Use of engineered nanomaterials to suppress crop disease and enhance yield

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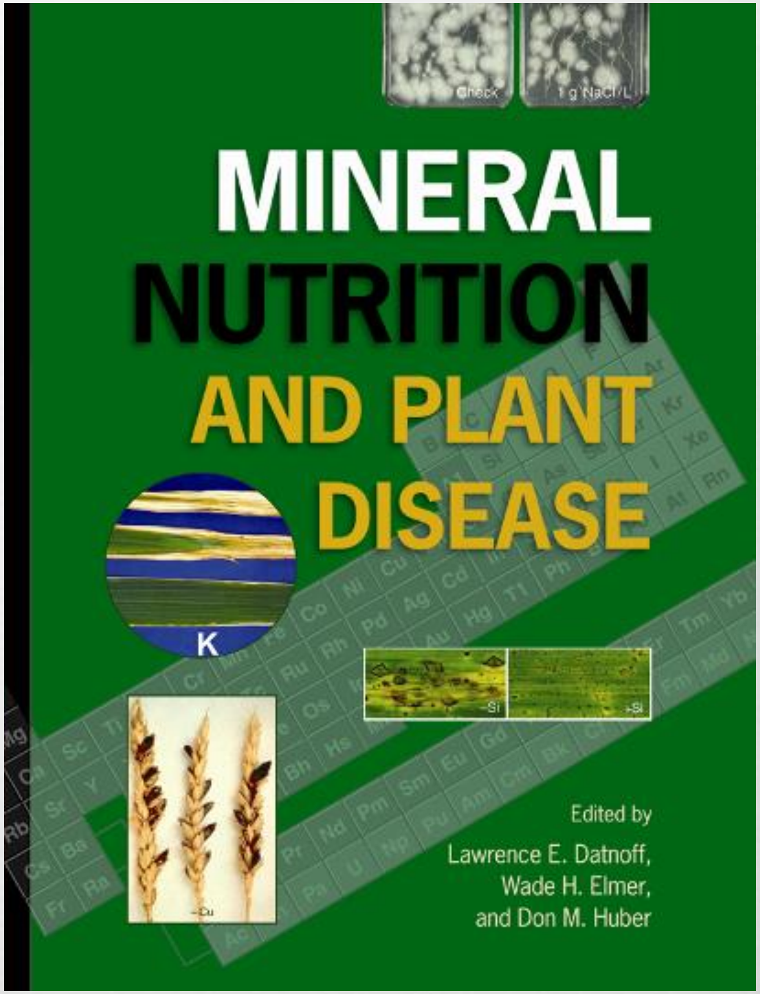
Introduction

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

Cu activates polyphenoloxidases

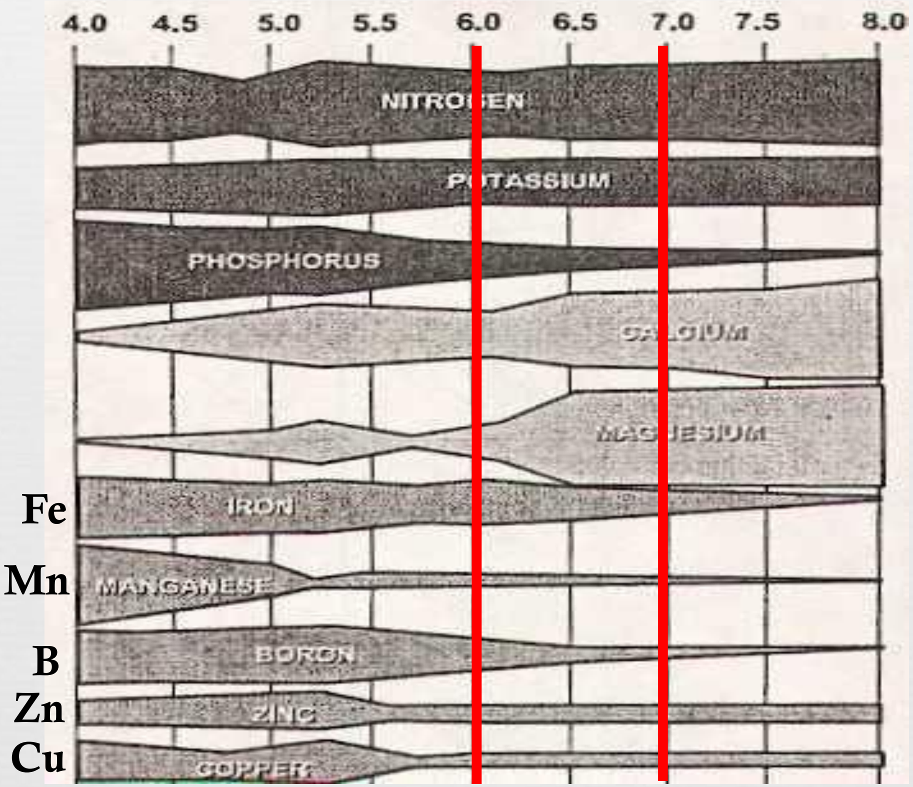
Mn activates enzymes in the Shikimic acid and Phenylpropanoid pathways

Zn activates superoxide dismutases



Obstacles

- Micronutrients are not basipetally translocated.
- When applied to soils (pH of 6-7), they precipitate as oxides and become unavailable to the plant.



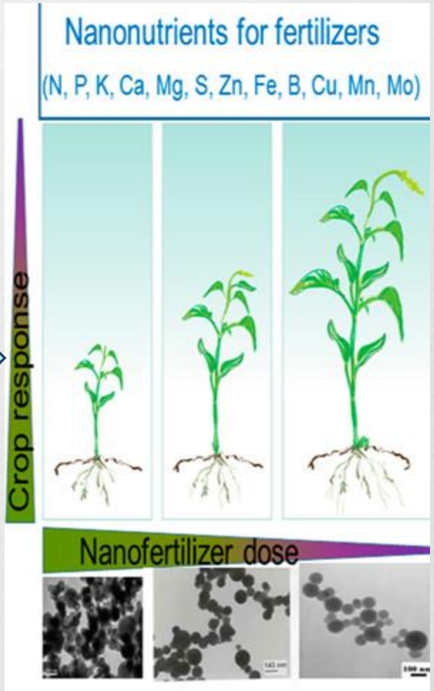
Significance

- Global food insecurity will worsen with a changing climate and an increasing population.
- Crop diseases reduce yield by up to 20%, resulting in billions of dollars in losses.
- Sustainable nanotechnology can have a potentially tremendous role in promoting food security.
- Our focus is on plant nutrition, which is critical to crop health.
- The availability of several micronutrients is often the major factor in determining a plant’s resistance or susceptibility to disease.
- This project will design nanoscale micronutrients that more effectively enhance nutrition, suppress disease and increase yield.

Nanofertilizers

- Challenges: low nutrient uptake efficiency for nitrogen- and phosphorus-based fertilizers;
  redundant fertilizers result in emission of harmful greenhouse gases and eutrophication.
- **Nanofertilizers** are nutrient fertilizers composed, in whole or part, of nanostructured formulation(s) that can be delivered to the plants, allowing for efficient uptake or slow release of active ingredients.

Conceptual overview of fertilizer advancement from bulk-scale to nanoscale production and application

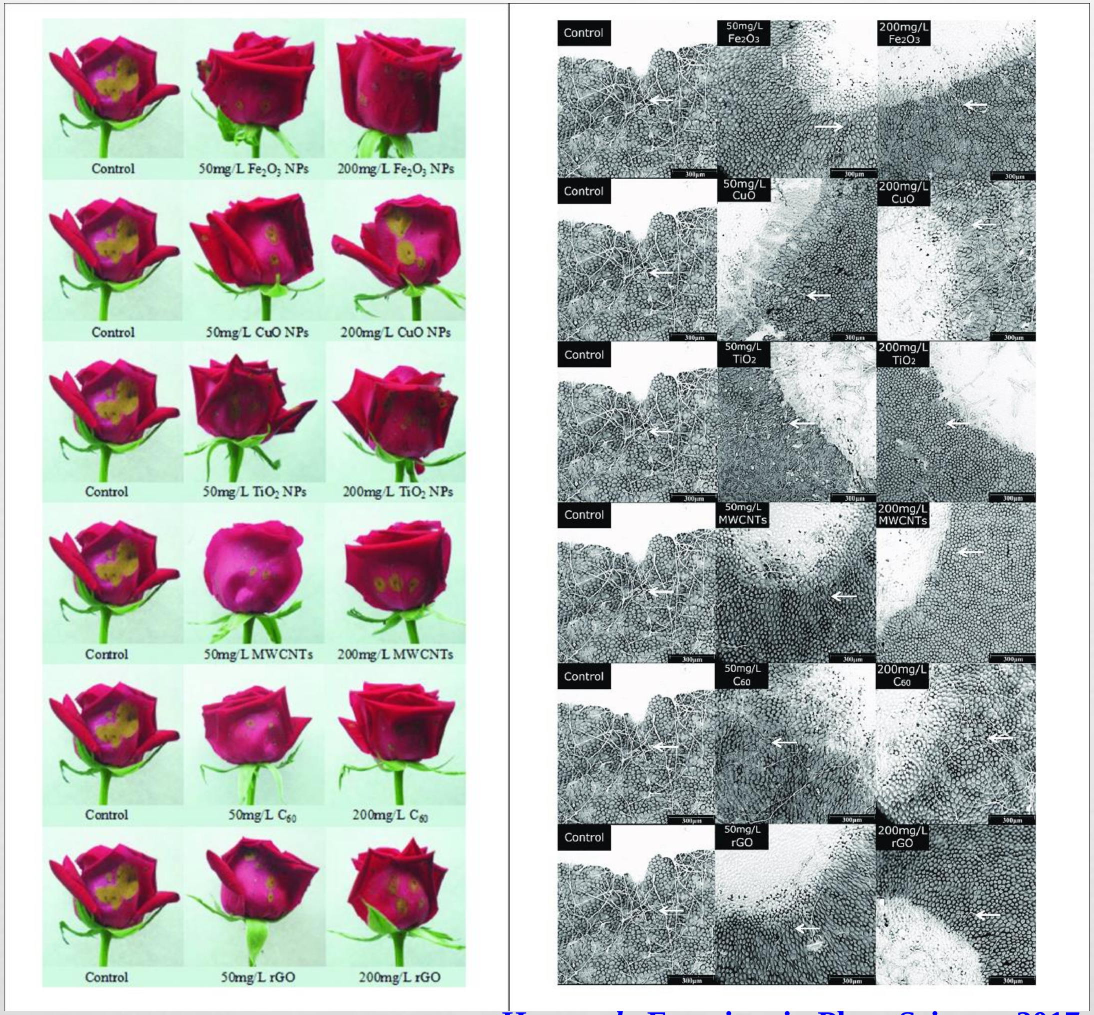


Growth enhancement

Raliya et al., *J. Agric. Food Chem, 2017;*
Dimkpa and Bindraban, *J. Agric. Food Chem, 2017.*

Antifungal properties of NMs

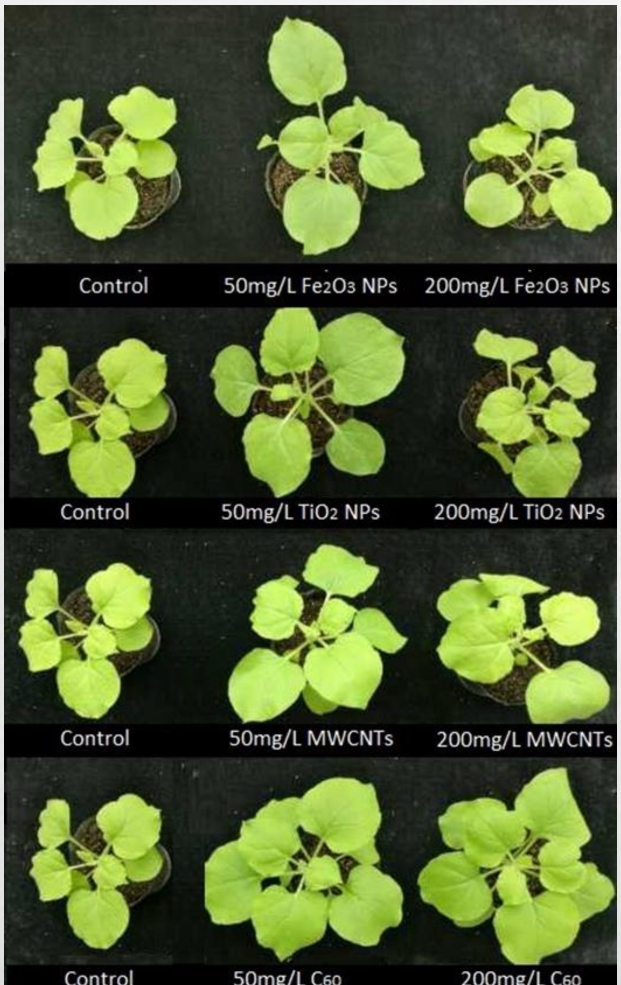
- *Botrytis cinerea* is a devastating disease of rose, resulting in at least a 30% loss of production annually;
- Foliar application of metal- and carbon-based NMs significantly inhibited *B. cinerea* growth in rose petals;
- SEM images also showed that exposure to different NMs notably suppressed the hyphae development as compared to the control.

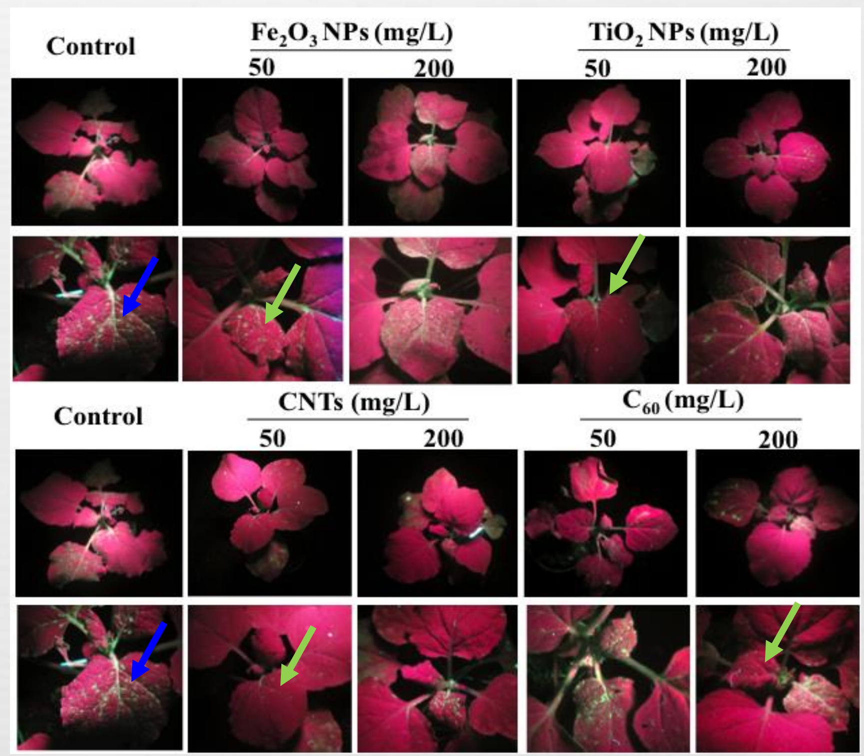


Hao *et al.*, Frontiers in Plant Science, 2017

Antiviral properties of NMs

- Different NMs were exposed to tobacco by foliar application. After 21-day exposure, *Turnip mosaic virus* (TuMV) was inoculated in tobacco leaves;
- Foliar application of NMs greatly enhanced tobacco growth in terms of the size of aboveground part and biomass. Fluorescent intensity suggested that the presence of NMs effectively inhibited TuMV invading to the newly-emerged leaves, the results were further confirmed at the protein level.





Ma *et al.*, In preparation, 2017

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)?

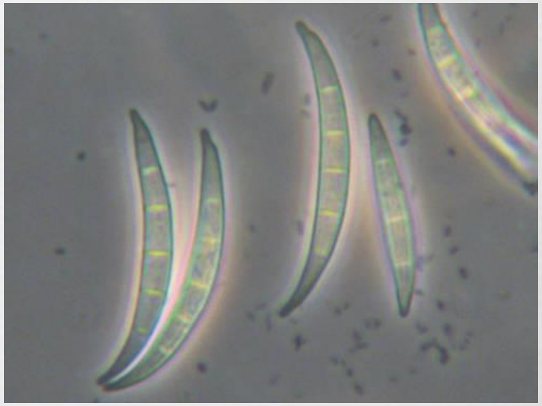
Nanoscale micronutrients (Cu, Zn, B, Si…)

Foliar application

Watermelon

Fusarium Wilt of Watermelon

2013 Florida Growables



Fusarium oxysporum
f. sp. *niveum* (*FON*)

Experimental design---Greenhouse trial

Watermelon at CAES in Nov., 2017

Dose Study for $Cu_3(PO_4)_2$ nanosheets and CuO NPs

Grow watermelon under greenhouse conditions in *Fusarium*-infested growth media. Add single amendment of $Cu_3(PO_4)_2$ nanosheets or CuO NP (plant "dip") at one of 10 concentrations.

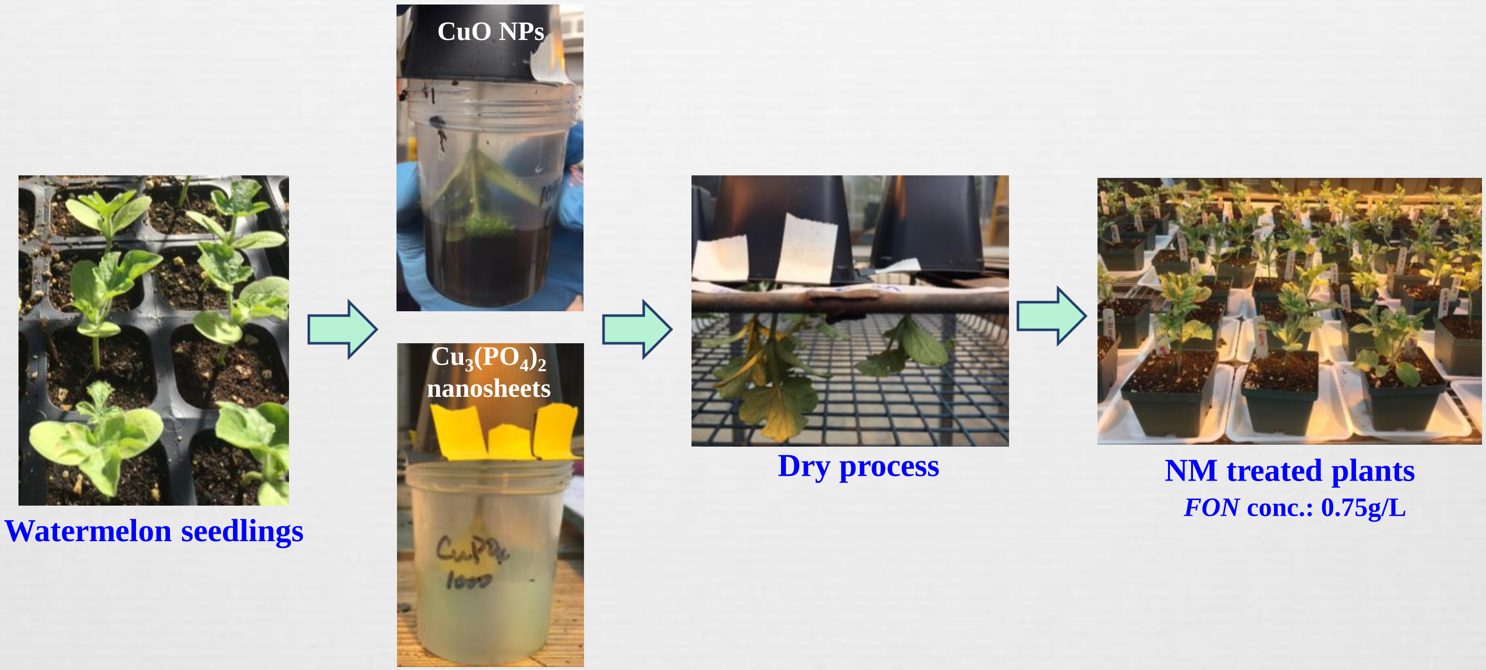
Conc.(mg/L)	$Cu_3(PO_4)_2$ w/ disease	CuO NP w/ disease
0	12	12
5	12	12
10	12	12
25	12	12
50	12	12
100	12	12
250	12	12
500	12	12
750	12	12
1000	12	12
	120 plants	**120 plants**

Watermelon seedlings at CAES

Endpoints

- Disease progress
- Yield/biomass
- Element content by ICP-OES/MS in roots/shoots.

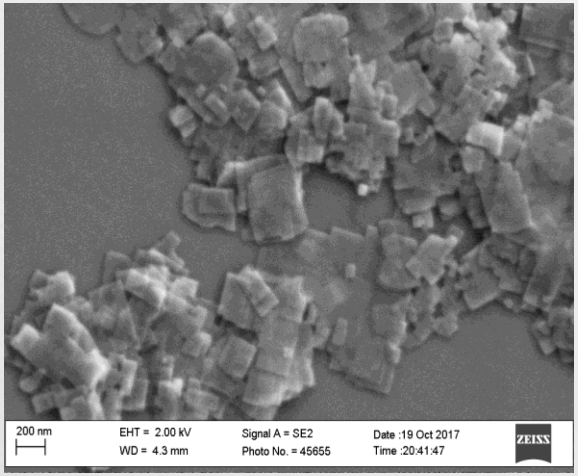
NM exposure setup



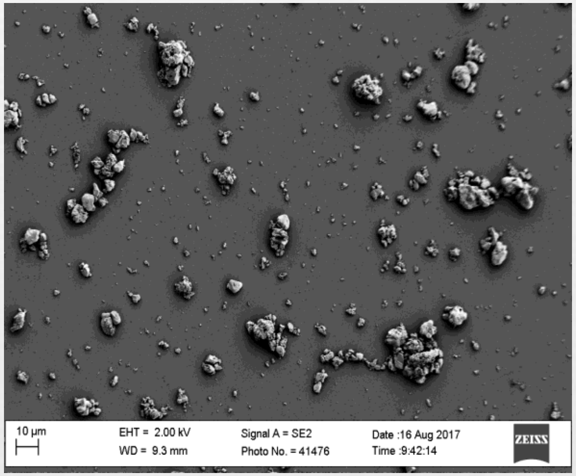
Preliminary results

$Cu_3(PO_4)_2$ nanosheets and CuO NPs characterizations

- SEM images show that $Cu_3(PO_4)_2$ has a thin sheet like morphology, while CuO shows micro and nanoscale features.



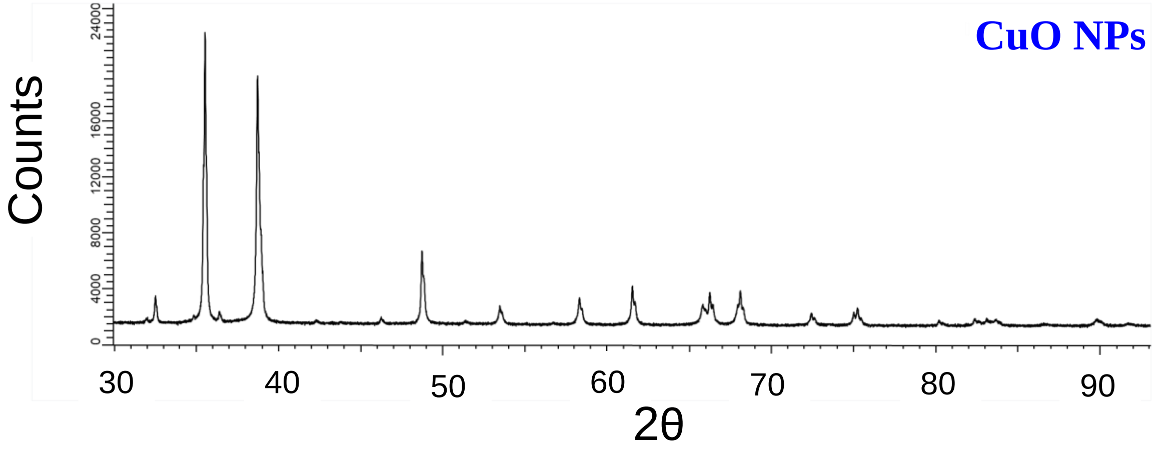
$Cu_3(PO_4)_2$ nanosheets



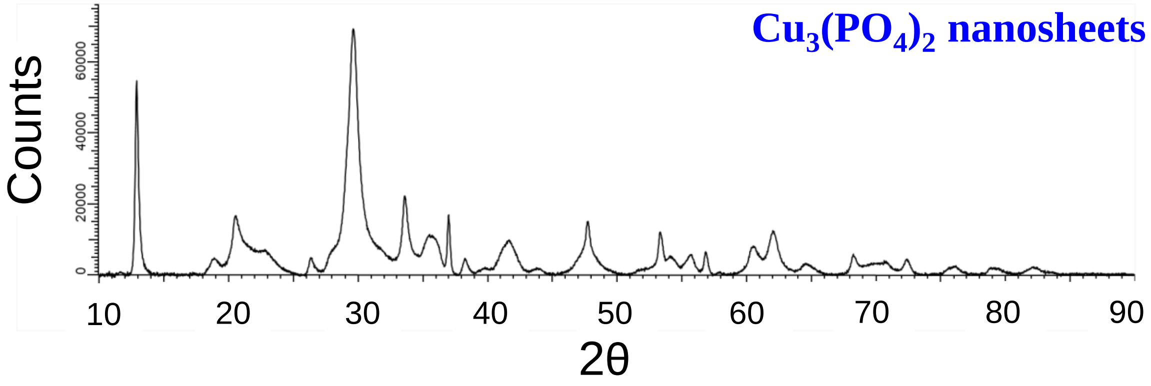
CuO NPs

Jaya Borgatta

X-ray diffraction analysis



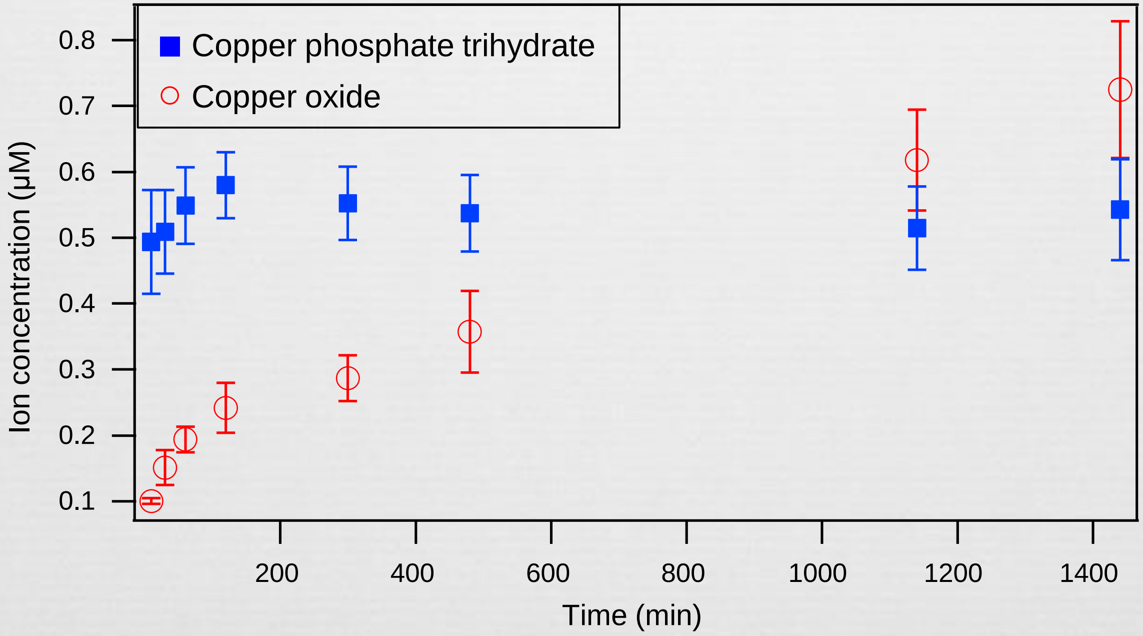
➢ The XRD pattern for copper oxide matches the pattern expected for CuO, tenorite.



➢ The XRD pattern for synthesized copper phosphate matches with $Cu_3(PO_4)_2 \cdot 3H_2O$, the broadening may represent some amorphous character.

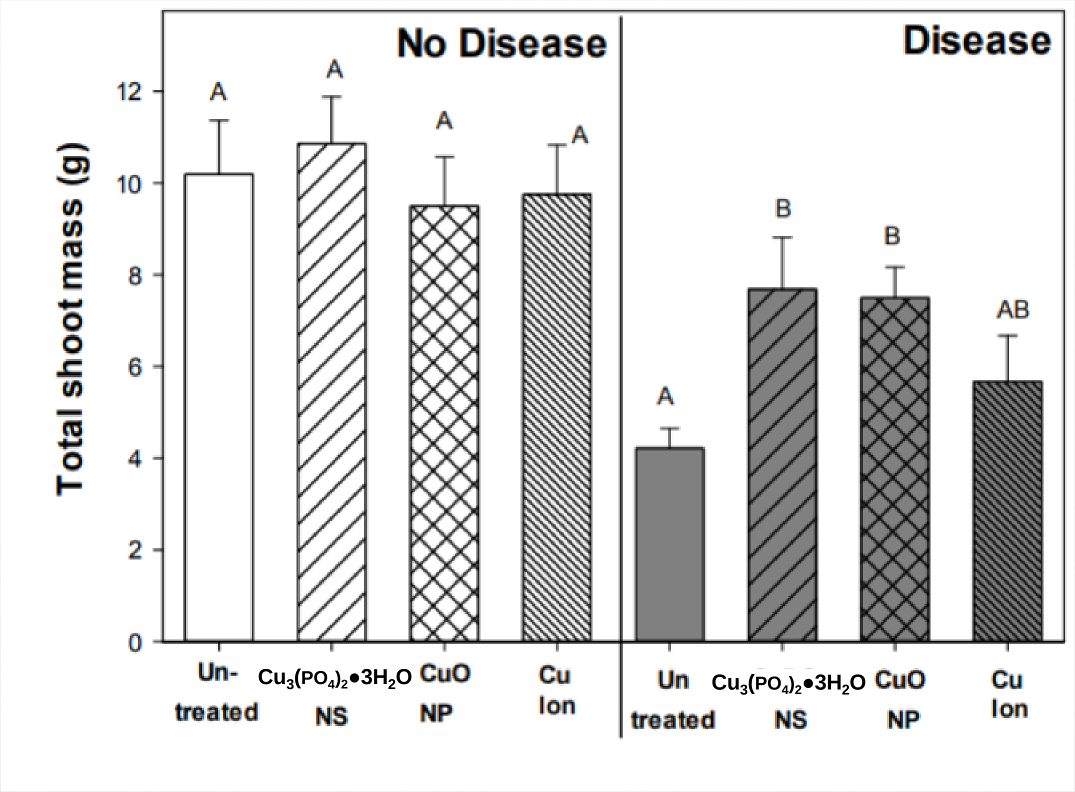
NM dissolution

- The dissolution experiments were performed in milliQ water, with 50 mg/L of particles;
- There is higher initial ion release from copper phosphate, followed by equilibration, while copper oxide appears to continue to dissolute.



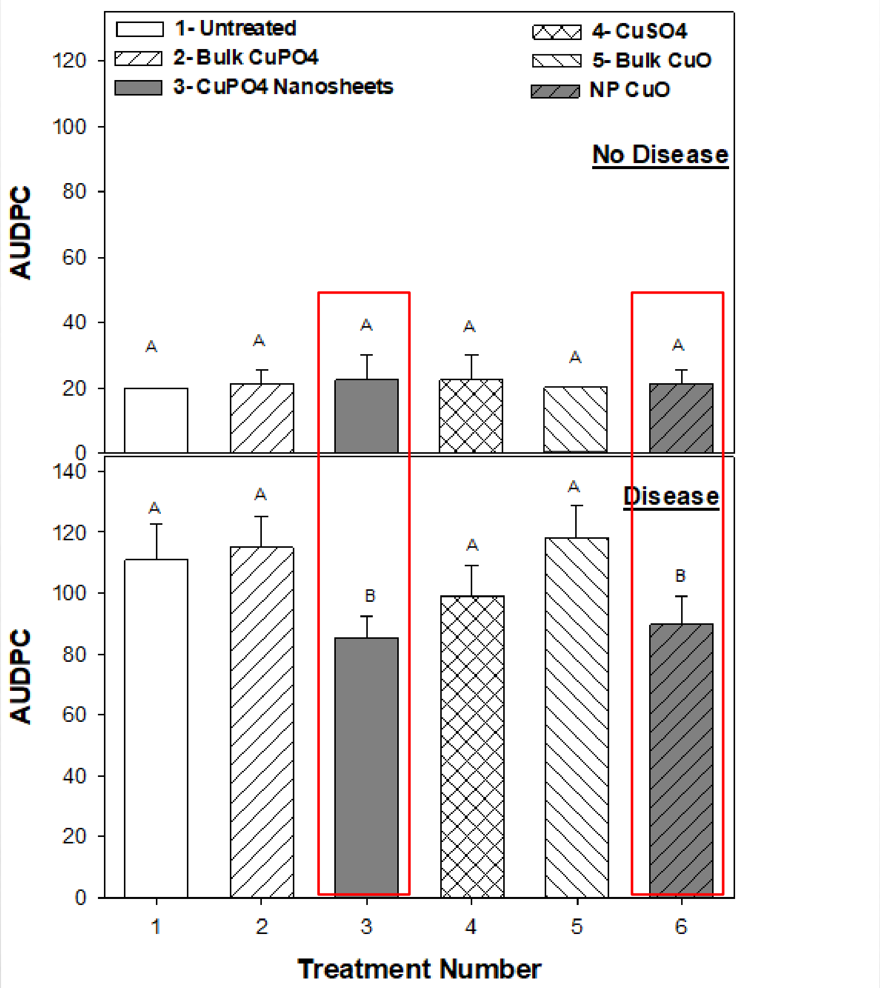
Fresh biomass as affected by NMs and *FON*

- Exposure to different forms of Cu has no impact on watermelon biomass as compared to the control;
- In the disease treatments, both Cu nanosheets and nanoparticles increase the shoot biomass by approximately 75% relative to the control.



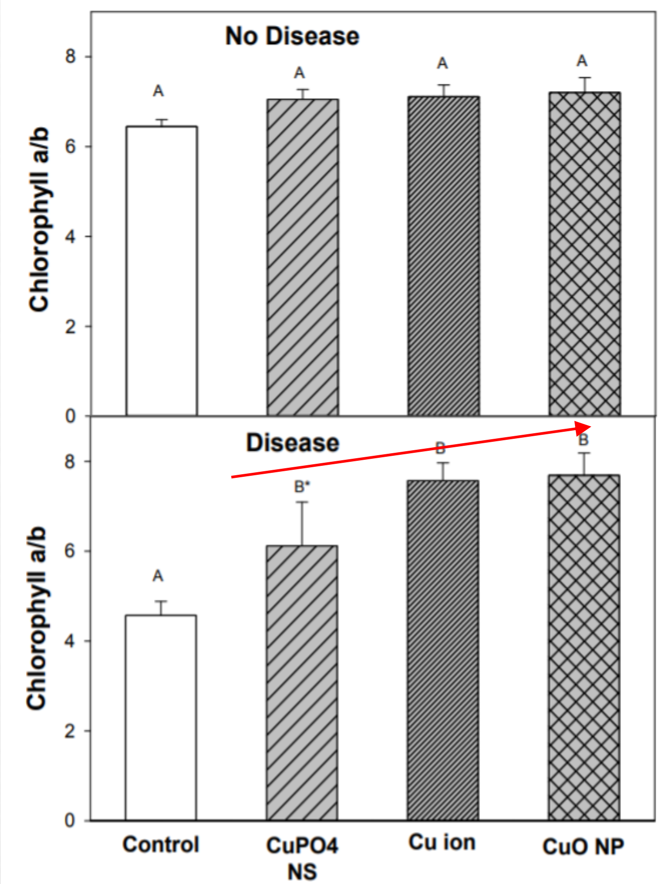
Disease rate assessment

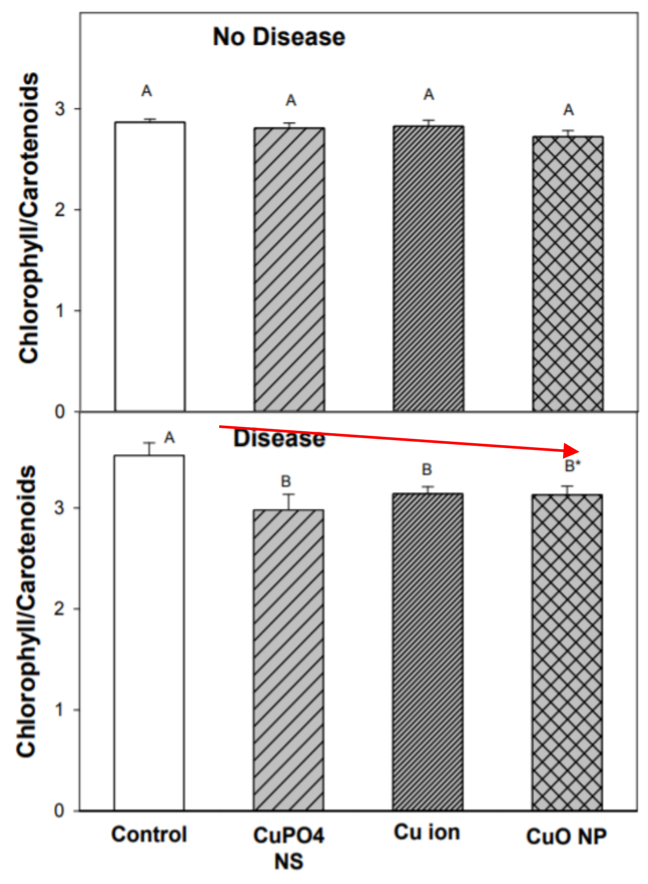
- AUDPC = area under the disease progression curve
- In the treatments with the absence of *FON*, no difference of AUDPC is observed;
- In the disease treatments, Cu nanosheets and nanoparticles significantly lower the reading of AUDPC by more than 20% relative to the control.



Pigment analysis

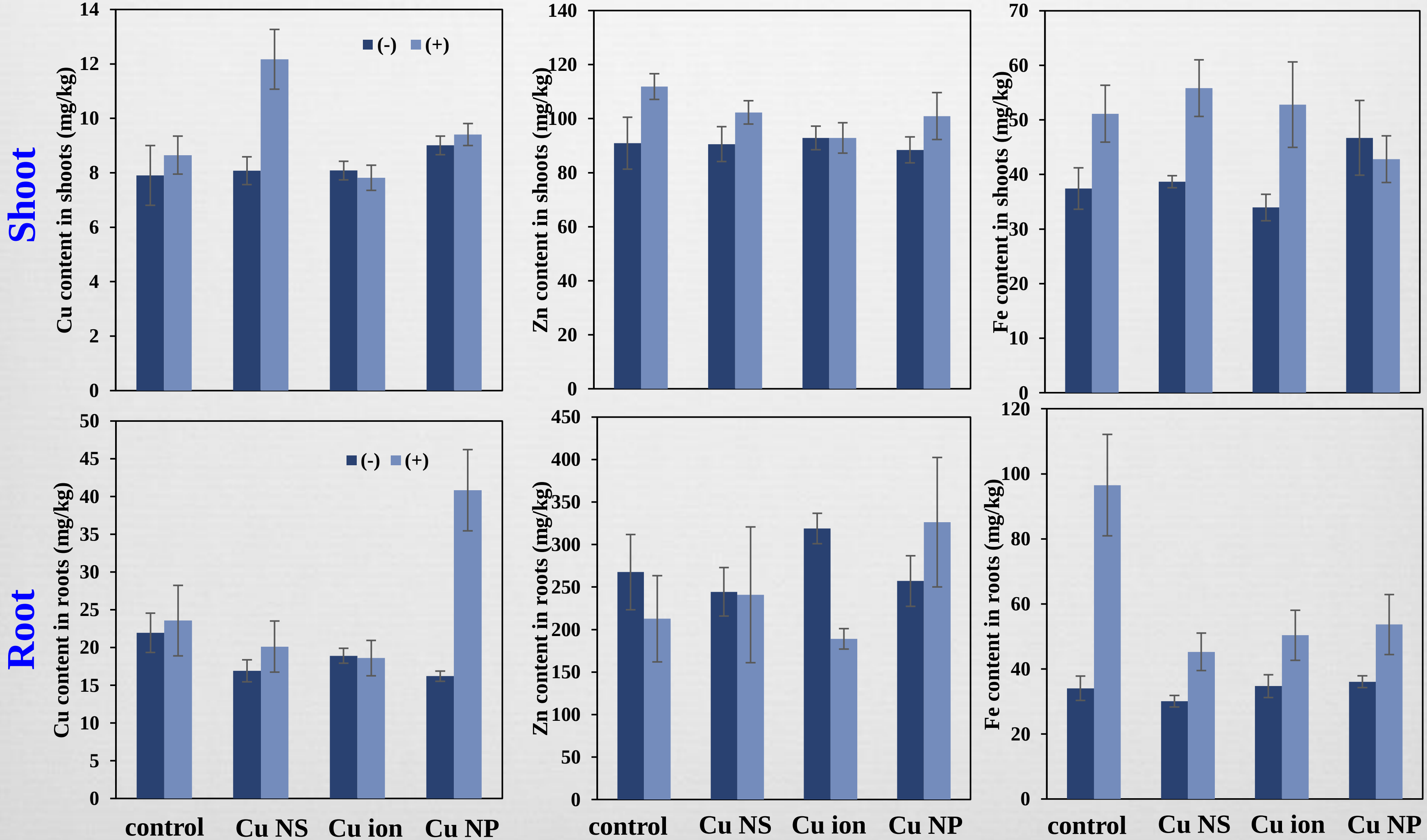
- The presence of NMs have no impact on the chlorophyll a/b ratio in the healthy watermelon; however, in the *FON* treatments, this ratio is significantly increased in all three Cu treatments, suggesting that Cu could reduce the *FON* induced biotic stresses;
- Similar results are evident in total chlorophyll/carotenoids ratio, indicating that the antioxidant defense mechanisms could be activated by Cu.





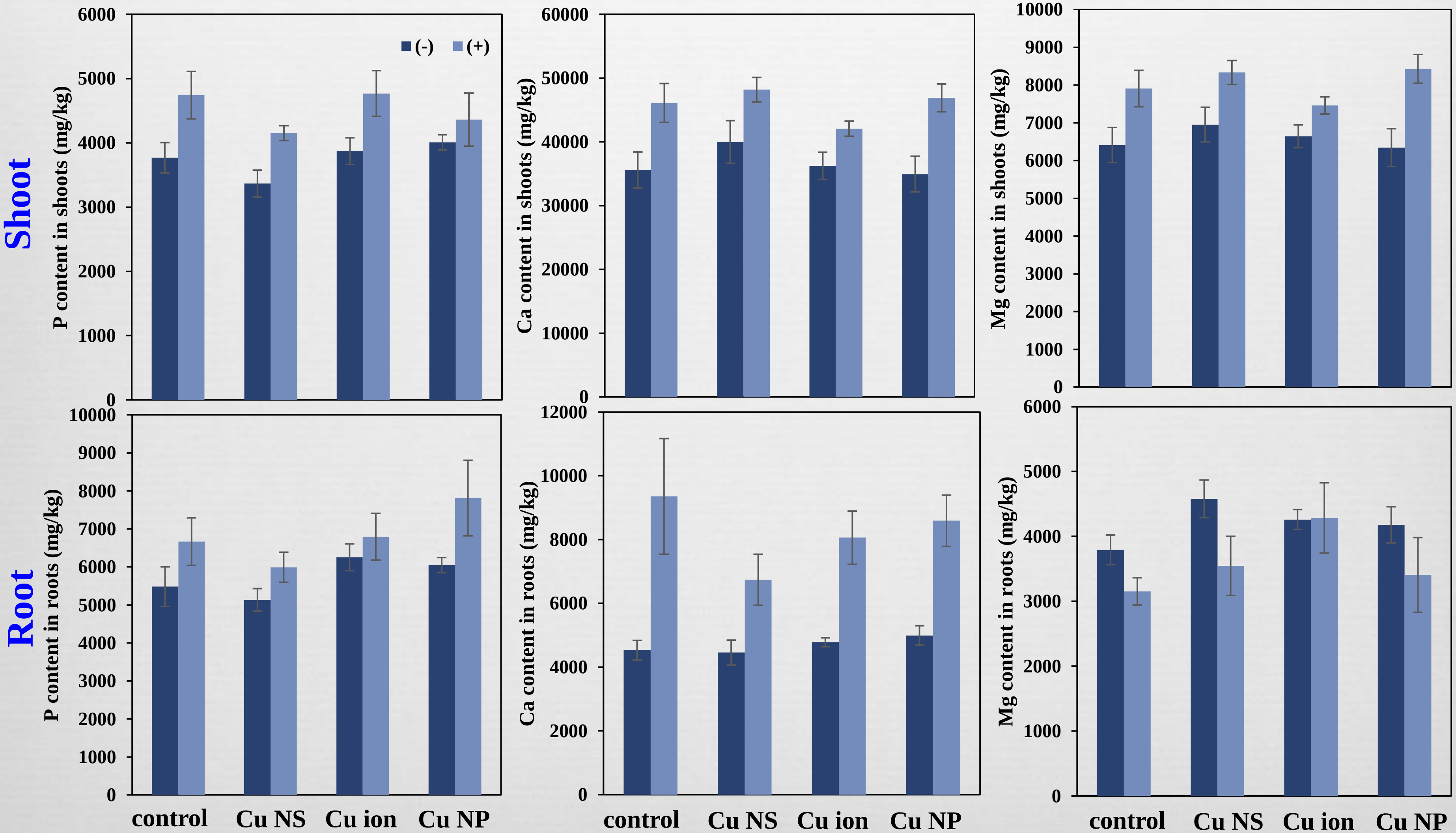
Micronutrient analysis

- Micronutrient levels can directly reflect whether stresses induced by NMs or *FON* occur in watermelon.
- Our preliminary results show that the micronutrient contents (Cu, Zn, and Fe) are not affected among all the Cu treatments. However, in the *FON* infested treatments, the Fe contents in both shoots and roots are notably higher as compared to the respective Cu alone treatment.



Macronutrient analysis

- Similar to the results of micronutrient contents, the presence of *FON* significantly alters the macronutrient contents in both shoots and roots;
- For example, the Ca contents in roots are increased by 44.4%-104% relative to the respective Cu alone treatment, but no difference is found among the Cu alone treatments.



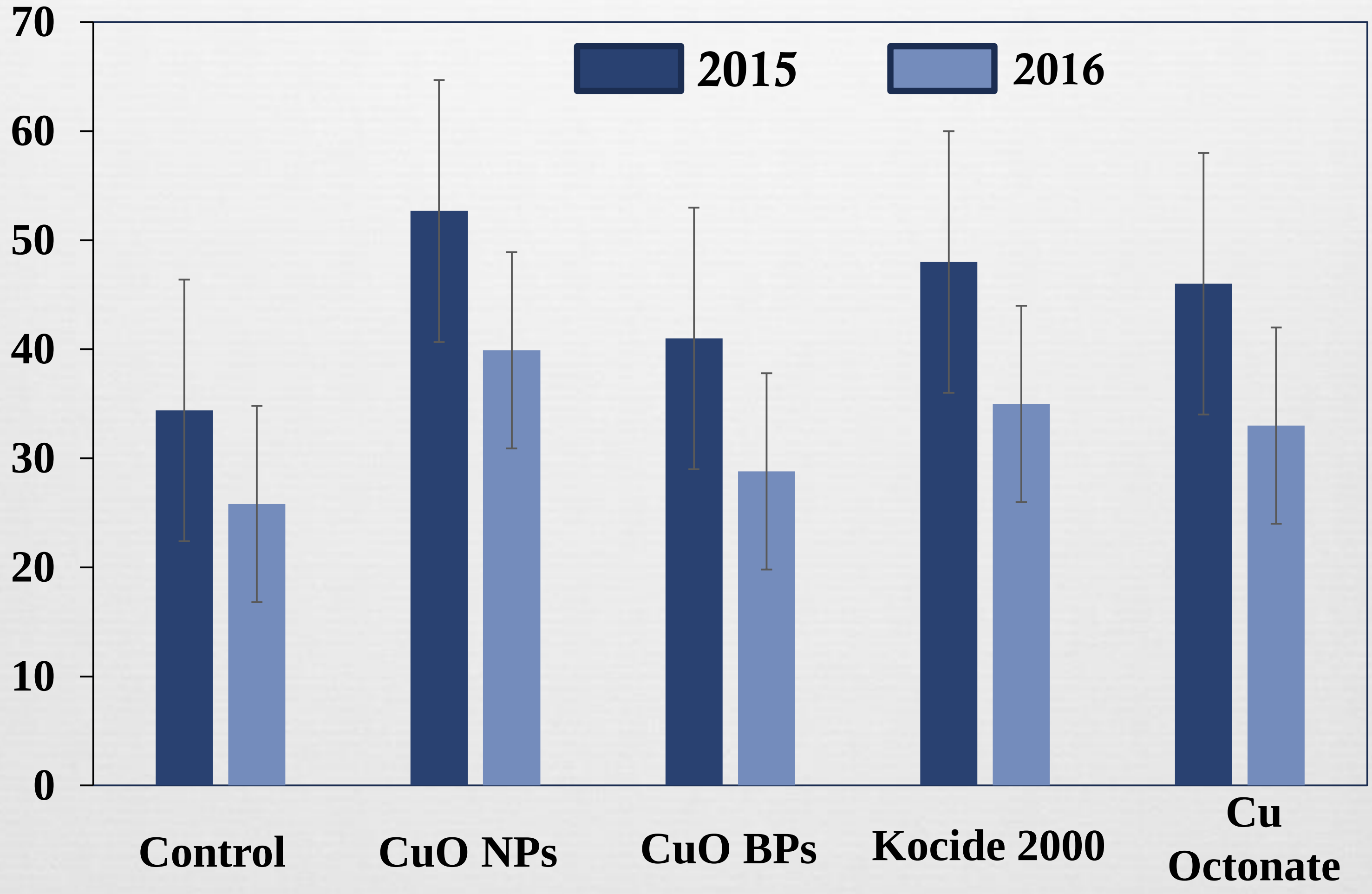
Field trials Hamden, CT

Effect of different sources of Cu

1. Control,
2. NP,
3. Bulked,
4. Kocide 2000
5. Cu Octonate

Applied once in the greenhouse at 400 µg/ml Cu; sprayed till run off.

Watermelon Yield (kg fruit/plot)



Conclusions

- Our preliminary results suggested that NM foliar application could enhance the plant growth in terms of aboveground biomass;
- Exposure to both nanosheets and nanoparticles significantly lowered the *FON* impact on watermelon;
- Pigment analysis showed that the presence of NMs could greatly alleviate the *FON* induced stresses in watermelon;
- Nutrient analysis seemed to suggest that mico- and macro-nutrient alteration in watermelon shoots and roots were resulted in by *FON* infection, not by NM exposure. Further study will be conducted to confirm our findings.

Acknowledgements

Advisors:

- Dr. Jason C. White
- Dr. Robert J. Hamers

Collaborators:

- Wade Elmer
- Christy Haynes

Lab members:

- Dr. Roberto DeLaTorreRoche
- Dr. Nubia Zuverza-Mena

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- Jaya Borgatta
- Natalie Hudson-Smith

Undergraduate students:

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- Eli Aliaga

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