

# Presymptomatic Disease Detection and Nanoparticle-Enhanced Electrical Capacitance Tomography

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**Abstract:** Here, we present a novel presymptomatic disease detection method by utilizing electrical capacitance tomography (ECT) for plant root monitoring. This was achieved by enhancing the signal with iron oxide nanoparticles (IONPs). IONPs were added to healthy and underdeveloped tomato plants during the course of one and a half months while ECT measurements were collected. The readings for the IONP-exposed samples showed an enhancement over the unexposed samples. This enhancement was greater in the healthy plants when compared with the underdeveloped plants. We also verified earlier claims that ECT can be used for presymptomatic disease detection in bell pepper plants inoculated with *Phytophthora capsici* (PCap). The healthy plants all showed a continuous increase in capacitance over a 2-week period, while the inoculated plants showed a sudden spike in capacitance 12-24 hours before wilting and browning of the stem occurred. The results from these studies indicate that IONP-enhanced electrical capacitance tomography is a non-destructive, *in-situ*, and rapid method to monitor relative root health, and that ECT can detect disease presymptomatically.

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## 1. INTRODUCTION

It is estimated that approximately 12% of the U.S. crop this year will be lost to disease (Agrios, 2005). Because of this, there is a real need for early detection of diseases in agricultural systems. This would allow for the timely application of interventions and the prevention of lost crops. Most of these diseases initially move to the roots and cause rapid decline of fibrous roots before showing any physical symptoms. Many of the diseases that affect crops begin in the roots. For example, huanglongbing (HLB) affected trees will lose up to 50% of their root mass before any symptoms appear above ground (Graham et al., 2013). As a result, farmers and plant biologists worldwide desire a rapid and non-destructive technique for measuring root systems and root health. Due to roots' delicate and intricate interaction with soil, overall root health is difficult to measure *in-situ*, and methods of separation from soil are difficult and often result in destruction of a portion of the root mass. Some of the current root measurement methods used today include: destructive soil cores, pits, and excavation (Böhm, 1979; Heerman, and Juma, 1993); image analysis (Pierret et al., 2002; Bouma et al., 2000); tracers (Kuzakov and Domanski, 2000); minirhizotrons (Majdi, 1996); X-ray (Gregory et al., 2003; Moran et al., 2000); and ground-penetrating radar (Barton and Montagu, 2004). These techniques do, however, suffer from their own limitations (Cao et al., 2010).

Electrical Capacitance Tomography (ECT) was first developed in 1972 by Chloupek (Chloupek, 1977). Chloupek used an impedance bridge to test the capacitance of several crop species including: potato tubers, carrots, onions, oats, and mustard plants. In a typical measurement, one electrode of an alternating current capacitance meter was inserted into a plant stem and the other placed into the surrounding soil. Root capacitance was shown to be directly correlated with root mass and surface area. In 1995, Dalton (Dalton, 1995) proposed a conceptual model to explain the capacitance of root systems. In his model, the root system can be treated like a resistance-capacitance circuit, where the roots act as cylindrical capacitors. The xylem and phloem sap within the root creates a low-resistance electrolyte solution separated from the "electrolyte solution" of the external soil by the root skin. The root surface acts as the dielectric in a capacitor. This results in a capacitance proportional to the charges on the opposite skin surfaces. ECT is centered on measuring the dielectric properties of a root system when they are polarized by the attachment of an external power source. The capacitance of a plant's root system is dependent on overall root structure and would be related to its size, similar to the addition of capacitors connected in parallel. It should be noted that one of the drawbacks of ECT is the sensitivity to soil type, water content, and electrode placement. This prevents the creation of a universal calibration curve for ECT across different plants and soil types. Previously, we

established that ECT is a rapid method for detection of relative root health (Sabo et al., 2016). By examining the relative capacitance changes of plants over time, healthy plants could be distinguished from unhealthy and even dying plants without the need for a calibration curve. The one drawback that was uncovered was the signal difference between healthy and dying roots was low for some time after the plants were killed. One way to improve the signal difference would be the absorption of a high-capacitance material by the healthy roots.

Iron oxide nanoparticles (IONPs) have been gaining attention in supercapacitor applications due to their non-toxic nature, low production cost, and high capacitance (Chen et al., 2009; Kulal et al., 2011; Wang et al., 2013). The capacitance of plant root systems that have absorbed these IONPs would likely increase due to the presence of a high-capacitance material in the xylem and phloem sap. It has been shown that pumpkin plants do, in fact, absorb IONPs through their roots, transporting them throughout the plant. A mass balance calculation was performed at the end of the experiment that showed approximately 45% of the IONPs exposed to the plants remained in the roots (Ma et al., 2010). This increase in capacitance readings would result in enhanced readings for healthy plants when compared with unhealthy ones, thus making it easier to identify plants that need lifesaving interventions.

Herein, we report the use of IONPs to enhance ECT measurements as to easily detect stunted and dying roots of tomato plants sooner. We also report the verification of the application of ECT to the presymptomatic disease detection of bell pepper plants infected with *Phytophthora capsici* (PCap).

## 2. MATERIALS AND METHODS

### 2.1 FeFe<sub>2</sub>O<sub>4</sub> Nanoparticle Synthesis

Magnetite (FeFe<sub>2</sub>O<sub>4</sub>) magnetic nanoparticles were prepared via co-precipitation. Ammonium hydroxide (NH<sub>4</sub>OH), 70mL, was added to 170 mL of deionized (DI) H<sub>2</sub>O and inert mixed using an argon stream. The solution was heated to 90°C for 1 hour. A solution of iron (II) chloride (FeCl<sub>2</sub>)/iron (III) chloride (FeCl<sub>3</sub>)/hydrochloric acid (HCl) at a ratio of 1:2:1, was added dropwise to the NH<sub>4</sub>OH solution over 10 minutes. The temperature of the solution was returned to 90°C, held for 2 hours, and then cooled to room temperature. The nanoparticles were separated using a magnet and washed three times in DI H<sub>2</sub>O. The concentration of the IONPs was calculated by evaporating the water from a 1mL sample of the solution and weighing the dried IONPs.

X-ray diffraction (XRD) of the nanoparticles was measured using a Bruker D8 Advanced X-Ray Diffractometer with a copper K $\alpha$  source over a 15°– 85° 2 $\theta$  range where 2 $\theta$  is the diffraction angle between the incident beam and the detector. The size of the nanoparticles was determined from the average peak broadening of the five strongest Bragg peaks using the Debye-Scherrer equation, assuming a shape factor of 0.9 (spherical).

### 2.2 Healthy vs. Stunted Roots Experiment

For the Healthy vs. Stunted roots study, 20 Better Boy tomato plants (*Lycopersicon esculentum*) were grown in August (mid-August to end of September) in Atlanta, Georgia. The tomatoes were planted in 5-gallon pots with a 70:30 mix of potting mix and garden soil (mixture of compost, sphagnum peat moss, and perlite). Ten tomato plants were kept in direct sunlight for a minimum of 6 hours every day, and watered in the evening, as needed. Ten plants were kept under a tarp that blocked all direct sunlight. They were also watered at the same time as the control plants. Five plants from each set of control and tarp-covered plants were exposed to ~10nm IONPs solutions twice a week. The solution consisted of ~300mg of nanoparticles suspended in 250mL of water, which was added to each plant in the evening (at the same time normal watering occurred) every 3-4 days. All tomato plants were allowed to grow for 2 weeks before any capacitance readings were recorded. The control group measurements were compared with the stunted group to monitor any differences in the readings.

Capacitance readings were collected every 3-4 days using a BK Precision 879B LCR meter. In a typical measurement, one electrode of the LCR meter was connected to a stainless steel 14-gauge needle that was inserted into the plant stem 1cm above the soil, while the second electrode was connected to a 3mm x 16.5cm stainless steel rod inserted 10cm deep in the surrounding soil, 3cm from the stem. One hour before capacitance measurements were taken, the plants were watered to field capacitance.

### 2.3 Healthy vs. Dead Roots Experiment

In this experiment, four tomato plants from the non-stunted control group of plants (two normal and two exposed to nanoparticles) were taken and sacrificed to study the effect of dead roots on IONP-enhanced capacitance readings. The plants were cut to the ground, leaving 3cm of stem and full root systems. IONPs continued to be added to the respective plants every 3-4 days. The capacitance was measured at the same time daily, using the same ECT setup (described above). The dying/dead group's capacitance readings were compared with the still growing control group.

### 2.4 Presymptomatic Disease Detection Experiment

The presymptomatic disease detection experiment was performed using ten bell pepper (*Capsicum annuum*) plants half of which were infected with *Phytophthora capsici* (PCap). The plants studied were 3-4 weeks old after emergence. The plants were inoculated a week prior to ECT measurements by applying 10ml of spore suspension (2000 spores/ml) to each plant as soil drench; ECT measurements were collected daily, approximately at 8am. The capacitance readings of the control group were compared with the inoculated group to distinguish any differences.

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