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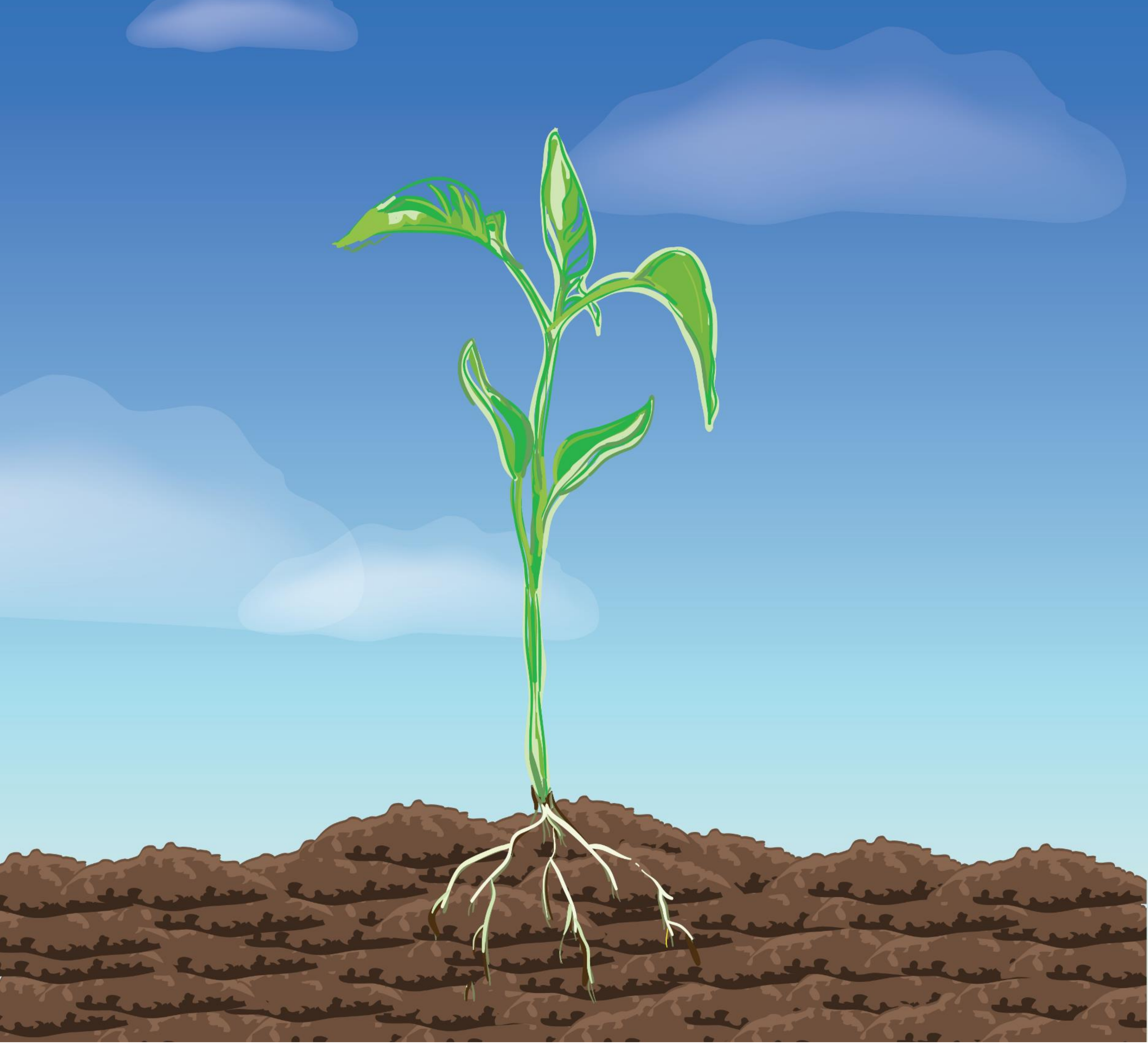
Nanoscale Micronutrients Suppress Disease



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

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Virtual Fertilizer Research Center

Connecticut Agricultural Experiment Station

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List of acronyms and abbreviations

AC	Activated Carbon
BC	Black Carbons
CNM	Carbon-Based Nanomaterial
CNT	Carbon Nanotube
CVD	Chemical Vapor Deposition
EFSA	European Food Safety Authority
IFDC	International Fertilizer Development Center
IPM	Integrated Pest Management
MS	Murashige and Skoog
MWCNT	Multiwall Carbon Nanotubes
NM	Nanomaterial
NP	Nanoparticle
OM	Organic Matter
PAH	Polycyclic Aromatic Hydrocarbon
QD	Quantum Dot
SWCNT	Single-Wall Carbon Nanotube
TCE	Trichloroethylene
VFRC	Virtual Fertilizer Research Center

1 Overview of nanotechnology in agriculture

The exponential growth in nanotechnology during the last decade has begun to revolutionize multiple industries and has a projected market value of $3 trillion by 2020 (Roco et al., 2011). Nanotechnology encompasses the production, characterization and application of materials with dimensions measured at the nanometer scale (10-9), typically less than 100 nm. The unique characteristics and high reactivity of nanoparticles (NPs) play a critical role in this discipline by providing enhanced performance over traditional bulk materials. The broad range of industries that have been impacted by nanotechnology includes disease treatment and drug delivery, electronics, cosmetics, food packaging and agriculture, among others (Naderi and Danesh-Shahraki, 2013).

Nanotechnology in agriculture has played and will play a key role in food production, food security and food safety throughout the world. The broad range of applications in agriculture includes nanofertilizers to increase plant growth and yield, nanopesticides for the control of crop pests and diseases and nanosensors for soil quality and plant health monitoring. Over the past decade, patents and products incorporating nanomaterials for agricultural practices (e.g., nanopesticides and nanofertilizers) have been developed. It has been previously reported that in 2011 over 3,000 patent applications dealing with nanopesticides were submitted (Kah et al., 2013). Selected products/patents acquired from patent databases using nano formulations in agriculture are listed in Table 1. The collective goal of all of these approaches is to enhance the efficiency and sustainability of agricultural practices by requiring less input and generating less waste than conventional products and approaches (Chinnamuth and Boopathi, 2009). The current report addresses the use of nanoscale amendment (metals, metal oxides, carbon) approaches to suppress crop disease and subsequently enhance growth and yield. Notably, this enhanced yield may be directly linked not only to the reduced presence of pathogenic organisms but also to the potential nutritional value of the NPs themselves as essential micronutrients necessary for host defense and likely a result of their greater availability in the “nano” form. Last, we will also offer comments on the current regulatory and economic perspective for such applications.

Table 1. *Nano-enabled products/patents in agriculture*

Name of Product/Patent	Product Type	Relevant NP Composition	Patent Number	Inventors
Active nano grade organic fine humic fertilizer and its production	Active organic fertilizer	Nano fermented active organic fertilizer	CN1472176-A	Wu et al.
Application of hydroxide nano rare earth to produce fertilizer products	Fertilizer	Hydroxide of nano rare earth	CN1686955-A	Wang et al.
Application of oxide nano rare earth in fertilizer	Fertilizer	Nano rare earth	CN1686957-A	Wang et al.
Biological organic compound liquid nanofertilizer and preparing process	Nano composite liquid fertilizer/pest resistant	Unclear	CN1452636-A	Ni J.
Coated slow-releasing fertilizer and its production	Fertilizer	Inorganic filler; polar bonding material	CN1854111-A	Ying et al.
Environment-friendly carbon-nano synergistic complex fertilizers	Fertilizer	Carbon nanomaterials	US 0174032-A1	Lui et al.
Liquid complex fertilizer which contains nanosilver and allicin and preparation methods to provide antibacterial effects thus to increase crop production	Fertilizer/antibacterial	Nanosilver	KR 000265-A	Kim et al.
Nano-composite superabsorbent containing fertilizer nutrients used in agriculture	Fertilizer	Nano-composite carbohydrate graft copolymer	US 0139347-A1	Barati et al.
Nano diatomite and Zeolite ceramic crystal powder	Fertilizer	Nano diatomite and zeolite	US 0115469-A1	Yu et al.
Nano-leucite for slow release nitrogen fertilizer and Green environment	Fertilizer	Potassium aluminium silicate (Leucite) NPs occluded by calcium ammonium nitrates	US 0190226-A1	Farrukh et al.
Nano long-acting selenium fertilizer	Fertilizer	Nano selenium	US 0326153-A1	Yin et al.
Nano-micron foam plastic mixed polymer fertilizer adhesive coating agent preparation method	Fertilizer	Nanomicron-foamed plastic organic compound mixed polymer	CN1631952-A	Zhang et al.
New method for preparation of controlled release special fertilizer comprises mixing and granulating Ximaxi clay minerals, coating with various fertilizers, trace elements and additives	Fertilizer	Nano clay	CN1349958-A	Li et al.

Name of Product/Patent	Product Type	Relevant NP Composition	Patent Number	Inventors
Non-metallic nano/micro particles coated with metal, process and applications	Fertilizer	Core of the non-metallic nano/micro particles are selected from inorganic material such as silica, barium sulfate. The metal coating is selected from Ag or transition/noble metals: copper, nickel, silver, palladium, osmium, ruthenium, rhodium.	US 0047546-A1	Malshe et al.
Plant growth liquid containing titanium dioxide nanoparticles comprises an aqueous titanium dioxide colloid solution incorporating a surfactant	Fertilizer	Nano titanium dioxide	BR03721-A	Lee et al.
Process compromises combining soil repairing technique and nanobiological fertilizer to promote growth of microbes, improve soil and remove residual herbicides	Biological fertilizer	Nano-class biological fertilizer	CN1413963-A	Min et al.
Production of novel precision customized control release fertilizers	Controlled release fertilizers	Transition metal silicates	US 8375629 B2	Prasad et al.
Production technology of nano-clay-polyester mixed polymer fertilizer coating cementing agent	Controlled releasing fertilizer; soil improver	Nano clay polyester mixed polymer	CN1414033-A	Zhang et al.
Production technology of coating cement for nano sulfonate lignin mixture fertilizer	Coating cement for controlled release fertilizer	Nano sulfonated lignin mixture water solution	CN1417173-A	Zhang et al.
Preparation of nanometer-scale olefin/starch mixed polymer fertilizer covering agent	Slow release fertilizer	Nano-level non-homogeneous phase mixed polymer of hydroxyethyl methacrylate	CN1546543-A	Zhang et al.
Silicon nanocarrier for delivery of drug, pesticides and herbicides, and for wastewater treatment	Pesticide	Nano silicon carrier	US 0225412-A1	Sardari et al.
Stable nanoparticulate composition for release of active agents	Fertilizer	Nano-sized particle of an active agent	WO 56866-A1	Bosch et al.
HeiQ AGS-20	Pesticide	Silver-silica composite material	US 0294919-A1 *Product available in the market	Company: HeiQ Materials
Nano-Argentum 10	Fertilizer/antifungal/bug repellent	Silver	*Product available in the market	Company: NanoSys GmbH

Note: Patents retrieved from FPO IP Research & Communities and Derwent Innovations Index in ISI Web of Knowledge.

1.1 Role of plant nutrition in crop diseases suppression

Prior to any discussion on the use of nanoscale micronutrients to suppress plant disease and enhance crop yield, a general overview of the relationship between plant nutrition and plant disease is first needed. A number of strategies for keeping crops disease-free have been developed, with common approaches being genetic breeding, cultural schemes with sanitation and host indexing, improved eradication procedures, new pesticides and implementation of integrated pest management (IPM) (USDA-ARS). The most successful strategy for soil-borne disease control is the development of host resistance. However, most crop species suffer from a lack of available resistance genes, and ongoing public discomfort with genetically modified food remains an issue of concern. As such, another viable strategy for maintaining crop health is to manage plant nutritional status. Nutrition often governs the fine line between crop susceptibility and resistance to a disease. One major confounding factor in providing proper nutrition is that many species vary significantly in their nutrient requirements, and the varying nutrient levels may affect the range of plant diseases in different ways. Furthermore, fertilization regimes necessary to maximize plant health often vary with the presence or absence of a significant pathogen.

Micronutrients play pivotal roles in the defense against plant diseases. Pathogen infection of plant tissue initiates a cascade of reactions, with a common response being the generation of inhibitory secondary metabolites. Importantly, many of these metabolites are produced by enzymes that are only activated by micronutrient cofactors. Copper, Mn and Zn are known to confer disease resistance by activating host defense enzymes such as phenylalanine ammonia lyase and polyphenol oxidases (Duffy, 2007; Evans et al., 2007; Huber and Thompson, 2007; Römheld V., 1991). In fact, the difference between plant resistance and susceptibility to infection is often the speed at which a cell can activate and produce the defense metabolites. Maintaining a sufficient supply of micronutrients in susceptible tissues ensures plant health in the presence of pathogenic populations. However, a significant limitation to micronutrient-based disease suppression is consistently low element availability in slightly acidic to neutral soils. In addition, micronutrients such as Fe, Mn and Zn become increasingly unavailable as soil pH approaches neutral, further limiting uptake by roots and maximizing root susceptibility to invasion (Sims, 1986). Finally, aerial application of nutrients with subsequent *in planta* delivery to roots is problematic since many micronutrients are not basipetally translocated as effectively as macronutrients such as N, P and K (Bukovac and Wittwer, 1957).

Nonessential elements such as Al and Si can also affect disease defense mechanisms in plants through several possible mechanisms. For example, plants that accumulate Si show resistance to a number of foliar and root diseases in both dicots and monocots (Datnoff et al., 2007). Most plants accumulate this element in great quantities (1-10% dry mass), but Si content in the plant often directly depends on availability in the soil, which may be quite limited. The role of Al in plant disease control has only begun to receive attention. Numerous field studies have demonstrated that soil amendments to acidify soils can be part of an integrated approach to managing root diseases and soil-borne pathogens. The use of Al as a soil amendment, however, has been limited because misapplication can lead to significant phytotoxicity and loss of yield or quality, while insufficient levels provided little or no disease suppression (Shew et al., 2007). Another aspect that has received little attention is the role elements might have in activating host defense mechanisms. For example, application of orthophosphates and calcium chloride increased plant production of phenolic compounds, which then conferred resistance to *Fusarium* wilt (Biswas et al., 2012). The role of inorganic metal oxides in inducing these responses has not been examined. However, similar issues associated with low Al and Si availability in soil and minimal shoot-absorption/translocation-to-roots limit the efficacy of these nonessential elements for plant protection. As discussed below, one of the most notable characteristics of metal and metal oxide NPs is the greatly enhanced availability to, and translocation within, plants. As such, application of NP-based micronutrient formulations may offer a new platform for disease management by much more targeted and effective nutrition-based manipulation of host resistance. We believe that the increased bioactivity and transportability of nanoscale micronutrients will result in enhanced disease suppression and crop productivity.

1.2 Nanomaterials synthesis

A nanomaterial (NM), such as carbon nanotubes (CNTs), has one dimension below 100 nm, whereas NPs have two dimensions at this scale. For the purposes of this report, these terms, although not synonymous, will be used interchangeably. The unique properties of NM are greatly influenced by their morphology, such as shape, size and crystalline phase. These are properties that can be controlled, and NM design and synthesis has consequently become an important field of research over the last decade. The scale of NM production is quite large. For example, titanium dioxide (TiO_2) is the NM with highest production, exceeding 10,000 tons worldwide each year, while other NMs such cerium dioxide (CeO_2), zinc oxide (ZnO) and CNTs are produced at levels ranging from 100 to 1,000 tons annually (Piccinno et al., 2012). A variety of chemical and physical methods have been developed and optimized for the synthesis of NPs; however, new techniques are being developed that focus on more "ecofriendly" platforms, such as the NM biosynthesis using plant extracts (Mittal et al., 2013). Currently, commercial synthesis of metallic NMs is typically prepared by "bottom-up" approaches, beginning with liquid-phase synthesis. The methods of synthesis generally rely on chemical reduction, since the process is efficient and enables control of the structure and growth of metal NP to optimize yield (Charitidis et al., 2014). The synthesis method relies on the reduction of metal particles by a variety of organic and inorganic reducing agents. Subsequent use of stabilizing agents is often necessary to avoid aggregation and ensure uniform particle size distribution. The most common reduction reagents used in the synthesis of metallic dispersions include sodium citrate, hydroxylamine, hydrogen peroxide, citric acid, cellulose, sodium carbonate and sodium hydroxide. The most common stabilizers used in this method are polyvinyl alcohol and sodium polyacrylate. Chemical reduction methods have been commonly reported in the literature, including for the synthesis of silver (Ag) (Guzmán et al., 2009), gold (Au) (Akbarzadeh et al., 2009) and platinum (Pt) (Charitidis et al., 2014). The synthesis of metal oxides (ZnO, CeO_2, TiO_2, CuO, Al_2O_3) traditionally relied on vapor-phase-based techniques, but the impact of lower production costs associated with solution phase production has been significant (Comini, 2013). More recently, hydrothermal techniques have more control of product properties and purity.

There are a large variety of physical techniques used for NM synthesis, including sonochemical reduction, supercritical fluids, laser ablation, gamma radiation and chemical vapor deposition (CVD) (Charitidis et al., 2014). With regard to carbon-based NMs, the most common synthesis method for fullerenes are the arc discharge and gas combustion methods (Swihart, 2003). It has been reported that gas combustion method can produce a high tonnage of fullerenes per year (Takehara et al., 2005), whereas the arc-discharge method produces low yield. Purification processes, such as soxhlet extraction, are often applied to remove hydrocarbon impurities. CNTs are commonly produced from CVD methods relying on the decomposition of gaseous hydrocarbon. The CVD method has proven to be an economical technique and to produce larger yields when compared to methods such as arc discharge and laser ablation. However, a major drawback in using CVD techniques is related to product purity, which decreases with increasing yield (Lai and Zhang, 2011).

The primary reason for discussing synthesis methods above is to highlight the multiple means during production by which impurities and byproducts can contaminate the final nanomaterial product in a way that can negatively impact the receiving environment (Petersen et al., 2014). As mentioned previously, the synthesis of carbon nanomaterials can result in heavy metal contamination that can alter surface coating structure and function. Commercial synthesis processes incorporate a range of organic solvents that need to be eliminated from the final product. Plants are an essential component of all ecosystems and will interact directly or indirectly not only with the NM but also with any contaminating solvents or metals. Given the sensitivity of many crop plants to select metals and solvents as well as concerns over potential food contamination, we note that it is important to consider the toxicological impacts of residual impurities as well as to characterize test materials adequately to understand NP fate and effects accurately.

1.3 NP effects on disease suppression and plant growth

1.3.1 Nanoparticle interactions with plants – Negative effects and toxicity

A range of nanofertilizers and nanopesticides are or will soon be commercially available (Table 1), while others are under development and soon to be on the market (Suppan, 2013). Prior to exploring the existing literature on the use of NPs for disease suppression, we first point out some areas of concern related to this topic. The rapid development of nanotechnology and specifically, the lack of particle size-specific regulatory framework, has raised concerns regarding NPs impacts on the environment and on human health. The environmental toxicity of metal NPs has been discussed in a number of recent reviews (Hawthorne et al., 2014; Ma et al., 2010; Miralles et al., 2012; Rico et al., 2011). Several studies have reported the toxicity of metal NPs on different crop species, for example: copper (Cu) NPs increased lipid peroxidation to 180% of the control level in *Elodea densa* Planch, while catalase and superoxide dismutase activities increased by a factor of 1.5-2.0 (Nekrasova et al., 2011). Similarly, studies with TiO_2 NPs demonstrated the reduction of cell wall pore diameters of *Z. mays* L. leaf from 6.6 nm (control) to 3.0 nm (1 g TiO_2 L^{-1}) (Asli and Neumann, 2009). Also, studies with onion (*Allium cepa*) reported a reduction in root growth (-4.81%) at 6 mM of TiO_2 NPs exposure in comparison with control plants (Ghosh et al., 2010). Other studies have shown that Ag NPs caused a complete germination inhibition for ryegrass (*Lolium*) and flax (*Linum usitatissimum*) at 750 and 1,500 mg L^{-1}, while only 13% germination rate was reported for barley (*Hordeum vulgare* L.) at 1,500 mg L^{-1} (El-Temsah and Joner, 2012). Stampoulis et al. (2009) reported that zucchini (*Cucurbita pepo*) biomass was reduced by 60% and 75% in the presence of Ag NPs and CNT. Another concern related to NM use in agricultural systems is the NP bioaccumulation and trophic transfer (Hawthorne et al., 2014). CeO_2 NPs were recently shown to penetrate the root tissue of mature soybean plants, and particles were transported to the soybean edible tissue (Hernandez-Viezcas et al., 2013). Although a number of studies have demonstrated NP phytotoxicity and accumulation, a similar number have reported negligible effects. As such, the consensus among the scientific community is that too little data exist to assess NP fate and effects accurately in the environment.

1.3.2 Nanoparticle interactions with plants – Disease suppression and enhanced growth

Plant diseases are responsible for billions of dollars in agricultural crop loss each year in the United States alone (USDA). In addition, over $600 million is spent annually on fungicides in an attempt to control pathogens (Gonzalez-Fernandez et al., 2010). Plant diseases are caused by fungi, bacteria, viruses or nematodes; the diseases incited by these pests cause economic losses by reducing attainable yields, product quality and/or shelf life. In addition, reports have indicated that one-quarter of food crops worldwide are affected by mycotoxins (Patel et al., 2014). Given the likely additional stresses on global agricultural productivity anticipated by an expanding population and a changing climate, the economic losses may in fact be of secondary concern.

It is clear that new strategies for plant disease management are greatly needed and will be a critical component to any long-term plan for sustaining or increasing agricultural productivity. The potential of nanotechnology and specifically, the use of NPs, in this effort has been a topic of discussion for several years. For example, Navarro et al. (2008) stated that nanoscale particles could retain nutrients due to their high specific surface area and thus serve as a longer-term nutrient source to biota. However, the focus of our interest is on the more direct effects of nanoscale amendments on disease-causing organisms. Specifically, can one treat plant diseases through the use of NPs that directly suppress pathogen activity, resulting in an enhanced crop growth and yield? Incidentally, many of the nanoscale elements that have been or could be effective are indeed required micronutrients, raising the possibility of further enhanced yield through nutritional benefit. Current conventional pesticide and fertilizer formulations often have active ingredients with poor water solubility, and availability to crops can be quite limited. Consequently, larger volumes/quantities of these formulations must be used to control pathogenic diseases effectively. Also, current fertilizer and metal ion-based pesticide formulations are subject to processes such as leaching, volatilization and precipitation by soil minerals. The result has been an exceptionally inefficient and costly approach to pathogen control and plant fertilization. However, the solubility and effectiveness of these agrichemical formulations could be greatly increased through the use of nano-enabled additives or carriers as well as by the incorporation of NPs themselves as the active ingredient (Naderi and Danesh-Shahraki, 2013). Additionally, nanofertilizers could include “nano-devices” to synchronize the release of the active ingredient over time and in concert with the uptake by crops. This would not only increase nutrient bioavailability but would also minimize wasteful interactions with soil or air that result in analyte losses from the system. As shown in Table 1, commercial companies are currently producing nano-enabled pesticides and fertilizers that are more soluble, better dispersed, less persistent and more specific with regard to target (Rai et al., 2012).

The existing literature shows that the influence of NMs varies with both plant species and nanomaterial type. Tables 2 and 3 contain a summary of a number of studies that investigated the positive impact of metal NPs on crop growth and pathogen suppression. Some NPs have a role in crop disease suppression directly as antimicrobial agents, such as Ag, ZnO, TiO_2, Mg and Si (Ram Prasad, 2014). Silver NPs display a strong inhibitory activity to microorganisms; therefore, they have been used in many industrial sterilization processes. The inclusion of Ag NPs in this report is not meant to advocate for its widescale application in agriculture; it is merely a reflection of the fact that the highly effective antimicrobial properties of the NP have led to much research into potential applications for phytopathogen control. Even though the bactericidal mechanisms of Ag NPs are still not fully understood, researchers have hypothesized that bacteria are killed through a combination of elemental NP Ag and released Ag+ ions. Silver ions interact with cysteine-containing proteins and enzymes on bacterial membranes, causing structural deformation and biochemical imbalance of the cell membrane. Subsequently, Ag+ ions penetrate the damaged cell membrane to inactivate cytoplasmic enzymes leading to inhibition of cell replication and finally cell death (Ocsoy et al., 2013). With regard to crop disease suppression, Jo et al. (2009) studied the inhibitory effect of Ag NPs on two plant-pathogenic fungi that infect perennial ryegrass (*Lolium perenne*). A 50% inhibition of colony formation was observed at 200 mg Ag NPs L^{-1}. Similarly, Lamsal et al. (2011a) reported Ag NPs could suppress anthracnose pathogen *Colletotrichum* spp. in field trials. Both studies (Jo et al., 2009; Lamsal et al., 2011a) reported that Ag NPs attached to and penetrated the microbial membrane, thereby reducing the magnitude of infection. Additionally, the later study reported that NP Ag reduces plant diseases more effectively when applied prophylactically, suggesting that the efficiency of suppression may be greatly influenced by treatment timing and/or the induction of resistance mechanisms. Similarly, Gajbhiye et al.

(2009) assessed the antifungal activity of Ag NP in combination with the fungicide fluconazole by a disk diffusion assay against several pathogenic fungi, including *Pleospora herbarum*, *Trichoderma* sp., *Candida albicans, Phoma glomerata* and *Fusarium*. Results showed that combined fluconazole-Ag NP had the greatest antifungal activity, achieving maximum zone inhibition against *C. albicans*, followed by *P. glomerata* and *Trichoderma* sp.

Recent studies have demonstrated the potential of other NPs such as ZnO for controlling pathogen growth. Given the lower overall metal phytotoxicity and the secondary benefits on soil fertility, ZnO NPs are likely a more appropriate choice for fungal pathogen control than is Ag (Dimpka et al., 2013). For example, ZnO NPs demonstrated higher inhibition (26%) against *F. graminearum* (a fungal plant pathogen) as compared to bulk ZnO and controls (~47%) for mung bean in broth agar medium (Dimkpa et al., 2013). Other studies with post-harvest pathogenic fungi showed that at 3-12 mmol l^{-1}, ZnO NPs caused significant inhibition of *Botrytis cinerea* (from 63-80%) and *Penicillium expansum* (61-91%) in microbiological plating (He et al., 2011). The authors reported that ZnO NPs inhibited growth by affecting broad cellular function within *B. cinerea* and *P. expansum*, thereby resulting in hyphal deformation and death, respectively. In a similar study, biosynthesized ZnO NPs (25 µg/mL) displayed higher inhibition rates against pathogenic bacteria and the fungal species *P. aeruginosa* (22 ± 1.8 mm) and *A. flavus* (19 ± 1.0 mm) (Jayaseelan et al., 2012). Wani and Shah (2012) investigated the potential of ZnO and MgO NPs to inhibit pathogens such as *Alternaria alternate, Fusarium oxysporum, Rhizopus stolonifer* and *Mucor plumbeus*. The authors reported a high inhibition rate in the germination of fungal spores upon exposure to the metal oxide NPs; the highest spore inhibition in germination (93.6%) was reported for *Mucor plumbeus* at 100 mg L^{-1} MgO NPs.

Published studies have also reported that the application of quantum dots (QDs) can significantly enhance plant growth and may offer selective activity against plant pathogens. Rispail et al. (2014) discussed the use of QDs and super-paramagnetic NPs for the detection and labeling of the fungal pathogen *Fusarium oxysporum*. The authors demonstrated intracellular internalization of NPs, suggesting that this technique could be applied for both detection and control platforms with this pathogen. Separately, the authors observed that 500 nM QDs reduced both fungal germination (20%) and hyphal growth (15.4%) (Rispail et al., 2014).

Alternatively, metal oxides such as TiO_2 have been used as nanoscale amendments in agriculture due to their photocatalytic properties. Field studies indicated that TiO_2 NPs (10-50 nm) inhibit pathogenic infection in cucumber by *Pseudomonas syringae* pv. *lachrymans* (68.6%) and *P. cubensis* (90.6%) (Cui et al., 2009); importantly, the authors also reported significant increases in photosynthetic activity (30%) as compared to control plants. Paret et al. (2013a); (2013b) showed that after photo-activation, NP TiO_2 amendment reduced bacterial spots (*Xanthomonas* sp.) on both roses and tomato at levels equivalent to or better than conventional approaches. Huang et al. (2005) used MgO NPs as an effective bactericide due to its strong interactions with negatively charged bacteria and spores. Copper oxide, Cu_2O NPs and Cu/Cu_2O composite NPs were selected for a field study using tomato (*Lycopersicon esculentum*) plants challenged with the pathogen *Phytophthora infestans.* Results from this study showed a higher protection (73.5%) against *P. infestans* when plants were treated with NP Cu as compared to currently available non-nano Cu formulations (57.8%) (Giannousi et al., 2013).

In the previously mentioned studies, the actual mechanism of enhanced plant growth is often unclear. Increased growth may be linked directly to reduced disease presence, which may be from direct action of the NP against the microbe itself or through the activation of key *in planta* defense pathways, which subsequently yield important defensive metabolites. Regardless, the end result is enhanced crop growth and yield, which may be occurring from a combination of reduced disease and from the fact that many of these nanoscale amendments are elements that may secondarily serve as plant micronutrients.

Table 2. *Summary of effects of NPs on disease suppression*

Nanoparticle	Plant Pathogen	Mode of Action	Ref.
Ag	*Bipolaris sorokiniana*	Inhibition of colony formation by 50%	Jo et al., 2009
	Magnaporthe grisea	Inhibition of colony formation by 50%	
	Colletotrichum	Suppressed pathogen attack (75%)	Lamsal et al., 2008
	Stem-end bacteria	Inhibited microbial growth and increased water uptake	Liu et al., 2009
	Phoma glomerata	Highest antifungal activity of was observed against *C. albicans* (26 mm) followed by *Trichoderma* sp. (22 mm) and *P. glomerata* (20 mm). No enhancement of the antifungal activity was recorded for *F. semitectum* and *P. herbarum*	Gajbhiye et al., 2009
	Phoma herbarum		
	Fusarium semitectum		
	Trichoderma sp.		
	Candida albicans		
Ag@dsDNA@GO	*Xanthomonas perforans*	Reduced the severity of bacterial spot disease by 40%	Ocsoy et al., 2013
MgO	*Alternaria alternate*	Suppressed spore germination (90%)	Wani et al., 2012
	Fusarium oxysporum	Suppressed spore germination (87%)	
	Rhizopus stolonifer	Suppressed spore germination (90%)	
	Mucor plumbeus	Suppressed spore germination (93%)	
ZnO	*Alternaria alternate*	Suppressed spore germination (78%)	Wani et al., 2012
	Fusarium oxysporum	Suppressed spore germination (58%)	
	Rhizopus stolonifer	Suppressed spore germination (76%)	
	Mucor plumbeus	Suppressed spore germination (68%)	
	Fusarium graminearum	Higher inhibition in comparison with ZnO bulk (26%) and control (~47%)	Dimkpa et al., 2013
	Penicillium expansum	Significantly inhibition to *P. expansum* (61% to 91%) and *B. cinerea* (63% to 80%)	He et al., 2012
	Botrytis cinerea		

Nanoparticle	Plant Pathogen	Mode of Action	Ref.
TiO_2	*Pseudomonas syringae pv. Lachrymans*	Bactericidal rate 68.6%, improved photosynthetic activity (30% increase)	Cui et al., 2009
	Pseudoperonospora cubensis	Bactericidal rate % 90.6%, improved photosynthetic activity (30% increase)	
MPA-QDs	*Fusarium oxysporum*	Detection and labeling of pathogen, effect on fungal germination (20%) and hyphal growth (15.4%)	Rispail et al., 2014
SiO_2 MNPs	*Fusarium oxysporum*	Detection and labeling of pathogen	

1.3.3 Positive effects of metal/metal oxide nanoparticles on agricultural crops: Nano vs. bulk

The current literature contains a number of reports of positive effects of NPs on plant germination, growth and yield. However, of great importance is the comparison in the interactions of plant-NPs against their equivalent bulk particles/ions. The differentiation between the two different sized materials is what will likely determine the effectiveness of NP implementation in crop production for disease suppression and nutrition enhancement. Thus, the literature discussed herein is restricted largely to studies involving a comparison of plants exposed to NPs and corresponding bulk/ion controls (Table 3).

There are several reports on the impact of metal and metal oxide NPs on plants as compared to the corresponding bulk particle or ion (Table 3). Zheng et al. (2005) reported that spinach *(Spinacia oleracea)* grown in 2.5% TiO_2 NPs (rutile) solution had more rapid germination and vigor than did bulk TiO_2-exposed seeds. NP TiO_2 also enhanced spinach growth (63% fresh weight, 76% dry weight), chlorophyll formation (28%), rubisco activity and the overall photosynthetic rate as compared to bulk TiO_2-exposed plants. A similar study using TiO_2 NPs in the anatase showed that spinach fresh and dry weight were increased by 58.2% and 69.8%, respectively (Linglan et al., 2008). In addition, the chlorophyll content and photosynthesis rate were enhanced by 19.0% and 29.9%, respectively, in comparison with controls. Plants treated with bulk-TiO_2 did not exhibit any physiological changes of statistical significance relative to the controls. Lastly, the activity of rubisco activase in the nano-anatase-treated spinach was 2.75 times higher than control and bulk TiO_2-treated plants. Jaberzadeh et al. (2013) conducted a field study to evaluate the foliar application of titanium (Ti) NPs on wheat (*Triticum aestivum*) under normal irrigation and water deficit stress. The authors showed that stem elongation and flowering were significantly enhanced relative to the corresponding bulk particles. In fact, 0.02% Ti NPs significantly enhanced nearly all measured agronomic traits, such as ear weight (g/m^2), ear and seed number, seed weight (g/1,000 seeds), yield (kg/ha), and biomass (kg/ha), as well as gluten and starch content. In another study under nitrogen deficient conditions, leaf greenness, total leaf protein content, fresh and dry biomass were all improved by nano-anatase TiO_2 treatments as compared to controls (Yang et al., 2007). The authors hypothesized that, under nitrogen-deficient conditions and in the presence of sunlight, nano-TiO_2 could directly reduce atmospheric chemi-sorbed nitrogen to ammonia, which might improve the overall growth of the spinach leaves.

Tarafdar et al. (2014) sprayed bulk and biosynthesized zinc NPs by foliar application at 10 mg L^{-1} to pearl millet (*Pennisetum americanum*) that was grown in the soil. The Zn NPs were synthesized via exposure of a precursor aqueous salt ZnO solution to fungal cell-free filtrate (*Rhizoctonia bataticola* TFR-6). When compared to the bulk amendment, Zn NPs treatment demonstrated significant increases in shoot length (10.8%), root area (18.4%), dry biomass (12.0%) and grain yield (29.5%). Moreover, biochemical parameters such as chlorophyll content and soluble leaf proteins were 18.4% and 19.9% higher, respectively, with the NPs amendment when compared to bulk Zn. In addition, Zn NPs enhanced the enzymatic activity of acid phosphatase (14.18%), alkaline phosphatase (22.58%) phytase (72.7%) and dehydrogenase (9.22%). Similar results were obtained by foliar treatment of cluster bean (*Cyamopsis tetragonoloba* L.) with biosynthesized ZnO NPs (synthesized from $ZnNO_3$ amendment with extracellular secretions of *Aspergillus fumigatus* TFR-8) (Raliya and Tarafdar, 2013). Cluster bean treated with ZnO NPs had shoot length, root length, total protein and chlorophyll that were 22.7%, 43.4%, 17.2% and 54.5% higher, respectively, than plants that received bulk ZnO. Moreover, phosphorus mobilizing enzyme activity (3.6-25.2%), rhizosphere microbial population (5.7-13.6%) and gum content were higher in plants treated with the ZnO NPs. In another study, peanut seeds treated with ZnO NPs (1,000 mg L^{-1}) demonstrated higher germination, chlorophyll, stem and root growth as compared to plants receiving the chelated bulk $ZnSO_4$ (Prasad et al., 2012).

Subsequently, foliar application to peanut plants in a field experiment with ZnO NP increased the pod yield up to 29.5% relative to bulk $ZnSO_4$. However, concentrations as high as 2000 mg L^{-1} caused inhibitory effects on crop growth and yield. Conversely, foliar spraying of a 1.5 mg L^{-1} ZnO NP aqueous solution to chickpea (*Cicer arietinum* L var. HC-1) promoted higher biomass than did bulk $ZnSO_4$ amendment (Burman et al., 2013). Notably, 10 mg L^{-1} ZnO NP induced adverse effects on root growth. The aforementioned studies on Zn highlight the importance of NPs concentrations that are applied and of the plant species being grown.

Alidoust and Isoda (2013) investigated the effects of NP Fe_2O_3, citrate-coated Fe_2O_3 NPs, bulk Fe_2O_3 and citrate-coated bulk Fe_2O_3 on soybean physiology after foliar or soil exposure. The authors reported that Fe_2O_3 NPs significantly increased root elongation and photosynthetic parameters compared to the corresponding bulk and citrate-coated Fe_2O_3 NPs via foliar application. Interestingly, the effects were less pronounced after soil application of the Fe_2O_3 amendments, likely due to rapid precipitation of the Fe ions. In contrast, *Spathyphyllum* (an ornamental species) treated by foliar and soil application with Fe_2O_3 NP, bulk Fe_2O_3, iron chelate EDDHA and Fe ethylenediaminetetraacetic acid (Fe-EDTA) did not express any significant physiological changes across treatments (Raziyeh Mohamadipoor, 2013). However, plants treated by foliar application of Fe_2O_3 NPs did contain higher concentrations of some key nutrients (N, P, K, Fe, Zn, Mn and Mg), suggesting perhaps more subtle positive effects of NP exposure. Similarly, NP presence in the growing medium of bean (*Phaseolus vulgaris*) was shown to have secondary impacts on the plant, including on the uptake of micronutrients such as Fe, Ca, Mn and Zn (Dimkpa et al., 2014a; Dimkpa et al., 2014b). Importantly, with exposure in the growing medium, the accumulation of these elements was largely reduced, which suggests that the mode of exposure (root zone vs. foliar) and perhaps the presence of rhizosphere microorganisms may control the extent and magnitude of changes in nutrient accumulation with NP exposure. Pradhan et al. (2013) studied the physiology and biochemical parameters of mung bean (*Vigna radiate*) after hydroponic treatment with NPs of Mn or $MnSO_4$. According to the authors, the highest doses (1 mg L^{-1}) of Mn NPs did not negatively affect the plants, whereas the corresponding salt did cause phytotoxicity even at lower concentrations. This may be due to the large initial burst of Mn release from the salt form, whereas dissolution from the NP is much slower and more controlled. Mung bean shoot, root, fresh weight, dry weight and rootlet number were 10-100% higher upon NP exposure as compared to $MnSO_4$. Mn NP treatment also enhanced chlorophyll content, carotene photophosphorylation, oxygen evolution and nitrogen metabolism (Pradhan et al., 2013; Pradhan et al., 2014). Almeelbi and Bezbaruah (2014) investigated the availability and release of phosphate and iron from phosphate-sorbed zero valent Fe NPs with spinach in hydroponic solution. Interestingly, both plant growth and biomass were increased by up to four times with the presence of the phosphate-sorbed Fe NPs, relative to controls; the Fe content increased in spinach roots, stem and leaves from 11 to 21 times.

Table 3. *Positive effects of nanoparticles in food crops: Nanoparticle vs. bulk*

NP	Particle Size	Bulk/Control Treatment	Concentration	Mode of Exposure	Plants	Growth Media	Effects	References
MWCNTs	Outer diameters 10-35 nm, a length of 6 μm	Activated carbon, graphene (2-5 nm)	50 ug mL^{-1}	roots	Tomato	Murashige and Skoog medium	• Enhanced fresh and dry biomass, Changes in gene expression (water channel protein)	(Khodakovskaya et al., 2011)
	Outer diameters 10-35 nm, an length of 6 μm	Activated carbon	0.1 to 500 ug mL^{-1}	Culture	Tobacco cells	Murashige and Skoog medium	• Enhanced cells growth and regulate cell division by activating water channel protein • Activated gene regulators of cell division and extension	(Khodakovskaya et al., 2012)
	Outer diameters 10-35 nm, a length of 6 μm	Activated carbon	50 and 200 ug mL^{-1}	Roots	Tomato	Germinated in Murashige and Skoog medium and transferred to soil mix	• Increased plant height, number of flowers and fruits	(Khodakovskaya et al., 2013)
SWNTs	Diameters 0.86 nm to 2.22 nm	Activated carbon, graphene	50 ug mL^{-1}	Roots	Tomato	Murashige and Skoog media	• Enhanced fresh and dry biomass	(Khodakovskaya et al., 2011)
ZnO	18.5 nm	Bulk TiO_2	10 mg L^{-1}	Foliar	Pearl millet	Soil	• Increased shoot length (15.1%), root length (4.2%) and area (24.2%) • Increased chlorophyll (24.4%), soluble leaf protein (38.7%) • Increased acid phosphatase (76.9%), alkaline phosphatase (61.7%) and phytase (>3x) • Enhancement of microbial population and activity in the rhizosphere	(Tarafdar et al., 2014)

NP	Particle Size	Bulk/Control Treatment	Concentration	Mode of Exposure	Plants	Growth Media	Effects	References
	1.2-6.8 nm	Bulk ZnO	10 mg L^{-1}	Foliar	Cluster bean	Soil	• Increased shoot length (31.5%), root area (73.5%), dry biomass (27.1%) and grain yield • Increased chlorophyll (~2.8 x) and soluble leaf protein (27.1%) • Increased enzyme activity of acid phosphate (73.5%), alkaline phosphate (48.7%), phytase (72.4%) • Increased rhizospheric microbial population (11-14%)	(Raliya and Tarafdar, 2013)
	???	Bulk ZnO and $ZnSO_4$	1.5 – 10 mg L^{-1}	Foliar	Chickpea		• Increased biomass accumulation compared to bulk and $ZnSO_4$	(Burman et al., 2013)
	????	Chelated bulk $ZnSO_4$	1000 mg kg^{-1} in soil	Foliar and root	Peanut plant	Soil	• Promoted both seed germination and seedling vigor • Early flowering manifestation and higher leaf chlorophyll content • Increased stem and root growth and yield	(Prasad et al., 2012)
Fe_2O_3	6 nm	Bulk and bulk citrated coated Fe_2O_3	Germination 50-200 mg L^{-1}. Soil and foliar 500 and 1000 mg L^{-1}.	Foliar and root	Soybean	Soil	• Increased root elongation and photosynthetic parameters by foliar application	(Alidoust and Isoda, 2013)
Mn	20 nm	Bulk $MnSO_4$	0.05-1 mg L^{-1}	Roots	Mung bean	Hoagland culture solution	• Increased shoot and root length, dry and fresh biomass, and rootlet number • Enhancement in chlorophyll, carotene photophosphorylation and oxygen evolution	(Pradhan et al., 2013)
	20.88 nm	Bulk $MnSO_4$	0.05-1 mg L^{-1}	Roots	Mung bean	Hoagland culture solution	• Increased nitrogen metabolism	(Pradhan et al., 2014)

NP	Particle Size	Bulk/Control Treatment	Concentration	Mode of Exposure	Plants	Growth Media	Effects	References
TiO_2		Bulk TiO_2	0.25%-6%	Roots	Spinach	Hoagland culture solution	• Accelerated seed germinantion, growth rate and chlorophyll • Enhanced rubisco activity and photosynthetic rate	(Zheng et al., 2005)
		Bulk TiO_2	0.25%	Roots	Spinach	Soil	• Enhanced growth rate and chlorophyll • Enhanced rubisco activity and photosynthetic rate	(Linglan et al., 2008)
		Bulk TiO_2	0.01%-0.03%	Foliar	Wheat	Field soil	• Increased ear weight, ear number, seed number, final yield and biomass • Increased gluten and starch	(Jaberzadeh et al., 2013)
		Bulk TiO_2	0.25%	Roots	Spinach	Hoagland culture solution	• Enhanced growth rate and chlorophyll • Enhanced total nitrogen, NH^{4+} and oxygen	(Yang et al., 2007)

2 Impact of soil on NP-enabled fertilization strategies

The mobility and activity of NPs are highly influenced by the chemical and physical characteristics of the surrounding environmental medium. Unfortunately, understanding the fate and effects of NPs in the soil is highly challenging, given the complex array of interactions that are likely and the overall lack of adequate detection platforms for nanomaterials in environmental matrices. As such, this is an area where the literature is somewhat sparse, although recent studies are shedding light on the importance of NP transformation in environmental media. The properties of the initially applied NPs can be altered or transformed through their interactions with the biotic and abiotic components of the soil, which in turn may subsequently control NP stability, aggregation, transport and availability to biota. For example, Tolaymat et al. (2010) reported that in "negatively" charged soils, Ag NPs have more mobility than in soils with an overall "positive" charge and that this interaction has significant impacts on the long-term transport of the NPs. Interestingly, the addition of various stabilizing agents (sodium citrate, polyvinyl pyrrolidone, amines, sugars and amides) can alter Ag NPs in ways that change the interaction with soil and dramatically impact overall mobility. Thalmann et al. (2014) showed that Ag NPs may undergo sulfidation reactions under both anoxic and oxic conditions and that the resulting particles may have different activity and mobility. Cornelis et al. (2012) was able to correlate Ag NP mobility in soil with the overall clay content. Hu et al. (2014) noted that the phytotoxicity of graphene to wheat under hydroponic conditions was almost completely alleviated by the addition of humic acid, a common constituent of natural organic matter. Thus, although much work remains to be done, it is clear that NP's properties, such as particle size and surface charge, as well as soil physical-chemical properties, will determine the fate and transport of NPs in soil systems and their bioavailability to crop plants.

As mentioned above, a significant limitation to micronutrient-based disease suppression is low element availability in soil, particularly those that are alkaline. For example, the nutrients Cu, Fe, Mn and Zn become increasingly less available as soil pH approaches neutral, a phenomenon known to limit root uptake and potentially compromise crop nutritional status (Sims, 1986). Also, studies on soil extracts have shown lower macro- and secondary nutrient concentrations, including soluble Ca, Mg and K, in acidic soil in comparison to alkaline soil (Watson et al., 2014). Importantly for this review, few studies have explored the effect of soil pH or other soil parameters on nanoscale amendments in soil. It is known that modifications in soil pH could progressively restrict the availability of many nutrients; as such, soil-based nanoscale amendment success for pathogen control may be impacted significantly by factors such as soil pH. Watson et al. (2014) studied the effects of soil properties on ZnO NPs' bioavailability and phytotoxicity to wheat (*Triticum aestivum*) grown in acidic (pH 4.5-5.4) and alkaline (pH ≥ 7.8) soils. Results showed a 200-fold higher soluble Zn content in the acidic soil, as well as 10-fold higher concentration in wheat shoots, compared to the alkaline soil. In addition, plants exposed to ZnO NPs (500 mg L^{-1}) in the alkaline soil showed increased lateral root production in comparison to plants grown in the acidic soil where root growth was decreased. These findings highlight the effect of soil properties on the availability and phytotoxicity of NPs; an understanding of these processes is clearly going to be necessary prior to the successful use of ZnO NPs as a soil amendment. Priester et al. (2012) reported high Zn accumulation (344 mg Zn kg^{-1}) in the leaves of soybeans after 50-day exposure to ZnO NPs in soil at pH 6.78. Although the impact of varying soil pH was not commented upon, the authors indicated that natural organic matter (OM) soil components would likely influence the stability and aggregation of these materials in the soil. Yang et al. (2009) reported that humic acid adsorption to nano metal oxides was pH-dependent; specifically, humic acid adsorption was observed with TiO_2, Al_2O_3 and ZnO NPs. Interestingly, SiO_2 NPs showed no adsorption due to the electrostatic interactions and ligand exchange between humic acid and NP oxide surfaces (Yang et al., 2009). Previous studies indicated an increased mobility of Ag NPs due to the presence of OM such as humic acid (Tian et al., 2010).

Another aspect that has to be taken into account is the presence of black carbons (BCs) such as biochar, which is widely found in soil and increasingly added as a soil amendment. Biochar is made from biomass that is burned in an enclosed environment with little oxygen. Biochar can be produced by a number of techniques and feedstock materials; these variations will influence the sorptive behavior of biochar production. It has been reported that biochar in soils is capable of sequestering other substances in terrestrial ecosystems (Chen et al., 2008; Elmer and Pignatello, 2011). Our studies have shown that biochar amendment, which significantly increases soil organic carbon, has little impact on the availability of NP CeO_2, but could significantly decrease the availability of other metal oxide NP and of plant-required nutrients in general.

3 Potential of foliar NP application for disease suppression

Given the many soil-based limitations on macro-, meso- and micronutrient availability, there has been interest in foliar-based fertilizer applications. Studies have indicated that particles can enter plant leaves through the stomata and cuticle structures of plants (Eichert et al., 2008; Schonherr, 2006). This entry or absorption behavior may well be more efficient for smaller diameter NPs. For example, Corredor et al. (2009) demonstrated that magnetic NPs penetrated the leaf cells of pumpkin through the stomata; however, no comparison to larger bulk particles was made. A number of studies have shown that nanoscale metals/metal oxides are translocated at significantly greater levels than corresponding bulk-scale elements and that this enhanced *in planta* transportability includes both xylem- and more importantly, phloem-based transport processes (Wang et al., 2012). Wang and colleagues (2013) developed an aerosol process for foliar delivery of NPs to watermelon. The authors evaluated the leaf-to-root translocation of NPs with both an aerosol and solution application method. The results showed higher percentages of NP-derived Ti, Mg and Zn in root tissue (5.45%, 21.17% and 13.93%, respectively) when compared to the corresponding NP solution method, which was still a foliar application (1.87%, 8.13% and 5.74%, respectively). The authors suggest that smaller aerosolized NPs (the peak diameters of TiO_2, MgO and ZnO aerosol were 27, 35 and 45 nm, respectively) more readily enter the stomata via “gas uptake” as compared with larger particles (the peak diameters of TiO_2, MgO and ZnO were found to be 150, 623 and 1,020 nm, respectively) in solution phase. Raliya and Tarafdar (2013) used ZnO NPs (1.2-6.8 nm) that were biosynthesized with extracellular secretions of the fungus *Aspergillus fumigatus* TFR-8 as a foliar spray on 14-day-old cluster bean plants and reported an increase in plant biomass (27.1%), shoot and root length (31.5% and 66.3%, respectively), chlorophyll content (276%), root area (73.5%), and P nutrient-mobilizing enzymes. Also, the authors indicated that the protein present in extracellular secretions acted as a capping agent, encapsulating ZnO NPs and increasing overall particle stability (Raliya and Tarafdar, 2013).

Similarly, Lu et al. (2002) reported an increase in the water absorption of soybean treated foliarly with a combination of TiO_2 and SiO_2 NPs, perhaps by increasing the activity of nitrate reductase antioxidative enzyme. Also, recent studies with CeO_2 NPs in suspension and nano ceria powder foliar applications were evaluated in cucumber plants (Hong et al., 2014). Here, the authors reported a higher leaf-to-root CeO_2 translocation when the oxide was applied as an NP suspension in contrast with the powder application, suggesting uptake through leaves and phloem-based transport to the other parts of the plant. It should be noted that larger particles could obstruct stomatal conductance in the leaf, causing alterations in transpiration rates and photosynthetic potential of crop plants, thereby limiting the effectiveness of a foliar application. Consequently, it is important to highlight that factors such as particle size, stability, concentration, plant species and method of application will influence foliar uptake of NPs (Wang et al., 2013).

With regard to foliar NP amendments for pathogen control, a small number of studies have been published. The inhibition of the powdery mildew foliar disease by Ag NPs was evaluated on cucumber and pumpkin plants under field cultivation conditions (Lamsal et al., 2011b). The results show that fungal inhibition increased with increasing NP concentration. The authors reported that the foliar application of Ag NPs at 100 mg L^{-1} was more effective at inhibiting powdery mildew than was the bulk treatment; the disease indices were 9.7% for Ag NPs and 35.2% for the corresponding bulk material. Mechanistically, the authors suggest that fungal inhibition was due to the interruption of cellular processes by accumulation of Ag in the fungal hyphae (Lamsal et al., 2011a). This assessment corresponds with the widely known phenomena of greater ion release from NP metals such as Ag, based on the much greater surface area in the NP form (Yin et al., 2011). In spite of the efficiency of Ag NP at inhibiting plant pathogens, its use and application does raise concerns over potential exposure and the adverse effects to human health and the environment. Ag NP's internalization into leaves tissues was noted after foliar exposure of lettuce, suggesting potential transfer to humans (Larue et al., 2014). Although the toxicity of Ag NP to humans is an ongoing topic of research, studies have found that particles can cause DNA and cell membrane damage (AshaRani et al., 2009; Gliga et al., 2014; Ivana Vinkovic´ Vrcˇ ek et al., 2014). Mesocosm studies revealed that Ag NP can cause greater toxicity to fish larvae than observed with corresponding $AgNO_3$ (Bone et al., 2014). Furthermore, the impact of particle weathering on toxicity is unknown, as well as the toxicity of subsequently generated transformation products (Levard et al., 2012).

Although a growing literature suggests significant potential for enhanced intra-plant NP translocation, there is no information on how NP micronutrients that are applied foliarly might uniquely affect root pathogens, either directly through toxic interactions or indirectly through promoted host resistance/defense. Preliminary data from our group have shown that foliar application of CuO suspensions onto tomato shoots resulted in significant subsequent disease (*Fusarium*) suppression (Figure 1), indicating a systemic activity of the NPs. Relative to untreated controls, disease progress was reduced by 23% with bulk Cu, but the NP form of the element reduced disease by 42%. A similar trend was evident with Mn amendment, where bulk and NP forms suppressed disease by 17% and 26%, respectively, but only the nanoscale form was significantly different from the control. More importantly, Cu NP foliar application resulted in higher levels of this element in the roots as compared to bulk material and controls. This may suggest that small particle size does indeed result in enhanced *in planta* (phloem-based) transport. This also raises the previously mentioned point that the nanoscale metal oxides may not only serve to directly inhibit pathogens but may also affect disease indirectly via improved plant nutrition. In fact, in unpublished field studies by our group, eggplant seedlings were transplanted into soil infested with *Verticillium dahliae*, a wilt-causing fungus, and showed increased marketable yield in 2013 and 2014 if seedlings were treated once in the greenhouse prior to planting with NP CuO. Bulk equivalents of CuO had no such effect on crop yield. A final note on the potential foliar application of nanoscale elements/micronutrients – although an exposure pathway comparison does not seem to have been made in the literature, there is reason to expect foliar nanoscale amendments may be more effective than soil-based treatment at both pathogen suppression, regardless of disease location in the plant and at enhancing nutritional status. Given the complexity of element interactions likely for a soil treatment route, the relative simplicity of foliar amendment and the known greater *in planta* mobility of NPs, greater effectiveness via foliar application may actually be anticipated, and future work should seek exploit this potential.

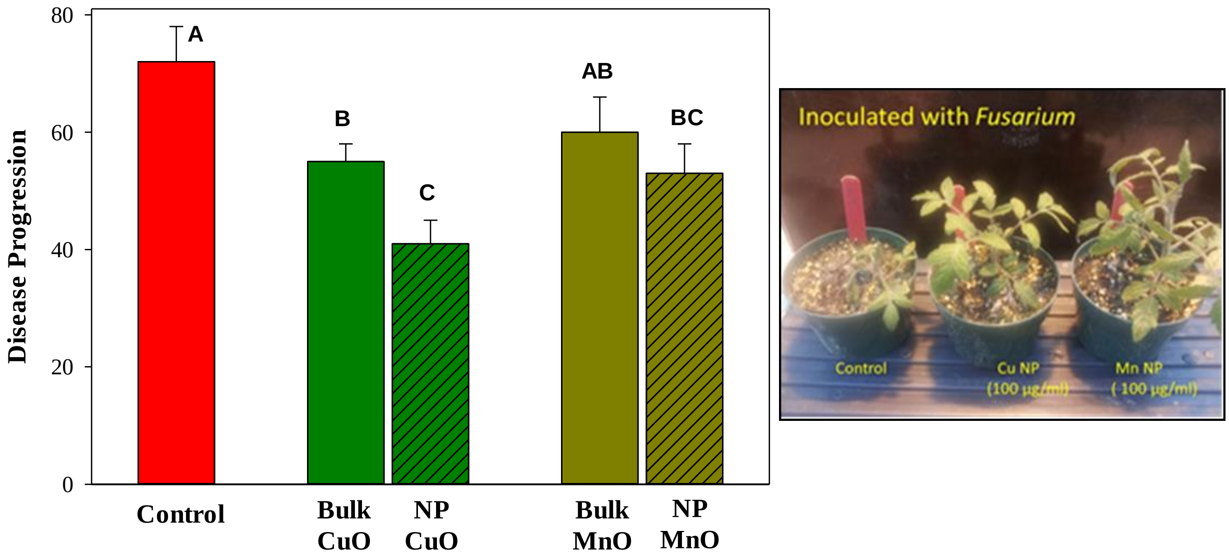

Figure 1. *Effect of NP or bulk equivalents MnO and CuO application on Fusarium disease progression on tomato grown in soil infested with* Fusarium oxysporum f. sp lycopersici *(left). Effect of NP Mn application on tomato biomass grown in soil infested with* Fusarium *(right).*

4 Carbon-based nano-formulations enhance plant growth

In agriculture, the extent of application of nanomaterials is still limited (Khot et al., 2012). Much of the focus has been on the potential of different metal/metal oxide nano-formulations and polymers (Gogos et al., 2012), as discussed above. However, discussions over the use of carbon-based NMs (CNMs) such as fullerenes, carbon NPs, fullerols and single/multiwall carbon nanotubes (SWCNT/MWCNT) as amendments have begun (Khot et al., 2012). Thus, although some preliminary findings discussed below appear promising in terms of enhanced growth and/or pathogen suppression, the mechanism of the interactions between different NMs with plants and soil-microbes is not fully characterized, and instances of phytotoxicity from CNM exposure do warrant caution.

Khodakovskaya et al. (2011) studied the role of MWCNT (98.5 ± 0.5% pure, outer diameter: 10-35 nm; Ave. length: 6 µm), SWCNT (SWCNT) (98% ± 0.7% pure; outer diameter: 0.86-2.22 nm; Ave. length: few microns), graphene (98% ± 0.5% pure; outer diameter: 100-200 nm; Ave. thickness: 2-5 nm) and bulk activated carbon (AC) on the physiology of tomato plants grown in Murashige and Skoog (MS) medium (Table 3). Fresh and dried biomass enhancements were observed only in the plants treated with SWCNT and MWCNT. Photothermal and photoacoustic mapping of MWCNT distribution in the tomato detected the materials in the roots, leaves and fruit down to the single NP and cell level. Interestingly, nano-scale graphene did not significantly affect plant growth rates, likely due to the inability of the sheets to penetrate plant tissues. Additional research with MWCNT found significant changes in total gene expression in exposed tomato, including the water channel proteins known as aquaporins. A follow-up study involving tobacco cells grown in MS medium found that MWCNT enhanced growth in the cell culture by 55-64% in comparison to untreated controls under a wide range of concentrations (5-55 µg mL^{-1}) (Khodakovskaya et al., 2012). On the contrary, AC induced tobacco cell growth only at low concentration (5 µg mL^{-1}) and was phytotoxic at higher levels (100-500 mg L^{-1}). The inhibition of cell growth in the medium was associated with AC at high levels absorbing key substances and nutrients from the medium, including plant growth regulators, vitamins, chelate, iron and zinc. Furthermore, molecular analysis revealed that carbon nanotubes likely stimulate cell division and plant growth by activating the water channels (aquaporins) and major gene regulators of cell division and extension. The same research group conducted soil-based life cycle experiments investigating the effect of MWCNT and AC exposure on tomato (Khodakovskaya et al., 2013). Plant height and number of flowers were higher in soil treated with MWCNT as compared to control and AC-exposed tomato. In addition, tomatoes watered with MWCNT solutions produced two times more fruit than did AC-amended and unamended plants, although the seed number and fruit size did not vary across experimental groups. Seeds of soybean, corn and barley exposed to MWCNT-agar medium (50-200 µg/mL) or by foliar spraying (25-100 µg mL^{-1}) exhibited faster germination and growth activation in comparison to control (Lahiani et al., 2013). Similar to previous work with tomato, the growth enhancement was linked to increased water penetration of the seeds and to increased activity of key water channel proteins. Although no bulk material controls were included in this study, the similarity of the results to the previous work does suggest that carbon nanomaterial-stimulated growth may occur across a number of crop species.

Tripathi et al. (2011) exposed *Cicer arietinum* to water-soluble citric acid coated-CNTs at 6.0 mg mL^{-1} for 10 days and observed intercellular uptake and growth stimulation. The authors hypothesized that, inside the plant's vascular tissue, the nanotubes formed an "aligned network" that increased water uptake efficiency (Tripathi et al., 2011). Lahiani et al. (2013) investigated the effects of MWCNT exposure (50, 100 and 200 µg mL^{-1}) on the germination and growth of soybean (*Glycine max*), corn (*Zea mays*) and barley (*Hordeum vulgare*) in agar medium for 10-11 days. Upon exposure, up to 50% increases in germination were noted and both soybean and corn experienced enhanced shoot development. Notably, internalization of MWCNTs was visualized by Raman Spectroscopy and transmission electron microscopy. Germination and growth enhancement were not limited to CNTs. There are various other CNMs that can have similar positive effects on crop growth and development. For instance, enhanced root growth in wheat was observed upon exposure to 150 mg L^{-1} of water-soluble carbon nano-dots exposure (Tripathi and Sarkar, 2014). In a life-cycle study, Sonkar et al. (2012) showed the growth enhancing effects of water-soluble carbon nano-onions (wsCNOs) in *Cicer arietinum*. A hydroponic germination study for 5-10 days at 10-30 µg mL^{-1} wsCNO revealed the increase in overall growth was proportional to the wsCNO dose in the growth media. Moreover, fullerol [$C_{60}(OH)_{20}$], a water-soluble fullerene derivative, was found to increase plant biomass and phytomedicinal content in bitter melon (*Momordica charantia*) (Kole et al., 2013). Fullerol uptake and translocation were confirmed by bright field imaging and FTIR spectroscopic studies. Treated bitter melon showed up to 54% and 128% increases in biomass and fruit yield, respectively as well as significantly increased anticancer (Cucurbitacin-B: 74% and lycopene: 82%) and antidiabetic compounds (Charantin: 20% and insulin: 91%) (Kole et al., 2013).

Although not directly related to disease suppression, it is worth mentioning that several studies have been published showing that CNM presence altered the fate and effects of contaminants in model and soil systems. For instance, Ma and Wang (2010) reported fullerene-dependent uptake of trichloroethylene (TCE) under hydroponic conditions; TCE accumulation increased by 26% and 82% at 2 and 15 mg L^{-1} of fullerene, respectively. In a soil-based study from our group (De La Torre-Roche et al., 2013), we found that MWCNT and fullerenes had differential effects on the uptake of weathered chlordane and DDx (DDT plus metabolites). MWCNT generally decreased pesticide uptake by four crops (zucchini, corn, soybean, tomato) across multiple CNM exposure levels. However, fullerenes showed mixed effects, ranging from suppressed uptake (similar to MWCNT) and no effect to a 35% increase in chlordane uptake by soybean and tomato. Recently, Hamdi et al. (2014) investigated the effects of surface modification of CNTs on the uptake of chlordane by lettuce (*Lactuca sativa* L.). Here, the authors reported that non-functionalized MWCNT decreased pesticide content in the roots and shoot by 78-88% but that the suppression was far more modest with amino-functionalized CNT (decrease in root: 57% and shoot: 23%).

Although most studies show enhanced plant growth in the presence of carbon nanomaterials, there are scattered reports of phytotoxicity (Stampoulis et al., 2009); however, most of the exposure levels are quite high when compared to the modeled “realistic” CNT flux of 0.01 µg/kg/y (Mueller and Nowack, 2008). Conversely, there are several reports indicating CNMs’ toxicity to soil microbes. Given the significant interplay between microbial and plant biota in agricultural systems, these findings are worth noting briefly. Chung et al. (2011) exposed two soils to 0-5,000 MWCNT for up to 11 days and noted decreases in microbial biomass and enzymatic activity. Jin et al. (2013) reported similar findings but noted that the microbial toxicity was more pronounced for SWCNT than for MWCNT. Rodrigues et al. (2013) noted that after soil exposure to functionalized SWCNT, the fungal community was significantly decreased and did not recover over an extended experimental period. In a 90-d exposure to MWCNT at 10-10,000 mg kg^{-1}, Shrestha et al. (2013) noted that although many populations of bacterial species were decreased, certain groups – such as those of the polycyclic aromatic hydrocarbon (PAH) degrading bacterial community – were increased. These results suggest a shifting toward more stress-tolerant bacterial community with increasing soil-MWCNT exposure.

From the above discussion, it is evident that CNMs have potential to enhance plant growth, nutrient uptake, seed germination and fruit quality. Among the CNMs, CNTs are the most extensively studied and showed promising positive effects on various crop plants. Low to moderate doses of CNTs seem to be advantageous in terms of improving overall plant growth. The mechanism of enhancing growth seems to center on more effective water consumption by up-regulating different water channel proteins. Given this and the fact that many required nutrients will be dissolved in the water that is being more effectively acquired, there may be significant potential for CNT use in climate-smart agricultural systems. This also raises the intriguing possibility of combining low-level carbon nanomaterial addition with nanoscale micronutrients to improve not only water acquisition/utilization efficiency but also to maximize nutrient cycling and disease suppression effects. However, the beneficial CNT response is largely dependent on the plant species, nature of the growth medium and growth conditions. Importantly, in the soil microbial community, carbon nanomaterial exposure often showed adverse effects across several populations. Consequently, caution is clearly warranted, particularly given the lack of mechanistic understanding with regard to toxicity. More research clearly needs to be done, but considering the available findings to date, CNMs may be considered as a promising nanoscale amendment for enhancing plant growth and crop quality/yield.

5 Regulatory and economic perspective of nanomaterials in agricultural systems

In the last decade, there has been a dramatic increase in scientific publications in the field of NMs application to agricultural systems (Gogos et al., 2012). Around 40% of the total number of published articles directly relate to CNMs, followed by TiO_2, Ag, SiO_2 and Al_2O_3 (Table 4) (Gogos et al., 2012). Although worldwide production and consumption of NMs has increased the risk of environmental exposure, insufficient information is available for NM-plant and NM-microbe interactions, particularly in soil, to characterize hazard or risk. Very little information is available regarding environmentally relevant concentration of NPs and thus, predicting toxicity based on studies conducted at high doses becomes problematic. There are a limited number of reports using theoretical modeling for NP fluxes, but uncertainty remains high (Table 4). It has been reported that theoretical "realistic exposure scenario" for TiO_2, Ag and CNTs are 0.4, 0.02 and 0.01 µg/kg/y, respectively (Mueller and Nowack, 2008; Gogos et al., 2012). However, the relationship between these predicted values and the actual concentration is unknown. Unfortunately, as a direct consequence of the lack of robust NP/NM environmental fate and effect data, a NP-specific regulatory framework has failed to develop. In 2009, the European Food Safety Authority (EFSA) published a guideline emphasizing the potential toxicity of NMs (Ganzleben et al., 2011). In 2010, the U.S. EPA approved the only NP-based antimicrobial pesticide, HeiQ AGS-20 (http://www.epa.gov/oppfead1/cb/csb_page/updates/2011/nanosilver.html), but regulations for NP/NM use in other agrichemicals remain elusive. Incidentally, a similar lack of regulatory guidance exists at the U.S. FDA for NP/NM use in food packaging and processing. Importantly, the lack of a regulatory framework has not restricted, and in the near future likely will not restrict, the use of nanomaterials in various agricultural applications.

Given the existing literature, it is clear that the use of nano-enabled formulations in agriculture could provide a higher efficiency in nutrient use and potentially offer a reduction in toxicity and other undesirable non-target effects when compared with bulk products. For example, the use of NPs in agriculture could greatly reduce the negative effects associated with "over dosage" application since small particle size enables more rapid entry into the plant; thus, providing higher efficient nutrient use (both of those applied and of those secondarily impacted) and requiring lower overall application rates. However, as with all agricultural amendments, it is important to note that NP dose will define the line between beneficial and negative effects on crops. Robust investigation will be needed to definitively characterize that line as a function of particle type, dose, application timing, soil characteristics and plant species.

A final comment on economics is likely in order. The high cost of conventional fertilizer and pesticide formulations, as well as secondary costs related to environmental contamination concerns, have stimulated interest and research in novel approaches for pathogen control and crop yield enhancement. Concerns over future stresses on agriculture from population pressure and a changing climate have also stimulated interest in alternative agricultural strategies. Regarding the cost of applying NPs as amendments, the total cost and associated benefits will vary among NPs, synthesis approach and crop species. High-production synthesis methods have been developed that are both low-cost and reliable. For example, in our field experiments with pathogen-infested soils, we treated an acre of eggplant transplants (3,500 plants) with 23 g of CuO NPs (100 mg/100 mL applied to 15 seedlings [0.1 g NPs*3500 plants/15 plants = 23 g NPs]); the estimated cost of those NPs was $44. Our preliminary data shows a 43-58% increase in fruit yield in 2013 and 2014 in comparison with control plants and a 17-31% increase over bulk equivalent CuO (the cost of the bulk CuO was $18.40). By way of some general assumptions, one can assume that the average eggplant produces five fruits, which equates to 17,500 fruits per acre. The cost per fruit in the store ranges from $1 to $5 per pound, assuming the grower sells for $1 per pound and each fruit weighs one pounds. That equates to $17,500 per acre of eggplant. The investment of an extra $44 per acre in the form of NP CuO could increase that dollar value by $10,150 or provide a return of $27,650 per acre. Obviously, these values are not direct profit as they ignore the inherent cost of planting, growth and harvest, but NP CuO could also reduce the need for some conventional pesticide and fertilizer inputs. In addition, our planting density (3,500 plants per acre) is significantly lower than that of commercial growers. Although this is only one crop with some large assumptions, the economics here and the science discussed above seem to warrant significant further investigation.

6 Conclusions

In U.S. agriculture, soil-borne pathogens reduce average crop yield by 10-20%, resulting in billions of dollars of losses. Although a number of disease management options exist for many crop species, all strategies (save host resistance) suffer from significant shortcomings. This fact, taken with the building pressure for greater food production and the potential challenges posed by a warming climate, highlights the need for new plant disease management tools. Plant microelements such as Cu, Fe, Ni, Mn, Si and Zn are known to play critical roles in plant disease resistance through enzyme activation for defense barrier production. However, low micronutrient availability in soil and poor *in planta* translocation inherently limit the utility of amendment strategies.

There is significant interest in applying nanotechnology to agriculture, with much focus on enhanced or more targeted delivery of agrichemicals and fertilizers, nanosensors to increase efficiency and novel treatment strategies to minimize waste production. However, one potential area that has not been adequately explored is the use of nanomaterials for disease suppression. Importantly, materials at the nanoscale possess unique chemical and physical properties not observed in equivalent bulk materials, and the literature clearly demonstrates enhanced availability and transport in biota, including plants, as a function of nanometer particle size. As such, NP forms of plant micronutrients such as those mentioned above and other nonessential elements, such as TiO_2 and Al_2O_3, may have significant use in disease management by directly interacting with pathogens or by affecting the systemic acquired resistance pathway. Preliminary data from our group and from others suggest significant potential for the use of nanoscale micronutrients, either by foliar or root application, to suppress disease and increase the quantity and quality of crop yield. In addition, economic projections based on our preliminary data suggest significant potential benefit in terms of increased revenue to the grower. Future research should be targeted at exploring the nature of these enhancements, including efforts to optimize treatment success and maximize yield. In addition, mechanistic investigations of these interactions will enable an assessment of particle fate and effects in the crop to address concerns of risk and food safety.

Table 4. *Modeled fluxes of different NMs and application rates of plant protection products (PPP) or fertilizers, selected from the scientific literature and patent applications*

NM Type	Modeled Flux Into Soil	Ref	Application Rate and Calcd Flux from PPP/Fertilizer[b]	Ref	Flux Ratio[c]
TiO_2	Realistic scenario: 0.4 μg/kg/y	Mueller and Nowack (2008)[d]	4.5–15 kg/ha ≈ 1607-5357 μg/kg/y	Ishaque et al. (2009)	334-1116
	High exposure scenario: 4.8 μg/kg/y		7.5 g/ha ≈ 2.7 μg/kg/y	Guan et al. (2008)	0.56
	0.28-1.28 μg/kg/y (US, EU and CH)	Gottschalk et al. (2009)[e]	Max 30 kg/ha ≈ 10714 μg/kg/y	Dookhith and Linares (1998)	2232
Ag	Realistic scenario: 0.02 μg/kg/y	Mueller and Nowack (2008)[d]	15 g/ha ≈ 5.4 μg/kg/y	Kim et al. (2008)	54
	High exposure scenario: 0.1 μg/kg/y				
	8.3-22.7 ng/kg/y (US, EU and CH)	Gottschalk et al. (2009)[e]			
CNT	Realistic scenario: 0.01 μg/kg/y	Mueller and Nowack (2008)[d]			
	High exposure scenario: 0.02 μg/kg/ y				
	0.56-1.92 ng/kg/y (US, EU and CH)	Gottschalk et al. (2009)[e]			

[a] Limited to those NM for which data due to usage in the anthroposphere was available.

[b] Assuming an application volume of 300 L ha^{-1}, 20 cm plow depth, a soil bulk density of 1.4 g/cm^3 (http://web.ead.anl.gov/resrad/datacoll/soildens.htm) and an application once per year.

[c] Calculated as flux from PPP/fertilizer divided by the value of the highest modeled flux.

[d] Based on an annual substance flow analysis from products to soil in Switzerland.

[e] Based on a probabilistic material flow analysis from a life cycle perspective of engineered NM-containing products.

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