Zinkicide for HLB:
Where Efficacy and Registration Stand

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Citrus Expo

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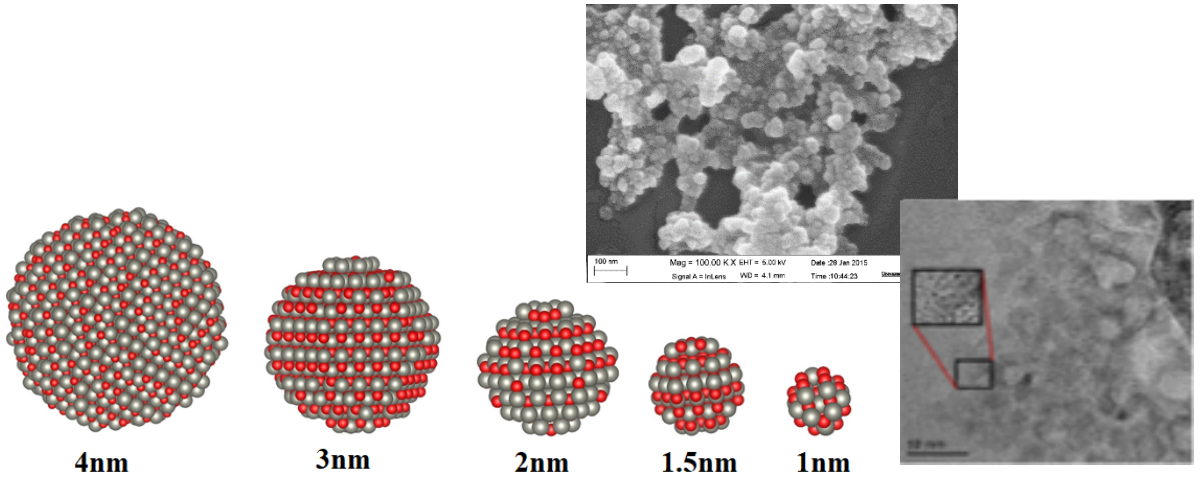
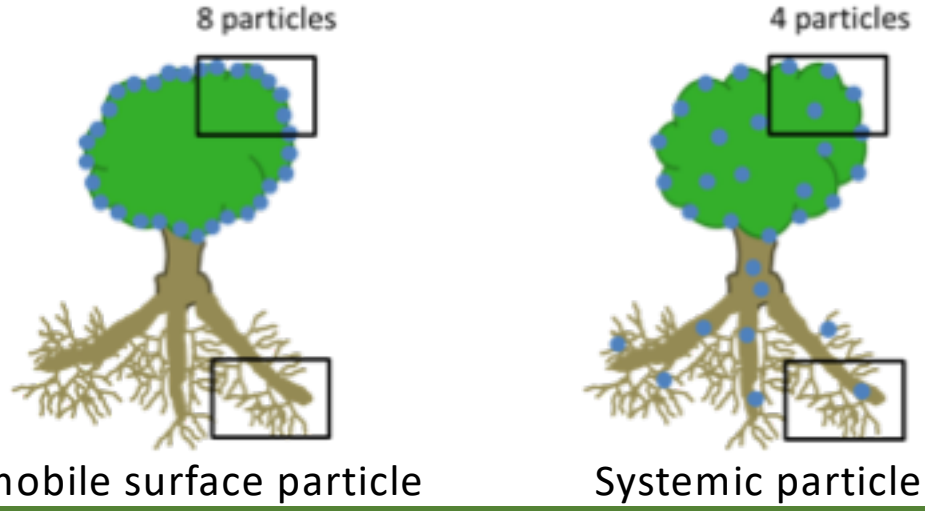
Zinkicide – what is it?

- Zinc oxide based nanomaterial
  - Developed by Dr. Swadesh Santra at UCF
- Plant metabolizable building blocks
- Designed for cell to cell movement
  - Size of GFP protein
  - <6 nm for systemic movement
- Stronger bacterial inhibition than copper



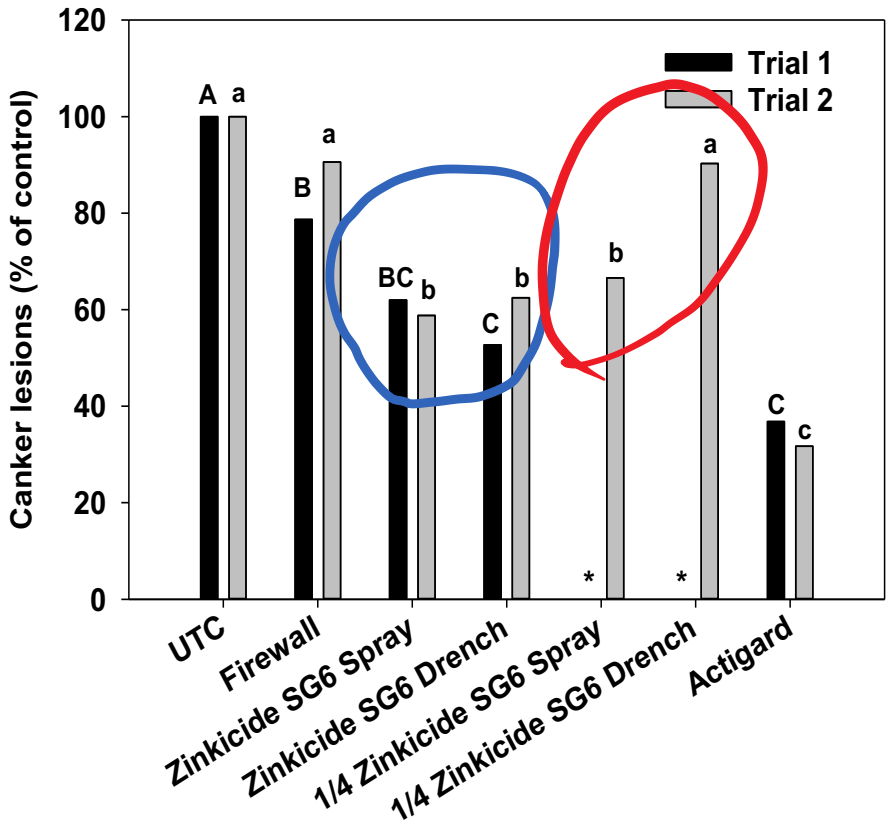


Immobile surface particle

Systemic particle

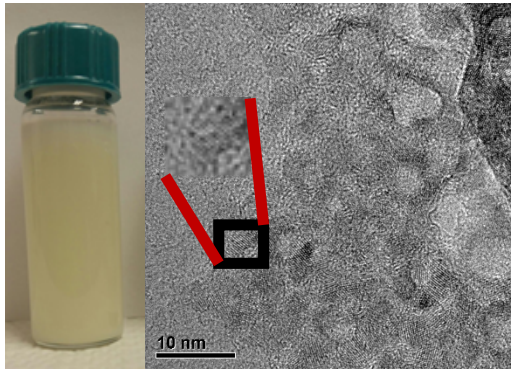
Zinkicide – Systemic activity

- Root uptake (soil drench)
  - Phloem availability confirmed in UCF experiments
- 4x reduction in total metal for local systemic activity
  - This data led to too low application rate in early field trials



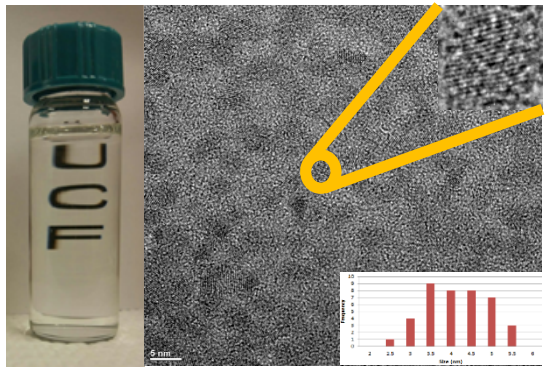
Zinkicide – Reagent to Ag grade materials

Reagent grade



- <10 nm particles
- Stability - Slowly settles

Agricultural grade – 110th attempt

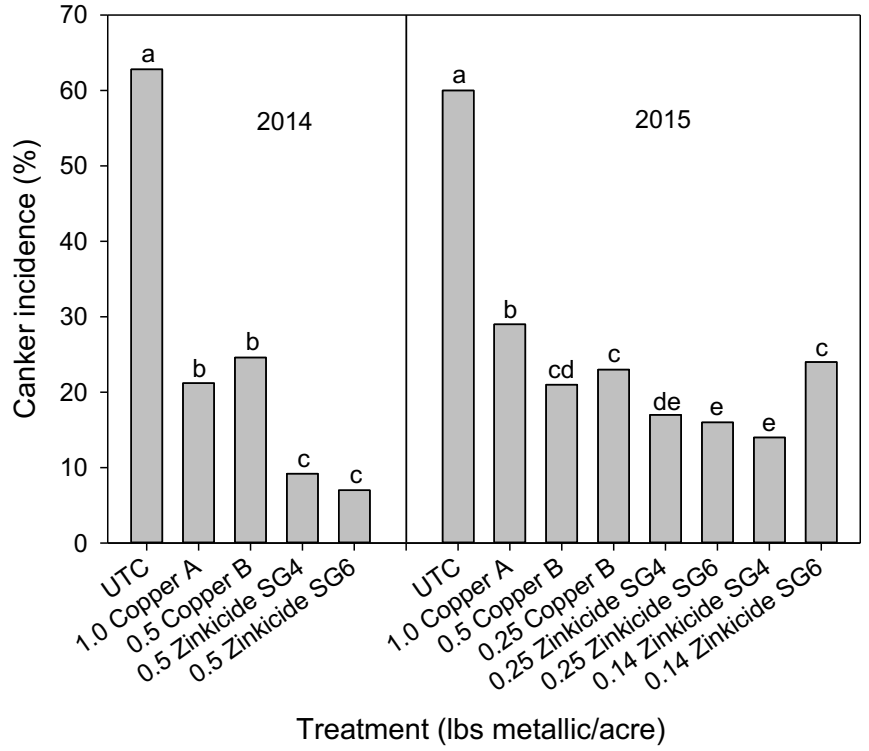


- ~4 nm particles
- Stability - No settlement
  - In proper storage container

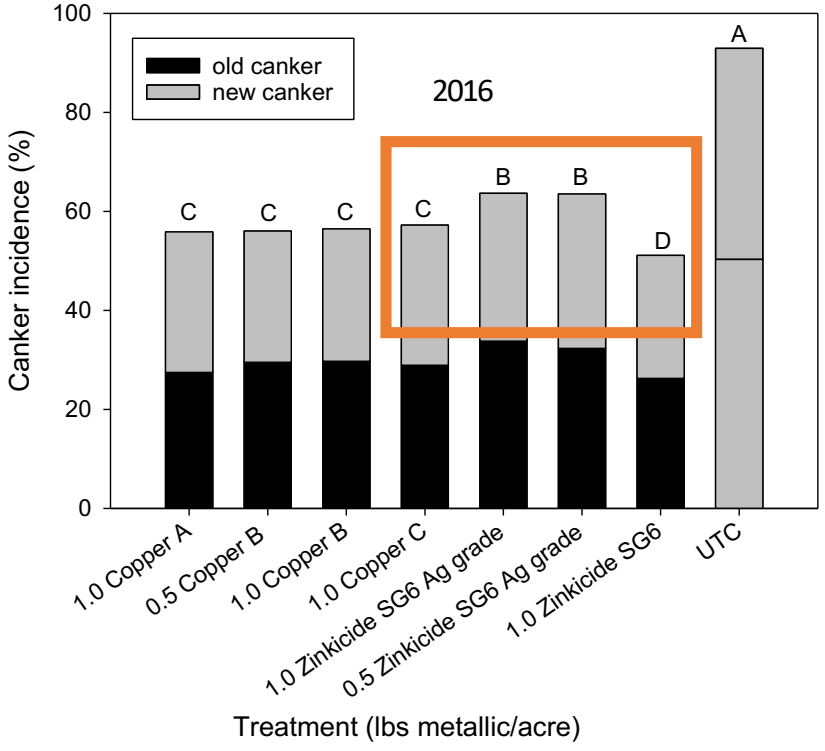
Zinkicide – Canker efficacy

Not all ZnO nanoparticles are the same

Reagent grade



Early Ag grade formulation

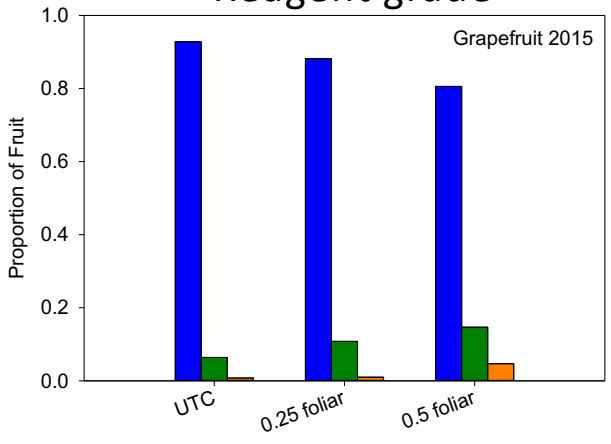


Zinkicide – HLB field Trials

- Monthly application year round
  - Expect management, not cure
- Grapefruit – 6 year old trees in Indian River
  - Randomized design with 25 trees in 5 plots
  - Spray only, drench only, spray+drench at 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

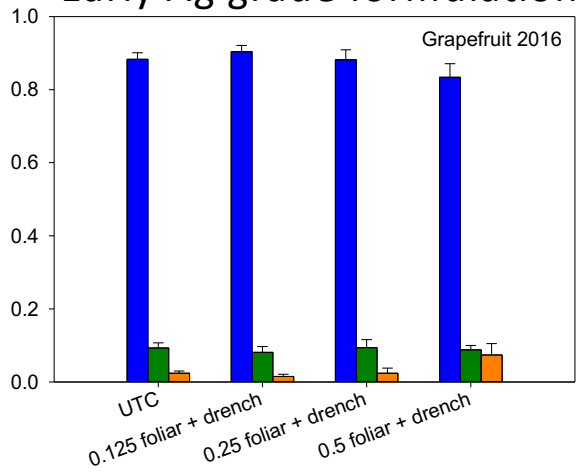
Zinkicide – Fruit size increase

Reagent grade



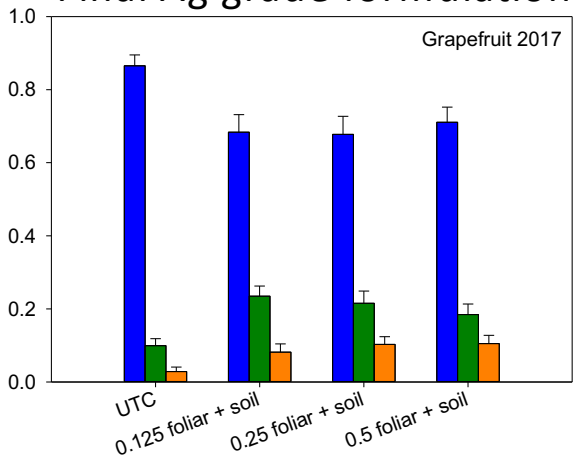
- Dose dependent fruit size increase
- Max effect not reached
- No yield increase

Early Ag grade formulation



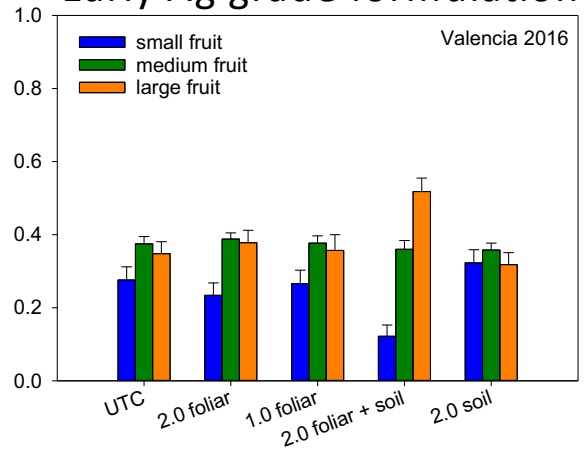
- Only highest rate spray+drench increased fruit size
- No yield increase

Final Ag grade formulation



- Strong fruit size effect

Early Ag grade formulation



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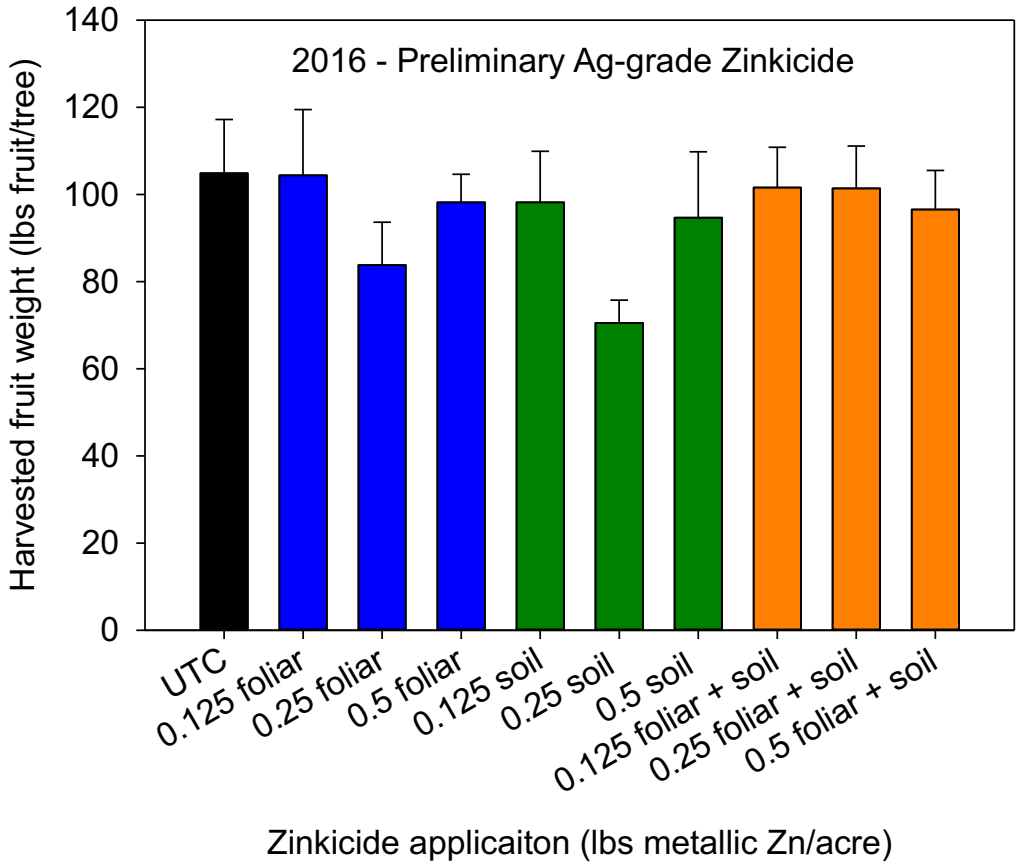
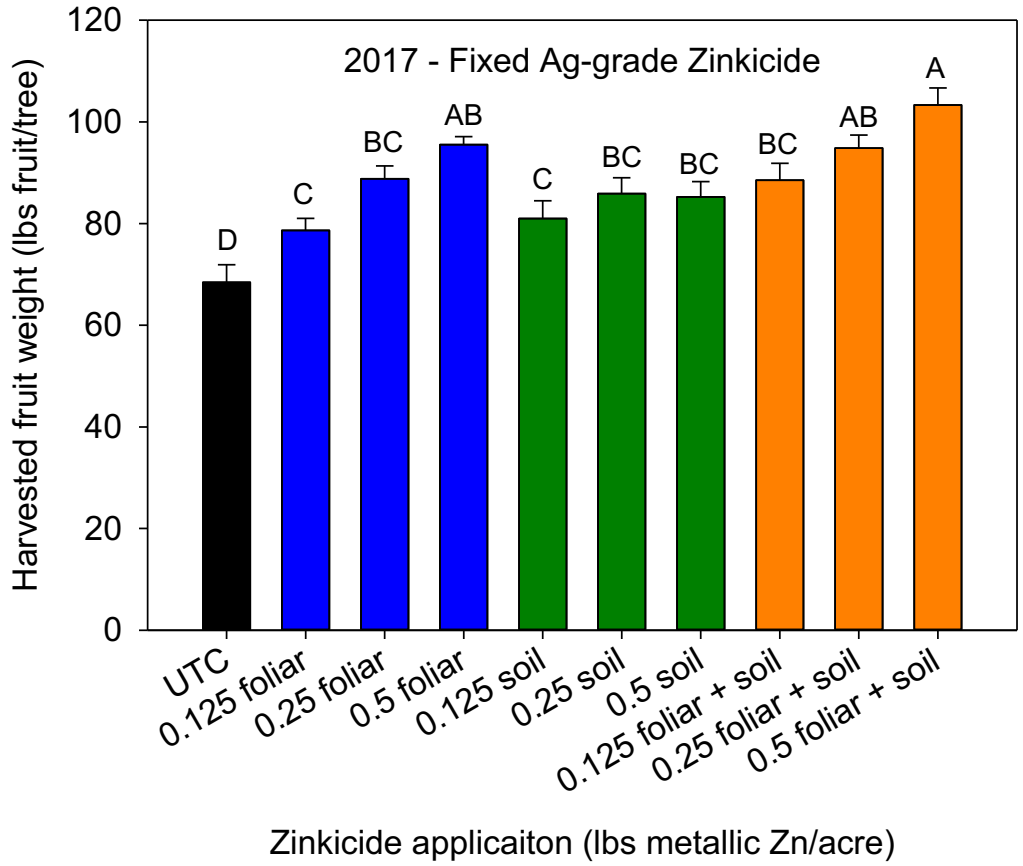
Zinkicide – HLB yield response

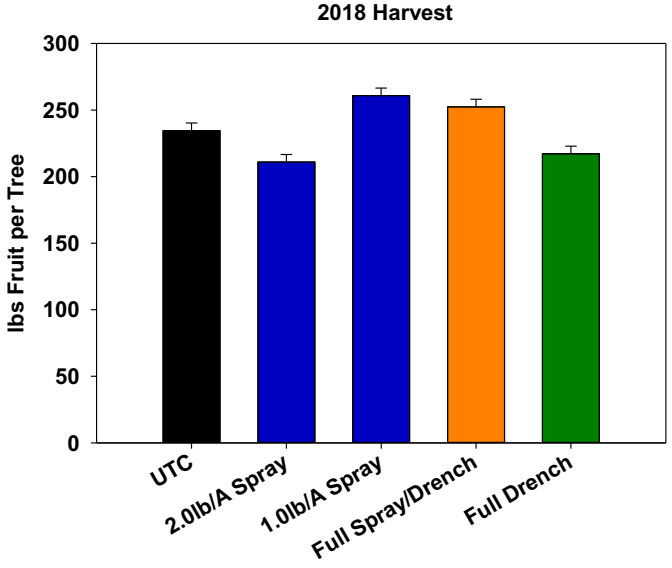
6 year old Grapefruit

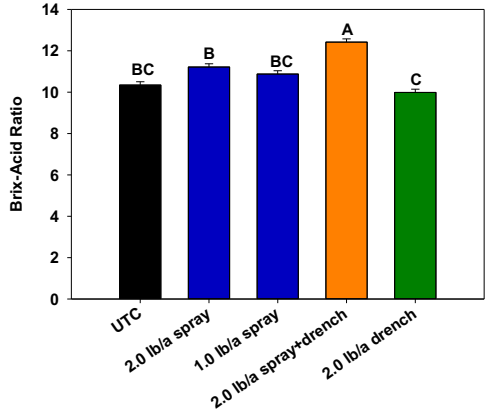




Zinkicide – HLB Yield Response

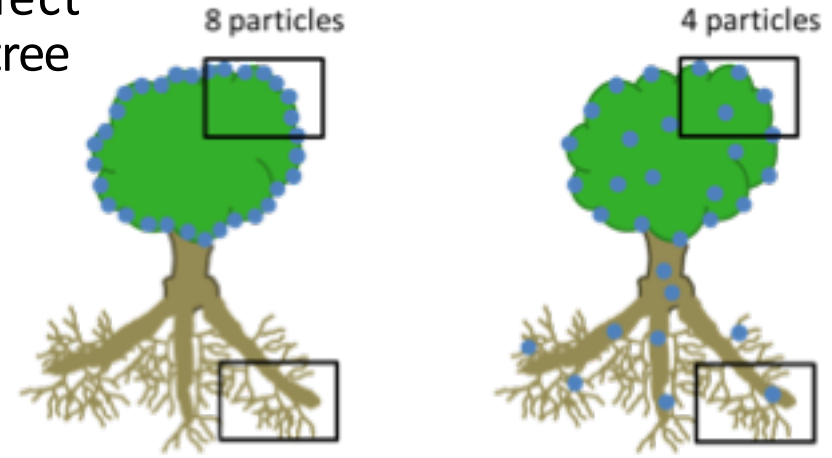
20+ year old Valencia





- Zinkicide improves crop
  - Yield
  - Juice quality
- Response is application dependent

- Why a less consistent response?
  - Dilution effect
    - Size of tree



Immobile surface particle

Systemic particle

- Phytotoxicity – limit to rate
  - (peel damage observed)
- Greater variability in HLB decline
- Application timing
- May have tree size limit

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

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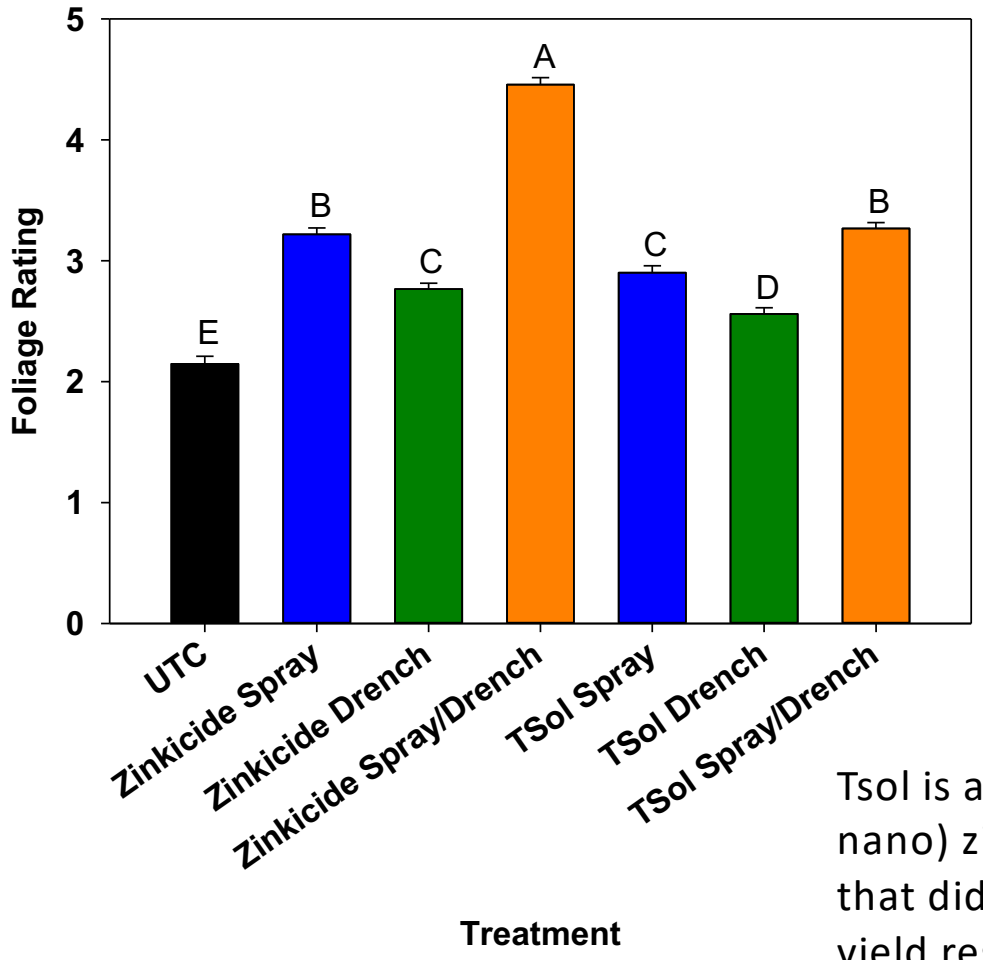


% foliage maintained
1 = 0-20%
2 = 21-40%
3 = 41-60%
4 = 61-80%
5 = 81-100%

Tsol is a soluble (non-nano) zinc formulation that did not show a yield response

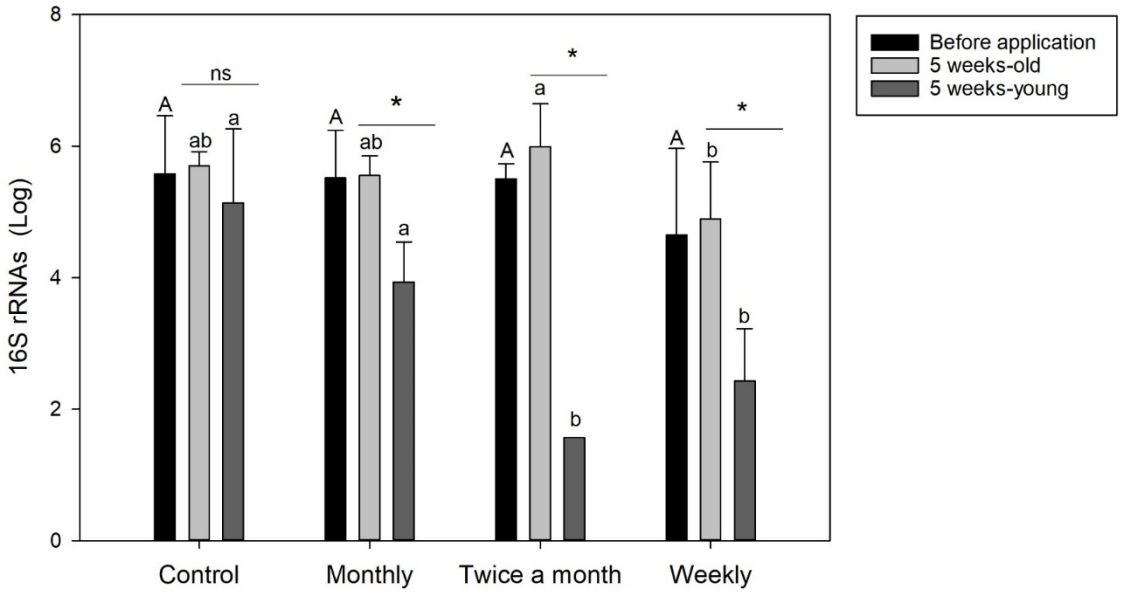
Efficacy is short-lived

- Loss of efficacy between sprays
- Preliminary evidence from greenhouse trials suggests efficacy lost after ~7 days
  - Timing depends on formulation
- Las populations may rebound in remaining 3 weeks

- Better for registration
  - Short active residual
- Need to optimize application schedule
  - Cluster few sprays
  - Would 4 or 8 sprays in 2 month interval be more effective?

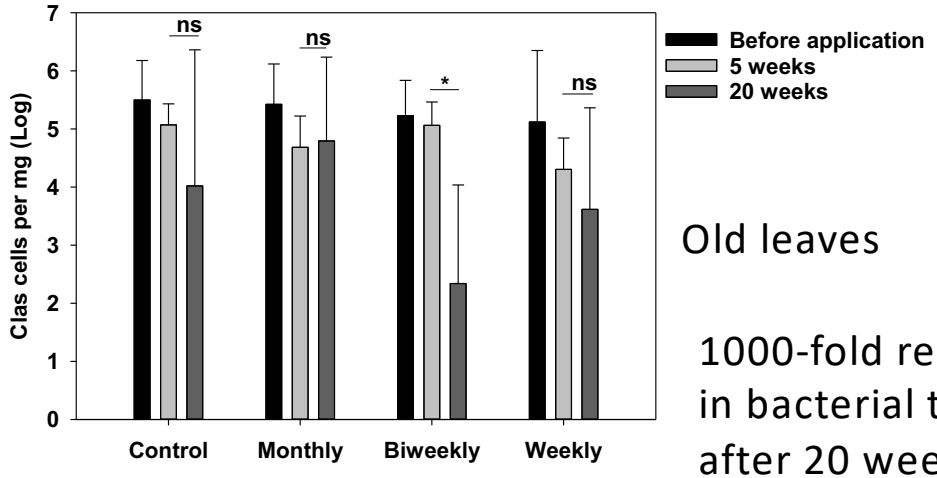
Zinkicide – Optimizing application

Bacterial activity – RNA based



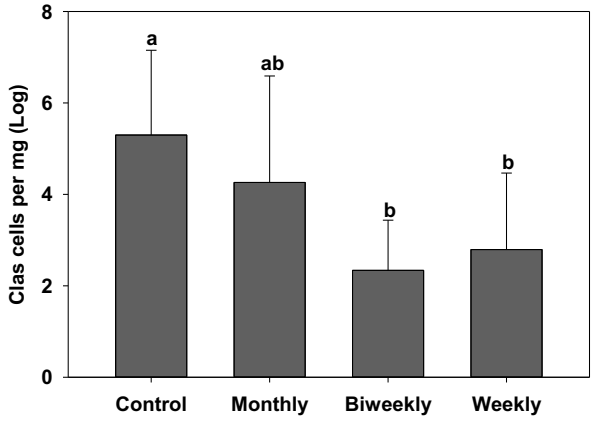
- RNA activity reduced in young leaves
- Plugging in old leaves may reduce access
- RNA activity in new growth predicts bacterial titer reductions
- Biweekly application is most effective

Clas titer – DNA based



Old leaves

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



Young leaves

Efficacy Conclusions

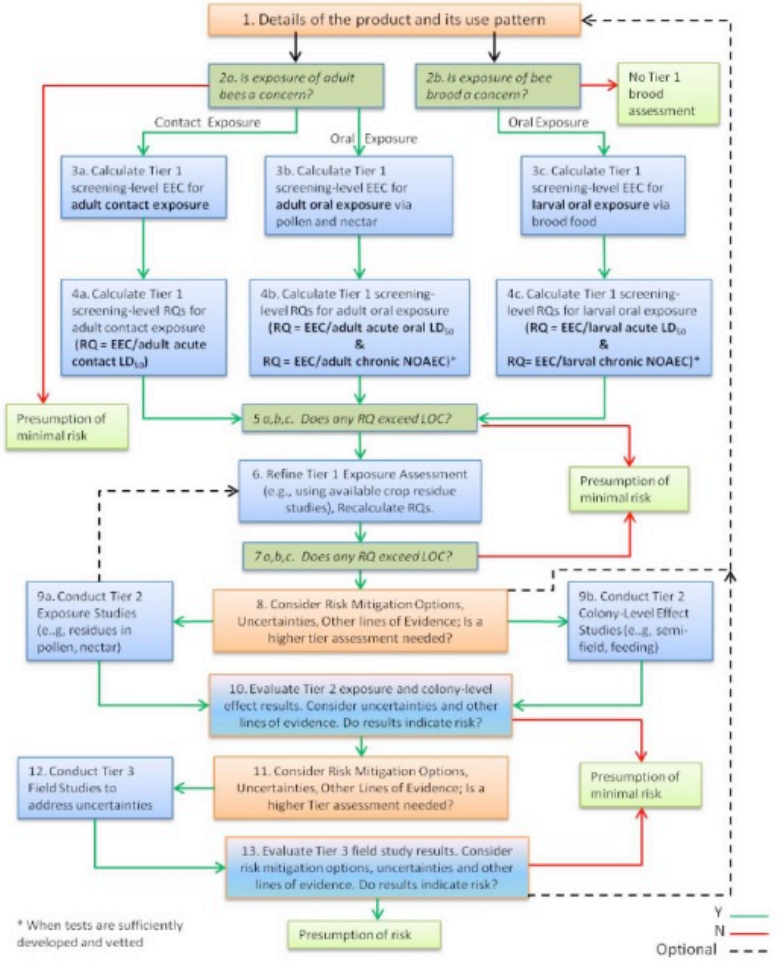
- Zinkicide has systemic activity
- Functional Agricultural-grade formulation developed
  - Not all ZnO nanoparticles act the same
    - Not just a Zinc response
  - Fixed for EPA registration purposes
- Zinkicide can improve yield on HLB-affected trees
  - Tree size dependent?
- Application rate and timing needs to be optimized
  - Cluster fewer sprays more frequently?

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 Process – Example: Honey Bees

- Difficult to predict timeline
- Depends on tier triggered in each case
- Existing literature on Zn and ZnO from non-agricultural uses may help



Tier 1: Laboratory studies on adults and larvae

Tier 2: Semi-field studies with whole colonies in tents

Tier 3: Full field studies with treated crop

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

Conclusions

- Zinkicide has systemic activity
- Zinkicide can improve yield on HLB effected trees
  - Tree size dependent
  - Application schedule needs optimization
- Functional Agricultural-grade formulation developed
  - Not all ZnO nanoparticles act the same
    - Not just a Zinc response
  - Fixed for EPA registration purposes
- Registration timeline difficult to predict
  - Preliminary data looks good

Team Effort

Monty Myers

Tony McIntosh

Mayara Murata

Diane Bright

Kayla Gerberich

Jian Wu

Johnathan Alexander

UCF

- Santra Lab – Design and characterization
- Tetard Lab – Detection and characterization
- Gesquire Lab – Detection and characterization

UF

- Dewdney Lab – Field Trials
  - Tracey Hobbs and Telva Aguilar
- Jim Graham
- Danyluk Lab/Packing house – Yield analysis
- Singerman Lab - Economics

Auburn University

- De La Fuente Lab – Mode of Action/Microfluidics

OSU

- Johnson Lab – Pollinator Toxicology

NMSU

- Gleason Program – Extension and outreach materials (www.zinkicide.org)

Oak Ridge National Laboratory

- Petridis Lab – Nanoparticle modelling

Funding

- CRDF

CRDF Citrus Research and Development Foundation, Inc.

- USDA-NIFA-SCRI-CDRE

USDA United States Department of Agriculture National Institute of Food and Agriculture