380N, A381G, D460V and Y463D. In addition, we identified 20 novel amino acid substitutions which seem only disseminated regionally. Since many of the substitutions are located in or close to the putative substrate binding site, in silico construction of *CYP51* protein variant models proved helpful to understand the interaction of the substitutions with the DMIs fungicide propiconazole. Strains with
increased azole resistance contained one or two insertions in the promoter region of the *PfCyp51* gene. The most frequently occurring insertion is comprised of repeat elements with a palindromic core correlating with altered gene expression. We also identified an additional insertion, that include a palindrome sequence in isolates from Philippines. The construction of two genetic linkage maps strongly suggest that *Cyp51* gene is the single major determinant of resistance towards DMI fungicides in *P. fijiensis*. These studies provide novel and important information to understand the development of resistance and for optimizing the use of DMIs fungicides to control Black Sigatoka.

Keywords: *Pseudocercospora fijiensis*, fungicide resistant, Cyp51, promoter insertion.
Potassium phosphite resistance and new modes of action for managing Phytophthora diseases of citrus in the United States

Abstract ID 112
H. Förster, W. Hao, M. Gray, and J. E. Adaskaveg

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Phytophthora diseases of citrus are caused by several species of *Phytophthora* and are economically important in citrus production worldwide. The main species occurring on citrus in California are *P. parasitica*, *P. citrophthora*, *P. syringae*, and the less common *P. hibernalis*. These pathogens can cause fruit brown rot, and some cause root rot, foot rot, or trunk cankers (e.g., gummosis). Phytophthora diseases are especially important in areas with high rainfall, poor soil drainage, or improper irrigation practices. In California, root and brown rot are observed together in the winter citrus harvest season when most of the annual rainfall occurs. Resistant rootstocks, cultural practices, and fungicide treatments are used to manage these diseases. Preharvest foliar and fruit applications of copper are effective against brown rot. Since the 1980s, phosphonates (e.g., fosetyl-Al; potassium phosphite, calcium phosphite) and phenylamide-acylalanine (e.g., mefenoxam) fungicides are registered as preharvest treatments for managing root and brown rot, and two to three applications are done per year. Phosphonates have been used more extensively due to phenylamide resistance, lower cost, ambimobility in the tree, and ease of application through chemigation or by foliar treatment. Baseline sensitivity studies before registration of phosphonates are lacking. In our initial testing, average EC₅₀ values for inhibiting mycelial growth were 7.4 mg/L for *P. citrophthora* and 20.6 mg/L for *P. syringae*. With increased sampling of isolates, a much wider range of sensitivities was detected. Agar-dilution assays indicated that for <8% of the isolates of each species collected, EC₅₀ values were up to 252 mg/L for *P. citrophthora* and 142 mg/L for *P. parasitica* and *P. syringae*. Growth of putative resistant isolates was similar to sensitive isolates in the absence of phosphite. To determine if these isolates were field resistant, Navel orange fruit were inoculated with sensitive (EC₅₀ = 7.6 mg/L) and putative resistant (EC₅₀ = 252 mg/L; Resistance Factor = 33) isolates of *P. citrophthora*. Using the sensitive isolate, brown rot was effectively managed with pre- or postharvest applications of potassium phosphite applied at 1000 or 6000 mg/L, respectively. Using a putative resistant isolate, these treatments failed to control the disease. The mode of action of phosphonate fungicides is still unknown. Although indirect effects such as increasing host resistance have been postulated for this FRAC group, direct toxicity to the pathogen appears to be the primary effect in providing brown rot control. Development of fungicides with new modes of action
is ongoing as a resistance management strategy. No cross resistance of the three Phytophthora species was detected to mefenoxam, as well as oxathiapiprolin, fluopicolide, or mandipropamid. The three new modes of action have baseline sensitivities (n = 62 isolates) with mean EC₅₀ values of <0.001, 0.06, or 0.0035 mg/L, respectively for mycelial growth inhibition of P. citrophthora. In field trials, they have reduced root rot to near zero levels; whereas oxathiapiprolin and mandipropamid are also highly effective against brown rot. All three are scheduled for registration on citrus in the United States.
Resistance of *Erysiphe necator* to SDHI fungicides: first identification in french vineyards, biological and molecular characteristics.

Abstract ID 70
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Succinate Dehydrogenase Inhibitor (SDHI) molecules are used in a wide variety of crops against various pathogens. In vineyard, SDHIs are used since 2006 against powdery mildew (*Erysiphe necator*) always in mixtures with QoI fungicides so far.

In 2014, we detected for the first time populations of *E. necator* with specific resistance to SDHI. Populations able to grow on leaf discs treated with a discriminatory rate of boscalid (100 mg/L) were collected from two vineyards located in the South East area in France. During the 2015 season, resistant populations were again isolated from the same locations but also in Languedoc and Champagne areas.

After single spore isolations from different resistant populations, characterization of the molecular targets was engaged. At this time, we have detected several mutations on different subunits of the Succinate deshydrogenase.

In parallel, *in-vivo* tests conducted on leaf vine discs were performed to determine the levels of resistance of the monoconidal resistant strains toboscalid and the cross resistance patterns to different molecules of the SDHI group (fluopyram and fluxapyroxad).

The presentation will focus on the different mutations identified and their relation with 1) the level of resistance and 2) the behavior against molecules of the SDHI group.
Adaptive Landscapes in Fungicide Resistance: Fitness, Epistasis, Constraints, and Predictability

Abstract ID 91
Nichola J. Hawkins, Bart A. Fraaije
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In the simplest cases, fungicide resistance evolves when a single mutation, conferring a high level of resistance with negligible fitness costs, emerges and is selected in a pathogen population. However, some fungicides such as the triazoles have proven more durable in the field precisely because this simplest resistance scenario has not occurred. Instead, mutations have arisen which each confer a smaller reduction in fungicide sensitivity, accumulating into multi-mutation haplotypes with generally increasing resistance levels but pervasive epistatic interactions between mutations. Through the evolutionary viewpoint of adaptive landscapes, combined with functional genetic tools to investigate the effects of mutations individually and in different combinations, it is possible to better understand the evolutionary trajectories available under fungicide selection, and the functional constraints limiting the evolution of resistance. We consider the adaptive landscape of Zymoseptoria tritici CYP51 under selection by triazole fungicides, an especially complex and rugged landscape with over 30 non-synonymous mutations in over 70 combinations, with epistatic interactions between mutations in terms of both fungicide resistance and enzyme function, and partial and in some cases negative cross-resistance between compounds. We look at the emergence of mutations over time, and consider the extent to which this is constrained by enzyme function or determined by the use of different azoles over time.
### FUNGICIDE RESISTANCE MONITORING: REGIONAL AND GLOBAL ASPECTS IV - Chair: Dr. Bart Fraaije

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<tr>
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<tr>
<td>14:00</td>
<td>Kleemann, J.</td>
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<td>Bayer CropScience</td>
<td>SDHI resistance in monocot and dicot pathogens</td>
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<tr>
<td>14:20</td>
<td>Olaya, G.</td>
<td>USA</td>
<td>Syngenta Crop Protection</td>
<td>Detection of the G143A Mutation that Confers Resistance to QoI Fungicides in <em>Alternaria tomatophila</em> isolates from Tomatoes.</td>
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<tr>
<td>14:40</td>
<td>Klosowski, A.</td>
<td>BR</td>
<td>Uni. Fed. Paraná</td>
<td>Detection of Mutations in Cyp51 and Cytb Genes of <em>Phakopsora pachyrhizi</em> Isolates and Competitive Fitness of Mutated and Wild Type Isolates</td>
</tr>
<tr>
<td>15:00</td>
<td>Lin, D.</td>
<td>CN</td>
<td>China Agricultur.. Universit.</td>
<td>Resistance to the Novel Fungicide Oxathiapiprolin in <em>Phytophthora capsici</em>: Risk Assessment and Molecular Mechanism</td>
</tr>
<tr>
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<td>Lopez, F.</td>
<td>AUS</td>
<td>Curtin University</td>
<td>Exploring the Molecular Basis Underlying Fungicide Resistance in <em>Pyrenophora teres f. Sp. teres</em> Infecting Barley in Australia</td>
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<td>15:40</td>
<td>Coffee break</td>
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### BIORATIONAL FUNGICIDES - Chair: Dr. Erich-C. Oerke

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<td>Schmitt, A.</td>
<td>D</td>
<td>Julius Kühn-Institut; Darmstadt</td>
<td>CO-FREE alternative test products for copper reduction in agriculture</td>
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<tr>
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<td>Ramseyer, J.</td>
<td>CH</td>
<td>University of Basel</td>
<td>Search for Alternatives to Copper in Organic Farming: Fungical Activity of a <em>Juncus effusus</em> Medulla Extract and its Active Constituent Dehydroeffusol against Downy Mildew and Apple Scab</td>
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<td>16:40</td>
<td>Glienke, C.</td>
<td>BR</td>
<td>Federal University of Paraná</td>
<td><em>Xylaria cubensis</em> Isolated from the Medicinal Plant <em>Maytenus ilicifolia</em> as a Biological Control of <em>Phylllosticta citricarpa</em></td>
</tr>
<tr>
<td>17:00</td>
<td>McDonald, M.R.</td>
<td>CA</td>
<td>University of Guelph</td>
<td>A food grade oil reduces foliar diseases of carrot</td>
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<td>17:20</td>
<td>Young, D.</td>
<td>USA</td>
<td>Dow Agro Sciences</td>
<td>Enhancing the Efficacy of Copper Fungicides through Synergism with Salicylaldehyde Benzoylhydrazones</td>
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<tr>
<td>17:40</td>
<td>Ryabchenko, A.</td>
<td>RUS</td>
<td>Main Botanical Garden</td>
<td>The effectiveness of chemical and biological agents against the pear scab pathogen <em>Fusicladium pyrorum</em></td>
</tr>
</tbody>
</table>
SDHI resistance in monocot and dicot pathogens

Abstract ID 41
Jochen Kleemann; Andreas Mehl
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Inhibitors of the complex II of the fungal respiratory chain, also known as succinate dehydrogenase inhibitors (SDHI), are of prime importance for crop protection worldwide. This calls for sound resistance research and management, in order to sustain the efficacy of this important chemical class. The molecular basis for the resistance is complex: numerous different sdh mutations in various plant pathogenic fungi lead to different levels of SDHI sensitivity. Some selected examples and recent findings in the area of our SDHI resistance research in monocot and dicot pathogens will be presented.
Detection of the G143A Mutation that Confers Resistance to QoI Fungicides in *Alternaria tomatophila* isolates from Tomatoes

Abstract ID 48
Olaya G¹, Stuerm C², Linley R¹, Edlebeck K¹, Torriani S²
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During the 2014 tomato growing season in Indiana the control of early blight caused by *Alternaria* was difficult to achieve. QoI fungicides are still part of the spray programs designed to control early blight and other tomato diseases, despite the fact that *Alternaria* isolates resistant to QoI fungicides have been reported since 2007 in tomato producing areas in the United States. The cytochrome *b* gene mutation associated with QoI resistance already documented is the F129L mutation (substitution of phenylalanine for leucine at position 129). Isolates of *Alternaria* were collected from tomato fields in Indiana and their response and resistance mechanism to the QoI fungicides azoxystrobin and famoxadone was determined. Isolates were identified as *A. tomatophila* based on conidial morphology and molecular tools. It was found that most of the isolates had the G143A (glycine for alanine at position 143). The detection of G143A mutation would explain the very low levels of early blight control obtained with the QoI fungicides. To our knowledge, this is the first report of the detection of QoI resistant isolates of *A. tomatophila* having the G143A mutation.
Detection of Mutations in Cyp51 and Cytb Genes of Phakopsora pachyrhizi Isolates and Competitive Fitness of Mutated and Wild Type Isolates

Abstract ID 49
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The Asian soybean rust, caused by Phakopsora pachyrhizi, is mostly controlled by sterol demethylation inhibitor (DMI) and quinone outside-inhibiting (QoI) fungicides in Brazil. A reduced efficiency of these fungicides for soybean rust control has been reported and has been associated with a lower DMI and QoI sensitivity. Mutations in CYP51 and CYTB genes can lead to pathogen resistance to DMIs and QoIs, respectively. Resistance mutations might reduce the efficiency of physiological and biochemical processes in the pathogen, leading to lower fitness. The occurrence of the mutations in both genes was investigated in 41 Brazilian isolates of P. pachyrhizi of 2012-2013 and 2013-2014 seasons and a pyrosequencing assay was developed for a rapid and quantitative detection of the F129L mutation. Additionally, we investigated if fitness costs are connected with mutations in the CYP51, and/or in the CYTB gene, in competition trials. For CYP51 analysis, the DNA of P. pachyrhizi spores was extracted and the pyrosequencing assay was applied to detect and quantify mutations. For CYTB analysis, besides DNA extraction, total RNA of P. pachyrhizi spores was extracted and reverse transcribed to cDNA and the whole CYTB gene was sequenced. For competition assays, seven isolates from BASF SE collection with different CYP51 and/or CYTB haplotypes. Spores of sensitive wild type isolate and isolates with different CYP51 and/or CYTB haplotypes were mixed and inoculated on detached soybean leaves. Frequency of relevant target site mutations were followed up by the pyrosequencing method over four disease cycles. In the analysis of CYP51 gene, only one P. pachyrhizi isolate was wild type, most of isolates showed the mutation F120L+Y131H, approximately 10% of isolates showed the mutation Y131F+I475T and two isolates showed a triple combination (F120L+Y131F+I475T), which was detected for this pathogen for the first time. The allele frequency of the mutations in CYP51 gene ranging 16 to 57%. The analysis of CYTB gene showed the presence of the F129L mutation and all isolates had an F129L frequency of either 0 or approximately 100%. Other mutations (G143A and G137R) were not found in this gene. The pyrosequencing was an effective method for detection and quantification of
the F129L mutation. In the competition assays, isolates with lower DMI sensitivity and different CYP51 haplotypes had competitive disadvantages compared with sensitive and CYP51 wild type isolates. The isolate with the F129L mutation in the CYTB competed equally well with the QoI sensitive and CYTB wild type isolate, under the conditions of this experiment. CYP51 and CYTB haplotypes were stable in all isolates over four disease cycles when cultivated alone. The competitive disadvantage of isolates with CYP51 mutations to sensitive isolates with CYP51 wild type in *P. pachyrhizi* might be used for resistance management strategies.
Resistance to the Novel Fungicide Oxathiapiprolin in Phytophthora capsici: Risk Assessment and Molecular Mechanism

Abstract ID 85
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Oxathiapiprolin was the first of the piperidinyl thiazole isoxazoline fungicides to be discovered and developed by DuPont in 2007. The results of binding assays and affinity chromatography have shown that the molecular target of oxathiapiprolin was the oxysterol binding protein (OSBP). Although oxathiapiprolin is in the process of being registered for the control of plant oomycete diseases in China, until now, there is no data available regarding the potential of Phytophthora capsici or any other oomycete pathogen to develop resistance to oxathiapiprolin, or any reports of possible resistance mechanisms. In this study, the baseline sensitivities of 175 isolates to oxathiapiprolin were initially determined and found to conform to a unimodal curve with a mean EC50 value of 5.61×10^{-4} μg/ml. Twelve stable oxathiapiprolin-resistant mutants were generated by fungicide adaption in two sensitive isolates, LP3 and HNJZ10, at a frequency of approximately 1×10^{-6}. The fitness of the LP3-mutants was found to be similar to or better than that of the parental isolate LP3, while the HNJZ10-mutants were found to have lost the capacity to produce zoospores. Taken together these results suggest that the risk of P. capsici developing resistance to oxathiapiprolin is moderate. Comparison of the PcORP1 genes in the LP3-mutants and wild-type parental isolate, which encode the target protein of oxathiapiprolin, revealed that a heterozygous mutation caused the amino acid substitution G769W. Transformation and expression of the mutated PcORP1-769W allele in the sensitive wild-type isolate BYA5 confirmed that the mutation in PcORP1 was responsible for the observed oxathiapiprolin resistance.
Exploring the Molecular Basis Underlying Fungicide Resistance in *Pyrenophora teres* f. sp. *teres* Infecting Barley in Australia

Abstract ID 96
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*Pyrenophora teres* f.sp. *teres* (*Pyrenophora teres* Drechsler (anamorph *Drechslera teres* [Sacc.] Shoem.) is a necrotrophic fungal pathogen and the cause of net form of net blotch (NFNB) that, together with spot form of net blotch (SFNB), is one of the most important diseases of barley (*Hordeum vulgare*). Net blotches are responsible for barley yield losses of 10 to 40%, although complete loss can occur with susceptible cultivars in the absence of fungicide treatment [1]. In Australia, the value of disease control is estimated at $98 million annually with average direct costs of $19 million annually, making it the most significant necrotrophic barley disease after SFNB [2].

Along with cultural practices, the main control measures are the application of effective fungicides and the use of cultivars with genetic resistance. However, due to the lack of highly resistant cultivars, net blotch diseases are widely controlled using fungicides from the triazole or demethylase inhibitor (DMI) group, which are site-specific systemic fungicides that inhibit the C14 demethylation step in ergosterol biosynthesis. Unfortunately, disease management has been compromised by the emergence of fungicide resistance in the last few years.

Alterations in the *Cyp51* gene in plant pathogens has been found to be one of the major mechanisms resulting in reduced sensitivity towards DMIs. Here we report the discovery of new mutations in the *P. teres* f.sp. *teres* *Cyp51* gene and dissect the contribution of these mutations to the final resistant phenotype. We also evaluate the potential of digital PCR for the high throughput detection of mutant populations in the field. The implications of these findings for net blotch fungicide resistance management are discussed.

References
CO-FREE alternative test products for copper reduction in agriculture

Abstract ID 93
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The project CO-FREE is funded by the European Commission, 7th framework program and aims to develop potent strategies to replace/reduce copper in organic, integrated and conventional farming. In the project, CO-FREE alternative test products (CTPs), decision support systems, susceptible and disease-tolerant varieties and innovative breeding goals (ideotypes) as well as cropping systems are integrated into management strategies. CO-FREE focuses on apple / Venturia inaequalis, grape / Plasmopara viticola, and, tomato and potato / Phytophthora infestans. More than ten CTPs are investigated: *Trichoderma atroviride* SC1 and protein extract SCNB, *Lysobacter* spp., yeast-based derivatives, *Cladosporium cladosporioides* H39, oligosacchadic complex COS-OGA, *Aneurinibacillus migulanus* and *Xenorhabdus bovienii*, sage extract, liquorice extract, PLEX and seaweed extract. Field trials were performed in different European countries in 2012-2015 following EPPO standards.

Field trials showed e.g.:

In apple / *V. inaequalis*
- CTPs were effective as stop treatments (leaves).
- In agroforestry production with high cultivar diversity, more than 50 % disease reduction was found (fruit).

In grape / *P. viticola*
- Disease was reduced up to 40 % (leaves and fruit).

In tomato / *P. infestans*
- Disease was reduced on leaves up to 58 % (field) and up to 70 % (greenhouse).

In potato / *P. infestans*
- Disease development was retarded (leaves and stem).
- Influence of cultivar was very important.
- Yield increase reached up to 40 %.

Besides this, further positive side-effects were observed with respect to yield quality (potato), support of beneficial mites (grape) and on reduced leaf fall caused by Marssonina coronaria (apple).

Results from four years with stand-alone applications and strategies using CTPs will be presented.
Search for Alternatives to Copper in Organic Farming: Fungicidal Activity of a *Juncus Effusus* Medulla Extract and its Active Constituent Dehydroeffusol against Downy Mildew and Apple Scab

Abstract ID 37
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Copper has been used since the 19th century for the control of plant diseases, and is still permitted in organic agriculture out of this tradition. In recent years, the utilization of copper has been criticized due to an unfavourable ecotoxicological profile. Therefore, considerable efforts have been made in organic agriculture to identify ecologically safer substitutes.

In this context, we screened an in-house library of plant and fungal extracts in vitro for an inhibitory effect against several plant pathogens (fungi, oomycetes, bacteria). As one of the hits, the ethyl acetate extract of *Juncus effusus* L. (Juncaceae) medulla showed strong inhibitory activity against *Venturia inaequalis* (apple scab) and *Plasmopara viticola* (grapevine downy mildew), with mean minimal inhibitory concentrations (MIC) (100%) of 35 µg/mL and 25 µg/mL, respectively. In a secondary assay on grapevine leaf discs inoculated with *P. viticola*, 94% inhibition was observed at a concentration of 0.5 mg/mL. When tested on grapevine and apple seedlings at a concentration of 0.5 mg/mL, the growth of these fungi was, on average, inhibited with 98% and 84% efficacy, respectively.

The major active constituent was identified as dehydroeffusol by a procedure referred to as HPLC-based activity profiling which combines biological activity data with chemoanalytical information. Structure elucidation was performed by a combination of ESI-MS and NMR spectroscopy. Dehydroeffusol showed mean MICs of 12 µg/mL against *V. inaequalis*, and 4.1 µg/mL against *P. viticola*, in vitro. Subsequent in vivo assessment of the pure compound revealed inhibition rates of 82% on grapevine seedlings, and 86% on apple seedlings at a concentration of 32 µg/mL.
**Xylaria cubensis** Isolated from the Medicinal Plant *Maytenus ilicifolia* as a Biological Control of *Phylllosticta citricarpa*

Abstract ID 55
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*Phylllosticta citricarpa* is the causal agent of Citrus Black Spot (CBS) and is subject to phytosanitary legislation in the EU. Despite of the intense use of fungicides, this disease has no effective treatment. In order to find out microorganisms to be used in biological control of CBS, in the present study we analyzed the biodiversity of endophytes from *Maytenus ilicifolia* and their capacity of secondary metabolites production. In this way two isolates belonging to the genus Xylaria were identified using morphologic and DNA sequence analysis. A multi-locus sequence analysis using ITS, β-tubulin, and α-actin genes revealed that the isolates LGMF1252 and LGMF1253 belong to the *Xylaria cubensis* aggregate. The secondary metabolites of LGMF1252 and LGMF1253 isolates were obtained by fermentation in Malt Extract Agar medium (MEA), and extracted with EtOAC. Crude extracts showed antagonistic properties against the mycelia and pycnidia of the phytopathogen *Phylllosticta citricarpa*. Thin layer chromatography (TLC) of these extracts revealed the presence of alkaloid and coumarin compounds, which probably are responsible for the biological activity observed. Isolate LGMF1252 was transformed, via *Agrobacterium tumefaciens*, with GFP and BAR expression cassettes using a binary plasmid vector based on pPZP201BK. The transformation of phytopathogens and endophytes is an important tool in biological control studies to understand the interaction of these microorganisms inside plants. This is the first report on the use of the endophyte *Xylaria* isolated from a medicinal plant for the control of *P. citricarpa*. 
A food grade oil reduces foliar diseases of carrot

Abstract ID 45
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A food grade oil product, in conjunction with various adjuvants (FGO+A), was evaluated in replicated field trials for the suppression of carrot leaf blights, caused by *Alternaria dauci* and *Cercospora carotae*. Trials were conducted in the Holland Marsh region of Ontario, Canada (44°02’ N, 75°35’ W) with carrot cv. Belgardo seeded on high organic matter soils (pH ~6.3, organic matter 73%). Trials were conducted in 2013 and 2014 and the individual products and combination were also evaluated in 2015. The products were applied 5 times a season beginning when blight symptoms were first observed. The standard fungicides boscalid was included in 2013, azoxystrobine + difenoconazole in 2015, and both fungicides in 2014. Incidence and severity were assessed before carrots were harvested and percent of dead leaves pre plant was recorded. Severity was rated on a scale of 0-5 and a disease severity index (DSI) was calculated. The DSI of the carrot leaf blights were 48, 52 and 44 DSI in the untreated check in the three years and incidence was over 90%. DSI was reduced to 35- 36% in each year by the FGO+A. In 2015, the FGO alone, reduced DSI to 24%. The FGO+A also reduced the percent of dead leaves in 2013 and 2015. Disease suppression was equivalent to that of fungicide boscalid. There was no advantage to combining the FGO+A with a fungicide, or doubling the rate. Azoxystrobin + difenoconazole was more effective than FGO+A in reducing leaf blight incidence and severity. The novel food grade oil shows promise for suppression of carrot leaf blights.
Enhancing the Efficacy of Copper Fungicides through Synergism with Salicylaldehyde Benzoylhydrazones

Abstract ID 104
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Copper salts are widely used as fungicides and serve a critical need for controlling fungal pathogens and managing resistance. However, there is growing concern about the environmental impact of copper, which is used at high rates. A series of salicylaldehyde benzoyl hydrazone (SBH) compounds which shows strong synergism with copper salts has the potential to increase efficacy while maintaining high disease control at reduced rates of copper. In vitro fungicidal activity of SBH compounds against Stagonospora nodorum and Phytophthora capsici was dramatically enhanced by increasing the concentration of Cu$^{2+}$ in growth media. SBH compounds form complexes with Cu$^{2+}$ (SBH-Cu) and the resulting complexes were much more potent than the free SBH ligands. SBH-Cu appears to act by delivering Cu$^{2+}$ into the cell. Synergism between SBH and copper salts was also demonstrated in greenhouse and field tests. SBH-Cu or mixtures of SBH with copper salts showed strong broad spectrum control of fungal diseases whereas free SBH was much less effective.
The effectiveness of chemical and biological agents against the pear scab pathogen *Fusicladium pyrorum*

Abstract ID 36
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Conidia of pear scab *Fusicladium pyrorum* (Lib.) Fuckel provide massive spread of the disease during the growing season. The aim of this study was to determine effectiveness of several new fungicides and agrochemicals against scab pome fruit crops and to find out the effect of the drugs on conidia *F. pyrorum*. We tested the efficacy Vitaplan (biological product that contains mixture of strains of *Bacillus subtilis*, titer $10^{10}$ CFU/g) 0.01%, growth regulator Amulet (composition of linear polyamino saccharides in a solution of succinic acid) 0.12% and mixed fungicide Strekar (25 g/l phytobacteriomycin and 70 g/l karbendazim) 0.15-0.2% on the infected leaves of pear cultivar Cathedral. Control plants were sprayed with water.

Morphology of *F. pyrorum* conidia were examined with scanning electron microscope LEO-1430VP equipped with 4QBSD electron detector and with refrigerating unit Deben Coolstage. The images were further processed using program ImageJ. Number of conidia on $10^4 \mu m^2$ area were counted.

The strongest damaging effect has been found when using mixed fungicide Strekar. Almost total destruction of the structures of the fungus has been noted. A small amount of non-viable conidial inoculum was observed on the surface of the cuticle. Conidia and conidiophores were shrunken and deformed.

Biological product Vitaplan significantly reduced sporulation and vitality of conidia. Treated conidiophores become brittle and break off at the base.

Growth regulator Amulet slowed down the development of the fungus, reduced sporulation frequency on the surface of plant cuticle, made conidia more shrunken and weakened. The number of intact conidiophores was less comparing untreated control, they were shorter, significantly deformed and had weakened turgor.

All tested drugs inhibited scab by different manners and were effective against mild and moderate fungi development as well as the effects on the proportion of infected plants and rates of fungi development. They may be used in protection system of pear from scab.
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<td>Graf, S.</td>
<td>D BASF SE</td>
<td>Status <em>In Vivo</em> and Molecular Diagnosis of Fungicide Resistance in Powdery Mildews</td>
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<td>Strobel, D.</td>
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<td>Field Performance of DMI Fungicides against <em>Zymoseptoria tritici</em> across Europe — Compromised by Further Sensitivity Shift?</td>
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<td>University of Guelph</td>
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<td>Glienke, C.</td>
<td>BR Federal University of Paraná</td>
<td>Sensitivity of <em>Monilinia fructicola</em> from Brazil to Thiophanate-Methyl</td>
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<td>Durable strategies for fungicides use: lessons from the past and leads for improving the future</td>
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<td>AUS CCDM, Curtin University</td>
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<td>USA N. Dakota State Univ.; U of Minnesota</td>
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Chair: Dr. Andreas Mehl

Chair: Dr. Helge Sierotzki
Status Of In Vivo and Molecular Diagnosis of Fungicide Resistance in Powdery Mildews

Abstract ID 83
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Powdery mildew fungi belong to the most important plant pathogens in various agricultural crops. A short generation time, the production of large amounts of conidia throughout the year and the airborne spread leads to a classification as moderate to high risk pathogens concerning the development of fungicide resistance. Sensitivity monitoring is therefore mandatory and performed for several species and different modes of action. Classical sensitivity tests with living fungal material can be challenging regarding sampling, transport and especially the maintenance of the living strains. Sensitivity monitoring of the “high risk” wheat powdery mildew (Blumeria graminis f.sp. tritici) to morpholines has been done now for 27 years using in vivo tests. This work serves as a classical example of adaptation leading to a limited level of sensitivity loss in commercial fields. Whenever possible, molecular genetic methods such as qPCR or pyrosequencing are preferred for a more efficient monitoring. Such methods require the knowledge of the genetic background of the resistance mechanisms, which are typically target site mutations. Sensitivity monitoring of the “high risk” cucurbit powdery mildews (Podosphaera xanthii and Golovinomyces cichoracearum) and the “moderate risk” grape powdery mildew (Erysiphe necator) to succinate dehydrogenase inhibitors (SDHIs) is currently done using in vivo assays. Adaptions were detected in these species at single commercial fields and the underlying molecular mechanisms (mutations in the SDH genes) have been partly elucidated. These studies contribute to the development of molecular genetic monitoring methods for SDHI sensitivity. Several years of monitoring of B. graminis f.sp. tritici to metrafenone using in vivo methods has shown two different resistance phenotypes that occur at low levels in wheat in commercial practice. Low levels of resistance to metrafenone have also been recently detected in grape powdery mildew in European vineyards. The mode of action of metrafenone is currently under closer investigation; its full elucidation would open the possibility for extensive monitoring work, including more efficient molecular methods.
Field Performance of DMI Fungicides against *Zymoseptoria tritici* across Europe – Compromised by Further Sensitivity Shift?

Abstract ID 44
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In recent years, epidemic levels of *Zymoseptoria tritici* in several key cereal-growing regions across Europe lead to significant yield losses. However, SDHI-based fungicide programs showed excellent activity whereas DMI-fungicides were more and more under discussion in light of eroding field performance. With a noticeable further shift in sensitivity detected via microtitre assays in some countries, the weaker activity of DMIs seemed to be explainable by an adaptation of the *Zymoseptoria tritici* population to this group of chemistry.

In this presentation, the field performance of various DMIs in major cereal-growing regions in 2015 is discussed. For each trial site, detailed molecular-biological analysis of the *Zymoseptoria tritici* population before and after fungicide application was made. With microtitre tests for different DMIs, a large variation in EC50 values and thus sensitivity of *Zymoseptoria tritici* isolates were found; with distinct differences between regions and countries. The observed differences in sensitivity can mostly be explained by cyp51 haplotypes and overexpression of cyp51 as well as efflux transporter. Interestingly, the sensitivity pattern differs for individual DMIs in untreated plots, suggesting a diverse population despite general cross-resistance. Furthermore, the results imply that the selective use of DMIs can drive further selection within a population. Despite the distinct sensitivity differences measured in microtitre plate assays for the various regions, field performance of DMIs against *Zymoseptoria tritici* does not seem to correlate well to these findings. This suggests that other factors such as disease pressure, weather conditions and application timing also have a significant impact on DMI performance.

Although sensitivity adaptations can decrease the activity of DMI fungicides especially under high disease pressure situations, most active candidates remain a valuable backbone for fungicide protection – especially in spray programs. To maintain reliable and consistent disease control including resistance management, a diverse portfolio of DMI and other fungicides is vitally important.
Strobilurin fungicides are effective tools for disease management that may also increase crop yield via changes in plant physiology, but are susceptible to rapid erosion of pathogen sensitivity. Replicated, small-plot field trials were conducted at a site in Ontario, Canada (44°02’ N 75° 35” W), to evaluate plant health effects on lentil, chickpea and field pea. The site was chosen because disease pressure was expected to be very low. The strobilurin fungicides pyraclostrobin and azoxystrobin were compared to chlorothalonil and an untreated check from 2013 to 2015. Fungicides were sprayed once, at early flowering. Two cultivars of each crop were assessed. Plots were rated for disease incidence, greenness (0 = yellow, 5= very green), height, lodging, shoot weight, and seed yield. On lentil, the incidence of foliar disease was low (< 6%) each year. Application of pyraclostrobin resulted in greener plants in 2013 and 2014, less lodging on cv. Maxim, higher seed weight on cv. Dazil in 2014, and taller plants on both cultivars in 2015. On chickpea, incidence ranged from < 5% in 2013 to 58% in 2014, but there were no differences in disease, plant growth or yield related to fungicide. On field pea, incidence was high in 2014 (47%) and 2015 (54%). Each fungicide reduced disease in 2014, as did pyraclostrobin and chlorothalonil in 2015. Increased plant height was related to improved disease control ($R^2 = 0.26$, $P = 0.003$). We conclude that lentil was more responsive to plant health effects of strobilurins than chickpea or field pea. About half of the isolates of Mycosphaerella pinodes (field pea) collected from 2013-2015 on the Canadian prairies were insensitive to strobilurin fungicides (up from 8% in 2012) and the entire population of Didymella rabiei (chickpea) was also insensitive.
Sensitivity of Monilinia fructicola from Brazil to Thiophanate-Methyl

Abstract ID 56
Fischer, J. M. M.; Araújo, H. E.; Glienke, C.; May De Mio, L. L.

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Stone fruits are produced worldwide and despite their economic significance, these crops are affected by many plant diseases. Brown rot is an important disease in the pre-harvest period, and it is caused by fungi from the genus Monilinia. M. fructicola is the prevalent species associated with brown rot in Brazilian orchards. The frequent use of site-specific fungicides increase the probability for fungicide resistance development in M. fructicola populations. The aim of this study was to investigate the sensitivity of Monilinia fructicola isolates to thiophanate-methyl (methyl benzimidazole carbamate) in 58 isolates collected from three states in Brazil from orchards with different histories of fungicide use. Sensitivity to fungicides was determined by inhibition of mycelial growth on fungicide-amended media. Partial sequencing of β-tubulin gene was used to determine the presence and frequencies of the mutation at codons 6 and 198 in strains exhibiting high (HR) and low (LR) resistance to thiophanate-methyl among sampled populations. Resistance to thiophanate-methyl was found in 70.7% of isolates collected from Brazil. The β-tubulin sequences showed that all five HR Brazilian isolates of M. fructicola isolates had the E198A allele conferring resistance to benzimidazole fungicides. Twenty-three LR isolates were identified as having codon 6 converted from CAT (histidine) to TAT (tyrosine), as expected, but thirteen LR isolates showed no mutation. These strains were characterized as S-LR and the mechanism of resistance should be better investigated.
Durable strategies for fungicides use: lessons from the past and leads for improving the future

Abstract ID 73
Anne-Sophie Walker for the FONDU working group
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The durability of strategies aiming to delay fungicide resistance evolution in populations of plant fungi relies on the skillful deployment in time and space of the various molecules registered for a specific usage. Therefore, the optimization of these strategies constitutes a major challenge of integrated pest management, all the more in the context of new regulations aiming to decrease pesticide use (e.g. in France, the “Ecophyto II” and “Agroécologie” plans). The respective interests and drawbacks of anti-resistance strategies (namely mixture, alternation, mosaic and dose modulation) are a matter of debate in the scientific community and should be more efficiently deployed in agricultural landscapes.

In this context, this paper reports the preliminary results of the FONDU project, aiming at (1) identifying and characterizing sustainable anti-resistance strategies and (2) disentangling the social and economic limits to their wide use on large territories. This project has a generic outcome but was first focused on Zymoseptoria tritici, the causal agent of septoria leaf blotch. Empirical data (e.g. the pluriannual dataset “Performance”, managed by the technical institute Arvalis-Institut du Végétal), as well as a specific model were mined to answer the first objective. Interviews with key French resistance managers, as well as economic models were carried to answer the second aim.

The FONDU working group is constituted by agronomists, plant pathologists, modellers, statisticians, economists and sociologists: Frédérique Angevin, Florence Carpentier, Sylvie Charlot, François Coléno, Marie-France Corio-Costet, Anne Dérédec, Frédéric Fabre, Mourad Hannachi, Stéphane Lemarié Hervé Monod and Anne-Sophie Walker
Fungicide resistance management in practice; mixtures, alternations and cross resistance patterns

Abstract ID 34
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Evolved resistance to fungicides is a critical issue for global food security. Resistance has emerged rapidly in some cases, and some so it is vital that the effective life of actives is prolonged as much as practicable. Recent theoretical models to account at least qualitatively for resistance evolution are backed up by extensive experimental data (reviewed in van den Bosch et al 2014, Ann rev Phytopath. 52 175-95). The challenge now is to combine these models with practical realities. The models suggest using other fungicides in mixtures or alternations with the threatened fungicide. This is believed to be effective if either neutral or negative cross resistance operates. Negative cross resistance presents the attractive scenario of using solo products on large areas for long periods until the resistant population has built up to a large enough level to warrant switching en masse to the second fungicide. The new fungicide is then used until resistance develops and the first fungicide is used again; the Merry Dance. This scenario has severe sociological and regulatory hurdles but may apply advantageously under certain circumstances. In many regions, the pathogen population has already developed resistance to different fungicides with a complex pattern of positive, zero and negative cross resistance. In these cases I will argue that prolongations of effective fungicide life can still be achieved by tactical use of fungicides to pull fungal populations into evolutionary dead ends. To maximise these possibilities, fungicides with enhanced negative cross resistance should be developed and encouraged by regulatory authorities.
Managing the evolution of fungicide resistance in potato blight

Abstract ID 32
Kevin Carolan; Joe Helps; Femke van den Berg; Frank van den Bosch
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Disease management relies, predominantly, on the application of fungicides and deployment of crop resistance genes; but such control measures exert selection pressures on pathogen populations. Maintaining effective control depends on managing two processes: firstly, the introduction of new resistance genes in the plant host and pathogen populations overcoming them; secondly, the introduction of new fungicide modes of action (MOA) and pathogen populations becoming insensitive to them.

Integrated control is widely believed to be more sustainable than reliance on one control option. There is however surprisingly little published evidence and mechanistic understanding of the combined deployment of fungicides and crop resistance genes. The evolution of fungicide insensitivity and the evolution of virulence are virtually always studied in isolation, whereas in practice the processes interact. This project studies the integrated use of fungicides and crop resistance genes to exploit evolutionary interactions to maximise the durability of crop protection.
Developing, Disseminating and Evaluating Management Strategies for Controlling *Rhizoctonia solani* on Sugar Beet in the United States

Abstract ID 103
Mohamed F. R. Khan

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2% of the United States population produces an abundant, safe, and reliable supply of inexpensive food for its 325 million inhabitants. Extension educators employed by Land Grant Universities throughout the USA and associated territories provide technical information to help producers. Federal, State and local governments provide funding for the Extension Service. Educators must record the impact or public good of the services provided. Extension educators play a vital role in helping growers in North Dakota and Minnesota produce about 60% of the US sugar beet which results in $4 billion of total economic activity. Growers listed root rot caused by *Rhizoctonia solani* as the most important production issue since 2009. The Extension educator conducted basic research to better understand the biology of the fungus to develop management strategies. Field research was conducted using penthiopyrad, an experimental SDHI fungicide, and azoxystrobin, a QoI fungicide, for managing the pathogen. Plot demonstrations were conducted to illustrate effectiveness of different fungicide treatments, research data collected, analyzed and developed into practical recommendations, which were disseminated directly to growers and other educators who advise growers at seminars and by using production guides, research reports, circulars, radio programs, the internet, and face-to-face meetings. Research indicated that azoxystrobin applied in-furrow at planting followed by a post application consistently resulted in significantly higher plant populations and significantly greater recoverable sucrose compared to the non-treated control. The practice of using starter fertilizer mixed with azoxystrobin at planting slowed planting operations, and multiple use of one mode of action was not conducive to resistance management. Penthiopyrad used as a seed treatment provided early season control by protecting plant populations compared to the non-treated check, but was not effective during the latter part of the season resulting in significantly reduced populations. Penthiopyrad followed by a post application of azoxystrobin resulted in higher populations and recoverable sucrose compared to the control. Penthiopyrad was registered in 2013 and became available in 2014 when it was used on about 70% of planted acreage. Usage increased to over 85% in 2015. Penthiopyrad seed treatment facilitated the use of starter fertilizers without adversely impacting speed of planting. Most growers also used a post-fungicide application of mainly azoxystrobin in 2014 and 2015. More research is need to determine better timing of post-fungicide applications and whether multiple post-fungicide applications with different chemistries will result in better disease control, higher yields, and reduces the risk of fungicide resistance.
Spraying Strategies Avoiding Selection of *Zymoseptoria Tritici* Triazole Resistance

Abstract ID 35
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After a drop in field performance of the triazoles epoxiconazole and prothioconazole in 2014 under high disease pressure, new recommendations for fungicide use for the control of *Zymoseptoria tritici* were formulated by Danish stakeholders to halt the development of triazole resistance. Denmark has compared with many other countries a reduced fungicide arsenal and there is therefore a major wish to stretch the longevity of products. The guidelines were based on preliminary results and current literature, recommending farmers i.a. to keep the total number of sprayings with DMIs in one season ≤ 3, not to use the same DMI more than twice, and to include products containing other modes of action, like boscalid and folpet.

In 2015 a two-year trial series was started to investigate the impact from the proposed recommendations on selection for CYP51 mutations under Danish field conditions to obtain a more profound background to encourage farmers to adapt these measures. Two trials were carried each consisting of 10 treatments. The occurrence of *Z. tritici* was assessed at 10-day intervals. At the end of the season, flag leaf samples were collected and plots harvested. Leaf samples were analysed for CYP51 mutations D134G, V136A/C, A379G, I381V, S524T by pyrosequencing and S524T by qPCR.

The preliminary findings support the current recommendations. It was shown, that strategies where the same triazole was used three times, increased point mutations D134G, V136A, and S524T significantly. By diversifying the strategy including different azoles, SDHIs and the multisite inhibitor Folpet, the selections for specific mutations was lowered. Applying azoles only twice or once per season held selection down as well, however, in some cases at the expense of disease control and yield. Including IPM elements is seen as important steps to minimise the need for treatments, which again could help holding down the resistance levels.
Abstracts

Poster Presentations
### New technologies and applications + New fungicides and new modes of action

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### Fungicide resistance monitoring: regional and global aspect I

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**Biorational fungicides**

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**Resistance management**

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1. Elucidation of a Novel Protein Kinase Target in Fungicide Research

Abstract ID 42

Samantha Hall, Dhaval Sangani, Rasmus Hansen, Shradha Singh, Fergus Earley, Andy Corran

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A chemical series has been highlighted with potent, broad spectrum fungicidal activity in Syngenta’s in-house biology screens and this glasshouse activity was shown to translate to field performance. Early assessment indicated inhibition of protein kinase enzymes might be responsible for the observed fungicidal effect. Subsequent protein kinase profiling screens revealed that the chemistry is selective; inhibiting only a small number of protein kinases. Chemical proteomics strategies were employed for target identification in Zymoseptoria tritici and continue to emerge as an attractive tool for probing ligand-protein interactions. Tools for structure activity determination were developed to support the lead generation pipeline. We will share some of the work done to understand the mechanism of action of this chemical class in fungi relevant to agriculture.
2. Novel chemical synthesized 2, 4-DAPG analogues: antifungal activity, mechanism and toxicology

Abstract ID 102
Liang Gong¹, Haibo Tan¹, Feng Chen², Xuewu Duan¹, Yueming Jiang¹
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2, 4-Diacetylphloroglucinol (2, 4-DAPG), a natural phenolic compound with excellent activities against various plant pathogens, was isolated from specific species of *Pseudomonas* including *P. fluorescens* and *P. protegens*. In order to improve its potential activity, 14 analogous of 2, 4-DAPG have been synthesized by Friedel–Crafts reaction using acyl chlorides and phloroglucinol. Among which we found some of them (such as MP4) with the highest antifungal activities against *Penicillium italicum* and *P. digitatum* than those of 2, 4-DAPG. In addition, one of the possible action mechanisms for control of *P. italicum* and *P. digitatum* was figured out via the expression profiles of all the cytochrome P450 genes in the genome of these two species underlying the pressure of MP4. Furthermore, the toxicology of MP4 was evaluated by cell activity assays using human normal lung epithelial cells and kidney 293 cells. Taken all, we found that novel synthesized MP4 giving a great promising as a fungicide used for postharvest citrus fruits.

Keywords: 2,4-DAPG analogues; fungicide; citrus pathogen; cytochrome P450; toxicity assess
3. High Throughput Imaging for Resistance Monitoring and Mode of Action Studies in *Botrytis cinerea* and Other Pathogens.

Abstract ID 66
Andreas Mosbach¹, Rob Lind², Dominique Edel¹, Dirk Balmer¹, and Gabriel Scalliet¹

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In *Botrytis*, resistance monitoring is currently done after propagation (sub-culturing) of the original samples. The fungicides are then tested on fresh spores with a wide range of fungicide concentrations for EC50 determinations. This two-step process proved to be reliable, but is time and resource demanding. Sub-culturing can also in some cases create a bias because bulked material on sub-cultured plates can sometimes show differences in relative resistance ratios when compared to the original sample. We are developing a platform for a high throughput evaluation of fungicide resistance frequencies in bulked samples. Our equipment enables 96 well-based spore germination imaging and analysis. The novel assays can determine the proportions of resistant isolates in bulked samples and the results show good consistency with frequencies inferred from molecular (pyrosequencing or qPCR) strategies.

Preliminary imaging with pathogens labelled at the subcellular level suggests that high content screening (HCS) will also enable mode of action related studies to be performed, making high throughput phenotyping a key asset in fungicide research.
4. Use of the method of chemical models for studying plant physiology due to fungicide treatment

Abstract ID 72
T.P. Yurina

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In this work we considered the use of the method of chemical models as a rapid test for monitoring the physiological condition of the plants when treated with systemic fungicides. The results demonstrated positive correlations of this method with other physical and biochemical methods. In the experiments 3,4-dioxiphenilalanin (DOPA) was used which is capable to be oxidized producing pigment melanin. It is reflected as a change of the optical density of the solution. Many years of application of this method with using 3,4-dioxiphenilalanin (DOPA) have shown its efficiency.
5. Monitoring of *Botrytis cinerea* Sensitivity to Fungicides in Strawberry Fields in Serbia

Abstract ID 87
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Gray mold is a common disease of strawberries caused by the fungus *Botrytis cinerea* Pers. Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel). Management of the gray mold in strawberry production in Serbia requires numerous treatments with fungicides, which intensified the risk of resistance development. Sensitivity of *B. cinerea* in strawberry fields in Serbia was monitored in 2014 and 2015 in two strawberry growing regions. Conidia from infected strawberry fruits with sporulating lesions were collected during the fruit ripening, from several fields per region and single spore isolates of *B. cinerea* were established. Sensitivity to thiophanate-methyl, fenhexamid, iprodione and fludioxonil were assessed based on the mycelial growth measurements at discriminatory concentration (DC) prepared on potato dextrose agar medium i.e. 1 μg ml-1, 0.6 μg ml-1, 25 μg ml-1, 0.2 μg ml-1, respectively. Spore germination assay was set up at water agar medium to examine the *B. cinerea* sensitivity to pyraclostrobin (DC= 5 μg ml-1; SHAM 100 μg ml-1). Isolates were classified as resistant if the colony growth or germination of spores on DC was ≥ 50% compared to control. In both inspected strawberry growing regions, the highest resistance of *B. cinerea* populations was expressed for pyraclostrobin ranging from 92.4% to 98.1%. Resistance to thiophanate-methyl, ranged from 12.2% to 18.2%, while for iprodione and fenhexamid in much lower rates from 5.8% - 7.6% and 1.7 – 4.5%, respectively. There are no resistant isolates detected for fludioxonil in either of the inspected regions. An intensive sensitivity monitoring conducted in strawberry fields in Serbia revealed prevalence of *B. cinerea* populations resistant to pyraclostrobin and significant incidence of resistance to thiophanate-methyl. Therefore, further usage of these fungicides should be reduced, while fungicides with lower resistance risk need to be more utilized in accordance with the anti-resistant strategy in order to control *B. cinerea* and delay the resistance development.

Abstract ID 97
Rafaele Regina Moreira¹; Camilla Castellar¹; Natasha Akemi Hamada²; Louise Larissa May De Mio¹

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Glomerella Leaf Spot is caused by species of the genus *Colletotrichum*, mostly by species of the *C. acutatum complex* (Cac) and *C. gloeosporioides* complex (Cgc). This disease occurs in all producing regions in Brazil causing symptoms on fruits and leaves, but the main damage is the premature defoliation. The disease control is carried out preventively with protector fungicides (average of 21 sprays per season), such as Dithiocarbamates (mancozeb, maneb and propineb) and also systemic fungicides (average of 8 sprays per season) such as Methyl Benzimidazole Carbamates (tiophanate-methyl). The aim of this study was to study sensitivity of *Colletotrichum* spp. to mancozeb and thiophanate-methyl fungicides and to classify the isolates in highly resistant (HR), resistant (R), moderately resistant (MR) or sensitive (S), according to Chung et al. (2006) comparing EC50 values of fungicide mancozeb when performed mycelial growth or spore germination assays. In total, 39 isolates were obtained during 2010 and 2011, from different parts of apple trees (buds, twigs, flowers and leaves). The concentrations of mancozeb and thiophanate-methyl for mycelial growth were: 0, 12.5, 50, 200, 400, 800 and 1,600 µg.mL-1; and for spore germination of mancozeb were: 0; 0.03; 0.10; 0.30; 10; 1.00; 3.00; 10.00; 30.00 µg.mL-1. The EC50 for the fungicide thiophanate-methyl ranged from 1.74 to values greater than 1,600.00 µg.mL-1 and the isolates were classified as 65% HR, 10% R, 19% MR. Mancozeb had a significant difference in EC50 comparing the two methodologies and it was no correlation between them. For mycelial growth the EC50 of mancozeb ranged from 218.00 µg.mL-1 to values greater than 1,600.00 µg.mL-1, and the isolates were classified as 68% HR and 32% R. For spore germination the EC50 ranged from 1.01 µg.mL-1 to 92.00 µg .mL-1, and the isolates were 16% MR and 84% S. Besides that, for mancozeb it was observed a significant difference in EC50 comparing the two complex of *Colletotrichum* studied; for Cac averaged 16.50 µg.mL-1 and, for Cgc the EC50 averaged 5.30 µg.mL-1.
7. Fungicide resistance dynamics and genetic diversity of *Botrytis* in German strawberry fields

Abstract ID 115
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*Botrytis cinerea* is the major pathogen on strawberries and causes high yield losses. Fungicides are used to control grey mould, but resistance frequencies in *Botrytis* field populations have dramatically increased over the last years. *Botrytis* isolates collected from German strawberry fields in 2012 to 2014 showed high resistance frequencies, including many isolates with resistance against several or all registered site-specific fungicides. Greenhouse and field experiments revealed that multiresistant strains could not be controlled anymore by fungicide treatments; in the laboratory, these strains showed only little evidence for fitness defects. Repeated samplings in strawberry fields revealed seasonal changes in resistance frequencies. Resistance frequencies increased after spraying and only slightly decreased after overwintering in the following spring. *Botrytis* populations on strawberries were genetically heterogeneous, consisting of several subgroups of *B. cinerea* and, in low abundance, the related species *B. pseudocinerea*. *B. pseudocinerea* was isolated more often in unsprayed fields, and in commercial fields before fungicide treatments. Surprisingly, all *B. pseudocinerea* strains behaved as ‘low-risk’ pathogens and almost never developed fungicide resistance.

In the course of these studies, a new species, called *Botrytis fragariae* sp. nov., was discovered on strawberries. The species was occasionally observed in German strawberry fields, but not found on other hosts. Fungicide resistance was common in *B. fragariae*, and similar resistance mutations as in *B. cinerea* were identified. Taken together, our data have revealed a remarkable genetic diversity of *Botrytis* on strawberries.
8. *In vitro* evolution of fluxapyroxad resistance in *Zymoseptoria tritici*

Abstract ID 63
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Emergence of resistance mechanisms and their fixation in field populations of *Zymoseptoria tritici* represents a constant threat to Septoria leaf blotch control in wheat by fungicides. A new generation of carboxamide fungicides, inhibiting succinate dehydrogenase, provides good Septoria control but previous studies have indicated that the risk of resistance development is high. The emergence and subsequent dynamics of genomic adaptive changes conferring resistance to the SDHI fluxapyroxad in *Z. tritici* was investigated *in vitro*. The sensitive isolate IPO323 was exposed to increasing concentrations of fluxapyroxad in replicate populations at three different starting concentrations with or without a short exposure to UV light at the start of the incubation. Target protein alteration was the most common resistance mechanism to fluxapyroxad and cross-resistance to both fluopyram and carboxin was often observed. There was successive replacement of fitter fluxapyroxad-resistant mutants carrying distinct amino acid substitutions in SdhB and SdhC during ten rounds of fluxapyroxad selection with SdhC-H152R emerging as the least sensitive mutant. However, in two of three populations not exposed to UV light only a single mutation arose, SdhB-H267Y, and reached high frequency without further replacement by other Sdh variants. In the remaining population not exposed to UV, a medium level of resistance developed and target-site changes were not detected.
9. The Resistance of *Verticillium dahliae*, Verticillium Wilt Causative Agent, to Chitosan Connected with Changes in Lipid Metabolism

Abstract ID 60
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The problem of phytopathogen resistance of to the unfungal compounds is very important for control of pathogens in agriculture. The adaptation of phytopathogen fungi to different unfavorable conditions and to the toxicants is directly combined with the reorganization of pathogen cell membranes, first of all with their lipid composition, i.e. polar lipids, sterols, fatty acids and their ethers.

The fungus *Verticillium dahliae* Kleb., the causal agent of Verticillium wilt in many plants, is one of the major phytopathogenes in any countries, that can cause serious losses in agricultural crops.

It has been shown, that the higher resistance of pathogenic strain of *V. dahliae* to chitosan in comparison with non-pathogenic strain was accompanied by considerable changes in lipid metabolism, causing changes of the permeability of cell membranes, which is in turn promoted the membrane complex stabilization.
10. Long-lasting Study of Fungicide Efficacy against Czech Cucurbit Powdery Mildew Populations

Abstract ID 90
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A total of 150 cucurbit powdery mildew (CPM) isolates (78 Golovinomyces orontii s.l. /Go/, 72 Podosphaera xanthii /Px/) from the Czech Republic from 2007 to 2011, were screened for fungicide efficacy to the four frequently used fungicides (fenarimol /formulated as Rubigan 12 EC/, dinocap /Karathane LC/, thiophanate-methyl /Topsin M 70 WP/, azoxystrobin /Ortiva/) and a control fungicide (benomyl /Fundazol 50 WP/). Fungicide efficacy was determined by a modified leaf-disc bioassay with five concentrations. Highly susceptible Cucumis sativus 'Stela F1' was used for preparation of leaf discs. Efficacy of the tested fungicides towards screened CPM isolates varied significantly during the studied period. From 2012 to 2013, efficacy of four new commonly used and registered fungicides (quinoxyfen /Atlas 500 SC/, propiconazole /Bumper 25 EC/, fenpropimorph /Corbel/, penconazole /Topas 100 EC/) was screened. As well as there has been continued monitoring of dinocap and azoxystrobin efficacy. Altogether 50 CPM Czech isolates (23 Go, 27 Px) were tested using a modified leaf-disc bioassay with three concentrations. Efficacy of fungicides towards screened CPM isolates varied significantly and there were observed also differences in efficacy of some fungicides between both CPM species and as well as between studied years. Fenpropimorph was 100% effective and showed phytotoxicity to C. sativus 'Stela F1' leaf discs. Propiconazole and penconazole were also highly effective. Dinocap expressed decreased efficacy from 2012 to 2013 (mainly for Go). Quinoxyfen appeared less effective. Azoxystrobin showed decreased efficacy and there was observed a shift towards prevalence of azoxystrobin-resistant strains in Czech CPM populations in 2013. This research was supported by the following grants: QH71229, MSM6198959215, IGA_PrF_2016_001.
11. Long-Lasting Study of Fungicide Efficacy against Czech Cucurbit Downy Mildew Populations

Abstract ID 89
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A total of 159 Czech *P. cubensis* isolates (from 2005 to 2011) were used for fungicide efficacy screening. Six commonly used and registered fungicides (except Ridomil Plus 48 WP that served as control) were tested for efficacy against *P. cubensis*. Fungicide sensitivity was determined by a floating leaf disc bioassay with five concentrations. Fosetyl-Al (Aliette 80 WP) and propamocarb (Previcur 607 SL) were the most effective. Metalaxyl (Ridomil Plus 48 WP) and metalaxyl-M (Ridomil Gold MZ 68 WP) were ineffective. However in 2010 efficacy of these fungicides increased slightly. In 2011, the same phenomenon appeared in reaction to metalaxyl-M. Cymoxanil (Curzate K) showed a very low efficacy during the period of study except the year 2009. Frequency of sensitive/moderately resistant/resistant strains to dimethomorph (Acrobat MZ WG) varied significantly during seven-years of screening and there was recorded a temporal shift towards higher sensitivity on all concentrations in Czech *P. cubensis* populations. From 2012 to 2014, efficacy of two new commonly used and registered fungicides (fluopicolide, propamocarb-hydrochloride /Infinito/, azoxystrobin /Ortiva/) was screened. As well as there has been continued monitoring of cymoxanil, dimethomorph and metalaxyl-M efficacy. Altogether 52 Czech *P. cubensis* isolates were screened using a floating leaf disc bioassay with three concentrations. Efficacy of fungicides against Czech *P. cubensis* populations varied among the years. Cymoxanil (Curzate K) was ineffective. In contrast to 100% efficacy of dimethomorph (Acrobat MZ WG) and Fluopicolide and propamocarb-hydrochloride (Infinito). Efficacy of metalaxyl-M (Ridomil Gold MZ Pepite) was high on contrary to very low efficacy of azoxystrobin (Ortiva). This research was supported by these projects: QH71229, MSM6198959215, IGA_PrF_2016_001.
12. Fungicides are relatively ineffective against *Stemphylium* leaf blight of onion

Abstract ID 47

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Stemphylium leaf blight of onion, caused by *Stemphylium vesicarium*, is a damaging foliar disease on onion in the Holland Marsh, Ontario, Canada ((44°02’’ N 75° 35” W). Genetic resistance is not available, so frequent application of fungicides is required to manage this disease. Replicated small-plot field trials were conducted to identify effective fungicides (2012-2015) and optimum application timing (2014 and 2015). The fungicides were: Pristine (pyraclostrobin 25.2%, boscalid 12.8%), Bravo 500 (chlorothalonil 50%), Manzate 750 F (mancozeb 75%), Switch 62.5 WG (cyprodinil 37.5%, fluodioxinil 25.0%), Fontelis 20 SC (pentiopyrad 20%), Inspire (difenoconzole 23.2%), Luna Tranquility (fluopyram 11.3%, pyrimethanil 33.8%), and Quadris Top (azoxystrobin 18.2%, difenoconazole 11.4%). Spray timing trials used Quadris Top with a non-ionic surfactant in 2014 and Luna Tranquility in 2015. In 2012 and 2014, disease severity was moderate, with 44-55% incidence by mid-August. In 2012, the first of 5 sprays was applied mid-July. Each of the fungicides reduced severity slightly compared to the untreated check (12-24% vs. 33%). Quadris Top was most effective. In 2013 and 2015, disease pressure was high, with 96-100% disease incidence by mid-August. Four sprays were applied in 2015 beginning mid-July. Only Luna reduced disease incidence compared to the untreated check (81 vs. 97%), and at only one sample date. In the spray timing trial in 2014, initiating the spray schedule between 3 and 17 July resulted in 3 to 5 fungicide applications and no differences in disease incidence (77-86%). In 2015, all of the spray timing treatments (6-10 sprays) resulted in a slight reduction in disease severity (42-46%) compared to the untreated check (65%). Lack of efficacy may indicate a need for very early fungicide application to prevent initial infection, and/or the role of toxins produced by the pathogen in season-long symptom development.
13. Genetic Diversity of *Phakopsora pachyrhizi* in Brazil

Abstract ID 82
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In the last 10 years the soybean cultivation increased at large scale in Brazil, making it the second largest producer worldwide. Asian soybean rust (ASR) was first identified in South America in Paraguay (2001) and in Brazil (2002). Agronomic strategies to control soybean rust have been adapted such as cultivation of resistant cultivars, elimination of secondary hosts and an interruption of the green bridge (no soybean cultivation during 3 months). The most effective solution to control *P. pachyrhizi* relates to the use of fungicides, mainly succinate-dehydrogenase inhibitors (SDHIs), demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs). During the past 10 years fungicide monitoring programs reported a sensitivity shift to DMIs. In the season 2013-14, molecular analysis reported the first occurrence of the presence of the F129L mutation in the target gene of QoI (*cytb*). Up until now, no adaptation to SDHI has been reported. The principal aim of this work is to better characterize the present Brazilian populations of *P. pachyrhizi*. Representative samples from the major soybean growing area were collected. Independent sequence alignments from different neutral molecular markers arising from the nuclear and mitochondrial were used to infer the genetic diversity of *P. pachyrhizi*. Moreover, intragenic variability of the target genes of major single site fungicide classes was assessed for a selection of samples. The results will be discussed in relation to the current planting, fungicide program and fungicide resistance management developed by FRAC.
14. Proposal for a unified nomenclature for target site mutations associated with resistance to fungicides

Abstract ID 116
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Evolved resistance to fungicides is frequently associated with substitutions in the amino acid sequence of the target protein. The convention for describing amino-acid substitutions is to cite the wild type amino acid, the codon number and the new amino acid, using the one letter amino acid code. It has frequently been observed that orthologous amino acid mutations have been selected in different species by fungicides from the same mode of action class, but the amino acids have different numbers. These differences in numbering arise from the different lengths of the proteins in each species. We propose a system for unifying the labelling of amino acids in fungicide target proteins. To do this we have produced alignments between fungicide target proteins of relevant species fitted to a well-studied “archetype” species. Orthologous amino acids in all species are then assigned numerical “labels” based on the position of the amino acid in the archetype protein.
15. Preliminary Results of Strobilurin Sensitivity of Zymoseptoria Tritici Italian Strains

Abstract ID 54
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*Mycosphaerella graminicola* (anamorph: *Zymoseptoria tritici*) is the causal agent of leaf blotch, the most important foliar disease of wheat in Northern and Central Europe. In Italy only during the last few years, the incidence of the disease has been increased. The most common strategies for the control of the disease is the use of fungicides in particular QoIs, DMIs, and more recently were introduced the SDHIs. The resistance to QoIs and DMIs was already common in the main wheat cultivated areas in the world (Cools and Fraaije, 2008; Estep et al., 2015; Fraaije et al., 2005; Gisi et al., 2005; Leroux et al., 2005; Stammler and Semar, 2011; Stewart et al., 2014) while in Italy no results coming from wide and specific sensitivity monitoring are available. The aim of this study was to test the sensitivity of *Z. tritici* strains to QoI in order to obtain the first data about the Italian scenario.

Leaves of bread and durum wheat were collected during 2015 from 10 fields with different use of fungicides (wild type, experimental centers and commercial ones) located in the North of Italy. The sensitivity of 60 isolates to azoxystrobin at different concentrations (0-0.001-0.01-0.1-1-2-10-20 mg/L of active ingredient) have been determined *in vitro* by microtiter assay (Stammler and Semar, 2011).

The EC50 values of the wild types ranged from 0.02 to 0.12 mg/L of azoxystrobin, while the first analyzed isolates collected from experimental plots and commercial fields showed EC50s variable from 1.92 to 5.1 mg/L. Only one isolate pointed out an EC50 of 20.6 mg/L of active ingredient.

On the base of these first results, we can suppose the presence of a slight decrease of sensitivity of *Z. tritici* isolates collected from Italian fields (according to Gisi et al., 2005). Molecular analysis on the presence of G143A substitution will be carried out.


16. Phostrol fungicide applied early was not effective against root rot of field pea, 2015

Abstract ID 64
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Root rot of field pea (Pisum sativum L.), caused by a disease complex that includes Fusarium spp., Pythium spp., and Rhizoctonia solani Kühn, is becoming an increasing important constraint to pea production on the Canadian prairies. Also, Aphanomyces euteiches Drechs. has recently been shown to be widespread and damaging across the region. No strong sources of root rot resistance are commercially available, and no fungicides have been identified that provide useful reduction in root rot caused by A. euteiches. Phostrol fungicide is one of the few fungicides that is translocated downward to plant roots, and so might protect the crop from root rot. Replicated small-plot field trials were conducted at five sites in 2015; the AAFC research farms at Saskatoon and Melfort, Saskatchewan, and at Lethbridge, Alberta, and two grower fields (one in Saskatchewan, one in Alberta) infested with A. euteiches. The treatments were calcium carbonate participate (4 and 8 T/ha), gypsum (2.2 T/ha), and granular Edge herbicide (13 and 26 kg/ha), broadcast prior to seeding, Phostrol (6 and 12 L/ha) applied ½ at seedling emergence and ½ 14 days later, and a nontreated check. Several treatments increased seedling emergence slightly at Melfort, but others reduced establishment at Saskatoon. There was no effect of treatment at the other three sites, and no positive effect on yield at any site. Spring and early summer conditions across the region in 2015 were drier than normal. As a result, disease pressure from seedling blight and root rot was generally low. Aphanomyces root rot caused substantial injury in the commercial field in Saskatchewan, but none of the treatments had an effect on emergence or yield. These results indicate that no treatment provided effective reduction in root rot severity.
17. Sensitivity of *Alternaria alternata* the causal organism of potato early blight to synthetic fungicides

Abstract ID 84
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Early blight caused by pathogenic fungus *Alternaria alternata* is an increasingly important potato disease in China and many parts of the world. No major gene resistance has been identified in potato host yet and the disease is controlled mainly by fungicide application. To understand the development of resistance to main synthetic fungicides on market, a total of 424 *A. alternata* isolates collected from 11 provinces in China between 2010 and 2013 were tested for their sensitivity to boscalid, azoxystrobin, procymidone and difenconazole. Frequency of resistance to boscalid, procymidone and difenconazole, which was determined by relative germination or relative mycelium growth rate greater than 50% at 10 μg/ml, 40 μg/ml and 2.5 μg/ml, was 53.2%, 3.0% and 0.9%, respectively. All isolates were sensitive to azoxystrobin. The frequency of resistance to boscalid was higher for isolates from Guizhou, Shandong, Henan and Neimenggu than other areas indicating that early blight control with boscalid may be not effective in these areas. Positive correlation of fungicide sensitivity was observed between boscalid and azoxystrobin, and difenconazole and procymidone (p < 0.0001), suggesting a high risk of developing cross resistance in these pairs of fungicides. Negative correlation of sensitivity was observed between boscalid and difenconazole (p < 0.0001) or procymidone (p = 0.0129), and azoxystrobin and difenconazole (p < 0.0001), indicating that these pairs of fungicides could be used as mixture or rotation to control potato early blight or other plant diseases.
18. Effect of Fungicide Applications on *Monilinia fructicola* Population Diversity and Transposon Movement

Abstract ID 76
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In this study we investigated whether previously reported, fungicide-induced mutagenesis in *M. fructicola* can accelerate genetic changes in field populations. Azoxystrobin and propiconazole were applied at half label rate to nectarine trees for two consecutive years in weekly intervals for about three months between bloom and harvest. Single spore isolates were obtained from blighted blossoms, corresponding cankers, and from fruit to investigate phenotypic and genotypic changes. In both years, isolate populations collected from fungicide-treated fruit and from untreated control fruit were not statistically different in haploid diversity (p>0.45 for 2013), (p> 0.46 for 2014), allele number (p=0.88 for 2013), (p=0.41 for 2014), and effective allele number (p>0.88 for 2013), (p>0.81 for 2014). Isolates from blossoms and corresponding cankers of fungicide treatments revealed no changes in Simple Sequence Repeat (SSR) size or evidence for induced *Mftc1* transposon translocation. No emergence of reduced sensitivity to azoxystrobin, propiconazole, iprodione, and cyprodinil that would indirectly indicate increased genetic diversity was detected. High levels of population diversity in all treatments provided evidence for sexual recombination of this pathogen in the field despite apparent absence of apothecia. Our results indicate that fungicide-induced, genetic changes may not occur as readily in field populations as they do in populations exposed continuously to sublethal doses in vitro.
Phoma stem canker, caused by intently related pathogens *Leptosphaeria biglobosa* and *L. maculans*, is an economically important disease on oilseed rape (*Brassica napus*). In 2015, in some regions of Germany relatively late disease symptoms were observed on the upper part of stems, causing the crown canker which was responsible for lodging of the plants and yield losses. The results of morphology and species-specific PCR assays revealed that *L. biglobosa* constituted 58% of all isolates obtained from the infected stems. In general, besides growing resistant oilseed rape cultivars, fungicide application showed a significant reduction in the percentage of blackleg disease incidence and severity but little is known about the differences of sensitivity of *Leptosphaeria* spp. isolates to different fungicides. At the present study, the effects of the most important groups of fungicides (QoI, SDHI, DMI and MBC) were examined on the germination of pycnidia and the inhibition of mycelial growth of both species. Fungicide sensitivity tests in vitro were conducted using fungicide amended agar plates at 0.0, 0.001, 0.01, 0.1, 1.0, 1.0 and 100.0 μg a.s. mL⁻¹ concentrations. The results show that the two pathogens did not differ significantly in their growth rates under in vitro conditions. Lower concentrations of all fungicides (0.001, 0.01, 0.1 and 1.0 μg a.s. mL⁻¹) have no or a low effect on conidial germination and mycelial growth inhibition in both species. In contrast, at higher concentrations significant differences were observed in the sensitivity to fungicides in *L. maculans* and *L. biglobosa*. *Leptosphaeria maculans* isolates were significantly more susceptible to all fungicides group than *L. biglobosa*.

Even at the highest concentrations (100 μg a.s. mL⁻¹) none of the fungicides could provide 50% control of *L. biglobosa* neither at the conidial germination test nor at the inhibition of mycelial growth assay.
20. Characterisation of SDHI and azole insensitive Zymoseptoria tritici UK field strains

Abstract ID 114
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Zymoseptoria tritici has rapidly developed resistance to Methyl Benzimidazole Carbamate (MBC) and Quinone outside Inhibitor (QoI) fungicides. Resistance evolved due to point mutations in the target site encoding gene resulting in amino acid substitutions, such as F129L and G143A in cytochrome b for the QoIs, or E198A in beta-tubulin for the MBCs. Cytochrome b G143A and beta-tubulin E198A became rapidly fixed in field populations due to high resistance factors and low fitness costs associated with these mutations. Azole resistance has developed much slower and is incomplete. Many different azole sensitivity phenotypes based on combinations of CYP51 mutations have been detected in field populations. CYP51 mutations can affect the enzyme function and the binding of different azoles with the target can also be differentially affected. Additional azole resistance mechanisms are overexpression of the target (CYP51) and a Major Facilitator Superfamily transporter (MgMfs1). Due to their importance in disease control and medium to high resistance risk, SDHI sensitivity monitoring has been carried out intensively. Here we report the latest genotype-to-phenotype relationships for a selection of the most azole and/or SDHI insensitive field strains isolated during 2015.
21. *Diaporthe endophytica* and *Diaporthe terebinthifolii* from Medicinal Plants in Biological Control of *Phylosticta citricarpa*

Abstract ID 57
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*Phylosticta citricarpa* is the causal agent of Citrus Black Spot (CBS) and is subject to phytosanitary legislation in the EU. CBS disease in Brazil is characterized by production losses and low price of fruit in the fresh fruit market. Currently the CBS is treated by several fungicides applications per year. One interesting alternative to CBS control without harming the environment would be the use of endophytes. *Diaporthe endophytica* and *D. terebinthifolii* isolated from medicinal plants *Maytenus ilicifolia* and *Schinus terebinthifolius* showed the inhibitory activity against *P. citricarpa in vitro* and in detached fruits. To optimize production of secondary metabolites, culture conditions including carbon and nitrogen source, pH and incubation time were studied. Bioactivities evaluation indicate that pH 5.8, malt extract and glucose as carbon sources, and peptone as a nitrogen source are the optimal production conditions. However, the incubation time varied between the two strains, 10 days for LGMF 907 and 21 days for LGMF 914. The compounds identification are being carried out. In order to verify the ability of these isolates to colonize the citrus plants, the DSRed fluorescente protein gene was introduced into their genomes using Agrobacterium tumefaciens system. Our results showed that *D. endophytica* and *D. terebinthifolii* were able to colonize citrus plants, and it was confirmed by reisolation of transformants from inoculated and uninoculated leaves. Microscopy analysis at the inoculation point showed the fungus colonize intercellular region and oil glands of citrus. These results suggest that these species are capable of colonize citrus plants and could be used in *P. citricarpa* biological control.
22. Assessing the risk of combination between MDR and specific resistance towards SDHI in *Zymoseptoria tritici*

Abstract ID 74
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*Zymoseptoria tritici* is responsible for leaf blotch, the most detrimental disease of wheat. This fungal pathogen is mostly controlled in Europe by the mean of fungicide treatments, largely including inhibitors of the sterol 14α-demethylase (DMIs class) and of the succinate dehydrogenase (SDHIs or carboxamides class). Resistance to DMIs is generalized over Western Europe, due to numerous combinations of mutations affecting the gene encoding the target enzyme (*CYP51*). Most recent *cyp51* genotypes exhibit high resistance factors to some DMIs and may lead to efficacy losses. Moreover, strains exhibiting multidrug resistance (MDR) are detected in populations since 2008 and induce high resistance towards all DMIs, due to a mechanism of increased efflux involving the membrane transporter MgMFS1. This new resistance mechanism, combined with target alteration resistance, lead to high resistance towards all DMIs, and also to low resistance towards SDHIs. SDHIs were recently introduced and only sporadic cases of specific resistance (CarR strains) are detected in European populations since 2012, with various genotypes, generally leading to low to moderate resistance factors, according to the molecule tested. At last, *Z. tritici* is largely recognized in literature for its high capacities of dispersion and recombination. Therefore, the risk of recombination between MDR and CarR strains is more likely to increase, due to the recent progression of MDR strains in populations and to the generalization of the use of SDHIs, as DMIs are partially loosing efficacy.

To anticipate this risk and mimic what may once occur in field populations, we crossed in our laboratory MDR and CarR parental strains, and produced progeny strains bearing at the same time the MDR and CarR alleles (multiple resistance). This poster presents this work and the preliminary characterization of the progeny.
Multidrug resistance (MDR) is a common trait developed by many organisms to counteract chemicals and/or drugs used against them. The basic MDR mechanism is relying on an overexpressed efflux transport system that actively expulses the toxic agent outside the cell. In fungi, MDR (or PDR) has been extensively studied in *Saccharomyces cerevisiae* and *Candida albicans*, but also plant pathogenic fungi are concerned by this phenomenon.

MDR strains are detected in septoria leaf blotch (*Zymoseptoria tritici*) field populations since 2008. These strains are cross-resistant to DMIs (high resistance factors, due to combination with target site resistance), QoIs and SDHIs (low resistance factors), due to active fungicide efflux.

In a previous study, we identified the *MgMFS1* gene overexpressed in all tested MDR field strains (Omrane et al., 2015). This gene encodes a major facilitator membrane transporter whose inactivation abolished the MDR phenotype in in two isolated strains (MDR6 and MDR7). To identify the mutation(s) responsible for MDR phenotype we applied bulk-progeny sequencing to MDR6 and MDR7 strains that allowed us to identify a 519 bp insert in the *MFS1* promoter in both strains. The insert, a reminiscence of a recent retrotransposition event, is responsible for MFS1 overexpression and the MDR phenotype. Genotyping of various field and MDR6 progeny strains revealed that at least one additional mutation is responsible for the MDR phenotype, *via MgMFS1* overexpression.

24. What is the best solution for fungicide resistance management in grapes: Sequences or Alternation?

Abstract ID 71
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Perennial crops such as grapes must be treated several times during the season to limit the damages of the major pathogens like grape downy mildew (*Plasmopara viticola*), grape powdery mildew (*Erysiphe necator*) or black-rot (*Guignardia bidwellii*). In France, the average numbers of downy mildew treatments vary from 5 to 10 depending the year and the area. The situation is similar with powdery mildew or black-rot. In parallel, the numbers of available mode of action is limited. So, the advisors should recommend repeated treatments with the same mode of action during the season but also over the years.

In this context, resistance to fungicide can develop quickly for most of the modes of action, especially for *P. viticola* but for *E. necator* too. Withdraw of the fungicide concerned by resistance is the worse situation. Application in mixture with another mode of action could be a reliable solution if there is no resistance for the partner and a good complementarity between the two molecules.

However, multiple applications of the same mode of action (alone or in mixture) must be done during the year. So, the key question is: should the fungicide spray in sequence (2 consecutive treatments for example) or must be applied in alternation with at least one application with another mode of action?

Based on the overview of the last 25 years of fungicide resistance evolution in grapes, this presentation will confirm that there is no a general rule. Sometimes, application of the fungicide at two different periods during the season is the best strategy to delay resistance (i.e. grape downy mildew and CAA fungicides). In other time, alternation of two QoI+SDHI fungicides with another mode of action is worse than a sequence of two treatments with the QoI+SDHI (i.e. grape powdery mildew).
25. Occurrence and Management of Cowpea (*Vigna unguiculata*) Root Diseases in Trinidad

Abstract ID 95
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Cowpea (*Vigna unguiculata*), one of the most widely grown legumes on the island nation of Trinidad, is a relatively fast growing and versatile crop. This crop, which is commonly referred to locally as “bodi” is susceptible to various bacteria, fungi and viruses that cause a variety of foliar and root diseases. In this investigation, field and greenhouse experiments were utilized to score disease incidence and to test the efficacy of treatments. In order to identify and score the incidence of disease in this crop several test fields were set up in strategic locations across the island of Trinidad. The field trials were carried out during both the dry and wet seasons and consisted of single and combination biocontrol treatments of *Trichoderma viride*, *Pseudomonas spp.*, and *Bacillus subtilis*. For the greenhouse trial emphasis was placed on the treatment of several fungal root diseases namely, *Sclerotium rolfsii* Sacc., *Fusarium oxysporum f. sp. tracheiphilum*, and *Pythium aphanidermatum*, with the identical biocontrol agents utilized during the field trials. The Percent Disease Incidence (PDI) for the field trials over both the wet and dry seasons was lowest for each root disease when treated with a combination of each bio-control agent (TV1+Bs1+Pf1).
26. Sensitivity of Venturia inaequalis to Different Fungicides

Abstract ID 69
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Venturia inaequalis causes apple scab or black spot and is the most important fungal disease in apples in most apple growing regions worldwide. It is classified as a pathogen with a higher resistance risk and therefore sensitivity monitoring for fungicides with a medium or high resistance risk is necessary. Several fungicides registered for the control of apple scab belong to the classes of quinone outside inhibitors (QoI), anilinopyrimidines (AP), sterol biosynthesis inhibitors (SBI) and succinate dehydrogenase inhibitors (SDHI). Various fungicides with a multi-site inhibition type play also an important role for the control of apple scab, including dithianon and the EBDC fungicides mancozeb and metiram. Many populations in various regions have developed resistance to QoIs in the last years caused by the target site mutation G143A in the cytochrome b gene. Using this knowledge, efficient monitoring programmes were developed based on pyrosequencing or qPCR. The situation of AP resistance is more difficult to document. It is recognised by different phenotypes, i.e. levels of resistance, but the resistance mechanism has not yet been linked to a target site mutation or other molecular mechanisms. Frequency of AP resistant strains has stabilised in the last decade and current data indicate a decrease of resistant strains during the 2014 and 2015 growing seasons. The shift to a lower sensitivity towards SBIs is well known in literature reported for a range of SBI fungicides. The shift led to a low efficacy of “older” SBIs in many regions. However, sensitivity data from the last 5 years indicate a stable situation for more recently introduced SBIs. The new class of SDHIs has also been introduced for scab control and there have been some strains found with a reduced SDHI sensitivity at a few trial sites in Europe. Such isolates have been characterised and the molecular mechanism have been identified (e.g. mutation H151R in the SDH-C subunit). As a result Pyrosequencing assays were then developed for a more efficient sensitivity monitoring in future. The monitoring of fungicides with a multi-site mode of action were carried out throughout the last 5 years with metiram and dithianon. No adaptations have been found for either AI since despite their market introductions over 50 years ago, confirming their invaluable importance for disease control and effective resistance management in commercial practice.
27. The Study of Influence of Aerotechnogenic Pollution on Lichenized Fungi by Means of Electron Microscopy

Abstract ID 61
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It is known that the phycobionts (lichenized fungi) are the main form-building components of lichens. The influence of aerotechnogenic pollution (heavy metals and SO2). The attention was focused on the changes of morphology and ultrastructure of lichen thalli caused by air pollution and on mineral element composition of outer and inner surfaces of lichen thalli. Being very sensitive to the atmospheric pollution, the lichens are considered as natural indicators of environmental conditions. The lichens of genus Cladonia: C. crispata, C. cryptochlorophaea, C. cyanipes, C. deformis, C. ecmocyna, C. stellaris were collected in heavily polluted areas of Cola peninsula (nearly from industrial town Monchegorsk). The lichens were examined by means of scanning electron microscopy. Electron microscopic study showed that dramatic alterations of lichen morphology were connected with alterations of hyphal growth. The presence of heavy metals and other mineral elements on lichen surfaces was determined by microscopic roentgen spectral analysis, compared and discussed. These observations demonstrate the significant effects of pollutants on lichen.
28. Effect of Salt and Drought Stresses on the Growth and Enzymatic Activity of *Ceratocystis radicicolathe* Causing Date Palm Black Scorch

Abstract ID 98
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*Ceratocystis radicicola* also has been reported to be associated with date palm in Qatar causing black scorch disease. Environmental factors such as stressed palms or senescent tree parts increase the incidence of the disease. Plant pathogenic fungi produce variety of enzymes that degrade plant cell wall. Extracellular enzymes secreted by fungi are able to soften tissues and degrade cell wall components. This current study aimed to examine the influence of salinity and drought stresses on the growth of *C. radicicola* in vitro and study the enzymatic activities of some enzymes in *C. radicicola*.

For salt stress, 4mm disc of *C. radicicola* was cultured in PDA or PDB media with different concentrations of NaCl. Regarding drought stress, 4mm disc of *C. radicicola* was cultured in PDA with different Poly Ethylene Glycol concentrations. To study enzymatic activity assay, Czapek media was prepared where the carbon source was substituted with 1% of the following: carboxymethyl cellulose (CMC), sucrose, pectin or xylan.

Results showed clear growth of *C. radicicola* in PDB media with NaCl concentrations of 0.26, 0.43, 0.6, 0.86, 1.03 and 1.2 M during the first three weeks while no growth was occurred in 1.37 M. Radial growth of the fungus did not show any changes with 0.26, 0.43 and 0.6 M while the diameter decreased significantly under 0.86, 1.03 and 1.2 M. Number of spores was decreased by increasing NaCl concentrations.

*C. radicicola* was able to survive under drought stress regimes up to 40% in the first seven days while it failed to grow at 60% of PEG4400. *C. radicicola* grow very well in czapek media supplemented with 1% both xylan and pectin as carbon sources while it showed weak growth in czapek media supplemented with 1% of CMC. On the other hand, it did not grow in czapek media with 1% sucrose. Both Xylanase and carboxymethyl cellulase had the highest enzymatic activities with 109 and 6.8 IU/ml, respectively when pectin was used as a carbon source at pH 8. In addition, high pectinase activity was recorded (61IU/ml) when pectin was used in the growth media at pH 7.5.
Active oxygen species are one of the main contributors of the signaling system by which wheat plants recognize an attack of the powdery mildew pathogen and activate immune responses. To model the oxidative stress leaves of wheat seedlings (*Triticum aestivum* L.) were treated with exogenous hydrogen peroxide and 3-amino-1,2,4-triazole, an inhibitor of peroxidase, contributing increase in the content of endogenous hydrogen peroxide. *Blumeria graminis* f. sp. *tritici* conidia germinate on the surfaces of leaves to produce a primary germ tube and a germ tube that develops an appressorium. Some proportion of conidia germinates abnormally and form appressoria with elongated germ tube or multiple germ tubs partially lost their orientation. Halo looking like concentric blue or rose colored circles 60–120 mkm in diameter after staining with amido black formed at the point of contact of primary germ tubes and appressoria with epidermis. Treatment with hydrogen peroxide and 3-amino-1,2,4-triazole inhibited development of pathogen colonies, increased the number of abnormal appressoria and average diameter of halo. Some halo in treated leaves changed the morphology and had internal rings.

After treatment with 1mM hydrogen peroxide in some sites of contact with pathogen, abnormal large halos up to 250–300 mkm were also observed. These observations suggest that increase of abnormal appressoria and the changes in halo morphology and size is a result of oxidative stress. Well-known formation of elongated appressoria in resistant plants was similar to abnormal development of the mildew pathogen at prooxidant treatment and, thus, may be associated with appearance of active oxygen species during plant resistance responses.
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