

Nanoscale nutrients to suppress disease and increase crop production



Jason C. White, Ph.D.

Director

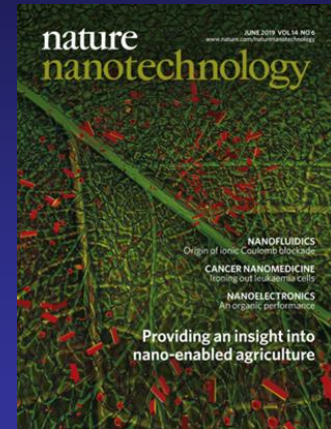
The Connecticut Agricultural Experiment Station, New Haven CT

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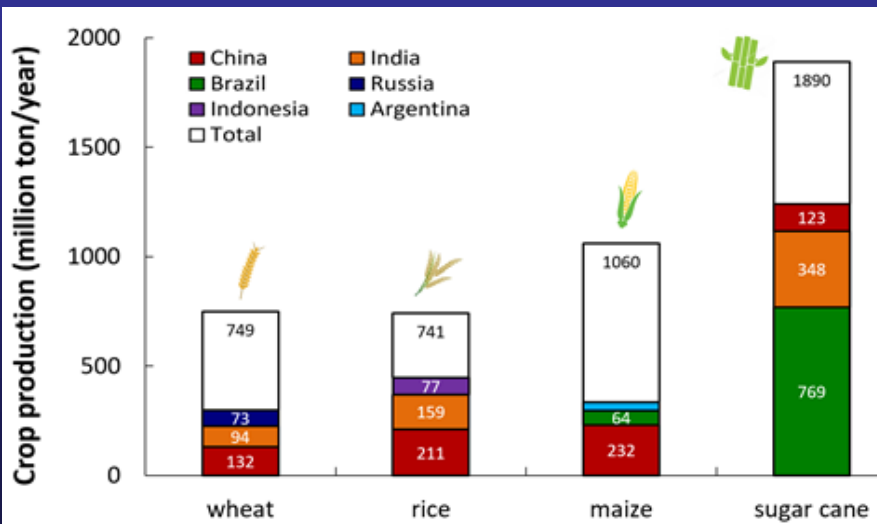
Agriculture: The Good and Bad

- Agricultural productivity has increased dramatically in the last 50 years (irrigation, agrichemicals). However, global agriculture is dominated by a small number of crops in a few countries.
- The rate of crop yield increase has declined since the 1980s.
- Poverty and hunger have decreased globally, but 800 million are chronically hungry; 2 billion suffer from micronutrient deficiencies.
- Agricultural systems in the much of the world have plateaued at 80% of yield potential; in more challenged areas, values may be 20%.
- The efficiency of agrichemical delivery is quite low; often below 25%

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Kah et al. 2019 *Nature Nano* 14:532-540.





Nanotechnology & Agriculture



➤ There has been significant interest in using nanotechnology in agriculture to:

- Increase production rates and yield
- Increase efficiency of resource utilization
- Minimize waste production

➤ Specific applications include:

- Nano-fertilizers, Nano-pesticides
- Nano-based treatment of agricultural waste
- Nanosensors

NANOTECHNOLOGY AND AGRICULTURE

Achieving food security through the very small

Nanotechnology could make agriculture more efficient and more sustainable, but more systematic understanding of the mechanisms involved is necessary to prove the potential of nano-enabled agrochemicals.

Jason C. White and Jorge Gardea-Torresdey

2018

NATURE NANOTECHNOLOGY | VOL 13 | AUGUST 2018 | 621-629 | www.nature.com/naturenanotechnology

Environmental Science Nano

2018

PAPER

View Article Online

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Environmental fate of nanopesticides: durability, sorption and photodegradation of nanoformulated clothianidin†

Melanie Kah, Helene Walch and Thilo Hofmann

REVIEW ARTICLE | INSIGHT

nature nanotechnology

Nano-enabled strategies to enhance crop nutrition and protection

Melanie Kah, Nathalie Tufenkji and Jason C. White

2019

Various nano-enabled strategies are proposed to improve crop production and meet the growing global demands for food, feed and fuel while practicing sustainable agriculture. After providing a brief overview of the challenges faced in the sector of crop nutrition and protection, this Review presents the possible applications of nanotechnology in this area. We also consider performance data from patents and unpublished sources so as to define the scope of what can be realistically achieved. In addition to being an industry with a narrow profit margin, agricultural businesses have inherent constraints that must be carefully considered and that include existing (or future) regulations, as well as public perception and acceptance. Directions are also identified to guide future research and establish objectives that promote the responsible and sustainable development of nanotechnology in the agri-business sector.

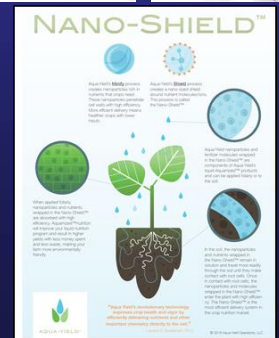
frontiers in Chemistry

2015

Nanopesticides and Nanofertilizers: Emerging Contaminants or Opportunities for Risk Mitigation?

Melanie Kah

Department of Environmental Geosciences, University of Vienna, Vienna, Austria



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Environmental Science Nano

2017

TUTORIAL REVIEW

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Nanotechnology for sustainable food production: promising opportunities and scientific challenges

Sónia M. Rodrigues, Philip Demokritou, Nick Dokoozlian, Christine Ogilvie Hendren, Barbara Karn, Meagan S. Mauter, Omowunmi A. Sadik, Maximilian Safarpour, Jason M. Unrine, Josh Viers, Paul Welle, Jason C. White, Mark R. Wiesner and Gregory V. Lowry

Environmental Science Nano

2019

CRITICAL REVIEW

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Recent advances in nano-enabled fertilizers and pesticides: a critical review of mechanisms of action

Ishaq O. Adisa, Venkata L. Reddy Pullagurala, Jose R. Peralta-Videa, Christian O. Dimkpa, Wade H. Elmer, Jorge L. Gardea-Torresdey and Jason C. White



Why Nano-Agriculture? Increasing Global Food Insecurity!!!

- Current estimates are that food production will need to increase by 70-100% by 2050 to sustain the population
- Negative pressure from a changing climate and a loss of arable soil
- And then there is COVID-19...
- Novel strategies and technologies are needed from “farm to fork” (and beyond) to sustainably solve the grand challenge of global food security
- Nanotechnology can and will play a significant role in this effort; particularly with the inefficiencies!!

CLIMATE CHANGE

Science Aug. 2018

Increase in crop losses to insect pests in a warming climate

Curtis A. Deutsch^{1,2,*†}, Joshua J. Tewksbury^{3,4,5,†}, Michelle Tigchelaar⁶, David S. Battisti⁶, Scott C. Merrill⁷, Raymond B. Huey², Rosamond L. Naylor⁸

Insect pests substantially reduce yields of three staple grains—rice, maize, and wheat—but models assessing the agricultural impacts of global warming rarely consider crop losses to insects. We use established relationships between temperature and the population growth and metabolic rates of insects to estimate how and where climate warming will augment losses of rice, maize, and wheat to insects. Global yield losses of these grains are projected to increase by 10 to 25% per degree of global mean surface warming. Crop losses will be most acute in areas where warming increases both population growth and metabolic rates of insects. These conditions are centered primarily in temperate regions, where most grain is produced.

ACS NANO

www.acsnano.org

EDITORIAL

At the Nexus of Food Security and Safety: Opportunities for Nanoscience and Nanotechnology

In a 2009 report, the United Nations Food and Agriculture Organization (UNFAO) presented the grand challenge “How to Feed the World in 2050”, as the number of people worldwide is estimated to grow to 9.1 billion.¹ This increase in population is largely centered in the developing world, and ensuring its food security is projected to require a 70% increase in food production. The needed increase in food production faces pressures from increasing urbanization and biofuel production and from climate change, which limit available land, water, biodiversity, and agriculture yield. In 2013, the UNFAO highlighted a “missed opportunity” in the quest for food security. One third of food produced (1.3 billion metric tons per year) is wasted in the supply chain; in the developing world, this is mostly due to poor quality, spoilage, and contamination, and in the developed world, this waste is largely

social policies and economic investment and, notably, new technologies.¹ Technologies are needed to enable sustainable and intelligent farming practices as the increased food production is forecasted to be achievable by increasing crop yield and intensity. These technologies must be accessible to the developing world where the increase in population will demand the greatest need for food. Improving the safety of food in the supply chain calls for new regulations and, again, new technologies. In the United States, the Food Safety and Modernization Act was signed into law in 2011, requiring greater surveillance in the food supply chain and a shift from response to prevention.² The Act calls for technologies to increase capabilities to detect and to respond to problems sooner and more effectively. Food packaging is integral to both reducing food waste and increasing food safety. Today, we rely

Opinion: To feed the world in 2050 will require a global revolution

Paul R. Ehrlich^{*,1} and John Harte^{*,1}

^{*}Department of Biology, Stanford University, Stanford, CA 94305; and ¹Energy and Resources Group, University of California, Berkeley, CA 94720

Achieving universal food security is a staggering challenge, especially in a world with an (and especially in combination) impede attempts to achieve progressive and effective policies

feed humanity makes the prospects seem slim for making the projected 9.7 billion population food-secure and healthy in 2050, and perhaps billions more beyond that (5).

Major Challenges

Humanity now faces severe biophysical con-

HOW TO FEED THE WORLD 2050

HIGH-LEVEL EXPERT FORUM

Rome 12-13 October 2009

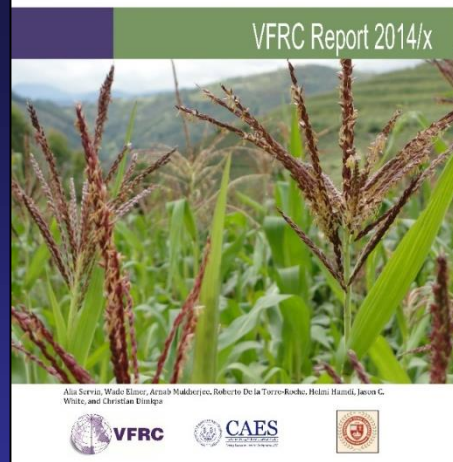


World Summit on Food Security



Nanoscale Nutrients and Root Disease

- In 2014, we began working on soil borne diseases; difficult to manage and reduce crop yields by 20%
- Fungal pathogens reduce US annual economic return by \$200 million; \$600 million on control
- Many micronutrients (Cu, Mn, Zn, Mg, B, Si...) stimulate or are part of plant defense systems
- However, these nutrients have limited availability in soil and limited efficacy when foliarly applied
- What about “nanoscale” nutrients? Will they be more effective at enhancing nutrition/suppressing disease? **Note- No direct toxicity to the pathogen**



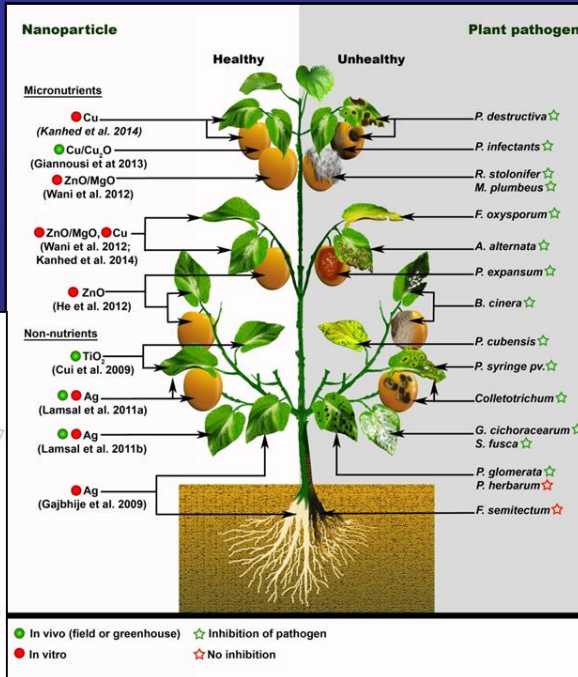
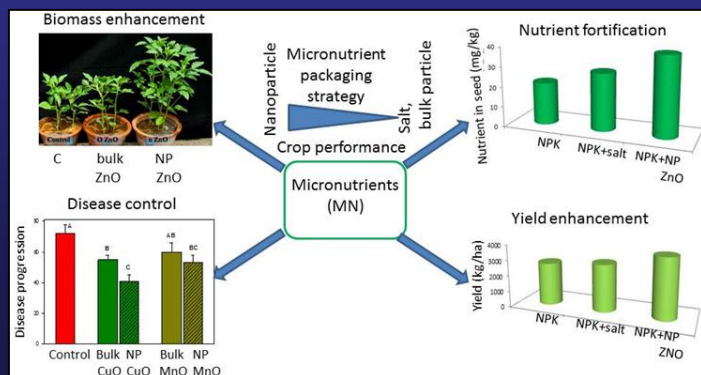
J Nanopart Res (2015) 17:92
DOI 10.1007/s11051-015-2907-7

REVIEW

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

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

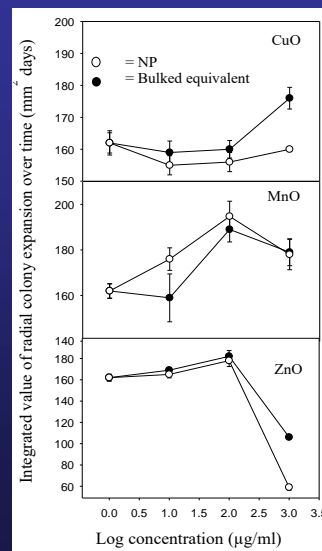
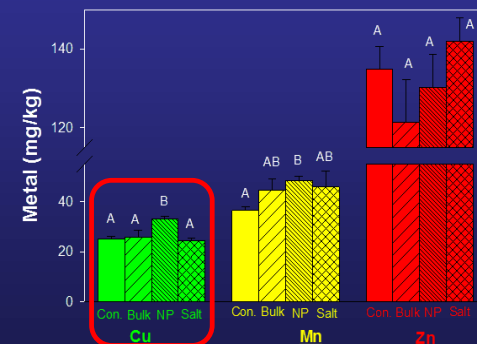
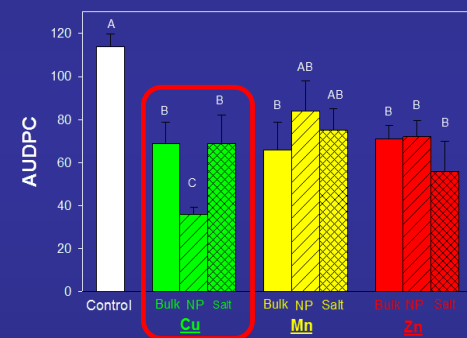
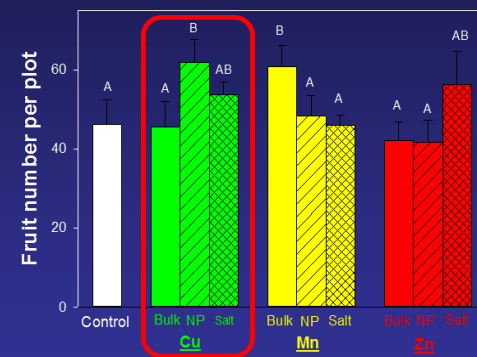
Servin et al. 2015. J. Nano. Res. 17:92.



Nanoscale Micronutrients for Disease Suppression

- 2014-2015- Greenhouse and field trials with eggplant and tomato; commercial NPs
- Single foliar application of NP (bulk, salt) **CuO**, **MnO**, or **ZnO** (100 mg/L; 1-2 mL treatment) to seedling; transplant to infested soil
- NP CuO had increased yield, greater disease suppression, and higher Cu root content. NP CuO had no direct toxicity on the pathogen

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



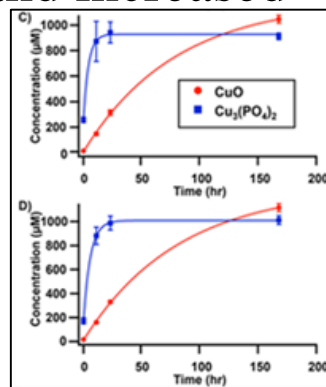
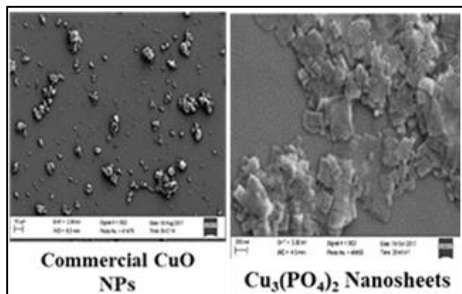


CAES Publications- 2020

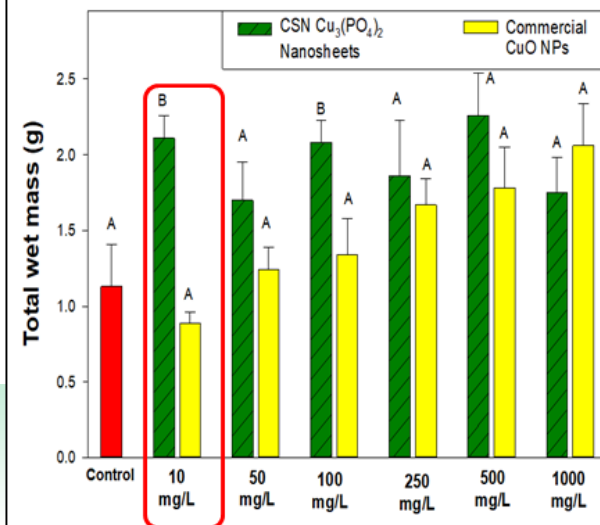
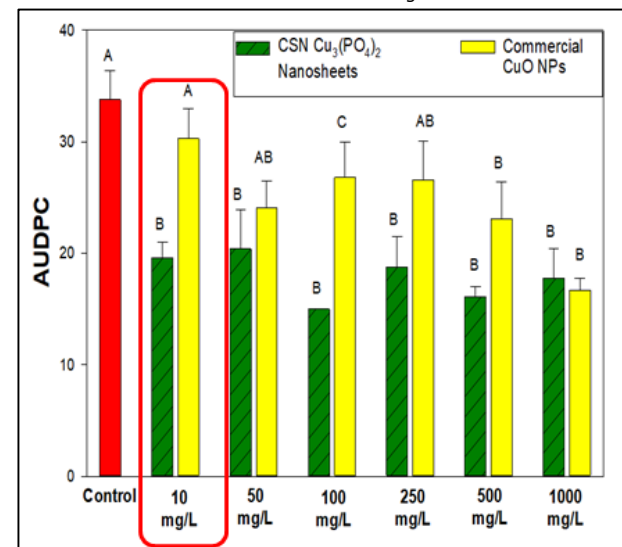


- Ma et al. 2020. Advanced material modulation of plant nutritional and phytohormone status suppresses soybean sudden death syndrome (SDS) and increases yield. *Nature Nanotechnol.* In press
- Hofmann et al. 2020. Moving forward responsibly in nanotechnology enabled plant agriculture. *Nature Food* 1:416–425
- De La Torre-Roche et al. 2020. Seed biofortification by engineered nanomaterials: A pathway to alleviate malnutrition? *J. Agric. Food Chem.* In press
- Shen et al. 2020. Copper nanomaterial morphology and composition control foliar transfer through the cuticle and mediate resistance to root fungal disease in tomato (*Solanum lycopersicum*). *J. Agric. Food Chem.* In press
- Salinas et al. 2020. Effects of engineered lignin-graft-PLGA and zein-based nanoparticles on soybean health. *Environ. Sci. Technol.* Submitted
- Song et al. 2020. Metabolic profile and physiological response of cucumber exposed to engineered MoS₂ and TiO₂ nanoparticles. *NanoImpact* Submitted
- Adeel et al. 2020. Carbon-based nanomaterial function suppress Tobacco Mosaic Virus (TMV) infection and induce resistance in *Nicotiana benthamiana*. *Small* Submitted
- Elmer et al. 2020. Influence of single and combined mixtures of metal oxide nanoparticles on eggplant growth, yield, and Verticillium wilt severity. *Plant Disease* In press
- **Shang et al. 2020. Copper sulfide nanoparticles suppress *Gibberella fujikuroi* infection in *Oryza sativa* seeds by multiple mechanisms: contact-mortality, nutritional modulation and phytohormone regulation. *Environ. Sci.: Nano* 7:2632-2643**
- An et al. 2020. Molecular mechanisms of plant salinity stress tolerance improvement by seed priming with cerium oxide nanoparticles. *Environ. Sci: Nano*. <https://doi.org/10.1039/D0EN00387E>
- **Xu et al. 2020. Enhancing agrichemical delivery and seedling development with biodegradable, tunable, biopolymer-based nanofiber seed coatings. *ACS Sus. Chem. Eng* 8, 25, 9537–9548**
- Dimkpa et al. 2020. Interactive effects of drought, organic matter, and zinc oxide nanoscale and bulk particles on wheat performance and grain nutrient accumulation. *Sci. Total Environ.* 722:137808
- Dimkpa et al. 2020. Facile coating of urea with low-dose ZnO nanoparticles promotes wheat performance and enhances Zn uptake under drought stress. *Front. Plant Sci.* 11:168

- Commercial CuO NPs vs $\text{Cu}_3(\text{PO}_4)_2$ nanosheets (NS) from the Center for Sustainable Nanotechnology (NSF CCI)
- Differences in morphology and composition lead to differences in dissolution
- Materials were foliar applied to watermelon grown in *Fusarium* infested soils (greenhouse, field)
- $\text{Cu}_3(\text{PO}_4)_2$ NS promote growth and inhibit disease more effectively than CuO NPs
- In the field, NS suppressed disease and increased yield at **10-fold lower dose**
- Effective management of risk!



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Borgatta et al. 2018. ACS Sustain. Chem. Eng. 6:14847-14856.



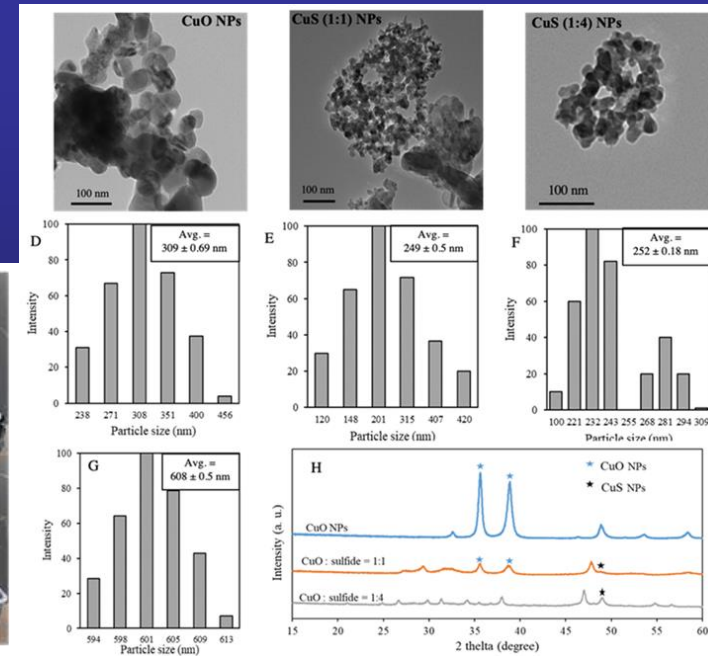
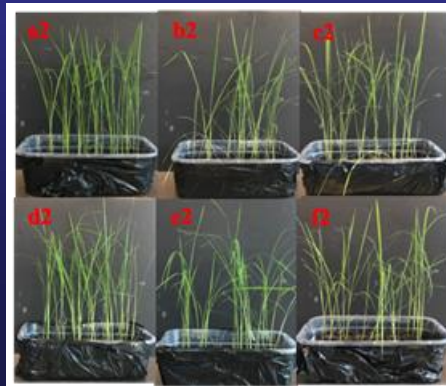


Shang et al. 2020



- Conducted with the University of Massachusetts Stockbridge School of Agriculture and the Hebrew University of Jerusalem
- Copper sulfide nanoparticles (CuS NPs) were synthesized at 1:1 and 1:4 ratios of Cu and S and antifungal efficacy was evaluated against *Gibberella fujikuroi* (Bakanae disease) in rice (*Oryza sativa*).
- A greenhouse study with rice seedlings that had been treated with 50 mg/L Cu-based NPs via seed exposure or foliar application (2 applications over 4 days) was conducted; CuO NP and Kocide 3000 were included for comparison.
- Measured endpoints after 21d included disease incidence, biomass, tissue element content and content of plant-defense related phytohormones

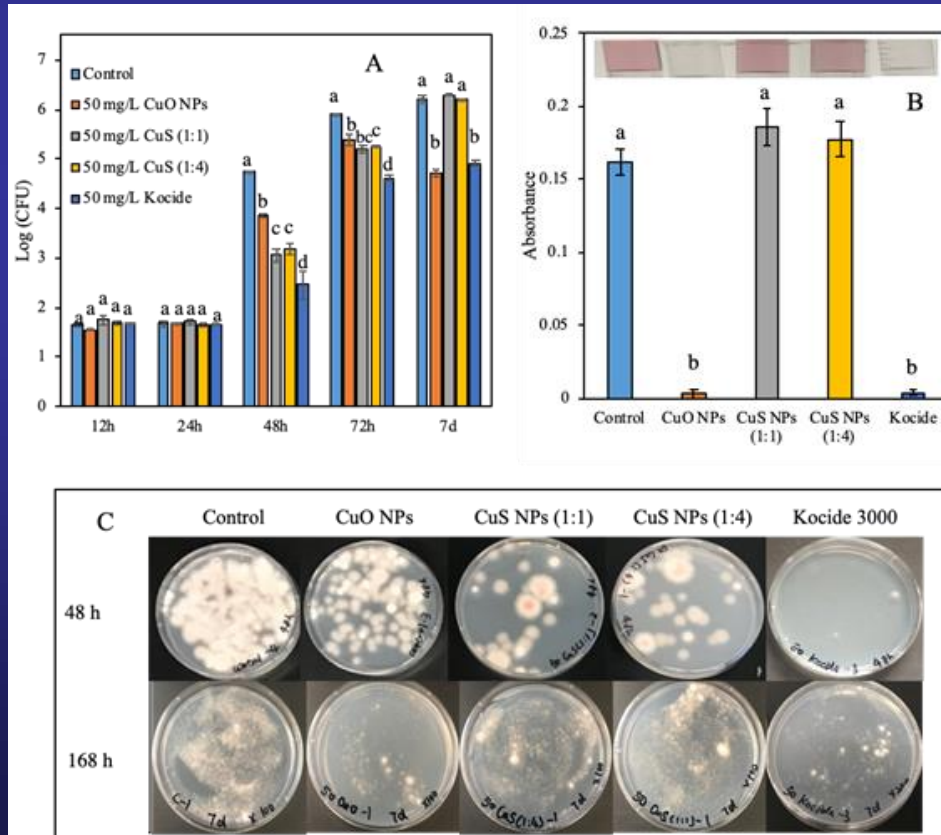
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In vitro Toxicity of CuS NPs

- An in vitro assay was done with 50 mg/L CuS or CuO NPs for 7 days (Kocide control).
- No difference was observed in the first 24 hours across all treatments (A).
- At 48 h, CuS (1:1) NPs decreased the CFU by 35.7 and 20.8% and CuS (1:4) NPs decreased the CFU by 33 and 17.6%, relative to the control and CuO NPs, respectively. Similar trends were evident at 72 hours.
- The antifungal activity of CuO NPs was significantly less than CuS NPs in the first 72 h, but that pattern was reversed at 168 h (A, B), at which point the CuS NP treatments were equivalent to the untreated control.
- Kocide 3000 exhibited the highest antifungal efficiency during the experiment.
- The pattern of dehydrogenase activity of *G. fujikuroi* at 168 hours across all treatments aligned with the fungal growth data (C).

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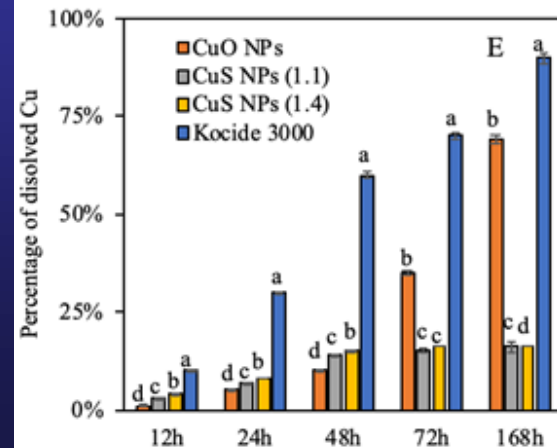
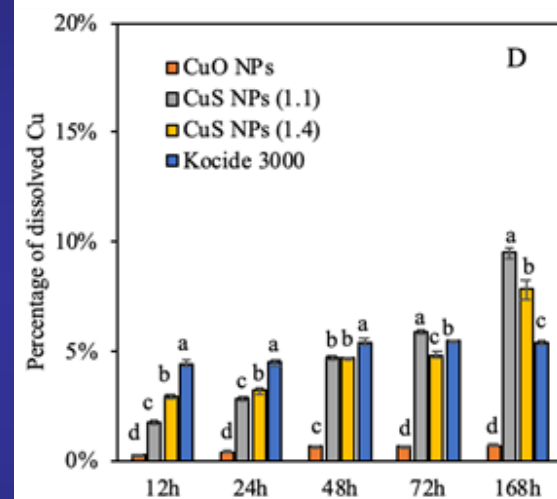


Cu NP Dissolution



- In water, Cu dissolution from CuO NP rose slowly from 0.27% to 0.64% within 48 hours and became stable afterwards.
- For CuS (1:1) and CuS (1:4) NPs, dissolution during 7d increased from 1.77% to 9.48% and from 2.89% to 7.82%, respectively (several-fold higher than CuO NPs [Figure D]).
- For Kocide 3000, Cu release was rapid in the first 12 hours but was then stable after at 5%.
- Cu dissolution kinetics were different in the PDB medium (Figure E). Both CuS NPs displayed significantly greater dissolution (14-15%) than did CuO NPs (10%) in the first 48 hours.
- However, at 72 hours, Cu release from the CuO NPs had increased to 35%, which was two-fold higher than the CuS.
- This reversed pattern continued to 168 hours, where 69.3% of total CuO NPs were dissolved (3-fold > CuS).
- This different pattern of dissolution in the two media was largely a function lower pH and organic ligands in the PDB, making dissolution more thermodynamically favorable.
- Release of Cu ions is likely critical role to antifungal activity.

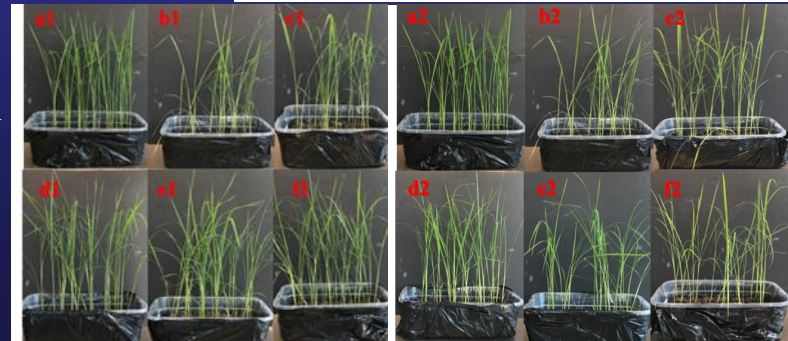
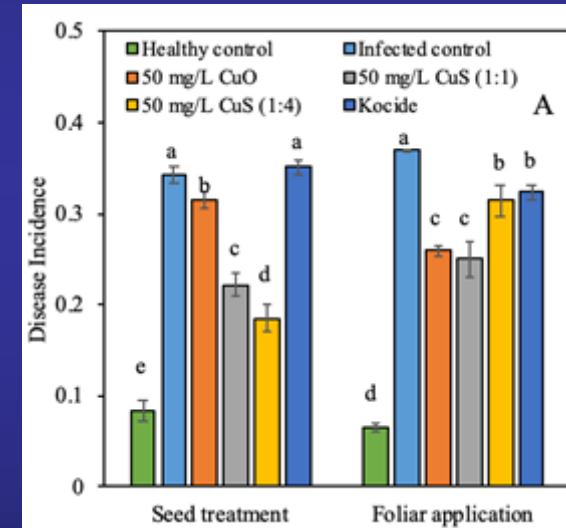
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Greenhouse study- 21 Days

- In the seed treatment, NP CuO, CuS (1:1) and CuS (1:4) significantly decreased disease incidence by 8.1%, 35.1, 45.9%, respectively, as compared to the diseased control (A).
- The findings demonstrate that the three types of Cu-based NPs significantly inhibited invasion by *G. fujikuroi*, although the two sulfide forms were most effective.
- Kocide 3000 (same [Cu]) did not impact disease incidence, although it is not intended for seed treatment.
- For foliar application, CuO NPs and CuS (1:1) NPs significantly decreased the disease incidence to the greatest extent; CuS (1:4) NPs and Kocide 3000 were less effective but still significantly reduced disease by incidence by 30 and 32.5%, respectively (A).
- These findings suggest that both seed treatment and foliar application of 50 mg/L Cu-based NPs can significantly reduce the severity of Bakanae disease, with efficacy that is significantly greater than Kocide 3000.
- The sulfide is more effective, which may be a function of particle size, Cu release, or S, which is involved in plant secondary metabolism and stress tolerance

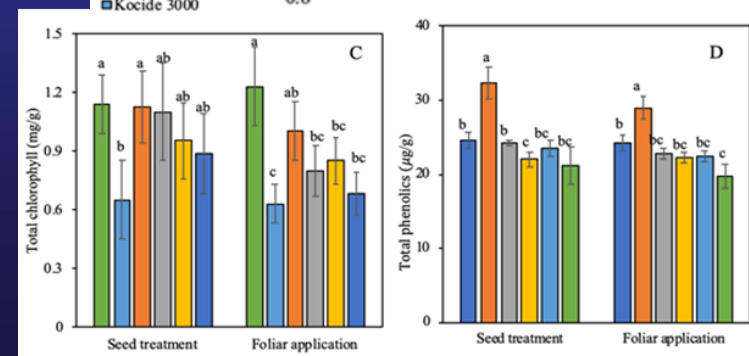
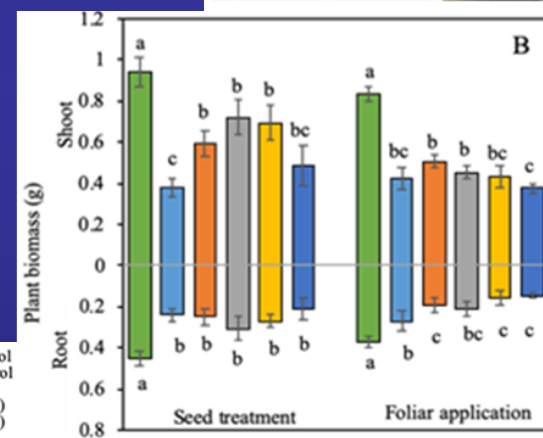
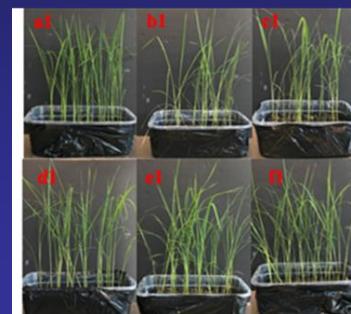
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Greenhouse study-21 Days

- In the seed treatment, biomass increased 56.9-89.6% for the Cu-based NPs (B).
- Kocide 3000 had no impact on the biomass of *G. fujikuroi*-infected plants
- For foliar application, infected rice shoot and root biomass were not impacted by the Cu-based NP treatment.
- The chlorophyll content in the CuO, CuS (1:1) and CuS (1:4) NPs-treated rice seed was increased by 72, 68 and 46%, respectively (C)(equal to healthy controls)
- Foliar exposure of Cu-based NPs to *G. fujikuroi* infected rice showed the similar but lesser pattern in total chlorophyll content.
- Phenolic compounds are important secondary plant metabolites and are involved in biotic stress-induced defense systems in plants.
- The seed/foliar treatment with Cu-based NPs significantly reduced the total phenolic content to that disease-free control levels (D).

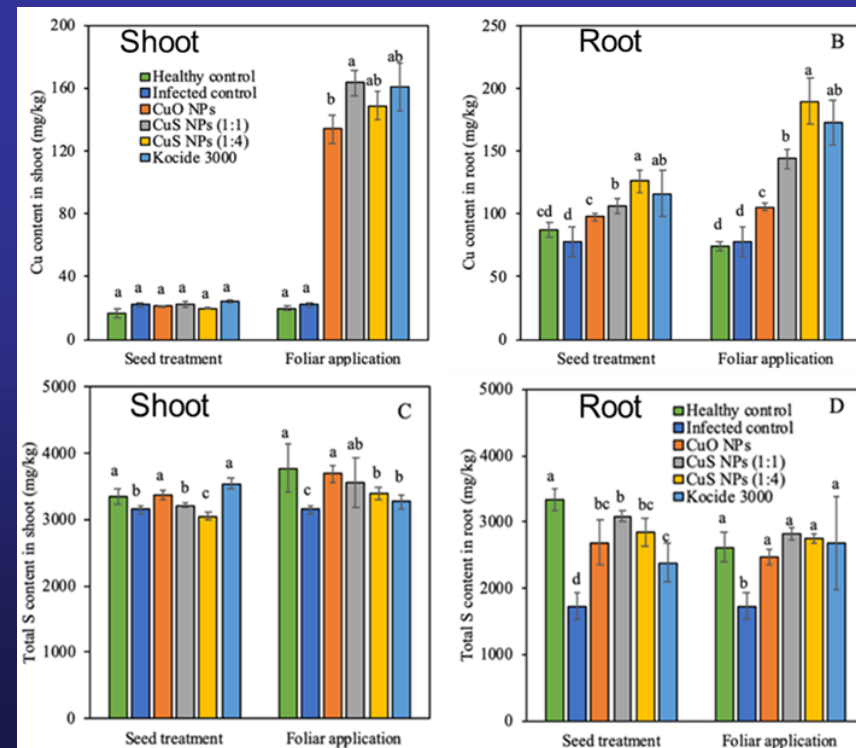
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Tissue Cu and S content

- In the seed treatment, Cu exposure did not significantly change the shoot Cu content (A)
- Cu-based particles increased the root Cu content by 25.7-62.9%. Importantly, the CuS (1:4) NPs delivered more Cu to the roots than the other particles (B).
- For foliar treatment, shoot Cu levels were increased in all NP treatments (A, B).
- CuS NPs transferred significantly more (36.4%) Cu to the roots (B).
- Increased Cu levels in NP-treated rice could suppress *Bakanae* disease directly by contact-killing and/or indirectly by stimulating plant defense and secondary metabolism.
- There was little difference in the shoot S content as a function of either nanoscale material exposure route or material type (C).
- In roots, all Cu-based NPs increased S content as compared to the diseased control (D).

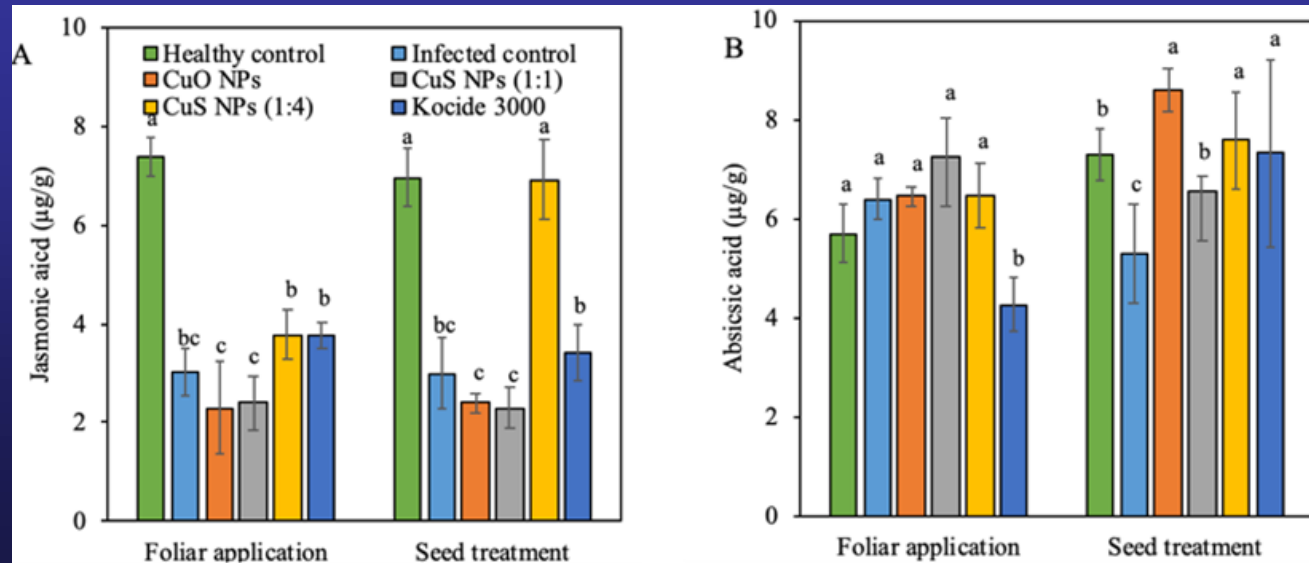
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Shoot phytohormone content

- Phytohormones are a critical to plant defense and response to biotic stress
- Foliar application of Cu NPs did not affect JA content in the infected shoots but did seem to increase ABA content (B), although differences were not statistically significant.
- In the seed treatment, CuS (1:4) NPs significantly increased JA and ABA content to levels of the healthy control (A)
- Our results suggest that select Cu-based NPs could increase the JA/ABA level in rice and further trigger rice defense systems

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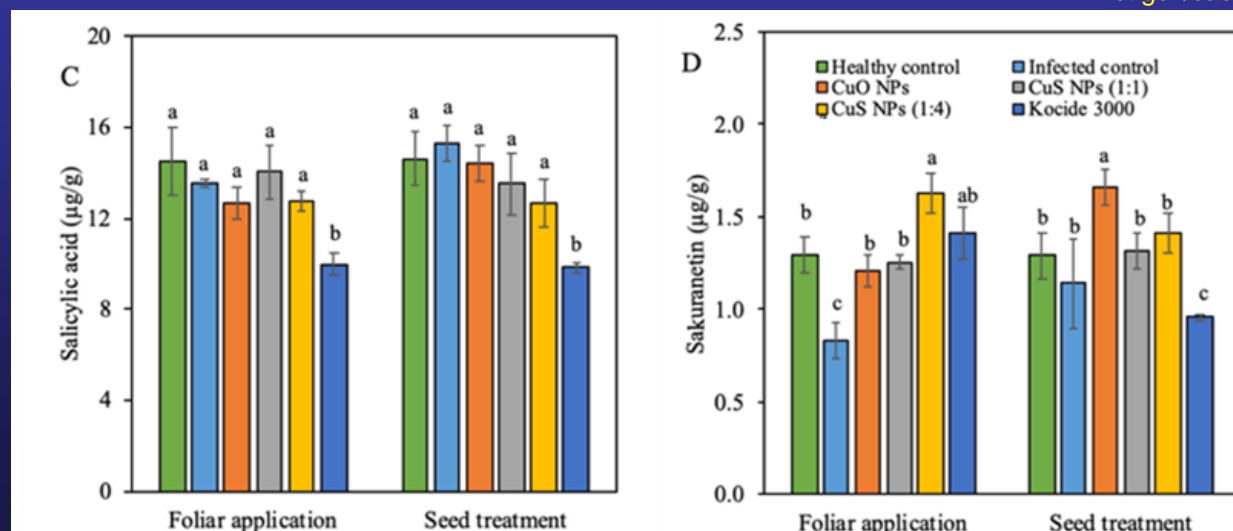


Shoot phytohormone content



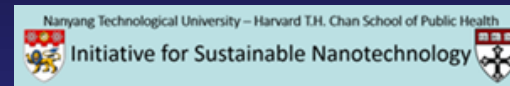
- Cu-based NP treatments had no impact on SA content (C); Kocide decreased SA content via both application routes
- Foliar exposure to all Cu-based particles significantly increased the SN content
- In particular, exposure to CuS (1:4) NPs increased the SN level by 96.4% relative to the diseased control.
- For the seed treatment, CuO NPs significantly increased the SN content over the diseased control
- It appears that nanoscale Cu-increased SN levels can contribute positively to defense-related systems in plants

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Xu et al. 2020

➤ Conducted as part of the Nanyang Technological University-Harvard University T.H. Chan School of Public Health Initiative for Sustainable Nanotechnology (NTU-HARVARD SUSNANO)

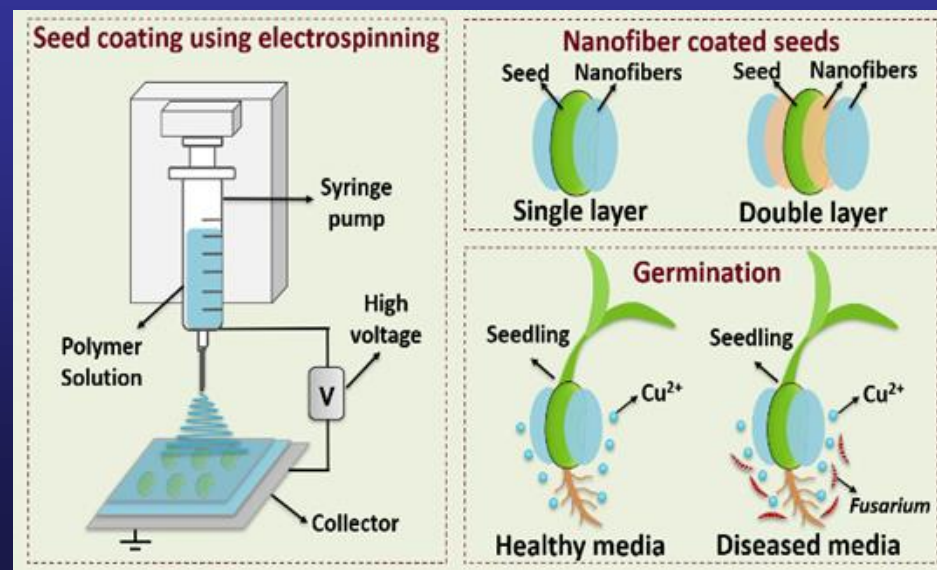


➤ Seed treatments have been used to deliver certain critical protective agents that promote seed storage and germination/seedling growth.

➤ However, current platforms are limited in terms of efficacy and versatility

➤ We developed a scalable, biodegradable, sustainable, “green” (non-toxic), biopolymer-based nanoplatform using electrospinning which can be used as a seed coating to enhance targeted and precision delivery of agrichemicals

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- Cellulose acetate/gelatin-derived electrospun nanofibers were synthesized that are bead free and of desired morphology/thickness, mechanical properties, and surface wettability
- The morphology of different electrospun Cu^{2+} loaded nanofibers and their diameter distribution ($n=50$) is shown below.
 - (a-b) CA/gelatin ratio of (a) 75/25 and (b) 50/50, without surfactant;
 - (c-d) CA/gelatin ratio of (c) 75/25 and (d) 50/50, with surfactant.
- The insert of the left of each image shows the freestanding electrospun nanofiber membranes ($2\text{ cm} \times 2\text{ cm}$).

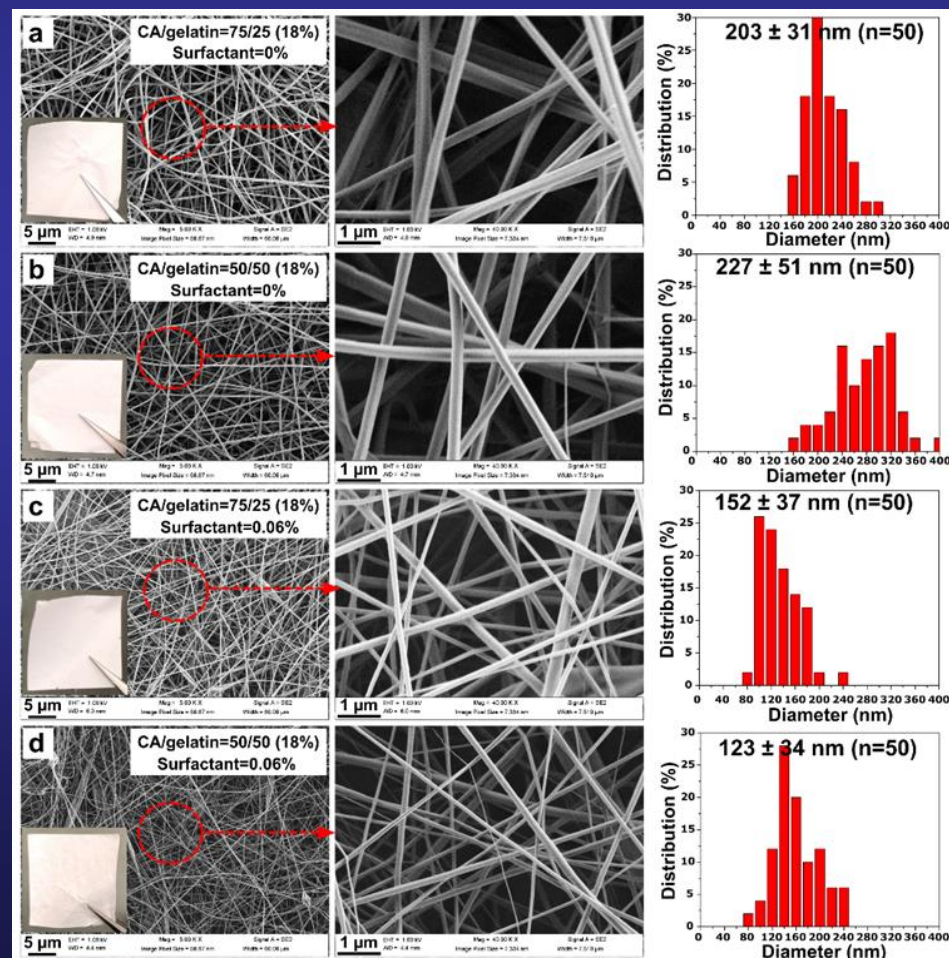


Table S1. Microstructure of electrospun Cu^{2+} loaded nanofibers.

	BET multipoint Specific Surface Area (m^2/g)	Average Pore Size (nm)	Total Pore Volume (cc/g)
CA/gelatin=50/50, surfactant=0.06%	18.12	4.23	0.019
CA/gelatin=75/25, surfactant=0%	18.59	5.32	0.025

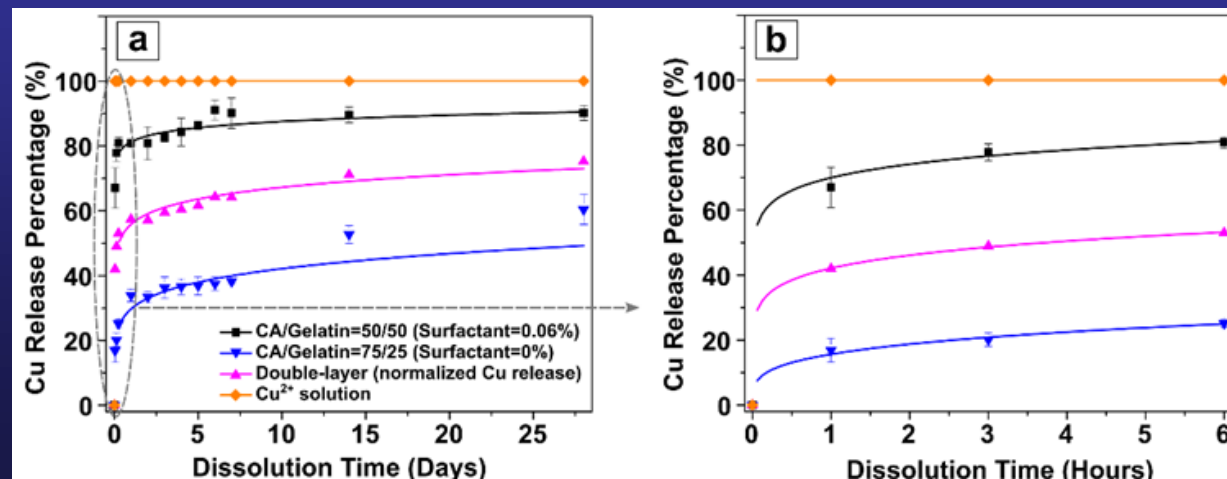


Cu Release: Tunability



- The Cu^{2+} release kinetics of different nanofibers and ionic control were measured
- “Fast” release is CA/gelatin=50/50, surfactant=0.06%; “Slow” release is CA/gelatin=75/25, surfactant=0%
- “Double-layer” is slow release on the inside of the fiber and fast release on the outside of the fiber
- The “fast” release nanofiber showed a burst of Cu^{2+} release due to the surfactant (increased wettability), with about 80% release in 3 h and 90% release at 28 d.
- The “slow” release nanofiber has a reduced gelatin ratio and no surfactant; only 20% Cu^{2+} was released in the first 3 hours, with a gradual release to 50% at 28 days.
- It important to develop a versatile biopolymer-based nanoplatform with tunable agricultural release kinetics, which can then be adapted for different types of seed

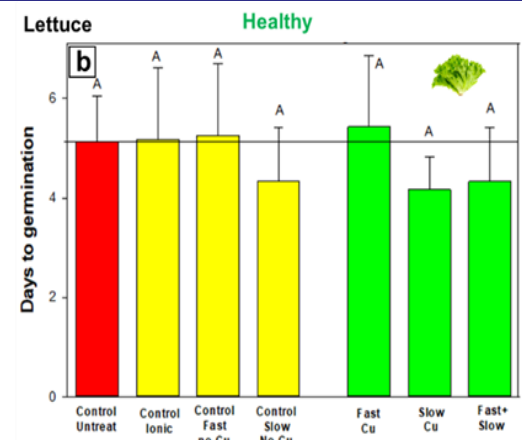
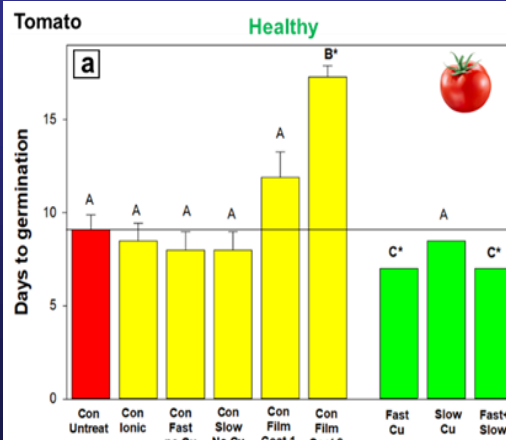
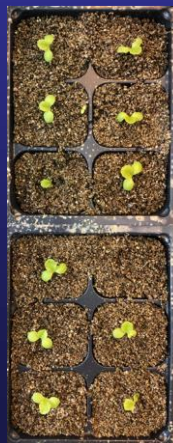
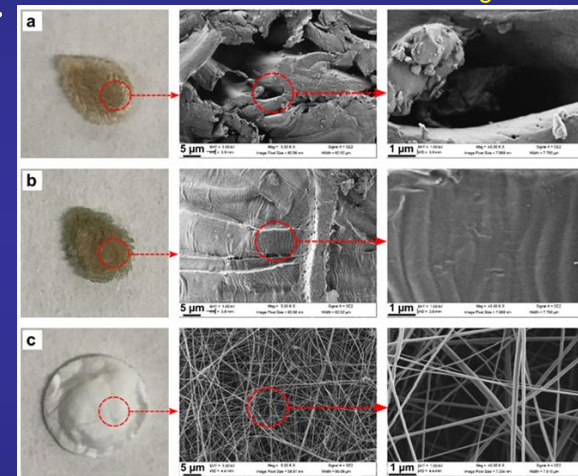
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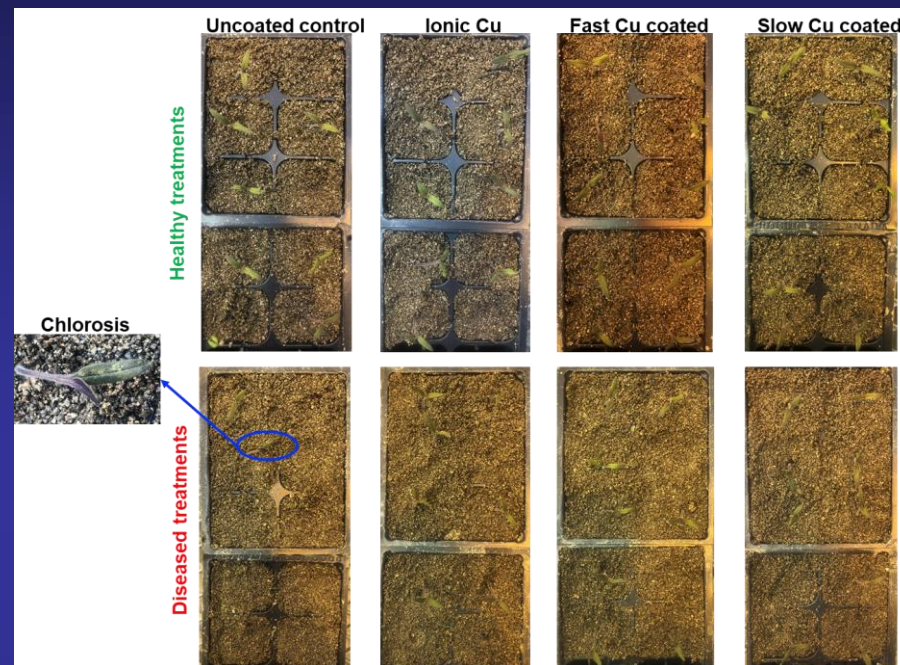
Time to Germination- Healthy

- Tomato and lettuce seeds coated with “fast,” “slow,” and “fast + slow” Cu release nanofibers, as well as ionic Cu and Cu-free nanofiber, and traditional film-coated controls were germinated
- For healthy tomato, the number of days to germination was decreased by 22% for the “fast” and “fast + slow” coated seeds (a).
- Ionic Cu and Cu-free nanofiber had no impact.
- The conventional Cu film coating increased the time to germination.
- For lettuce, there was no effect, although there were trends for reduced time to germination with treatment
- The fiber coating gives targeted and precision delivery of Cu^{2+} closer to the seeds and the growing root system.

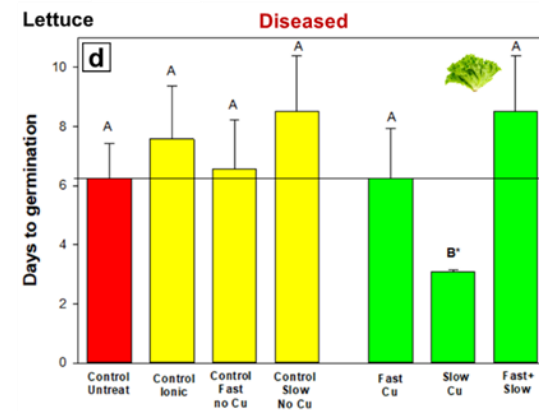
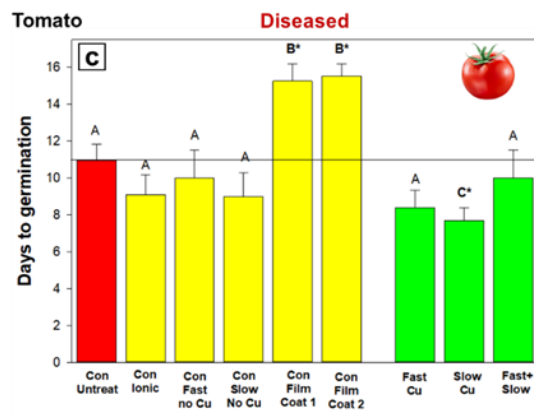
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- The presence of *Fusarium* **increased** the time to germination by approximately 20%.
- The “slow” release coated seeds significantly **reduced** the time to germination by 30% for tomato (c).
- Ionic Cu and Cu free nanofibers had no effect
- However, both film coatings significantly **increased** the time to germination for tomato.
- Similar results were found for lettuce, with the “slow” Cu release coating significantly **decreasing** the germination time by 51% (d).



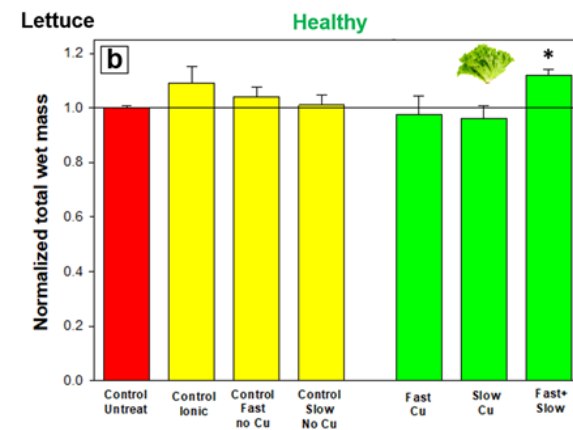
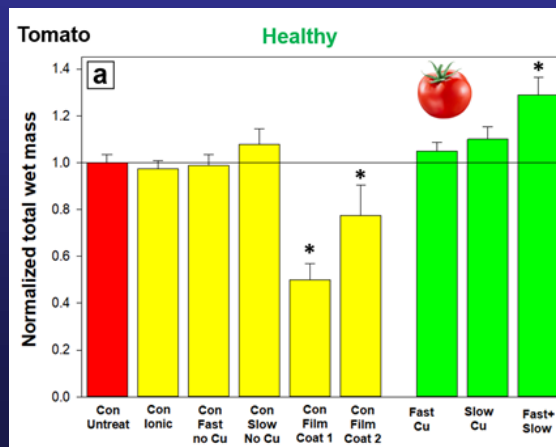
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- At 15 days, the root, shoot and total biomass of all treated seedlings was determined. Data were normalized to the untreated controls.
- Without incorporating (NPK) fertilizer in the nanofiber coating, Cu alone is not expected to increase biomass. However, many of the decreases in “time to germination” translated to increased biomass.
- The “fast + slow” nanofiber coating significantly increased total biomass of both tomato and lettuce by 12-29%, possibly because the double layer coating delivered Cu to the right place perhaps at the right time.
- Ionic Cu and Cu free nanofibers had no impact
- Conventional film coatings decreased the total tomato biomass; no such impact on lettuce.



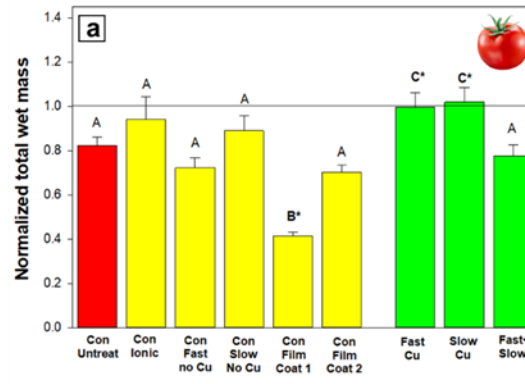
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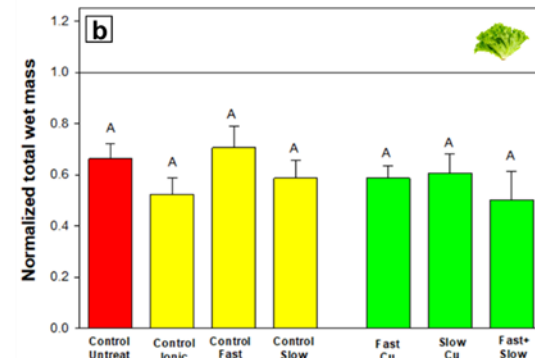
- *Fusarium* infection reduced biomass by 18% (tomato) to 36% (lettuce)
- For tomato, the “fast” and “slow” release Cu nanofiber coatings significantly increased biomass by 18% and 20% respectively (a).
- Ionic Cu and Cu-free nanofibers had no effect and one conventional film coating significantly decreased the total biomass.
- For lettuce, none of the treatments significantly impacted the biomass of infected lettuce seedlings (b), possibly because the disease caused by *Fusarium* (different species from that infects tomato) is more intense or has an altered infection cycle for lettuce.
- This suggests that the impacts of Cu loaded nanofiber coating on plant growth is pathogen/plant species dependent and that the nanofiber composition and agrichemical may need to be tuned to accommodate individual systems.



Tomato Diseased- Normalized to untreated healthy control



Lettuce Diseased- Normalized to untreated healthy control

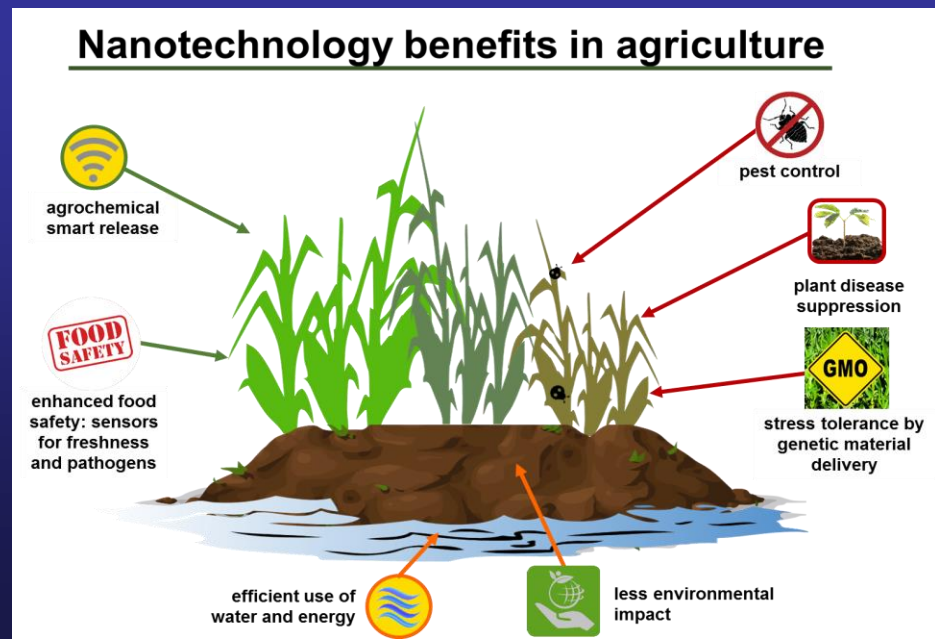




Conclusions

- Nanotechnology has the potential to dramatically improve agriculture; to literally help feed the world
- There is significant evidence that nanoscale nutrients can be used to promote plant nutrition and health to deter/suppress disease (viruses, bacteria, fungi)
- The use of nanoscale biopolymers as delivery vehicles for seed treatments and foliar amendments has great potential
- Because of this and because of widespread use of nanomaterials in other sectors, exposure in the food supply could be significant and applications must be safe and sustainable!
- As such, an understanding of **mechanisms of action/interaction** is needed to enable accurate assessment of risk

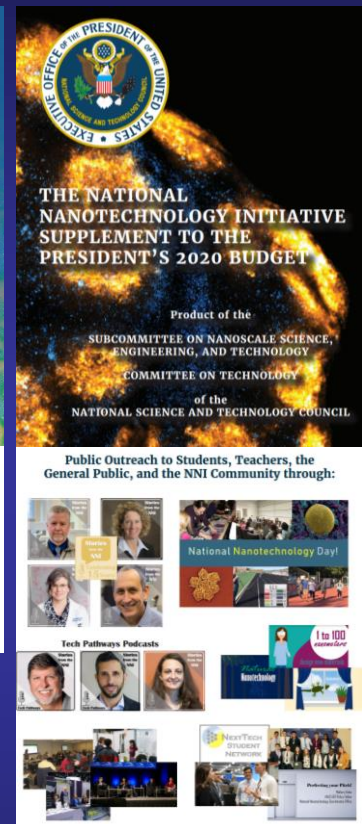
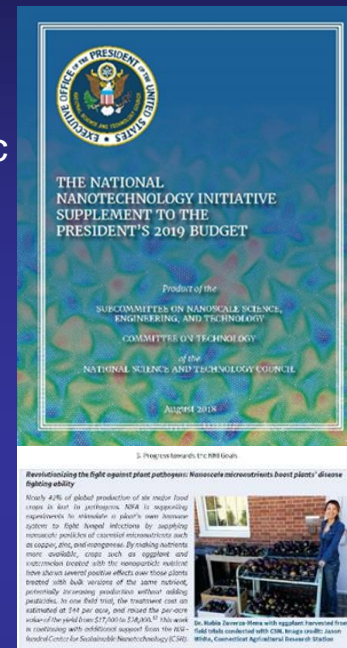
White and Gardea-Torresdey, 2018
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