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 <u>first exotherm</u> (Fig. 18)

2. Result of extracellular freezing? [Explain with respect to Ψ_w gradients.]

a. Cytoplasmic solute conc. _____

b. Result: Prevention of <u>intra</u>cellular freezing which is usually fatal

3. Free Energy is less at surface of ice crystal:

   a. Less kinetic and vibrational energy

   b. Resultant Ψ_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	Ψ_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_2O 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

LONGER NIGHTS → P_{fr} → ABA synthesis in leaves → a) bud dormancy
b) leaf abscission
c) membrane permeability

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

a. Sugars and proline –compatible solutes that protect proteins from denaturation

b. 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