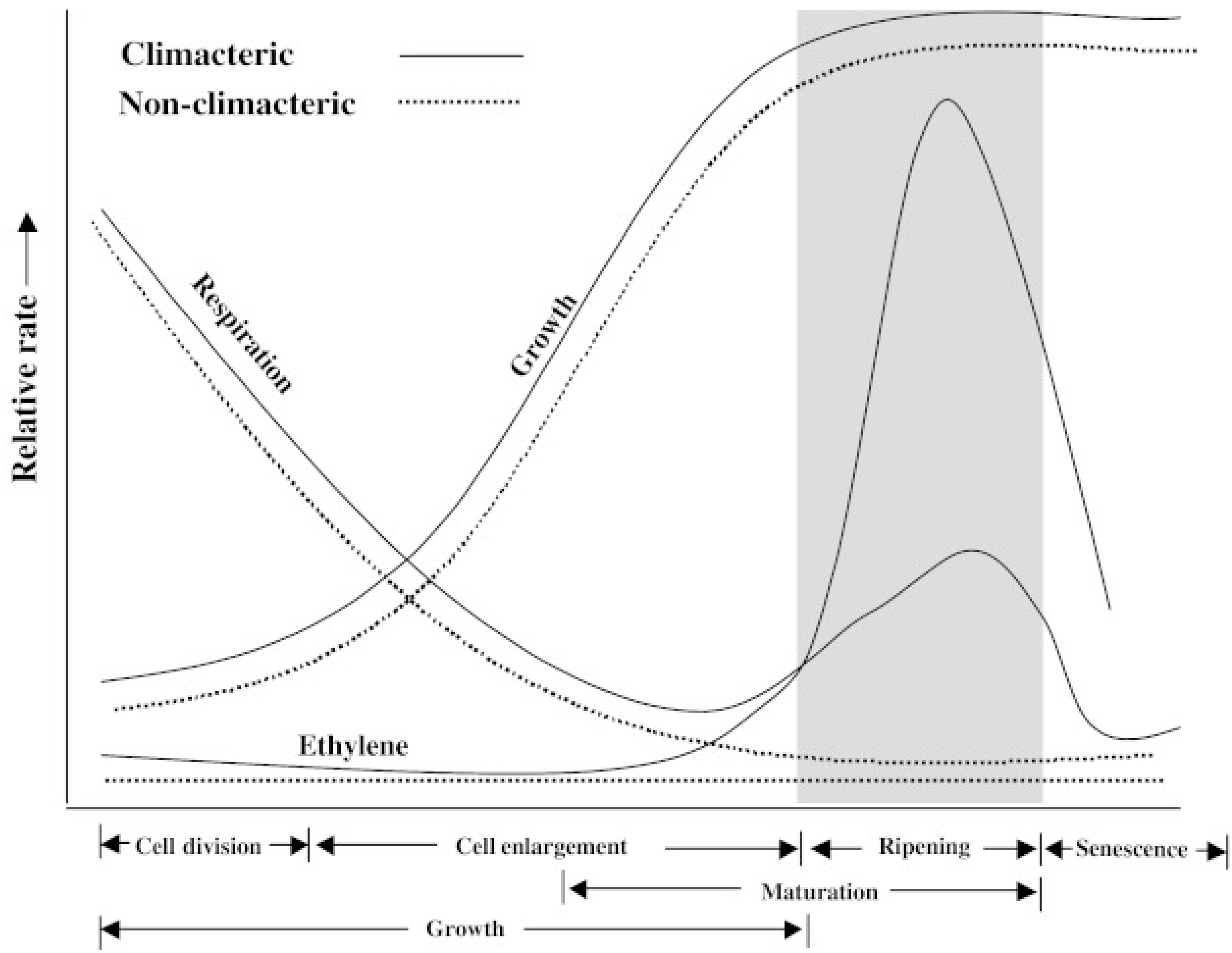
5th Lecture: Physiology
Respiration and ethylene production

Climacteric versus non-climacteric fruit

- Climacteric stage is defined as a rapid increase in respiration and ethylene production in parallel to ripening process of the fruit

- Non-climacteric fruits are considered those fruits that do not show the dramatic increase in respiration and ethylene production after harvest

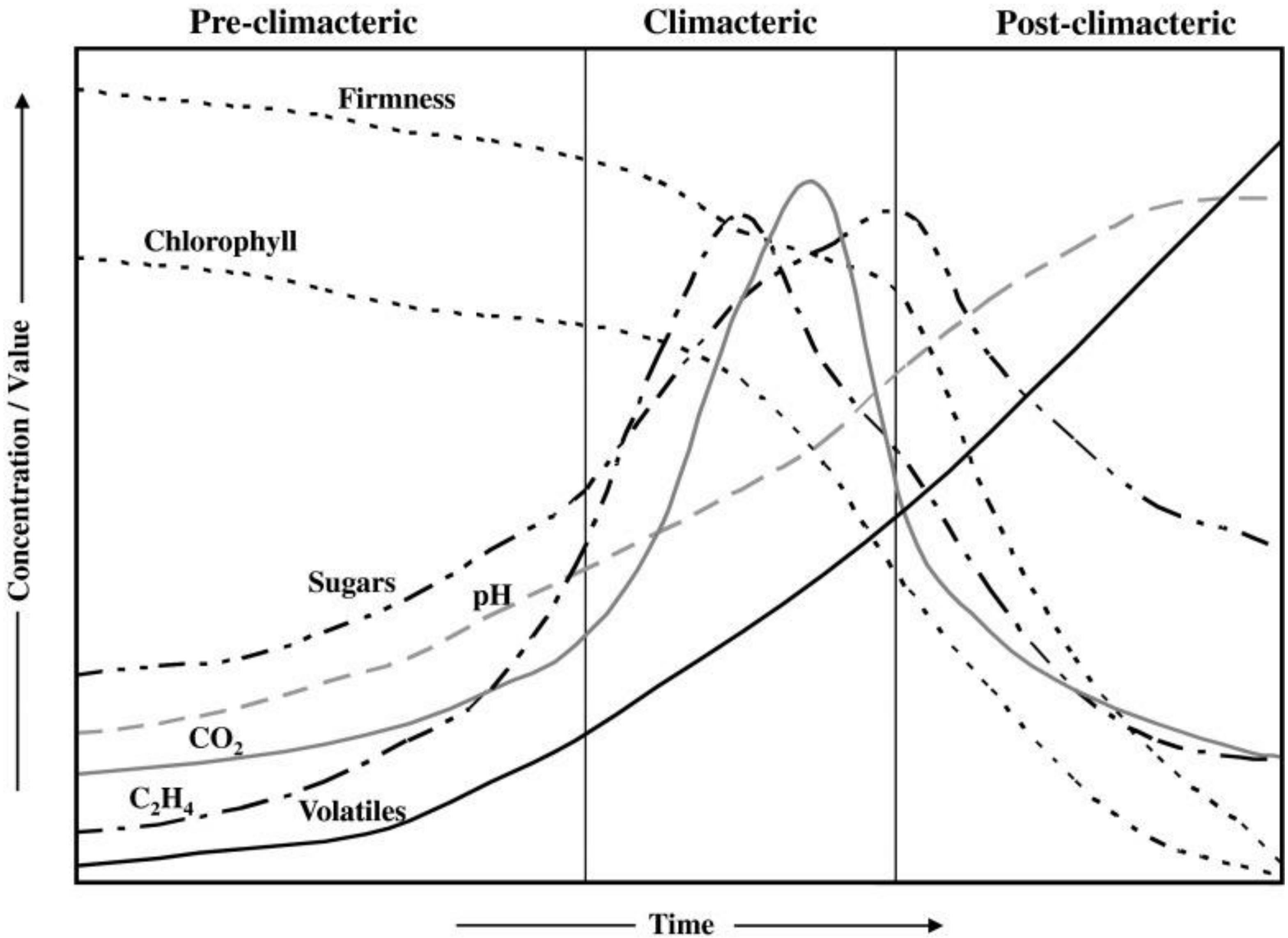


Climacteric
Non-climacteric
Relative rate
Respiration
Growth
Ethylene
Cell division
Cell enlargement
Ripening
Senescence
Maturation
Growth

The ethylene burst occurring during the climacteric fruit

The ethylene burst closely follows the rise in climacteric respiration and in turn is followed by over ripening phenotypes:

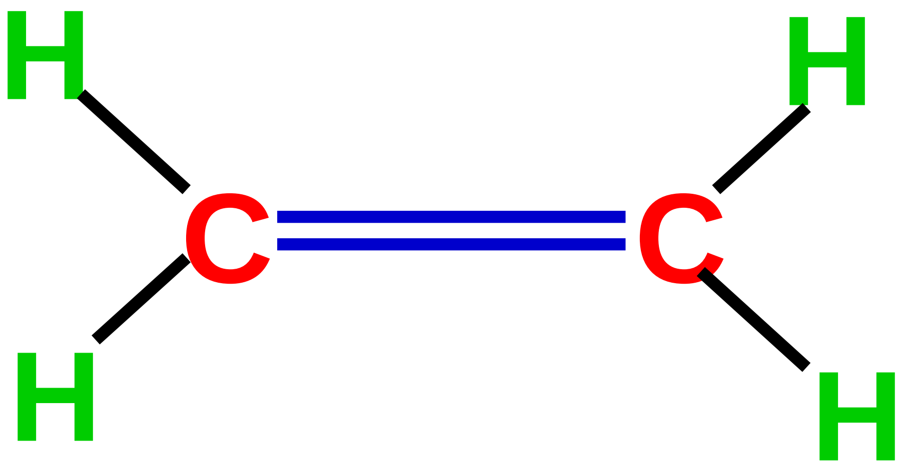
1. Chlorophyll loss (resulting in degreening)
2. Carotenoid accumulation (resulting in the characteristic red coloration of tomato)
3. Softening
4. Altered acidity
5. Conversion of starch to sugars, and
6. Evolution of aroma volatiles



Postharvest changes in physicochemical properties and volatile constituents of apricot (Prunus armeniaca L.). Characterization of 28 Cultivars

C_2H_4 – Ethylene

- **A simple gaseous organic molecule. The only plant hormone in a gas-shaped**
- **Plant hormone involved in regulating growth, maturation, aging and fruit detachment.**
- **Naturally synthesize in the plant, but produced by combustion engines (mainly diesel).**
- **Biologically acting at very low concentrations at the level of part per millions and even part per billion**



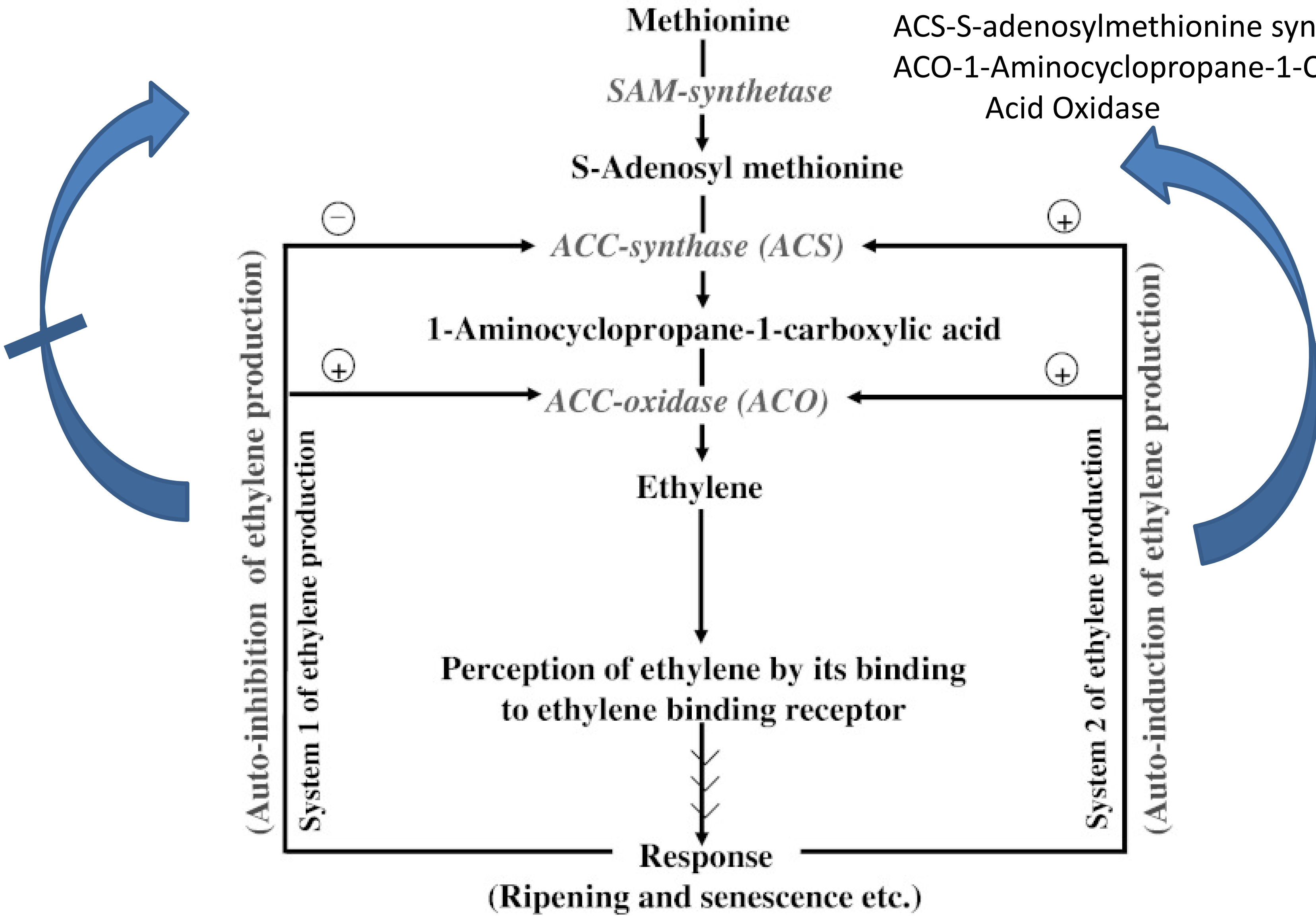
Ethylene Biosynthesis

1. **Ethylene can be produced by almost all plant parts**
2. **Aging tissues and ripening fruits produce more ethylene than young tissues**
3. **The amino acid methionine is the basis for the synthesis of ethylene.**

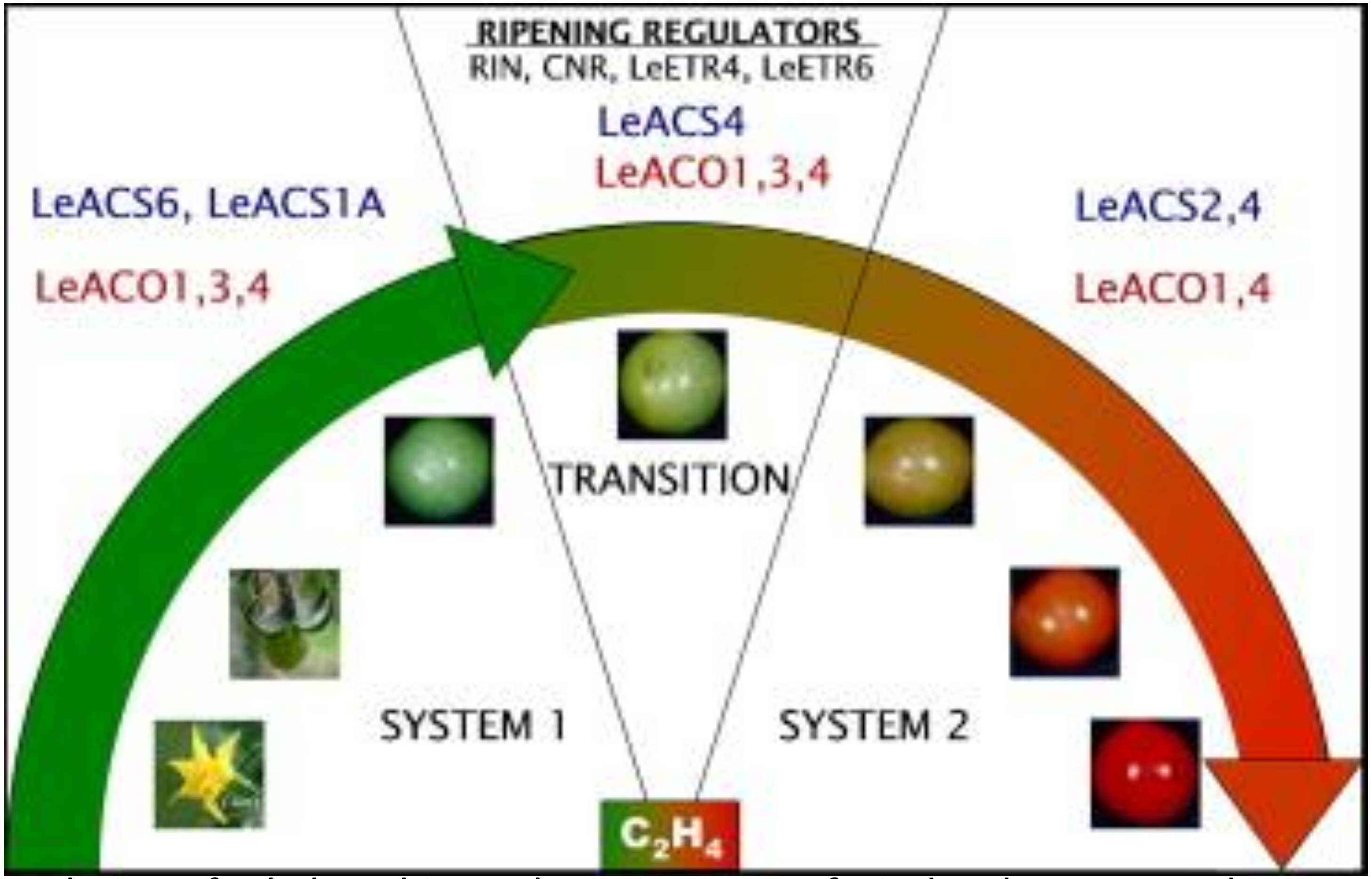
Classification of produce according to the production of ethylene (µl C_2H_4/Kg-h)

Produce	Quantity of ethylene (ppm)	Level
Artichoke, asparagus, cauliflower, citrus, grapes, pomegranate, leaf spices, root vegetables, flowers	Less than 0.1	Very low
Cucumber, blueberries, eggplant, okra, pepper, pumpkin, pineapple, persimmon, watermelon	1.0 - 0.1	Low
Banana, Fig, Guava, Tal Honey Melon, Mango, Tomato, Lychee	10.0-1.0	Moderate
Apple, apricot, avocado, Gallia melon, kiwi, papaya, nectarine, peach, pear, plum	100.0-10.0	High
Annona, passiflora, Sapota	More than 100.0	Very high

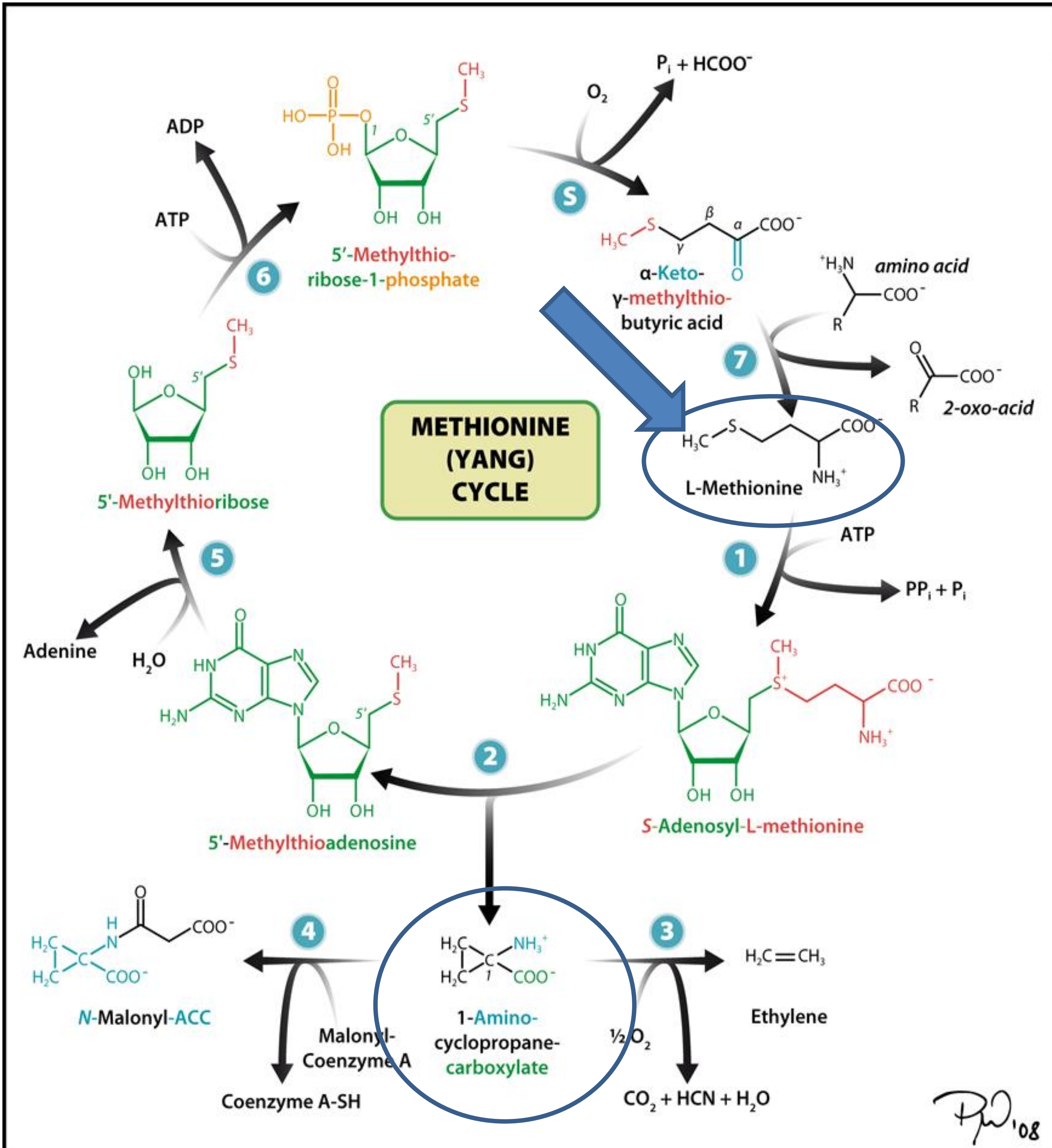
ACS-S-adenosylmethionine synthase
ACO-1-Aminocyclopropane-1-Carboxylic Acid Oxidase



Simplified pathway of ethylene biosynthesis in plants **showing auto-inhibition** (inhibiting its own production) and auto-induction of ethylene (**inducing its own production**). These systems are referred as system 1 and system 2 of ethylene production respectively. In system 1, ethylene inhibits its own production by inhibiting (⊖) ACS expression/activity. It may be noted that the ACO activity is enhanced during system 1 but due to the absence of any enhancement in the activity of ACS there is no auto-induction. In system 2, ethylene induces more of its own production by stimulating (⊕) the expression/activity of both of the enzymes (ACS and ACO) simultaneously



Regulation of ethylene biosynthesis in tomato fruit development and ripening. During development (System 1) lower and auto-inhibitory ethylene is synthesized byLeACS1A,6 and LeACO1,3,4. At the transition stage, the ripening regulators indicated play critical roles. LeACS4 is induced and a large increase of auto-catalytic ethylenestarts, resulting in negative feedback on System 1. LeACS2,4 and LeACO1,4 are thenresponsible for the high ethylene production through System 2.



Ethylene Biosynthesis in Plants

Enzymes

1. SAM synthetase
2. ACC synthase
3. ACC oxidase
4. ACC *N*-malonyl-transferase
5. MTA nucleosidase
6. MTR kinase
7. Transaminase

S. Spontaneous reaction

Abbreviations

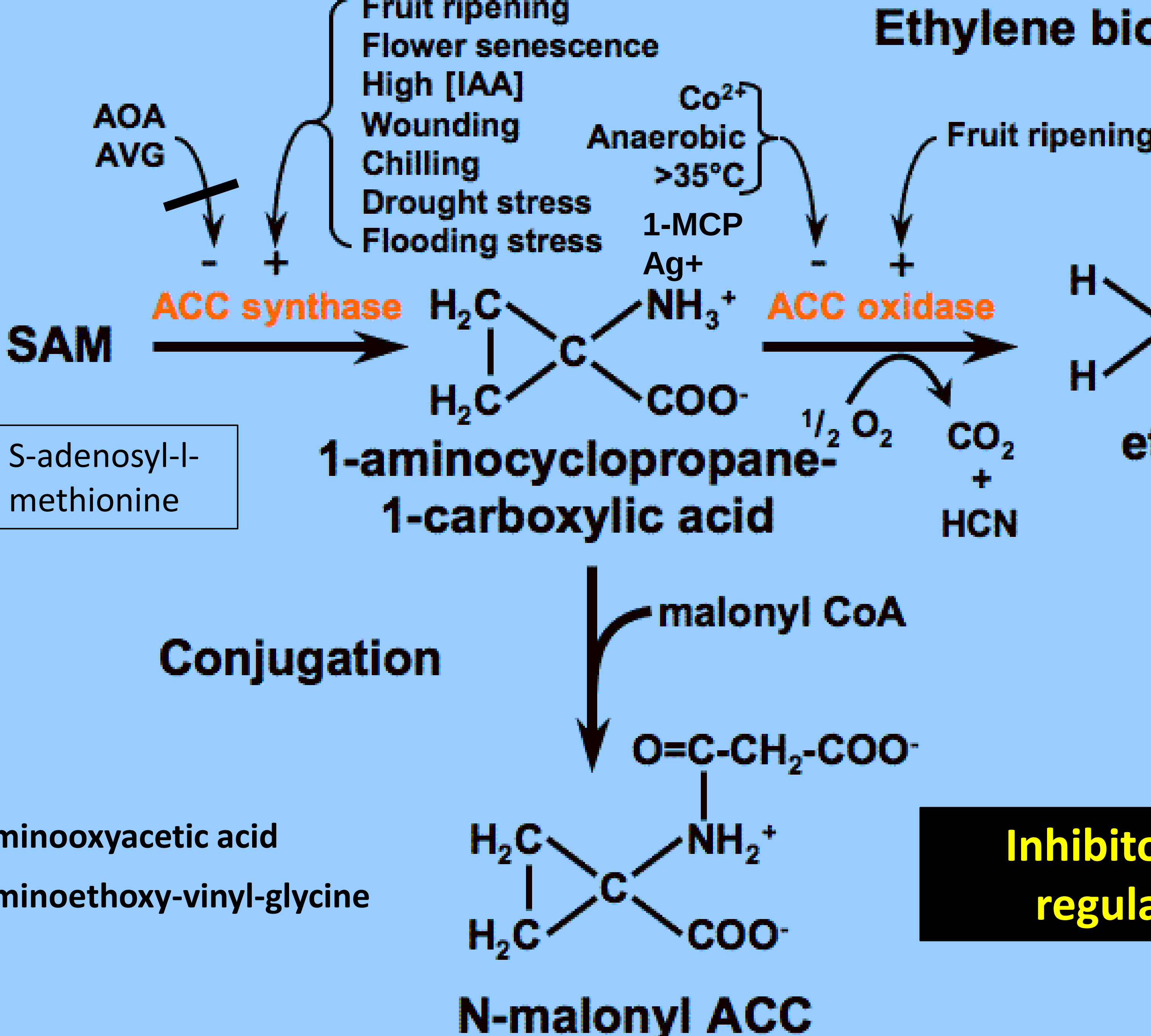
ATP	Adeninnucleotidtriphosphate
ADP	Adeninnucleotiddiphosphate
ACC	1-Aminocyclopropane-carboxylate
HCN	Hydrocyanide acid
MTA	5'-Methylthioadenosin
MTR	5'-Methylthioribose
PP_i	Diphosphate (Pyrophosphate)
P_i	Phosphate
SAM	S-Adenosyl-L-methionine

Sources

Buchanan BB, Gruissem W, Jones RL (2000). Biochemistry and Molecular Biology of Plants. *Am Soc Plant Phys* (Rockville).

Wang K C-L, Li H, Ecker JR (2002). Ethylene Biosynthesis and Signalling Networks. *Plant Cell* (Supplement) S131-S151.

Ethylene biosynthesis



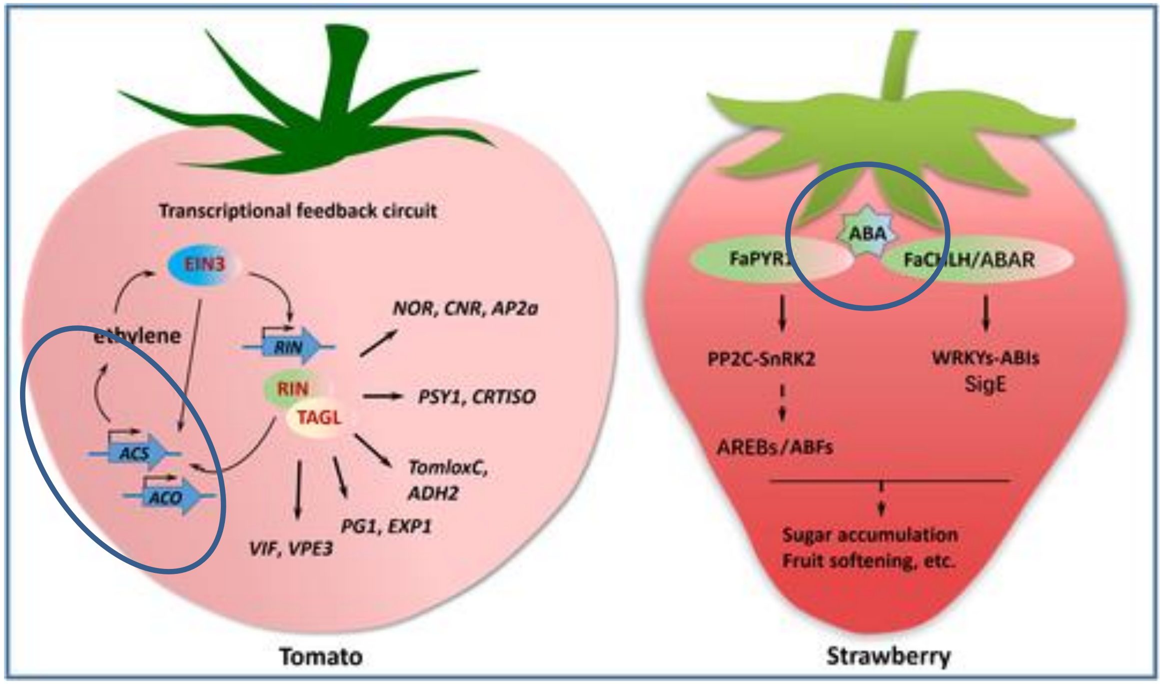
S-adenosyl-l-methionine

aminooxyacetic acid

aminoethoxy-vinyl-glycine

Inhibitors and regulators

Summary and new updates

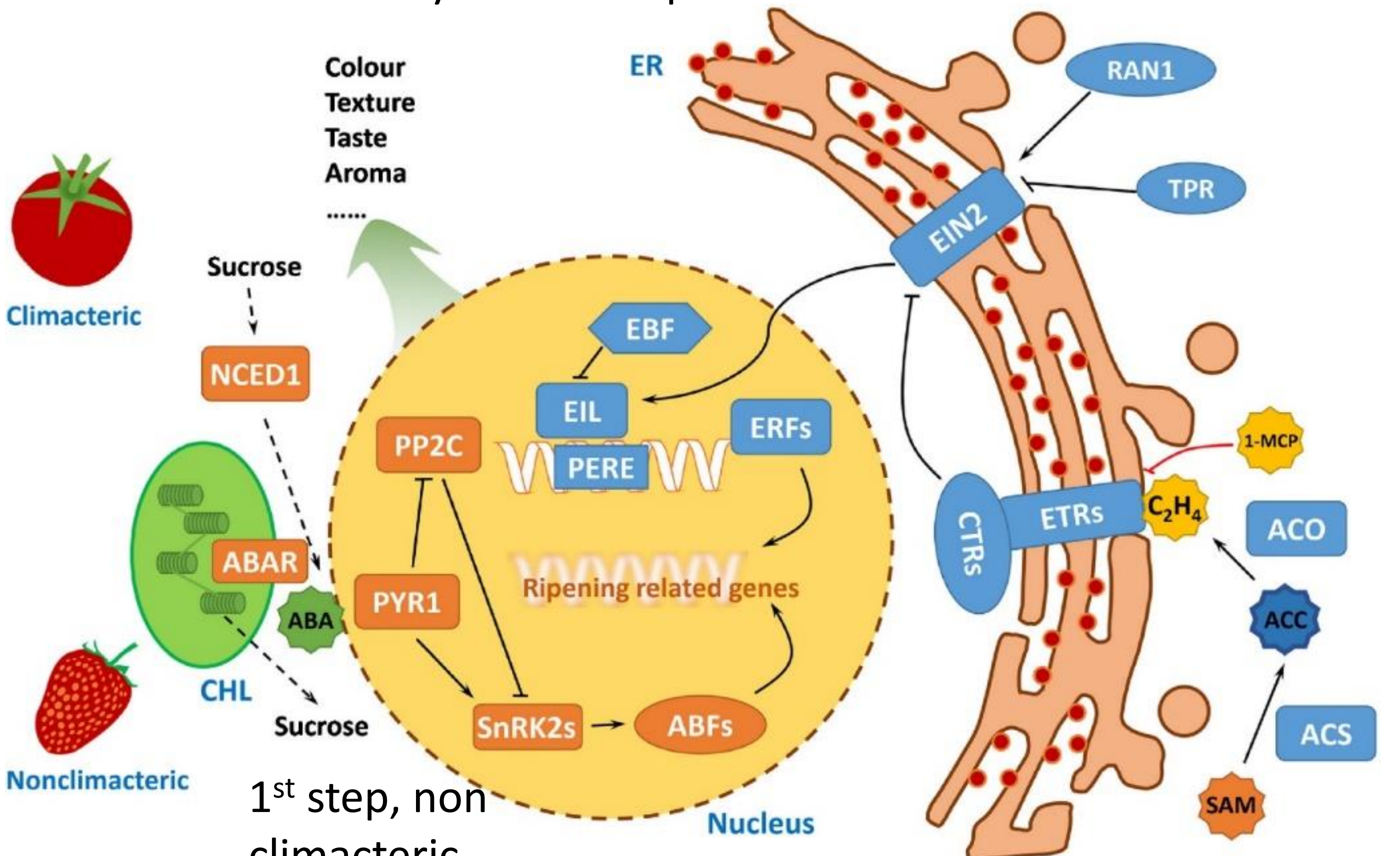


Schematic diagram of the transcriptional network involved in fruit ripening

For tomato fruit, RIN and TAGL1 function as a complex, activating ethylene biosynthesis genes, thus forming a positive feedback circuit to generate autocatalytic ethylene (Lü *et al*., 2018). This MADS-type circuit further activates downstream ripening-related genes.
In immature fruit, the transcriptional feedback loop in tomato is repressed with key genes associated with DNA hypermethylation and H3K27me3 modification.

For strawberry fruit, the Abscisic acid (ABA) signal is mainly perceived by two putative ABA receptors, namely FaPYR1 and FaCHLH/ABAR, which further employ FaPYR1-PP2C-SnRK2 and FaABAR-WRKYs-ABIs or sigma factor E (SigE) modules to modulate sugar accumulation, fruit softening and other ripening-related events.

Summary and new updates

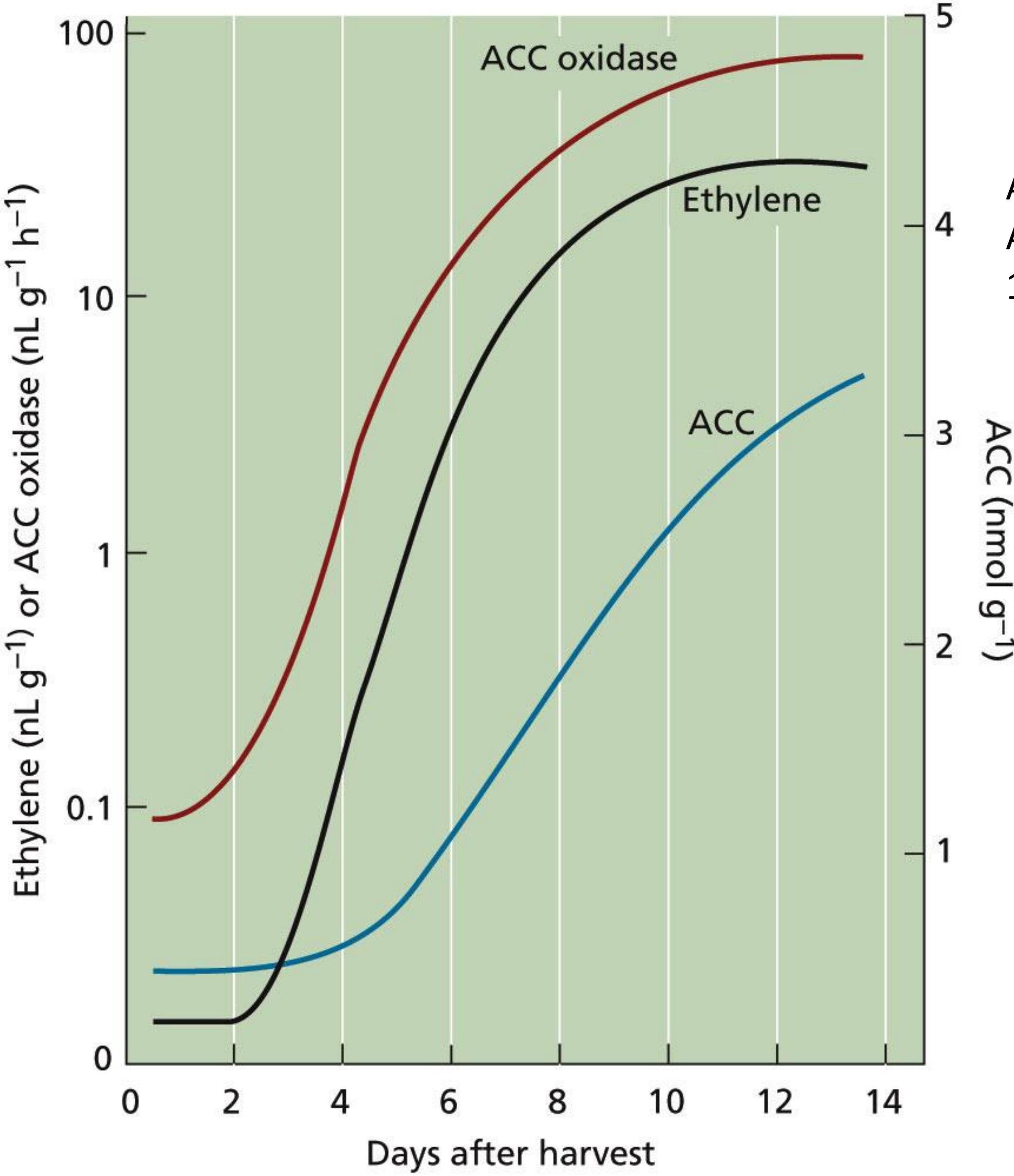


1st step, non climacteric

1st step, climacteric

Key players involved in ethylene and Abscisic acid (ABA) signaling in regulating fruit ripening. **Autocatalytic ethylene synthesis is catalysed by *S*-adenosyl-L-methionine synthetase (SAMS), ACC synthase (ACS) and ACC oxidase (ACO). In the absence of ethylene, the signal from ethylene receptors (ETRs) to CONSTITUTIVE TRIPLE RESPONSE (CTR) is blocked; ethylene binding induces the inactivation of ETRs and switches off the inhibition of CTR1 on ETHYLENE-INSENSITIVE 2 (EIN2).** The activated EIN2 stabilises EIN3/Ethylene Insensitive3-Like (EIL) transcription factors (TF), which can activate the expression of target genes by binding to primary ethylene response elements (PEREs), including the genes encoding ethylene response factor (ERF) TFs. ERFs modulate the ethylene-regulated genes by binding to their promoters. TPR and RAN1 are also required for ethylene binding and ETR degradation respectively. **In terms of nonclimacteric fruit ripening, sucrose serves as a signal for accelerating fruit ripening by promoting ABA biosynthesis** via FaNCED in strawberry, whereas the PYR1–PP2C–SnRK2 module directly regulates the expression of ABA-responsive genes via the phosphorylation of ABF TFs.

ACC concentrations, ACC oxidase activity, and ethylene during ripening of apples

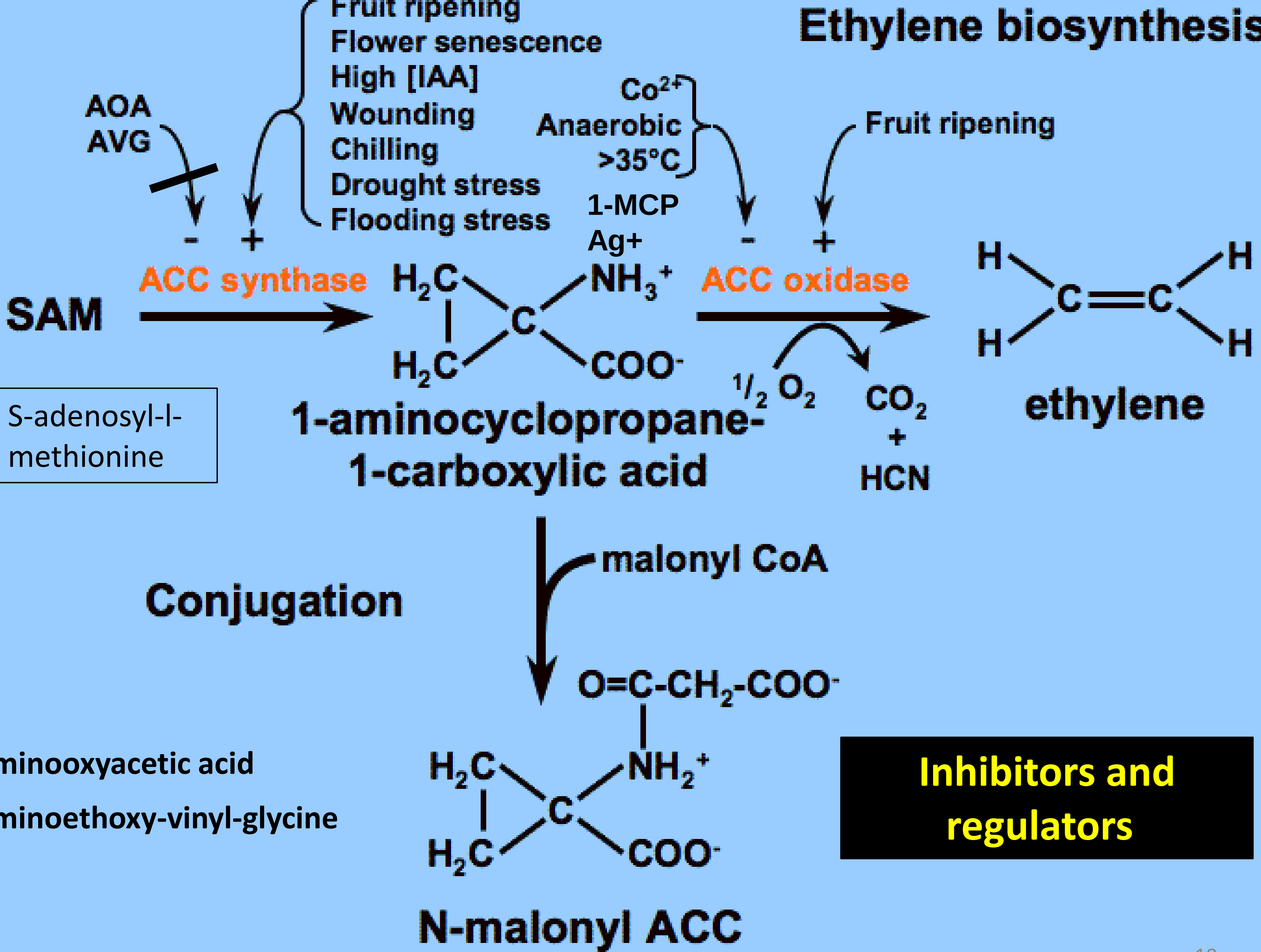


ACO-1-Aminocyclopropane-1-Carboxylic Acid Oxidase

ACS-S-adenosyl methionine synthase

PLANT PHYSIOLOGY, 5e, Figure 22.3

Ethylene biosynthesis



S-adenosyl-l-methionine

aminooxyacetic acid

aminoethoxy-vinyl-glycine

Inhibitors and regulators

Inhibitors of the ethylene biosynthetic pathway are available for further testing the activity of ACC activity:

Aminooxyacetic acid (AOA)
and
Aminoethoxyvinylglycine (AVG)

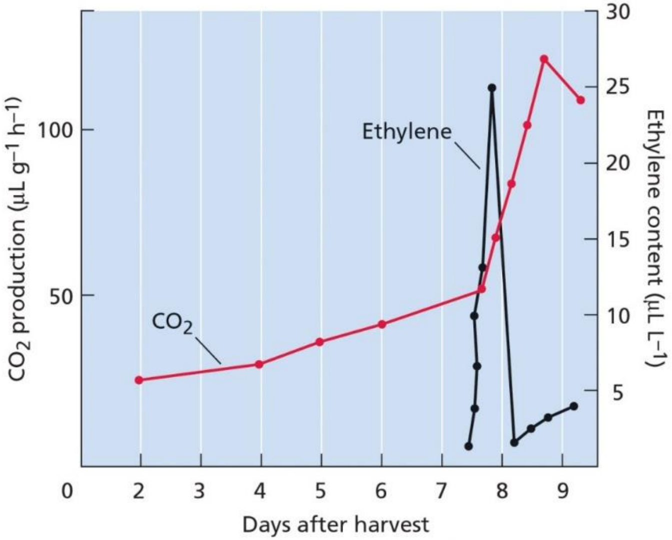
block the synthesis of ACC, whereas CO^{2+} prevents its conversion to ethylene.

AOA and AVG, were found to inhibit the synthesis and export of ACC from anaerobic roots, whereas CO^{2+} had no effect, as predicted from their respective sites of action.

Ethylene induce ripening in climacteric fruits

Climacteric fruit: Fruits ripened in response to ethylene show a characteristic increase in respiration before the ripening phase. Such fruits show a sharp increase in the formation of ethylene immediately before the increase in respiration.

Non-climacteric fruit: Fruits that do not show an increase in respiration and production of ethylene.

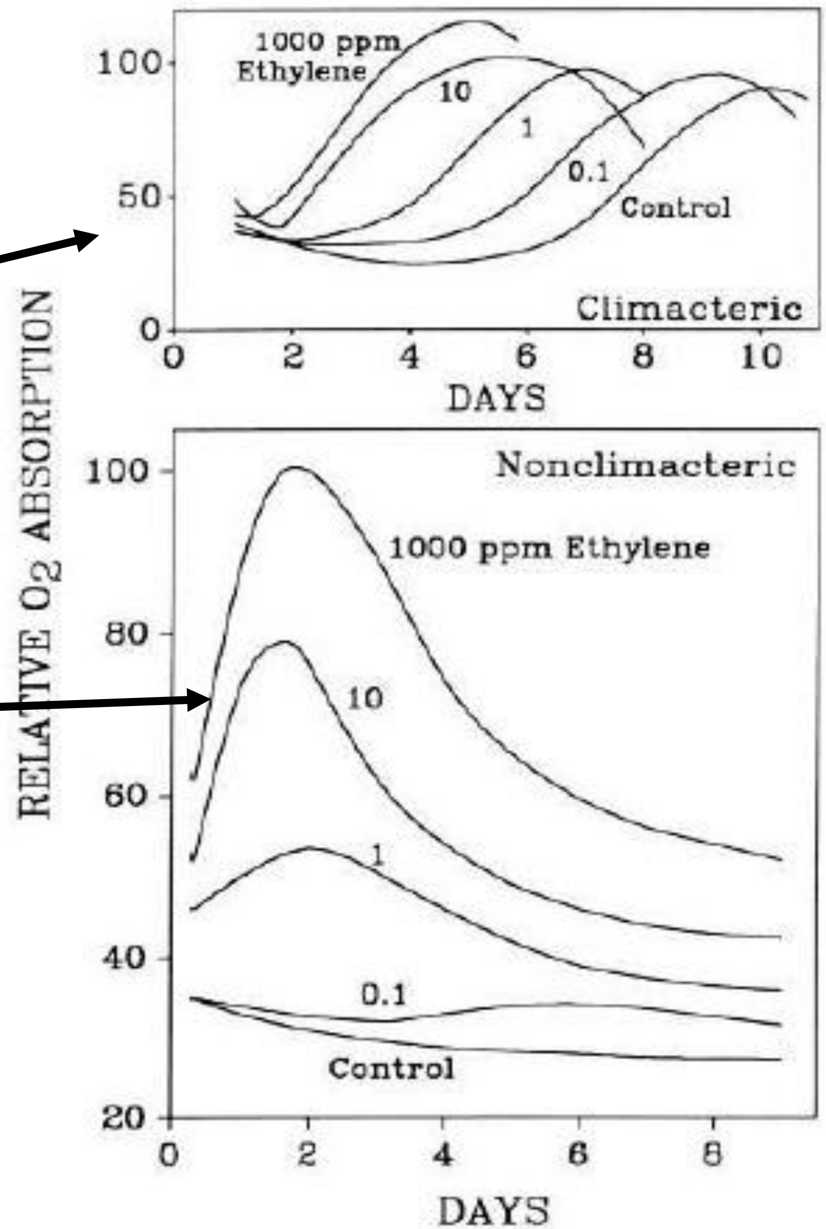


PLANT PHYSIOLOGY, Third Edition, Figure 22.4 © 2002 Sinauer Associates, Inc.

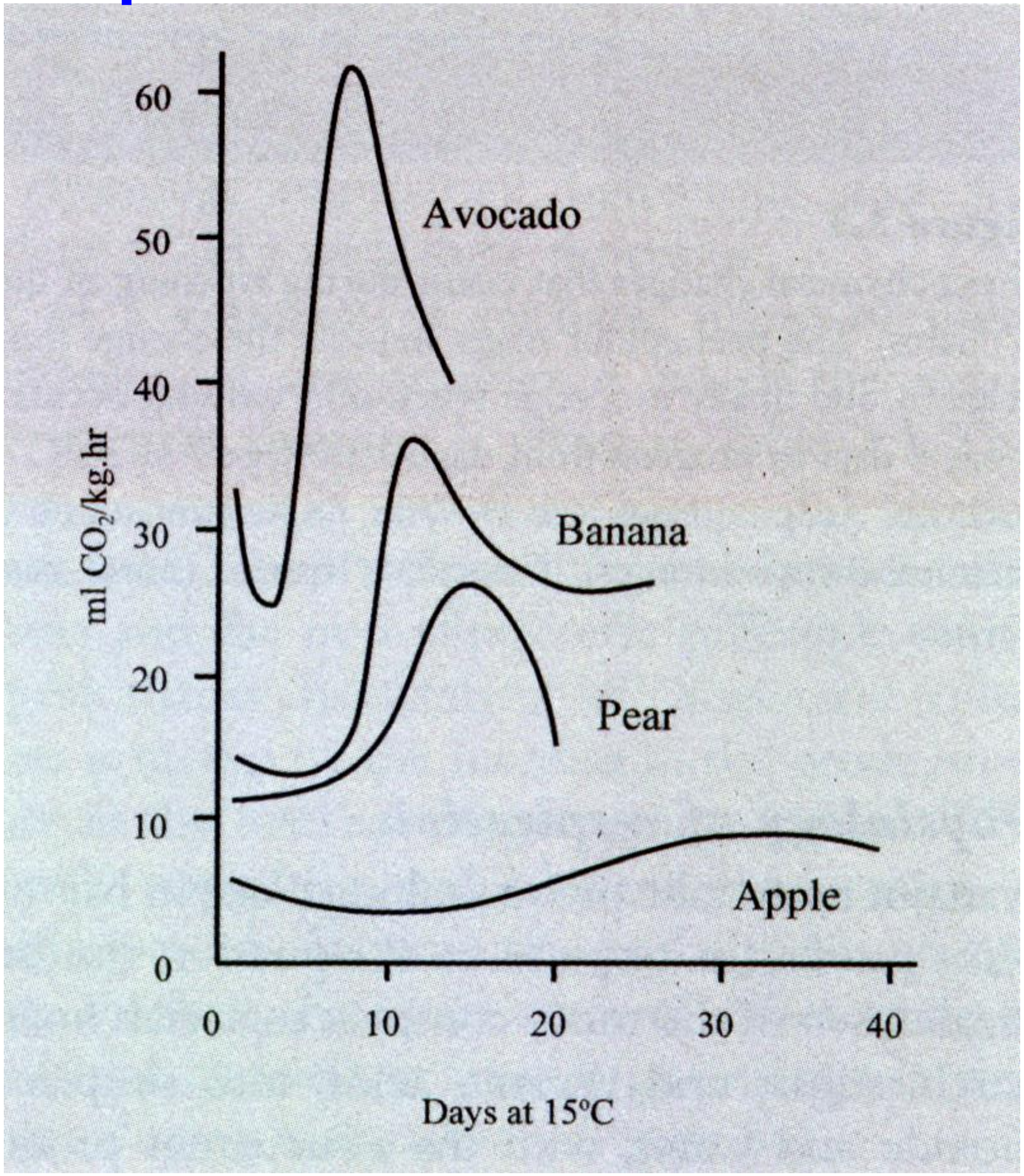
Autocatalytic effect – Ethylene induced the fruit to produce more ethylene

The differences between climacteric and non-climacteric fruit in the ethylene synthesis and their response to external ethylene

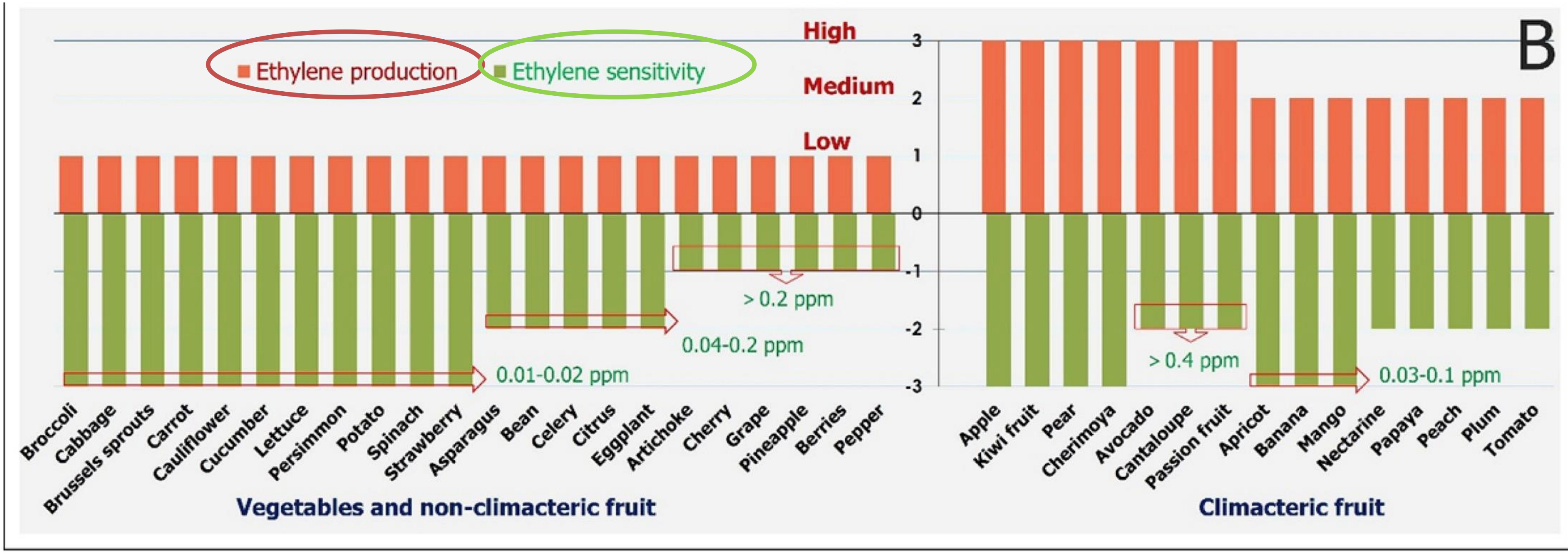
	Climacteric	Non climacteric
Response to external ethylene	Increases respiration just before the increase in respiration of the control	Increase respiration during the whole shelf life
Respiration response	Regardless of ethylene concentration	Depending on the ethylene concentration
Autocatalytic response	Present (ethylene presence increases ethylene production)	Not present
Internal conc. of ethylene	May change from very low to very high	Low



Respiration of climacteric fruits



Production of ethylene and sensitivity to ethylene in a number of fruits and vegetables

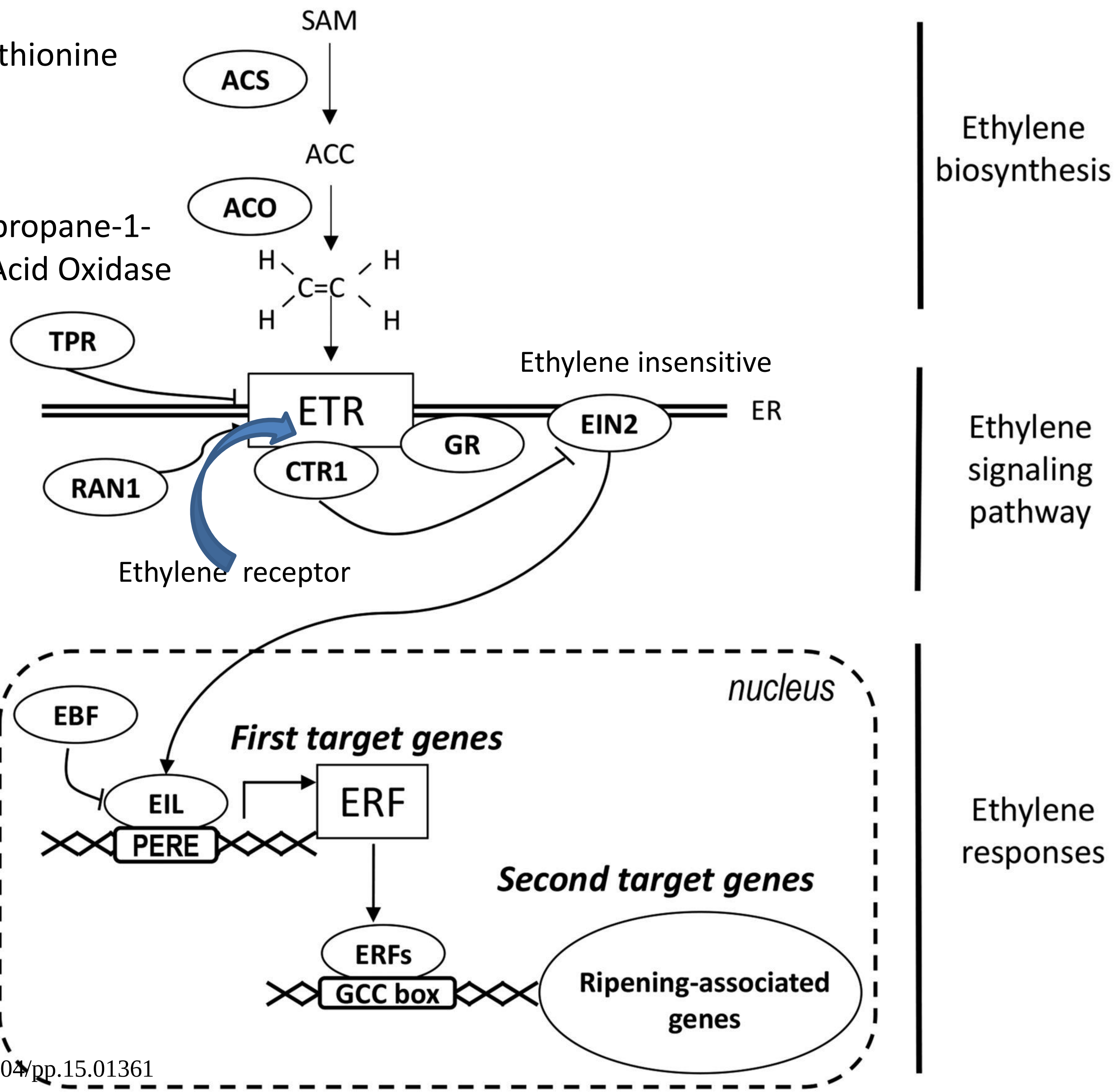


Bingxue et al. 2019

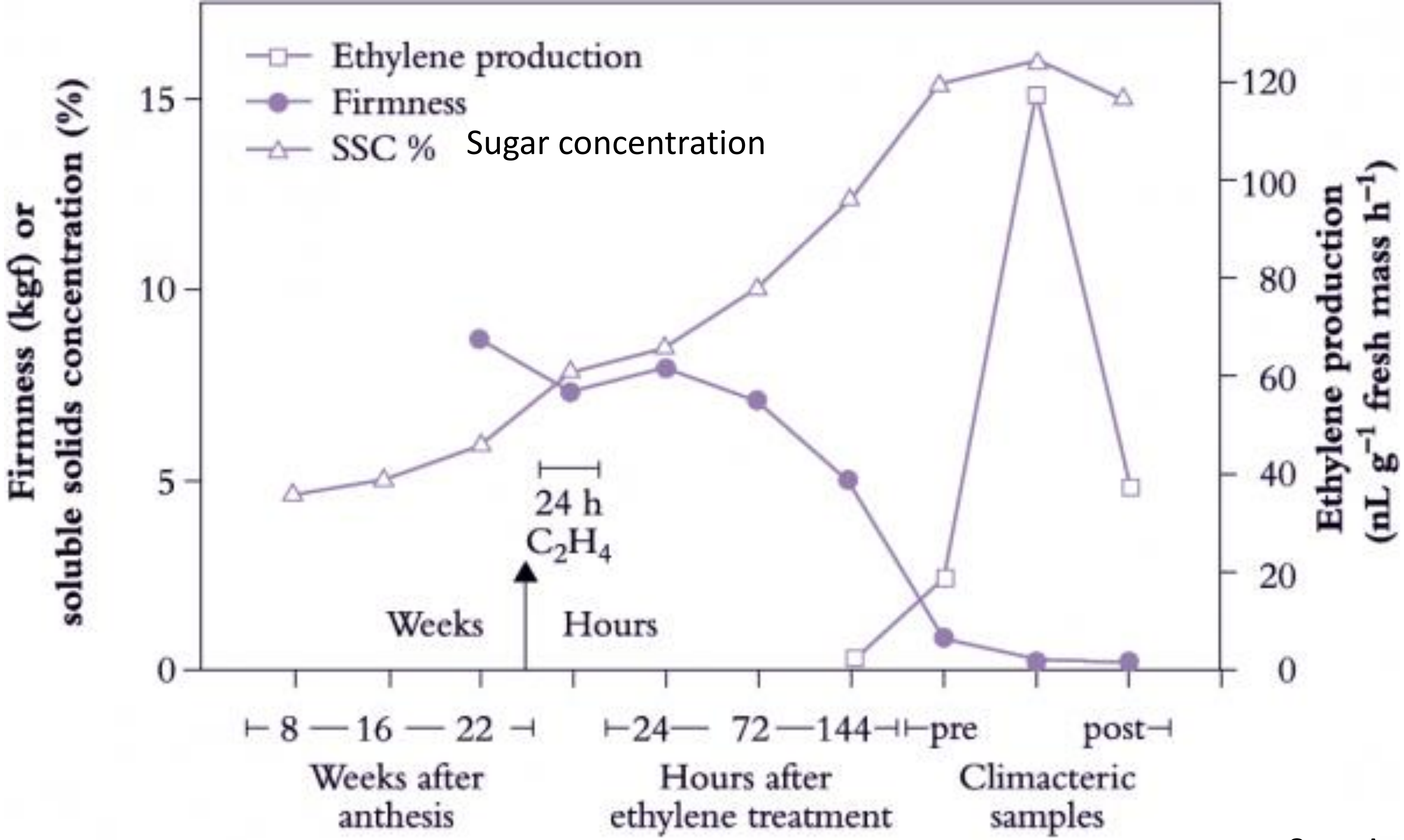
Model for the transfer of the ethylene signal

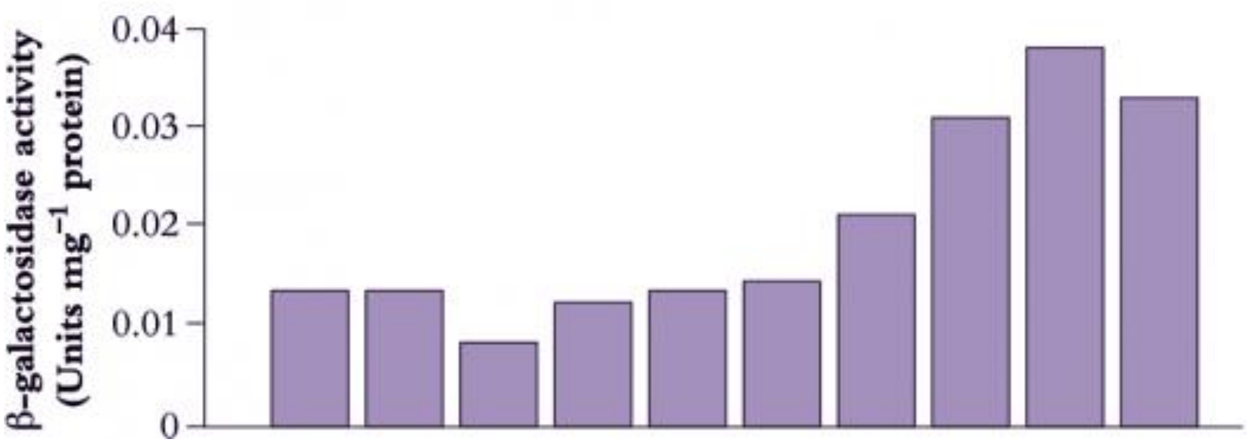
ACS-S-adenosylmethionine synthase

ACO-1-Aminocyclopropane-1-Carboxylic Acid Oxidase



DOI: https://doi.org/10.1104/pp.15.01361

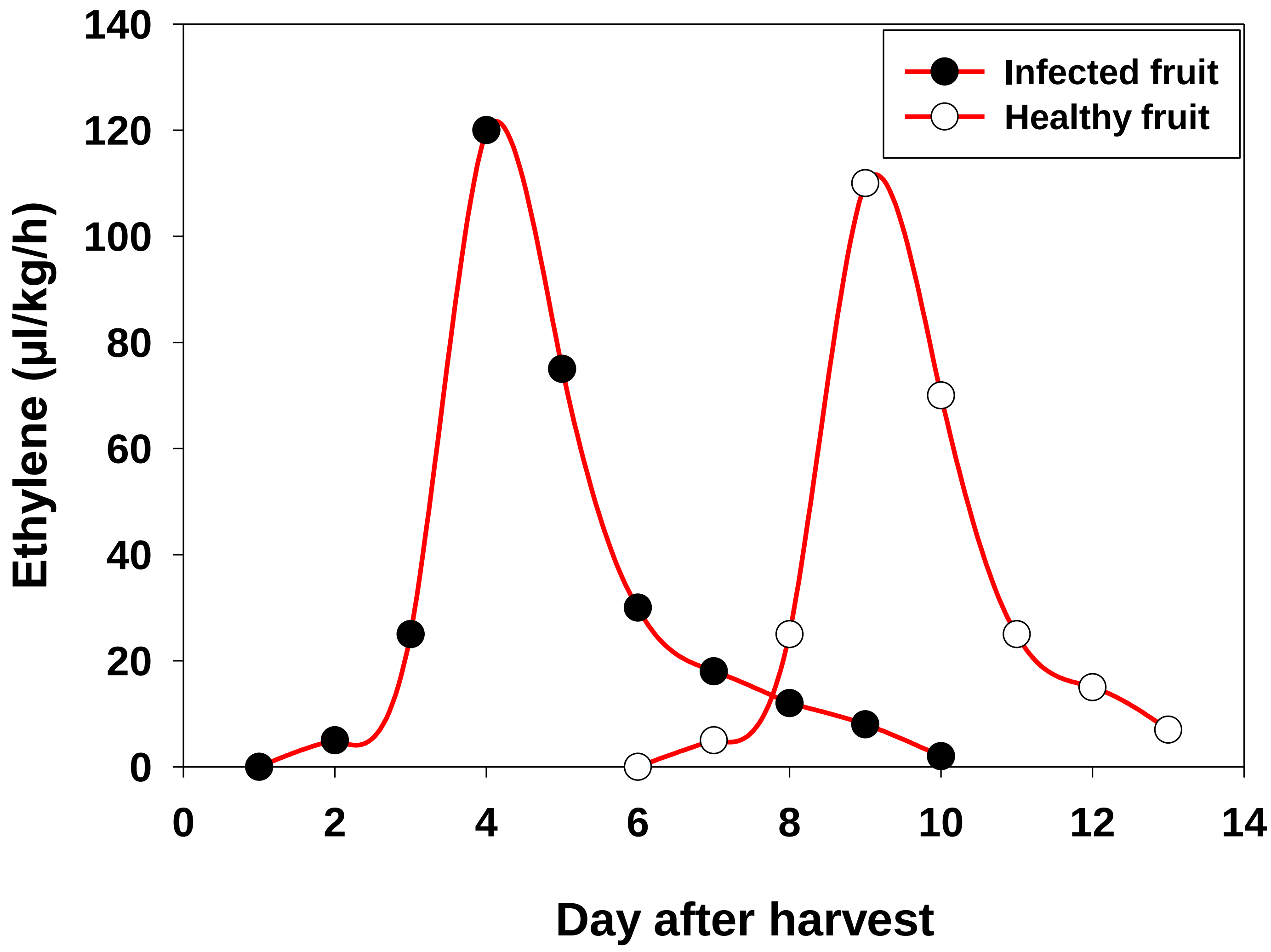




β-galactosidases catalyze the hydrolysis of terminal galactosyl residues from carbohydrates and degrade cell wall components during senescence

The effect of banana ripening on the firmness of the fruit and the activity of the enzyme B-galactosidase

Effect of decay of avocado on ethylene production



Ethylene in the Air

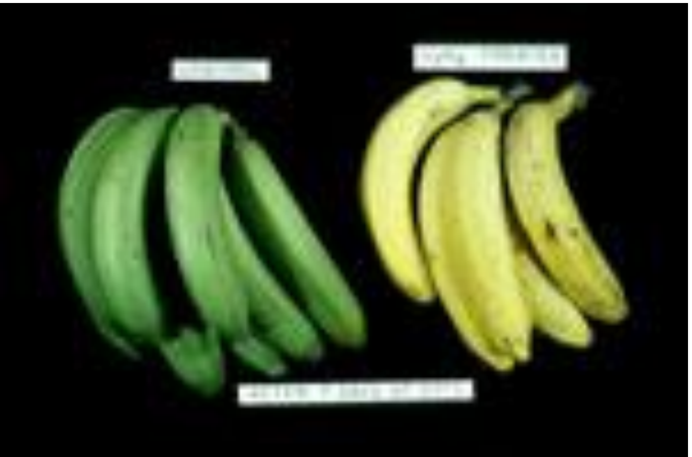
Beside it self-biosynthesis, the harvested product can be exposed to atmospheric ethylene that include:

- **Engine gases from trucks and forklifts.**
- **Industrial pollution**
- **Pollution from burning fuels.**
- **Climacteric ethylene emissions**
- **Smoke**

The commercial use of ethylene

Commercial uses:

- Allows the uniform and absolute ripening of banana, avocado and ripe green tomato.
- Enables the change and development of color (de greening) in citrus fruits.



The best conditions for uniform ripening with ethylene

- Temperature between 18 and 25 C
- Relative humidity of 95%
- Ethylene concentration of 1-100ppm
- Length of treatment between 1-5 days
- Air spin in the ripening room
- Ventilation exchange to prevent accumulation of CO_2

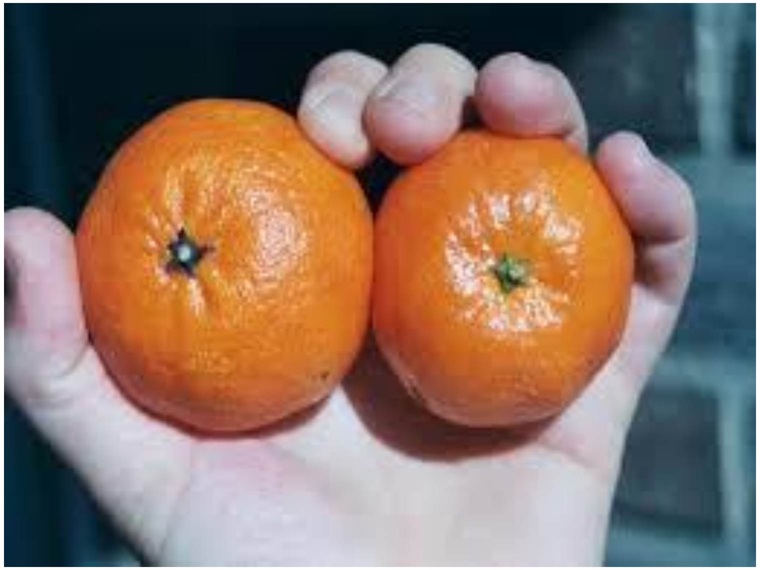
De-greening process of citrus (early harvesting of fruits during autumn) early fruit)

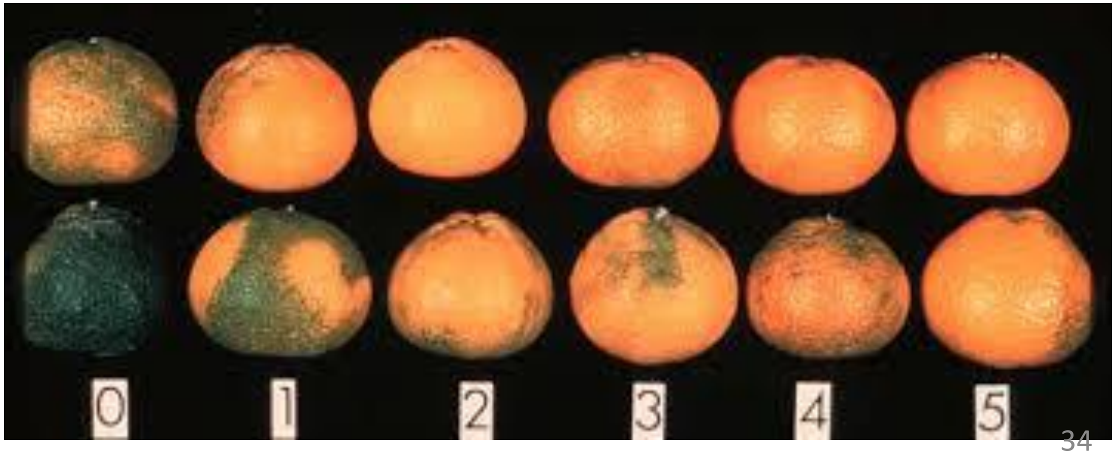
Ethylene -1-5 ppm
Temperature treatment: 20 C
Humidity 90-95%
Ventilation: One full change of air once a week

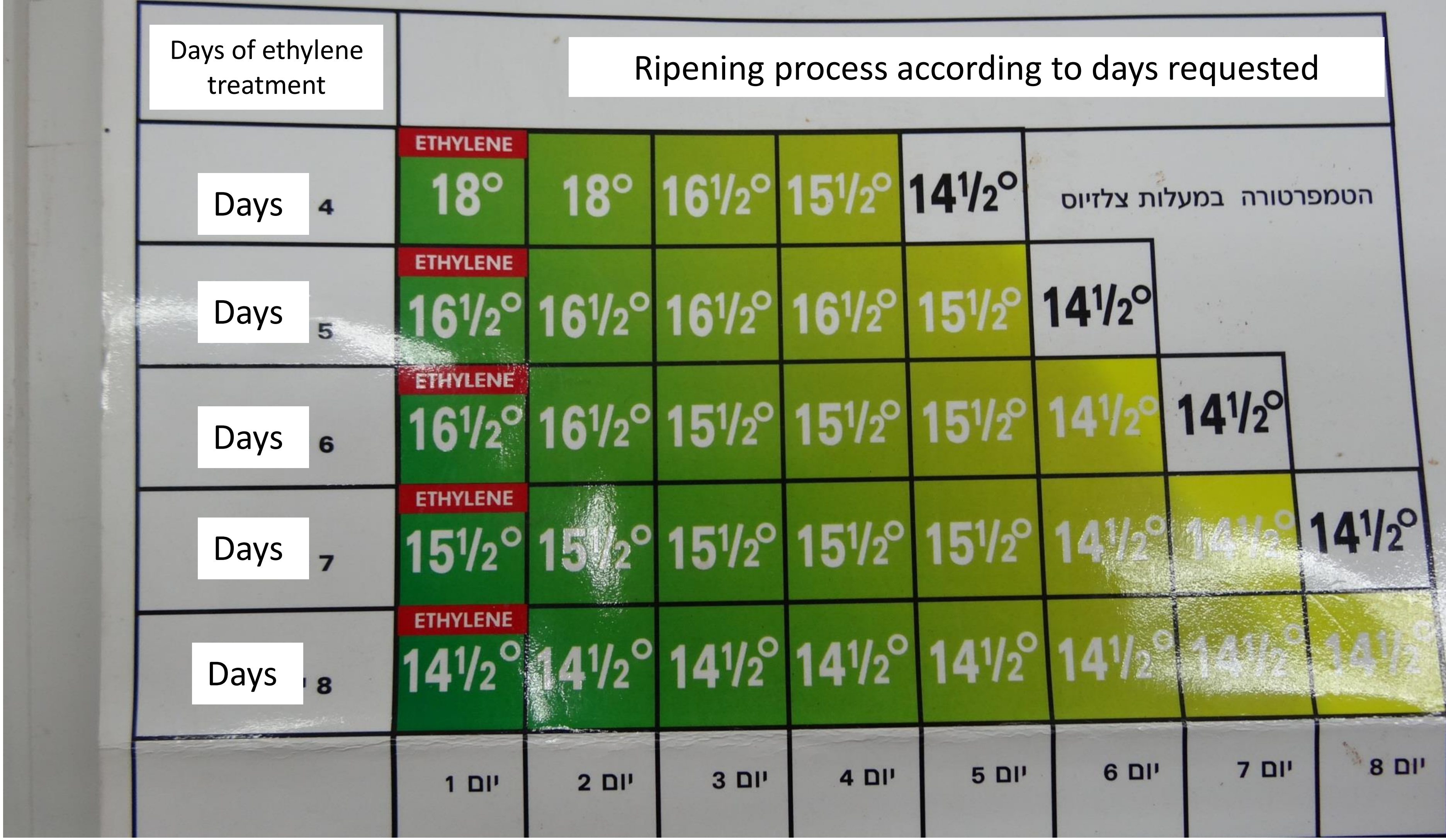
Factors that may affect the process

- Unripe fruit will affect the development of normal color
- A strong growth development inhibit the process of color change.
- Color change is induced by night temperatures of 7 to 13 degrees C.
- Scion of the tree –intensity of the tree's growth can affect the color change of the fruit
- Spraying program – summer spraying with oils against insects can reduce color development.
- Fertilization – a high nitrogen level increases tree growth that affects color development.

De-greening process of citrus (early harvesting of fruits during autumn) early fruit







Days of ethylene treatment	Ripening process according to days requested							
	יום 1	יום 2	יום 3	יום 4	יום 5	יום 6	יום 7	יום 8
Days 4	ETHYLENE 18°	18°	16½°	15½°	14½°			
Days 5	ETHYLENE 16½°	16½°	16½°	16½°	15½°	14½°		
Days 6	ETHYLENE 16½°	16½°	15½°	15½°	15½°	14½°	14½°	
Days 7	ETHYLENE 15½°	15½°	15½°	15½°	15½°	14½°	14½°	14½°
Days 8	ETHYLENE 14½°	14½°	14½°	14½°	14½°	14½°	14½°	14½°

הטמפרטורה במעלות צלזיוס

Banana ripening in the presence of 100 mm ethylene, and 95% humidity, depending on the ripening stage of the fruit


1
ירוק
ירוק טבעי
2
ירוק בהיר
שינוי ראשוני בצבע
כתוצאה מהבחלה
3
ירוק בהיר
עם
צהוב בהיר
שינוי ברור בצבע
מוכן לשיווק
במזג אויר חם
4
צהוב
עם
מעט ירוק
מוכן לשיווק
במזג אויר קר
5
צהוב
עם
ירוק בקצוות
צבע אידיאלי
לשיווק קמעונאי
6
צהוב מלא
מוכן
למכירה ולאכילה
7
צהוב
עם
נקודות חומות
בשל לגמרי
עם ארומה
www.i-m-b.org.il


א.מ. בננות
1851000


RED PRM
כאן

Negative effects of ethylene

Ethylene is a very explosive gas

Blend of ethylene with air at concentrations of 3% to 32% is very explosive

Sources of ethylene

In the ripening room the sources of ethylene might be:

- Commercial cylinders (contains a mixture of inert gas to prevent explosion, without the presence of oxygen).
- Generators to produce ethylene by drying ethanol by heat. $C_2H_5OH - H_20 = C_2H_4$
- Ethylene releasers like Ethaphone, Calcium Carbide $+H_2O$

Negative effects of ethylene

As the hormone of ripening and ageing, ethylene has a negative effect on stored produce

- Induce senescence of leaves
- Induce ripening and softening fruit
- Inhibit flower opening (symptom of "sleep“)
- Catalyzes the creation of stem detachment
- Induces potato germination
- Causes peel defects
- Increases bitterness (isocoumarins accumulation) in carrot
- Increases rust stains in lettuce
- Increases firmness of asparagus

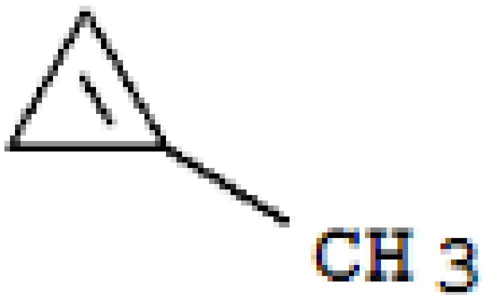
Ways to prevent negative effects

- Avoid keeping ripe fruit next to ethylene-sensitive produce
- Avoid using an electric forklift inside store rooms
- The truck loading area must be isolated from the treatment and storing area.
- Avoid throwing rotten produce inside the packing house
- Avoid smoking and/or burning in the packing house.
- Keep proper ventilation.
- Use scrubber of ethylene as Potassium permanganate
- Block ethylene activity by the use of 1-MCP


+ ETHYLENE
– ETHYLENE

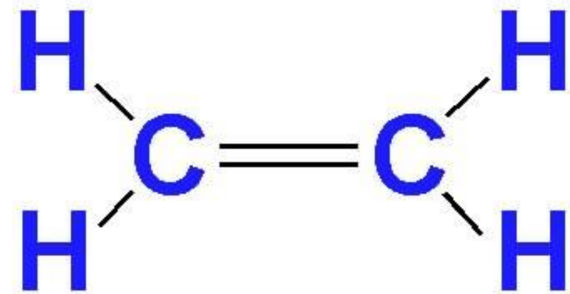

ISL

Scheming mechanism of ethylene and smarter fresh (1-MCP) during ripening/aging of fresh produce

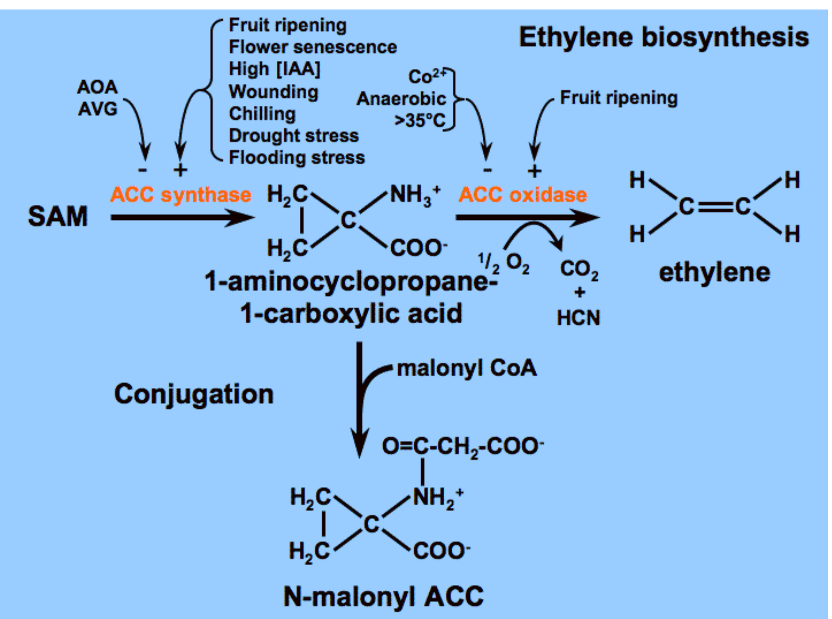


1-Methylcyclopropene

SmartFresh™

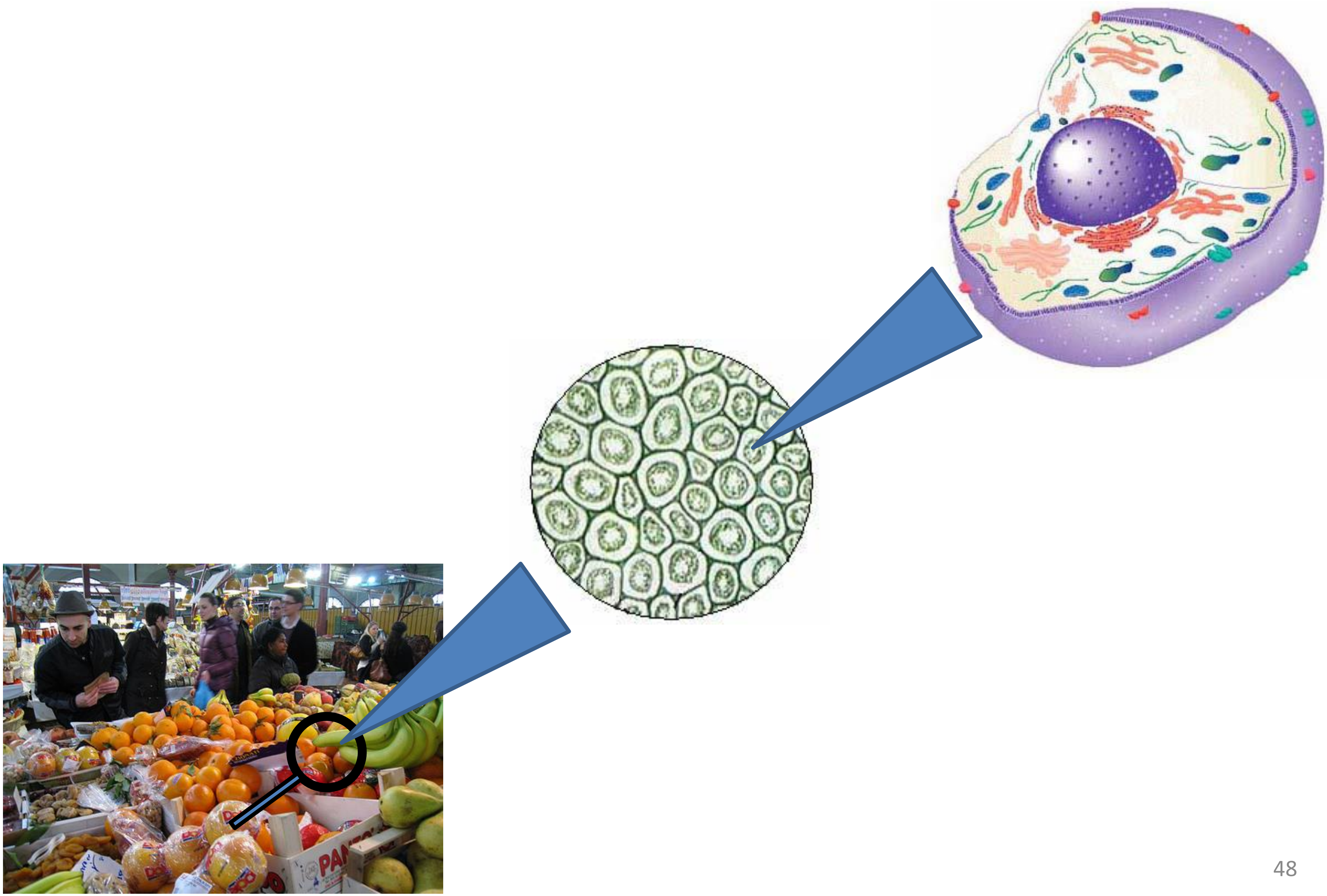


Ethylene

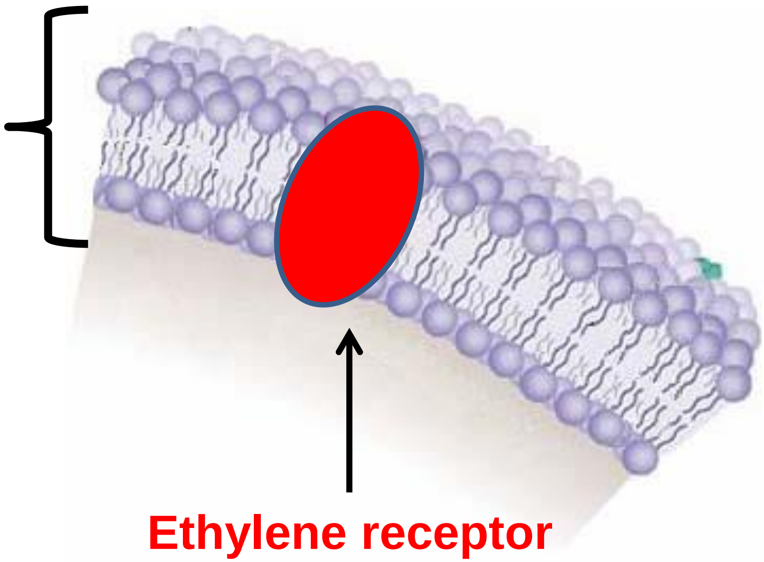


Inhibit ACC oxidase

Both the ethylene and the 1-MCP operate at the cell level



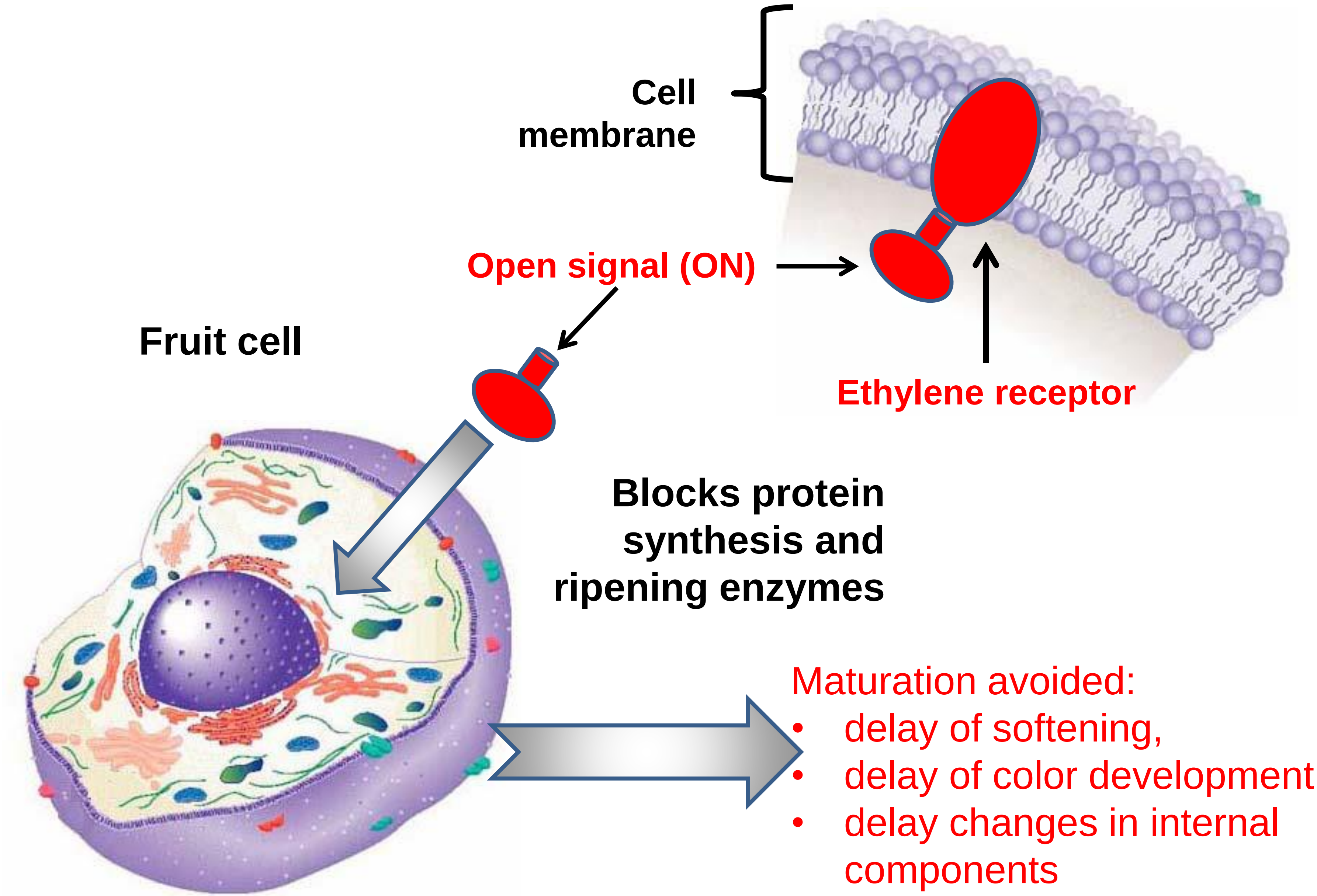
Mechanism of action of ethylene and 1-MCP



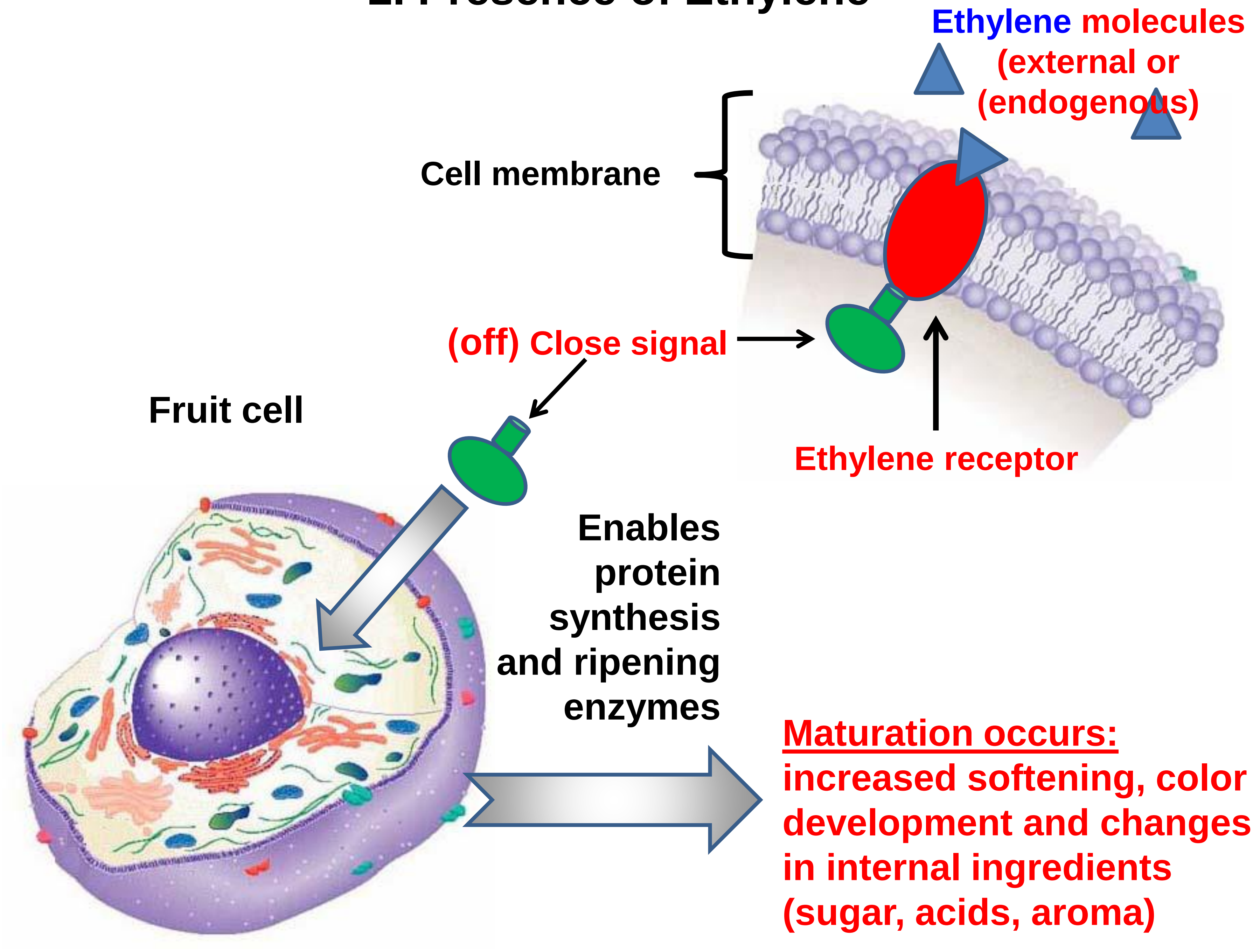
Possibilities:

1. No or low level of ethylene
2. Ethylene is present
3. 1-MCP is present
4. Ethylene+1-MCP are present
5. Effect of 1-MCP

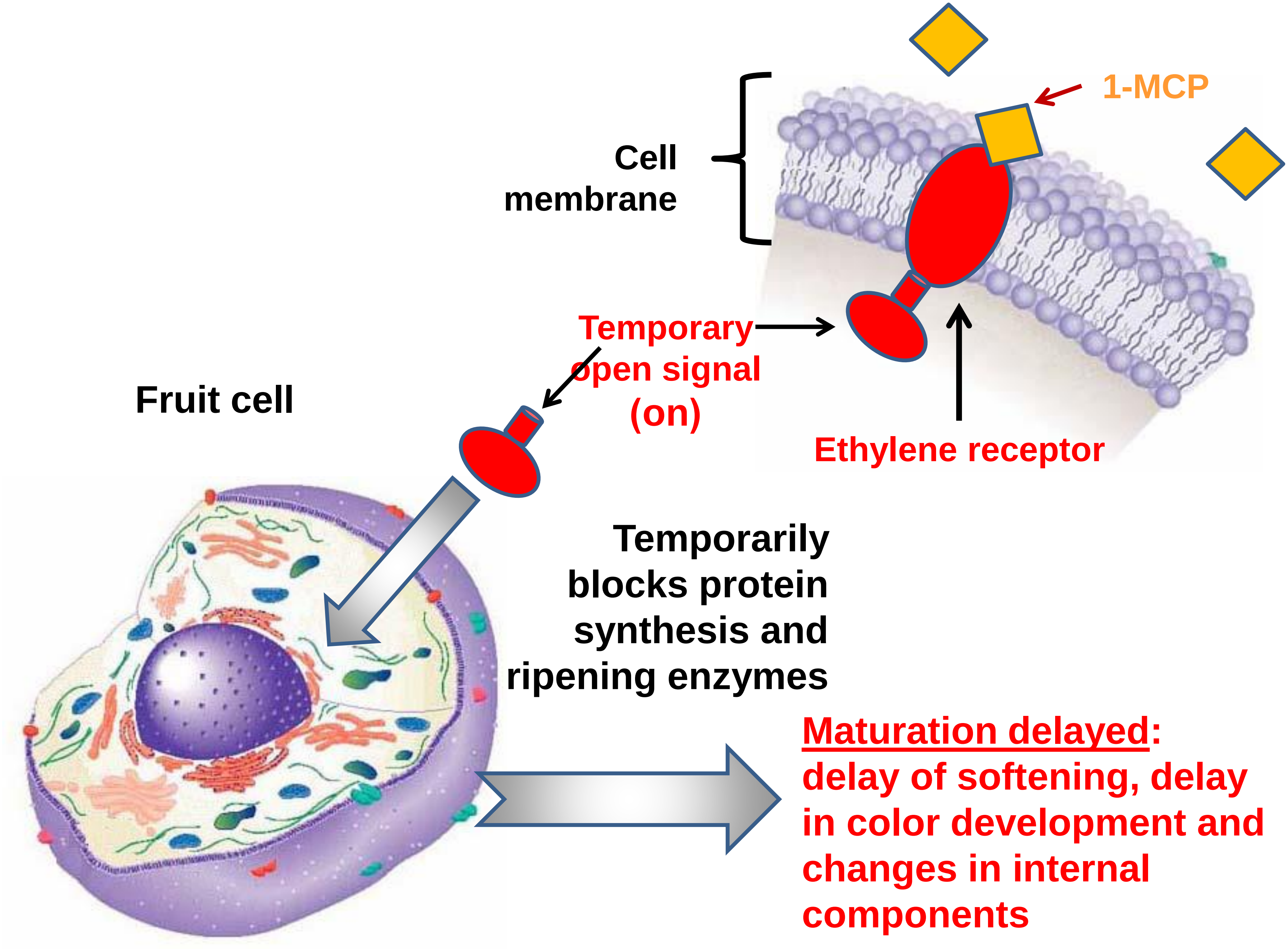
1. No, or low levels of ethylene



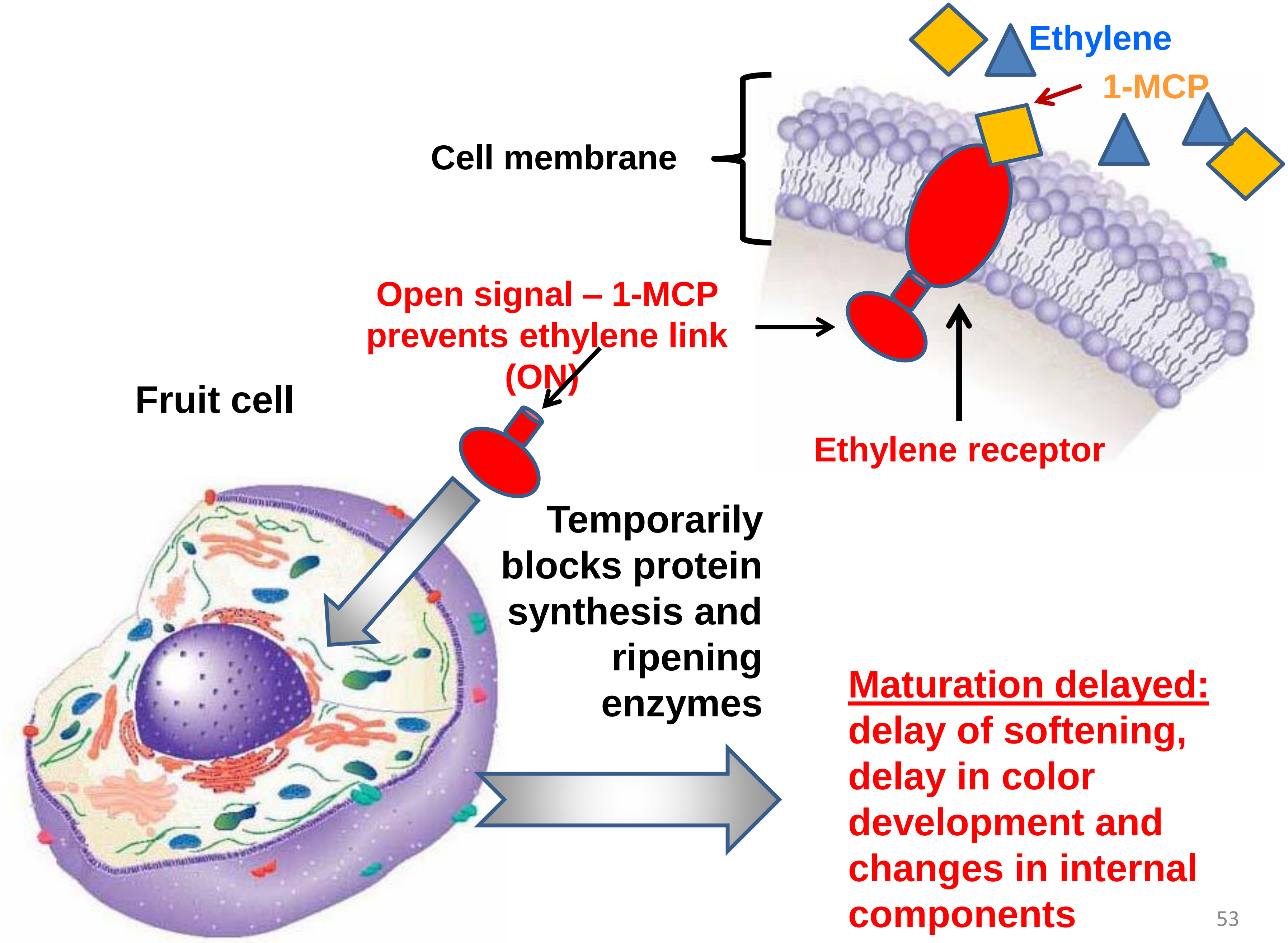
2. Presence of Ethylene



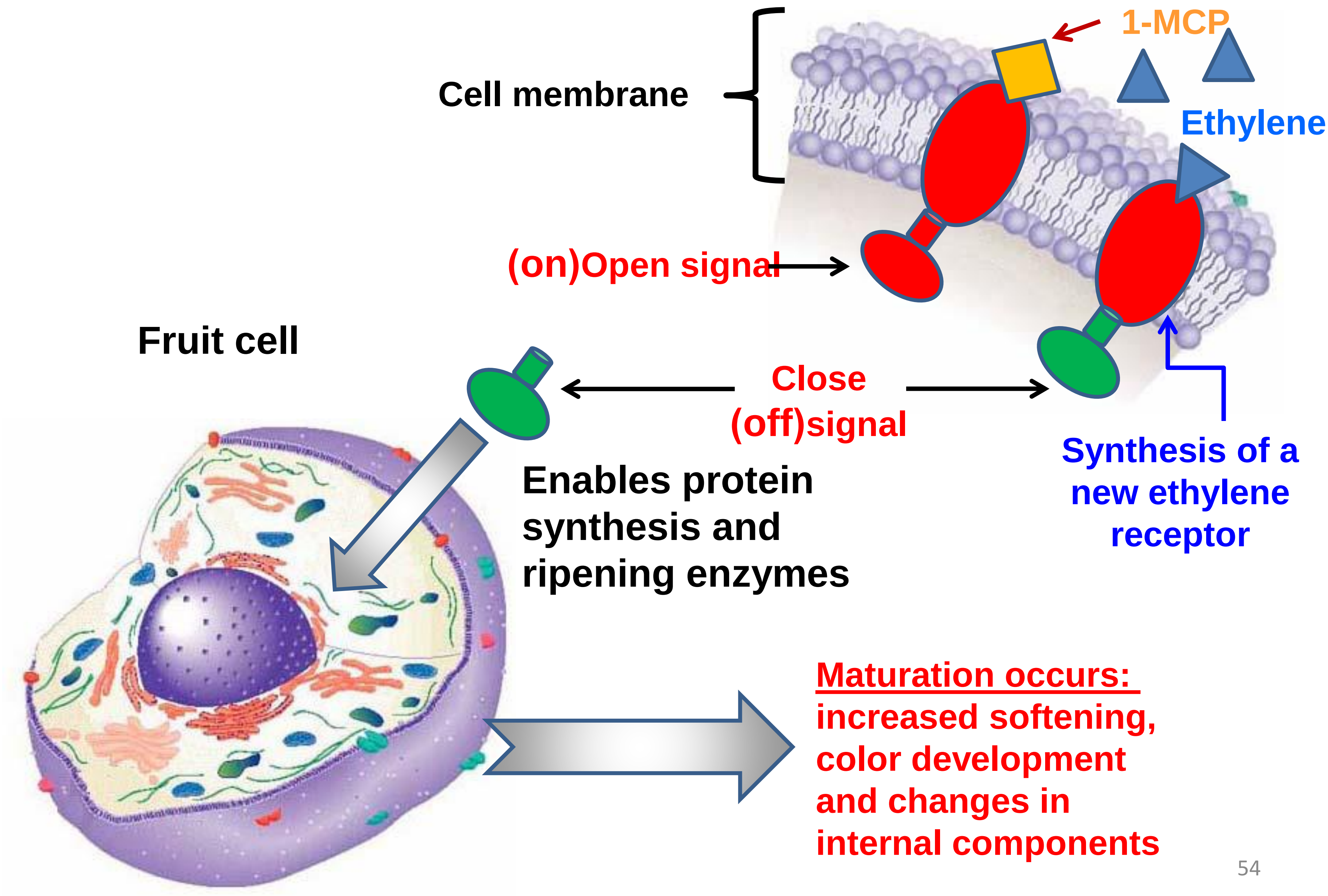
3. Presence of 1-MCP



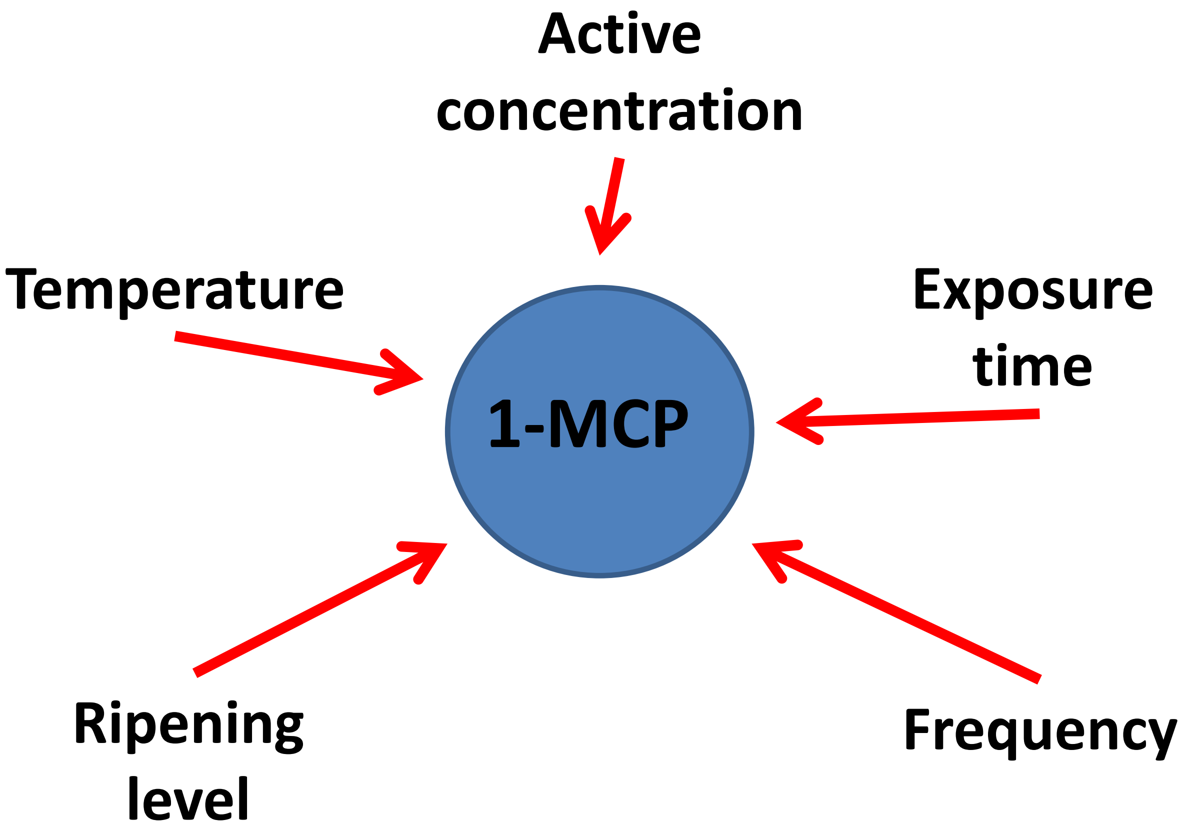
4. 1-MCP and ethylene are present



5. Reversing the effect of 1-MCP



Factors affecting 1-MCP efficiency




SUNON
0504PFB3-8
0.4W
MADE IN TAIWAN

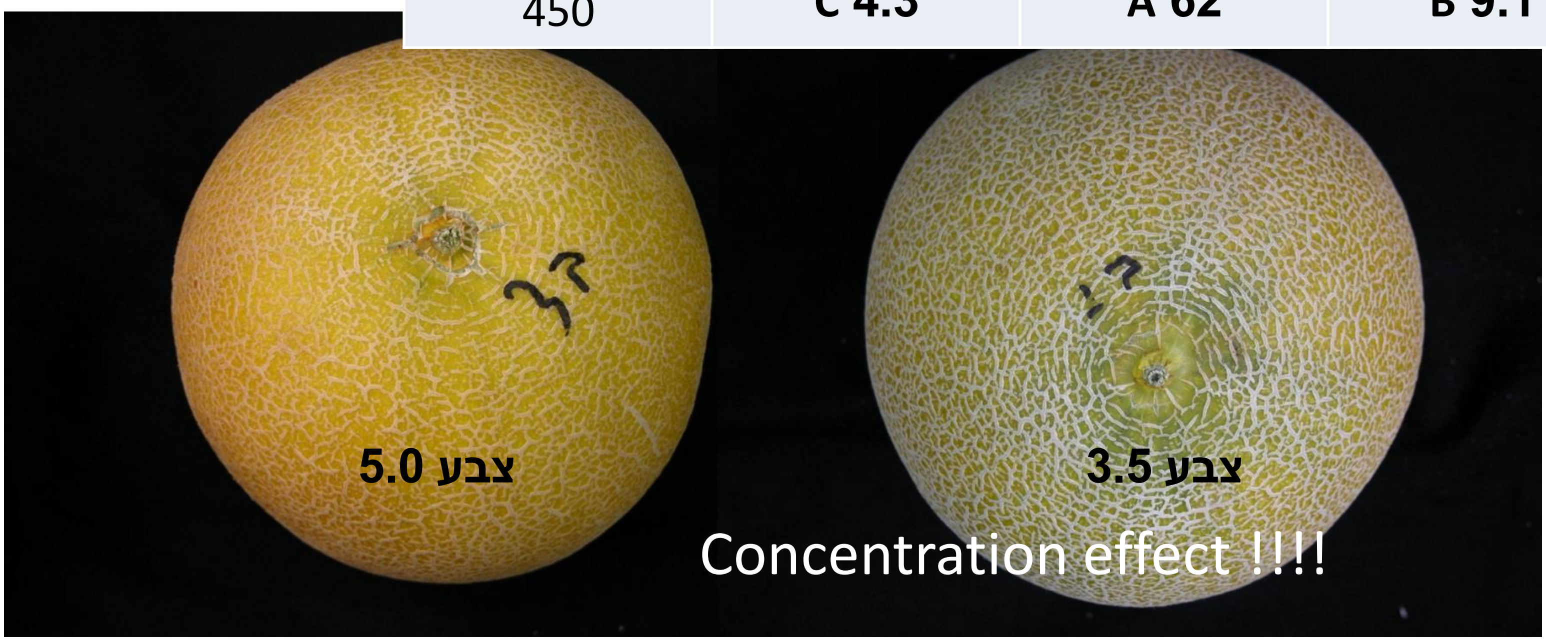
1-MCP treatment


RIMI
SMARTFRESH™ ACTIVATOR KIT
Dispose of by landfilling, incineration, or chemical sewer according to federal, state and local laws.
La disposición final por enterrado, incineración o tratamiento químico debe hacerse de acuerdo con la legislación local, estatal o federal.
O descarte final em aterro, por incineração ou tratamento químico deve ser feito de acordo com a legislação local, estadual ou federal.
Per lo smaltimento mandare questo materiale a una discarica o a un inceneritore osservando la legislazione vigente.

The effect of 1-MCP concentrations on color development, fruit firmness and sugar content in the melons, after 15 days at 5 C and 3 day shelf life at 20 C.

Fruit were treated with 1-MCP, 150, 300 and 450 nannoliters/liters, at 20 s C for 24 hours. The fruit was harvested at a color value of 3.5 color and firmness of about 80 newton's.

Treatment (nanoliter/lt MCP)	Color (1-6)	Firmness (Newton)	Sugar (%)
Control	A 5.6	D 32	A 10.0
150	B 4.9	C 52	A 9.8
300	C 4.5	B 56	A 9.7
450	C 4.3	A 62	B 9.1



The effect of 1-MCP applied at three different temperatures on the quality of melons after 15 days storage at 5 C and another 3 days shelf life at 20 C. The fruit was treated with 1-MCP, at a concentration of 300 nano-liters/liters, each treatment lasting 24 hours.

Treatment (nanoliter/lt MCP)	Color (1-6)	Firmness (Newton)	Decay(%)
Control	A 5.6	D 45	A 5.2
MCP 5 C	AB 5.3	C 49	B 3.4
MCP 10 C	B 5.1	B 55	C 1.9
MCP 20 C	C 4.7	A 71	D 1.1

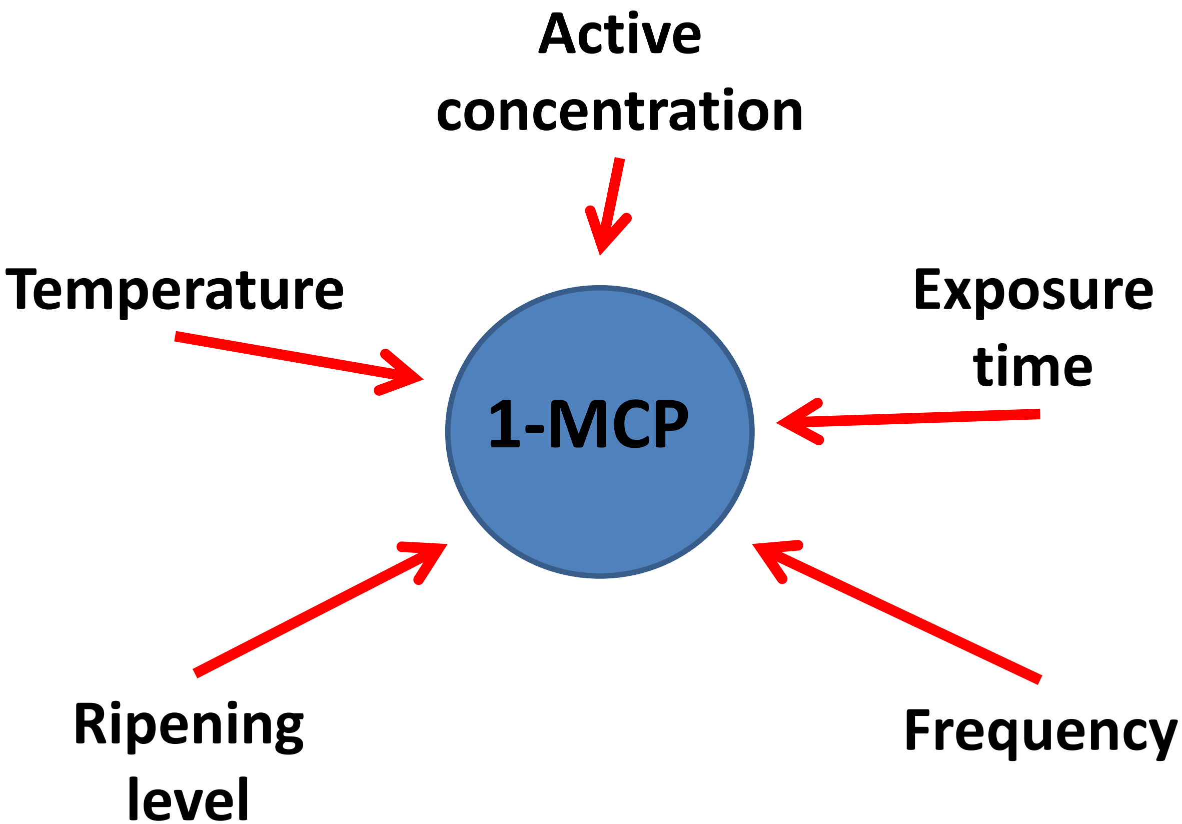
Temperature effect !!!!

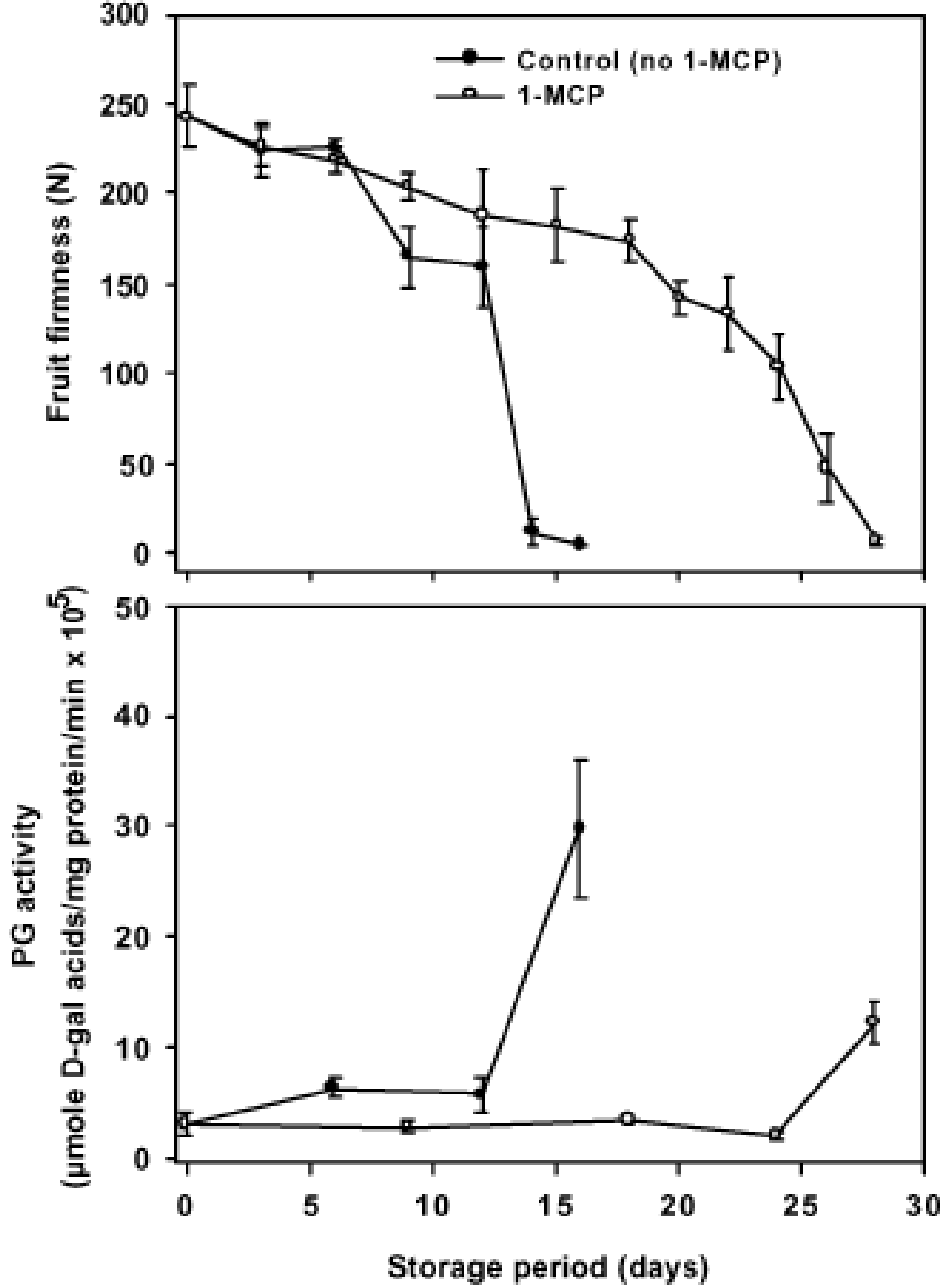
The effect of 1-MCP at a concentration of 300 nanoliters/liters on the development of color and melon firmness harvested in three different ripe states (2 - ripe green; 3.5 - yellow/green; 5 - yellow) at the end of 15 days of storage at 5 C followed by 3 days at 20C

Quality and colour	O time not treatment			After storage and shelf period without treatment			After storage and shelf life treated with MCP		
Control	Green (2)	Yellow/green (3.5)	Yellow (5)	Green (2)	Yellow/green (3.5)	Yellow (5)	Green (2)	Yellow/green (3.5)	Yellow (5)
Color (1-6)	2	3.5	5.0	3.5	5.4	5.8	2.5	4.7	5.5
Firmness (newton's)	95	75	50	64	40	25	90	58	32

Level of ripening effect !!!!

Factors affecting 1-MCP efficiency





Avocado fruit firmness and polygalacturonase activity treated with 1-MCP for 12 hours at 20 degrees C and stored in 13 degrees C for 28 days

Summary,

- Growth, including ripening and ageing, are coordinated and active processes that occur in fresh produce after harvesting affected by ethylene.
- Fruits and vegetables are classified into two main groups: climacteric and non-climacteric.
- The ability to control the maturation and ageing processes (ethylene production, respiration, water loss, nutritional changes, etc.) are the milestones for maintaining the quality of fresh produce after harvesting.

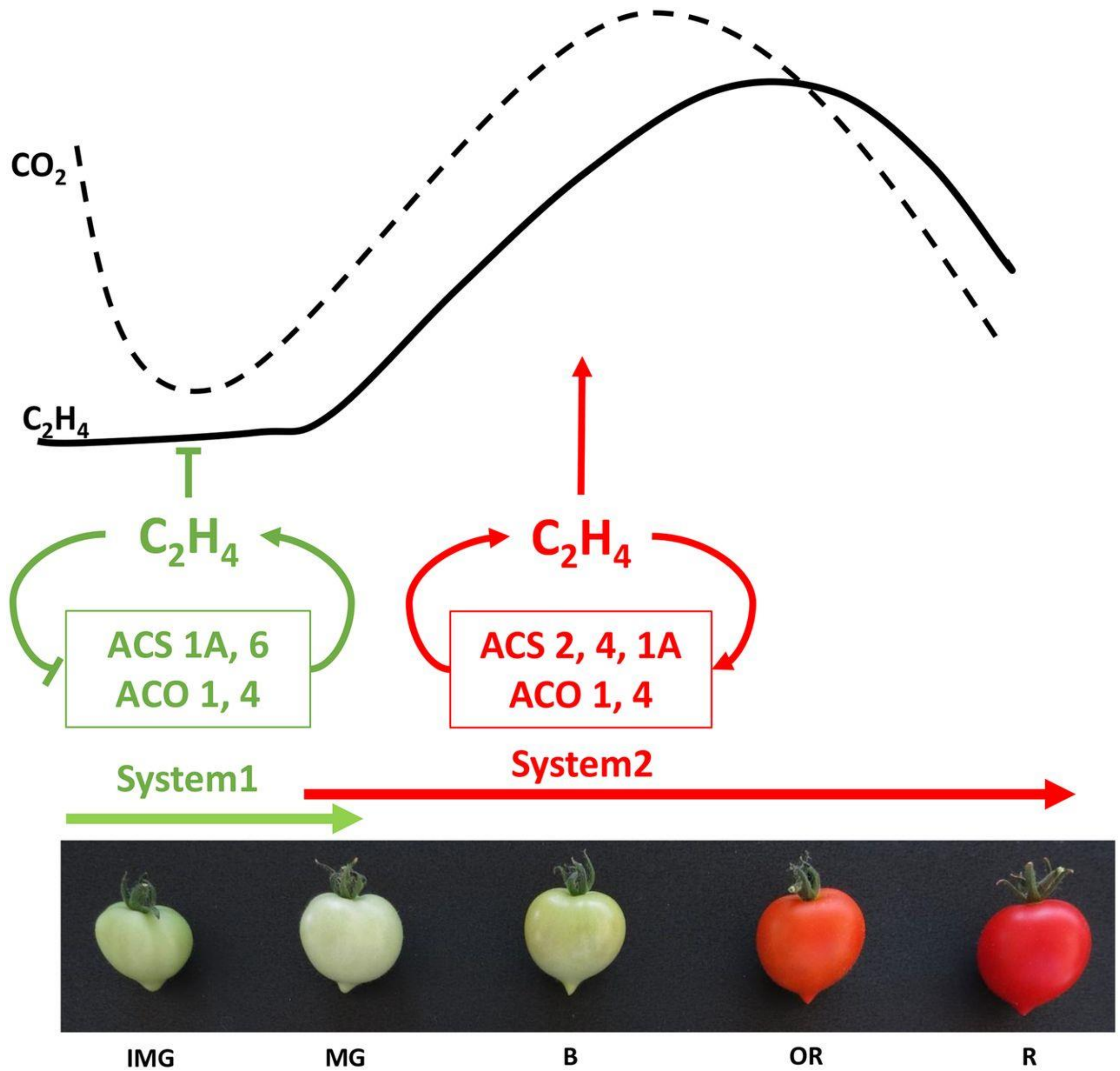
Summary
Regulating the synthesis of ethylene

The increase of ethylene occurs:

1. During fruit ripening
2. After physical damage (wounding, harvesting)
3. Fruit decay
4. Increase of temperature over 30 C
5. Stresses (water, cold and high temperature, atmospheric limitations).

The decrease of ethylene occurs:

1. Decline in temperature
2. Decline of O_2 under 8%
3. Increase of CO_2 over 2%



שתי מערכות של אתילן פועלות במהלך התפתחות והבשלת הפרי. בשלב של טרום-הבשלה, הביוסינתזה של האתילן נעשית על ידי מערכת 1, כאשר מערכת-2 מאופיינת על ידי אוטוקטליזה של אתילן במהלך ההבשלה. הגנים העיקריים המעורביפ במערכת-1 הנם ACS6 ו-ACS1A, פעילותם של גנים אלה מוקטנים על ידי אתילן. בשלב הירוק בשלב והלאה, יצירת מערכת-2 משופעלת בעיקר על ידי ACS2 ו-ACS4, ובא לידי ביטוי בהשראת האתילן. תוצרי ACS1A מראים עלייה חולפת בזמן הבשלת הפרי, דבר המעיד כי גן זה חשוב בויסות המעבר ממערכת-1 חמערכת-2. רמת תוצרי ACO1 ו-ACO4 נמוכים במצב טרום-הבשלה ירוק, אבל עולים בצורה חדה בשיא הקלימקטרי כאשר מערכת-2 ביצירת האתילן עובדת.

ראשי חץ מראים ויסות חיובי וראש "שטוח" מייצג ויסות שלילי