

Cold Acclimation and Freezing Damage



Avoidance vs. Tolerance

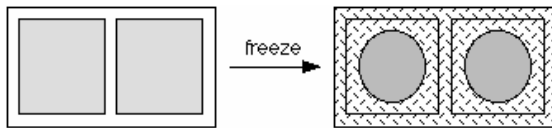
Avoidance- supercooling

Tolerance – tolerate loss of water to extracellular ice

Cellular Effects Under Prolonged Freezing

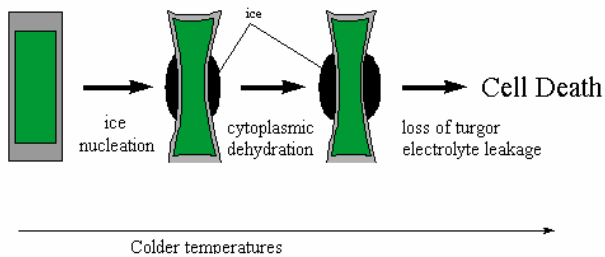
As ice formation advances during a prolonged freeze there is a continuing decrease in Ψ in the apoplast. Water moves from the cell leading to plasmolysis. pH changes are observed and dehydration injury is present.

As freezing continues the ice crystals move to protoplast and that is quite lethal.



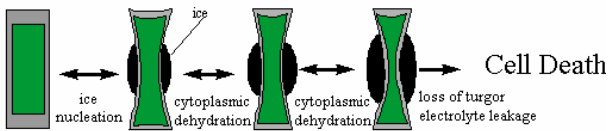
As ice forms in the walls, water exits the cell. The solute concentration of the cytoplasm increases and the protoplast shrinks (becoming plasmolyzed).

Freezing process in a non-acclimated cell



Ice nucleation draws water out of cytoplasm, the cell collapses as ice expands. In acclimated tissue the process is reversible, to a point.

Freezing process in an acclimated cell strategy: cell tolerates freezing and dehydration



Surviving Freezing

Ice Recrystallization – (e.g. carrot) can freeze without being damaged. They produce proteins that slow ice crystal formation, leading to the formation of small round particles of ice that are less damaging.

Antifreeze compounds lower the freezing point of the cytosol by changing **thermal hysteresis**. Some examples are amino acids like proline and other small molecules.

Supercooling– protoplasmic water is liquid until -35 to -40°C. This is accomplished by limiting ice nucleation centers. Pure water will spontaneously crystallize at -40°C.

Surviving Freezing with Supercooling

To limit freezing injury many temperate plants utilize supercooling– various mechanisms to limit ice nucleation.

Most flower buds in temperate plants depend on supercooling.

Some organs do not supercool at all (vegetative buds) so it is possible to have avoidance via supercooling and tolerance in the same plant.

Freeze injury still occurs if freezing is rapid, as there is no ability to partition resources fast enough to allow supercooling.

Freezing Rate and Damage

The extent of damage depends on the rate of temperature decrease. If freezing is rapid then the ice formation is rapid leading to:

Cavitation of cell– air bubble formation. Damages membranes in the absence of ice formation.

Immediate intracellular ice formation– no time for ice to start in apoplast, the whole thing freezes at once.

**What is rapid? <2°C/h leads to extracellular ice formation
>5.6°C/h leads to intracellular ice formation
shifts of 9.4°C/h have been recorded in MN**

CRYOPRESERVATION– fast, small ice bodies, usually not damaging

Freezing Rate and Damage

Once ice nucleation begins then it progresses very quickly, leading to almost instantaneous formation of ice crystals.

Mature field peaches will have ice spread throughout an entire tree in 16 minutes!

The rate of ice crystal formation increases as the extent of supercooling increases. Ice crystals rely on coordination of water molecules which is favored at lower temps.

Although good for freeze avoidance, it may be a limitation in some species in cold climates.

Supercooling Not a Strategy for All Plants

Since supercooling can be detrimental in some climates some plants that are adapted to extreme cold (such as boreal plants) **do not** survive by supercooling. Rather, they tolerate extracellular ice formation and they are extremely tolerant to dehydration stress. These plants have very large extracellular spaces.

Physical Barriers to Ice Formation

When an organ or tissue freezes plants must prevent that ice from propagating to other organs that are still in the process of supercooling.

Physical barriers are useful:

Xylem discontinuity – prevents ice from spreading through the apoplast. Dormant flower buds of many species lack functional mature xylem vessels, so there is no connection to the rest of the plant. Buds can supercool to a greater extent. Lower supercooling potential occurs with development of xylem vessels in primordia of deacclimated buds.

There is also a decrease (or lack) of intercellular space in flower bud tissue which also serves as a physical barrier to ice propagation.

Physical Barriers to Ice Formation

PECTINS- water insoluble carbohydrates within the cell wall and intercellular spaces.

In peach floral buds pectin accumulation parallels ability to supercool.

Adopting Avoidance and Tolerance

Polylepis tarapacana

High altitude plant in Bolivia

During cold dry season is cold tolerant

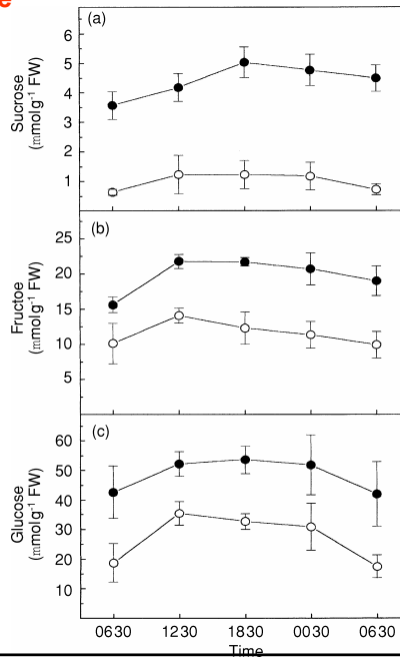
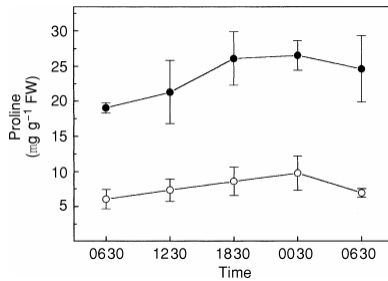
During cool wet season is cold avoiding. Enhanced supercooling capacity.



Adopting Avoidance and Tolerance

Polylepis tarapacana

Sugar and proline levels are highest at night



Ice Nucleating Agents

Certain molecules orient water molecules into energetically favorable orientations that crystallize into ice.

Ice Nucleating Bacteria – INB's. 70's-80's

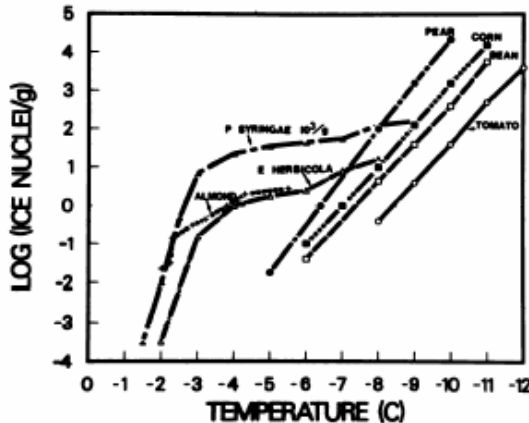
Pseudomonas syringae

Erwinia herbicola

Both are ubiquitous, non-pathogenic

Ice Nucleating Bacteria

Plant cells do not contain nucleation sites, so ice typically does not form until -8°C . However plant tissues harbor bacteria that possess ice nucleating proteins, shifting the freezing point and causing damage at higher temperatures. How do nucleating proteins work? Coordinating water?



Mutant strains missing a 150 Kd protein can be sprayed on plants to compete with natural strains and lower the freezing point.

Lindow *et al.*, 1982

Types of Freezes

Radiation- calm clear conditions. Wind <3 mph, radiational cooling and temperature inversion (colder air at ground level, warmer air slightly above).

Advective – Large, cold air mass with wind > 3 mph

Protection from Freeze

1. Passive
2. Active

Passive Freeze Protection

- 1. Site Selection – most important decision**
 - a. Planting on slope – high on slope brings into warm air**
 - b. water -- planting near water buffers from temp extremes.**
- 2. Cultivar selection**
- 3. Rootstock selection**
- 4. Planting date (for field annuals)**
- 5. healthy plants**

Active Freeze Protection

- 1. Irrigation – protects in two ways**

- 1. specific heat – irrigation with warm water will transfer heat to air and ground.**
- 2. Heat of fusion releases heat if cold.**

Heat of fusion is a good remedy for radiation freezes IF humidity is not too low. At low relative humidity overhead irrigation becomes risky because more chance of losing heat to evaporative cooling.

(Evaporation will remove 6x heat as the same quantity of water deposits when it freezes: heat of fusion = -6 kJ/mol; evaporation = -41kJ/mol)

Active Freeze Protection

- 1. Heaters – not used much, expensive**
- 2. Wind Machines – push down the inversion**
- 3. Trunk wraps, soil banks – limits temperature deviation**
- 4. Row covers, microirrigation**
- 5. Straw/snow**