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Title: Metal Nanoparticles for Management of Phytophthora fruit rot and Fusarium wilt of watermelon.

Summary and Project Request: Nanoparticles (NPs) in the recent years have emerged as a powerful tool in material science and chemistry as it exhibits significant improvement compared to corresponding bulk materials due to their significantly high surface-to-volume ratio, and size-dependent qualities. Although the effect and fate of NPs in plants has only recently started to receive attention, the most recent literature clearly demonstrates enhanced availability and transport as a function of nanometer particle size. Toxicity considerations, including negative environmental effects has led to the re-design of many NPs by tuning the size and shape and by surface modification leading to increased antimicrobial activity, as well and decreased ecological toxicity. There has been a huge thrust on the use of nanotechnology for development of novel antimicrobials for management of plant pathogens affecting agricultural crops in the last decade. Examples include use of Ag, Cu, CuO, ZnO, TiO_2, carbon-based nanomaterials, such as carbon nanotubes, and other metallic NPs formed on silica. Mathews Paret's plant pathology lab at the University of Florida is one of the leading labs in the US conducting research on use of NPs in plant disease management. Most recently, our group conducted an extensive study of the photocatalytic activity of titanium dioxide NPs including TiO_2/Zn, TiO_2/Ag and TiO_2 and DNA-directed Ag NPs on *Xanthomonas* sp. which indicated to the potential of using NPs in managing bacterial spot of tomato and bacterial leaf spot of roses (Paret et al., 2013a; Paret et al., 2013b; Ocsoy et al., 2013). Wade Elmer's plant pathology lab at the Connecticut Agricultural Experimental Station is also actively conducting research on metal NPs for management of Fusarium wilt of tomato and Verticillium wilt of Eggplant. Both of these research projects have indicated to improved benefit of using Cu and Mn NPs compared to bulk materials in reducing impact of these soil-borne diseases. Studies also indicated that NPs of these metal oxides applied foliar may allow a slow release of ions and/or particles that may be loaded into the phloem for transport to susceptible root tissues. The mechanisms of NP absorption in plant leaves and roots could significantly impact crop health, yield and plant disease management against Phytophthora fruit rot and Fusarium wilt of watermelon. Utilizing this preliminary information and the high level of expertize in handling NPs in plant disease management at Paret and Elmer labs, this project will conduct field trials in Florida and Connecticut exploring impact of NPs of metal oxides against Phytophthora fruit rot and Fusarium wilt of watermelon. The project funding requested is $24,000 for 1-year from December 1, 2014 - November 30, 2015. The lead PI is Mathews Paret; and Wade Elmer and Josh Freeman, the vegetable specialist at the University of Florida will serve as the Co-PIs. This project will be supported by collaboration of Paret lab with Weihong Tan at the Department of Chemistry, University of Florida and Wade Elmer's collaboration with Alia Servin and Jason White in the Department of Analytical Chemistry at the Connecticut Agricultural Experiment Station.

Objectives/goals of the proposal

1. To examine effect of NPs of metal oxides of Al, Cu, Fe, Ni, Mn, Si, Ti and Zn against *Phytophthora capsici* and *Fusarium oxysporum* f. sp. *niveum* in greenhouse conditions.

2. To evaluate the best NPs of metal oxides from objective 1 for management of Phytophthora fruit rot and Fusarium wilt of watermelon in field trials in Florida and Connecticut.

The goal of the project is to develop a novel strategy for effective management of Phytophthora fruit rot and Fusarium wilt on watermelon by utilizing the latest advances in NP technology.

Materials and Methods

Relevant to objective 1: Greenhouse trials will be conducted (December 2014 - February 2015) using 3-4 week old transplants of watermelons variety Melody (Syngenta Inc.). Plants will be transplanted into potting medium in 10-cm diameter pots. NP suspensions of the metal oxides of Al, Cu, Fe, Ni, Mn, Si, Ti and Zn at rates of 0, 100, and 1000 µg/ml will be applied to the transplants till runoff at weekly intervals (4 applications). For the root application treatments, transplants will receive 50 ml of 0, 100, and 1000 µg/ml of the same NP suspensions at weekly intervals (4 applications). Five days after the first treatment, plants will be inoculated with well-characterized isolates of *P. capsici* ($5x10^4$ sporangia/mL) and *Fusarium oxysporum* f. sp. *niveum* (1×10^6 micro conidia/ml; Zhou et al., 2010) in separate trials using standard procedures. Disease severity will be monitored based on a 0-11 HB scale (Horsfall and Barratt, 1945) for 2 months at weekly intervals. Studies will include bulked equivalents of the NPs subjected to the same protocols. This will be conducted by Paret lab. There will be 20 plants per treatment and the experiment will be conducted as a randomized complete block design. Five watermelon transplant roots will be digested in acid two times (2 and 4 weeks after treatment with NPs) during the experiment, and analyzed for mineral composition by ICP-MS at Elmer lab.

Relevant to objective 2: The best two metal NPs for each of the pathogens, and the most effective and 5X the effective concentration from objective 1 will be selected for field trials to be conducted in Quincy, FL and New Haven, CT. Drip or foliar application (8 applications) will be selected based on the most effective method for disease management from greenhouse experiments. The Phytophthora fruit rot trials will be conducted in the spring and fall of 2015 at Quincy, FL. The Fusarium wilt trial will be conducted in the spring of 2015 at Quincy and summer of 2015 at New Haven. Fields natural infected with the pathogens will be used for the trials. Field trials will be conducted using 4-5week old transplants of watermelon variety Melody and Sp6 as pollenizer. There will be 8 plants of Melody and 2 plants of Sp6 in each plot. The seedlings will be transplanted at an in-row spacing of 3 feet. Raised bed plots will be prepared and the trial maintained for weeds, diseases, insects and nutrient management as per current watermelon production guidelines in Florida. Each plot will be replicated 4 times and the experiment will be arranged as a Randomized Complete Block Design. Disease severity data will be collected at weekly intervals based on Horsfall-Barratt scale; the mid point averages of disease severity scale will be used to calculate the total Area Under Disease Progress Curve (AUDPC). Yield will be assessed for all trials at the end of the experiment. Data will be statistically analyzed using SAS.

References:

Ocsoy I, Paret ML, Ocsoy MA, Kunwar S, Chen T, You M, Tan W. 2013. Nanotechnology in plant disease management: DNA-directed silver nanoparticles on graphene oxide as an antibacterial against *Xanthomonas perforans*. ACS Nano 7:10 8972-8980

Paret ML, Vallad GE, Jones, JB, Averett D, Olson SM. 2013. Photocatalysis: Effect of light-activated nanoscale formulations of TiO_2 on *Xanthomonas perforans* and control of bacterial spot on tomato. Phytopathology 103(3): 228-236

Paret ML, Palmateer A, Knox G. 2013. Evaluation of a light-activated nanoparticle formulation of TiO_2/Zn for management of bacterial leaf spot on Rosa 'Noare'. HortScience 48(2): 189-192

Zhou, X. G., Everts, K. L., and Bruton, B. D. 2010. Race 3, a new and highly virulent race of *Fusarium oxysporum* f. sp. *niveum* causing Fusarium wilt in watermelon. Plant Dis. 94:92-98.

Dates for project reports to be submitted to the NWA: First report: August 2015: Final Report: December 2015

Amount of money requested: $24,000

List all parties involved with the project, including the Lead Investigator

Mathews Paret, PI, University of Florida, NFREC, Plant Pathology, $12,000
Wade Elmer, Co-PI, Connecticut Agricultural Experimental Station, $6,000
Josh Freeman, Co-PI, University of Florida, Plant Pathology, $6,000

Matching funds: $15,482.44

10% of Mathews Paret time Annual salary ($88,024.96): $8,802
20% of Laura Ritchie Salary (Ms. Ritchie is the biological scientist who will be managing the greenhouse and field trials in Florida) ($33,402): $6,680.44

Emphasize which NWA priority area your project is based on

Disease management of Phytophthora fruit rot and Fusarium wilt

Addendum to proposal - a layman's brief on what the project is projected to accomplish

Expected Outcomes from this project

1. The efficacy of Metal NPs against Phytophthora fruit rot and Fusarium wilt will be established which will be a novel approach in disease management against these two major soil-borne disease on watermelon
2. The developed NPs will lay the platform for information pipeline for commerical fungicide companies to incoporate effective materials into the research and commercialization portfolio with support from Paret and Elmer lab research efforts.
3. Metal NPs generated will have multi-site mode of action and will be a major addition to the very small library of multi-site and effective fungicides available in the market for control of Phytophthora fruit rot and Fusarium wilt. Thus these materials will be a major boost to the industry, which is dealing with increased cases of fungicide resistance development.
4. The effective NPs will be only required in small quantities compared to bulk materials and thus highly sustainable in production.