Ethylene Induced Nitrile and VOC Synthesis by Soil Microbes; Improved Root Elongation & Reduced Risk of Fungal Infection in Plants

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ABSTRACT:

The scope of the project was to develop a method to induce soil microbes to inhibit fungal infection and improve root elongation. The study was randomized. Gladiolus bulbs selected for the study were visibly inspected for viability and visible signs of infection. Two trials were conducted from Aug. 5th – Sept. 5th 2014 with 4 replicates per condition over a 7-d period in damp outdoor conditions in late summer. A mixed culture of plant growth promoting rhizobacteria (PGPR) were collected from soil surrounding the roots of young fruit bearing trees. Microbes were mixed with minimal media (no-carbon source), and cultured with an ethylene and used as potting soil. Bulbs planted in ethylene induced soil displayed 0% visible fungal growth, while 38% of bulbs grown in control conditions displayed some form of fungal growth and/or infection. Ethylene induced soil increased root length by 225% in bulbs in 7-d period. GC Mass Spectrophotometry data suggest ethylene may induce soil microbes to synthesize several VOCs including (ethanol, 3-methyl-1-butanol, pentanol) and esters (ethyl acetate), that may have synergistic benefits to lower the risk of fungal infection by soil mold, while nitrile compounds improve root elongation. The findings are preliminary, additional studies are required to understand the mechanism.

Keywords: Fertilizers, Organic, Plant Growth Promoting Rhizobacteria, Crops, Peas, Flowers
INTRODUCTION:

The farming industry is a $618 billion-dollar business, that is built on an unhealthy reliance on chemical fungicides and chemical fertilizers to maintain crop health and increase yields (Al-Busaidi, 2013; Johnson, 2014). However, recent implications of the negative impact these chemicals have on our environment have forced the agricultural industry to search for effective and ecofriendly alternatives (Ribaudo et al., 2011; Banayo et al., 2012; Ishfani, 2012; Aggani, 2013). Plant growth promoting rhizobacteria (PGPR) are soil microbes that biosynthesize numerous organic compounds that can improve plant development and reduce pathogenic infections, (DeBont and Albers, 1976; Miller et al., 1987; Vandeputte et al., 2005; Arshad et al., 2007). These microbes maybe the ecofriendly answer to harsh chemical fertilizers and fungicides.

Studies conducted by Flematti and later on Abeles suggest in nature during a forest fire vegetation release large portions of ethylene and/or cyanohydrins as a pollutant, (Flematti et al., 2011; Abeles, 2012; Nelson et al., 2012; Downes et al., 2013). Abiotic reactions convert ethylene and/or cyanohydrins to nitriles that enhance plant growth (Boubel et al., 1994; Brust, 2009).

This study will force microbes to convert ethylene into a nitrile and other volatile compounds (VOCs) that will improve plant development and health. The process will include the use of co-inducers, urea and cobalt under high pressure and low O2 conditions. Ethylene will induce microbes to express a monooxygenase enzyme used to convert ethylene to an epoxide then to an ethanol for cells to metabolize (DeBont et al., 1974; DeBont & Perk, 1980; Germon and Knowles, 1988; Saeki et al., 1999). The present report investigates whether soil microbes cultured on ethylene as a carbon source will convert the compound to VOCs, esters, and aldehydes that improve plant development and reduce the risk of pathogenic infections.
MATERIALS AND METHODS:

Soil Collection/ Induction

Elsgaard demonstrated that efficiency for degrading hydrocarbons (i.e. ethylene) can be improved by exposing bacteria to increasing concentrations of ethylene for extended periods of time, (Elsgaard, 1998). After several days of exposure, the bacteria were able to remove over 30% ethylene from the headspace. This study, builds on those findings. Soil microbes were collected from the roots of young fruit bearing trees, a pear tree (*Pyrus communis*), black beauty egg-plant tree (*Solanum melongen*), and peach tree (*Prunus persica*).

The mixed culture soil was suspended in a minimal media, cofactors urea and cobalt were added (urea 7.5 g; CoCl$_2$ 0.201 g) (Dietz *et al.*, 1980; Shadowen *et al.*, 1989; Pierce *et al.*, 2008; Perry, 2011; Perry, 2016). For minimal media preparation see Perry, 2011. The soil and minimal media were placed into a closed air tight container and ethylene was added 20% (v/v). No continuous aggregation was performed, cells metabolized ethylene as the carbon source under low oxygen conditions for 4-d at room temperature.

Planting/Harvest

Gladiolus species (mixed) bulbs selected for the study were visibly inspected for viability and visible signs of infection. Two trials were conducted from Aug. 5$^{th}$ – Sept. 5$^{th}$ 2014 with 4 replicates per condition (control, non-induced, and induced) over 7-d in outdoor conditions. Bulbs were planted 3 inches below the soil and watered every 3 days. On the 7$^{th}$ day, bulbs were removed from the soil and imaged. A concurrent study was conducted with *Pisum sativum L.* (sweet pea) seeds from Aug. 5$^{th}$ – Oct. 5$^{th}$ 2014 with 23-25 seeds for each condition over 8-wk in outdoor conditions. Plants were imaged on 8$^{th}$ week, see supplemental data for comparison data for study.
GC-MS Head Space Analysis of Soil Samples

GC-MS analysis was performed by Millis Scientific, Inc. in Baltimore, MD. Samples were analyzed for the presence of ethylene, cyanide, cyanohydrin, and various volatiles. Collection, 25 ml of soil/media mixture were aliquot into 40 ml vials equipped with septum. Assay Preparation, samples were incubated at 50°C for two hours, then 2ml of 10M HCl was added to acidify soil for HCN assay. Assay Parameters, Poraplot Q Plot column by Chrompack (10M 0.32 mm), carrier gas helium at (2.5 ml/min splitless), He; injection volume, 1 ml; injection temperature, 200°C; initial temperature, 50°C for 1 min, increasing by 10°C min⁻¹ to a final temperature of 220°C, hold 1 min; ion source temperature, 150°C; and transfer line temperature 250°C. Mass-to-charge ratios (m/z values) from 10 to 400 were monitored using the scan mode. Waters/Micromass Quatro GC mass spectrometer interfaced to a Thermo Electron Trace gas chromatograph was utilized for the analysis, identified with the aid of AMDIS software using the NIST mass spectral library.

RESULTS

Comparison Root Length/ Pathogenic Infection

Microbes were mixed with minimal media (no-carbon source), and cultured with an ethylene and used as potting soil. Bulbs were placed in the soil for 7-d, removed, then imaged. Ethylene induced soil reduced risk of pathogenic infection from soil molds. For each trial 12 bulbs were planted, ethylene induced soil displayed 0% visible fungal growth while 38% of control sets displayed visible pathogenic growth on exterior of bulbs, See Image 1. Ethylene induced soil enhanced root elongation. Control, Median Root Length 0.38 mm. Non-Induced Soil, Median Root Length 0.75 mm. Induced Soil, Median Root Length 1.15 mm. Ethylene induced soil increased root length by 225% in bulbs in 7-d period, See Image 2.
Image 1: **Ethylene Induced Soil Reduced Risk of Pathogenic Infection:** (A) Control, 38% displayed visible fungal growth. (B) Induced Soil, 0% displayed fungal growth.

Image 2: **Ethylene Induced Enhanced Root Elongation:** (A) Control, Median Root Length 0.38 mm. (B) Induced Soil, Median Root Length 1.15 mm.
Comparison Root Length/ Flower Formation

A concurrent study was conducted with *Pisum sativum* L. (sweet pea) seeds from Aug. 5\(^{th}\) – Oct. 5\(^{th}\) 2014, see supplemental data for additional comparison. Pea plants grown in induced soil displayed over 120% increase in the number of flowers formed per plant at 6 weeks, See Image 3.

At 8 weeks, pea plants grown in induced soil averaged 9 peas per plant, compared to the 3 peas per plant for both the control and non-induced soil. Ethylene induced soil increased the number of peas per plant by 300%. Induced soil also displayed increase in number of germinated plants, healthier color, and uniform appearance, See Image 3.

**Image 3:** Comparison of Pea Plants 6 weeks: Ethylene increased the number of flowers per pea plant at 6 weeks by 120%.
Image 4A: Comparison of Retention Time in Minutes. (A) Non-Induced Soil (B) Induced Soil. GC-Mass Spec analysis, Ethanol (7.0), Acetonitrile (7.1), Ethyl Acetate (11.08), 2-Methyl, 1-propanol (11.76), Butanol (12.4), 2-Pentanone (13.1), 2-Pentanol (13.7), and 3-Methyl,1-butanol (14.26), only Induced Soil suggest significant amounts of VOCs in the soil.
Image 4B: **Comparison of Retention Time in Minutes.** (A) Non-Induced Soil (B) Induced Soil. GC-Mass Spec analysis, Ethanol (7.0), Acetonitrile (7.1), Ethyl Acetate (11.08), 2-Methyl, 1-propanol (11.76), Butanol (12.4), 2-Pentanone (13.1), 2-Pentanol (13.7), and 3-Methyl,1-butanol (14.26), only Induced Soil suggest significant amounts of VOCs in the soil.
DISCUSSION/ CONCLUSION

Soil microbes can be induced to efficiently convert ethylene into cellular metabolites (Hartmans et al., 1991; Elsgaard 1998; Elsgaard 2000; Perry, 2011). This method induces a monooxygenase enzyme to convert ethylene into an epoxide that may be converted into ethanol, aldehydes, nitriles, esters, or VOCs, See Image 5 (Ensign et al., 2003; Perry 2016). These VOCs include ethanol, acetonitrile, 2-methyl 1-propanol, butanol, 2-pentanone, 2-pentanol, and 3-methyl 1-butanol. Compounds shown in prior literature to inhibit the growth of pathogenic fungi in plants (Rezende et.al, 2015; Jantasorn et al., 2016; Liarzi et. al, 2016). Whether the compound benefits are effective independently or only synergistically (Strobel et. al, 2001; Fialho et. al, 2011). Additional experimentation is required to understand the metabolic pathway used by the microbes.

![Figure 5: Proposed Pathway, based on GC Mass Spec Data](image)

Ethanol may be converted to VOCs by microbes in low oxygen and high pressure conditions.
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