Nanoscale micronutrients suppress plant disease and increase crop yield

Sanghamitra Majumdar[1]

Jason C. White[1], Wade Elmer[2]

[1]Department of Analytical Chemistry, [2]Department of Plant Pathology
The Connecticut Agricultural Experiment Station, New Haven CT

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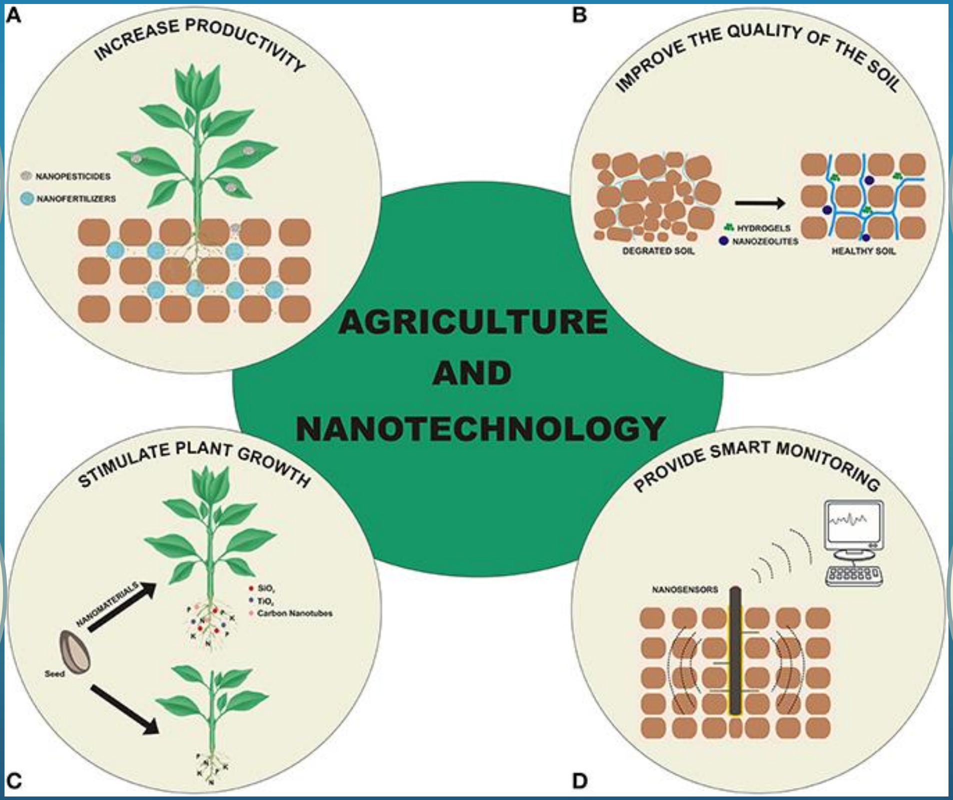
Nanomaterials and Agriculture

The goals fall into several categories:

Transport and controlled release of bioactive substances

Nanosensors and Indicators For Detecting Pathogens



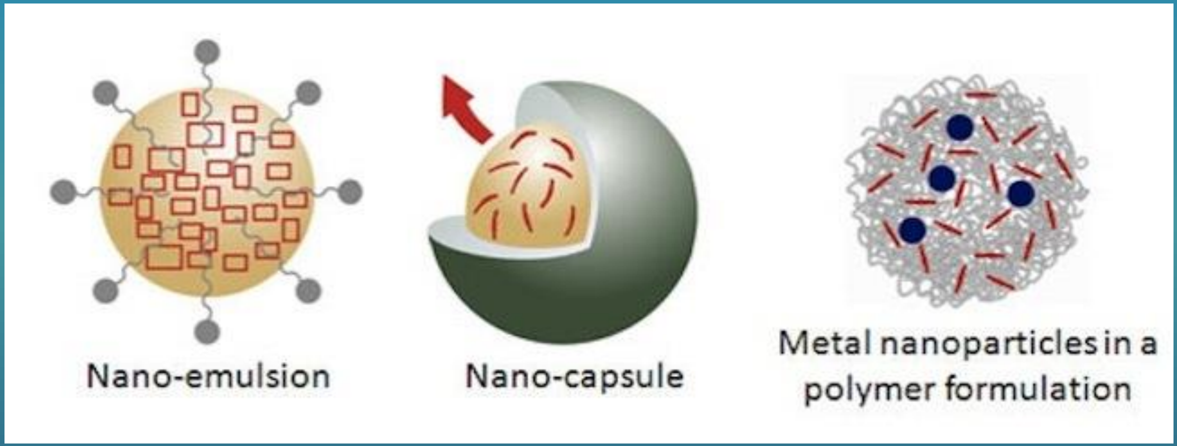
Water Purification

Resource utilization and Waste Minimization

Fraceto, L.F et al. (2016) Nanotechnology in Agriculture: Which Innovation Potential Does It Have?. *Front. Environ. Sci.*, 4: 20.
Sekhon B.S (2014), Nanotechnology in agri-food production: an overview*Nanotechnology, Science and Applications*, 7, 31–53

Nanomaterials and Agriculture contd.

- Nano-fertilizers often contain nutrients/growth promoters encapsulated in nanoscale polymers, chelates, or emulsions
- Nano-pesticides: active pesticidal (insecticide, fungicide,…) ingredient associated with or within a nanoscale product or carrier

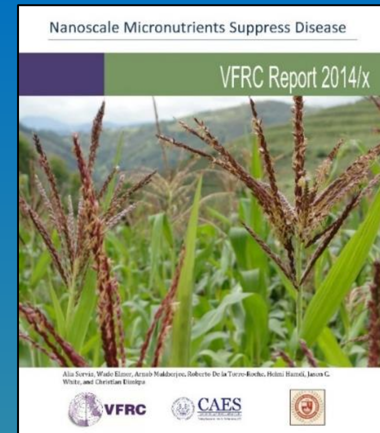


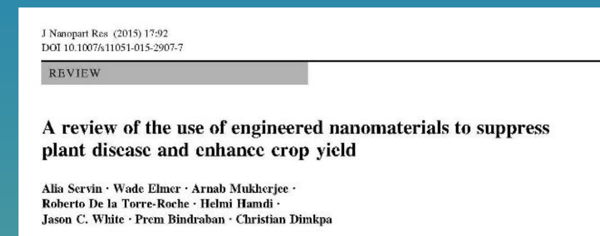
Kah et al. (2013) *Critical Reviews in Environmental Science and Technology*, 43 (16) , pp. 1823-1867

- Slow, targeted, efficient release becomes possible
- Increased stability/solubility
- Lower required amounts of active ingredients

CAES Initiatives

- Nanoscale based micronutrients for disease suppression (particularly **root disease**)
- Started with a small grant from Virtual Fertilizer Research Center/ International Fertilizer Development Center (VFRC/IFDC) to write a report and a review article (*J. Nano. Res.* 2015, 17:92) on nanoscale nutrients and crop disease
- Generated some interesting data (Elmer and White. 2016. Environ. Sci.: Nano, 3, 1072-1079); USDA Grant- $480,000; 3/16-2/19.

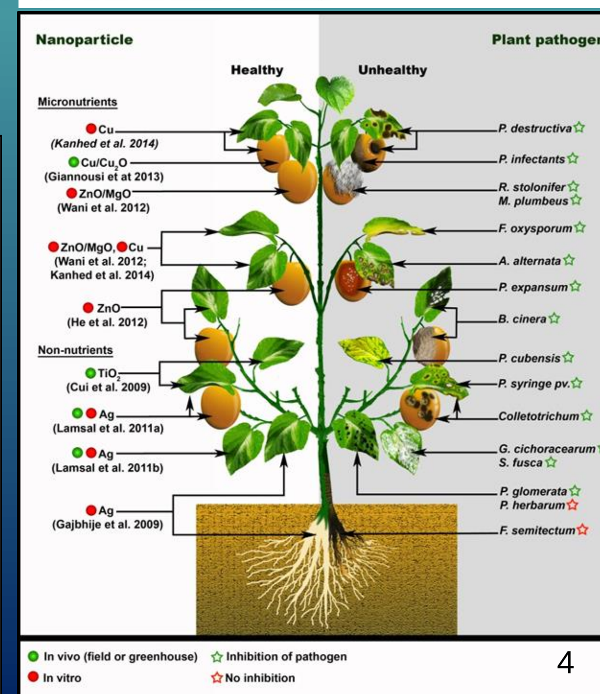


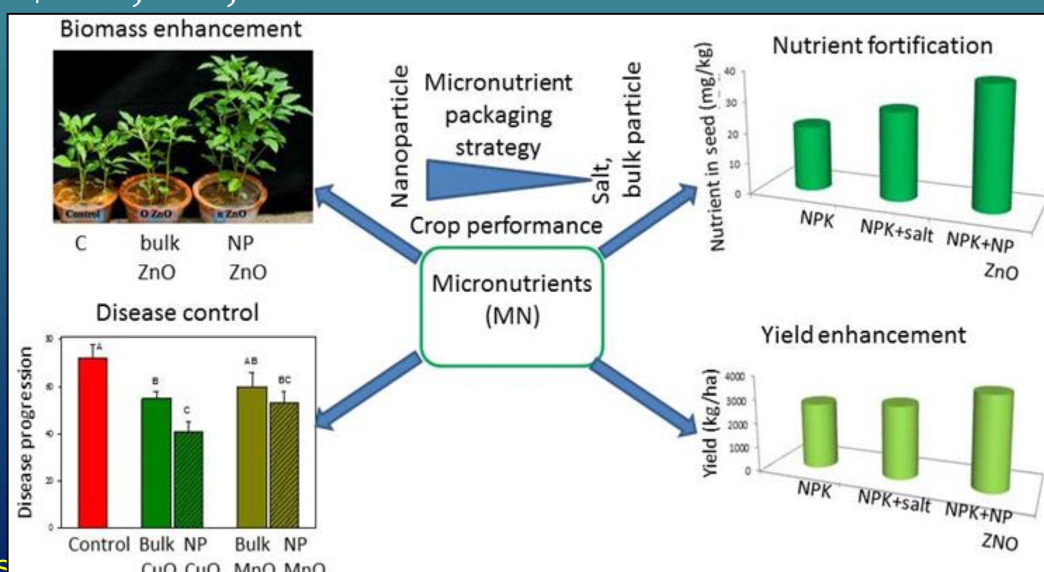

J Nanopart Res (2015) 17:92
DOI 10.1007/s11051-015-2907-7

REVIEW

A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield

Alia Servin · Wade Elmer · Arnab Mukherjee · Roberto De la Torre-Roche · Helmi Hamdi · Jason C. White · Prem Bindraban · Christian Dimkpa





Why Micronutrients?

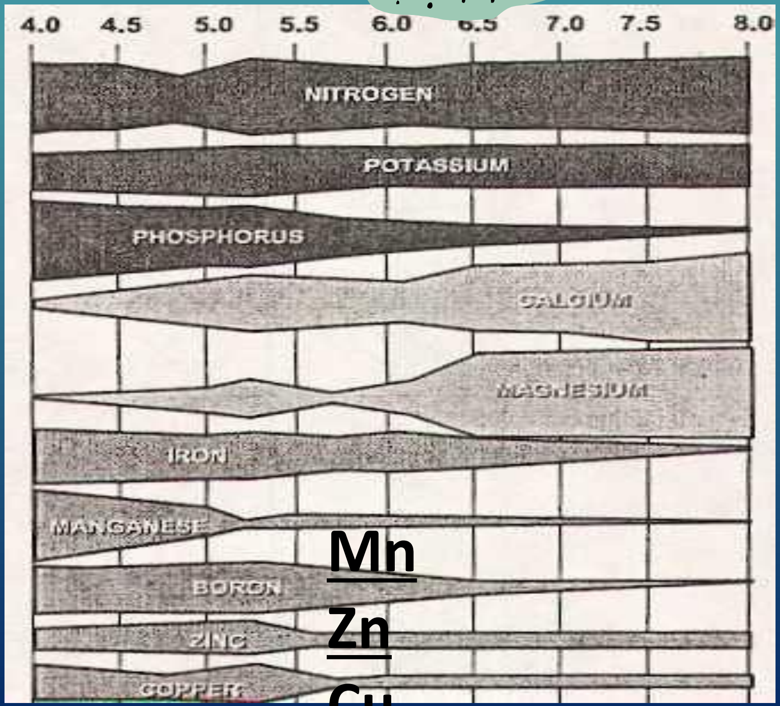
Nutrition is the first line of defense against disease. Micronutrients protect **roots** against soilborne diseases by activating enzymes to create defense products.

- Cu: activates polyphenol-oxidases
- Mn: activates enzymes in the Shikimic acid and Phenylpropanoid pathways
- Zn: activates superoxide dismutases

Micronutrient Availability?

- Increasing micronutrient levels in roots is problematic in neutral soils.
- Micronutrients are not basipetally (shoot to root) translocated.

- When applied to soil they frequently precipitate and become unavailable to the plant
- Limited options for preventing and treating root disease (host resistance, fumigation)



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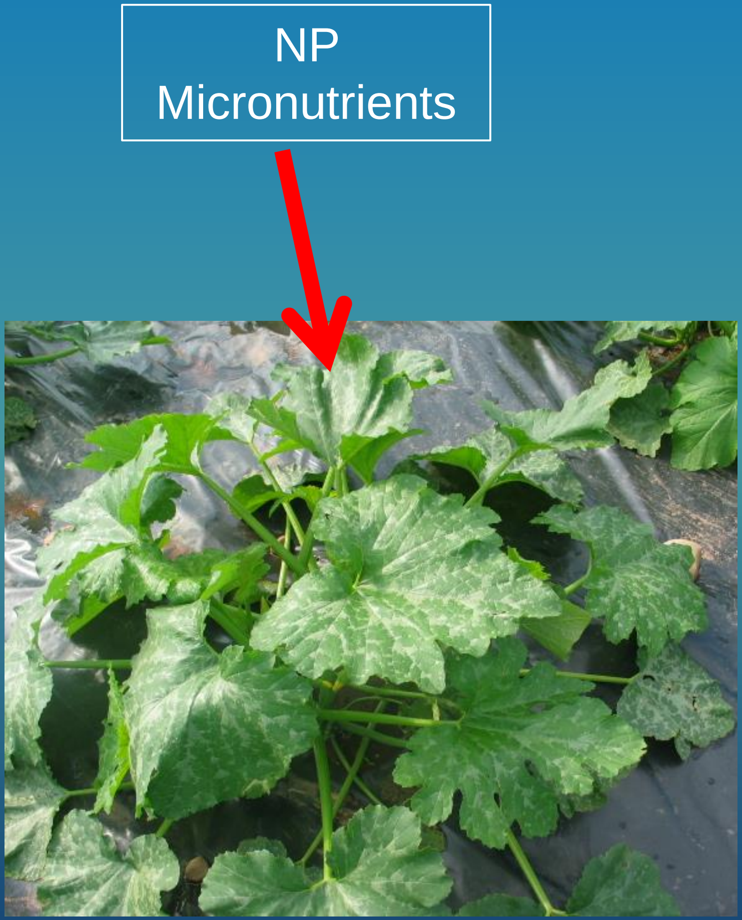
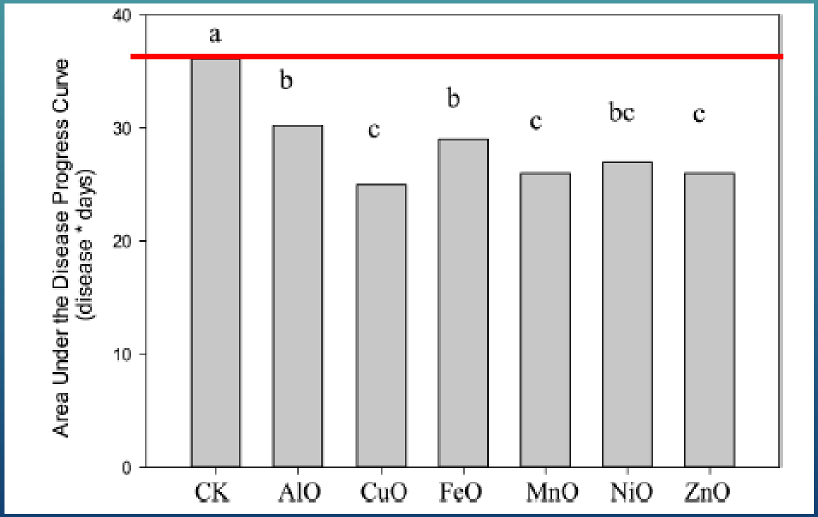
The Hypotheses?

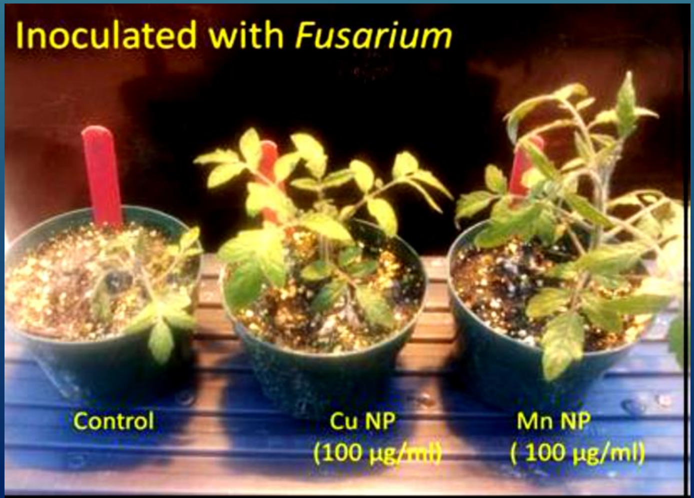
- Would applying nanoscale micronutrients to leaves affect growth?
- Would these metals be translocated to roots?
- Could these translocated nutrients stimulate plant defense and suppress root disease (mostly fungi)?



Fusarium wilt of Tomato Greenhouse study

- Used Tomato and *Fusarium* (fungal root pathogen)
- Two concentrations (100 or 1,000 mg/L) of NP Al, Fe, Cu, Mn, Ni, or Zn oxides were sprayed onto tomato seedlings in the greenhouse.
- Plants were inoculated with *Fusarium* and disease was measured

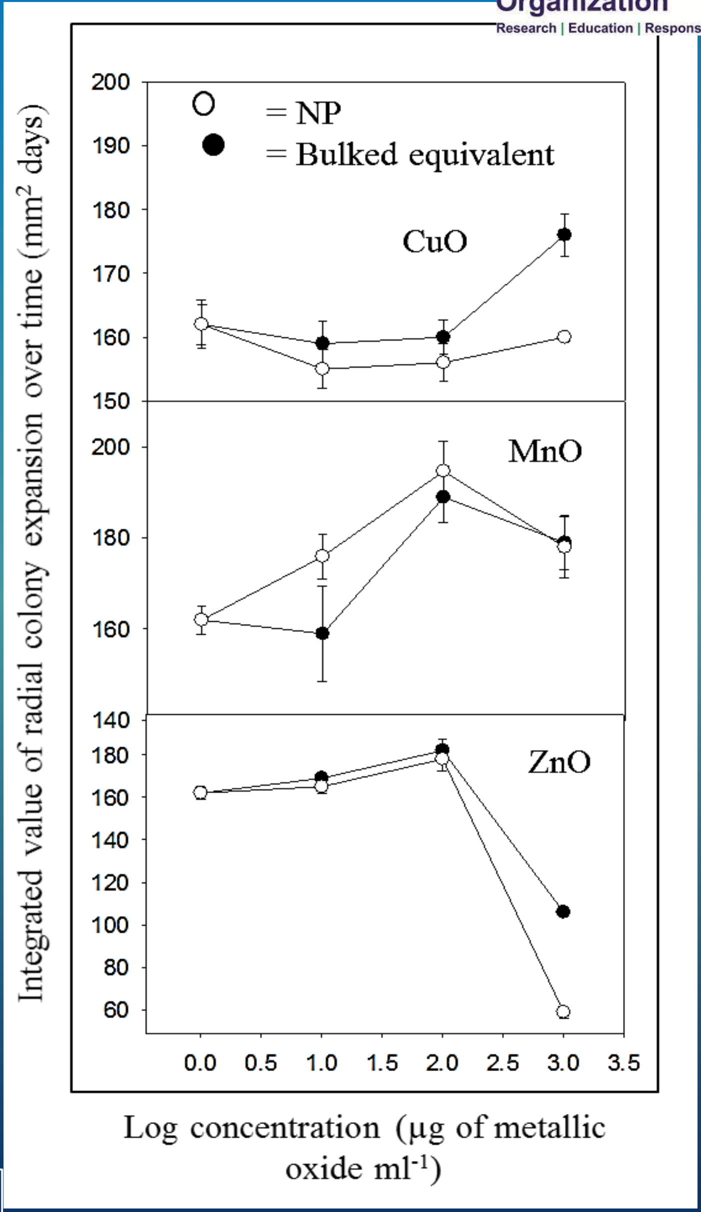




Elmer and White. 2016. *Environ. Sci.: Nano*, 3, 1072-1079

Direct Effect on the Pathogen?

- There has been work on nano-fungicide formulations to **directly** suppress fungal pathogens (Ag, Zn, Cu), although not a lot on root pathogens.
- We ran *in vitro* assays with NP and bulk metal oxides against *Fusarium* (25% potato dextrose agar).
- Bulk and NP ZnO had significant toxicity but MnO and CuO either had no effect or promoted fungal growth
- Our CuO effects are driven by nutrition and disease resistance



Elmer and White. 2016. *Environ. Sci.: Nano*, 3, 1072-1079

Verticillium Wilt of Eggplant

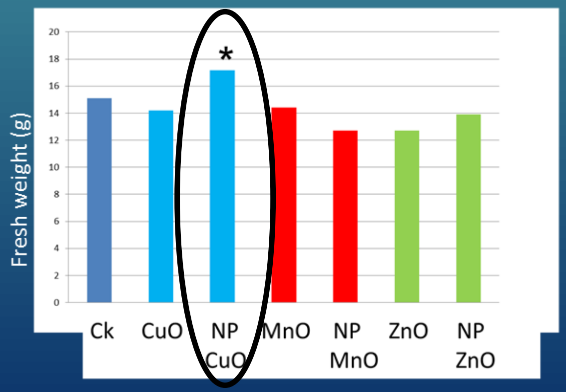
- Caused by soilborne fungus, *Verticillium dahlia*; can reduce yields by 30%
- In greenhouse trials, would foliarly applied NPs of Cu, Mn, or Zn suppress *Verticillium*?
- Would they behave the same as their bulk oxide equivalents?

Elmer and White. 2016. *Environ. Sci.: Nano*, 3, 1072-1079

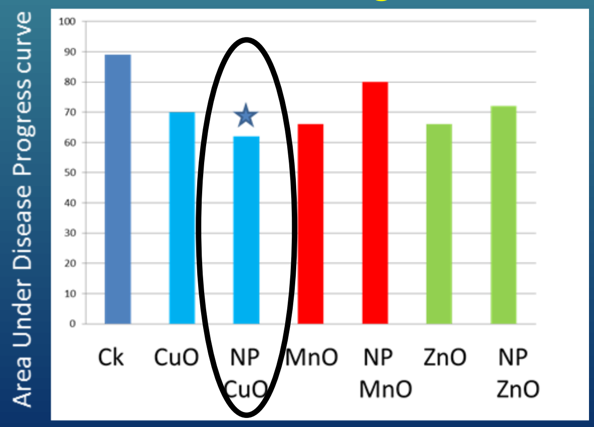
Verticillium Wilt of Eggplant

- NP of Cu, Mn, and Zn oxides were compared to the bulk oxide equivalent (1000 mg/L).
- Plants were sprayed (15ml), allowed to dry and grown in soil with *V. dahliae*.
- CuO NP treated plants had greater biomass (left), less disease progress (center) and higher Cu root content (right)

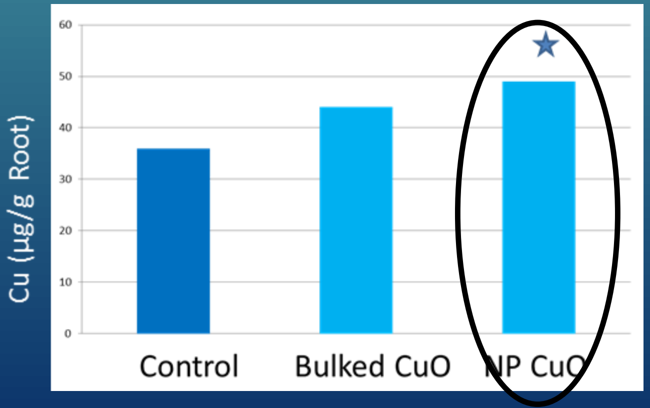
Biomass



Disease Progress



Root Cu Content



Elmer and White. 2016. *Environ. Sci.: Nano*, 3, 1072-1079

Verticillium Wilt of Eggplant Field Trials 2013-2014

- Treatments included NP or bulk CuO, MnO, and ZnO
- Single application in greenhouse followed by transplantation to infested field soil
- Yield and fruit element content measured

Elmer and White. 2016. *Environ. Sci.: Nano*, 3, 1072-1079

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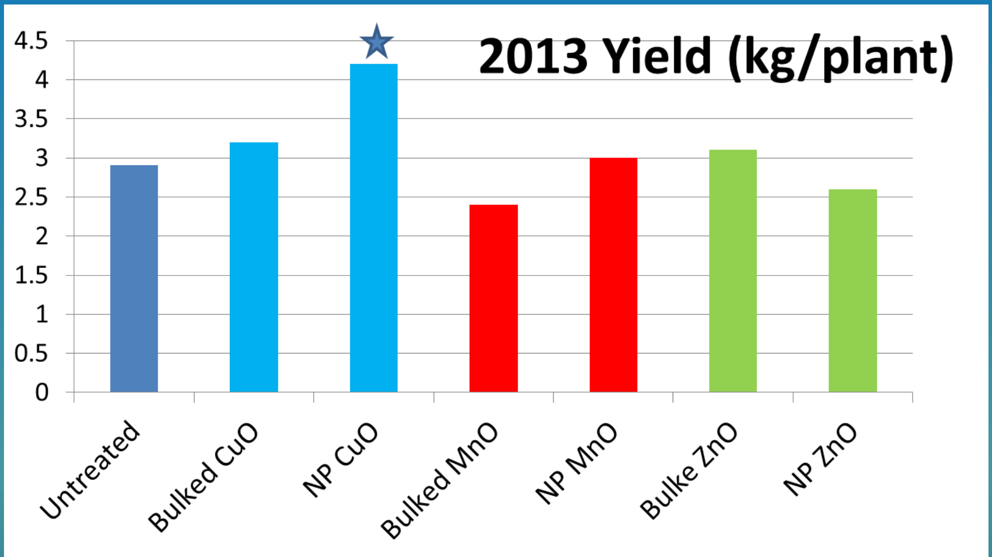
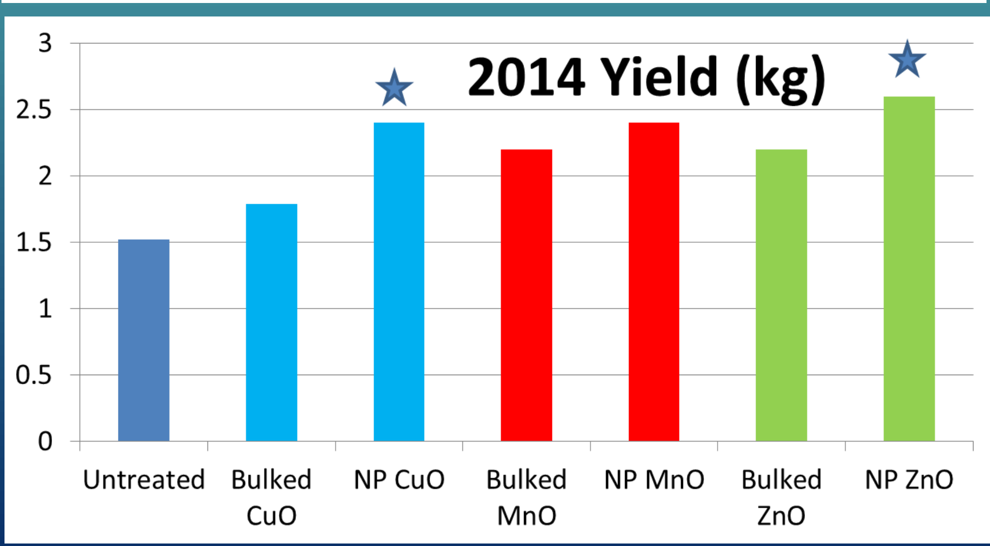
Verticillium Wilt of Eggplant Field Trials 2013-2014

- In two separate field trials, NP CuO increased fruit yield, decreased disease, but did not increase fruit Cu content

- $44 per acre investment for NP CuO suppressed a root pathogen of eggplant, increasing yield from $17,500/acre to $27,650/acre.

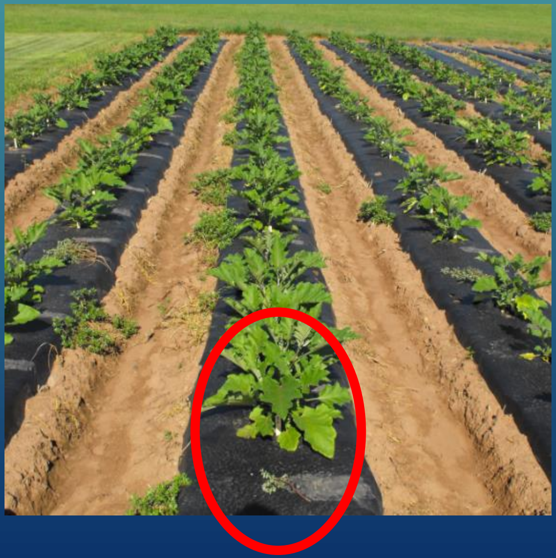
Elmer and White. 2016. *Environ. Sci.: Nano*, 3, 1072-1079



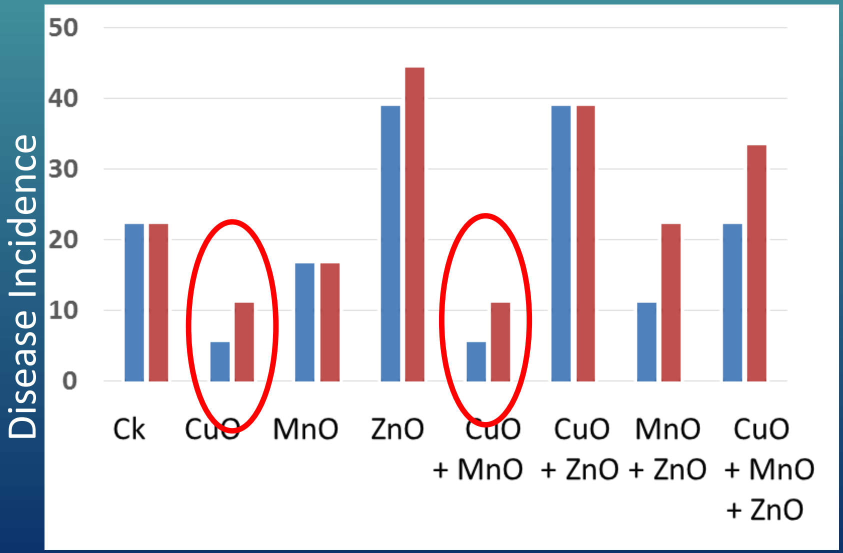


Verticillium Wilt of Eggplant Field Trial 2016

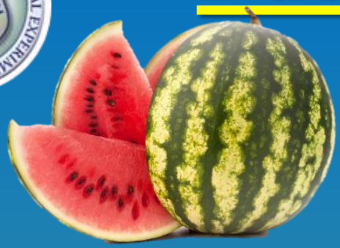
- Treatments include NP CuO, MnO, ZnO, CuO + MnO, CuO + ZnO, MnO + ZnO, CuO + MnO + ZnO
- Greenhouse application (1000 mg/L) followed by transplant to infested soil; periodic applications in field ongoing at 2 farms
- Yield and fruit elemental to be content measured
- Initial disease progress data taken on 2 occasions (blue and red bars)



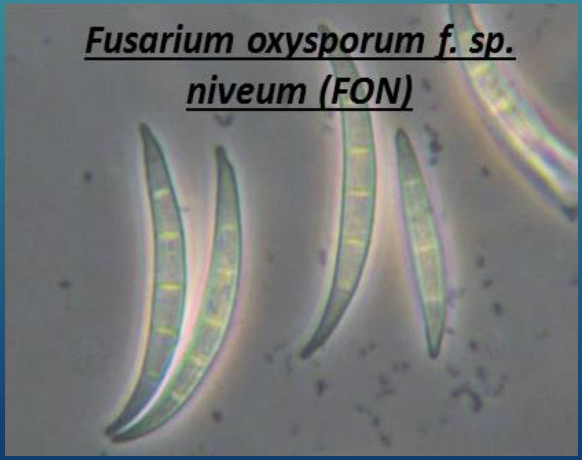
NP CuO



Fusarium Wilt of Watermelon-Greenhouse study 2015



- Another *Fusarium* pathogen attacks watermelons; increased occurrence in Florida has been reported (significant economic impact)
- Similar infection through roots causing whole plant wilt
- Host resistance options limited
- Chemical control ineffective



Fusarium Wilt of Watermelon
Greenhouse study 2015-2016

- A greenhouse study with single foliar application of 100 or 1000 mg/L prior to growth in soil containing FON
- NP CuO significant promoted plant growth and significantly suppressed disease progress at both treatment levels
- Others affected disease only

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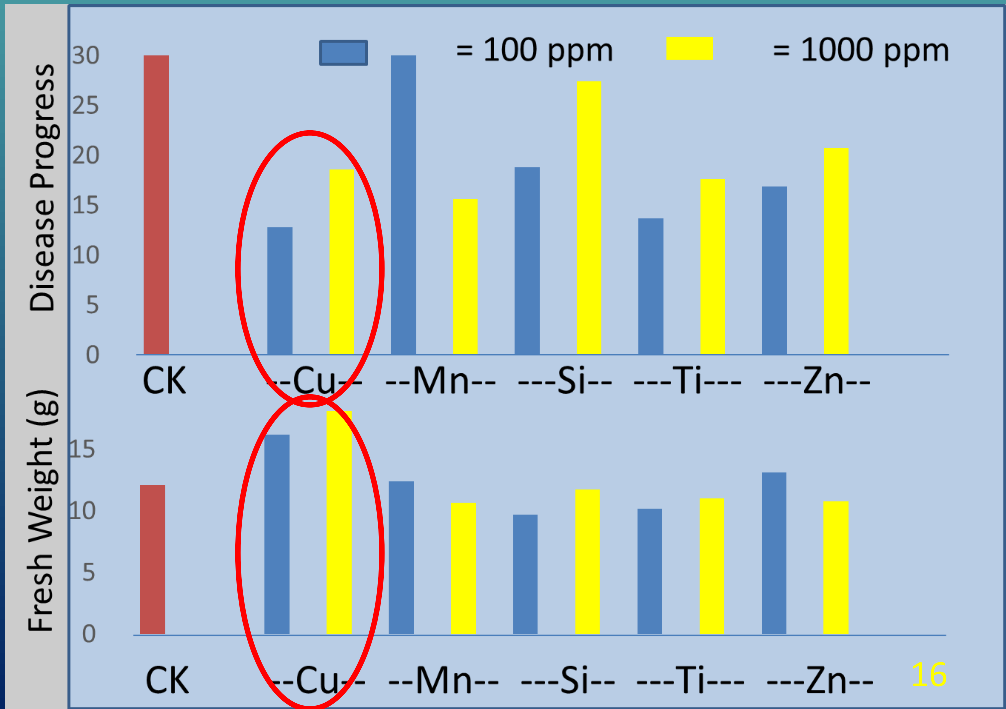


Fusarium Wilt of Watermelon- Field 2016

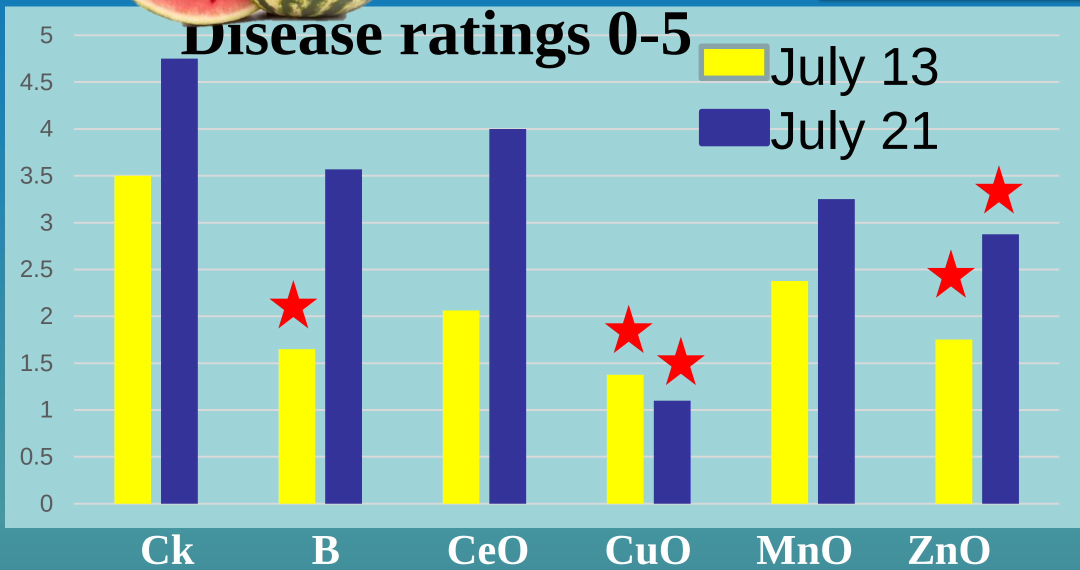
Treatments- Multiple foliar applications made during growth at 2 farms

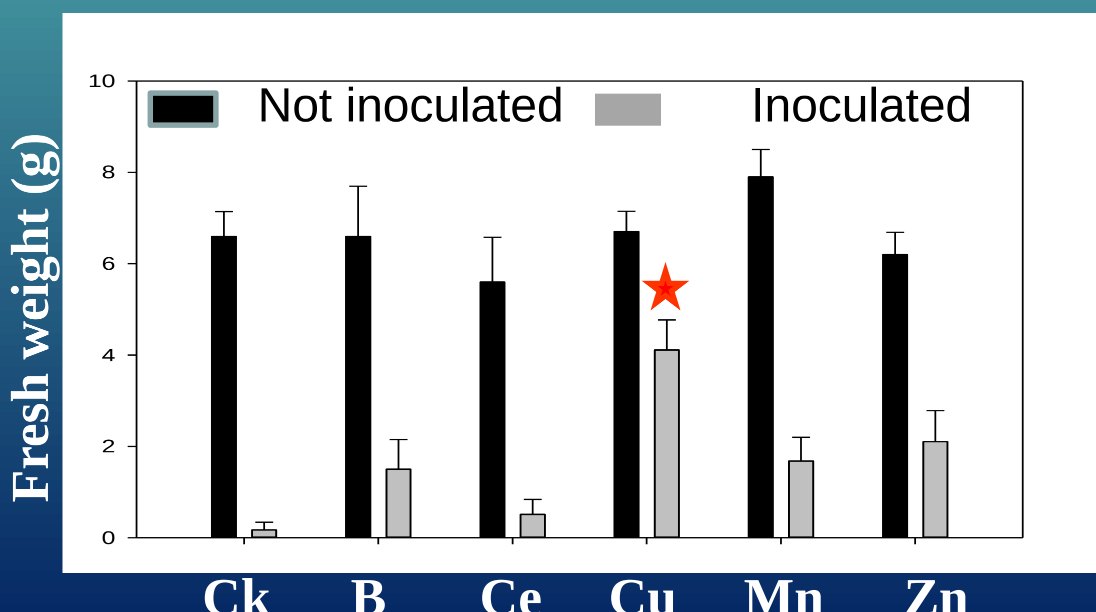
Treatments applied once the greenhouse and once in the field

- Control
- B NP
- CeO NP
- CuO NP
- MnO NP
- ZnO NP

Fusarium Wilt of Watermelon
Field 2016

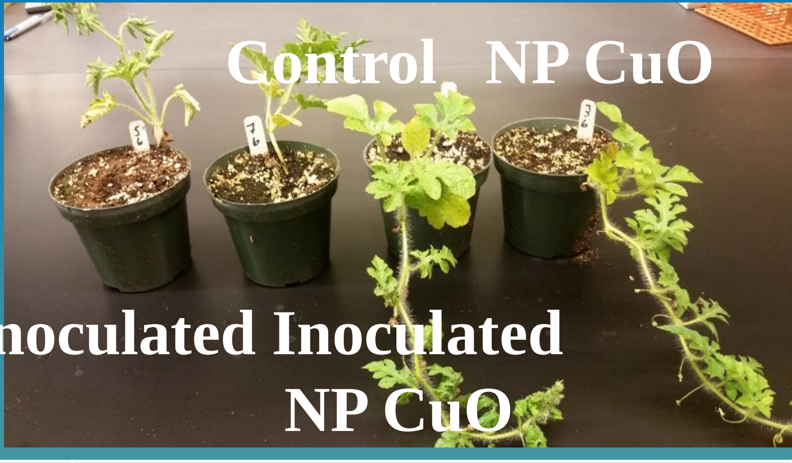
Yield was unaffected

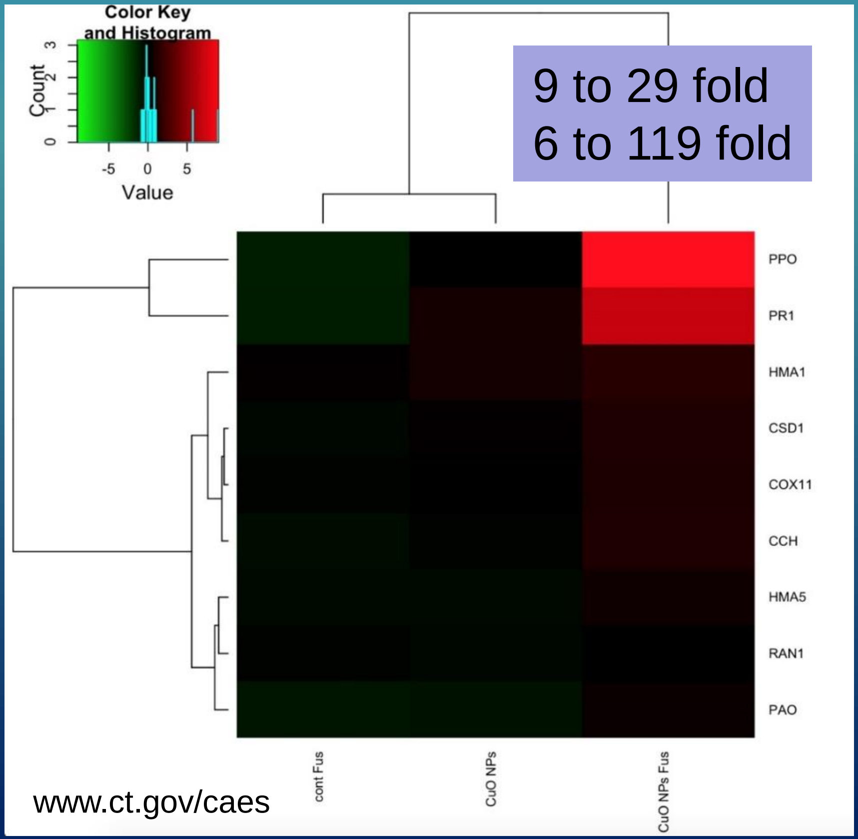




Fusarium Wilt of Watermelon- Field 2016: Transcriptomic analysis of roots

Strong up-regulation of Polyphenol oxidase genes and PR 1 protein genes when both Fusarium and NP of CuO are present.





CCH	Cla020497	Copper chaperone, cch
COX 11	Cla002392	Cytochrome c oxidase assembly protein ctag / Cox11 family
HMA 1	Cla006819	Heavy metal atpase 1, hma1
HMA 5	Cla011458	Heavy metal atpase 5, hma5
RAN1	Cla009875	Heavy metal atpase 7, hma7, ran1
CSD1	Cla011299	Copper/zinc superoxide dismutase 1, csd1, sod1
PAO	Cla015262	Polyamine oxidase 1, pao1
PPO	Cla019486	Polyphenol oxidase chloroplastic-like
PR1	Cla001623	Pathogenesis-related gene 1

Conclusions

- Treating seedlings with nanoscale CuO had positive effects on the growth and yield of
  - Tomato in the presence of *Fusarium* (greenhouse)
  - Eggplants in the presence of *Verticillium* (greenhouse, field)
  - Watermelons in the presence of *Verticillium* (greenhouse, field)
- Season long effects were observed following single or double applications to young transplants.
- Mechanism of action is either improved plant nutrition or stimulated disease response (or both); little direct NP activity on the pathogens.
- Associated with NP CuO was upregulation of polyphenoloxidase and PR 1 genes in root tissue .

Acknowledgements

- **Wade Elmer, Ph.D**- CAES
- **J. Gardea-Torresdey, Ph.D.**- UTEP
- **Christian Dimkpa, Ph.D.**- VFRC
- **At CAES**- R. De la Torre-Roche, P. Thiel,

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F. Pasquali (Univ. of Parma), S. Younas (SCSU)

Funding- USDA AFRI Foundational Program Area A1511 “Nanotechnology for Agricultural and Food Systems;” Grant 2016-67021-2498 “Nanoscale elements suppress plant disease, enhance micronutrient use efficiency, and increase crop yield.” Also, USDA Hatch and FDA FERN

VFRC Nourishing plants for healthy people in a green world

FERN Uniting Federal, State and Local Laboratories for Food Emergency Response

USDA National Institute of Food and Agriculture

UTEP The University of Texas at El Paso

Nanomaterials and Food Products

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➢ Food Safety

- ➢ Antimicrobials in food packaging
- ➢ Nanocoatings for food and equipment
- ➢ Nanosensors for pathogen detection

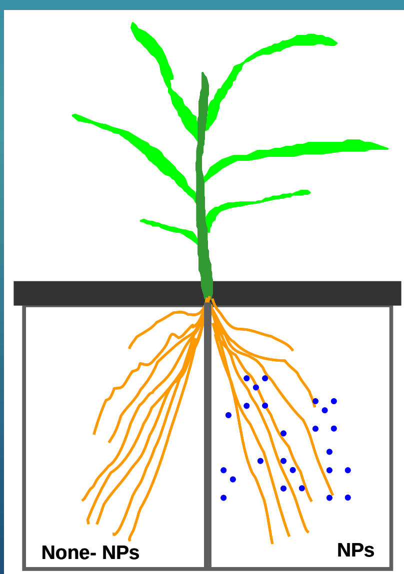
Nanocor ®

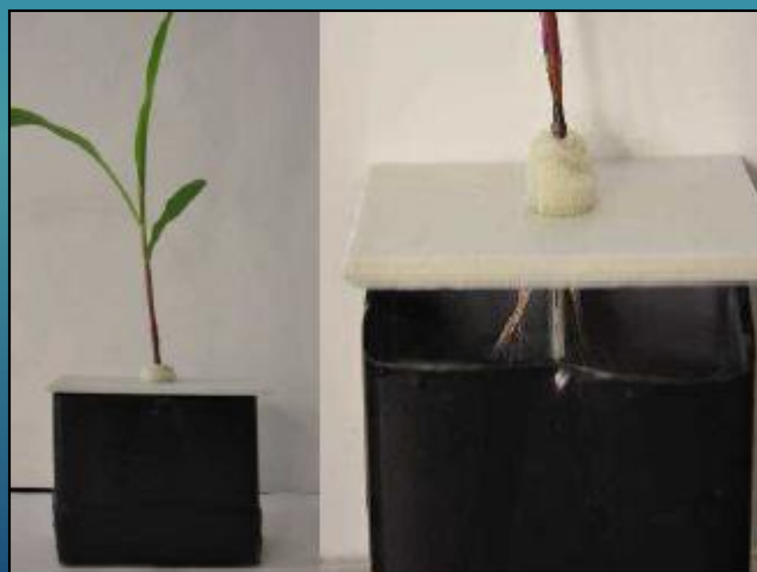
➢ Food Defense

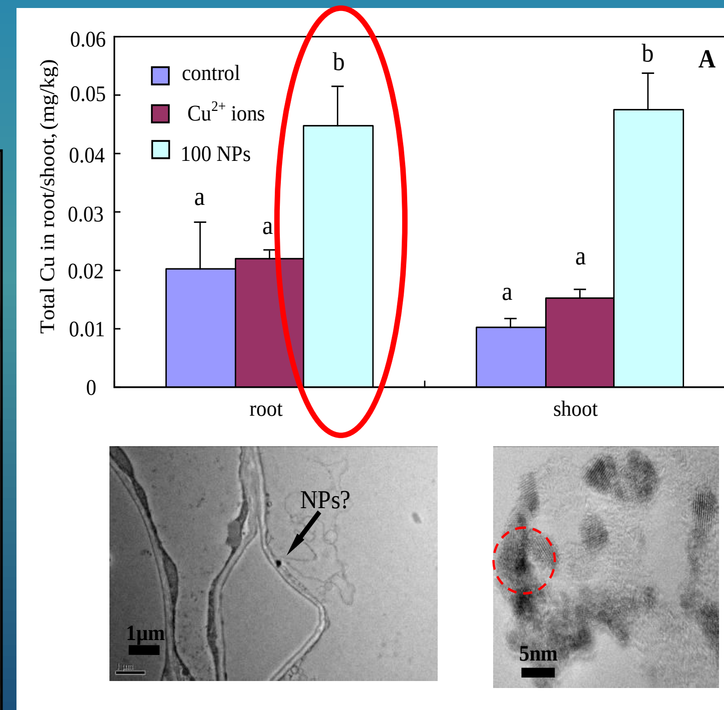
Nanosensors for specific agents of concern (biological weapons such as *B. anthracis*, Ebola and others; plant proteins such as ricin and abrin.

When Chemists talk to Plant Pathologists…

- NP CuO (and other metal NPs?) can move basipetally whereas bulk equivalents do not.



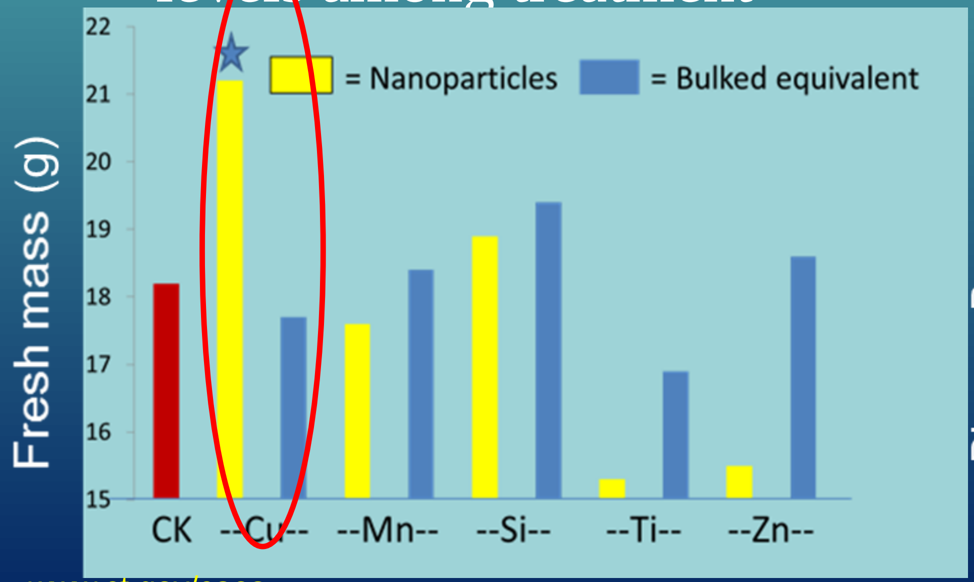


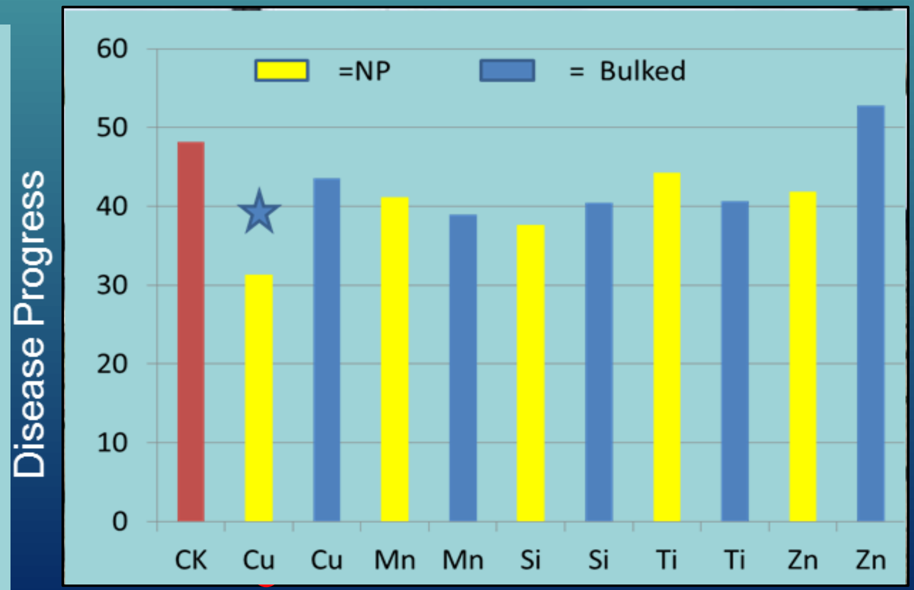


Wang, White et al. 2012. Xylem- and phloem-based transport of CuO nanoparticles in Maize (*Zea mays* L.) *Environ. Sci. Tech*. 46:4434-4441.

Fusarium Wilt of Watermelon- Greenhouse study 2015-2016

- Greenhouse study with single foliar application of 1000 mg/L prior to growth in soil containing FON
- Again, NP CuO significant promoted plant growth (left) and significantly suppressed disease progress (right) (Estimates of disease progress=disease * days)
- ICP-MS analysis of edible flesh found no differences in Cu levels among treatment



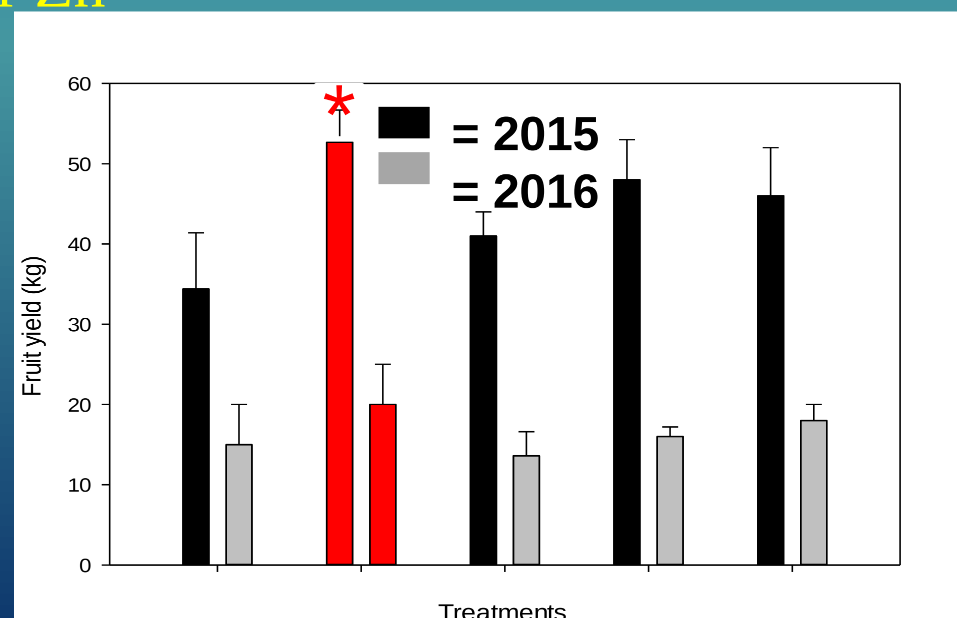


Fusarium Wilt of Watermelon
Field trial 2015

Treatments- Applied twice to seedlings in greenhouse

1. Control
2. CuO NP
3. Bulk CuO
4. Kocide 2000
5. Organic Cu soap (Cu octanoate)

- Fruit yield increased by NP
- No difference in fruit Cu content
- Did not affect levels of P, K, Ca, Mg, Mn, S or Zn



Effect of Bulked vs NP of Cu, Mn, Si, Ti, and Zn oxides on Cu levels in roots of watermelons.