Advanced Copper Composites Against Copper-Tolerant *Xanthomonas perforans* and Tomato Bacterial Spot

A. Strayer-Scherer, Y. Y. Liao, M. Young, L. Ritchie, G. E. Vallad, S. Santra, J. H. Freeman, D. Clark, J. B. Jones,[†] and M. L. Paret[†]

First, second, and ninth authors: Department of Plant Pathology, University of Florida, Gainesville 32611; third author: NanoScience Technology Center and Burnett School of Biomedical Science, University of Central Florida, Orlando 32826; fourth, seventh, and eighth authors: North Florida Research and Education Center, University of Florida, Quincy 32351; fifth author: Department of Plant Pathology, Gulf Coast Research and Education Center, University of Florida, Wimauma 33598; sixth author: NanoScience Technology Center, Department of Chemistry, Department of Materials Science and Engineering and Burnett School of Biomedical Sciences, University of Central Florida, Orlando; and tenth author: Department of Plant Pathology and North Florida Research and Education Center, University of Florida, Quincy. Accepted for publication 3 October 2017.

ABSTRACT

Bacterial spot, caused by *Xanthomonas* spp., is a widespread and damaging bacterial disease of tomato (*Solanum lycopersicum*). For disease management, growers rely on copper bactericides, which are often ineffective due to the presence of copper-tolerant *Xanthomonas* strains. This study evaluated the antibacterial activity of the new copper composites core-shell copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu) as potential alternatives to commercially available micron-sized copper bactericides for controlling copper-tolerant *Xanthomonas perforans*. In vitro, metallic copper from CS-Cu and FQ-Cu at 100 µg/ml killed the copper-tolerant *X. perforans* strain within 1 h of exposure. In contrast, none of the micron-sized copper rates (100 to 1,000 µg/ml) from Kocide 3000 significantly reduced

copper-tolerant *X. perforans* populations after 48 h of exposure compared with the water control ($P < 0.05$). All copper-based treatments killed the copper-sensitive *X. perforans* strain within 1 h. Greenhouse studies demonstrated that all copper composites significantly reduced bacterial spot disease severity when compared with copper-mancozeb and water controls ($P < 0.05$). Although there was no significant impact on yield, copper composites significantly reduced disease severity when compared with water controls, using 80% less metallic copper in comparison with copper-mancozeb in field studies ($P < 0.05$). This study highlights the discovery that copper composites have the potential to manage copper-tolerant *X. perforans* and tomato bacterial spot.

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops in the United States. In 2015, the United States harvested 37,312 ha of fresh-market tomato and 125,695 ha of processing tomato, valued at over \$2.6 billion (USDA 2016). Bacterial spot, caused by *Xanthomonas euvesicatoria*, *X. gardneri*, *X. perforans*, and *X. vesicatoria*, is one of the major bacterial diseases affecting tomato worldwide (Jones et al. 2004). Infection typically results in necrotic leaf and fruit lesions, and defoliation (Potnis et al. 2015; Ritchie 2000). Necrotic fruit lesions and sunscald due to defoliation can reduce fruit quality (Ritchie 2000). Under optimal disease conditions, bacterial spot can cause yield losses of up to 50% in warm, humid regions within a single season (Louws et al. 2001; Pohronezny and Volin 1983; Scott et al. 1989). Unfortunately, once a field becomes infested with bacterial spot, it is extremely difficult to have success in disease control.

Although streptomycin was the dominant bactericide used to manage bacterial spot in the 1950s, copper-based bactericides became the chemical of choice due to the emergence of streptomycin-resistant xanthomonads (Marco and Stall 1983; Thayer and Stall 1962). Due to continuous use, copper-tolerant *X. euvesicatoria* strains became prevalent in the late 1960s in Florida and reduced the efficacy of copper-based bactericides (Marco and Stall 1983). As a result of copper-tolerant strains, control of bacterial spot was only achievable with the addition of ethylene-bis-dithiocarbamates to the tank mix with copper bactericides (Conover and Gerhold 1981; Marco and Stall 1983). Copper-tolerant xanthomonads were sensitive to copper-mancozeb, in

part due to the release of more soluble copper than in a copper suspension alone (Marco and Stall 1983). Thus, copper-mancozeb has been the grower's standard for decades in managing bacterial spot of tomato. Currently, growers utilize an integrated pest management (IPM) strategy, which typically includes using pathogen-free certified seed, disease-free transplants, and multiple applications of copper-mancozeb (Gitaitis et al. 1992; Momol et al. 2008; Ritchie 2000). However, under optimal disease conditions, copper-mancozeb fails to adequately control bacterial spot when copper-tolerant strains are present (Jones and Jones 1985; Obradovic et al. 2005). Thus, an effective disease management strategy for bacterial spot is required for maintaining the tomato industry in the United States.

Several other disease-management technologies, including plant inducers that activate systemic acquired resistance (SAR), biological control agents, light-activated nanoparticles, and a silver-based nanomaterial, have been evaluated as potential alternatives to copper-based bactericides. Acibenzolar S-methyl (ASM) (Actigard 50W; Syngenta Crop Protection, Greensboro, NC), a synthetic SAR inducer, does not have direct antibacterial activity but triggers the plant's natural defense responses to increase resistance to the bacterial spot pathogens and reduce disease severity compared with nontreated controls (Kessmann et al. 1994; Obradovic et al. 2005). However, full-season application of ASM is not recommended due to its association with yield depression (Graves and Alexander 2002; Romero et al. 2001). Several studies have demonstrated that foliar applications of bacteriophages, a biological control agent, provided comparable or significantly improved disease control when compared with copper-mancozeb (Balogh et al. 2003; Flaherty et al. 2000; Momol et al. 2008; Obradovic et al. 2004). However, bacteriophages are highly sensitive to environmental factors, which are deleterious to phage survival and efficacy (Flaherty et al. 2000; Iriarte et al. 2007). Paret et al. (2013)

[†]Corresponding authors: M. L. Paret; E-mail: paret@ufl.edu; and J. B. Jones; E-mail: jbjones@ufl.edu

analyzed the antibacterial activity of a light-activated, nanoscale form of titanium dioxide (TiO₂) alone and in combination with silver (Ag) or zinc (Zn) against *X. perforans* and bacterial spot of tomato. Although TiO₂/Zn significantly reduced disease severity compared with copper and the nontreated controls, field-grown tomato plants exhibited phytotoxicity following the sixth application at approximately 500 µg/ml. In addition to light-activated nanoparticles, a silver-based nanocomposite, Ag-dsDNA-GO, was recently evaluated as a potential alternative to copper to manage bacterial spot of tomato (Strayer et al. 2016). In vitro assays revealed that Ag-dsDNA-GO at 10 µg/ml killed all bacterial cells of a copper-tolerant *X. perforans* strain within 1 h. Under greenhouse conditions, tomato plants treated with Ag-dsDNA-GO at 75 µg/ml significantly reduced bacterial spot disease severity when compared with copper-mancozeb. However, this nanocomposite is not currently available commercially and the results need to be further validated in field trials.

Currently, commercially available copper-based bactericides typically contain micron-sized metallic copper in the form of insoluble copper compounds, such as copper (II) hydroxide, copper (II) oxide, or copper (II) oxychloride (Young and Santra 2014). For instance, Kocide 3000 (DuPont, Wilmington, DE), a commercialized copper bactericide, contains micron-sized metallic copper at approximately 5 µM in the form of copper (II) hydroxide (Adeleye et al. 2014; Nufarm 2013). Several studies have revealed that nanometer-sized metallic compounds have higher antibacterial activity when compared with their micron-sized counterparts (Gunawan et al. 2011; Jiang et al. 2009; Panáček et al. 2006; Yamamoto 2001). More specifically, in those studies, as the size of metallic particles decreased, their antibacterial activity increased. In general, the increased antibacterial activity of metallic nanoparticles is likely due to their small size and high surface-to-volume ratio. These two characteristics are thought to allow metallic nanoparticles to penetrate microbial membranes and release more metal ions into solution more efficiently than their micron-sized counterparts (Baker et al. 2005; Morones et al. 2005; Panáček et al. 2006). Furthermore, copper hydroxide and copper oxide particles are inherently hydrophobic and aggregate in water. This aggregation reduces the overall surface area of metallic particles, which decreases their antibacterial activity (Bae et al. 2010; Owens et al. 2016). To prevent aggregation of copper particles, three advanced copper composites were designed to improve overall antimicrobial efficacy of metallic copper particles: core-shell copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu) (Maniprasad and Santra 2012; Santra et al. 2014; Young and Santra 2014). In all of these composites, copper particles are embedded in hydrophilic silica gel matrix to reduce particle-particle aggregation (Young and Santra 2014). Sol-gel silica is hydrophilic and negatively charged at neutral to slightly basic pH conditions, and serves as a weak chelator of copper ions (Owens et al. 2016). When copper particles are embedded into the silica gel, aggregation is prevented. As a result, bioavailability of copper ions is increased (Santra 2012; Santra et al. 2014). Therefore, these copper composites in comparison with micron-sized metallic copper from currently available copper bactericides could have higher antibacterial activity against copper-tolerant *Xanthomonas* strains.

Thus, the goal of this study was to compare the antibacterial activity of three newly designed copper composites (CS-Cu, MV-Cu, and FQ-Cu) with micron-sized metallic copper alone and in combination with ethylene-bis-dithiocarbamate. The specific objectives of this study were to compare three copper composites with micron-sized metallic copper for (i) reducing in vitro bacterial growth of copper-tolerant and copper-sensitive strains of *X. perforans*, (ii) reducing bacterial spot disease severity caused by a copper-tolerant strain of *X. perforans* and assessing the risk of phytotoxicity under greenhouse conditions, and (iii) reducing bacterial spot disease severity caused by a copper-tolerant strain of *X. perforans* and assessing the risk of phytotoxicity and marketable yield on treated tomato plants under field conditions.

MATERIALS AND METHODS

Bacterial strains and storage. Two *X. perforans* strains—GEV485 (copper-tolerant) and 91-118 (copper-sensitive)—were used in this study. For short-term storage and experiments, bacteria were grown on nutrient agar (NA) medium (BBL; Becton Dickinson and Co., Cockeysville, MD) at 28°C and grown for 24 h. For long-term storage, purified cultures were stored in a sterile 30% glycerol solution at -80°C.

Design of the advanced copper composites. Three advanced copper composites (CS-Cu, MV-Cu, and FQ-Cu) were designed to improve overall antimicrobial efficacy of metallic copper. In all of these composites, copper particles are embedded in a hydrophilic, porous silica gel matrix to reduce particle-particle aggregation during the sol-gel synthesis process (Young and Santra 2014). Copper was chelated by the silica gel and partially converted to crystalline copper hydroxide/oxide particles at pH 7.5 and above. The silica layer around copper crystals minimizes particle aggregation and improves overall dispersion of copper composites. As previously described by Maniprasad and Santra (2012), colloidal silica particles (Stöber et al. 1968) were used as a filler to further disperse copper-silica gel particles in the CS-Cu composite (Fig. 1A and D). In the MV-Cu composite, copper is present in mixed-valence states (+1 and +2 oxidation states) along with metallic Zn (approximately 10 wt%) (Fig. 1B and E) (Young and Santra 2014). As previously described by Santra et al. (2014), the silica gel in the FQ-Cu composite is embedded with both a quaternary ammonium compound (Fixed-Quat) and metallic copper (Fig. 1C and F). The purpose of using Fixed-Quat is to (i) further improve the dispersion of copper-silica gel particles and (ii) improve overall antimicrobial efficacy of the materials through additional biocidal activity of Fixed-Quat. In all three of the copper composites, copper ions are released in a slow manner. Thus, all of these copper composites can be considered somewhere between the soluble (such as copper salts) and insoluble (such as copper oxides and copper hydroxides) forms of copper.

In vitro assays. Two *X. perforans* strains, GEV485 and 91-118, were grown on NA plates for 24 h at 28°C. The bacterial strains were then transferred to NA plates containing copper (II) sulfate pentahydrate (CuSO₄ · 5H₂O) (Sigma-Aldrich, St. Louis) at 0.08 µM/liter and incubated for 24 h at 28°C. Bacterial suspensions were prepared in sterilized deionized water amended with 0.01 M MgSO₄, adjusted to absorbance at 600 nm = 0.3 (approximately 5 × 10⁸ CFU/ml), then diluted to 10⁵ CFU/ml. For each strain, 20 µl of each bacterial suspension was transferred to sterile glass tubes containing 2 ml of each of the following: metallic copper prepared from CS-Cu, MV-Cu, and FQ-Cu at rates of 100, 200, 500, and 1,000 µg/ml; and metallic copper prepared from Kocide 3000 (DuPont, Wilmington, DE) at rates of 100 µg/ml (0.334 g/liter), 200 µg/ml (0.668 g/liter), 500 µg/ml (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter). Kocide 3000 contains 30% metallic copper (Kocide 3000 at 1 g/liter contains copper at approximately 300 µg/ml) in the form of copper hydroxide (Cu(OH)₂). Additionally, three sterilized glass tubes containing 2 ml of sterilized deionized water amended with 0.01 M MgSO₄ and 20 µl of each bacterial suspension served as the controls. The tubes were incubated on a shaker (150 rpm) at 28°C. Bacterial populations were measured by plating 50 µl from each tube on NA after 1, 4, 24, and 48 h of incubation. The plates were incubated for 48 h at 28°C; then, the colonies on each plate were counted to calculate the CFU per milliliter for each time point. Each treatment consisted of three replicates and each in vitro assay was conducted two times.

Greenhouse experiments. The following treatments were prepared in 200 ml of sterile tap water for the first and second greenhouse experiments: metallic copper prepared from CS-Cu, MV-Cu, and FQ-Cu at rates of 100, 500, and 1,000 µg/ml; metallic copper prepared from Kocide 3000 at rates of 100 µg/ml (0.334 g/liter), 500 µg/ml (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter); metallic copper from Kocide 3000 at 630 µg/ml (2.1 g/liter) in combination

with Penncozeb 75DF (United Phosphorus, Inc., King of Prussia, PA) at 1.2 g/liter; and sterile tap water. For the third greenhouse experiment, the following treatments were prepared in 200 ml of sterile tap water: metallic copper prepared from CS-Cu, MV-Cu, and FQ-Cu at 75, 100, 200, and 500 µg/ml; metallic copper prepared from Kocide 3000 at rates of 75 µg/ml (0.251 g/liter), 100 µg/ml (0.334 g/liter), 200 µg/ml (0.668 g/liter), and 500 µg/ml (1.67 g/liter); metallic copper from Kocide 3000 at 630 µg/ml (2.1 g/liter) in combination with Penncozeb 75DF at 1.2 g/liter; and sterile tap water. The chemical suspensions were applied as foliar sprays onto the abaxial and adaxial leaf surfaces of 2- to 3-week-old (four- to five-true-leaf stage) Bonnie Best tomato plants (four plants per treatment) until run-off. Four plants were sprayed with sterile tap water, which served as the negative control. The chemical suspensions were allowed to air dry on the leaf surface completely before inoculation, which took approximately 4 h. The copper-tolerant *X. perforans* strain GEV485 was used in the greenhouse experiments. A bacterial suspension (5×10^8 CFU/ml) was sprayed onto both sides of the tomato leaves. The inoculated plants were placed in plastic bags to maintain high humidity and incubated in a growth chamber at 28°C for 48 h. The bags were then carefully removed and the plants were incubated in the growth chamber for three additional days before being transferred to the greenhouse. In the greenhouse, tomato plants were subjected to natural light conditions (approximately 12 h of light per day), average nightly temperatures of 21°C, and average daytime temperatures of 32°C. For irrigation, water was applied daily to the soil using a garden hose to avoid wetting the foliage. At the end of the experiment, tomato plants were at the six- to seven-true-leaf growth stage. Beginning at 5 days postinoculation, disease and phytotoxicity were assessed using the Horsfall-Barratt disease severity scale every other day and ending at 14 days postinoculation (Horsfall and Barratt 1945). The area under the disease progress curve (AUDPC) and progression of phytotoxicity over time were both calculated by directly taking the midpoint of the percent range for each disease severity value (Madden et al. 2007).

Field experiments. Three field trials were conducted in Florida during 2015 and 2016. Two of these field trials were conducted at the University of Florida, North Florida Research and Education Center (NFREC) in Quincy during fall 2015 and spring 2016. The third field trial was conducted at the University of Florida, Gulf Coast Research and Education Center (GCREC) in Wimauma during spring 2016. BHN602 and HM1823 tomato plants susceptible to bacterial spot were arranged in a completely randomized block design in Quincy and Wimauma, respectively. There were four plots per treatment at each location that contained 10 and 15 plants per plot at GCREC and NFREC, respectively. Tomato plants were staked, produced, and managed for weeds, insects, and fungal pathogens following current tomato production standards, as described by Freeman et al. (2016). Experimental plots in Quincy consisted of fumigated raised beds covered with black polyethylene mulch for spring plantings and white polyethylene mulch for fall plantings. At the Wimauma location, white polyethylene mulch was used. For both fall 2015 and spring 2016 Quincy trials, each plot was 7.6 m in length, plants were spaced 50.8 cm apart, and the distance between rows and plots was 0.9 m. For the spring 2016 Wimauma trial, each plot was 6.1 m in length, plants were spaced 60.1 cm apart, and distance between rows and plots was 1.5 m. The following treatments were sprayed on foliar parts of transplanted tomato plants 1 week prior to inoculation and in weekly intervals postinoculation: metallic copper from CS-Cu, MV-Cu, and FQ-Cu at 100 and 200 µg/ml; Kocide 3000 at 1.95 kg/ha; a combination of Kocide 3000 at 1.95 kg/ha and Penncozeb 75DF at 1.11 kg/ha; and water. Following the initial spray applications, the first, middle, and last plants in each plot were spray inoculated with approximately 50 ml of a bacterial suspension (5×10^8 CFU/ml) made from 24-h cultures of GEV485 grown on NA. There were eight additional treatment applications during the fall 2015 Quincy trial, nine additional treatment applications during the spring 2016 Wimauma trial, and six additional treatment applications during the spring 2016 Quincy trial. Treatments were applied at a rate of 562 liters/ha for weeks 1 through 6, 843 liters/ha for weeks 7 through 9, and 937 liters/ha in week 10. Disease was assessed using

Fig. 1. Scanning electron microscopy (SEM) and high-resolution transmission electron microscopy (TEM) images of three copper composites: core shell silica copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu). **A**, SEM image of CS-Cu revealed roughly spherical, submicron (400 to 600 nm) particles with uneven coating. **B**, SEM image reveals that MV-Cu display large, sharp, and jagged multimicron-size gel morphology. **C**, SEM image reveals that FQ-Cu has smooth, large, multimicron-size gel morphology. **D**, High-resolution TEM image of CS-Cu focuses on an individual particle with a distinct, visible shell. **E**, High-resolution TEM image confirms that MV-Cu is a large, irregular gel with darker contrast of electron rich areas. **F**, High-resolution TEM image confirms that FQ-Cu is a large, irregular gel with darker contrast of electron-rich areas.

the Horsfall-Barratt disease severity scale once a week postinoculation until harvest (Horsfall and Barratt 1945). AUDPC was calculated by taking the midpoint of the percent ranges for each disease severity value (Madden et al. 2007). Mature green or early breaker stage fruit was harvested to simulate Florida tomato production standards and graded based on United States Department of Agriculture grades (Paret et al. 2013; USDA 1976). Plants were harvested twice for the fall 2015 Quincy trial and once for the spring 2016 trials in Quincy and Wimauma.

Statistics. All statistical analyses were completed using IBM SPSS Statistics (version 22; IBM Corp., Armonk, NY). The data collected from in vitro, greenhouse, and field experiments were analyzed for statistical significance using analysis of variance (ANOVA) followed by pairwise comparison with the Student-Newman-Keuls (SNK) method with a P value of 0.05 and analyzed for outliers (Pallant 2013).

RESULTS

The effect of copper composites on in vitro *X. perforans* growth. The in vitro assay was conducted two times but there was no significant interaction effect of the experimental repeat or interaction of the experimental repeat with the treatments on bacterial recovery of a single strain in vitro, as shown by ANOVA with a P value of 0.05 (data not shown). Thus, the data from the two experiments were combined and analyzed for statistical significance using SNK method with a P value of 0.05. There was a statistically significant difference between groups as determined by one-way ANOVA (F [67,136] = 5,170.9, P < 0.001) and no outliers were identified. In vitro, all the metallic copper bactericides demonstrated antibacterial activity against a copper-sensitive strain of *X. perforans*, 91-118 (Fig. 2A). All the metallic copper concentrations (100, 200, 500, and 1,000 µg/ml) from CS-Cu, MV-Cu, FQ-Cu, and Kocide 3000 completely inhibited bacterial growth within 1 h of exposure. In contrast, only the three copper composites demonstrated antibacterial activity against a copper-tolerant strain of *X. perforans*, GEV485 (Fig. 2B). All metallic copper concentrations (100, 200, 500, and 1,000 µg/ml) from CS-Cu and FQ-Cu completely inhibited bacterial growth within 1 h of exposure. Additionally, all metallic copper concentrations from MV-Cu completely inhibited bacterial growth within 24 h of exposure. In contrast, equivalent rates of micron-sized metallic copper from Kocide 3000 did not significantly (P < 0.05) reduce the bacterial population when compared with the water control.

The effect of copper composites on bacterial spot disease severity under greenhouse conditions. In the first greenhouse experiment, there was a statistically significant difference between groups as determined by one-way ANOVA and no outliers were identified for both the AUDPC (F [13,28] = 36.0, P < 0.001) and phytotoxicity (F [13,28] = 108.2, P < 0.001) data. All metallic copper concentrations (100, 500, and 1,000 µg/ml) from the three copper composites provided significant (P < 0.05) suppression of bacterial spot disease severity when compared with the water control (Fig. 3A). In contrast, only the 1,000-µg/ml micron-sized metallic copper concentration prepared from Kocide 3000 provided significant suppression of bacterial spot when compared with the water control. Copper-mancozeb also significantly reduced bacterial spot disease severity when compared with the water control. Additionally, when compared with copper-mancozeb, metallic copper at rates as low as 100 µg/ml from CS-Cu and 500 µg/ml from either MV-Cu or FQ-Cu significantly reduced bacterial spot disease severity. However, none of the metallic copper rates prepared from Kocide 3000 significantly reduced bacterial spot disease severity when compared with copper-mancozeb. Furthermore, phytotoxicity occurred on plants treated with metallic copper at rates of 500 and 1,000 µg/ml from CS-Cu and 1,000 µg/ml from MV-Cu and FQ-Cu (Fig. 4A). However, none of the other metallic copper-based treatments resulted in significantly (P < 0.05) higher phytotoxicity in comparison with the water control. Although bacterial spot and

phytotoxicity symptoms would appear on the same leaves, they could be distinguished from one another in most cases because each caused distinct symptoms. Early symptoms of phytotoxicity on tomato leaves included chlorosis, distortion, necrotic spots, and necrosis at leaf tips. In some cases, necrotic spots would coalesce, resulting in full tissue necrosis on tomato leaves. In contrast, bacteria spot lesions are initially small, circular, water-soaked or yellow-green areas that expand rapidly and later dry and turn brown to black in color, with a wet-to-greasy and angular appearance (Miller et al. 1996; Vallad et al. 2004). However, in the cases where full tissue necrosis occurred due to copper applications, disease and phytotoxicity ratings would cease because the two could no longer be differentiated from one another based on symptoms alone.

In the second greenhouse experiment, there was a statistically significant difference between groups as determined by one-way ANOVA, and no outliers were identified for both the AUDPC (F [13,28] = 18.0, P < 0.001) and phytotoxicity (F [13,28] = 24.0, P < 0.001) data. All metallic copper concentrations (100, 500, and 1,000 µg/ml) from the three copper composites provided significant (P < 0.05) suppression of bacterial spot when compared with the water control (Fig. 3B). Copper-mancozeb also significantly reduced bacterial spot disease severity compared with the water control. However, none of the Kocide 3000 treatments significantly reduced bacterial spot disease severity when compared with the water control or copper-mancozeb. Additionally, metallic copper at rates as low as 100 µg/ml from CS-Cu, 500 µg/ml from MV-Cu, and 1,000 µg/ml from FQ-Cu significantly reduced bacterial spot disease severity when compared with copper-mancozeb. Furthermore, phytotoxicity was observed on plants treated with metallic copper at rates as low as 100 µg/ml from CS-Cu and 500 µg/ml from MV-Cu and FQ-Cu (Fig. 4B). However, none of the other metallic copper-based treatments resulted in significantly (P < 0.05) higher phytotoxicity in comparison with the water control. Because the 1,000-µg/ml metallic copper concentration from all three copper composites caused phytotoxicity, this concentration was not tested in the third greenhouse experiment.

In the third greenhouse experiment, there was a statistically significant difference between groups as determined by one-way ANOVA, and no outliers were identified for both the AUDPC (F [17,36] = 43.3, P < 0.001) and phytotoxicity (F [17,36] = 14.9, P < 0.001) data. All metallic copper concentrations (75, 100, 200, and 500 µg/ml) from the three copper composites provided significant (P < 0.05) suppression of bacterial spot compared with copper-mancozeb and the water control (Fig. 3C). Additionally, copper-mancozeb and micron-sized metallic copper at rates as low as 200 µg/ml from Kocide 3000 significantly reduced bacterial spot disease severity compared with the water control. Furthermore, visible, significant (P < 0.05) phytotoxicity was observed on plants treated with metallic copper at rates as low as 75 µg/ml from CS-Cu and 500 µg/ml from MV-Cu (Fig. 4C). However, none of the other metallic copper treatments resulted in significantly higher phytotoxicity compared with the water control.

The effect of copper composites on bacterial spot disease severity under field conditions. In the fall 2015 field trial conducted in Quincy, there was a statistically significant difference between groups as determined by one-way ANOVA for the AUDPC (F [8,27] = 2.8, P = 0.021) data, and no outliers were identified. The application of MV-Cu with metallic copper at either 100 or 200 µg/ml significantly (P < 0.05) reduced bacterial spot disease severity (i.e., AUDPC) compared with the water control (Table 1). None of the CS-Cu or FQ-Cu rates significantly reduced bacterial spot disease severity when compared with the water control. Additionally, both metallic copper concentrations (100 and 200 µg/ml) from the three copper composites provided statistically equivalent disease control in comparison with the grower's standard, copper-mancozeb. However, Kocide 3000 and copper-mancozeb applications did not significantly reduce bacterial spot disease severity when compared with the water control. Furthermore, none of the copper-based treatments had a significant impact on tomato fruit yield in this trial. In contrast to

greenhouse experiments, application of the copper composites did not cause any phytotoxicity on the tomato plants (data not shown). Additionally, bacterial spot fruit symptoms were observed in less than 1% of the fruits in all the field trials and were not significant enough for reliable assessments (data not shown). Thus, disease incidence was not reported in this study.

In the spring 2016 field trial conducted in Quincy, there was a statistically significant difference between groups as determined by one-way ANOVA for the AUDPC ($F [8,27] = 6.5, P = 0.001$) data, and no outliers were identified. Both metallic copper concentrations (100 and 200 µg/ml) from the three copper composites provided significant ($P < 0.05$) suppression of disease compared with the water control and provided statistically equivalent disease control in comparison with the grower's standard (Table 2). In contrast, Kocide 3000 and copper-mancozeb applications did not significantly reduce bacterial spot disease severity when compared with the water control. Furthermore, none of the copper-based treatments had a significant impact on tomato fruit yield in this trial. Applications of the

copper composites did not cause any phytotoxicity on the tomato plants (data not shown).

In the spring 2016 field trial conducted in Wimauma, there was a statistically significant difference between groups as determined by one-way ANOVA for the AUDPC ($F [8,27] = 2.7, P = 0.025$) data, and no outliers were identified. Application of metallic copper at rates of 100 µg/ml for CS-Cu, 200 µg/ml for MV-Cu, and 100 and 200 µg/ml for FQ-Cu caused a significant ($P < 0.05$) reduction in bacterial spot disease severity compared with the water control, as indicated by AUDPC (Table 3). Additionally, both metallic copper concentrations (100 and 200 µg/ml) from the three copper composites provided statistically equivalent disease control in comparison with the grower's standard, copper-mancozeb. However, Kocide 3000 and copper-mancozeb applications did not significantly reduce bacterial spot disease severity when compared with the water control. Furthermore, none of the copper-based treatments had a significant impact on tomato fruit yield in this trial. Applications of the copper composites did not cause any phytotoxicity on the tomato plants (data not shown).

Fig. 2. In vitro activity of various concentrations of metallic copper from three copper composites—core shell silica copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu)—and Kocide 3000 (DuPont, Wilmington, DE) on *Xanthomonas perforans* survival over time (black bars = 1 h, light gray bars = 4 h, dark gray bars = 24 h, and white bars = 48 h). There were three replicates per treatments and the treatments were as follows: metallic copper at 100, 200, 500, and 1,000 µg/ml from CS-Cu, MV-Cu, and FQ-Cu; metallic copper prepared from Kocide 3000 at 100 (0.334 g/liter), 200 (0.668 g/liter), 500 (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter); sterilized deionized water amended with 0.01 M MgSO₄. **A**, 91-118 (Cu-sensitive strain) and **B**, GEV485 (Cu-tolerant strain). Error bars = standard deviation. A P value of 0.05 was used in the IBM SPSS Student-Newman-Keuls statistical analysis. The in vitro experiments were conducted twice and the effect of experiment was not significant. Therefore, the data were pooled for analysis and the analysis was among all treatments.

DISCUSSION

In this study, we describe for the first time the antibacterial properties of three new copper composites against copper-tolerant *X. perforans*. These composites showed potential as alternatives to commercially available, micron-sized metallic copper bactericides used in the management of bacterial spot disease. This finding is significant because, currently, all of the *X. perforans* strains present

in Florida are copper tolerant (Horvath et al. 2012; Momol et al. 2008). When copper-tolerant strains are present in warm, humid environments, bacterial spot is difficult to control with commercially available copper bactericides (Momol et al. 2008; Obradovic et al. 2004). In this study, micron-sized metallic copper at of Kocide 3000 rates as high as 1,000 µg/ml had no effect in vitro on the copper-tolerant *X. perforans* strain even after 48 h of exposure. However, an increase in antibacterial activity against a copper-tolerant

Fig. 3. Effect of various metallic copper concentrations from three copper composites: core shell silica copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu); Kocide 3000 (DuPont, Wilmington, DE); and copper-mancozeb on bacterial spot of tomato based on the area under disease progress curve (AUDPC) under greenhouse conditions. **A**, Experiment 1 consisted of the following treatments: metallic copper at 100, 200, 500, and 1,000 µg/ml from CS-Cu, MV-Cu, and FQ-Cu; metallic copper prepared from Kocide 3000 at 100 (0.334 g/liter), 200 (0.668 g/liter), 500 (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter); metallic copper prepared from Kocide 3000 at 100 (0.334 g/liter), 200 (0.668 g/liter), 500 (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter); a combination of metallic copper at 540 µg/ml from Kocide 3000 (2.1 g/liter) and Penncozeb 75DF (United Phosphorus, Inc.)v(1.2 g/liter) (Cu-Man); and sterilized tap water. **B**, Experiment 2 consisted of the following treatments: metallic copper at 100, 200, 500, and 1,000 µg/ml from CS-Cu, MV-Cu, and FQ-Cu; metallic copper prepared from Kocide 3000 at 100 µg/ml (0.334 g/liter), 200 µg/ml (0.668 g/liter), 500 µg/ml (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter); a combination of metallic copper at 540 µg/ml from Kocide 3000 (2.1 g/liter) and Penncozeb 75DF at 1.2 g/liter (Cu-Man); and sterilized tap water. **C**, Experiment 3 consisted of the following treatments: metallic copper at 75, 100, 200, and 500 µg/ml from CS-Cu, MV-Cu, and FQ-Cu; metallic copper prepared from Kocide 3000 at 75 µg/ml (0.251 g/liter), 100 µg/ml (0.334 g/liter), 200 µg/ml (0.668 g/liter), and 500 µg/ml (1.67 g/liter); a combination of metallic copper at 540 µg/ml from Kocide 3000 (2.1 g/liter) and Penncozeb 75DF at 1.2 g/liter (Cu-Man); and sterilized tap water. Disease severity was calculated using the Horsfall-Barratt scale, a nondimensional 12-point scale, to assess the percentage of canopy affected by bacterial spot. Values were converted to midpercentages and used to generate AUDPC. Error bars = standard deviation. *P* value of 0.05 was used in the IBM SPSS Student-Newman-Keuls statistical analysis. There were four plants per treatment in each experiment.

strain of *X. perforans* was observed when the size of metallic copper was reduced from micrometers to nanometers. For instance, metallic copper from CS-Cu and FQ-Cu at rates as low as 100 µg/ml completely inhibited bacterial growth of a copper-tolerant strain of *X. perforans* within 1 h of exposure in vitro. Although the mechanism is unknown, this increase in antibacterial activity is expected to be a result of the small size and high surface-

to-volume ratio of the copper composites (Baker et al. 2005; Morones et al. 2005; Panáček et al. 2006; Santra 2012; Santra et al. 2014). Regardless of the mechanism, copper composites are more effective against a copper-tolerant strain of *X. perforans* than micron-sized metallic copper in vitro.

Greenhouse and field experiments revealed that all three copper composites were either more effective at reducing bacterial spot

Fig. 4. Effect of various metallic copper concentrations from three copper composites: core shell silica copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu); Kocide 3000 (DuPont, Wilmington, DE); and copper-mancozeb on tomato plants under greenhouse conditions. **A**, Experiment 1 consisted of the following treatments: metallic copper at 100, 200, 500, and 1,000 µg/ml from CS-Cu, MV-Cu, and FQ-Cu; metallic copper prepared from Kocide 3000 at 100 µg/ml (0.334 g/liter), 200 µg/ml (0.668 g/liter), 500 µg/ml (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter); a combination of metallic copper at 540 µg/ml from Kocide 3000 (2.1 g/liter) and Penncozeb 75DF (United Phosphorus, Inc.) at 1.2 g/liter (Cu-Man); and sterilized tap water. **B**, Experiment 2 consisted of the following treatments: metallic copper at 100, 200, 500, and 1,000 µg/ml from CS-Cu, MV-Cu, and FQ-Cu; metallic copper prepared from Kocide 3000 at 100 µg/ml (0.334 g/liter), 200 µg/ml (0.668 g/liter), 500 µg/ml (1.67 g/liter), and 1,000 µg/ml (3.34 g/liter); a combination of metallic copper at 540 µg/ml from Kocide 3000 (2.1 g/liter) and Penncozeb 75DF at 1.2 g/liter (Cu-Man); and sterilized tap water. **C**, Experiment 3 consisted of the following treatments: metallic copper at 75, 100, 200, and 500 µg/ml from CS-Cu, MV-Cu, and FQ-Cu; metallic copper prepared from Kocide 3000 at 75 µg/ml (0.251 g/liter), 100 µg/ml (0.334 g/liter), 200 µg/ml (0.668 g/liter), and 500 µg/ml (1.67 g/liter); a combination of metallic copper at 540 µg/ml from Kocide 3000 (2.1 g/liter) and Penncozeb 75DF at 1.2 g/liter (Cu-Man); and sterilized tap water. Phytotoxicity was calculated using the Horsfall-Barratt scale, a nondimensional 12-point scale, to assess the percentage of canopy affected by bacterial spot. Values were converted to midpercentages and used to generate the progression of phytotoxicity overtime. Error bars = standard deviation. *P* value of 0.05 was used in the IBM SPSS Student-Newman-Keuls statistical analysis. There were four plants per treatment in each experiment.

disease severity or at least provided statistically equivalent disease control when compared with micron-sized metallic copper on tomato plants inoculated with a copper-tolerant strain of *X. perforans*. Under greenhouse conditions, metallic copper at rates as low as 100 µg/ml from the copper composites significantly reduced bacterial spot

disease severity in comparison with equivalent rates of micron-sized metallic copper. Additionally, at least one or more of the three copper composites significantly reduced bacterial spot disease severity in comparison with the water control under field conditions, whereas copper-mancozeb did not. Although management plans are

TABLE 1. Effect of copper (Cu) composites—core shell silica copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu)—and concentration (metallic Cu) on bacterial spot disease severity on tomato BHN602, based on area under the disease progress curve (AUDPC ± standard deviation), and the yield of tomato in a field trial conducted during fall 2015 in Quincy, FL

Treatment, metallic Cu	AUDPC ^y	Yield (kg/ha) ^x			
		Medium	Large	Extra large	Total ^z
CS-Cu, 100 µg/ml	599±74 ab	15,258	28,533	24,825	68,616
CS-Cu, 200 µg/ml	588±94 ab	16,204	30,709	27,378	74,291
MV-Cu, 100 µg/ml	480±31 a	15,087	26,318	21,193	62,598
MV-Cu, 200 µg/ml	538±63 a	14,310	25,488	17,387	57,185
FQ-Cu, 100 µg/ml	590±74 ab	13,731	21,432	17,974	53,137
FQ-Cu, 200 µg/ml	662±69 ab	16,345	22,669	18,822	54,836
Kocide, 540 µg/ml	671±99 ab	17,308	24,506	19,211	61,025
Copper-mancozeb, 540 µg/ml	596±185 ab	14,130	22,718	16,582	53,430
H ₂ O	774±140 b	16,043	23,028	21,042	60,113

^x None of the copper-based treatments had a significant ($P < 0.05$) impact on tomato fruit yield based on the IBM SPSS Student-Newman-Keuls (SNK) statistical analysis in this trial.

^y Disease severity was calculated using the Horsfall-Barratt scale, a nondimensional 12-point scale, to assess the percentage of canopy affected by bacterial spot. Values were converted to midpercentages and used to generate AUDPC. Column means indicated with the same letters are not significantly different ($P < 0.05$) based on the IBM SPSS SNK statistical analysis.

^z Total marketable yield (USDA 1976).

TABLE 2. Effect of copper (Cu) composites—core shell silica copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu)—and concentration (metallic Cu) on bacterial spot disease severity on tomato BHN602, based on area under the disease progress curve (AUDPC ± standard deviation), and the yield of tomato in a field trial conducted during spring 2016 in Quincy, FL

Treatment, metallic Cu	AUDPC ^y	Yield (kg/ha) ^x			
		Medium	Large	Extra large	Total ^z
CS-Cu, 100 µg/ml	837±46 a	3,157	6,445	11,624	21,526
CS-Cu, 200 µg/ml	838±48 a	4,162	7,175	11,624	22,961
MV-Cu, 100 µg/ml	887±16 a	2,440	6,745	16,216	25,401
MV-Cu, 200 µg/ml	832±87 a	2,153	4,162	17,507	23,822
FQ-Cu, 100 µg/ml	869±7 a	3,444	7,893	12,628	23,965
FQ-Cu, 200 µg/ml	938±87 a	1,435	5,023	15,785	22,243
Kocide, 540 µg/ml	1,135±60 ab	3,014	6,171	13,202	22,387
Copper-mancozeb, 540 µg/ml	1,188±144 ab	2,296	5,166	11,767	19,229
H ₂ O	1,402±112 b	3,014	6,171	12,915	22,100

^x None of the copper-based treatments had a significant ($P < 0.05$) impact on tomato fruit yield based on the IBM SPSS Student-Newman-Keuls (SNK) statistical analysis in this trial.

^y Disease severity was calculated using the Horsfall-Barratt scale, a nondimensional 12-point scale, to assess the percentage of canopy affected by bacterial spot. Values were converted to midpercentages and used to generate AUDPC. Column means indicated with the same letters are not significantly different ($P < 0.05$) based on the IBM SPSS SNK statistical analysis.

^z Total marketable yield (USDA 1976).

TABLE 3. Effect of copper (Cu) composites—core shell silica copper (CS-Cu), multivalent copper (MV-Cu), and fixed quaternary ammonium copper (FQ-Cu)—and concentration (metallic Cu) on bacterial spot disease severity on tomato HM1823, based on area under the disease progress curve (AUDPC ± standard deviation), and the yield of tomato in a field trial conducted during spring 2016 in Wimauma, FL

Treatment, metallic Cu	AUDPC ^y	Yield (kg/ha) ^x			
		Medium	Large	Extra large	Total ^z
CS-Cu, 100 µg/ml	687±97 a	521	2,824	29,794	28,249
CS-Cu, 200 µg/ml	779±132 ab	512	1,509	27,605	27,190
MV-Cu, 100 µg/ml	833±70 ab	574	2,092	25,584	29,626
MV-Cu, 200 µg/ml	728±49 a	662	2,462	24,066	33,139
FQ-Cu, 100 µg/ml	690±94 a	865	2,815	32,265	35,945
FQ-Cu, 200 µg/ml	713±82 a	653	2,321	32,450	35,424
Kocide, 540 µg/ml	972±140 ab	547	1,968	29,653	32,168
Copper-mancozeb, 540 µg/ml	773±69 ab	424	1,774	29,864	32,062
H ₂ O	1,137±70 b	424	1,818	26,449	28,691

^x None of the copper-based treatments had a significant ($P < 0.05$) impact on tomato fruit yield based on the IBM SPSS Student-Newman-Keuls (SNK) statistical analysis in this trial.

^y Disease severity was calculated using the Horsfall-Barratt scale, a nondimensional 12-point scale, to assess the percentage of canopy affected by bacterial spot. Values were converted to midpercentages and used to generate AUDPC. Column means indicated with the same letters are not significantly different ($P < 0.05$) based on the IBM SPSS SNK statistical analysis.

^z Total marketable yield (USDA 1976).

designed to ensure minimal variability among experiments, the ability of each copper composite to reduce bacterial spot disease severity when compared with the water control varied among field trials. Many complex factors, including environmental conditions and disease, insect, and weed pressure, could have all played a role in the observed variation among field experiments. All these factors are different at each field trial location and within sites between growing seasons. Thus, variability in treatments among field trials is expected for this type of research on bacterial spot. Although reductions in disease severity were observed with at least one of the composites in each field trial, as shown by the AUDPC data, applications of copper composites did not significantly increase fruit yield in these studies. Due to the small scale of these bacterial spot trials, increases in yield are seldom observed in trials of this scale (Huang et al. 2012; Louws et al. 2001; Paret et al. 2013). Furthermore, the variation in fruit size among the three field trials is also expected because different tomato varieties were used in each location. Tomato variety HM1823 was used in the spring 2016 Wimauma trial and produces both large and extra-large round fruit, whereas BHN602, the Quincy trial variety, produces large, globe-shaped fruit (Freeman et al. 2016). When comparing the fall 2015 and spring 2016 Quincy trials, the tomato plants produced enough fruit to allow for an additional harvest at the end of the fall 2015 Quincy trial, whereas tomato plants in the spring 2016 Quincy trial only produced enough fruit for one harvest.

Because copper bactericides have been used for the past five decades to control bacterial spot of tomato, the accumulation of copper ions in the soil is an environmental concern (Marco and Stall 1983; Momol et al. 2008). This is a major concern in areas that have a long history of copper applications, because copper ions are not degraded in soil and can accumulate to high levels (Köller 1999). Since 1998, Florida has harvested 665,800 acres of fresh-market tomato (USDA 2016). Based on grower information pertaining to the number of copper applications (10 to 22 applications per acre per season), tomato growers in Florida applied approximately between 1 and 3 million kg of metallic copper (30% of Kocide 3000 is metallic copper) from 1998 to 2016. An advantage of the copper composites is that bacterial spot disease severity was significantly reduced when using 50 to 90% less metallic copper from the three copper composites in comparison with equivalent rates of micron-sized metallic copper under greenhouse conditions. Furthermore, the copper composites also contained 80% less metallic copper and provided statistically equivalent disease control in comparison with field-applied rates of metallic copper in copper-mancozeb. More specifically, the amount of metallic copper could be reduced from 5.8 kg/ha to 1.2 kg/ha in a single growing season (10 applications/season). If copper composites are labeled for use in the near future, the average amount of metallic copper applied to fresh-market tomato would be reduced to approximately 15,000 kg/year in Florida. Hence, the use of copper composites could potentially reduce the accumulation of metallic copper released into the environment.

In general, tomato plants are highly sensitive to copper-based bactericides and phytotoxicity is a concern. Due to the slow release of copper from the composites, phytotoxicity studies of these composites on ornamental plants (*Vinca* sp.) revealed that phytotoxicity is reduced in comparison with copper salts such as copper sulfate (Young and Santra 2014). However, phytotoxicity becomes more prominent as the copper concentration is increased (Martins and Mourato 2006; Sonmez et al. 2006; Young and Santra 2014). Under greenhouse conditions, tomato plants treated with metallic copper from any of the three copper composites exhibited phytotoxicity. Because phytotoxicity increased as the concentration of metallic copper was increased, the 500 and 1,000 µg/ml rates of metallic copper were eliminated from the field trials. Under field conditions, applications of the copper composites did not cause phytotoxicity and did not reduce fruit yield in tomato plants.

Another key question is whether *Xanthomonas* strains could develop resistance to copper composites as a result of commercialization.

Based on prior knowledge of heavy use of micron-sized copper bactericides and the development of copper resistance in *Xanthomonas* spp., it is likely that the bacterium could develop resistance to copper composites. To combat fungicide resistance, it is often recommended to use at-risk fungicides in a mixture or in rotation with a fungicide of a different type. To reduce the risk of resistance to copper composites, best-management practices, including the use of SAR inducers, biopesticides, and cultural practices, can be incorporated with copper composites as tank mix or in rotation programs. However, the efficacy of copper composites within an IPM program requires further study.

To the best of our knowledge, this is the first study to evaluate copper composites for the management of bacterial spot of tomato. Based on this study, the newly designed copper composites appear to be a promising alternative to micron-sized metallic copper bactericides. The copper composites exhibited high antibacterial activity against both copper-tolerant and -sensitive strains of *X. perforans*, whereas micron-sized metallic copper had no in vitro inhibitory effect against a copper-tolerant *X. perforans* strain. These copper composites were formulated to provide the maximum abundance and sustained release of ionic copper for long-term disease protection and improved adherence of the materials to plant surfaces to improve rainfastness (Santra 2012; Santra et al. 2014). These characteristics allow for the reduction in the amount of metallic copper needed to manage copper-tolerant *X. perforans* strains by 80% in comparison with existing micron-sized metallic copper bactericides. All three copper composites are being evaluated for commercial readiness by the University of Central Florida Office of Research and Commercialization in collaboration with a leading agrochemical company through a sponsored research agreement. This evaluation process includes (i) transition from reagent-grade chemicals to agriculture-grade chemicals, (ii) scalability, (iii) shelf-life, (iv) cost effectiveness, (v) regulatory requirements, and (vi) efficacy in other crops, including crop safety. Studies are also in progress to explore the ability of copper composites to manage bacterial spot caused by *X. euvesicatoria*, *X. gardneri*, and *X. vesicatoria*. Additionally, the fate of copper composites in the environment is being evaluated to understand how the run-off of copper composites affects soil microbial populations in comparison with applications of micron-sized metallic copper.

ACKNOWLEDGMENTS

Funding for this work comes from the United States Department of Agriculture (USDA) Specialty Crop Research Initiative (2015-51181-24312), USDA Agriculture and Food Research Initiative (2016-67021-24992), USDA Current Research Information System (HATCH FLA-NFC-005242), and the Florida Tomato Committee Grant 2015-2016. We thank G. V. Minsavage and R. E. Stall for the technical support in conducting greenhouse experiments; and B. S. Hughes, H. Adkison, and R. Willis for their assistance in maintaining and harvesting fruit from the spring 2016 field trial in Wimauma, FL.

LITERATURE CITED

- Adeleye, A. S., Conway, J. R., Perez, T., Rutten, P., and Keller, A. A. 2014. Influence of extracellular polymeric substances on the long-term fate, dissolution, and speciation of copper-based nanoparticles. *Environ. Sci. Technol.* 48:12561-12568.
- Bae, E., Park, H. J., Lee, J., Kim, Y., Yoon, J., Park, K., Choi, K., and Yi, J. 2010. Bacterial cytotoxicity of the silver nanoparticle related to physicochemical metrics and agglomeration properties. *Environ. Toxicol. Chem.* 29:2154-2160.
- Baker, C., Pradhan, A., Pakstis, L., Pochan, D. J., and Shah, S. I. 2005. Synthesis and antibacterial properties of silver nanoparticles. *J. Nanosci. Nanotechnol.* 5:244-249.
- Balogh, B., Jones, J. B., Momol, M. T., Olson, S. M., Obradovic, A., King, P., and Jackson, L. E. 2003. Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. *Plant Dis.* 87:949-954.
- Conover, R. A., and Gerhold, N. R. 1981. Mixtures of copper and maneb or mancozeb for control of bacterial spot of tomato and their compatibility for control of fungus diseases [*Phytophthora infestans*, *Stemphylium solani*, *Xanthomonas campestris* pv. *vesicatoria*, Florida]. In: *Proc. Annu. Meet. Fla. State Hortic. Soc.*

- Flaherty, J. E., Somodi, G. C., Jones, J. B., Harbaugh, B. K., and Jackson, L. E. 2000. Control of bacterial spot on tomato in the greenhouse and field with H-mutant bacteriophages. *HortScience* 35:882-884.
- Freeman, J. H., Vallad, G. E., and Dittmar, P. J. 2016. Vegetable Production Handbook of Florida 2016-2017. IFAS Extension University of Florida, Gainesville, FL.
- Gitaitis, R., McCarter, S., and Jones, J. 1992. Disease control in tomato transplants produced in Georgia and Florida. *Plant Dis.* 76:651-656.
- Graves, A., and Alexander, S. 2002. Managing bacterial speck and spot of tomato with acibenzolar-S-methyl in Virginia. Online publication. *Plant Health Prog.* doi.org/10.1094/PHP-2002-0220-01-RS
- Gunawan, C., Teoh, W. Y., Marquis, C. P., and Amal, R. 2011. Cytotoxic origin of copper (II) oxide nanoparticles: Comparative studies with micron-sized particles, leachate, and metal salts. *ACS Nano* 5:7214-7225.
- Horsfall, J. G., and Barratt, R. W. 1945. An improved grading system for measuring plant diseases. *Phytopathology* 35:655.
- Horvath, D. M., Stall, R. E., Jones, J. B., Pauly, M. H., Vallad, G. E., Dahlbeck, D., Staskawicz, B. J., and Scott, J. W. 2012. Transgenic resistance confers effective field level control of bacterial spot disease in tomato. *PLoS One* 7:e42036.
- Huang, C.-H., Vallad, G. E., Zhang, S., Wen, A., Balogh, B., Figueiredo, J. F. L., Behlau, F., Jones, J. B., Momol, M. T., and Olson, S. M. 2012. Effect of application frequency and reduced rates of acibenzolar-S-methyl on the field efficacy of induced resistance against bacterial spot on tomato. *Plant Dis.* 96:221-227.
- Iriarte, F., Balogh, B., Momol, M., Smith, L., Wilson, M., and Jones, J. 2007. Factors affecting survival of bacteriophage on tomato leaf surfaces. *Appl. Environ. Microbiol.* 73:1704-1711.
- Jiang, W., Mashayekhi, H., and Xing, B. 2009. Bacterial toxicity comparison between nano-and micro-scaled oxide particles. *Environ. Pollut.* 157:1619-1625.
- Jones, J. B., Lacy, G. H., Bouzar, H., Stall, R. E., and Schaad, N. W. 2004. Reclassification of xanthomonads associated with bacterial spot disease of tomato and pepper. *Syst. Appl. Microbiol.* 27:755-762.
- Jones, J. B., and Jones, J. P. 1985. The effect of bactericides, tank mixing time and spray schedule on bacterial leaf spot of tomato. *Proc. Fla. State Hortic. Soc.* 98:244-247.
- Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S., and Ryals, J. 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annu. Rev. Phytopathol.* 32:439-459.
- Köller, W. 1999. Chemical approaches to managing plant pathogens. Pages 337-376 in: *Handbook of Pest Management*. J. R. Ruberson, ed. M. Dekker, New York.
- Louws, F. J., Wilson, M., Campbell, H. L., Cuppels, D. A., Jones, J. B., Shoemaker, P. B., Sahin, F., and Miller, S. A. 2001. Field control of bacterial spot and bacterial speck of tomato using a plant activator. *Plant Dis.* 85:481-488.
- Madden, L. V., Hughes, G., and van den Bosch, F. 2007. *The Study of Plant Disease Epidemics*. American Phytopathological Society, St. Paul, MN.
- Maniprasad, P., and Santra, S. 2012. Novel copper (Cu) loaded core-shell silica nanoparticles with improved Cu bioavailability: Synthesis, characterization, and study of antibacterial properties. *J. Biomed. Nanotechnol.* 8:558-566.
- Marco, G. M., and Stall, R. E. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. *Plant Dis.* 67:779-781.
- Martins, L. L., and Mourato, M. P. 2006. Effect of excess copper on tomato plants: Growth parameters, enzyme activities, chlorophyll, and mineral content. *J. Plant Nutr.* 29:2179-2198.
- Miller, S. A., Rowe, R. C., and Riedel, R. M. 1996. Bacterial spot, speck, and canker of tomatoes. The Ohio State University Extension, Columbus, Ohio.
- Momol, T., Jones, J., Olson, S., Obradovic, A., Balogh, B., and King, P. 2008. Integrated management of bacterial spot on tomato in Florida. Online publication. University of Florida. <http://ufdc.ufl.edu/IR00003017/00001>
- Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., and Yacaman, M. J. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology* 16:2346-2353.
- Nufarm. 2013. Champ® Formula 2 Flowable. Online publication. http://www.nufarm.com/Assets/19430/3/Champ2F_PIB_13-AG-0020.pdf
- Obradovic, A., Jones, J. B., Momol, M. T., Balogh, B., and Olson, S. M. 2004. Management of tomato bacterial spot in the field by foliar applications of bacteriophages and SAR inducers. *Plant Dis.* 88:736-740.
- Obradovic, A., Jones, J. B., Momol, M. T., Olson, S. M., Jackson, L. E., Balogh, B., Guven, K., and Iriarte, F. B. 2005. Integration of biological control agents and systemic acquired resistance inducers against bacterial spot on tomato. *Plant Dis.* 89:712-716.
- Owens, G. J., Singh, R. K., Foroutan, F., Alqaysi, M., Han, C. M., Mahapatra, C., Kim, H. W., and Knowles, J. C. 2016. Sol-gel based materials for biomedical applications. *Prog. Mater. Sci.* 77:1-79.
- Pallant, J. 2013. *SPSS Survival Manual*. McGraw-Hill Education, London, United Kingdom.
- Panáček, A., Kvítek, L., Pucek, R., Kolář, M., Večeřová, R., Pizúrová, N., Sharma, V. K., Nevečná, T., and Zbořil, R. 2006. Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. *J. Phys. Chem. B* 110:16248-16253.
- Paret, M. L., Vallad, G. E., Averett, D. R., Jones, J. B., and Olson, S. M. 2013. Photocatalysis: Effect of light-activated nanoscale formulations of TiO₂ on *Xanthomonas perforans* and control of bacterial spot of tomato. *Phytopathology* 103:228-236.
- Pohronezny, K., and Volin, R. B. 1983. The effect of bacterial spot on yield and quality of fresh market tomatoes. *HortScience* 18:69-70.
- Potnis, N., Timilsina, S., Strayer, A., Shantharaj, D., Barak, J. D., Paret, M. L., Vallad, G. E., and Jones, J. B. 2015. Bacterial spot of tomato and pepper: Diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Mol. Plant Pathol.* 16:907-920.
- Ritchie, D. F. 2000. Bacterial spot of pepper and tomato. Online publication. *Plant Health Instruct.* doi.org/10.1094/PHI-I-2000-1027-01
- Romero, A., Kousik, C., and Ritchie, D. 2001. Resistance to bacterial spot in bell pepper induced by acibenzolar-S-methyl. *Plant Dis.* 85:189-194.
- Santra, S. 2012. Silica-based antibacterial and antifungal nanoformulation. U.S. Patent No. 8,221,791. U.S. Patent and Trademark Office, Washington, DC.
- Santra, S., Bazata, J., and Young, M. 2014. Core-shell quaternary ammonium nanomaterials, methods and applications. U.S. Patent No. 14/248,725. U.S. Patent and Trademark Office, Washington, DC.
- Scott, J. W., Somodi, G. C., and Jones, J. B. 1989. Resistance to bacterial spot fruit infection in tomato. *HortScience* 24:825-827.
- Sonmez, S., Kaplan, M., Sonmez, N. K., Kaya, H., and Uz, I. 2006. High level of copper application to soil and leaves reduce the growth and yield of tomato plants. *Sci. Agric.* 63:213-218.
- Stöber, W., Fink, A., and Bohn, E. 1968. Controlled growth of monodisperse silica spheres in the micron size range. *J. Colloid Interface Sci.* 26:62-69.
- Strayer, A., Ocoy, I., Tan, W., Jones, J. B., and Paret, M. L. 2016. Low concentrations of a silver-based nanocomposite to manage bacterial spot of tomato in the greenhouse. *Plant Dis.* 100:1460-1465.
- Thayer, P. L., and Stall, R. E. 1962. Streptomycin resistance of the bacterial spot pathogen and control with streptomycin. *Plant Dis.* 46:389-392.
- USDA. 1976. United States standards for grades of fresh tomato. United States Department of Agriculture (USDA) Marketing Service, Washington, DC.
- USDA. 2016. Vegetables: Acreage, production, and value. Online publication. United States Department of Agriculture (USDA) National Agricultural Statistics Service. https://www.nass.usda.gov/Quick_Stats/index.php
- Vallad, G., Pernezny, K., and Momol, T. 2004. A Series on Disease in the Florida Vegetable Garden: Tomato. University of Florida, IFAS Extension, Gainesville, FL.
- Yamamoto, O. 2001. Influence of particle size on the antibacterial activity of zinc oxide. *Int. J. Inorg. Mater.* 3:643-646.
- Young, M., and Santra, S. 2014. Copper (Cu)-Silica nanocomposite containing valence-engineered Cu: A new strategy for improving the antimicrobial efficacy of Cu biocides. *J. Agric. Food Chem.* 62:6043-6052.