



# III INTERNATIONAL SYMPOSIUM ON POSTHARVEST PATHOLOGY

*Using science to increase food availability*

## *Programme & Abstracts*

BARI (ITALY), 7 - 11 JUNE 2015



**Dipartimento di Scienze del Suolo  
della Pianta e degli Alimenti**

*Under the auspices of:*

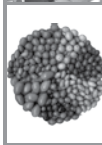
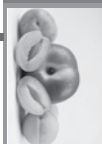


**REGIONE PUGLIA**

*Patrocinio dell'Assessore alle  
Risorse Agroalimentari della Regione Puglia*



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## International Scientific Committee

|                               |   |
|-------------------------------|---|
| <b>Asgar Ali</b>              | <i>CEPB, University of Nottingham, Malaysia</i>           |
| <b>Paolo Bertolini</b>        | <i>University of Bologna, Italy</i>                       |
| <b>Vincenzo De Cicco</b>      | <i>University of Molise, Italy</i>                        |
| <b>Samir Droby</b>            | <i>ARO, Israel</i>  |
| <b>Deena Errampalli</b>       | <i>Agriculture and Agri-Food, Canada</i>                  |
| <b>Kerry R. Everett</b>       | <i>Institute for Plant and Food Research, New Zealand</i> |
| <b>Luis González Candelas</b> | <i>IATA, Spain</i>  |
| <b>Maria Lodovica Gullino</b> | <i>University of Turin, Italy</i>                         |
| <b>Antonio Ippolito</b>       | <i>University of Bari, Italy</i>                          |
| <b>Pervin Kinay Teksur</b>    | <i>Ege University, Turkey</i>                             |
| <b>Lise Korsten</b>           | <i>University of Pretoria, South Africa</i>               |
| <b>Jia Liu</b>                | <i>Hefei University, China</i>                            |
| <b>Leon Terry</b>             | <i>Cranfield University, England</i>                      |
| <b>Shiping Tian</b>           | <i>National Academy of Sciences, China</i>                |
| <b>Josep Usall</b>            | <i>IRTA, Spain</i>  |
| <b>Silvana Vero</b>           | <i>University of the Republic, Uruguay</i>                |
| <b>Michael Wisniewski</b>     | <i>USDA-ARS, WV, USA</i>                                  |
| <b>Juan Pablo Zoffoli</b>     | <i>Pontificia Universidad Católica, Chile</i>             |

## Local Organizing Committee

|                                |                                  |
|--------------------------------|----------------------------------|
| <b>Antonio Ippolito</b>        | <i>University of Bari, Italy</i> |
| <b>Simona Marianna Sanzani</b> | <i>University of Bari, Italy</i> |
| <b>Franco Nigro</b>            | <i>University of Bari, Italy</i> |
| <b>Angela Ligorio</b>          | <i>University of Bari, Italy</i> |
| <b>Stefania Pollastro</b>      | <i>University of Bari, Italy</i> |

## Organizing Committee

|                             |   |
|-----------------------------|---|
| <b>Raffaello Castoria</b>   | <i>University of Molise, Italy</i>                        |
| <b>Gabriella Cirvilleri</b> | <i>University of Catania, Italy</i>                       |
| <b>Donato Di Venere</b>     | <i>ISPA-CNR, Bari, Italy</i>                              |
| <b>Giuseppe Lima</b>        | <i>University of Molise, Italy</i>                        |
| <b>Marta Mari</b>           | <i>University of Bologna, Italy</i>                       |
| <b>Massimo Reverberi</b>    | <i>Sapienza – University of Rome, Italy</i>               |
| <b>Gianfranco Romanazzi</b> | <i>Marche Polytechnic University, Italy</i>               |
| <b>Leonardo Schena</b>      | <i>Mediterranean University of Reggio Calabria, Italy</i> |
| <b>Davide Spadaro</b>       | <i>University of Turin, Italy</i>                         |
| <b>Thaer Yaseen</b>         | <i>IAMB, Italy</i>  |

**14:00-18:00** Participant registration

**18:00-18:20** Informal welcome

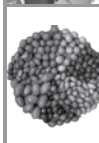
## **Symposium history and perspectives**

**Samir Droby, Michael Wisniewski, Josep Usall, Pervin Kinay, Antonio Ippolito**

**18:20-19:20** Inaugural Invited Lectures

- |           |                    |   |
|-----------|--------------------|---|
| <b>L1</b> | <b>18:20-18:50</b> | <b>Next Generation Sequencing for next generation crops</b><br>Massimo Delledonne, Università degli Studi di Verona, Italy                                      |
| <b>L2</b> | <b>18:50-19:20</b> | <b>Science and social media: how to avoid feeding the troll and save your time</b><br>Lorenzo Mannella, Università degli Studi di Modena e Reggio Emilia, Italy |

**Welcome cocktail (Villa Romanazzi Carducci)**



## Programme - June, 8 2015

**08:30-09:00 Welcome Ceremony and Opening remarks**

**09:00-11:00 Session I: Studies on Host-Pathogen interactions**

Moderators: Massimo Delledonne, Massimo Reverberi

### Invited lectures

**L3 09:00-09:30 Role of effector proteins in pathogenicity of postharvest pathogens**

Samir Droby, ARO, The Volcani Center, Israel

**L4 09:30-10:00 Genomic tools for developing markers for postharvest disease resistance in rosaceae fruit crops**

Michael Wisniewski, USDA-ARS, USA

**L5 10:00-10:30 Omics technologies to unravel pathogenicity mechanisms in *Penicillium* spp.**

Luis Gonzalez-Candelas, IATA-CSIC, Spain

### Oral communications

**O1 10:30-10:45 Simultaneous RNA deep-sequencing of *Colletotrichum gloeosporioides* arms and tomato fruits defense strategies at different stages of fruit-fungal interactions**

Noam Alkan, ARO Volcani Center and Weizmann Institute of Science, Israel

**O2 10:45-11:00 Proteomic and oxi-proteomic response of apple to a compatible (*Penicillium expansum*) and a non-host (*P. digitatum*) pathogen**

Rosario Torres, IRTA, XaRTA-Postharvest, Catalonia, Spain

**11:00-11:30 Coffee break**

**11:30-12:30 Session I continued**

Moderators: Michael Wisniewski, Luis Gonzalez-Candelas

### Invited lecture

**L6 11:30-12:00 Function of small GTPase Rho3 in regulating growth, conidiation and virulence of *Botrytis cinerea***

Shiping Tian, Chinese Academy of Sciences, China

### Oral communications

**O3 12:00-12:15 *Botrytis cinerea* and grapevine inflorescence interaction**

Zeraye M. Haile, Fondazione Edmund Mach, Italy

**O4 12:15-12:30 May strawberry volatile emission influence *Botrytis cinerea* growth?**

Fiorella Neri, Università degli Studi di Bologna Alma Mater, Italy

**12:30-13:30 Lunch**

**13:30-14:30 Poster session 1 (PM 1-30)**

**14:30-16:30 Session II: Microorganisms as Biocontrol Agents**

Moderators: Josep Usall, Marta Mari

*Invited lectures*

- L7 14:30-15:00 Ecological fitness of yeasts to control postharvest diseases of fruits and its impact on formulation and practical application**  
Haissam Jijakli, Gembloux Agro-Bio Tech, Université de Liège, Belgium
- L8 15:00-15:30 Unraveling the mechanisms used by antagonistic yeast to control postharvest pathogens on fruit**  
Davide Spadaro, Università degli Studi di Torino, Italy

*Oral communications*

- O5 15:30-15:45 DNA-based methodologies to detect and quantify the postharvest biocontrol agent *Pantoea agglomerans* CPA-2 applied on oranges**  
Neus Teixidó, IRTA, XaRTA-Postharvest, Catalonia, Spain
- O6 15:45-16:00 Effect of two biological control agents on apple postharvest diseases in long-term storages in Canada**  
Deena Errampalli, Agriculture and Agri-Food Canada, Canada
- O7 16:00-16:15 Yeasts as biological agents to control *Botrytis cinerea* on roses**  
Alba Marina Cotes, Colombian Corporation for Agricultural Research, Colombia
- O8 16:15-16:30 In vitro and in vivo screening of antagonistic bacterial strains isolated from vineyards to control *Botrytis cinerea* in grapevine tissues**  
Rana Haidar, Université de Bordeaux, France

**16:30-17:00 Coffee break**

**17:00-18:00 Session III: The Microbiome and Its Relation to Postharvest Pathology**

Moderators: Samir Droby, Davide Spadaro

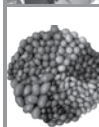
*Invited lecture*

- L9 17:00-17:30 Analysing the plant microbiome for control of pathogens**  
Gabriele Berg, Gratz University of Technology, Austria

*Oral communications*

- O9 17:30-17:45 Metabarcoding analysis of beneficial and detrimental fungi in aerial plant parts**  
Leonardo Schena, Università Mediterranea di Reggio Calabria, Italy
- O10 17:45-18:00 Diversity of *Botrytis cinerea* isolates and variability of microflora in noble rotted grape berries in Eger wine region**  
Júlia Kaló-Hegyí, Eszterházy Károly College, and Bethune-Cookman University, Hungary

**Open discussion**



## Programme - June, 9 2015

### 09:00-11:00 Session I: Toxic Fungal Metabolites and Postharvest Pathology

Moderators: Corrado Fanelli, Simona M. Sanzani

#### Invited lectures

- L10 09:00-09:30 Regulated alternariol biosynthesis by *Alternaria alternata* is important for successful substrate colonization**  
Rolf Geisen, Max Rubner Institute, Germany
- L11 09:30-10:00 Lipid signals in the interaction between mycotoxigenic fungi and their hosts**  
Massimo Reverberi, Sapienza - Università degli Studi di Roma, Italy

#### Oral communications

- O11 10:00-10:15 Non-fermenting yeast strains are able to control vegetative growth and sporulation of *Aspergillus carbonarius* and to adsorb Ochratoxin A from grape juice**  
Quirico Migheli, Università degli Studi di Sassari, Italy
- O12 10:15-10:30 Identification and mycotoxigenic capacity of fungi associated with pre- and postharvest fruit rots of pomegranates**  
Loukas Kanetis, Cyprus University of Technology, Cyprus
- O13 10:30-10:45 De novo sequencing and detection of secondary metabolite gene clusters of *Penicillium griseofulvum***  
Houda Banani, Università degli Studi di Torino, Italy
- O14 10:45-11:00 Facing the problem of fungal and Ochratoxin A contamination of fresh grape and raisins in Algeria**  
Siheem Fodil, Mediterranean Agronomic Institute of Bari, Italy

**11:00-11:30 Coffee break**

### 11:30-13:00 Session II: Epidemiology and Detection of Postharvest Pathogens

Moderators: Quirico Migheli, Leonardo Schena

#### Invited lecture

- L12 11:30-12:00 Recommendations to current postharvest practices to prevent fresh fruit contamination by *Listeria monocytogenes***  
Dumitru Macarasin, U.S. Food and Drug Administration, USA

#### Oral communications

- O15 12:00-12:15 Population structure and fungicide resistance profile of *Botrytis* spp. associated with stem end rot of pomegranate fruit in California and Greece**  
George S. Karaoglanidis, Aristotelian University, Thessaloniki, Greece
- O16 12:15-12:30 Bitter rot of apples caused by *Colletotrichum acutatum*, a predictive model for infection and inoculum release**  
Kerry R. Everett, The New Zealand Institute for Plant & Food Research Limited, Mt Albert, Auckland, Australia



**O17 12:30-12:45 Identification and characterization of fungi causing bull's eye rot on apple in Poland**

Monika Michalecka, Research Institute of Horticulture, Skierniewice, Poland

**O18 12:45-13:00 Citrus-associated *Alternaria* species in the mediterranean areas**

Francesca Garganese, DISSPA, Università degli Studi di Bari Aldo Moro, Italy

**13:00-14:00 Lunch**

**14:00-15:00 Poster session 2 (PT 1-28)**

**15:00-16:30 Session II continued**

Moderators: Kerry R. Everett, George S. Karaoglanidis

*Invited lecture*

**L13 15:00-15:30 Epidemiology of *Botrytis cinerea* as basic knowledge of gray mold control on postharvest storage**

Juan Pablo Zoffoli, Pontificia Universidad Católica, Chile

*Oral communications*

**O19 15:30-15:45 Natural infection moments of bull's eye rot in Belgian orchards**

Wendy Van Hemelrijck, Research Station for Fruit Cultivation (pcfruit npo), Belgium

**O20 15:45-16:00 Postharvest storage rots of apples and pears in The Netherlands**

Marcel Wenneker, Wageningen University & Research Centre, The Netherlands

**O21 16:00-16:15 Heart rot and soft rot of pomegranate fruit in southern Italy**

Roberto Faedda, Università degli Studi di Catania, Italy

**O22 16:15-16:30 Influence of floral morphology and fruit development on internal fruit rot (*Fusarium* spp.) in bell pepper**

Mario Frans, KU Leuven Campus Geel, Belgium

**O23 16:30-16:45 Role of two inoculation methods in expression of anthracnose resistance genes in chili (*Capsicum annuum*)**

Patcharaporn Suwor, Khon Kaen University, Thailand

**16:45-17:15 Coffee break**

**17:15-18:30 Round table**

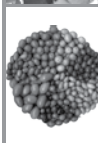
**Innovations in the Management of Table Grape Diseases**

Moderators: Juan Pablo Zoffoli, Antonio Ippolito

*Oral communications*

**O24 17:15-17:30 To melt or not to melt: the significance of postharvest disinfection for prevention of decay of table grapes after storage**

Amnon Lichter, ARO, The Volcani Center, Israel



## Programme - June, 9 2015

**O25 17:30-17:40 Efficacy of a new SO<sub>2</sub> generator pad in maintaining postharvest quality of table grapes**

Francesco Saporito, Decco, Italy

**O26 17:40-17:50 Effect of postharvest treatment and quarantine procedure on organic table grape cv. Italia**

Flutura Lamaj, Mediterranean Agronomic Institute of Bari, Italy

**O27 17:50-18:00 Impact of ventilation area of the liner bag, in the performance of SO<sub>2</sub> generator pads in boxed table grapes**

José Luis Henríquez, Universidad de Chile, Chile

**18:00 Open discussion**

**09:00-11:00 Session I: Alternative Means for the Management of Postharvest Pathogens**

*Moderators: Amnon Lichter, Gianfranco Romanazzi*

*Invited lecture*

**L14 09:00-09:30 Natural compounds: an alternative in postharvest diseases control**

Marta Mari, Università degli Studi di Bologna Alma Mater, Italy

*Oral communications*

**O28 09:30-09:45 Electrolyzed salt solution mode of action in controlling green mould of citrus fruit**

Simona M. Sanzani, DISSPA, Università degli Studi di Bari Aldo Moro, Italy

**O29 09:45-10:00 Potassium silicate: a new organic tool for the control of citrus postharvest green mold**

Lluís Palou, Institut Valencià d'Investigacions Agràries (IVIA), Spain

**O30 10:00-10:15 In vivo application of garlic extracts for the management of postharvest decay in apples**

Cheryl L. Lennox, Stellenbosch University, South Africa

**O31 10:15-10:30 UV-C light to reduce biotic and abiotic stresses of stored fruit and vegetables: a brief review**

Franco Nigro, DISSPA, Università degli Studi di Bari Aldo Moro, Italy

**O32 10:30-10:45 Ripening degree influences development of postharvest fungal decay on European plum more than preharvest applications of calcium and fungicides**

Jorunn Børve, Norwegian Institute for Agricultural and Environmental Research, Norway

**O33 10:45-11:00 Alternative technology: using plant volatiles to control anthracnose in avocados**

Dharini Sivakumar, Tshwane University of Technology, South Africa

**11:00-11:30 Coffee break**

**11:30-13:00 Session I continued**

*Moderators: Paolo Bertolini, Deena Errampalli*

*Invited lecture*

**L15 11:30-12:00 New heat treatments as alternative means to control postharvest pathogens on fruits**

Josep Usall, IRTA, XaRTA-Postharvest, Catalonia, Spain

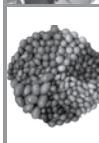
*Oral communications*

**O34 12:00-12:15 Investigating the control of green mould on sweet oranges subjected to steam treatment**

Abiola T. Aborisade, The Federal University of Technology, Nigeria

**O35 12:15-12:30 New tools to improve the shelf life of chestnut fruits during storage**

Michelina Ruocco, Istituto per la Protezione Sostenibile delle Piante, CNR, Portici (NA), Italy



## Programme - June, 10 2015

**O36 12:30-12:45 Preharvest treatments with alternatives to conventional fungicides to control postharvest decay of strawberries**

Gianfranco Romanazzi, Università Politecnica delle Marche, Italy

**12:45-13:45 Lunch**

**13:45-14:45 Poster session 3 (PW 1-63)**

**14:45-16:15 Session II: Integrated Approaches and New Products to Reduce Food Waste**

*Moderators: Pervin Kinay, Giuseppe Lima*

### Invited lecture

**L16 14:45-15:15 Avocado fruit quality management during the postharvest supply chain**

Lise Korsten, University of Pretoria, South Africa

### Oral communications

**O37 15:15-15:30 Combined treatments based on biocontrol yeasts and agrochemicals or GRAS compounds to control postharvest decays of different fruit**

Giuseppe Lima, Università degli Studi del Molise, Italy

**O38 15:30-15:45 The flooder, an alternative Imazalil application method for postharvest citrus green mould control**

Arno Erasmus, Citrus Research International, Nelspruit, South Africa

**O39 15:45-16:00 BTH induced resistance against postharvest diseases in muskmelon fruit and its mechanisms of action**

Bi Yang, Gansu Agricultural University, China

**16:00-16:30 Coffee break**

**16:30-17:30 Session II continued**

*Moderators: Neus Teixidò, Shiping Tian*

### Invited lecture

**L17 16:30-17:00 Using platinum group metals to reduce postharvest waste**

Leon A. Terry, Cranfield University, UK

### Oral communications

**O40 17:00-17:15 Control of apple bitter rot and blue mold, and peach brown rot by the *Citrus reticulata* and *C. aurantium* extract-based product, biolasting®**

Marta Almazán, Futureco Bioscience S.A., Spain

**O41 17:15-17:30 Management the whole processes of fresh Egyptian sweet potatoes prepared for export against soft rot**

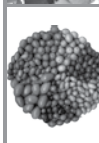
Saneya M. El-Neshawy, Plant Pathology Research Institute, Egypt

**17:30-18:15 ISHS Business Meeting**

**18:15 Closing Ceremony**

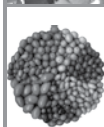
**20:30 Gala dinner (Sala Zonno, Bari)**

|            |   |           |
|------------|---|-----------|
| <b>L1</b>  | <b>NEXT GENERATION SEQUENCING FOR NEXT GENERATION CROPS</b><br>Massimo Delledonne, Italy  | <b>26</b> |
| <b>L2</b>  | <b>SCIENCE AND SOCIAL MEDIA: HOW TO AVOID FEEDING THE TROLL AND SAVE YOUR TIME</b><br>Lorenzo Mannella, Italy   | <b>27</b> |
| <b>L3</b>  | <b>ROLE OF EFFECTOR PROTEINS IN PATHOGENICITY OF POSTHARVEST PATHOGENS</b><br>Samir Droby*, Elena Levin, Dila Beno, Michael Wisniewski, John Norelli, Ana-Rosa Ballester, Luis Gonzalez-Candelas, Israel                      | <b>28</b> |
| <b>L4</b>  | <b>GENOMIC TOOLS FOR DEVELOPING MARKERS FOR POSTHARVEST DISEASE RESISTANCE IN ROSACEAE FRUIT CROPS</b><br>Michael Wisniewski*, John Norelli, Samir Droby, Ana-Rosa Ballester, Elena Levin, USA                                | <b>29</b> |
| <b>L5</b>  | <b>OMICS TECHNOLOGIES TO UNRAVEL PATHOGENICITY MECHANISMS IN <i>PENICILLIUM</i> SPP.</b><br>Luis Gonzalez-Candelas, Spain   | <b>30</b> |
| <b>L6</b>  | <b>FUNCTION OF SMALL GTPASE RHO3 IN REGULATING GROWTH, CONIDIATION AND VIRULENCE OF <i>BOTRYTIS CINEREA</i></b><br>Shiping Tian*, Guozheng Qin, Boqiang Li, Zhanquan Zhang, Bang An, China                                    | <b>31</b> |
| <b>L7</b>  | <b>ECOLOGICAL FITNESS OF YEASTS TO CONTROL POSTHARVEST DISEASES OF FRUITS AND ITS IMPACT ON FORMULATION AND PRACTICAL APPLICATION</b><br>Haissam Jijakli*, Rachid Lahlali, Belgium  | <b>32</b> |
| <b>L8</b>  | <b>UNRAVELING THE MECHANISMS USED BY ANTAGONISTIC YEAST TO CONTROL POSTHARVEST PATHOGENS ON FRUIT</b><br>Davide Spadaro, Italy  | <b>33</b> |
| <b>L9</b>  | <b>ANALYSING THE PLANT MICROBIOME FOR CONTROL OF PATHOGENS</b><br>Gabriele Berg, Austria  | <b>34</b> |
| <b>L10</b> | <b>REGULATED ALTERNARIOL BIOSYNTHESIS BY <i>ALTERNARIA ALTERNATA</i> IS IMPORTANT FOR SUCCESSFUL SUBSTRATE COLONIZATION</b><br>Rolf Geisen*, Katharina Buhl, Christoph Weinert, Markus Schmidt-Heydt, Sabine Kulling, Germany | <b>35</b> |
| <b>L11</b> | <b>LIPID SIGNALS IN THE INTERACTION BETWEEN MYCOTOXIGENIC FUNGI AND THEIR HOSTS</b><br>Massimo Reverberi, Italy   | <b>36</b> |
| <b>L12</b> | <b>RECOMMENDATIONS TO CURRENT POSTHARVEST PRACTICES TO PREVENT FRESH FRUIT CONTAMINATION BY <i>LISTERIA MONOCYTOGENES</i></b><br>Dumitru Macarisin*, Peter Evans, Yi Chen, USA  | <b>37</b> |
| <b>L13</b> | <b>EPIDEMIOLOGY OF <i>BOTRYTIS CINEREA</i> AS BASIC KNOWLEDGE OF GRAY MOLD CONTROL ON POSTHARVEST STORAGE</b><br>Juan Pablo Zoffoli, Chile  | <b>38</b> |
| <b>L14</b> | <b>NATURAL COMPOUNDS: AN ALTERNATIVE IN POSTHARVEST DISEASES CONTROL</b><br>Marta Mari*, Fiorella Neri, Italy   | <b>39</b> |
| <b>L15</b> | <b>NEW HEAT TREATMENTS AS ALTERNATIVE MEANS TO CONTROL POSTHARVEST PATHOGENS ON FRUITS</b><br>Josep Usall*, Maria Sisquella, Carla Casals, Neus Teixidó, Rosario Torres, Inmaculada Viñas, Catalonia                          | <b>40</b> |
| <b>L16</b> | <b>AVOCADO FRUIT QUALITY MANAGEMENT DURING THE POSTHARVEST SUPPLY CHAIN</b><br>Lise Korsten, South Africa   | <b>41</b> |
| <b>L17</b> | <b>USING PLATINUM GROUP METALS TO REDUCE POSTHARVEST WASTE</b><br>Leon A.Terry, UK  | <b>42</b> |



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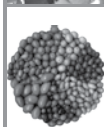






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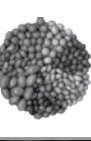
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## NEXT GENERATION SEQUENCING FOR NEXT GENERATION CROPS

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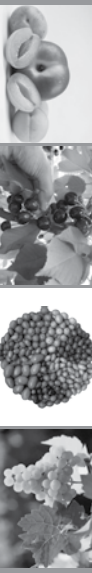
Resistance to storage diseases is much more difficult to obtain than other forms of resistance, because the organisms causing preharvest and postharvest disease may be quite different and the susceptibility to infection may occur only after the ripening process. The process of obtaining resistance is however the same: one must identify the disease and the organisms responsible for it, detect resistance in cultivated plants or wild species, breed resistance into the crop lines and then select the plants with superior resistance. Nowadays, the very long task of identifying desirable characteristics (genes or alleles) and to transfer these traits into new varieties is facilitated by the the availability of very powerful genomic tools. Now Generation Sequencing (NGS) technologies are allowing the mass sequencing of genomes and transcriptomes, which is producing a vast array of genomic information. Genomic approaches like TILLING and EcoTILLING make possible to screen mutant and germplasm collections for allelic variants in target genes, and re-sequencing of genomes is very useful for the genome-wide discovery of markers like SSRs and SNPs and for the construction of high density genetic maps. However, re-sequencing does not allow the characterization of genes not shared with the reference genome that may be involved in the trait of interest. A significant improvement in sequencing technology is therefore desirable in order to allow high quality de-novo genome assembly at a reasonable cost. During my talk, I'll describe how NGS technologies progressed during the last few years, and were they are evolving. The Next Generation Sequencing technologies are almost ready, and we must be prepared for it.

## SCIENCE AND SOCIAL MEDIA: HOW TO AVOID FEEDING THE TROLL AND SAVE YOUR TIME

**Lorenzo Mannella**

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Scientists are committed to face many issues in post-harvest physiology and food safety, thus we wonder how they have ended up in dealing with internet trolls too. Being a troll a person who overruns social media and websites' comments section with taunting and offensive content, the scientific community has quickly become a typical target of trolling. On the other hand, social media have turned into a thriving environment suitable for scientist-to-scientist and scientist-to-public communication. Facebook, Twitter and Google+ gather hundreds of millions of users worldwide, including scientists and science students, while ResearchGate, LinkedIn and other online services have evolved into professional tools for researchers. Moreover, social media can also help scientists debunk fake news, disseminate clear scientific knowledge and support debates over key issues of public interest (e.g. GMOs, organic agriculture and food safety). Indeed, public discussion on social media is no more a "one-way" interaction. A social media user can freely interact with public profiles of research institutions and scientists, urging them to build a solid online presence to communicate science. Therefore, researchers should pay great attention to the management of social media, although it is a time-consuming task. Eventually, how are scientists going to cope with social media and benefit from it?





## ROLE OF EFFECTOR PROTEINS IN PATHOGENICITY OF POSTHARVEST PATHOGENS

Samir Droby<sup>1\*</sup>, Elena Levin<sup>1</sup>, Dlila Beno<sup>1</sup>, Michael Wisniewski<sup>2</sup>, John Norelli<sup>2</sup>, Ana-Rosa Ballester<sup>3</sup>, Luis Gonzalez-Candelas<sup>3</sup>

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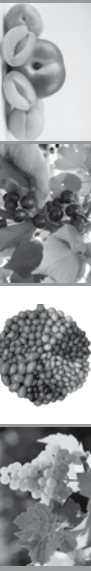
Fruits have evolved an array of defense mechanisms that inhibit or restrict infection by postharvest pathogens. Concomitantly, pathogens have also evolved a variety of mechanisms to manipulate fruit defense mechanisms and establish infections. In this presentation, a general overview of the role of effector proteins in pathogenicity of plant fungal pathogens will be presented. Specific focus will be placed on two major postharvest pathogens, *Penicillium expansum* and *P. digitatum*, the causal agents of green and blue mold on apple and citrus fruit, respectively. Research has demonstrated that in the citrus-*P. digitatum* interaction, the pathogen is capable of actively suppressing fruit defense response through modulating the oxidative burst that occurs in host cells. *P. digitatum* produces high amounts of citric acid during of the decay process that potentially inhibits H<sub>2</sub>O<sub>2</sub> production in fruit tissue, thus enhancing pathogenicity. Additionally, secreted proteins extracted from culture filtrate of the pathogen were demonstrated to be capable of manipulating host defenses and render citrus fruit susceptible to other non-compatible pathogens, such as *P. expansum*. In the apple-*P. expansum* interaction, the pathogen deploys a wide array of pathogenicity factors in order to overcome or inhibit fruit defense response. In our current research, we have documented the secretion of effector proteins during the initial stages of spore germination and infection. Bioinformatic analysis indicates that there are twelve genes containing a conserved LysM domain in the genome of *P. expansum*. Eight of these genes are predicted to be secreted. Genes coding for three of the secreted proteins were found to be actively transcribed during apple infection. Expression levels of the three putative effectors: PELysM4, PELysM11, and PELysM13 were examined during apple infection and we also determined the effect of deleting these genes on the pathogenicity of *P. expansum*. The role of two NLP effectors on the pathogenicity of *P. expansum* was also studied. The role of these effectors in pathogenicity will be discussed.

## GENOMIC TOOLS FOR DEVELOPING MARKERS FOR POSTHARVEST DISEASE RESISTANCE IN ROSACEAE FRUIT CROPS

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A wealth of new plant genomic information and molecular tools have been developed over the past ten years and now the challenge is to learn how to apply this information to address critical production problems, such as disease resistance and abiotic stress tolerance. *Malus sieversii*, an apple species native to Kazakhstan and a progenitor of the modern domesticated apple (*Malus x domestica*) is considered a reservoir of genetic diversity for many economically important traits. Earlier research has demonstrated that some genotypes of *M. sieversii* are highly resistant to postharvest pathogens such as *Penicillium expansum*. One such genotype, PI613981 was crossed with 'Royal Gala' to create a mapping population, GMAL4593. In addition *P. expansum* resistance, this population also segregates for fire blight and apple scab resistance, water use efficiency, and codling moth and oblique banded leaf roller resistance. We have spent four years creating a genetic framework map for this population and phenotyping the harvested fruit for *P. expansum* resistance from 171 individuals. This has led to the identification of a QTL for blue mold resistance on LG 10. The identification of such markers is a first step in providing new markers for use in marker-assisted-breeding. We are currently validating these markers and transferring blue mold resistance to a higher quality genetic background (known cultivars) using a transgenic, early-flowering apple system. Along with identifying a QTL, transcriptomic analyses have been conducted in an attempt to identify genes specifically associated with a resistance response and potential gene-specific markers. These studies were also conducted to obtain a more complete understanding of the host-pathogen interaction in resistant and susceptible genotypes in regards to their response to *P. expansum*. In one experiment, NGS data using Illumina sequencing was used to compare the transcriptome of a single resistant and susceptible individual genotype in response to wounding and wounding plus inoculation. Samples were taken at time 0 and 24h. Differential expression, gene ontology (GO), and pathway analysis (KEGG) were conducted using CLC Genomics Workbench software and free online tools, while Qlucore was used for PCA analysis. Another set of samples consisted of five pooled resistant and five pooled susceptible genotypes. Additional, time-course transcriptomic studies have been conducted on the parents of the GMAL4593 population. Results of these analyses will be presented.



## OMICS TECHNOLOGIES TO UNRAVEL PATHOGENICITY MECHANISMS IN *PENICILLIUM* SPP.

**Luis González-Candelas**

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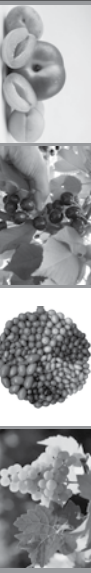
The genus *Penicillium* comprises many species of great impact both in human health and food industry that are able to produce a wide array of secondary metabolites with either beneficial or detrimental effects to humans. Three *Penicillium* species are amongst the most important postharvest pathogens of pome (*P. expansum*) and citrus (*P. digitatum* and *P. italicum*) fruits. We have sequenced the genomes of these three species. Comparative genomic analysis revealed that *P. expansum* has the greatest potential for secondary metabolite production. In fact, *P. expansum* is well known for its capability to produce the mycotoxins patulin and citrinin, whereas the other two species do not produce the mycotoxins. We have investigated the role of patulin and citrinin in *P. expansum*'s pathogenicity through the generation of gene knockout mutants that no longer produce either patulin or citrinin. None of the deletion mutants is affected in pathogenicity towards apple fruits, at least under controlled laboratory conditions. These results raise the question of the role of these two mycotoxins in the virulence of *P. expansum*. Interestingly, a time-course genome wide analysis of gene expression of *P. expansum* genes during infection of apple fruit revealed the induction of another putative secondary metabolite cluster, which seems to have been acquired by horizontal gene transfer. The transcriptomic analysis also revealed the induction of different gene families that can be relevant for virulence, including genes coding for specific plant cell wall degrading enzymes (CWDE), proteases, putative effectors or enzymes involved in redox metabolism. Interestingly, some of these gene families are also induced in *P. digitatum* during the infection of citrus fruit, as is the case for proteases and CWDE. The role in virulence of some of these genes has also been addressed in *P. digitatum* by generating gene knockout mutants.

## FUNCTION OF SMALL GTPASE RHO3 IN REGULATING GROWTH, CONIDIATION AND VIRULENCE OF *BOTRYTIS CINEREA*

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*Botrytis cinerea* Pers. Fr. is an important plant pathogen attacking over 200 crop species worldwide and causing serious decay in mature or senescent tissues of dicotyledonous hosts. There is an extensive ROS generation in *B. cinerea*, which could induce a significant oxidative burst and even hypersensitive response (HR) in its host plant. Small GTPase of the Rho subfamily play important role in regulating the ROS generation by NADPH oxidases in animal, plant, and fungi. In this study, we mainly investigated the biological functions of Rho3 in *Botrytis cinerea*, and found that deletion of the *rho3* from *B. cinerea* significantly suppressed vegetative growth and conidiation, reduced appressorium formation and decreased virulence. Microscopy analysis revealed that the distance between septa was increased in the  $\Delta\rho3$  mutant. In addition, our results suggested that mitochondria may be the main sources of intracellular reactive oxygen species (ROS) in *B. cinerea* based on dual staining with 2', 7'-dichlorodihydrofluorescein diacetate and MitoTracker orange. The  $\Delta\rho3$  mutant showed less accumulation of ROS in the hyphae tips compared to the WT strain of *B. cinerea*. Our findings provide the novel evidence to ascertain the function of small GTPase Rho3 in regulating growth, conidiation and virulence in *B. cinerea*.



## ECOLOGICAL FITNESS OF YEAST'S TO CONTROL POSTHARVEST DISEASES OF FRUITS AND ITS IMPACT ON FORMULATION AND PRACTICAL APPLICATION

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Ecological studies are focused on the influence of environmental parameters on the growth and biocontrol properties of a Biocontrol Agent (BCA). For a post-harvest application, the ecological studies will evaluate if the BCA is well adapted to the existing storage conditions. Moreover, the comparison with pathogen's niche will allow the selection of the most appropriate storage condition to control the pathogen, favoring BCA growth and limiting pathogen's growth. For a pre-harvest application, ecological studies will highlight the adverse environmental parameters hampering the establishment and the growth of the BCA. This knowledge will allow the development of a formulation limiting this negative influence. We have studied *in vitro* and *in vivo* the influence of UV radiation, relative humidity (or water activity *in vitro*) and temperature on the growth of *P. anomala* strain K and *C. oleophila* strain O. Both strains are two antagonistic yeast's previously selected for their high and reliable efficacy after their postharvest application on apples against *Penicillium expansum* and *Botrytis cinerea*. Results of the sensitivity of antagonistic strains to some conditions (UV exposure and low RH) will be presented as well as the development of appropriate formulation to protect the strains against such detrimental conditions. Furthermore, *in vitro* and *in vivo* studies were also undertaken to develop models based on Box-Behnken matrix predicting the combined effects of relative humidity, temperature, and initial applied concentration for both strains. Our model is capable of predicting the yeast population densities on the apple surface 48 h after field spraying of BCA and might be useful to determine the number of treatments to obtain efficient control of pathogens. Finally efficacy of Nexy (registered product based on *C. oleophila* strain O) tested in practical conditions against *P. expansum* and *B. cinerea* on apples and pears will be discussed.

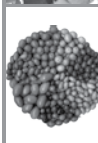


## UNRAVELING THE MECHANISMS USED BY ANTAGONISTIC YEAST TO CONTROL POSTHARVEST PATHOGENS ON FRUIT

**Davide Spadaro**

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Biological control using microbial antagonists is one of the most promising alternatives for reducing fungicide use during the postharvest life of fruit. A good understanding of the mode of action of biocontrol agents (BCAs) towards plant pathogens is essential to develop appropriate selection strategies, production, formulation and methods of application, and to facilitate registration procedures. Several possible antagonistic mechanisms have been suggested to operate against post-harvest rots on fruit including antibiosis, parasitism or direct interaction with the pathogen, production of lytic enzymes, induced resistance and competition for limiting nutrients and space. Competition for nutrients and space is considered to be a primary mode of action against postharvest fungal pathogens. In particular, competition for iron is believed to play a significant role in biocontrol interactions. Various hydrolases, including chitinase, beta-1,3-glucanase and protease, are released by yeast BCAs against postharvest pathogens. Microbial biocontrol agents interact with wounded tissue and they can induce various biochemical and molecular changes in fruit tissues. The mechanisms of some strains of *Metschnikowia pulcherrima*, *M. fructicola*, *Aureobasidium pullulans* and *Pichia guilliermondii* were recently elucidated in the control postharvest diseases of fruits. Information on the mechanisms of action for most of the antagonists investigated is still incomplete, because of the difficulties encountered during the study of the complex interactions between fruit host, pathogen, antagonist and others microorganisms present in the site of interaction. Advanced microbiological, microscopic, biochemical and molecular techniques and technologies are currently available and can be utilized effectively to improve our knowledge about the mechanisms of action of microbial antagonists. Moreover, the availability of efficient omics technologies, along with bioinformatics, has provided new tools to obtain deeper and more accurate insights about the biocontrol interactions.



## ANALYSING THE PLANT MICROBIOME FOR CONTROL OF PATHOGENS

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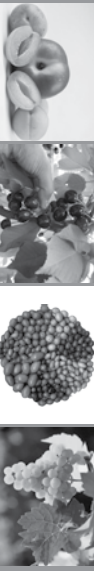
The importance of microbial inhabitants for plant growth and health has been recognized already 100 years ago. Since that time, much has been learned about microorganisms and their close symbiotic relationship with plants (Berg *et al.* 2015, e-book: <http://journal.frontiersin.org/ResearchTopic/15431>). Comparable to humans and other eukaryotic hosts, plants also may be realized as meta-organism that harbors a “second genome”. Plant-associated microbes can help plants fend off disease, stimulate growth, occupy space that would otherwise be taken up by pathogens, promote stress resistance, and influence crop yield and quality. Therefore, the plant microbiome is a key determinant of plant health and productivity. Plant microbiome discoveries could fuel progress in sustainable agriculture, such as the development of microbial inoculants. Although we recognize a growing market for these bio-products, they still have their problems, e.g., short shelf-life, inconsistent effects under field conditions, and risk predictions. The application of “omics”-technologies has allowed for an enormous progression in the development of so-called next-generation bio-products (Berg *et al.*, 2013, *Agronomy* 3: 648-656). On the other hand, plant metagenomes can be used to detect novel substances to control pathogens. Here, microorganism-derived volatiles are an interesting source especially for postharvest diseases. Advances in these aspects could open new perspectives for sustainable agriculture.

## REGULATED ALTERNARIOL BIOSYNTHESIS BY *ALTERNARIA ALTERNATA* IS IMPORTANT FOR SUCCESSFUL SUBSTRATE COLONIZATION

**Rolf Geisen\*, Katharina Buhl, Christoph Weinert, Markus Schmidt-Heydt, Sabine Kulling**

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During the colonization process fungi have to interact with the plant tissue. Several plant pathogenic fungi are able to produce mycotoxins. For several of these mycotoxins, like for example the trichothecenes or patulin, it has been shown that they can act as pathogenicity factors supporting the colonization of the respective plant tissue by the fungus. This activity was also demonstrated for alternariol biosynthesis in *A. alternata*. Mutant strains of *A. alternata* not able to produce alternariol had a greatly reduced capacity to colonize tomatoes compared to the wild type. After addition of external alternariol the capacity was re-established in a concentration dependent manner. Because the alternariol biosynthesis by the fungus is important for its colonization, the biosynthesis of alternariol must be carefully controlled to ensure successful colonization. Usually, tomato tissue has a high water activity and a low pH. This means that alternariol biosynthesis must be possible under these conditions. Changes in the osmotic conditions, that mean changes in water activity, are sensed by the HOG signaling cascade pathway, whereas changes in pH are mediated to the transcriptional level by the *pacC* signal transduction system. We could show that both signaling transduction pathways play an important role in ensuring that alternariol is produced at conditions which initially signal optimum substrate conditions. However, after initial colonization, the tomato tissue can defend the colonization by certain inhibitory secondary metabolites. According to our results, chlorogenic acid, a natural component of resistant tomato phenotypes, can inhibit the expression of the alternariol polyketide synthase gene, thereby inhibiting alternariol biosynthesis and further colonization. Moreover, certain other plant secondary metabolites, which occur in tomato tissue, like the polyamines, have a profound, but metabolite specific influence on alternariol biosynthesis. According to these results, a carefully tuned action/reaction between the tomato tissue and the fungus determines if the colonization is successful or not.



## LIPID SIGNALS IN THE INTERACTION BETWEEN MYCOTOXIGENIC FUNGI AND THEIR HOSTS

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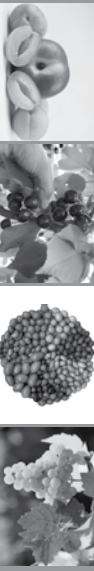
Lipid molecules are gaining momentum as signals exchanged by interacting organisms during pathogenic and/or symbiotic deals. Some class of lipids, especially those over-represented in the interaction interfaces actively drive the fate of plant-microorganism interactions. Notably, lipid compounds may reProgramme the transcriptome of the pathogen as well as of the host, leading to defence responses such as Programmed cell death in plants or mycotoxin synthesis in the pathogen. In relation to this, host-cuticle components such as sphingolipids and oxylipins may contribute to drive host-pathogen interactions. According to available studies, sphingolipids are involved in signalling pathways that promote hypersensitive response and associated Programmed cell death in plants whilst some phyto-oxylipins may affect virulence and the production of secondary metabolites in pathogenic fungi.

## RECOMMENDATIONS TO CURRENT POSTHARVEST PRACTICES TO PREVENT FRESH FRUIT CONTAMINATION BY *LISTERIA MONOCYTOGENES*

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Foodborne listeriosis has been commonly associated with the consumption of ready-to-eat meat and dairy products; however, fresh fruits emerged as a new concern for *Listeria monocytogenes* contamination. In the 2011 listeriosis outbreak associated with cantaloupes, the 2014 recall of stone fruits, and the 2014-2015 listeriosis outbreaks associated with caramel apples, clinical isolates of *L. monocytogenes* were traced back to the fruit processing facilities, indicating that fruits were contaminated during postharvest processing. Moreover, in several incidences during the 2011 cantaloupe outbreak, the onset of listeriosis symptoms took place within 24h after consuming the fruit, suggesting that ingested fruit portions contained an extremely high level of pathogen. The objective of the current study was to identify the risk factors in postharvest practices facilitating fruit colonization by *L. monocytogenes*. Due to the simplicity, effectiveness, and low cost, hydrocooling remains a popular technique to remove heat load from fruits after harvest. Water chlorination (100-150 ppm) is generally considered sufficient to prevent the accumulation of decay microorganisms and human pathogens in circulating chilled water. During peak operating hours; however, the buildup of organic matter may cause dramatic fluctuation in concentration and availability of active chlorine which strongly reduces the sanitation efficiency. The potential of *L. monocytogenes* to internalize into fruits during the process of hydrocooling was investigated. We demonstrated that *L. monocytogenes* can infiltrate into cantaloupe and avocado fruits during hydrocooling. Bacteria infiltration primarily took place through the stem scar and then distributed within the whole fruit, in some instances reaching the calix area. Populations of internalized *L. monocytogenes* colonized edible portions of the fruit reaching eight Log CFU/g within two weeks after hydrocooling at storage temperature of 3°C. These studies will help to define contamination risks associated with commonly used post-harvest handling practices in the production of tree fruits and fruit vegetables.





## EPIDEMIOLOGY OF *BOTRYTIS CINEREA* AS BASIC KNOWLEDGE OF GRAY MOLD CONTROL ON POSTHARVEST STORAGE

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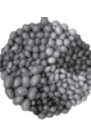
Gray mold caused by *Botrytis cinerea* Pers. Fr., (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) is a worldwide disease affecting a large number of economically important agricultural and horticultural crops. Postharvest expression of the disease is mainly associated to the infections that occur in the orchards and remain quiescent or latent until the suppression is released by the host tissue. *B. cinerea* is a filamentous fungus and necrotrophic pathogen that occurs in orchards as sclerotia, conidia and mycelium; affecting leaves, flowers and fruits. Epidemiology studies of *B. cinerea* done in grapes, have allowed understanding the infection and developing successful strategies for the gray mold control. Different infection pathways have been described for *B. cinerea* on grape berries, namely through stigmata, pedicels, natural opening, wounds or direct penetration of the cuticle. Direct penetration of *B. cinerea* through the berry resulting in the 'slip skin' symptom and it is mainly associated with infection under a wet skin after a rain. Otherwise, berry cuticle is very tolerant to the infection when airborne dry conidia contaminate the berry surface. Symptoms of gray mold during commercial storage of table grape with SO<sub>2</sub> are mainly concentrated at the base of the berry, epidemiology studies demonstrated that gray mold is related with latent infection that occurs by conidia dispersed in the berry pedicel attachment zone. In other fruits such as blueberries, gray mold observed during storage is mainly associated to infection occurring directly through the skin at advanced stage of berry maturity and from conidia contaminating the fruit scar left by the pedicel removal at harvest. In strawberries the role of latent mycelia of *B. cinerea* in leaves is well established affecting the disease during storage. Differences in the epidemiology of *B. cinerea* can change in relation to environmental conditions and pre harvest practices, therefore the management of the gray mold should be adapted considering those changes.

## NATURAL COMPOUNDS: AN ALTERNATIVE IN POSTHARVEST DISEASES CONTROL

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Plant organs produce an amazing diversity of secondary metabolites having a wide range of biological activities. In particular, recent studies have shown as they can play important roles as ‘infochemicals’ in plant-pathogen interactions, being associated with the defence system and function as fungal inhibitors. A large number of studies on the use of natural compounds have demonstrated their role in the control of fruit postharvest diseases. Natural compounds can be grouped in different categories such as flavour compounds, essential oils, plant extracts, jasmonates etc., most of them inhibit directly pathogens; others can enhance fruit defence system. In the review, advantages and disadvantages in the use of natural compounds will be discussed. The high volatility of some natural compounds makes them suitable for fruit biofumigation, despite their use could confer off-odours or off-flavours in fruit, since many natural compounds have strong or unpleasant odours. *In vivo* trials, the most fungicidal activity was found with isothiocyanates, trans-2-exenal, carvacrol, thymol, citral and trans-cinnamaldehyde etc. against the main postharvest fruit pathogens (*Botrytis cinerea*, *Penicillium* spp., *Monilinia* spp. etc.) however different sensitivity to treatments was found among fruit species and cvs. Essential oils are concentrated mixtures of volatile compounds and most of their components have no specific cellular targets avoiding the appearance of resistant pathogen strains. Nevertheless, their composition can be influenced by many factors, such as climatic and seasonal conditions or harvested period as observed in thyme. Plant extracts from *Acaya seyal* and *Withania somnifera* have received attention as non-chemical control means of postharvest diseases for farmers in some African regions that are dependent on locally available disease control measures, however the high variability in composition and concentration of active substances makes difficult to obtain a standard product for possible formulation. More investigations are required to implementing an eco-chemical approach in postharvest disease control.



## NEW HEAT TREATMENTS AS ALTERNATIVE MEANS TO CONTROL POSTHARVEST PATHOGENS ON FRUITS

**Josep Usall<sup>1\*</sup>, Maria Sisqueña<sup>1</sup>, Carla Casals<sup>2</sup>, Neus Teixidó<sup>1</sup>, Rosario Torres<sup>1</sup>, Inmaculada Viñas<sup>2</sup>**

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The growing public concern over the health and environmental hazards associated with high levels of pesticide use, have resulted in a restrictions in their use imposed by legislation and distribution companies. This situation increases the interest in the development of alternative non-chemical control methods. In this presentation we evaluate the use of several heat treatments as hot water dips, curing, radiofrequency and microwave treatments in order to control the main postharvest pathogens on fruits. Most of our studies were focused in citrus and stone fruits, but interesting information is also available in other fruits. Some of the results are already used in commercial conditions as the combination of hot sodium bicarbonate dips in citrus, and others are very news as the radiofrequency or microwave heats treatments, with very interesting results.



## AVOCADO FRUIT QUALITY MANAGEMENT DURING THE POSTHARVEST SUPPLY CHAIN

**Lise Korsten**

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Increased economic and environmental pressures have resulted in a global need for more accurate and safe crop protection measures. Avogreen® (*Bacillus subtilis*) has been South Africa's first pre-harvest biological control product registered against the avocado postharvest diseases anthracnose (*Colletotrichum gloeosporioides*). Although this biological disease control product offers an environmentally friendly alternative to the use of chemical pesticides, few growers have opted for this approach, mainly due to ineffective marketing and uncertainty over the successful performance of *Bacillus subtilis* under different production regions. Alternative approaches to postharvest applications integrating green products have been evaluated successfully in laboratory, semi commercial, and under commercial conditions. Essential oils, chitosan treatments, edible coatings and biocontrol agents were evaluated and the impact of the different treatments on host defence mechanisms assessed. Host-specificity studies also focussed on cross-inoculation of 350 *C. gloeosporioides* isolates on mango, strawberries, peppers, guavas, papayas and citrus. Successful biocontrol can be measured against scientific, social, economic, political, ecological and biological factors. This review assesses the various factors that impact on biocontrol efficacy and commercial viability in an integrated disease control scenario.



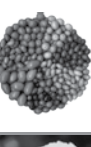
## USING PLATINUM GROUP METALS TO REDUCE POSTHARVEST WASTE

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Postharvest waste has always been a significant global problem and one which has become increasingly recognised as such by policy makers, food producers, retailers and indeed consumers. Ethylene is known to be involved in many postharvest plant/pathogen interactions and often its control can reduce disease incidence. Platinum group metals have in recent years been employed to reduce ethylene in storage environments, yet there remains an opportunity to not only better implement them throughout the fresh produce supply chain, but also to use them as tools to improve understanding on the role of ethylene in both climacteric and non-climacteric systems. Herein, recent examples on banana, avocado and strawberry will be used to demonstrate how a better understanding of ripening/senescence can be used to implement practical technological solutions to reduce postharvest waste.



# ORAL COMMUNICATIONS

## SIMULTANEOUS RNA DEEP-SEQUENCING OF *COLLETOTRICHUM GLOEOSPORIOIDES* ARMS AND TOMATO FRUITS DEFENSE STRATEGIES AT DIFFERENT STAGES OF FRUIT-FUNGAL INTERACTIONS

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*Colletotrichum gloeosporioides* is a widely distributed fungus that causes economically significant postharvest fruit disease called anthracnose. *C. gloeosporioides*-tomato fruit interaction is characterized by three major junctures; appressoria, quiescence and necrotrophic stages. *C. gloeosporioides* breach the unripe fruit cuticle using appressoria whence the fungi adopt a biotrophic-like morphology and remain quiescent until fruit ripening. When fruit ripen the fungus switches to necrotrophic growth and causes the anthracnose disease. The fruit and pathogen transcriptome were characterized simultaneously at each stage. Fungal germination and appressoria formation metabolism prior to penetration was characterized. Concomitantly, the fruit PAMP receptors recognize the fungi and activate a massive fruit defense response involving JA, ethylene, and ABA pathways and cuticle synthesis. The quiescent stage was characterized by the appearance of distinct fungal morphology: dendritic like structures in the fruit cuticle and swollen hyphae in the first epidermal cell. The quiescent fungal transcriptome portrays activation of chromatin remodeling and cell cycle arrest in the fungus while the fruit continued with the massive up-regulation of defense pathways including phenylpropanoids and phytoalexins. When the fruit ripens, necrotrophic growth ensues and the fungus using pacC activated an arsenal of pathogenicity factors including proteases and cell wall degrading enzymes. In contrast to the resistant response shown by the mature green fruit, the ripe fruit activates a susceptible fruit response mediated by salicylic acid pathway culminating in cell death and anthracnose disease. Altogether, simultaneous fruit-fungal transcriptome analysis and the validation of our conclusions by tomato-fruit phytohormones transgenes and fungal mutants we highlights our perception of unfolding the complex synchronized fungal-fruit arms and defense race that occurs during postharvest.

## PROTEOMIC AND OXI-PROTEOMIC RESPONSE OF APPLE TO A COMPATIBLE (*PENICILLIUM EXPANSUM*) AND A NON-HOST (*P. DIGITATUM*) PATHOGEN

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Despite the current use of chemical fungicides, *Penicillium expansum* still is one of the most devastating pathogen of pome fruits. In particular, *P. expansum* enters tissues through wounds causing large economic losses worldwide. To obtain new rational and environmentally friendly control alternatives, it is fundamental to unravel the molecular mechanisms underlying the fruit defence responses and the pathogen virulence factors. In this study, we examine the protein abundance changes (proteome), as well as the protein carbonyls (oxi-proteome) formed by reactive oxygen species (ROS) in ‘Golden Smoothee’ apple in response to wounding, *P. expansum* (compatible) and *P. digitatum* (non-host) pathogen. In addition, we evaluated the correlation between the transcript and protein abundances of six genes involved in wound and pathogen responses. Our study identified 26 proteins whose abundance changed in response to both abiotic and biotic stress. While many of these changes are constitutively observed in response to abiotic and biotic stresses, other proteins, as Mal d 1.03E and EF-Tu, were specifically induced in response to the non-host pathogen. Using our protein carbonyl detection method based on fluorescent Bodipy, we also identified 27 proteins as sensitive ROS targets (oxidized). These ROS target proteins were related to metabolism processes, suggesting a relevant role in apple fruit defense response against abiotic and biotic stresses. ACC oxidase and two glutamine synthetases showed the highest protein oxidation level in response to *P. digitatum* infection. Importantly, only one of the six studied genes showed a significant correlation at the transcript and protein level: Mal d 1.03E. This result supports the idea that studies only based on transcriptional changes may provide a partial view of the fruit response against external stresses. Documenting changes in the apple proteome and oxi-proteome, can provide useful information to better understand how impaired protein functions affect fruit defense mechanisms.

*This research was supported by the national project AGL2011-30519-C03/AGR and by a PhD grant BES-2009-027752 (G. Buron-Moles)*



**BOTRYTIS CINEREA AND GRAPEVINE INFLORESCENCE INTERACTION**

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*Botrytis cinerea*, a cosmopolitan necrotrophic fungus, is the cause of gray mold disease in a wide range of crops including grapevine, where it affects both fruit quality and yield. The pathogen is difficult to manage because of its quiescent, asymptomatic infections that often occur at the flowering stage. It is believed that following early colonization of the flowers, the fungus enters a quiescent phase until ripening onset. Then, it activates from quiescence by further colonizing the ripening berry without visible signs and finally it egresses. The asymptomatic stage of the disease makes the pathogen difficult to control as disease symptoms appear only after colonization has largely progressed. In order to verify the proposed infection model and shed light on the molecular mechanisms of the grapevine/fungus interaction, inflorescences of *Vitis vinifera* (cv. Pinot Noir) were inoculated with a GFP-labelled B05.10 strain at cap falling stage (EL25/26). Samples were taken at 12, 24, 48, 72 and 96 hours post inoculation (hpi) and subjected to plating on selective medium, targeted secondary metabolite analysis (mainly phenols) and RNA sequence analysis. By surface sterilizing infected berries, we observed that most of the spores germinate and then enter quiescence before penetrating into the lower cell layers. The analysis of phenols content showed a significant induction of several stilbenoids, including oligomeric ones and of some phenylpropanoids such as caftaric acid. The RNA sequence analysis showed a larger number of genes (807) modulated at the early time point (24 hpi) than at the later one (194, 96 hpi) suggesting that at this time the pathogen becomes quiescent. The genes were classified according to their annotated functional role and provided further understanding of the biology of the interaction between fungus and plant during the infection.

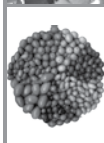
## MAY STRAWBERRY VOLATILE EMISSION INFLUENCE *BOTRYTIS CINEREA* GROWTH?

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Many fungal pathogens that infect fruit in the field cause symptomatic disease during storage and marketing as ripening progresses; the development of some diseases is also favoured by the occurrence of mechanical injuries. As an increase in volatile emission generally occurs during fruit ripening and following wounding, we have assumed that volatile organic compounds (VOCs) could be involved in disease development, and we have tested this hypothesis in strawberry-*Botrytis cinerea* interaction. The infection process of this pathogens starts frequently in flower, but it remains quiescent in unripe tissue, and the presence of fruit wound facilitates grey mould development. Thirty-five strawberry VOCs were tested on *B. cinerea* *in vitro* and fruit volatile emission was analysed in 'Monterey' strawberries harvested at four ripening stages by headspace solid-phase microextraction/gas chromatography-mass spectrometry and proton transfer reaction- time of flight-mass spectrometry. Results showed that key strawberry aroma compounds stimulated *B. cinerea* conidial germination at concentrations naturally detected in ripe strawberry, and some 'green leaf volatiles' emitted from wounded fruit stimulated pathogen' conidial germination or mycelial growth. The results of our work provide advances in understanding the functional role of fruit VOCs and suggest that *B. cinerea* could use some volatiles of strawberry as chemical signals to: i) recognize the ripening of fruit host and resume its growth from the latent phase, and ii) recognize the presence of host damaged tissues and increase its invasive growth. In particular, ethyl butanoate and furaneol could signal strawberry ripening, and the green leaf volatiles *trans*-2-hexenol, *trans* 2-hexenyl acetate and *cis*-3-hexenyl acetate could signal the presence of damaged tissues, that are easier sites for penetration by *B. cinerea*. Future investigations are needed to elucidate molecular, biochemical, and cellular mechanisms involved in fruit VOCs-pathogen interaction.





## DNA-BASED METHODOLOGIES TO DETECT AND QUANTIFY THE POSTHARVEST BIOCONTROL AGENT *PANTOEA AGGLOMERANS* CPA-2 APPLIED ON ORANGES

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*Pantoea agglomerans* strain CPA-2 is an effective biocontrol agent (BCA) for postharvest diseases of citrus and pome fruits. However, to implement their use as control strategy is necessary to study the traceability and survival of BCAs in the environment application, for registration issues. The main objective of this work was to evaluate the persistence and quantify the population of CPA-2 after its postharvest application on orange cv. Valencia Late by molecular techniques. After treatment, the persistence of CPA-2 was evaluated by sampling the packing line and storage chambers, as well as on working clothes by conventional PCR. The results showed that the maximum persistence of CPA-2 was lower than 3 days in surfaces of packing line. Furthermore, CPA-2 did not survive more than 1 day on working clothes, while in the environmental or on different storage chamber surfaces was not detected. In addition, the CPA-2 populations were quantified by quantitative PCR (qPCR) combined with a DNA intercalating reagent, propidium monoazide dye (qPCR-PMA) to quantify the CPA-2 viable cells on fruit surface. The qPCR-PMA method was compared with qPCR and dilution plating method. Results showed that CPA-2 populations quantified by PMA-qPCR were significantly different compared with those obtained by qPCR during the time-course of the assay; however, no significant differences were observed between PMA-qPCR and dilution plating. In conclusion, the persistence of CPA-2 was low at different sampling areas, suggesting that it cannot grow and survive on the sampled surfaces. Furthermore, PMA-qPCR method can be a quick and specific tool to monitor the viable population of CPA-2 on fruit. This methodology gives valuable information on viable population behavior, as well as entry into the VBNC (Viable but non-culturable) state at short times under cold storage conditions and the presence of residual DNA from dead cells.

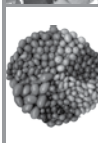
*This research was supported by the national project RTA2009-00053-00-00 and with the scholarship 198363 of CONACyT (L. Soto)*

## EFFECT OF TWO BIOLOGICAL CONTROL AGENTS ON APPLE POSTHARVEST DISEASES IN LONG-TERM STORAGES IN CANADA

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Blue mould caused by *Penicillium expansum* and gray mould caused by *Botrytis cinerea* are the two important postharvest diseases of apples in Canada in long term storages. The objective of this study was to test the efficacy of two antagonistic rhizobacteria isolated from cold Saskatchewan soils for control of postharvest diseases under controlled atmosphere (CA) or cold air storage at two locations in Canada using local apple varieties and storage conditions. Two bacterial antagonists, *Pseudomonas fluorescens* 4-6, *P. fluorescens* 1-112 were tested against the blue mould and gray mould on fruit from two apple cultivars, 'Empire' and 'McIntosh' in Ontario (ON) and 'Gala' and 'Jonagold', in British Columbia (BC). The wounded apples were co-treated with  $1 \times 10^4$  conidia/ml of pathogen of either TBZ-resistant *P. expansum* or *B. cinerea* and/or combination with  $1 \times 10^8$  CFU/ml of *Pseudomonas fluorescens* antagonist 4-6, *P. fluorescens* antagonist 1-112, a 1:1 mixture of the two antagonists ( $1 \times 10^8$ /ml). Positive controls, a bacterial biocontrol Biosave and chemical fungicide fludioxonil (Scholar) were applied at recommended rates. Control treatment had no antagonists or fungicide. Treated fruit were incubated in cold storage at 2°C for up to 6 months, or in controlled atmosphere (CA) storages for up to 5 months ('McIntosh'; 3 °C, 1.5% O<sub>2</sub> and 2.5 % CO<sub>2</sub>) and 6 months ('Empire'; 1.7 °C., 2.5% O<sub>2</sub> and 2.5 % CO<sub>2</sub>) and in a subsequent shelf-life study at 20°C for 7 days. The antagonists 4-6 and 1-112, Bio-Save, and Scholar reduced disease incidence by 71-96% compared to the controls after 2 months in CA storage. Both antagonists were found effective against blue and gray moulds on apples in cold and CA storages in both ON and BC provinces. While neither of the antagonists was as effective as the chemical fungicide Scholar under air storage, they showed promise for comparable control of postharvest decay under CA storage and were as effective as BioSave under air cold storage for up to two months. Further studies, with different apple cultivars, are underway.



## YEASTS AS BIOLOGICAL AGENTS TO CONTROL *BOTRYTIS CINEREA* ON ROSES

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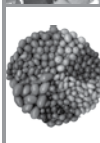
Gray mold, caused by *Botrytis cinerea* is a common disease of greenhouse roses (*Rosa hybrida* L.). However, the infection often cannot be detected at harvest and it develops rapidly during storage and shipment. Infection first appears as water-soaked spots or flecks on the flower petals. As the lesions coalesce, the infected petals turn brown and wither. Many growers dip cut flowers in fungicides solution to prevent development of *B. cinerea*. The use of naturally occurring yeasts has attracted special interest because of their special attributes, nevertheless it has been recognized that the efficacy and consistent performance of the biocontrol agents are affected by different factors. In order to select a biocontrol agent for controlling gray mold, 23 indigenous phyllosphere yeasts were tested accordingly to different criteria, as biocontrol activity and adherence on rose petals, tolerance to UVB radiation, and growth at 25 and 30 °C. Results showed that all the evaluated yeasts significantly reduced gray mold development down to 17 to 64% and severity down to 2.5 to 30%, in comparison with the untreated control (100%). Yeast adherence ranged from 55 to 99%. Thirty percent of the tested yeasts showed less than 45% growth inhibition after UVB radiation exposure. Yeast growth significantly differed among isolates at 25 and 30°C. Accordingly to the above criteria five of the yeast isolates were identified as *Rhodotorula mucilaginosa* (Co3), *Rhodotorula glutinis* (Si6), *Debaryomyces hansenii* (Si29) and *Pichia onychis* (F11 and F14). Taking into account that *R. glutinis* fulfilled other criteria for product development and commercialization, a liquid biopesticide formulation was developed. Further experiments will be conducted to determine the efficacy of this product under commercial rose production.

## **IN VITRO AND IN VIVO SCREENING OF ANTAGONISTIC BACTERIAL STRAINS ISOLATED FROM VINEYARDS TO CONTROL *BOTRYTIS CINEREA* IN GRAPEVINE TISSUES**

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Botrytis bunch rot caused by *Botrytis cinerea* is an important fungal disease of grapevine, with high economic importance in wine grape production and postharvest storage of table grapes. Biological control by antagonistic bacteria is a promising strategy for reducing the common use of synthetic and specific fungicides to control this pathogen. A total of 45 bacterial strains, isolated from grapevine tissues in Bordeaux vineyards were screened *in vitro* for their potential antifungal activity against two major vineyard subpopulations of *B. cinerea*, *i.e.* *transposa* and *vacuma*. These two transposon genotypes differ significantly in virulence on grape berries. The inhibitory effects of the bacterial strains on the mycelium growth of the two subpopulations were tested *in vitro* for detecting the potential production of diffusible metabolites and volatile organic compounds. Furthermore, ten strains among the most effective ones were selected and evaluated *in vivo* on detached grapevine host organs: leaf discs and grape berries. We showed that some of the bacterial strains tested strongly inhibited *B. cinerea* mycelial growth under *in vitro* conditions, and also significantly reduced rot severity *in vivo*. The results suggest that some bacterial strains, naturally associated with the grapevine host, could be possibly used to control postharvest gray mold.



## METABARCODING ANALYSIS OF BENEFICIAL AND DETRIMENTAL FUNGI IN AERIAL PLANT PARTS

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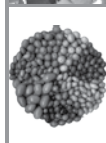
Metabarcoding analysis is a powerful, culture-independent technique that can be used to investigate the composition and relative abundance of community members in environmental samples. In the case of fungal communities, analyses are almost exclusively based on the amplification of the ITS regions of ribosomal DNA (rDNA). A major drawback of using these regions is the difficulty encountered in discriminating between closely-related species that may have DNA sequences which are identical or differ only by a few nucleotides. This weakness may be particularly relevant to the identification of fungal biocontrol agents and plant pathogens, since related species with very similar ITS sequences can exhibit completely different biological characteristics. The phylogenetic analysis of representative reads, along with selected validated reference sequences, may represent a valuable approach for revealing genetic variations and enabling the identification of detected *taxa* with a higher level of accuracy. This approach proved to be very useful in the analysis of fungal communities associated with the olive phyllosphere and carposphere, since the majority of the sequence types (STs) were identified at the species level and the remaining sequences were associated with a restricted number of *taxa*. While it is possible to anticipate the future use of more variable barcode genes, the single copy nature of most alternative markers and difficulties in designing universal primers, may complicate analyses. The lower number of available reference sequences also represents an important issue. Regardless of the barcode gene utilized, primer selection greatly affects the results of the analyses. Universal fungal primers are valuable in ecological studies, since they enable the broad characterization of diversity. Primer sequences that are more specific, for example targeting relevant fungal genera, however, may be more appropriate in specific studies. Metabarcoding analysis of epiphytic and endophytic microbioma of plants is rapidly emerging as powerful tool in studying the complex interactions between pathogens, biocontrol agents, and plant tissue.

## DIVERSITY OF *BOTRYTIS CINEREA* ISOLATES AND VARIABILITY OF MICROFLORA IN NOBLE ROTTED GRAPE BERRIES IN EGER WINE REGION

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The filamentous ascomycete fungus *Botrytis cinerea* (grey rot) has two remarkable characters: on the one hand, it can cause heavy losses in vineyards and in the agriculture in general, but on the other hand, it is the microbe responsible for the noble rot of grape. The unique ‘aszú’ berries and the possibility of noble rot are very rare in the wine growing territories of the world. This economic importance of *B. cinerea* has inspired an extensive research activity into its biology and disease management. Based on these considerations, phenotypic and genetic properties of *B. cinerea* and the microbial diversity of ‘aszú’ berries were studied. More than 150 isolates were collected in the sampling years, which were then characterized morphologically and genetically. The isolates originated from vineyards where only two single grape varieties, Olaszrizling and Turán, have been cultivated. Identification of microbe samples was performed by sequencing of their ITS region, whilst genetic diversity of *B. cinerea* population was determined by the analysis of MSB1 minisatellite sequences located within the intron of the ATP synthase. Microflora of ‘aszú’ berries was dominated by *B. cinerea*. Significant genetic variability was found among the isolates collected in different years, but no difference was found among isolates collected in the same year but from different grape varieties. The minisatellite allelic map revealed striking heterogeneity between the alleles. The high proportion of sclerotium demonstrates the good adaption ability of *B. cinerea* to the various environmental conditions. These results are in contrast with those of earlier experiments and the latest observations in the identification of the speciality of this local population.





## NON-FERMENTING YEAST STRAINS ARE ABLE TO CONTROL VEGETATIVE GROWTH AND SPORULATION OF *ASPERGILLUS CARBONARIUS* AND TO ADSORB OCHRATOXIN A FROM GRAPE JUICE

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Ochratoxin A (OTA) contamination in processed beverages such as wine and grape juice is caused by *Aspergillus* spp. grape infection. In order to meet the Islamic dietary laws concerning the absence of alcohol in *halal* beverages, the biocontrol potential against the pathogenic fungus and OTA-producer *A. carbonarius* of two non-fermenting (*Cyberlindnera jadinii* 273 and *Candida friedrichii* 778) and two low-fermenting (*Candida intermedia* 235 and *Lachancea thermotolerans* 751) yeast strains was tested. The two low-fermenting strains showed a significant antagonistic behaviour against *A. carbonarius* both on grape berries and in *in vitro* experiments, while the filtrate and autoclaved filtrate culture broth of the yeast strains had no significant effect on pathogen growth. Volatile organic compounds (VOCs) produced by all four yeast inhibited pathogen sporulation *in vitro* and VOCs produced by strain 778 also reduced significantly *A. carbonarius* vegetative growth. The ability of the four yeast strains to remove OTA from grape juice was also tested: three of them (235, 751, and 778) were able to efficiently adsorb artificially spiked OTA from grape juice. Autoclaving treatment improved OTA adsorption capacity by all the four tested strains.

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## IDENTIFICATION AND MYCOTOXIGENIC CAPACITY OF FUNGI ASSOCIATED WITH PRE AND POSTHARVEST FRUIT ROTS OF POMEGRANATES

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Pomegranate fruit rot, is considered worldwide an important burden for the industry, affecting the produce both quantitatively and qualitatively. Thus, during 2013, 280 fungal isolates were collected from orchards in GR and CY, while additional 153 isolates were collected from cold-storage in GR. Molecular identification revealed that preharvest fruit rots were caused predominately by species of the genera *Aspergillus* (*A. niger* and *A. tubingensis*) and *Alternaria* (*A. alternata*, *A. tenuissima*, and *A. arborescens*). While, postharvest rots were caused mainly by *Botrytis* spp., followed by *Pilidiella granati* and *Alternaria* spp. Considering, that a significant portion of the causal agents are known for their mycotoxigenic potential in other crops, their mycotoxin potential was examined. Alternariol (AOH), alternariol methyl-ether (AME) and tentoxin (TEN) production was estimated among *Alternaria* isolates, while ochratoxin A (OTA) and fumonisin B<sub>2</sub> (FB<sub>2</sub>) production was assessed within the black aspergilli. Overall in both countries, 89% of the *Alternaria* isolates produced AOH and AME *in vitro*, while TEN was produced only by 43.9%. *In vivo* production of AOH and AME was restricted to the 54.2 % and 31.6 % of the GR and CY isolates, respectively, while none of the isolates produced TEN *in vivo*. Among black aspergilli 21.7% of the GR and 17.8% of the CY isolates produced OTA *in vitro*, while *in vivo* OTA was detected in approximately 8.8% of the isolates from both countries. FB<sub>2</sub> was present *in vitro* in 42.0 % of the GR and 22.2% of the CY isolates, while *in vivo* the production was limited to 27.5% and 4.5% of the GR and the CY isolates, respectively. Our data imply that mycotoxigenic *Alternaria* and *Aspergillus* species constitute a significant subset of the fungal population associated with pomegranate fruit rots, but also pose a potential health risk factor for consumers of pomegranate-based products.



## DE NOVO SEQUENCING AND DETECTION OF SECONDARY METABOLITE GENE CLUSTERS OF *PENICILLIUM GRISEOFULVUM*

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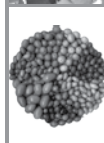
*Penicillium* spp. is one of the most common fungi occurring in a wide range of habitats, with large economic impact on human life. Among 350 recognized species in *Penicillium* genus, *Penicillium griseofulvum* (syn. *P. patulum* Bain.; *P. urticae* Bain.) is worldwide distributed, and has been associated with blue mould decay in storage apple fruits. *P. griseofulvum* is known to produce a wide array of important secondary metabolites, including patulin, penicillin, and griseofulvin. To gain insight into secondary metabolite synthesis in *P. griseofulvum* and assess its potential in terms of biotechnological applications and threats for food safety, we have sequenced for the first time the genome of the strain PG3 which has been isolated from rotted apples harvested in Italy in 2013. Sequence analysis of PG3 showed that the estimated size of the genome is about 29.3 Mb, and gene annotation revealed that 9,631 proteins were encoded in the genome. Genome-wide analysis of *P. griseofulvum* PG3 genes revealed three putative gene clusters for penicillin, griseofulvin and patulin biosynthesis. The patulin gene cluster was identified for the first time in *P. griseofulvum*, and contains 15 genes gathered together. Gene expression analysis and patulin production by PG3 were detected *in vitro* and on apple, confirming that PG3 is a high patulin producer strain and it has developed different mechanisms for regulating patulin production compared to *P. expansum*. In addition to the patulin and penicillin gene clusters, the partial griseofulvin gene cluster was identified compared to the gene cluster described in *P. aethiopicum*. The chemical analysis and the expression of three genes from the griseofulvin cluster confirm griseofulvin production by PG3 and revealed the exact griseofulvin gene cluster which provides the basis for genetic and biochemical studies of the pathway.

## FACING THE PROBLEM OF FUNGAL AND OCHRATOXIN A CONTAMINATION OF FRESH GRAPE AND RAISINS IN ALGERIA

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Ochratoxin A (OTA) contamination in grapes is related to the presence of black Aspergilli. In this work fresh table grapes and raisin samples collected from different regions in Algeria, were surveyed for the presence of OTA and OTA-producing black Aspergilli. No black Aspergillus species was found in fresh table grapes, whereas in raisins black Aspergilli were found in all the tested varieties, with differences between varieties and sampling regions. Generally, *Aspergillus carbonarius* was the most frequently isolated species among black Aspergilli. Furthermore, the 85% of *A. carbonarius* isolates and 75% of *A. niger* isolates were OTA producers. We investigated the effect of ozone (O<sub>3</sub>) treatment at 0.3 ppm on conidia germinability during a period from 12 hours to 14 days, through optical microscope. O<sub>3</sub> significantly controls conidia germinability of both OTA producer and non-producer isolates. These results underline the need to establish a limit for OTA contamination in grapes in Algeria, and highlight O<sub>3</sub> potential to control fungal contaminants, especially those responsible for OTA production.



## POPULATION STRUCTURE AND FUNGICIDE RESISTANCE PROFILE OF *BOTRYTIS* SPP. ASSOCIATED WITH STEM END ROT OF POMEGRANATE FRUIT IN CALIFORNIA AND GREECE

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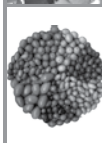
Stem end rot is the main postharvest disease of pomegranate fruit worldwide and is considered to be the outcome of latent infections. In total 121 and 96 *Botrytis* spp. isolates originating from decayed fruit were collected in 2013 from California and Greece, respectively, and used to investigate the disease etiology and the fungal population structure. Identification of causal agents showed that, in both countries, stem end rot is caused by a complex of *Botrytis* spp. In California 52, 34.7 and 13.6% of the isolates were identified as *B. cinerea*, *B. pseudocinerea* and *Botrytis* group S, respectively, while in Greece the same 3 pathogens were identified at frequencies of 44.7, 39.5 and 15.6%, respectively. *B. pseudocinerea* is reported for first time as an agent of stem end rot of pomegranates. The population structure was investigated using as marker the presence of the two transposable elements (TEs) Boty and Flipper. Results showed that Boty and transposa isolates (both TEs present) were predominant in the *B. cinerea* subpopulations in Greece and California, respectively, while Vacuma (both TEs absent) and Boty isolates were found to be predominant in the *B. pseudocinerea* subpopulations in Greece and California, respectively. Measurements of fungicide sensitivity revealed the complete absence of fungicide resistance in any of the Greek isolates, while in the Californian subpopulations high frequencies of fungicide resistance was observed in *B. cinerea* isolates but not in *B. pseudocinerea* and *Botrytis* group S. The higher frequencies of resistance were observed for QoIs, SDHIs and benzimidazoles with values of 61.9, 57.1 and 20.6%, respectively, while frequencies of resistance to anilinopyrimidines and hydroxylanilides, were lower. Measurements of susceptibility in the 4 main varieties cultivated in Greece ("Acco", "Kolindrou", "PG 116-117" and "Wonderful") revealed that fruit of "Kolindrou" was the most susceptible in isolates of all the 3 *Botrytis* spp., while fruit of "Wonderful" was the most resistant.

## BITTER ROT OF APPLES CAUSED BY *COLLETOTRICHUM ACUTATUM*, A PREDICTIVE MODEL FOR INFECTION AND INOCULUM RELEASE

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*Colletotrichum acutatum* causes the bitter rot apple disease in New Zealand that expresses both in the orchard and postharvest resulting in significant fruit loss. Three years of study of inoculum release and infection of apples in the laboratory and orchards has resulted in the derivation of a simple predictive model based on temperature and rainfall. This model has been validated using industry data for one apple growing region in New Zealand. In addition a series of spray trial investigating the timing of application were conducted. In combination with the results of these spray trials, use of the model has improved the control of this disease so that it is no longer a problem for New Zealand growers.



## IDENTIFICATION AND CHARACTERIZATION OF FUNGI CAUSING BULL'S EYE ROT ON APPLE IN POLAND

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Bull's eye rot is the most important storage disease of apples, causing up to 30-40% crop yield losses on susceptible cultivars in Poland. Due to the intensive plant material exchange, the knowledge about the species composition of causal agents of the disease needs to be updated. In this study the available multiplex PCR assay (Gariépy et al. 2003, *Mycol. Res.*, 107, 528-536) was modified to increase its specificity and applied for the identification of the fungi both in pure cultures and directly in symptomatic apple fruits. In years 2011-2012, on a total of 550 samples of symptomatic fruits collected from 9 cold storages located in different regions of Poland, three pathogenic species were detected. *Neofabraea alba* was detected as a predominant species causing bull's eye rot of apple, in 96% of 286 (year 2011) and 92% of 264 (year 2012) tested samples. *N. perennans* was found only in 4% and 7% of apple samples in 2011 and 2012, respectively. Just in two apple samples *Cryptosporiopsis kienholzii* was found, while *N. malicorticis* was not detected in any sample tested. Additionally, the new multiplex PCR protocol was applied for the detection of bull's eye rot fungi directly in apparently healthy fruits, proving its utility. Additionally, 26 isolates of *N. alba*, 22 of *N. perennans* and 10 of *C. kienholzii*, derived from diseased apples after cold storage in 2014, were tested in ISSR PCR assay, and their genetic diversity was determined using 5 microsatellite markers. The resulting dendrogram, comprising results of all five ISSR reactions, segregated all strains according to their species affiliation. No relations was found between the observed amplification pattern and the geographical origin of the strain.

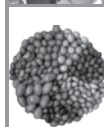


## CITRUS-ASSOCIATED *ALTERNARIA* SPECIES IN THE MEDITERRANEAN AREAS

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*Alternaria* brown spot is one of the most important diseases of tangerines and their hybrids worldwide. Recently, disease outbreaks in Southern Italy, Spain, and Greece refocused the attention on the disease. Twenty representative cultures of *Alternaria* were selected from a collection of more than 150 isolates from leaves and fruits of cvs Fortune, Nova, Valencia, and Tangerine. They were characterized with specimen strains of *A. tenuissima*, *A. alternata*, *A. arborescens*, *A. citri*, *A. toxicogenica*, and *A. limoniasperae* ('small-spored' *Alternaria* species) to determine the etiology of the disease and evaluate the virulence of different isolates/species. Morphological characteristics and sporulation patterns separated most *Alternaria* isolates into three main groups corresponding to *A. alternata*, *A. arborescens*, and *A. tenuissima*, whose the first was the most abundant one. Phylogenetic analyses based on endopolygalacturonase (endoPG) and beta-tubulin genes, two anonymous genomics regions (OPA 1-3 and OPA 2-1), and the internal transcribed spacer (ITS) region produced a clustering of isolates largely confirming morphological results. The OPA 1-3 region was more suitable than the other tested regions for separating closely related 'small-spored' *Alternaria* species and revealed the existence of intra-species molecular variability. Investigated isolates showed different levels of virulence on leaves and fruits but it was not possible to identify a direct correlation between virulence and genetic/morphological groupings of isolates. The toxigenic potential of *Alternaria* strains was investigated. The twenty strains were assayed for the production of tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT) and tentoxin (TEN). The TeA was the most abundant toxin, produced *in vitro* in the range 0.2-20 mg/L. Most of the strains were able to synthesize AOH, AME and ALT, although at a lesser extent. The widespread occurrence of *Alternaria* in citrus and its ability to produce mycotoxins might represent a serious concern for producers and consumers.





## NATURAL INFECTION MOMENTS OF BULL'S EYE ROT IN BELGIAN ORCHARDS

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Species of the genus *Neofabraea* are the causal agent of Bull's eye rot in many countries across the world and are responsible for major economic losses. In Belgium the most susceptible apple variety is Pinova and the most prevalent species causing this disease on apples is *N. alba*. At this moment, little is known about the epidemiology of this pathogen in Belgian orchards. To unravel important moments for natural infestation of apples, field trials on 'Pinova' were performed at the pcfruit research station during several years. In these trials fruits were bagged at specific moments prior to harvest. These trials indicated that fruits are already naturally infested with *N. alba* as early as 12 weeks prior to the first harvest period. Fruit rot evaluations 5 months after storage pointed out that on bagged fruits infection levels of 5 till 10% were noticed. Furthermore, it was observed that infection levels increased as the harvest period approached. Next to this, also unbagged fruits on individual trees were analyzed for the appearance of Bull's eye rot. Out of this study it became clear that there are big differences in infestation between individual trees, but no correlations between specific positions of fruits in a tree were observed. For fruit growers it is important to adapt their management strategy towards storage diseases according to these findings.

## POSTHARVEST STORAGE ROTS OF APPLES AND PEARS IN THE NETHERLANDS

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Postharvest diseases are a major problem in long storage of apples and pears in the Netherlands. Despite intensive preharvest spraying Programmes significant losses occur. Over 150 heavily affected lots of apples (mainly cv. Elstar) and pears (mainly cv. Conference) from packing houses in different regions of the Netherlands were evaluated for decay symptoms and causal organisms. Assessments showed that the most important pathogens are *Neofabraea* spp. (apples and pears) and *Cadophora* spp. (pears). Infection by these two pathogens occurs in the orchard but remains latent until storage. Other pathogens such as *Botrytis* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., and *Cladosporium* spp. were isolated at low frequencies and are considered of minor importance. However, new problems with sooty blotch and lenticel rot of apple were noticed, most likely caused by other, not yet identified, pathogens. Pathogenicity testing and characterization of isolates are on-going. For major pathogens, qPCR assays are available. Samples of substrates (e.g. leaves, cankers, soil) were monthly taken from 10 apple and 10 pear orchards in 2012. Samples were assessed using the qPCR assays for presence and dynamics of pathogen populations. This information on the pathogen life cycles is needed for the development of innovative strategies (e.g. sanitation practices) to prevent postharvest losses. Storage conditions may significantly influence disease development. Recently, the project 'KWALIFRUIT' was launched to identify the optimum harvest stage of pome fruit and optimal storage conditions for maximum fruit quality and storage life and minimal postharvest losses.



## HEART ROT AND SOFT ROT OF POMEGRANATE FRUIT IN SOUTHERN ITALY

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Pomegranate (*Punica granatum* L.) has become a commercial fruit crop in southern Italy during the last years. In 2013, specialized pomegranate cultivation extended over 130 ha. Leading producing regions are Apulia, Sicily, Sardinia, Calabria and Latium. Two types of rot of mature pomegranate fruit have been detected in these regions, heart rot and soft rot. Heart rot, or black rot, was found mostly in fruit of cv. Wonderful and consisted in an internal decay of the arils, sometimes confined to part of the fruit compartments, while the rind was unaffected. Fruit remained firm, but was lighter than healthy one. When it was cut open, a dark grey to black mold emerged. The incidence of the disease varied from 1 to 9 %. The causal agent of this disease was identified as *Alternaria alternata* (Fr.) Keissl on the basis of morphological traits as well as of sequencing of the ITS and TEF1-alpha gene DNA regions. Very probably, *A. alternata* gains entrance into the fruit during bloom and fruit set. Control measures before harvest are being tested. Heart rot represents a serious concern for pomegranate industry due to the difficulty in screening infected fruits on the basis of external symptoms. A soft rot was observed on mature fruit of the cv. Mollar de Elche, after dryness periods followed by intense rains. Species of *Penicillium*, *Alternaria* and *Aspergillus*, as identified on the basis of both morphological traits and sequencing of the ITS,  $\beta$ -tubulin and TEF1-alpha gene DNA regions, were associated with this type of rot. The etiological role of these fungi has not been clearly defined. However, they seem to be secondary invaders colonizing wounds. Before harvesting oviposition punctures of the Mediterranean fly, *Ceratitis capitata* (Wiedemann) are a very frequent cause of fruit wounding.

## INFLUENCE OF FLORAL MORPHOLOGY AND FRUIT DEVELOPMENT ON INTERNAL FRUIT ROT (*FUSARIUM* SPP.) IN BELL PEPPER

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Bell pepper is an important vegetable cash crop grown worldwide including Belgium. In the last decade, internal fruit rot caused primarily by members of the *Fusarium lactis* species complex and to a lesser extent by *Fusarium oxysporum* and *Fusarium proliferatum*, became a major disease in greenhouse-grown bell peppers. In addition, recent reports showed also disease incidence in field-grown bell peppers. After initial infection through the flower, the disease is latent until the green mature stage of the fruit. During the coloring stage, the fungus can start to proliferate on the inside of the fruit, as mycelium on the ovary, and/or cause necrosis. Later, sunken lesions appear on the outside. Nearly all growers are confronted with this problem and average yield losses are estimated at 5% with seasonal peaks up to 20%. Observations by growers suggested differences in susceptibility between pepper varieties. In this study, we report and discuss the differences in floral morphology and fruit development of different bell pepper varieties and their potential correlation with internal fruit rot. To evaluate the susceptibility differences, the floral morphology of three varieties was compared by measuring flower diameter, the dehiscence of the anthers, and the number of petals. Additionally, the longevity of the petals and styles and the position of the flowers were evaluated in relation to internal fruit rot incidence. To account for the influence of fruit development, the number of fruits was also considered in relation to their maturation.



## ROLE OF TWO INOCULATION METHODS IN EXPRESSION OF ANTHRACNOSE RESISTANCE GENES IN CHILI (*CAPSICUM ANNUUM*)

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Pre- and postharvest anthracnose fruit rot is a major disease of chili (*Capsicum* spp.) in tropical production systems. Anthracnose can cause substantial postharvest losses in fruit quantity and quality by creating an entry point for aflatoxins. *Colletotrichum acutatum* is the most prevalent in North East Thailand causing chili anthracnose. Identification of germplasm resistant to anthracnose requires reliable techniques of *C. acutatum* inoculation. The two inoculation methods (microinjection and spray inoculation) on anthracnose resistance were investigated with the cross between resistant (AVPP0207; P<sub>R</sub>) and susceptible (KKU-P31118; P<sub>S</sub>) *Capsicum annuum* L. parents. Fifteen green mature fruits of six populations (P<sub>R</sub>, P<sub>S</sub>, F<sub>1</sub>, F<sub>2</sub>, BC<sub>1</sub>P<sub>S</sub> and BC<sub>1</sub>P<sub>R</sub>) were inoculated by spray and microinjection methods using *C. acutatum* - Ca153 at concentrations of 1x10<sup>7</sup> and 5x10<sup>5</sup> conidia/ml, respectively. Lesion diameters were measured and recorded five days after inoculation (DAI) for the microinjection method and the percent disease severity (PDS) was recorded seven DAI for the spray method. Frequency distribution of the disease score in F<sub>2</sub> and backcross plants suggested that a single dominant gene was responsible for resistance when plants were challenged with the spray method. However, a single recessive gene provided resistance when plants were challenged with the microinjection method. Linkage analysis between these two monogenic resistance genes identified through the two inoculation methods showed both genes inherited independently at 47.45 cM distance. A possible reason for different modes of inheritance will be discussed, as well as the appropriate inoculation method from this study in our anthracnose resistance breeding Programme to reduce postharvest losses of chili.

## TO MELT OR NOT TO MELT: THE SIGNIFICANCE OF POSTHARVEST DISINFECTION FOR PREVENTION OF DECAY OF TABLE GRAPES AFTER STORAGE

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Table grapes are highly susceptible to postharvest losses due to infection by the gray mold fungus *Botrytis cinerea*. During the last few years there were reports from commercial storage of decay which was expressed by melting of the berries and the involvement of an unknown pathogen was considered. 'Scarlotta' is a new variety and its storage properties have not been established so far. Storage of 'Scarlotta' was performed for 6, 10, and 14 weeks with 3 days at shelf life. The grapes were either dipped in 32% ethanol before storage or treated during storage with one or two SO<sub>2</sub> sheets. After 6 weeks of storage the control grapes suffered 60% decay while SO<sub>2</sub> treated grapes suffered mainly melting-like decay without expression of typical gray mold symptoms. Ethanol-treated grapes had similar decay as two SO<sub>2</sub> sheets whereas a combined treatment of ethanol and SO<sub>2</sub> had no decay. The results of storage after 10 and 14 weeks as well as the identity of the decay agent will be reported.



## EFFICACY OF A NEW SO<sub>2</sub> GENERATOR PAD IN MAINTAINING POSTHARVEST QUALITY OF TABLE GRAPES

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Gray mold is the most damaging postharvest disease of table grapes, due to the ability of the causal agent, *Botrytis cinerea* Pers., to adapt to different environmental conditions. A success factor in long-term storage of table grapes is the application of SO<sub>2</sub> generator pads. Our research aimed at testing the activity of a new SO<sub>2</sub> generator pad (Decco, Italia) on gray mold and quality of table grapes cv. Italia and Red Globe stored for 90 days. The test involved the application of the pad in three different moments: in the field, prior to precooling, and immediately after precooling. In addition, three different bags were used for packaging: perforated and unperforated plastic liners and the new Decco MAP (modified atmosphere packaging) bag. At 30 days of storage, 'Red Globe' packed in the field or just before precooling with SO<sub>2</sub> generator, showed no infection by *B. cinerea*; while 23-33% of clusters were infected when the pad was inserted after precooling, with no significant differences among the various plastic liners used. Clusters cv. Italia showed a similar trend but a lower disease incidence as compared to 'Red Globe', with a maximum of 10% of infection in presence of SO<sub>2</sub>. The highest incidence of rots was recorded at 90 days on cv. Red Globe with increasing values moving from perforated to unperforated plastic liners and the new Decco MAP bag. In absence of SO<sub>2</sub> generators, the infections on both cultivars reached 100%. Clusters of 'Italia' and 'Red Globe' packed with Decco MAP showed the best quality.

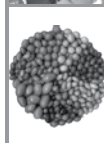


## EFFECT OF POSTHARVEST TREATMENT AND QUARANTINE PROCEDURE ON ORGANIC TABLE GRAPE CV. ITALIA

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Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is regarded as one of the most destructive agricultural pests worldwide causing severe effects on trade to sensitive local and international markets. The establishment of a quarantine protocol for organic table grapes is essential to overcome phytosanitary barriers and to have open access for organic table grapes to foreign markets. An experiment to evaluate the mortality assessment of *Ceratitis capitata* (MFF) was carried out in 2011 in Apulia region. Application of cold treatment at  $1 \pm 0.5^\circ\text{C}$  for 11 days was performed as a quarantine treatment against MFF. However, during cold storage the grape quality might decrease due to the development of *Botrytis cinerea*. Organic table grapes are more vulnerable to fungi infestation as it is not permitted to treat organic commodities with fungicides. Quality assessment of cv. Italia table grapes treated with  $\text{CO}_2$  (30% for 48 hours) or continuous  $\text{O}_3$  (0.8-1.2 ppm) for the control of the development of grey mould was carried out in a cold room ( $0 \pm 1^\circ\text{C}$  and 90-95% RH) for 27 days and at  $12^\circ\text{C}$  for 2 more days to evaluate shelf life. The evaluation was done weekly by chemical, physical, and sensory analyses. Mortality assessment of MFF showed that cold treatment is effective as a quarantine method in postharvest. The most tolerant insect stage resulted first instar larvae requiring more than 11 days at  $1 \pm 0.5^\circ\text{C}$ , followed by second and third instar larvae. Most susceptible stage was pupae, which requires less than 7 days to achieve 99% of mortality. Quality assessment of table grapes showed no differences among treatments, however,  $\text{O}_3$  -treatment significantly reduced micro-flora on the berry skin and  $\text{CO}_2$  increased the methanol, ethanol, and acetaldehyde contents without damaging the quality of grapes. Pre-treatments with  $\text{CO}_2$  and  $\text{O}_3$  prevented the development of *B. cinerea* and preserved the commercial quality up to 28 days of storage plus 2 day of shelf life.





## IMPACT OF VENTILATION AREA OF THE LINER BAG, IN THE PERFORMANCE OF SO<sub>2</sub> GENERATOR PADS IN BOXED TABLE GRAPES

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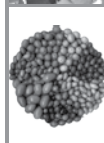
SO<sub>2</sub> generator pads are an important tool to prevent rotting of table grapes, caused by fungal pathogens, mainly *Botrytis cinerea*, during storage and shipping. Sodium metabisulfite salt contained in the pad reacts with water vapor and releases SO<sub>2</sub>. Due to market regulations, the liner bag used in the table grape packaging can have 0.3, 0.9 or 2.7 % ventilated area. Switching to higher ventilated areas has been associated with higher decay. The objective of the study was to determine the dynamic of concentration of SO<sub>2</sub> inside the boxes, during cold storage, and relate it with rotting and bleaching of table grapes. Different experiments were conducted from 2011 to 2013; in general, grapes were packed in boxes with liners of different ventilation areas, silicon hoses were set for weekly measurements of the gas concentration inside the box, then they were cold stored for 35 to 94 days, and after a period of three days at room temperature, simulating shelf life, the percentage of rotting and bleaching was determined. In a first experiment, rotting of Red Globe grapes due to *B. cinerea* averaged 32.7 % and 4.6 % in control boxes (without generator pads) with 0.3 and 0.9 % of ventilation, respectively. Similarly, boxes with 0.9 % ventilation had 76.1 % of rotting compared to a 24.3 % in boxes with 2.7 % ventilation. Differences of grey mold were also observed in boxes packed with generator pads, where 16.8 % rotting was significantly different to a 5.0 % rotting in boxes with 2.7 % and 0.9 % ventilation area, respectively. Bleaching of grapes was affected negatively with increasing ventilation reaching 10.3, 1.9 and 0.5 % in boxes with 0.3, 0.9 and 2.7 % ventilated area, respectively. SO<sub>2</sub> concentration inside the boxes was lower throughout the storage period, as the ventilation area of the liner increased.

## ELECTROLYZED SALT SOLUTION MODE OF ACTION IN CONTROLLING GREEN MOULD OF CITRUS FRUIT

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Electrolysed sodium bicarbonate ( $\text{eNaHCO}_3$ ) using thin film diamond electrodes has been demonstrated to effectively manage postharvest rots of citrus fruit. In the present study, the effect of this treatment on *Penicillium digitatum* growth and/or green mould was investigated. Results indicated that spore germination and germ tube elongation of *P. digitatum* were strongly inhibited (80%) within 15 min of  $\text{eNaHCO}_3$  treatment, as compared to not only untreated control, but also to electrolyzed water (EW) or non-electrolyzed  $\text{NaHCO}_3$ . Moreover, 45 min of electrolysis completely suppressed spore germination. Electrolyzed  $\text{NaHCO}_3$  also triggered the accumulation of reactive oxygen species (ROS), causing an oxidative stress in *P. digitatum* spores, followed by a collapse of mitochondria membrane potential and a decrease in intracellular ATP. Results of assays on citrus fruit showed that  $\text{eNaHCO}_3$  proved to control significantly *P. digitatum* infections when applied in wounds other than but close to the one inoculated with the pathogen. The induction of resistance hypothesis seemed confirmed by the up-regulation of defence-related genes including *chitinase* and *peroxidase* at 6 h, and *phenylalanine ammonia lyase* (PAL) at 12 h post-treatment (hpt). As further confirmation,  $\text{eNaHCO}_3$  proved to increase the activity of related enzymes. Moreover, an increase in  $\beta$ -1,3-glucanase activity was observed at 12 hpt, suggesting an immediate host response against pathogen by limiting tissue colonization. Overall, the defence response seems related to the induction of phenyl propanoid pathway. In conclusion, both the direct inhibition of *P. digitatum* growth and the induction of fruit resistance should be considered an important aspect of the multiple mode of action of  $\text{eNaHCO}_3$  in controlling postharvest citrus diseases.



## POTASSIUM SILICATE: A NEW ORGANIC TOOL FOR THE CONTROL OF CITRUS POSTHARVEST GREEN MOLD

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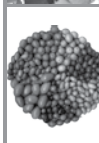
The curative activity of postharvest treatments with potassium silicate (PSi,  $K_2SiO_3$ ) against one of the most economically important citrus postharvest diseases, green mold caused by the fungus *Penicillium digitatum*, was evaluated in laboratory trials. Oranges (*Citrus sinensis* L.) cv. Lanelate were artificially inoculated in rind wounds with the pathogen and 24 h later immersed in aqueous solutions of 90 mM PSi at 20 or 50°C for 60 or 150 s. This PSi concentration had been selected as the most effective in previous tests. Treated fruit were incubated for 7 days at 20°C, at which time disease incidence (% of infected wounds) and severity (lesion diameter) were recorded. The best overall performance was achieved with dips at 20°C for 60 s. These treatments were then applied to oranges cv. Valencia inoculated 24 h before and subsequently stored at 5°C and 90% RH for up to 6 weeks. At the end of the cold storage period, PSi dips significantly reduced the incidence of green mold by 45% with respect to control fruit (dipped in water). Green mold severity at the end of the 6-week cold storage period on control and PSi-treated oranges was 200 and 98 mm, respectively. In conclusion, PSi showed potential as a new reduced-risk chemical treatment for cost-effective control of citrus green mold. It might be of use in integrated disease management Programmes to replace or reduce the usage of polluting conventional postharvest fungicides.

## IN VIVO APPLICATION OF GARLIC EXTRACTS FOR THE MANAGEMENT OF POSTHARVEST DECAY IN APPLES

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Postharvest losses due to decay can be significant in the South African apple industry. The use of fungicides to manage postharvest decay is becoming increasingly restricted, and consequently alternatives need to be researched. In this study, the application of garlic extracts both directly or through volatile exposure were tested *in vivo* for potential to inhibit decay caused by postharvest pathogens *Botrytis cinerea*, *Penicillium expansum* and *Neofabraea alba* on three apple cultivars, Granny Smith, Golden Delicious and Pink Lady. Curative application of the extracts by direct exposure was more effective than a protective application for decay management of *B. cinerea* and *P. expansum* on all three cultivars, whereas direct exposure of fruit that were artificially inoculated with *N. alba* did not result in any inhibition compared to the control treatments. Extract volatiles did not inhibit postharvest decay on any of the apple cultivars, and in some cases, resulted in increased lesion diameters. This study demonstrated that garlic extracts have the potential to reduce postharvest decay caused by *B. cinerea* and *P. expansum*, when applied directly to the fruit.





## UV-C LIGHT TO REDUCE BIOTIC AND ABIOTIC STRESSES OF STORED FRUIT AND VEGETABLES: A BRIEF REVIEW

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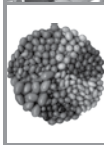
New and expanding trends in food and agriculture for chemical-free techniques have prompted the search for alternative means to control postharvest decay of fruit and vegetables. Among the alternative means, ultraviolet-C light (UV-C, 190-280 nm) is one of the most attractive. Illumination with UV-C light increase the content of health-promoting compounds in several crops, thus improving the sensory and nutritional quality of several commodities. The use of UV-C light at 254 nm, determines a reduction of postharvest biotic and abiotic stresses, when applied at low doses. However, radiation intensity affects the effectiveness of the treatment, as it can maximize the benefits of UV-C on fruit quality, while significantly reducing the treatments time. The reductions of postharvest diseases have largely been attributed to induced resistance effects, related to the production of substances (mainly phenolics) toxic to the pathogens, and incited by an increase in the activity of key synthetic enzymes (*i.e.* phenylalanine ammonia lyase). With appropriate exposure time, UV-C light can cause weak stress responses, thus acting as an abiotic elicitor triggering systemic defense response in several plants species. It is well known that UV-C light reduces the activity of enzymes causing postharvest senescence (*i.e.* lipoxygenase, polyphenol oxidase) and stimulate the biosynthesis of phytoalexins and antifungal compounds in several fruit and vegetables. Enzymes such as chitinases, glucanases, and other pathogenesis-related protein are also induced. Activation of defence related genes by UV-C light has been reported in various commodities, among which the key enzymes in phenylpropanoid/flavonoid pathway are included. Today, the availability of reliable and more sensitive molecular techniques, (*e.g.* Next Generation Sequencing, Microarray, real-time PCR) greatly improve the identification and characterization of UV-C regulated genes and pathways, thus contributing to a better understanding of the induced resistance mechanisms and quality improvement in harvested commodities.

## RIPENING DEGREE INFLUENCES DEVELOPMENT OF POSTHARVEST FUNGAL DECAY ON EUROPEAN PLUM MORE THAN PREHARVEST APPLICATIONS OF CALCIUM AND FUNGICIDES

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Shelf life of plum (*Prunus domestica* L.) is limited by several factors, including development of fungal decay. In either one or two seasons different European plum cultivars were exposed to different combinations of calcium or fungicide applications before harvest or were left unsprayed. On the experimental trees fruits were harvested as commercial practice giving a sample of fruit with a range in maturity acceptable for sale. The yield was divided into two groups, less and more ripened fruit. Samples from each group were stored for 10-14 days at 4°C followed by a simulated shelf life period of 2-3 days at 20°C. Fruit quality was assessed at harvest and after storage. Number of fruit with fungal decay was counted at the end of storage and after simulated shelf life. At harvest more ripen fruit had higher fruit weight, soluble solids content, background and cover colour, and lower firmness in most of the experiments. Fruit from trees sprayed six times with calcium had higher fruit weight in first year, but not in second, was less ripen as measured with colour and firmness on some cultivars, but not on others. Time of fungicide application had no effect on fruit quality at harvest. Differences in fruit quality at harvest were most often similar after storage. Fruit grouped as more mature at harvest developed more fungal decay after simulated shelf life than less mature fruit in five of eight experiments. In one of six experiment calcium applications reduced development of fungal postharvest decay. Fungicide applications had no effect on postharvest fungal decay in either of four experiments. The present results indicate that the ripening degree of plum fruit is more important for development of fungal decay than preharvest applications of calcium or fungicides.





## ALTERNATIVE TECHNOLOGY: USING PLANT VOLATILES TO CONTROL ANTHRACNOSE IN AVOCADOS

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Major postharvest losses are encountered in avocado (*Persea americana*) throughout the supply chain mostly due to anthracnose (*Colletotrichum gloeosporioides*). Owing to the increasing consumer concern regarding food safety, the importing countries have enforced stringent regulations regarding the maximum residue limits in the skin of the fruit. Additionally, due to the development of fungicide resistant strains, postharvest fungicide applications are not considered to be a long-term solution for the fruit industry. Although acidified prochloraz treatment helped to reduce the concentration of active prochloraz, the disposal of a low pH solution remains a problem. Apart from the above mentioned, commercial Avoshine<sup>®</sup> canuba wax coating is used for avocados. “Green-skinned” cultivars may develop surface discolouration if the proper wax formulation and application methods are not employed. Therefore, all these factors have stimulated the search for eco-friendly novel alternative decay control strategies that can be easily implemented in the commercial packing line as a standard practice for all the export cultivars. Essential oils/fruit volatiles and their components are gaining increasing interest due to their volatility, relatively safe status, low risk of developing resistance to decay-causing pathogens, eco-friendly and biodegradable properties. During preventative treatment, freshly harvested avocados ‘Hass’ and ‘Ryan’ from three orchards were fumigated with thyme oil (960  $\mu$ L/10 L) for 24h at 20°C and thereafter, inoculated with 10<sup>6</sup> spores/ml and incubated at 20°C for 5-6 days. The untreated fruit and the commercial prochloraz treatments were included for comparison. The results showed the thyme oil treatment (fumigation) significantly reduced the anthracnose incidence by ~ 65% compared to the untreated control fruit and by 23% in comparison to the prochloraz treatment in all three cultivars. In addition, the fruits subjected to thyme oil treatment were firmer than the prochloraz treated fruits. Thyme oil treatment did not affect the sensory properties; however, ripening patterns under artificial conditions must be investigated.

## INVESTIGATING THE CONTROL OF GREEN MOULD ON SWEET ORANGES SUBJECTED TO STEAM TREATMENT

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Steam treatment of 'Amber' sweet orange (*Citrus sinensis* L.) fruits prior to storage at tropical ambient temperature controlled *Penicillium digitatum*. To investigate the basis of decay control by steam in this cultivar, equal numbers of fruits were treated as follows: wound inoculated with the pathogen and exposed to steam at 50°C; uninoculated but heated by steam; uninoculated and unheated; wounded and steam treated; wounded and unheated. Control fruits were inoculated but not exposed to steam. All fruits were stored at 28°C and 95% relative humidity. All control fruits decayed. Dichloromethane extracts of flavedo tissue from fruits that remained healthy afterwards were fractionated and tested for biological activity. Fractions from all treatments showed inhibition of fungal spore germination, indicating the presence of antifungal compounds in all the fruits. Gas chromatography–mass spectrometry analysis of the active fractions revealed that hydrocarbons (substituted and unsubstituted aliphatic, aromatic and alicyclic), esters and terpenes were some of the components common to all fruits. Some of the compounds are known for antioxidant, antibacterial or antifungal action. However, carveol, an oxidation product of limonene, trans-p-mentha-1(7), 8-dien-2-ol, an oxygenated monoterpene, and 9, 12, 15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z), were present only in the wound inoculated steam treated fruits, suggesting that they were produced in response to infection. Carveol, being antifungal itself, possibly synergized other components of the essential oil increasing antifungal action in addition to the spore kill effect of steam treatment. The results suggest the presence of both preformed and induced antifungal compounds in inoculated fruits.



## NEW TOOLS TO IMPROVE THE SHELF LIFE OF CHESTNUT FRUITS DURING STORAGE

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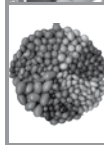
Chestnut (*Castanea sativa* Mill.) is an economically important fruit and timber crop worldwide, including Italy. The quality of chestnut fruits can be affected in pre- and postharvest by insects and molds. Since 2005, chestnut growers in Italy have been suffering increasing yield losses, due to the rotting of the ripe nuts both before and after picking. In 2014 chestnut losses reached more than 50% in some zones of southern and northern Italy. The factors that are influencing the growing incidence of the diseases can be related to climate changing. Abundant rain during winter and springtime are favoring the development of chestnut pathogenic fungi. Moreover, in Italy chestnuts are normally subjected to “curatura”, a practice used with the purpose of killing the larvae of pests (mainly *Curculio elephas* and *Cydia* spp.). This technique consists in submerging the chestnuts in hot water (50°C) for 45 min and then cool them in cold water (15-18°C) for the same time. This procedure, although effective against the pests, may favour the development of molds during postharvest phase. During 2 years of investigation on chestnut postharvest problems in the Campania region (South Italy) we found that from both healthy and rotting chestnut fruits, the most commonly isolated fungus was *Gnomoniopsis castanea*, followed by *Fusarium* spp. and *Aspergillus* spp. It is known that these last two genera include several species of strong mycotoxin producers. The aim of our study was to find possible solutions to prevent chestnut yield losses during postharvest. Therefore, we investigated the possibility to add to the water used for “curatura” some biological products derived from the biocontrol fungus *Trichoderma harzianum* and from plant extracts. We managed to find some compounds and mixtures that incorporated into the treatment can significantly increase the shelf life of chestnuts during postharvest.

## PREHARVEST TREATMENTS WITH ALTERNATIVES TO CONVENTIONAL FUNGICIDES TO CONTROL POSTHARVEST DECAY OF STRAWBERRIES

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Strawberries (*Fragaria×ananassa*) are particularly perishable commodity, especially during postharvest storage, when decay, mainly gray mold and Rhizopus rot, caused by the spoilage fungi *Botrytis cinerea* and *Rhizopus stolonifer*, frequently occur. The aim of the study was to test the effectiveness in the control of postharvest decay of strawberry fruit of field applications of chitosan, laminarin, benzothiadiazole, calcium + organic acids, and extracts of *Abies* spp., *Polygonum* spp., and *Saccharomyces* spp. These treatments were compared with a water control and with a strategy based on the use of conventional fungicides ascyprodinil+fludioxonil, pyrimethanil and fenhexamid. Compounds were sprayed every 5 days on the canopy of strawberry cvs. Alba and Romina, from flowering to the ripening, during 2012 and 2013. After harvest, the strawberries were stored 7 days at  $0.5 \pm 1^\circ\text{C}$ , and then exposed to shelf life, when decay evaluation was carried out. The treatments with compounds alternative to the conventional fungicides significantly reduced postharvest decay of strawberry, mainly gray mold followed by Rhizopus rot, as compared with the water control. Among alternative treatments, chitosan and benzothiadiazole were overall the most effective, while the lowest disease values were observed in both years applying the fungicide strategy. None of the treatments had detrimental effects on strawberry quality parameters, including fruit firmness and color. The preharvest treatment with these alternatives to synthetic fungicides could complement the conventional strategies in the control of postharvest decay of strawberry, especially when the disease pressure is low.



## COMBINED TREATMENTS BASED ON BIOCONTROL YEASTS AND AGROCHEMICALS OR GRAS COMPOUNDS TO CONTROL POSTHARVEST DECAYS OF DIFFERENT FRUIT

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Postharvest losses of fruit and vegetables are caused by different pathogenic fungi and some of them are also responsible for the production of toxic secondary metabolites known as mycotoxins. Currently postharvest decays are essentially managed by application of synthetic fungicides. Because of the side effects of chemicals and the possible risks posed on consumers and on the environment, in the last decades biocontrol agents (BCAs) were selected and proposed as safer alternatives. However, under commercial conditions the control of postharvest pathogens achieved by BCAs was sometimes unsatisfactory and resulted variable over time and/or level of disease pressure. To overcome these constraints and to pave the way to a large-scale implementation of biological control, our research was aimed to improve and stabilize the activity of selected biocontrol yeasts. With this in mind, we report and discuss results of experiments aimed at the optimization of biocontrol activity of selected BCAs and to reduce accumulation of mycotoxins in fruit by combining or alternating these beneficial microorganisms with agrochemicals or GRAS (Generally Recognized as Safe) compounds and/or by including BCAs in a suitable control schedule taking into account host and pathogen biology.

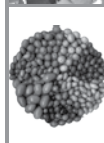
## THE FLOODER, AN ALTERNATIVE IMAZALIL APPLICATION METHOD FOR POSTHARVEST CITRUS GREEN MOULD CONTROL

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The majority of South African packhouses use a dip tank to apply imazalil (IMZ) for the control of green mould (*Penicillium digitatum*). JBT Corporation in California developed an alternative to the dip tank that has been in use for the past decade. The flooder applies fungicide in an aqueous solution by means of a number of weirs that creates a seamless laminar flow that falls onto the fruit over rotating brushes. The flooder has not yet been scientifically assessed. Dip was compared to flooder application; the effect of number of weirs and solution temperature on IMZ residue loading and green mould control was investigated. Similar results for dip and flooder were obtained for fruit treated at solution temperatures 25 and 35°C in terms of residue loading (0.5 – 1.0 µg.g<sup>-1</sup>) and curative control (>90%). In addition, the flooder generally gave better protective control levels compared to the dip application. Higher temperatures tended to give better residue loading (1.0 – 3.0 µg.g<sup>-1</sup> and < 1.0 - < 2.0 µg.g<sup>-1</sup> for 25 – 60°C in 250 and 500 µg.mL<sup>-1</sup>, respectively), with curative control on all treatments >80%. Applications using three to five weirs gave more consistent curative control compared to one and two weirs. Lemon and soft citrus fruit treated at temperatures higher than 45°C developed rind injury. Results show that the flooder is an effective alternative IMZ application method to the dip tank.



## BTH INDUCED RESISTANCE AGAINST POSTHARVEST DISEASES IN MUSKMELON FRUIT AND ITS MECHANISMS OF ACTION

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Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH, Bion®, Actigard®) is known as acibenzolar-S-methyl (ASM), supposedly a structural and functional analogue of salicylic acid, has been acknowledged as an effective activator of systemic acquired resistance during interaction of plants and pathogens. Some studies indicated that BTH induced a broad-spectrum resistance against postharvest diseases in fruits. Our results showed how preharvest application of BTH significantly decreased latent infection caused by *Alternaria alternata* and *Fusarium semitectum*, and postharvest BTH treatment dramatically reduced *Fusarium* rot caused by *F. semitectum* and Pink rot caused by *Trichothecium roseum* in muskmelon fruit. The mechanism of action of BTH involved activation of reactive oxygen species (ROS), phenylpropanoid metabolism, and accumulation of pathogenesis-related proteins (PRs) at biochemical level. Proteomic level results showed that 69 spots changed abundance significantly in muskmelon fruit treated with BTH. Fifty-two spots out of 69 were identified using MALDI-TOF/TOF by blasting against NCBI database. Functional classification revealed that the protein species identified were related to defense and stress responses (23.1%), protein synthesis-destination-storage (23.1%), energy metabolism (13.5%), primary metabolism (9.6%), cell structure (9.6%), secondary metabolism (5.8%), signal transduction (3.8%) and transporters (3.8%). These proteins, involved in the major biological processes, confirmed that BTH could induce resistance against postharvest diseases in muskmelon fruits.

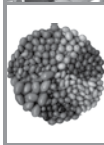


## CONTROL OF APPLE BITTER ROT AND BLUE MOLD, AND PEACH BROWN ROT BY THE *CITRUS RETICULATA* AND *C. AURANTIUM* EXTRACT-BASED PRODUCT, BIOLASTING®

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Reduction of postharvest fruit losses is a major agricultural goal affected by withdrawal of effective chemicals used due to environmental or health concerns. Novel clean solutions are necessary. Activity of BIOLASTING®, a citrus extract based formulation, was tested on *Colletotrichum gloeosporioides* (Bitter Rot) and *Penicillium expansum* (Blue Mold) on apples, and *Monilia laxa* (Brown Rot) on peaches. In controlled laboratory tests (18°C, 80% RH), apples cv. Golden and peaches cv. Amarillo-Calanda were disinfected, wounded, and inoculated (I) with spore solutions of pathogens. Two hours after inoculation, fruits were submerged into different water dilutions of BIOLASTING® (1% to 4%). Lesions diameter was measured at 21, 31 or 26 DAI and in the Brown Rot test, quality parameters were determined (Firmness, pH, Soluble Solid Content). In a second trial, control of Brown Rot on peach cv. Amarillo-Tardío was assessed in a commercial packinghouse during 30 days. The product was applied by immersion and spraying at two doses (1% and 1.5%). A conidial suspension of *Monilia* sp. was injected on wounds just before treatments. Fruits were kept refrigerated (2-5°C) under controlled atmosphere (CO<sub>2</sub>, O<sub>2</sub> and RH). In the tests on apples BIOLASTING® applied at 2% reduced 66.8% the area infected by *C. gloeosporioides*, but was unable to control *P. expansum* at any doses. In peach laboratory tests, BIOLASTING® at 3 and 4% reduced *M. laxa* disease by 72 and 78.4% respectively, and improved all the quality parameters assessed. Under commercial conditions, the BIOLASTING® exhibited more than 90% reduction of rot surface with no significant difference between doses or type of application. The incidence was also reduced with better results (93%) at the 1.5% dose (immersion). Results suggested that postharvest applications of BIOLASTING® might significantly reduce Bitter Rot on Apples and Brown Rot on Peaches.

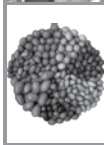


## MANAGEMENT THE WHOLE PROCESSES OF FRESH EGYPTIAN SWEET POTATOES PREPARED FOR EXPORT AGAINST SOFT ROT

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Soft rot caused by *Rhizopus stolonifer* to sweet potatoes is a remarkable phenomenon in Egypt. During handling system in presence of high moisture content, the tuber's tissues are attacked by *R. stolonifer*, which ultimately causes full deterioration of tuber's body. The whole process chain for exporting the native sweet potato cv. Abees to England market (including immediate curing after harvest, trimming, washing, packing, transportation and sea or air shipping) are properly applied by cooperative work with AGRO FOOD CO (LTD). Sterilizing tubers with UV-C treatment (254 nm) at 20 cm height for 1, 2, and 3 h, achieved marked reduction of either the microbial load on tuber surfaces, or the contamination potential in internal atmosphere of cold storage room. Subsequently reduced tuber soft rot after three days of exposure to UV-C to almost 0% infection and maintained export tuber characteristics were observed. By the UV-C light treatment, healing, induction of PAL peroxides and polyphenol oxidase activity were observed in tuber tissues. As the exposure time to UV-C light increased, a higher quantity of phenol content was detected, associated to a slight decrease of sugar content. Quality assessment of tubers through a traceability system of the whole export channel, up to reaching the final destination, was determined including tolerance to major and minor defects due to infection by *R. stolonifer*. The overall impact was a significant reduction in postharvest losses of sweet potatoes and increase of the export capacity.



# *POSTERS*



## INCREASED ANTHOCYANIN AND FLAVONOIDS IN MANGO FRUIT PEEL ARE ASSOCIATED WITH COLD AND PATHOGEN RESISTANCE

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Mango fruit (*Mangifera Indica* L., cv. Shelly) developing at the exterior of canopy and exposed to sunlight acquires a red peel color on the sun-exposed side compared to the green peel fruit that develop within the canopy. Measurements of the red tissue showed a significant increase in total anthocyanin and flavonoids accumulation but not in chlorophyll. The ripening parameters between red and green mango fruit harvested at the same day from the same orchard, including; brix, firmness and titratable acidity were similar at harvest, during cold storage and further shelf life. However, fruit with a red side or that were mostly green showed a varied response to biotic and abiotic stresses. After three weeks of cold-storage at 5°C ‘green fruits’ showed significantly more lipid peroxidation and developed significantly more chilling injury symptoms, such as black spots and pitting, than the ‘red fruits’. Furthermore, ‘red fruits’ were found to be more resistant to a challenge of *Colletotrichum gloeosporioides* fungal inoculation and showed reduction in general decay incidence. Thus, mango fruit with more red color in their peel correlate to anthocyanin and flavonoids accumulation, and showed increased resistance to chilling injuries and pathogens related rots. The results point to new agro-technological approaches to extend shelf life quality in mango.

## DEVELOPMENT OF A $\beta$ -RECOMBINASE/SIX-BASED SYSTEM FOR MARKER RECYCLING IN *PENICILLIUM DIGITATUM*

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*Penicillium digitatum* is a fungal necrotroph that causes a common citrus postharvest disease known as green mold. The development of new and safe control methods alternatives to fungicides would benefit from the knowledge of the fungal pathogenicity and the fruit defense mechanisms. Recently we have published the complete genome of *P. digitatum* and have identified some genes putatively involved in pathogenesis (Marcet-Houben et al. 2012, BMC Genomics 13:646; López-Pérez et al. 2015, Mol. Plant Pathol. doi:10.1111/mpp.12179). A good approach to study the involvement of different genes in pathogenesis is the use of knockout mutants. However, to produce multiple gene deletions different selection markers are needed, limiting the number of genes to be deleted. Szewczyk et al. (2013, J Microbiol. Methods 92:236-243) described the functionality of a bacterial recombination system employing  $\beta$ -recombinase actin on *six* recognition sequences ( $\beta$ -rec/*six*) in *Neurospora crassa*, which allowed repetitive site-specific gene deletion and marker recycling. The aim of our study is to develop a marker recycling system for sequential targeted gene deletions in *P. digitatum*. To demonstrate the functionality of the  $\beta$ -rec/*six* system in *P. digitatum* we describe the generation of a polygalacturonase *pg1* deletion strain, recycling of the marker cassette, and the subsequent deletion of the polygalacturonase *pg2* gene. Our results confirm the functionality of the recyclable marker cassette in *P. digitatum*. Orange *in vivo* assays of the simple knockout mutants ( $\Delta pg1$  and  $\Delta pg2$ ) and the double knockout mutant ( $\Delta pg1 \Delta pg2$ ) confirm the involvement of both polygalacturonases in the *P. digitatum* pathogenicity in citrus fruits.

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## GENOMIC CHARACTERIZATION REVEALS MOLECULAR MECHANISM OF PATULIN BIOSYNTHESIS AND VIRULENCE IN *PENICILLIUM EXPANSUM*

Boqiang Li\*, Shiping Tian, Guozheng Qin, Yuanyuan Zong, Yong Chen, Zhanquan Zhang

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*Penicillium expansum* Link is a widespread postharvest pathogen that infects a variety of horticultural crops resulting in globally economic losses. Besides its pathogenicity to fruits, *P. expansum* is the most important producer of mycotoxin (patulin), which can induce immunological, neurological and gastrointestinal diseases, leading to a serious health risk to consumers. Although the biosynthetic pathway of patulin was previously proved to be ten steps based on several biochemical studies and the identification of several mutants in other *Penicillium* species, a cluster of genes responsible for patulin biosynthesis in *P. expansum*, however, has not been identified. Based on a whole-genome shotgun sequencing strategy, we sequenced the genome of *P. expansum* strain T01 and identified to be 33.52 Mb with 11,770 predicted protein-coding genes. We predicted 71 backbone genes and 55 gene clusters related to secondary metabolism, which is distinctly larger than that identified in other sequenced *Penicillium* species. Further, we identified a cluster of 15 genes responsible for the biosynthesis of patulin. The importance of all the genes in patulin cluster is firstly ascertained using a gene knockout approach. Deletion of 8 genes completely blocked patulin production. Among them, PePatL may act as a pathway specific transcription factor, and play essential role in patulin biosynthesis. We also found that patulin production did not contribute to the virulence of *P. expansum*. Our findings indicate previously unknown roles of all the genes in the patulin cluster and cast insight into the molecular mechanism in patulin biosynthesis of this economically important fungal pathogen.

## AVOCADO “NATIVO MEXICANO” FRUIT TRANSCRIPTOME (ESTs) IS DOMINATED BY STRESS AND INNATE IMMUNITY GENES

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México is the first producer, exporter and consumer of avocado fruit in the world. Michoacán is the main state producer of this fruit. Plants of avocado “nativo mexicano” *Persea americana* var *drymifolia* are used like rootstock for the principal commercial varieties (Hass for example). Despite that great importance for fruit production little information exist about this avocado variety. We generated and analysed ESTs libraries of different structures of avocado “nativo mexicano” fruit. We founded that the fruit transcriptome is dominated by the expression of stress and innate immunity genes. We shown that the protein product of two immune innate isolated genes; defensin and snakins, have antimicrobial activity. Works against avocado postharvest pathogens is doing. This finding could be very important for develop biotechnology strategies to protect fruits and horticultural products during postharvest period.







## METABOLOMIC RESPONSE OF *FRAGARIA X ANANASSA* DURING THE INITIAL STAGE OF FRUIT-PATHOGEN INTERACTION

Ellaine Grace Nagpala<sup>1\*</sup>, Michela Guidarelli<sup>1</sup>, Mattia Gasperotti<sup>2</sup>, Domenico Masuero<sup>2</sup>, Urska Vrhovsek<sup>2</sup>, Elena Baraldi<sup>1</sup>

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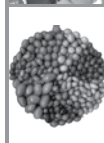
Secondary plant metabolites, specifically polyphenols, have been reported to play a significant role in plant defense. In strawberry fruits, a number of compounds have been found to be positively correlated with the concentration and resistance against some pathogens. It is known that major pathogens of strawberry such as *Colletotrichum acutatum* and *Botrytis cinerea*, while infecting fruits at white unripe stages, become quiescent early after infection. As strawberry fruit is known to be rich in antioxidants, this study was conducted to investigate the role of polyphenols in the early stages of fungal infection in strawberry fruits. In addition, the influence of polyphenols on the latency of fungal infection in the fruits was also determined. White and red fruits of *Fragaria x ananassa* were inoculated with *C. acutatum* and *B. cinerea*. The infection was arrested at 24 and 48 h from inoculation. A targeted metabolomics analysis of polyphenols was conducted with a triple-quadrupole mass spectrometry, while a spectrophotometric assay was performed for high-molecular mass proanthocyanidins. A total of forty-six compounds resulted from the metabolomic analysis. Results showed different responses in white and red fruits between groups of polyphenols, extending to individual concentration of compounds. Higher concentration of total polyphenols was particularly noted in white strawberry fruits after 48 h of inoculation, both for *C. acutatum* and *B. cinerea*. This finding suggests the involvement of polyphenols in the resistance of white strawberry fruit against pathogens. Quantitative real-time reverse transcription PCR of genes in the phenylpropanoid and flavonoid pathway was carried out and related with the concentration of compounds from the metabolomic assays.

## PROTEOMIC ANALYSIS OF *PENICILLIUM EXPANSUM* IN RESPONSE TO EXOGENOUS NITRIC OXIDE

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Application of chemical fungicides to control diseases brings the concern about food and environmental safety. Screening new antimicrobial compounds and exploring involved mechanisms have great significance to development of new disease management strategies. *Penicillium expansum*, a widespread filamentous fungus, is a major causative agent of fruit decay and has a potential public health significance since it produces the mycotoxin patulin. In the present study, we found sodium nitroprusside (SNP) as nitric oxide (NO) donor could effectively inhibit the germination of *P. expansum* spores and lower its pathogenicity on apple fruits. Through two dimension electrophoresis (2-DE) and mass spectrometry (MS) analysis, the responses in proteome of *P. expansum* on exogenous NO were characterized, and ten differentially expressed proteins were identified. Of them, five proteins including glutamine synthetase (GS), amidohydrolase, nitrilases, nitric oxide dioxygenase (NOD), and heat shock protein 70 were up-regulated, and others including tetratricopeptide repeat domain, UDP-glucose pyrophosphorylase (UGP), enolase (Eno), heat shock protein 60, and K homology RNA-binding domain were down-regulated. Expressions of four genes associated with the identified proteins (GS, NOD, UGP, and Eno), were also evaluated at the mRNA level by RT-PCR. We speculate that NO partly exert its inhibitory function on *P. expansum* by poisoning cellular energy production in cytoplasm. The outcome may provide novel evidence for understanding the mechanism of NO regulating virulence of the fungal pathogen.





## DIFFERENTIAL APPLE TRANSCRIPTOMIC RESPONSES TO *PENICILLIUM EXPANSUM* (PATHOGEN) AND *P. DIGITATUM* (NON-HOST PATHOGEN) INFECTION

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*Penicillium expansum* is the causal agent of blue mould of pome fruits and is responsible for important economical losses during postharvest handling in all producing countries. Although control of this important pathogen can be achieved by using chemical fungicides, the appearance of resistant strains and increasing public concern about the use of chemicals in food products have motivated the study of host-pathogen interactions. To develop a better understanding of disease resistance mechanisms in apples, a comprehensive transcriptional analysis of apple gene expression in response to a compatible (*P. expansum*) and non-host (*P. digitatum*) pathogen was conducted using an apple microarray of approximately 40,000 probes. The obtained data provide further evidence that apples inoculated with *P. expansum* exhibit significant upregulation of defense-related genes and genes involved in detoxification of reactive oxygen species. In contrast, apples inoculated with *P. digitatum*, a non-host pathogen, exhibited upregulation of genes involved in phenylpropanoid metabolism. To confirm the accuracy of the expression profiles obtained with the microarray, reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) was conducted for four genes involved in the phenylpropanoid pathway (*PAL1*, *PAL2*, *COMT2* and *POX64*). Expression data was obtained for different time points after inoculation and fruit maturity stages. The highest expression level of the phenylpropanoid genes was detected 48 h after inoculation with *P. expansum* in both immature and mature apples. Collectively, the results of the present study support the hypothesis that apples exhibit a more complex and diverse defense response to the compatible pathogen than to the non-host pathogen. *P. expansum*, however, is able to overcome these defenses and successfully infect apples.

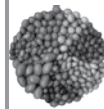
*This research was supported by two national projects AGL2008-04828-C03-01/AGR and GL2011-30519-C03/AGR and by a PhD grant for INIA (L. Vilanova)*

## A COMPARISON OF REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN SUSCEPTIBLE AND RESISTANT POTATO CULTIVARS INOCULATED WITH *FUSARIUM SULPHUREUM*

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Dry rot is an important postharvest disease of potato tubers that causes serious economic losses. Some *Fusarium* involved in dry rot, *F. sulphureum* is the most important pathogen in Gansu province of China. The effect of *F. sulphureum* inoculation was studied on reactive oxygen species production of susceptible cultivar “Longshu No 3” and resistant cultivar “Qingshu No 168”. The results showed that lesion diameters on inoculated slices of “Longshu No 3” were significantly larger than those of “Qingshu No 168”, indicating resistant cultivar had a stronger resistance to *F. sulphureum*. *F. sulphureum* inoculation induced significant production of  $H_2O_2$  and  $O_2^-$ . With the accumulation of ROS, lipid peroxidation and loss of cell membrane integrity were observed in inoculated slices of both cultivars. The content of ROS, lipid peroxidation and loss of cell membrane integrity in inoculated slices of resistant cultivar were less than that of susceptible cultivar. The activities of NADPH oxidase (NOX), catalase (CAT), peroxidase (POD), glutathione reductase (GR) significantly increased after inoculation, the activities of super oxide dismutase (SOD), ascorbate peroxidase (APX) also increased at early infection stages, but decreased rapidly and were lower than that in the control at later infection stages. The activities of CAT, SOD, POD, GR, APX in inoculated slices of resistant cultivar were higher than those of susceptible cultivar. These findings suggested that overproduced ROS involved in the pathogenicity of *F. sulphureum* in potato tubers, and the resistance cultivar had a stronger antioxidant system, leading to less accumulation of ROS and membrane damage.





## PUTATIVE ROLE OF HYDROGEN PEROXIDE IN *TRICHOTHECIUM ROSEUM*

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*Trichothecium roseum* is an important postharvest pathogen, causing very serious decay of fruit and vegetables in China. However the pathogenic mechanisms of the pathogen remain largely unknown. In this study, we analyzed the roles of  $H_2O_2$  generated by the pathogen by using horseradish peroxidase-red phenol assay and  $CeCl_3$  staining method. The accumulation of  $H_2O_2$  was investigated in spores treated with dimethylthiourea (DMTU), a scavenging agent of  $H_2O_2$ . The relationship between spore pathogenicity and  $H_2O_2$  accumulation was studied in challenged apple fruits. The results indicated that  $H_2O_2$  generated by germinal tube during growth and germination of spores, cytoplasm, and cell wall of germinal tube. The level of  $H_2O_2$  was significantly decreased by DMTU. The growth of mycelium was also suppressed. Intriguing, reduction of  $H_2O_2$  led to decrease of pathogenicity in apple fruits. These results suggest that decreased effects of *T. roseum* pathogenicity by  $H_2O_2$  are likely to inhibit the growth of mycelium.

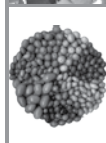
*This work was supported by National Natural Science Foundation of China (No. 31201665).*

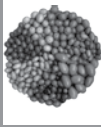
## THE CONTRIBUTION OF CUTICULAR WAX TO PRE-PENETRATION GROWTH OF *ALTERNARIA ALTERNATA* ON PEAR FRUIT

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Morphology and chemical composition of cuticular wax layers, represent the first site of contact with fungal pathogens, affect initial infection processes including conidial germination, appressoria and infection hyphae formation which are necessary for subsequent cuticular penetration of host surfaces. Dual role (prevention or facilitation of fungal invasion) of cuticular wax layers have been shown to be involved in the process of infection. *Alternaria alternata*, the causal agent of Alternaria rot of Asian pears, could initially infect the fruit via the styles or peel of the fruit during the growing season and then remain in a latent state; also, the incidence of *A. alternata* colonization in the fruit peel was positively correlated with cuticle thickness. The results showed that cuticular wax inhibited spore germination and mycelium growth of *A. alternata*, but stimulus infection structure including appressoria and infection hyphae formation, this function was also related to wax hydrophobicity (e.g. contact angle). Meanwhile the attachment, growth and appressoria formation of *A. alternata* were inhibited by the wax of early period fruit development, the appressoria formation of *A. alternata* was induced by the wax of mature period fruit. Studies are underway to elucidate the regulatory mode of the chemical composition or morphology of cuticular wax on infection behavior of *A. alternata* on Asian pears.





# DISRUPTION OF MELANIN SYNTHESIS GENES (*BCBRN1* AND *BCPKS13*) OF *BOTRYTIS CINEREA* CAUSES DEFICIENCY IN CONIDIATION AND MELANIZATION BUT ENHANCES VEGETATIVE GROWTH RATE AND VIRULENCE

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*Botrytis cinerea* is a necrotrophic pathogen that causes gray mold disease in a broad range of plants. Melanin has been shown to be an important component of the extracellular matrix of *B. cinerea*, and melanin is known to be required for pathogenicity of several plant pathogenic fungi which specially depend on mechanical penetration of host epidermis. In this study, we investigated the function of *bcbn1* and *bcpks13*, respectively encoding tetra-hydroxynaphthalene (THN) reductases and polyketide synthase of *B. cinerea*. These two enzymes are involved in dihydroxynaphthalene (DHN) melanin biosynthesis in many fungi. Deletion mutants of each gene were created by homologous recombination. The  $\Delta bcpks13$  and  $\Delta bcbn1$  mutants showed white and orange pigmentation respectively but no melanin accumulation. Noticeably, both  $\Delta bcbn1$  and  $\Delta bcpks13$  mutants were deficient in conidiation, but enhanced in growth rates and virulence on various hosts. Moreover, the mutants displayed elevated acidification and secretion of cell wall degrading enzymes, and preferably utilized the plant cell wall components as the carbon sources for mycelium growth in vitro. In contrast, overexpression of *bcbn1* (OE::*bcbn1* strain) resulted in attenuated hydrolytic enzyme secretions, acidification abilities, and pathogenicity. Taken together, these results indicate that *bcbn1* and *bcpks13* are required for diverse cellular and developmental processes like melanization and conidiation in *B. cinerea*, and above all, these two genes negatively regulate virulence of this pathogen.



## RNA-SEQ ANALYSIS OF UNRIPE AND RIPE STRAWBERRY FRUITS INTERACTING WITH *BOTRYTIS CINEREA*

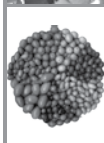
Lisa Zoli<sup>1</sup>, Paolo Sonego<sup>2</sup>, Marco Moretto<sup>2</sup>, Kristof Engelen<sup>2</sup>, Paul Tudzynski<sup>3</sup>, Claudio Moser<sup>4</sup>, Elena Baraldi<sup>1\*</sup>

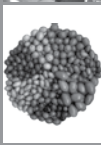
<sup>1</sup>Department of Agricultural Sciences (DIPSA), University of Bologna, Viale Fanin 46, 40127 Bologna, Italy. <sup>2</sup>Department of Computational Biology, Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Trento (TN) 38010, Italy.

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Grey mould caused by *Botrytis cinerea* is a major disease of strawberry, causing huge fruit losses worldwide. This fungal pathogen can infect fruits both at unripe or ripe stages, but the disease symptoms develop only on red ripe fruits, mostly during the postharvest storage phase, since, on white unripe fruits, *B. cinerea* stops its growth early after infection and becomes quiescent until the fruit ripen. To investigate the molecular bases of the low susceptibility of unripe fruit stages, RNA-seq analysis was performed on white and red strawberry fruits after 24 h infection with *B. cinerea*. The fruits of diploid woodland strawberry (*Fragaria vesca*) were used instead of the octoploid *Fragaria x ananassa*, because of the availability of genome sequence. RNA-seq analysis showed that a total of 712 genes were significantly regulated in the white and red non-inoculated fruits during ripening, whereas 54 and 41 genes resulted differently expressed in white and red infected fruits, with respect to their mock-inoculated counterparts. These genes were classified according to their annotated functional role and their possible involvement in strawberry fruit response to *B. cinerea* is discussed.





## CLONING, CHARACTERIZATION AND OVEREXPRESSION OF A NOVEL CHITINASE GENE (*MFCHI*) FROM *METSCHNIKOWIA FRUCTICOLA* AP47 AND IDENTIFICATION OF ITS BIOLOGICAL ACTIVITY AGAINST BROWN ROT OF PEACHES

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Biological control using antagonistic yeasts has been explored as a promising alternative to chemical fungicides to control postharvest diseases of fruits. The yeast antagonist *Metschnikowia fructicola* strain AP47 showed a high efficacy in controlling brown rot on stone fruits, however its mechanism against postharvest pathogens is still unclear. AP47 was able to produce chitinase enzymes in the presence of *Monilinia* spp. cell wall, and a novel chitinase gene *MfChi* (GenBank accession number HQ113461) was cloned from the genomic DNA of *M. fructicola* AP47. Sequence analysis showed lack of introns, an open reading frame (ORF) of 1,098 bp encoding a 365 amino acid protein with a calculated molecular weight of 40.9 kDa and a predicted pI of 5.27 was determined. *MfChi* is highly induced in *M. fructicola* after interaction with *M. fructicola* cell wall, suggesting a primary role of *MfChi* chitinase in the antagonistic activity of the yeast. *MfChi* gene was overexpressed in *Pichia pastoris* and showed a high chitinase activity towards p-(GlcNAc)<sub>3</sub> which is a suitable substrate for endochitinase activity detection. The antifungal activity of the recombinant MfChi was investigated against *M. fructicola* and *M. laxa* *in vitro* and on peaches. MfChi significantly inhibited *M. fructicola* mycelial growth in PDA plate and no conidia sporulation was observed in the growing side of the pathogen mycelium closer to the chitinase treatment. In PDB medium, MfChi is highly effective in reducing spore germination and germ tube length of *Monilinia* spp.. The enzyme, when applied on peaches, successfully reduced brown rot severity, but its antifungal activity mainly depends on the chitinase concentration and time since treatment application. This work shows that the chitinase *MfChi* could be developed as a postharvest treatment with antimicrobial activity for fruit undergoing a short storage period.

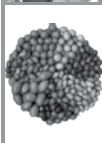
## OPTIMIZATION OF DRY FORMULATIONS FOR THE BIOCONTROL AGENT *CANDIDA SAKE* CPA-1 USING FLUIDISED-BED DRYING

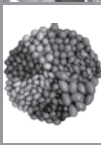
Anna Carbó<sup>1\*</sup>, Rosario Torres<sup>1</sup>, Josep Usall<sup>1</sup>, Cristina Solsona<sup>1</sup>, Elena Costa<sup>1</sup>, Inmaculada Viñas<sup>2</sup>, Estanislau Fons<sup>2</sup>, Neus Teixidó<sup>1</sup>

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The biocontrol agent *Candida sake* CPA-1 has been demonstrated to be effective against several diseases as blue and grey moulds caused by *Penicillium expansum* and *Botrytis cinerea*, respectively in pome fruit or *Botrytis* bunch rot in grapes. Consequently, to optimize a dry formulation for *C. sake* to improve shelf life and manipulation is essential to increase its potential for future commercial applications. The aim of this investigation was to optimize the conditions of a dry formulation for *C. sake* by fluidised-bed drying. Initially, drying conditions (temperature and time) were optimized. Then, several additives used as carriers or as protective compounds were evaluated in order to improve survival. In addition, the effect in cells survival of two rehydration media and different ratios of time and temperature of rehydration were tested. Finally, biocontrol efficacy as well as product shelf life at different storage conditions were studied. The optimal conditions for drying process were found to be 40 °C during 45 minutes. Concerning the additive substances, potato starch used as carrier significantly enhanced the viability compared to the other tested, but none of the protective compounds tested increased the viability of dried cells. Room temperature and short rehydration time with phosphate buffer were considered the optimum conditions to recover dried formulates. Cells survival was significantly dependent on the storage temperature when dried powders were not stored under vacuum conditions; therefore dried formulates were suggested to be stored at 4 °C and air packaged; shelf life assays were made for twelve months with good results. Formulated products maintained biocontrol efficacy.

*This research was supported by a national project RTA2012-00067-C02-01 and by a PhD grant from INIA (A. Carbó).*





## UNDERSTANDING THE MECHANISM OF BIOLOGICAL CONTROL OF POSTHARVEST PHYTOPATHOGENIC MOULDS PROMOTED BY FOOD ISOLATED YEASTS

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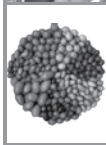
The knowledge of the mode of action of antagonists, which are able to provide a significant reduction in disease incidence and severity of postharvest pathogenic moulds, is an important tool for improving their performances and reliability, for developing formulations apt to enhance the expression of such traits, and for establishing screening criteria in the selection of new biocontrol agents. In this study, a reduction of postharvest decay of citrus, table grape and strawberries, caused by *Penicillium digitatum*, *P. italicum* and *Botrytis cinerea* strains, was demonstrated by using in *in vivo* experiments four food-isolated yeasts strains, *Wickerhamomyces anomalus* BS91, *Metschnikowia pulcherrima* MPR3, *Aureobasidium pullulans* Pi1, and *Saccharomyces cerevisiae* BCA61. Mechanisms of fungal inhibition were elucidated in *in vitro* experiments. All yeast strains demonstrated antifungal activity against *P. digitatum*, *P. italicum* and *B. cinerea* based on competition for nutrients at a different level depending on species and medium. The competition for iron, the ability to form biofilm and to colonize fruit wounds were hypothesized as the main mechanisms of action for *M. pulcherrima*. The biocontrol abilities of *S. cerevisiae* and *W. anomalus* strains was proved to be correlated with killer phenotype. The production of glucanase, chitinase and protease, and the ability to colonize the wounds were the most important mechanisms for biocontrol activity in *A. pullulans* and *W. anomalus*, which also showed high ability to form biofilm. The production of VOCs with *in vitro* and *in vivo* inhibitory effect was observed for all the tested species. Furthermore, peroxidase and superoxide dismutase activity assays were conducted to evaluate the ability to induce systemic resistance. It is concluded that the understanding of the multiple and different modes of action of the tested yeast species represents a key step to explain the excellent control of postharvest penicillium and botrytis moulds of oranges, grapes and strawberries fruits.

## EFFICACY OF FIELD APPLICATIONS OF BIOCONTROL AGENTS AGAINST GREY MOULD ON TABLE GRAPE IN POSTHARVEST

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<sup>1</sup>*Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", Locorotondo (Ba), Italy.* <sup>2</sup>*Osservatorio Fitosanitario, Regione Puglia, Italy.* <sup>3</sup>*Dipartimento di Scienze del Suolo, delle Piante e degli Alimenti dell'Università degli Studi di Bari, Aldo Moro, Bari, Italy. E-mail: francesco.faretra@uniba.it*

Grey mould (*Botrytis cinerea*) causes heavy yield losses on late ripening table-grape, both in the field and in post-harvest, and often its control requires intensive spray schedules. In 2013-2014, two trials were carried out in arbour vineyards ("tendone", trellising system), table-grape cvv Red Globe and Italia, covered with plastic sheets to delay harvesting time, located in South Italy. Bio-control Agents (BCAs), *Aureobasidium pullulans*, *Bacillus amyloliquefaciens* and *B. subtilis*, were applied alone or in alternation with chemical fungicides, to verify the possibility of reducing the number of chemical sprays as well as fungicide residues on bunches. As expected, grey mould always increased after 4 days of simulated shelf-life at room temperature after cold storage. In 2013, after 10 days of storage symptoms were absent on untreated bunches but after shelf-life almost 80% were rotted; after 30 days of cold storage, 34% bunches (96% after shelf-life) were rotted. In 2014, after 10 days of cold storage 58% of untreated bunches showed symptoms, and prevalence increased to 100% after 20 days. BCAs, applied alone, never allowed any significant containment of grey mould infections as compared to the untreated check. No significant differences of efficacy were observed between the strategies in which applications of BCAs preceded or followed chemicals sprays. In 2013, the protection schedules based on the integration of BCAs with chemical fungicides always showed good efficacy levels even after 30 days of cold storage and shelf-life, which were not statistically different from those obtained with the exclusive use of chemicals (fluopyram or alternation of different fungicides). In 2014, the best results were obtained when *B. subtilis* was applied previously or subsequently to consecutive applications of different fungicides. The use of BCAs always significantly reduced fungicide residues in bunches at harvesting time.





## ISOLATION AND SELECTION OF YEASTS FOR BIOLOGICAL CONTROL OF POSTHARVEST DECAY OF MANGO

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Consumers concerns about food safety led to great restrictions for postharvest application of fungicides, especially for fruits and vegetables. So, it is very important to develop alternative strategies for the control of postharvest diseases. In this study, 163 yeast were isolated from fruits cultivated at São Francisco River Valley (Brazil) using different approaches and evaluated about their potential of control of mango decay. The largest number of isolates was obtained from table and wine grapes, followed by mango and melon. The isolates were examined as antagonists against major ethiological agents of mango decay in the region (*Colletotrichum gloeosporioides*, *Fusicoccum aesculi* and *Lasioidiplodia theobromae*). Six to twelve percent of them were able to inhibit disease symptoms progress when co-inoculated with the pathogens in mango fruits along 10 days incubation. The data collected were analyzed by the Kaplan-Meyer success/failure method and it was found that the isolates LF, L7K and L10 showed incidence curve of anthracnosis, stem-end rot and *Fusicoccum* rot significantly lower than control treatment (GBW test;  $p > 0,05$ ). In the second experiment, the mango fruits were submitted to the common handling of postharvest operations (washing/drying, wax and cold storage in paper box) and inoculated with the yeasts and pathogens isolates. All yeasts significantly reduced the incidence of anthracnosis, while only the isolate L10 significantly reduced stem-end rot and *Fusicoccum* rot incidence by Dunnet test ( $p > 0.05$ ). Additionally, the inoculation of all the yeast isolates reduced severity of anthracnosis and stem end rot to up to 90% than the control treatment. For *F. aesculli*, the innoculation of the isolates L7K and L10 showed a reduction of severity higher than 80% in relation to the control.

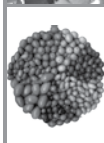


## TOLERANCE OF YEASTS BIOCONTROL AGENTS TO TEMPERATURE, UV RADIATION AND OSMOTIC STRESS

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Biocontrol became an alternative to control of postharvest decay of table grape, however due to the minimal handling in the packing-house its application is limited to field. Therefore, considering the semi-arid climate of the region of the São Francisco River Valley (Brazil), tolerance to climate must be included in the selection process of biocontrol agents. In this work the yeast isolates L7K and L10, which were previously selected for control of postharvest decay of grapes, were evaluated for their natural tolerance to UV-B light, temperature and low water availability. In a first experiment, isolates were incubated on fruit surface and kept in growth chamber adjusted to temperatures ranging from 6 to 40 °C. In the second experiment, the isolates were inoculated in culture media added with polyethylene glycol (PEG) 6000 in order to achieve osmotic potential from 0 to -20 MPa. In a third experiment, both isolates were grown in culture media containing increasing amounts of PEG 6000 and incubated at temperature ranging from 20 to 35 °C in order to evaluate their tolerance to multiple stress. In the last experiment the isolates were exposed to increasing doses of UV-B radiation in order to evaluate their natural tolerance to UV. All isolates showed optimal growth around 20-30 °C and negligible growth from 35 °C onward. Isolate L10 showed highest resistance to osmolite addition, maintaining high cell counts up to -5 MPa. The surface curves obtained showed that L10 was linearly susceptible to increasing temperature and reduction in water availability in the culture medium. Isolate L7K however, showed a paraboloid surface curve, with high tolerance to the initial combination of temperature and osmolite. Overall, the isolates were highly susceptible to UV exposure, reducing viable cell counts in fruit surface to lower than 50% in the minor radiation dose tested (2,050.0 mJ.cm<sup>-2</sup>).







## DEVELOPMENT OF A SCAR MARKER AND A STRAIN-SPECIFIC GENE MARKER FOR THE DETECTION OF THE BIOCONTROL AGENT *BACILLUS AMYLOLIQUEFACIENS* CPA-8

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The strain CPA-8 is an effective biocontrol agent against brown rot caused by *Monilinia* spp in stone fruit. The registration of biological control agents requires the development of monitoring systems to detect and identify the agent in the environment. In this work, a reliable tool for the detection of this *Bacillus* strain was carried out by DNA amplification techniques. The RAPD technique was applied to a collection of 30 *B. subtilis* strains and 47 related *Bacillus* species. Among the 30 primers tested, the primer pair OPG1/OPG6 amplified a fragment specific to the strain CPA-8. The PCR product (668 bp) was sequenced and used to design 6 SCAR primer pairs that were again evaluated on all strains collection. A SCAR marker (named SCAR 4) amplified a semi-specific fragment of 665 bp for the strain CPA-8 but also for other 12 strains with phenotypically differences from CPA-8. In order to distinguish among these 13 strains, strain-specific genes related to ecological adaptations of *Bacillus amyloliquefaciens* species were analysed. Three genes were proposed for being involved in adaptation processes: RBAM 007750, RBAM 007760 and trp E (G). On the basis of these genes, 7 primer pairs were designed and tested against genomic DNA from all strains. The primer pair F2/R2 obtained from RBAM 007760 gene amplified a fragment of 265 bp specifically for strain CPA-8. Our results reveal that combination of two molecular markers (SCAR 4 and F2/R2 fragment from RBAM 007760) provides a suitable monitoring tool to specifically identify CPA-8. Furthermore, the homology of the sequences studied in this work indicated that the biocontrol agent CPA-8 belongs to *Bacillus amyloliquefaciens* species instead of *B. subtilis* that was the first classification.

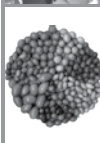
*This research was supported by the European project BIOCOTES FP7-612713 and by a PhD grant from AGAUR (2014-FI\_B 00367\_Amparo M. Gotor).*

# EFFICACY SEMI-COMMERCIAL TRIALS OF NATIVE YEASTS: *PICHIA MEMBRANIFACIENS* AND *CRYPTOCOCCUS VICTORIAE* ON CONSERVATION MEDIUM AND LENGTH PEARS, IN NORTHERN PATAGONIA, ARGENTINA

Maria Cecilia Lutz<sup>1,2\*</sup>, Christian Ariel Lopes<sup>1</sup>, María Cristina Sosa<sup>2</sup>, Betina Gramisci<sup>1</sup>, Marcela Paula Sangorrin<sup>1</sup>

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Antagonistic behavior of native yeasts: *Pichia membranifaciens* NPCC1250 and *Cryptococcus victoriae* NPCC1263 on *Botrytis cinerea* and *Penicillium expansum*, was evaluated in semi-commercial conditions in two organic packinghouse (A and B) of Neuquén, Argentina. In line-packaging, applications were performed on D'Anjou and Packham's pear fruit. Yeast biomass was produced in cane molasses (12.8 g/L) and urea (0.6 g/L). About 400 kg of fruit were sprayed with yeast suspension ( $10^{11}$  CFU/mL). Treated fruit was packaged and stored in commercial storage chamber ( $-1/0^{\circ}\text{C}$ , 95% RH). During 2012, the effect of yeasts alone and with  $\text{CaCl}_2$  (2% w/v) was evaluated. At 90 days, in (B) on D'Anjou pears, yeasts with  $\text{CaCl}_2$  reduced the incidence of *P. expansum* more than 60% and *Cr. victoriae* plus  $\text{CaCl}_2$  fully controlled to *B. cinerea*. Packham's pears were evaluated at 160 days. The  $\text{CaCl}_2$  improved the antagonism of *Cr. victoriae* against *P. expansum*. In (A), *P. membranifaciens* controlled 83% both pathogens and *Cr. victoriae* 100% to *B. cinerea*. In (B), *P. membranifaciens* controlled 50% and 56% the incidence of *B. cinerea* and *P. expansum*, respectively; while, *Cr. victoriae* controlled 72% of *B. cinerea*. In 2013, the effect of yeasts ( $10^{11}$  CFU/mL) alone or in mixture (1:1) with  $\text{CaCl}_2$  2% (w/v) was evaluated. At 90 days, in D'Anjou pears, *P. expansum* was controlled by the three treatments in 82% and *B. cinerea* by *P. membranifaciens* and yeast mixture. In general, the mixture of yeasts was more effective than alone. While, in Packham's, *P. expansum* was completely controlled by *Cr. victoriae* and the mixture, and by *P. membranifaciens* alone, in 88%; while *B. cinerea* was totally controlled by *P. membranifaciens*. The number of cells sprayed of yeast was reduced by the delivery system employed in at least two orders of magnitude. The yeasts colonized the fruit surface, reaching  $10^7$  and  $10^8$  CFU/cm<sup>2</sup> of fruit. This safe technology based on yeasts, could significantly reduce the incidence of postharvest diseases of pears under commercial conditions.





## EFFECT OF DIFFERENT FILM FORMING AGENTS ON THE EFFICACY OF *CANDIDA SAKE* FOR BIOCONTROL OF *BOTRYTIS CINEREA* IN GRAPES

Anna Marín<sup>1</sup>, Maite Cháfer<sup>1</sup>, Lorena Atarés<sup>1</sup>, Amparo Chiralt<sup>1</sup>, Rosario Torres<sup>2\*</sup>, Josep Usall<sup>2</sup>, Neus Teixidó<sup>2</sup>

<sup>1</sup>Instituto de Ingeniería de Alimentos para el Desarrollo. Camí de Vera s.n. 46022. Universitat Politècnica de València, València, Spain. <sup>2</sup>IRTA, XaRTA-Postharvest, Edifici Fruitcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, 25003 Lleida, Catalonia, Spain. E-mail: rosario.torres@irta.cat

The aim of this work was to study the efficacy improvement of the biocontrol agent *Candida sake* CPA-1 on grapes applied in combination with different film forming dispersions (FFDs). The FFDs were prepared with several biopolymers: corn starch (S), hydroxypropylmethylcellulose (HPMC), sodium caseinate (NaCas) and pea protein (PP) with and without different surfactants: oleic acid (OA), Span 80 (S80) and Tween 85 (T85). CPA-1 was incorporated to the FFDs at a concentration of  $5 \times 10^7$  CFU/mL and treatments were sprayed on the grapes using an air brush. An additional treatment of *C. sake* without FFD was also applied (CS). In the adherence and survival assays, the population of *C. sake* was quantified after 24 h and 7 days of incubation at 20° C and 85% RH. For the efficacy assays, after the application of the FFD, a conidial suspension of *Botrytis cinerea* at  $10^4$  conidia/mL was sprayed. The reduction of rot incidence and severity was visually determined after 7 and 12 days.. The results were expressed referred to a control serie of infected grapes treated with water. The initial adherence of CPA-1 was significantly improved when was applied with S, NaCas and PP OA based coatings. After 7 days, an increase of *C. sake* population was observed in all treatments. The coatings which achieved a significantly higher survival of the BCA respect to CS were those obtained with NaCas with or without surfactants and PP with OA and T85. In general, all treatments resulted on a similar or higher reduction of incidence after 7 days than CS. The best results were obtained with S T85, HPMC S80 and PP OA, with values around 80% reduction. Protein based coatings significantly improved the results of CS. Results suggest that FFDs, especially proteins, contribute to better fix *C. sake* on grapes surface and allow a higher survival over time. Similarly, coatings seem to enhance the biocontrol activity.

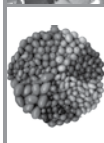
*This research was supported by a national project RTA2012-00067-C02-01 and by a PhD grant from Conselleria d'Educació from València, Spain (A. Marín).*

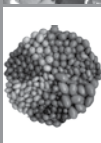
## EFFECT OF ANTAGONISTIC *SACCHAROMYCES CEREVISIAE* ON QUALITY OF RED GLOBE GRAPES, SAN JUAN, ARGENTINA

**María Cristina Nally<sup>1,2\*</sup>, David Sebastian Radicetti<sup>1</sup>, Virginia Mercedes Pesce<sup>1,2</sup>, Leticia Anahi Rodríguez<sup>1,2</sup>, Yolanda Paola Maturano<sup>1,2</sup>, María Eugenia Toro<sup>1</sup>, Fabio Vazquez<sup>1</sup>**

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Red Globe (*Vitis vinifera* L.) is a non-climacteric fruit with a short postharvest life. Loss of quality in this fruit is mostly due to its sensitivity to fungal decay as grey and sour rots. The use of yeasts as biocontrol agents has become a sustainable strategy for controlling postharvest diseases of fruit. We determined that *S. cerevisiae* BSc203 decreased fungal disease incidence *in vitro* and at field conditions. However there are no data about the effects of this biofungicide on the quality of the table grape. The aim of this work was to evaluate the effect of this biofungicide on quality parameters of Red Globe grapes, in an organic vineyard. Field assay: BSc203 ( $10^8$  cells/mL) + xanthan gum + glycerol was sprayed in four stages during ripening of the grapes. Matured fruit were harvested and transferred to the laboratory. Bunches were weighed using a digital balance. Berries equatorial diameters were measured with a digital caliper. Total soluble solids (TSS) were determined by hand-held refractometer. Surface color was measured at two points around the equatorial zone of fruit by a colorimeter. Results: BSc203 had no significant effect on weight of bunches, TSS and color of berries. Equatorial diameter of treated berries with biofungicide was significantly higher than the control ( $p \leq 0.05$ ). Conclusion: the spraying with BSc203 in organic vineyard before harvest had no significant deleterious effects on the quality of Red Globe grapes. Future research will be done about the effect on size of berries owing to spraying BSc203.





## RED YEAST ISOLATES AND SIDEROPHORES PRODUCTION TO CONTROL BOTRYTIS STORAGE ROT OF TABLE GRAPES

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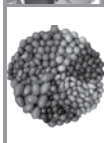
Grey mould is one of the most severe postharvest disease of fruit and vegetables. The use of fungicides is restricted in most countries, and there are problems due to the negative effects they may have both on the human and environmental health, and on the selection of fungicide-resistant strains. The use of naturally occurring antagonists to control storage decay and increase product quality represents a suitable alternative to chemical fungicides. However, the modes of action for most of the antagonists has not yet been fully elucidated, because of the difficulties arising from the complex interactions between host, pathogen, antagonist, and others microorganisms occurring in the site of interaction. Among the desirable characteristics of antagonists, the ability to produce siderophores is included. Several phyllosphere yeasts species are known to produce hydroxamate-type siderophores, iron-binding compounds in response to Fe-stress conditions. In this research, more than 100 red yeasts were isolated from the surface of organically and conventionally trained table grape berries and leaves, orange fruits, and olive drupes. Siderophores production was scored qualitatively on CAS-blue agar plates, and the most active isolates were selected for further quantitative assessments. Among the selected isolates some resulted very active hydroxamate-type siderophores producers, the best reaching  $0.7 \text{ g L}^{-1}$ . A selection among high, medium and low siderophores-producer strains was as then further evaluated for biocontrol activity against Botrytis storage rot on apple fruits and table grapes; moreover, the ability to survive on the table grape berries, at  $0-1^\circ\text{C}$ , for the storage duration was also determined. Results indicated that strains R50 and R51, identified as *Rhodotorula* spp., were the most effective in reducing Botrytis storage rot, both on table grapes and apples, although with an intermediate hydroxamate-type siderophores production. R50 and R51 were also able to maintain high population level on fruit surface after 30-days storage at  $3^\circ\text{C}$ .

## ACTIVITY OF ENDOPHYTIC FUNGI FROM *ARTEMISIA ABSINTHIUM* ON *BOTRYTIS CINEREA*

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The main postharvest decay of table grapes (*Vitis vinifera*) is gray mold, caused by *Botrytis cinerea*. An alternative to the use of synthetic fungicides to control postharvest diseases relies in the use of naturally occurring microorganisms, and endophytes have been proposed as promising biocontrol agents. These organisms reside in the plant tissues as symbiotic microbes or opportunistic pathogens, and they can produce a plethora of compounds. This research aimed to study the activity on *B. cinerea* of 12 endophytic fungi isolated from wormwood roots (*Artemisia absinthium*). All isolates except B16C39 were not able to infect intact or wounded berries. In dual culture with *B. cinerea*, endophytes reduced radial fungal growth from 33 to 50%, with the highest inhibition halo found with isolate B11C29. Single table grape berries were wounded with a needle (2 × 2 mm), then inoculated with 40 µl of a conidial suspension (10<sup>5</sup> and 10<sup>6</sup> spores ml<sup>-1</sup>) of the biocontrol agent. After 24 h incubation at 20°C, on the wound it was deposited a 40 µl drop of a conidial suspension (10<sup>4</sup> spores ml<sup>-1</sup>) of *B. cinerea*. All biocontrol agents were more effective when used at 10<sup>6</sup> spores ml<sup>-1</sup>. Four of fungal strains, B14C35, B5C15, B6C18 preliminarily identified as *Penicillium* sp., and B9C22 (still not identified) significantly reduced both percentage of infected berries and lesion diameter as compared to the water treated control. Full identification of these promising biocontrol agents is in progress.







## ANTIFUNGAL ACTIVITY OF *BACILLIS SUBTILIS* HK2 AGAINST *TRICHOTHECIUM ROSEUM* CAUSING PINK ROT OF MELON AND WHITE STAIN SYMPTOM ON GRAPE

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Pink rot on melon and White stain symptom on grape are caused by *Trichothecium roseum*, one of the most important diseases of grape and melon. These diseases occur national-wide in Korea and causes irreversible damage on grape and melon, at harvest season. This work presents the evaluation of the capacity of *Bacillus subtilis* HK2 to protect melon and grape against *T. roseum* and establishes its role as a biocontrol agent. In this study, we isolated a *Bacillus* strain HK2 from rhizosphere soil, identified it as *Bacillus subtilis* by 16S rRNA analysis and demonstrated its antifungal activity against *T. roseum*. Under I-plate assay it was observed that the effect of hyphal growth inhibition was not due to production of volatile compounds. The optimum culture condition of HK2 was found at 30°C and initial pH of 7.0. Application of HK2 culture suspension reduced 90.2% of white stain symptom on grape as compared to control, resulting in greater protection to grape against *T. roseum* infestation. Butanol extract of HK2 culture purified using flash column chromatography. The antifungal material was a polar substance as it showed antifungal activity in polar elute. Therefore, our results indicated a clear potential of *B. subtilis* HK2 to be used for biocontrol of Pink rot in melon and white stain symptom on grape caused by *T. roseum*.

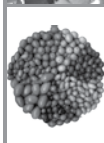


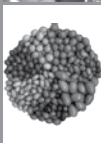
## EFFICACY OF *CANDIDA OLEOPHILA* STRAIN O IN PREVENTING POSTHARVEST DISEASES OF FRUITS

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Biological control of postharvest diseases is of uppermost interest comparatively to chemical fungicides: absence of chemical residues in the food chain and effluents, and low risk in appearance of fungicide-resistant pathogens. A yeast *Candida oleophila* strain O-based solution has been developed. This yeast was recently added to the list of approved active substances by the EU. Several studies have shown that *Candida oleophila* strain O (that will be soon commercially available under the trade name NEXY®) is an effective antagonist of *Penicillium expansum* and *Botrytis cinerea* in stored apples and pears, of *Penicillium* spp. in stored citrus and *Colletotrichum musae* in stored bananas. NEXY®, combination of *Candida oleophila* strain O with calcium gluconate (ratio 1/6) was applied at 233g/100 L by drenching or dipping to fruits wounded. 24h later, the fruits were inoculated with pathogens previously mentioned. 3 to 24h after the inoculation, fruits were placed in cold storage conditions (4-6 °C for Pome and Citrus fruits, and 13°C for bananas). After 42 to 85 days of cold storage, the percentage of infected Pome and Citrus fruits is significantly higher in untreated fruits (30 to 70 %) than in fruits treated with NEXY® (10 to 40%) or chemical reference (imazalil or thiabendazole)(0 to 50%). After 7 to 12 days of storage, the percentage of infected bananas is significantly higher in untreated fruits (60 to 100 %) than in bananas treated with NEXY® (0 to 85%) or chemical reference (thiabendazole) (0 to 65%). These results support that combination of *Candida oleophila* strain O with calcium gluconate has a broad spectrum of activity against a number of postharvest pathogens on a variety of fruits. NEXY® is a good alternative to synthetic fungicides in particular in low and moderate disease pressure conditions.





## BIOLOGICAL CONTROL OF *BOTRYTIS CINEREA*, *PENICILLIUM EXPANSUM* AND *MUCOR PIRIFORMIS* ON GALA AND MCINTOSH APPLES USING *PSEUDOMONAS FLUORESCENS* STRAINS

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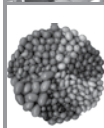
Postharvest disease causes major losses for apple producers and packinghouses worldwide. Three major postharvest fungal pathogens, *Penicillium expansum*, *Botrytis cinerea*, and *Mucor piriformis*, commonly infect and rot apples in storage in British Columbia, Canada. Fungicides have been applied extensively to reduce postharvest loss, but pathogen resistance is emerging and public pressure to reduce fungicide use has led to increased research for safer alternatives such as biocontrols. Three strains of *Pseudomonas fluorescens* 4-6, 1-112 and 2-28, isolated from the rhizosphere of pulse crops in Western Canada, were studied as potential biocontrol agents under commercial cold and Controlled Atmosphere (CA) storage with two apple varieties, Gala and McIntosh. Percent infection of apples inoculated with each of the three pathogens and biocontrol strains was determined after 15 weeks in commercial cold storage or 17-22 weeks in CA storage and compared with the fungicide Scholar<sup>®</sup> (fludioxonil) and the biocontrol agent Bio-Save<sup>®</sup> (*P. syringae*). All three isolates inhibited the growth of *B. cinerea*, *P. expansum*, and *M. piriformis* *in vitro*. Efficacy of the *P. fluorescens* strains varied with pathogen, apple variety and storage environment. Percent infection by *P. expansum* and *M. piriformis* was lower in Gala and McIntosh apples treated with *P. fluorescens* strain 4-6 and stored in CA than for those in cold storage. Strain 2-28 decreased the percent infection of Gala and McIntosh apples infected with *B. cinerea* and *P. expansum* in CA storage compared to those in cold storage. McIntosh apples, which had higher titratable acidity than Gala, exhibited greater percent infection than Gala. Strain 4-6 showed the most consistent efficacy against all three pathogens and on both apple varieties. The disease control was comparable to Bio-Save<sup>®</sup> but less effective than Scholar<sup>®</sup>. These results suggest that *P. fluorescens*, has potential to control common postharvest fungal pathogens during cold and CA storage.

## EFFECT OF CHITIN ON THE BIOCONTROL EFFICACY OF *RHODOTORULA MUCILAGINOSA* ON POSTHARVEST DECAY OF PEACHES

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Antagonistic yeasts have shown great potential as an alternative to synthetic fungicides for the control of postharvest decay of fruits. However, for biological control to be accepted as an economically viable option, consistency and efficacy of antagonistic yeasts in controlling postharvest diseases must be enhanced. In the present investigation, the influence of chitin amendment to the culture media on the efficacy of the biocontrol yeast *Rhodotorula mucilaginosa* in controlling postharvest blue mold decay and *Rhizopus* decay of peaches were investigated. The biocontrol activity of *R. mucilaginosa* grown on NYDB (Nutrient Yeast Dextrose Broth) with chitin 0.5% (w/v) was significantly enhanced as compared to the respective control. The population of *R. mucilaginosa* harvested from NYDB amended with chitin at 0.5% or from NYCB (chitin as the sole carbon source instead of dextrose in the media of NYDB) increased rapidly in peach wounds compared to that harvested from NYDB without chitin at the whole storage period except 1 d. *In vitro* test showed that, the addition of chitin (0.1-2.0 % w/v) in the culture media had no significant effect on the growth of *R. mucilaginosa* on NYDB compared with the case that without chitin. Conversely, the population of *R. mucilaginosa* on NYCB was significantly lower than that of the NYDB after 24 h incubation. In summary, our results showed that the biocontrol activity of *R. mucilaginosa* could be enhanced by chitin induced incubation, which may offer great practical potential in reducing the postharvest diseases of peach fruit. The mode of action may be involved in its ability to enhance growth of the antagonistic yeast in fruits. Of course, other mechanisms, alone or collectively, may also be involved. All these should be further studied.





## PREHARVEST SPRAYING OF BIOTIC AND ABIOTIC ELICITORS REDUCES POSTHARVEST DECAY CAUSED BY *MONILINIA FRUCTICOLA* AND *RHIZOPUS STOLONIFER* IN PEACHES

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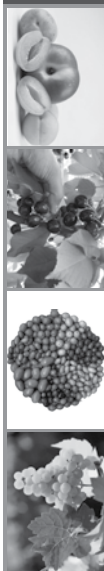
Decay caused by *Monilinia fructicola* and *Rhizopus stolonifer* results in substantial postharvest loss of peaches in southern Brazil. This research was carried out to evaluate *in vivo* control of *M. fructicola* and *R. stolonifer* by spraying an orchard of peach trees (cultivar Chimarrita) with biotic and abiotic elicitors. The experiment consisted of the following treatments: control (spraying with water); acibenzolar-S-methyl (ASM; 50 mg L<sup>-1</sup> and 100 mg L<sup>-1</sup>); *Saccharomyces cerevisiae* (SC; 1 mL L<sup>-1</sup> and 2 mL L<sup>-1</sup>); *Bacillus subtilis* (10 mL L<sup>-1</sup>); and chitosan (10 g L<sup>-1</sup>). Treatments were sprayed four times, with an interval of seven days, starting one month before the predicted fruit harvesting date. The experiment followed the randomized block design. Before the storage, fruits were separated in to four lots. Two lots were inoculated with *M. fructicola* by two different methods, one lot was inoculated with *R. stolonifer* and one lot was left not inoculated. Fruits were then cold stored (0±1 °C; RH = 95±2%) for 30 days. Decay incidence and severity were assessed after removal from cold storage, followed by 0, 1, 2 and 3 days at 20 ± 2 °C (shelf life). Spores production and viability were assessed at the third day of shelf life. At 0 day of shelf life, the treatment with SC 2 mL L<sup>-1</sup> showed best control of decay severity caused by *M. fructicola* (82%). Also at 0 day of shelf life, all elicitors provided 100% control of decay incidence and severity caused by *R. stolonifer*, with the exception of ASM at 50 mg L<sup>-1</sup> (which reduced severity by 89% and incidence by 85%). Attributes of fruit quality and ripening were not different between treatments. The results show that the elicitors assessed were efficient to reduce postharvest decay in 'Chimarrita' peaches caused by *M. fructicola* and *R. stolonifer*.

## POSTHARVEST DISEASES OF *TUBER MELANOSPORUM*

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Black truffle (*Tuber melanosporum*), also called black Périgord truffle, is well known as high value product and appreciated for its characteristic taste and aroma. It is harvested on spontaneously mycorrhized plants in several areas of Europe (e.g. Italy, France, Spain), and it is grown on artificially inoculated plants here and in several other areas of the world (other European Countries, Chile, Australia, New Zealand, South Africa, North America). After harvest, black truffles suffer from dehydration, then it is stored at 2-4°C within paper towels, that need to be changed every 1-2 days, useful to absorb excess of water. Storage can last at highest 7-10 days, later there is development of fungi and bacteria. Black truffle survive in the soil in symbiosis with a high number of microorganisms useful for its growth. Once harvested, those microorganisms starts to live using the truffle as a substrate. Agent of postharvest decay of black truffle include filamentous fungi, yeasts and bacteria. Among filamentous fungi, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium* and *Trichoderma* are the most common genus, some of them able to produce mycotoxins. Among bacteria, we can find *Pseudomonas* spp., *Clostridium* spp., lactic acid bacteria, coliforms (*Escherichia coli*, *Listeria monocytogenes*), and Enterobacteriaceae (*Raoultella terrigena*, *Enterobacter intermedius*), and some of these are in the list of foodborne pathogens and/or can produce toxins. The development and application of strategies to improve the shelf life of black truffle is important to extend their postharvest life and reduce the development of pathogens that can be harmful for consumers.





## INHIBITORY ACTIVITY OF SARDINIAN PEAR ON *PENICILLIUM EXPANSUM* PATULIN BIOSYNTHESIS

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Patulin is a tetraketide mycotoxin with carcinogenic and teratogenic effects produced by different filamentous fungi affecting fresh produce. *Penicillium expansum* is the main species responsible for patulin production in pome fruits. Based on previous studies, performed to rank natural resistance within the Sardinian pear germplasm collection, we focused our research on the natural resistance of two endemic accessions (*Vacchesa* and *Sarmentina*) and one national cultivar (*Abate fetel*) against *P. expansum* infection and on patulin production. During a 7 day survey, *P. expansum* pathogenesis and patulin accumulation was monitored: *in vitro* based on natural pear-based media (NPBM) obtained from *Vacchesa* and *Sarmentina* accessions and from *Abate fetel* cultivar, and *in vivo* on artificially wound-inoculated fruit. *P. expansum* radial growth was monitored and hyphal morphogenesis observed by scanning electron microscopy (SEM) on pear fruit and media. A significant reduction of patulin accumulation was found in *Vacchesa* and *Sarmentina* accessions compared to *Abate fetel* using high-pressure liquid chromatography-mass spectrometry (LC-MS). qRT-PCR analysis of *patL*, *patN* and *patK* genes involved in the biosynthetic pathway of patulin was carried out. Analysis of patulin gene expression during *P. expansum* growth on PDA and NPBM evidenced a positive correlation between gene expression and patulin production. Comparative metabolic profiling of the endemic accessions and the national cultivar by high-field NMR spectroscopy provided useful data regarding the chemical classes that may be involved in the inhibitory activity. Based on these findings these two Sardinian pear accessions exert a higher inhibitory activity on pome blue mold decay growth and patulin production compared to the national *Abate fetel* pear cultivar.



## TRANSCRIPTOMIC APPROACH TO ELUCIDATE THE MOLECULAR MECHANISMS ACTIVATED BY *SPOROBOLOMYCES* SP. IN RESPONSE TO THE MYCOTOXIN PATULIN

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Patulin (PAT) is produced by *Penicillium expansum*, the causal agent of blue mold of stored pome fruits. This mycotoxin has genotoxic, teratogenic and immunotoxic effects, and its presence in pome fruits and derived products represents a serious health hazard. Biocontrol agents (BCAs) belonging to Pucciniomycotina red yeasts, such as *Rhodosporidium kratochvilovae* LS11 and *Sporobolomyces* sp. IAM 13481, are able to resist PAT and degrade it into the less toxic compounds desoxypatulinic acid and ascladiols. We previously applied a forward genetics approach and found that resistance of the BCAs to PAT toxicity is a crucial step for its degradation, which biochemical studies confirmed to be an inducible enzymatic process. In this work, we investigated the changes of gene expression in *Sporobolomyces* sp. exposed to PAT through a transcriptomic approach based on RNA sequencing (RNAseq). The majority of genes upregulated were those involved in oxidation-reduction process(es) and transport, thus suggesting that *Sporobolomyces* activates defense mechanisms to oxidative stress to resist PAT toxicity and expel the mycotoxin out of the cells. Other upregulated genes encoded transcription factors and proteins involved in glutathione and methionine biosynthesis. Conversely, PAT treatment decreased the expression of genes involved in the processes of protein synthesis and modification, such as those involved in transcription, RNA processing, translation, protein phosphorylation and biosynthesis of amino acids. This indicates a reduction of metabolic activity probably due to the high energy requirement of the yeast cells, which need to recover from insult caused by PAT to overcome its toxicity. Although PAT degradation needs to be further investigated through gene/protein discovery, this study outlines the complex mechanisms activated by a BCA in response to the mycotoxin and set the basis for i) the biodegradation of PAT in fruit juices, and ii) the development of a user-friendly biosensor for its rapid and cost-effective detection.





## EFFECTS OF SPRAY PROGRAMMES IN VINEYARD AND DIFFERENT TYPE OF SUN-DRYING SYSTEM ON FORMATION OF OCHRATOXIN A ON RAISINS

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Turkey is the biggest raisin producer and exporter country in the world. The major problem on raisins is not only residue on but also ochratoxin A produced by fungi. OTA, which is common in raisins, is a mycotoxin produced by two main genera of fungi, *Aspergillus* and *Penicillium*. In this study, effects of preharvest spray Programmes in vineyards and different type of sun-drying systems on formation of OTA on raisins in Manisa, Sarigöl. The spraying Programme, farmer's Programme and control were compared in three separate parcels. The spraying Programme was started after the last fungicide applications for powdery mildew in the vineyard. After each fungicide application, microbial load were tested on fresh field-treated grapes. All grapes were dried in concrete and soil ground under the open sun after harvesting. In the other group, the grapes were harvested and stored as fresh with SO<sub>2</sub> and without SO<sub>2</sub> in cold storage conditions for two months. Disease assessments and quality parameters were analyzed in bunches in vineyard also. Raisins were stored in normal storage conditions and in cold storage room in the sacks and polyethylene bags for 8 months. It was found that after analyses of amount of OTA was found below the legal limits at the beginning and the 2nd month of the study in dried grapes. Generally, preharvest fungicides applications was reduced the growth of *Aspergillus* spp. *Aspergillus* spp. population were found very high on dried in soil ground.

## PATULIN AND PATULIN-PRODUCING *PENICILLIUM* SPP. IN APPLES AND APPLE-BASED PRODUCTS MARKETING IN QATAR

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In this study, forty-five samples of undamaged fresh apple fruit, apple juice and apple-based baby food products sold in different markets in Qatar were surveyed for both fungal and patulin contamination. Twenty-five *Penicillium* spp. isolates were selected, including 23 *P. expansum* and one isolate each of *P. brevicompactum* and *P. commune*. All the tested *Penicillium* spp. isolates produced patulin *in vitro* (from 40 to 100 µg/g of malt yeast extract agar medium). Patulin was detected by LC/MS/MS methodology in all 20 tested apple juice samples at levels ranging from 5.3 to 82.2 µg/L. Only 5 samples contained patulin levels higher than the European Union (EU) maximum limit (50 µg/kg). The average patulin contamination was 30.7 µg/L and 10.9 µg/L in baby apple juice and in baby apple compote, respectively (EU maximum level: 10 µg/kg). Our results indicate that the incidence of patulin in apple juice does not represent a serious risk for the adult consumer since the mean level of contamination is below the limit recommended by the EU. However, the significant contamination of apple-derived baby food (juice and compote) marketed in Qatar constitutes a matter of concern. We recommend that a high level of awareness of the protection of infant and children groups is needed and that strict measures to control the quality of baby apple food imported in the Gulf Countries must be taken.

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## EFFECT OF *KLUYVEROMYCES THERMOTOLERANS* ON *SACCHAROMYCES CEREVISIAE* DURING FERMENTATION PROCESSES

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Fungal diseases are one of the main reasons for economic losses in viticulture. *Aspergillus* section *Nigri* species have been reported as agents of black grape rot. Antagonistic microorganisms are a sustainable alternative to synthetic fungicides. In previous studies (Ponsone et al., 2013) showed that two yeast strains, *Kluyveromyces thermotolerans* RCKT4 and RCKT5, inhibited *Aspergillus* growth and decreased OTA accumulation in wine grapes. However, there are no data about the effects of *K. thermotolerans*, potential biofungicides, during oenological fermentations. The aim of this work was to evaluate the impact of two selected potential biofungicides in mixed cultures with *S. cerevisiae* BSc203 (an oenological strain) during microfermentations. Pure and mixed cultures (1%BSc203-99%biofungicide; 50%BSc203-50%biofungicide; 99%BSc203-1%biofungicide) were inoculated in commercial must. All fermentation samples were periodically withdrawn and spread on WLN-Agar plates ( $\text{Log}_{10}\text{UFC/ml}$ ), in which both strains colonies can be differentiated. In co-cultures *Saccharomyces* (BSc203)/*Kluyveromyces* (RCKT5, RCKT4), BSc203 yeast concentration was no significantly different to pure BSc203 concentration, except in co-culture 99%RCKT4-1%*S. cerevisiae*, at the end of fermentations (22 days). Cells concentration of BSc203 (in 99%*K.th.*/1%*S.c.*) was 1.38  $\text{Log}_{10}$  cycle lower than in its pure culture. In all mixed cultures assayed basic quality of wines obtained were according to OIV (2013). Biofungicide yeasts did not affect the fermentative process. Conclusions: *K. thermotolerans* RCKT4 affected BSc203 growth when was inoculated in high proportions. RCKT5 did not affect *S. cerevisiae* growth in all conditions assayed.

## SEED-BORNE MYCOFLORA AND MYCOTOXINS OF POSTHARVEST WHEAT IN SAUDI ARABIA

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One hundred samples of wheat grains were collected in 2013 and 2014 from wheat-cultivated fields at different governorates in Saudi Arabia and screened for their seed-borne mycoflora. A total of 28 genera and 53 species of fungi were recovered from the collected samples using agar plate (AP) and standard moist blotter (SB) methods. The two methods differed as regards the frequency of recovered seed-borne fungi. SB technique effectively detected the seed-borne saprophytes e.g., *Rhizopus stolonifer* (68%), *Penicillium* spp. (61%), *Aspergillus flavus* (57%) and *A. niger* (29%). *Alternaria alternata*, *Ulocladium* spp., *Cladosporium* spp., *Penicillium* spp. and *Bipolaris* spp. were the most abundant. *Fusarium verticillioides* and *F. chlamydosporum* were the most dominant species among all *Fusarium* species (39.5, 35.5% in SB and 40, 37% in AP techniques), followed by *F. graminearum* and *F. incarnatum*. Obtained results revealed that wheat grains were infected with several pathogenic fungi such as *A. alternata*, *B. sorokiniana*, *F. verticillioides* and *F. graminearum*. On the other hand, *Ustilago tritici* was the most commonly observed smut fungus on wheat samples. Using seed washing technique, 92 samples were found to be infected with loose smut (chlamydospores). The distribution of wheat seed-borne fungi was also investigated throughout the sampling area. In this concern, Al-Riyadh, Al-Jouf and Tabuk governorates recorded the highest incidence of seed-borne mycoflora of wheat. Mycotoxins such as aflatoxins (AFB1, AFB2 and AFG1, AFG2), ochratoxin A (OTA), zearalenone (ZON), deoxinivalenol (DON) and fumonisins (FB1 and FB2) were also assessed in wheat samples. The results showed that many mycotoxins such as aflatoxins, ochratoxin A, zearalenone, deoxinivalenol and fumonisin FB2 were detected and the fumonisin FB1 were present. Therefore, there is a serious need to increase public awareness on aspects related to seed health to develop suitable management's practices for improving the quality of wheat grains.



## **PATHOGENICITY AND MYCOTOXIGENICITY OF *PENICILLIUM EXPANSUM* AND *P. GRISEOFULVUM* ON TEMPERATE FRUIT**

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Blue mould is the most common postharvest disease of cold stored apples and pears. Besides *P. expansum*, *Penicillium griseofulvum* has been reported as agent of blue mould on apple in the United States, Brazil, and Italy. Both *P. expansum* and *P. griseofulvum* can be producers of patulin. The aim of the present study was to evaluate the virulence of eight strains of *P. expansum* and two strains of *P. griseofulvum* on 17 species and cultivars of pome and stone fruit. Also the capacity to produce patulin on the same fruit species was assessed. Compared to *P. expansum*, the strains of *P. griseofulvum* showed a higher preference for apples. The virulence of *P. griseofulvum* strains on pear, peach, plum, and apricot was significantly lower than that of *P. expansum*. The results obtained permit to hypothesize a broader spectrum of hosts for *P. expansum*, and a specific host range for *P. griseofulvum*, restricted to apple. Virulent strains produced larger rots on all the species and cultivars. Accumulation of patulin in infected apple tissue and blue mould of eight strains of *P. expansum* tested on four cultivars of apple were negatively correlated. Very high values of patulin contamination corresponded to very low virulence on apples. Moreover, the most virulent strains of *P. expansum* produced lower levels of patulin, and the less virulent strains of *P. expansum* were the highest patulin producers. Though not as strongly pathogenic as *P. expansum*, the strains of *P. griseofulvum* were high patulin producers, particularly on apples and on apricots 'Aurora'. Also stone fruit, and particularly apricots and peaches, were susceptible to patulin contamination. In conclusion, besides *P. expansum*, *P. griseofulvum* can be a significant patulin producer on apple, and *P. expansum* can contaminate other fruit species, including stone fruit.

## PLANT FOOD SUPPLEMENTS AND FOOD COLORING AGENTS DERIVED FROM *VITIS VINIFERA*, A NEW SOURCE OF HUMAN EXPOSURE TO OCHRATOXIN A

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Ochratoxin A (OTA) is an extremely harmful mycotoxin, having nephrotoxic, immunosuppressive, teratogenic and carcinogenic properties. *Aspergillus carbonarius* was identified as the main species responsible for OTA accumulation in grape and derived products. During winemaking, 95% of OTA originally present in grapes remains adherent onto pomaces, the main by-product. Grape pomaces are increasingly being used as starting material in the industrial production of plant food supplements (PFS), food colouring and tartrates. The occurrence of OTA in commercially available PFS (24 samples), food colouring (13 samples) and leavening agents containing tartrates (4 samples), all derived from *Vitis vinifera* was investigated in this study by using an improved HPLC-FLD method. The occurrence of OTA in 32 samples of grape pomaces collected from vineries of Apulia and Basilicata during 2013 and 2014 was also investigated. OTA was found in 75% of commercial PFS samples and 69% of food colouring samples at levels of 0.50–20.23 µg/Kg and 0.50–32.00 µg/kg, respectively. The four commercial leavening agents containing tartrates were negative for OTA. Ninety-six percent of grape pomaces samples collected in 2013 contained OTA at levels of 3.60–140.90 µg/kg. All samples collected in 2014 contained OTA at level of 2.80–47.30 µg/kg. Higher levels of OTA (up to 849.10 µg/kg) were measured in samples of grape pomaces collected in Apulia in the past. The high incidence of positive samples as well as the high variability of OTA levels in grape pomaces makes it imperative to check the OTA level in this starting material if used for production of PFS and food colouring agents. Maximum permitted level(s) of OTA should be established in commercial PFS and food colouring agents derived from *V. vinifera* due to the high incidence of OTA contamination in these products.





## T-2 TOXIN INDUCED RESISTANCE AGAINST DRY ROT IN POTATO TUBERS BY ACTIVATING REACTIVE OXYGEN SPECIES AND PHENYLPROPANOID METABOLIC PATHWAY

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Dry rot, caused by *Fusarium* spp., is one of the most important postharvest diseases of potato tubers. Our previous results showed that the trichothecenes, Fus-X, 3ADON, DAS and T-2, were found accumulation in dry rotten tubers. In general, these trichothecenes are thought to be a virulence factor, but little is known about effect of T-2 on the defense response in hosts. In this study, the effects of T-2 toxin treatment at 1 µg/g on induced resistance were investigated against dry rot in potato tubers (cv. Longshu No.3) inoculated with *F. sulphureum*. The results showed that T-2 toxin significantly reduced lesion diameter of inoculated tuber slices, and stimulated H<sub>2</sub>O<sub>2</sub> and generation O<sub>2</sub>, simultaneously enhanced the related-enzymatic activities of reactive oxygen species scavenging system, including oxidized glutathione (GSSH), glutathione (GSH), ascorbate peroxidase (APX), ascorbic acid (ASA), dehydroascorbate (DHA) and glutathione reductase (GR). Meanwhile, T-2 toxin treatment increased the related-enzymatic activities of the phenylpropanoid metabolic pathway such as phenylalanine ammonia-lyase (PAL) and 4-coumarate CoA ligase (4CL), and the contents of the related-metabolic products including total phenolics, flavonoids and lignin in treated tubers. These results suggested that T-2 toxin at low concentration could be as an elicitor to induce resistance against dry rot of potato tubers by activating ROS and phenylpropanoid metabolic pathway.



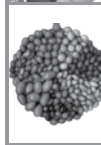
## CHEMICAL AND PHYSICAL METHOD TO REDUCE AFLATOXIN CONTAMINATION IN DRIED CHILI PRODUCTS IN THAILAND

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Aflatoxin contamination due to *Aspergillus flavus*, is the major problem of dried chili in tropical countries, like Thailand. The simple and applicable procedures for dried chili producers and farmers are urgently needed. Two experiments with 2 chili varieties were investigated during dry season between January and April and during rainy season between May and August 2014. Fresh chili fruits, cv. 'KKU#2', the improved cultivar of Plant Breeding Research Center for Sustainable Agriculture KKU, and cv. 'Super Hot', the commercial variety of East West Seeds Co., Ltd., were harvested at ripe mature stage. For the 1<sup>st</sup> experiment in dry season, fresh fruits were treated with the chemical by soaking in a commercial bleach solution, Sodium hypochloride (NaOCl), 0 (control treatment), 0.25, 0.5, 0.75 and 1%, for 5 minutes and rinsed with running water for 10 minutes. On the 2<sup>nd</sup> experiment in rainy season, fresh fruits were treated with physical method by soaking in boiling water (100°C) for 0 (control treatment), 1, 2 and 3 minutes. The treated fruits of both experiments were sun-dried and oven-dried at 80°C thereafter. Dried fruits were stored in plastic bag at room temperature for 3 months. Aflatoxin and pungency contents as well as color of dried products were monthly determined. The results showed that both chemical and physical methods did not affect pungency contents of dried chili products. Chemical treatment did not affect color while physical method enhanced the darker red color of dried chili products than control treatment. Nevertheless, all treatments used did not clearly reduce the contents of aflatoxin. Thus the effects of season on drying and period of time for chemical soaking is currently discussed. Besides, the appropriate concentration of NaOCl is 0.5 - 0.75%, while soaking time in boiling water is 2 minutes.



## BLUE MOLD ON POME FRUIT IN SOUTHERN ITALY AND CHARACTERIZATION OF ASSOCIATED *PENICILLIUM* SPP.

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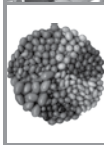
Blue mold is one of the most important postharvest diseases of pome fruit in all producing countries. Its causal agent, *Penicillium expansum*, is also known to produce the mycotoxin patulin, with mutagenic, immunotoxic, and neurotoxic properties. Aims of the present study were to identify *Penicillium* isolates associated with blue mould decay of pome fruits in Apulia region (South Italy), verify if their genetic potential to produce patulin corresponded to actual toxin contamination, and compare their *in vitro* and *in vivo* toxigenicity. Twenty-nine isolates of *Penicillium* spp. were recovered from apples and pears with blue mold symptoms. Fruits were analyzed for patulin content and results were compared with *in vitro* toxin production. In general, patulin production was more conspicuous *in vivo* (particularly on Golden Delicious apples) than *in vitro*, although the stronger *in vivo* producer did not correspond to the stronger *in vitro* producer. Isolate identification was based on both morphological characters and DNA analysis by PCR amplification with *P. expansum* species-specific primers and sequencing of beta-tubulin gene. Furthermore, fungal isolates were tested for the occurrence of gene (*patN*) coding the enzyme isoeopoxydon dehydrogenase (IDH), involved in the patulin metabolic pathway and considered an useful indicator of critical control points for patulin contamination. All 25 isolates identified as *P. expansum* were *patN* and patulin production positive. Moreover, 4 pear isolates belonging to other *Penicillium* spp. were found, whose identification is being confirmed. They were positive for the *patN* gene, but only two actually produced patulin. It can be concluded that blue mold of pome fruits in Apulia is mainly associated with toxigenic *P. expansum* isolates, thus a rapid detection is important to avoid patulin contamination beyond the regulatory limits. Nevertheless, it seems that *patN* gene alone cannot be considered a predictive assay for production of patulin. An evaluation of its expression level should be carried out.

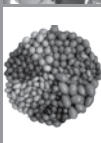
## CHARACTERISATION OF FUNGAL PATHOGENS ASSOCIATED WITH STEM-END ROT OF AVOCADO FRUIT IN ITALY

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The Sicilian coasts provide suitable environmental conditions for production of high-quality tropical and subtropical fruits. In particular, avocado (*Persea americana*) and mango (*Mangifera indica*) orchards increased in last years on this area. Postharvest infections of tropical and subtropical fruits commonly occurs wherever the crops are cultivated. Several fungal species are reported as causal agents of anthracnose and stem-end rot. Among these, *Botryosphaeria* spp. and *Colletotrichum* spp. are the mostly spread worldwide. In Mediterranean environment, decay caused by several fungal pathogens are reported on plants and fruits of mango, but extensive surveys on avocado orchards were never done. The aims of this study were to determine the occurrence of stem-end rot disease in one of the major avocado growing areas in southern Italy and to identify the fungal species associated with fruits symptoms basing on morphological and molecular analysis. Approximately 100 avocado fruits cv. Hass were collected in four orchards in Catania province and incubated in laboratory. Stem-end rot developed from 5 to 10 days. Small pieces of symptomatic flesh from the margin of infected area were placed onto potato dextrose agar. A total of 47 isolates were recovered. Conidia characteristics and colony morphology were determined. Multilocus sequences analysis was performed using beta-tubulin gene, internal transcribed spacers of the ribosomal DNA and translation elongation factor gene (for *Botryosphaeria* spp.) or histone 3 gene (for *Colletotrichum* spp.). The molecular analysis allowed the identification of 68% of isolates as *Neofusicoccum parvum*, 17% as *Colletotrichum gloeosporioides* and 15% as *C. fruticola*. To our knowledge, these are the first data on occurrence of *N. parvum*, *C. gloeosporioides* and *C. fruticola* associated with stem-end rot of avocado in Europe. Further studies on pathogenicity ability of these species, in pre and postharvest conditions, should be carried out.





## CHANGES IN ANTIOXIDANTS COMPONENTS DURING COLD-STORAGE ON APPLE (*MALUS DOMESTICA*, CV. GRANNY SMITH) GROWN UNDER DIFFERENT SOLAR RADIATION

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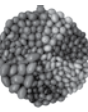
The intensity of solar radiation affects the antioxidant system of the fruit tissues. High solar radiation increases oxidative metabolism generating an excess in Reactive Oxygen Species (ROS) that, if not effectively detoxified by the cell defense systems, leads to membrane peroxidation, tissue senescence and general disorders. In this study, changes in flesh firmness, ethylene production rate (ERP), oxidative stress and evolution of the antioxidants response were evaluated at harvest and during cold storage (0, 90 and 150 days) of apple peel tissues that were developed exposed and non-exposed to high solar radiation. Flesh firmness of exposed tissues (ET) was higher than in non-exposed tissues (NET) and in both cases decreased during cold storage, whereas ERP increased and no significant difference ( $P < 0.05$ ) was detected between different tissues. The chlorophyll concentration at harvest was  $28.5 \mu\text{g}\cdot\text{g}^{-1}$  FW in ET and  $62.1 \mu\text{g}\cdot\text{g}^{-1}$  FW in NET and decreased during storage reaching 21.1 and  $58.6 \mu\text{g}\cdot\text{g}^{-1}$  FW, respectively; the same pattern for Photosystem II efficiency (Fv/Fm) was observed. The content of Thiobarbituric acid reactive substances (TBARS) was higher in ET at harvest; however, it increased in both tissues during cold storage and the value was lower in ET. Antioxidant activity (DPPH radical-scavenging) and Superoxide dismutase (SOD) and Ascorbate peroxidase (APX) activities were higher in ET. SOD activity at harvest was  $38.5 \text{ UI}\cdot\text{g}^{-1}\text{g FW}$  in ET and  $4.71 \text{ UI}\cdot\text{g}^{-1}\text{g FW}$  in NET, and increased to 352.8 and  $13.8 \text{ UI}\cdot\text{g}^{-1}\text{g FW}$ , respectively, after 150 days of storage. APX activity was 9.65 and  $3.28 \text{ UI}\cdot\text{g}^{-1}\text{g FW}$  at harvest in ET and NET, and increased to 12.6 and  $6.36 \text{ UI}\cdot\text{g}^{-1}\text{g FW}$ , respectively, during storage. These results indicate that apple tissues exposed to solar radiation increase antioxidant cell defenses systems, which persist during cold storage.

## CHARACTERIZATION OF *DIAPORTHE* SPECIES ISOLATES CAUSING POSTHARVEST ROT ON KIWIFRUIT CV. HAYWARD DURING COLD STORAGE IN CHILE

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Species of *Diaporthe* have a worldwide distribution causing diseases on a wide range of hosts, including seeds, crops, ornamental, forest and fruit trees. Recently, species of *Diaporthe* were identified from postharvest rot of kiwifruit during cold storage in Chile. The objective of this study was to characterize isolates of *Diaporthe ambigua*, *D. australafricana*, *D. novem* and *D. rudis* by the production of reproductive structures (perithecia and pycnidia), radial growth at different temperatures, virulence on mature kiwifruits cv. Hayward, and in vitro sensitivity to fungicides as Benomyl, Tebuconazole and Pyraclostrobin by reduction of radial growth as measured by ED50 values. The results obtained indicate that *D. rudis* and *D. australafricana* produced high number of perithecia and pycnidia on stem alfalfa and pine needle when compared against to *D. ambigua* and *D. novem*. The isolates of *D. ambigua* grew at temperatures between 5 and 35°C, whereas that the isolates of *D. australafricana*, *D. novem* and *D. rudis* grew between 5 and 30°C. All the species grew at 0°C on PDA, when were stored for 120 days, but at different growth rates. Mature Hayward kiwifruits were susceptible to the infection at 0°C being *D. ambigua* isolates, the most aggressive. All species of *Diaporthe* were highly sensitive to the three fungicides, obtaining low ED50 values of <0.008 µg a.i./mL for Benomyl, <0.001 µg a.i./mL for Pyraclostrobin, and <0.140 µg a.i./mL for Tebuconazole. These results suggest that isolates of *D. ambigua* has a different fitness than other species of *Diaporthe* isolated from kiwifruit rot.





## FUNGI CAUSING ROT OF CACTUS PEAR FRUIT IN SOUTHERN ITALY

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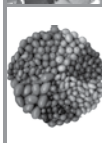
In this study, *Alternaria alternata* and *Macrophomina phaseolina* are reported for the first time as causal agents of post-harvest decay of fully-ripe fruits of cactus pear cv. Sulfarina in southern Italy. The species were identified by sequencing the ITS, GAPDH and TEF1-alpha gene DNA regions. To fulfill Koch's postulates, *A. alternata* and *M. phaseolina* were inoculated on fruits of cactus pear cv. Sulfarina. The symptoms observed on fruits with natural infections were reproduced and the inoculated fungi were reisolated only from symptomatic fruits. *A. alternata* induced a dry rot at 5°C but did not cause symptoms at 20°C, while *M. phaseolina* induced a basal soft rot on fruits both at 5 and 20° C. Dry rot caused by *A. alternata* is a relevant postharvest problem for cactus pear fruits during cold storage while the decay caused by *M. phaseolina* has been observed only sporadically.

## EFFECT OF IRRIGATION MANAGEMENT ON FIELD AND POSTHARVEST QUALITY OF ORGANIC TABLE GRAPES

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Irrigation management is considered as a key element that contributes to enhance and sustain grapevine health and improve crop productivity and quality. Actually, a Regulated Deficit Irrigation (RDI) strategy can modify vine canopy microclimate, through control and reduction of vegetative growth, providing a good air circulation and sunlight penetration. The present study evaluates the effect of two different water regimes (V1 and V2, corresponding to 100 and 80% of estimated vine evapotranspiration, respectively) on vine health, berries' growth and postharvest quality of organic table grapes (cv. Italia). To the purpose, field and postharvest surveys were conducted. In particular, during cold storage in semi-commercial conditions, the development of rots, as well as microbiological and organoleptic characteristics of the berries, were weekly assessed. Results showed that V1 favored vine development and induced an increase in vegetative and productive growth, while V2 appeared sufficient to achieve a complete table grape development. In addition, the titratable acidity did not change between the two irrigation types, but V2 berries have higher penetration index. This higher total berry compactness resulted in a lower susceptibility to abiotic and biotic stresses. The presence of fungi, mainly *Penicillium* spp., *Botrytis cinerea*, *Cladosporium* spp. and *Aspergillus* spp., showed some fluctuation over time, with a greater incidence on V1 berries than on V2 ones. In general, the disease index had an upward trend during the various assessments. Concluding, the V2 (RDI) could provide satisfactory results concerning overall organic table grape quality. Moreover, reducing water demand for irrigation, the saved water can be diverted for alternative uses.





# POSTHARVEST PHYSIOLOGY OF *VERBENA BONARIENSIS*

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Fernando Luiz Finger<sup>1</sup>

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The substitution of exotic ornamental species for native species is a trend in landscaping. *Verbena bonariensis* has a great potential as a native ornamental specie. The use of native plants provides environmental benefits due to the requirement for maintaining resources and wild species have highest resistance to pathogen infection. In general, microorganisms can adapt to different conditions affecting the shelf life of flowers. This study evaluated post-harvest variables stems of *V. bonariensis* to determine the useful life and possible prevalent infestations. Flower stems were collected on the UFOP campus, Brazil (20°23'47.71"S, 4330'28.20"W), voucher specimen OUPR26753, in the morning and patterned on 30cm stems that were placed in containers with 40mL of distilled water, with eight replications. The post-harvest analyses consisted of relative fresh weight, rate of absorption of vase solution, evaluation the number of open flowers, longevity and xylem blockage. Data were analyzed for 15 days. The diameter of the stems was  $1.275 \pm 0,1669\text{cm}$  (10 cm above the base), average weight  $3.3233 \pm 0,5326\text{g}$ , inflorescence number of  $40 \pm 14$ . During the experiment the ambient average temperature was  $21, 33 \pm 1,16^\circ\text{C}$  and relative humidity of  $75.67 \pm 7.10$ . The floral abscission is growing with is maximum in 4<sup>th</sup> day, down 47,5%, keeping stable throughout the experiment. Fading flowers (lilac color to violet) occurs from the 7<sup>th</sup> day, where was greater absorption of vase solution and consequent physiological stress. The xylem blockade began on the 4<sup>th</sup> day with peak on the 15<sup>th</sup> day with total occlusion. On the 7<sup>th</sup> day were visualized aphids and black fungus. After 10 days of experimental conduction occurring maturation of seeds. It can be concluded that the prevailing aphid infestations does not preclude commercialization of stems of *V. bonariensis*, because the appearance occurs in the senescence.

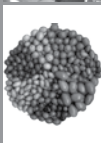
## POSTHARVEST PHYSIOLOGY OF STEMS FLORAL OF *AGERATUM CONYZOIDES*

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*Ageratum conyzoides* L. is native to tropical America, distributed in tropical and subtropical areas of the world and has high allelopathic and ornamental potential. The flowers are arranged in terminal inflorescence in corymb, variable color between white, pink to purple. The objective of this study was to investigate the life of cut stems of *A. conyzoides* in aqueous solution in pot, evaluating possible recurring infestations and the marketing of life as ornamental flowers. The species was collected on the campus of UFOP, Brazil, in november 2011. Postharvest analysis consisted of weight relative; absorption rate of the vase solution; transpiration rate; assessing the number of open flowers; and longevity of floral stems (22 cm), with 21 inflorescences placed in 20mL of distilled water for 21 days. Each experimental unit will consist of one plant per pot evaluated over time. In fresh weight on analysis, the average weight of the buds was  $3.4609 \pm 0.5495$ g. The average final weight of the rods was  $2.2414 \pm 0.5633$ g, a decrease of 32.55% of weight [ $\hat{y} = -0.0059x^2 + 0.0706x + 3.3282$  ( $R^2=0.9861$ )]. The average diameter of the stems was  $1.3250 \pm 0.1669$ mm. The open inflorescence number of analysis of variance was expressed by the equation  $\hat{y} = 0.0354x^2 - 0.486x + 14.803$  ( $R^2=0.923$ ). In the absorption rate of vase solution was symmetrical behavior as a function of time and the decrease of absorption related to blockade of the xylem. The transpiration rate was 4.0; 1.86 and 1.6 times lower than the original, on the 15th day and senescence, indicating adaptability of the stems to the water environment. The longevity of *A. conyzoides* was high (10 days). Systematic xylem occlusion was represented regression equation  $\hat{y} = 0.5054x - 0.7865$  ( $R^2=0.9921$ ). During this experiment, were not observed weed agents on the stems or flowers.





## DETERMINATION OF RESISTANCE LEVELS OF THE *BOTRYTIS CINEREA* ISOLATES AGAINST SOME FUNGICIDES AND THEIR MOLECULAR CHARACTERIZATION ON GRAPES IN AEGEAN REGION

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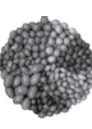
*Botrytis cinerea* is the most destructive pathogen on grapes. The usage of very intensive fungicides to control the disease causes risk of acquiring the pathogen resistance. In this study, *Botrytis cinerea* isolates were collected from the vineyards in the Aegean Region. Isolates were tested for their sensitivity to fungicides (pyrimethanil, iprodione, fenhexamid, cyprodinil+fludioxonil, boscalid) *in vitro* and *in vivo* conditions. Genetic differences between these isolates by molecular methods were also determined with specific primers. According to ED<sub>50</sub> value of fungicides pyrimethanil and iprodione were found to low effective while fenhexamid and cyprodinil+fludioxonil were highly effective against mycelial growth of isolates. The efficacy on spore germination of *B.cinerea* isolates was determined at different doses of the fungicides. Cyprodinil+fludioxonil at the dose of 3 µg/ml inhibited spore germination of all *B.cinerea* isolates. It was observed that pyrimethanil did not inhibit mycelia growth of 50% of *B.cinerea* isolates and iprodione 70% in the dose range of 5-10 µg/ml. Both *in vitro* and *in vivo* conditions it was determined that cyprodinil+fludioxonil mixture was the most effective active ingredients to inhibit mycelial growth of isolates. All isolates had a point mutation (G143A). Therefore it was detected with allele-specific primers (BcAR-F; 5'-GGC AAA TGT CAC TGT GAG C-3', BcAR-R; 5'-ACC ATC TCC ATC CAC CAT ACC T-3'). Furthermore, to determine the genetic differences among *B.cinerea* isolates molecular analysis was carried out. In seven isolates of *B.cinerea* were obtained band 604 bp in gel electrophoresis of PCR products.

## SURVEYS FOR *MONILINIA* SPP. ON STONE FRUITS IN CENTRAL-EASTERN ITALY

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The brown rot disease caused by *Monilinia* spp. is a serious disease that leads to significant losses on stone and pome fruits, both before and after harvest. Three species can be agent on brown rot on stone and pome fruits: *M. laxa*, *M. fructigena* and *M. fructicola*. Up to few years ago, *M. laxa* and *M. fructigena* were widespread in Italy, the first one mainly on stone fruits and the second one on pome fruit. In 2009, it was reported in Italy the presence of *M. fructicola*, that is particularly aggressive and it is listed as quarantine pathogen in Europe. Therefore, during this work we surveyed the area to isolate and identify the species of *Monilinia* involved in brown rot of stone fruits in Central-eastern Italy. During the period 2011–2015, samples from decayed stone fruits were collected from commercial fields and local markets of Marche region, central-eastern Italy. Isolates were morphologically characterized, and identified by polymerase chain reaction (PCR). Most isolates were identified as *M. laxa*. However, few isolates of *M. fructigena* and *M. fructicola* from sweet cherries were also found, last one in several samples collected during 2013. This finding is useful to report the presence of *M. fructicola* on sweet cherry in the Marche region. Further investigations are needed to assess the actual distribution of this pathogen in central-eastern Italy.





## THE OCCURRENCE OF *NEOFABRAEA* SPP. IN 'PINOVA' APPLES CAN BE REDUCED BY INCREASED STORAGE TEMPERATURE

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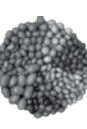
The apple variety 'Pinova' is highly susceptible to fungal decay, mainly caused by *Neofabraea alba* and *N. perennans* (syn. *Gloeosporium* spp.). The aim of this work was to evaluate the influence of different storage temperatures in combination with modern storage technologies on the occurrence of fungal diseases in 'Pinova' apples. The experiment was conducted over five years and carried out in three identical storage rooms (à 11 tons) at the Competence Center for Fruit Growing - Lake Constance, comparing ULO conditions (1.0kPa O<sub>2</sub> + 2.5kPa CO<sub>2</sub>) at 1°C with ULO at 3° to 5°C in combination with 1-MCP, or with dynamic controlled atmosphere (DCA; ~0.5 kPa O<sub>2</sub> + 2.5 kPa CO<sub>2</sub>), controlled by chlorophyll fluorescence, at 1° and 3°C. After 6 to 7 months storage four bins (~ 300kg) per treatment were classified in rotten or healthy fruit using a fruit grading line with manual touch sensors. Results show a significant reduction of fungal decay in higher storage temperatures (3° to 5°C) regardless of storage condition (ULO, ULO + 1-MCP or DCA) and compared to storage at 1°C. At the same time increased storage temperature had no negative influence on fruit quality. As 'Pinova' is a slow softening variety firmness of the apples stored at recommended temperature of 1°C did not differ from 'Pinova' apples stored at 3°, 4° or 5°C. In conclusion, increased storage temperatures might be an effective way to reduce the occurrence of *Neofabraea* spp. and to maintain fruit quality during storage of 'Pinova' apples. Additionally increased storage temperatures provide a significant reduction of energy consumption during storage, as multi-year experiments have shown.

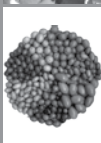
## DEVELOPMENT AND APPLICATION OF A TAQMAN REAL-TIME PCR ASSAY FOR RAPID DETECTION OF *PHYTOPHTHORA SYRINGAE* ON APPLES AND PEARS

Khanh Pham\*, Marcel Wenneker

Wageningen University & Research Centre, Applied Plant Research, Research Unit Fruit, P.O. Box 200, 6670 AE Zetten, The Netherlands. E-mail: marcel.wenneker@wur.nl.

*Phytophthora syringae* and *P. cactorum* commonly occur in apple and pear orchard soils and are the cause of a collar rot and fruit rot. The genus *Phytophthora* depends on free water at various critical stages of its life cycle, particularly for the production and dispersal of zoospores. The association of significant levels of *Phytophthora* fruit rot with wet weather is well known. Fruit infected early by *Phytophthora* spp. may develop symptoms before harvest. If the storage period is sufficiently long, comparatively low levels of primary rots, can give rise to substantial secondary rotting through contact spread during storage. Fruit infected by *Phytophthora* spp. near harvest may develop symptoms in storage only. Storage bins contaminated with soil particles from *Phytophthora* infested orchard soils could contaminate grading water when entering the water dump, and lead to infections of healthy fruits. Recirculated water in particular is likely to become contaminated with these disease spores. *P. syringae* is considered as a quarantine organism in a number of countries (e.g. China). Therefore, discriminating *P. syringae* from *P. cactorum* is important for exporting pears. The identification of *P. syringae* is not possible based on host symptoms alone because other *Phytophthora* spp. can produce similar symptoms. *P. syringae* has distinct morphological characteristics which allows to discriminate between other *Phytophthora* spp. when isolated in culture. It is, however, time-consuming to culture the pathogen from symptomatic plant material. Also, the identification of *Phytophthora* spp. based on its morphology requires specialist training and experience. The newly developed TaqMan PCR allows the detection of *P. syringae* in naturally infected material (fruits and stems) and substrates such as grading water of packing houses. PCR amplification was monitored in real-time and semi-quantitative detection was possible.





## POSTHARVEST LOSSES BY COMPLEX OF *PHYTOPHTHORA* SP. AND *BOTRYTIS CINEREA* IN LONG STORAGE PEAR FRUIT IN NORTH PATAGONIA, ARGENTINA

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In 2013-2014, economic losses by decay in Packham's Triumph pears were recorded in a packinghouse of the Alto Valle de Río Negro. Fruit had been treated against *Penicillium* spp. and *Botrytis cinerea* with captan and pyrimethanil, and stored into carton packets with nylon in controlled atmosphere. After 9 months, 64 packets with 5500 fruits corresponding to seven different lots were evaluated. Severe symptoms of decay on fruit were observed. The losses were quantified and the etiology of the disease studied. By observation of symptoms and microscopy, *B. cinerea* and *Phytophthora* sp. were identified. Koch's postulates and inoculation of single or combined pathogens in fruit were performed. Affected fruit came from orchards of two production's zones of Río Negro and Neuquén. Incidence percentage of this decay (% DI) in all fruit reached 15-20%. The most affected lots showed % DI distributed: (i) 1.3 - 3% of *B. cinerea*; (ii) 0.5 - 1.2% of *B. cinerea* and *Phytophthora* sp. and (iii) 1.2 - 1.6% of *Phytophthora* sp.. The fruit decay of the combined pathogens was firm to touch, practically mummified, with two distinct brown colorations, diffuse water soaked margins, and without formation of *B. cinerea* nests. Cultures of *B. cinerea* in APD-N and *Phytophthora* sp. in V-8 media showed typical characteristics of each pathogen. In laboratory, detection of *Phytophthora* in samples (71-75%) of orchard's soil with pear baits was verified. Since post-harvest diseases are a combination of latent infections settled during the growing season and post-harvest wounds, asymptomatic fruit taken 30d prior to harvest and at physiological ripeness was processed with ONFIT (overnight freezing incubation technique) detecting 5 and 2% of *Phytophthora* and 0-2% of *B. cinerea*. This is the first report for the region of post-harvest pear fruit decay caused by *B. cinerea* associated to *Phytophthora* sp. with detection of latent infections.

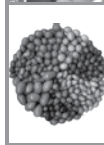


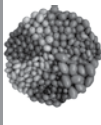
## CALYX AND STEM MOLD, AFFECTING PEAR FRUIT COSMETIC QUALITY: DIAGNOSTIC, ETIOLOGY AND MANAGEMENT STRATEGIES

**María Cristina Sosa<sup>1\*</sup>, María Cecilia Lutz<sup>1</sup>, Nadia Condoplo Lefort<sup>1</sup>, Lucas Vera<sup>1</sup>, Pamela Fagotti<sup>2</sup>**

<sup>1</sup>Fitopatología, <sup>2</sup>Tecnología de Frutas, IBAC-Facultad de Ciencias Agrarias, Universidad Nacional del Comahue, Km 11.5, Ruta 151, Cinco Saltos, Río Negro, Argentina. E-mail: mcristinasosa10@gmail.com

Argentina is the largest producer and exporter in the Southern Hemisphere of short, medium and long shelf life pears. During 2014's storage and retail, European markets claimed the presence of a white-grayish mold in calyx and stem affecting fruit's cosmetic quality. In Packham's Triumph and Beurre D'Anjou fruits, observation by light microscopy was performed, typical structures of *Alternaria* and *Cladosporium* spp. were identified. Koch's postulates were carried out. Fruits, with mold, which were incubated in moist chamber (22°C-7days) did not developed decay; however, in pathogenicity tests in pear fruits all isolates of *Alternaria* spp. were pathogenic. The hypothesis is that calyx and stem mold by *Alternaria* spp. and *Cladosporium* spp. originates in the orchard from spores that infect, and remain dormant until the tissues become senescent during storage. Hence, monitoring of the fungal micro flora of stem, sepals and fruit bottom (calyx) was performed. Both pathogens were from setting fruit, with prevalence in sepals of *Cladosporium* spp. (20%) at 15 days after full bloom (DAFB) and *Alternaria* spp. (76%) at 60 DAFB. In the search for putative management strategies, effectiveness of pyraclostrobin and boscalid (Bellis, Basf) was evaluated. This fungicide inhibited the mycelial development of *Alternaria* spp. (98%), and of *Cladosporium* spp (100%). In 2014-2015, to assess the effectiveness of these fungicides in controlling mold of pear fruit, they were applied in the orchard 7 days before harvest. Furthermore, the effect of disinfectants over cross contamination in pool immersion (line of packing) was evaluated. Peracetic acid was the best disinfectant since reduced the incidence of mold in about 33%, even 30ds at -1/0°C plus 7ds at 22°C. The calyx and stem mold of pear fruit by *Alternaria* and *Cladosporium* can be controlled using combined strategies pre and post-harvest that improves the cosmetic quality of the fruit.





## EFFECT OF PREHARVEST SOLAR RADIATION ON APPLE SKIN OXIDATIVE DISORDERS DURING COLD-STORAGE

**Laura I. Vita, Nidia F. Gonzalez, Graciela M. Colavita\***

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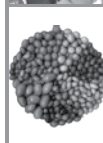
Sunscald and superficial scald are physiological disorders of apple that develop during low temperature storage. Sunscald is exclusively observed on sun-exposed section, while superficial scald is manifested on non-exposed skin. *Malus domestica*, cv. Granny Smith sun-exposed (ET) and non-exposed tissues (NET) were compared during 0, 90, 120, 150 and 180 days of cold-storage (DCS) in terms of sunscald and superficial scald incidence,  $\alpha$ -farnesene (AF) and conjugated trienes (CT), antioxidant activity (DPPH radical-scavenging), lipid peroxidation (thiobarbituric acid-reactive substances, TBARS) and maturity indices. After 90 DCS and 1 week at 20°C, sunscald was observed in ET and affected 41.4% of the fruits. Superficial scald was detected in NET and affected 66.5% of the fruits. At 180 DCS these disorders enhanced to 100%. In advancing storage, AF concentration increased. The highest level (125.5 nmol.cm<sup>-2</sup> and 95.8 nmol.cm<sup>-2</sup> for ET and NET, respectively) was measured at 120 days of storage, decreasing at 180 days. CT in ET were lower during all storage period, but increased significantly in NET after 90 days in consistent with superficial scald symptoms. Along all cold-storage DPPH was significantly higher ( $P<0.05$ ) in ET than NET. At harvest ET showed significantly more TBARS (59.8 nmol.g<sup>-1</sup>FW) than NET (43.8 nmol.g<sup>-1</sup>FW). During storage lipid peroxidation increased, and reached at 180 days, 101.8 nmol.g<sup>-1</sup>FW in NET and 75.5 nmol.g<sup>-1</sup>FW in ET. There were significant differences in fruit maturity between ET and NET. Flesh firmness and soluble solid concentration were higher in ET than NET throughout the sampling period. These results showed that alternative physiological oxidative process may be occurring in exposed and non-exposed tissues during cold-storage and the higher antioxidants activity found in sun-exposed tissue were ineffective in preventing sunscald.

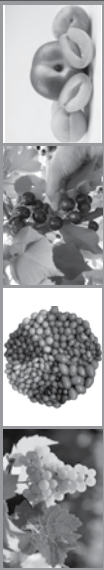
## MONITORING OF VOLATILE ORGANIC COMPOUNDS FOR EARLY DETECTION OF STORAGE DISEASES IN ONIONS

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Postharvest losses of onions are caused by spoilage from microbial infection, sprouting and mass loss after harvest. During storage, onions spoil when microorganisms grow and spread from already infested but often healthy-looking tissues, even without noticing. Early detection of diseases and physiological changes can thus minimise the economic losses if methods allow for remedial action. Methods based on emission of volatile organic compounds could provide a feasible solution for quality assurance during storage of onion if methods are related to detection of diseases. The objective of this work was to evaluate the volatile organic compound (VOC) method for early detection of diseases and postharvest changes of onions. In this study, one onion cultivar was harvested, dried and stored for 5 months at commercial conditions and then transferred to experimental conditions at a time when quality began to deteriorate due to disease development. Onions were incubated in glass jars during three months and lids were periodically closed for collection of VOCs. At the same time, visual quality of onions was evaluated. VOCs were extracted by solid-phase micro-extraction (SPME) and separated and identified by GC/MS. In preliminary experiments, the SPME-GC/MS technique could distinguish between the VOC profiles of healthy and slightly wounded onions stored at 20°C. Data on the VOC profile of stored onions will be presented and discussed in relation to disease development.





**DEVELOPMENT OF A METHOD FOR PROFILING OF VOLATILE ORGANIC COMPOUNDS TO MONITOR HEAT STRESS IN HEAT TREATED APPLES**

**Martin Himmelboe<sup>1\*</sup>, Alexandru Luca<sup>1</sup>, Renata de Paulo Rocha<sup>2</sup>, Marianne G. Bertelsen<sup>1</sup>, Merete Edelenbos<sup>1</sup>**

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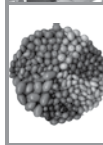
Storage rot is a major contributor to losses of organic apples due to restrictions in spraying in orchards. Postharvest pre-treatments such as hot water dipping (HWD) has been proven successful to reduce storage rot. However, excessive heat during dipping easily induces heat stress and physiological disorders which show up first after several weeks in storage. To further develop the HWD technology, a method for early detection of physiological disorders is desirable. Apples emit volatile organic compounds (VOCs) in response to stress and ripening which can be collected and measured on GC-MS. Several techniques have been developed for sampling of VOCs. Static headspace sampling is by far the simplest method but the method lacks sensitivity. However, the sensitivity can be greatly enhanced if the samples are analyzed in selected ion monitoring (SIM) mode as long as relevant compounds are included in the Programme from the beginning of the analysis. In this study, a SIM Programme was developed for analyzing VOCs from apples of cv. Ingrid Marie and Pinova dipped in water at 20 and 56 °C for 3 min. VOCs were sampled by solid-phase microextraction and analyzed on GC-MS in total ion current mode. From these results, a SIM Programme was developed which enabled detection of trace VOCs collected by static headspace sampling. The results showed that apples treated with 20 °C for 3 min differed in VOC profile from apples treated with 56 °C for 3 min, which all showed physiological disorders during storage. This work provides a solid basis for a future development of a VOC method for early detection of physiological disorders in apples caused by HWD and for improving the HWD technology to better control storage rot.

## GROWTH CONDITIONS INFLUENCE GROWTH AND VOC PRODUCTION OF ONION SPOILAGE FUNGI

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Onion is a perishable product with high post-harvest losses caused by mechanical injuries, improper curing, rotting, water loss and microbial spoilage. Important diseases, related with fungal spoilage, are *Botrytis* neck rot or grey mold rot caused mainly by *Botrytis allii*, black mold caused by *Aspergillus niger* and blue mold caused by *Penicillium* spp. However, timely detection of fungal contamination of onion bulbs is a difficult task, often leading to unreliable results. The use of volatile organic compounds (VOCs) as an early-warning tool to detect onion bulbs disease related to fungal proliferation may be a solution for spoilage monitoring during storage. The aim of this study was to evaluate the effect of medium (PDA: potato dextrose agar, OM: onion medium) and temperature (4°C, 15°C, 25°C) on growth and production of VOCs from selected spoilage fungi. Mycelial radial distribution was used as a measure of growth at each of the growth conditions. To evaluate the influence of temperature and media on the production of volatiles, 20-ml vials with PDA or OM were inoculated with 35 µl of a spore suspension and incubated at the respective temperatures. VOCs profiles were recorded (SPME/GC-FID and GC-MS) at 3, 7 and 12 days after inoculation. Fungal growth rate was strictly dependent on both temperature and medium. Preliminary VOC data indicates that unique volatiles are produced by the different studied spoilage fungi.





## EFFECT OF *MENTHA PIPERITA* AND *SYZYGIUM AROMATICUM* ESSENTIAL OILS ON POSTHARVEST QUALITY AND VASE LIFE OF *GERBERA JAMESONII*, CVS RED EXPLOSION AND PINK ELEGANCE, CUT FLOWER

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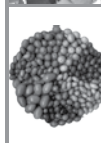
Concerns related to safety of chemical compounds and their bad effect on human and environmental being have increased the interest in using natural compound, such as herbaceous essence compounds, for controlling bacterial and fungal contaminations and reducing the wastage after harvest of Gerbera cut flower. The effects of *Mentha piperita* L. and *Syzygium aromaticum* (L.) Merr. & Perry essential oils on postharvest quality and vase life of Gerbera (*Gerbera jamesonii* Bolus) cut flower (cv Red explosion and Pink elegance) was evaluated using completely randomized factorial design with 16 treatments, 3 replications, and 5 flowers in each experimental unit. Treatments included *M. piperita* and *S. aromaticum* essential oils at 4 concentrations (0, 50, 100, 300 mg/l) which were used as mixture in plants' preservative solutions. Sucrose 4% was added to each preservative solution. Distilled water added with 4% sucrose was used as a control. The flowers were cut from a commercial greenhouse and immediately transferred to experiment place. The experiment conditions were: temperature  $22 \pm 1^\circ\text{C}$ , relative humidity 65-75%, normal light. The flowers were placed in flowerpots containing 400 ml of preservative solutions. The obtained results suggested that *M. piperita* and *S. aromaticum* had positive effects on flowers vase life so that most treatments groups had better results than the control one. The comparison of groups showed that the best life treatments were 11.5 and 13.0 for *M. piperita* L. and *S. aromaticum* in 100 mg/l and 300 mg/l, respectively. Parameters such as water absorption, fresh weight, and flower diameter increased in most treatments. *M. piperita* and *S. aromaticum* treatments were also very effective in reducing stem bending, antocyanin leakage, and ion leakage. The water content of petals was significantly affected by essential oils, being that of *S. aromaticum* the most effective in increasing petal's water content.

## INHIBITION OF POSTHARVEST DECAY OF SATSUMA MANDARIN DURING STORAGE BY NON-THERMAL PLASMA TREATMENT

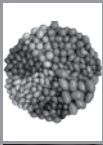
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The occurrence of postharvest decay in Satsuma mandarin (*Citrus unshiu* Marc.) is mainly effected by the infection of *Penicillium digitatum*, *P. italicum* and *Aspergillus flavus*. To prevent the postharvest decay of Satsuma mandarin, various treatments were introduced such as the chemical fungicide, CA and MA storage, etc. Activity of non-thermal plasma converts stable gas to ionized gas called a discharge or plasma. This ionized gas has the antimicrobial activity. Also, evaluation of plasma generator caused the economical and convenient application. This study was focusing on storage enlargement of Satsuma mandarin by non-thermal plasma at atmospheric pressure and room temperature. Total soluble solids and acidity of Satsuma mandarin were not showed significant difference between non-treatment(NT) and non-thermal plasma treatment(NTP) during 40-days storage period. The hardness of fruits treated by NTP was higher after 20 days. But weight loss of fruits treated by NTP was 2% higher during 40 days. The occurrence of decay was significantly lower in NTP during storage. Especially green and blue mold were occupied about 80% on NT, but the infection on NTP was less than 10%. That is, treatment of non-thermal plasma prevents postharvest decay caused by fungi and it is the efficacious alternative to extend the shelf-life of Satsuma mandarin.







## CONTROL OF ALTERNARIA DECAY OF SWEET ORANGE USING THYME EXTRACT AND THIABENDAZOLE

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In order to evaluate the effect of Thyme extract and Thiabendazole on postharvest decay of Valencia sweet orange was conducted an investigation in completely randomized design as factorial arrangement with four replications. The first factor was storage type including cool (4°C) and room temperature (25°C) storages and the second factor was treatment type including unwounded control; wounded control; wounded control inoculated by *Alternaria* fungus; Thyme extract 150, 300 and 450 mgL<sup>-1</sup>; and Thiabendazol 1.0, 1.5 and 2.0 gL<sup>-1</sup>. The wounded fruits were treated by extracts and fungicide separately and then were inoculated by *Alternaria* fungus. After treating, the treated fruits were kept in different storages for two months and then the percentage of decay, TSS, vitamin C and total acid were measured in them. According to the results, thyme extract could compete with Thiabendazole to control *Alternaria* decay and keep fruit quality.

## EFFECT OF SEVERAL PHENOLIC ACIDS OBTAINED FROM GRAPE POMACE ON HYPHAL GROWTH OF *BOTRYTIS CINEREA*

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*Botrytis cinerea*, also known as “gray mold fungus”, cause serious pre- and postharvest diseases in at least 200 plant species. The broad host range of *B. cinerea* results in great economic losses not only during growth but also during storage and transport of the Chilean export products. Traditionally, chemical control remains the main way to reduce the incidence of gray mold. However, in many countries the regulatory authorities have started to restrict the use of chemical pesticides due to generation of resistant isolates caused by the indiscriminate use of these compounds. In addition, use of synthetic fungicide has generated a negative public perception regarding the safety of pesticides. Based on the described previously, it is reasonable to assume that the development of a set of new alternatives to disease control is urgent and necessary. One approach to explore and to found new kind of fungicides could be to study phenolic compounds present in grape pomaces. Among them, catequin, caffeic acid, sinapic acid and *p*-coumaric acid are the most representative. The objective of this work was to evaluate the antifungal activity of catequin, caffeic acid, sinapic acid and *p*-coumaric acid on hyphal growth of *B. cinerea* and to study a possible action mechanism. The antifungal activity studies were assessed *in vitro* using the radial growth test on malt-yeast extract agar and the IC<sub>50</sub> value of the phenolic compound was calculated. The *p*-coumaric acid presented higher inhibitory effect than the other compounds. In addition, with the purpose to identify a possible action mechanism, apoptotic-gene expression studies were performed. The expression studies were implemented on specific apoptotic gene in presence of *p*-coumaric acid. The genes assessed were *Nma* that encode to protease related to apoptosis-induction and *Cas-1* that encode to a protein similar to metacaspases.

The authors thank FONDEF IDeA CA12i10054 and FONDECYT 1130389 for their financial support.



## EXTRACTS FROM WILD EDIBLE HERBS FOR CONTROLLING POSTHARVEST ROTS OF FRUIT AND VEGETABLES

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The use of natural compounds with antimicrobial activity may be a viable alternative to the use of synthetic fungicides to control pathogens attacking fresh fruit and vegetables during postharvest storage. This paper reports results on the *in vitro* and *in vivo* antifungal activity of total and fractionated phenolic extracts obtained from wild edible herbs (*Borago officinalis*, *Orobancha crenata*, *Plantago coronopus*, *P. lanceolata*, *Sanguisorba minor*, *Silene vulgaris*, *Sonchus asper*, *S. oleraceus* and *Taraxacum officinale*) against some of the most important postharvest diseases: gray mold (*Botrytis cinerea*), brown rot (*Monilinia laxa*), blue mold (*Penicillium italicum*, *P. expansum*), green mold (*P. digitatum*), and black mold (*Aspergillus carbonarius*, *A. niger*). The extracts obtained from *S. minor* and *O. crenata* completely inhibited conidial germination of *M. laxa*, *P. digitatum*, *P. italicum*, and *A. niger* and greatly reduced that of *B. cinerea* and *P. expansum*. The extracts of both species reduced the elongation of the germ tube even in cases in which there was a lack of inhibition of conidial germination. The same extracts were tested *in vivo* on stone fruits (apricots, nectarines, sweet cherries), oranges, and grapes with good results. Some phenolic compounds present in the extracts were identified as potential active components. Studies are in progress to isolate and purify the potentially active compounds and to test their activity as pure principles, which might be used as natural active ingredients for new formulations able to control postharvest rotting of fruit and vegetables.

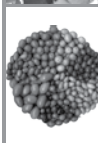
## EXPLORING THE EFFECTS OF GASEOUS OZONE AND 1-METHYLCYCLOPROPENE TREATMENTS ON GRAY MOLD OF APPLE FRUIT AT PHYSIOLOGICAL AND PROTEOMIC LEVEL

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Gray mold caused by *Botrytis cinerea* Pers., is one of the most important postharvest rots of apple fruit. Control of the disease is achieved by fungicide treatments. However, development of fungicide resistance and social concerns regarding pesticide residues, necessitate research for alternative control methods. In the current study, the effect of gaseous ozone (O<sub>3</sub>) exposure (0.3 µL L<sup>-1</sup>) and/or 1- methylcyclopropene (1-MCP – 0.5 µL·L<sup>-1</sup>, 24 hour, 0°C), an inhibitor of ethylene perception, on the development of gray mold on apple fruit (“Granny Smith” and “Red Delicious”) was investigated. Artificially inoculated apple fruit, treated or not with 1-MCP, subjected for 2 or 4 months to cold storage (0 °C, RH 95%) either in an O<sub>3</sub> enriched atmosphere or in a conventional cold chamber. Results showed that on both cultivars, on 1-MCP treated fruit a higher disease incidence was observed compared to the untreated fruit. In contrast, exposure to ozone in the presence or absence of 1-MCP, resulted in a decrease of disease severity by more than 50%. Ripening features, including ethylene production, fruit firmness and soluble solids content, were remarkably depressed by 1-MCP application, particularly evidenced in “Granny Smith”. Using two-dimensional gel electrophoresis (2-DE) analysis, we compared the protein expression in fruit mesocarp of “Granny Smith” subjected to 1-MCP, ozone and inoculation with *B. cinerea*. This analysis revealed that 1-MCP treated fruit that had been inoculated with the pathogen differed to more than 34 protein spots compared to non-inoculated fruit, while fruit exposed to ozon and had been inoculated with *B. cinerea* differed in 21 protein spots compared to non-inoculated fruit. Overall, the results suggest that ozone application may contribute to the reduction of gray mold on apple fruit, while MCP treatments may contribute to increased fruit susceptibility.



## INFLUENCE OF HOT WATER TREATMENT ON FRUIT QUALITY AND SHELF-LIFE OF 'TOPAZ' APPLES FROM ORGANIC PRODUCTION

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One of the main problems during storage of organically produced apples is their limited storage life due to fungal decay. A method to reduce the occurrence of these diseases is the treatment of apples with hot water (45 to 60°C) before storage. Although the effect of hot water treatments (HWT) on fungal decay during storage has been widely investigated, however, there is little information about its impact on fruit quality and shelf-life. Therefore, the aim of this work was to assess quality changes of organically grown 'Topaz' apples after HWT compared to untreated fruit (no HWT). Fruit were picked from two harvest dates at the Competence Center for Fruit Growing - Lake Constance. For half of the fruit batch HWT was applied for 2 minutes at 52°C using a commercial device. The fruit were stored at 1°C under regular atmosphere (RA) and controlled atmosphere (1.0kPa O<sub>2</sub> + 2.5kPa CO<sub>2</sub>). Results show a reduction of fungal decay in hot water treated fruit after RA as well as CA storage compared to untreated control. Concerning fruit quality (firmness, TSS, TA) no differences were observed between hot water treated and untreated apples. Quality losses and fungal decay were more intense in RA compared to CA storage.

## DEFENCE RESPONSES TO THYME OIL VAPOURS IN *PRUNUS PERSICA* DURING POSTHARVEST STORAGE

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Brown rot (*Monilinia* spp.) affects the fruit quality, marketability and shelf life of peaches (*Prunus persica*). Increasing consumer concern regarding food safety and quality makes it necessary to search for natural environmentally friendly alternative products for postharvest disease control. Thyme oil and cinnamon oil are listed as a minimum risk pesticide and therefore exempt from pesticide residue tolerance requirements. In this investigation, peach cultivars Spring Princes and Sonnet at commercial maturity were tested for each postharvest treatment for *in vivo* preventive and curative applications. For the preventive application, fruits were exposed to thyme oil or cinnamon oil vapours (960 µL/10L) for 24 h, at 25 °C. Peaches were then inoculated with a drop of conidial suspension ( $1 \times 10^6$  spores/mL) of *Monilinia laxa* and incubated for 3-5 days at 25 °C. For the curative treatment, fruits were inoculated with the same spore suspension and incubated for 12 h at 25 °C, then exposed to thyme oil or cinnamon oil vapours (150 µL/1.5 L) at 25 °C, 75% RH up to 3 days. Iprodione (100 mL/L) and untreated control were included for comparative purposes. Brown rot incidence and severity (lesion diameter) were recorded after 3 days. The activity of enzymes linked to defence mechanisms (chitinase,  $\beta$ -1,3-glucanase, phenylalanine ammonia-lyase, polyphenol oxidase), antioxidant enzymes (superoxide dismutase and catalase) and total phenolic content were determined. Preventive and curative thyme oil applications significantly reduced the incidence and severity of brown rot in peaches. The effect of thyme oil on brown rot control was significantly higher in cv. Spring Princes than in cv. Sonnet. Thyme oil application significantly increased the activity of defence enzymes and total phenolic content in cv. Spring Princes. Therefore, the thyme oil application shows potential to control brown rot in peaches, and its effectiveness can vary among the cultivars.





## EFFECT OF BLUE LIGHT ON *IN VITRO* GROWTH OF *PENICILLIUM DIGITATUM* AND *PENICILLIUM ITALICUM*

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*Penicillium digitatum* and *P. italicum* are the major pathogens of citrus fruit after harvesting. The continuous use of chemical fungicides on citrus postharvest has contributed to the development of resistant strains against the commercial fungicides. Recently, it has been reported that blue LED light with emissions between 390 and 450 nm may reduce *P. digitatum* development. We hypothesized that the effect of blue light inhibiting fungal growth may increase with light fluence and that the light treatment may be shortened by increasing such fluences to induce the same fungistatic, or even fungicidal, effect. To test such a hypothesis, the effect of different periods and blue light fluences on the growth of *P. digitatum* and *P. italicum* strains cultured on potato-dextrose agar (PDA) at 22 °C was evaluated. Results showed the potential of blue light controlling the growth of both pathogens and that the treatment efficacy increases with its duration and the light fluence. Growth of selected strains was completely avoided after exposure of the cultured plates to high blue light intensity (700  $\mu\text{molm}^{-2}\text{s}^{-2}$ ). Results also showed that blue light may have a fungistatic effect when applied at a lower fluence (120  $\mu\text{molm}^{-2}\text{s}^{-2}$ ) although longer treatments were required. Furthermore, results revealed the potential of blue light of controlling *Penicillium* fungal growth once germination of spores has been produced. Global results indicate that blue light may be a tool to avoid contaminations and infections caused by *P. digitatum* and of *P. italicum* strains during postharvest handling of citrus fruits.



# **IN VITRO EFFECT OF CRAB SHELL CHITOSAN AGAINST MYCELIAL GROWTH OF *BOTRYTIS CINEREA*, *PENICILLIUM* SPP. AND *PILIDIELLA GRANATI* FROM POMEGRANATE**

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Postharvest diseases due to fungal infections cause serious economic losses for the pomegranate industry during storage, transport and marketing. Microbial biocontrol agents such chitosan have shown potential as green alternatives to control to synthetic. The objectives of this study were to identify causal agents of pomegranate latent infection and asses the use of chitosan as a potential postharvest bio-control agent. The study established the presence three major fungal genera along different phenological stages of pomegranates: *Botrytis cinerea*, *Penicillium* spp. and *Pilidiella granati*. The antifungal activity of chitosan and fludioxonil (a registered postharvest fungicide) was tested *in vitro* against these three pathogens. Mycelial growth of the pathogens was inhibited by the fludioxonil and chitosan in a dose-dependent manner. The concentration causing a 50% (EC<sub>50</sub>) and 95% (EC<sub>95</sub>) reduction in percentage were determined. This study has demonstrated that crabshell could be used in the control of postharvest pathogens caused by *B. cinerea*, *P. expansum* spp. and *Pilidiella garanati*. However, to achieve complete inhibition for three days, chitosan (30 mg/L) required close to 30 times that (1mg/L) for Fludioxonil.





## EVALUATION OF ANTIFUNGAL AGAINST *BOTRYTIS CINEREA* OF LACCASE SYNTHETIZED COMPOUNDS

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*Botrytis cinerea*, also known as “gray mould fungus”, attacks a broad range of host resulting in great economic losses on Chilean export products. However, despite of many varieties of botrycides, the fungus has generated resistance against these chemical products, therefore is imperative to develop new molecules with antifungal activity. One approach to solve this problem is to utilize enzymes as laccase to improve of the antifungal capacity of certain compound. Laccase is a copper-containing oxidase that catalyzes reduction of molecular oxygen to water and the oxidation of a phenolic compound. The objective of this work was to evaluate the antifungal activity against *B. cinerea* of the products of the reaction among the flavonoid 3-methyl galangin and *p*-chloroaniline catalyzed by the enzyme laccase. The laccase-mediated reaction contained aniline:flavonoid in a molar ratio of 2:1, 16.8 mM of flavonoid, 200 rpm, 15 h, and 2.15 U of enzyme. After purification by semi-preparative thin layer chromatography, the synthesized products were analyzed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopy. Two products were obtained: a homomolecular compound (benzidine) and heteromolecular compound (3-(*p*-chlorophenylimino)-5,7-dihydroxy-2-phenyl-4-chromanone). The effect of benzidine on mycelial growth of *B. cinerea* was assessed *in vitro* using the radial growth test on malt-yeast extract agar and presented an  $\text{IC}_{50}$  value higher than the commercial fungicide, iprodione while a mixture of benzidine and 3-(*p*-chlorophenylimino)-5,7-dihydroxy-2-phenyl-4-chromanone at 60 ppm inhibited in a 34% the mycelial growth of the fungus.

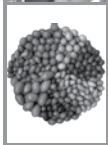
*The authors thank FONDECYT 1130389 and FONDEF IDEa CA12i10054 for their financial support.*

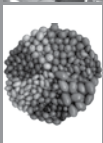
## ACTIVITY OF GREEN BEANS EXTRACT IN CONTROLLING POSTHARVEST BROWN ROT OF APRICOT AND PEACH

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Brown rot caused by *Monilinia* spp. is a common and destructive disease of plum, peach, apricot, and sweet cherry fruits. Young fruits are normally resistant, but may become infected as they mature, even in the absence of wounds. Post-harvest losses routinely occur during storage and transport, in some cases even affecting fruit during the processing stage. When conditions are conducive to the disease, post-harvest losses can be very high, reaching in some cases values of 80-90%. The use of natural substances is one of the emerging technologies for controlling postharvest diseases. In recent years, interest in natural substances has increased and numerous studies on the antifungal activity of a wide range of secondary plants metabolites have been reported. Reports on the antifungal activity of peptides and proteins isolated from medicinal plants have mainly concerned species of the *Fabaceae* family, and a variety of antifungal proteins and peptides, involved in plant defence and belonging to the family of pathogenesis-related proteins, has been isolated from leguminous plants. Water-soluble extract from green beans, cv Pinto, was prepared by grinding 50 g of fresh seeds in distilled water. In *in vivo* tests, green beans extract determined a significant reduction of colony diameter and conidia germination of *Monilinia laxa*, as compared to the untreated control. Brown rot incidence of apricot and peach fruits, either incubated at 25°C or stored at 4°C, showed also significant reduction. Fruits inoculated with *M. laxa* and treated with the green beans extract 3 hours later, showed the lowest lesion diameter and percentage of infected fruits. Instead, fruits treated with the green beans extract first and then inoculated with the pathogen, showed lesion diameter and decay incidence similar to the control fruits, thus suggesting that the extract do not induce resistance mechanism.





## ANALYSIS OF ESSENTIAL OIL FROM *CITRUS UNSHIU* AND ITS ANTIMICROBIAL ACTIVITY AGAINST POSTHARVEST DISEASES

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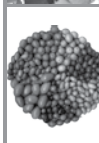
Essential oil of Satsuma mandarin (*Citrus unshiu* Marc.) peel is the valuable material for medical and industrial purposes, because of its antioxidant and antimicrobial activities. This study was focusing on analysis of essential oils extracted by cold press extraction (CPE), steam distillation extraction (SDE) and supercritical fluid extraction (SFE) with CO<sub>2</sub> for the efficacious application on industry, also testified its antimicrobial activity against postharvest diseases. *Citrus unshiu* essential oils by extraction methods were analyzed by GC-MS and HPLC. On CPE and SDE methods, monoterpene hydrocarbon was mostly occupied with limonene and  $\gamma$ -terpinene. Germacrene A and  $\alpha$ -farnesene, that are sesquiterpene hydrocarbon, were also detected. However, Limonene and  $\gamma$ -terpinene were quantified slight amount in extract by SFE. But carvacrol, that is monoterpenoid phenol, was highly detected by GC-MS. The extraction efficiency of SFE methods was the highest, and more various compounds were detected. On HPLC analysis, nobiletin, tangeretin and auraptene were identified in extracts by CPE and SDE methods. Whereas narirutin and hesperidin were in plenty in extract by SFE method, and the extraction efficiency of quercetin and tangeretin was also the highest. Antimicrobial activity of essential oil from *Citrus unshiu* was conducted *in vitro* against citrus postharvest pathogens, *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold). Citrus essential oils were compared with non-treatment and the chemical fungicide (imazalil). Essential oil was showed the antimicrobial activities against green and blue mold, but less than chemical fungicide. That is, the application of essential oil is able to reduce the usage of chemical fungicide and become the alternative mean to control postharvest pathogen.

## RECENT ADVANCES TO CONTROL SPOILAGE MICRORGANISMS IN WASHING WATER OF FRUITS AND VEGETABLES: THE USE OF ELECTROLYZED WATER

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Washing water used for processing fruits and vegetables can convey spoilage fungi and bacteria. The common procedure to reduce microbial contamination involve the use of chlorine based compounds. Recently, electrolyzed water (EW) has been evaluated as an alternative measure in controlling microbial spoilage contamination occurring during washing steps. This work reviews results related to the application of EW for controlling microbial viability responsible for decay development during storage period. EW produced with sodium bicarbonate as electrolyte reduced *Penicillium* spp. population in water and, consequently, green mould decay in citrus fruits; the use of sodium chloride in EW production inactivated spores of *Fusarium* sp. in water and reduced pineapple decay during storage at 12°C for 20 days as well as controlled yeast and mould population in date fruits up to six months of cold storage. EW was also found effective in controlling spoilage bacteria on ready-to-eat produce. *Pseudomonas fluorescens*, *Pantoea agglomerans*, and *Rhizobium aquatilis* were undetectable in electrolyzed process water amended with sodium chloride although similar treatment slightly reduced the *Erwinia carotovora* load inoculated onto lettuce. EW at low free chlorine concentration reduced viability of *Pseudomonas* spp. and psychrotrophic bacteria in both simulated and industrial washing water. EW treatment of fresh cut lettuce dipped in microbial contaminated water reduced *Pseudomonas* spp. of about 1 log cfu/g delaying spoilage symptoms early occurred in untreated vegetables. These results demonstrate that the use of EW can control spoilage microorganisms in washing water, reduce cross-contamination phenomena and delay fruit and vegetable decay.





## INFLUENCE OF POSTHARVEST OZONE TREATMENT ON CATALASE AND LIPOXYGENASE ACTIVITY AND ON ANTHOCYANIN CONTENT OF “RED GLOBE” TABLE GRAPES

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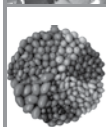
Ozone (O<sub>3</sub>) is a strong oxidizer (oxidation potential 2.07 eV) that is highly reactive with a broad spectrum of biomolecules such as lipids and nucleic acids. Even if the high reactivity of O<sub>3</sub> prevents it from penetrating deeply into the tissues, when O<sub>3</sub> comes in contact with plant tissues it may induce oxidative stress which can affect the antioxidant plant cell defences. The type and extent of such responses depend on various factors, including the duration of exposure and the oxidative status of the cell. This study investigated the effect of O<sub>3</sub> treatment of Red Globe grapes on some enzymatic activities involved in antioxidant plant defences: superoxide dismutase (sod), catalase (cat), glutathione peroxidase (gpx) and lipoxygenase (lox); moreover, the level of anthocyanins and flavonols present in grape berries was analyzed. Grapes were stored for 20 days at 1°C±1 and then maintained for 4 days at 23°C±1 to simulate the store/market environment. O<sub>3</sub> was applied at 0.2 µl/L (De Nora generator) during the storage period. Grapes were naked or packed in plastic bags, and the latter were tested with and without SO<sub>2</sub> pads. The activity of the considered enzymes was analyzed through spectrophotometric assays. Anthocyanins and flavonols were analysed by HPLC equipped with a Diode Array Detector. O<sub>3</sub> treatment led to a significant increase in cat activity, whereas sod, gpx and lox activities were not significantly affected, and anthocyanins and flavonols level decreased. The results showed that even if O<sub>3</sub> may induce oxidative stress in a cell, this does not imply the development of cellular damage.

## POMEGRANATE AND SUMAC EXTRACTS AS ROT PREVENTIVE AGENTS ON TABLE GRAPE BERRIES, CV. ITALIA

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Gray mold caused by *Botrytis cinerea* is the main postharvest decay of table grapes and it is generally considered the most important postharvest pathogen. Due to the increased consumer demand for healthy/organic food products, natural inhibitors for pathogenic microorganisms have been explored from plant extracts. In the present investigation, the ability of pomegranate (*Punica granatum* L.) peel and sumac (*Rhus coriaria* L.) fruit and leaf extracts to inhibit the decay of table grape (cv. Italia) berries was assessed. Different extraction methods were applied on plant tissues and extracts were chemically characterized for their total phenol and anthocyanin contents. Furthermore, qualitative analyses of the same compounds were obtained through UHPLC-PDA-ESI/MS<sup>n</sup>. Both *in-vitro* and *in-vivo* essays were performed in order to assess the antifungal activity of the extracts. The ethanolic pomegranate peel extract was the richest in phenols (66.97 g Gallic Acid Equivalents/kg) while the ethanolic extract and the aqueous extract from sumac fruit showed the highest anthocyanin amounts (171.96 and 94.92 mg Cyanidin 3-Glucoside Equivalents/kg, respectively). The ethanolic pomegranate peel extract was the most efficient against *Botrytis* rot on artificially inoculated berries completely inhibiting the pathogen at different intervals of time between treatment and pathogen inoculation (0-12-24 hours). The concentrated sumac leaf extract reached the 100% of rot inhibition when *B. cinerea* spores were inoculated after 24 hs from treatment, while sumac fruit extract at the same time interval showed a 75% of rot reduction.







## ALTERNATIVE MEANS TO CONTROL POSTHARVEST BLUE MOLD DECAY OF 'ROCHA' PEARS

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Postharvest diseases occurring during storage, transport and commercialization have a considerable economic impact in fruit-producing countries. In pome fruits, fungal diseases are the main responsible for limiting storage period. Synthetic fungicides, traditionally used in managing the postharvest decay, present health and environmental risks and make necessary a shift towards safer and more eco-friendly alternatives. Biological control using bioactive plant compounds has proved to be a promising alternative to chemicals in fighting decays. This work reports the use of *Origanum vulgare*, *Mentha pulegium* and *Satureja montana* for controlling *Penicillium expansum*, responsible for blue mold decay in 'Rocha' pear. Artificially wounded pears were inoculated with the pathogen, treated with different concentrations of the plant extracts and incubated at room temperature during 7 days. The disease incidence and the lesion diameters were measured daily. The overall results disclosed that caprylic acid, an *Origanum vulgare* constituent selected due to its satisfactory antifungal efficacy and safety, was the most efficient in controlling *P. expansum*. In curative or prophylactic treatments, a dose dependent reduction of disease incidence was observed when caprylic acid was applied. Regarding *Mentha pulegium* and *Satureja Montana* essential oils, preliminary tests showed interesting antifungal effectiveness under shelf life conditions; however more studies are needed in order to confirm their antifungal activity during long-term cold storage of pears.

*This work was funded by the FCT project PTDC/AGR-ALI/118477/2010 from MCTES, Portugal.*

## POTENTIAL APPLICATION OF PORTUGUESE PROPOLIS TO CONTROL BLUE MOLD DISEASE IN 'ROCHA' PEAR

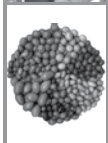
Claudia Sánchez<sup>1\*</sup>, Paula Duarte<sup>2</sup>, Paula Vasilenko<sup>1</sup>, Mário Santos<sup>1</sup>, Margarida Gonçalves<sup>2</sup>

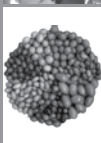
<sup>1</sup>Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Oeiras, Portugal.

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Blue mold decay caused by *Penicillium expansum* is one of the most important postharvest diseases in 'Rocha' pear, causing considerable economic losses. The use of synthetic fungicides has been the main method used in managing this postharvest decay. However, the rising concern for health risks and environmental pollution due to the use of chemicals makes necessary the development of new and safer strategies. Biological control using natural compounds has proved to be a promising alternative to chemicals in fighting decays. Propolis is a natural resinous substance collected by *Apis mellifera* bees from the leaf buds and barks of trees. One of the most important properties is its antimicrobial activity against many bacteria, yeasts and fungi. In this context, the present study aims to evaluate the antifungal properties of a portuguese propolis extract against *P. expansum*, alone or as a coadjuvant of the antagonist *Aureobasidium pullulans*. The extract studied was obtained from propolis collected in different regions of Portugal and was extracted with 96% ethanol, in the dark, during 12h, at room temperature. The ratio crude propolis to solvent was 1:10 (w/v). The extract was characterized in terms of its phenolic composition (Folin-Ciocalteu assay), antioxidant capacity (FRAP and DPPH) and *in vitro* antifungal activity. For the *in vivo* assays, artificially wounded pears were inoculated with the pathogen or with the pathogen and the antagonist, treated with the propolis extract and incubated at room temperature during 7 days. The disease incidence and the lesion diameters were measured daily. Preliminary results showed interesting antifungal effectiveness under shelf life conditions. Propolis extract delayed fungus development and reduced the diameter of the lesions. No toxic effects were observed on fruits. However, the combination propolis extract-antagonist does not seem to exert any significant synergistic action.





# REDUCTION OF INFECTION CAUSED BY *ALTERNARIA* SP. AND *LEPTOSPHAERIA* SP. AFTER USING EFFECTIVE MICROORGANISMS PREPARATION ON YELLOWSEED FORMS OF *BRASSICA NAPUS* OBTAINED *IN VITRO* FROM "INTERSPECIFIC HYBRIDS"

Elizabeth Starzycka-Korbas<sup>1</sup>, Michal Starzycki<sup>1\*</sup>, Wojciech Rybinski<sup>2</sup>, Lidia Lyczko<sup>3</sup>

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<sup>3</sup>Koszalin University of Technology, Mechanical Engineering Department, Raclawicka 15-17 St. 75-620 Koszalin, Poland. E-mail: m.starzycki@ihar.edu.pl

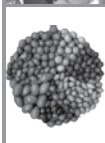
The study used *Brassica napus* L. yellowseed forms, which can be used as an additive to mustard. The primary objective of the study was to determine whether the protective spray after flowering plants using effective microorganism (EM) is effective in the field on the new genotypes. The new forms of rapeseed (RS *in vitro*) were obtained after interspecific crossing *B. oleracea* (n = 9), and *B. campestris* (n = 10). To confirm the presence of *B. oleracea* in the cytoplasm of the received plant (the BC4 generation), DNA PCR analysis was performed. For the BC4 generation the amplified DNA pattern was identical to plant with cabbage cytoplasm. But the biggest perceived DNA differences were observed in the F1 of hybrid generation. After vernalisation and backcrossing of the giving small yield yellow seed forms of winter oilseed rape (4 backcross) obtained a *B. napus* (n = 19), with high, but insufficient resistance to dangerous pathogens: *Alternaria* sp. and *Leptosphaeria* sp. After harvest ITS DNA sequencing was performed to identify pathogens. On the upper parts of plant have been identified pathogens *Alternaria* sp. and on lower parts of the stems *Leptosphaeria* sp. The field experiment was conducted in IHAR-PIB in Malyszyn involving 20 breeding yellow seeds forms sprayed EM and 20 of the same forms as a control without spraying. The occurrence of *Alternaria* sp. and *Leptosphaeria* sp. was entirely confined after application of EM which was statistically demonstrated. During the phytopathological inspection of yellow seeds observed a small percentage of dangerous pathogens.

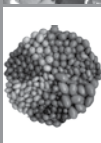
## SAFE POSTHARVEST TREATMENT WITH PROTECTIVE EFFECT IN ORANGES INOCULATED WITH *PENICILLIUM DIGITATUM*

**Valeria C. Bordagaray, Lauro Bordenave Imer, Julieta Bof, Gustavo D. Suárez\*, Delia E. Locaso**

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The protective effect of antifungal treatments in citric fruits has been evaluated opposite to conventional fungicides of chemical synthesis, a substitute to conventional treatments has evaluated, since its negative effects on health and environment, both fungicides and residues. Untreated "Valencia" oranges, export quality, were obtained from a citrus packhouse in the Entre Ríos, Argentina. Two coatings -coatQ1 and CoatQ2- with different concentrations of fatty acids C24/C16 and chitosan were tested against fruit treated with Imazalil - 250  $\mu\text{g} \cdot \text{mL}^{-1}$  - and commercial wax with 18 % solids, as a positive control (C); and uncoated fruit as a control (T). The application of the coatings was carried out in two layers in a line of industrial packhouse. For the first treatment, A1 the fruit was treated with coatQ1 and then with coatQ2; on the second treatment, A2 the order of application was interchanged. All the fruits were inoculated with 10  $\mu\text{L}$  of a suspension of  $10^6$  spores. $\text{mL}^{-1}$  of *Penicillium digitatum*, and stored for 10 days at 25 °C and 95% RH . The strain used was isolated from fruits affected by the disease obtained from the region. The efficacy of the disease control of was expressed as percentage of reduction in the number of infected fruit compared to the fruits used as a control. Additionally, the infection index was used to quantify the surface of the fruit covered with green spores. The greater efficacy was achieved in fruit with conventional treatment (89.0 %), followed by A1 with 72.9 %. The lowest infection index was for the A1 with 6 %, front C (10.5 %) and T (41.5 %). On the frame of an integrated management of postharvest diseases, the proposed treatment might turn out to be promising to replace synthetic fungicides with protective against on *P. digitatum*.





## EXPLOITATION OF DITTANY (*ORIGANUM DICTAMNUS*) OIL FOR THE TOMATO FRUIT PRESERVATION AGAINST *BOTRYTIS CINEREA*

Andriana Stavropoulou<sup>1</sup>, Kostas Loulakakis<sup>1</sup>, Dimitris Lydakakis<sup>1</sup>, Naresh Magan<sup>2</sup>, Nikos Tzortzakakis<sup>3\*</sup>

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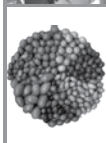
Grey mould (*Botrytis cinerea*) development *in vitro* or in cherry tomato (*Solanum lycopersicum* L.) fruit was evaluated after treatment with dittany (*Origanum dictamnus* L.) oil (0-50-100-250 ppm) and storage at 12°C and 95% relative humidity during or following exposure to the volatiles. *In vitro*, fungal colony growth, spore production and spore germination in PDA, inhibited with the application of dittany oil with greater effects recorded in higher dittany concentrations. However, fungal biomass growing in PDB accelerated with oil > 50ppm but spore production was inhibited with oils oil > 50ppm, highlighting a more detail study needed for the oil action onto the fungi *per se*. *In vivo*, vapour-treated fruits reduced lesion growth after 7 days of exposure, and greater effects marked at the higher concentration (250 ppm). Examining the fungal reproductive phase, essential oil volatiles decreased spore production comparing to the control treatment, but this was not evidence for spore germination. The benefits associated with dittany volatiles-enrichment was maintained in fruit pre-exposed to vapours, resulting in suppression in lesion growth while no differences observed in spore germination and spore production. Fruits exposed to essential oil did not differ in quality related attributes such as fruit firmness, total soluble solids, titratable acidity, weightloss, colour (L, C, h) and respiration rates. The results of this study indicate that dittany volatiles may be considered as an alternative to the traditional postharvest sanitizing techniques. Each commodity needs to be individually assessed, and the volatile concentration and sanitising technique optimised, before the volatile treatment is used commercially.

## METHYL JASMONATE, VINEGAR AND SAGE OIL VAPOUR SUPPRESS GREY MOULD DEVELOPMENT IN PEPPER FRUIT

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Grey mould rot (*Botrytis cinerea*) development *in vitro* or in pepper (*Capsicum annuum* L.) fruit was evaluated after treatment with methyl jasmonate (MJ; 44.8 µl/l), vinegar (VIN; 16 ml/l), or sage oil (SAG; 0.4 ml/l) and storage at 11°C and 95% relative humidity during or following exposure to the volatiles. Fruit treated with VIN and SAG vapours reduced lesion development while no differences observed on fungal spore germination/production. Fruit lesion development was suppressed after fruit exposure to pure (100% v/v) SAG vapours. Moreover, exposure to pure VIN- and SAG-vapours reduced (up to 92%) spore germination *in vitro*, but no differences were observed in the MJ treatment. The benefits associated with volatiles-enrichment was maintained in fruit pre-exposed to SAG oil vapours, resulting in suppression in lesion growth with no effects on fungal reproductive stage (spore germination/production). Studies performed on fungi grown on Potato Dextrose Agar (PDA) revealed colony growth suppression and spore production for direct SAG vapours application or PDA pre-exposed to SAG following *B. cinerea* inoculation, implying that suppression of pathogen development was due in a large part to the impact of volatiles on fruit-pathogen interactions and/or “memory” effects on fruit tissue and/or medium culture. Work is currently focussing on the mechanisms underlying the impacts of volatiles on fruit quality related attributes. The results of this study indicate that volatiles may be considered as an alternative to the traditional postharvest sanitizing techniques. Each commodity needs to be individually assessed, and the volatile concentration and sanitising technique optimised, before the volatile treatment is used commercially.







## **BOTRYTIS CINEREA POSTHARVEST TREATMENTS WITH BRASSICA DERIVED ALLYL-ISOTHIOCYANATE IN DIFFERENT HOST FRUIT**

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Grey mould decay, caused by *Botrytis cinerea* Pers: Fr., is a worldwide postharvest disease causing serious losses during fruit storage. In the past fungicides were widely applied to control grey mould, however their intense use contributed to the development of resistant strains and increased the issues on fungicide residues in fruit. In order to find alternative approaches, natural compounds with antimicrobial activity, such as glucosinolate (GL)-derived isothiocyanates (ITCs), obtained great attention with promising results. Allyl isothiocyanate (AITC), formed by the endogenous sinigrin-myrosinase system of Brassica defatted seed meal, seems to deserve particular interest due to its known high biological activity and its physico-chemical characteristics, as the low vapour pressure. In the present work the *in vivo* effect of AITC on grey mould decay, on two different host fruits, was valuated and compared. Fruit considered were the highly perishable strawberry and the kiwifruit, usually subjected to a long storage time. They were treated with AITC vapours produced from Brassica defatted seed meal at specific concentrations and treatment time, stored and finally valuated for pathogen infection, AITC residues and fruit quality. The AITC treatment (0.1 mg ml<sup>-1</sup> for 4 hours) demonstrated to inhibit *B. cinerea* development on two naturally infected 'Tecla' and 'Monterey' strawberries by over the 45% on six trials out of seven, without affecting their phenolic content and antioxidant capacity. The effects of the same technique preliminarily applied to artificially inoculated 'Hayward' kiwifruit are under investigation and the results of microbiological, biochemical and molecular analyses on fruit will be discussed.

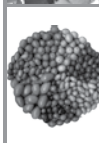


## PHYSICOCHEMICAL PROPERTIES OF ORGANIC GROWN "TOPAZ" APPLES AS AFFECTED BY HOT WATER TREATMENT AND STORAGE ATMOSPHERE

Emil Zlatić<sup>1</sup>, Janez Hribar<sup>1</sup>, Petra Terpinc<sup>1</sup>, Stanislav Tojanko<sup>2</sup>, Rajko Vidrih<sup>1\*</sup>

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Nowadays a lot of efforts are put to minimise the use of fungicides in preventing fungal rots. In addition to resistant cultivars, more advanced postharvest treatments are used, it seems that implementation of more rigorous storage conditions (dynamic atmosphere) or physical postharvest treatments may be quite promising. Hot water treatment is already widely used for some agricultural commodities, totally replacing the usage of harmful chemicals. In this study organic grown 'Topaz' apples were treated with hot water and stored in normal and controlled atmosphere. Apples from orchard that was not exposed to any fungicide treatment were also included in this experiment. Thermally untreated fruits represented the control. Organic apples treated with hot water showed less fungal decay than other treatments but only when stored in controlled atmosphere. Apples treated with hot water and stored in normal atmosphere exhibited more fungal rots than non treated control. After storage, regardless of cultivation technique and hot water treatment no significant differences were found for fruit firmness, acidity and vitamin C content between treatments.





## POSTHARVEST TREATMENT WITH SODIUM SILICATE INHIBITS BLUE MOLD OF APPLE FRUIT AND ITS EFFECT ON METABOLISM OF HYDROGEN PEROXIDE

Junjie Wang<sup>1,2,3</sup>, Jingjing Zheng<sup>1</sup>, Yang Bi<sup>2\*</sup>, Yunfei Wang<sup>2</sup>, Zhichun Ma<sup>1</sup>, Liuqing Jiang<sup>1</sup>

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The objective of this study is to investigate the inhibition of blue mold and the effect of metabolism of hydrogen peroxide ( $H_2O_2$ ), and to illuminate the role of  $H_2O_2$  on induce resistance on apple fruit (*Malus domestica* cv. Fuji) treated with sodium silicate. Fruit was dipped in  $200\text{ mmol}\cdot\text{L}^{-1}$  sodium silicate for 10 min, and then inoculated with diphenylene iodonium (DPI), an inhibitor of  $H_2O_2$  producer on membrane, and with *Penicillium expansum*. Sodium silicate treatment resulted in alleviation of incidence of disease, and inhibition of development of lesion of inoculated fruit. Compared with the control, both superoxide dismutase (SOD) and NADPH oxidase (NOX) activities in the treated fruit were significantly higher at 3 and 5 d after treatment, while catalase (CAT) and ascorbate peroxidase (APX) activities were significantly lower from 3 to 7 d which were associated with the significant increase of superoxide ( $O_2^{\cdot-}$ ) production at 1 and 3 d and of hydrogen peroxide ( $H_2O_2$ ) content from 5 to 7 d after treatment respectively. Furthermore, the inhibition of the activity of NOX by DPI also resulted in inhibition of  $H_2O_2$  content on membrane, and accelerating development of lesion. It is suggested that the blue mold of apple fruit was inhibited through inducing the accumulation of  $H_2O_2$  on membrane with sodium silicate treatment after harvest.

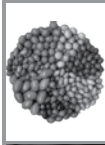
## CHITOSAN CONTROL OF POSTHARVEST BLUE MOLD DECAY OF APPLE FRUIT AND POSSIBLE MECHANISMS INVOLVED

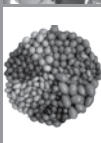
Huan Li<sup>1</sup>, Yadan Wang<sup>1</sup>, Fei Liu<sup>1</sup>, Ziming Wu<sup>1</sup>, Jun Li<sup>2</sup>, Hongyin Zhang<sup>1</sup>, Yun Wang<sup>1\*</sup>

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Blue mold decay, caused by species of *Penicillium expansum* Link, is one of the most important postharvest diseases of apples and pears worldwide. Chitosan is known to be a nontoxic, biodegradable polysaccharide, and also possesses antifungal effects on various plant pathogenic bacteria and fungi. In the present study, the effectiveness of chitosan in controlling blue mold decay of apple fruit caused by *P. expansum* and the possible mechanisms involved in were investigated. The application of chitosan significantly reduced the blue mold decay of the postharvest apple fruit and patulin accumulation, and no negative effect of chitosan treatment on fruit quality was observed after 15 days of storage. Changes of protein expression profiles of apple fruit upon chitosan treatment were analyzed by two-dimensional electrophoresis and twenty differentially expressed proteins were identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry analysis. Several proteins involved in defense, carbohydrate catabolism and protein biosynthesis were up-regulated by chitosan treatment. Activated defense response and increased energy supply in chitosan treated fruit may constitute the molecular basis for increased resistance to the possible future pathogen infection. This study contributes to a better understanding of the cellular events in apple fruit under chitosan treatment in view of a promising alternative in controlling postharvest disease.





## INFLUENCE OF POSTHARVEST OZONE TREATMENT ON DECAY OF "RED GLOBE" TABLE GRAPES

**Thaer Yaseen<sup>1\*</sup>, Alessandra Ricelli<sup>2</sup>, Pietro Albanese<sup>1</sup>, Isabella Nicoletti<sup>3</sup>, Salwa Essakhi<sup>1</sup>, Cristian Carboni<sup>4</sup>, Anna Maria D'Onghia<sup>1</sup>**

<sup>1</sup>*Centre International de Hautes Etudes Agronomiques Méditerranéennes/Mediterranean agronomic Institute, Via Ceglie 9, 70010 Valenzano, Bari, Italy.* <sup>2</sup>*Istituto di Chimica Biomolecolare-CNR, P.le Aldo Moro, 5 I-00185 Rome, Italy.* <sup>3</sup>*Istituto di Metodologie Chimiche-CNR, Area della Ricerca di Roma 1, Via Salaria, km 29,300 Montelibretti, Rome, Italy.* <sup>4</sup>*De Nora NEXT-Industrie De Nora S.p.A. Via Bistolfi, 35- 20134 Milano Italia. E-mail: y.thaer@iamb.it*

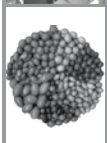
Ozone (O<sub>3</sub>) is a strong antimicrobial agent, that is Generally Recognized As Safe (GRAS) and therefore lightly regulated. An investigation was conducted to assess the effect of O<sub>3</sub> on postharvest decay of "Red Globe" table grape stored for 20 days at 1°C±1 then maintained 4 days at 23°C±1 to simulate the store/market environment. O<sub>3</sub> was applied at 0.2 µl/L (MET S.r.l. generator). Grapes were naked or packed in plastic bags; the packed grapes were tested with and without SO<sub>2</sub> pads. Decay severity was expressed through the McKinney index that gives the weighted average of the disease severity as an actual percentage in terms of the maximum disease severity. The quantification of fungi and yeasts was done by the Colony Forming Units (CFUs) counting. One kilogram of grape berries from each replicate were shaken at 270 rpm for 1hr in 500mL of sterile distilled water then a serial dilution of the suspension from D<sub>2</sub> to D<sub>100</sub> was made. An amount of 100µl of the suspension was plated on NYDA medium containing streptomycin sulfate (250mg/L) and ampicillin (250mg/L) and CFUs were counted after 3 days of incubation at 24°C±1. A significant reduction of table grape decay was observed in the samples treated with O<sub>3</sub> packed in plastic bags with SO<sub>2</sub> pads and in naked samples. In particular O<sub>3</sub> at 0.2 µl/L inhibited the aerial growth of the mycelia and reduced the CFUs of fungi and yeasts commonly present on grape berries such as *Botrytis* sp., *Penicillium* sp. or *Mucor* sp. Thus, O<sub>3</sub> prevented rot from spreading from decayed fruit to adjacent healthy fruit. Therefore, O<sub>3</sub> treatment could be an alternative to, or integrated with, the use of SO<sub>2</sub>.

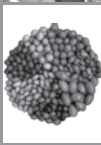
## EFFECTS OF POSTHARVEST BRASSINOLIDE DIPPING ON THE QUALITY PARAMETERS AND ANTIOXIDANT ACTIVITY IN PEACH FRUIT

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*Penicillium expansum* is the casual agent of blue mould of peach fruit, and causes severe losses after harvest. The effects of brassinolide (BL) at different concentrations (0, 2, 5, 10  $\mu\text{mol}\cdot\text{L}^{-1}$ ) against *P. expansum* in peach fruit and on storage quality were studied in this paper. This study also determined the effect of BL on main enzymes activity and antifungal compounds in phenylpropanoid pathway metabolism of peach fruit. Fruit were treated with BL immediately after harvest and storage at 20 °C for 5 days. The results indicated that BL significantly decreased ( $P<0.05$ ) lesion diameter of peach fruit inoculated with *P. expansum*, and the optimal concentration is 5  $\mu\text{mol}\cdot\text{L}^{-1}$ . BL at 5  $\mu\text{mol}\cdot\text{L}^{-1}$  concentration delayed the increase of weight loss, and the decrease of flesh hardness, soluble solids content (SSC) and titratable acid (TA) content in peach fruit. Ascorbic acid (AsA) content was significantly induced ( $P<0.05$ ) by BL treatment. The results also indicated that BL treatment significantly enhanced ( $P<0.05$ ) the activities of phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumarate/coenzyme A ligase (4CL), and the contents of total phenolic compounds, flavonoids and lignin in peach fruit. These results suggest that BL reduced fruit decay caused by *P. expansum* may be associated with induction of disease resistance in fruit and delay of senescence.





## LIGHT-DEPENDENT EXPRESSION OF BCUVE1 LEADS TO OPTIMIZED FUNGICIDAL EFFECT OF UV-C AGAINST *BOTRYTIS CINEREA* IN VITRO AND ON SURFACES OF POSTHARVEST CROPS

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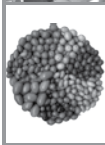
Ultraviolet-C light (UV-C, 190-280 nm wavelength) offers interesting possibilities for controlling postharvest diseases as safe alternative to conventional chemical fungicide. *Botrytis cinerea* is an important necrotrophic plant pathogen causing grey mould disease in a variety of economically important crop plants. Light as an important environment factor causes universal changes in gene expression of this fungus. Here, we found that a gene predicted to encode for endonuclease in *B. cinerea*, *Bcuve1*, is significantly induced in response to light. *In vitro* assay shows that germination of the wild type spores incubated in dark can be totally inhibited by UV-C (150  $\mu\text{J}/\text{cm}^2$ ), while those spores in light can survive this UV-C dosage. In contrast, the  $\Delta bcuve1$  mutants are hypersensitive to UV-C irrespective of light condition. Furthermore, the ortholog of blue light receptor white collar 1 in *B. cinerea*, BcWC1, is required for photo-induction of *Bcuve1* expression and UV-C resistance, indicating a light dependent endonuclease excision repair pathway to protect this fungus against DNA damage. Additionally, when inoculated on surfaces of postharvest crops, UV-C (150  $\mu\text{J}/\text{cm}^2$ ) application abolishes decay development by  $\Delta bcuve1$  in both light and dark conditions, but this UV-C dosage can only prohibit the virulence of wild type in dark condition, although virulence of  $\Delta bcuve1$  is comparable to wild type strain when UV-C is not applied. More interestingly, some common postharvest fungal pathogen species of *Fusarium*, *Alternaria*, and *Penicillium* genera are also more sensitive to UV-C in dark than in light. In general, we propose that fungicidal function of UV-C can be enhanced when it's applied in dark due to the light-dependent UV-C resistance of plant pathogenic fungi, and thus the beneficial effect of UV-C for postharvest disease management can be improved by optimizing application moment.

## ALTERNATIVE MEASURES TO CONTROL POSTHARVEST GRAY MOLD IN GRAPES WITHOUT CHANGES IN FRUIT QUALITY

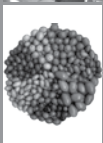
Said Ennahli<sup>1\*</sup>, Hafida Hanine<sup>2</sup>, David Scurlock<sup>3</sup>

<sup>1</sup>National School of Agriculture, Meknes, Morocco. <sup>2</sup>Laboratory of Bio-interface, Faculty of Science, University of beni-Mellal, Morocco. <sup>3</sup>Ohio State University, Columbus, OH, USA. E-mail: ennahli.1@osu.edu

This study aims at controlling post-harvest disease caused by gray mold in organically grown table grapes while maintaining quality attributes. Grape clusters were artificially infected with *Botrytis cinerea*. Treatments consisted of combined concentrations of ethanol (%) / Chitosan (%) (10/0.5, 30/0.5, and 50 /0.5) with or without ozone (1ppm), and control (no treatment) were applied. Quality parameters were measured at 3 different dates (10, 30, and 45 days) during storage at 0.5°C. The incidence of infected berries and the quality of the grape fruit were investigated. The immersion of berries in 30% ethanol (v/v) with 0.5% chitosan treated with 2% ozone resulted in a significant synergetic reduction of gray mold (from 56% in control to 5%) without any change in color or in fruit quality. Ozone treatment increased the efficiency of ethanol/chitosan in gray mold control by an average of 23% as compared to ethanol/chitosan treatment only. No significant changes were observed in all individual sugars, titratable acidity, flavonoids, and in total anthocyanins at any sampling dates. The ozone treatment resulted in significant change of color only after 45 days of storage at 0.5°C in all treatments. The treatment 30%/0.5% coupled with 2ppm ozone exhibited the best control of gray mold without noticeable changes in fruit quality.







## HEAT TREATMENTS FOR KILLING *PSEUDOMONAS SYRINGAE* PV. *ACTINIDIAE* ON CONTAMINATED KIWIFRUIT POLLEN

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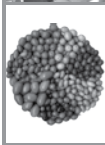
The bacterial pathogen, *Pseudomonas syringae* pv. *actinidiae* strain V (Psa-V), has caused a devastating epidemic of the gold variety of kiwifruit, *Actinidia chinensis* 'Hort 16A', in New Zealand. Psa-V is causing a global pandemic of kiwifruit and has also spread to kiwifruit vines in Italy, Greece, Portugal, France, Japan and Chile. The origin of the strain of Psa causing the pandemic is hypothesised to be China. Kiwifruit have male and female flowers on sexually differentiated vines. Application of pollen by artificial means has been shown to improve pollination compared with pollination by bees. The result is higher yields and larger sized fruit. Pollen from the Bay of Plenty in New Zealand is contaminated with Psa-V. In order to reduce the introduction of new inoculum by pollen to orchards that are not yet infected, a Programme was initiated to identify protocols that kill Psa-V but retain pollen viability. A combination of heat and dessication in anoxic conditions was shown to reduce the population of Psa-V on artificially contaminated pollen by 7.9 log-fold, whilst retaining pollen viability. Results so far suggest that kiwifruit pollen is naturally contaminated with  $10^5$  -  $10^7$  cfu/g, and therefore our treatment should kill all Psa-V on this pollen.

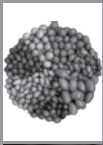
## EFFECT OF *ALOE VERA* GEL COATING AS POSTHARVEST TREATMENT TO REDUCE DECAY AND INCREASE THE STORAGE QUALITY OF TABLE GRAPE CV. ASKARI

Mehdi Hosseini Farahi\*, Masoud Zadebagheri

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Grape is one of the most important fruit in iran and the world both as fresh fruit (table grape) and processed in wine, grape juice, molassa, and raisins. The aim of this study was the effects of aloe vera gel on improvement of quality and reduces of decay percentage of table grape cv Askari. For this study, a factorial experiment base on randomize completely block design was conducted with twenty treatments and three replication. The first factor dipping of grape clusters in four ratios of *Aloe vera* (AV): distill water (dW) (0:1, 1:1, 2:1 and 3:1 v/v) and second factor were including the storage time in five times (0, 15, 30, 45 and 60 day) after harvest in 4°C and 85±5% RH. Traits such as weight loss percentage, berry browning percentage, berry-shriving percentage, and berry decay percentage were measured. Results showed that use of AV gel reduced weight loss percentage, berry decay percentage, berry shriving percentage and berry browning percentage. The highest weight loss percentage, berry decay percentage and berry browning percentage were observed in uncoated grapes (control) in 60 days after storage. Therefore, application of postharvest AV gel in 2:1 ratio for improvement of quality and shelf life of grape cv Askari is useful.





## EFFECT OF EDIBLE COATING OF CHITOSAN COMBINED WITH CALCIUM SULPHATE ON IMPROVEMENT OF POSTHARVEST QUALITY OF PEACH FRUIT (*PRUNUS PERSICA*) DURING COLD STORAGE

Mehdi Hosseini Farahi<sup>1\*</sup>

<sup>1</sup>Young Researchers and Elites Club, Yasouj Branch, Islamic Azad University, Yasouj, Iran. E-mail: m.h.farahi@iauyasooj.ac.ir

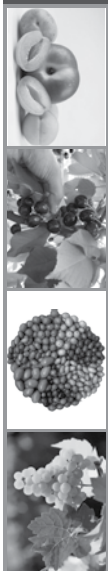
In this study, the influence of chitosan (CS) coating combined with calcium sulphate ( $\text{CaSO}_4$ ) treatment on fruit quality, control of weight loss and maintaining quality of peach was investigated. Fruits were coated with CS 2%, 3%, CS 2%+  $\text{CaSO}_4$  5% and CS 3%+  $\text{CaSO}_4$  5% as well as treated with distilled water (control). Fruits were stored at 4°C and  $80 \pm 2\%$  relative humidity for 60 days. The fruit weight loss percentage (FWL %), fruit decay percentage (FD %), fruit firmness (FF) and fruit shriving percentage (FSH %) were followed at an interval of 15 days up to 60 days. Results showed that fruits untreated exhibited significantly increase in FWL%. CS coating treatment significantly decreased FWL% ( $P>0.05$ ). Uncoated fruits exhibited significantly decrease in FF. CS coating significantly increased FF. The highest FF was observed in fruit coated with CS 3%+  $\text{CaSO}_4$  5% as compared to uncoated fruits. Fruit coated with CS significantly decreased the FD%. The lowest FD% was observed in peach fruit coated with CS 3%+ $\text{CaSO}_4$  5% as compared to uncoated fruits. Utmost of FSH% was obtained in peach fruit coated with CS 3% as compared to other treatments. Therefore, use of CS edible coating treatment is an effective technique for kipping and maintaining organoleptic characteristics and as well for the prolonging of postharvest life in peach. Finally, the use of CS +  $\text{CaSO}_4$  for increasing of postharvest quality of peach cv Alberta is useful.

## POMEGRANATE PEEL EXTRACT TO CONTROL POSTHARVEST ROT OF LEMONS AND SWEET CHERRIES

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<sup>1</sup>Dipartimento di Agraria, Università Mediterranea di Reggio Calabria, Località Feo di Vito, Reggio Calabria, 89122, Italy. <sup>2</sup>Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Corso Savoia, 190 - 95024 Acireale (CT), Italy. <sup>3</sup>Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via G. Amendola 165/A, Bari 70126, Italy. E-mail: giulia.lidestri@unirc.it

The efficacy of an ethanolic extract from pomegranate peel was evaluated against postharvest rots of lemons and sweet cherries. On artificially inoculated lemons, both *Penicillium digitatum* and *P. italicum* were completely inhibited by preventive treatments (extract applied before pathogens) even when the extract was highly diluted (100 times). Significant reductions were also obtained with the extract diluted 1000 times. Interestingly, a high level of protection was also obtained with diluted and undiluted extract in curative treatments i.e. by inoculating the pathogen 1, 12 or 24 hours before the extract. Furthermore, the extract induced resistance in treated lemon tissues since rots were significantly reduced when extract and pathogens were applied in spatially separated wounds. In semi-commercial trials conducted by dipping sweet cherries, cvs Bigarreau moreau and Giorgia, in diluted solutions of the extract (1:10, 1:50 and 1:100) a high efficacy against natural rots (mainly caused by *Monilinia laxa* and *Botrytis cinerea*) was revealed after 15 days of storage and 5 days of shelf-life. In particular, the development of rots was completely inhibited on sweet cherries of cv Giorgia dipped in the ten-time diluted extract. Data suggest pomegranate peel extract as a powerful natural compound to control postharvest rots. Important features are its mechanism of action (a direct action against the pathogen and the induction of resistance in the host), the ability to penetrate host tissues and block already established infections (curative effect) and the high level of protection in semi-commercial conditions against natural infections.





## ELICITATION OF RESISTANCE RESPONSES IN APPLE AND GRAPEFRUIT BY POMEGRANATE PEEL EXTRACT

Sonia Pangallo<sup>1</sup>, Ginat Raphael<sup>2</sup>, Elena Levin<sup>2</sup>, Oleg Feygenberg<sup>2</sup>, Flora V. Romeo<sup>3</sup>, Paolo Rapisarda<sup>3</sup>, Leonardo Schena<sup>1\*</sup>, Samir Droby<sup>2</sup>

<sup>1</sup>Dipartimento di Agraria, Università Mediterranea di Reggio Calabria, Località Feo di Vito, Reggio Calabria, 89122, Italy. <sup>2</sup>Department of Postharvest Science, ARO, The Volcani Center, P.O.Box 6, Bet Dagan 50250, Israel. <sup>3</sup>Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM), Corso Savoia, 190 - 95024 Acireale (CT), Italy. E-mail: lschena@unirc.it

Pomegranate (*Punica granatum* L.) peel extract (PGE) have the capability to inhibit infection and development of *P. expansum* on apples and *P. digitatum* and *P. italicum* on grapefruits. The aim of the current work was to investigate the possible mechanism of action by which PGE inhibit the infection apple and citrus pathogens. Although PGE exhibited direct inhibitory effect on spore germination and germ-tube elongation *in vitro*, it strongly elicited typical resistance responses in the treated fruit tissue. Interestingly, stronger responses were evident in fruit tissue treated with PGE and inoculated with the pathogen indicating the possible involvement of priming effect of the treatment. To gain a better understanding of the biochemical and molecular changes that are taking place in grapefruit tissue, we used q-PCR to investigate the expression of key genes involved in induced resistance in the fruit. Results indicated that PGE induced the expression of genes encoding mitogen-activated protein kinase (MAPK) and mitogen-activated protein kinase kinase (MAPKK), chitinase (CHI), phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS). Expression of these genes was examined at 4 time points (0, 6, 24, and 48 h) following PGE treatment with or without inoculation of the pathogen. In non-inoculated surface wounds, PGE treatment caused significant increase in transcript levels of PAL, CHI, MAPKK and CHS after 6 h and only PAL and CHS expression levels continued to increase at 48 h relative to controls. With regard the expression of the genes in PGE-treated and inoculated wounds, level expression of all examined genes found to be higher especially at 24 and 48 h as compared to controls. Moreover, the ability of pomegranate peel extract to activate reactive oxygen species (ROS) was also demonstrated. PGE applied in grapefruit wounds showed high level of ROS, especially after 24 and 48 h; however on apple increased intensity was present only after 24 h and decreased after 48 h. Collectively, the results show that induction of resistance may have a major role in the mechanism by which pomegranate peel extract inhibit the infection and development of postharvest pathogens of apples and citrus fruit.

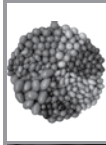
## NOVEL EFFECTS OF HEAT TREATMENT ON PEACH FRUIT: THE INVOLVMENT OF VOCs

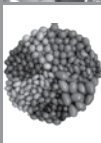
Alice Spadoni<sup>1\*</sup>, Fiorella Neri<sup>1</sup>, Michela Guidarelli<sup>1</sup>, Luca Cappellin<sup>2</sup>, Alberto Algarra Alarcon<sup>2</sup>, Flavia Gasperi<sup>2</sup>, Franco Biasioli<sup>2</sup>, Marta Mari<sup>1</sup>

<sup>1</sup>Criof-DipSA, University of Bologna, via Gandolfi, 19, 40057 Cadriano Bologna, Italy.

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The effect of hot water treatment (HWT) for the control of peaches brown rot was investigated. Peaches were dipped in water at 60°C for 60 s and artificially inoculated with *Monilinia fructicola* conidia suspension. HWT failed to control *Monilinia* rot if applied before inoculation and microscopic observations revealed a stimulatory effect on germ tube elongation of *M. fructicola* conidia placed immediately after HWT on fruit surface, compared to the control. The influence of fruit volatile emission due to HWT was performed on pathogen conidia exposed to the headspace surrounding peaches. The results showed an increase of *M. fructicola* conidia germination ranging from 33% to 64% for 'Lucie Tardibelle' and 'Redhaven' heat-treated peaches, respectively, compared to the control. The volatile blend emitted from heat-treated fruit was analyzed by solid-phase microextraction/gas chromatography-mass spectrometry (SPME/GC-MS) and proton transfer reaction time of flight-mass spectrometry (PTR-ToF-MS). Fifty compounds were detected by SPME/GC-MS in volatile blends of 'Lucie Tardibelle' peaches and significant differences in volatile emission were observed among heated and control fruit. By PTR-ToF-MS analysis acetaldehyde and ethanol resulted fifteen and twenty-eight fold higher in heated fruit compared to unheated ones, respectively. *In vitro* assays confirmed the stimulatory effect (60% and 15%) of acetaldehyde (0.6  $\mu\text{L L}^{-1}$ ) and ethanol (0.2  $\mu\text{L L}^{-1}$ ) on *M. fructicola* conidial germination and mycelia growth, respectively. For the first time, our results showed that volatile organic compounds (VOCs) emitted from heat-treated peaches could stimulate *M. fructicola* conidia germination, increasing brown rot incidence in treated peaches when the inoculation occurs immediately after HWT.





## NATURAL COMPOUNDS AS ANTIMICROBIAL AGENTS AND THEIR IMPACT ON SENSORIAL QUALITY OF PACKAGED ORGANIC LEAFY GREENS

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Microbial growth is one of the main causes of deterioration and postharvest loss of fresh vegetables. Their control is essential especially in the case of organic vegetables, which are more susceptible to microbial attack as they are produced without agrochemicals. Application of natural antimicrobial compounds in packaging seems to be an innovative and safe solution as these compounds can inhibit microbial growth and help maintain quality. The aim of this study was to select natural compounds based on their antimicrobial activity and to evaluate their impact on sensory quality of packaged organic wild rocket. Eugenol, carvacrol, *trans*-cinnamaldehyde, *trans*-anethole and  $\alpha$ -pinene were tested against selected pathogens for their antimicrobial activity in *in-vitro* study. Only eugenol, carvacrol, *trans*-cinnamaldehyde, and *trans*-anethole showed antimicrobial activity. Based on these results and preliminary sensory tests, eugenol, carvacrol and *trans*-anethole were selected for the study. Ten percent of active compound was incorporated into biodegradable pellets, which were inserted in biodegradable non-woven sachets. One sachet with 1g pellets was placed in an empty tray. The tray was filled with 100g of organic wild rocket and wrapped with laser perforated polypropylene film. After 7 days of storage at 5 °C, sensory descriptive analysis was performed. A trained panel consisting of 9 panellist evaluated visual appearance (visual freshness, green color, discoloration, brown cut-edges and rotten leaves) and aroma (fennel, clove, oregano, rotten, sulphur, and off-odor). No significant differences were found for the visual attributes. However, for aroma attributes, packages with notes of eugenol and *trans*-anethole were significantly different from control and carvacrol packages. Eugenol and *trans*-anethole masked the off-odor released by the wild rocket during 7 days storage. The study showed that natural antimicrobials can mask off-odors that impair sensory quality of packaged leafy greens.

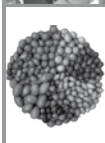


## USE OF TRAMETANO® FOR ELICITING DEFENCE REACTIONS IN WHEAT AND MAIZE PLANTS AGAINST FUNGAL PATHOGENS

**Chiara Pietricola<sup>1\*</sup>, Angela Iori<sup>2</sup>, Valentina Farina<sup>1</sup>, Valeria Scala<sup>1</sup>, Marzia Scarpari<sup>1</sup>, Marcello Scrosati<sup>3</sup>, Massimo Reverberi<sup>1</sup>, Corrado Fanelli<sup>1</sup>**

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Durum and common wheat and maize are amongst the most cultivated cereals worldwide. They are the base of human and animal food and used in livestock industry. Durum wheat is of great importance in Italy and in the Mediterranean area for producing semolina and its derived products such as pasta and couscous. Worldwide production of these cereals achieve approximately 620 Mton and 36 Mton for common and durum wheat respectively. Up to 20-30% of this production is wasted because of foliar diseases due to fungi such as *Septoria tritici* and *Stagonospora nodorum*. Another important aspect to consider is the contamination of cereals with mycotoxins. These substances are secondary metabolites produced by fungi that can have several toxic effects in both humans and animals. *Aspergillus flavus* and *Fusarium verticillioides*, producers of mycotoxins, are the main cause of maize diseases. Phytochemicals treatment in field can partially control these diseases but generating pollution and health hazards. Moreover, starting from 2014 EC has banned several pesticides (EC/129/2009) posing severe constraint to cereal farmers for using such products. If confirmed, the application of this directory will worst the current situation concerning the wheat production leading to concrete and severe losses. The aim of our study is to exploit the eliciting aspect of Trametano®, an exo-polysaccharide produced by the edible mushroom *Trametes versicolor*, for priming the defences of durum wheat and maize against pathogens of these cereals. The onset of plant defences were evaluated by combining multiple techniques of molecular biology and analytical chemistry into leaf samples deriving both from greenhouse and in the field experiments.





## PROTEIN HYDROLYSATES AGAINST *PHYTOPHTHORA* SPP. ON CITRUS

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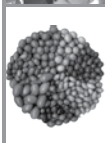
Citrus is an important subtropical crop, cultivated in nearly 135 countries, and is vulnerable to more than 100 diseases, of which Phytophthora diseases are among the most serious and economically important. The use of biotechnological tools associated with conventional citrus breeding was until now the most efficient approach to face the disease as they help developing cultivars with desirable characteristics. However, the setup of alternative control means efficient and eco-friendly would be important whenever the above-mentioned preventive strategies would be not feasible. Recently, protein hydrolysates proved to induce resistance against the oomycetes *Plasmopora viticola* in grapevine plants and, concerning citrus fruit, against *Penicillium digitatum* through eliciting defense responses. In this preliminary study, both *in vitro* and *in vivo* approaches were held to test soybean and casein hydrolysates ability to control brown rot of citrus fruit caused by *Phytophthora citrophthora* and *P. nicotianae*. In *in vitro* tests, a dose-dependent effect was observed. Soybean hydrolysate proved to be the most efficient treatment and *P. nicotianae* the most susceptible pathogen, with a reduction of growth up to 45%. The hydrolysates proved effective even *in vivo*, although further trials are in progress. These preliminary results suggest that hydrolysates might represent an interesting alternative to chemical fungicide application against Phytophthora diseases of citrus fruit.

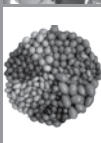
## POSTHARVEST DECAY CONTROL OF A “LONG-STORAGE” TOMATO LANDRACE USING DIFFERENT PREHARVEST TREATMENTS

**Raffaele Carrieri<sup>1a\*</sup>, Mario Parisi<sup>2a</sup>, Francesco Di Dato<sup>2</sup>, Paola Tarantino<sup>1</sup>, Francesco De Vita<sup>2</sup>, Raffaele Perreca<sup>3</sup>, Riccardo Riccardi<sup>3</sup>, Ernesto Lahoz<sup>1</sup>**

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*\*these authors contributed equally to this work*

A cherry-like tomato landrace known as “Pomodoro del piennolo del Vesuvio” is cultivated without irrigation on the slopes of the Vesuvio volcano. Fruits are characterized by high firmness and good thickness of pericarp, no jointless trait and long shelf-life. Following the harvest, they are conserved in the typical hung-shaped appearance up to 250 days under no-conditioned atmosphere. Since remarkable incidences of wilted or rotten fruits have been reported, mainly occurred in the early stage of “natural” home-made preservation, reducing the decay in postharvest is highly desirable for a very profitable crop as the “piennolo” tomato. Recently, alternative sources for controlling postharvest rots have been proposed. In this study, seven commercial products (essential oils of thyme and oregano, Chitoplant, propolis, Karma, Signum, Amylo-X) were sprayed on the plants at 5, 15, 25 and 35 days before the harvest. The control was not treated. Harvested fruits from each experimental plot were conserved in an unconditioned and well-ventilated shed up to 200 DAH. Starting from 80 DAH, the number of healthy and rotten berries was evaluated every 40 days. The decayed fruits showing typical symptoms of fungal diseases, were collected and the fungal pathogens were isolated and morphologically characterized. The data on the efficacy in the control of eight relevant fungal pathogens, in field and in postharvest stage, are reported. Commercial product Signum showed the highest efficacy in the control of the diseases (54.8%) and resulted also to be the most efficient treatment in reducing the postharvest decay showing at 200 DAH a considerable incidence of healthy fruits (56.0% respect to 41.0% recorded for the control). In general, all the products showed more efficacy in the control of fungi, in particular *Penicillium* spp. and *Aspergillus* spp., in postharvest stage.





## CONTROL OF *PENICILLIUM DIGITATUM* ON TAROCCO ORANGE BY COMBINED APPLICATION OF *PSEUDOMONAS SYRINGAE* AND RESISTANCE INDUCERS

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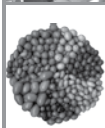
*Penicillium digitatum* is the causal agent of green mould of citrus fruits, that often causes extensive decay losses during storage and transportation, thus limiting the commercial life of harvest fruits. Accordingly, sustainable control strategies may prevent postharvest diseases and may be a useful alternative to pesticide applications. The aim of this study was to evaluate the effectiveness of *Pseudomonas syringae* strain 48SR2 (PS), sodium bicarbonate (SBC) heated to 45°C, acibenzolar-S-methyl (ASM) and chitosan, applied alone and in combination, on oranges cv. "Tarocco" artificially inoculated with *P. digitatum* 24 h before, simultaneously, and 24 h after treatments. Combinations of PS and SBC simultaneously applied with the pathogen effectively controlled green mould of "Tarocco" oranges, whereas treatments applied alone were less effective. When treatments, alone and in combination, were applied 24 h after pathogen inoculation, the efficacy was considerably lower. However, ASM and chitosan, alone and in combination with PS, did not show any efficacy when applied simultaneously or 24 h before *P. digitatum*. PS and SBC applied 24 h before pathogen inoculation significantly reduced disease incidence and severity. Also in this case, the combination of treatments improved the protection provided by PS and SBC treatments alone. Surprisingly, combinations of PS and ASM resulted in significant synergistic inhibition of the green mould when applied 24 h before pathogen inoculation as preventive treatments. Also PS and chitosan in combination significantly reduced incidence and severity of green mould when applied as preventive treatments, and the control was more effective than treatments with chitosan alone. These experiments suggest that the combination of *P. syringae* biocontrol agents and resistance inducers could be considered a recommended approach to improve pest management strategies in citrus packinghouses.

## THE PIPELINE FOR PRODUCTION OF STONE FRUIT-BASED JUICE RESULTS IN ZERO FUNGICIDE RESIDUE BY IMPLEMENTING BIOLOGICAL AND INTEGRATED CONTROL OF BROWN ROT

Filippo De Curtis<sup>1</sup>, Assunta Raiola<sup>2</sup>, Alberto Ritieni<sup>2</sup>, Giuseppe Lima<sup>1\*</sup>, Raffaello Castoria<sup>1</sup>

<sup>1</sup>Dipartimento Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, Via De Sanctis s.n.c. - 86100 - Campobasso, Italy. <sup>2</sup>Dipartimento di Scienza degli Alimenti, Università di Napoli Federico II, Parco Gussone, 80055 Portici, Napoli, Italy. E-mail: decurtis@unimol.it

Brown rot is a major disease of stone fruit. It is caused by *Monilia* spp., and in South and Central Italy causes severe fruit losses. The spores of the fungal pathogen penetrate also through fruit wounds caused by environmental factors and agricultural practices. Disease symptoms appear during storage, when pathogen quiescence ceases and fruit colonization restarts. At present, chemical control is the main strategy against brown rot. We show the results achieved in 3 years of experiments carried out in 5 orchards in Southern Italy with different climatic patterns. Biological and integrated control protocols were applied for preventing brown rot of peaches to be used for producing juices. These protocols led to the achievement of fruit juices with zero fungicide residues.





## EFFICACY OF HEATED SOLUTIONS OF FLUDIOXONIL AND PROPICONAZOL IN CONTROLLING POSTHARVEST DECAY OF CITRUS FRUIT

Raphael Ginat<sup>1</sup>, Oleg Feygenberg<sup>1</sup>, Sawsen Sidri<sup>2</sup>, Valentin Turegano-Meneses<sup>2</sup>, Samir Droby<sup>1\*</sup>

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*Penicillium digitatum*, *P. italicum* and *Galactomyces citri-aurantii*, the cause of green mold, blue mold and sour rot, respectively, are the most common world-wide and cause serious losses in citrus fruit after harvest. Their control relies mainly on the use of several fungicides such as Imazalil, TBZ and OPP or SOPP. In recent years, however, new wide-spectrum low risk fungicides were approved for use against postharvest pathogens of citrus fruit. Among them phenylpyrrole Fludioxonil and DMI-triazol Propiconazole. Efficacy of Fludioxonil and Propiconazole was a subject of different studies in the past and proved effective against various postharvest pathogens of citrus fruit. The aim of this study was evaluate the efficacy of heated solutions of both fungicides in controlling artificial and natural infections of green and blue mold as well as sour rot, in small scale and packhouse trials. The application of non-heated solutions of Fludioxinil (400-700 µg/ml) at 24 h after inoculation with either green or blue mold pathogens reduced infection by more than 70% compared to the non-treated control. In packhouse trials, fruit dipping for 20 seconds in heated solution (50°C) of Fludioxonil at 500 µg/ml followed by coating with polyethylene wax containing 1000 µg/ml of Fludioxonil was more effective that the standard packhouse treatment consisted of heated solution of Imazalil at 500 µg/ml and 1000 µg/ml Imazalil in the wax coating. Navel oranges treated with non-heated solutions of Propiconazole (300-600 µg/ml) at 24 h after inoculation with *G. citri-aurantii* showed that significant control of infection was achieved at concentration above 400 µg/ml. Heated solution (50°C) of Propiconazole at 400 µg/ml, however, was very effective in inhibiting infection and development of sour rot. When Propiconazole was combined with OPP at 1000 µg/ml, it was very effective against sour rot at all concentrations tested (400-600 µg/ml).

## EFFECTS OF ELECTROLYZED WATER (VERDEVIVA) ON APPLE: FIELD TREATMENT AND POSTHARVESTING APPLICATION

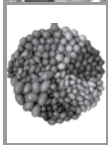
**Violetta Ferri<sup>1\*</sup>, Eade Damara<sup>3</sup>, Giancarlo Colelli<sup>3</sup>, Thaer Yaseen<sup>2</sup>**

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The effect of electrolyzed water on bacterial count was evaluated on freshly harvested apple fruits and on the washing water of working line. Verdeviva, containing 50 to 400 ppm available chlorine, was generated by electrolysis of a KCl solution using an electrolyzed water generator. Apple fruits were treated with electrolyzed water containing 50 ppm available chlorine by immersion and 400 ppm by spraying. These treatments reduced the total microbial count (mesophilic aerobic bacteria) by 0.9 to 1.0 log<sub>10</sub> colony forming units (CFU)/g on fruits surface when compared with untreated samples after 32 days of storage in controlled atmosphere (1 °C, 1% CO<sub>2</sub>, 2% O<sub>2</sub>, and 90% relative humidity, similar effect was recorded on the washing water of working line. Rinsing with Verdeviva containing 50 ppm chlorine and storing in ozone enriched atmosphere increased fruit shelf-life and decreasing bacterial count when compared with the water-rinsed control. Microbial populations increased on untreated fruits stored at 1°C for 7, 14, 21 or 32 days. Furthermore, we reported that a high concentration of chlorine (400 ppm) in water was able to reduce pesticide residue on apple 50% to 100% after washing the apple comparing with untreated fruits. Verdeviva was also applied on field before harvesting in order to protect the apples against *Gloesporium* spp. The apple cv. Cripps Pink was stored in controlled atmosphere or dynamic controlled atmosphere and the rot incidence was evaluated after 4 months. The preliminary results showed that Verdeviva have an activity similar or higher compared to the reference standards.







## EFFECT OF PACKAGING AND STORAGE CONDITIONS ON SOME BIOCHEMICAL PARAMETERS AND MICROBIOLOGICAL SAFETY OF SEMI-DRY TOMATO

Maria Antonia Gatto<sup>1</sup>, Lucrezia Sergio<sup>1\*</sup>, Maria Pieralice<sup>1</sup>, Vito Linsalata<sup>1</sup>, Luigina Spremulli<sup>2</sup>, Donato Di Venere<sup>1</sup>

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High water activity ( $a_w$ ) can affect maintenance and quality of semi-dry products during storage. In the present work, the evolution of physico-chemical, biochemical, and microbiological parameters of semi-dry tomato in different conditions of packaging and storage is reported. Tomato fruits, cv. Ikram, were washed with sodium hypochlorite 0.01% and cut into quarters; slices were sprinkled with a solution of 2% (w/v) ascorbic acid and 4% (w/v) sodium chloride. The semi-dry product was obtained using a heat pump dehydration system, up to 75% weight loss and  $21.0 \pm 0.5\%$  dry matter. Semi-dried tomato slices were packed in air or in modified atmosphere (MA, 30% CO<sub>2</sub> + 70% N<sub>2</sub>), packed in PP trays with a OPA+PP/EVOH/PP cover film, and stored at 4 °C or 12 °C for 30 days. Every 10 days during storage, dry matter,  $a_w$ , pH, colour, texture, content in sugars (glucose, fructose) and lycopene, polygalacturonase activity, and total microbial load were evaluated. As this latter, it was assessed to be much below the limit established for spoilage microorganisms in food products (5 log CFU/g f.w.). The semi-dry product stored in MA at 4 °C maintained the best quality characteristics and good microbiological stability. In particular, after 20 days of storage, a slight increase in the fungal component (i.e. *Botrytis cinerea*, *Mucor* spp., and *Cladosporium* spp.) compared to the starting level (from 3 to 4 log CFU/g f.w.) was observed. Moreover, the bacterial component remained on the initial levels (3 log CFU/g f.w.) during the whole storage period. The results showed that the used MA conditions are able to preserve quality and hygienic properties of the product for 20 days at 4 °C.

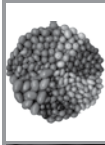
*This research was financed by MIUR - Research Project "High-Convenience Fruits and Vegetables: New Technologies for Quality and New Products" (OFRALSER), PON 01\_01435.*

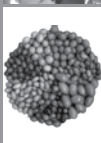
## ALTERNATIVES TO SYNTHETIC FUNGICIDES IN CONTROLLING POSTHARVEST ANTHRACNOSE AND STEM-END ROT IN MANGO

**L. Chathurika Karunanayake<sup>1\*</sup>, Ganga Devi Sinniah<sup>2</sup>, Nimal K.B. Adikaram<sup>3</sup>, Dulanjani S. Wijayasekara<sup>3</sup>, Charmalie L. Abayasekara<sup>3</sup>**

*<sup>1</sup>Department of Botany, Faculty of Natural Sciences, Open University of Sri Lanka, Nawala, Sri Lanka. <sup>2</sup>Plant Pathology Division, Tea Research Institute, Talawakelle, Sri Lanka. <sup>3</sup>Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka. E-mail: kokar@ou.ac.lk*

Two common postharvest diseases affecting the mango industry in Sri Lanka and other South and Australasian countries are anthracnose and stem-end rot (SER). Immature mangoes remain unaffected due to a formidable constitutive defence system. However, ripening leads to decline of antifungal activity causing postharvest disease development. Control of postharvest anthracnose and SER of mango was attempted with alternatives to fungicides. Retention of latex with intact pedicel in harvested fruit proved to be an effective and completely chemical free mean, which reduced both incidence and severity of anthracnose and SER. Inoculation studies showed that, postharvest treatment with defence elicitors, Bion®, Salicylic acid and Kasil® reduced anthracnose development, Bion® being significantly effective with over 80% reduction in lesion area. In particular, 50 ppm Bion® was effective in the more susceptible cultivars, while 25 ppm was effective in a more resistant cultivar. Concerning Salicylic acid, 500 ppm was necessary to reduce anthracnose development and higher concentrations slightly damaged fruit. Kasil® applied as a postharvest dip at 1000 and 5000 ppm induced antifungal activity (on TLC bio-assay) and reduced both anthracnose and SER development; however, reduction was significant only at the onset of disease development. Field application of salicylic acid 100 ppm and 500 ppm was conducted at mid-fruit fill (6 weeks) and mature stage (9 weeks). Mid fruit fill stage application proved to be more effective with significant reduction in anthracnose development. Kasil® applied as a soil drench at 1000 ppm reduced both incidence and severity of postharvest anthracnose development. The effect was significant only at the beginning of disease. Application of high doses of potassium fertilizer to mango trees also effectively reduced SER development in fruit at postharvest stage, percentage reduction ranging from 24 to 45%. Results indicate that alternatives to fungicides are available to control postharvest diseases in mango fruit.





## EVALUATION OF THE EFFICACY OF FUNGICIDE FLUDIOXONIL IN THE POSTHARVEST CONTROL OF 'BULL'S EYE' ROT (*NEOFABRAEA ALBA*) IN CHILE

**Mauricio Lolas<sup>1\*</sup>, Gonzalo Díaz<sup>1</sup>, Raúl Mendez<sup>1</sup>, Marcela Cáceres<sup>1</sup>, Benjamín Valiente<sup>2</sup>, Luis Neubauer<sup>3</sup>**

<sup>1</sup>Laboratorio de Patología Frutal, Departamento de Producción Agrícola, Facultad de Ciencias Agrarias, Universidad de Talca, 2 Norte 685, Talca, Chile. <sup>2</sup>ANASAC Chile S.A., Almirante Pastene 300, Santiago, Chile. <sup>3</sup>PACE International, Av. Presidente Eduardo Frei Montalva 6001, Santiago, Chile. E-mail: mlolas@utalca.cl

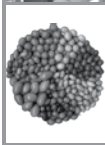
'Bull's eye' rot, caused by *Neofabraea alba*, is a serious disease affecting apples, causing annual losses to the Chilean apple industry during postharvest. The effectiveness of fungicide fludioxonil (Starter Pro 230 SC, ANASAC Chile S.A.; and Scholar 230 SC, Syngenta) in the control of 'bull's eye' rot in apples cv. Cripps Pink, was determined by applications via drenching after harvest. The apples used for evaluations were obtained from a commercial orchard which historically presented a high pressure of 'bull's eye' rot, located in Longaví, Chile. Treatments, which were applied, using an experimental drencher with a recirculating system, consisted in 100 L of fungicide solution showering 6 boxes of 80 apples for 30 s. Then, boxes were removed; air dried and stored at 0°C for 75 and 120 days in a completely randomised design. At each period of storage, the boxes were kept at 22°C for 7 days, after each apple being evaluated for the presence of rot lesions. Finally, after a week out of cold storage, the prevalence of rot lesions in each box was assessed. Treatments consisted in Starter Pro 230 SC at 200 and 100 ml/hL; Scholar 230 SC at 200 ml/hL and, Starter Pro 230 SC at 100 ml/hL + Tecto 500 SC (thiabendazole) at 125 ml/hL. Only water was used as a control treatment, in which the prevalence of 'bull's eye' rot reached 42.9% in those apples, cold stored for 120 days + 7 days at 22°C. Starter Pro 230 SC and Scholar 230 SC at doses of 200 ml/hL, significantly reduced 'bull's eye' rots prevalence in apples evaluated after 120 days of storage at 0°C and also, after 7 days at 22°C. However, at doses of 100 ml/hL, Starter Pro 230 SC was not effective compared with the control. The mixture of Starter Pro 230 SC at dose of 100 ml/hL and Tecto 500 SC at dose of 125 ml/hL, was significantly effective in reducing the 'bull's eye' rot prevalence.

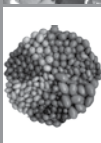
## EFFICACY OF NEW FUNGICIDES ON POSTHARVEST “BOSC” PEAR DECAY BY *ALTERNARIA* AND *CLADOSPORIUM* IN NORTH PATAGONIA, ARGENTINA

María Cecilia Lutz<sup>1\*</sup>, María Cristina Sosa<sup>1</sup>

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In recent years, decay by *Alternaria* spp. and *Cladosporium* spp. constituted a hazard to “Bosc” pear fruit during cold storage associated with significant economic losses (about 50%) in the Rio Negro and Neuquén Valleys. Studies with new fungicides that potentially have good activity against these key pathogens of “Bosc” pears were completed. In 2011, sensitivity to: pyraclostrobin (pyr) + boscalid (bosc) and cyprodinil (cyp) + fludioxonil (flu) in *A. alternata* and *C. herbarum* isolates was assayed according to FRAC. Doses tested were 0, 0.1, 1, 10 and 100 µg/mL. Both fungicides efficiently controlled “in vitro” the colony mycelia growth of *A. alternata* (98% with cyp+flu; ED<sub>50</sub> 0.0014 µg/mL) and *C. herbarum* (100% with pyr+bosc, ED<sub>50</sub>: 0.00049 µg/mL). Furthermore, spore’s germination of both pathogens was significantly reduced. Efficacy of fruit decay control was evaluated. Sets of 10 fruits (times 3) were wounded, treated by immersion with fungicide (1min) and inoculated with mycelia (3x3 mm). After 7 days at 22±2°C, both fungicides (100 µg/mL) reduced significantly the incidence by *C. herbarum* (85%) and *A. alternata* (50%). Post-harvest control assays were done by spraying with pyr+bosc and cyp+flu prepared to 0.6 and 1 gr/L, respectively on fruits in packing line in 2012. Sets of wound artificially inoculated and control fruits were stored 4 months at -1/0°C. Protective effect by fungicides in wounds inoculated with *Alternaria* and *Cladosporium* was demonstrated (%IR=98). Natural infection on non-artificially wounded fruit was reduced in 35%. The presence of inoculum in the orchard and the need to diminish rates of infection, directed the evaluation of pre-harvest spray. A set of fruits was wounded at harvest and cold stored for 2 months. Decay incidence was significantly reduced by pyr+bos (into 90%). These results are promising for the control of decay by *Alternaria* and *Cladosporium* on “Bosc” pears in Argentina.





## POSTHARVEST PRESERVATION OF APPLE FRUITS USING INTEGRATION OF GAMMA IRRADIATION AND BIOCONTROL AGENT

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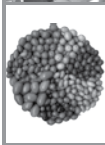
Apples have the second highest free radical scavenging-linked antioxidant activity, whereas antioxidants activity of fruits is important for both postharvest preservation and human health benefits. Postharvest blue mold caused by *Penicillium expansum* is the major factor limiting the storage life of apples. The purpose of the present study was to investigate changes in antioxidant activity and lesion diameter of cv. Golden Delicious apples in order to determine the optimum dose of gamma irradiation in combination with *Pseudomonas fluorescens* and to avoid blue mold during 9 months cold storage. Since, the possibility of changes in quality value caused by irradiation depends on irradiation dose, the minimum dose should be applied to control postharvest diseases on apple fruits. Treated fruits were irradiated at doses of 0, 200, 400, 600 and 800 Gy and then inoculated with *P. fluorescens* suspension. Samples were evaluated at 3 months intervals. In non-irradiated and irradiated samples, antioxidant activity was significantly increased after 6 months, but in pathogen-treated non-irradiated samples, it was observed earlier. In antagonist-treated samples, *P. expansum* was inhibited similar to irradiation at 200-400 Gy. So, the best result was observed through integrated effect of antagonist and irradiation (at 200- 400 Gy) after 9 months storage. Also, data showed that, lesion diameter of pathogen-treated non-irradiated apples was significantly increased after 3, 6 and 9 months storage. However, antagonist effect was similar to irradiation at 200 and 400 Gy that could prevent lesion diameter in pathogen-treated apples. *P. fluorescens* inhibited *P. expansum* similar to irradiation at 200-400 Gy and conclusively can reduce apple fruits losses during postharvest preservation. It is clear in this study that integrated treatment of gamma irradiation and biocontrol agent has potential as an alternative means of postharvest disease control.

## **'CONIELLA GRANATI' - A NEW PATOGEN OF POMEGRANATES IN ISRAEL - POSTHARVEST FUNGICIDE SCREENING FOR CONTROL OF STORAGE DECAY**

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Pomegranate storage duration is limited by postharvest physiological and pathological problems. The latter include a wide range of pathogenic fungi: *Botrytis cinera*, *Alteranria* sp., *Penicillium* sp., *Aspergillus* sp., *Rhizopus stolonifer* and *Mucor* sp. In 2010, a new pathogen causing decay appeared in storage, *Coniella granati*. The fungus has been found in the orchard on the bark of dry twigs and may infect the young fruit growing on the tree. During storage irregular, yellow-brown patches appear on the fruit peel, upon which black, hard fruiting bodies - pycnidia - develop and the decay spreads from the peel to the arils. The aim, of our study was to find a postharvest, fungicidal treatment to control decay development during storage. Pomegranate fruit picked in early November were wound inoculated with a *C. granati* spore suspension. After overnight incubation at 20°C and 95% RH, the fruit were divided into 9 lots and dipped in 8 different fungicides, or left untreated as a control. The tested fungicides were: pyrimethanil, captan, fludioxinil, prochloraz, thiabendazole, fluopyram + tebuconazole, trifloxystrobin + tebuconazole. When dry, the fruit were cooled to 7°C and packed in LDPE liners. After 3 months' storage at this temperature decay incidence was monitored. The results showed that among the fungicides tested, prochloraz (not permitted in Europe) and the two combinations containing tebuconazole were the most effective in controlling the *C. granati* decay.





## NEW METHOD TO CONTROL POSTHARVEST DISEASES USING BIOLOGICAL FUNGICIDE POLYVERSUM CONTAINING *PYTHIUM OLIGANDRUM* BY FOGGING IN STORAGE CHAMBERS

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Polyversum, biological fungicide containing *Pythium oligandrum* M1 is well known as a very effective against several fungi, like *Botrytis* spp., *Penicillium* spp., *Rhizopus* spp. etc. which causes also important postharvest diseases. Since 2012 we have started testing of the new method for postharvest treatment in various crops in storage chambers. We used hot fog generator PulsFog Bio for application of Polyversum. Tests were performed as on long term storage crops like apples, pears, Chinese (nappa) cabbage, cabbage or celery as on limited term storage crops like strawberry and broad bean. In all performed tests we received spectacular results in suppression of those pathogens compared to untreated control. We received variable results in the root vegetables, due to remnants of soil present in storage crates, which probably influenced penetration of fog. We expect the first minor crop authorization of the product for postharvest application in Poland in 2015 as we received positive toxicological assessment report from the competent authority.

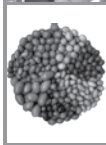


## INTEGRATED TREATMENT WITH EDIBLE COATINGS AND THYME OIL ON THE CONTROL OF IMAZALIL RESISTANT *PENICILLIUM DIGITATUM* OR *P. ITALICUM* STRAINS AND *GEOTRICHUM CITRI-AURANTII*

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Significant postharvest losses are encountered during storage and transportation of citrus fruit due to green mold (*P. digitatum*), blue mold (*P. italicum*) and soft rot (*G. citri-aurantii*). Use of synthetic fungicides for postharvest application is becoming a challenge due to various reasons such as: higher consumer preference for organic fresh produce that is free from toxic residues, importing countries enforcing strict regulations regarding the minimum pesticide residue levels in the edible portion of the fresh produce, postharvest pathogens developing resistance to synthetic fungicides, and waste disposal of fungicides in the pack houses. In this investigation, nine essential oils were screened (*in vitro*) in order to see the antifungal activity on imazalil resistant *P. digitatum* or *P. italicum* strains and *G. citri-aurantii*. Gum arabic or *Aloe vera* or chitosan coating alone or in combination with thyme oil were tested in *in vitro* in order to determine the effectiveness of the coatings on the three pathogens separately. Curative and preventative applications of chitosan coating and thyme oil (1:3 v/v) were carried out on soft citrus cv. Empress at 20 °C. Although 100% disease incidence was observed with respect to all the treatments adopted in this investigation after postharvest storage at 7 days, significant reduction of the severity of soft rot, green and blue mold rot was noted with chitosan treatment. Chitosan treatment was observed to increase the PAL, PPO activities as well as the total phenolic content in fruits from preventative application. Therefore, chitosan coating is suitable for organic soft citrus fruit although the decay severity was similar to that of the commercial fungicide application. Furthermore, the findings revealed the negative effect of integrated treatment of thyme oil and chitosan application on imazalil resistant *P. digitatum* or *P. italicum* strains and *G. citri-aurantii*. Although lemon grass oil showed 100% radial mycelial growth inhibition in *in vitro*, it failed to reduce the disease severity of the three tested pathogens during curative and preventive treatments.





## SU.SA.FRUIT: LOW PESTICIDE IPM IN SUSTAINABLE AND SAFE FRUIT PRODUCTION

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European agricultural policy requires the implementation of integrated pest management (IPM) to promote a sustainable use of pesticides with regard to regional growing conditions. European Member States are developing National Action Plans to reduce the use of pesticides wherever possible. Due to the hazardous effects of agrochemicals both on humans and the environment, there is a growing trend towards the management of ecological interactions in agro-ecosystems. IPM has become the mainstream strategy for plant protection in the EU, as it is an important tool to maintain food security, while increasing the environment protection. The overall objective of the LIFE.SU.SA. FRUIT project is to develop, apply and demonstrate an economically viable strategic plan to implement IPM, by promoting the use of low chemical field and post-harvest fruit production practices, in typical Croatian and Italian agro-ecosystems. The project aims to create an environmentally friendly management system for fruit production and storage by making more efficient use of resources, while ensuring food safety. Innovative field (i.e. insect exclusion nets; autoconfusion; biocontrol agents) and post-harvest (i.e. hot water treatments) fruit production practices aim at reducing pesticides, leading to lower the environmental impact and the risk of worker exposures. The project will be performed in Croatia and Italy. The partners involved are Zagreb, Torino and Bologna Universities, a producer association (Apofruit), and two private companies (Agra and XEDA International). The target pests are codling moth and peach moth while the target pathogens are apple scab, brown rot and other apple, peach and nectarine postharvest pathogens.

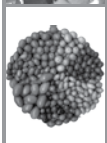
## BIOCOMES: NEW BIOLOGICAL PRODUCTS FOR SUSTAINABLE FARMING AND FORESTRY

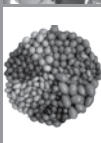
**Neus Teixidó<sup>1\*</sup>, Antonieta De Cal<sup>2</sup>, Josep Usall<sup>1</sup>, Belén Guijarro<sup>2</sup>, Inmaculada Larena<sup>2</sup>, Rosario Torres<sup>1</sup>, Maribel Abadías<sup>1</sup>, Jürgen Köhl<sup>3</sup>**

<sup>1</sup>IRTA, XaRTA-Postharvest, Edifici Fruitcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, 25003 Lleida, Catalonia, Spain. <sup>2</sup>INIA, Departamento de Protección Vegetal, Carretera de la Coruña Km 7, 28040 Madrid, Spain. <sup>3</sup>DLO (Wageningen UR) The Netherlands. E-mail: neus.teixido@irta.cat

The growing interest in biological control has been reflected during last decades in a big number of scientific publications, books and symposia. However, biocontrol commercial application at a European level is limited and biological control products are not currently available for the control of important pests and diseases that cause high economic losses. The objective of BIOCOMES is to develop 11 new biocontrol products to control key pests and diseases present in agricultural and forestry crops, as a suitable and environmental-friendly alternative strategy to chemical products. BIOCOMES includes 13 private companies (most of them SEMs) and 14 research centres from 14 different countries, working together to develop tools and give solutions to the Integrated Pest Management in Europe. Main targeted pests and diseases are: gypsy moth and large pine weevil in forestry, tomato leaf miner, potato moths, whitefly in vegetables, aphids in fruit tree crops, cabbage moth, brown rot in stone fruit, fungal root diseases in nurseries, Fusarium in cereals, soil-borne Verticillium wilt in Brassica and powdery mildew in cereals. Developed biocontrol products are parasitoids, entomopathogenic nematodes, viruses, bacteria and fungi, and at the end of the project most of them will be at a stage close to market implementation. Brown rot in stone fruit is the only postharvest disease studied in BIOCOMES and the objective is to complete the development of the biocontrol agents *Penicillium frequentans* 909 (INIA) and *Bacillus subtilis* CPA-8 (IRTA) to obtain products that provide an effective strategy to control brown rot in stone fruit production.

*This research is supported by a European Union's VIIth Framework Programme project (FP7-612713).*





## VAPORIZATION OF BIOLOGICAL CONTROL ORGANISMS IN COLD STORAGE ROOMS TO CONTROL POSTHARVEST DISEASES

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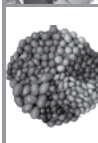
Storage diseases can cause important losses on pome fruits. Disease management to control storage diseases includes several treatments with different fungicides in the weeks prior to harvest. Nowadays, the presence of residues on fruits becomes more and more a public and governmental concern. In order to reduce the chemical residue on fruits to a minimum, more and more research is performed on alternative disease management. In this respect, in 2013, a project concerning the 'Vaporization of biological control organisms (BCOs) in cold storage to control postharvest diseases', which was funded by the Agency for Innovation by Science and Technology, has started at the pcfruit research institute in collaboration with ILVO and the University of Leuven. In a first step, the influence of fungicides applied in the months prior to storage on different BCOs was tested *in vitro* and *in vivo*. Besides that, also the influence of additives on the efficiency of biological control organisms in their control of storage diseases caused by *Neofabraea alba* or *Botrytis cinerea* was investigated after artificial inoculation experiments. This pointed out that some fungicides can have important negative effects on the BCOs tested and that additives, like calcium chloride and calcium nitrate, can enhance the efficacy of BCOs in the control of fungal storage diseases. Furthermore, out of different tests the vaporization device 'Swingtec Fontan Starlet' was selected for the vaporization of the BCOs and research is going on to optimize the homogeneous distribution of the BCOs in the cold storage room with the help of a Computational Fluid Dynamics (CFD) model.

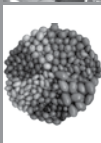
## BTH PROMOTED WOUND HEALING ON POTATO TUBERS

**Wang Yi\*, Bi Yang, Li Yongcai, Wu Juetian, Li Changjian, Jiang Hong, Bao Gaihong**

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Wound healing is one of the most effective strategies for reducing postharvest disease of potato tubers. Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH, Bion<sup>®</sup>, Actigard<sup>®</sup>), is also known as acibenzolar-S-methyl (ASM), supposedly a structural and functional analogue of salicylic acid, has been acknowledged as an effective activator of systemic acquired resistance, it was reported that BTH could reduce postharvest diseases in some fruits. In this study, the effects of BTH treatment at 100 mg/L on wounded potato tubes (cv. Longshu No.3) was investigated by using weight loss, disease incidence and index, and suberin poly (phenolic) (SPP) domain and suberin poly (aliphatic) (SPA) domain depositions as indicators of wound-healing efficiency. The results showed that weight losses, disease incidence and index in wounded tubes treated with BTH were lower than those of untreated control. The wounded tubers treated with BTH promoted accumulation of SPP and SPA. In addition, BTH treatments obviously enhanced the activities of phenylalanine ammonialyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumarate/coenzyme A ligase (4CL), cinnamyl alcohol dehydrogenase (CAD), peroxidase (POD), and increased the content of lignin in wounded tubers. The results suggested that BTH could accelerate the suberization of wounded potato tubes by inducing the phenyl propane metabolism.





## EFFECT OF ELECTROLYZED WATER, OZONIZED WATER AND CONTINUOUS OZONE EXPOSURE ON APPLE DURING CONSERVATION

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In this work, the effect of ozonized water (O<sub>3</sub>) 2ppm and electrolyzed water (EW) 400 ppm of active chlorine as disinfectant of apple washing water of working line was evaluated. Washed fruits were stored in ozone-enriched atmosphere (0.5ppm) to determine the effect of the combination of both methods on the quality of apple fruit during postharvest, untreated apple fruits stored in conventional conditions were used as controls. The amount of fungi and yeasts Colony Forming Units (CFUs) present on fruit surface and shelf life (McKinney index) were evaluated after washing and during storage. O<sub>3</sub> concentration and EW dose were set in a previous *in vitro* experiment. The results showed that EW was more efficient than ozonized water in reducing the quantity of contaminants present on fruit surface; this effect was more evident in stored fruits in ozone-enriched atmosphere. Similar reduction was recorded on yeasts, with the difference that ozonized water was less efficient in reducing CFU of yeast. An extension of fruit shelf-life was also observed at the end of the trial. The results lead to suggest a correlation between the antimicrobial effect of ozone and electrolyzed water treatment to counteract a pathogenic attack or, in general, a stress event.

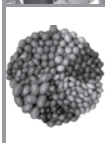
## EFFECT OF MANAGING PROCESSES IN STONE FRUIT PACKINGHOUSES ON *MONILINIA* SPP. DEVELOPMENT

**Maria Bernat<sup>1\*</sup>, Joan Segarra<sup>2</sup>, Antonieta de Cal<sup>3</sup>, V. Llorens<sup>1</sup>, Carla Casals<sup>4</sup>, Josep Usall<sup>1</sup>**

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The brown rot caused by *Monilinia* spp. produced important economical losses in postharvest. Catalonia (Spain) has a high production of stone fruits and an important part is exported to other European countries. Therefore, it is necessarily increase stone fruit shelf life. The objective of this study was evaluating development of disease caused by *Monilinia* spp. on each of different managing processes on a stone fruit packinghouse. Peaches and nectarines were infected with *Monilinia laxa* at  $10^3$  conidia/ml in three different moments (0, 24 and 48 hours) before the processes. Then, inoculated fruits were subjected to each different process. The different managing processes were: hydrocooling, cool rooms at 0 and 4 °C, water dump at 4 and 15 °C with either sodium hypochlorite (40ppm) or without it (0 ppm), sorting at 15 and 25 °C during 5 hrs and air cooling tunnel. After each postharvest process fruits were incubated 8 days at 0°C and then 5 days at 20°C. Disease incidence and lesion diameter were recorded during incubation period. All fruits inoculated at 48 hours before each process has developed the disease independently of the management process done. However, we found significant differences between some management processes on fruits inoculated at 0 and 24 hours. Water dump with 40 ppm of sodium hypochlorite and hydrocooling were the only processes where brown rot was reduce. The most important factors to reduce disease incidence on hydrocooling were exposure time and sodium hypochlorite concentration.

*This research was supported by the Ministry of Economy and Competitiveness (Government of Spain) with the project AGL2011-30472-C02-02 and by a PhD grant BES-2012-059949 (Maria Bernat).*







## **PATHOLOGICAL AND PHYSIOLOGICAL RESPONSE OF 'JINTAO' KIWIFRUIT TO INCREASING CONCENTRATION OF CARBON DIOXIDE PRODUCED BY MODIFIED ATMOSPHERE PACKAGING**

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Modified atmosphere packaging (MAP) is widely used to extend the storage of 'Hayward' kiwifruit. Softening and fruit dehydration are reduced in an environment of high humidity and high CO<sub>2</sub> concentration provided by modified atmosphere bags. Furthermore, controversial results on control of stem end rot, produced by *Botrytis cinerea*, have been reported regarding to CO<sub>2</sub> concentration. Jintao, an *Actinidia chinensis* kiwifruit specie, recently introduced as new supply of yellow cultivar of kiwifruit worldwide, requires basic knowledge on pathological and physiological deterioration to extend the storage at low temperature. The aim of this research was to understand the effect of increasing concentration of CO<sub>2</sub> on deterioration of 'Jintao' kiwifruit. High CO<sub>2</sub> concentration in an atmosphere non-restrictive in O<sub>2</sub> (CO<sub>2</sub>/O<sub>2</sub>; 3.5%/18%; 5.0%/16%; 8.0%/11%) around 'Jintao' kiwifruits was attained by passive MAP and fruit quality was compared with perforated bags stored for 60, 90 and 120 days at 0°C. The firmness retention was the main physiological effect produced by increasing concentration of carbon dioxide. Instead of stem end rot, calix end rot produced by *Botrytis cinerea* was the main disease associated with decayed kiwifruit at every storage period. MAP increased the prevalence of the disease compared with perforated bag but the high concentration of CO<sub>2</sub> reduced the percentage of rotten fruit during storage. Future studies of epidemiology the disease will be able to introduce an effective disease control which qualifies the benefits of modified atmosphere packaging on 'Jintao' kiwifruit.

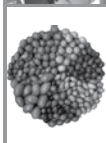
*This research was supported by Kiwigold Chile Spa.*

## EFFECT OF PREHARVEST SALT TREATMENT AND OZONE LOW CONCENTRATION ON POSTHARVEST DECAY PEACHES

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To meet the increasing demand for safe and high quality fresh peaches and the recent food safety regulations is important to embrace a holistic approach combining pre- and postharvest practices in view of attaining maximum quality and satisfaction at the consumer level. Modern sanitation techniques relying on application of physical methods and/or Generally Recognized As Safe (GRAS) compounds are desired for reducing microbiological spoilage. Ozone has attracted considerable commercial interest, especially because it does not leave any residue on produce and is accepted by many organic grower organizations. The activity of preharvest application of 1% calcium chloride and Fortisol (Citrosol, Valencia, Spain) in combination with postharvest O<sub>3</sub> (150 ppb) supply on postharvest rots of peaches cv Notarangelo is reported. Water and fenhexamid (500g/ha a.i.) were used as untreated and chemical control, respectively. Three days after in the field treatment, fruits were harvested and cold-stored for 15 days (3±2°C, 92±5% RH) in presence of conventional or ozonized atmosphere. At the end of storage in both storage conditions no rotted fruits were observed. After three days of storage at 20°C shelf-life, calcium chloride and Fortisol, compared to the untreated control, resulted in a reduction of rots of 34% and 70%, respectively, instead in presence of O<sub>3</sub> the reduction was 25 and 48%. Furthermore, in ozonized atmosphere the weight losses was significantly lower as compared to the conventional atmosphere and, consequently, fruits on the whole showed a more fresh appearance. Ozone influenced microbial epiphytic population, being bacteria and filamentous fungi reduced and yeasts increased at the end of cold storage. In addition, the activities of beta-1,3-glucanase, peroxidase, chitinase, and peroxidase were increased in salt treated peach fruits. The combined application of salts and O<sub>3</sub> could be an acceptable strategy for preserving postharvest quality of peach fruits.





## APPLE DISINFECTION: PREVENTING OR INTRODUCING BIAS?

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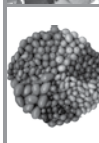
Fruit material used in postharvest phytopathology research is generally disinfected prior to subjecting it to treatments. Although this is common practice, many different protocols exist, most of them with ethanol or sodium hypochlorite. A targeted investigation into the effects these treatments might have on the fruit and, hence, on the research outcome, is lacking. Therefore, the aim of this research was to investigate the effect of ethanol (70%, 2 min), sodium hypochlorite (8°C1, 2 min), and hot water (55°C, 4 min) treatments on macroscopic quality parameters of apple fruit (cv. Jonagold) in order to select a disinfection protocol suited for research purposes. Treated apples were stored at 18°C for 15 days during which respiration was measured regularly. On day 15, firmness, brix, color, and weight loss were measured. All treated apples exhibited a significantly ( $p < 0.05$ ) higher respiration rate compared to the control. Ethanol had a significant effect on the apple fruit color attributes and sodium hypochlorite treatment caused an increase in brix. No significant changes were detected for firmness or weight loss. The same setup was then used to test different sodium hypochlorite concentrations ranging from 0.3 to 15°C1 (2 min) in order to find an optimum at which no changes occur for the measured variables. Although respiration rates and brix values were correlated with sodium hypochlorite concentration, no significant changes were found in contrast to previous results. Treatment with 15°C1 did exhibit a significantly higher weight loss, indicating changes in the apple's cuticle. This study demonstrated that prevalent disinfection methods used in postharvest phytopathology studies induce changes in macroscopic fruit quality parameters. Therefore, they can possibly introduce a bias on the research outcome. No significant or apparent effects were detected for sodium hypochlorite concentrations of 1°C1.

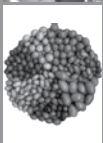
## THE EFFECTIVENESS OF *BACILLUS SUBTILIS* ON THE CONTROL OF THE MOST COMMON POSTHARVEST PHYTOPATHOGENS

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Food preservation concerns all growers around the world and involves the prevention of bacterial and fungal infections that may occur during the growing season and at harvest time, but also during food storage, transport, and marketing. Microspore's research activities and trials have proved that Sublic, a formulation based on *Bacillus subtilis*, can be used to control postharvest diseases. *B. subtilis* is a PGPR (plant growth-promoting rhizobacteria) that can be found in soil and already used in various biocontrol systems. The formulation based on *B. subtilis* enhances foliar microflora rebalancing, in order to prevent stresses caused by pathogen attacks. In particular, this research aimed at testing Sublic effectiveness to control some of the fungi causing postharvest diseases, like *B. cinerea* and *Fusarium* spp. *In vitro* antagonism trials showed a high-level of inhibition for both phytopathogens. An analysis of the fungicide activity of subtilisin produced by *B. subtilis* has been also conducted by cultivating *B. cinerea* and *Fusarium* sp. in increasing concentrations of bacterial cultural filtrates. The fungal performance (biomass) was evaluated by determining the dry weight: the filtrates of *B. subtilis* inhibited the growth of both fungi, being *B. cinerea* the most sensitive. At a later stage, some *in vivo* tests were made through foliar application of Sublic. In addition to a significant control of both fungi, the formulation determined an increase in fruit weight and yield.





## SOURCE AND SPREAD OF FUNGAL PATHOGENS CAUSING CROWN ROT DISEASE IN ORGANIC BANANAS

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Bananas are harvested while still green and many packaging processes are carried out until arriving to the market. Preparing banana hands by dividing the big cluster (dehanding), then cleaning and removing the accumulated latex (delatexing) that exudes directly after trimming the crown tissues. One of the most important postharvest disease affecting bananas during this stage is crown rot. The infection mainly occurs at harvest time, but the symptoms appear after overseas transportation. It is a complex disease where different fungal pathogens are involved and varying from one region to another. The use of synthetic fungicides to control such a disease is restricted and regulated in organic production. Over a period of two years (2013-2014), samples were collected directly on site in Dominican Republic from five different organic banana plantations and their corresponding packing stations, as symptomless samples covering all steps. A total of 3181 fungal colonies were obtained from crown tissues and 600 representative colonies were purified, characterized, and identified using morphological and molecular methods. Fungi were found in all analyzed samples representing eleven genera and the etiological agents of crown rot on organic banana were mainly *Fusarium* (38%), *Colletotrichum* (10%) and, *Lasiodiplodia* (2%). Fungi were isolated in high rate from flowers as well as crown parts, mainly occupied external part but some of them were isolated from the inner tissues. The diffusion of pathogens occurs when the bananas are processed through the dehanding and washing tanks. The final crown trimming followed by washing step and the application of protective products are the critical points.

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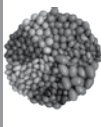
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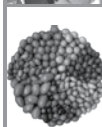
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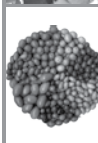
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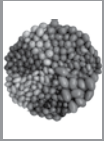
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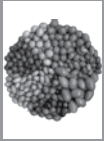
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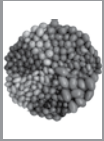


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