FINAL PROJECT REPORT
WTFRC Project Number: AP-06-605B

Project Title: Testing of a sticker for ethylene release from apples

PI:	Mark Riley, PhD	**Co-PI(2):**	Robert Klein, PhD
Organization:	U of Arizona	**Organization:**	Rediripe ©
Telephone/email:	520-626-9120, riley@ag.arizona.edu	**Telephone/email:**	RKlein@rediripe.com
Address:	1177 E. 4th St	**Address:**	1506 San Carlos SW
Address 2:	Shantz Room 403	**Address 2:**	
City:	Tucson	**City:**	Albuquerque
State/Province/Zip	AZ 85721	**State/Province/Zip:**	NM, 87104

Cooperators: Dr. James Mattheis, USDA/ARS

Other funding Sources

Agency Name: USDA, SBIR phase I, awarded in 2006
Amount awarded: $80,000
Notes:

Total Project Funding: WTFRC $43,060 + USDA $80,000 = $123,060

Budget History:

Item	**Year 1:** 2006	**Year 2:** 2007	**Year 3:**
Salaries	11283	11283	
Benefits	372	372	
Wages			
Benefits			
Equipment			
Supplies	9000	6500	
Travel	1000	1000	
Miscellaneous			
Total	**23905**	**19155**	

Objectives: The goal of this research program is to develop a simple and inexpensive device to provide a noninvasive means to determine ethylene release from apples. This objective will be met through design of a sticker based device that presents a gradual color change indicative of the amount of ethylene released by an individual apple. Specifically, the proposed device is a flat, inexpensive, thin permeable membrane sandwich in the form of a patch or "sticker" that self-adheres to the surface of the apple. The sticker 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 external surface of the detector. Ethylene is a demonstrated fruit ripeness indicator and its release correlates with ripening. The stated objective would be met by addressing the following aims.

Specific aim 1: Develop a stable and reproducible device using ethylene sensitive reagents integrated into membranes.

Specific aim 2: Tune the responsiveness of the device for fast (hours) to slow (days to weeks) response to ethylene.

Specific aim 3: Test the devices in the laboratory, orchard, and packinghouse.

Timeline:

Year 1: 1/06 – 12/06

Improve stability of device based on results of small field trials from Fall 2005.
Begin development of slow responding device by modifying reagents and supports.
Perform field trials in the orchard and packinghouse

Year 2: 1/07 – 12/07

Improve stability of device based on results of small field trials from Fall 2006.
Improve time of response of devices.
Perform moderate scale field trials in the orchard and packinghouse

Significant findings: Over the past year, we have made substantial progress in the development and testing of this device. To summarize accomplishments of 2007:

1) Improved the device performance. Advances were made in the device in how the reagents are encapsulated and interface with the adhesive. This greatly diminished the impact of varying humidity (elevated or reduced), improved storage capabilities, and decreased temperature sensitivity. This encapsulation did, however, decrease the sensitivity to ethylene levels. Detection limit is approximately 1.0 ppm ethylene.
2) Developed and modified device prototypes for orchard use; made improvements based on orchard findings.
3) Assessed potential reagent toxicity. Reagents cause less of a biological response than do copper or zinc in soluble forms.
4) Performed laboratory and orchard trials. Laboratory testing was done in conjunction with development and focused on improving stability. Orchard trials were performed by Ines Hanrahan, Ph.D., on a variety of apple cultivars during the July-October 2007 harvest season. Briefly, the device performed reasonably well with good stability and response to ethylene levels ranging from 0.1 to more than 10 ppm in the orchard. Unfortunately, no useful correlation could be found between ethylene release (and sticker color) and other measures of fruit maturity. Full color development was obtained after normal harvest time. These studies are summarized below.

Research and discussion: Our goals for 2007 activities were to continue the progress made in the previous year in improving device stability and sensitivity, to develop a prototype with separate components with variable response to ethylene, to run additional trials in the orchard and packing house, and to explore opportunities for manufacture of the device.

Device development

Significant efforts were made in altering the reagent composition to improve stability, in providing a means to adhere the sticker to fruit surfaces, and improving contrast for ease of assessment in the field. The major advances were produced through adding hygroscopic materials to the reagents, drying the reagents to a lower final water content, and providing a more air tight seal preventing air contact from outside the fruit surface. These efforts are summarized briefly below.

We evaluated a large number of potential support materials with the goal of providing an easy to use, easy to apply to the fruit, and stable approach for detection of ethylene. Support materials that received significant attention include:

> Evaluated included polymeric membranes, papers (filter paper, printer paper), and glass filters. Particulates evaluated include a wide range of materials including TiO_2, SiO_2, $ZnSO_4$, $CaCl_2$, $NaSO_4$, metal oxides, metal chlorides, perlite, and earthstone.

We performed extensive trials on membranes including but not limited to: Nylaflo, GH polypro, polyethersulfone, Tuffryn, nitrocellulose, polypropylene, Biodyne, glass, Durapore (wetted and non wetted), Gore-tex, nylon, tyvek (wetted, non-wetted, food grade), and Whatman filter numbers 1 and 50. For most of this work we focused on utilizing the commercial membrane material, called "Durapore" from Millipore, a PVDF film, as this provides the greatest stability against unintended color development. Membranes were loaded with reagents, allowed to dry, and then exposed to ethylene and other volatiles. For nearly all membranes, no significant benefit was observed over use of Durapore. Wetting of normally hydrophobic membranes was a challenge as our reagents begin as an aqueous solution. Wetted Durapore and the glass membrane showed the most promise.

Due to the high cost of the Durapore membrane, an important part of recent experimentation has involved testing other brands and other membranes. Of the numerous membranes tested, the two that were of most interest from a commercial perspective were Biotrace PVDF and GLA-5000 PVC Membrane Filter, both made by Pall Corporation. However, both of these membranes are highly hydrophobic, making use of a wetting agent necessary. In order to find the best wetting agent, several were tested, including ethyl alcohol, methyl alcohol, and isopropyl alcohol. After thorough experimentation, it was determined that neither of the new membranes was a suitable substrate for reagents, as both reacted (with no ethylene exposure) on the bench as much as or more than Durapore. Figure 1 shows an image of stickers using the wetter Durapore on apples in the laboratory.

Additional studies were run to identify solvents for improved wetting characteristics on membranes including a non-wetted Durapore which has a greater potential stability. Ethanol turns the solution green. All chlorides reduce the sensitivity of the solution. Palladium chloride may not be substituted for palladium sulfate. Sodium sulfate and magnesium sulfate may not substitute for sulfuric acid.

Our prior plans for utilizing a white powder instead of a membrane system led to the development of an easy to apply material (similar to "white out"). This method was tested in the spring and found to have lower sensitivity to ethylene and greater interference due to increased humidity. The approach was set aside and not used in field trials.

Figure 1: Stickers on apples after improvements in the drying process.

Toxicity tests were performed on the chemical reagents using human cell cultures. It was found that the Pd had an LD50 of approximately 0.5 mM and the Mo had an LD50 of approximately 0.7 mM. More testing needs to be performed, however, these cell response levels place the reagents in a similar category as exposure to soluble Fe, and less hazardous than soluble Cu or Zn. This is encouraging as Fe is considered to be of minimal health concern at the dosage present in our stickers. Further testing is required in order to satisfy requirements for FDA review. We continue to asses mechanisms of cellular response in order to validate these measurements.

Orchard tests - Performed by Ines Hanrahan, Ph.D.

During the July-October 2007 growing season the WTFRC and RediRipe jointly evaluated field performance of ethylene stickers when placed on apples. The objectives were to ensure reliable color change of the stickers under various field conditions and to relate sticker color change to fruit ethylene emission and common harvest maturity parameters.

General trial procedures
Stickers were shipped from Arizona via FedEx overnight and typically arrived in Yakima, WA, in the late morning of the following day. Upon arrival stickers were inspected for color change and signs of damage. Apart from the first set (10% of stickers had turned color) stickers arrived without discernable color change. For transport stickers were kept in the folders provided by Riley's lab and care was taken to avoid any contact with ethylene, such as ripe fruit in the car and office. Typically, stickers were applied to fruit within 24 hours of arrival. Across all trials stickers were placed on fruit of similar size and maturity on the north facing side of the apple (Figure 2).


Figure 2: Position of stickers on single fruit, and within the tree

Positive controls were placed on leaves (upper and lower side), bark and posts approximately one foot away from any fruit (Picture 2). Stickers typically did not change color.

Figure 3: Negative controls on a leaf and a post (bark not shown)

Responses of stickers to changing relative humidity have been a concern in the past. The first two sets of stickers tested in 2007 were split into 4 groups: orchard with or without overhead irrigation, fruit indoors and outdoors. No differences in response patterns were observed (Tables 1-5). The last two sets of stickers experienced 5 separate rain events. About 80% of the stickers remained functional and retained adhesiveness. The position of the stickers on the fruit was inconsequential. We tested all horizontal and vertical. In order to be consistent, we chose to apply stickers around the equator of the fruit facing north.

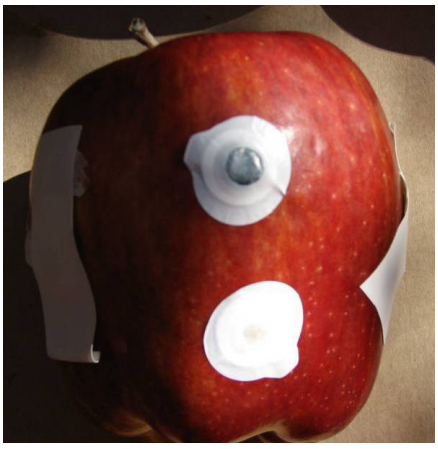

Figure 4: Sticker response to ethylene 24 hours after application

Summary of trial results:
Assessments of ethylene release and sticker color development were performed over the course of the July-October 2007 apple harvest season in WA. Raw data and more details can be provided if needed. Below we summarize the more relevant results.

First we assessed the correlation between IEC (internal ethylene concentration (in ppm)) and the ethylene release rate (in uL / kg –hr). There is a strong correlation ($R^2 = 0.97$) which demonstrates that quantifying the amount of ethylene released by the fruit can be a reliable indicator of the internal ethylene levels (Figure 5)

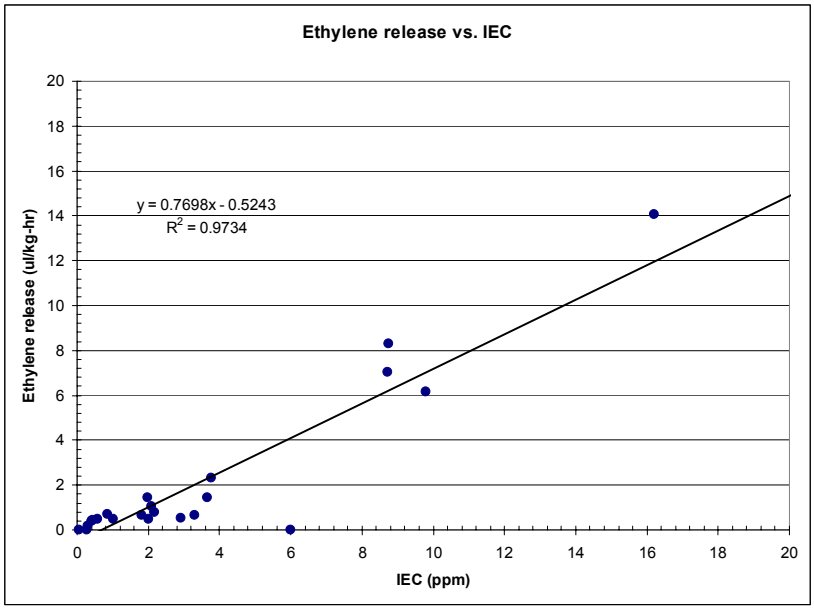

Figure 5: Correlation between IEC and ethylene release rate as measured for the variety of apples shown in Figure 6.

Measurements were performed from August to October. One test performed on August 16, showed a good correlation between sticker color and IEC and ethylene release is shown below (Table I).

16-Aug	wt(kg)	time	ppm	ul/kg/hr	Color?	IEC
Pacific outside for 6 days	0.237	40	3.079	38.39	XXXX	49.4
	0.228	42	2.074	25.60	XXXX	27.7
	0.258	43	0.187	1.99		3.3
	0.26	45	0.227	2.29	XX	1.5
	0.261	46	0.221	2.18		3.5
Pacific indoors for 6 days	0.183	67	4.088	39.41	XXXX	59.9
	0.201	73	0.136	1.10		2.1
	0.226	75	0.123	0.86		1.8
	0.247	77	1.321	8.21	XX	15.2
	0.197	79	5.186	39.39	XXXX	47
Pacific harvested today	0.238	76	0.232	1.52		3
	0.226	77	0.322	2.19		3.6
	0.235	78	0.32	2.06		2.7
	0.227	79	0.427	2.81		5.3
	0.213	80	0.376	2.61		5.5
Imperial harvested today	0.206	81	0	0.00		0.3
	0.208	89	0.075	0.48		1.1
	0.189	65	0.075	0.72		1
	0.173	66	0	0.00		0.2
	0.19	67	0	0.00		0.4

XXXX	stickers had turned blue
XX	lighter color

Later in the season, after improvements in device stability, multiple quantitative assessments of sticker color were performed. A summary of one data set is shown below. Sticker color related to ethylene released had a correlation coefficient of 0.59 when sticker color is compared with a 1-8 scale (1 being white, 8 being a dark blue). Ethylene released at a level as low as 0.1 ppm could be detected with the device. The results shown in Figure 6a are for only fruits that were not damaged; Fuji's are also not shown here. Figure 6b shows a breakdown of all cultivars tested in this set. There appear to be some cultivar-specific effects.

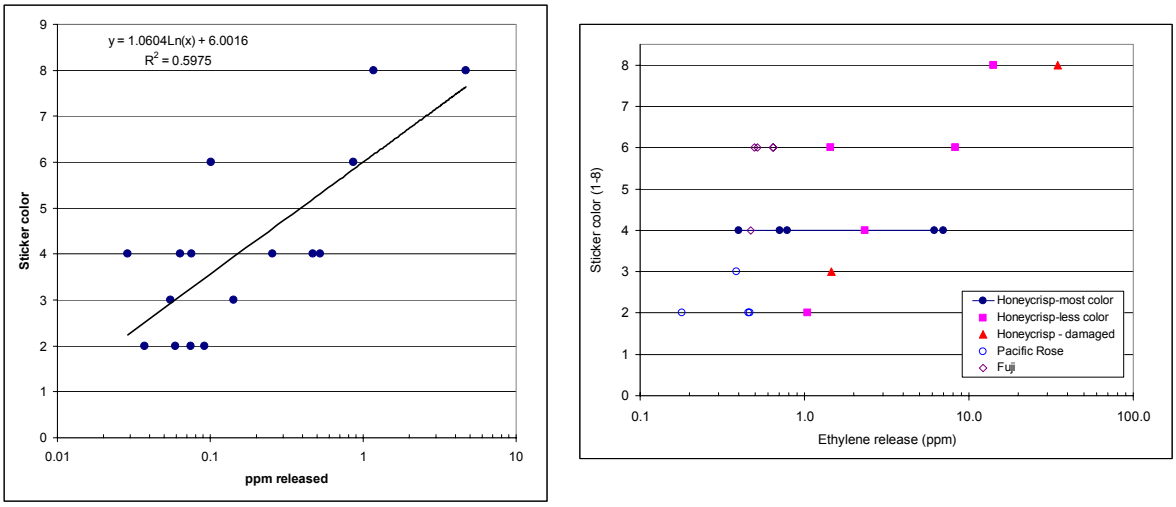

Figure 6: Sticker color development as a function of ethylene release in ppm. This shows a variety of response behaviors based on the fruit cultivar. Measurements performed on 9/28/2007-3 days after application, 2nd pick timing.

An aspect of this work which we believe is critical to assessing the utility of the sticker device is the connection between ethylene release and other standard measures of fruit maturity. These are shown in Figure 7 for the late September test. In short, there appears to be no significant correlation between ethylene release and fruit weight, firmness, starch, color, or SSC.

Conclusions from Dr. Hanrahan:

> Based on our observations, the stickers do pick up ethylene emitted by apples. Environmental factors, especially relative humidity, did not interfere with proper readings in our study. Once apples start to emit ethylene, it appears to dissipate evenly across the fruit surface. Hence, the actual position of the stickers on the fruit does not interfere with the ethylene readings. For the purpose of repeatability we suggest placing stickers around the equator, on the north-facing side of the fruit.
>
> Besides the Honeycrisp trial or when fruit was not intended for storage, commercial harvest always preceded any kind of sticker reaction. Hence, with the current sensitivity the stickers would not be suitable as harvest prediction tool for apples in Washington State. Replicating RediRipe's benchtop tests under field conditions to establish reliable sticker readings in the ethylene range found in apples around commercial harvest timing (0-1ppm) would be of utmost importance.

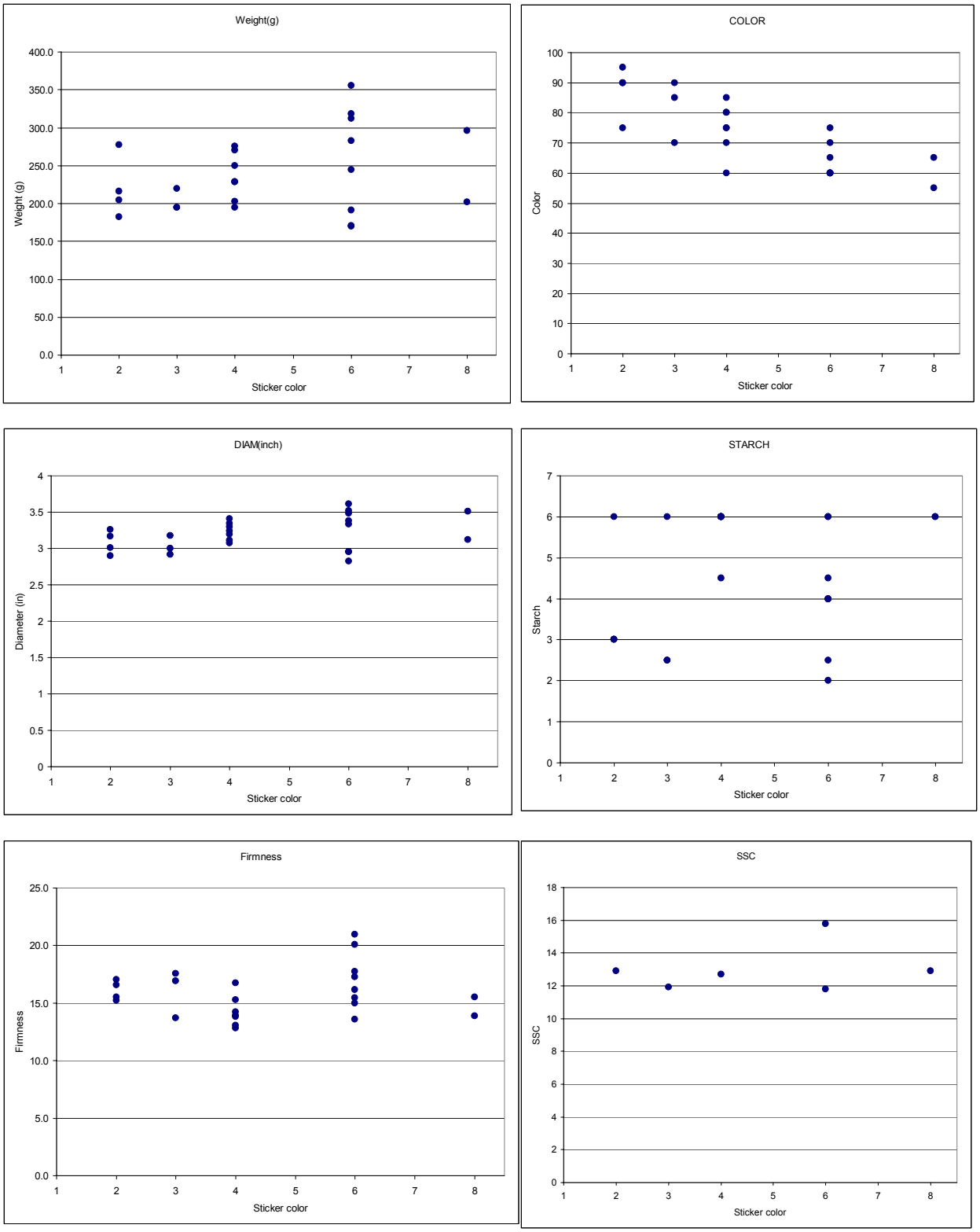

Figure 7: Maturity data with development of sticker color.

Following are suggestions for sticker utility improvement from Dr. Hanrahan:

Without direct negative control, correct reading of the stickers in the field is difficult, even with available color scales on paper. In order to simplify the reading, color gradients could be placed directly next to the sticker and the sticker itself should have a bigger surface area.

Ethylene is indicative of physiological maturity, which does not always correlate well with commercial maturity. Other factors such as background color, starch movement/amount of soluble solids/firmness are widely used by the fruit growing community instead. Current research suggests that starch depletion as well as background color are the closest related to actual maturity. To date researchers were unsuccessful when trying to relate ethylene production, or maybe more precisely the onset of ethylene production to commercial maturity.

It seems like the most practical use for the sticker would be in any kind of scenario that requires presence/absence readings of ethylene such as:

- After treatment of fruit with ethylene synthesis or action inhibitors (i.e. ReTain, Smartfresh), for example: determine exact ReTain timing in the field; assess if and when a certain lot of fruit has 'awaken' after storage and Smartfresh treatment in the warehouse
- To verify when to start preconditioning of fruit such as winter pears
- Document effectiveness of ethylene applications in the field and post-harvest for example to assess: return bloom on apples, stem loosening of sweet cherries prior to mechanical harvest, or preconditioning of winter pears.

Another use would be to explore how each apple variety reacts. For example, Gala apples generally produce about 1ppm right around commercial maturity, hence would be a candidate for the sticker, given reliable sensitivity in that range. We do not know a lot about newer varieties, and one would have to look at ethylene curves closely. For example, some variety might have an ethylene peak much smaller than the climacteric a certain time before commercial maturity, for example Honeycrisp.

Discussion

In the past several years we have developed a simple device that responds to ethylene released by individual fruits. We have improved the device formulation with much of the progress occurring as a result of orchard trials performed in 2006 and 2007. The results indicate that the Durapore stickers are reasonably well able to respond to 1 ppm ethylene while requiring 24-48 hours for full color development. The missing element here is the inability to correlate sticker color with other indices of fruit maturity. It appears that utilizing this device for apple orchard assessment provides minimal advantage to the grower for the cultivars and conditions tested here. We are willing to address other applications of the device, including those suggested by Dr. Hanrahan.

We wish to thank the Washington Tree Fruit Research Commission and its Manager James McFerson, PhD, for their encouragement and generous support.