# ANNUAL REPORT- AWARD 2016-67021-24985

## Year 1 Annual Year Report: March 1, 2016- February 28, 2017

## "NANOSCALE ELEMENTS SUPPRESS PLANT DISEASE, ENHANCE MACRONUTRIENT USE EFFICIENCY, AND INCREASE CROP YIELD"

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## 1. BRIEF REPORT OVERVIEW-

**A. Grant Objectives**- The primary goal is to use nanoscale elements as a comprehensive agricultural amendment strategy to suppress crop disease, improve nutritional content, and enhance overall yield. Soil pathogens significantly limit agricultural production, reducing crop yield by 10-20% and resulting in billions of dollars in annual losses. Separately, pathogen control efforts by fungicides exceed $600 million per year in the US. At the same time, macronutrient utilization remains low due to environmental losses and low availability. This shortfall in food production will worsen with a changing climate and an increasing population. Nanotechnology can play a critical role in maximizing global food production and achieving food security. Current approaches have focused on nano-enabled conventional agrichemicals, nanosensors, and waste treatment strategies. However, little is known about the effects of nanoparticle (NP) elements on disease suppression, macronutrient uptake, and crop growth. For example, plant micronutrients are pivotal in disease resistance through activation of defense barrier production and by affecting the systemic acquired resistance pathway. Unfortunately, element availability in soil is limited and foliarly applied micronutrients are not significantly translocated to roots. Although NP intra plant translocation has been reported, there is no information on whether enhanced translocation of NP elements can deter root or shoot pathogens. Based on preliminary data and a recent review of the literature, our central hypothesis is that NP elements can be used to strategically suppress disease, improve nutritional status and enhance crop growth and yield. Our four objectives are:

Objectives-

1. Demonstrate NP efficacy upon foliar or root application at suppressing fungal pathogen infection in model vegetable and grain species.
2. Determine the role of soil type and NP source in disease suppression and crop yield.
3. Determine the impact of NP element treatment on macronutrient (NPK) utilization.
4. Characterize NP absorption and translocation mechanisms in plant tissues by scanning/ transmission electron microscopy (S/TEM-EDS).

**B. General Statement of Progress-** This progress report covers the first year of our three year project. We feel that significant progress has been made on all objectives.

1. Objective 1- Objective one addresses the effect of NP foliar or root application on fungal infection in crops. At CAES, experiments investigating eggplant and tomato were initiated during the current period. First, a series of greenhouse and field experiments were established to assess combinations of nanoparticles (NP) of $CeO_2$, CuO, MnO, and ZnO on eggplant growth upon

infection with *Verticillium* wilt. In an analysis of preliminary data, all treatments performed better than control in total yield, and combinations of Mn and Zn were the most effective at suppressing the effects of disease. Tissue samples of fruit, roots, stems, and leaves are currently being processed for analysis of elemental composition by ICP-MS. A second set of greenhouse experiments with watermelon were conducted to compare NP CuO to other products. Two greenhouse studies showed that NP CuO were superior to NP B, $CeO_2$, MnO, TiO, SiO, or ZnO in suppressing *Fusarium* wilt on watermelon and for promoting growth. ICP-MS showed twice as much Cu in the roots of plants sprayed with NP of CuO than controls or other treatments.

Three field trials have been completed at CAES on watermelon. A two year study that began in 2015 was completed in Hamden, CT and compared the efficacy of NP CuO to bulk CuO, Kocide 2000 (a CuOH-containing fungicide), Cu octanoate (an organic Cu fungicide) and an untreated control. Although yield differences were not statistically significant, in both years seedling transplants sprayed with NP CuO produced the highest yield and had lowest disease ratings. ICP-MS analysis of fruit from found no differences in elemental content of the edible tissue. The third study was conducted in Griswold, CT and compared NP B, CeO, CuO, MnO, or ZnO to untreated watermelon plants in *Fusarium*-infested soil or in non-infested soil. As before, plants treated with NP of CuO produced the highest yield in infested soil, but significant differences were not observed. Transcriptomic analysis was performed on roots of watermelons treated with Cu, *Fusarium*, the combination, and then were compared to controls in three separate experiments. Significant upregulation of polyphenol oxidase genes (a Cu-activated enzyme associated with host defense) and of the Plant Resistance 1 protein (associated with resistance) were consistently observed when NP CuO and *Fusarium* were combined. This data supports our hypothesis that NP CuO may activate defense mechanisms in plants, likely via basipetal translocation of the nanoscale nutrient. This watermelon study is currently being written up as a peer reviewed submission to *Plant Disease*.

At UTEP, an experiment was set up in the greenhouse in July 2016 to examine the efficacy of root and foliar application of ZnO nanoparticle and $ZnSO_4$ to control *Fusarium* on tomato. Three week old seedlings were treated with the Zn products and were transplanted to soil; after growth for one week, seedlings were inoculated with *Fusarium oxysporum*. In this initial study, no signs of fungal infection were found in any of the treatments. This was due to either a small amount of inoculum or the strong resistance of the cultivar Tiny Tim Cherry Tomato used for the experiment. A second greenhouse experiment was established in November 2016 using the tomato variety Bush Beefsteak, which is more susceptible to *Fusarium* infection. Plants were transplanted in late November and were inoculated with a high amount of the pathogen. Different variables such as disease severity, photosynthetic parameters, production, and fruit quality were collected until full maturity; data analysis is currently underway. Co-PD Elmer visited UTEP in February of 2017 to provide additional training and guidance on handling and inoculating the fungal pathogens.

2. <u>Objective 2-</u> Objective two is investigating the importance of soil type and NP source on disease suppression and crop yield. As noted under objective one, experiments at CAES are occurring at two separate farms with two separate soil conditions. The Hamden CT farm is a fine sandy loam whereas the Griswold soil is a Windsor loamy sand. Initial data analysis is focusing on the specific trials within each farm; once that analysis is complete, a comparison of effects across the two different farms/soils will be completed. Regarding the importance of NP source, CAES has begun informal collaborations with colleagues at Washington University in St. Louis and the University of Texas-Austin; both colleagues have synthesized specific micronutrient

nanomaterials that are being evaluated along-side the commercially available materials that we have purchased.

3. Objective 3- Objective three is assessing the impact of NP amendment on macronutrient (NPK) utilization. At IFDC, a greenhouse study was established in July 2016 to evaluate the effect of NP ZnO versus Zn salts on the growth, yield and NPK use efficiency of sorghum as a function of soil versus foliar application. The plants were harvested in late November and data analysis is underway. Samples for microscopic analysis to determine presence or absence of ZnO NPs in plant shoots were sent to CAES for imaging. ICP-MS is ongoing for nutrient content. In a second experiment, a winter wheat crop was established during late November 2016 to evaluate the effect of residual ZnO nanoparticles (and Zn salt) from prior soil application versus new ZnO application on growth, yield, NPK use and Zn content enhancement in grain. In a third trial, another winter wheat crop was established during late November 2016 to evaluate the effect of NP $Mn_2O_3$ versus Mn salt and bulk Mn on growth, yield and NPK use efficiency when applied as either a soil or foliar treatment. Last, a project conducted in conjunction with US AID was completed. The study was on the effect of composite NP micronutrients (ZnO, CuO and $B_2O_3$) versus salts of these elements on soybean vegetative and reproductive development, as well as NPK use, as a function of drought stress.

4. Objective 4- Objective four is characterizing NP presence in plant tissues by electron microscopy. Tissues from the various experiments conducted at CAES and IFDC have been acquired and will be analyzed by electron microscopy. The microscope at the CT Department of Public Health has been offline for the last month; once the system is back on line, samples will be sectioned, embedded, and analyzed by CAES staff.

## 2. SCIENCE IMPACTS-

During the first year our project, we feel that significant scientific impacts were achieved. As highlighted below, eight peer reviewed publications were published that acknowledge this grant for funding; additional publications are currently under preparation. In addition, since March of 2016, investigators (or students/Post-doctoral Associates) presented project research on 19 occasions at national and international venues. Lastly, the website for our USDA NIFA AFRI Center of Excellence entitled The Center for Nanotechnology and Agricultural Pathogens Suppression (CeNAPS) has been established (http://www.ct.gov/caes/cwp/view.asp?a=4898&q=585400 ).

### A. **Publications**- (Papers that acknowledge this grant since March 2016)


1. Elmer, W., White, J.C. 2016. The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. *Environmental Science: Nano.* 3,1072-1079.

2. Peralta-Videa, J.R., Huang, Y., Parsons, J.G., Zhao, L., Lopez-Moreno, M.L., Hernandez-Viezcas, J.A., Gardea-Torresdey, J.L. 2016. Plant-based green synthesis of metallic nanoparticles, scientific curiosity or a realistic alternative to chemical synthesis? *Nanotechnology for Environmental Engineering* 1(1):4, doi: 10.1007/s41204-016-0004-5.

3. Pullagurala Venkata L. R., Hernandez-Viezcas, J.A., Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2016. Lessons learned; are engineered nanomaterials toxic to terrestrial plants? *Science of the Total Environment* 568, 470-479
4. Majumdar, S., Peralta-Videa, J.R., Trujillo-Reyes, J., Sun, Y., Barrios, A.C., Niu, G., Flores-Margez, J.P., Gardea-Torresdey, J.L. 2016. Soil organic matter influences cerium translocation and physiological processes in kidney bean plants exposed to cerium oxide nanoparticles. *Science of the Total Environment* 569-570, 201-211.
5. Dimkpa, C., Bindraban, P., Fugice, J., Agyin-Birikorang S., Singh, U., Hellums D. 2017. Composite micronutrient nanoparticles and salts decrease drought stress in soybean. *Agronomy for Sustainable Development.* DOI: 10.1007/s13593-016-0412-8.
6. Du, W., Gardea-Torresdey, J.L., Xie, Y., Yin, Y., Zhu, J., Zhang, X., Ji, R., Gu, A.K., Peralta-Videa, J.R., Guo H. 2017. Elevated $CO_2$ levels modify $TiO_2$ nanoparticle effects on rice and soil microbial communities. *Science of the Total Environment* 578, 408-416.
7. Tassi, E., Giorgetti, L., Morelli, E., Peralta-Videa, J.R., Gardea-Torresdey, J.L., Barbafieri, M. 2017. Physiological and biochemical responses of sunflower (*Helianthus annuus*) exposed to nano-$CeO_2$ and excess boron: Modulation of boron phytotoxicity. *Plant Physiology and Biochemistry* 110, 50-68.
8. Tan, W., Du, W., Barrios, A. C., Armendariz Jr. R, Zuverza-Mena, N., Ji, Z., Chang, C. H., Zink, J.I., Hernandez-Viezcas, J.A. Peralta-Videa, J.R., Gardea-Torresdey, J.L. 2017. Surface coating changes the physiological and biochemical impacts of nano-$TiO_2$ in basil (*Ocimum basilicum*) plants. *Environmental Pollution* 222, 64–72.

Manuscripts currently under review or in preparation

1. Elmer, W., Pagano, L., Gardea-Torresdey, J., Dimkpa, C., White, J.C. 2017. Impact of metal oxide nanoparticles on Fusarium suppression and watermelon growth. *Plant Disease* In preparation.

**B. Presentations**- (Note- Alternative funds were used to support much of this travel)

1. Dr. J.C. White presented a lecture entitled “Nano-related work at the CT Agricultural Experiment Station” at the SETAC Nanotechnology Advisory Group meeting as part of the SETAC Europe 26th Annual Meeting in Nantes France (May 22-26, 2016).
2. Dr. J.C. White attended the USDA Nanotechnology for Agricultural Systems Project Director Program review at Pennsylvania State University in State College and presented a posted entitled “Nanoscale nutrients suppress plant disease, increase macronutrient use efficiency, and increase yield” (June 5-7, 2016).
3. Dr. J.C. White presented an invited lecture entitled “Nanomaterials and the Food Supply: Assessing the Balance Between Applications and Implications” at the “Environmental Sciences: Water” Gordon Conference in Holderness NH (June 28-30, 2016).
4. Dr. W. Elmer gave a lecture entitled “Metal Oxide nanoparticles for management of soil-borne diseases of vegetables” at a Symposium on Nanoparticles in Agriculture held during

the Annual Meeting American Phytopathology Society in Tampa FL (July 30-August 3, 2016).

5. Dr. J.C. White presented an invited lecture entitled "Engineered Nanoparticles in Food: Implications for Food Safety and Consumer Health" at the International Association for Food Protection (IAFP) annual meeting in St. Louis MO (August 1-4, 2016).
6. Dr. J.C. White presented a lecture entitled "Nanoscale Nutrients Suppress Plant Disease and Increase Crop Yield" at the 252$^{nd}$ American Chemical Society (ACS) annual meeting in Philadelphia PA (August 21-24, 2016).
7. Dr. J.L. Gardea-Torresdey gave an invited lecture entitled "Environmental implications of nanomaterials in the environment. Effects of nanoceria in common bean: A spectroscopic and proteomic analysis" at the 13th International Phytotechnologies Conference Hangzhou, China (September 26-29, 2016).
8. Dr. W. Elmer gave a lecture entitled "The effect of CuO nano particles on *Fusarium* wilt of watermelon" at the Northeastern Division of the American Phytopathological Society meeting in Ithaca, NY (October 19-21, 2016).
9. A CAES Post-doctoral Associate presented a lecture entitled "Nanoscale micronutrients suppress plant disease and increase crop yield" at the 5th annual meeting of the Sustainable Nanotechnology Organization in Orlando FL (November 10-12, 2016).
10. A UTEP graduate student (I.A. Medina-Velo) presented at poster entitled "Comparison of the effects of ZnO nano/bulk materials on bean plants grown in different soil types" at the Fifth Annual Sustainable Nanotechnology Organization Conference (SNO) in Orlando, FL (November 10-12, 2016).
11. A UTEP graduate student (S. Rawat) presented at poster entitled "Phyto-toxicological effects of copper nanoparticles on bell pepper (Capsicum annuum) plants" at the Fifth Sustainable Nanotechnology Organization Conference (SNO) in Orlando, FL (November 10-12, 2016).
12. Dr. J.C. White presented an invited lectured entitled "Nanomaterials and the Food Supply: Assessing the Balance Between Applications and Implications" at the International Conference on Nanotechnology Applications and Implications of Agrichemicals Towards Sustainable Agriculture and Food Systems" in Beijing China (November 17-18, 2016).
13. Dr. C. Dimkpa presented an invited lectured entitled "Nanotechnology in Crop Fertilization: What Would Motivate the Fertilizer Industry?" at the International Conference on Nanotechnology Applications and Implications of Agrichemicals Towards Sustainable Agriculture and Food Systems" in Beijing China (November 17-18, 2016).
14. Dr. J.L. Gardea-Torresdey presented an invited lecture entitled "Environmental implications of nanomaterials in the environment. Effects of nanoceria in common bean: A spectroscopic and proteomic analysis" at the NANOMXCN-2016 "Mexico-China Workshop II on Renewable Energy and Environmental Remediation" which was held at the City University of Hong Kong, Hong Kong, China (December 4-6, 2016).
15. Dr. J.L. Gardea-Torresdey presented an invited lecture entitled "Environmental Implications of nanomaterials in the environment. Effects of nanoceria on common bean:

A spectroscopic and proteomic analysis" at the Department of Environmental Science and Engineering, Nanjing Agricultural University, Nanjing, China (January 11, 2017).

16. Dr. J.C. White presented an invited lecture entitled "Nanomaterials and the Food Supply: Assessing the Balance Between Applications and Implications" at the University of Connecticut Department of Pathobiology and Veterinary Science Seminar Series in Storrs CT (January 19, 2017).
17. Dr. J.C. White presented an invited lecture entitled "Nanomaterials and the Food Supply: Assessing the Balance Between Applications and Implications" at the University of Nebraska Water Center Seminar Series in Lincoln NE (January 31-February 1, 2017).
18. Dr. W. Elmer gave an invited lecture entitled "Metal Oxide nanoparticles for management of soil-borne diseases of vegetables" at the University of Texas El Paso (February 17, 2017).
19. Dr. J.C. White presented an invited lecture entitled "Nanomaterials and the Food Supply: Assessing the Balance Between Applications and Implications" at the University of Connecticut Department of Nutritional Sciences Seminar Series in Storrs CT (February 27, 2017).

**C. <u>Discoveries</u>**- Several significant discoveries were made during the first year of the project. First, in a greenhouse study investigating watermelon growth when infected with *Verticillium*, plants were pretreated with nanoscale oxides of Cu, Mn, Si, Ti, or Zn prior to planting in soils (Figure 1). The findings show that NP CuO significantly suppressed the fungal disease, leading to a 30-40% increase in plant biomass. In a follow up study in the field, amendments of NP B, $CeO_2$, ZnO, MnO, and CuO were made as foliar applications during growth (Figure 2); data analysis is ongoing. Figure 3 shows a recent experiment being conducted by the UTEP graduate student and Figure 4 is a photograph of Dr. W. Elmer (CAES CoPI) during a recent presentation at UTEP.

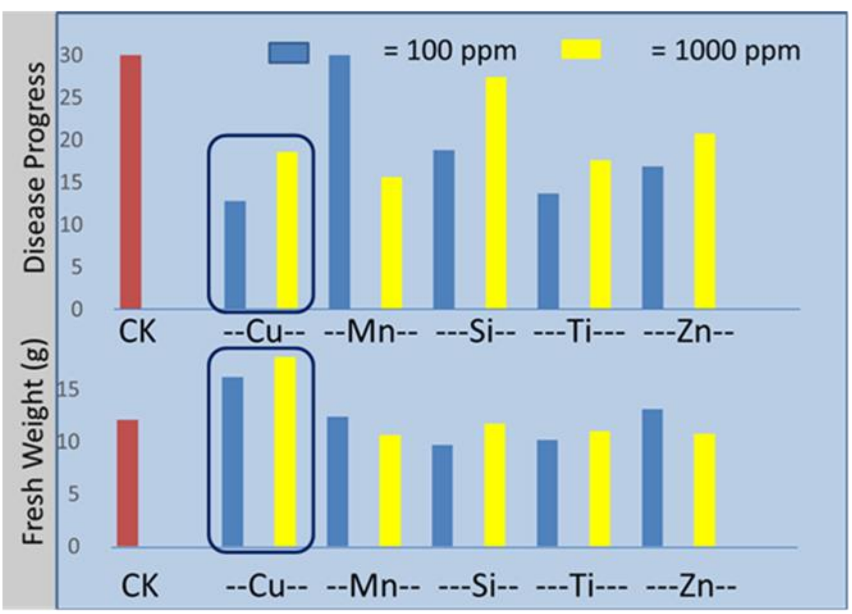



Figure 1. Impact of NP micronutrient oxides on watermelon infected with Verticillium wilt in a greenhouse study.

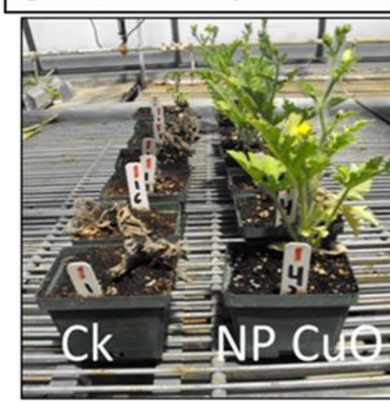


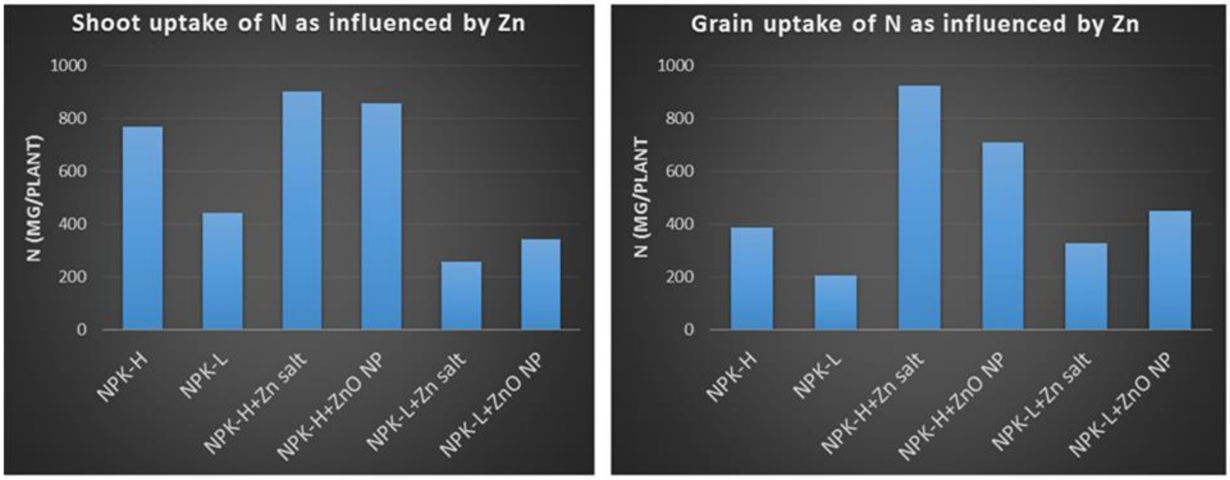
Figure 2. Field study involving watermelon growth in *Verticillium* infested soil.

Figure 3. Greenhouse study at UTEP involving ZnO NPs, Tiny Tim Cherry Tomato, and the fungus *Fusarium*. This figure shows the greenhouse cultivation of the tomato seedlings and the transplant for further plant development.

Figure 4. Dr. Wade Elmer presentation at UTEP. Dr. Elmer explains recent results about the crop disease suppression and potential yield increase with the use of nanoparticles.


Last, in IFDC greenhouse growth studies involving sorghum, preliminary data show that shoot (Figure 5; left panel) and grain (Figure 5; right panel) uptake of nitrogen (N) were influenced by ZnO NPs. In the shoot, ZnO NP promoted N uptake by 11% (and Zn salt by 17%) at high (NPK-H) N application rate, but lowered uptake at low (NPK-L) N rate. However the lowering was less strong with ZnO NPs (by 23%) than with Zn salt (by 42%). In the grain, ZnO NP promoted N uptake at both high (by 84%) and low (by 122%) N applications. Zn salt showed stronger effect of increasing N uptake (by 140%) at high N rate, while ZnO NPs were stronger at low N rate. Shoot uptake of N was determined mid-way into the plant growth duration, while grain N uptake was assessed at full maturity. These findings indicate that ZnO NPs may be applied to increase N use efficiency in high N input cropping systems. In low N input systems, grain N nutritional quality may be enhanced by Zn application.

Figure 5: Effect of Zn application as ZnO nanoparticles (NP) and salt ($ZnSO_4.7H_2O$) on the uptake of nitrogen in sorghum.




**D. <u>Education/Training</u>-**

A Ph.D. student (Mr. Ishaq Adisa) at UTEP and Post-Doctoral Associate (Dr. Roberto De La Torre Roche) at CAES are actively working on this project. In addition, three undergraduate chemistry students from Southern CT State University (Ms. Sadia Younas, Mr. Jared Simpson, Ms. Alessia DiPaolo) are actively working on the project at CAES. A CAES Post-doctoral Associate was sent to the fifth annual meeting of the Sustainable Nanotechnology Organization and gave a platform presentation entitled "Nanoscale micronutrients suppress plant disease and increase crop yield." Additional project results were presented in both platform and poster form at a number of professional scientific meetings (see Section 2B). One of these venues was an international conference hosted by the Chinese Academy of Agricultural Sciences and USDA that focused on sustainable nanotechnology in agriculture. In addition, CAES project results (including an ongoing field demonstration) were presented at the agency annual open house (Plant Science Day) held in August of 2016; the event was open to the public and over 1,100 attendees were present. The CAES investigators spoke to three separate reporters during the current period; the links are:

https://www.chemistryworld.com/news/metal-micronutrients-get-to-the-root-of-antifungal-defence/1017334.article

http://www.ozy.com/fast-forward/could-nanotechnology-end-hunger/70771

http://www.nhregister.com/health/20161226/connecticut-scientists-studying-nanoparticles-to-help-grow-disease-resistant-food

Figure 6. Photo from the New Haven Register story describing research as part of this USDA NIFA funded project. Dr. J.C. White is on the left; Dr. W. Elmer is on the right.

Finally, we have created a website (http://www.ct.gov/caes/cwp/view.asp?a=4898&q=585400 ) for our USDA NIFA AFRI Center of Excellence entitled The Center for Nanotechnology and Agricultural Pathogens Suppression (CeNAPS).

## 3. MILESTONES REACHED-

Below is the project management timetable from our original proposal; we have made significant progress on achieving scheduled deliverables/objectives. We are ahead of schedule on the field studies and particle source experiments, as well as objective three. We are on schedule with the greenhouse studies, as well as ICP work for samples generated under objectives 1-2. For objective four, as noted above, the instrument is currently under repair.

**PROJECT MANAGEMENT TIMETABLE/TIMELINE**

| | OBJECTIVE | MON. 1-6 | MON. 7-12 | MON. 13-18 | MON. 19-24 | MON. 25-30 | MON. 31-36 |
|---|---|---|---|---|---|---|---|
| 1 | Sand-based greenhouse studies | X | X | X | | | |
| | Field-based studies | | X | X | X | | |
| | ICP-OES/ICP-MS | | X | X | X | X | X |
| 2 | Soil "type" field experiments | | | X | X | X | |
| | NP source experiments | | | | X | X | X |
| 3 | NP/macronutrient utilization studies | | | X | X | X | X |
| 4 | S/TEM-EDS work | | X | X | X | X | X |

## 4. CHANGES IN SCIENTIFIC DIRECTION-

During the current reporting period, there were several changes/amendments in scientific direction, all of which will increase project output and impact. First, sorghum has been added as a crop to replace corn; this new crop better aligns with IFDC goals and related projects. At UTEP, additional investigations looking at the effect of cultivar type on treatment effects have been added. This was in part based on initial data showing the first selected cultivar was highly resistant to infection. Additionally, $CeO_2$ nanoparticles and its salt (cerium acetate) have been added to the library of compounds to test in the greenhouse experiments. Third, at CAES transcriptomics has been added as another parameter to measure plant response to infection and nanoscale nutrient amendment. Lastly, we have begun an informal collaboration with colleagues at the University of Texas Austin and Washington University in St. Louis; collaborators there are synthesizing nanoscale micronutrients of interest for use in greenhouse and field trials focusing on the importance of NP source/design. The original plan was to purchase different products commercially; by having materials synthesized for us, we will have greater knowledge of and control over the products used.

## 5. SIGNIFICANT ACTIVITIES AND/OR CHANGES IN PROGRAM MANAGEMENT-

One of the CoPDs on this project, C. Dimkpa, has moved from the VFRC in Washington DC to the parent organization, IFDC, which is in Muscle Shoals Alabama. This move has allowed the investigator more direct and continual access to greenhouse facilities (the original plan was remote supervision), which will increase project output.

## 6. SCIENCE CHALLENGES-

During initial studies at UTEP, we had some difficulty cultivating and inoculating the fungal pathogens. This was in part related to problems that occurred during shipment. In February 2017, CoPD Elmer travelled to UTEP and trained the student on relevant techniques so as to ensure project success.

## 7. NEXT STEPS-

Over the next 6-12 months, ongoing greenhouse trials described above at CAES, UTEP, and IFDC will be completed, including extensive analysis by ICP-MS for element content in various tissues and by electron microscopy on specific tissues to detect the presence of nanoparticle forms of amended nutrients. The CAES eggplant trials described above will be repeated in 2017 to confirm findings. At UTEP, additional cultivars of tomato will be investigated under the current protocols. Finally, preliminary studies assessing the impacts of soil type and NP source will be planned. A manuscript describing the CAES greenhouse and field work with watermelon will be prepared for submission to a peer reviewed journal, as will the IFDC work on micronutrients and drought stress.

## 8. ADDITIONAL INFORMATION-

PDF copies of the manuscripts produced in during the current period are available by request. Similarly, PowerPoint or PDF versions of any of the presentations can also be made available.