Pre- and postharvest control strategies to prevent decay and meet quarantine requirements for Phytophthora brown rot

Updates on current disease management

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Phytophthora diseases of citrus affect roots, tree trunks, fruit

Healthy tree
Foot rot & Gummosis
Brown rot
Tree decline, dieback, yield reduction
Root rot
Phytophthora diseases of citrus affect roots, tree trunks, fruit

Mainly 4 species of Phytophthora are involved:
- *P. citrophthora*
- *P. parasitica (P. nicotianae)*
- *P. syringae*
- *P. hibernalis*

These species may all cause fruit brown rot, but root rot and gummosis are mainly caused by *P. citrophthora* and *P. parasitica*.

Occurrence of the 4 species is seasonal:
- Winter: *P. citrophthora, P. syringae, P. hibernalis*
- Summer: *P. parasitica*

Epidemiology of Phytophthora root rot

- Phytophthora root rot causes a slow decline of the tree.
- The disease destroys the feeder roots of susceptible rootstocks. The pathogen infects the root cortex, which turns soft and separates from the stele.
- If the destruction of feeder roots occurs faster than their regeneration, the uptake of water and nutrients will be severely limited and stored energy reserves will be depleted.
- Disease symptoms are often difficult to distinguish from nematode, salt, or flooding damage; only a laboratory analysis can provide positive identification.
Epidemiology of Phytophthora root rot

- Mostly caused by *P. citrophthora* and *P. parasitica*
- *Phytophthora* species are present in most citrus groves.
  - *P. citrophthora*: Active during cool seasons when citrus roots are inactive and their resistance to infection is low.
  - *P. parasitica*: Active during warm weather when roots are growing.
- Survival of adverse conditions in the soil as persistent spores.
- Source of water may be a factor for disease severity – well water vs. surface water (i.e., rivers)

Citrus Brown Rot

- Species of *Phytophthora* involved:
  - *P. citrophthora*
  - *P. parasitica* (*P. nicotianae*)
  - *P. syringae*
  - *P. hibernalis*
- Occur in all growing regions of CA.
- *P. citrophthora* and *P. parasitica* are considered most important.
- Losses are associated with periods of high rainfall.
- Other species are quarantine or potential quarantine problems – *P. syringae, P. hibernalis*
Citrus Brown Rot – Symptoms and Economic Impact

- Develops mainly on mature fruit in the lower tree canopy
- Olive-brown discoloration of the rind
- Distinctive pungent odor
- Fruit remain firm and leathery, unless invaded by secondary decay organisms.
- At high humidity, fruit become covered by a delicate white growth of the fungus.
- The most serious aspect: Fruit infected before harvest may not show symptoms. If infected fruit get mixed with healthy fruit, the disease may spread quickly from fruit to fruit in storage and during transit.
- Citrus brown rot: pre- and postharvest losses

Citrus Brown Rot – Economic Impact

- *P. syringae* recently has become a regulatory issue in the exportation of California orange fruit to China -
  - *P. syringae* has been considered of limited distribution and minor importance as compared to *P. citrophthora* or *P. parasitica*. 
Citrus Brown Rot – Distribution of causal pathogens

- Survey on the geographic distribution of *Phytophthora* species causing brown rot of citrus.
- Isolates are characterized using morphological and molecular methods.
- In winter samplings, we found *P. citrophthora* and *P. syringae*.
- *P. syringae* may be more widely distributed than previously thought.

Disease cycle of *Phytophthora* spp. – The root rot and brown rot phases are connected

- 18 h of wetness required for sporangia production and zoospore release.
- 3 h of wetness required for infection.
- Zoospores from sporangia on the ground may be splashed up onto low-hanging fruit and the trunk.
- Inoculum levels and wetness length are the most important predictors of brown rot epidemics.
- Sample *P. parasitica* July through September, *P. citrophthora* January through March.
- *Phytophthora* populations >15 to 20 propagules/g root zone soil may warrant treatment.
Wetness and temperature effects on infection of Navel oranges by *P. citrophthora*

- Zoospores encysted within an hour at incubation at 20C.
- Germ tubes were observed after 1 h.

Fruit inoculation studies at 20C:
- 1 h wetness - no disease
- 1.5 h wetness - 10% incidence
- 2 h wetness - 70% incidence

Experiments with *P. syringae* are currently being done

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Management of Phytophthora Diseases

- Cultural practices
  - Resistant rootstocks
  - Planting on berms for adequate drainage
- Avoid over-irrigation
  - Alternate side irrigation
  - Drainage tiles
  - Skirting of trees
- Fumigation
- Surfactants
- Fungicides
  - Preharvest
  - Postharvest
### Management of Phytophthora Diseases - Rootstocks

<table>
<thead>
<tr>
<th>Type</th>
<th>Rootstock</th>
<th>Root rot</th>
<th>Gummosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon</td>
<td>Macrophylla</td>
<td>Tolerant</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>Rough lemon</td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>Volkameriana</td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>Yuma ponderosa</td>
<td>Tolerant</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Orange</td>
<td>Taiwanica</td>
<td>Susceptible</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>Sour orange</td>
<td>Intermediate</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>Sweet orange</td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
<tr>
<td></td>
<td>Trifoliate</td>
<td>Tolerant</td>
<td>Tolerant</td>
</tr>
<tr>
<td>Hybrids</td>
<td>C-35 citrange</td>
<td>Tolerant</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>C-32 citrange</td>
<td>Tolerant</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>Carrizo citrange</td>
<td>Intermediate</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>Troyer citrange</td>
<td>Intermediate</td>
<td>Tolerant</td>
</tr>
<tr>
<td></td>
<td>Swingle</td>
<td>Tolerant</td>
<td>Tolerant</td>
</tr>
</tbody>
</table>

Source: UC ANR Publication 21477

### Alternate Side Irrigation to Control of Phytophthora Root Rot

#### A
- Single-line microsprinkler system
- Alternating irrigation

#### B
- Dual-line microsprinkler system
- Alternate row irrigation

a b a b
Management of Phytophthora root rot with chemicals

Evaluation of soil application of fungicides & surfactants for managing Phytophthora root rot

Water

100 mg/L Naiad

Ridomil 2E

20 mg/L Naiad
Tree excavation and determination of root weight

Selected Fungicides with Activity Against Oomycetes

<table>
<thead>
<tr>
<th>Class</th>
<th>FRAC</th>
<th>Common Name</th>
<th>Target</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic*</td>
<td>M1</td>
<td>Coppers, Bordeaux</td>
<td>Foliar</td>
<td>Foliar</td>
</tr>
<tr>
<td>Phosphonates*</td>
<td>33</td>
<td>Fosetyl-Al, phosphorous acid</td>
<td>Foliar, soil</td>
<td>Foliar, soil, postharvest</td>
</tr>
<tr>
<td>Phenylamides*</td>
<td>4</td>
<td>Metalaxyl, mfenoxam</td>
<td>Foliar, soil</td>
<td>Soil, trunk</td>
</tr>
<tr>
<td>Qols*</td>
<td>11</td>
<td>Azoxystrobin, fenamidone, others</td>
<td>Foliar</td>
<td>Foliar, postharvest</td>
</tr>
<tr>
<td>CAAs</td>
<td>40</td>
<td>Dimethomorph, mandipropamid**, iprovalicarb</td>
<td>Foliar</td>
<td>Postharvest</td>
</tr>
<tr>
<td>Benzamides</td>
<td>43</td>
<td>Fluopicolide**</td>
<td>Foliar (soil)</td>
<td>Foliar (soil)</td>
</tr>
<tr>
<td>Carbamates</td>
<td>28</td>
<td>Propamocarb</td>
<td>Foliar, soil</td>
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<tr>
<td>Piperidinyl thiazole isoxazoline</td>
<td>New group</td>
<td>Oxathiapiprolin</td>
<td>Foliar (soil)</td>
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<tr>
<td>Surfactants***</td>
<td>---</td>
<td>Various</td>
<td>Soil</td>
<td>Soil</td>
</tr>
</tbody>
</table>

* - Registration on tree crops including citrus.
** - Proposed to IR-4 for tree crops (e.g., citrus).
*** - Soil amendment, not labeled for disease control.

Fungicide classes evaluated for root rot management
Target sites of chemicals active against *Phytophthora parasitica*

- Zoospores
- Sporangia
- Oospores
- Chlamydospores
- Mycelium
- surfactants, fenamidone, phosphonates
- phosphonates, mefenoxam, iprovalicarb
- mefenoxam phosphonates

Target sites of newer fungicides are currently being evaluated.

Effect of a single soil application of fungicides on root rot of citrus in greenhouse studies

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenoxam</td>
<td>D</td>
</tr>
<tr>
<td>Fenamidone/iprovalicarb</td>
<td>CD</td>
</tr>
<tr>
<td>Fenamidone</td>
<td>C</td>
</tr>
<tr>
<td>Iprovalicarb</td>
<td>BC</td>
</tr>
<tr>
<td>Propamocarb</td>
<td>AB</td>
</tr>
<tr>
<td>Water check</td>
<td>A</td>
</tr>
</tbody>
</table>

- Citrus seedlings were transplanted from flats into peat pots containing soil inoculated with chlamydospores (32/g soil). Fungicides were prepared in water at a concentration of 2 ppm and applied to seedlings (200 ml/pot). Check plants received 200 ml water. There were seven single-seedling replicates per treatment. The experiment began in August 2001 and concluded 90 days later.

- Mefenoxam is highly effective, but resistance occurs at many locations.
- New soil fungicides are currently being evaluated in a large-scale field study.
### Conclusions – Management of root rot

Fungicides can be effective against species of *Phytophthora* causing citrus root rot in California -

- Mefenoxam was the most effective fungicide. Citrus root rot was effectively controlled in greenhouse and field experiments.
- Phosphonates were also very effective against citrus root rot.
- Efficacy of fenamidone, iprovalicarb, and propamacarb was lower and thus, these fungicides were not developed for use on citrus.
- New fungicides are currently under investigation.

### New fungicides for management of Phytophthora root rot

- Mandipropamid (evaluated, but is planned only for postharvest applications)
- Fluopicolide
- Oxathiapiprolin

A field trial with navel orange on Carrizo rootstock was established at UC Riverside in Sept. 2013
New treatments for brown rot and root rot

In vitro fungicide sensitivities against mycelial growth

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>FRAC</th>
<th>P. parasitica (ppm) (16 isolates)</th>
<th>P. syringae (ppm) (28 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefenoxam</td>
<td>4</td>
<td>0.08 – 0.28</td>
<td>0.004 – 0.03</td>
</tr>
<tr>
<td>Fluopicolide</td>
<td>43</td>
<td>0.04 – 0.08</td>
<td>0.02 – 0.05</td>
</tr>
<tr>
<td>Mandipropamid</td>
<td>40</td>
<td>0.003 – 0.008</td>
<td>0.002 – 0.006</td>
</tr>
<tr>
<td>Oxathiapiprolin</td>
<td>New*</td>
<td>0.0004 – 0.0011</td>
<td>&lt;0.001</td>
</tr>
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- Mandipropamid and oxathiapiprolin are most effective
- Oxathiapiprolin: new class - piperidinyl thiazole isoxazoline
- Additional isolates and isolates of *P. citrophthora* are being evaluated
- Toxicity against other stages in the life cycle (zoospore production, cyst germination, oospore production) are also being evaluated

In vitro activity of potassium phosphite against *P. citrophthora* and *P. syringae*

Three isolates of each species.
Regressions of mycelial growth inhibition of *P. citrophthora* and *P. syringae* on concentration of potassium phosphite

Concentrations of potassium phosphite were from 0 to 4000 mg/L. Regressions were performed using analysis of variance procedures.

Management of Phytophthora Brown Rot - Preharvest Fungicide Sprays -
Management of Phytophthora Brown Rot  
- Preharvest Fungicide Sprays -

**Current guidelines:**
- One spray of copper fungicide between October and December before or just after the first rain.
- Apply at 400-700 gal/A.
- If frequent and high rainfall after the first application, repeat applications in January or February.
- Spray the skirts to about 4 feet above ground; whole tree applications may be necessary for some varieties or in orchards with a history of the disease.
- Spraying the ground underneath the trees may also reduce brown rot infections.

Management of Phytophthora Brown Rot  
- Preharvest fungicide sprays -

**Most effective orchard sprays:**
Bordeaux mixture (copper sulfate + lime) with 0.6 to 0.8 lb of metallic copper/100 gal.
Neutral (fixed) coppers (copper hydroxide, copper oxide)

Phosphonates (e.g., fosetyl-al - Aliette 5 lb/A, potassium phosphite - Prophyt 4 pints/A, etc.) are systemic and provide effective control when applied up to 5 weeks prior to infection.

Phenylamides (e.g., mefenoxam) – Ridomil Gold 0.5-3 qt/A applied to soil
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### New preharvest treatments for control of brown rot caused by Phytophthora spp.

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<th>Application</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Revus 8 fl oz</td>
<td>Oxathiapiproline 4.8 fl oz</td>
</tr>
<tr>
<td>Oxathiapiproline 4.8 fl oz</td>
<td></td>
</tr>
<tr>
<td>Badge 7 pts + Lime 3.5 lb</td>
<td></td>
</tr>
</tbody>
</table>

Fruit harvested 6 weeks after field treatment and inoculated with *P. citrophthora*
Persistence of new preharvest treatments for control of brown rot caused by *Phytophthora* spp. 2013

- Revus (Mandipropamid), Prophyt (Phosphorous acid), and Badge-Lime (copper oxychloride- copper hydroxide) were very effective when fruit were harvested 2 weeks after application.
- After 21 days, Presidio was no longer effective, whereas efficacy of Prophyt was reduced after 4 weeks.
- Copper effective for ca. 6 wk; whereas Revus was still very effective for 8 wk.

New preharvest treatments for brown rot - 2014

- Treatments applied at 400 gal/A on Jan. 3, 2014.
- Fruit were harvested weekly for 8 weeks and inoculated with *P. citrophthora*.
- Total precipitation during trial period 55 mm.
New preharvest treatments for brown rot - 2014

Temporal efficacy of preharvest treatments to navel oranges

Trial at UC Riverside

Treatments applied at 400 gal/A on Feb. 3, 2014.
- Fruit were harvested at selected intervals and inoculated
- Total precipitation during trial period 41.9 mm.

Management of Phytophthora Brown Rot
- Postharvest Fungicide Treatments -
New postharvest treatments for control of brown rot caused by *Phytophthora* spp.

Laboratory studies: Treatment of fruit 15 h after inoculation

• Azoxystrobin, mandipropamid (Revus), fluopicolide (Presidio), and potassium phosphite (Prophyt, Fungiphite) were highly effective as pre-infection treatments.
• Only potassium phosphite was effective as a post-infection treatment.
• Potassium phosphite was also highly effective against brown rot caused by *P. syringae* and *P. hibernalis* in other studies.

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![Graph showing incidence of brown rot](image)

**New postharvest treatments for control of brown rot caused by *P. citrophthora***

- Post-infection activity:
  - Dip treatments 15 h after inoculation
- Pre-infection activity:
  - Inoculation 6 h after treatment

- Water (control)
- Potassium phosphite 630 mg/L
- Potassium phosphite 1260 mg/L
- Mandipropamid 300 mg/L
- Fluopicolide 300 mg/L
- Azoxystrobin 600 mg/L

Incidence of brown rot (%)

0 20 40 60 80 100

0 20 40 60 80 100

- Azoxystrobin, mandipropamid (Revus), fluopicolide (Presidio), and potassium phosphite (Prophyt, Fungiphite) were highly effective as pre-infection treatments.
- Only potassium phosphite was effective as a post-infection treatment.
- Potassium phosphite was also highly effective against brown rot caused by *P. syringae* and *P. hibernalis* in other studies.
Potassium phosphite and heat treatments for control of brown rot caused by *P. citrophthora* in the laboratory

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Temperature</th>
<th>Fruit coating spray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (control)</td>
<td>ambient</td>
<td>---</td>
</tr>
<tr>
<td>Water</td>
<td>56C</td>
<td>---</td>
</tr>
<tr>
<td>Imazalil 1000 mg/L</td>
<td>ambient</td>
<td>Imazalil 1000 mg/L + TBZ 3500 mg/L</td>
</tr>
<tr>
<td>Potassium phosphite 2000 mg/L</td>
<td>ambient</td>
<td>---</td>
</tr>
<tr>
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<tr>
<td>Potassium phosphite 2000 mg/L + imazalil 1000 mg/L</td>
<td>56C</td>
<td>---</td>
</tr>
</tbody>
</table>

15-sec dip treatments done 20 h after inoculation. 56C = 133F

Slight negative interaction between K-phosphite and fruit coating, but treatment at higher temperature was effective.

brown rot caused by *P. citrophthora* in the laboratory

<table>
<thead>
<tr>
<th>Aqueous dip 15 sec</th>
<th>Dip temperature</th>
<th>Wax spray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>ambient</td>
<td>---</td>
</tr>
<tr>
<td>K-phosphate 2000 ppm</td>
<td>ambient</td>
<td>---</td>
</tr>
<tr>
<td>K-phosphate 2000 ppm + imid. 1000 ppm</td>
<td>ambient</td>
<td>Imid 1000 ppm + TBZ 3500 ppm</td>
</tr>
<tr>
<td>K-phosphate 2000 ppm + imid. 1000 ppm</td>
<td>140F</td>
<td>Imid 1000 ppm + TBZ 3500 ppm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aqueous dip 15 sec</th>
<th>Dip temperature</th>
<th>Wax spray</th>
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<tr>
<td>Water</td>
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<tr>
<td>K-phosphate 2000 ppm + imid. 1000 ppm</td>
<td>130F</td>
<td>Imid 1000 ppm + TBZ 3500 ppm</td>
</tr>
</tbody>
</table>

Lab studies, 15-sec dip treatments 20-24 h after inoculation.

Treatments at 140F were consistently highly effective. Treatments at 130F were variable in efficacy.
Fruit were inoculated with zoospores of *Phytophthora citrophthora* and treated after 20 h in a heated (average temperature 55º C) flooder for approximately 11 sec. This was followed by a spray application with 1000 mg/L imazalil and 3600 mg/L thiabendazole (TBZ) in a carnauba-based packing fruit coating. Fruit were incubated for 8 days at 20º C and then evaluated for the incidence of brown rot.

Treatments were applied in a heated flooder at 125-136F. Each treatment had 1000 fruit. Fruit were evaluated after two weeks of incubation at 68F (20C).

Percent control was based on the reduction of brown rot incidence of each treatment as compared to the control treatment.
New postharvest treatments for Phytophthora brown rot

Brown rot studies with inoculated fruit:
- 15-sec water dips at 140F were highly effective
- 15-sec water dips at 130F were moderately and inconsistently effective
- 15-sec dips with **K-phosphite** at 2000 ppm were highly effective
- Negative interaction with imazalil and subsequent application of fruit coating
- Heated K-phosphite treatments are the best option: contact activity and residual activity.

Summary: New treatments for Phytophthora brown rot of citrus

- Potassium phosphite is highly effective against Phytophthora brown rot and can be used preharvest and postharvest to prevent decay.
- Several phosphite products are registered. They have exempt status in the US.
  - IR-4 residue studies are being done to obtain international MRLs/FAT
  - As always, check MRL databases for the limits of a specific export country. (Some countries consider phosphites as pesticides).
- The postharvest fungicide Graduate A+ can also be used as a protective treatment to prevent the spread of brown rot.
- Additional materials (mandipropamid, fluopicolide, oxathiapiprole) have been identified for pre- or postharvest use and are considered for registration.
Implementation of Results by Industry

• Two approaches to solving Phytophthora brown rot for orange export to China -
  – Potassium phosphite postharvest treatments
  – Hot water postharvest treatments

• Preharvest Management Practices –
  – Skirting
  – Foliar and fruit applications of copper
  – Development of new fungicides for season-long control
  – Managing soil populations of pathogens and root rot control with fungicides – (mefenoxam, phosphonates, and new fungicides)

• Results will have to be approved by APHIS and China?
  – Systems approach allows for achieving 99% control (Probit 9 >99.9% is not a requirement for plant diseases)

Questions?
Update on postharvest activities for decay management of citrus

- Evaluation of new chemistries
- Resistance monitoring
- Development of anti-resistance strategies
- Supporting current registrations
- Developing new registrations and MRL/FATs in international markets

Postharvest fungicides for citrus in the US

<table>
<thead>
<tr>
<th>Phenols</th>
<th>Benzimidazoles</th>
<th>DMI-imidazoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium ortho-phenyl phenate (SOPP)</td>
<td>Thiabendazole (TBZ)</td>
<td>Imazalil</td>
</tr>
<tr>
<td>Phenylpyrroles 1930s</td>
<td>Strobilurins (Qols) 1970s</td>
<td>DMI-triazoles 1980s</td>
</tr>
<tr>
<td>Fludioxonil (Graduate) 2006</td>
<td>Azoxystrobin (Diploma) 2010</td>
<td>Propiconazole (Mentor) New</td>
</tr>
<tr>
<td>Anilinopyrimidines 2006-2010</td>
<td>Phosphonates 2013</td>
<td>EXP-13</td>
</tr>
<tr>
<td>Pyrimethanil (Penbotec) 2012</td>
<td>Potassium Phosphite (Prophyt, Kphos) New requirements</td>
<td></td>
</tr>
</tbody>
</table>

- Reduced risk fungicides
- Goal is to prevent resistance to fludioxonil and azoxystrobin and to define usage pattern for fungicides with resistance.
- New mixture partners for other fungicides

Fungicide with resistance in Penicillium spp. populations
Imazalil is still highly needed in citrus IPM programs:

- Has a different mode of action from other registered fungicides, except propiconazole
- Can be used in rotations and mixtures – resistance management
- Has international MRLs and FAT
- Resistant populations of *Penicillium* spp. are less fit.

Inevitably imazalil may be canceled if it stays on the Prop 65 list.

Questions?