



Discussion of Penicillin, Antimicrobial R&D and Bactericide Now Initiative

Prepared for CPDC

Lake Alfred, FL

June 12, 2014

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Outline

- Perspective – science, business, regulatory, market aspects of current challenge
- Resources and knowledge available today
- Major classes of antibiotics with efficacy
- Bactericide Now Initiative

Requirements for Management

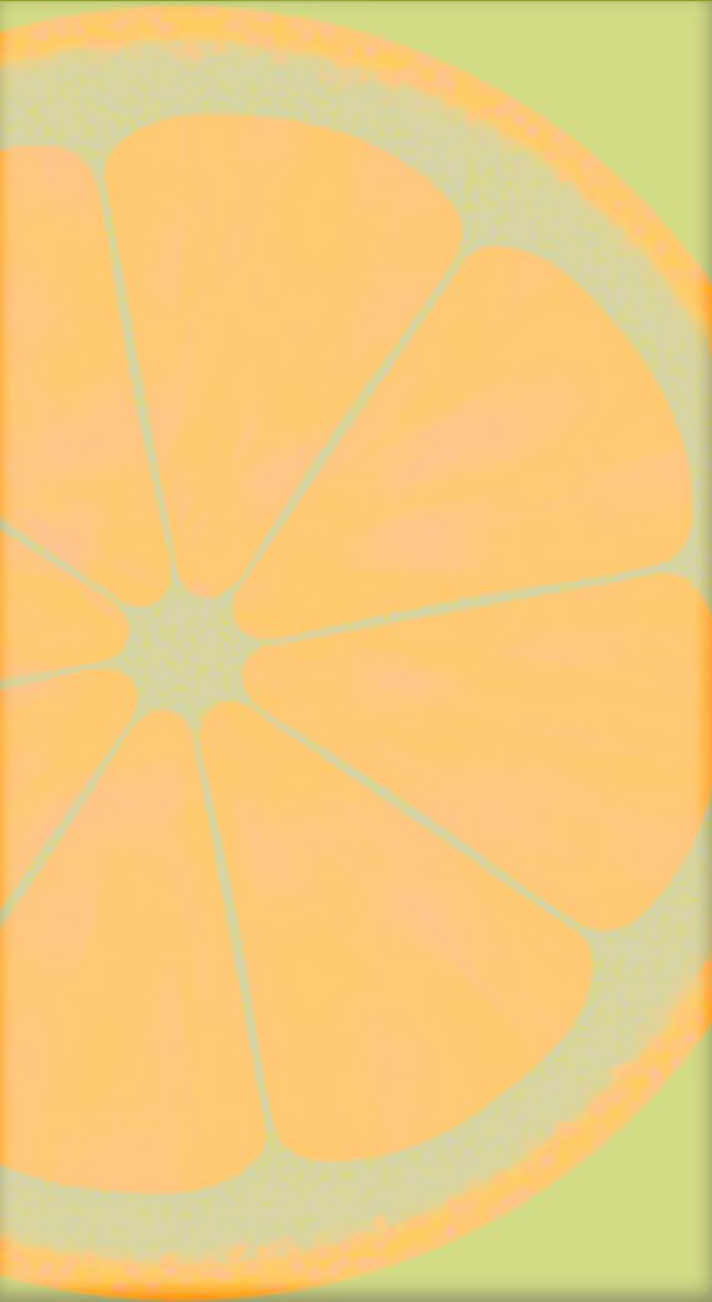
- Slow spread of disease – CHMA and insect control
- Treat existing infected trees
- Protect new plantings
- Provide long-term sustainable genetic and biological solutions

Requirements for Management

- Treat existing infected trees
 - Enhanced nutritionals
 - Plant growth regulators
 - Naturally occurring microbes
 - Thermal therapy
 - Antibacterial compounds

National Research Council 2010

- Research and Technology Recommendations
- Long-Term Objective 2
 - “Support development and testing of bactericides, therapeutics or SAR activators.”
- Less than 5 yrs for “proof of principle and demonstration of HLB mitigation” and greater than 5 yrs for development



Citrus Greening Disease (Huanglongbing)

SEARCH FOR AGRICULTURAL THERAPEUTICS

Biological Assays

- Graft-based assay
 - Infected scion soaked in test solution and grafted onto uninfected rootstock, follow by PCR
 - Slow, low-throughput
 - Evidence of efficacy *in planta* and first look at phyto-toxicity
- *Liberibacter crescens* culture-based assay
 - Much faster, higher-throughput followed by *in planta* confirmation on *CLas*

Prior Results

- Compounds screened
 - ~100+ by graft graft assay and
 - ~400+ by culture assay
- Wide variety of categories of chemicals
 - Antibiotics and agricultural antibiotics
 - Polycation polymers
 - Biopesticides, plant essential oils, terpenoids
 - New actives and non-antibiotic derivatives
 - Host immune modulators

What are we looking for?

- HLB treatment; effects on tree symptoms through CLas titre reduction
- Sources: Solutions Page, Research, Companies
- Chemical properties
 - MW: < 450 g/mol preferably < 250 g/mol
 - Log Kow: 2 to 4
 - pKa: 2 to 6.5 preferably 3 to 5.5
- Volume of phloem: 10 ft tree, 1000 L
- ? pH: 8.0 – 6.0 stylectomy vs fractionation

Current Testing Resources

Liquid Culture Assay on *L. crescens* ⇔ MIC90
(Dr. Erik Triplett Laboratory)

Citrus Flush Assay on *C. asiaticus* ⇔ Confirmation
(Dr. Claudio Gonzalez)

Trunk Injection ⇔ Dose
(Dr. Nian Wang)

Requirements and Results

Liquid Culture Assay on *L. crescens* ⇔ MIC90
< 1 mg of compound, ~ 2 weeks for results

Citrus Flush Assay on *C. asiaticus* ⇔ Confirmation
~ 100 mg of material, ~ 1 month

Trunk Injection ⇔ Dose
> 10 gr of material, 2-7 months

Top 25 Antibiotics in *L. crescens*

- Criteria used to make the list
 - Phloem mobility.
 - Inhibits *L. crescens* at a level of 10 μ M or less.
 - Inhibits growth of *L. crescens* by at least 98%.

Top 10 antibiotics; Tetracyclines

Antibiotic	μM	% inhibition
Tet #9	0.8	100
Tet #11	1.7	100
Tet #34	5.0	100
Tet #35	5.0	100
Tet #8	8.6	100
demeclocycline	8.6	100
Tet #5	9.8	99.8
oxytetracycline	10.0	99.8
Tet #33	1.0	99.8
Tet #2	2.0	99.8

Top 10 antibiotics; Tetracyclines

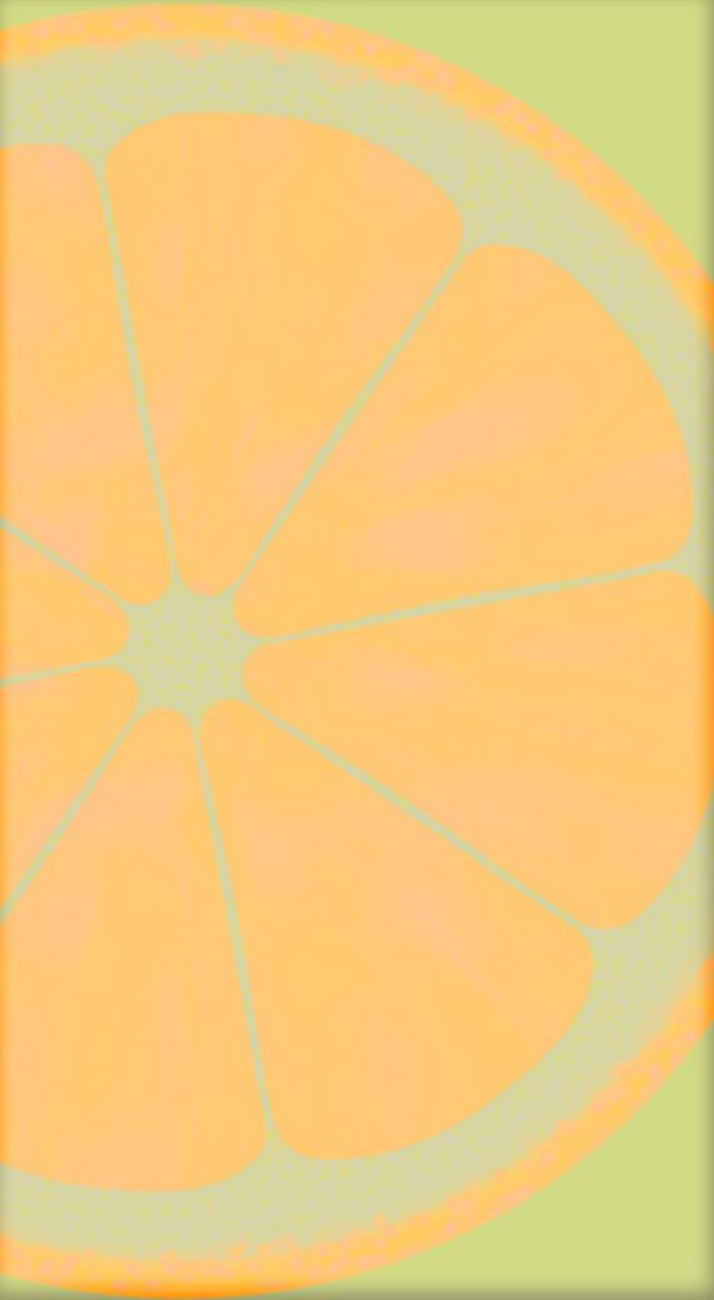
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Tet #33	1.0	99.8
Tet #2	2.0	99.8

Next antibiotics (11-20)

Antibiotic	μM	% inhibition
Tet #18	9.0	99.6
Tet #36	5.0	99.6
Tet #10	1.9	99.4
cephaprin	3.5	99.4
penicillin G	1.8	99.3
cefotaxime	0.7	99.3
penicillin V	0.1	99.1
cefmetazole	4.2	98.9
cefmenoxime	1.2	98.8
ticarcillin	5.2	98.5

Next antibiotics (21-25)

Antibiotic	μM	% inhibition
ampicillin	0.7	98.5
cefoxitin	4.7	98.4
Tet #24	8.6	98.3
cefdinir	6.3	98.3
cefcapene	1.4	98.3

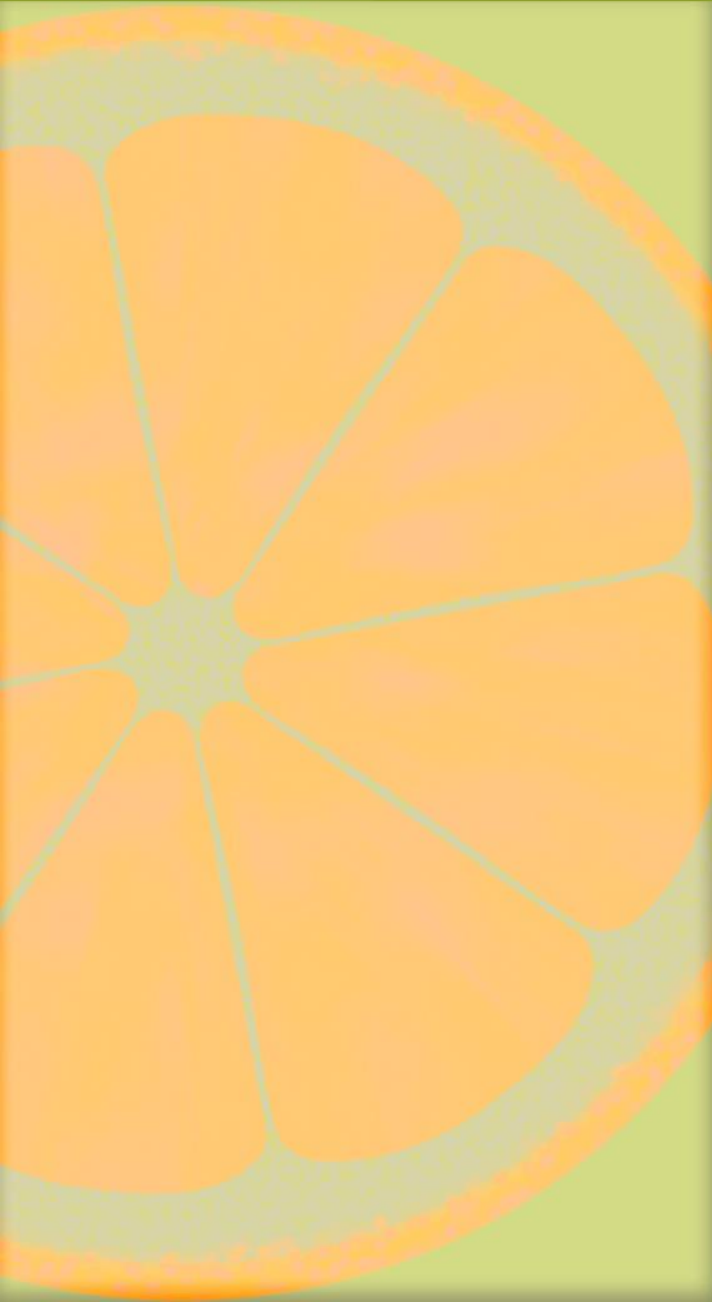


Plans for FY2014-2015

BACTERICIDE NOW INITIATIVE

Possibilities

- Listed in order of increasing regulatory constraints
 - GRAS-like and 25(b) minimum risk pesticides
 - Commercial antibiotics currently registered for use in food crops
 - Copper and Zinc pesticide formulations
 - Biopesticides
 - “Repurposing” existing products
 - Non-antibiotic tetracycline derivatives



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THANK-YOU