# Ethylene induced Soil Delays Ripening in Organic Bananas

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## ABSTRACT:

The scope of the project was to develop a method to induce soil bacteria to biosynthesize compounds that retard the effects of ethylene induced ripening in climacteric fruits. The study was randomized. Organic bananas selected for the study were visibly inspected to ensure the fruit was unripen with no visible signs of bruising, spotting, or infection from a local distributor. Four trials were conducted from June 5$^{th}$ - August 5$^{th}$ 2014 with 3 replicates (3-4 bananas per experimental unit) in 4 trial studies for 3 days at room temperature. 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 no-carbon source media, and cultured with an ethylene for 3 d at room temperature in a closed container. Induced soil was used to delay ripening. Microbes induced with media and ethylene delayed ripening 100% of the time in all experimental units compared to control samples, while microbes cultured with media (no ethylene) delayed ripening less than 10% of the time compared to the control. These cells also appeared to increase the incidence of fungal infection in the fruit. The findings suggest induced microbes may convert ethylene into ethanol then acetaldehyde. The two compounds may form an acetaldehyde/ethanol vapor that delays ripening, and a secondary nitrile compound that inhibits fungal growth.

**Keywords:** Ripening; Ethylene; Ethanol; Acetaldehyde; Plant Growth Promoting Bacteria



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# INTRODUCTION:

The U.S.D.A. estimates over 34 % of all harvested produce is loss to spoilage, deterioration, mechanical injuries, sprouting, and/or physiological disorders (15% of these effects are caused by ripening) (Grolleaud 2002; Kader 2005; Barth et al., 2010). However, consumer demand for fresh unspoiled organic fruits and vegetables has increased by almost 30% since the 1980's, (Johnson, 2014). This requires the current $618 billion-dollar US domestic farming industry to search for innovative and ecofriendly alternatives to prevent fruit ripening, (Blankenship, 2001; Kader, 2005; Barth *et al.*, 2010; Johnson, 2014). This is a challenge as climacteric ripening can be initiated in organic bananas with less than 124 mg/m$^3$ ethylene (Rasori *et al.,* 2002; Theologis 2004). Current methods to deter ripening include scrubbers and filters, and application of 1-MCP (Morretti *et al.,* 2002; Singh *et al*., 2008). 1-MCPis the most effective, but permanently alters the fruit physiological process and taste, (Sisler *et al*., 2001). Other alternatives may include acetaldehyde. Preliminary finding suggests the aldehyde may inhibit ethylene production by inhibiting ACC synthase and ACC oxidase enzymes (Pesis and Marinansky, 1993; Beaulieu et.al, 1997; Ritenour et. al, 1997; Pesis, 2005; Podd and Staden, 1998).

Plant growth promoting rhizobacteria (PGPR) may offer a more cost effective and ecofriendly, means to produce the acetaldehyde and ethanol components to delay ripening (Perry, 2011). Ethylene may induce microbes express a monooxygenase enzyme that converts ethylene to ethanol under higher pressure and high CO2 conditions, the ethanol may be converted into either acetaldehyde or acetonitrile compounds for the cells to metabolize (DeBont *et al.,* 1974; DeBont &Perk, 1980; Germon and Knowles, 1988; Saeki *et al.,* 1999). The present report investigates whether *R. rhodochrous DAP 96253* cultured on alkene hydrocarbon (ethylene) can convert ethylene to acetaldehyde to inhibit ripening.



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# MATERIALS AND METHODS:

## Soil Collection/ Induction Method

Elsgaard demonstrated soil bacteria can be induced to degrade ethylene by gradually exposing the bacteria to ethylene at increasing concentrations for extended periods of time, (Elsgaard, 1998; Elsgaard & Anderson, 1998). In this study, PGPR induced to degrade ethylene. A mixed culture of PGPR were collected from soil surrounding the roots of young fruit bearing trees. The fruit bearing plants included flordahome pear tree (*Pyrus communis*), black beauty eggplant tree (*Solanum melongen*), and peach tree (*Prunus persica* grown in a nursery in Albany, Ga. Soil and microbes were mixed with no-carbon source minimal media containing cofactors urea 7.5 g; $CoCl_2$ 0.201 g; 20% (v/v) ethylene for 4 d at room temperature, (Dietz *et al.,*1980; Shadowen *et al.,* 1989; Pierce *et al.,* 2008; Perry, 2011; Perry, 2016). Minimal M9 Stock: 64 g $Na_2HPO_4$-$7H_2O$, 15 g $KH_2PO_4$, 2.5 g NaCl, 5.0 g $NH_4Cl$ in 1 L distilled $H_2O$ and (autoclave), 200 mL M9 Stock, 2 mL of 1 M $MgSO_4$ (sterile), & 100 uL of 1 M $CaCl_2$ (sterile) in 1 L).

## Ripening Experiment

The induced soil mixture was aliquoted into 80 ml volumes placed in a closed container with unripen fruit. Soil was placed in close proximity, but not directly in contact with the fruit, SEE Image 1. Organic bananas selected for the study were visibly inspected to ensure the fruit was unripen with no visible signs of bruising, spotting, or infection from a local distributor, (Green Tomato Organic Market) and imaged (Sony “Cyber Shot’, 7.2 mega pixels, automatic flash).



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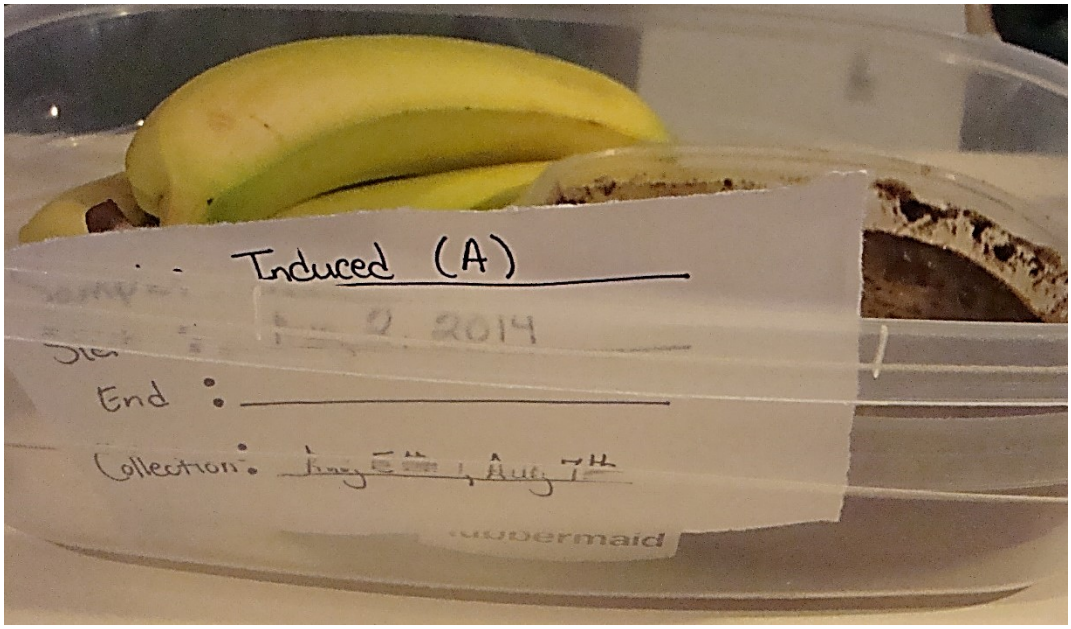


**Image 1:** Bananas and Induced Soil in a bin. Fruit and soil were placed in sealed container for 3 days and imaged. Images were compared to controls.

**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.



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# RESULTS

### Comparison of Induced Soil, Non-Induced Soil, & Control Soil

Induced soil was used to delay ripening. The data compares the three experimental conditions, control (no soil/microbes), non-induced (induction media without ethylene), and induced (induction media with ethylene). Microbes induced with media and ethylene delayed ripening 100% of the time in all experimental units compared to control samples, while microbes cultured with media (no ethylene) delayed ripening less than 10% of the time compared to the control. These cells also appeared to increase the incidence of fungal infection in the fruit.


**Image 2:** Comparison of 3 Conditions, bananas stored with induced soil displayed reduced spotting, reduced change in size, reduced change in firmness, and maintained color.



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**Image 3:** Comparison of 3 Conditions, (A) compares bananas on Day 0, all bananas are firm without visible spotting. (B) compares bananas on Day 3, the induced soil preserved the bananas color and firmness with no visible spotting. (C) compares the peeled bananas, the induced soil preserved the bananas mass, firmness, and no bruising from handling. R1 (Control), R2 (Non-Induced Soil), & R3 (Induced Soil).

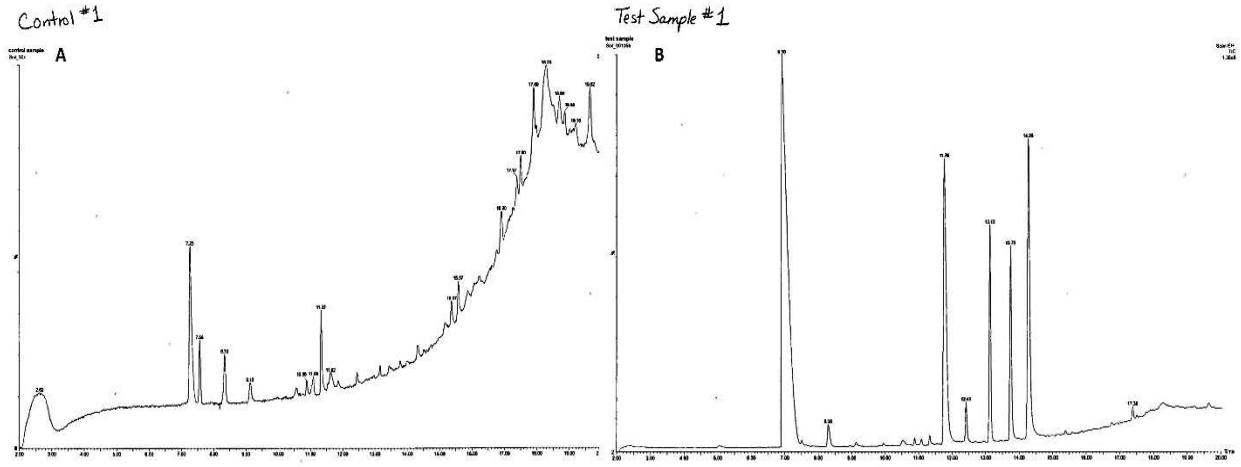


**Image 4:** Comparison of 2 Conditions, (A) Non-Induced Soil (B) Induced Soil. GC-Mass Spec analysis, shows Ethanol Retention Time 7.0 min, Acetonitrile Retention Time 7.1 min, Butanol Retention Time 12.4 min. Both samples showed peaks at those periods, only Induced Soil suggest significant amounts of both solvents were in the soil.



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# DISCUSSION/ CONCLUSION

The data suggest ethylene, cobalt, and urea can be used as inducers to induce soil microbes to efficiently convert ethylene into compounds delay the effects of ripening in fruit, (Hartmans *et al.,* 1991; Elsgaard 1998; Elsgaard 2000; Perry, 2011). This induction method may be facilitated by a monooxygenase like enzyme that converts ethylene into an epoxide (Ensign *et al.,* 2003; Perry 2016). The epoxide is highly reactive and unstable and in an aqueous solution and maybe converted to an ethanol that is subsequently converted to an acetaldehyde or acetonitrile compound, (SEE Figure 5). Such a process may have significant industrial applications. Additional experimentation is required to further understand and examine the potential metabolic pathway used by the bacteria.

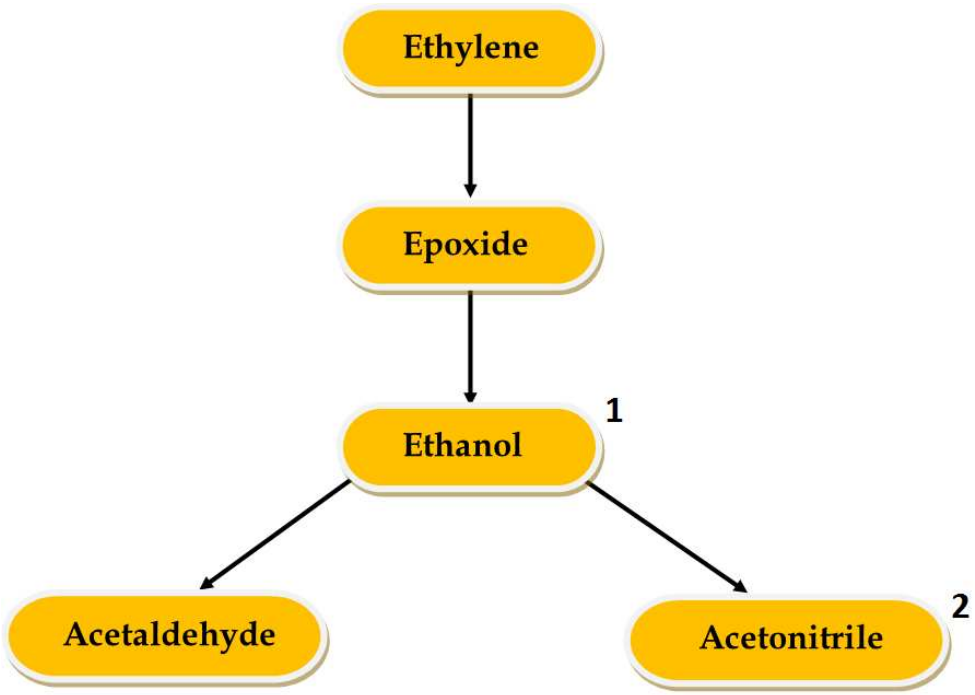


**Figure 5:** Proposed Pathway, based on GC Mass Spec data. (1,2) Ethanol, RT 7.0 min & Acetonitrile RT 7.1 min. To formulate a more conclusive proposed pathway additional data and findings are required. The only proposed enzyme in this pathway is a monooxygenase enzyme that converts the ethylene to an epoxide molecule that can then be converted to ethanol.



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