FINAL PROJECT REPORT
WTFRC Project Number: PR-06-609

Project Title: Near real-time ethylene sensor for pear post-harvest applications

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Budget History:

Item	Year 1:	Year 2:	Year 3:
Salaries	5250		
Benefits	5000		
Wages	3000		
Benefits			
Equipment	1000		
Supplies	500		
Travel	250		
Subcontract	2500		
Miscellaneous	0		
Total	17500		

I. PROJECT OBJECTIVES

This was a one year project with a single objective, and that is to qualify sensor accuracy and measurement repeatability under controlled-atmosphere environmental conditions. The response of the sensor to various ethylene concentrations under different temperatures, relative humidity, and atmospheric conditions ($O_2/N_2/CO_2$ ratios) were also to be examined.

II. SIGNIFICANT FINDINGS

Several major accomplishments are highlighted below and further discussions of the results will follow in the upcoming sections.

1. *Impact of Temperature:* Our results indicate that the ethylene sensor generates a stronger signal at higher temperatures and as the temperatures approach the CA room storage temperatures, the sensitivity reduces two almost half of that observed at room temperatures.
2. *Impact of Oxygen to Nitrogen Ratio:* The sensor shows low sensitivity to the ratio of oxygen to nitrogen, and even at a completely oxygen-free environment, the signal is less than 20%.
3. *Impact of Humidity:* The sensor appears to show some sensitivity to humidity, although little measurable differences were observed between normal atmospheric conditions (~50% RH) to the high humidity conditions of CA rooms (>90%RH).
4. *Impact of Interferents:* The sensor appeared to show little to no sensitivity to interferents that might be present in the CA room, such as CO.
5. *CA Chamber Tests for Pear Ethylene Production:* The data for ethylene concentrations measured for Bartlett pear under CA condition show very close agreement to measurements obtained with a GC during the same tests and under the same conditions.

III. JUSTIFICATION AND METHODS FOR ETHYLENE SENSING

Ethylene production rate and the amount of ethylene present in the surrounding environment of a single apple and pear (or in general for climacteric fruit) have been shown to affect their quality during various stages of ripening. Further, this information can be used as an indication of the stage of ripeness (or maturity) of the fruit.[1,2] This is especially true in post-harvest where the rate of ripening, scalding, browning, and other issues could prevent high quality fruit from reaching the market.

A number of researchers are currently using various methods supported by a Gas Chromatography system (GC) to research the different aspects of interaction of ethylene and fruit quality at various pre- and post-harvest stages. While significant amount of data has been accumulated and a large of body of literature exists on varieties such as Bartlett pears (and golden delicious apples), little information is available for some of the newer varieties such as Comice pears (and Honey Crisp apples). Research performed on Bartlett pears[3] suggested that very low ethylene concentrations of

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[1] Kupferman, E., 1986, "The Role of Ethylene in Determining Apple Harvest and Storage Life," Post Harvest Pomology Newsletter, Vol. 4, No. 1. http://postharvest.tfrec.wsu.edu/pgDisplay.php?article=N4I1C

[2] Sansavini, S., F. Donati , F. Costa, and S. Tartarini, 2004, "Advances in Apple Breeding for Enhanced Fruit Quality and Resistance to Biotic Stresses: New Varieties for the European Market," *Journal of Fruit and Ornamental Plant Research,* vol. 12, 2004 Special ed.

[3] Bower, J.H., W.V. Biasi, E.J. Mitcham, 2003, "Effect of ethylene in the storage environment on quality of Bartlett pears," *Postharvest Biology and Technology* 28 (2003) 371_/379.

less than 1-ppm have to be maintained to control fruit quality, which is difficult due to high ethylene production of fruit even at -1 °C storage temperatures. For such tight control, it is required to continuously monitor the ethylene levels in the storage facilities. There is currently no cost-effective real-time ethylene sensor in the market that can produce reliable measurements at 0.1 ppm levels required for control in CA and RA rooms.

Fluid Analytics has recently developed a cost-effective electrochemical sensor for monitoring ethylene in air at concentrations of 0.1 ppm and lower. In our electrochemical sensor, selective adsorption of ethylene to a nanoporous gold surface takes place as a prerequisite to sensing. Adsorption onto the surface of gold is restricted to very few compounds with specific molecular structure, namely pi-bond. The nanoporous gold is deposited onto a polymer electrolyte membrane such as Nafion® (registered trade mark of duPont) and acts as the anode for the oxidation of ethylene at a given voltage. A flowing stream of air is passed over the ethylene adsorbing gold electrode. When an electrical potential is applied across the anode and cathode, ethylene that is adsorbed on the gold surface is oxidized to acetaldehyde at the triple-phase boundary, as formulated below:

$$C_2H_4 + H_2O \rightarrow CH_3CHO + 2H^+ + 2e^-$$

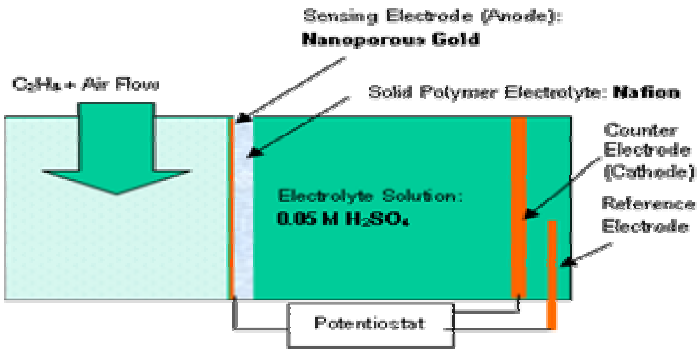


Figure 1. Current ethylene sensing approach.

A sensitive galvanometer is used to measure the current flowing in the cell as a result of the electrons produced by the oxidation of ethylene on the gold anode. The migration of protons through the membrane completes the cell circuit. The current, i, generated by the sensor is a function of a number of parameters, but notably can be linked directly to the partial pressure (or concentration, C) of ethylene in the freestream.

$$i = S \cdot C$$

In the above equation, S is the sensor sensitivity and that is what discriminates our sensor from most other commercially available electrochemical sensors. As S becomes larger, the threshold of detection becomes lower while the resolution of the instrument improves. Our laboratory measurements show that the sensor response is extremely linear for concentrations between 10-ppb and 10-ppm. At the lower end of this range, the sensor provides high resolution down to 10-ppb. More details will be provided in the following sections.

IV. RESULTS AND DISCUSSION

IV.1. Task 1: Prototype Development and Packaging for CA Tests

All engineering, assembly and testing on this system was done at Fluid Analytics. Figure 2 shows a photograph of the completely packaged system. The complete ethylene sensing instrument is packaged in an engineered sheet metal enclosure. The enclosure serves as s a rugged framework for mounting the internal components, while providing a suitable, field-usable protective shell.

Figure 2. Photograph of the packaged system.

Internally, the enclosure is divided into three distinct sections, separated by metal barriers that are integral to the enclosure, and provide additional structural stability. There are provisions on top of the sensor box for electrolyte access ports and access to the fluidics compartment in which the electrochemical cell, the air pump, and all of the associated tubing are located. This section was segregated from the other two as a way of preventing contact between the liquid electrolyte and the electronics. The metal barrier also serves as an electromagnetic shield, to minimize the amount of electrical noise coupled into the sensor cell. The center or the electronics compartment contains the bulk of the electronics: the potentiostat, the main control board, and the front-panel display. The compartment on the right, the power conditioning and management compartment, contains the AC-to-DC power supply. In the future versions, the batteries and corresponding power electronics will be placed in this section. This section is segregated from the main electronics for safety reasons, to avoid the possibility of AC line voltage coming in contact with the electronics. All user-accessible components (air connectors, control buttons, data link, etc.) are located on the exterior, creating a system that does not need to be opened during normal usage.

IV.2. Task 2: Research in Controlled Atmosphere Room at ARS

Prior to any testing in the cold storage facilities, a series of tests were performed to simulate the CA environment in a more controlled laboratory setting. The following sections cover the results of these tests.

2.1. Impact of Temperature Variation on Sensor Response

Knowing that reactions and diffusion are affected significantly by temperature changes, we expected a correlation between sensor sensitivity and temperature. This correlation could then be built into our calibration curves for temperature correction of the data.

In order to test the dependence of sensitivity on temperature, we constructed a controlled temperature box in which to place the sensor; this was to insure that the cell electrolyte was at a controlled

temperature. We also established a stainless steel section of tubing through which all gas would pass prior to entering the sensor; the tubing insured that the gas entering the sensor was at the same temperature as the electrolyte as verified by the inline gas temperature/humidity sensor. Additional temperature probes were placed in the controlled temperature box. A cell was filled with water, placed in the controlled temperature box, and the box temperature was set. The water temperature was monitored over time to establish how long it took for the water to reach the same temperature as the air in the box. Although this time varies depending on the starting temperature of the water and the set temperature of the controlled temperature box, it was found that an equilibrium time of ~ 1 hour was sufficient for all planned temperature tests. Then the cell was filled with the proper electrolyte solution, and temperature testing commenced. Testing included operating at four different temperatures between 10°C and 40°C at 10°C increments. A wide range of ethylene concentrations were tested in order to establish a well defined sensitivity line. A schematic and photo of the temperature test setup is shown in Figure 3.

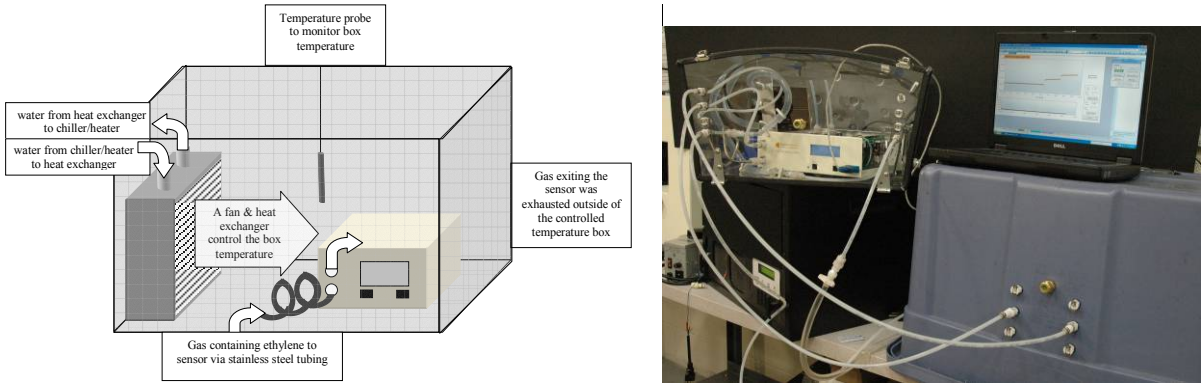


Figure 3. Schematic and photo of test setup for controlled temperature tests.

Using these tests, we were able to find a strong and clear correlation between sensor sensitivity (response) and temperature. The sensitivity of the sensor seems to be linearly correlated to temperature and increased with increasing temperature. These results are shown in Figure 4.

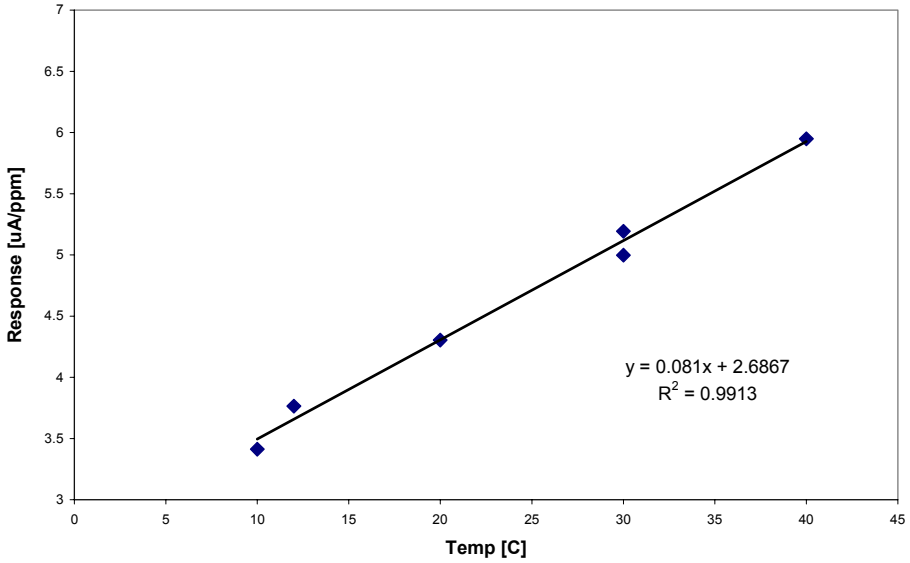
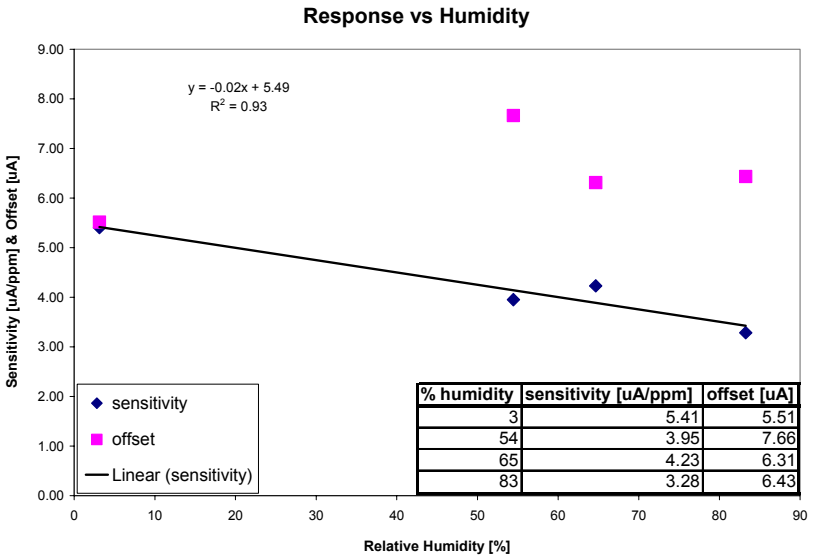


Figure 4. Sensitivity appears to increase with increasing temperature.

2.2. Response of Sensor to Varying Humidity

Varying humidity tests were performed to attempt to assess a correlation between humidity and sensitivity to ethylene for humidity dependent correction. In these tests the flow rate was kept at a constant 500 sccm and was provided from a gas mixing system and cylinder gas. The experiment was setup with gas flowing from the gas mixing system through cells with moistened plain Nafion membranes for humidification. Humidity was varied by varying the number of humidification cells and by changing the water feed rate in those cells. Humidity was recorded using the standard temperature/humidity sensor. Temperature was maintained between 25 °C and 26 °C. Five different concentrations were tested at each humidity level to give an accurate representation of sensitivity. R-squared values of the linear fit of concentration vs cell response at each humidity level exceeded 0.997 in all cases. The plot below shows the results of these humidity tests revealing an inverse relationship between sensitivity and humidity.



% humidity	sensitivity [uA/ppm]	offset [uA]
3	5.41	5.51
54	3.95	7.66
65	4.23	6.31
83	3.28	6.43

Figure 5. Sensitivity appears to decrease with increasing humidity.

2.3. Response in Air

In order to investigate the differences in sensor response at CA condition versus regular atmosphere with high oxygen levels, we constructed an apparatus by which we could mix room air with standard concentrations of ethylene in order to produce a mixture primarily containing room air, but also having a known concentration of ethylene. This was done using a fully functional prototype and relying on the pump to maintain a constant flow rate through the sensor. The outlet of the gas mixing apparatus was placed in a small chamber open to the air and the inlet to the sensor was attached directly to this chamber. When the flow rate of the gas coming from the mixing apparatus was higher than the flow rate of the pump, the chamber would fill with our mixed gas recreating the same conditions as when the sensor is directly connected to the mixing apparatus. When the flow rate of the gas coming from the mixing apparatus was lower than the flow rate of the pump, that gas would mix with room air creating a known concentration of ethylene in what was primarily room air. The flow rate of the pump was measured using an upside down graduated cylinder in a large bath of water. The oxygen-free tests were performed by mixing nitrogen standards with ethylene standards using precision flow controllers in both streams prior to mixing.

The results of these tests are shown in Figure 6. The y-axis is in counts, units proportional to current that are measured by the control board in the sensor prototype. Note that approximately 20% higher sensitivity is realized in the normal atmosphere as opposed to CA condition. However, because our

sensor is sufficiently sensitive, the reduction of 20% in the sensitivity does not affect our ability to measure 100-ppb or lower ethylene levels if desired.

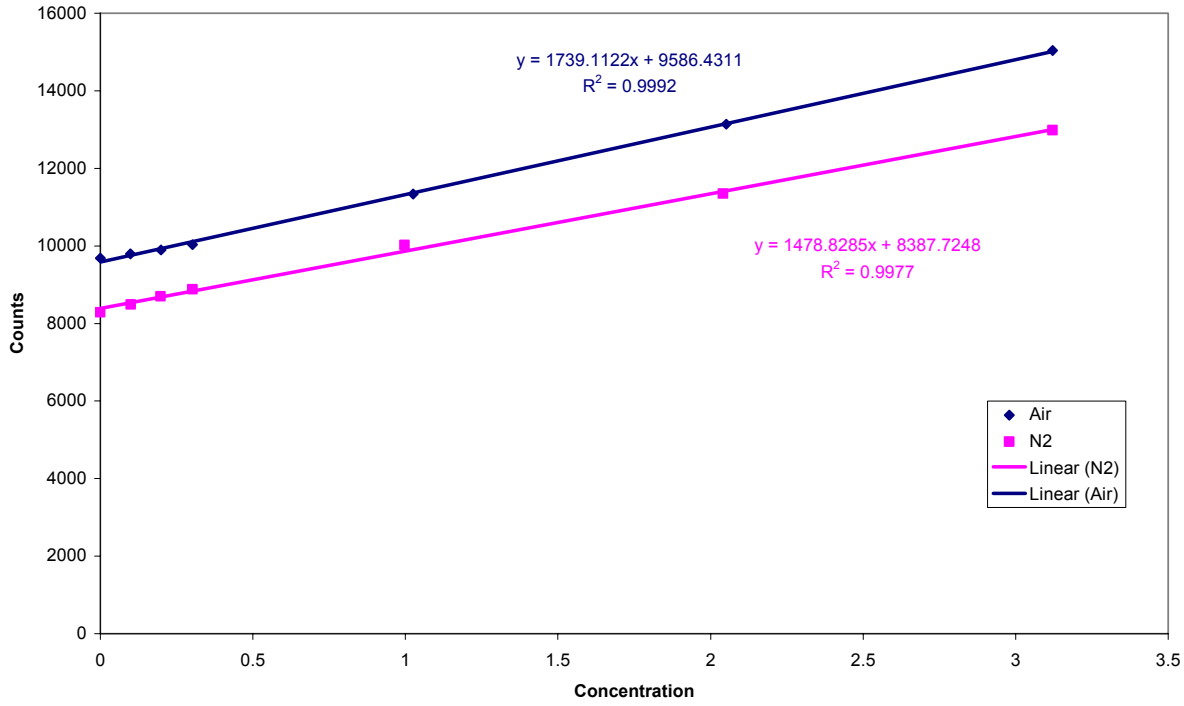


Figure 6. Sensitivity in oxygen-depleted environment (CA) versus normal atmosphere.

2.4. Response of Sensor to CO versus Ethylene

In addition to ethylene we expect that our sensor will respond to other chemicals of similar structure. However, the chemicals that we are interested in testing are those that may be present at the same time while measuring the ethylene levels for postharvest applications. One of the chemicals is CO, knowing that CO has an affinity for adsorption on the surface of gold. A series of tests was performed using the mixing apparatus and an additional cylinder containing 10 ppm carbon monoxide standard mixed with nitrogen to dilute to different known concentrations. A full step test scale of ethylene was performed before and after exposure to carbon monoxide to evaluate possible hysteresis.

The results of these tests are shown in Figure 7. The results of the carbon monoxide testing show that although there is some sensitivity to carbon monoxide, the sensor is more than 40 times more sensitive to ethylene than to CO. This suggests that interference from carbon monoxide is not a significant issue, unless the levels of carbon monoxide are extremely high (higher than the EPA set hazard threshold) or the ethylene measurement requirements are in the 10-ppb levels (which is highly unlikely). Further, no hysteresis effects were observed in our measurements suggesting that the sensor is not “poisoned” by CO as might be the case with platinum-based catalysts.

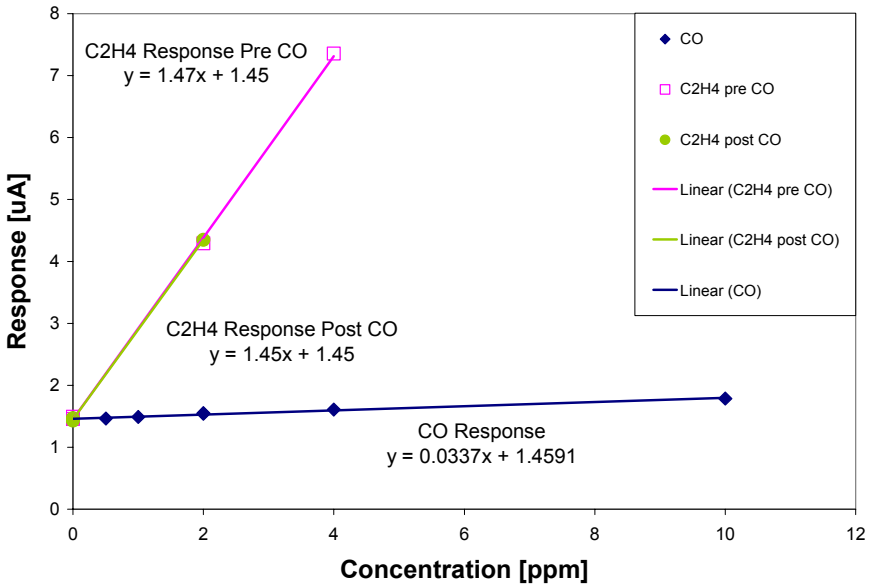


Figure 7. Sensitivity to carbon monoxide in relation to ethylene.

2.5. Summary of CA Room Measurements with Bartlett Pears

A series of tests were performed at USDA-ARS Tree Fruit Research Laboratory in Wenatchee, Washington. Three different CA chambers each with an empty volume of 151 Liters were used. Two of the chambers contained 50 mature green 2006 season Bartlett pears (*Pyrus communis* L.*)* and one chamber was kept empty as control. In order to simulate the humidity condition in the pear-containing chambers, moist paper towels were maintained in the control chamber.

The measurement process included two GC samples that were taken before and after the ETH-1010 measurements with our electrochemical sensor. Careful measured were taken to minimize the reading differences between the two GC samples for each run.

The tests started with the chambers filled with the Bartlett pears and allowed to sit under the recommended CA conditions for more than 24 hours. The gas composition was maintained at 1.5% O_2, 0.5% CO_2, and 98% N_2 and the temperature was kept at 1 °C. Since the background ethylene was low, additional ethylene gas was injected into the chamber in order to make quick comparison between the GC and Fluid Analytics ethylene sensor at the CA conditions. The ethylene injections were performed every 15 minutes at increments of 3 to 5-ppm using a 14,680-ppm ethylene standard, followed by a 0.5 ml sample tested in a HP 5880A GC with flame ionization detector, continuous sampling through the ETH-1010 sensor, and again followed by a second GC sample.

Interestingly, the data from the empty chambers show a good agreement within ±10% of the GC results. Some of the data for chambers filled with Bartlett pear appeared to show a higher level of spread, especially around 10-ppm, where the uncertainty was closer to 15% between the two measurement methods. It is not clear at this point whether or not GC readings were low, ETH-1010 readings were high, or both. The general trend, however, appeared to have excellent agreement.

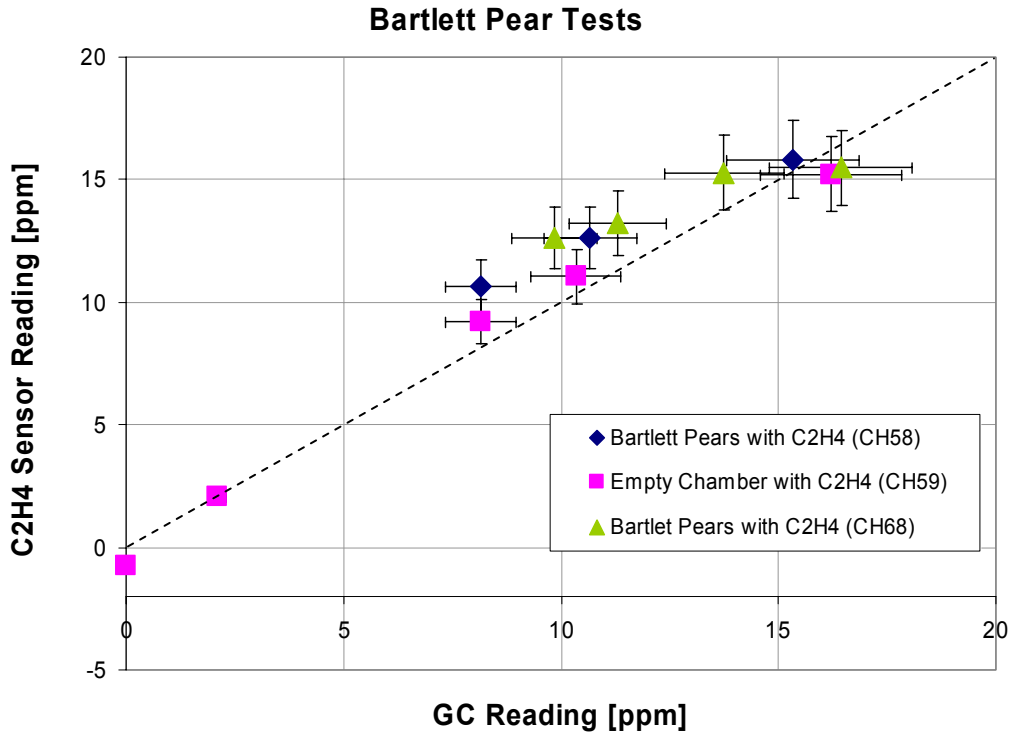


Figure 8. Comparison of HP 5880A GC and ETH-1010 ethylene measurements for empty and Bartlett-filled CA chambers. The error bars are +/- 10% of the reading.

In a separate set of tests with single store-bought organic Bosc pears, we were able to observe a measurable ethylene production rate at room temperature as evidenced in Figure 9. The measured ethylene production rates were approximately 500 ppb/min, which represents the initial part of this curve.

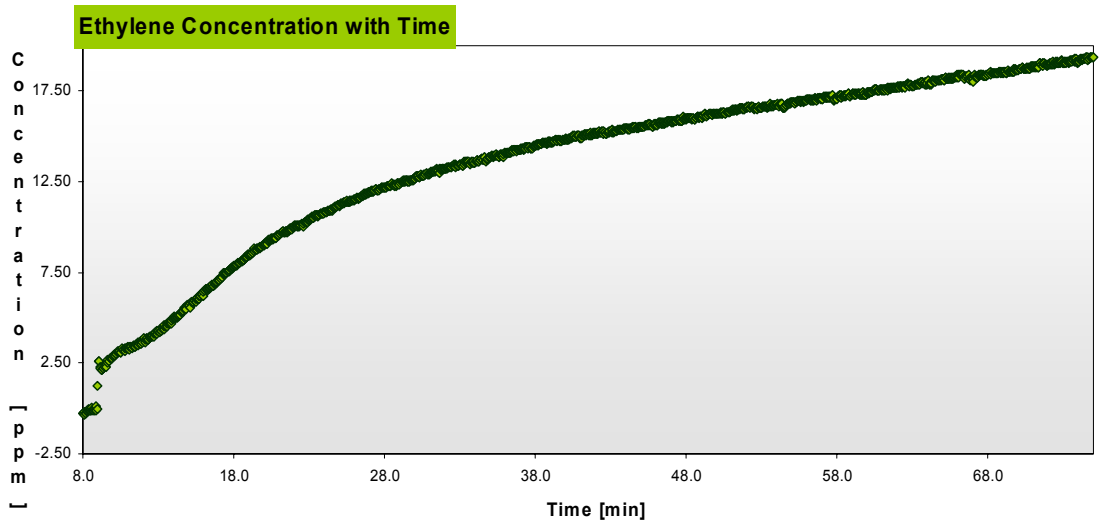


Figure 9. Ethylene production rate for a Bosc pear kept in a 0.5 L container at standard temperature and pressure.

V. SUMMARY

In summary, our technology is differentiated from the competitive technologies in several ways:

1. Accuracy: The current system is able to measure concentrations as low as 10-ppb, which is significantly lower than what other commercially available instruments are capable of, and it is comparable to a GC.
2. Response time: The current sensor is able to make one reading in just a few seconds. This is a marked improvement over a gas chromatography system.
3. Cost: The current price for a scientific grade instrument is <$5,000 and the actual price depends on the added functions and features. Future mass produced instrument is expected to have significantly lower price.
4. Size: Our system is portable and handheld. It measures approximately 10.5”Wx4”Hx10” D and it weighs less than 5 lbs.
5. Ease of use:
    a. It displays the ppm/ppb or concentration of ethylene;
    b. It does not require any additional hardware;
    c. It comes with attachments for different applications;
    d. It is accompanied with appropriate data acquisition and display software that allows the user to store data (internally as well as on a laptop).

VI. ACKNOWLEDGEMENTS

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