Zinkicide[™] - a systemic nano-ZnO based bactericide/fungicide for crop protection

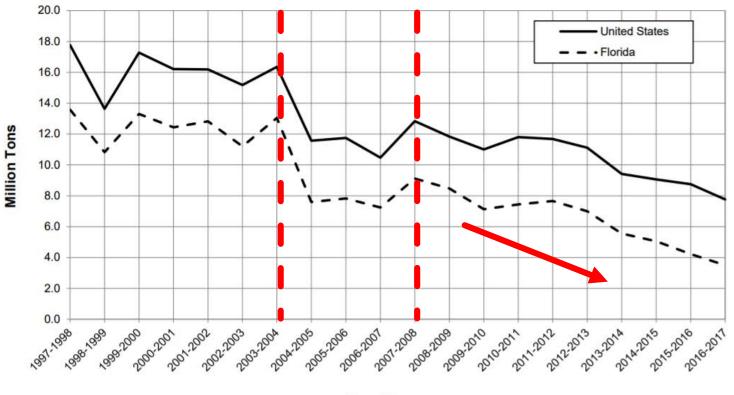
Swadeshmukul Santra

QEEN II: 2nd Quantifying Exposure to Engineered Nanomaterials from Manufactured Products Workshop

> Washington D.C. October 10-11, 2018

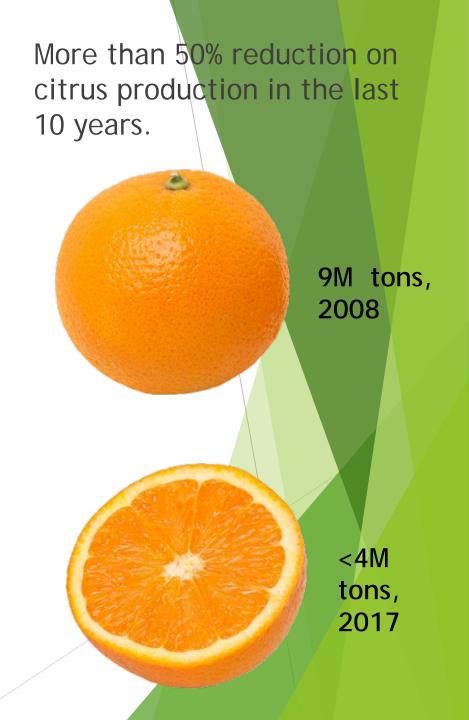
Citrus Production in Florida

Citrus Production - United States and Florida: Crop Years 1997-1998 through 2016-2017



Crop Year

Source: Florida Citrus Statistics 2016-2017 (March 2018) USDA, National Agricultural Statistics Service.



Citrus Greening (HLB)

- Huanglongbing (HLB) is a deadly bacterial disease.
- Main symptoms include reduction on fruit size and intense fruit drop.
- It is caused by the phloem-limited bacterium 'Candidatus Liberibacter asiaticus' (CLas).
- Transportation of sugar from leaves to plants parts is compromised by the presence of CLas in the phloem tissue.
- CLas bacteria are vectored by the Asian citrus psyllid (ACP: Diaphorina citri).



Asian Citrus Psyllid (ACP)

- Diaphorina citri is a 4 mm sap-sucking bug that feeds on plants, using their sucking and piercing mouthparts.
- CLas multiplies inside the insect's gut and is transmitted from tree to tree.
- Eggs are laid (800/life time of female) on the tips of growing shoots, between and near the unfolding leaves.



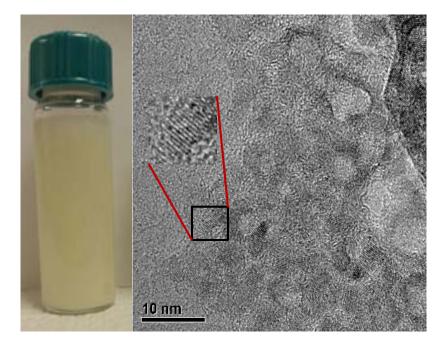
A systemic bactericide capable of reaching phloem tissue is desperately needed!

ZinkicideTM

https://www.zinkicide.org/

Zinkicide - Reagent to Ag grade materials

Reagent grade



- <10 nm particles</p>
- Stability Slowly settles

Potential of Nano-Formulated Zinc Oxide for Control of Citrus Canker on Grapefruit Trees, J. H. Graham, E. G. Johnson, M. E. Myers, M. Young, P. Rajasekaran, S. Das, and S. Santra, Plant Disease 2016 100:12, 2442-2447

Agricultural grade - 110th attempt



- ~4 nm particles
- Stability No settlement
 - In proper storage container

Zinkicide[™] - HLB Field Trial

Two separate HLB Field trials

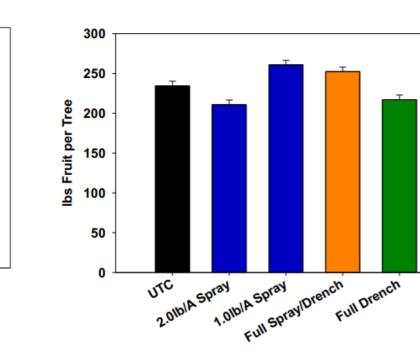
- Grapefruit 6 year old trees in Indian River,
- Randomized design with 25 trees in 5 plots
- Spray only, drench only, spray+drench
- Multiple rates
- Valencia 20+ year old trees on Ridge,
- Randomized complete block design
- 2 rates for spray only, 1 rate for drench and spray+drench
- Yield, fruit size, and juice quality assessed at harvest



Yield Response - dose dependent

Grapefruit

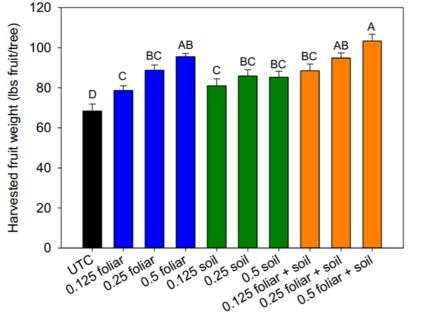
Harvested 2017



Harvested 2018

Valencia

Acknowledge to Dr. Evan Johnson University of Florida/Lake Alfred

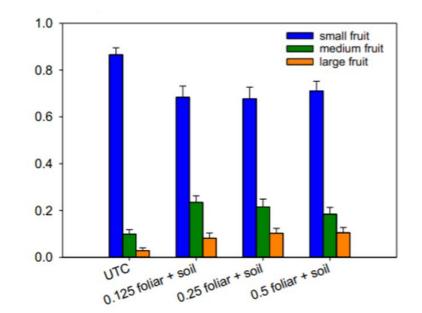


Zinkicide applicaiton (lbs metallic Zn/acre)

Fruit size and juice quality

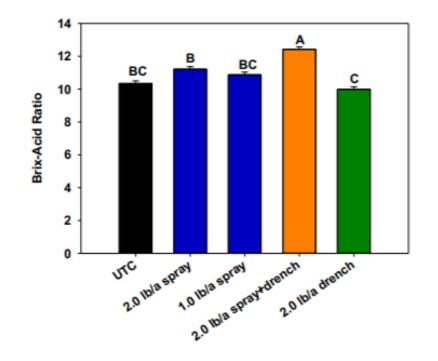
► Grapefruit

Dose dependent fruit size effect



Valencia

Dose and application method dependent juice quality effect



Acknowledge to Dr. Evan Johnson University of Florida/Lake Alfred

Zinkicide reduced hurricane damage in young trees

- ▶ New planting trial Ft. Pierce
 - Trees treated monthly since planting
 - Originally with SG4, 2017 with fixed ag-grade formulation
- Spray + Drench Zinkicide treatment kept majority of leaves and fruit
- Most other trees stripped of leaves, some of fruit



Zinkicide[™] Antibacterial efficacy Bacterial activity- RNA

Bacterial activity – RNA based Before application 5 weeks-old 5 weeks-young 6 16S rRNAs (Log) 2 Control Monthly Twice a month Weekly

- Greenhouse experiment
- Effect of Zinkicide rate: monthly, biweekly and weekly applications
- RNA activity reduced in young leaves
- RNA activity in new growth predicts bacterial titer reductions
- Biweekly application is most effective

Acknowledge to Dr. Mayara Murata University of Florida/Lake Alfred

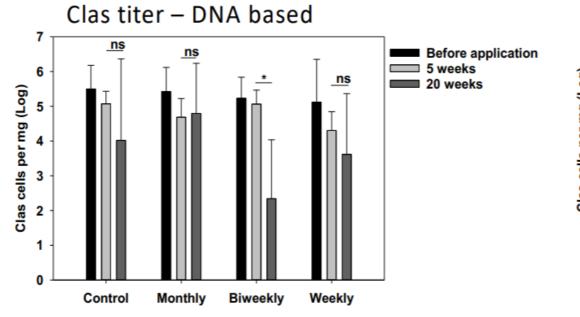
Zinkicide[™] Antibacterial efficacy - DNA

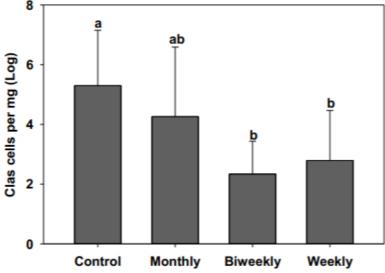
Old leaves

1000-fold reduction in bacterial titer after 20 weeks of biweekly application

Young leaves (after 20 weeks)

Experiment is being repeated and titer reduction has been observed after 5 weeks in young leaves.





Acknowledge to Dr. Mayara Murata University of Florida/Lake Alfred

Registration Status

- Zinc and Zinc oxide are not registered as actives for any pesticide use
- Full registration requires a Registrant (Ag-chem company)
 - Original registrant backed out when couldn't get a "me too" registration (existing active)
 - Final negotiations underway for new registrant
- Full registration process (GLP certified lab testing)
 - Toxicology
 - Residue
 - Environmental Fate

Registration Status - Prelim Data

Not GLP certified

Toxicology

- Adult Honeybees not affected by Zinkicide
- ► Honeybee larva sensitive to feeding on rates 10 times in field rate
- Less toxic to aquatic animals (Fathead minnow larvae) than commercial copper products

Residue

- ▶ No significant increase in Zinc content of juice or peel oil
- Preliminary evidence from greenhouse trials suggests efficacy lost after ~7 days

Conclusions

- Zinkicide has systemic activity
- Zinkicide can improve yield on HLBaffected trees
- Zinkicide application rate and timing affect the efficacy
- Agricultural-grade formulation is ready for EPA registration
- Bee toxicity and residual analysis of Zinkicide are being studied





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Thank you!