

Fresh-Cut Product Biology

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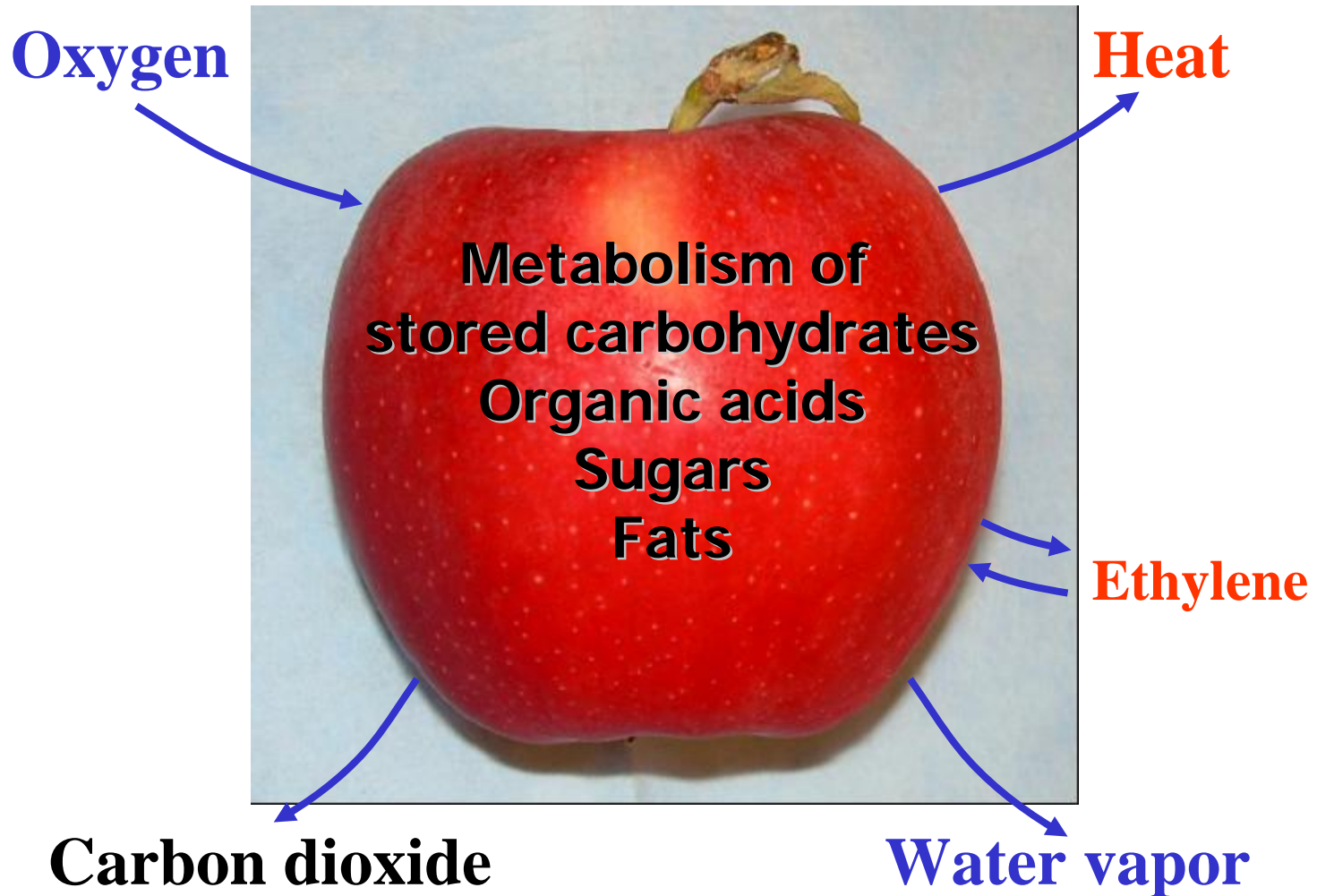
CONCLUSION

1. Fresh-cut fruits and vegetables often respond differently than the whole commodity.
2. Knowledge of basic biology of whole and fresh-cut commodities helps optimizes quality retention during handling and marketing.
3. Unexpected things will happen when fresh-cut fruits and vegetables are produced and marketed.
4. Genetic modifications may prevent deleterious wound-induced changes, but it may also make the plant more susceptible to stresses during growth.

Introduction

- *Fresh-cut requires wounding* (e.g., lightly processing, minimally processed) the plant tissue.
- *Types of wounds include:* abrasion, chopping, cutting, dicing, grating, mincing, peeling, scraping, slicing, shredding, tearing, etc.
- *Plants have evolved a limited number* of genetically preset physiological responses to wounding.
- *The response depends on* the type of wound and other previous or subsequent stresses.

Harvested Crops are Living Organisms



Product Structure

A commodity is made up of different tissue systems

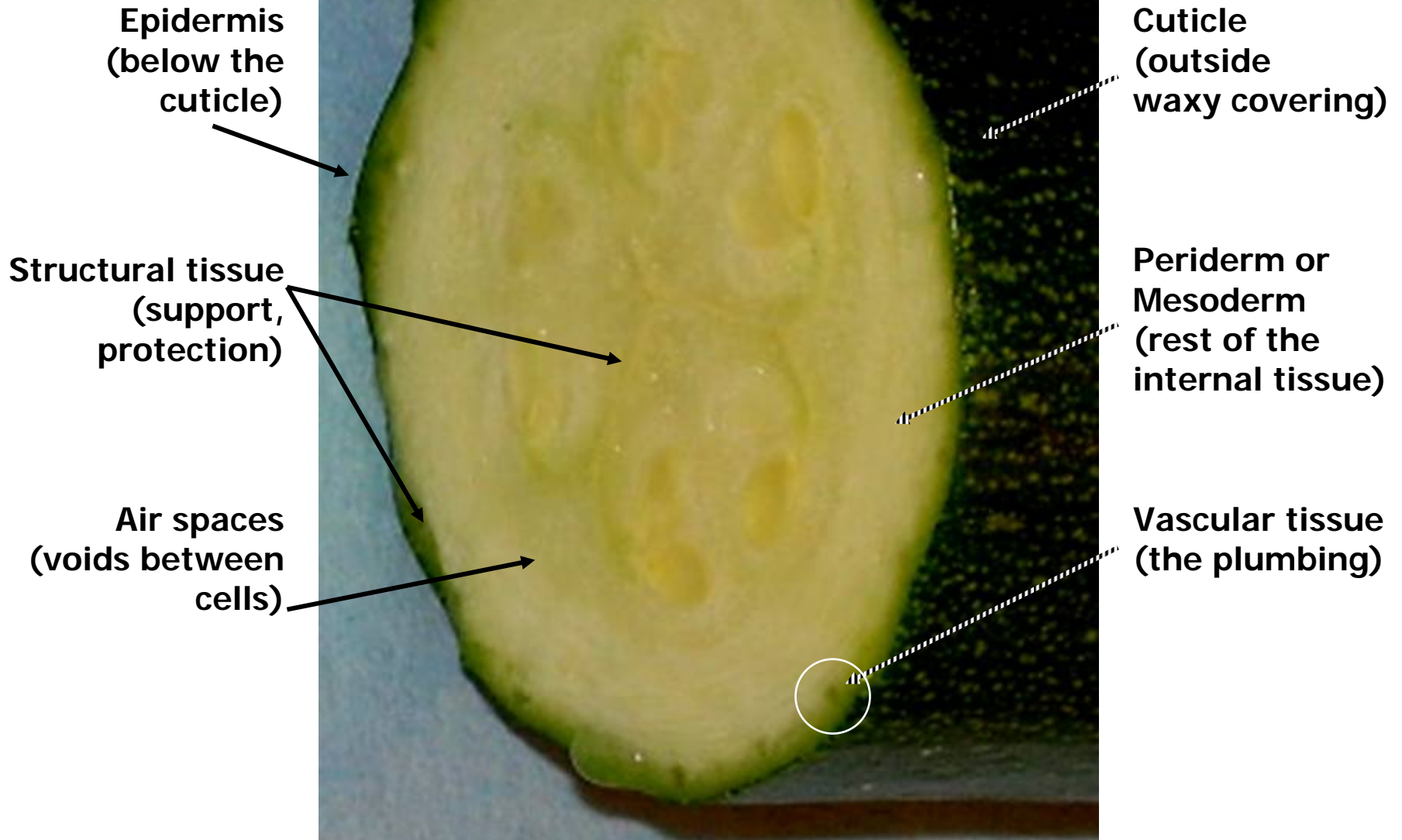
- **The cuticle** is a layer of waxes on the outside and is a barrier to water loss and pathogen entry.
- **The epidermis** is the first layer of living cells. It produces the cuticle and also acts as a barrier.
- **The inner tissues** comprises most of what we eat and is called the periderm and/or mesoderm.

Product Structure

A commodity is made up of different tissue systems

- **Structural tissue** supports the cells and gives the commodity its shape and texture.
- **Vascular tissue** is interspersed in the commodity and provides the plumbing to move material around.
- **Air spaces** abound between the cells and are needed to move O₂ into the tissue and CO₂ away.

Parts of a Whole Commodity



Epidermis
(below the
cuticle)

Cuticle
(outside
waxy covering)

Structural tissue
(support,
protection)

Periderm or
Mesoderm
(rest of the
internal tissue)

Air spaces
(voids between
cells)

Vascular tissue
(the plumbing)

Cellular Components

Plant cells are divided into many compartments that isolate reactions and substrates.

This isolation protects the cell from unwanted reactions that may damage the cell.

Cellular Membranes

Membranes are boundaries that divide the cell into many compartments

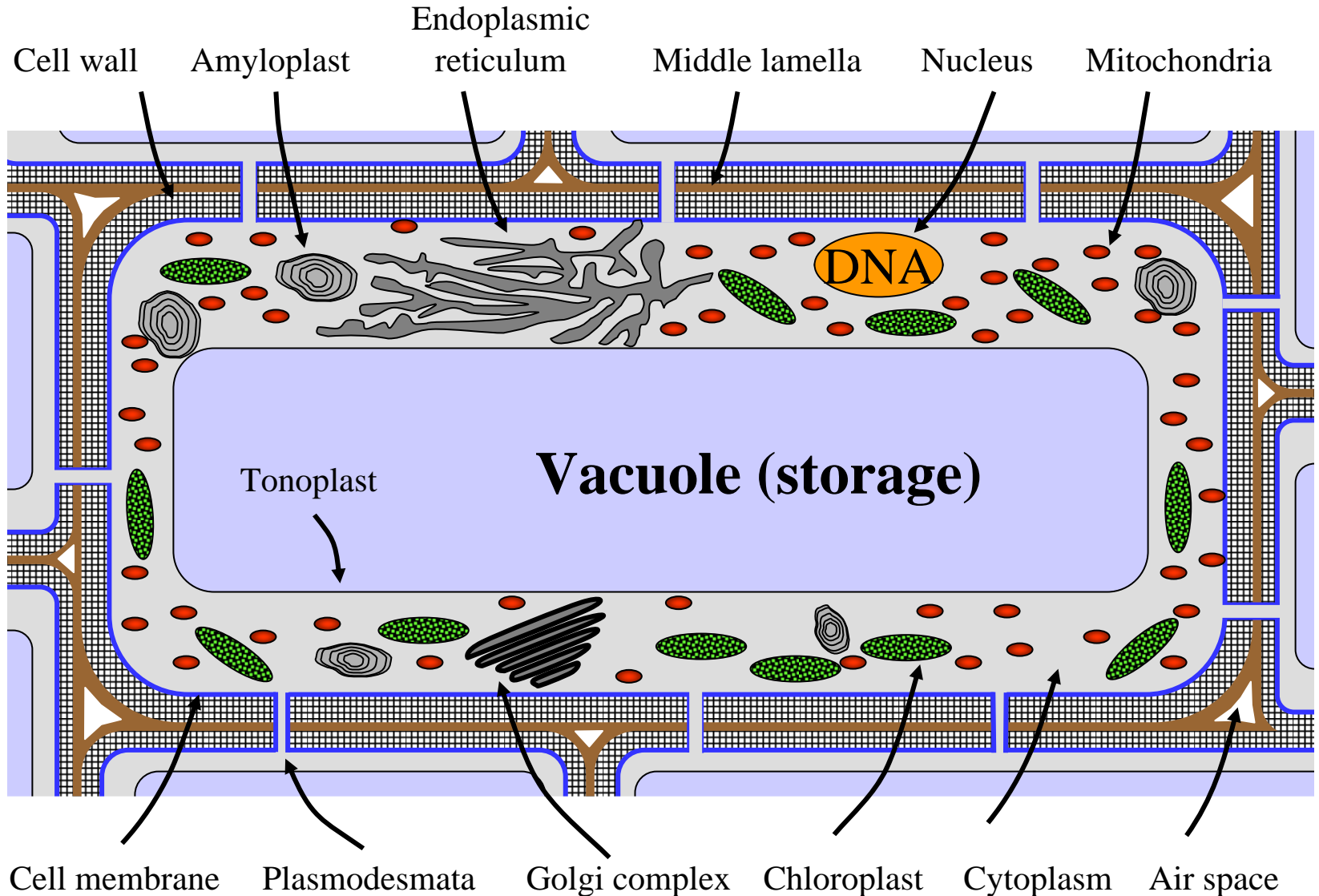
Cell membrane,
Vacuole membrane,
Chloroplast membrane,
Mitochondria membrane,
Other membrane bound spaces:
ER, Golgi, Amyloplast, Lysosome.

Cellular Membranes

Maintaining membrane integrity is crucial to maintaining cell vitality and product quality.

- The cell membrane isolates and protects the cell from the environment.
- The vacuole membrane isolate stored organic acids, sugars and pigments.
- The mitochondria membrane isolate reactive energy producing reactions.
- The lysosome membrane isolates degrading enzymes from the rest of the cell.

Generalized Plant Cell



The Opportunities for Wounding are Many and Varied

Harvesting

Sufficient injury to separate from parent plant

Transporting

Vibration, compression, temperature abuse, etc.

Handling

Unloading, sorting, cleaning, packaging, etc.

Processing

Trimming, abrading, segmenting, etc.

The Responses to Wounding are Many and Varied

Physical response

Mechanical changes to existing tissue

Biochemical response

Reactions with existing cellular components (substrates and enzymes)

Physiological response

Induction of the synthesis or activation of enzymes and synthesis of substrates

Examples of Fresh-cut Responses to Wounding

Carrot - 'Baby' Carrots

Surface whitening

Edible coatings (make coatings from commodity)

Celery - Petiole Segments

Pithiness, vascular elongation, flaring of ends

Tomato - Ripening Mature-green Slices

Altered water activity and gaseous atmosphere

Unexpected sprouting of seeds (vivipary)

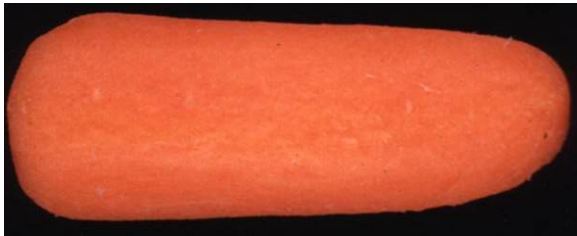
Lettuce - Browning Disorders

Russet spotting

Wound-induced browning – cause & wound signal

White-blush development on fresh-cut carrots

Is it a physical
(e.g., surface debris)



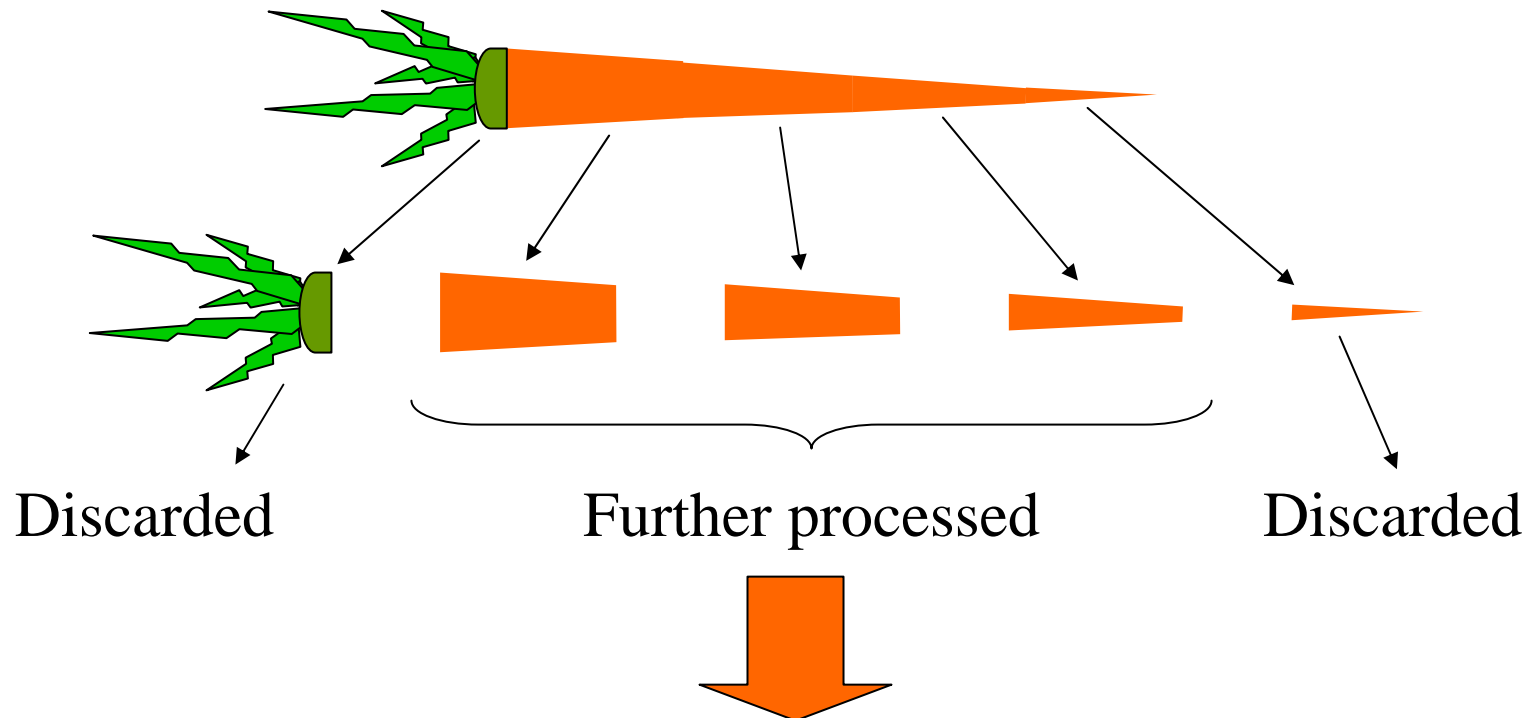
Or



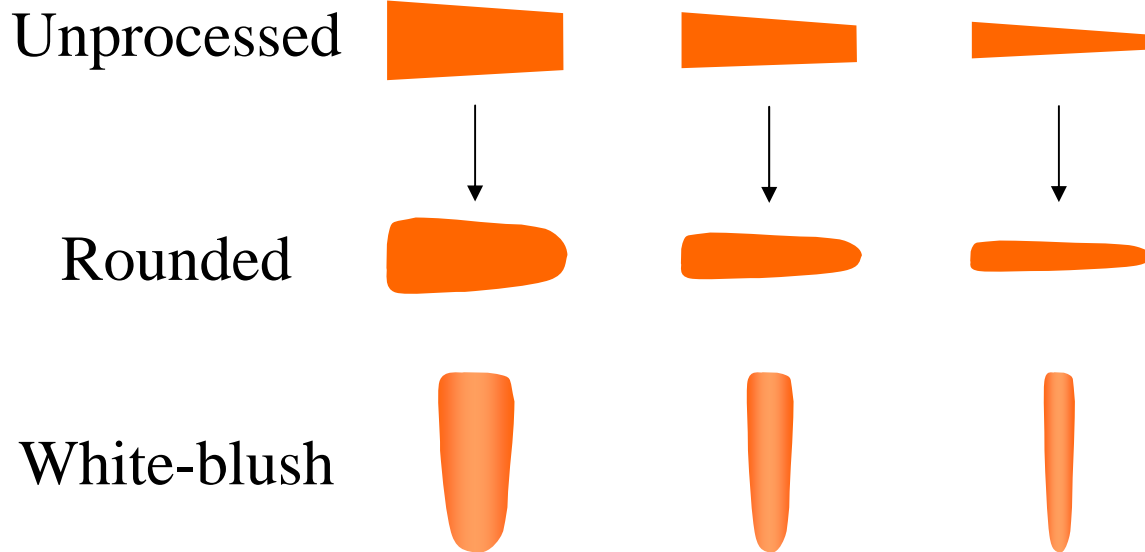
a physiological change?

(e.g., wound-induced lignin synthesis)

Fresh-cut carrots are produced from 3 inch long segments cut from longer carrots.



Abrading the surface of the carrot pieces produces rounded edges but also leaves cellular debris on the surface.



Reducing white-blush development on fresh-cut 'baby' carrots

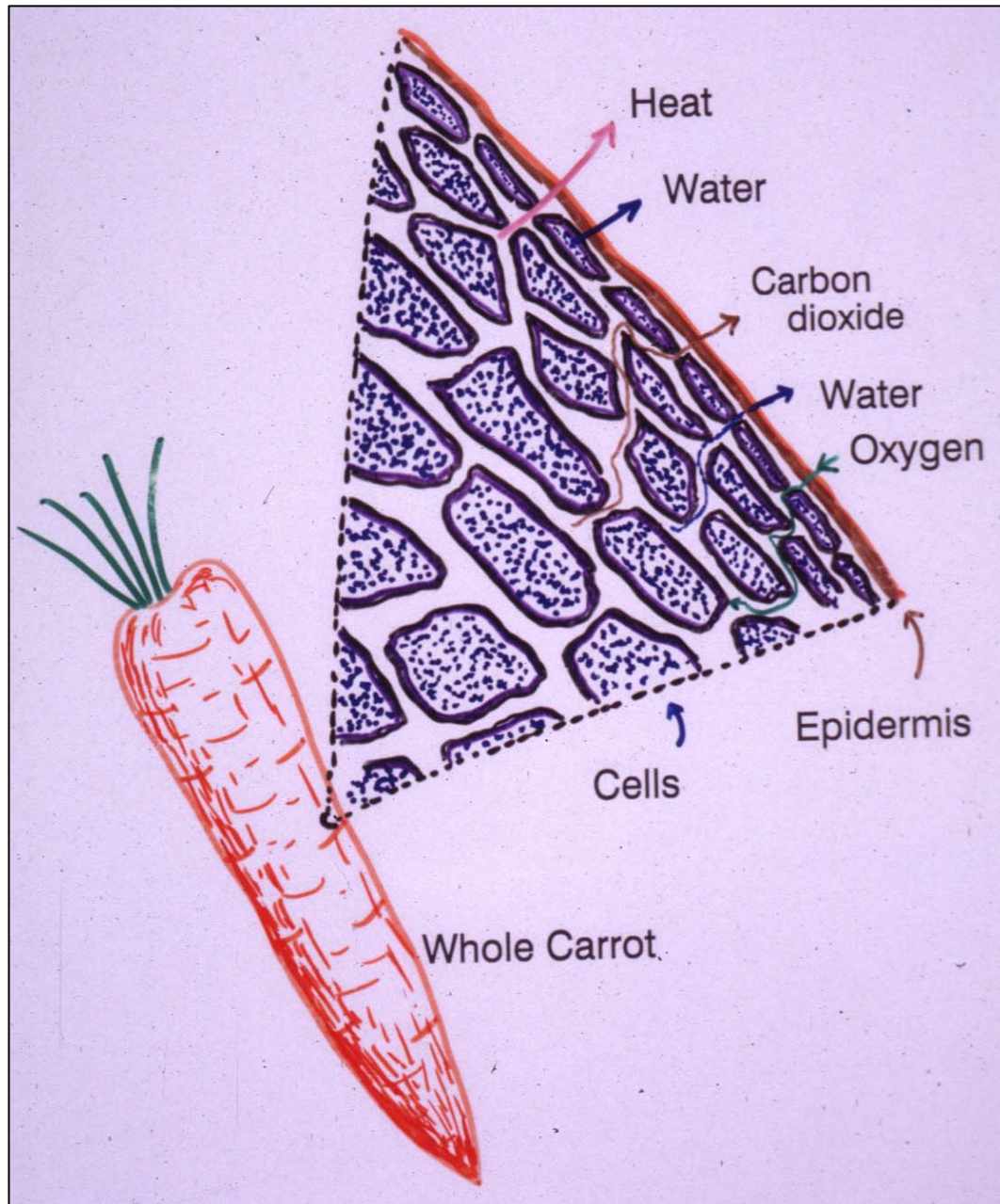


Water
loss

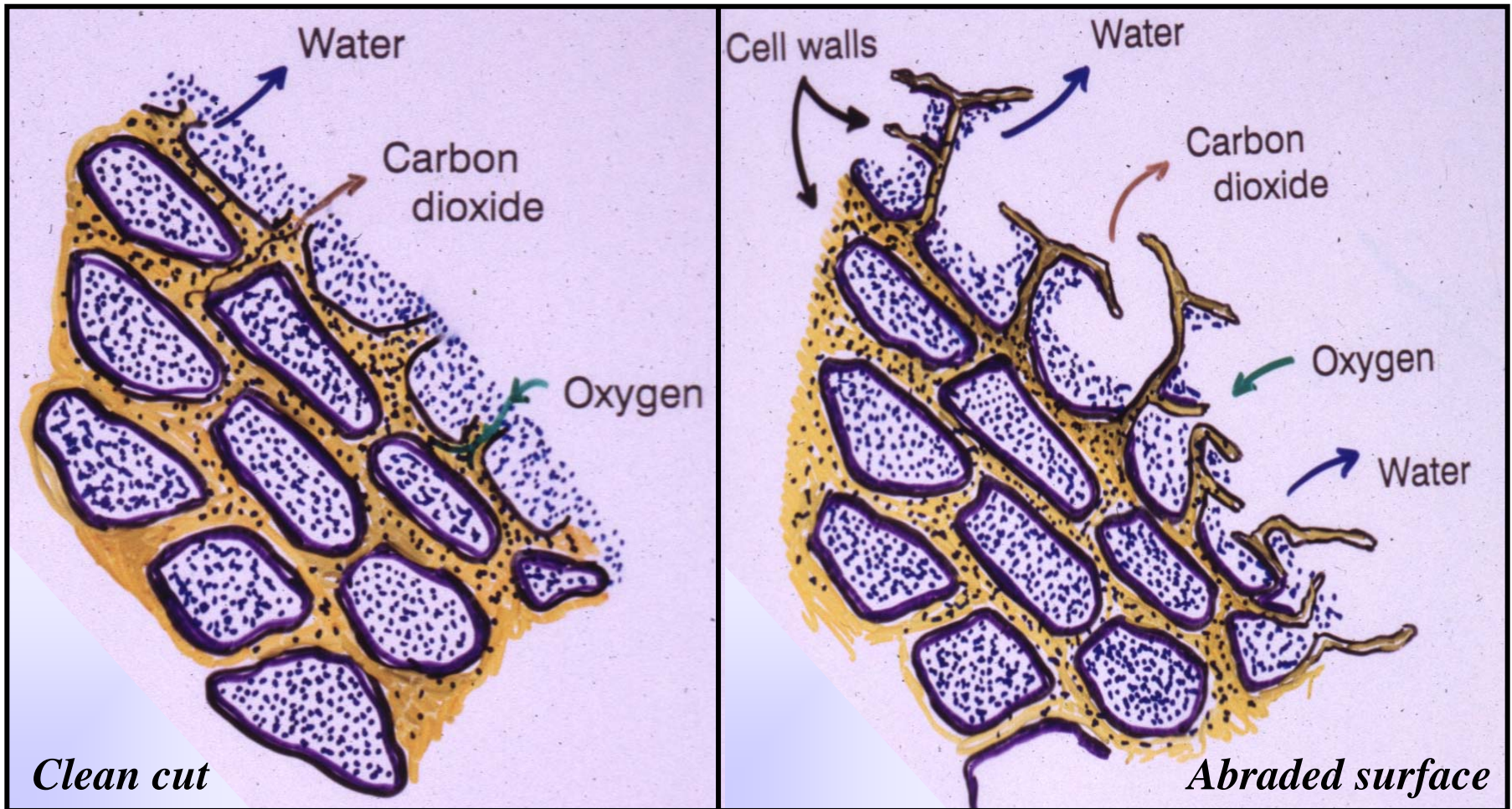


Notice surface
debris



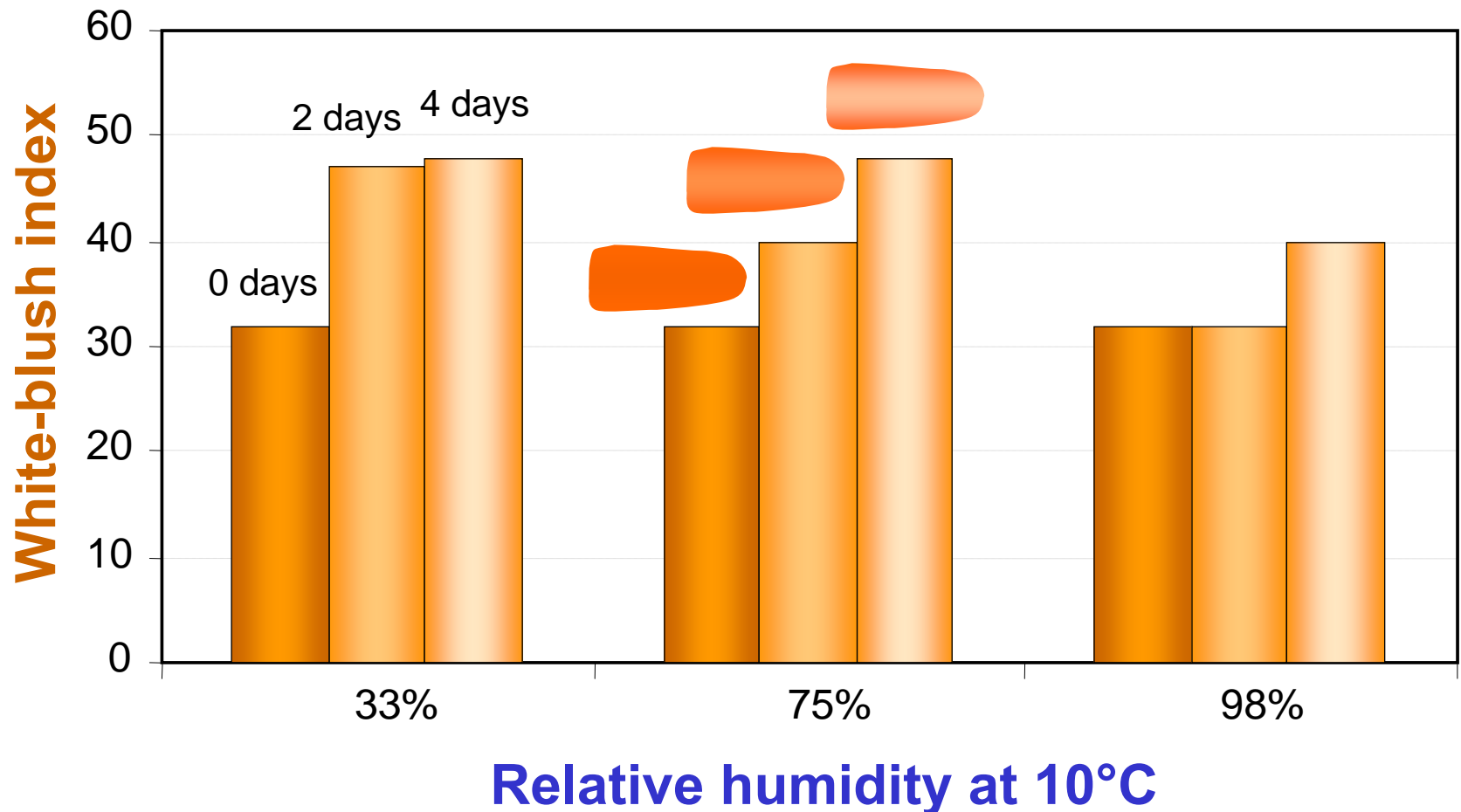


Cellular debris (cell wall remnants) on the cut surface

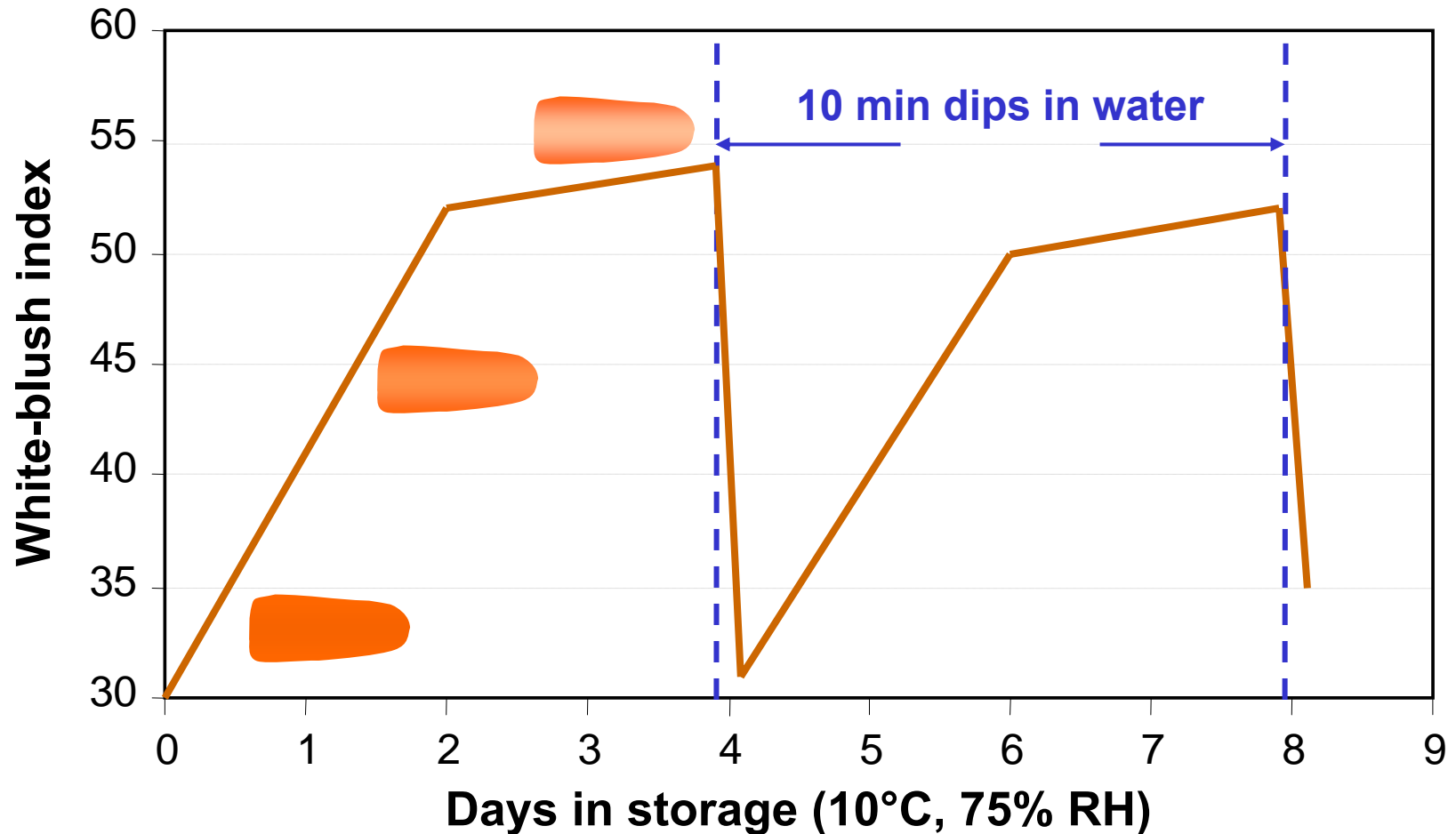


Abrading the carrot's surface leaves cellular debris that forms the white-blush when adhering moisture is lost

White-blush develops more rapidly when the fresh-cut carrot pieces are stored at a low relative humidity



Re-wetting the carrot's surface reverses white-blush the development on fresh-cut carrot pieces



*Water on the surface of 'baby' carrots
prevents white-blush development*



White-blush can develop during storage

Movement of water from respiring carrots to the inner surface of a closed bag can remove enough water to produce white-blush.

Moisture on the carrot's surface reduces white-blush formation during storage and marketing



Notice water droplets

Package of fresh-cut made from a thin plastic film

Cold room at 0 °C

Warm air, 100% RH

Heat water of vaporization carries off water vapor and heat energy

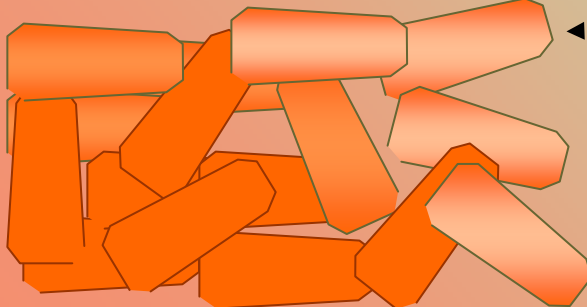
Heat released during the condensation of water vapor on the cold surface of the film moves heat out of the package, but the water droplets remain

Heat

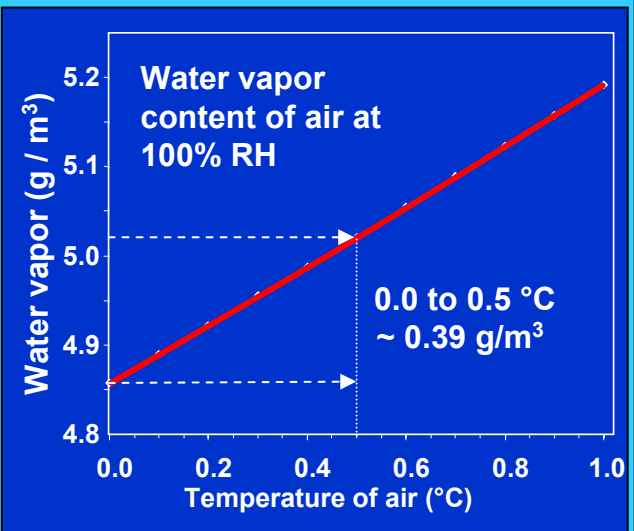
Warm air

Cold air

Heat of respiration



Cold air, 100% RH



Celery petiole segments

**Why aren't celery petiole
segments as popular as 'baby'
carrots?**

Problems and Solutions with Celery Petiole Segments

Problem

Solution

Pithiness

Sort by density

Flaring of cut ends

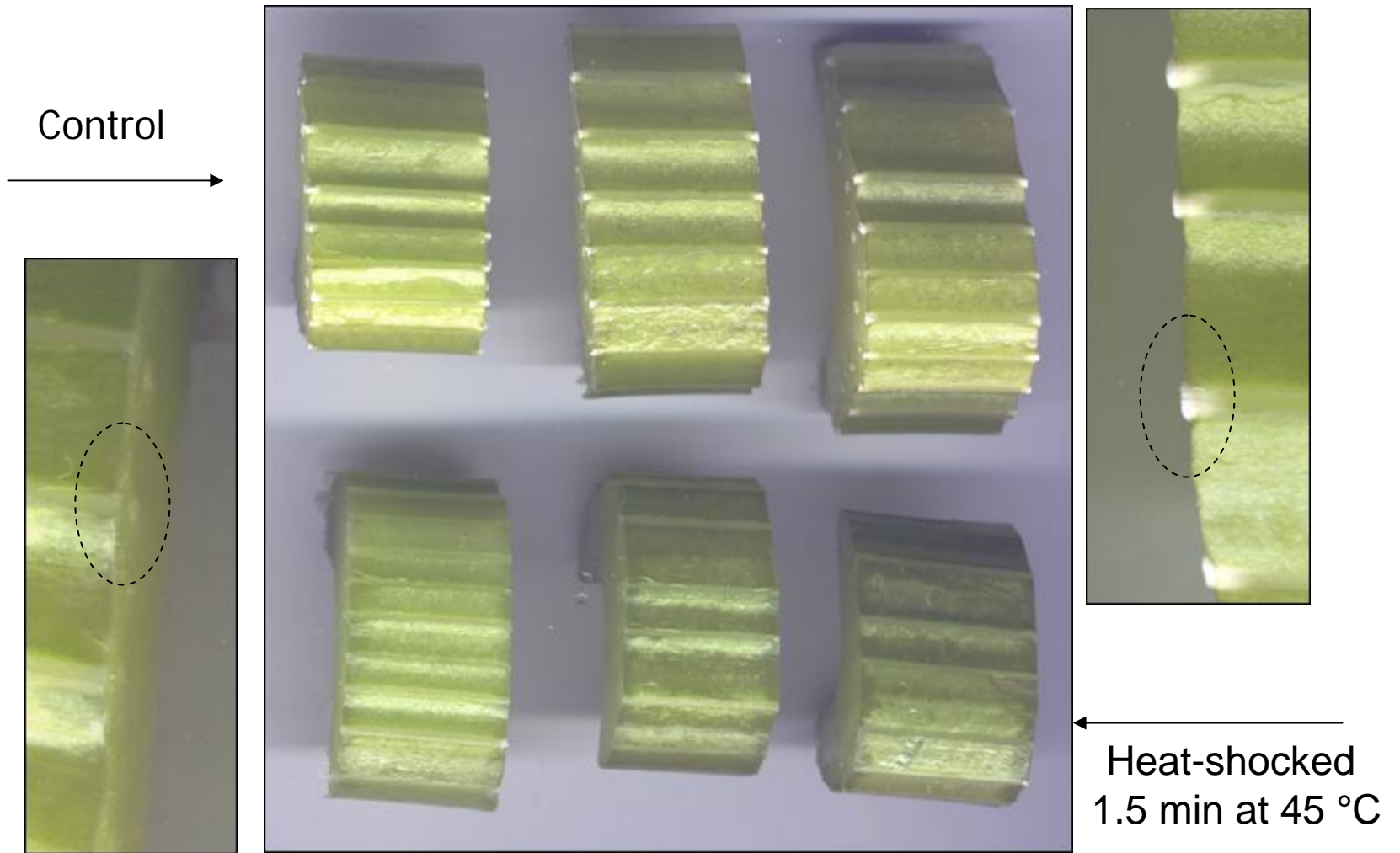
Edible coatings

Protruding vascular bundles

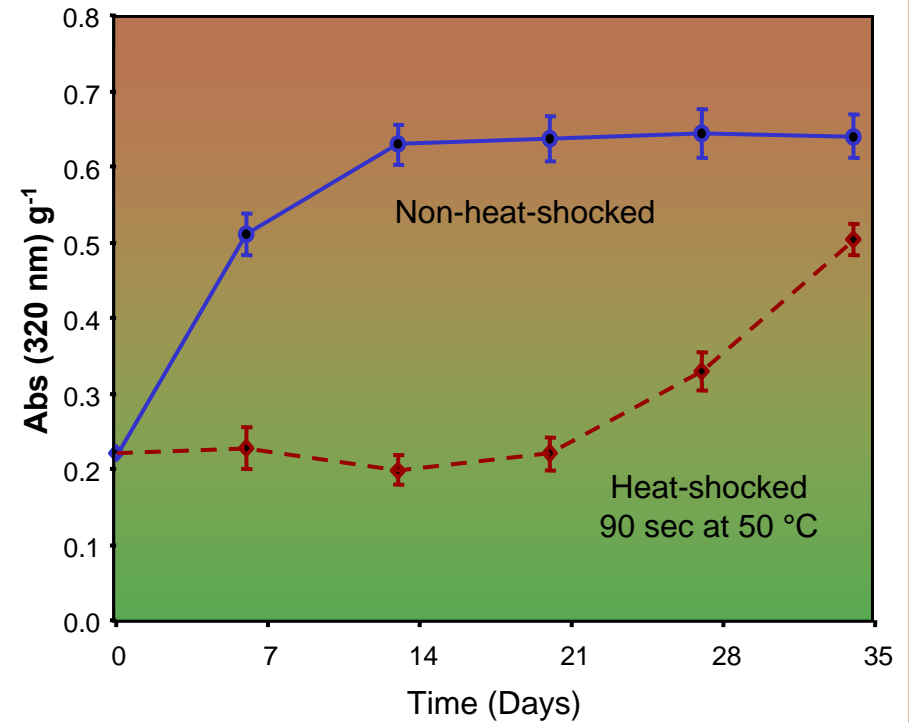
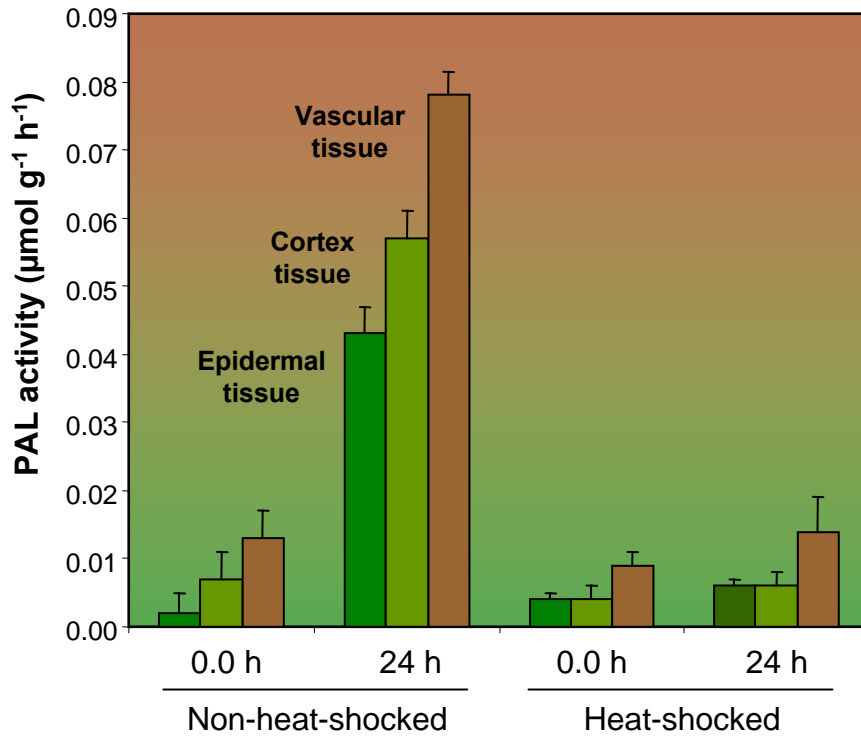
Heat-shock

Tissue browning

Heat-shock



Excised celery petioles stored for 2 week at 0 °C



Celery petioles heat-shocked at 50 °C for 90 sec, and stored at 0 °C.

Exposing excised celery petioles to a heat-shock reduces the subsequent activity of wound-induced PAL and the accumulation of wound-induced phenolic compounds.

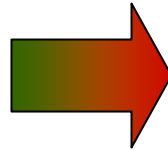
Seed germination in slices of ripened mature-green tomatoes

Many responses to wounding can produce unexpected and unwanted effects.

Ripening Slices of Mature-green Tomato Fruit



Mature-green tomato



Red-ripe tomato

Seeds germinated during ripening



Changing Internal Gas Concentrations can Produce Unexpected Effects

Altering water activity and internal gas composition can stimulate responses that were eliminated during domestication and breeding of commercial cultivars

Gas Exchange in Whole Commodities Takes Place Through Natural Openings

Openings in the epidermis

Stomata, hairs, trachomes, lenticels

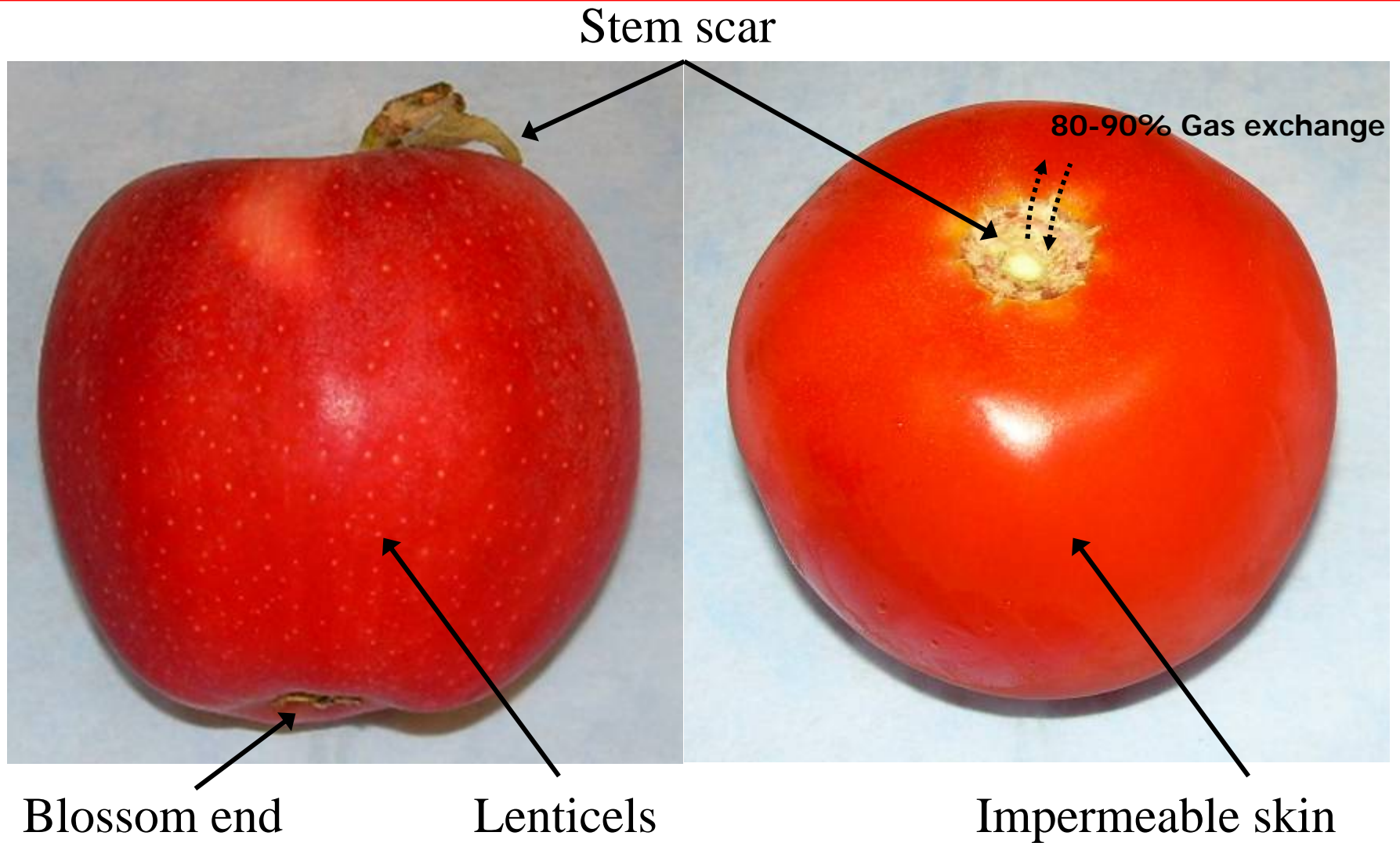
Openings at the stem or blossom ends

Stem scars, pericle, open calex

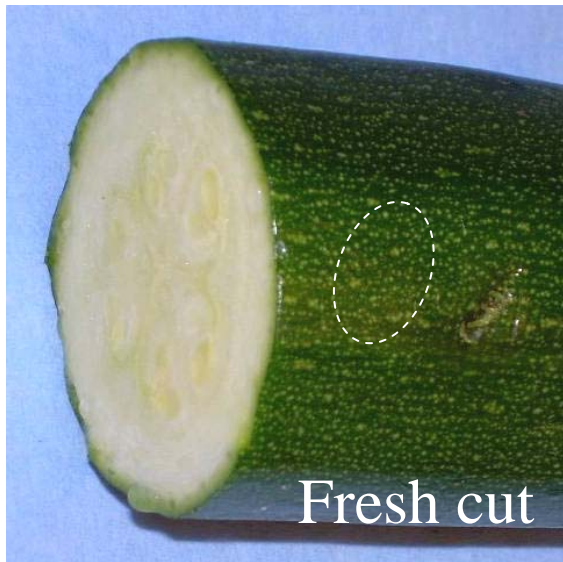
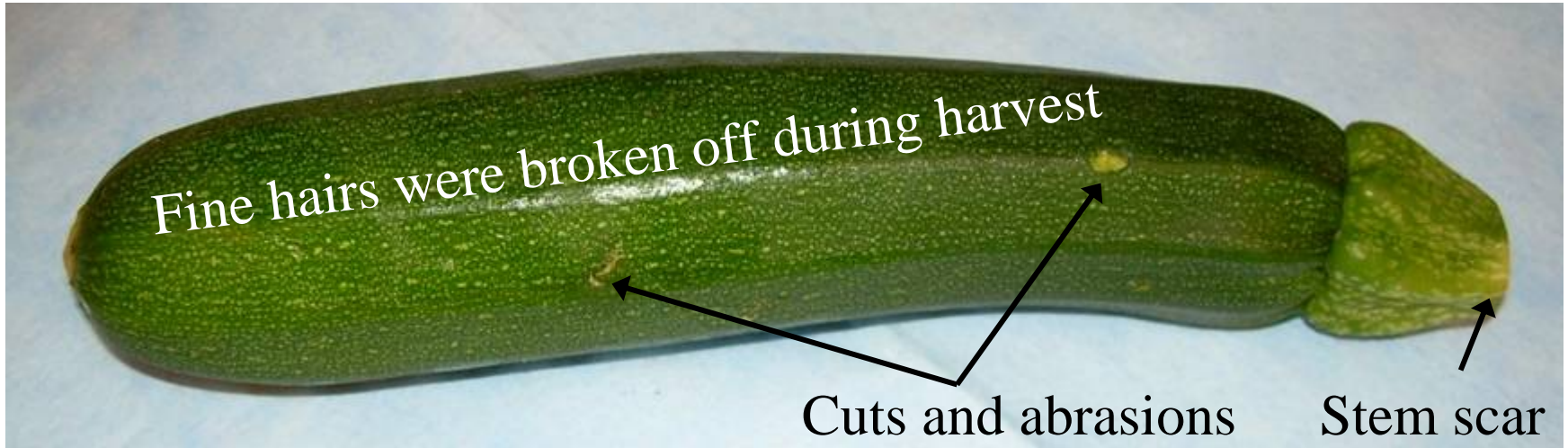
Injuries provide additional openings

Unrestricted gas movement

Gas Exchange



Sites of Gas Exchange and Water Loss



Water
loss



Wounding Greatly Alters Rates of Gas Exchange

Removal of the epidermal layer and blocking the surface of the tissue with rinse water has

TWO EFFECTS

First; it inhibits gas diffusion

(gas moves through air 10,000 times faster than through water)

_____ and _____

Second; it enhances gas diffusion

(the unprotected surface is exposed as the liquid is re-absorbed or evaporates)

Immediate and Subsequent Physical Effects

- *Mechanical shock to tissue*

- Bruises, cracks, fractures in tissue
 - Hydraulic shocks propagate through tissue

- *Removal of cuticle and epidermis*

- Alter gas diffusion
 - Increase or decrease of
 - Carbon dioxide levels
 - Oxygen levels
 - Accelerated water loss
 - Entry of contaminants
 - Loss of structural integrity

- *Elimination of natural barriers*

- *Surface debris changes appearance*

- White blush formation

- *Liquid on cut surface blocks pores*

- Reduced gas diffusion
 - Elevated CO₂, Reduced O₂
 - Accelerates water loss
 - Provides substrate for microbes

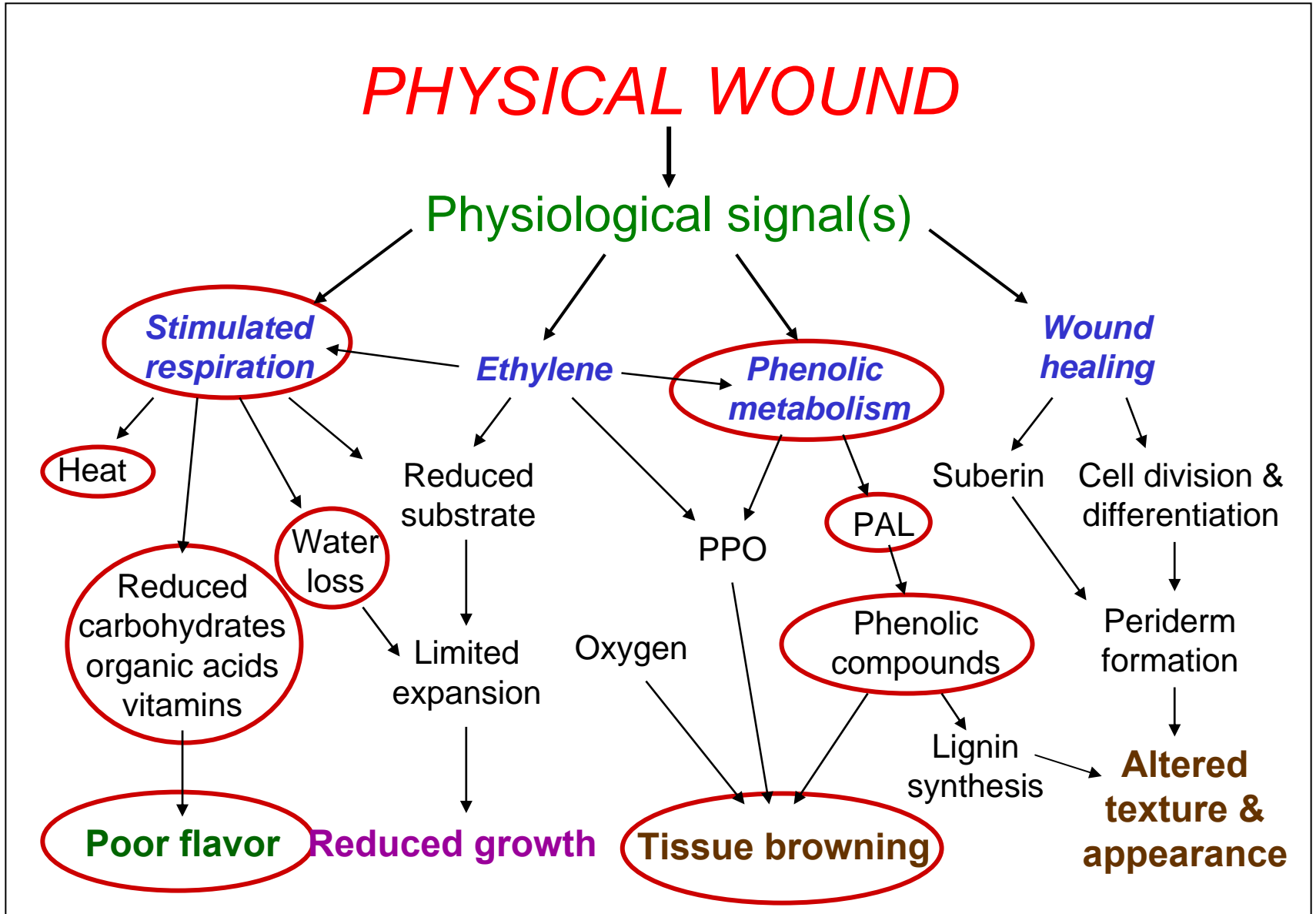
- *Exposure to contaminants*

- Microbes, pesticides, dust, etc.

Subsequent Biochemical and Physiological Changes

- **Wound signal (nature, speed)**
Hormone (traumatin), Wall fragment
Hydraulic change, Bioelectrical wave
- **Membrane depolarization**
Increased permeability
Mixing of cellular compounds
Calcium & signal transduction
Vacuole contents
- **Loss of protoplasmic streaming**
- **Membrane disorganized**
Lipids oxidized
Free fatty acids produced
- **Elevated respiration**
CO₂, heat production, O₂ consumption
- **Oxidative reactions**
Non-respiratory O₂ consumption,
phenolic oxidation and tissue browning
- **Compositional changes**
Polysaccharides, organic acids, aroma,
sugar to acid balance, loss of flavor,
toughening
- **Induction of wound healing**
- **Induction of enzymes**
- **Altered protein synthesis**
- **Altered phenolic metabolism**
Phytoalexins, browning substrates
- **Elevated ethylene production**

PHYSICAL WOUND



Wounding Stimulates Respiration and Tissue Browning

Increased respiration reduces quality and shelf-life by:

Increasing metabolism of stored food

Increasing heat production

Increasing water loss

Increased tissue browning reduces quality and shelf-life by:

Increasing phenolic metabolism and oxidation

Increasing production of brown pigments

Tissue browning and reduced quality

Reducing Wound-induced increased in Respiration

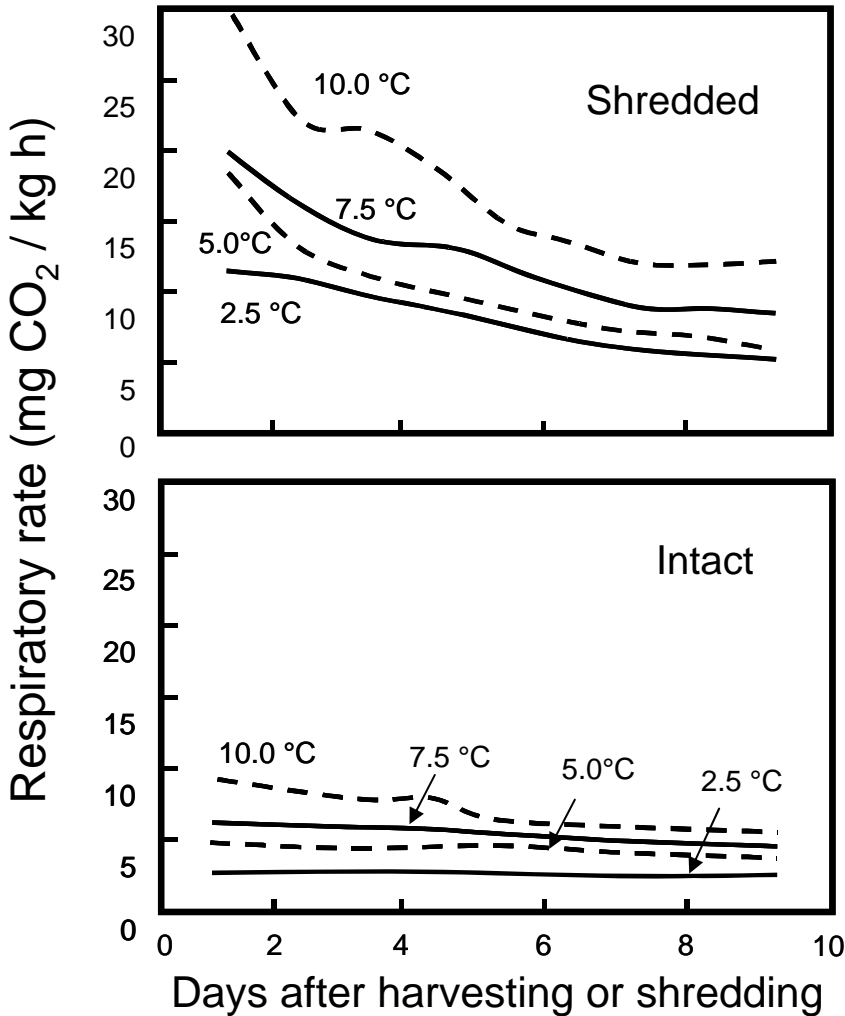
Wound-induced increases in respiration:

Are usually short lived
(can adversely affect MA and MAP)

Can be reduced by cold temperatures

Chilling injury is less important in fresh-cut
(rapid use, fully ripe),
so even chilling sensitive tissue can be stored near 0°C

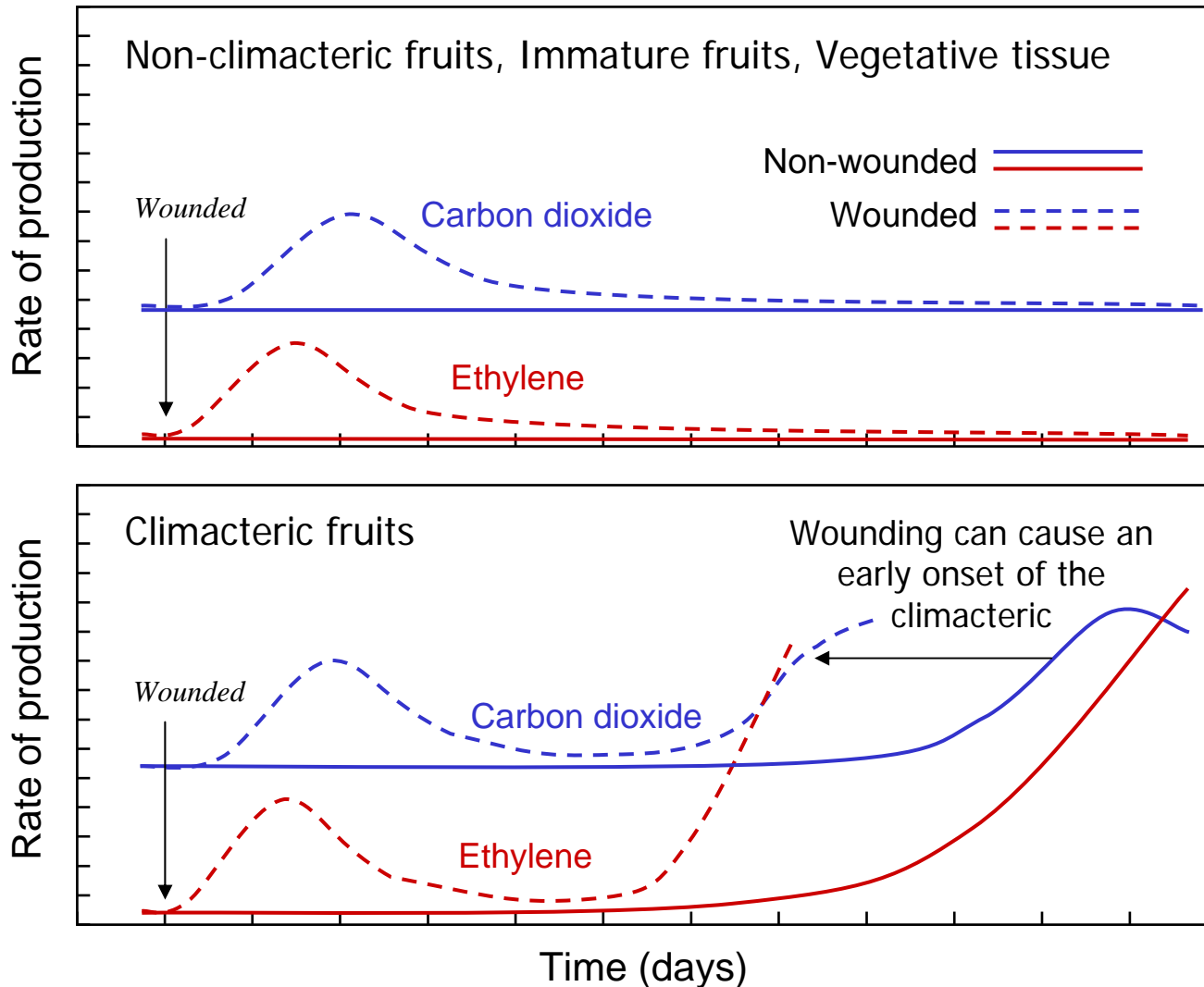
Wound-induced increases in respiration are usually short lived



Respiration rates of intact and shredded cabbage stored at four temperatures (Cantwell, 1991).

Wounding greatly increased the rate of respiration, but it declined with time in storage

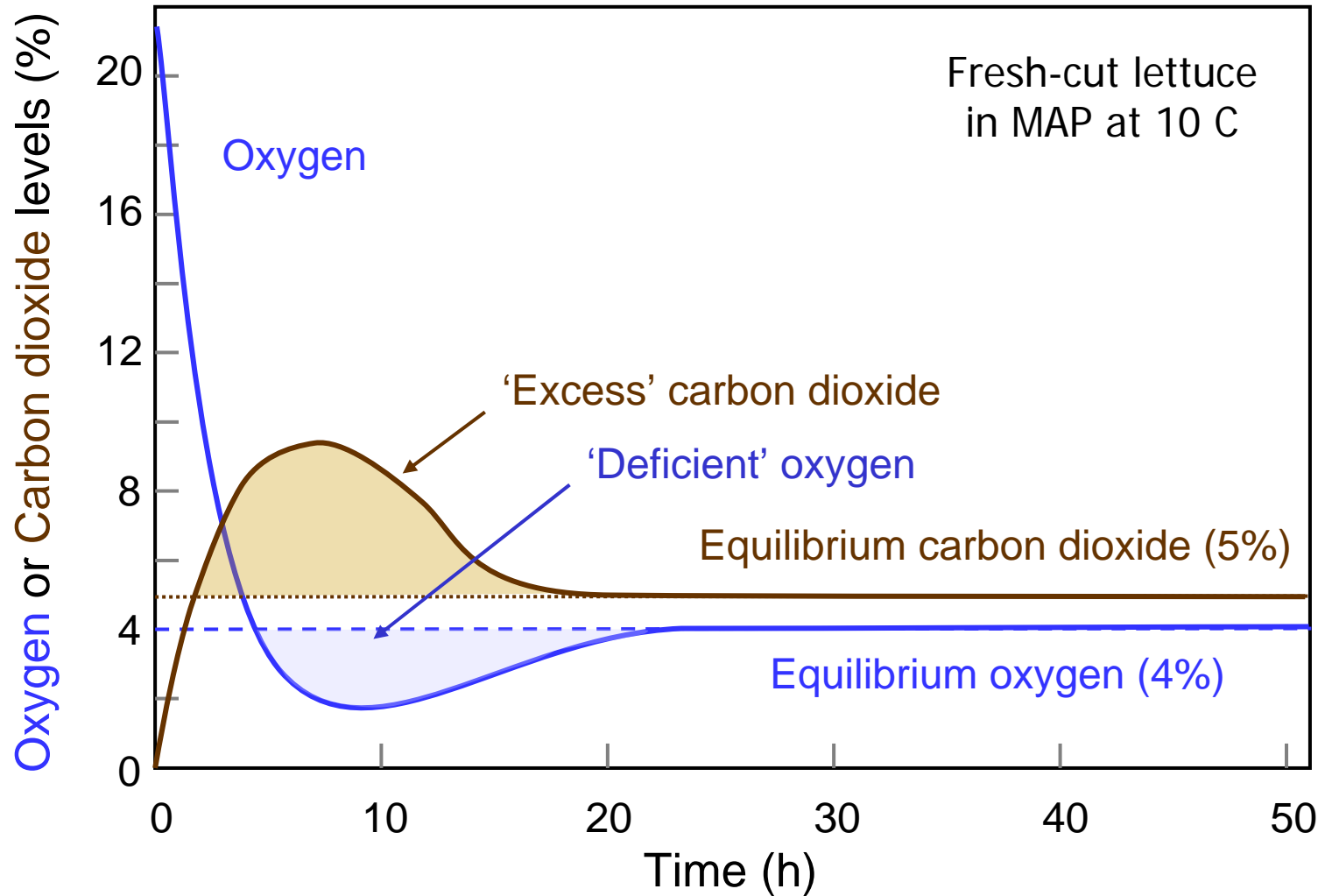
Effect of Wounding on the Respiratory Climacteric



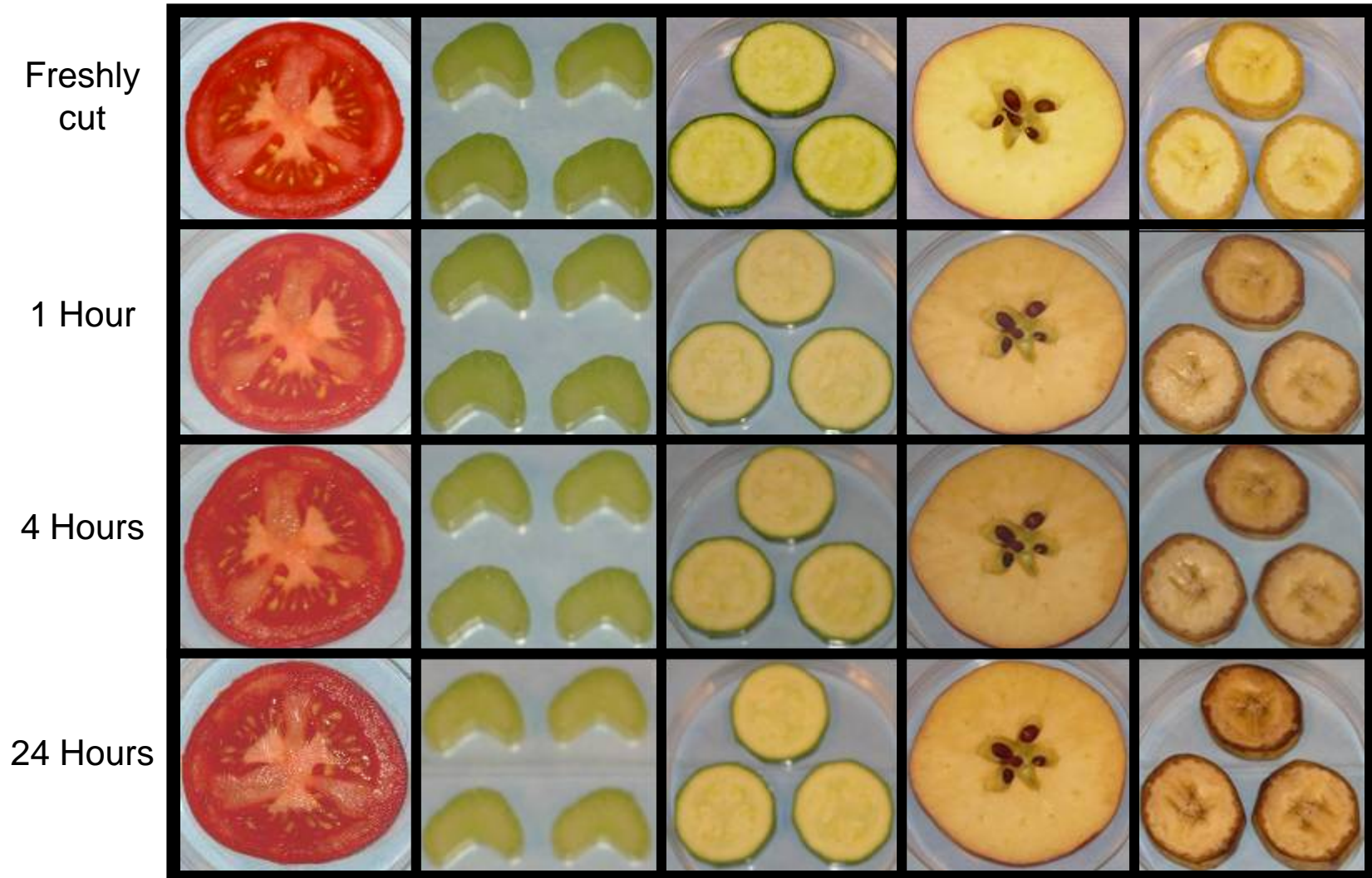
Variations in Respiration Complicate Designing Modified Atmosphere Packaging

- Modified Atmosphere Packages (MAP) are designed so respiration of the commodity establishes and maintains the desired atmospheric composition of oxygen and carbon dioxide within the package.
- Plastic films, package dimensions, and product amounts are combined to produce a package that will establish and maintain the desired atmosphere.
- Plastic films that have the correct gas permeability to maintain an establish atmosphere when tissue is respiring at a constant rate will be inadequate to accommodate the large burst of respiration following wounding.
- Oxygen levels may initially decline, and carbon dioxide levels may initially increase to injurious levels before equilibrium is established.
- The rapid increase, and later decline in respiration makes it very difficult to design MAP packages for fresh-cut produce.

Respiration of Wounded Tissue in MAP



Color changes after cutting



Not all tissues respond similarly to wound-induced browning

Tissues that brown rapidly have:

High levels of phenolic compounds
Low levels of antioxidants
Adequate PPO, POD activity
'Leaky' membranes

Tissues that brown slowly have:

Low levels of phenolic compounds
High levels of antioxidants
Low levels of PPO, POD activity
Intact membranes

Different procedures are used to prevent browning in the two tissue types

Reduce levels of phenolic compounds
(genetic selection, cultural practices)
Supply antioxidants, exclude oxygen
Reduce PPO, POD activity
Increase membrane stability

Prevent phenolic accumulation
(reduce signal, enzyme, and
phenolic synthesis)
Increase membrane stability
Exclude oxygen

Wounds Induce Phenolic Metabolism Leads to Tissue Browning

Lettuce leaves usually have low levels of phenolic compounds and it takes a few days after wounding for the enzymes of phenolic metabolism to be synthesized, and for them to synthesize phenolic compounds that accumulate and subsequently cause tissue browning.



1

3

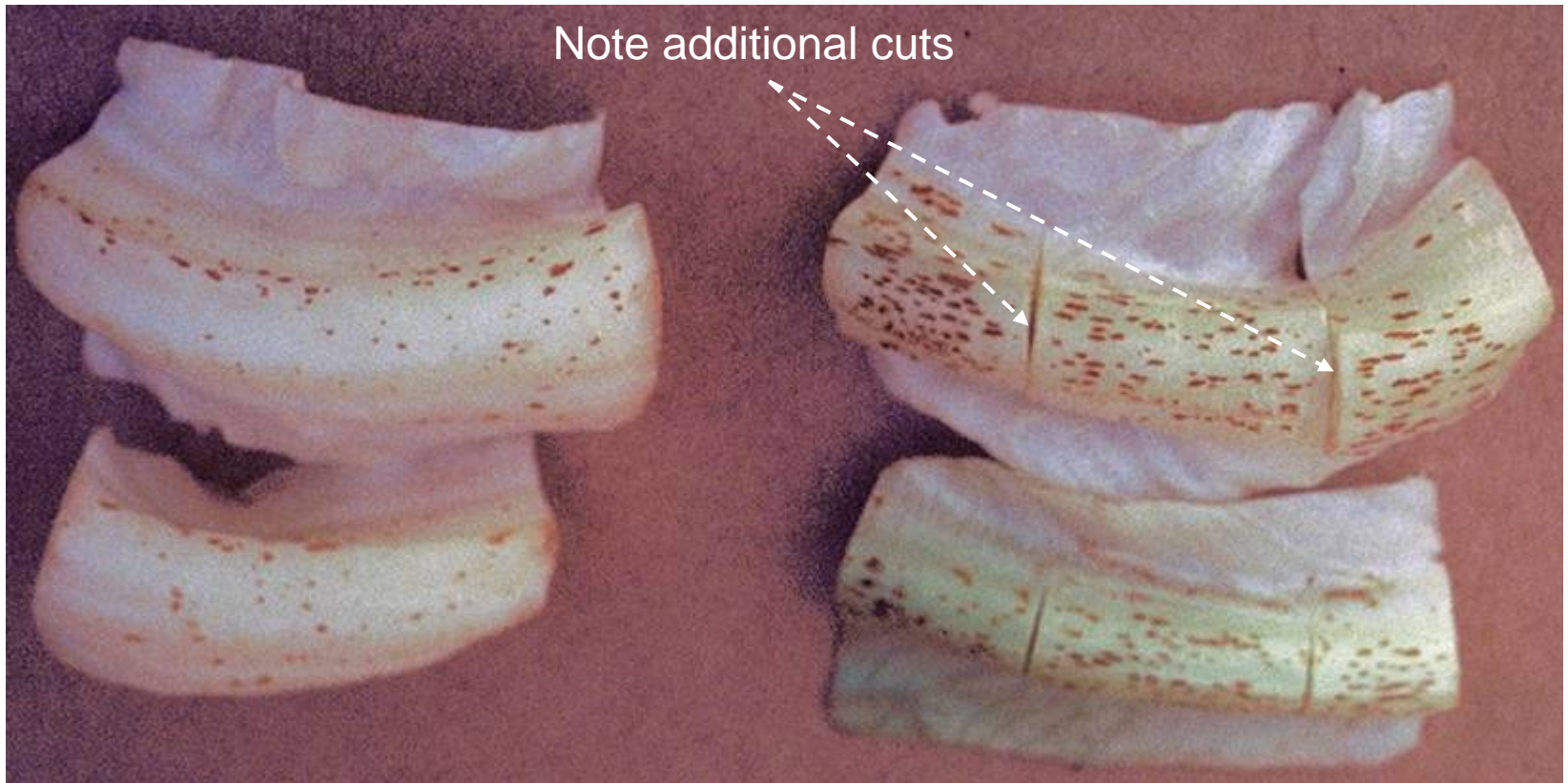
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Days

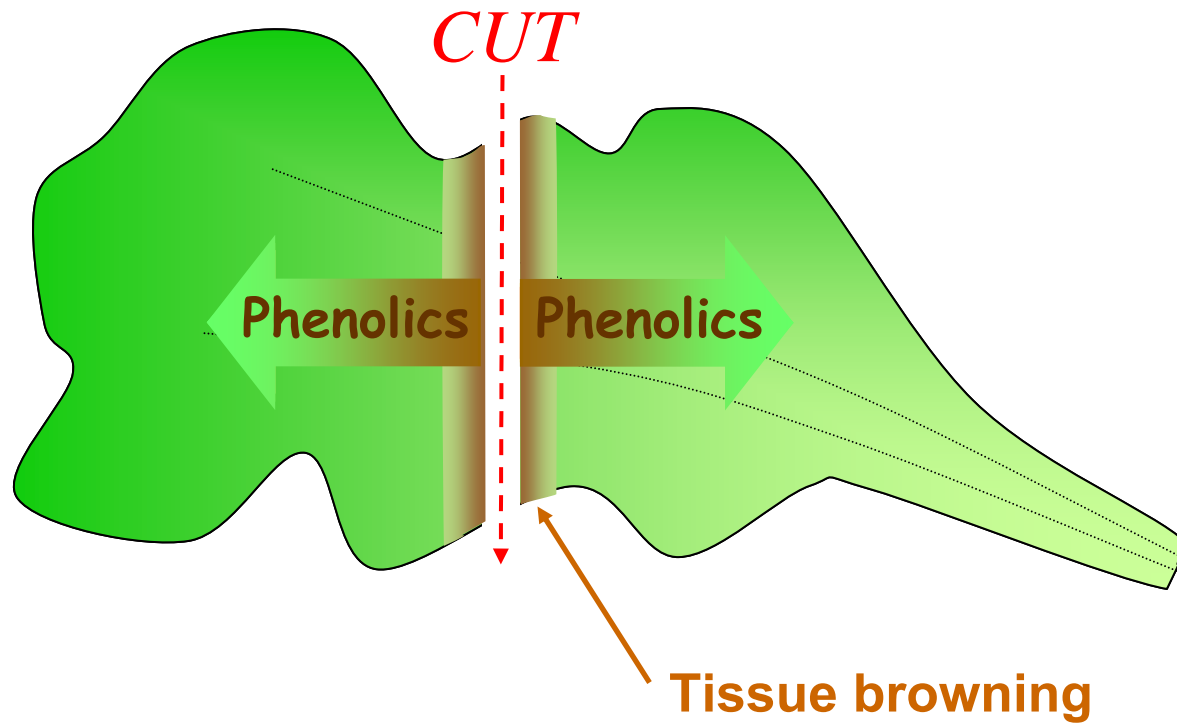
Wound responses of lettuce

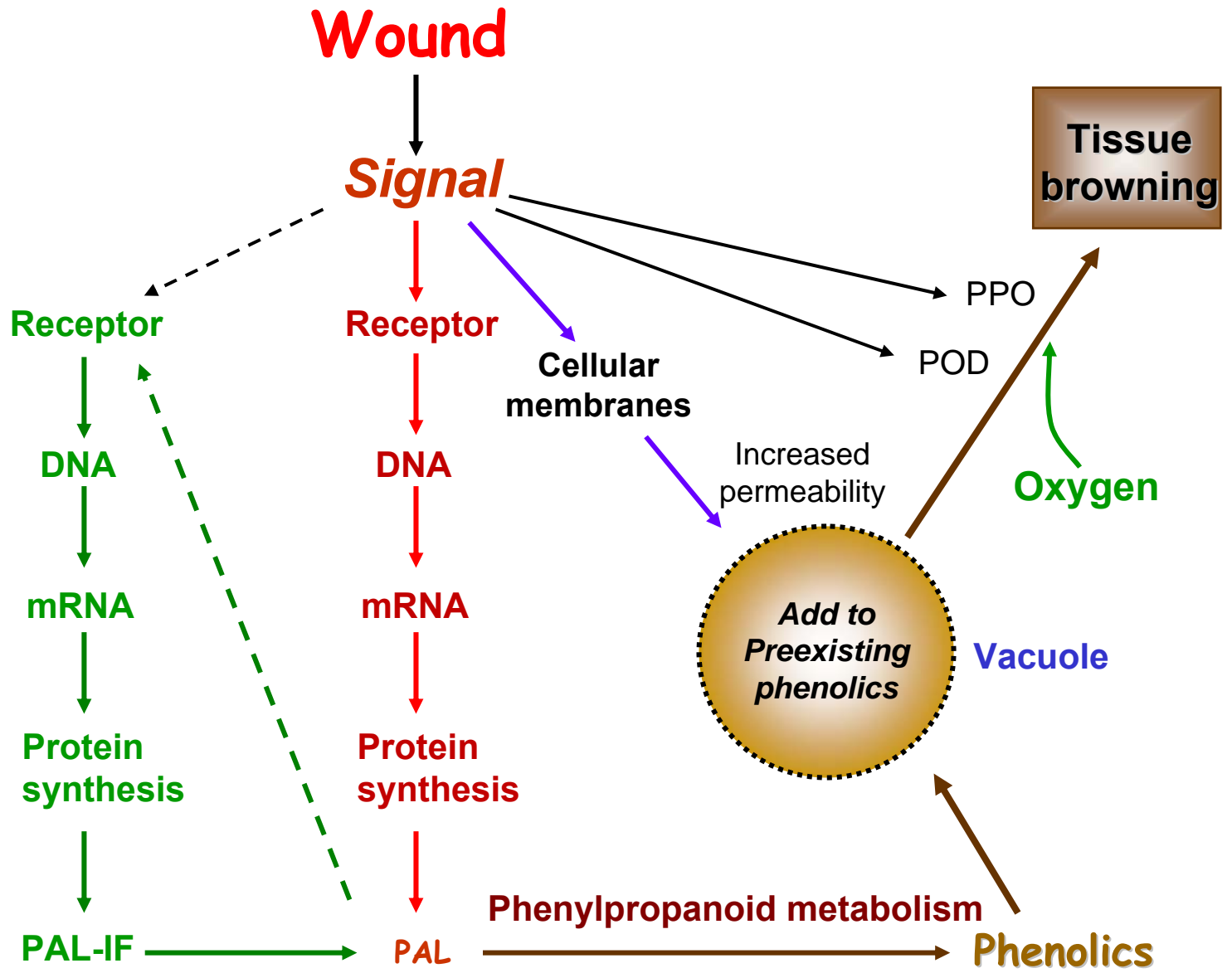
Wounding and ethylene both promote PAL activity and the accumulation of phenolic compounds and tissue browning.

However, the wound response does not appear to involve ethylene, but is initiated by a *de novo* synthesized wound signal that diffuses into adjacent tissue from the wound site and stimulates phenolic metabolism.



Russet spotting on whole or wounded lettuce mid-rib segments induced by exposure to 1.0 ppm ethylene in air at 5 °C. Wounding greatly increased the occurrence of russet spotting.

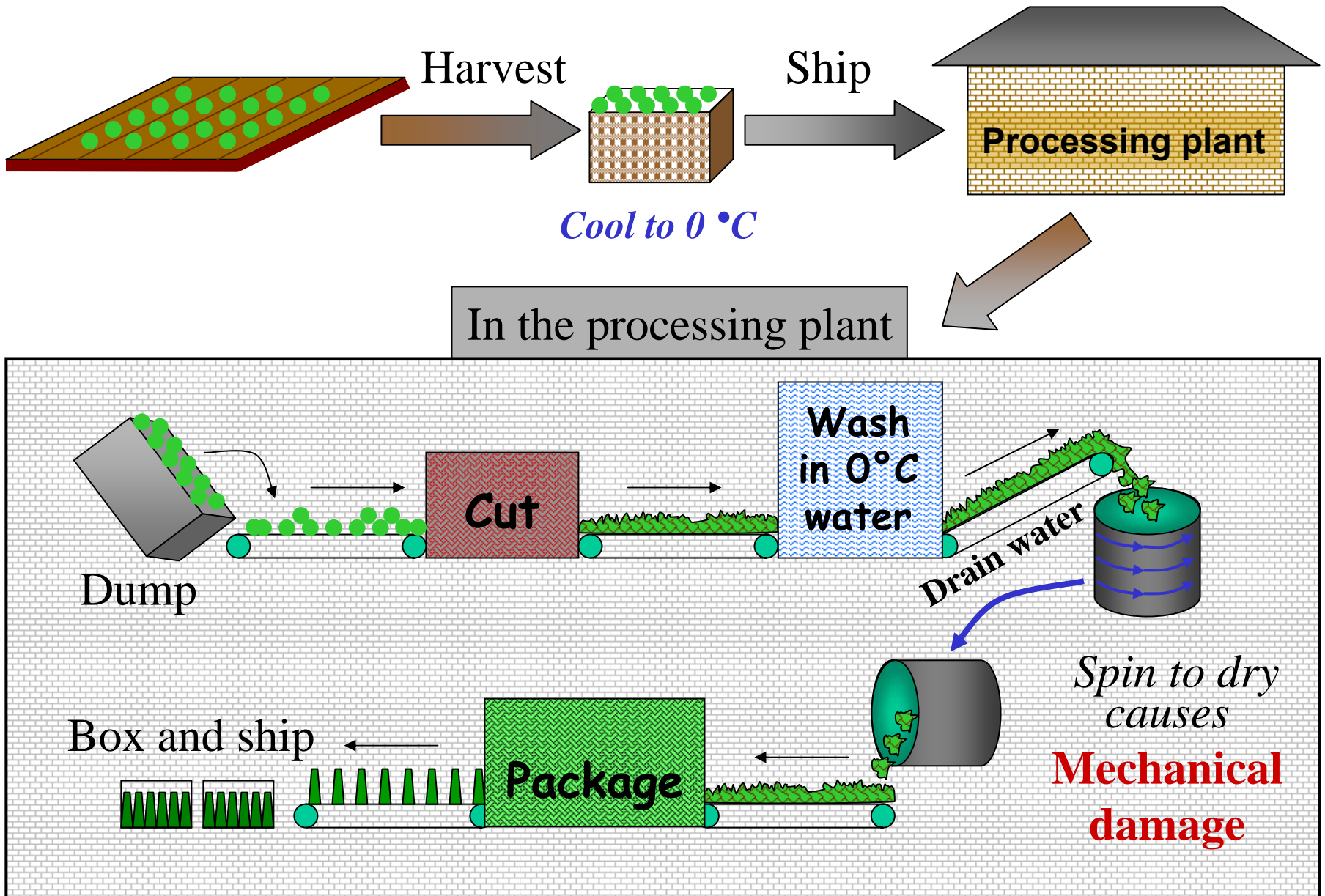




Controlling Browning in Lightly Processed Lettuce & Celery

- Minimize stress before & after harvest
- Use sharp knives during processing
- Market the product rapidly
- Store and ship at 0 °C
- Use packaging that excludes oxygen
- Increase carbon dioxide in package

There are Many Opportunities for Wounding



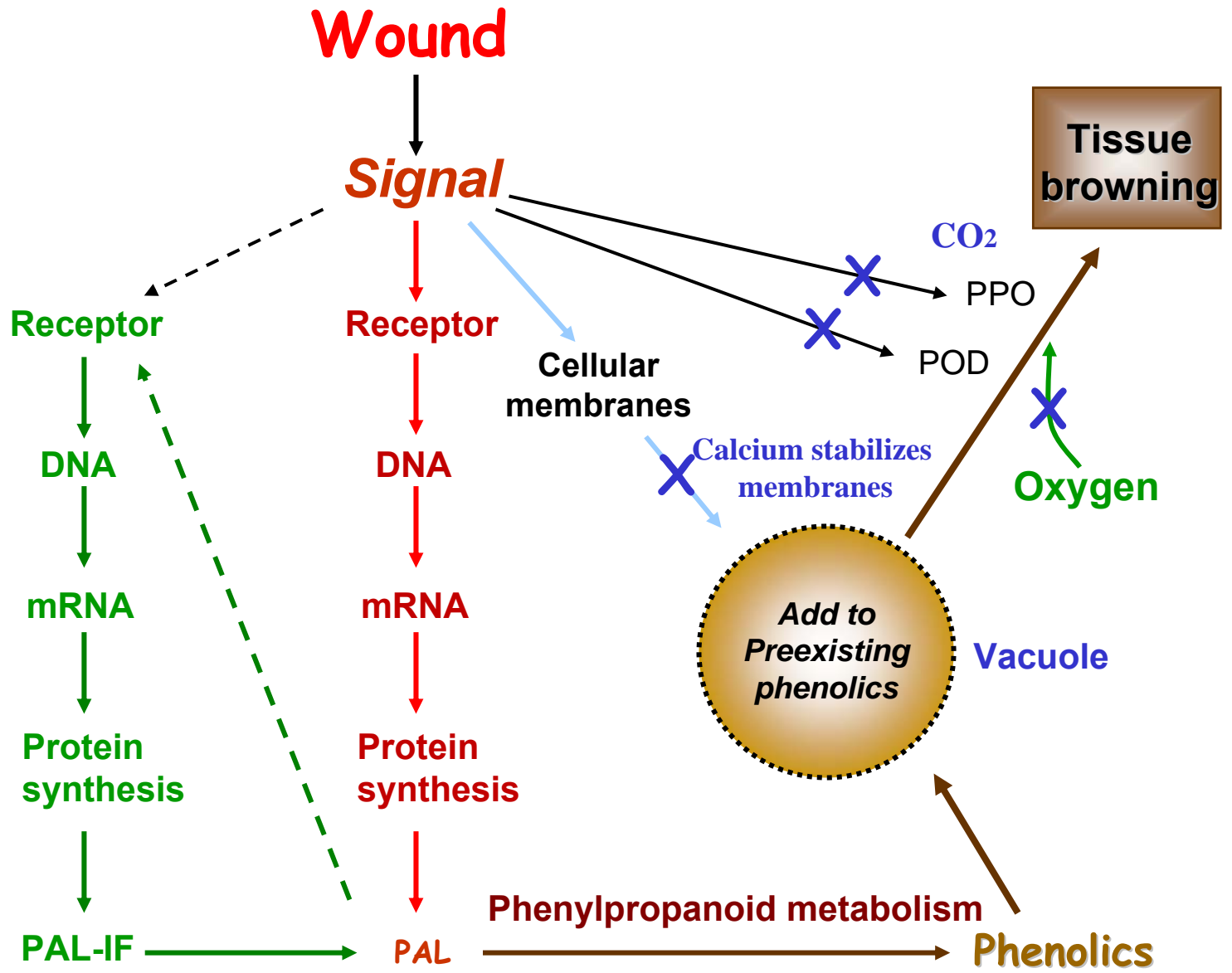
Wound-induced edge browning



Controlling Browning in Lightly Processed Lettuce & Celery

- Minimize stress before & after harvest
- Use sharp knives during processing
- Market the product rapidly
- Store and ship at 0 °C
- Use packaging that excludes oxygen
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Controlled or modified atmospheres



Ethylene Should be Minimized to Maintain Quality

Wounding can stimulate ethylene production and accumulation in enclosed spaces

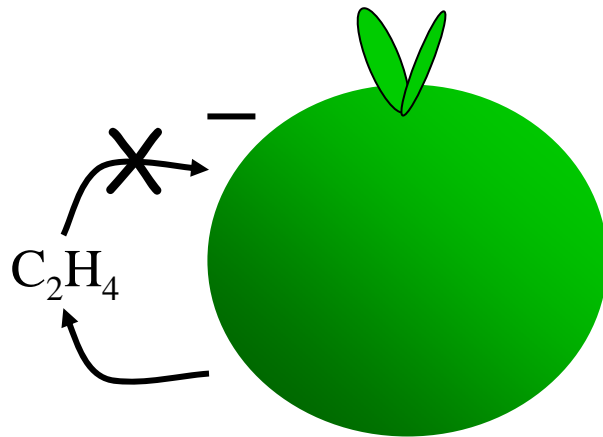
Low levels of ethylene promote the ripening of climacteric fruit and vegetables

Even small amounts of ethylene (e.g., $0.02 \mu\text{l}\cdot\text{l}^{-1}$) can adversely affect the storage life of non-climacteric vegetables

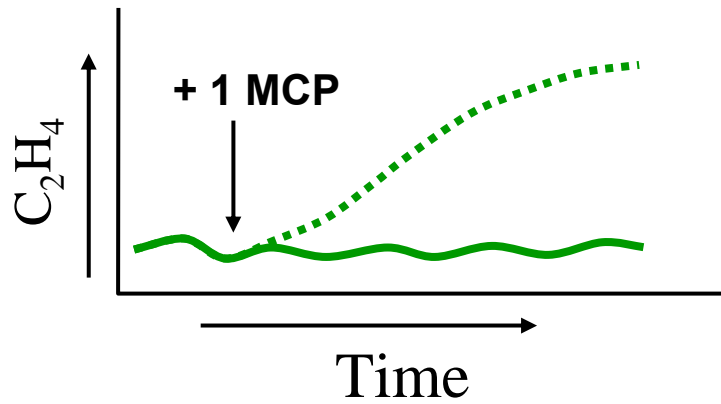
There is a **Positive** and a **Negative** Feedback of Ethylene on Ethylene Production

- Vegetable tissue and immature fruit have negative feedback, where ethylene inhibits its production
- **Mature and ripening fruit** have positive feedback, where ethylene promotes its production

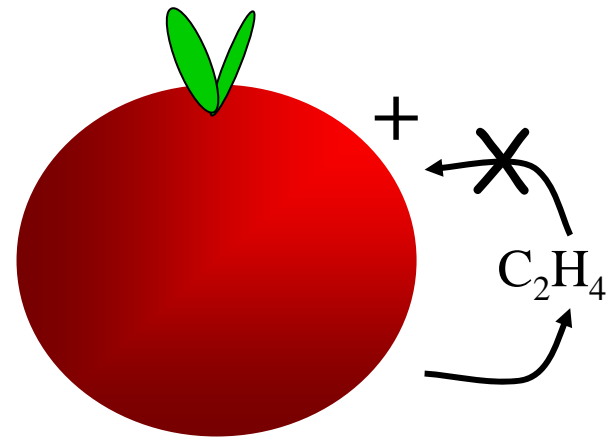
Negative feedback



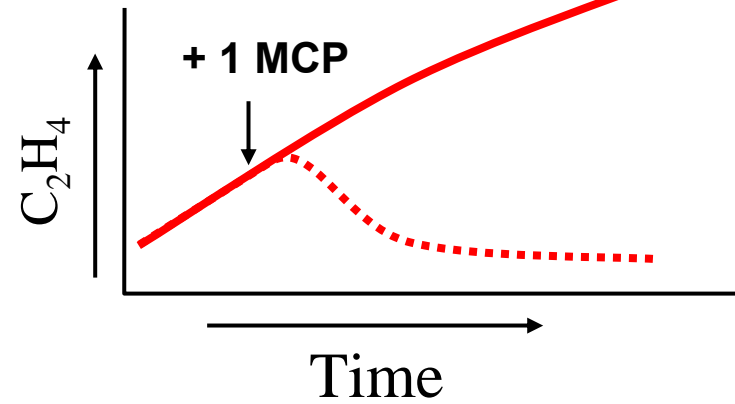
Immature



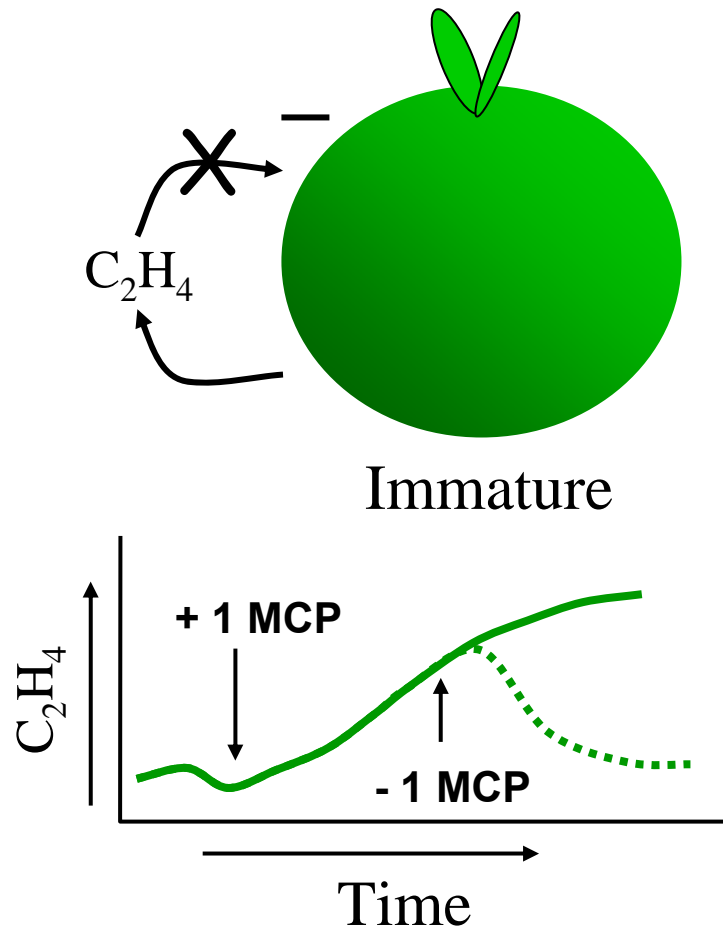
Positive feedback



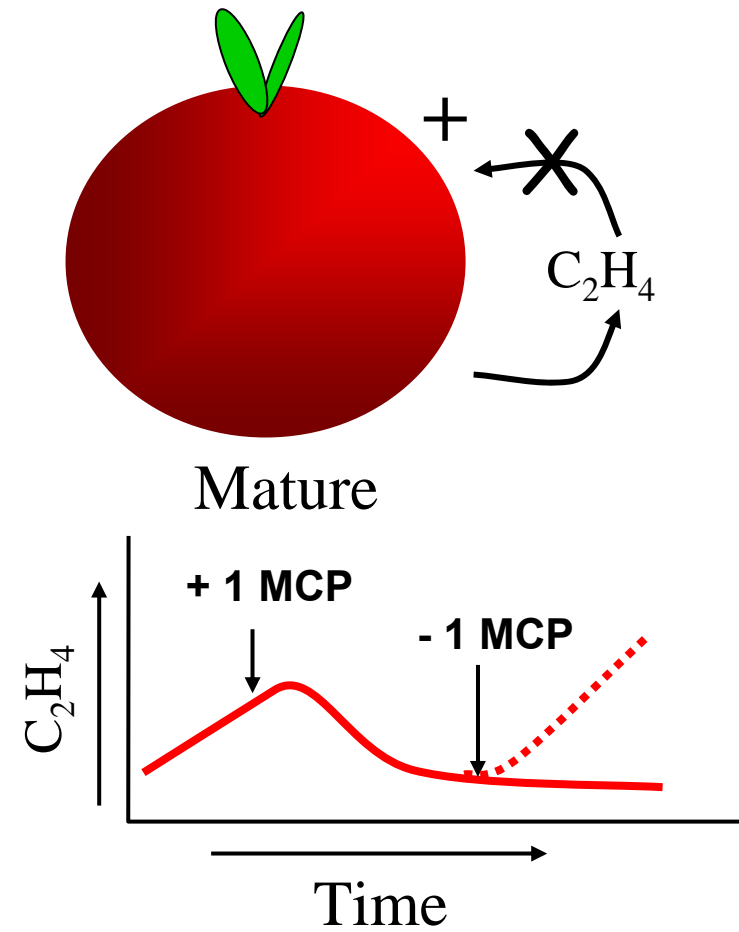
Mature



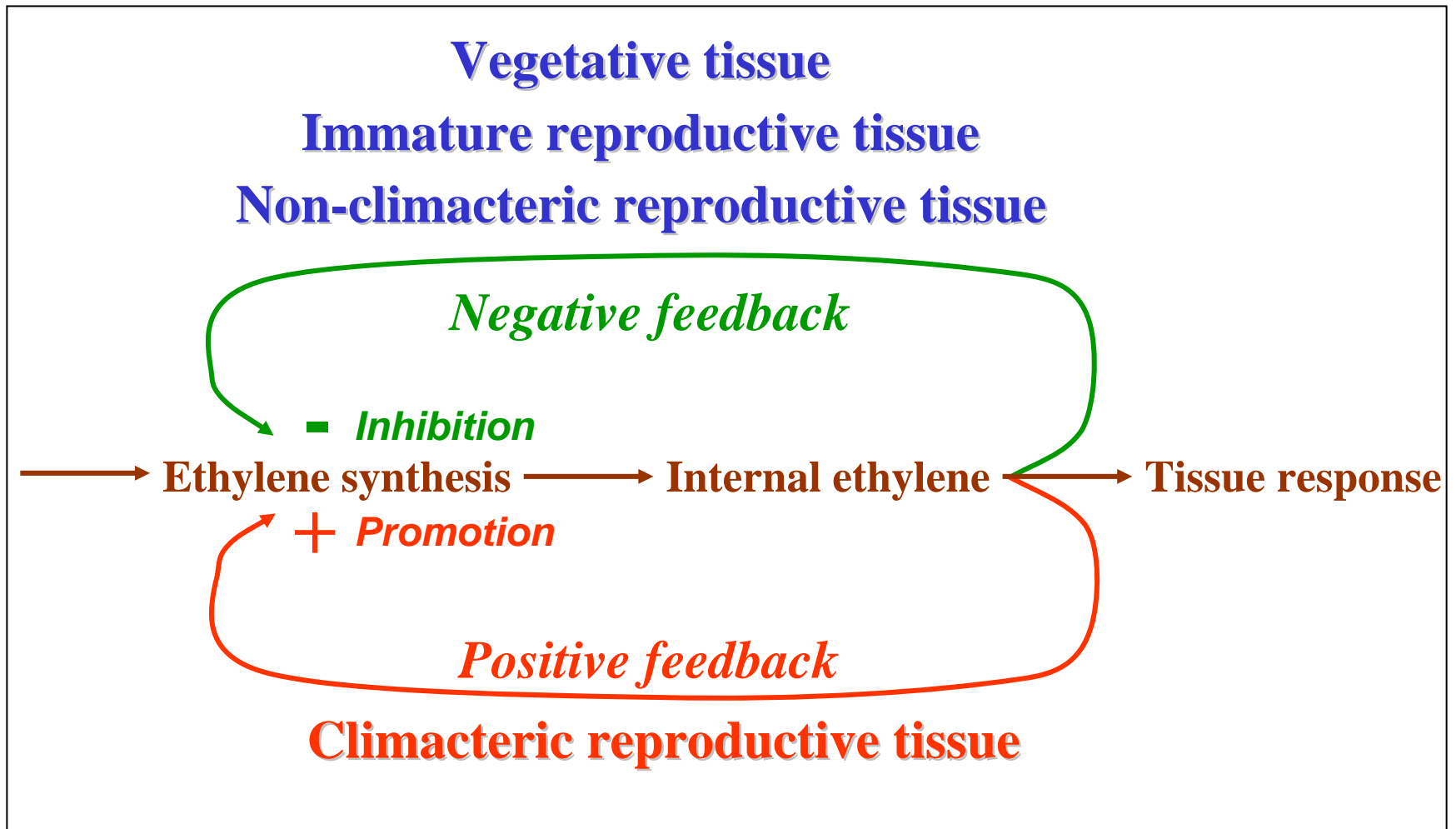
Negative feedback



Positive feedback



Feedback Control of Ethylene on Ethylene Production



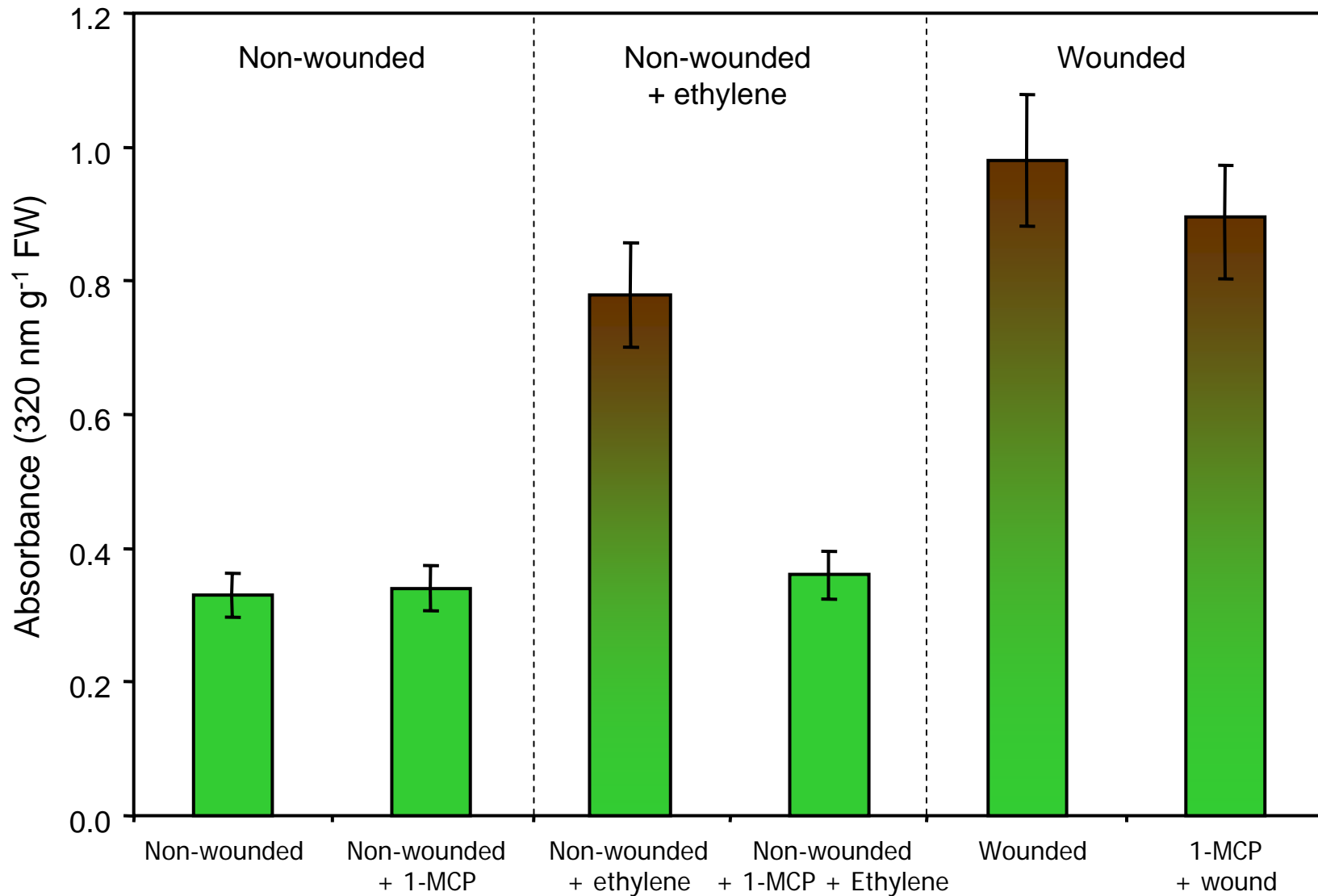
Controlling the effects of ethylene

Should ethylene be removed from the atmosphere, or should the tissue be made less sensitive to ethylene ?

- Ventilation may be impractical in CA or MA
- One of the beneficial effects of traditional CA is that low oxygen reduces ethylene synthesis, and low oxygen and elevated carbon dioxide reduces sensitivity to ethylene
- Pre-storage treatments such as heat-shock and 1-MCP can render the commodity insensitive to ethylene

Ethylene is not involved in all wound responses

- Negatively regulated in non-climacteric tissues
- Wound-induced increase is usually transitory
- PAL increased before inductive C₂H₄ rise
- Response even when C₂H₄ action blocked



Lettuce leaves of excised mid-rib tissue (wounded) treated with 1-MCP or ethylene and then held at 10 °C for 0 to 48 h before assaying phenolic content (Abs 320 nm/g FW).

Controlling Browning in Lightly Processed Lettuce & Celery

- Minimize stress before & after harvest
- Use sharp knives during processing
- Market the product rapidly
- Store and ship at 0 °C
- Use packaging that excludes oxygen
- Increase carbon dioxide in package

Are there other methods to control browning?

Through evolution plants acquired an impressive, but limited ability to respond to stresses

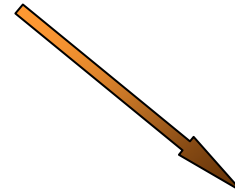
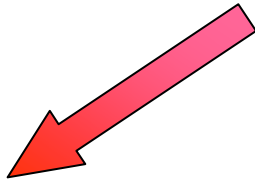
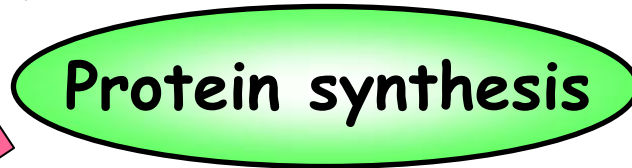
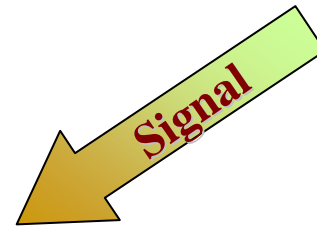
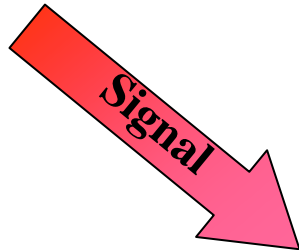
Some responses take precedence over others

The metabolic response induced by one stress (e.g., heat-shock) can therefore be used to redirected the response to another stress (e.g., wounding).

Heat-shock

**General
Metabolism**

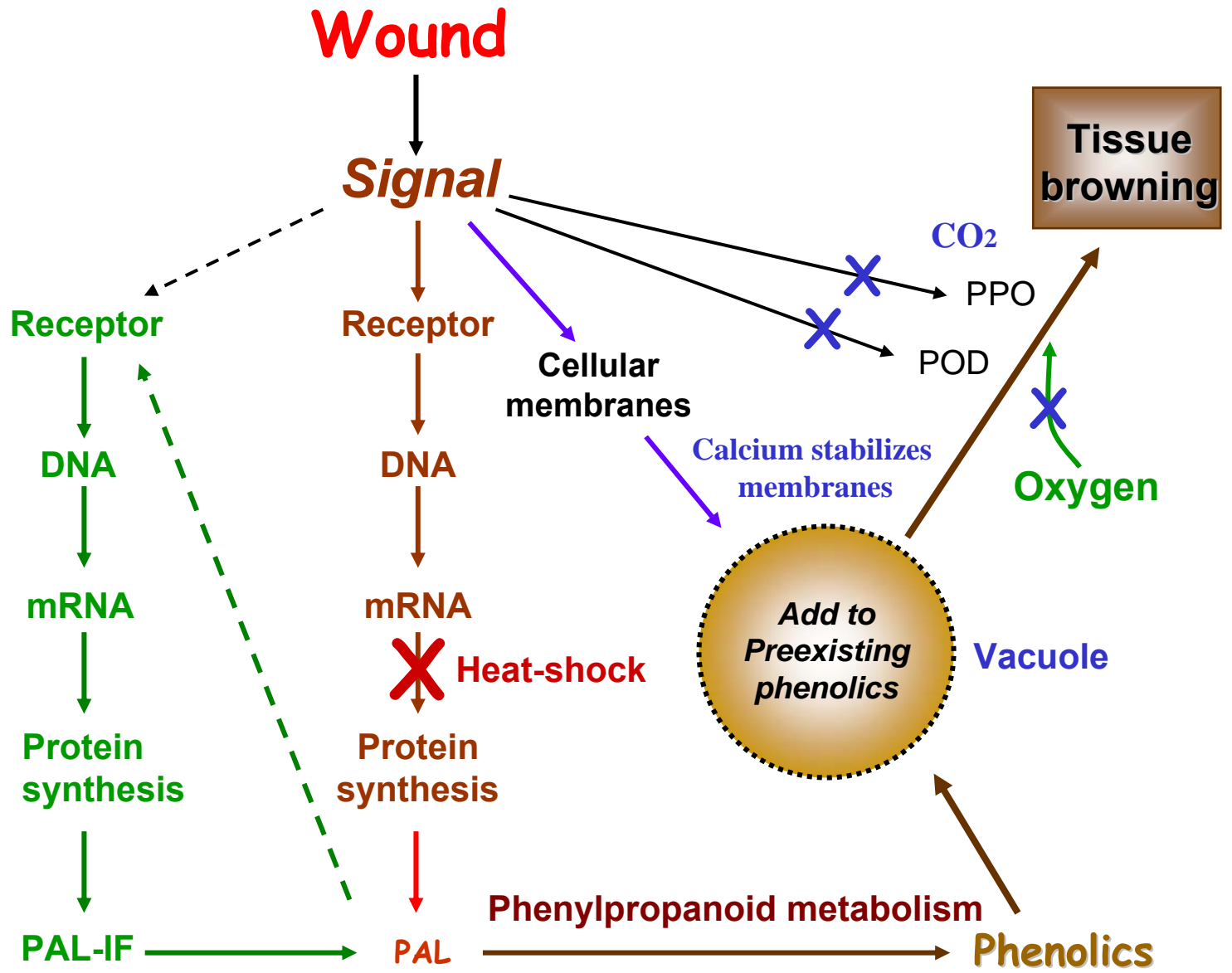
Wounding



**Heat-shock
proteins**

**Very Reduced Rate
of Protein Synthesis**

PAL



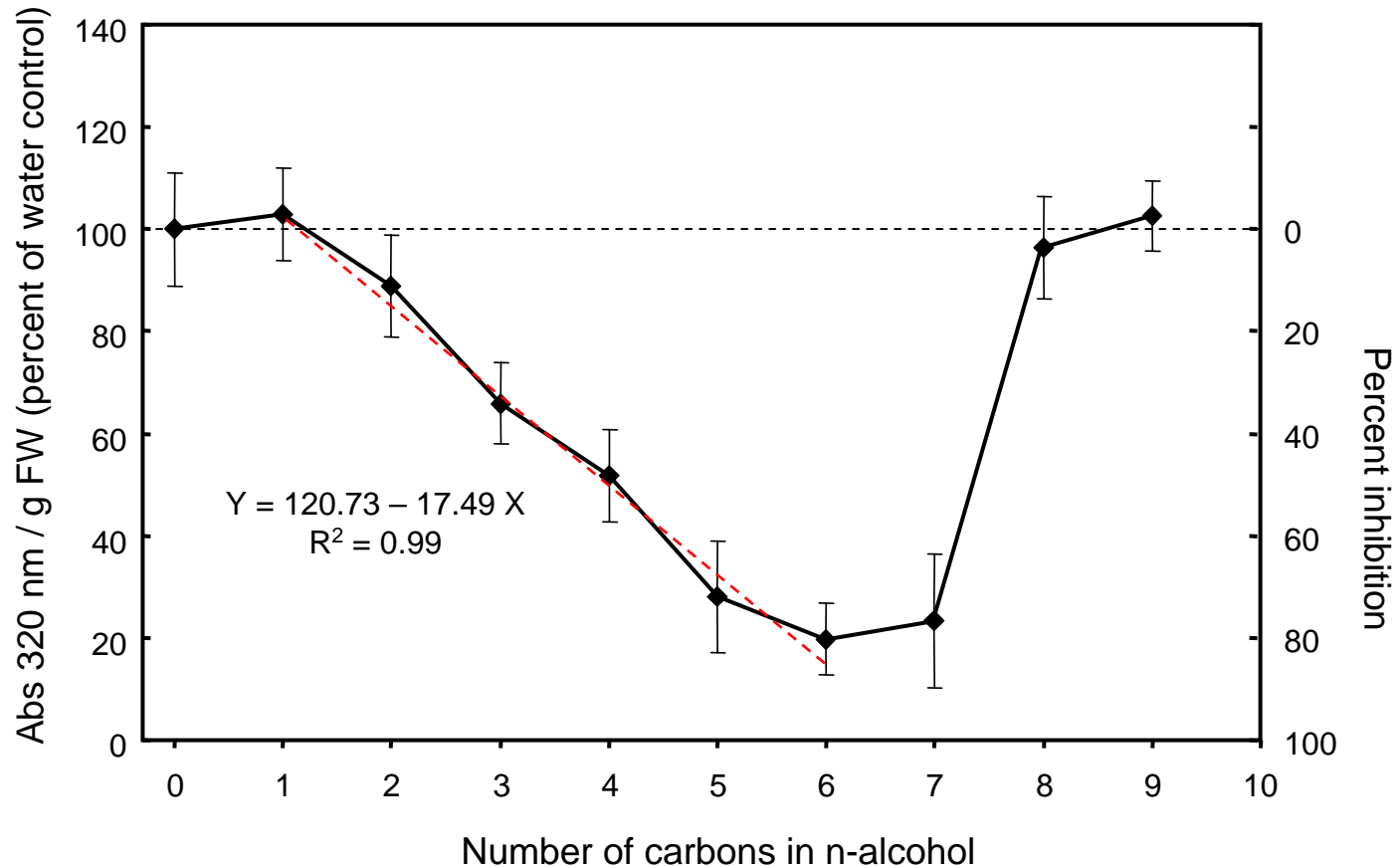
If ethylene does not
signal wound-induced
tissue browning

What Does?

Wounding Produces a Wound Signal

- Signal produced at site of injury
- Diffuses or propagates into adjacent tissue
- Diminishes with time and distance from wound
- Induces wound responses in non-injured tissue
- Induces PAL activity and phenolic synthesis
- Phenolic compounds accumulation
- Tissue browning

Activity depends on 'size' of alcohol

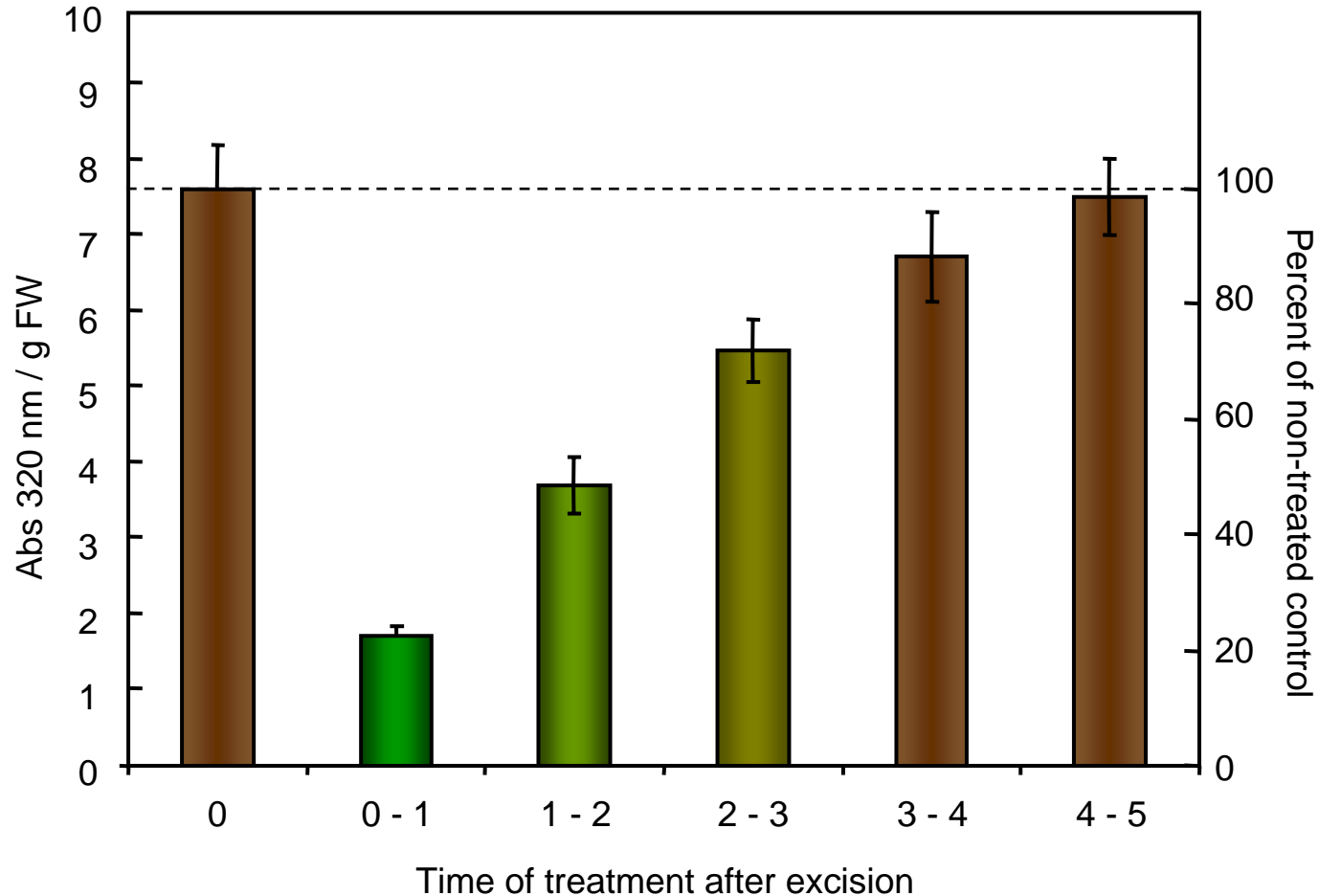


n-Alcohols applied for 12 h as vapors (20 $\mu\text{mol/g FW}$) to lettuce mid-rib tissue subsequently held at 10 °C for 48 h before assay

n-Alcohols reduce tissue browning



Activity depends on time of application

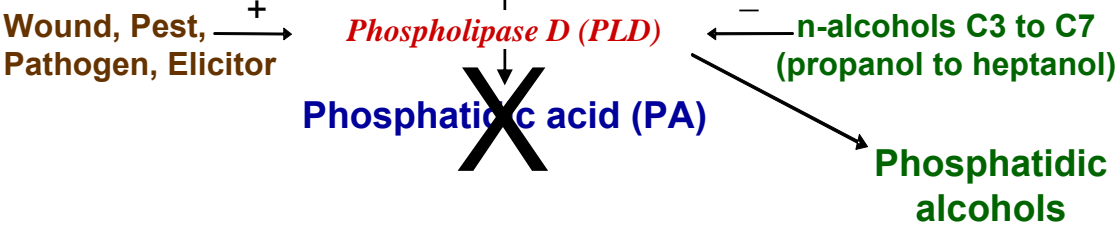


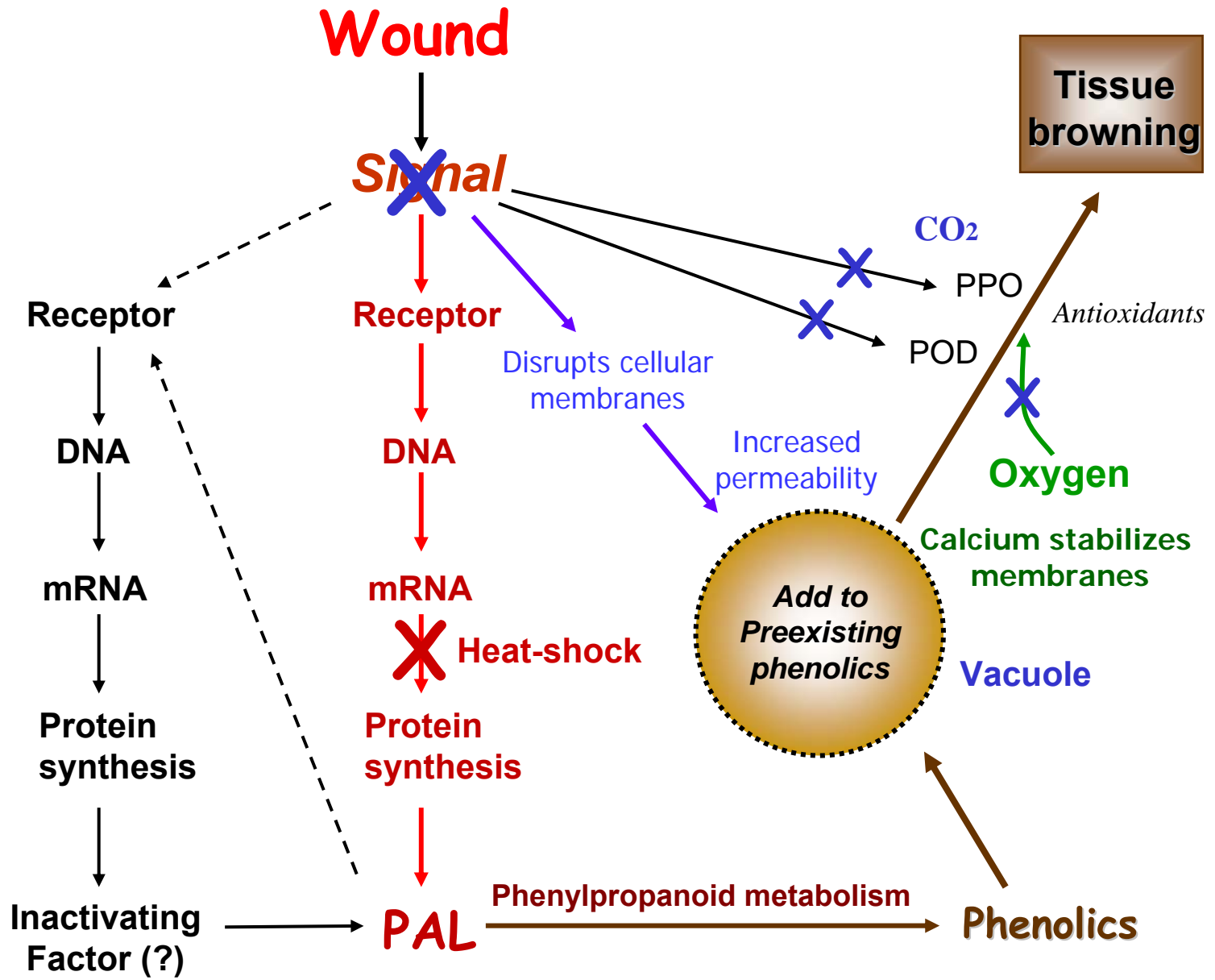
n-Hexanol applied as 20 mM solution (pH 7.0) to lettuce mid-rib tissue for 1 h at 0, 1, 2, 3, 4, or 5 h after excision or subsequently held at 10 °C for 48 h before assay

How does this help us find the wound signal?

n-Alcohols (C2 to C7) selectively inhibit phospholipase D (PLD), an enzyme in a known metabolic pathway.

PHOSPHOLIPIDS IN MEMBRANE





But some phenolic compounds are antioxidants, and antioxidants are good for you.

Do we really want to prevent the accumulation of phenolics, or just prevent tissue browning?

CONCLUSION

1. Fresh-cut fruits and vegetables often respond differently than the whole commodity.
2. Knowledge of basic biology of whole and fresh-cut commodities helps optimizes quality retention during handling and marketing.
3. Unexpected things will happen when fresh-cut fruits and vegetables are produced and marketed.
4. Genetic modifications may prevent deleterious wound-induced changes, but it may also make the plant more susceptible to stresses during growth.