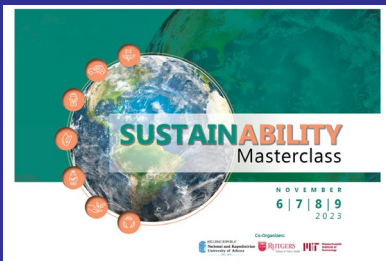


# Sustainable Agriculture: Nano-enabled strategies for food security in a changing climate



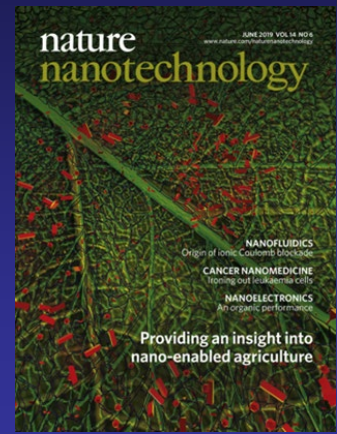
**Jason C. White, Ph.D.**

Director, The Connecticut Agricultural Experiment Station, New Haven CT

Presented at the SUSTAINABILITY Masterclass, National and Kapodistrian University of Athens, Athens, Greece, November 6-9, 2023

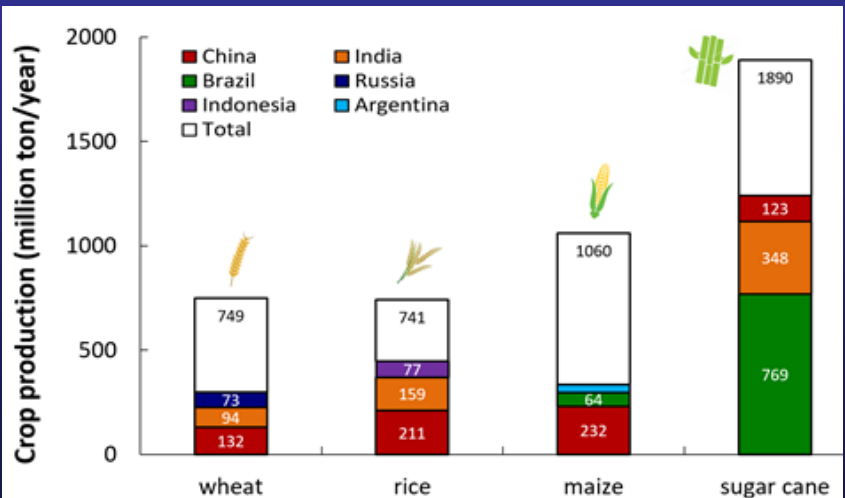
# Agriculture: Current Perspective

- 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 micronutrient deficiencies.
- Agricultural systems in the much of the world have plateaued at 20-80% of yield potential
- Agrichemical delivery efficiency is often only 1-25% (Nanotechnology!)



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

[www.ct.gov/caes](http://www.ct.gov/caes)





# Why Nano-Agriculture? Declining Global Food Security!!!

- 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...
- 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!!

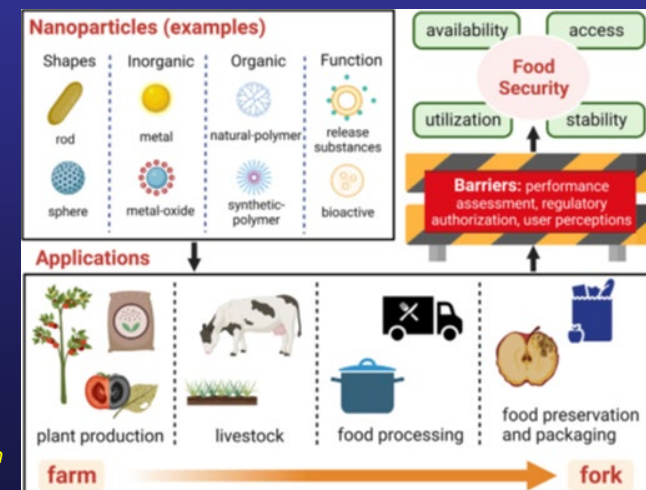


PNAS January 2019  
**Decline in climate resilience of European wheat**  
Helena Kahiluoto<sup>1,3</sup>, Janne Kaseva<sup>1</sup>, Jan Balek<sup>4,5</sup>, Jørgen E. Olesen<sup>6</sup>, Margarita Ruiz-Ramos<sup>1</sup>, Anne Gobin<sup>1</sup>, Kurt Christian Kernebaum<sup>7</sup>, Jozef Tóth<sup>8</sup>, Françoise Ruget<sup>9</sup>, Roberto Ferris<sup>8</sup>, Pavol Bezák<sup>10</sup>, Gemma Capellades<sup>11</sup>, Camilla Dibari<sup>12</sup>, Hanna Mäkinen<sup>13</sup>, Gaas Hendel<sup>14</sup>, Domènec Ventrella<sup>15</sup>, Alfredo Rodríguez<sup>16</sup>, Marco Binetti<sup>17</sup>, and Mirek Trnka<sup>18</sup>

CLIMATE CHANGE Science Aug. 2018  
**Increase in crop losses to insect pests in a warming climate**  
Curtis A. Deusch<sup>1,2,3,4</sup>, Joshua J. Tewksbury<sup>3,4,5,6,7</sup>, Michelle Tigchelaar<sup>8</sup>, David S. Battisti<sup>9</sup>, Scott C. Merrill<sup>7</sup>, Raymond B. Huey<sup>2</sup>, Rosamond L. Naylor<sup>10</sup>

ACS NANO  
**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.<sup>1</sup> This increase in social policies and economic investment and, notably, new technologies.<sup>2</sup> Technologies are needed to enable sustainable and intelligent farming practices as the increased food production is forecasted to be achievable by increasing crop

PNAS  
**Opinion: To feed the world in 2050 will require a global revolution**  
Paul R. Ehrlich<sup>1,2</sup> and John Harte<sup>3,4</sup>  
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  
Major Challenges  
Humanity now faces severe biophysical con-





# “Nano” Research at the CAES



## 1. Applications: Nano-enabled agriculture

- Nano-enabled micro/macronutrient delivery platforms
- Nanoscale micronutrients to modulate crop nutrition for disease suppression (ROS!)
- Nanoscale materials to enhance abiotic stress tolerance (ROS!), photosynthesis, induce RNA interference



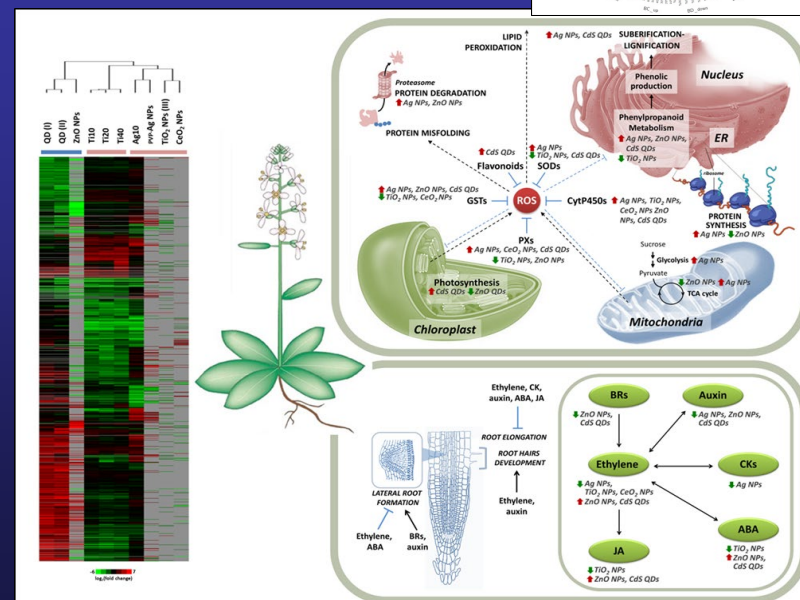
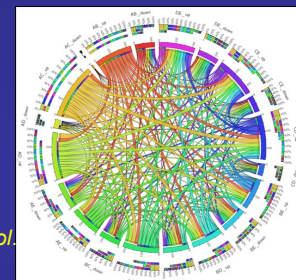
[www.ct.gov/caes](http://www.ct.gov/caes)

ROS ↑↓ ROS ↑↓ ROS ↑↓ ROS ↑↓ ROS ↑↓ ROS ↑↓ ROS ↑↓ ROS

## 2. Implications: Nanotoxicology

- Fate and effects of nanomaterials (NM) on plants and related biota (ROS!)
- Investigating the molecular basis of plant response; needed to ensure accurate risk assessment and safe use (ROS!)
- NM trophic transfer and transgenerational impacts in the food chain (ROS!)
- NM Co-contaminant interactions with pesticides, pharmaceuticals, metals (ROS!)

Ruotolo et al. 2018. *Environ. Sci. Technol.* 52:2451-2467.







# 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**



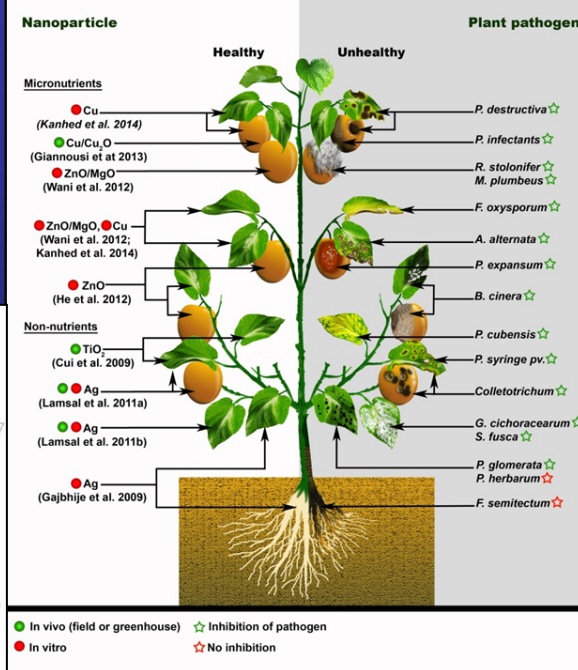
Alia Servin, Wade Elmer, Arnav Mukherjee, Roberto De la Torre-Roche, Helmi Hamdi, Jason C. White, and Christian Dimkpa  
VFRC CAES

J Nanopart Res (2015) 17:92  
DOI 10.1007/s11051-015-2807-7

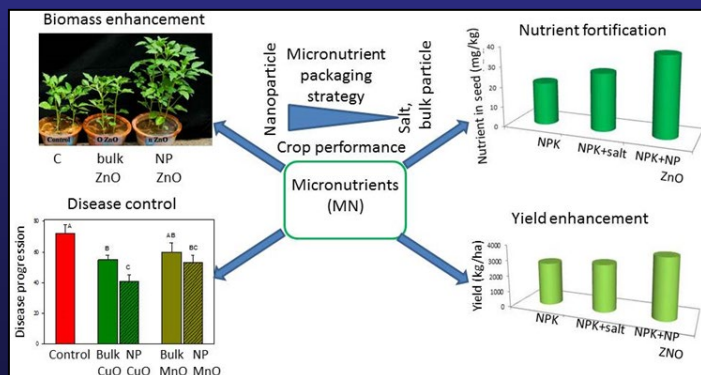
### REVIEW

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

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



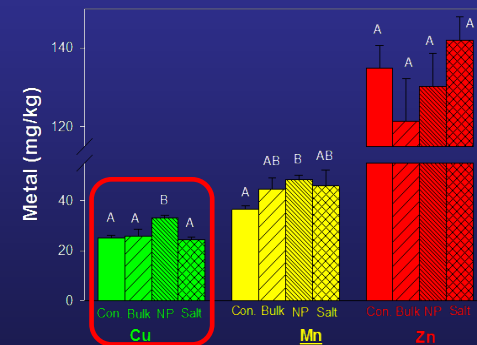
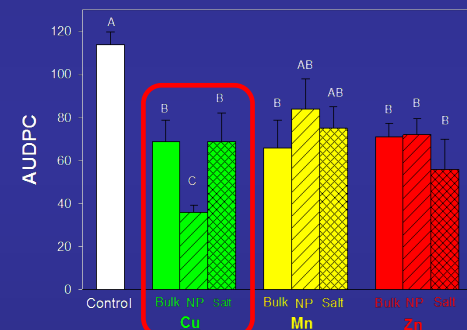
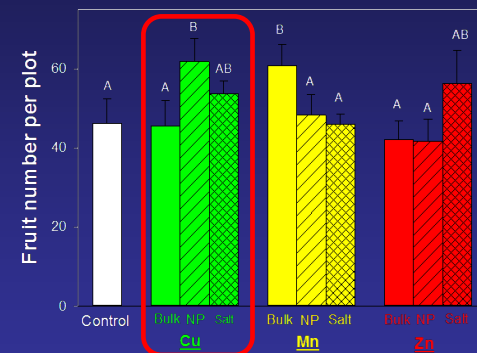
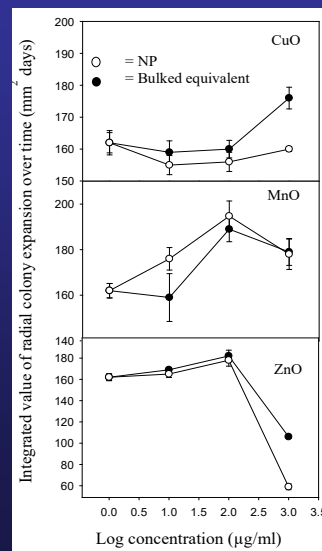
Servin et al. 2015. J. Nano. Res. 17:92.



# 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

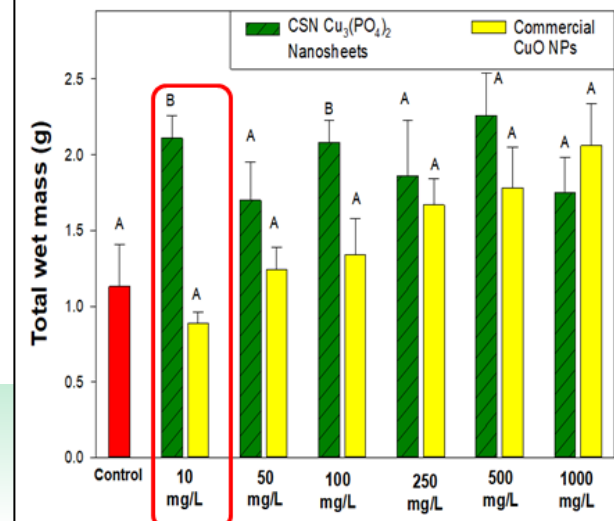
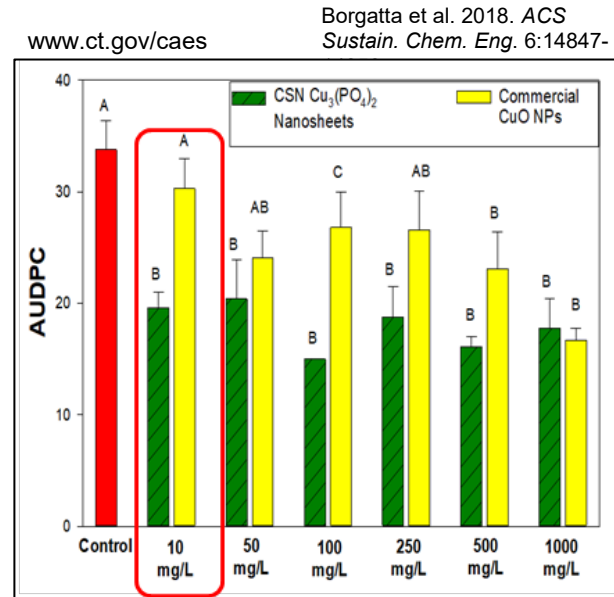
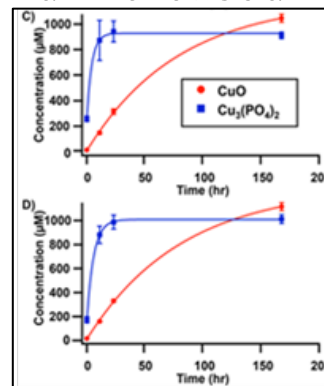
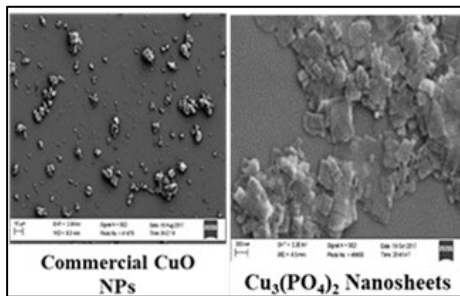
- \$44 per acre for NP CuO suppressed a root pathogen of eggplant, increasing yield from \$17,500/acre to \$27,650/acre



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

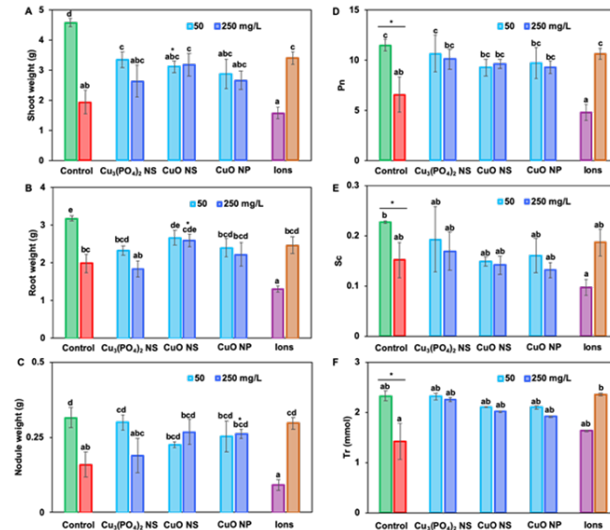
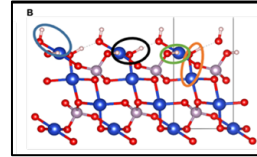
# Tuning Particle Properties

- Commercial CuO NPs vs  $\text{Cu}_3(\text{PO}_4)_2$  nanosheets (NS) from the NSF 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!

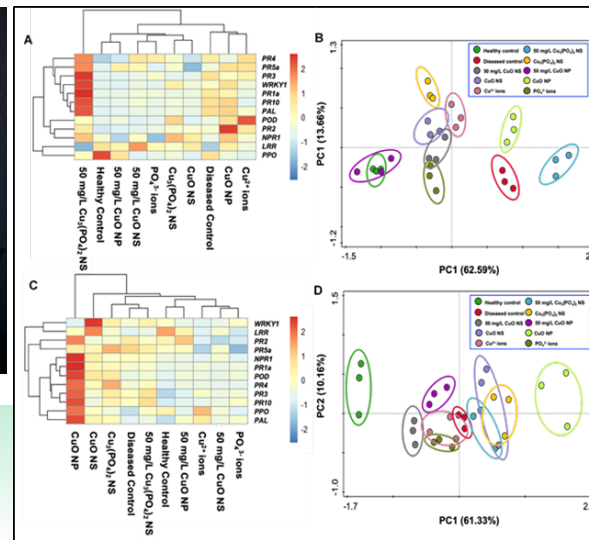


# Tuning Particle Properties

- Custom  $\text{Cu}_3(\text{PO}_4)_2$  and  $\text{CuO}$  nanosheets (NS) and commercial  $\text{CuO}$  nanoparticles (NPs) were investigated with soybean sudden death syndrome (SDS).
- Infection reduced biomass and photosynthesis by 60-70%; foliar application of nanoscale Cu reversed this damage.
- Disease-induced changes in antioxidant enzyme activity and fatty acid profile were also alleviated by Cu-amendment.
- The transcription of two dozen defense- and health-related genes correlated nanoscale Cu-enhanced innate disease response to reduced pathogenicity and increased growth.
- $\text{Cu}_3(\text{PO}_4)_2$  NS exhibited greater disease suppression than  $\text{CuO}$  NPs due to greater leaf surface affinity and Cu dissolution as determined computationally and experimentally.
- The findings highlight the importance and tunability of NM properties such as morphology, composition, and dissolution.

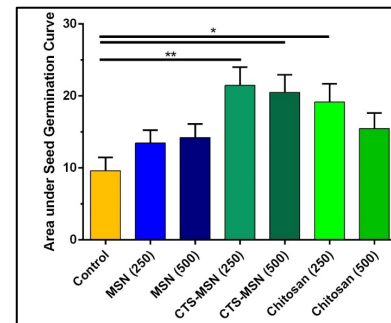
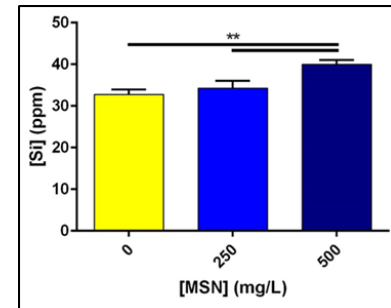
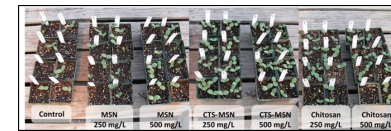


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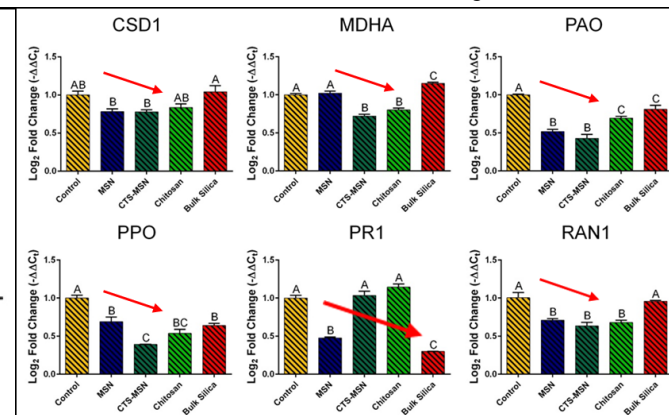
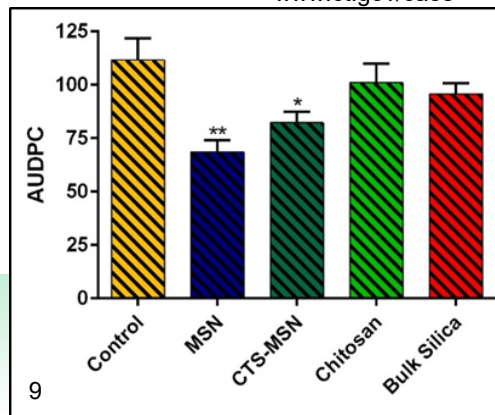


- Si is a non-essential element that helps plant response to biotic/abiotic stress
- The potential of mesoporous silica nanoparticles (MSN) with or without a chitosan coating to suppress *Fusarium* wilt in watermelon was evaluated
- Materials were seed treated or foliar applied (0-500 mg/L) to watermelon grown in *Fusarium* infested soils (greenhouse, field)
- Seed Si content increased by 7-20%; germination was increased and disease was suppressed
- For many genes related to stress (CSD1, PAO, PPO, RAN1, MDHA), treatment with MSNs, CTS-MSNs, or chitosan showed decreased expression.
- The decreased expression suggests alleviation of some of stress of disease



www.ct.gov/caes

Buchman et al. 2019 ACS Sus. Chem. Eng. 7:19649-19659



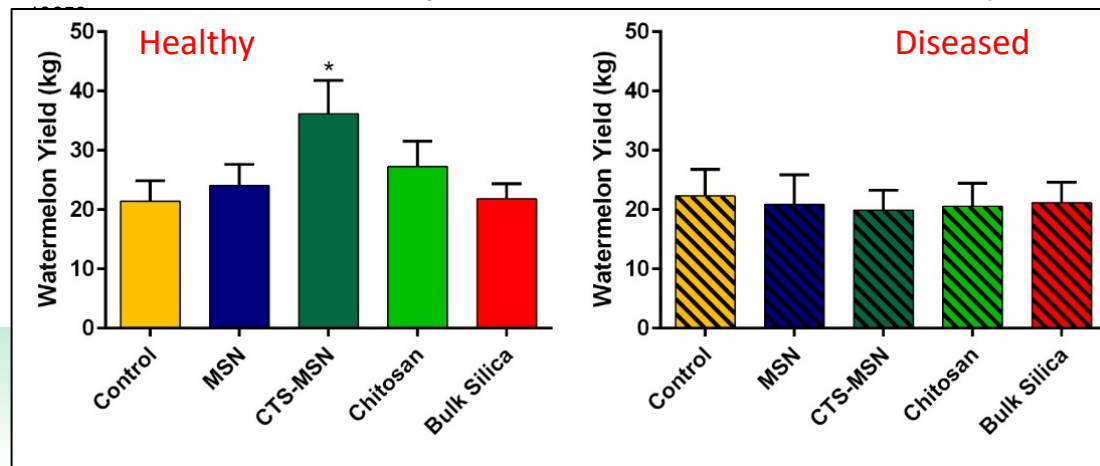


# Tuning Particle Properties

- In the field, the impact of seedling treatment on fruit yield was measured.
- For diseased-infected plants, treatment had no impact.
- For healthy plants, a single application of 1-2 mL of 500 mg/L via seedling dipping led to a 70% increase in watermelon yield
- The cost of this amount of CTS-MSN is approximately \$0.02/seedling or \$19 per acre of watermelon.
- Assuming an average watermelon yield per acre of 31,800 pounds (USDA, 2014) and a sale price of \$0.40/pound, this \$19 could increase yield to 54,000 pounds
- This equates to an increase from \$11,100 to \$18,900 per acre!

Buchman et al. 2019 ACS Sus. Chem. Eng. 7:19649-

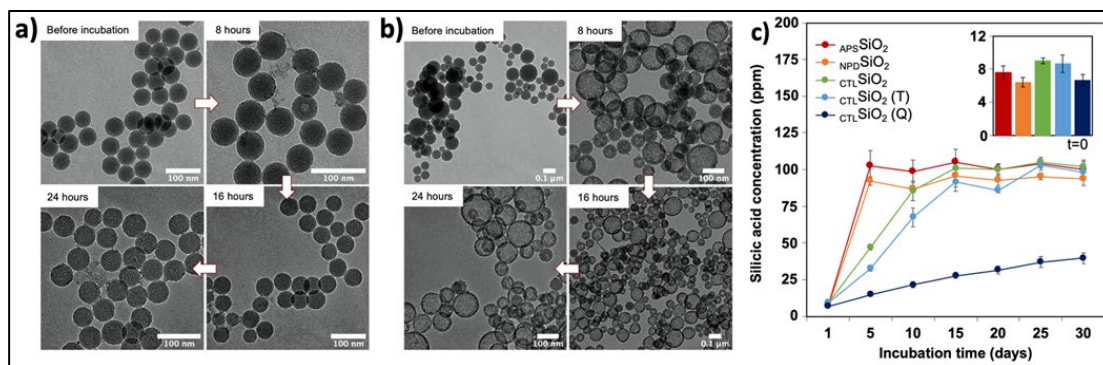
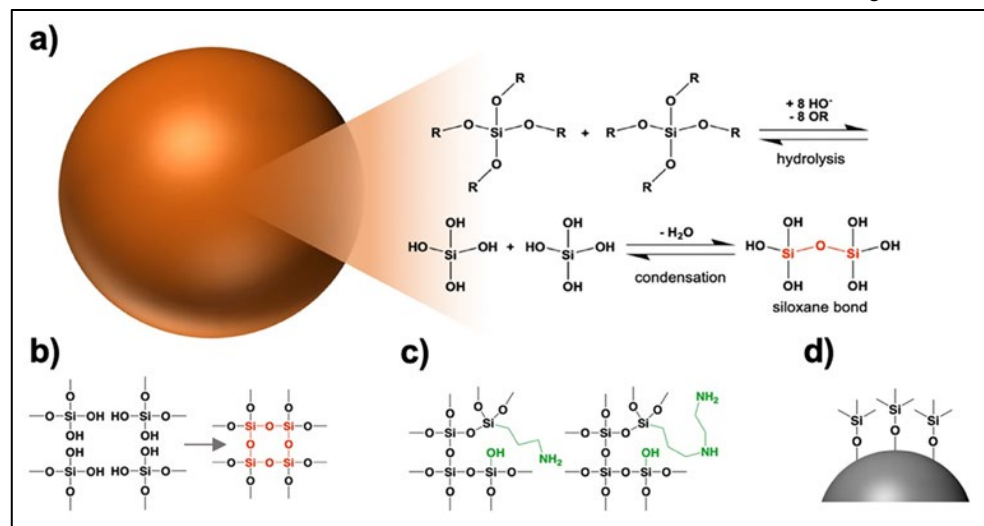
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# Tuning Particle Properties

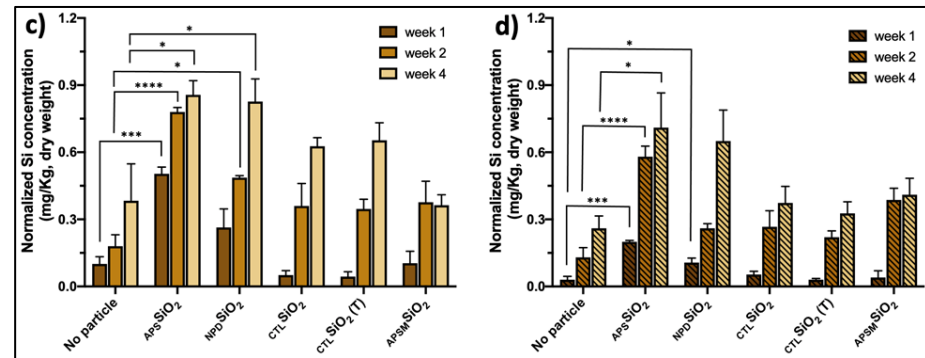
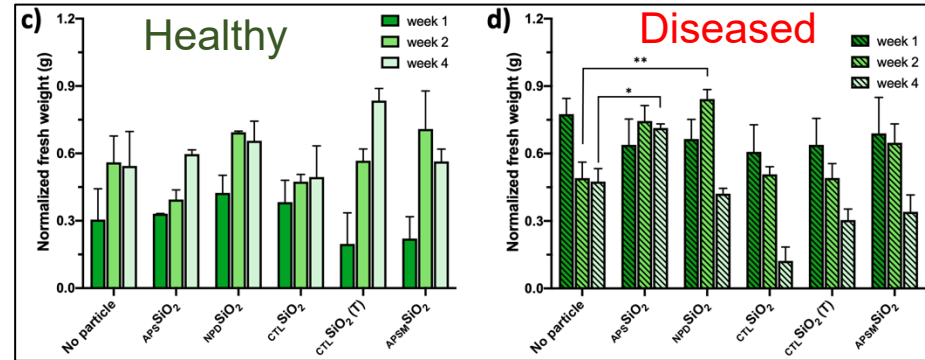
- In this study, silica nanoparticles (NPs) with tunable dissolution rates were synthesized and applied to watermelon (*Citrullus lanatus*) to enhance plant growth while mitigating development of the Fusarium wilt disease caused by *Fusarium oxysporum*.
- The hydrolysis rates of the silica particles were controlled by the degree of condensation of or the catalytic activity of aminosilane.
- In greenhouse and field studies, plants were foliar treated with 1.8 ml of a 1500 mg/L solution of the SiO<sub>2</sub> NPs, equating to about 2.7 mg of material

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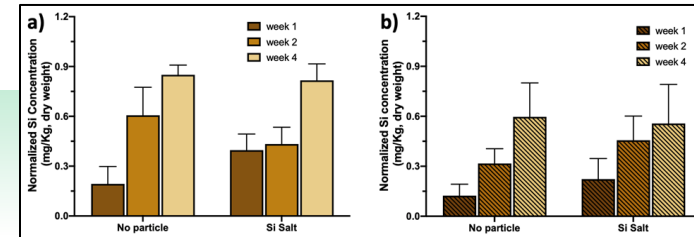
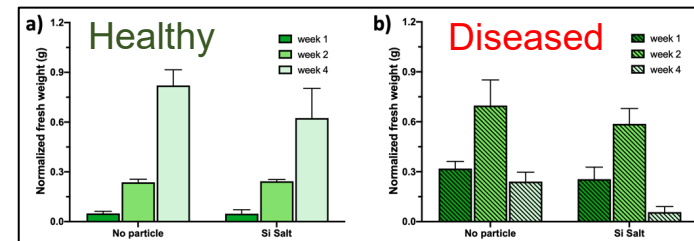


# Tuning Particle Properties

- The results demonstrate that the plants treated with fast dissolving NPs maintained or increased biomass whereas the particle-free plants had a 34% decrease in biomass.
- Further, a higher silicon concentration was measured in root parts when the plants were treated with fast dissolving NPs, indicating effective silicic acid delivery.
- Interestingly, foliar application of silicic acid had no beneficial impact
- In a follow-up field study over 2.5 months, the fast-dissolving NP treatment enhanced fruit yield by 81.5% compared to untreated plants.
- These findings indicate that the colloidal behavior of designed nanoparticles can be critical to nanoparticle-plant interactions leading to disease suppression and plant health, as part of a novel strategy for nano-enabled agriculture

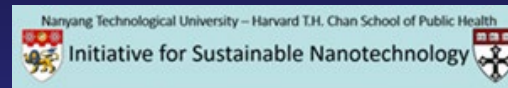


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➤ Conducted as part of the Nanyang Technological University-Harvard University T.H. Chan School of Public Health Initiative for Sustainable Nanotechnology



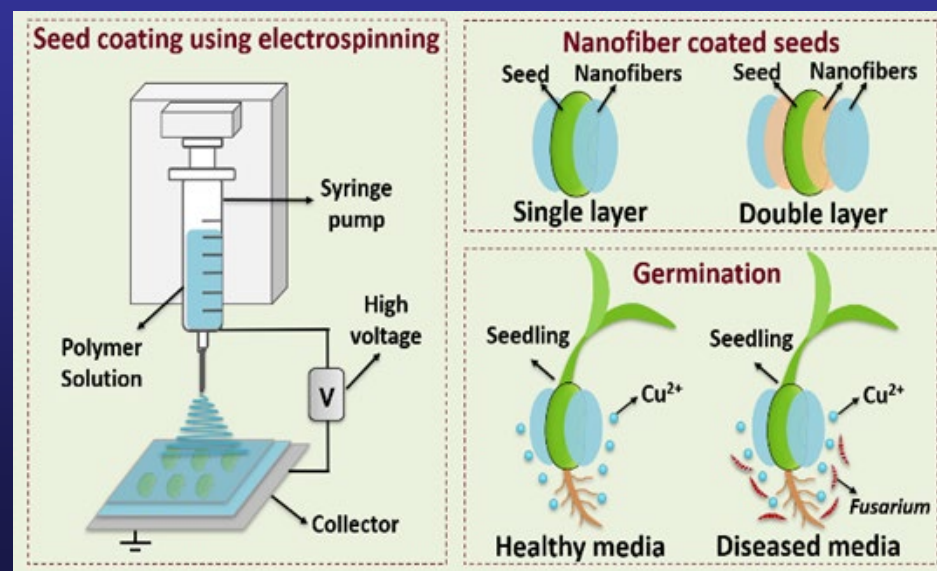
➤ Seed treatments have been used to deliver certain critical protective agents that promote seed storage, germination, and seedling growth.

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

➤ We developed a scalable, biodegradable, sustainable, “green” (non-toxic), biopolymer-based nanoplatfrom using electrospinning which can be used as a seed coating to enhance targeted and precision delivery of agrichemicals (the 3 Rs).

➤ Tested under healthy and diseased (*Fusarium*) conditions

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➤ Cellulose acetate/gelatin-derived electrospun nanofibers were synthesized that are of desired morphology/thickness, mechanical properties, and surface wettability

➤ The morphology of different electrospun **Cu<sup>2+</sup> loaded** nanofibers and their diameter distribution (n=50) is shown below.

[www.ct.gov/caes](http://www.ct.gov/caes)

➤ (a-b) CA/gelatin ratio of (a) 75/25 and (b) 50/50, without surfactant;

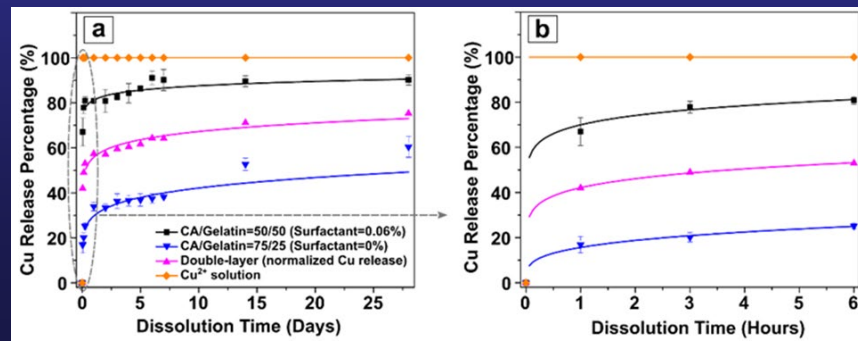
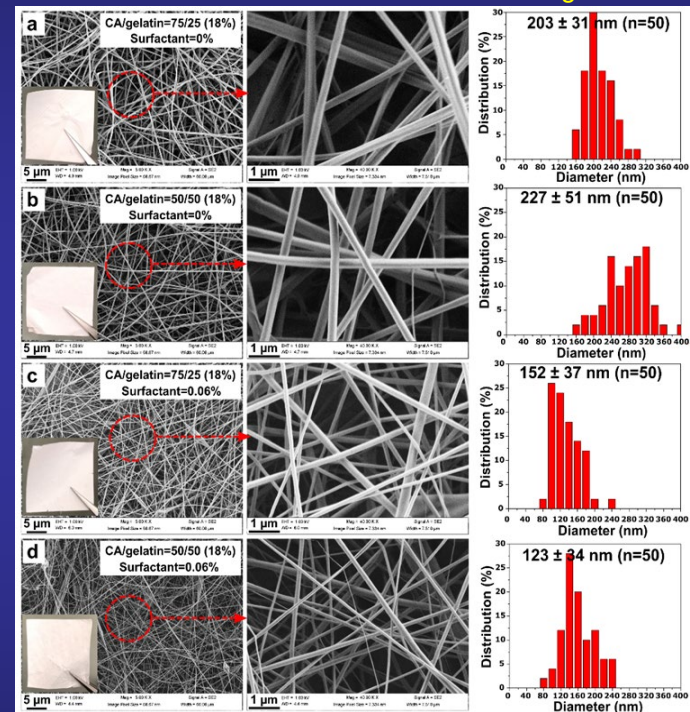
➤ (c-d) CA/gelatin ratio of (c) 75/25 and

➤ (d) 50/50, with surfactant

➤ The insert of the left of each image shows the free-standing electrospun nanofiber membranes

➤ The Cu<sup>2+</sup> release kinetics of were measured

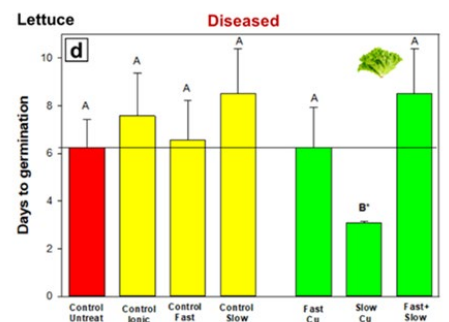
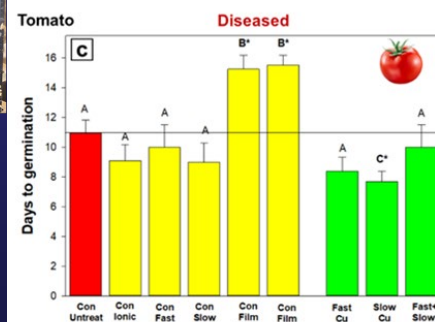
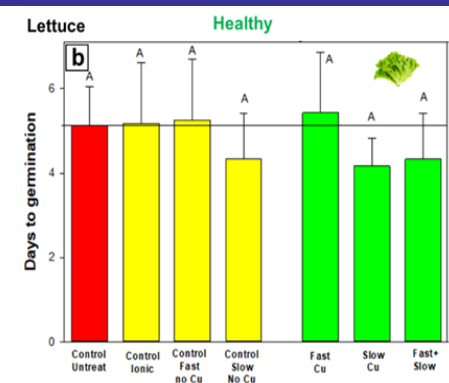
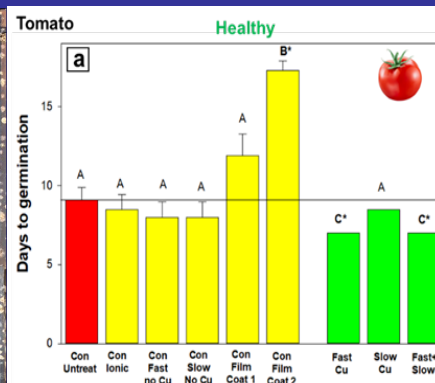
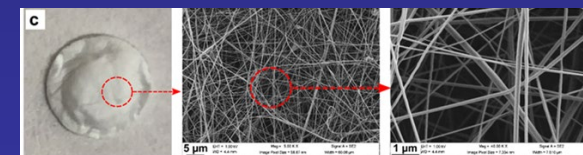
➤ “**Fast**” release is CA/gelatin=50/50, surfactant=0.06%; “**Slow**” release is CA/gelatin=75/25, surfactant=0%; “**Double-layer**” is “fast” on the outside and slow in the inside



# Time to Germination

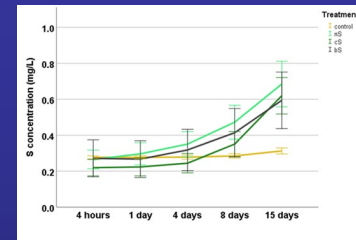
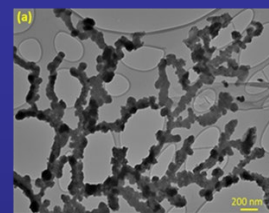
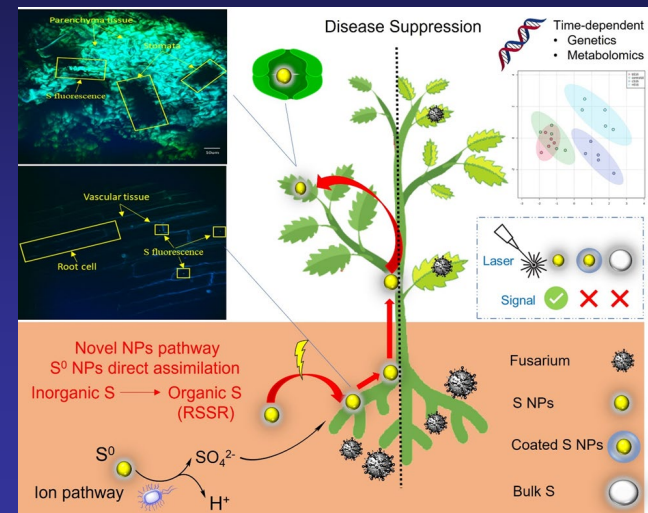
- Tomato and lettuce seeds coated with “fast,” “slow,” and “fast + slow” Cu release nanofibers, as well as ionic Cu and Cu-free nanofiber, and traditional film-coated controls were germinated
- For healthy tomato, the number of days to germination was decreased by 22% for the “fast” and “fast + slow” coated seeds (a).
- For lettuce, there was no effect, although there were trends for reduced time to germination with treatment
- Fusarium increased the time to germination by 20%.
- The “slow” release coated seeds significantly reduced the time to germination by 30% for tomato
- For lettuce, with the “slow” Cu release coating significantly decreasing the germination time by 51% (d).
- ***The increased rate of germination led to greater biomass at 15 days!***

[www.ct.gov/caes](http://www.ct.gov/caes)

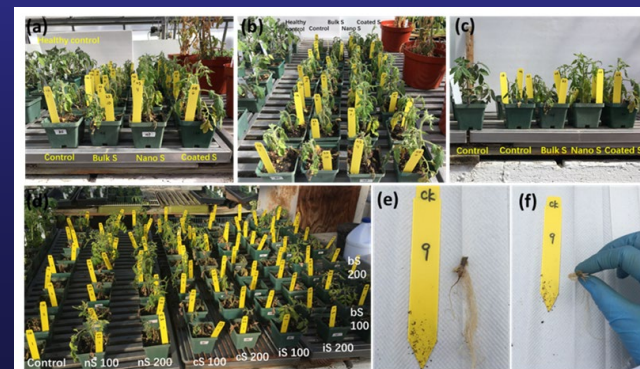




- Wang et al 2022. Therapeutic delivery of nanoscale sulfur to suppress disease in tomato: In vitro imaging and orthogonal mechanistic investigation *ACS Nano* 16, 7, 11204–11217
- Pristine (nS) and stearic acid coated (cS) sulfur NPs added at 200 ppm to soil planted with tomato and infested with *Fusarium oxysporum*.
- Bulk sulfur (bS), ionic sulfate (iS), and healthy controls were included.
- Orthogonal endpoints measured included agronomic and photosynthetic parameters, disease severity/suppression, mechanistic biochemical and molecular endpoints including the time-dependent expression of 13 genes related to two S bioassimilation and pathogenesis-response, and metabolomic profiles by LC-MS

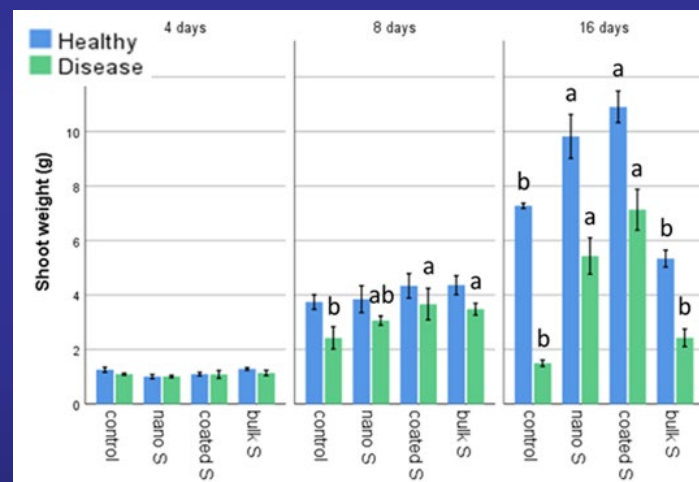
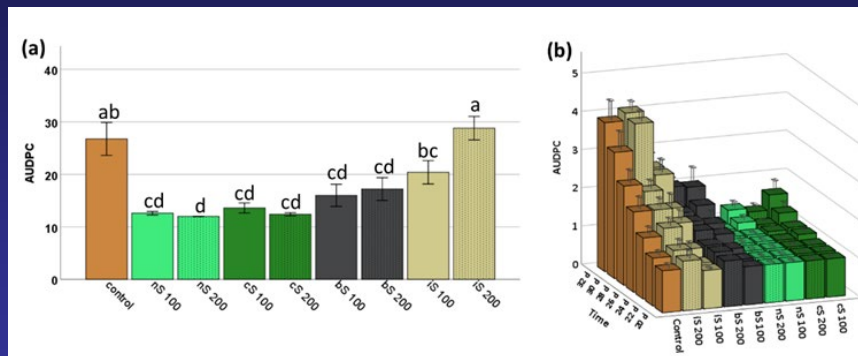


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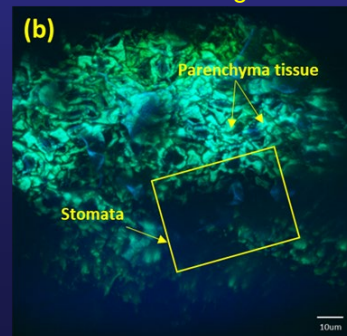
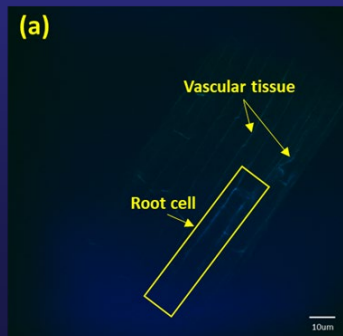




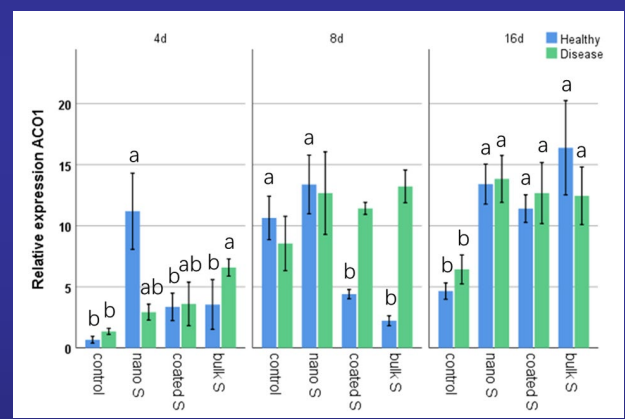
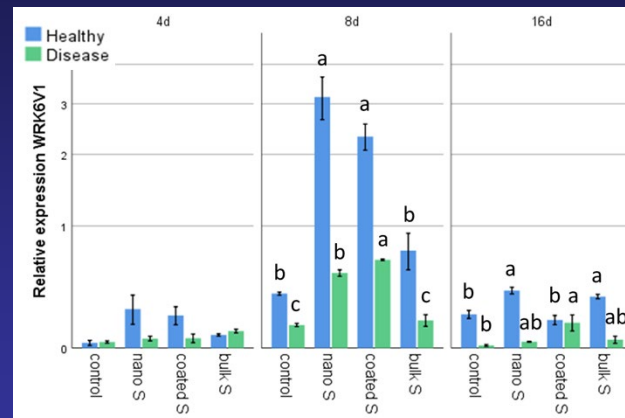
- Disease decreased plant biomass by up to 87%, but nS and cS amendment significantly reduced disease (AUDPC) by 54 and 56%, respectively.
- Increased in planta S accumulation was evident, with size-specific translocation ratios suggesting *different* uptake mechanisms.
- In vivo two-photon microscopy and time-dependent gene expression revealed a nanoscale-specific elemental S bioassimilation pathway within the plant that is separate from traditional sulfate accumulation.



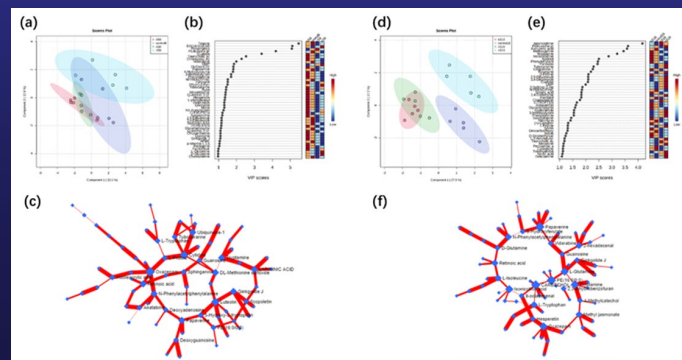
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- These findings align with time-dependent metabolomics, which exhibited increased disease resistance and plant immunity related metabolites only with nanoscale treatment.
- For example, the expression of WRKY6v1 and ACO1 (both are defense-related genes) was upregulated in a time dependent fashion as a function of S size and coating
- The linked gene expression and metabolomics data demonstrate a time-sensitive physiological window where nanoscale stimulation of plant immunity will be effective.
- These findings provide mechanistic understanding of nanosulfur-mediated disease suppression and advance sustainable nano-enabled agricultural strategies to increase food production.

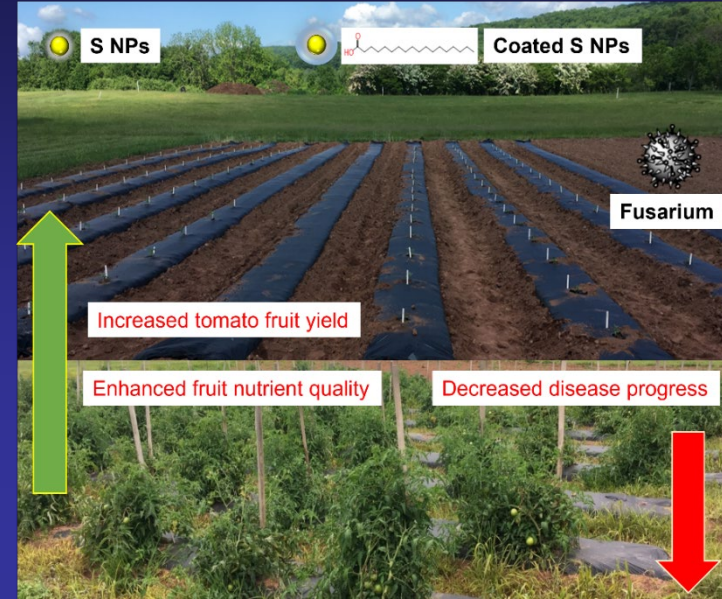


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# Nanoscale Sulfur and Disease

- Wang et al. 2022. Surface coated sulfur nanoparticles suppress Fusarium disease in field grown tomato: Increased yield and nutrient biofortification *J. Agric. Food Chem.* 70, 45, 14377–14385
- In the infested treatments, cS significantly reduced disease severity compared to other treatments.
- Ca, Cu, Fe, and Mg content in the S-amended fruit were increased over diseased controls.
- On a per acre basis, a **\$33** investment in nanoscale sulfur led to an increase in marketable yield from **4,920 kg per acre to 11,980 kg/acre** for healthy plants and from **1,135 kg/acre to 2,180 kg/acre** for Fusarium-infested plants
- These increases equate to an additional **\$6,700-12,200** in economic return per acre.

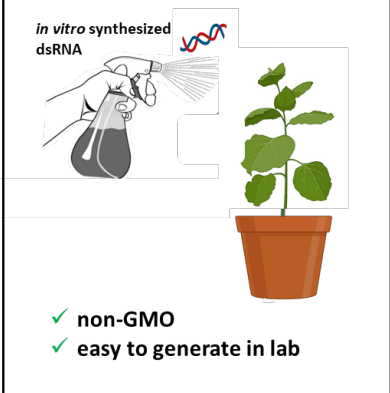




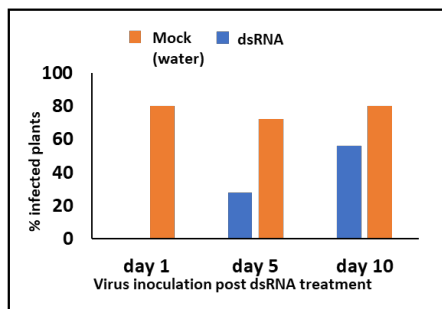
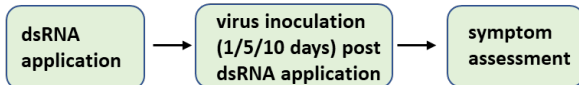
# RNA Interference and Viral Infection

Typically applied double stranded RNA (dsRNA) provides protection against target plant virus

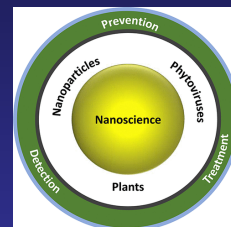
dsRNA application triggers RNAi, a natural host defense mechanism against the virus



Concern: Longevity of protection window?



dsRNA provides small protection window due to easy degradability of nucleic acid



ACS Nano 2021, 15, 6030–6037

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## Nanotechnology and Plant Viruses: An Emerging Disease Management Approach for Resistant Pathogens

Tahir Farooq,<sup>#</sup> Muhammad Adeel,<sup>#</sup> Zifu He, Muhammad Umar, Noman Shakoor, Washington da Silva, Wade Elmer, Jason C. White,<sup>#</sup> and Yukui Rui<sup>#</sup>

REVIEW

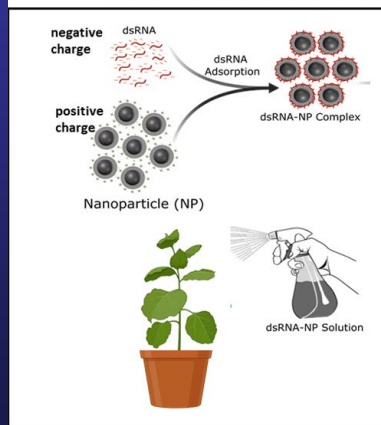
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“Tunable release of dsRNA molecules into plants from sustainable nanocarriers: A novel management tool for viral Pathogens” Da Silva et al.

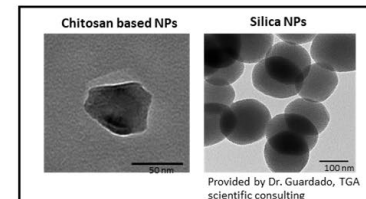


Nanoparticles as dsRNA carriers

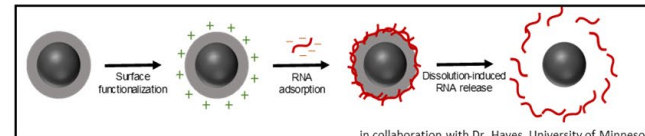
- Protection against nucleic acid degradation
- Sustained/controlled release of dsRNA



Nanoparticles being tested for dsRNA absorption and release



Synthesize silica NPs for a more controlled dsRNA release



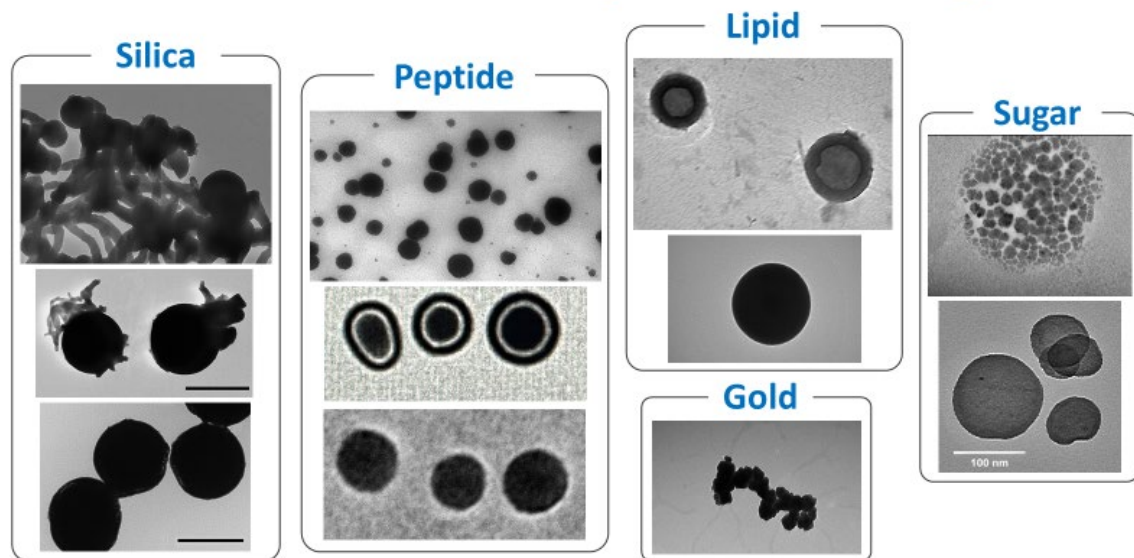
In collaboration with Dr. Hayes, University of Minnesota

Identify most efficient dsRNA delivery system for virus control



- A wide range of materials are being investigated as potential carriers to deliver and provide controlled long-term release of various genetic cargo
- Current model system is potato and the PVY
- PVY is a single stranded RNA virus that infects mainly Solanaceous plants including, potato, tomato, pepper,...

## Nanocarriers of various compositions are being tested

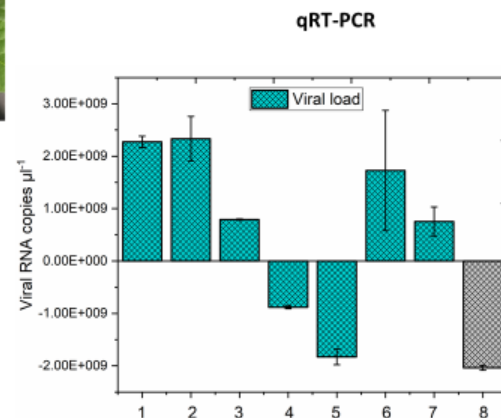


## Peptide-dsRNA nanocomplex treated with PVY infection?



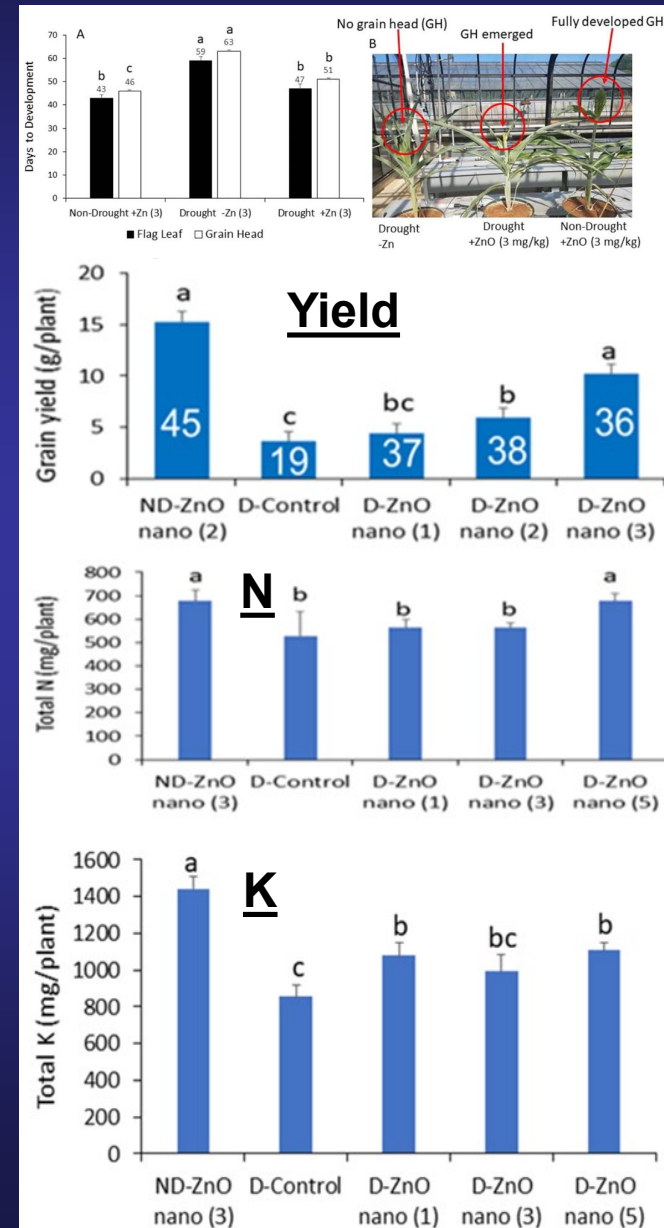
a) Positive control, b) dsRNA treated, c) & d) Peptide-nano dsRNA treated

1. PVY-Positive control
2. PVY+peptide 1%
3. PVY+peptide nanoparticles
4. PVY+Peptide-dsRNA (100µg)
5. PVY+Peptide-dsRNA (200µg)
6. PVY+DsRNA (100µg)
7. PVY+DsRNA (200µg)
8. PVY-Negative control



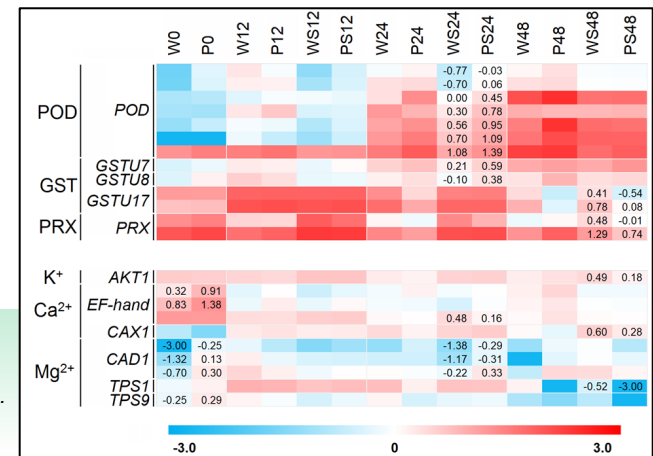
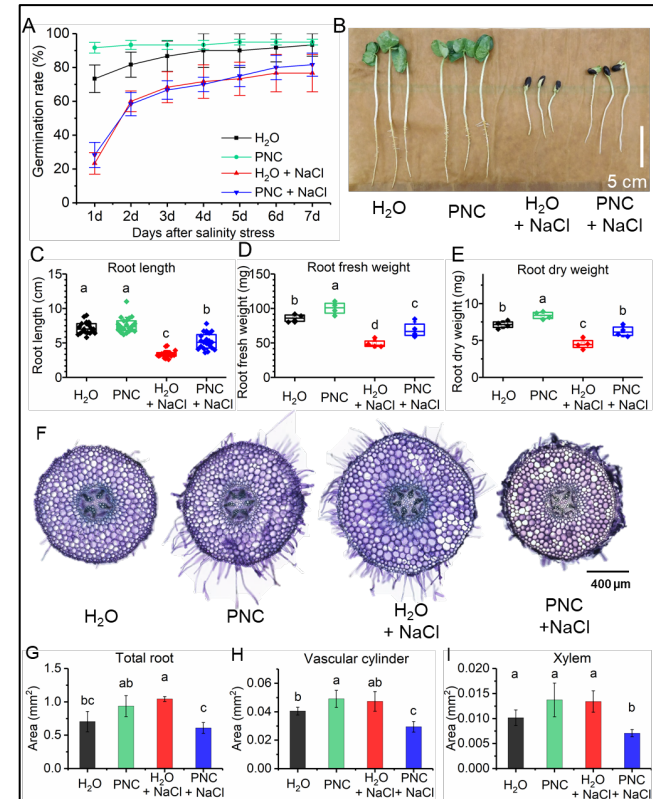
# NP ZnO alleviates drought-induced damage

- Soil amended with ZnO-NPs at 1, 3, and 5 mg Zn/kg; drought imposed 4 weeks after sorghum seed germination (40% field moisture capacity).
- Leaf and grain head emergence delayed 6-17 d by drought; delays were reduced to 4-5 days by ZnO-NPs
- Drought reduced grain yield (76%); ZnO-NPs improved grain yield under drought by 22-183%.
- Drought lowered grain Zn content by 32%; ZnO-NPs improved (89-100%) grain Zn under drought.
- Drought inhibited total N acquisition by 22%; ZnO-NPs (5 mg/kg) restored total N levels.
- K by 41%; ZnO-NPs improved total K acquisition by 16-30%.



# Nanoscale CeO<sub>2</sub> and Salinity

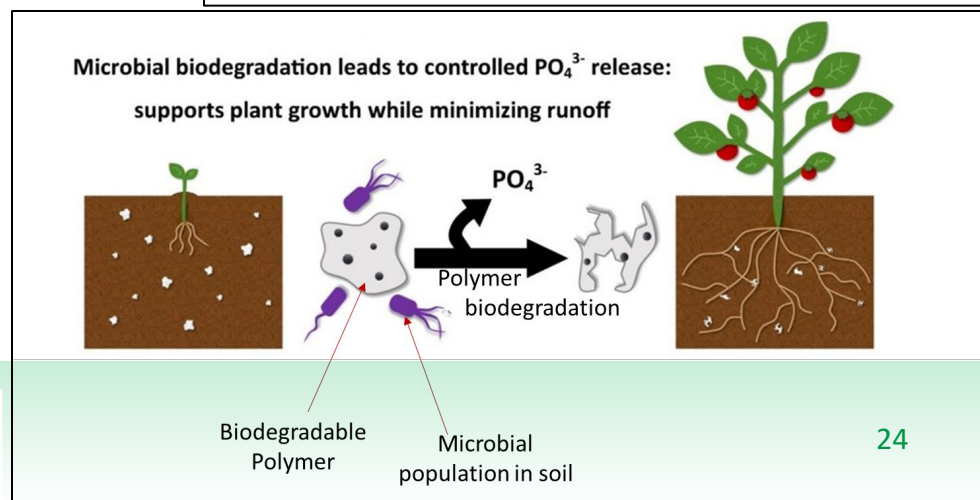
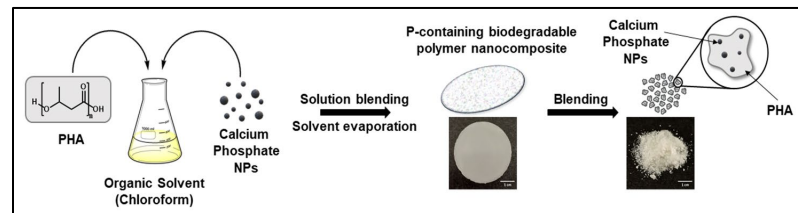
- Cotton seeds were primed with 500 mg/L PNC poly(acrylic acid)-coated cerium oxide nanoparticles (PNC) (24 h in water) and germinated under salinity stress (200 mM NaCl)
- PNC were in the seed coat, cotyledon, and root apical meristem.
- Priming increased root length (56%), mass (39%), modified root structure, and increased root vigor (114%) under salt stress.
- Priming decreased root ROS accumulation (46%) and alleviated root morphological/physiological changes induced by salinity stress.
- Roots from exposed seeds had similar Na, decreased K (6%), greater Ca (22%) and Mg content (60%) compared to controls.
- 4779 root transcripts were differentially expressed by priming relative to controls; DEGs were associated with ROS pathways (13) and ion homeostasis (10)
- Seed priming with NMs provides a sustainable and scalable tool to improve plant stress tolerance.





# Polymer Nanocomposites- P Delivery

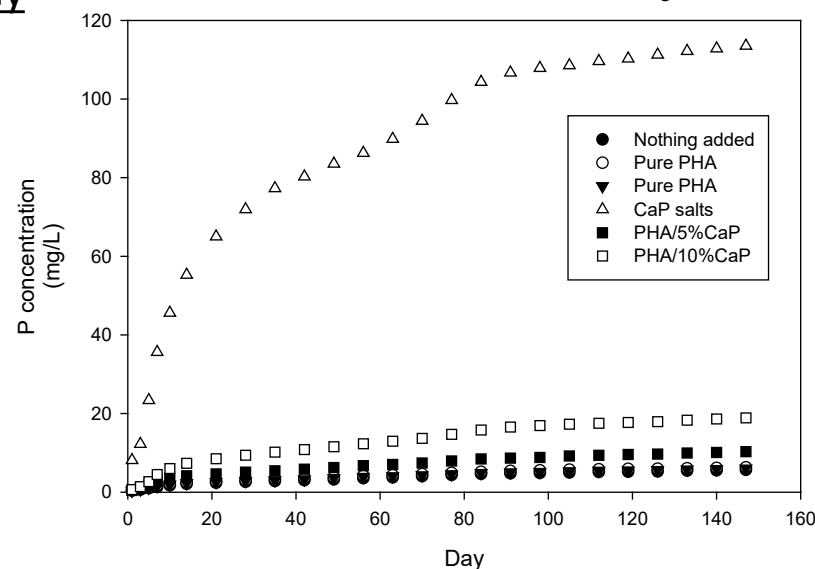
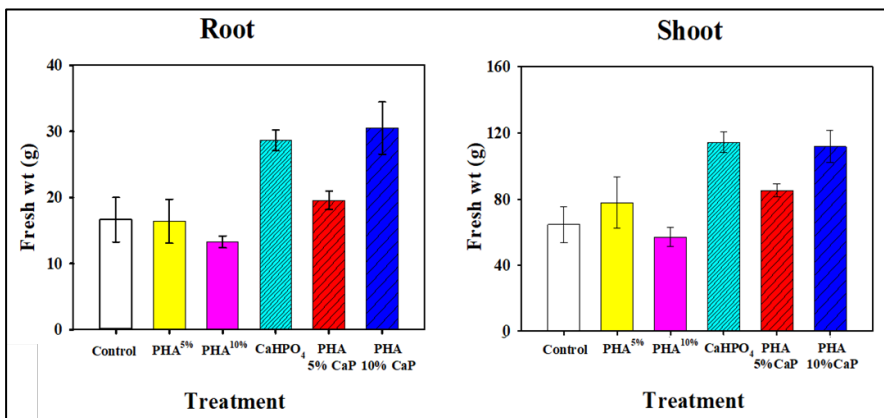
- We propose to make a **tunable suite** of biodegradable polymer nanocomposite fertilizers that will release P to plants as desired rates.
- Polyhydroxyalkanoate (PHA) is a highly biodegradable polymer made by bacteria.
- We used solution blending to make composites of PHA and calcium phosphate (CaP) nanoparticles (NPs); then we mix that composite into soil with plants.
- As native bacteria in soil biodegrade the PHA, CaP is released from the polymer matrix and becomes available to plants.
- There is little or no P run-off because CaP is retained in the PHA until it is biodegraded and released.
- This responsive platform is tunable (changing polymers or co-polymer ratios).



- Polymer nanocomposites added to soil with tomato plants; compared to CaP salts that mimic traditional fertilizers for 150 days (full life cycle).
- Leachate (i.e., runoff) was collected periodically and P in runoff was measured with ICP-OES
- The nanoscale polymers reduced P “run-off” by **10-fold!**
- Plant biomass, chlorophyll, fruit yield, nutritional content, total protein, and lycopene content were all statistically equivalent between conventional P and the nanocomposite P materials.



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# Polymer Nanocomposites- P Delivery

➤ **Sigmon et al. 2023** “Role of phosphorus and biodegradable polymer type on phosphorus fate and efficacy in a plant-soil system” *J. Agric. Food Chem.*  
<https://doi.org/10.1021/acs.jafc.3c04735>

➤ **Goal-** To understand how the properties of biodegradable polymer-nanoparticle composites vary with different P type fertilizer

## Greenhouse experiment with Tomato (50 d, 40 mg P per replicate)

- |   |                |
|---|----------------|
| 1) Control (Nothing added)                    | 2) PHA Control |
| 3) Calcium phosphate monobasic (Mono)         | 4) PHA-Mono    |
| 5) Calcium phosphate dibasic(Di)              | 6) PHA-Di      |
| 7) Hydroxyapatite nanoparticles(HAN)          | 8) PHA-HAN     |
| 9) Hydroxyapatite microparticles (HAM)        | 10) PHA-HAM    |
| 11) Calcium pyrophosphate nanoparticles (CAP) | 12) PHA-CAP    |

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➤ **Endpoints-** Amount of P released: leachate, soil column, and dissolution; Plant growth and health: biomass, tissue P concentration; Total and available P in soil after plant growth





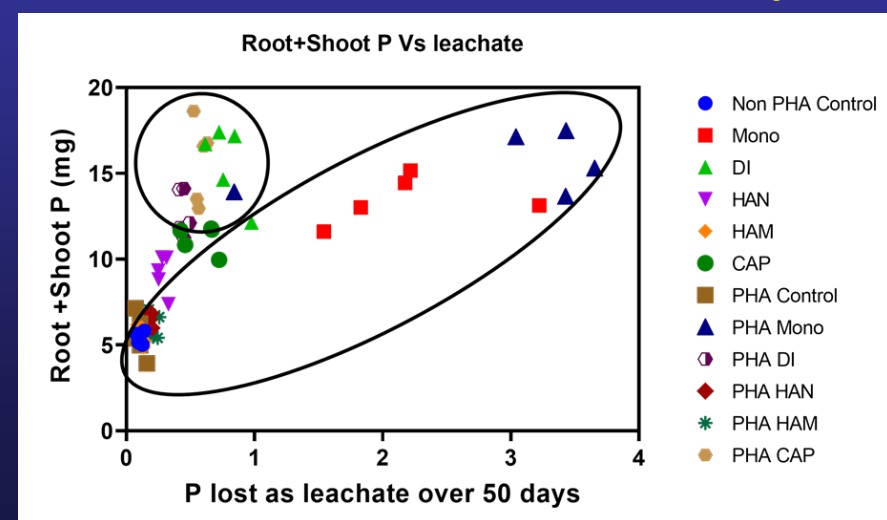
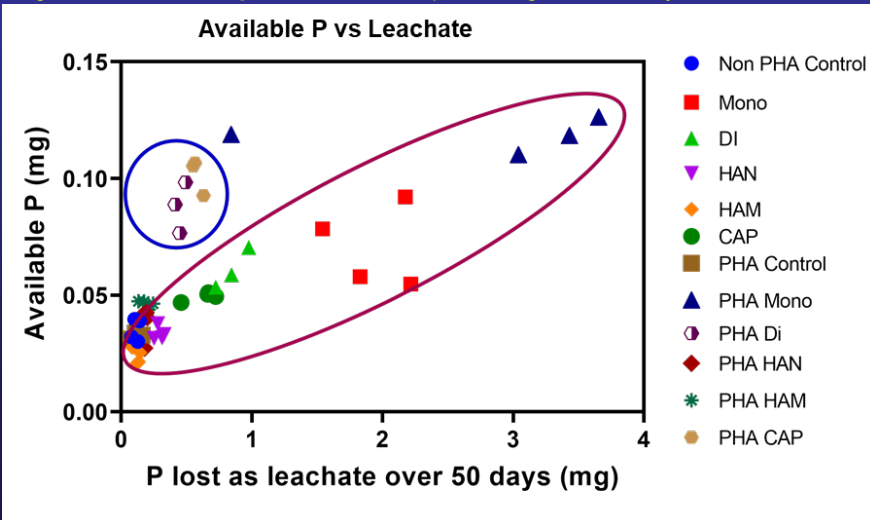


# Polymer Nanocomposites- P Delivery

- Equivalent biomass across all P types, regardless of PHA presence
- Correlation analysis identified 3 performance classes :
  - (i) HAM and HAN did not increase bioavailable P, plant P-uptake, or change P in runoff/leaching compared to controls;
  - (ii) MCP, Di, CAP, and PHA-MCP increased P-uptake and/or bioavailable P but also increased P loss in runoff/leaching; and
  - (iii) PHA-Di and PHA-CAP where increased bioavailable P and plant P-uptake were achieved with minimal P loss in runoff/leaching.**
- Biopolymers can promote plant P-uptake and improve bioavailable soil P, mitigating the negative environmental impacts of P loss from agricultural systems

Sigmon et al. 2023 *J. Agric. Food Chem.* <https://doi.org/10.1021/acs.jafc.3c04735>

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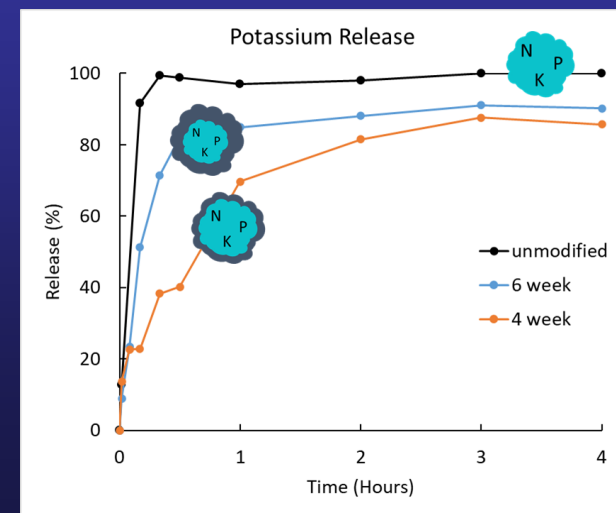
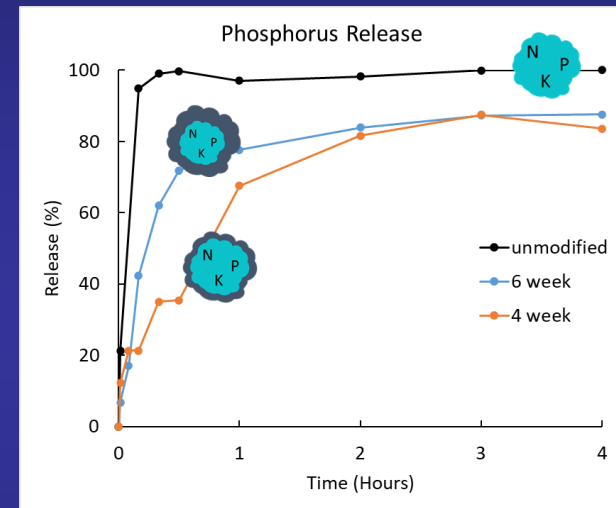




# Controlled NPK Release- Nanocellulose

- **Gomez Maldonado et al. 2023** “Slowing soluble NPK release with hydrophobized nanocellulose-based hydrogels for enhanced efficiency fertilizers” *Environ. Sci.: Nano*. Submitted (Auburn, Hopkins, CAES)
- Cellulose nanofibrils-based hydrogels (CNFs) were regenerated from mixed softwood in acidic media and were loaded with NPK
- NPK-loaded CNFs were functionalized using the gas phase esterification; reaction time was varied to tune the hydrophobicity of NPK-CNF
- The more-esterified sample (6 weeks) released K and P more quickly than the 4-week despite a greater functionalization.
- This result counters the expectation that greater hydrophobicity should slow release; esterification beyond a critical point make break down the micro-nano structure, creating channels for rapid nutrient dissolution

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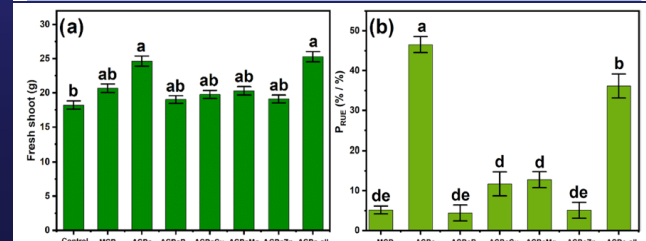
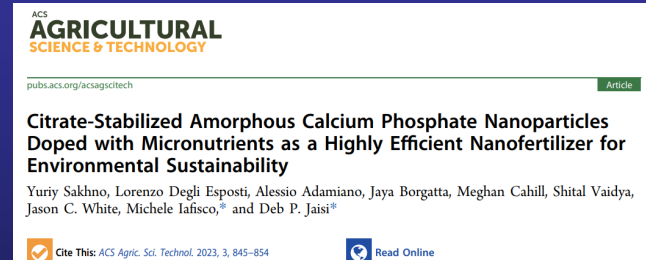
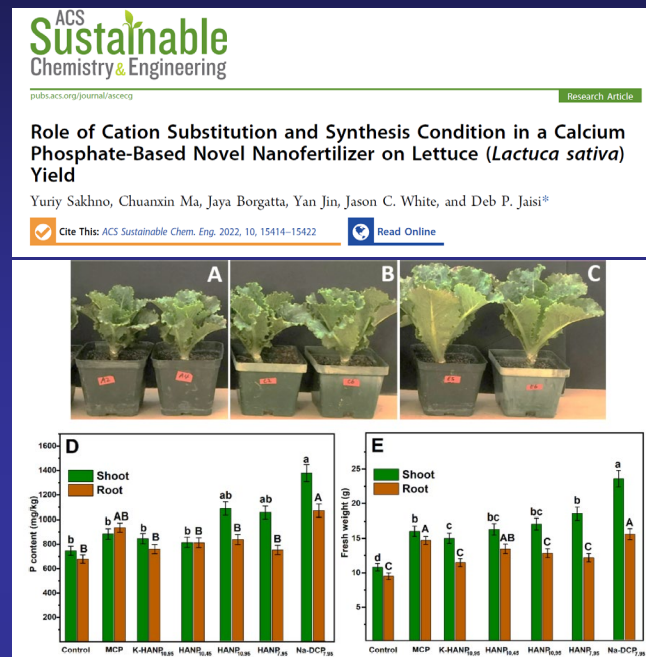


## Sakhno et al. 2022

- HANPs and dicalcium phosphate anhydride (DCPA) under varying crystallization conditions (temperature, pH, and cation substitution) were tested on lettuce
- HANPs and DCPA stimulated lettuce growth, but the extent of enhancement was a function of synthesis condition

## Sakhno et al. 2023

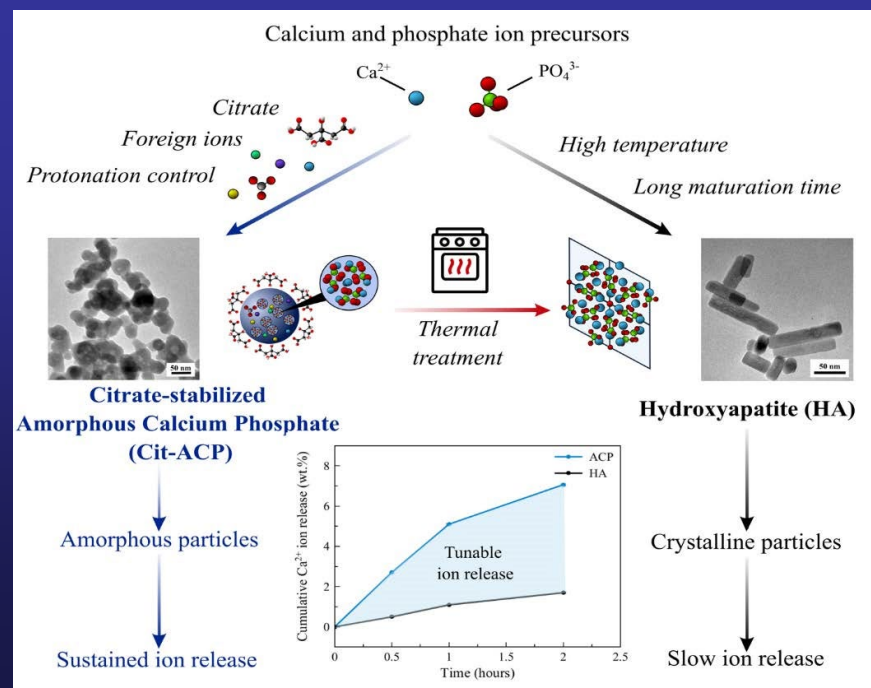
- Citrate-stabilized ACP nanoparticles (ACPcs) doped with micronutrients (B, Cu, Mg, and Zn)
- P resource use efficiency (RUE), accounting for crop yield and P lost in leachate, was higher in all ACPc than conventional MCP (monocalcium phosphate) and was about a log order higher in multi-micronutrient doped ACPcs





## Calcium phosphate nanocomposites for improving nutrient use efficiency and crop yield and reducing loss: PD- Jaisi (UDeI); CoPDs- White/Steven (CAES), Iafisco (NRC Italy)- 6/1/24-5/31-27

- Synthesize amorphous and crystalline calcium phosphate (ACC) nanocomposite as a micronutrient-doped novel nanofertilizer from waste recycled P.
- Develop a mechanistic, structure-function relationship of P release kinetics with structural and surface and interfacial characteristics of ACCPs and tune for optimal P release.
- Greenhouse experiments with plant species and apply the isotope tracking method to discriminate P uptake from ACCPs vs. other soil P pools and compare nutrient and resource use efficiencies (NUE and RUE) with conventional and slow-release fertilizers.
- Analyze microbiome and rhizosphere nutrient chemistry to quantify the positive and synergistic impacts of ACCPs against conventional and slow-release fertilizers.



# Responsive Nanocapsules

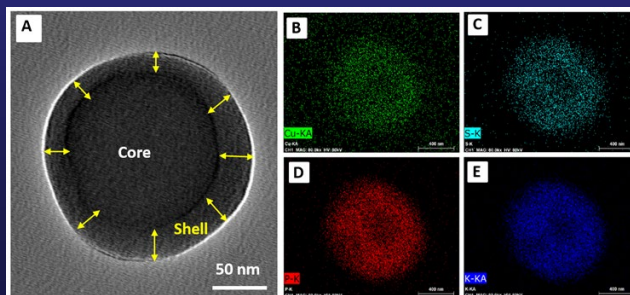
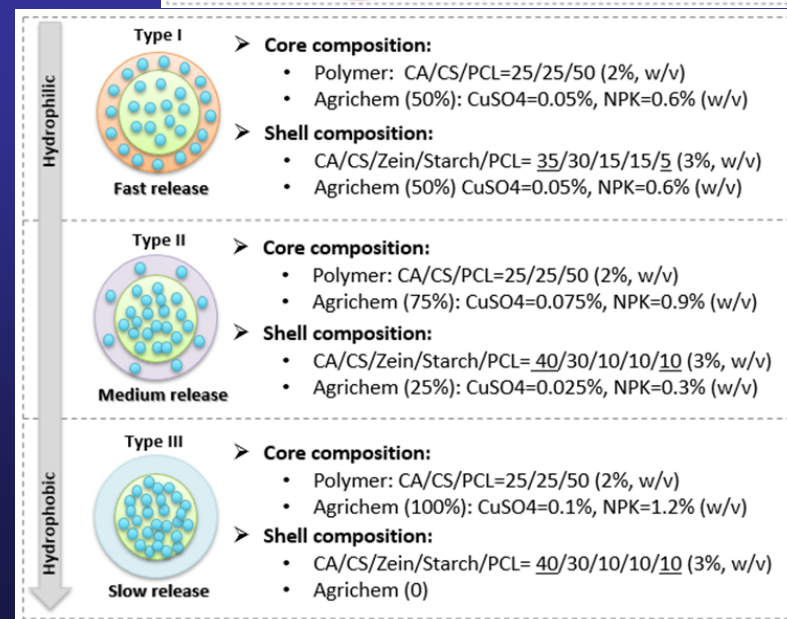
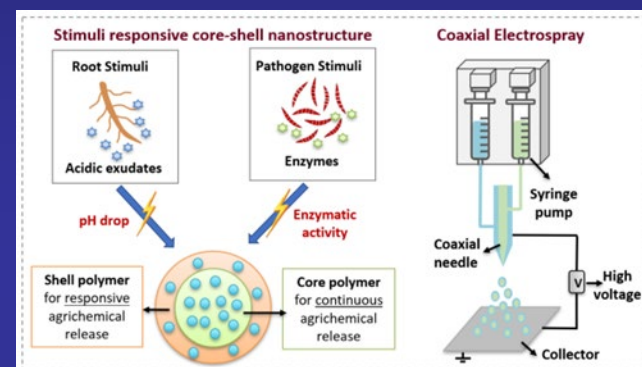
- Biopolymer-based multi stimuli responsive nanoplatform (i.e., core/shell nanostructure) was developed by a “green” electrospray approach for smart agrichemical delivery.
- The shell polymer was designed to be responsive to different triggers such as pH and microbial enzyme activity, and the core polymer was designed to continuously release the agrichemicals over the longer term.
- NPK and Cu were loaded at 100% and 25% label rates
- The pH and enzyme responsiveness was demonstrated by the analyte release kinetics as a function of chemical composition.
- Efficacy was evaluated in soil-based greenhouse studies using soybean and wheat.

Nanyang Technological University – Harvard T.H. Chan School of Public Health  
Initiative for Sustainable Nanotechnology

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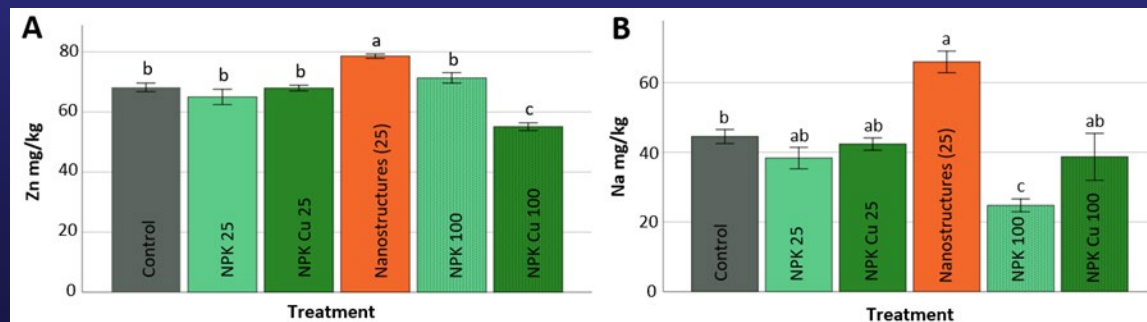
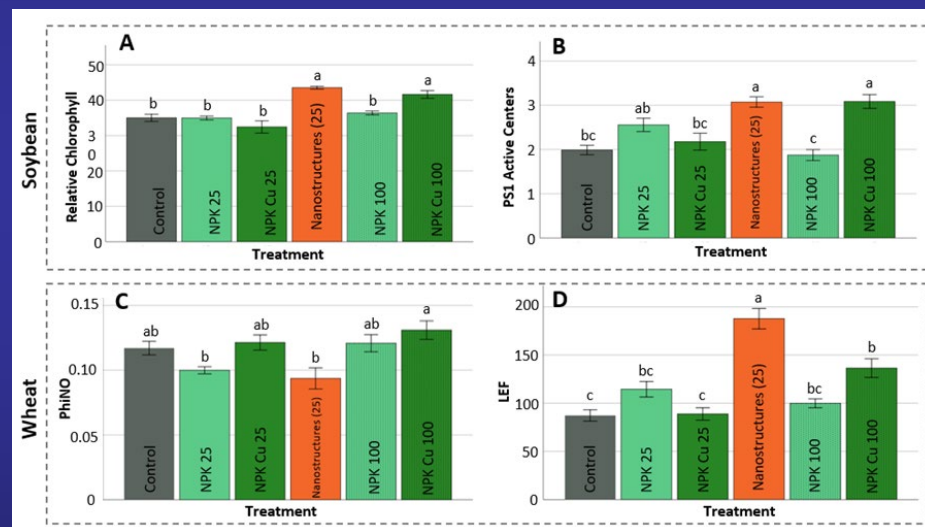


# Responsive Nanocapsules



- Amendment of the responsive nanostructure at 25% NPK/Cu resulted in enhanced photosynthetic parameters in both soybean and wheat, as compared to conventional fertilizer controls at 100% the label rate.
- Moreover, the Zn and Na content in the leaves of 4-week old soybean seedlings were significantly increased with nanostructure amendment, indicating that NPK and Cu in this nanoscale form can potentially be used to modulate the accumulation of other important micronutrients as part of a potential biofortification strategy.
- This responsive core/shell nanostructure represents a novel and significant advance in the development of precision sustainable agriculture

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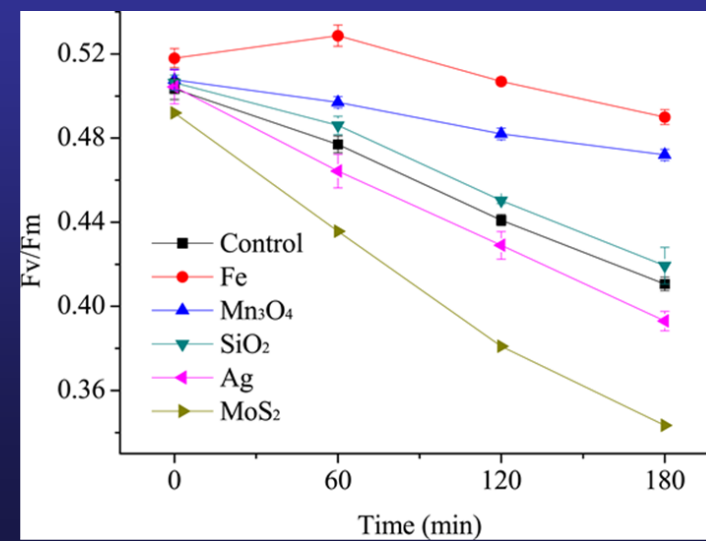
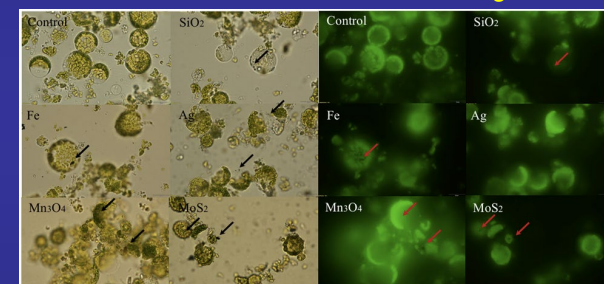


# Enhancing Photosynthesis

- Conducted in collaboration with Nanjing Univ., Nanjing Technical Univ., and the Univ. of Texas El Paso
- Some NPs have exhibited potential for promoting photosynthesis and this could potentially enhance crop productivity.
- Understanding the fundamental interactions between NPs and plants is crucial for the sustainable development of nano-enabled agriculture.
- Spinach leaf mesophyll protoplasts were cultivated with NPs (Fe,  $Mn_3O_4$ ,  $SiO_2$ , Ag, and  $MoS_2$ ) at 50 mg/L for 2 hours under illumination.
- Endpoints- maximum quantum yield, ATP production, photoelectrochemical measurements and GC-MS based metabolomics
- Whole plant exposure for comparison
- Photosynthetic efficiency (maximum quantum yield) was significantly increased by  $Mn_3O_4$  and Fe NPs and decreased by NP Ag and  $MoS_2$



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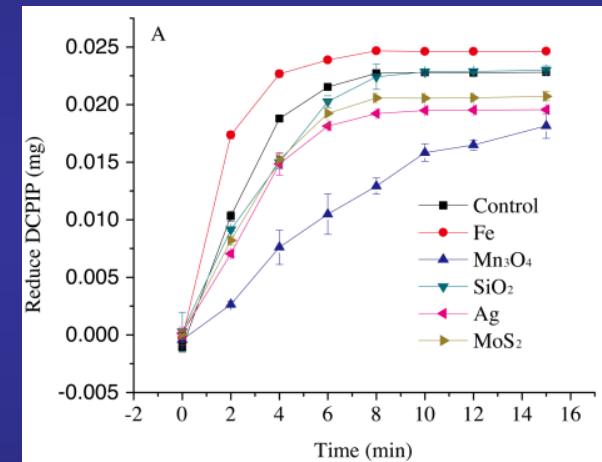




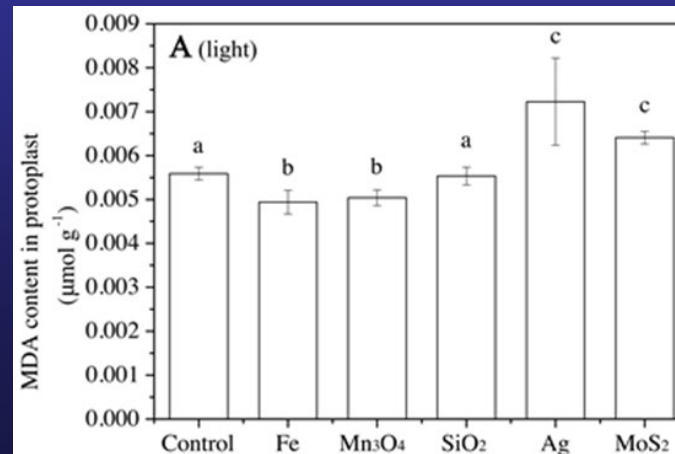
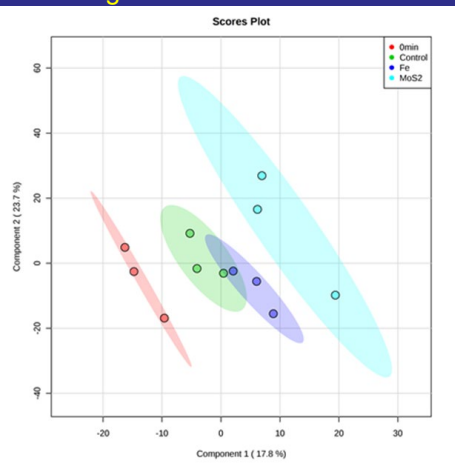
# Mechanism of Enhanced Photosynthesis?

- The Hill reaction was performed; the dye DCPIP intercepts electrons in the thylakoid membrane and is an indicator of photosynthesis.
- NP Fe increased DCPIP reduction; NP Fe and  $Mn_3O_4$  increased ATP production
- NP Ag and  $MoS_2$  decreased ATP production
- NP Fe and  $Mn_3O_4$  decreased lipid peroxidation; NP Ag and  $MoS_2$  increased lipid peroxidation
- Clear separation of metabolite profiles with NPs

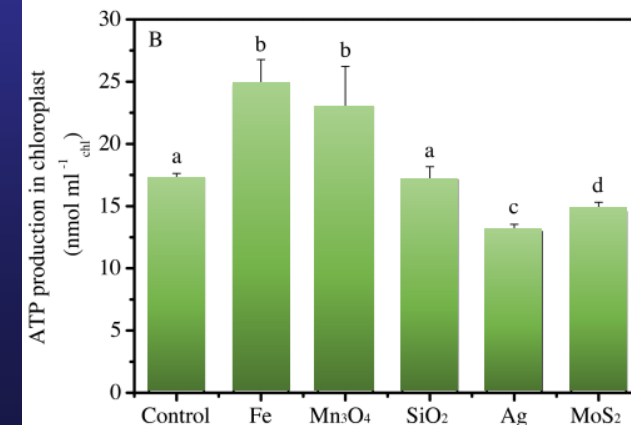
Wang et al. 2020 *J. Agric. Food Chem.* 68:3382-3389.



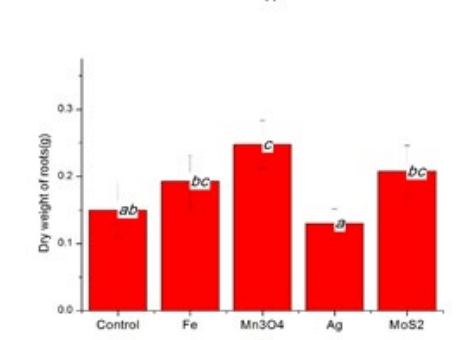
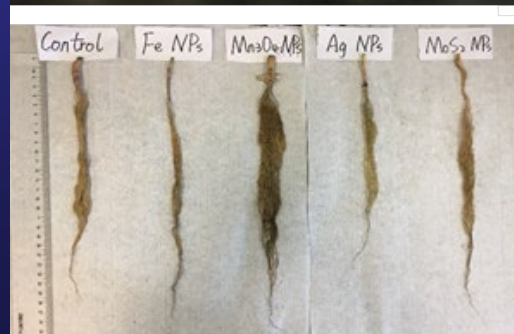
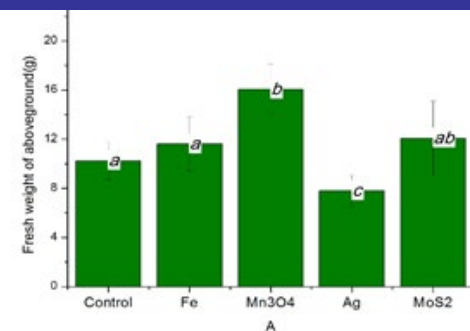
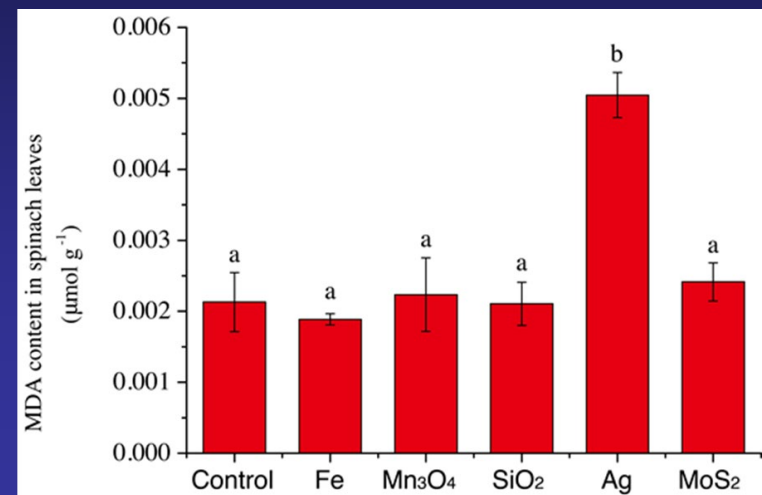
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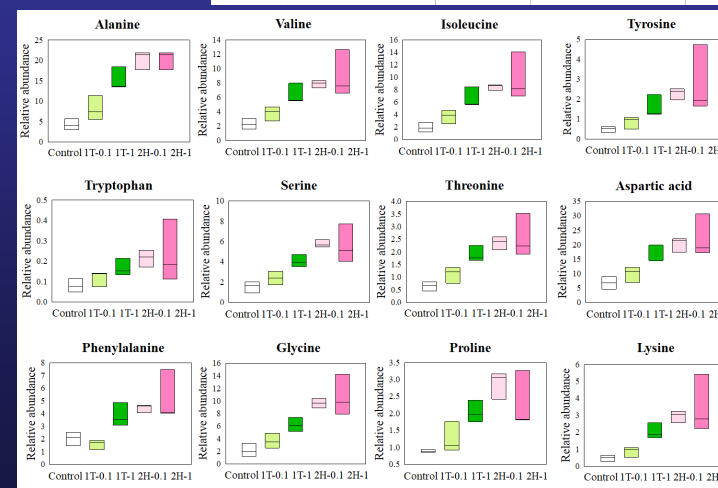
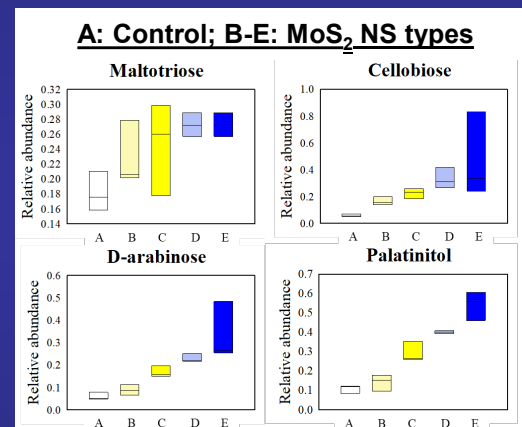
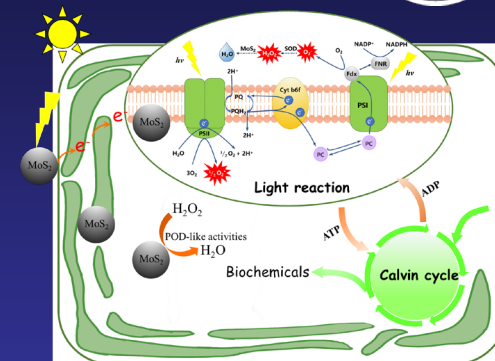
- MDA content was measured in leaves; Ag induced significant lipid peroxidation.
- Phenotypic images of spinach leaves and roots (A and B); fresh biomass of leaf and root (C and D).
- $Mn_3O_4$  significantly increased spinach leaf biomass by 57%.
- NP Ag significantly decreased plant shoot biomass by 24%
- The impact of different NPs on root biomass exhibited a similar pattern to the leaves.  $Mn_3O_4$  NPs significantly increased root biomass by 65% and Ag decreased mass 13%.
- Mesophyll protoplasts are a good model





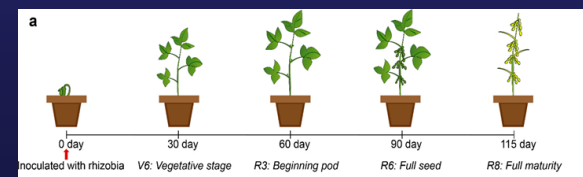
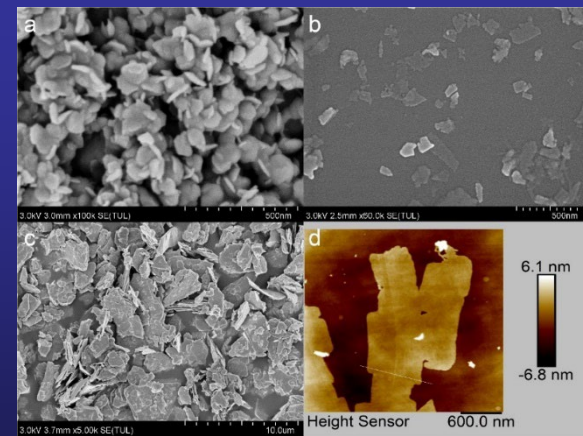
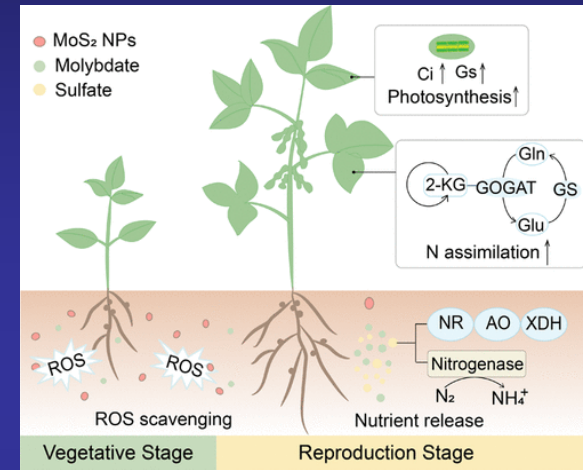
# MoS<sub>2</sub> Nanosheets and Metabolism

- The effects of molybdenum disulfide (MoS<sub>2</sub>) nanosheets (NS) on a N<sub>2</sub>-fixation cyanobacteria by monitoring growth and metabolome changes.
- MoS<sub>2</sub> NS did not exert overt toxicity at 0.1 and 1 mg/L.
- Intracellular semiconducting MoS<sub>2</sub> nanosheets absorb light and generate photo-excited electrons that are transferred to the chloroplast electron transport chain and supply reducing power
- These semiconducting properties and the enzyme-like activities of MoS<sub>2</sub> NS promoted *Nostoc* metabolism, including enhancing carbon fixation via accelerating the Calvin Cycle.
- The altered C metabolism subsequently drove proportional changes in N metabolism.
- These intracellular metabolic changes in C and N cycling could be highly useful in agriculture
- **New \$750,000 USDA AFRI grant started May 1, 2023!**



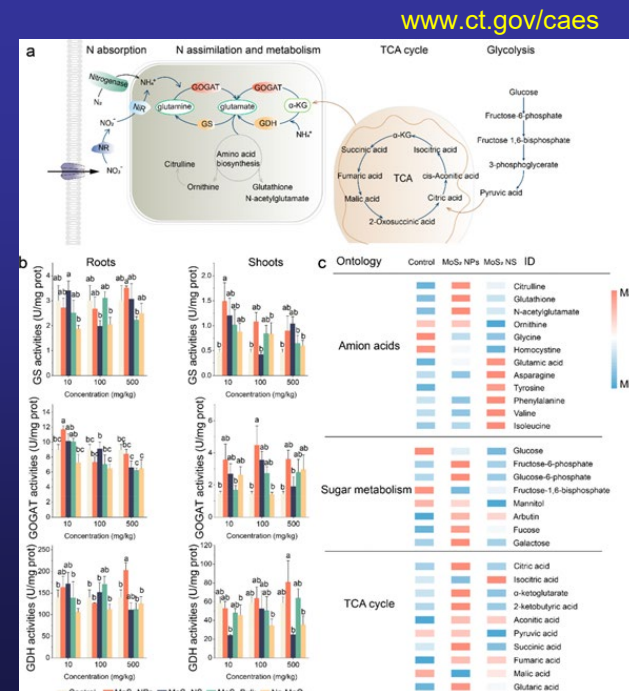
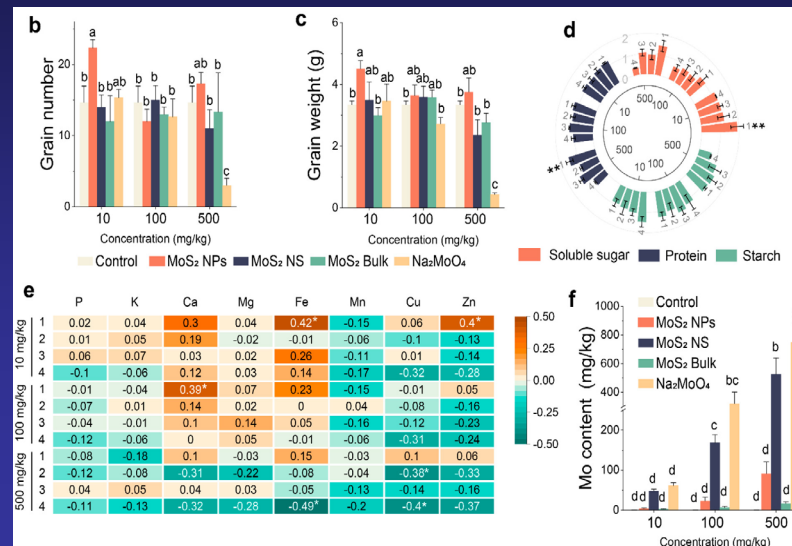
# Nanoscale MoS<sub>2</sub> and Soybean

- **Li et al. 2023.** Molybdenum nanofertilizer boosts biological nitrogen fixation and yield of soybean through delaying nodule senescence and nutrition enhancement. *ACS Nano* 17, 15, 14761–14774.
- Soybean biological nitrogen fixation (BNF) is reduced by abiotic stresses.
- Enhancing BNF can alleviate global food insecurity and reduce the environmental impact of agriculture.
- This has proven challenging using current genetic modification or bacterial nodulation methods.
- Multifunctional MoS<sub>2</sub> NP and NS, MoS<sub>2</sub> Bulk, and Na<sub>2</sub>MoO<sub>4</sub> were added to soil at 10, 100, 500 mg/kg; plants were harvested at 30, 60, 90 and 115 days
- Biomass, photosynthetic endpoints, tissue element content, nitrogen fixation enzymes, and expression of Mo-related genes were measured



# Nanoscale MoS<sub>2</sub> and Soybean

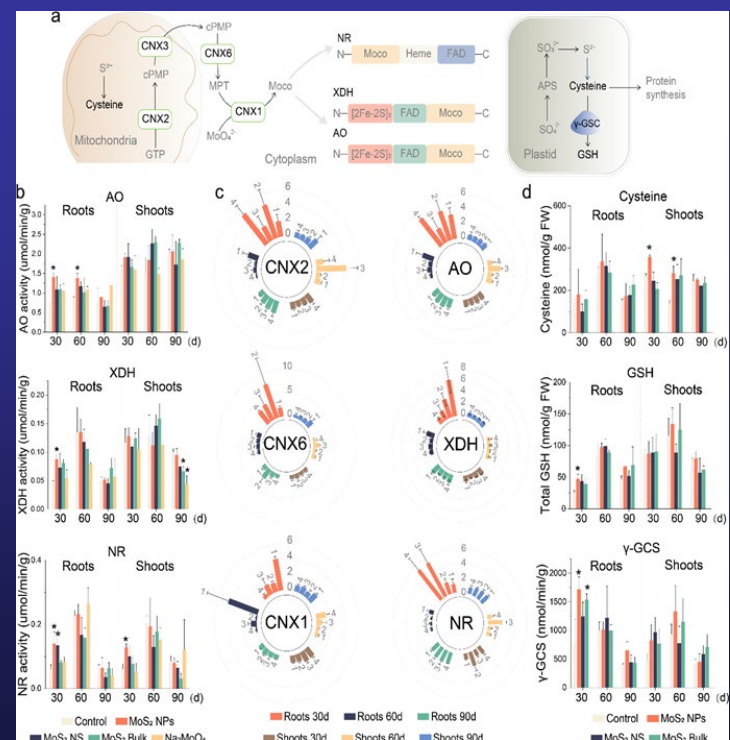
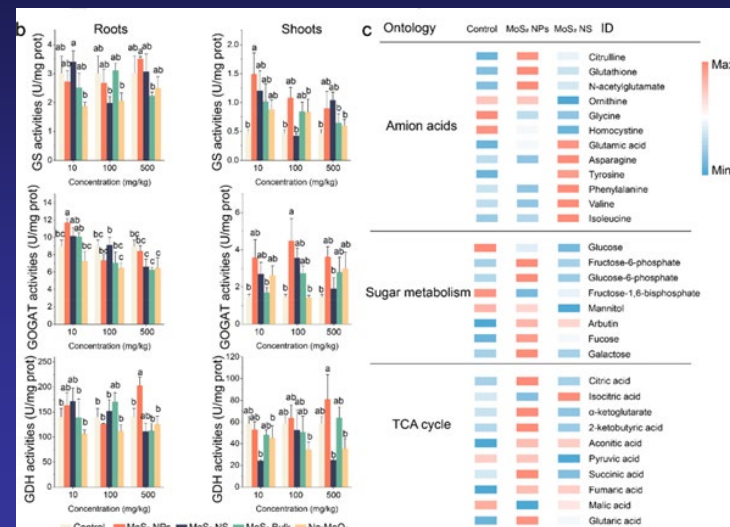
- MoS<sub>2</sub> NPs (10 mg/kg) increased the grain number and weight by 46 and 30%; protein by 46% (500 mg/kg); soluble sugar by 91% (10 mg/kg).
- The remaining treatments had little effect
- N uptake/assimilation involves glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate dehydrogenase (GDH).
- MoS<sub>2</sub> NPs (10 mg/kg) increased root GOGAT activity by 2.2- and 1.5-fold; GOGAT activity by 2.08-fold (100 mg/kg),
- MoS<sub>2</sub> NPs (10 mg/kg) increased shoot GS and GOGAT activity by 0.3 times; GDH by 0.45-fold (500 mg/kg)
- MoS<sub>2</sub> NPs (10 mg/kg) increased shoot GS and GOGAT activity by 2.2- and 1.5-fold; GOGAT activity by 2.08-fold (100 mg/kg),
- Enhanced GS-GOGAT cycle accelerated N assimilation and promoted photosynthesis through a cascade reaction, facilitating growth.





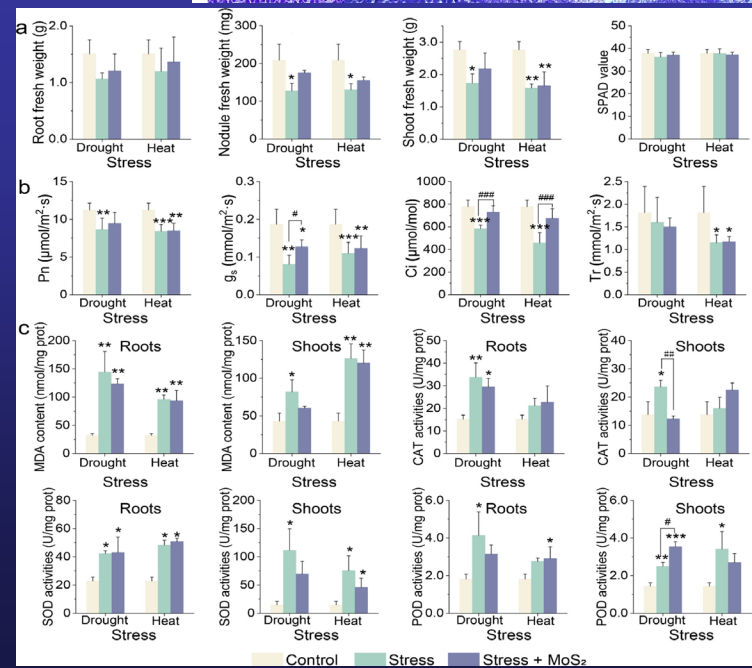
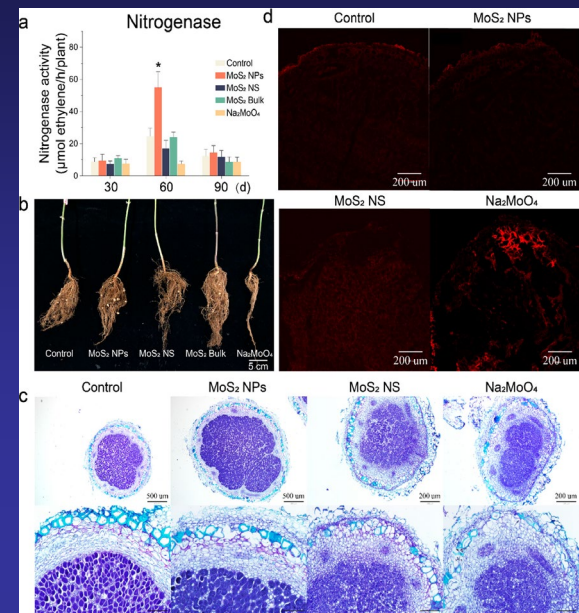
# Nanoscale MoS<sub>2</sub> and Soybean

- The leaf metabolome (500 mg/kg MoS<sub>2</sub> NPs/NS) was enriched in amino acid (N) and carbohydrate pathways (C)
- MoS<sub>2</sub> NPs increased sucrose synthesis and TCA metabolites
- MoS<sub>2</sub> NS enriched amino acids for stress response and increased sucrose breakdown
- Mo is the metal center for nitrogenase, nitrate reductase (NR), aldehyde oxidase (AO) and xanthine dehydrogenase (XDH); all are involved in N assimilation and other key pathways
- MoS<sub>2</sub> NPs increased tissue AO, XDH, NR by 64-138%; MoS<sub>2</sub> NS increased the NR 129%;
- MoS<sub>2</sub> bulk and Na<sub>2</sub>MoO<sub>4</sub> had no effect
- MoS<sub>2</sub> NPs increased CNX1 expression (310%), which mediates insertion of Mo into several proteins to achieve biological activity



# Multifunctionality of MoS<sub>2</sub> NPs

- MoS<sub>2</sub> NPs enhanced nitrogenase activity by 122% at 60 days and increased nodule growth.
- At this stage, nodule senescence had started, and N<sub>2</sub> fixation was declining but nodules treated with MoS<sub>2</sub> NPs had higher symbiont density and reduced nodule ROS
- MoS<sub>2</sub> NPs demonstrated a clear potential to enhance soybean tolerance to abiotic stress (heat, drought) by capturing the ROS and providing essential Mo and S nutrients.
- The level of oxidative damage was also reduced, as demonstrated by the reduced MDA levels and antioxidative responses
- Soybean biomass, height, and photosynthetic output were significantly improved by MoS<sub>2</sub> NPs.



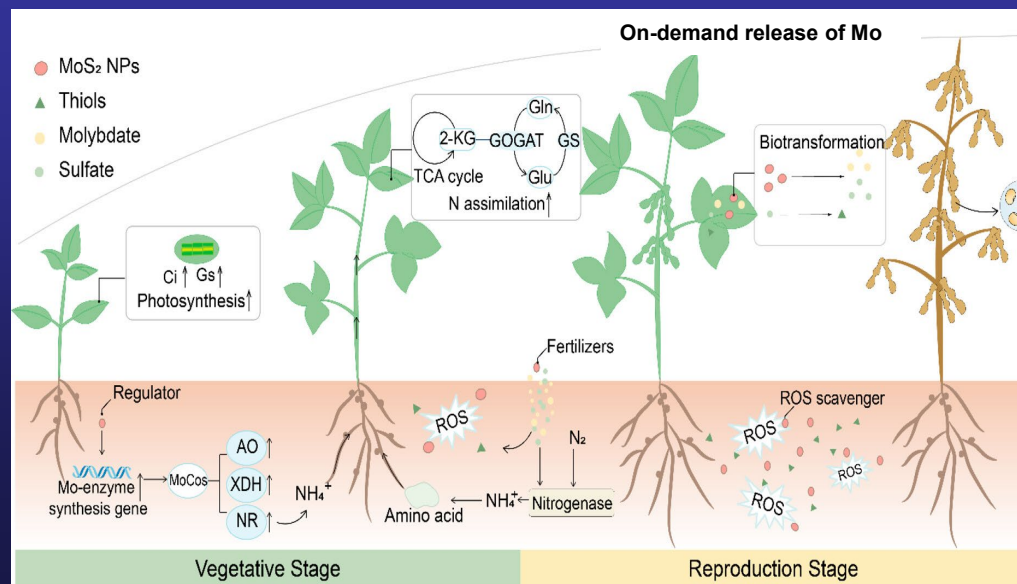


# Multifunctionality of MoS<sub>2</sub> NPs



- MoS<sub>2</sub> NPs release Mo in a responsive fashion to support BNF and capture ROS at different growth stages to promote soybean C and N assimilation.
- In the early stages (vegetative), seedlings are vulnerable to stress and MoS<sub>2</sub> NPs remain as intact particles to function as enzymes to capture the ROS while releasing a small portion of Mo to support nodule formation and N<sub>2</sub>-fixation.
- At later stages (reproductive), soybean requires large amounts of nitrogen, and nodule function starts to decline due to senescence. However, MoS<sub>2</sub> NPs continuously dissolve and release more Mo to support N<sub>2</sub>-fixation.
- The MoS<sub>2</sub> NPs act as nano-enzymes and maintain enzyme mimetic function, protecting the nodule cells and maintaining N<sub>2</sub>-fixation ability by capturing ROS and delaying nodule senescence.

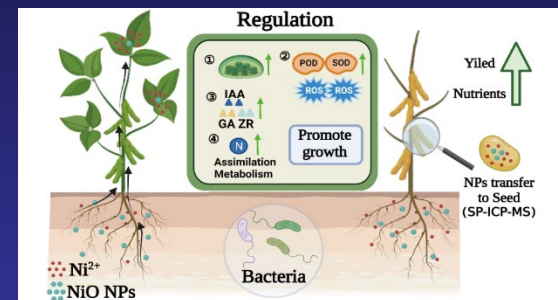
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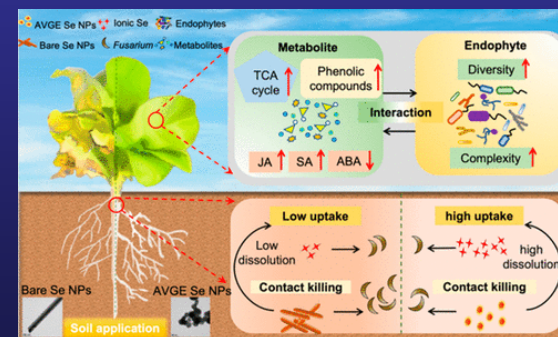
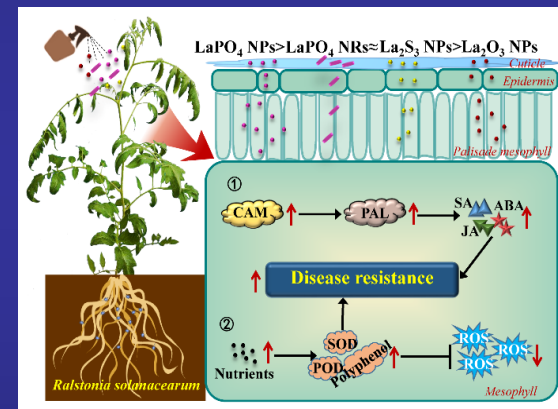
## Other Nutrients

- Zhou et al. 2023. Nickel oxide nanoparticles improve soybean yield and enhance nitrogen assimilation. *Environ. Sci. Technol.* 57, 19, 7547–7558.
- Liao et al. 2023. Potential of novel magnesium nanomaterial to manage bacterial spot disease of tomato in the greenhouse and field conditions. *Plants* 12, 1832.
- Wang et al. 2023. Lanthanum-based nanomaterials suppress bacterial wilt in tomato: Importance of particle morphology and dissolution profile. *Environ. Sci.: Nano* 10, 747-760.



## Green/Biosynthesis

- Shang et al. 2023. Aloe vera extract gel-derived selenium nanoparticles enhance disease resistance in lettuce by modulating the metabolite profile and bacterial endophytes composition *ACS Nano* 17, 14, 13672–1368472.
- Karmous et al. 2023. Biologically synthesized zinc and copper oxide nanoparticles using *Cannabis sativa* L. enhance soybean (*Glycine max*) defense against *Fusarium virguliforme*. *Pest. Biochem. Physiol.* 194, 105486
- Noman et al. 2022. Bio-functionalized manganese nanoparticles suppress *Fusarium wilt* in watermelon (*Citrullus lanatus* L.) by infection disruption, host defense response potentiation and soil microbial community modulation. Small 2205687.
- Prakash et al. 2022. Hybridization of chitosan and biosynthesized silver nanoparticles to enhance antimicrobial activity against phytopathogens in tomato (*Solanum lycopersicum*). *ACS Agric. Sci. Technol.* 2:719-733.
- Ahmed et al. 2022. Bioengineered chitosan-iron nanocomposite controls bacterial leaf blight disease by modulating plant defense response and nutritional status of rice (*Oryza sativa* L.). *Nano Today* 45, 101547



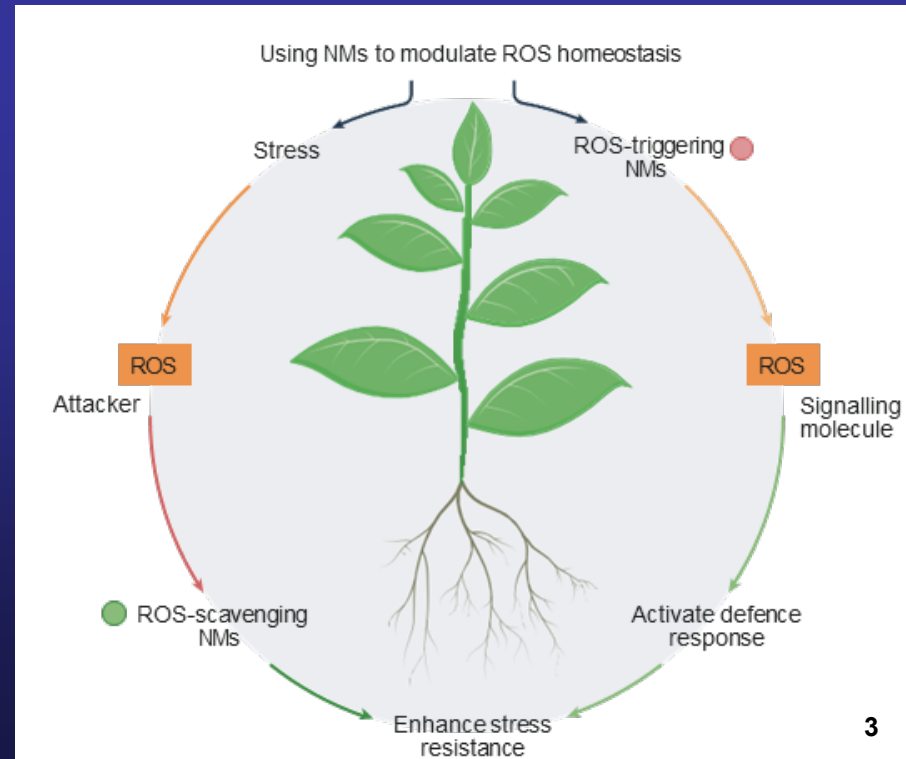


# Nanomaterial Exposure and ROS

- Reactive oxygen species (ROS) are generated during basic cell metabolism but also as a function of xenobiotic exposure
- ROS can damage biomolecules (DNA, proteins) and organelles
- Although cells have systems to quench ROS, they are also important intracellular signaling molecules that can stimulate plant defense pathways

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- Can we use ROS-scavenging and ROS-triggering nanomaterials to modulate ROS homeostasis for stress tolerance enhancement?
- The left pathway is “recovery or curative” (**lots of data here**)
- The right pathway is a “preventive” strategy (?)



# ROS-Triggering/Stress Training?

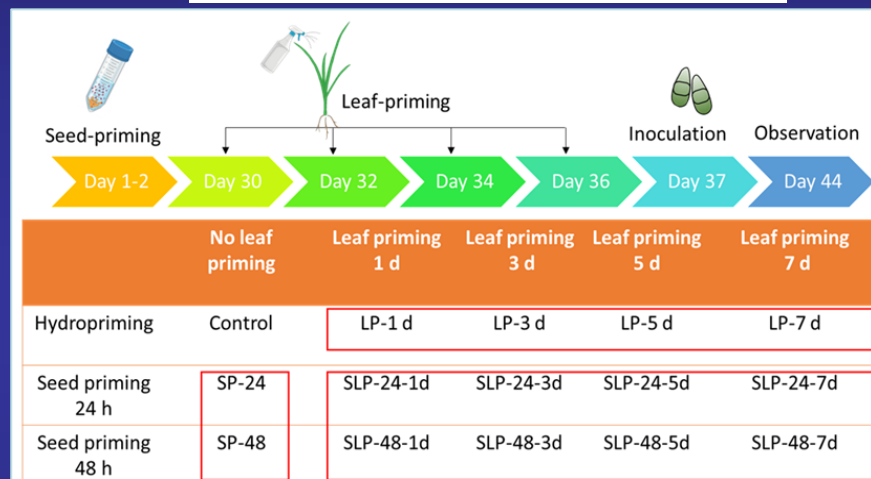
- Chen et al. 2023. *ACS Nano* 17, 11, 10760–10773
- Three regimens of AgNPs-based “stress training”: seed priming (SP), leaf priming (LP), and combined seed- and leaf- priming (SLP) (40 mg/L)
- Trained rice seedlings were subsequently exposed to either rice blast fungus (*M. oryzae.*) or cold stress (10°C).
- Metabolomic and transcriptomic profiling were used to understand the mechanisms and kinetics of plant response.

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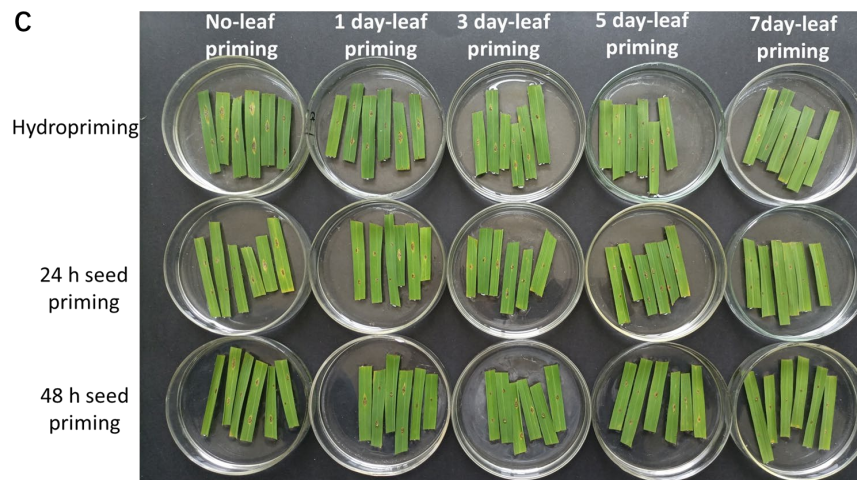
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## Engineering Climate-Resilient Rice Using a Nanobiostimulant-Based “Stress Training” Strategy

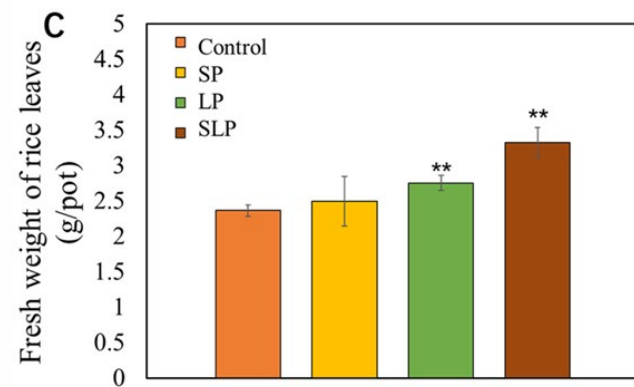
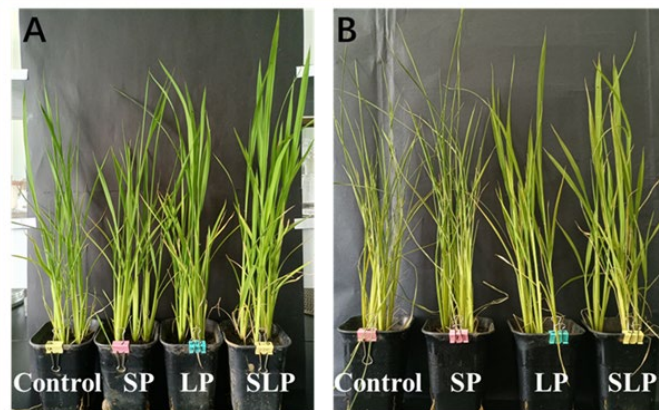
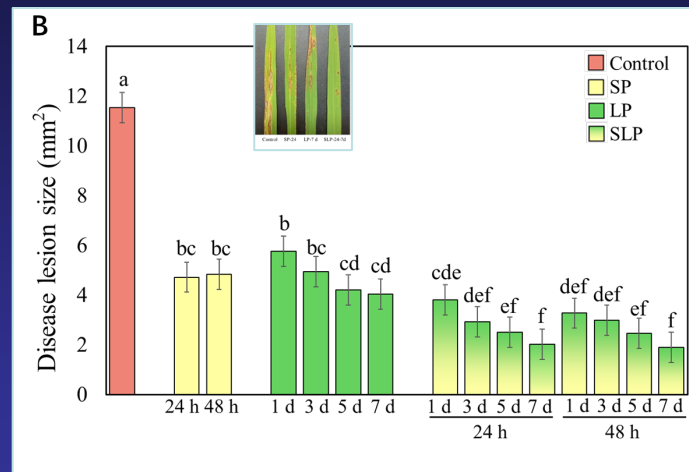
Si Chen, Zhengyan Pan, Weichen Zhao, Yanlian Zhou, Yukui Rui, Cong Jiang, Yi Wang, Jason C. White, and Lijuan Zhao\*



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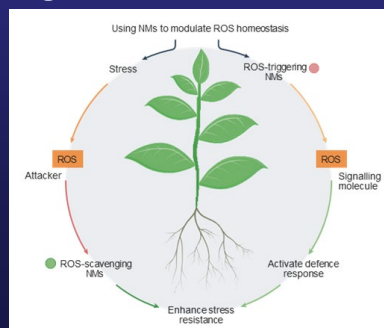
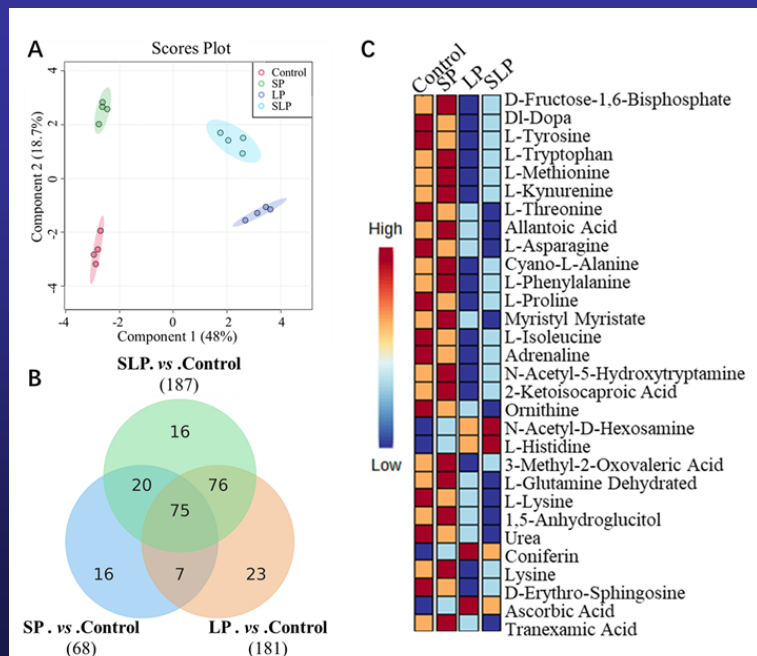
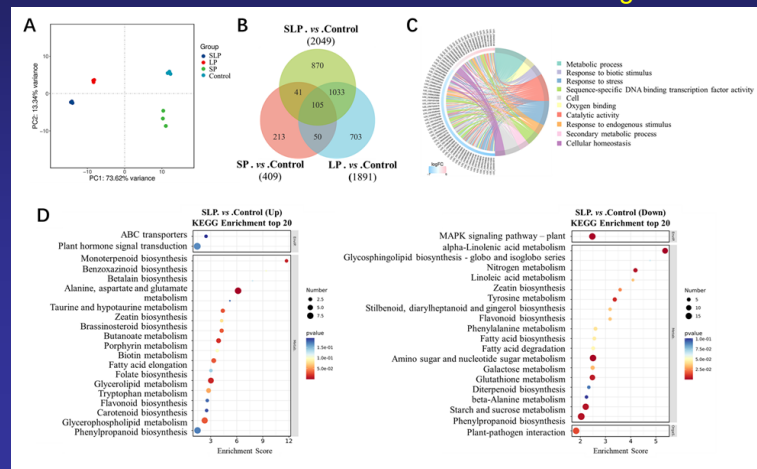


- All “stress training” regimes, particularly SLP, significantly enhanced fungal pathogen resistance (lesion size reduced by 82% relative to un-trained control).
- Tolerance to cold stress was also significantly enhanced (leaf biomass increased by 35%).
- Metabolomic and transcriptomic profiling shows that “stress training” induced considerable metabolic and transcriptional reprogramming in rice leaves.



- AgNPs-boosted ROS activated stress signaling pathways by oxidative post-translational modifications of stress related kinases, hormones, and transcriptional factors (TFs).
- These signaling pathways subsequently modulated defense gene expression, including specialized metabolites (SMs) biosynthesis, cell membrane lipid metabolism, and pathogen-plant interaction.
- AgNPs-triggered metabolic and transcriptional reprogramming enable a more rapid and intense response to future stresses.

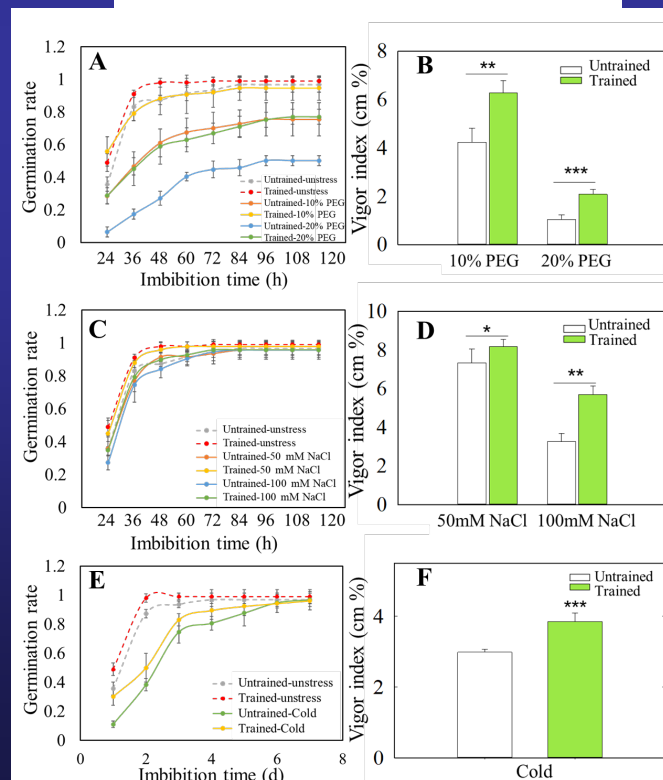
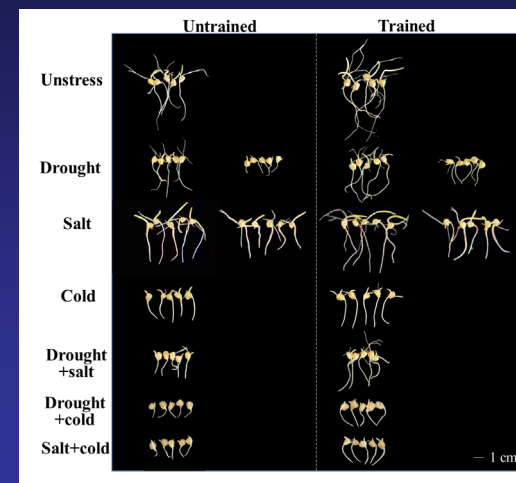
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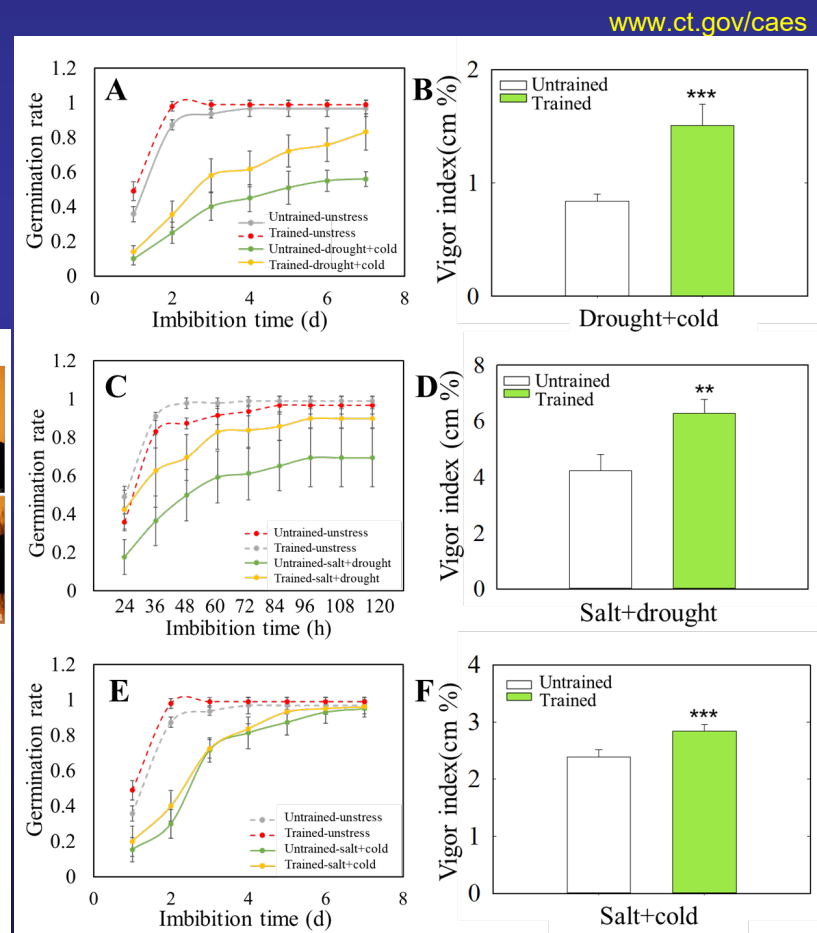
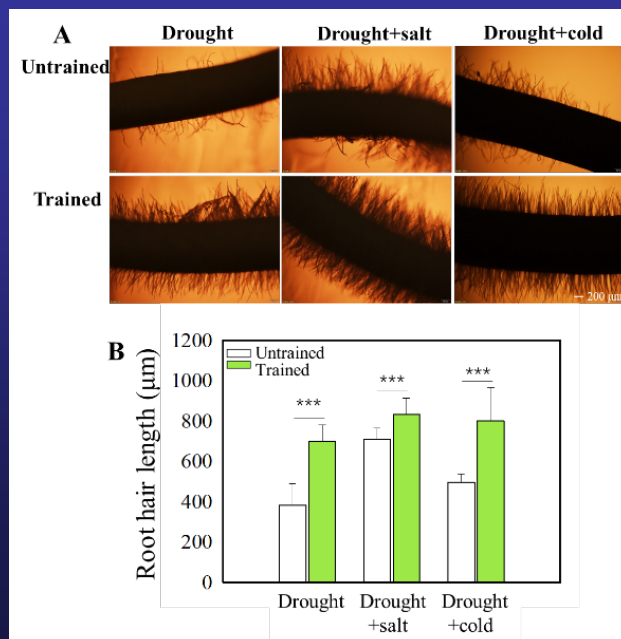
# ROS-Triggering/Stress Training

- Chen et al. 2023. Seed-priming with ROS-generating nanoparticles enhanced maize tolerance to multiple abiotic stresses. *Environ. Sci. Technol.* In press
- Maize seeds treated or primed with 40 mg/L nanoscale Ag, Fe<sub>2</sub>O<sub>3</sub> or Fe<sub>3</sub>O<sub>4</sub> were subjected to single (drought, salinity, cold) or multiple (drought + cold, drought + salt, salt + cold) stresses.
- Seeds exhibited accelerated germination speed; increased germination rate, seedling vigor, and seedling growth under drought (10%-20% PEG), salinity (50-100 mM NaCl), and cold (15 °C) stress
- Demonstrates enhanced resilience to diverse stress.



# ROS-Triggering/Stress Training

- Importantly, maize resistance to simultaneous multiple stresses was significantly enhanced.
- Under drought conditions, seed training significantly boosted root hair density and length (%), which enabled greater tolerance to water deficiency in combination with salinity or cold stress.

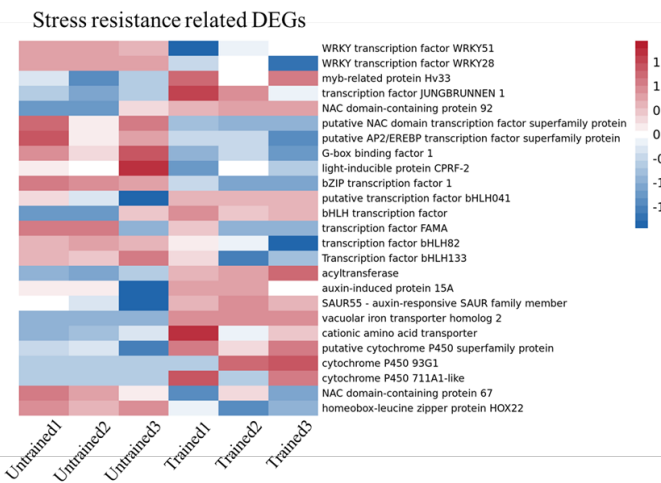
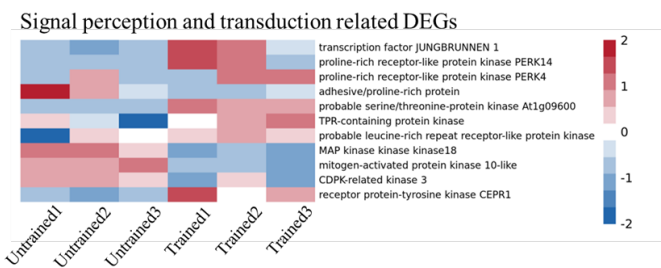


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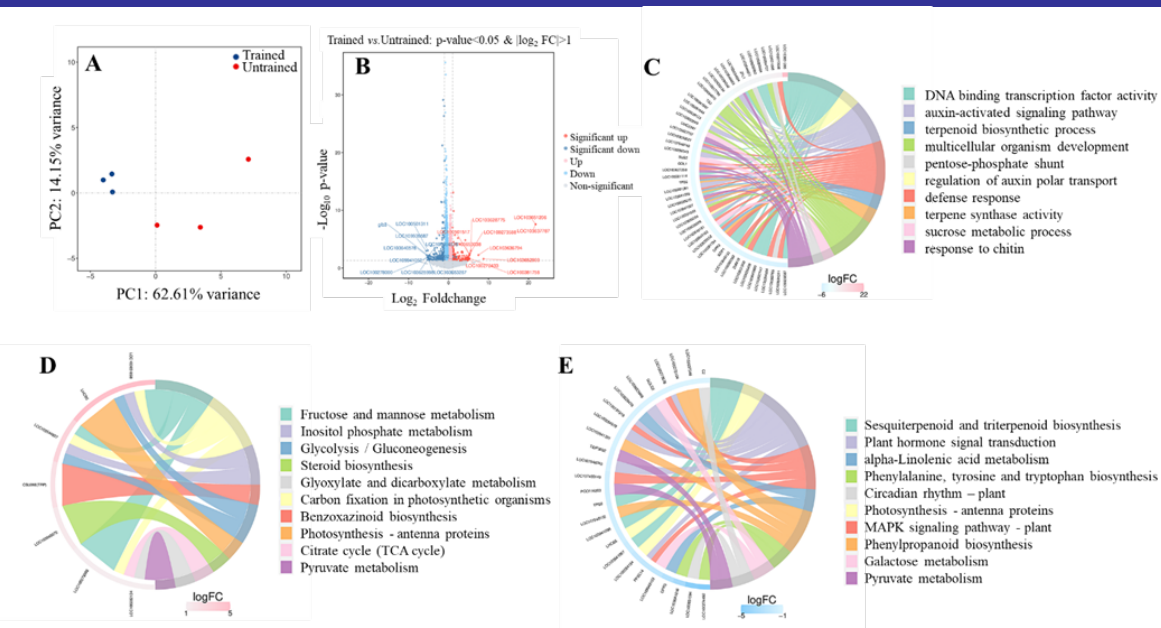
# ROS-Triggering/Stress Training

- RNA-seq analysis reveals that Ag/Fe NPs seed training induced transcriptomic shift in maize seeds.
- Plant hormone signal transduction and MAPK signaling pathways were activated and maintained through seedling growth.

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Chen et al 2023 *Environ. Sci. Technol.* In press



➤ Nano-enabled responsive nanopesticides for sustainable agriculture and global food security

Dengjun Wang, Navid B. Saleh, Andrew Byro, Richard Zepp, Endalkachew Sahle-Deemessie, Todd P. Luxton, Kay T. Ho, Robert M. Burgess, Markus Flury, Jason C. White, and Chunming Su

➤ A meta-analysis on the key properties of nanopesticides in controlling agricultural pests compared to their conventional analogs (36,658 Google Patents; 500 peer-reviewed papers between 2015 and 2021).

➤ The analysis shows that compared to conventional pesticides, their overall efficacy against target organisms is 31.5% higher, including an 18.9% increased efficacy in field trials.

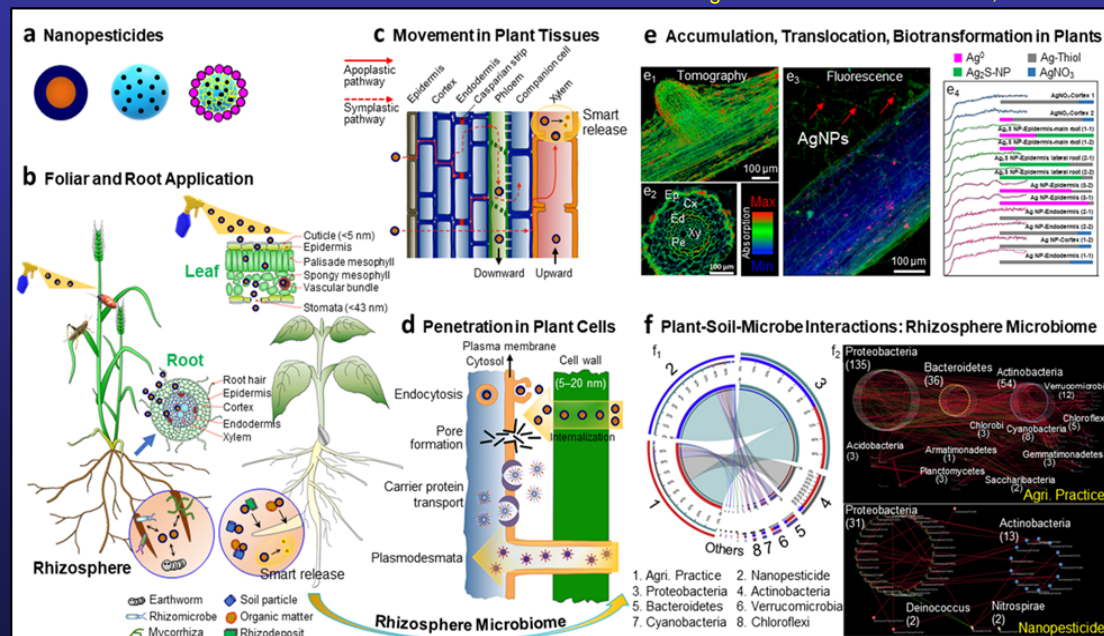


**nature nanotechnology** ANALYSIS  
<https://doi.org/10.1038/s41565-022-00083-8>  
 Check for updates

**Nano-enabled pesticides for sustainable agriculture and global food security**

Dengjun Wang<sup>1,2,3,4</sup>, Navid B. Saleh<sup>5,6</sup>, Andrew Byro<sup>7</sup>, Richard Zepp<sup>8</sup>, Endalkachew Sahle-Deemessie<sup>9</sup>, Todd P. Luxton<sup>3</sup>, Kay T. Ho<sup>3</sup>, Robert M. Burgess<sup>4</sup>, Markus Flury<sup>10</sup>, Jason C. White<sup>8</sup> and Chunming Su<sup>11,12</sup>

Deng et al. 2022 *Nature Nano.* 17, 347–360



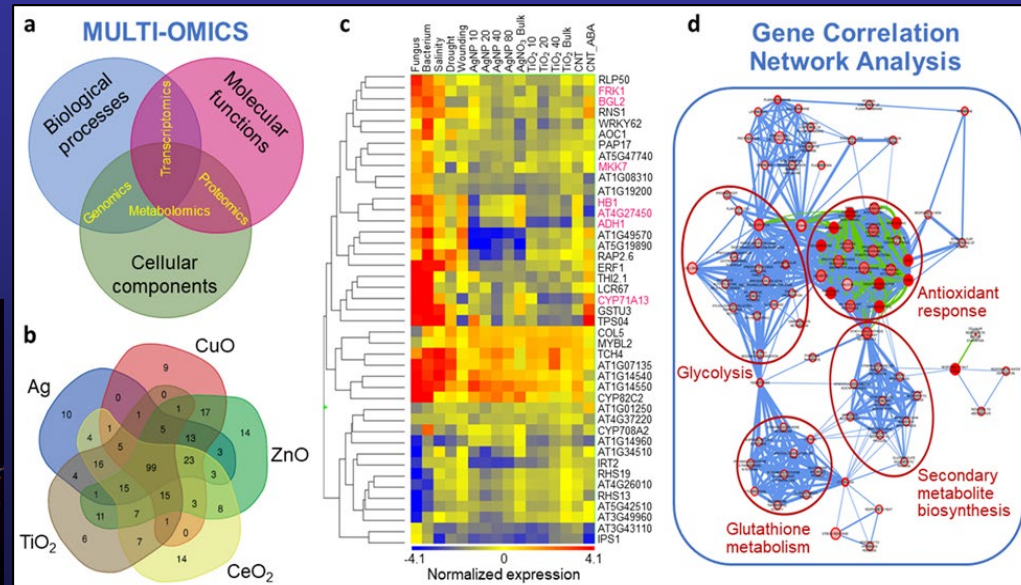


# Nanopesticide Efficacy- US EPA 2022

- Nanopesticides toxicity toward nontarget organisms is 43.1% lower
- The premature loss of AIs prior to reaching target biota is reduced by 41.1%, paired with a lower leaching potential of AIs by 22.1% in soils.
- Other benefits include enhanced foliar adhesion, improved crop yield and nutrition, and intelligent/responsive nanoscale delivery platforms of AIs to mitigate biotic and abiotic stresses (e.g., heat and drought).
- Uncertainties associated with the adverse effects of some nanopesticides are not well-understood and require further investigation.

Deng et al. 2022 *Nature Nano.* 17, 347–360

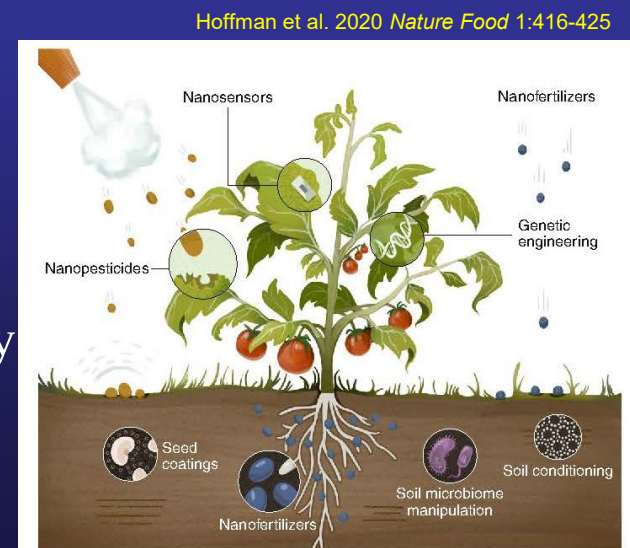
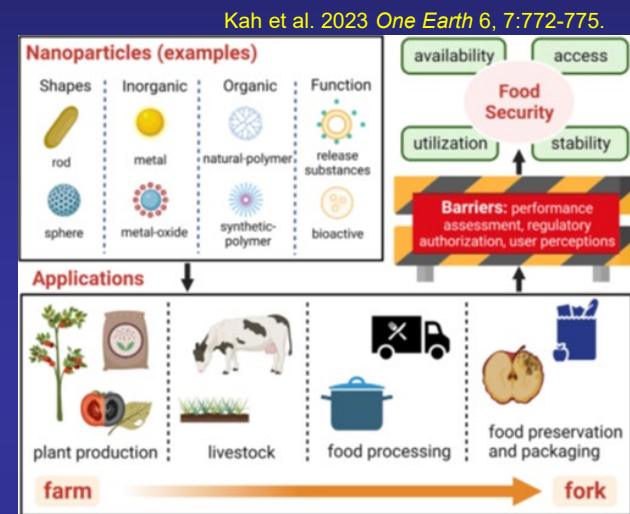
- Overall, nanopesticides are potentially more efficient, sustainable, and resilient with less environmental impact





# Conclusions

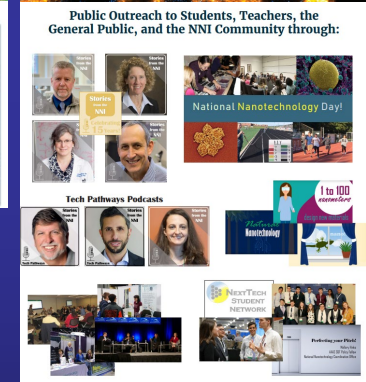
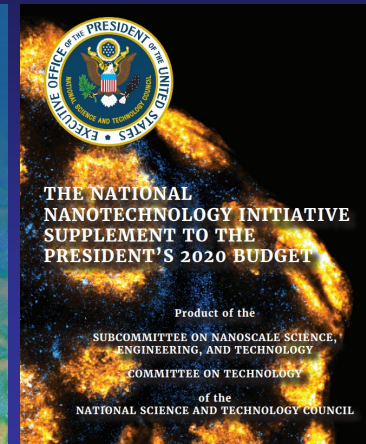
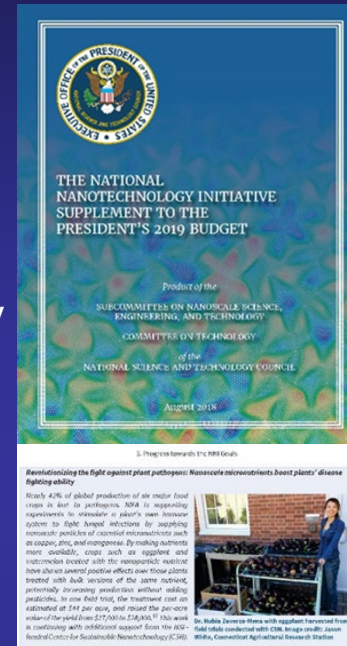
- Nanotechnology has the potential to dramatically improve agriculture; to literally help feed the world
- Nanoscale materials can be used to promote plant health to deter/suppress disease, to more precisely and efficiently deliver nutrients, promote photosynthesis, and increase abiotic stress tolerance
- 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!
- An understanding of mechanisms of action/interaction is needed to enable accurate risk assessment
- This includes an understanding of potential secondary yet significant effects, such as those in the microbiome





# Acknowledgements

- **Demokritou et al.-** Rutgers/Harvard Univ. TH Chan School Public Health
- **Jaisi et al.-** UDel; **Ma et al.-** Guangdong Univ.of Technol.
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- **Hamers et al.-** NSF Center for Sustainable Nanotechnology
- **Gardea-Torresdey et al.-** UTEP; **Keller et al.-** UCSB
- **Marmioli et al.-** Univ. of Parma, Italy; **Liu et al.-** CAS
- **Cao et al.-** CAAS; **Wang et al.-** Jiangnan Univ.
- **Paret et al.-** Univ. Fl.; **Lin et al.-** Zhejiang Univ.
- **Rui et al.-** China Agricultural Univ.; **Chen et al.-** RISF CAF
- **Tang et al.-** Guangxi Univ; **Wang et al.-** Huazhong Univ. of Sci. and Technol.;
- **At CAES-** Elmer, Dimkpa, Wang, Deng, Vaidya, De la Torre-Roche, Servin, Ma, Mukherjee, Zuverza-Mena, Shen, Tamez, Adisa, Borgatta, Majumdar, Wang, Pagano, Hawthorne, Musante, Thiel
- **Funding-** USDA NIFA AFRI, USDA Hatch, FDA FERN, CSN/NSF



5. Progress towards FY1988 Goals

**Revolutionizing the fight against plant pathogens: Nanoscale microstructures boost plants' disease fighting ability**

Nearly 40% of global production of six major food crops is lost to pathogens. NIFA is supporting experiments to measure a plant's own defenses to fight fungal infections by applying nanoscale particles of calcium microstructures such as copper, zinc, and manganese. By making nutrients more available, crops such as eggplant and tomatoes treated with the nanoscale particles show above average positive effects over those plants treated with bulk versions of the same nutrient, potentially increasing production without adding pesticides. In one field trial, the treatment cost an additional \$14 per acre, and raised the per-acre value of the yield from \$27,500 to \$28,600. This work is continuing with additional support from the Agricultural Center for Sustainable Nanotechnology (CSN).

Dr. Sukho Joo and team with eggplant harvested from field trials conducted with CSN. Image credit: Jason Wilhoit, Connecticut Agricultural Experiment Station.

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