Postharvest disease management in organic farming: integration of strategies

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Postharvest pathogens

**Latent pathogens:**
natural openings or directly

**Wound parasites:**
accidental openings
Postharvest disease management

- Disease monitoring
- Disease prevention
- Disease control
Best agricultural practice

- Choice of the cultivar
- **Balanced fertilization**
- Adequate irrigation
- Pruning systems
- Removal of mummies (Monilia spp.)
- Harvest time
Balanced fertilization

Cadophora luteo-olivacea

After 3-4 months storage

Correlation of skin pitting with dry matter and nitrogen content.
Brown rots on different peach and nectarine cultivars

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Cultivar</th>
<th>Total rots (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>after 4 weeks storage</td>
<td>after 8 weeks storage</td>
<td>1st harvest</td>
<td>2nd harvest</td>
</tr>
<tr>
<td>Peach</td>
<td>Rubi Rich</td>
<td>10.8</td>
<td>13.8</td>
<td>*68.4</td>
<td>85.5</td>
</tr>
<tr>
<td></td>
<td>Vista Rich</td>
<td>*4.0</td>
<td>9.8</td>
<td>*56.4</td>
<td>72.6</td>
</tr>
<tr>
<td></td>
<td>Red Moon</td>
<td>0.6</td>
<td>0.6</td>
<td>*13.8</td>
<td>83.4</td>
</tr>
<tr>
<td></td>
<td>Rome Star</td>
<td>*1.2</td>
<td>4.6</td>
<td>*38.2</td>
<td>52.2</td>
</tr>
<tr>
<td>Nectarine</td>
<td>Big Top</td>
<td>*5.8</td>
<td>7.4</td>
<td>*40.6</td>
<td>52.6</td>
</tr>
<tr>
<td></td>
<td>Diamond Ray</td>
<td>*0.6</td>
<td>3.4</td>
<td>*13.8</td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>Orion</td>
<td>0.4</td>
<td>1.6</td>
<td>*4.0</td>
<td>9.4</td>
</tr>
</tbody>
</table>
- Clean boxes, bins and storage chambers
- Elimination of waste waters
- Industrial equipment provided with sterilization programmes
- Workers with adequate technical clothes
Best storage practice

- Low temperature
- Curing
- Control of relative humidity
- Controlled atmosphere
- Ethylene control

Curing:
24-48 h at room temperature to avoid Botrytis cinerea infections on kiwifruit
Controlled atmosphere for kiwifruit

C. luteo-olivacea

<table>
<thead>
<tr>
<th>Packinghouse</th>
<th>CA(^a) conditions</th>
<th>CA(^b) establishment (days)</th>
<th>C. luteo-olivacea incidence (%) on kiwifruit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{O}_2) (%)</td>
<td>(\text{CO}_2) (%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.0</td>
<td>4.5</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>4.5</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>4.0</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>5.0</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>4.5</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>3.3</td>
<td>4.7</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>3.3</td>
<td>4.7</td>
<td>12</td>
</tr>
</tbody>
</table>

In fruit with low dry matter and high N:

- Shorter storage time
- Slower rate of C.A. and temperature establishment (pull down)
Postharvest disease management

Disease monitoring

Disease prevention

Disease control
Disease monitoring

Development of molecular diagnostic tools

Monilinia spp. in Italy

Three species: multiplex PCR
Postharvest disease management

- Disease monitoring
- Disease prevention
- Disease control
Disease control strategies

- Thermotherapy
- Biocontrol agents
  - Natural products:
    - silicates,
    - chitosan,
    - VOCs,
    - acetic acid,
    - plant extracts,
    - glucosinolates,
    - essential oils.
- Resistance inducers
Thermotherapy

Application methods

- Hot water spray
- Hot air
- Hot water dip

Temperature > 40°C (45-60°C)
Time: 1-60 minutes

Effects: pasteurization / induction of resistance
- increase of lignin in tissues,
- HSPs,
- PRPs (Porat et al., 2000),
- phytoalexins (e.g. scoparone and scopoletin).

Citrus against P. digitatum
Thermotherapy on peaches cv Cresthaven

**TT: advantages**

- Wound cicatrisation
- Maintenance of the organoleptic characteristics
- Ease of use
- Lack of residues
- Lack of toxicity
- **Possibility of integration with available strategies**
Effect of hot water dipping against Monilinia laxa on plums cv Angeleno stored at 1°C for 30 days

Max: 48-52°C for 3 min
No phytotoxicity
**TT: damages**

**Damages due to hot air**

**Apricots cv Ninfa artificially inoculated with Monilinia laxa.**

- a. Fruit dipped in water at 48±1°C for 3 minutes.
- b. Fruit dipped in water at 56±1°C for 3 minutes.
Commercial HWD

Prototype and commercial equipment (Courtesy Marta Mari)
## Biological control

### Ideal antagonist

- Genetically stable
- Effective at low concentrations
- Effective against a wide range of pathogen species
- Effective on various host species

- Simple in its nutritional requirements
- Able to grow in cheap substrates
- Able to be formulated with a long shelf life
- Easy to be applied and distributed

- Compatible with other chemical and physical treatments
- Compatible with commercial processing procedures
- Able to survive in adverse environmental conditions

- Not pathogenic for the host plant
- Not toxic for humans
- Not able to grow at 37°C

#### Yeast

- tolerant to extreme environmental conditions of storage (T close to 0°C, high RH, low O₂)
- adapted to fruit (high sugar, high osmotic pressure, low pH).
- rapid growth in fermenters,
- lack of production of toxic metabolites.
Development of BCAs

Isolation

Selection

Efficacy

Mechanism of action

Molecular characterization

Patent

Production

Stabilization and formulation

Toxicological studies

Patent

Registration
Mechanisms of action

Why?
- Formulation
- Methods of application
- Registration

A tritrophic interaction

Antagonist
- Microbiology
- Microscopy
- Biochemistry
- Molecular biology
- NG sequencing
- Proteomics
- Metabolomics
- Bioinformatics

Host fruit → Pathogen
BCAs under development

**Metschnikowia pulcherrima**
BI O126 and MACH1

**Metschnikowia fructicola**
AL27 and AP47

**Pichia guilliermondii** M8

**Pseudozyma fusiformata**
AP6

**Aureobasidium pullulans** PL5
Metschnikowia spp.

Active against *Botrytis cinerea*, *Penicillium expansum*, *Monilia* sp., *Alternaria alternata*

Ascomycetous yeast
(an.: *Candida pulcherrima*)
1. No growth at 37°C.
2. No production of antibiotics on apple.
3. Present on fruit, buds and flowers of apple trees.
4. Initial fermenter in the cider process.
5. Early fermentation in grape must.

BI O126
Spadaro et al 2002, PBT

MACH1
Saravanakumar et al 2008, PBT
**P. guilliermondii vs B. cinerea on apples**

Pichia guilliermondii M8
Isolated from maize rhizosphere
vs Botrytis cinerea

Apples were treated with the $10^8$ cells ml$^{-1}$ by dipping for 1 min, stored at 1°C for 120 days.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control of grey mould on apples</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease incidence (%)</td>
<td>Inhibition efficacy (%)</td>
</tr>
<tr>
<td>P. guillermondii M8</td>
<td>20.0 ± 4.0 a</td>
<td>55.8 ± 8.8 b</td>
</tr>
<tr>
<td>Tebuconazole*</td>
<td>10.7 ± 2.3 b</td>
<td>76.5 ± 5.1 a</td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>45.3 ± 4.0 c</td>
<td>---</td>
</tr>
</tbody>
</table>

Zhang et al., 2011 BIO CON
**BCAs on stone fruit**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP6</td>
<td>Apple</td>
<td>Pseudozyma fusiformata</td>
</tr>
<tr>
<td>AP47</td>
<td>Apple</td>
<td>Metschnikowia fructicola</td>
</tr>
<tr>
<td>PL5</td>
<td>Plum</td>
<td>Aureobasidium pullulans</td>
</tr>
</tbody>
</table>

Efficacy against brown rot of 3 antagonists applied at $10^8$ cells ml$^{-1}$ on peaches cv Redhaven stored at 1$^\circ$ C for 21 days.

Zhang et al., 2010 PBT
A. pullulans on stone fruit

Efficacy of A. pullulans PL5 against M. laxa on plums cv Angeleno and on peaches cv Redhaven stored at 1.2°C for 28 and 21 days.

No effect on fruit quality: firmness, TSS, ascorbic acid or titratable acidity.
### Postharvest biofungicides

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Product name</th>
<th>Activity</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>Boniprotect</td>
<td><em>Penicillium, Botrytis, Monilinia</em></td>
<td>EU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(preharvest)</td>
</tr>
<tr>
<td><em>Candida sake</em></td>
<td>Candifruit</td>
<td><em>Penicillium, Botrytis, Rhizopus</em></td>
<td>Spain</td>
</tr>
<tr>
<td><em>Pantoea agglomerans</em></td>
<td>Pantovital</td>
<td><em>Penicillium, Botrytis, Monilinia</em></td>
<td>Spain?</td>
</tr>
<tr>
<td><em>Pseudomonas syringae</em></td>
<td>Biosave</td>
<td><em>Penicillium, Botrytis, Mucor</em></td>
<td>U.S.A</td>
</tr>
<tr>
<td><em>Cryptococcus albidus</em></td>
<td>Yield plus</td>
<td><em>Botrytis, Penicillium, Mucor</em></td>
<td>South Africa</td>
</tr>
<tr>
<td><em>Metschnikowia fructicola</em></td>
<td>Shemer</td>
<td><em>Botrytis, Penicillium, Rhizopus, Aspergillus</em></td>
<td>Netherlands?</td>
</tr>
<tr>
<td><em>Candida oleophila</em></td>
<td>Nexy</td>
<td><em>Botrytis, Penicillium</em></td>
<td>Belgium, EU</td>
</tr>
</tbody>
</table>

AvoGreen (*Bacillus subtilis*) and Aspire (*Candida oleophila*), no more on the market.
Natural products

Inorganic compounds:
- Silicates

Animal products:
- Chitosan

Plant products:
- volatile organic compounds,
- glucosinolates,
- plant extracts,
- essential oils.
Silicates

Chitosan

Animal origin: polymer with antifungal and protective properties on plants.

1. It activates mechanisms of resistance against pathogens (PRPs, phenolics, phytoalexins, lignin deposition)
2. It creates a semi-permeable barrier, protecting from postharvest rots.
Over 130 compounds distributed in the Capparales order.

Antimicrobial activity: hydrolysis of glucosinolates by myrosinases with release of isothiocyanates.

On pome and stone fruit (Mari et al., 2002):
B.cinerea, M.laxa, P.expansum, M.piriformis and R.stolonifer
DISTRIBUTION
Present in Around 50 botanical families
(Lamiales, Asterales, Rutales)

LOCALIZATION
Flowers, leaves, roots, wood, rhizome, fruit, bark, seeds

QUANTITY
Generally lower than 1% (range: 0.01-2.00%).

Mixture of volatile aromatic compounds.
  • Terpenes
  • Aldehydes
  • Ketones
  • Fatty acids
  • Phenols
  • Esters
  • Alcohols
Chemical composition through GC-MS
10% more effective than 1% but...

Efficacy of EO treatments expressed as rot diameter (mm) caused by *Monilinia laxa* on nectarines (45 fruit per treatment) cv Big Top and cv Nectarross stored at 1.0±1°C for 28 days after artificial inoculation. Treatments were performed with EO of *Ocimum basilicum* (BAS), *Foeniculum sativum* (FEN), *Lavandula officinalis* (LAV), *Origanum majorana* (MAR), *Origanum vulgare* (ORE), *Mentha piperita* (PEP), *Rosmarinus officinalis* (ROS), *Salvia officinalis* (SAG), *Satureja montana* (SAV), *Thymus vulgaris* (THY) and *Mentha arvensis* (MIN) at 1% or 10% (v/v) concentrations.
**Phytotoxicity**

Thyme EO at 10% on apples cv Granny Smith and on apples cv Golden delicious.

Thyme EO at 10% on a nectarine cv Big Top.
Spray, dip or fumigation

The \textit{vapour phase activity} makes EOs potential fumigants of fruit and vegetables in storage chambers.

Spray of an emulsion of grapefruit EO at 0.1% on peach cv Cresthaven
Preharvest use

- *Satureja montana*
  - Essential oil (10%)

- *Thymus vulgaris*
  - Essential oil 10%

- Potassium silicate $K_2SiO_3$ 2%

**Field Treatment**

**Brown rot on peaches**

**Organoleptic characteristics**
Postharvest disease management

- Disease monitoring
- Disease prevention
- Integrated disease control
- BCAs
- HWD
- EOs
Biological means cannot at the moment solve all the problems of postharvest rots.

**Traditional methods**

- Temperature, humidity and CA of storage
- Fungicides

**Alternative methods**

- Thermotherapy
- Ultraviolet rays
- Natural products
- GRAS substances
- Essential oils
- Resistance inducers
- Antagonist
Conclusions

- Efficacy (crops, pathogens, time)
- Cost
- Toxicology
- Phytotoxicity
- Shelf life
- Scaling up (BCAs: production and formulation)

Industrial partners: HWD machines, EO and BCA producers,…

Public-private partnership: spin off companies
Cooperation with companies

To build confidence, pilot tests should be conducted in commercial packinghouses. Extension and technical services are essential. Marketing product & knowledge necessary.
Thank you for your attention!