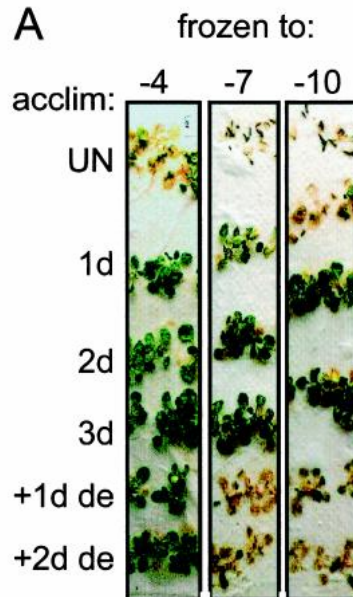


Cold Acclimation

Warner and Juntilla
Plant Physiol. 1999 June 1; 120(2): 391–400.



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Physiological Changes During Cold Acclimation

Antifreeze proteins- slow water crystallization

Chaperones– stabilize protein conformation during cold stress and associated dehydration

Membrane Stabilizing Proteins maintain membrane integrity

Dehydration resistance proteins – increase in osmolytes that decrease water potential, bringing water to tissue

Antioxidant enzymes – scavenge free radicals accumulating due to oxidative stress.

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Changes in Carbohydrate Profiles

Soluble sugars increase in tissues during cold acclimation.

Mostly sucrose, also raffinose (glu-glu-fru) and stachyose (gal-glu-glu-fru)

Most accumulation is in cell walls

Helps slow ice crystal formation

Acts as an osmolyte in the cytosol.

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Changes in Membrane Lipid Composition

Decreasing temperatures cause membranes to lose fluidity. They contract and solidify.

An increase in desaturated fatty acids changes the fluidity of the membrane so that the threshold of temperature damage is lowered.

Starting with a more fluid membrane delays the loss of fluidity to a critical point.

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Association Between Hardiness and Dormancy

Endodormancy is a prerequisite for maximum cold hardiness in deciduous plants. Cold hardiness and dormancy development allow plants to withstand cold temperatures.

If buds don't go dormant soon enough and an adequate level of cold hardiness does not develop, plants can be damaged by early frosts and freezes. However, if plants go dormant too early there is a shortened growing season and generally reduced potential.

Timing of bud dormancy is a tradeoff between growth and survival

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Dehardening does not occur usually until end of dormancy

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The Razor's Edge Between Growth and Survival

Sour cherry crop in MI is severely damaged 1 of every 3 years due to late winter, early spring freeze injury.

Most critical time for tissue injury due to freeze is in early winter before the plant can fully develop cold acclimation (planting by water helps). Late winter/early spring warm spells can trick plants into development and leave tissues vulnerable (again, planting by water helps).

Also, Northern and Southern ecotypes have different sensitivities.

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Ice Nucleation

The concept of nucleation is the “seed” that allows ice crystal formation.

Requires coordination of several hundred water molecules

At a critical temperature at a critical osmolarity

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The CELLULAR Basis of Freezing

Extracellular vs. Intracellular Freezing

Solute concentration is lower in the apoplast, so freezing usually starts there.

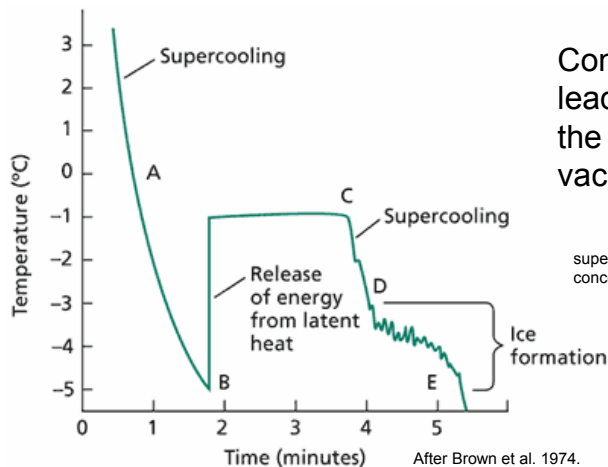
Ice decreases Ψ in apoplast, so it becomes a sink for water (more like a black hole).

This is not normally lethal to hardy plants, and tissue recover on rewarming, but injury can occur under prolonged or rapid freezes.

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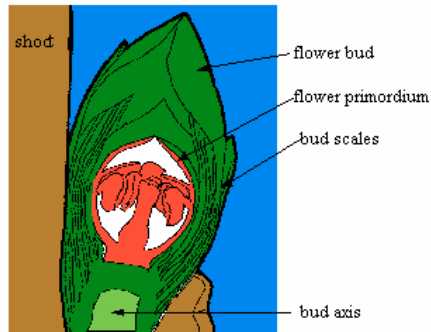
Ice Formation

The temperature drops in the cytosol and vacuole until ice forms in apoplast, then there is an increase from heat of fusion (point B) that will maintain cell temp.

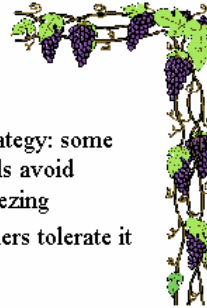


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Freezing process in flower buds



- strategy: some cells avoid freezing
- others tolerate it



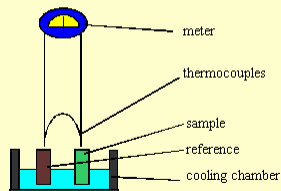
Ashworth, 1982; Ashworth et al., 1989

In peach, ice forms first in the scales surrounding the bud, and then the bud axis. The meristematic tissues are surrounded by a layer of cells with high waxy content, and the meristem cells themselves have little nucleation potential.

At a critical temperature the meristems will present nucleation sites.

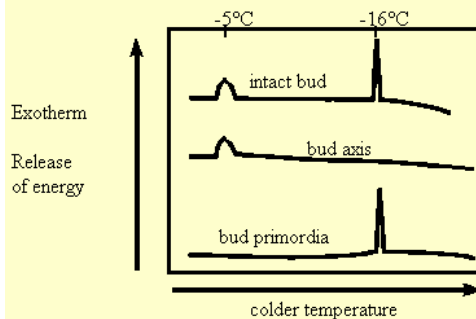
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Differential thermal analysis the equipment



Differential thermal analysis

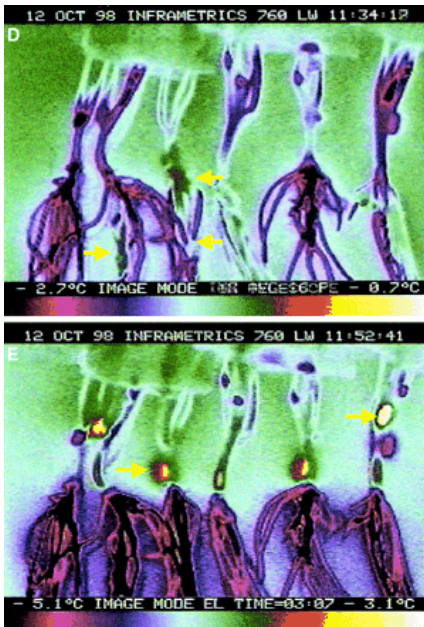
the results in peach buds



Freezing protection is developmentally regulated

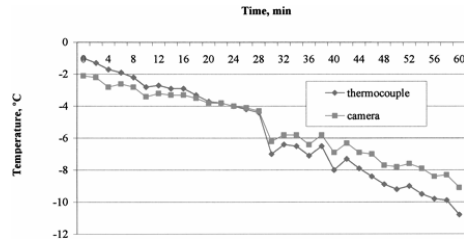
Vegetative buds not as susceptible to freezing. A cold snap in On March 12, 1993 in Niagra, Ontario destroyed 90% of the grape crop for some regions with a value of \$60 million dollars. Vegetative tissues not affected and plants were productive in subsequent years.

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Imaging freezing tissues in real time

It is possible to visualize the heat of fusion temperature increase

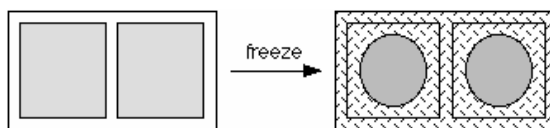


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Cellular Effects Under Prolonged Freezing

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.

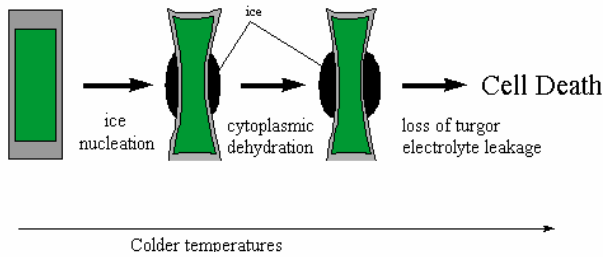
As freezing continues the ice crystals move to protoplast and that is quite lethal.



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

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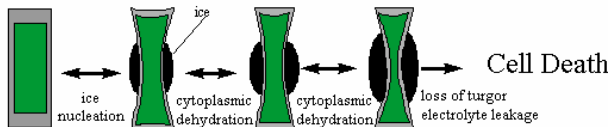
Freezing process in a non-acclimated cell



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



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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 .

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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.

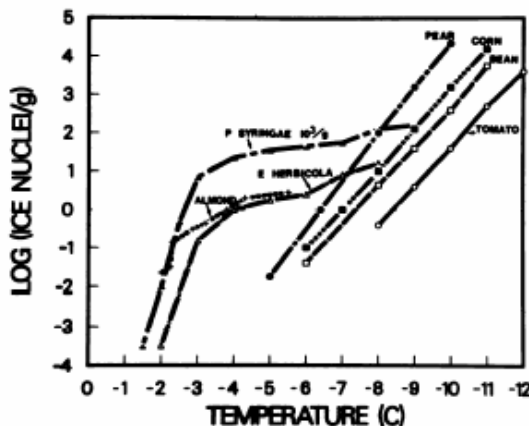
What is rapid? $2^{\circ}\text{C}/\text{h}$ leads to extracellular ice formation
>math>5.6^{\circ}\text{C}/\text{h}</math> leads to intracellular ice formation
shifts of

CRYOPRESERVATION– fast, small ice bodies, usually not damaging

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Ice Nucleating Bacteria

Plant cells do not contain nucleation sites, so ice typically does not form until



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

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