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The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium

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Nanoparticles (NP) have great potential in agriculture. For example, micronutrients have poor mobility in plants and poor availability in neutral soils, yet they play pivotal roles in root health. We investigated whether foliar sprays of micronutrient NP could affect plant health in disease infested soils. In the greenhouse, NP of AlO, CuO, FeO, MnO, NiO, and ZnO were sprayed on tomatoes and grown in soilless medium infested with the *Fusarium* wilt fungus. NP of CuO, MnO, or ZnO reduced disease estimates [area-under-the-disease-progress-curve (AUDPC)] by 31%, 28%, or 28%, respectively, when compared to untreated controls. When NP of CuO, MnO, or ZnO, their bulked equivalents, or their sulfate salts were compared to untreated eggplants and held in the greenhouse in soilless medium infested with the *Verticillium* wilt fungus, NP of CuO increased fresh weights by 64%, reduced AUDPC values by 69%, and had 32% more Cu in the roots. These same amendments were sprayed onto the foliage of tomato and eggplant transplants and set in field plots in soil heavily infested with the *Verticillium* wilt fungus. Compared to untreated controls, yields of tomato were 33% or 31% greater with NP of CuO or the bulked MnO, respectively. NP of CuO or ZnSO₄ increased eggplant yields by 34% or 41% when compared to controls, respectively. *In vitro* studies found NP of CuO were not inhibitory to the *Fusarium* wilt fungus, suggesting host defense was being manipulated.

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Nano impact

Micronutrients, such as Cu, Mn and Zn, protect plants from root disease, but foliar applications are not basipetally translocated to roots. Furthermore, soil treatments are usually ineffective due to poor availability in near neutral pH soils. The potential for using nanoparticles of micronutrients to nourish roots in disease infested soils is great, but a dearth of information exists on efficacy and application. The current study was designed to investigate whether or not foliar applications of metallic oxides of known micronutrients could affect plant health when grown in soil with known plant pathogens. After prescreening several NP, we focused on NP of CuO, MnO, or ZnO. Of these, CuO NP was the most effective treatment and consistently increased plant growth and yield in the presence of virulent pathogens. Root digests revealed increased Cu levels, suggesting that application of the NP may allow for increased basipetal translocation. These findings suggest plant health could be improved following applications of low levels of NP to young plants, thereby minimizing human and environmental exposure to these particles.

1. Introduction

The unique size and properties of NP result in enhanced performance in biological systems when compared to traditional bulk or ionic materials.¹ In agriculture, there is great interest in the ability of this technology to improve food production, storage, security, and safety through its use in nanofertilizers, nanopesticides, and nanosensors.¹ However, the effect and fate of NP in plants has only begun to receive attention. Most

studies have examined NP role in toxicity to plants,^{2,3} but their possible role in providing nutritional benefits to plant organs to promote plant health has more recently been explored.^{4,5}

The literature demonstrates enhanced effectiveness toward plant pathogens as a function of nanoscale size. The role of NP of Ag,^{6–10} Cu,^{5,12,18} Si,¹⁴ Ti,¹⁵ and Zn¹¹ in plant defense has been explored; several creative methods for utilizing nanoparticles have been employed. For example, Ali *et al.*⁶ synthesized NP of Ag with plant extracts and used them as foliar sprays to suppress *Phytophthora* infection on plants grown in a growth chamber. Paret *et al.*¹⁵ used a novel photocatalytic reaction to produce reactive oxygen species from NP of doped formulations of Ti oxides that suppressed bacterial leaf spot and increased tomato yield when applied foliarly.

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The literature also shows enhanced intra-plant NP translocation. NP of Fe, Cu, Ag, Si, and Zn are absorbed by plants at rates frequently greater than their bulked equivalents.^{3,11,16–18} Wang *et al.*¹⁸ first reported on the movement and distribution of NP of CuO in maize plants. Dimkpa *et al.*¹¹ similarly showed systemic transport of NP of CuO and ZnO. Stampoulis *et al.*¹⁷ reported that the uptake of NP of Ag in zucchini plants was 4.7 times greater than plants exposed to the bulk equivalent. Although many of these reports show transport and toxicity of NP,³ there is less information on basipetal transport of NP of micronutrients applied foliarly.

The role of micronutrients in suppression of crop disease is well documented,¹⁹ but most micronutrients (Cu, Mn, Zn) are poorly translocated to the roots following foliar applications.²⁰ These applications can correct foliar deficiencies, but rarely impact root health. Root tissues under attack by plant pathogens rapidly activate enzyme systems to generate defense products.²¹ Micronutrients such as Cu, Mn, and Zn have each been implicated in activating defense reactions that lead to resistance mechanisms.^{22–24} Other metals like Al, Fe, and Ni have also been implicated in disease suppression, although their impact as NP have not been examined.^{26–28}

This research examines the effects of foliar applications of NP metal oxides on tomatoes grown in soil and soilless mix infested with *Fusarium oxysporum* Schltdl.:Fr. f. sp. *lycopersici* (Sacc.) W. C. Snyder & H. N. Hansen, the cause of Fusarium wilt and on eggplant grown in soil and soilless mix infested with *Verticillium dahliae* Kleb., the cause of Verticillium wilt. Both diseases are common in the Northeastern USA and are persistent threats to tomato and eggplant cultivation.^{29,30} Although resistant cultivars are available, many of the horticulturally desirable cultivars are susceptible. Current strategies are growers are forced to fumigate, or relocate to new land.^{29,30} Specifically, our objective was to compare the effect of single foliar applications of NP to untreated plants or to plants treated with the larger bulk equivalent or the sulfate salt of each metal for effect on disease progression, growth, and yield. We also determined element tissue levels to assess whether significant *in planta* transport occurred for the metal oxide in question.

2. Materials and methods

2.1 Nanoparticles and plant materials

NP of Al (20 nm), Cu (30 nm), Fe (20–40 nm), Mn (40 nm), Ni (10–20 nm), and Zn (10–30 nm) oxides were obtained from US Research Nanomaterials (Houston, TX). Bulk oxide equivalents and sulfate salts were obtained from Fisher Scientific (New Jersey, USA). NP solutions were prepared at 0.1 or 1.0 mg ml⁻¹ distilled water and were sonicated for at least 10 min in a FS20H Ultrasonic cleaner (Fisher Scientific Inc., Pittsburgh, PA) prior to application. Bulk suspension were applied at the same concentrations as the respective NP. Sulfate salt were adjusted so the respective metal concentration was constant. No surfactants were used in these studies.

2.2 Plant culture, inoculum, and disease assessment in the greenhouse

Tomato cv. Bonnie Best and Eggplant cv. Black Beauty (Comstock Ferre Seed Co., Wethersfield, CT) seeds were germinated in soilless potting mix (ProMix BX, Premier Hort Tech, Quakertown, PA, USA) and fertilized once after three weeks with 40 ml of Peter's soluble 20–10–20 (N–P–K) fertilizer (R. J. Peters Inc., Allentown, PA). When plants reached the 3- to 4-leaf stage, medium size plants were selected. Initially, plant roots were washed free of all soilless mix, inverted, and all above-ground foliage was immersed into the treated suspensions (bulk, NP, salt) or distilled water. Plants were drained, keeping roots unexposed, dried, and transplanted into 10 cm pots filled with 300 cc of soilless mix. For tomato, the soilless medium was infested with the pathogenic fungus, *Fusarium oxysporum* f. sp. *lycopersici* (race 2 isolate FRC O-0113, Fusarium Research Center, Penn State Univ., University Park, PA) (1 g inoculum per liter soilless mix = yields approximately 1 × 10⁵ colonies per g potting mix as determined by serial dilution onto 25% potato-dextrose-agar (Difco, Corpus Christi, TX)). The inoculum was prepared on autoclaved millet seed (1:1 millet to H₂O) that had been seeded with an agar plug colonized by the virulent isolate of *F. oxysporum* f. sp. *lycopersici* and allowed to incubate for 7 days. Inoculum was dried, ground in a mill, and passed through a 1 mm sieve. Eggplants were inoculated by drenching pots with 40 ml of a spore suspension of *V. dahliae* (5 × 10⁵ spore ml⁻¹). The inoculum was produced on 25% potato-dextrose-agar (Difco, Corpus Christi, TX). The inoculum was held at 22–25 °C for 10 days, washed from the plate and counted using a hemocytometer. An equal number of plants were grown in non-infested soilless mix for tissue analyses. All plants received 50 ml of a complete fertilizer solution (20–20–20 NPK) once per month.

As symptoms of disease developed, plants were rated for severity approximately twice per week on a scale of 1 to 5 where 1 = no disease, 2 = slightly stunted plants, 3 = stunted and or partially wilted plants, 4 = completely wilted plants, and 5 = dead plants. The progress of disease developed was plotted over time and the area-under-the-disease-progress-curve (AUDPC) was calculated using the trapezoid rule where: $AUDPC = \sum (Y_i + Y_{i+1})/2 \times (t_{i+1} - t_i)$, where Y_i = disease rating at time t_i .³¹ The pathogen was re-isolated from wilted stem tissue to confirm its association with the disease.

2.3 Experimental field plots

NP, bulk oxides, or sulfate salts of Cu, Mn and Zn were applied to 6 week-old tomatoes and eggplants in the greenhouse. One week later, plants were transplanted into experimental field plots at Lockwood Farm in Hamden, CT. The soil was a Cheshire fine sandy loam (Typic Dystrocrept) (pH 6.1). The field had been planted with eggplants and other solanaceous hosts for over 20 years and has high populations of *V. dahliae* that remain constant at approximately 13 ± 5 microsclerotia per g soil as determined by the method of

Huisman and Ashworth.²⁵ Both eggplant and tomato are highly susceptible to *Verticillium* wilt. The fields were cover-cropped each year with winter rye and plowed under on 31 May 2013 or 25 May 2014. Granular fertilizer (10–10–10, N–P–K) was broadcasted at 112 kg N ha⁻¹. Although soil tests for micronutrients were not done, no evidence of Cu, Mn, or Zn deficiencies have ever been observed on plants at Lockwood farm. Raised beds were prepared 1.5 m apart and covered with 4 mil black plastic. Irrigation drip tape was laid down. Experiments were separated by 3.6 m. Plants were set in plots 0.75 m spacing. An application of carbaryl or pyrethroid insecticides was applied at label rates in the first few weeks to suppress flea beetles. Plots were irrigated to ensure weekly rainfall was at least 2.5 cm. Tomatoes were evaluated in 2013, but eggplants were evaluated in both 2013 and 2014. In 2014, another treatment was added. MgSO₄ was applied at the rate where the SO₄²⁻ ion was the same as the mean of the other sulfate salts.

Three weeks after planting, plants were rated for disease severity by visually estimating the percentage of the canopy exhibiting symptoms of chlorosis and wilt. Tomato fruit number and fruit weight were recorded on two occasions during growth and plant weight was recorded at harvest. For eggplants, canopy size (CS) was estimated every 1 or 2 weeks for 13 weeks in 2013 and for 9 weeks in 2014. Details for estimating CS are described elsewhere.³¹ The CS values were integrated over time to produce the area under the plant canopy growth curve (CGC, m² × days) in analogy with the formula used for AUDPC.³⁰ Disease assessments were also taken and integrated to produce AUDPC as described above. Marketable eggplant fruit were harvested, counted, and weighed every 4 to 5 days from early August through early October. Total fruit number and weight were computed along with average fruit size.

2.4 Elemental analysis

Dried root tissue from greenhouse experiments were ground in a Wiley mill, passed through a 1 mm sieve, and composited according to block to yield three bulked samples per treatment. In some experiments, leaf tissue was similarly bulked and assayed. In 2013, one medium eggplant fruit from each plot was assayed to determine if the edible flesh portions had greater uptake of the test elements. Digests on ground samples (0.5 g) were done in 50 ml polypropylene digestion tubes with 5 ml of concentrated nitric acid at 115 °C for 45 min using a hot block (DigiPREP System; SCP Science, Champlain, NY). The Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn content was quantified using inductively coupled plasma emission spectrometry on an iCAP 6500 (Thermo Fisher Scientific, Waltham, MA), and is expressed as µg g⁻¹ (dry weight) plant tissue.

2.5 Experimental design and statistical analyses

Greenhouse repetitions had 10 to 12 replicates in randomized blocks of 2 to 3 replicates per block. Data sets were combined when no interaction between treatment and experiment was detected. Greenhouse and field studies were analyzed as ran-

domized complete block designs and means were separated using Fisher LSD tests at $P = 0.05$ or the non-parametric rank test Kruskal–Wallis. Treatment effects were tested using the SYSTAT V.10 (Cranes Software International Limited, Bangalore, Karnataka, INDIA) procedure for mixed model ANOVA with year and replication as random effects. The data from MgSO₄ plots were removed from the field analyses in 2014 to provide a balanced design for analysis.

2.6 *In vitro* assessment of NP and bulked equivalent on *Fusarium oxysporum* f. sp. *lycopersici*

NP or bulk equivalents of CuO, MnO, or ZnO were amended into 25% potato dextrose agar at 0, 10, 100, or 1000 µg ml⁻¹ agar autoclaved, poured into petri dishes (10 cm diam.), and allowed to cool (Difco, Corpus Christi, TX). Three replicate agar plates were seeded in the center by mycelial transfer with a 4 mm agar plug colonized by *F. oxysporum* f. sp. *lycopersici* and held at 25 °C in the dark. The colony was measured twice at right angles from the edge of the mycelial front to the opposite end on day 3 and day 6. The geometric mean was calculated for each plate at each time and those values were integrated over the three time points (0, 3, and 6) to produce a single value reflecting the area under radial colony expansion curve in analogy to the AUDPC described above. Means were plotted over the Log metal concentration.

3. Results

3.1 Effect of NP on tomato and eggplant growth, disease, and elemental composition in greenhouse trials

Since significant interactions between the repetitions of the greenhouse tomato studies and the treatments were not detected ($P = 0.243$), experiments were combined. No difference was detected between the two rates (0.1 and 1 mg test nanoparticle L⁻¹ H₂O) ($P = 0.973$) so only the larger rate is shown (Fig. 1). *Fusarium* wilt symptoms developed in the untreated plants about three weeks after inoculation. All six NP were effective in reducing the AUDPC, but suppression was the greatest for NP of CuO, MnO, and ZnO when compared to untreated plants. No evidence of phytotoxicity was noted in these trials. Root tissue digests revealed tomatoes treated with NP of CuO had elevated levels of Cu when compared to other treatments (Table 1). Applying NP of MnO and ZnO did not affect root levels of Mn or Zn. The other elements Ca, Fe, K, Mg, P, and S were unaffected by the any of the treatments (data not shown).

In the greenhouse, wilt symptoms developed one week after inoculation in control eggplants. Plant weights were strongly affected by inoculation ($P < 0.001$). In the absence of disease, no treatment differed from the controls (Table 2). However, when the plants were inoculated, only NP of CuO increased fresh weight compared to controls. AUDPC values revealed that most treatments (except NP of MnO and MnSO₄) were effective in suppressing *Verticillium* wilt. No phytotoxicity was noted with any of the treatments.

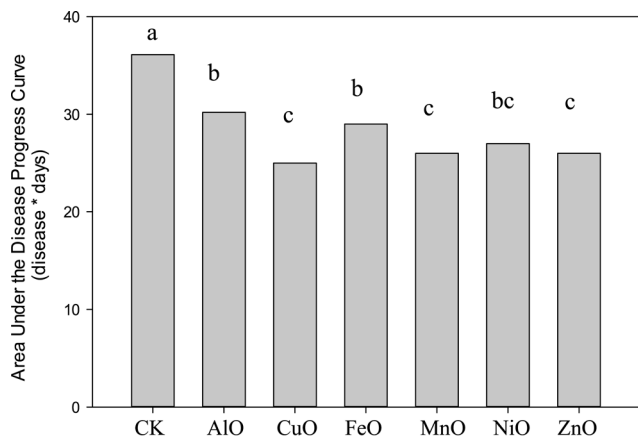


Fig. 1 The effect of foliarly applied nanoparticles of metallic oxides (1 mg l^{-1}) on the integrated estimates of disease ratings over time (area-under-the-disease-progress-curve) of tomato plants grown in soil with *fusarium oxysporum* f. sp. *lycopersici*.

Table 1 Cu, Mn, and Zn levels in roots of tomato plants treated with nanoparticles (NP) of CuO, MnO, and ZnO

NP	Cu ($\mu\text{g g}^{-1}$ tissue)	Mn ($\mu\text{g g}^{-1}$ tissue)	Zn ($\mu\text{g g}^{-1}$ tissue)
Untreated	21.2 ab	107.2	116.1
CuO	28.1 b	124.0	86.0
MnO	18.7 a	174.8	88.8
ZnO	19.8 a	102.9	147.5
	Fisher LSD	Ns	Ns

Elemental analysis of Cu, Mn, and Zn in plant roots and leaf tissue revealed difference were associated with treatment (Table 3). Root concentrations of Cu were higher in plants treated with NP of CuO when compared to untreated control or to the bulk equivalent of CuO. Cu levels were high on leaves of plants treated with NP of CuO or CuSO_4 , but not on leaves treated with the bulk equivalent of CuO. Compared to controls, Mn levels were elevated in roots of plants treated with NP of MnO and NP of ZnO. All Mn treatments increased leaf levels. We detected no increase in Zn in roots of plants treated with any of the Zn compounds, but leaf tissue showed significant increases for all three Zn sources.

3.2 Effect of NP on tomato and eggplant yield in field trials

Tomatoes planted in *V. dahliae* infested soil did not develop typical symptoms of wilt so disease estimates are not presented. Plants treated with NP of CuO and bulked oxides of MnO produced the greatest number and yield (kg) of fruit per plot (Table 4). Copper sulfate and ZnSO_4 also produced plants with greater fruit weight and larger sizes when compared to controls.

Verticillium wilt symptoms on eggplant appeared approximately four weeks after planting. No interactions between treatment and year were observed for the canopy-growth-progress-curve values or for the AUDPC, so data from both years were combined (Table 5). Estimates of the plant canopies integrated over the season for each treatment did not differ from the control, although the two largest canopies

Table 2 Effect of nanoparticles (NP) of Cu, Mn, and Zn oxides on growth of greenhouse-grown eggplant transplants in soil infested with *Verticillium dahliae*. Values are in g (dry weight)

Treatment ^d	Inoculated with <i>V. dahliae</i>		Area under the disease progress curve ^c
	Non-inoculated	Fresh weight (g)	
Control	14.2 ab	8.9 a ^b	114 a
CuO	14.2 ab	10.6 ab	69 b
bulk			
CuO NP	17.2 b	14.6 b	36 b
CuSO_4	14.7 ab	12.6 ab	69 b
MnO	14.3 ab	9.9 a	66 b
bulk			
MnO NP	12.8 a	9.7 a	84 ab
MnSO_4	15.1 ab	9.7 a	75 ab
ZnO	12.7 a	10.0 ab	71 b
bulk			
ZnO NP	14.0 ab	9.5 a	72 b
ZnSO_4	13.6 ab	10.5 ab	56 b

^a Treatments were applied as foliar sprays at $1.0 \text{ mg metal oxide per ml}$ distilled water. Sulfate salts were applied at the same metal equivalent per ml distilled water. ^b Values represent the mean of six replicates; values followed by differing letters are significantly different at Fisher LSD at $P = 0.05$. ^c Area under the disease progress curve calculated using the equation: $\text{AUDPC} = \sum (Y_i + Y_{i+1})/2 \times (t_{i+1} - t_i)$, where Y_i = disease rating at time t_i .

were those treated with NP of CuO and ZnSO_4 . Given the wide variation among plots, the estimates of disease progress did not differ for the treatments when compared to untreated controls. The treatment \times years interaction terms were significant for marketable yield ($P = 0.008$), so both years are presented separately (Table 5). When compared to the 2013 yield, productivity was less than half in 2014, compared to 2013. However, the only treatments that affected yield in both years were NP of CuO and ZnSO_4 . In 2014, the treatment MgSO_4 was included to evaluate a possible contribution of S to growth and disease development, but no significant differences were observed when compared to the 2014 controls. Fruit concentrations of Cu, Mn, and Zn were not affected by treatment (Table 5). Other elements in the fruit skin and flesh were also assayed and found to be unaffected by treatment (data not shown).

When the NP of CuO, MnO, or ZnO were compared to their respective bulked equivalents for their effect on the integrated value of radial expansion of *F. oxysporum* f. sp. *lycopersici*, over 6 days *in vitro*, only NP of ZnO and its bulked equivalent were inhibitory and only at the highest concentration of $\log 3$ (Fig. 2). The bulk equivalent of ZnO was not as toxic as the NP form. On the other hand, the NP of CuO were stimulatory at the highest concentration of $\log 3$ ($1000 \mu\text{g ml}^{-1}$ PDA) while the bulked equivalent did not differ from the untreated control ($\log 0 \mu\text{g ml}^{-1}$ PDA). The concentration of MnO at $\log 2$ ($100 \mu\text{g ml}^{-1}$ PDA) was the most stimulatory for both forms of MnO. The stimulation declined at $\log 3$ for the both forms of MnO, the growth was still greater than the untreated control ($\log 0 \mu\text{g ml}^{-1}$ PDA).

Table 3 Concentration of Cu, Mn, and Zn in roots and leaves of eggplants treated with oxides of Cu, Mn, or Zn as nanoparticles (NP), as larger bulked equivalent of CuO, MnO, and ZnO, or as sulfate salts of Cu, Mn, or Zn

Treatment ^a	Roots ($\mu\text{g g}^{-1}$)			Leaves ($\mu\text{g g}^{-1}$)		
	Cu	Mn	Zn	Cu	Mn	Zn
Control	25.1 b ^b	36.5 a	135.0	4.2 a	101.6 a	88.8 a
CuO bulked	25.9 b	37.0 a	131.4	7.5 a	95.4 a	98.7 a
CuO NP	33.2 c	44.6 ab	129.9	25.0 b	113.7 ab	92.0 a
CuSO ₄	24.5 b	39.0 a	151.6	24.0 b	100.6 a	99.3 a
MnO bulked	26.3 b	44.7 ab	134.6	5.0 a	131.1 b	98.4 a
MnO NP	22.9 ab	48.4 b	143.0	4.5 a	128.4 b	110.5 a
MnSO ₄	28.7 b	45.8 ab	141.9	4.7 a	135.4 b	102.0 a
ZnO bulked	21.6 ab	48.7 b	121.5	4.1 a	115.0 ab	143.7 b
ZnO NP	20.9 a	41.4 ab	130.3	4.5 a	104.9 a	137.9 b
ZnSO ₄	18.7 a	41.0 ab	142.1	4.6 a	99.7 a	130.6 b

^a Treatments were applied as foliar sprays at 1000 μg (test metal) per ml distilled water. ^b Values represent the mean of 2 bulked replicates (3 plants per bulked replicate); values followed by differing letters are significantly different by Tukey's test at $P = 0.05$.

Table 4 Yield components of tomatoes treated with nanoparticles (NP) or the larger bulked equivalents of CuO, MnO, and ZnO or with sulfate salts of Cu, Mn, or Zn

Treatment	No. of fruit per plot	Yield (kg per plot)	Average fruit mass (g)
Untreated	46.3 a ^a	3.2 a	70.9 a
Bulked CuO	45.5 a	3.1 a	70.3 a
NP CuO	61.8 b	4.4 b	71.2 a
CuSO ₄	53.7 ab	4.1 b	76.4 b
Bulked MnO	60.8 b	4.2 b	69.0 a
NP MnO	48.3 a	3.4 a	71.2 a
MnSO ₄	46.0 a	3.0 a	62.3 a
Bulked ZnO	42.2 a	3.2 a	71.8 a
NP ZnO	41.7 a	3.3 a	75.2 ab
ZnSO ₄	56.2 ab	4.5 b	83.8 b

^a Values followed by different letters are significant different by Fisher LSD.

4. Discussion

The use of NP of metal oxides to suppress *Fusarium* and *Verticillium* wilt is a novel strategy for pathogen control. The unique size and properties of NP offers new uses for disease suppression and fertilization.⁵ While the performance of these NP varied across our studies, we observed that NP of CuO were more frequently associated with increases in growth and yield of plants when grown in pathogen-infested soils than any other the other treatments. In certain experiments, the ionic salt of Cu stimulated growth. In the case of Zn, the sulfate salt was frequently more effective than either the NP or bulk oxide form. In all cases, the results were surprising given that lasting, often season-long, effect was achieved with a singular dose to a young transplant. These findings are extremely important for future field applications, in that small amounts of NP could be applied under controlled and safe conditions to young transplants, thereby reducing concerns over NP exposure to humans and the environment.

In the greenhouse study, we observed greater levels of Cu in the roots of plants treated with NP of CuO, as well as reductions in disease. In the field, we observed increases in yield on tomatoes and eggplants treated with NP of CuO when compared to untreated controls. Since care was taken

to ensure that no CuO product were applied to the soil, we do not believe root absorption of Cu applied from the foliar treatment was significant. Therefore, we suggest basipetal translocation of NP of CuO may have occurred. Future studies will examine the actual fate of the NP in plants to determine whether or not the NP is being loaded into the symplast or whether the NP is being biologically dissolved into its ionic form and then translocated to the roots.

A few reports have also found that NP of CuO were effective in suppression of other crop diseases. In a field study with tomato (*Lycopersicon esculentum*), late blight (*Phytophthora infestans*) was suppressed more with NP of CuO than other applications of Cu-based hydroxide fungicide (Kocide 2000).¹² NP of CuO applied to paper discs at 20 μg per disk were more inhibitory to the pathogens *Phoma destructiva*, *Alternaria alternata*, *Curvularia lunanata*, and *Fusarium oxysporum* than the fungicide carbendazim.¹³ These findings conflict with our *in vitro* studies where NP of CuO were ineffective in reducing the radial expansion of *F. oxysporum* f. sp. *lycopersici*. We recognize in our study that mixing the NP of CuO in the agar might have alter its inhibitory effect. However, given that the greatest concentration of 1000 μg NP CuO per ml had no inhibitory effect on the radial expansion of the pathogen, we propose it is unlikely that NP

Table 5 Canopy growth, disease, and yield of eggplants grown in soil with *Verticillium dahliae* and treated with oxides of Cu, Mn, or Zn as nanoparticles (NP), as larger bulked equivalents, or as sulfate salts of Cu, Mn, or Zn

	Canopy growth progress ^a	AUDPC ^b	Yield 2013 (kg per plant)	Yield 2014 (kg per plant)	2013 fruit ($\mu\text{g g}^{-1}$)		
					Cu	Mn	Zn
Control	15.1 ab ^c	383 ab	3.0 a	1.5 a	9.4	13.5	13.1
CuO bulked	16.5 b	370 ab	3.2 ab	2.0 ab	4.9	9.3	8.4
CuSO ₄	15.1 ab	330 ab	2.3 a	2.4 ab	8.0	16.9	17.5
CuO NP	16.7 b	540 b	4.2 b	2.6 b	7.0	15.9	14.5
MnO bulked	14.8 ab	308 ab	2.2 a	2.0 ab	7.3	15.7	16.1
MnSO ₄	15.3 ab	365 ab	2.2 a	2.3 ab	8.9	16.7	18.9
MnO NP	15.0 ab	294 a	3.0 ab	2.5 ab	8.0	16.0	18.2
ZnO bulked	15.1 ab	262 a	3.2 ab	2.4 ab	7.5	1.11	15.0
ZnSO ₄	16.9 b	385 ab	5.0 b	2.7 b	6.7	15.8	14.8
ZnO NP	13.6 a	421 ab	2.8 ab	2.2 ab	7.3	14.7	13.3
MgSO ₄ ^d	—	—	—	2.2 ab	—	—	—

^a Canopy growth curve ($\text{m}^2 \times \text{days}$); an integrated estimate of the plant canopy size (dm^2) measured weekly during 2013 and 2014. ^b Area under the disease progress curve based on weekly estimates of disease severity measured in 2013 and 2014. ^c Values represent the mean of five replicate field plots (3 plants per plot) for 2013 and 2014; values followed by differing letters are statistically different by Tukey's test at $P = 0.05$. ^d MgSO₄ was included only in 2014.

of CuO was directly acting as a fungicide. Alternatively, the detection of greater levels of Cu in the roots of greenhouse plants foliarly treated with NP of CuO offers the possibility that increased Cu levels may furnish the root cells with im-

proved nutrition, which may in turn, enhance resistance to infection. Root infecting fungi like *Fusarium* and *Verticillium*, infect early in the season and remain in low density in the roots until symptoms appear at anthesis.³² At this time, carbohydrates are diverted to developing flowers and fruits, and fungi must begin invading tissue for nutrients. Supplemental Cu may enhance defense products.²³ In soybean, applying Cu increased polyphenol oxidases even though no Cu deficiency symptoms were evident.³³ At present, we are testing the hypothesis that NP of CuO may affect the production of defensive secondary metabolites. Alternatively, Cu ions could suppress disease by initiating the hypersensitive response (HR) in plant activated disease resistance. Although we did not note any obvious necrosis on treated leaves, the HR response may have initiated.

The ZnSO₄ treatment also increased yield in eggplant and tomato and tended to be better than the ZnO NP in reducing disease. In general, elevated Zn concentrations are associated with higher levels of *Fusarium* disease on tomatoes, but those studies were conducted where ionic salts were fed to tomato roots hydroponically.²² Duffy²² did not cite *Verticillium* as a disease affected by Zn. More research is needed to understand why ZnSO₄ promoted yield. Although we did not measure the onset of flowering and fruit set, it is unlikely that NP of CuO or ZnSO₄ treatment would promote early harvest since most growth prompting treatment have the effect of delaying anthesis and the onset of fruiting so plants remain vegetative longer and produce flowers and fruit later in the season.³²

Although Zn oxides (bulked and NP) were inhibitory to the radial growth of the pathogen *in vitro*, the effect was only observed at 1.0 mg ml^{-1} . Given that this concentration is very high, it is unlikely that it was acting as a direct fungicide. We also recognized that the NP formulation of ZnO may agglomerate upon exposure to water and despite sonication, the availability of Zn was less than in salt form. Using coated NP and/or surfactants may increase bioavailability.³⁷

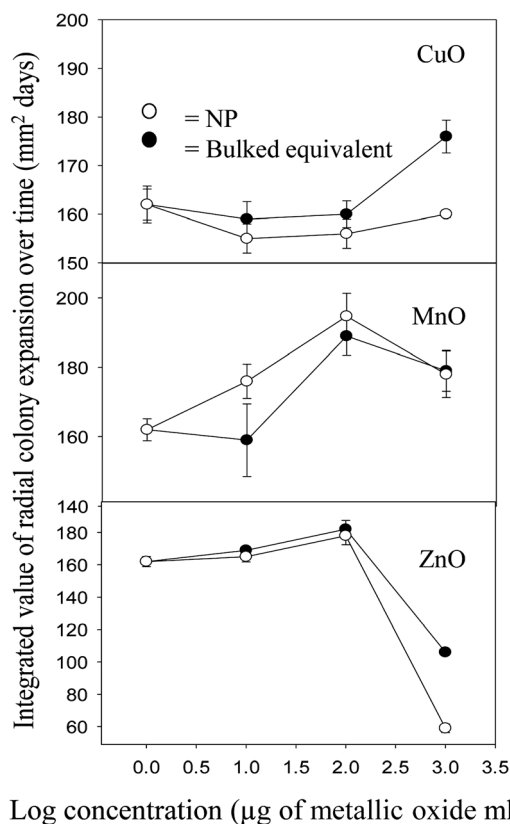


Fig. 2 The effect of nanoparticle (NP) or bulked equivalent rates (log concentration) of CuO, MnO, or ZnO on the integrated values of the radial colony expansion of *Fusarium oxysporum* f. sp. *lycopersici* on 25% potato dextrose agar over three time points. Error bars represent the standard error of the mean.

A consensus exists that soil conditions that increase micronutrient availability are associated with suppression against soilborne diseases caused by *Fusarium* and *Verticillium*.^{32,34–36} Delivering sufficient micronutrient fertilization to roots of established plants is problematic because soil applied micronutrients are not very available in neutral or slightly acidic soils and foliarly applied micronutrients are basipetally translocated.²⁰ NP may offer an improved method of delivering essential micronutrients to the roots of plants in disease stressed soils. Although we found no detectable increase in Cu, Mn, or Zn in the eggplant fruit from treated plants, we recognize that caution needs to be exercised until the safety of NP application on food crops has been thoroughly examined.³⁸

5. Conclusion

We investigated in the greenhouse and field whether foliar sprays of micronutrient NP on tomatoes and eggplants could affect plant weight and yield when grown in disease infested soils. Initial screens in the greenhouse with NP of AlO, CuO, FeO, MnO, NiO, and ZnO that were sprayed on tomatoes and grown in soil infested with the *Fusarium* wilt fungus found that NP of CuO, MnO, or ZnO reduced disease estimates when compared to untreated controls. In field experiments, NP of CuO and ZnSO₄ were more frequently associated with increases in growth and yield of both tomatoes and eggplants than NP of MnO or ZnO, bulked forms of CuO, MnO, or ZnO, or sulfate salts of Cu or Mn. *In vitro* studies found NP of CuO were not inhibitory to the *Fusarium* wilt fungus suggesting host defense was being manipulated. Use of NP of CuO could be used to boost vigor and yield in plants grown in disease-stressed soils.

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