

Reading: Marchand pages 41-56

I. WHEN PLANT TISSUES FREEZE -- WHAT HAPPENS ?

A. EXPERIMENTAL SETUP – What is the role and placement of the following?

1. PLANT TISSUE SAMPLE – See Marchand, Appendix B
2. THERMOCOUPLE – See Marchand, Appendix A
3. REFRIGERATED WATER BATH, or DRY ICE

B. COOLING CURVE -- Figure 16

1. SUPERCOOLING – \_\_\_\_\_

Water rarely freezes as temperature reaches 0°C:

Rather \_\_\_\_\_

2. Effect of additives to water:
  - a. Solutes (colligative property) \_\_\_\_\_
  - b. Ice-nucleators – form “centers” around which crystals can grow
3. FLASH FREEZING – rapid, widespread crystallization throughout tissue
  - a. Causes measureable release of heat seen as an \_\_\_\_\_
  - b. Source of the thermal energy is \_\_\_\_\_

C. CELLULAR EVENTS -- Figure 18

1. Extracellular water freezes first – Why? \_\_\_\_\_  
> Causes first exotherm (Fig. 18)
2. Result of extracellular freezing? [Explain with respect to  $\Psi_w$  gradients.]
  - a. Cytoplasmic solute conc. \_\_\_\_\_
  - b. Result: Prevention of intracellular freezing which is usually fatal

3. Free Energy is less at surface of ice crystal:
  - a. Less kinetic and vibrational energy
  - b. Resultant  $\Psi_w$  gradient drives osmosis across membrane to the extracellular ice crystals
4. Result: Frost Plasmolysis → delay or prevention of \_\_\_\_\_  
 Conclude: Extracellular ice is GOOD!

D. SUMMARIZE: Can you explain the events of plant cell freezing with respect to the following concepts?

Supercooling	Exotherm	Osmosis	Solutes
Flash freezing	Vibrational energy	$\Psi_w$ gradient	

E. CELL DEATH – consequence of intracellular freezing

1. Escaping death means – \_\_\_\_\_  
 Membranes must be permeable to allow "free water" to escape  
 >> RESULT: Tissue may survive -20 to -30 C w/o dying (Fig 16)
2. SIGN OF DEATH -- second exotherm -- Fig. 16  
 This apparently suggests freezing of \_\_\_\_\_
3. Two theories to explain cause of CELL DEATH:
  - a. Membrane integrity lost -- prevents some water from exosmosis  
 -- RESULT: frozen inside
  - b. "Vital water" hypothesis (Weiser) -- death when "bound water" freezes  
 -- denaturation of enzymes/memb. ptn.  
 -- here, less focus on membranes

F. CHALLENGE OF PLANT CELLS

1. AVOID \_\_\_\_\_
2. MOST CHALLENGING CONDITIONS -- \_\_\_\_\_
3. Exception: "Glass Formation" [amorphous (no reorient. of H<sub>2</sub>O to crystal) solidification]  
 e.g. *Populus balsamifera* showed solidification of intracell. fluid after slow cool to -28C

## II ACCLIMATION TO RESIST FREEZING:

A. Acclimation – process in which \_\_\_\_\_

B. EXPERIMENT -- collect twig samples from July to December

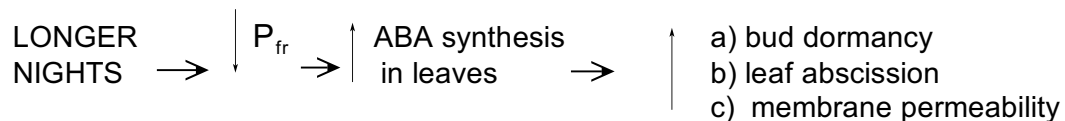
-- threshold temperature for survival becomes lower  
e.g. light frost in early fall may be devastating

C. PHASE I – of acclimation induced by the following two ENVIRONMENTAL STIMULI:

1. \_\_\_\_\_

2. \_\_\_\_\_

MECHANISM: Environmental cue --> photoreceptor --> hormone --> physiological effect



NOTE: Dormancy is an active process (e.g. requires availability of sugars, etc.)

D. PHASE II CHANGES --> induced by occurrence of freezing temperatures

1. Increased lipid unsaturation --> increased membrane permeability  
--> decrease crystallization point of lipids

2. Changes in sugars and protein configurations

- Sugars and proline –compatible solutes that protect proteins from denaturation
- Sugars/organic acids may counteract against dehydration (see 1.)

E. FREEZE TOLERANCE AND GEOGRAPHIC RANGE

1. Low temperature threshold of tolerance corresponds to average minimum temp. of location of northern limit of the species range

2. SUGGESTS temperature is KEY factor in limiting distribution

3. STUDY Tables 2, 3, 4

## III. ANIMAL CELL RESPONSE TO SUBZERO -- see Marchand, pp 125-141 [Assignment]

A. MOST INSECTS ARE \_\_\_\_\_ AND \_\_\_\_\_ like plants

> INSECTS often overwinter in association with plants leaves, stems, litter

B. INSECTS ACCLIMATIZE under similar stimuli as those causing plants to acclimate

C. NOTE: Acclimatization is active process in insects, too; involves biochemical changes