FINAL REPORT
WTFRC Project #: PH-04-449

Project title: Non-invasive colorimetric measurement of ethylene release for in-orchard evaluation of apple readiness for harvest

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This report describes activities in the second year of a two year project funded by the WTFRC.

Objectives: The goal of this research program is to develop a simple and inexpensive means to evaluate ethylene release from apples pre- and post-harvest. Specifically, the proposed device is a flat, inexpensive, micro-thin permeable membrane sandwich in the form of a patch or "sticker" that self-adheres to the surface of the apple. The patch detects the emissions of ethylene from an individual apple (rather than the atmosphere around many apples) and consequently displays a color change indicating ripeness on the visual (external) surface of the detector. Ethylene is a demonstrated fruit ripeness indicator and its release correlates with the onset of readiness for harvest.

By translating the apple fruit's natural ripening timetable into a simple, easy-to-read colorimetric form, this research offers a unique, previously undeveloped response to the commercial apple market's need for a quick, inexpensive, reliable measurement of apple fruit ripeness in the orchard. The sensor is designed to measure ripeness of each individual apple on the tree or a selected sampling of apples. Preliminary estimates indicate the sensor may be the approximate diameter of a US quarter.

In order to be a feasible technology, the final device must meet several strict requirements. In descending order of importance these include detecting low levels of ethylene (0.1 ppm is our target), stability and insensitivity to confounding factors, rapid response (preferably within minutes), high contrast to permit easy detection, safety and minimal environmental impact, and low cost. Our efforts to date have focused on the priorities of low detection limits, stability, and rapid response.

Significant findings:
Development of this sensor has proceeded in several stages: designing of a testing chamber, identification of suitable colorimetric reagents, evaluation of colorimetric reagents, design of a membrane system to encapsulate the chemistry, and optimization of formulations and detection.

Our results for the past year are summarized below:

1) evaluated a number of potential chemistries for sensitivity and selectivity to ethylene,
   identified one ideal chemistry using a molybdenum compound (Mo) with a Pd catalyst
2) investigated the impact of interfering factors (humidity, temperature, light, CO2, O2, N2),
   these factors all found to be negligible, except for light. We continue to work with uv protective films which have strong potential for success.
3) evaluated several potential support materials (membranes, solids, gels),
   identified three potentially feasible support materials and continue to compare based on stability, manufacturability, and cost;
4) investigated potential toxicity
   in-house toxicity experiments showed no negative response to chemistry when immobilized on supports. Some cell response was initially observed due entirely to the low pH of chemistry used earlier in the work. This has now been neutralized.
5) developed an integrated device;
6) initiated limited field trials (results not yet obtained).

In summary, we have a working device which is responsive to ethylene concentrations of at least 1 ppm; a significant color change is obtained in the span of several hours. This color remains constant for at least 24 hours.

Challenges to be addressed:

We are submitting a request for funds for a new project that in effect is a continuation of the current two-year work plan. The work that remains to be done to fully optimize and validate the device includes the following.

1) Complete development of integrated device with full protection from uv light.
   We have been advised by researchers at Sandia National Laboratories (Albuquerque, NM) on several new approaches and are in the middle of evaluations.
2) Increase ability to manufacture stickers.
   Currently we can make dozens of devices at a time; however, larger throughput is needed to be able to perform extensive field trials.
3) Perform a larger set of field trials.
   James Mattheis, PhD, USDA, ARS, Tree Fruit Research Laboratory, has assisted on our initial trials. We aim to further evaluate ease of use, ability to visually discriminate color change, sensitivity, selectivity, impact of environment, etc.
4) Perform an external evaluation of device toxicity to ensure safety.
   Potential partners include other University of Arizona research groups (Dr. Stuart Williams, Head of Biomedical Engineering) and connections with Sandia National Laboratories.
5) Modify speed of color change.
   Develop a means to integrate ethylene measurement over many days with a slow color change. This can be accomplished through modification of the membrane separating the chemistry from the fruit surface.
6) Improve device performance based on results of field trials.

Methods:

Chemistries

We have evaluated a number of chemistries for sensitivity to the fruit-ripening hormone, ethylene (C_2H_2), while providing a visually distinct color change. Our initial list included 15 candidate schemes based on information collected from the literature, from our prior experience, and from discussions with chemists. Candidates have included schemes involving reactions with metals such as palladium and manganese.

We have evaluated both liquid phase chemistries and solid phase. Liquid phase involved dissolving the reagents in a suitable solvent (water or acetone), introducing ethylene into the head space of a closed vial and then mixing. Solid phase methods involved precipitating or covalently attaching chemistries to a solid support phase including silica, activated charcoal, under an agarose layer, and on several membrane materials. One of the primary challenges we faced in evaluating these chemistries is the low solubility of ethylene in solvents that are appropriate for dissolving the chemistries. The $KMnO_4$ is soluble in aqueous solutions, but when ethylene was introduced at varying concentrations, the level of conversion of the reaction was not always consistent, thus leading to variability in degree of color change. This variability initially was ascribed to experimental error and poor mixing; however, improvements in these techniques led to only modest increases in reproducibility. We believe that the primary limiting factor is the low water solubility of ethylene. This led to our evaluation of solid phase chemistries including attachment and precipitation of the chemistries on and within membranes (Teflon, nylon, and agarose) and on solid support materials (several types of silica and activated charcoal).

The initial plan was that the attachment to membranes would be ideal as this would simplify manufacture of the eventual device. Unfortunately (as discussed in more detail below), such formations proved less stable than desired. Undesired reactions and color changes were observed after exposure of membrane preparations to oxygen or upon drying. In some cases these reactions were reversible, in others they were not. Attachment to silica support phases proved to be the most stable and provided the most reliable response to ethylene. Below we discuss the most successful schemes we have evaluated.

We have evaluated a number of materials as support for the indicator chemicals. There are two potentially feasible systems for the material supports: membranes and silica. Based on investigations of both sensitivity and stability of chemicals with respect to color change with various types of membranes, the desired characteristics of the material supports are the following:

1. Gas permeable (allow ethylene gas access to the chemistry).
2. Acid tolerance (The Pd-Mo chemistry is highly acidic).
3. No interference from other materials (The indicators can react with organic compounds and provides a color change without ethylene exposure).
4. Hydrophilic
5. UV resistant
6. Adhesive resistant
7. Permit visual analysis (Preference: opaque color).
8. Not fragile

Membrane system

The primary design of the device is in a membrane form that would be easy to manufacture and apply the final device to a commercial product. We have selected and tested several kinds of membranes which may be suitable for the indicator systems while providing the color change with ethylene. The following lists are membranes that have been tested:

1. Nitrocellulose membrane (Millipore, 40)
2. Durapore membrane Filter (Millipore, 40)
3. Fluoropore membrane (Millipore, 40)
4. Nylon membrane (Millipore, 40)
5. Qualitative filter paper membrane (Whatman, 41)
6. Fiber Glass membrane
7. Tyvek membrane:
8. Nuclepore Polycarbonate filtration
9. PTFE

Table 1: Description of membranes

Types of membranes	**Composition**	**Qualities**	**Color**	**Wettability**	Max. allowed temp. (C°)
Nitrocellulose Membrane	Mixed cellulose esters	- Widely use in analytical and research applications	opaque white	Hydrophilic	75
Durapore Membrane	Polyvinylidene fluoride	- broad chemical compatibility - Mesh size: 125 μm	opaque white	Hydrophilic	85
Fluorppore membrane	Supported PTFE bonding to polyethylene	- broad chemical compatibility	white	Hydrophobic	130
Nylon membrane	Nylon	- Compatible with a broad range of solvents - Pore size: 0.22 - 0.45μm		Hydrophilic	75

Types of membranes	Composition	Qualities	Color	Wettability	Max. allowed temp. (C°)
		- Mesh size: 11-180 µm			
Qualitative Filter membrane	Cellulose	- Use for clarifying liquid - Diameter: 150 mm	opaque white	Hydrophilic	N/A
Tyvek membrane	Spundbonded Olefin	- Ultraviolet light resistance	opaque white	Hydrophilic	N/A
Nuclepore Polycarbonate filtration	Microporous Polycarbonate film	- Transparent and smooth flat surface		Hydrophilic	140
Polytetrafluoro ethylene (PTFE)	PTFE on a polypropylene support	- Wide chemical compatibility - Wide temperature compatibility - Diameter: 5.0 cm	opaque white	Hydrophobic	60

Results and discussion:

We have evaluated a number of reagents and the above membrane systems. Below we summarize the most productive experiments and how they support the development of this device.

We have evaluated the ammonium molybdate and palladium sulfate reagents with activate silica gel, silica sand and silica gel. We coated several proportions of ammonium molybdate and palladium sulfate solution on the material and dry at room temperature. Only activated silica gel did change color from yellowish to blue with high concentration of ethylene (Figure 1, table 2). The color change of the reaction is very stable and obviously distinctive. The challenge with the silica gel is that the material is sensitive to the oxygen. We may eventually return to this approach; however, the oxygen reaction makes this formulation less than ideal.

Figure 1: Palladium molybdate on activated silica gel with (right) and without (left) ethylene.

Mo : $PdSO_4$	Ethylene concentration (ppm)						
	Control*	Tank**	0.1	0.5	1.0	1.5	2.5
5 : 1	yellow	Dark blue	Green yellow	Green yellow	Green blue	Blue	Blue

Table 2: The color change of palladium molybdate solution on activated silica gel after exposure to ethylene. * Control: addition of 5 ml of air; ** Tank: addition of 5 ml of ethylene gas from the tank

Pd:Mo	Silica gel			Silica sand			Activated silica**		
	High conc.*	2ppm	Control	High conc.*	2ppm	Control	High conc.*	2ppm	Control
1:3	black	yellow	yellow	black	yellow	Yellow	black	yellow	yellow
1:5	black	yellow	yellow	black	yellow	Yellow	black	yellow	yellow
1:7	black	yellow	yellow	black	yellow	Yellow	black	yellow	yellow
1:10	black	yellow	yellow	black	yellow	Yellow	black	yellow	yellow

Table 3: The color change of palladium molybdate solutions on solid materials with ethylene exposure.
* High concentration is a high concentration of ethylene directly from the tank.
** Activated silica gel is a material from the commercial ethylene detection tube which was washed out the chemical.

We have focused our evaluation of membranes on three types of membranes: filter membrane, durapore, and nitrocellulose membrane. Initial experiments were tested with 3 potential compounds (table 3).

1. $(NH_4)_6Mo_7O_{24} * 4H_2O$
2. $(NH_4)_6Mo_7O_{24} * 4H_2O + Na_2Si\ F_6$
3. $(NH_4)_6Mo_7O_{24} * 4H_2O + Na_2Si\ F_6 + PdSO_4$ (palladium sillicomolybdate)

Based on the experiment, ammonium molybdate on a membrane provided the color change from white to blue when exposed to ethylene, but the control also turned to blue color with air exposure in absence of ethylene. Ammonium molybdate and sodium sillicofluoride compound on membrane did not change color with any gas. Palladium silicomolybdate on membrane provided the color change from yellow to gray with ethylene exposure. The membrane turned a gray-yellowish color with air exposure after 5 days later. The membrane in form of sticker was also destroyed by adhesive compound (table 4).

Therefore, compound 1 and 2 on the membrane did not provide the color change with ethylene exposure. Only compound 3 has a potential in the color change for ethylene detection, but the stability of the control and the degradation of the membrane in the sticker form were initially a problem (Figure 2).

Membrane type	chemistry	Control	Ethylene exposure			Sticker form	Sticker form on the apple
			High	1.5 ppm	5.5 ppm		
Filter membrane	Compound1	blue	blue	blue	blue	blue	Blue
	Compound2	white	white	white	white	white	white
	Compound3 (Pd:Silico-1:3)	yellow	gray	yellow	gray	chewed up by itself	N/A
Nitrocellulose (Millipore)	Compound1	white	white	white	white	white	White
	Compound2	white	white	white	white	white	white
	Compound3	N/A	N/A	N/A	N/A	N/A	N/A

Table 4: Palladium sillicomolybdate compound on membranes in various conditions.

Glass fiber paper, Nitrocellulose and Durapore, which are inorganic membranes, were selected in this experiment. Various proportions of palladium and molybdate solution were coated on the membranes. Even all proportions changed to the blue color with ethylene exposure (Figure 3 and 4). But, Ex1 provides the best results in stability and sensitivity of the color change.

Sample	Control		High conc. of ethylene	Low conc. of ethylene
	1hr	1 day		
Ex0	Pale yellow	Gray-yellow	Dark blue	Blue
Ex1	Pale yellow	Pale yellow	Dark blue	Light blue
Ex2	Pale yellow	Light blue	Dark blue	Light blue
Ex3	Pale yellow	Light blue	Dark blue	Slightly blue
Ex4	White	White	Slightly blue	White

Table 5: The color change of Ex 0-4 on the membranes in various conditions.

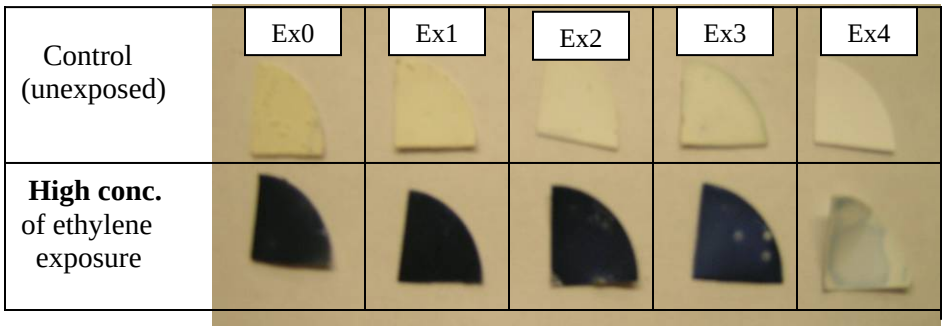


Figure 2: Ex 0-4 on the nitrocellulose membranes with ethylene exposure (bottom) and non-exposure (control, top).

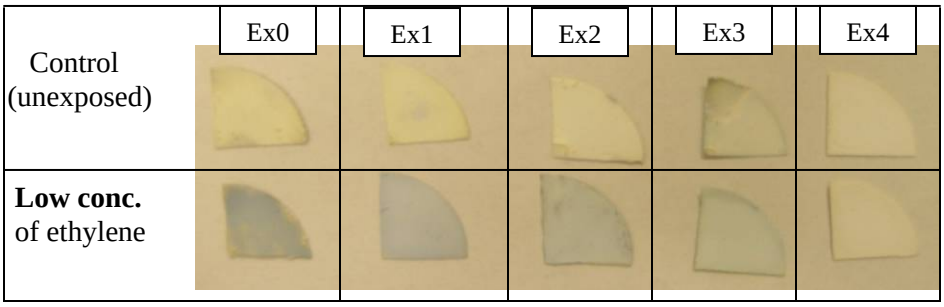


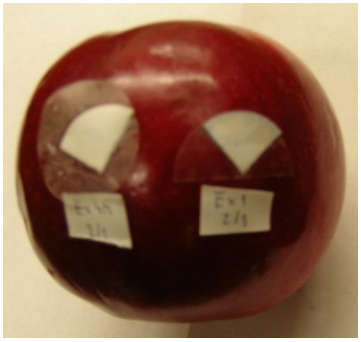
Figure 3: Ex 0-4 on the nitrocellulose membranes with 5 ppm ethylene exposure (bottom) and non-exposure (control, top).

Evaluation of the color change for various kinds of apple fruits was tested on nitrocellulose membrane with chemistry proportions of Ex 1. Stickers placed on every species of the apples turn the blue color within 24 hours after exposure. Granny Smith and Mcintosh species had a very small color change. These apples were purchased at a grocery store in Tucson, AZ, in February. Subsequent experiments with other samples of these same species displayed ethylene release rates that were barely detectable as quantified through standard analytical means. More experiments need to be done with apples producing a greater amount of ethylene.

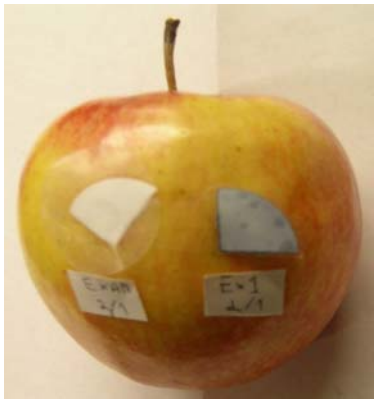
There is a significant trade off that needs to be made between having good sensitivity to ethylene, represented by a deep color change, and having a device which is overly sensitive to interfering compounds (light, non-ethylene hydrocarbons). We have focused primarily on obtaining the best specificity possible at this time rather than obtaining the largest color change that might have less meaningfulness. Our continuing work focuses on how best to improve the magnitude of the color change and improve (and specify) the speed with which the change occurs.

1

2



3



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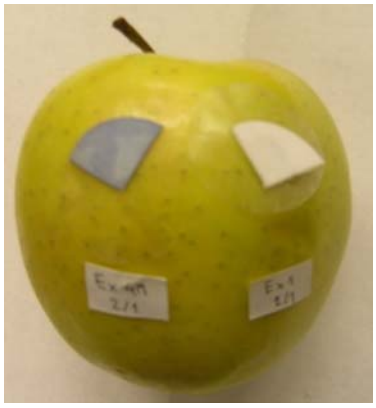

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Figure 4: Ex 1 on nitrocellulose membranes exposed to 7 kinds of apple fruit over 24 hours as the follow.

1. American Cameo
2. Granny Smith
3. Mcintosh
4. Gala
5. Golden Delicious
6. Red Delicious
7. Fuji

The white membranes on the apple are the controls. Note, apples were purchased from a local grocery store in Tucson, AZ in February, 2005. Ethylene release from these apples was fairly low as determined using an electronic ethylene sensor.

Figure 5 shows a more quantitative analysis of the sticker response. The intensity of blue, red, and green pixels in an image of the sticker was quantified (and baseline of a neutral background subtracted). Blue pixels are not shown since although blue changes significantly, there is little discrimination between ethylene concentrations. Red and green pixels (and total luminosity) change significantly over time of exposure and display a dose dependent response. The greatest separation in color is apparent after ethylene exposure from 3 to 6 hours and so this is deemed to be the optimal time for quantification.

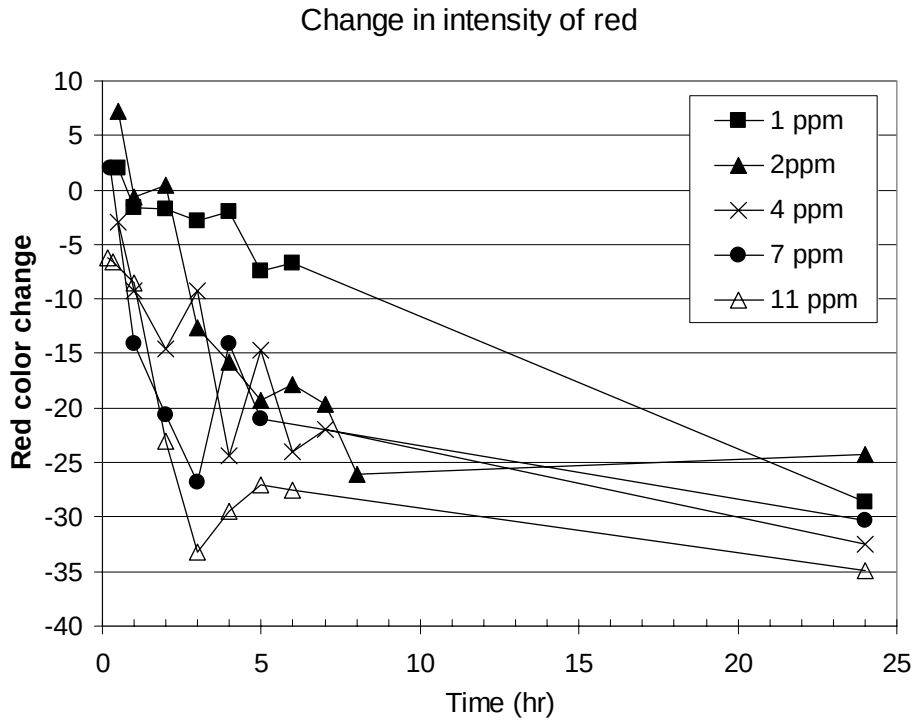

Figure 5a: Change in the intensity of red pixels in a nitrocellulose loaded sticker in response to ethylene.

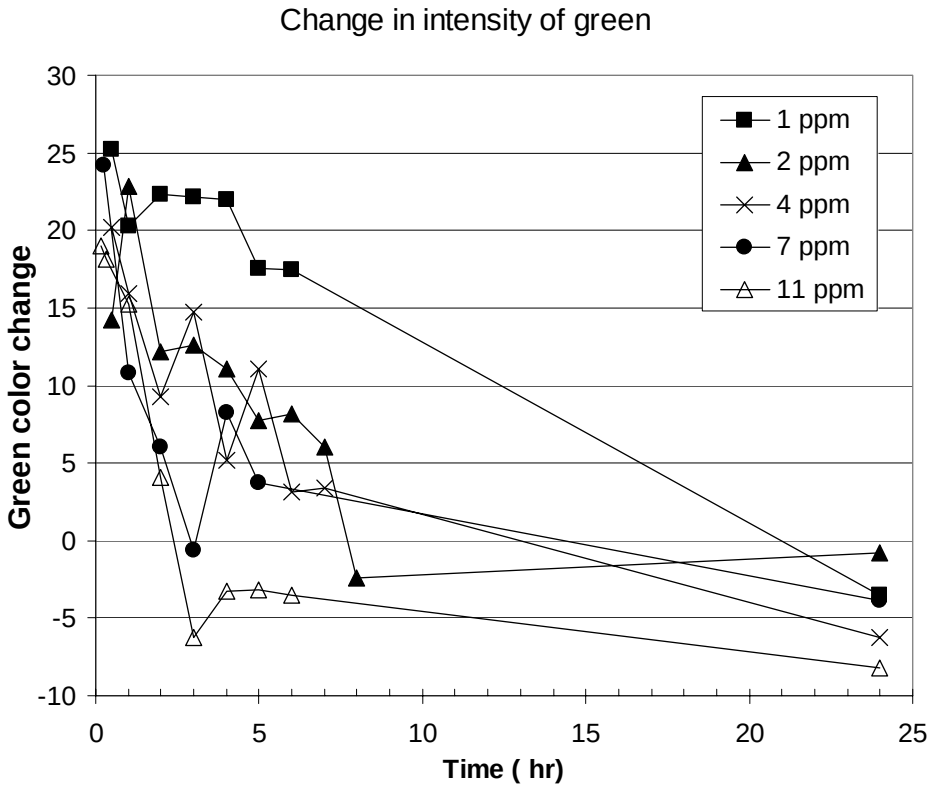

Figure 5b: Change in the intensity of green pixels in a nitrocellulose-loaded sticker in response to ethylene.

Humidity
The impact of humidity on the sensor response was quantified using a sealed glass chamber into which was placed a humidity sensor and a beaker of water with varying amounts of salt.

Ex 1 on Membrane	Humidity test			Control (without test)
	Wet condition	Dry condition		
		unexposed	Exposed ethy.*	
Nitrocellulose	Slightly gray yellow	Pale yellow	Blue with slightly gray	Pale yellow
Durapore	Gray *	Pale yellow	Blue	Pale yellow

Table : Humidity test of Ex 1 on nitrocellulose and durapore membrane.
* exposed to ethylene at a high concentration

A few drops of water were wet on the nitrocellulose membranes and dry by the room temperature air. With the membrane in dry condition, they still provide the same color as the one without the humidity test and change to blue color with high concentration of ethylene. But the color reversed back to pale yellow a day later.

When durapore membranes were exposed to up to 84% humidity for over 100 hours (4 days), membranes became wet. These were taken out of the chamber and exposed to ethylene. The gray color appears on the membrane, but when the membrane dries, the color is still pale yellow. If membrane is dried and then exposed to ethylene, a pale yellow will turn to blue color. Therefore, the humidity has minimal effect on the color change when the membrane itself is dry, but when the membrane is wet, the color change is muted.

Color Scale
We have developed the color scale by obtaining the color from real exposed membranes at various concentrations of ethylene. This calibration needs to be updated and modified for each of the combinations of chemistries and support materials. Images of membranes exposed to varying amounts of ethylene will be used to develop the color scale. This will also need to be adapted as we improve and modify the use of a uv protective film.

Summary
After two years of development supported by the WTFRC, we have a reasonably successful working device that is responsive to ethylene concentrations of at least 1 ppm; a significant color change is obtained in the span of several hours. There remain a number of challenges still to be addressed in order to ensure that the sensor works properly under field conditions and in improving the intensity of the color change. We are submitting a request for funds for a new project that in effect is a continuation of this current two year work plan. This one-year request includes work to complete development, to run more extensive field trials, and to make modifications based on the results of field trials. We also aim to modify the device to alter the speed of color change so that a slower response, such as during storage, could be implemented.

Personnel
Navaporn Srinavakul, MS Candidate, Ag. and Biosystems Engineering, Univ. of Arizona
Dominic DeCianne, Research Technician, Ag. and Biosystems Engineering, Univ. of Arizona

Budget Summary

Item	Year 1 - 2003	Year 2 - 2004
Salary (1 Grad student, hourly, $16.50)	$ 8,863	$ 8,863
ERE (benefits) 3.3% (as of 2005)	$ 1,170	$ 1,170
Salary (1 Under grad stud, hourly)		
ERE (benefits) 3.3%		
Subtotal on personnel		
Travel	$ 2,000	$ 2,000
Supplies	$ 4,000	$ 4,500
Overall Coordinator (Off-campus)	$ 4,500	$ 3,000
SubTotal	$ 20,532	$ 19,532
Indirect Cost (51% of MTDC)	$ 8,176	$ 8,431
Total	$ 28,709	$ 27,964