UCDAVIS POSTHARVEST TECHNOLOGY

Ethylene Inhibition and Control

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Fruit Ripening and Retail Handling Workshop
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Thanks to Michael Reid, UC Davis for many slides

Some References

- Ethylene effects. M.E. Saltveit. 2004. USDA handbook 66. Commercial Storage of Fruits, Vegetables…… http://www.ba.ars.usda.gov/hb66/contents.html
- Recent advances in Ethylene Research. Z.F. Lin, S. Zhong and D. Grierson. 2009. J. Expt. Bot. 60: 3311-3336.
- Postharvest Technology Center; postharvest libraries: ethylene and 1-MCP; http://postharvest.ucdavis.edu/
- The use of 1-Methylcyclopropene (1-MCP) on fruits and vegetables. C. Watkins. 2006. Biotech. Adv. 24: 389-409.

The Dual Role of Ethylene in Postharvest Handling

- **Useful:**
  - **Accelerates ripening**
  - **Chlorophyll degradation**
  - **Causes abscission**
- **Problematic:**
  - **Accelerates ripening**
  - **Accelerates senescence and chlorophyll degradation**
  - **Causes abscission**
  - **Other undesirable changes**

Ethylene Production Rates by Fresh Produce at 20°C (68°F)

Range (µL/kg-h)	Product
0.01-0.1	Citrus, grape, cherry strawberry **MOST VEGETABLES**
0.1-1.0	Pineapple, blueberry, cucumber
1.0-10.0	Banana, mango, tomato, honeydew melon, fig
10-100	Apple, avocado, cantaloupe, nectarine, papaya, pear
>100	Cherimoya, passion fruit, sapotes

Ethylene production and sensitivity information for specific products:
Produce Facts: http://postharvest.ucdavis.edu/PF/
USDA Handbook 66: http://www.ba.ars.usda.gov/hb66/

Abscission of snapdragon flowers in response to ethylene shows a typical threshold and plateau response; cultivars to differ in threshold response

Similar threshold for ethylene effects on lettuce and carrots

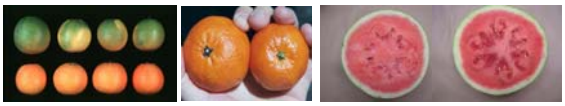
From M. Reid, UC Davis

Postharvest Compatibility Issues

- **Temperature**
- **Relative Humidity**
- **Ethylene**
- **Odor**

Ethylene can be detrimental for Fruits

Fruit	Symptoms
Kiwifruit	Softening; as little as 50 ppm induces softening
Avocado, Fuyu Persimmon	Exposure to 1ppm at 5°C increases chilling injury symptoms
Citrus	Use of ethylene for de-greening may increase senescence of peel and button
Stone fruits	Increase in decay associated with accelerated softening
Watermelon	Tissue softening and breakdown

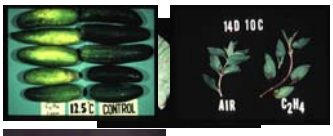


Detrimental Ethylene Effects on Vegetables

The lower the temperature, less ethylene is produced by fruits
At lower temperatures, vegetables are less affected (exceptions: lettuce, carrots)

Problematic:

- **Accelerates ripening**
- **Accelerates senescence**
- **Causes abscission**
- **Bitterness in carrots**



Manage Ethylene Effects

1. **Temperature and Time**
2. **Avoidance**
3. **Removal**
4. **Inhibition of production**
5. **Inhibition of action**
6. **Germplasm modification**

1. Temperature & Time

- Low temperature slows reaction rates by slowing enzyme activity
- Ethylene perception is slowed and ethylene responses are slowed by low temperature
- Time is required for product to perceive ethylene and cause response
  - How long an exposure before detrimental effect?
  - Ethylene concentration, temperature, time

2. Avoidance

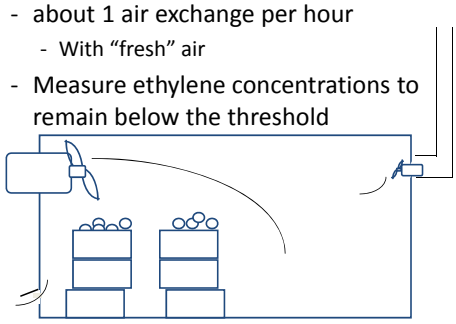
Keep ethylene sources away from sensitive products

- Electric forklifts; floor polishers
- Ethylene producing products
- Smoking
- Decaying produce

3. Removal

Ventilation

- about 1 air exchange per hour
  - With "fresh" air
- Measure ethylene concentrations to remain below the threshold



3. Removal

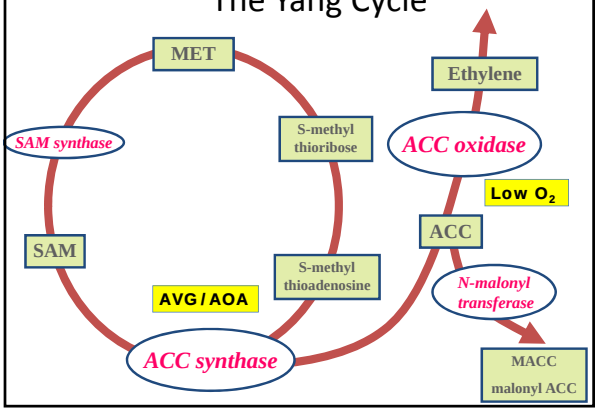
- Absorption (act. carbon, filters)
- Oxidation – $KMnO_4$, U.V., Ozone

http://postharvest.ucdavis.edu/yellowpages
Ethylene scrubbers and action inhibitors

4. Inhibition of Production

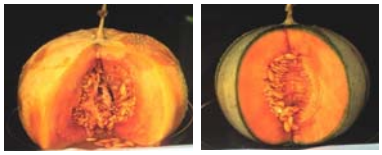
Low temperature
Low O2
Chemical inhibitors of enzymes
-AVG, AOA, others
Antisense technology
-gene/DNA inserted reverse order
-nonfunctional
Natural mutants

The Yang Cycle



Molecular manipulation of ripening

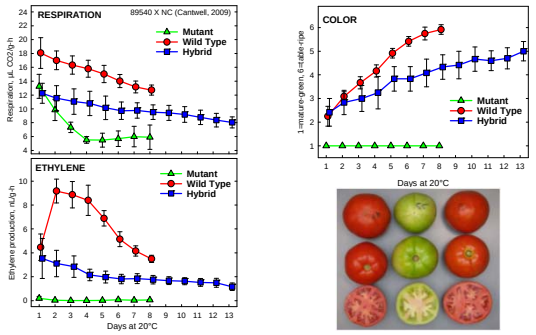
- Anti-sense ACC synthase; Anti-sense ACC oxidase
- Result - fruits that ripen very slowly, require ethylene treatment to ripen or to produce aroma volatiles



Non-modified wild type Anti-sense ACC Oxidase

Charentais melons harvested 38 days post-pollination
Stored at 25°C for 10 days (J.C. Pech and colleagues)

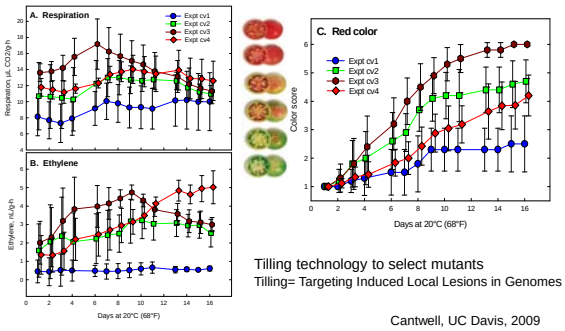
Ripening mutants retard the ripening process
Example: DFD (Delayed Fruit Deterioration) naturally occurring mutant



WT 8d Mutant Hybrid 18d

Cantwell, UC Davis, 2009

Tomato cultivars and their ripening physiology and ability to ripen
Hybrids based on mutants can have greatly retarded ripening off the plant



Tilling technology to select mutants
Tilling= Targeting Induced Local Lesions in Genomes

Cantwell, UC Davis, 2009

5. Inhibition of action

Low temperature
high CO_2
low O_2
STS- silver thiosulfate (cut nonedible flowers)
Cyclopropenes
1-MCP (Smartfresh™; Ethylbloc™)

Low O_2 Delays Ripening of 'Santa Rosa' Plums

Air 1% O_2 + 5% CO_2

5 weeks at 10ºC

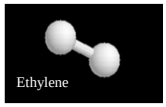
6 Months Storage of Bartlett Pears

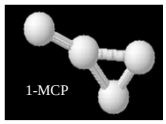
Low O_2 Retards Ripening of Partially Ripe Tomato Fruit

CA slows fruit ripening

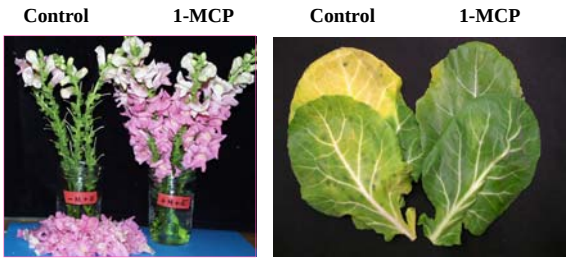
Inhibition of ethylene action
1-MCP (SmartFresh™) Treatment

- Gas liberated from a substrate in closed space much like an ethylene treatment
- Irreversibly binds to ethylene receptor
- Concentration sufficient to saturate receptors (~1000 ppb = 1 ppm)
- Treatment time sufficient for gas to penetrate tissues (hours)
- Treatment temperature (0-20°C)
- Single or multiple applications
- Product and stage of maturity

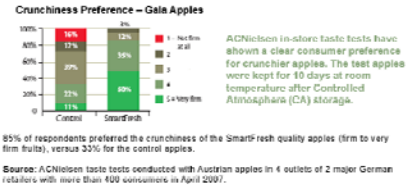




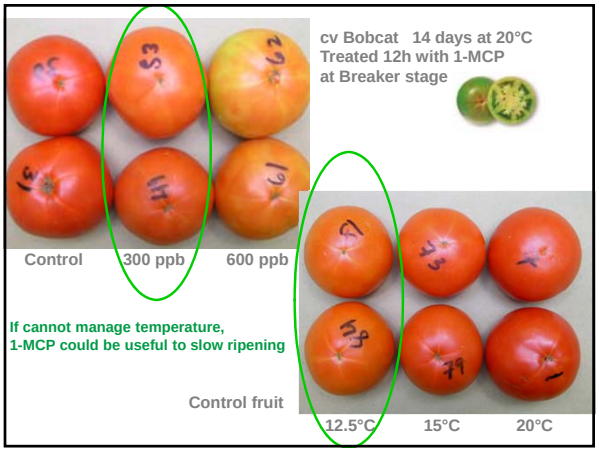
1-MCP - a gaseous ethylene inhibitor



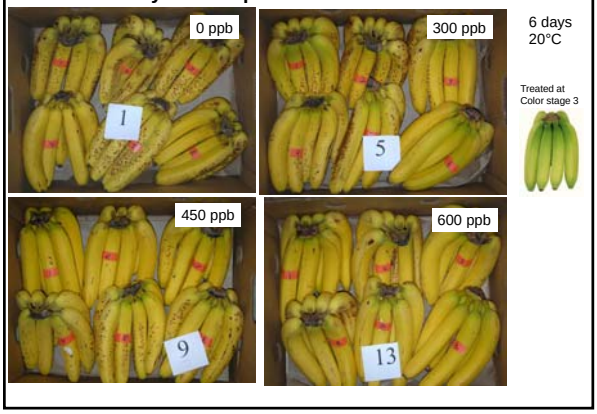
Major commercial use to date-keep apples crispy; 50% or more of US apples treated with 1-MCP



http://www.agrofresh.com/



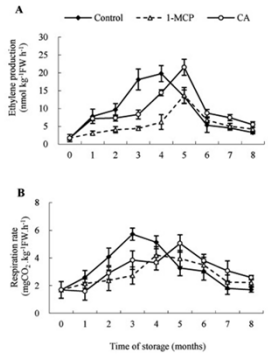
1-MCP to delay brown spots on bananas at retail markets



1-MCP & Melons

- Western shipping cantaloupes-not much benefit on firmness at storage temperature.
- Eastern shipping cantaloupes-reduce softening at at warm temperatures.
- Galia; extend shelf-life, reduce firmness loss
- Watermelon-clear benefit; reduce firmness loss and internal breakdown

1-MCP, CA and pear ripening



From M.S. Reid, UC Davis

Fruits and SmartFresh™ (1-MCP)

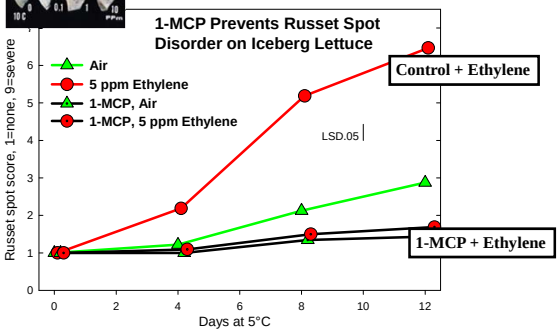
- SmartFresh™ is a powerful regulator of climacteric fruit ripening
- Its use is complicated for fruit because results depend on:
  - Fruit maturity/ripeness at application
  - 1-MCP concentration and exposure time
  - Temperature during and following treatment
- Important to determine where SmartFresh™ can add value to the fruit category

Review Paper: The use of 1-MCP on fruits and vegetables.
C.B. Watkins. 2006. Postharvest Biology Technology 24: 389-409.

EthylBloc sachets (1-MCP)
in shipping boxes

Photos: George Staby

For many vegetables, 1-MCP effective for ethylene control but not cost-effective



Test#2, midribs; 1000 ppb 1-MCP; Tarraza and Cantwell, 2002, UC Davis

6. Germplasm selection & engineering

Natural variation in susceptibility to ethylene

Creation of germplasm insensitive to ethylene

Ripening of Plum Varieties

Ethylene Production Rate	Ripening Rate	Selected Varieties
Very low	Very slow	Black Beaut, Casselman, Kelsey, Late Santa Rosa, Nubiana, Queen Ann, Roysum
Low	Slow	Durado, El Dorado, President, Red Beaut, Santa Rosa, Simka
Moderate	Moderate	Ambra, Friar, Grand Rosa, July Santa Rosa, Laroda, Queen Rosa, Wickson
High	Fast	Frontier

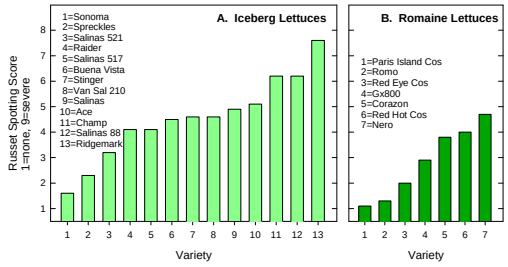
From Kader, UC Davis

Avoiding ethylene effects

Selection of resistant varieties

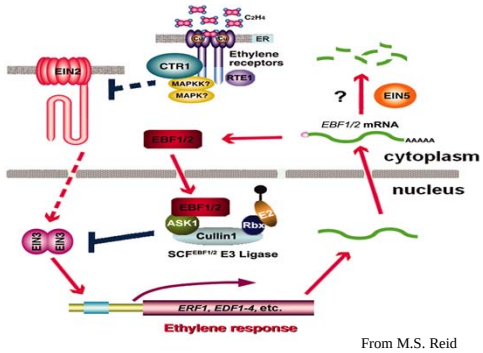
From M.S. Reid

Performance of Lettuce Cultivars in Ethylene-induced Russet Spotting

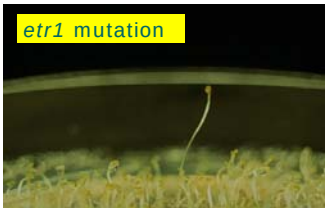


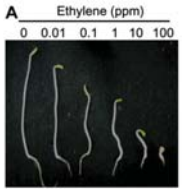
Development of Russet Spot Disorder on Iceberg and Romaine Lettuces. Intact heads were stored 3 days in 5ppm ethylene at 5°C (41°F) plus 1 week in air.

The ethylene response cascade

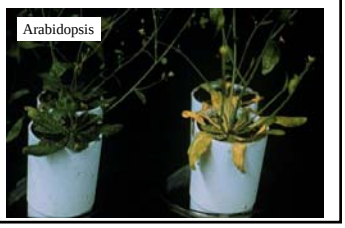


From M.S. Reid





Anthony Bleecker1988



Use of the ethylene receptor gene

- Insert mutated gene in sensitive plant - becomes insensitive
- Use tissue-specific, or stage-specific 'promoter' - ethylene action is inhibited only in that tissue or at that time (e.g. mature flowers, ripe fruits)
- Already done with petunias, carnations, other flowers

Transcriptome Changes Associated with Delayed Flower Senescence on Transgenic Petunia by Inducing Expression of etr1-1, a Mutant Ethylene Receptor. H. Wang, G. Stier, J. Lin, G. Liu, Z. Zhang, Y. Chang, M.S. Reid & C-Z. Jiang. 2013. Plos One 8(7): e65800.

New technology – use inducible promoters

- Ethylene has many functions in plants
- Need to control ethylene in a specific tissue
- Application of a simple chemical or physical stress
  - Alcohol, dexamethasone, copper, heat
- Drives synthesis of a gene
  - For example, DEX/*etr1*

Ethylene Summary

- Ethylene has both positive and negative effects in postharvest handling of fruits and vegetables
- Know which fresh products are sensitive to ethylene
- Do not mix high ethylene producing crops with ethylene sensitive crops
- Ventilation and low temperatures help control most detrimental ethylene effects
- SmartFresh™ (1-MCP) can retard ripening and can be useful for mixed loads
- Molecular manipulation for specific control