The Physiology Of Higher Plants
An Outline

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Introduction

All animal life on this planet depends ultimately on plants—especially the green autotrophic plants—for the oxygen we breathe and the food we eat. There could hardly be a more important group of organisms to study and attempt to understand.

This compilation is derived from the University of Washington Plant Physiology (Biology 425) course lectures given by Professor Elizabeth Van Volkenburgh and from the textbook, Lincoln Taiz and Eduardo Zeiger, *Plant Physiology, 4th Ed.*, publ. Sinauer Associates, Inc., 2006. Online components of this textbook including "Web Topics" and "Web Essays" referenced throughout this outline may be found at http://4e.plantphys.net/index.php. Page numbers expressed as TZ252 refer to pages in this edition of this textbook. Although most of this document follows the chapter and section sequences of the textbook, rearrangements have occasionally been made, sections have been freely retitled for clarity, and additional materials have been included from other sources where appropriate and feasible.

The subject matter of this course and textbook includes how plants work: the mechanical, physical, and biochemical functioning of living plants, primarily the vascular seed-bearing higher plants (mostly angiosperms and gymnosperms), but some references are made to physiology of other eukaryotic plants such as algae, fungi, and mosses, and to prokaryotes such as bacteria, in order to contrast or show similarities. Another definition of plant physiology is "What plants do: Metabolism, Assimilation and transport of water and nutrients, Growth and development, and Responses to the environment".[1] Compared to my previous experiences with human physiology, this course involves considerably more details at the biochemical level.

These notes present selected facts and concepts that are especially interesting or fundamental, much of which I was not previously well versed on. I am a retired physician—not a biologist, botanist, or physiologist—and I don't claim authoritative knowledge of this material. (Disclaimer: I am not in a position to evaluate or make medical recommendations regarding various health-related claims made about purportedly beneficial or harmful phytochemicals such as isoflavonoids, carotenoids, tannins, or other herbal substances. You should seek qualified advice before deciding to ingest or be treated with any of the biochemicals described in this outline.)

I am preparing these notes primarily as a learning aid for myself, but some of the material could be useful to others. In some cases, I have added basic review material on subjects for which prior knowledge is presumed for the proper understanding of plant physiology, particularly topics in biochemistry, physics, and geobiology. To add personal interest, I have also tried to at least mention many of the specific plants, plant etymologies, plant compounds, plant phenomena, and other plant trivia and lore that my wife or I have had direct or indirect experience with, or that seem to be of general or medical interest (for example, the psychoactive and poisonous plant products).

The superb textbook is extremely detailed and technical, so these notes only scratch the surface of this amazingly complex subject. For the sake of brevity, many of the experimental details and qualifying comments that make scientific assertions more accurate have often been omitted. Also for brevity and to avoid copyright problems, I have not included illustrations or diagrams in this document, only text paraphrases and (hopefully) "fair use" quoted text and formulas. Many of the cited references include some of the diagrams needed to understand this material, but there is no substitute for having access to the textbook—you should buy this book!

Viewing this document in a Web browser or in a recent generation Adobe Acrobat Reader allows following the Web URL hyperlinks, just as with a normal Web page. In some instances, multiple separate sources are combined into one footnote (in which case the individual sources are demarcated by a bullet "• "). Inclusion of material from *Wikipedia* and other Web sources does not imply any assumption of authoritativeness of these resources.

[1] The website http://www.uwyo.edu/botany4400/ using the same textbook to present a quite different plant physiology course outline.

All text and Web resources were accessed in January to June 2008 unless otherwise stated. Corrections and comments are welcome—send these to mcgoo at u period washington period edu [after converting this eaddress to standard format].

Instructor And Course Website

Professor: Elizabeth "Liz" Van Volkenburgh, Ph.D. = "EVV"
Websites:
Course Website: http://courses.washington.edu/biol425/index.html [expired]
Course Web Forum: https://catalysttools.washington.edu/gopost/board/lizvanv/4598
(restricted access)
Textbook Website: http://4e.plantphys.net/index.php (or http://www.plantphys.net/)

My wife and I both express our thanks to Professor Van Volkenburgh for allowing us to audit her excellent course.

Sugar Chemistry Highlights

This review[2] is intended to aid the study and understanding of sucrose, cellulose, starch, and the carbon sugar-related produts of photosynthesis. (For a general look at bioactive chemicals, see web essay 1.1, Exploring Chemical Space in the Plant World. See also Web Topic 8.11 Glossary of carbohydrate biochemistry. Proteins and Enzymes are discussed in chapter 2.)

- The C*O* or C=*O* group is called a **carbonyl** group[3]. It appears in **aldehydes** RCH*O*, **ketones** RC*O*R', **Carboxylic acids** RC*O*OH, **esters** RC*O*OR', and **amides** RC*O*NR'R''
- **Monosaccharides** have the chemical formula $(CH2O)_{n+m}$ with the chemical structure $H(CHOH)_nC=O(CHOH)_mH$. If n or m is zero, it is an **aldehyde** and is termed an **aldose**, otherwise it is a **ketone** and is termed a **ketose**. Monosaccharides contain either a ketone or aldehyde functional group, and hydroxyl groups on most or all of the non-carbonyl carbon atoms.[4] [This describes the **linear form** of a monosaccharide.]
- A monosaccharide is a **triose, tetrose, pentose, or hexose**, etc. based on the number of carbons (e.g., 3, 4, 5, or 6). Sedoheptulose is one of the few heptoses, sialose is a nonose.
- Linear (chain) forms of monosaccharides are aldehydes [e.g., glucose = C1 aldose] or ketones [e.g., fructose= C2 ketose]. Carbon numbering begins at the end closest to the aldehyde or ketone C.
- The **linear stereoisomers** (in this case, mirror images = enantiomers) of monosaccharides are D- or L- based on the *asymmetric* C furthest from the aldehyde or ketone group, e.g., C5 [not C6] for glucose, in comparison to D-glyceraldehyde or L-glyceraldehyde. Most natural sugars are D-stereoisomers.
- The **aldohexose** stereoisomers[5] are **glucose**, **mannose**, **galactose** (plus 5 uncommon or unnatural forms—allose, altrose, gulose, idose, and talose). The common **ketohexose** stereoisomers are D-**fructose** and D-**sorbose**
- **Ring formation (Cyclization)**: The aldehyde and ketone functional groups in monosaccharides are reactive and react reversibly with nearby hydroxyl functional groups to form intramolecular **hemiacetals** [R-CHOH-O-R'] or **hemiketals**, respectively. The resulting 6 member ring structure is related to **pyran** [more closely to saturated tetrahydropyran], and is termed a **pyranose**. If the ring has 5 members (similar to **furan**), it is a **furanose**. (For example, arabinose may occur in furanose and pyranose forms.)[6] As a monosaccharide in solution, the ring spontaneously opens and closes, allowing rotation to occur about the bond between the carbonyl group and the neighboring carbon atom, yielding two distinct stereoisomers or **anomers** (α and β). This process is termed **mutarotation**. For instance, in the familiar "chair" form of glucose in cellulose, the β-D-glucopyranose has the -OH arising at the anomeric carbon [C1] pointing out rather than down.

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[2] Sugar chemistry: http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/sugar.htm
[3] Carbonyl: http://en.wikipedia.org/wiki/Carbonyl
[4] Monosaccharide: http://en.wikipedia.org/wiki/Monosaccharide
[5] Aldohexoses: http://en.wikipedia.org/wiki/Hexose
[6] Arabinose forms: http://www-jmg.ch.cam.ac.uk/data/molecules/nameindex.html

- The aldehyde [glucose = C1 aldose] or ketone [fructose= C2 ketose] linear forms of monosaccharides (glucose, fructose) are energetically disfavored over the cyclized forms—only 0.02% of glucose in solution is in chain form.
- **Glycosidic bonds and Polymers**: In a **1→4-O-glycosidic bond**[7], a C1-O-C4 bond is made involving the C1 of one sugar molecule and the C4 of the other. Likewise a C1-O-C6 bond involving the C1 of one sugar molecule and the C6 of the other is called a 1→6-O-glycosidic bond. Glycosidic bonds allow formation of disaccharides such as **sucrose** [α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside], polysaccharides such as cellulose and starch, and other sugar polymers. Examples include:
  - **Starch (amylose)**: **α-D-1,4** glycosidic bonds, or **(1→4)α-D** glycosidic bonds [forming linear chains]
  - **Starch (amylopectin)**: **α-D-1,6** glycosidic bonds, or **(1→6)α-D** glycosidic bonds [forming side chains]
  - **Cellulose**: **β-D-1,4** glycosidic bonds, or **(1→4)β-D** glycosidic bonds [forming linear chains]
- In **sugar acids**, the aldehyde at C1, or the hydroxyl on the terminal carbon, is oxidized to a carboxylic acid. Examples are **gluconic acid and glucuronic acid**.
- A **sugar alcohol** is a hydrogenated form of carbohydrate, whose carbonyl group (aldehyde or ketone, reducing sugar) has been reduced to a primary or secondary hydroxyl group. Examples include **glycol, glycerol, sorbitol, mannitol**.
- **Polysaccharides**: A **glycan** is a polysaccharide polymer. A **glucan** is a glycan of glucose, a **xylan** is a xylose polymer, **galactan** is a glycan of galactose, etc. **Xyloglucan** is a primary glucan with side chains of one or more xyloses. **Arabinoxylan** is a xylan with arabinose side chains (apparently in furanose 5-member ring form). Naming is not always consistent. In polysaccharides, the anomeric form (α or β) is stabilized and does not mutarotate.

Chapter I. Plant Cells
Review of Plant Anatomy and Cellular Components

Meristems And Vessels; Anatomy Of Stems and Trunks

Above the soil level is the major **vegetative** organ, the **shoot**, and below is the **root** system. The attachment or transition point between the stem or shoot and the root is termed the **base**. (See Web essay 19.2 regarding confusion in the terminology for root apex and base in embryos versus seedlings.) The term *vegetative* signifies that part of a plant's life other than the reproductive phase. The major plant tissues are dermal, ground, and vascular.

Plant tissues grow at rapidly dividing tissue sites called **meristems**, including **apical meristems** at **shoot apex**, **root apex**, and **axillary bud**[TZ2]. Meristematic tissue, which contain totipotent undifferentiated cells analogous to animal stem cells, may also be found in stems (as intercalary meristems arising at nodes) and in reproductive structures such as **floral (inflorescence) meristems**. Vegetative plant growth is indeterminate, due to the permanent growth of meristem tissues in the shoot and root apices (but reproductive growth is determinate). The vascular cambium is a later meristem that generates the xylem and phloem. These vascular structures are positioned in a ring located inside the **cortex** and outside the **pith**.

Woody trees and shrubs:[8] the vascular cambium initially produces **primary xylem** (inside) and **primary phloem** (outside). In subsequent years the vascular cambium adds **growth rings**: additional layers of **secondary xylem** (which forms immediately inside the vascular cambium) and of secondary phloem (which forms immediately outside the vascular cambium). Older (more centrally positioned) layers of xylem become the annual tree rings—these stop conducting water but provide mechanical support. The older layers of primary and secondary phloem are crushed by outward expansion of the secondary xylem. (fig. 10.2) "Since the phloem is constantly being pushed outward and crushed, only the innermost layers [of

[7] Glycosidic bond: http://en.wikipedia.org/wiki/Glycosidic_bond
[8] Anatomy of trunks of trees and shrubs: http://plantphys.info/plants_human/secondary.html

secondary phloem] adjacent to the vascular cambium are functional phloem."[9] The secondary xylem cells produced in the spring and early summer of the year are larger in radial dimension. In late summer, fall, and winter the secondary xylem cells are much smaller in the radial dimension, thus generating the alternating bands of growth rings. **Girdling** a tree destroys the active phloem layer preventing distribution of sugars, but does not stop transpiration from water transport located in the more deeply positioned xylem.

Cork cambium[10] **(phellogen, bark cambium)** generates **cork (phellem)** to the outside (as part of the **bark** or **periderm**) and in some plants the **phelloderm** or **phellem layer** (inside the cork cambium).

The plant circulatory system (in vascular tissues) is not a closed loop as in animals with hearts. It may be considered divided into:

- **Xylem vessels**: which transport water and soil nutrients from the roots to the shoot, and, separated from the xylem by the **vascular cambium**, the
- **Phloem vessels**: which transports **photosynthate** (organic products of **photosynthesis**, also called **photoassimilate**) from **sources** to **sinks** (such as meristems) regardless of the up/down direction of flow in the plant.

The axially (longitudinally) elongated vessels of the xylem consist of **tracheids** (found in all spermatophytes) and **vessel elements** (found primarily in angiosperms). These **tracheary elements** are composed of apoptotically dead cells with rigid perforated walls stiffened with **lignin**, thereby capable of remaining open at negative pressure (called "**tension**" throughout this book). They are interconnected by **simple pits** and **end wall perforations**. They have no cytoplasm, organelles, or plasma membranes. (See further discussion in Chapter IV.)

In contrast, the phloem vessels consist of living **sieve cells** interconnected through **sieve areas** and (in angiosperms only) **sieve plates**, with adjacent **companion cells**. (See Chapter X)

For flower parts and the angiosperm life cycle, see TZ Webtopic 1.2

For more details on plant tissue systems, see TZ Webtopic 1.3 and chapters pertaining to xylem and phloem.

Cell Wall and Intracellular Components

The **cell wall** is semi-rigid and consists of a matrix of **cellulose** [(1→4)β-D-glucan] microfibrils interspersed with **hemicelluloses** and **pectins** (sugar acids). It is relatively porous to water and many molecules, and adjacent cell walls are glued together by the **middle lamella**. See chap 15 for full description. Because it has great tensile strength and limited distensibility, it serves to limit cell expansion, allowing hydrostatic pressure and turgor to build up. The cell wall is of course outside the plasma membrane and it can be debated whether it is extracellular or an intrinsic part of the cell—EVV and many authors [and MCM] consider it to be a part of the cell (as it is with bacteria). The **primary cell wall** does not contain **lignin**, but the **secondary cell wall** does, and is the basis of **wood** formation.

The **plasma membrane** (a term favored for plants over **cell membrane**) consists of an 8 nm-thick **phospholipid bilayer** which is **hydrophilic** on both surfaces and **hydrophobic** inside. (Many membranes are phospholipids, but the bounding membrane of chloroplasts and other plastids is a **glycosylglyceride** which does not contain phosphate). Proteins embedded in or attached to this membrane can be **integral**, **anchored**, or **peripheral**.[TZ6] Membranes are maintained in a **semi-permeable** state by living organisms through the expenditure of energy—this property disappears in dead cells. Inside the plasma membrane is the **cytosol** and organelles.

The **nucleus** is bounded by the **nuclear membrane**, which has **nuclear pores** that allow selective passage of **ribosomes** (synthesized in the **nucleolus**), **transcribed mRNA**, and certain proteins, etc. The genetic material of condensing **chromatin** at mitosis is hierarchically organized as

[9] Secondary and primary phloem: http://arnica.csustan.edu/boty1050/Secondary/secondary.htm
[10] Cork cambium: http://en.wikipedia.org/wiki/Cork_cambium

- **DNA** double helix of hydrogen bonded single strands (overall 2 nm diameter)
- **Nucleosomes** ("beads on a string") of DNA wrapped around histone proteins (11 nm diam)
- **Chromatin** fibers of closely packed nuclesomes, 6 nucleosomes per turn (overall 30 nm diam)
- Looped domains of chromatin (300 nm)
- Condensed **chromatid** of chromosomes (700 nm)
- Condensed metaphase **chromosome** with 2 chromatids joined at the **centromere** (1400 nm diamter for both chromatids combined)

Protein synthesis takes place by **translation** of mRNA and amino acids at free or bound ribosomes in the cytoplasm, especially on the **rough endoplasmic reticulum (RER)**. (Lipids are synthesized on **smooth ER (SER)**.[TZ11]

The **Golgi apparatus, body, complex, or stacks** (named after Camillo Golgi) create secretory vesicles which discharge to the extracellular space at the plasma membrane.

Other membrane-bound **organelles** include:
- **Mitochondria**[TZ17], the sites of intracellular respiration, in which energy is released from sugar metabolism and H^+ gradient with creation of ATP from ADP and inorganic phosphate P_i. (See chapter 11 for more details regarding mitochondria.)
- **Chloroplasts**[TZ18] have **grana** (which are stacks of membranes called **thylakoids** having an internal lumen), and **stroma** (the region lying outside the thylakoid lumen), and are where photosynthesis occurs.
  Both organelles are probably **endosymbionts** (based on the presence of extra bounding membranes, metabolism, intrinsic circular chromosome of DNA typical of bacteria, eubacterial RNA polymerases, etc.. They are hypothesized to have derived from **alpha-proteobacteria** and **cyanobacteria**, resp.[11]
  Other plastids besides chloroplasts include non-pigmented **leucoplasts**, chloroplast precursors (**etioplasts)**, starch-storing **amyloplasts**, and pigmented **chromoplasts**. Plastids can in certain circumstances interconvert.[TZ20] (See also web essay 7.1 on a novel view of chloroplast structure.)

The **central vacuole**,[TZ17] which is bounded by the **tonoplast** membrane, may occupy up to 95% of the volume of the mature cell. It has many functions: stores water, nutrients, inorganic ions including salts and Ca^{+2}, sugars, malate, organic acids, enzymes, proteases, toxic metals including cadmium, waste products, secondary compounds or metabolites such as proteins, pigments, oxalic acid, toxic plant alkaloids (e.g., strychnine, cocaine, and caffeine), nucleic acids, and major lipids. The vacuole, by distending the cell against the restraining cell wall, helps to maintain the hydrostatic pressure and turgor of the cell, thereby contributing to a dynamic stiffening of plant parts such as the stem or leaf, etc. in the absence of rigid substances such as **lignin**. Smaller vacuoles may store protein (in seeds) or hydrolytic enzymes (**lytic vacuoles**).

Microbodies play a role in leaves and seeds and include **peroxisomes** (which remove O_2 in PS cells from organic substrates producing H_2O_2, which is broken down) and **glyoxysomes** (for converting fatty acids into sugars in oil-storing seeds) and **oleosomes** (oil bodies, for oil storage).

The intracellular **cytoskeleton**[TZ23] consists of
- **Microtubules**: 25 nm diameter composed of **tubulin subunits α** and **β**. These play a role in **mitosis** (forming the box-like **mitotic spindle**, as plants do not have centrosomes from which the spindle radiates), and in **cytokinesis** (the partitioning of the cell in late mitosis after nuclear division at the site of the **phragmoplast** and subsequent **cell plate**, see web topic 1.5),
- **Microfilaments**, 7 nm diameter, composed of **globular actin** (**G-actin**). These combine with **myosins** (motor proteins) to cause **cytoplasmic streaming** (as in Chara with high speeds of up to 75 μm/sec; and
- **Intermediate filaments**, 10 nm diameter, composed of various linear polypeptide monomers, etc.

The first two of these listed can dynamically assemble and disassemble to meet the cell's minute by minute needs.

[11] For Woese's phylogenetic tree of life of eubacteria, archaea, and eucarya and a vascular plant phylogenetic trees, see TZ web topic 1.1

The **cell cycle** is regulated by **cyclin dependent protein kinases (CDKs)** and consists of the longer **Interphase** (during which DNA transcription is possible and optimized) and a shorter phase, **Mitosis** (during which transcription is thought to cease):

- **G_1** part of **Interphase**: The letter G = Gap. **START** is passed committing the cell, and CDK, activated by ATP, will stimulate DNA synthesis during early S.
- **S** part of Interphase: The letter S = Synthesis. DNA of chromatin is **replicated** into **2 identical sister chromatin strands** (terminology varies, but generally these strands are not termed chromatids at this point, though they are sometimes called chromosomes).
- **G_2** part of Interphase
- **M** : The letter M = **Mitosis**. Chromatin condenses to **chromosomes** in **prophase**, with each chromosome consisting of 2 identical sister **chromatids** connected at the centromere. In **metaphase**, the chromosomes line up along the metaphase plate. In **anaphase**, the proteins binding sister chromatids at the centromere are cleaved, and microtubules connected to kinetochores in the centromere region pull the two chromatids of a chromosome apart (at which point the strands are called "**daughter chromosomes**")...

The **karyotypes**[12] of humans and other eukaryote organisms diplay chromosomes at arrested cell division using colchicine. Humans are diploid organisms and somatic and zygote human cells (i.e., not gametes, the sperm or egg) have 22 pairs of homologous but non-identical chromosomes plus the XX or XY pair—each of these 23 pairs consisting of a chromosome derived from each parent [aside from the mixing of **crossover** that occurs during Prophase 1 of meiosis]. For humans, the **monoploid number** of chromosomes in each mutually homologous set is said to be 23. Each single chromosome of a 46-chromosome karyotype consists of 2 identical sister chromatids united at the centromere.

Plant cells like animal cells are frequently **diploid** (having 2 sets of homologous but non-identical chromosomes), but plants may be **tetraploid** (4 sets of homologous chromosomes) or **polyploid** (many sets) after 2 or more cycles of DNA replication occur without intervening mitosis (i.e., cell division). Diploidy, which applies to homologous nonidentical chromosomes, is not the same as the replication of identical sister chromatids which can be seen in metaphase chromosomes. (Polyploidy is uniformly fatal in humans.)

Plasmodesmata, Symplast, and Apoplast

Plasmodesmata[TZ29] are tubular extensions of the plasma membrane, 40-50 nm diameter, which cross the cell walls and provide cytoplasmic interconnections between cells. The continuous cytoplasm of two or more such interconnected cells is called the **symplast** and movement of water and solutes in this space is called **symplastic transport**. The connected cells may or may not be clonally related. A **plasmodesma** has 2 channels:

- **the desmotubule**: a complex centrally positioned inner membranous structure connecting the ER of adjacent cells (of uncertain actual functionality, since it is so narrow)
- **the cytoplasmic sleeve** interconnecting the cytosol of adjacent cells. It is a narrow space between the desmotubule and the plasma membrane, with a **size exclusion limit SEL** of c. 1.5 to 2 nm and c. 700 to 1000 daltons.[13] **Actin** and **myosin** may regulate the actual pore size.

Movement in the symplast through plasmodesmata allows intercellular signaling but also transmission of viruses.

Apoplast: The space within the plant but outside the regions bounded by plasma membranes—much of this space being within or adjacent to the porous cell walls—is called the **apoplast**. According to the textbook, the apoplast includes the **air spaces** between cells, and the xylem vessels of dead cells.

Chapter II. Energy And Enzymes: Entropy, Free Energy, Redox And Electrochemical Potentials

[12] Karyotype: http://en.wikipedia.org/wiki/Karyotype
[13] Dalton: 1 dalton or Da = 1 atomic mass unit, 1/12 of the mass of ^{12}C

This entire chapter is available online at http://4e.plantphys.net/pdf/ch2.pdf

The purpose of this chapter is to gather together and explain recurring thermodynamic concepts found in the text.

Thermodynamics: Work, Free Energy, Enthalpy, and Entropy

Energy is the capacity to do work, which may be mechanical, osmotic, chemical, or electrical, etc.

W = F Δl
Mechanical work W is the product of the opposing force F exerted while displacing an object by distance Δl. It has dimensions m l^2 t^{-2}. Expansion during a reaction of an initial volume against atmospheric pressure to volume V + ΔV performs work PΔV. In biology, work is any displacement performed against an opposing force: electric, osmotic, chemical. It consists of a product of a potential/intensity factor independent of size (such as force) and a capacity factor proportional to size (such as distance). These **potential|capacity factor pairs** include: **Force|Distance**, **Pressure|Volume**, **Electric Potential|Charge**, **Chemical Potential|Mass**, **Concentration|Mass**, and **Temperature|Entropy**. Work or Energy may be expressed as calories or Joules (1 calorie = 4.186 Joule or kg m^2/s^2). 1 mol charge moved against a potential of 1 volt involves 96,500 J work.

First Law of Thermodynamics[14]

Total energy of a closed system is always conserved. Some work goes to heat, so in general

ΔU = ΔQ + ΔW where
- **ΔU** is the net internal energy[15] added to a system
- **ΔQ** is the amount of heat absorbed by the system from its surroundings
- **ΔW** is the amt. of work done *on the system by its surroundings*

For a leaf, the energy absorbed from light equals the amount of energy stored plus the amount emitted. Alternatively ΔW and ΔU may be expressed as:

ΔU = ΔQ - ΔW where
- **ΔU** is the net internal energy added to a system
- **ΔQ** is the amount of heat absorbed by the system from its surroundings
- **ΔW** is the amt. of work done *by the system on its surroundings*

For a **thermodynamically reversible process** (one in which the opposing forces are so nearly balanced that an infinitesimal change in one or the other could reverse the direction of the process, and defined in terms of exact differentials), a maximum amount of work is performed.

Second Law of Thermodynamics[16]

The **entropy**[17] represents the amount of energy in a system not available for doing work, and corresponds to the degree of randomness in the system. Systems do not always move spontaneously to lower internal energy—e.g., melting ice in water yields a higher internal energy (at the expense of increasing entropy). The direction of all **spontaneous processes** moves a system plus its surroundings to a state of higher entropy—this is the Second Law. Mathematically, the **isothermally unavailable energy** of a system (that energy which cannot be given up to do work without a drop in temperature) is **ST**, where T is temperature °K and S is entropy (e.g., in $J \cdot K^{-1}$). It represents the part of the system's energy required just to be at its

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[14] First law of thermodynamics: http://en.wikipedia.org/wiki/First_law_of_thermodynamics
[15] Internal energy:
"In thermodynamics, the internal energy of a thermodynamic system, or a body with well-defined boundaries, denoted by U ... is the total of the kinetic energy due to the **motion of molecules** (translational, rotational, vibrational) and the **potential energy** associated with the vibrational and electric energy of atoms within molecules or crystals. It includes the energy in all the **chemical bonds**, and the energy of the free, **conduction electrons** in metals... Internal energy does not include the translational or rotational kinetic energy of a body as a whole."
http://en.wikipedia.org/wiki/Internal_energy
[16] Second law of thermodynamics: http://en.wikipedia.org/wiki/Second_law_of_thermodynamics
[17] Entropy: http://en.wikipedia.org/wiki/Entropy

current temperature. This may also be stated: "In any process where the system gives up energy ΔE, and its entropy falls by ΔS, a quantity at least $T_R\Delta S$ of that energy must be given up to the system's surroundings as unusable heat (where T_R is the temperature of the system's external surroundings)."[18] Clausius stated his law as follows: $\int \partial Q/T \geq 0$ for real spontaneous cyclic processes.

Approaching **absolute zero** 0°K, the entropy of a pure [?crystalline] substance approaches zero (otherwise it approaches at least a minimum value dependent only on the temperature)—this is sometimes called the **Third Law**).[19]

Gibb's Free Energy and Enthalpy (Heat Content)

In order to express the work that chemical reactions perform within organisms, use **Gibbs' Free Energy**: the energy in a system that is available for doing work under isothermal conditions:

ΔH = ΔG + TΔS, or
ΔG = ΔH - TΔS where
ΔH = **enthalpy**[20] (**heat content**), the total energy change including possible volume changes
ΔG = Gibbs' free energy

The relationship between H and U is given by:
dH = dU + (PdV + VdP)
But when volume changes are negligible,
ΔU ≈ ΔG + TΔS, and
ΔG ≈ ΔU - TΔS
For all spontaneous changes at constant temperature, **ΔG < 0**. This can be used as a criterion of feasibility. When this condition is met,
ΔU - TΔS < 0, or
TΔS > ΔU, or
TΔS > 0
[If we may assume that ΔU = 0 in the case of spontaneous isolated reactions occurring within an organism?]
A chemical reaction cannot proceed spontaneously unless **ΔG is negative**. A chemical reaction for which ΔG = 0 is at **chemical equilibrium**.

The relationship between the maximum amt. of work that a reversible reaction at constant temp and pressure can perform and the change in free energy ΔG is given by
$\Delta W_{max} = -\Delta G$
Since ΔG must be negative for all spontaneous reactions (at constant temperature and pressure), ΔW_{max} is a positive quantity as expected. If the reaction in not reversible, the work yield will be lower. If there is pressure-volume work, the work available otherwise is lower.

A process for which ΔG < 0 is **energy releasing** (**exergonic**), and can proceed spontaneously.
A process for which ΔG > 0 is **energy-consuming** (**endergonic**), and cannot proceed spontaneously unless it is coupled to (i.e., accompanied by) an exergonic reaction, so that net ΔG < 0.

Standard free energy change ΔG° is the change of free energy when the **reactants and products are at 1M**. The related **ΔG°'** is the change of free energy when the **reactants and products are at 1M** and at **pH=7**. These conditions are highly non-physiologic.

The **equilibrium constant K** for a reaction $\alpha A + \beta B + \ldots \leftrightarrow \sigma S + \tau T + \ldots$ at equilibrium is given by

$K = \{A\}^{\alpha}\{B\}^{\beta}\ldots / \{S\}^{\sigma}\{T\}^{\tau}\ldots$

and relates to ΔG° as follows:
ΔG° = -RT ln K
q = [C][D]/[A][B] (the **mass action ratio**)
ΔG = ΔG° + RT ln q = **-2.3 RT log (K/q)**

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[18] Entropy: http://en.wikipedia.org/wiki/Entropy
[19] Third law of thermodynamics: http://en.wikipedia.org/wiki/Third_law_of_thermodynamics
[20] Enthalpy: http://en.wikipedia.org/wiki/Enthalpy

Thus ΔG is determined by the displacement from equilibrium, and work must be done to displace the system from equilibrium.

The enthalpy change measures the energy transferred as heat.
Processes that generate heat are said to be **exothermic** (e.g., oxidation of glucose to CO_+ and H_20).[21]
Processes that absorb heat are said to be **endothermic**.

Oxidation / Reduction (Redox) reactions

For **Oxidation/Reduction Redox** reactions, the Free energy change is expressed as the standard Redox potential in electrochemical units.

Oxidation is the transfer of electrons from an **electron donor** (**reductant or reducing agent or reducer**) to an **electron acceptor** (**oxidant or oxidizing agent or oxidizer**). The donor is **oxidized** by losing electrons and the acceptor is **reduced** by gaining electrons. In this textbook, oxidation is also used to refer to the removal of hydrogen atoms from a substance, and reduction to the incorporation of hydrogen by a substance.

	Donor of Electrons (e.g., Fe^{2+}) **or of Hydrogen** (e.g., H_2 or NADPH)	**Acceptor of Electrons** (e.g., O_2) **or of Hydrogen** (e.g., $C_6H_{6)}$
Pre-Reaction Reactant Synonyms	**Reductant, Reducing Agent, Reducer, Electron Donor (or Hydrogen Donor)**	**Oxidant, Oxidizing Agent, Oxidizer, Electron Acceptor (or Hydrogen Acceptor)**
Reaction Effect on Reactant	• **Oxidized** (loses electrons, ends with higher oxidation number), or in some cases • **Diminished in hydrogen** (e.g., NADPH→$NADP^+$) or reactant hydrogen has been consumed	• **Reduced** (gains electrons, ends with lower oxidation number), or in some cases • **Increased in hydrogen** (e.g., $C_6H_6 + 3\ H_2 \rightarrow C_6H_{12}$)

E.g., for the inorganic oxidation of ferrous ion to ferric:

$$2Fe^{2+} + 1/2O_2 + 2H^+ \leftrightarrow 2Fe^{3+} + H_20$$

- **Oxidation Half Reaction**: $2Fe^{2+} \leftrightarrow 2\ Fe^{3+} + 2e^-$ [Fe^{2+} or Fe(II) is oxidized by losing electrons to form Fe^{3+} ferric or Fe(III)]
- **Reduction Half Reaction**: $1/2O_2 + 2H^+ + 2e^- \rightarrow H_2O^-$ [O^0 is reduced to O^{-2} by gaining electrons]

In organic chemistry (and this textbook), reduction also refers to the addition of hydrogen to an organic molecule by a reducing agent which provides those hydrogens.[22] For example, benzene is reduced to cyclohexane by the reducing agent H_2 in the presence of a platinum catalyst:

$$C_6H_6 + 3\ H_2 \rightarrow C_6H_{12}$$

The tendency of a substance to donate electrons (its "electron pressure") is measured by its $\mathbf{E_0}$ or $E^{ø}$ (superscript should be a circle with a horizontal bar through it), which is its **standard redox potential** (or **Standard Electrode Potential SEP**) measured at 1M concentrations and pH = 0. It may be expressed as $\mathbf{E'_0}$, the standard redox potential measured at 1M concentrations and at pH = 7.

SEP is measured in a half-cell by comparison with the voltage in a separate half cell connected by a salt bridge and containing a **standard hydrogen electrode**[23] (of platinum surrounded by hydrogen bubbles at 1 atmosphere) which can reversibly accept electrons and has reference redox potential always defined as zero.[24] Standard electrode potentials are expressed as standard reduction potentials with the reduced

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[21] Enthalpy of reactions: http://webbook.nist.gov/chemistry/name-ser.html
[22] Reduction is gain of electons or of hydrogen: http://en.wikipedia.org/wiki/Reductant
[23] Hydrogen electrode: http://en.wikipedia.org/wiki/Hydrogen_electrode
[24] Absolute electode potential of Standard Hydrogen Electrode is given as either
• 4.44 V at 298.15K: http://goldbook.iupac.org/S05917.html and

species on the right of the reaction equation. For example, , with Fe^{2+} and ferrous sulfate in the other half-cell, and the reaction written as a reduction:

Fe^{2+} (aq) + $2e^- \leftrightarrow Fe^0$ (solid)

the measured E_o = -0.42 V. This means that the ferrous Fe(II) half-cell has a more negative voltage (electric potential) than the standard hydrogen electrode half cell, so that the Fe(II) here acts as an electron donor (reductant) to the other cell.[25] For ferric Fe(III), the E_o = -0.04 V, a much weaker reductant.

Similarly, with Zn and zinc sulfate in the other half-cell, and the reaction written as a reduction:

Zn2+ (aq) + $2e^- \leftrightarrow Zn^0$ (solid)

the measured E_o = -0.786 V. This means that the zinc half-cell has a more negative voltage (electric potential) than the standard hydrogen electrode half cell, so that the zinc here acts as an electron donor (reductant) to the standard half cell.[26]

When comparing the redox potential of reactants, the more negative the value of E_0, the greater the reducing strength (ability to donate electrons) of this reactant. Thus, Fe(III) has a SEP of -0.04 V. Its E_0 is less negative than that of Fe(II). The redox potential of the reduction of oxygen to water is +0.82 V. The E_0 of the reduction of Fe(III) to Fe(II) is +0.77 [at pH = 1].... [MCM: some aspects of these numbers are unclear and appear to be inconsistent.] A solution of Fe(II) and Fe(III) and O_2 is not at equilibrium, and has free energy given (see below) by:

$\Delta G^{0'} = -nF\ \Delta E'_0$ where

n = number of electrons transferred

F = Faraday constant.

Thus the redox potential can give the change in free energy on a redox reaction.

For nonstandard conditions and a redox pair, the measured voltage E_h relates to E'_0 as follows:

$E_h = E'_0 + (2.3RT/nF) \log ([\text{Oxidant}]/[\text{Reductant}])$ where

n = number of electrons transferred

R = Ideal Gas Constant

F = Faraday constant.

The **mid-point potential E_m** is defined as the electrochemical potential for which the concentrations of the oxidized and reduced forms of the redox pair are equal though not necessarily 1 molar.[27] This measure is useful in biological systems for which attaining 1M concentrations may not be feasible. When conditions are nonstandard, the measured redox potential will differ substantially from standard reduction potential. E_m (like E_h) measures the tendency of a compound or ion to donate or receive electrons from other compounds/ions. A large positive E_m means that the compound/ion is a strong oxidant and attracts and wants to accept electrons, whereas a large negative value means that the compound/ion is a strong reductant and repels and wants to donate electrons. In either case, E_m are measured relative to the standard hydrogen electrode.[28]

When the oxidant and reductant concentrations are equal (but not necessarily 1M each) and pH = 7,

$E_h = E'_0 = E_m$

Electrochemical Potential

Transporting an uncharged solute uphill against its concentration gradient (from lower to higher concentration) decreases entropy and requires the input of free energy. To transfer 1 mol of solute requires

$\Delta G = 2.3RT \log ([C_2]/[C_1])$

If $C_2 > C_1$, then $\Delta G > 0$ and work is required to make this transfer. However, movement such as by diffusion can proceed spontaneously from C_2 to C_1 when $C_2 > C_1$, since this increases entropy and $\Delta G < 0$.

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http://en.wikipedia.org/wiki/Hydrogen_electrode

• possibly -0.42 V according to the textbook

[25] Redox measurement: Clugston and Flemmin, *Advanced Chemistry*, 2000

[26] *Ibid.*

[27] Midpoint potential: *Plant Energetics*, Octavian S. Ksenzhek, Alexander G. Volkov, 1998

[28] Midpoint potential: TZ web topic 7.6

If the solute is also charged, the work done to move it across a membrane electric potential depends on the **membrane potential ΔE**. This is defined as the work required by an agent to move a test charge from one side of a membrane to the other. The change in free energy when moving one mole or ion against a membrane potential ΔE is given by:

$\Delta G = zF\,\Delta E$ where

ΔG = change in free energy of the ion

ΔE = membrane potential

z = valence or charge of the ion

F = Faraday's constant

Moving a cation (positively charged ion such as Ca^{+2}) to a higher (more positive) voltage requires input of work from another source (e.g., "pumping", as with moving H^+ outside the relatively electronegative cell cytosol, a process coupled with ATP hydrolysis)

Moving an anion (negatively charged such as Cl^-) to a more positive voltage can proceed spontaneously and output (do) work.

The **Electrochemical Potential Difference** Δμ [printed mu with a tilde over it] for a particular ion takes into consideration the work required or performed arising from both the voltage ("electro-") and concentration ("-chemical") gradients, and is given by

$\Delta\mu = \Delta W_{max} = \Delta G\ (kJ\ mol^{-1}) = zF\,\Delta E + 2.3RT \log([C_2]/[C_1])$

It is the partial molar Gibbs free energy of the substance at the specified electric potential [and concentrations].[29] Ions tend to flow from areas of higher to lower Electrochemical Potential so that ΔG is negative. If ΔG is negative, it defines the maximal work output a reaction can perform; if negative, it defines the minimum work input a reaction requires to proceed. To express Δμ in terms of mV, use

$\Delta\mu/F\ (in\ mV) = z\,\Delta E + 2.3(RT/F) \log([C_2]/[C_1])$

In the special case of protons, the total energy available for ATP synthesis is given by the **proton motive force** (see TZ p. 106 and 148):

$\Delta p\ (in\ mV) = \Delta E - 59(pH_i - pH_o)$

For permeant ions distributed at equilibrium across a membrane, $\Delta G = \Delta\mu = 0$ and we have

$\Delta E = -2.3(RT/zF) \log([C_2]/[C_1])$

which is the Nernst equation for the permeant ionic species. At equilibrium, a permeant ion will be so distributed across the membrane that the chemical driving force will be balanced by and equal magnitude but opposite electric driving force.

A transmembrane electric potential may arise merely from a **diffusion (Donnan) potential** down a preexisting concentration gradient (when the counterion is impermeant), but more commonly arises from diffusion plus electrogenic ion pumps that require an external energy source.

Proteins and Enzymes

The **alpha amino acids** have an **-NH_2 amino** group and a **carboxyl** group (-COOH) attached to the same terminal carbon (called the **α-carbon**), plus an organic substituent R group also attached to this alpha carbon (simply -H for glycine, -CH_3 for alanine, -CH_2SH for cysteine, etc). The 20 usual proteinogenic amino acids may be divided into 4 groups based on the chemical behavior of the R group:

- **Hydrophobic (nonpolar) R group: Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan, Methionine, Cysteine, Glycine**
- **Hydrophilic (polar) Neutral R group: Asparagine, Glutamine, Serine, Threonine, Tyrosine**
- **Hydrophilic (polar) Basic R group: Lysine, Arginine, Histidine**
- **Hydrophilic (polar) Acidic R group: Aspartate (and Aspartic Acid), Glutamate (and Glutamic Acid)**

Proteins are made of **polypeptides** consisting of chains of **amino acids**. Amino acids are linked in **peptide bonds**, which form when the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water (H_2O)—a **dehydration** synthesis reaction (also known as a **condensation** reaction)—which usually but not always occurs between amino acids. The resulting -C(=O)NH- bond is called a **peptide bond** and the resulting group an **amide group** or **peptide group**.

[29] Electrochemical potential: http://goldbook.iupac.org/E01945.html

Peptide bonds may be broken by **amide hydrolysis**, the adding of water, to reform the $-NH_2$ and -C(=O)OH groups.

Peptide bonds have two resonance forms and have a **partial double bond** character and can form hydrogen bonds.[30] Because the bond between the carbonyl carbon and the nitrogen (-CO-NH-) has a partial double bond character, rotation around this bond is restricted. "Thus, the peptide unit is a planar, rigid structure and rotation in the peptide backbone is restricted to the bonds involving the α- carbon."[31]

Enzymes are proteins that have high **specificity** and high **efficiency** for catalyzing (accelerating) specific reactions. (Some RNAs and protein-RNA complexes also catalyze reactions, so-called **ribozymes**.) They increase rates of exergonic reactions by lowering the free energy barrier between reactants and products (the energy of the transition state), usually by forming complexes and intermediates. This effectively raises the ground state ΔG and lowers the ΔG of the transition state. Enzymes however cannot convert an overall-endergonic reaction to an overall-exergonic one. Enzymes often **couple** a favorable exergonic reaction ($\Delta G < 0$) with an unfavorable endergonic ($\Delta G > 0$) reaction, where the coupled reactions have net $\Delta G < 0$. The commonest protein on earth is the enzyme rubisco (used in C_3 photosynthesis), comprising 50% or more of plant leaf proteins. Many enzymes function optimally in narrow pH and temperature ranges, some in extreme conditions.

The levels of protein and specifically enzyme organization are as follows:

- **Primary structure**: polypeptide chain are comprised of amino acids linked by covalent peptide bonds.
- **Secondary structure**: The polypeptide chain folds spontaneously into a 3-dimensional conformations linked by noncovalent bonding (hydrogen, electrostatic, and van der Waals bonds, and hydrophobic "bonds", etc.), initially as α-helices (singular: helix) and β-pleated sheets. There may also be covalent disulfide bonds (-C-S-S-C-) formed usually by the oxidation of sulhydryl (thiol) -SH groups from cysteine (but not methionine).
- **Tertiary structures**: The secondary structures pack together via further conformational changes
- **Quaternary structure**: The final form of enzymes consists of combining one or more protein polypeptide tertiary structures as subunits (protomers) in an oligomeric complex.

The **active site** of an enzyme is often located at the interface of two or more independently folding units called **domains**, typically of size c. 10^4 daltons. Binding of the substrate is non-covalent. The catalytic groups may be $-NH_2$, -COOH, imidazole, $-S^-$, -OH, or cofactors such as metal ions etc. A conformational change may result from substrate binding, affecting the binding of a 2nd substrate (e.g., hexokinase). Isoenzymes (isozymes) are enzymes in a family having similar catalytic properties but different structures. Enzymes frequently require **cofactors**:

• **coenzymes** such as metal ions (Mo or Zn ions) or small non-protein non-permanently-bound organic compounds such as Coenzyme A and NAD^+/NADH, or

• **prosthetic groups** (non-protein components that are bound tightly to enzymes, such as heme and Ca^{2+})

Enzymes are typically highly stereospecific—e.g., β-glucosidase hydrolyzes organic β-glucosides formed by β-glycosidic bonds to D-glucose but not α- anomers or L-glucose compounds.

Enzyme kinetics often follow **Michaelis-Menten kinetics**:

$$\mathbf{v\ (initial\ velocity) = (V_{max}\ [S]) / (K_m + [S])}$$

where V_{max} is the asymptotic maximum velocity attained at saturation by substrate S. A small K_m is associated with a high affinity of the enzyme for the substrate.

These kinetics are also seen by some **membrane transport processes** such as (reversible) mitochondrial or chlorplastic ATP-ase pumps. The reactants are the solute outside (or inside) the membrane and the carrier [pump] and the products are the solute inside (or outside) the membrane and the carrier [pump]. A maximum rate of carrier mediated transport is exhibited.

Enzymes are subject to activation and inactivation including inhibition. The **Lineweaver-Burk double reciprocal plots** allow graphical determination of $1/K_m$ and of $1/V_{max}$. These graphs may also be used to demonstrate **competitive inhibition** (K_m increased but V_{max} unchanged) versus **noncompetitive inhibition** (K_m unchanged but V_{max} reduced). Competitive inhibition often results direct competition from a resemblance of the inhibitor to the substrate, or indirectly in allosteric enzymes for which binding of an

[30] Peptide bonds and hydrogen bonding: http://en.wikipedia.org/wiki/Peptide_bond

[31] Rotation at peptide bonds: http://webhost.bridgew.edu/fgorga/proteins/resonance.htm

effector distant from the active site alters the conformation of the active site. In noncompetitive inhibition, the inhibitor does not compete for binding with the substrate for the active site, but may block the substrate's access, etc. Inhibition may also be **mixed**.

Enzyme kinetics may be strongly dependent on pH—typically bell-shaped curves having a peak optimal value of pH—or temperature (in which a peak occurs at an optimal temperature and increasing **denaturation** at temps typically of 40 - 50 °C. Tightly regulated enzymes are often **cooperative systems** in which a small change such as in an inhibitor or activator concentration causes a large change in reaction velocity. These are often **allosteric systems** with oligomeric enzymes, in which the inhibitor induces a conformational change in the enzyme. The cooperation may arise from binding of a **ligand** at the substrate binding site (**homoallostery**) or at other sites (**heteroallostery**).

Enzyme activity may also be controlled by altering:

- **concentrations** controlled by genetic processes
- **phosphorylation** or **adenylylation**
- **compartmentalization** in vacuoles, chloroplasts, mitochondria, membranes, cell walls, etc.
- **feedback inhibition** in which intermediate or final products negatively feedback on the initial committed step (as in glycolysis and gluconeogenesis)

Chapter III. Water And Plant Cells

Water is frequently a limiting resource for plants. Crop yields are directly affected by the number of days of high water availability during the growing season, or by annual precipitation. Plants must **transpire** water at the leaves as a consequence or tradeoff needed for obtaining atmospheric CO_2—the H_20:CO_2ratio is 500:1 in some healthy well-watered plants. The cell wall is porous to water.

Water moves passively in the plant down gradients of water potential—there are no water "pumps".

Water: Heat Properties

Water is a polar molecule. The hydrogens form an angle of 105° with the oxygen. The O is more electronegative than the hydrogens. The weak separation of partial charges and resulting electrostatic attraction between opposites allows water to form hydrogen bonds. Water is an excellent solvent due to its small size and polar nature, especially for ionic substances and molecules with -OH or -NH_2 groups. The electrostatic attraction increases the solubility of polar substances in water.

Water requires an unusually large energy input to raise its temperature (high **specific heat capacity** c_p).[32]

Water (liquid) c_p = 4.1855 J g–1 K–1 (or about 1 calorie / gram -°C)
Water (liquid) C_p = 75.327 J mol^{-1} K^{-1} (or about

The **latent heat of vaporization** for water[33] is c. 44 kJ mol^{-1} or 2272 J/g, a very high value (e.g., ethyl alcohol is 855 J/g, and ammonia is 1369 J/g).
These properties help plants buffer temperature fluctuations intrinsic heat capacity and by evaporation.

Water: Cohesion, Adhesion, Surface Tension, and Capillarity

Water has a high **cohesivity** due to the mutual attraction of its polar molecules—this can be seen with water droplets.

[32] Specific heat capacity: Specific heat capacity c_p is defined as (T/N) ($\partial S/\partial T$) where T is the temperature, N is the number of molecules being heated, and $\partial S/\partial T$ is the ratio of heat input to temperature change: http://en.wikipedia.org/wiki/Heat_capacity#Heat_capacity
[33] Latent heat of vaporization J/g: http://en.wikipedia.org/wiki/Latent_heat

At an air-water interface, there is a greater attraction between water molecules compared to between air and water molecules, and the lowest energy configuration is one which minimizes the surface area of the interface.

Water's **adhesivity** (attraction to an adjacent solid phase) varies with the solid material and is quantified via the **contact angle**. A low contact angle ($\theta < 90^o$) indicates relatively high adhesion to a **hydrophilic** substance such as glass (higher "**wettability**") and higher **capillarity** (i.e., higher level in a capillary tube compared to the baseline water level). A high contact angle $\theta > 90^o$ indicates relatively low adhesion to a **hydrophobic** substances such as a waxed surface, lower wettability, and lower capillarity (i.e., lower level in a capillary tube compared to the baseline water level).

In order to overcome the force of cohesion and adhere to a larger curved surface area interface with a hydrophilic substance, additional energy is required to exceed the minimum energy configuration. This quantity is called **surface tension**,[34] and is measured in energy/area J/m^2 or in Force/distance N/m)

The surface tension produces a net pressure differential at a curved air-water interface. The pressure is a function of the surface tension and the radii of curvature of the surface, and is given by[35]

$\mathbf{\Delta P = \gamma (1/R_x + 1/R_y)}$ where

ΔP is pressure difference in the water

γ is surface tension [72.0 mN m-1 for water at 25 ºC][36]

R_x and R_y are radii of curvature in each of the axes that are parallel to the surface and positive when directed into the fluid.

When R_x and R_y are positive (as with hydrophobic surfaces), the internal liquid pressure is increased allowing the surface to bow out in the center and thereby diminish contact with a hydrophobic surface. When R_x and R_y are negative, the internal liquid pressure is decreased, allowing the water to extend further up the hydrophilic surface.

The pressure differential that develops in water from an air-water interface due to surface tension increases with decreasing radius. For interstices between hydrophilic surfaces (such as inside leaves or soils), lower water content translates to smaller water collections having smaller radii of curvature, leading to a greater **tension** (more negative pressure change, $\Delta P<0$) within this water.

Pressure Unit Comparisons:

1 atmosphere = 14.7 PSI or 0.1013 MPa

1 MPa = 9.9 atmospheres

Tire pressure and home plumbing pressure are c. 0.2 MPa

A water column of 15 feet has positive pressure at the bottom of 0.05 MPa.

Water has a high tensile strength arising from its surface tension. This allows water to be pulled up a tube by tension above. Capillarity in xylem vessels contributes < 1 meter rise, so cannot explain water transport in tall trees (Web topic 3.1). In a thin capillary, the negative pressure (tension) sustainable before **cavitation** (breaking into bubbles) occurs can be as high as -30 MPa. Microbubbles greatly increase the likelihood of cavitation. (see chapter 4)

Diffusion Rates of Dissolved Substances

Substances diffuse down a concentration gradients according to Fick's first law:[37]

$J = - D \partial\varphi/\partial x$ where

J is the diffusion flux density (rate of transport) in (amount of substance) $length^{-2}$ $time^{-1}$,

D is the diffusion coefficient or diffusivity in $length^2$ $time^{-1}$

φ (for ideal mixtures) is the concentration in (amount of substance) $length^{-3}$

Diffusion rate is proportional to concentration gradient. D depends on the substance diffusing, the medium through which it diffuses, and Temperature. Diffusion can be rapid over short distances, but is

[34] Surface tension: http://en.wikipedia.org/wiki/Surface_tension

[35] Laplace-Young equation for surface tension: http://en.wikipedia.org/wiki/Young-Laplace_equation

[36] Water/air surface tension: http://www.kayelaby.npl.co.uk/general_physics/2_2/2_2_5.html

[37] Fick's law: http://en.wikipedia.org/wiki/Fick's_law_of_diffusion

very slow over long distances and not adequate, for instance, to move glucose between parts of a plant (which would require 32 years to diffuse 1 meter—see web topic 3.2).[38]

Bulk Flow of Water, Water Potential, and Osmosis

Water moves passively in the plant down the gradient of water potential (if semi-permeable membranes are involved) or the gradient of pressure potential (if bulk flow not involving semi-permeable membranes). Because diffusion would be so slow, water containing solutes generally moves by **bulk (mass) flow**. The volume flow rate is given by Poiseuille's equation:

Volume flow rate (in $m^3\ s^{-1}$) = $\mathbf{(\pi r^4/8\eta)\ (\Delta\Psi_p/\Delta x)}$ where

r = radius of tube

η = viscosity

$\Delta\Psi_p/\Delta x$ = gradient of hydrostatic pressure potential

Thus such flow is very dependent on radius of the conducting tube. Bulk flow accounts for long-distance transport in the xylem.

While chemical potential expresses free energy per mole, **water potential** is usually used, and is given in terms of the partial molal volume of water (thus a measure of the free energy of water per unit volume, in unit of $J\ m^{-3}$). 1 mole of water occupies $18x10^{-6}\ m^3\ mol^{-1}$. Water potential in plants is primarily a sum given by

$\mathbf{\Psi_w = \Psi_s + \Psi_p + \Psi_g}$ where

Ψ_w = water potential

Ψ_s = solute potential

Ψ_p = hydrostatic pressure potential

Ψ_g = gravity potential

The units of these potentials are therefore energy per unit volume $J\ m^{-3} = N\text{-}m\ m^{-3} = N\ m^{-2}$ = **Pa**. (Other pressure units such as **MPa** may also be used). The potentials must be given relative to a reference, usually pure water at ambient temperature and atmospheric pressure and at some defined height relative to the plant. (See Topic 3.3 for Alternative Conventions for Components of Water Potential and Topic 3.6 on measuring water potential.)

Ψ_g gravity potential is given by

$\mathbf{\Psi_g = \rho_w g h}$ where

g = gravitational constant = $9.8\ m\ s^{-2}$

ρ_w = water density = $1000\ kg/m^3$

$\rho_w g$ = water density times gravitational acceleration (9800 Pa = 0.0098 MPa ≈ 0.01 MPa m^{-1})

h = height (m)

For a height of 1 m, the Ψ_g is 9800 Pa or 0.0098 MPa or c. 0.01 MPa.
For a height of 10 m, the Ψ_g is c. 0.1 MPa.
For a height of 100 m, the Ψ_g is c. 1 MPa.

Solute or osmotic potential Ψ_s is usually negative because solutes reduce the free energy of water by diluting it and increasing entropy. It is given by the van 't Hoff equation:

$\mathbf{\Psi_s = -RTc_s}$ where

R = gas constant ($8.32\ J\ mol^{-1}\ K^{-1}$)

T = degrees Kelvin

c_s = solute concentration in osmolality (moles of osmotically active solute such as ions per liter of water, or $mol\ L^{-1}$)

Hydrostatic Pressure Ψ_p: Inside cells it is called **turgor pressure** and is usually positive. (It might conceivably be negative at times in lignified living cells, but not in more flexible living cells, see Web Topic 3.4.) It is often negative in the xylem.

It can be hard to distinguish between solute potential Ψ_s and hydrostatic potential Ψ_p in dry soils and dry plant tissues such as seeds, and these terms are sometimes combined as **matric potential Ψ_m** ($\Psi_m = \Psi_s + \Psi_p$, see web topic 3.5).

[38] Diffusion rates: see TZ Webtopic 3.2

Measure water potential with psychrometer or pressure chambers, solute potential with cryoscopic osmometer, pressure potential with pressure probes,

Water moves across a semi-permeable (selectively permeable) membranes from areas of higher water potential to areas of lower water potential—this process is called **osmosis**. (NOTE: In the absence of the membrane, the movement of water is controlled simply by hydrostatic pressure differences.) Cells receive water by reducing their internal water potential.

The transport flow rate of water across a membrane $\mathbf{J_v}$ is given by

$\mathbf{J_v} = \mathbf{Lp}\ \mathbf{\Delta\Psi_w}$ where

$\mathbf{J_v}$ = transport flow rate of water across a membrane ($m^3\ m^{-2}\ s^{-1}$ or $m\ s^{-1}$)

$\mathbf{L_p}$ = **hydraulic conductivity** of the membrane ($m\ s^{-1}\ MPa^{-1}$)

$\Delta\Psi_w$ = transmembrane water potential difference $\Delta\Psi_w$

Osmosis across semi-permeable membranes is driven by water potential gradients, combining both the solute potential Ψ_s and the hydrostatic pressure Ψ_p. Plants do not have active transmembrane water pumps, but can change intracellular (or intraluminal xylem) solute potentials in order to influence water flow. Water can move in a plant against its water potential only when coupled to the movement of solutes, in which case the decrease of free energy of the solute exceeds the gain in the water's free energy—the overall net change of free energy is negative as expected.[TZ47] Flow across membranes is passive in response to water potential differences, and occurs primarily through water-selective **aquaporins** (integral membrane proteins) rather than directly through the otherwise nearly impermeable membrane. Aquaporins can be gated reversibly, so that plants may be able to control their plasma membrane water permeability.

(For full details of experiments that follows, see fig. 3.9) A wilted or flaccid plant cell has low turgor (hydrostatic) pressure, though its negative solute potential contributes to a typically negative water potential. The cell wall collapses along with the plasma membrane and remains in contact with it. A flaccid cell with high internal solute content placed in 0.1 M sucrose will attract water to its interior and into the vacuole, and may end up with increased hydrostatic pressure as a result of distension, especially of the vacuole, pressing against the restraining cell membrane. Cells placed in unusually high concentrations of glucose may exhibit **plasmolysis** from water loss—a separation of the plasma membrane from the cell wall with interposed fluid [or air, somewhat like a pneumothorax]. (This phenomenon rarely occurs in nature except in extreme conditions such as air entry through the cell wall. See web topic 3.8) External pressure applied to a cell may squeeze water out of it, make its internal solute concentration higher and its solute potential more negative. Small changes in water content of a cell may result in large changes in hydrostatic pressure, as depicted in **Höffler diagrams**. If cell volume decreases by only 10-15%, the turgor pressure may drop to 0.

In **cacti**, the **water-storing cells** are much more distensible than the photosynthetic cells, due to more flexible walls, so are less prone to have rising hydrostatic pressure.[TZ48] These cells give up their water more readily, allowing the photosynthetic cells to maintain higher water content during water stress.

The Ψ_s of the apoplast is usually very low.

Well watered plants have water potentials of 0 to -1 MPa, but with higher degrees of **water stress** develop water potentials down to -3 of -4 MPa, with extreme cases down to -10. **Halophytes**, which grow in saline conditions, are able to survive by generating a very low intracellular Ψ_s, allowing them to maintain a lower intracellular water potential to attract water. Water stress affects many processes as follows (listed in order from earlier to later to occur based on degree of water stress):

Cell expansion < Wall synthesis < Protein synthesis < Stomatal Conductance < Photosynthesis < Solute accumulation < Abscisic acid accumulation

Positive turgor pressure is important to stretch the cell walls, provide support for nonlignified tissues, and facilitate plant growth.

Chapter IV. Water Balance Of Plants

The need for CO_2 gas exchange exposes plants to risk of dehydration. The plant does not use metabolic energy to pump water in from the soil, but rather uses spontaneous movement of water to regions of lower free energy and water potential.

Soil Water

Soils range from sandy soils (with c. 1 mm particles, low overall surface area, and low "**field capacity**" to retain water) to clays (with particles < 1 µm, high overall surface area, and high capacity to retain water). Soil has air in between the particles. Soils typically have a nearly zero water potential Ψ_w due to low concentrations of dissolved solutes (but may be more negative if solutes are high). In addition, Ψ_p is near zero for wet soils, but more negative for dry soils. The negative potential arises from the relationship of surface tension and curvature of the water/air interface in the soil interstices as previously discussed. Soil water tension may be estimated at

$\mathbf{\Psi_p = -2T/r}$ where

Ψ_p = hydrostatic pressure (tension in this case)

T = surface tension of water (7.28×10^{-8} MPa m)

r = radius of curvature at air/water interstitial interfaces

As the soil dries out and water recedes into the smallest cracks, the radii of curvature become smaller (c. 1 µm) and soil Ψ_p becomes more negative to as low as -1 to -2 MPa.

Water moves in soil by bulk flow along pressure gradients $\Delta\Psi_p$, and also by diffusion, especially in drier soils. Plants deplete the water (and nutrients) next to the roots and root hairs, locally reducing Ψ_p near the root surface. This water deficit is replenished at a rate dependent on the **hydraulic soil conductivity** (see web topic 3.7). This quantity decreases as water content decreases, due primarily to presence of soil air and narrowing of conduction channels. In very dry soils, the Ψ_w may fall to the **permanent wilting point** (c. 1.5 MPa, see chapter 26) at which point the plant cannot maintain turgor pressure (web topic 4.2).

For more on irrigation, see Web topic 4.1

Root Absorption And Water Transport

Root hairs greatly increase the surface area available for absorption. Water enters primarily near the **root tip**. Older root areas are sealed off by hydrophobic materials and protective outer tissues (exodermis, etc.)—this facilitates water entry preferentially from new regions that have not already been depleted of nutrients. The delicate connections between new root tips and hairs and soil water are easily disrupted, as with **transplanting seedlings**.

Water enters the root via both apoplastic and symplastic pathways under the driving force of differences in Ψ_p. In the apoplast pathway, water travels across the epidermis and along the root cortex. At the **endodermis** (surrounding the vascular bundle), apoplastic water encounters the Casparian strip, an impervious barrier of **suberin**, forcing this water to cross a cell membrane (through aquaporins) and enter the symplast. This transport process requires actively metabolizing root cells and is inhibited when these cells are not receiving adequate O_2 (such as when the root is submerged, since water diffusion of oxygen is much slower). The permeability of aquaporins may decrease from increased pH [sic, may actually be acidosis][39] resulting from decreased rates of respiration. Thus shoots may **paradoxically wilt** when roots are flooded with water and hypoxic.

Water crossing a cell membrane and entering directly into the symplast traverses the cytosol of cells via their desmoplasts until the xylem conduits are reached. There is also a possible "transmembrane" pathway involving multiple membrane crossings, including the tonoplasts. Both of these pathways depend on well-oxygenated root cells that can be impaired by root hypoxia/anoxia.

Roots can generate "**root pressure**", a positive Ψ_p in the root xylem as high as 0.05 to 0.5 MPa. This occurs primarily at night and early morning when soil water potential is high, humidity is high and

[39] Effect on aquaporins of cell acidosis in anoxia: p. 757 of http://www.biolcell.org/boc/097/0749/0970749.pdf

transpiration rates are low—absorbed solutes build up in the xylem, lowering Ψ_s and decreasing Ψ_w in the xylem, providing a driving force like an osmotic cell for water absorption. Positive root pressure is associated with **guttation** or "**dewdrops**" of xylem sap forming at **hydathodes** at vein endings at the tips of leaves. Positive root pressure may help to dissolve xylem bubbles developed during the day.

Water Transport Through The Xylem

See web essay 4.1 for a history of the study of water movement in the xylem.

Xylem consists of dead lignified (and thus rigid) **tracheary elements**: **tracheids** and **vessel elements** (see also TZ Chapter I discussion). The **simple pits** connect adjacent tracheids and vessel elements, and the latter also have connecting **end wall perforations** (which provide a larger and lower resistance connection). The simple pits have a porous **pit membrane** (with a thickened **torus** in conifers) that acts as a safety valve which closes in the event of gas bubbles and air embolism.

Water movement in xylem is by bulk flow along pressure gradients $\Delta\Psi_p$, and is facilitated by the relatively low resistance. A velocity of 4 mm s^{-1} and vessel radius 40 µm requires by Poiseuille's equation a gradient of 0.02 MPa m^{-1}, but actual resistance may be twice as high. To move water across cell membranes at this rate would require $2x10^8$ MPa m^{-1}—thus xylem transport is vastly more efficient. (See web topic 4.3 for more on calculating velocities of water movement in the xylem.)

The pressure differential required to lift water 100 m from the base to the top of a tall tree is estimated at 2 MPa, or 3 MPa allowing for friction—the top of the tree must have a water potential of c. -3 MPa (see web essay 4.3). The still somewhat debated **cohesion-tension theory of sap ascent** explains how this pressure differential is achieved (web essay 4.2). Root pressure (0.05 to 0.5 MPa maximal under optimal conditions) is not sufficient, and most of the pressure differential arises from **tension** (negative pressure) that develops in the leaves and that "pulls" the water up. The water in the interstices between the hydrophilic mesophyll cell cell walls is like a fine capillary network, forming curved air-water interfaces which induce a tension transmitted to the cells. The sun provides the energy by heating and evaporating water that is transpired.

But can xylem cells really maintain such high tensions without **cavitation**? Apparently so, though some vessels do cavitate (see web essay 4.4) The lignin prevents collapse of the xylem vessels under tension, and denser wood is required to sustain higher tensions. The tension creates a **metastable** state in the xylem, since the saturated vapor pressure of water is c. 0.002 MPa at 20 ºC[40] [MCM: how can this data be applied?]. Why does the water not boil? The cohesion and adhesion make the activation energy of the transition to the vapor phase very high, and the xylem structure minimizes the existence of nucleating sites for gas bubbles. [In contrast, the highest sustainable water column at 1 atmosphere (0.1013 MPa) is about 10.3 m =33.9 ft in larger-diameter non-biological pipes[41]—above this height, the water boils or "cavitates".]

Water Movement From The Leaf To The Atmosphere

The waxy impervious **cuticle** prevents water diffusion across much of the leaf surface—95% of water exits by diffusion via the **stomata**. The factors affecting this transpiration movement are
(1) a gradient in water vapor concentration between the internal leaf air spaces (5-40% of the leaf) and the air in the boundary layer immediately outside the stomata, and
(2) Diffusion resistance

The internal surface area inside the leaf and from which water evaporates is quite large, 7-30x the leaf surface area. This allows water potential equilibration inside the leaf between the air spaces and the cell wall spaces. The water potential for inner air is c. -1.38 MPa, for inside air next to the pore is -7 MPa, and for air just outside the pore is -103 MPa. Thus water vapor wants to move down this potential gradient to the outside.

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[40] Water vapor pressure by temperature: http://en.wikipedia.org/wiki/Vapor_pressure_of_water
[41] Maximum sustainable in vitro water column: http://en.wikipedia.org/wiki/Atmospheric_pressure

The **boundary layer** water content, and thus the potential gradient, can be strongly influenced by wind speed ("still air" diminishes exchange), leaf size and shape, fluttering of leaves (as in quaking aspen), leaf hairs, and sunken configuration of stomata. The opening of the stomata is done to lower resistance to CO_2 diffusion and increase uptake, but water loss inevitably accompanies this. When a plant attempts to prevent dehydration by keeping its stomata closed, the availability of CO_2 inside the leaf diminishes, and therefore photosynthesis diminishes. Movement of water in outside air away from the boundary layer is by **convection**, a type of bulk flow.

The **stomata pore** size is regulated by **guard cells**, which are found in liverworts, mosses, and higher plants. They guard cells are flanked by subsidiary cells. The cellulose microfibrils of guard cells vary with species but tend to fan out radially from the pore [or are oriented like barrel hoops per LVV], so that when the cells enlarge from increased turgor pressure, they primarily elongate Since the ends are tethered together, and the inner and outer cell wall may differ in thickness, elongation results in bowing outward, opening the pore. Swelling results from lowering of Ψ_w inside the cell compared to adjacent cells resulting from lowering of Ψ_s by ion uptake and biosynthesis of organic molecules in the guard cell. (Guards cells close in water stress conditions under the influence of the stress hormone, abscisic acid ABA.)

The **transpiration ratio** expresses water moles transpired / CO_2 moles assimilated. This ratio is up to 500 in C3 plants, as a result of:

- higher water than CO_2 gradients
- lower CO_2 concentrations compared to water
- slower rate of CO_2 diffusion
- CO_2 must cross several membranes to be assimilated.

The C_4 plants have more favorable transpiration ratios of c. 250, and CAM desert plants achieve much more efficient use of scarce water, with ratios of c. 50.

For more on leaf transpiration and water vapor gradients, see web topic 4.4

Summary Of Water Movement In The Soil-Plant-Atmosphere Continuum

A tug-of-war for water exists in the **soil-plant-atmosphere continuum** as follows:

- **Soil**: Dry soils have very negative water potential Ψ_w and may attract water from the roots.
- **Root xylem**: Lowered solute potential Ψ_s and lowered overall Ψ_w by solute accumulation in the root xylem attracts water into the roots (sometimes increasing xylem Ψ_p in the roots under optimal conditions).
- **Shoot xylem and leaves**: Tension (low Ψ_p) and resulting low leaf Ψ_w draw water up the xylem to the leaves.

Chapter V. Mineral Nutrition: Soil Nutrients And Their Absorption By Roots

Although they can manufacture carbohydrates and other related organic compounds from water and CO_2, plants require mineral nutrients (N, P, K, other inorganic ions) from the soil. They are like miners of the earth, and are aided in this process by mycorrhizal fungi and nitrogen fixing bacteria. (Some insectivorous plants such as the pitcher plants receive nutrients from insects.)

Essential Nutrients And Deficiencies

Essential nutrients are those minerals (elemental or inorganic ions, customarily excluding C H O in water and CO_2) for which the absence leads to severe abnormalities in growth, development, or reproduction. The essential nutrients include:

Essential Nutrients:

- **Derived from Water and CO_2** (soil concentrations are 6%, 45%, 45%):
  C H O
- **Soil Macronutrients** (typically concentration 1.5 - 0.1 % of dry soil, listed in decreasing %):
  N K Ca Mg P S Si
- **Soil Micronutrients** (typically 100 - 0.1 ppm in dry soil, listed in decreasing abundance):
  Cl Fe B Mn Na Zn Cu Ni Mo

Nutrients By Biochemical Function:
Another classification of Nutrients is by biochemical function as follows:

- Part of Carbon compounds (other than C H O): N S
- Important in Energy Storage or Structural Integrity: P Si B
- Remain in Ionic Form: K Ca Mg Cl Mn Na
- Involved in Redox Reactions: Fe Zn Cu Ni Mo

The essentialness of a nutrient can be determined with **hydroponic** growing conditions. **Hoagland solution** (p. 78) was formulated to allow rapid plant growth, and contains all the known essential mineral elements. Here is a sample recipe (all concentrations in units of millimoles/liter):[42]

0.4 $NH_4H_2PO_4$ [Ammonium phosphate]
2.4 KNO_3
1.6 $Ca(NO_3)_2$
0.8 $MgSO_4$
0.1 Fe as Fe-chelate [e.g., DTPA]
0.023 B as $B(OH)_3$ [boric acid]
0.0045 Mn as $MnCl_2$
0.0003 Cu as $CuCl_2$
0.0015 Zn as $ZnCl_2$
0.0001 Mo as MoO_3 or $(NH_4)_6Mo_7O_{24}$ [Ammonium heptamolybdate]
Cl as chlorides of Mn, Zn, and Cu listed above

N must be supplied both as **ammonium** (NH_4^+) and as **nitrate** (NO_3^-) for optimal growth. Iron tends to precipitate as insoluble iron hydroxide ($Fe(OH)_2$ or $Fe(OH)_2$) or iron phosphate ($Fe_3(PO4)_2$ or $FePO_4$), and Fe must be present in "available" form, typically **chelated** with citric acid, tartaric acid, or improved chelators such as EDTA or DTPA.

For a discussion of plant **deficiency disorders**, see textbook and web topic 5.1. Common symptoms include **chlorosis** (leaf yellowing, sometimes in characteristic patterns involving new vs. old, intervenous areas, etc.) or leaf whitening; purple, dark green, bronze discoloration, growth stunting (sometimes localized, such as to internodal area), **necrotic** areas, susceptibility to root rot, leaf curl and crinkle, bent stem (lodging), premature abscission, etc.

Analysis of plant tissues, not just of soil, provides the most useful data for assessing possible deficiencies.

Treatment of deficiencies may involve soil application (rarely, leaf application) as follows:

- Correcting soil pH—some nutrients are less available at altered pH, typically raised pH.
- Inorganic fertilizer addition (including micronutrients, especially in leached sandy acid soils)
- Organic fertilizers (these require breaking down, or mineralization before they are fully available)
- Mycorrhizal fungi

Soil, Roots, and Microbes

Soil particles tend to have negative charges on their surfaces, due to exchange of Al^{3+} and Si^{4+} cations with cations of lower charge: K^+ Mg^{2-} Ca^{2+}. Organic particles also have negative charge due to dissociation of H^+ from carboxylic and phenolic groups. Mineral cations such as NH_4^+ and K^+ adsorb to these negative charges, preventing their ready leaching and maintaining their availability. "The degree to which a soil can adsorb and exchange ions is termed its **cation exchange capacity** (CEC)..." Soils with higher CEC tend to

[42] Hoagland solution: http://www.soils.wisc.edu/~barak/soilscience326/hydropon.htm

have a larger reserve of mineral nutrients. But anions such as NO_3^- are easily leached out and can become depleted. Phosphate ($H_2PO_4^-$) can be tightly bound to Fe^{2+}, Fe^{3+}, and Al^{3+}, and therefore of limited availability.

Soil pH affects availability and growth. Root growth is usually favored in slightly acid soils (which fungi prefer) compared to more alkaline soils (which bacteria like). Acidity promotes weathering of rock and release of K^+, Mg^{2+}, and Mn^{2+}... Acidity arises from CO_2 conversion to carbonic acid. Bacterial decomposition can convert ammonia and hydrogen sulfide to nitric and sulfuric acids. In arid climates, K^+, Mg^{2+}, and Ca^{2+}, and Mn^{2+} are released but do not leach out, leaving the soil alkaline...

Soils are categorized by **particle size**:
- Gravel < 2 mm
- Coarse sand: 0.2 - 2 mm
- Fine sand: 0.02 - 0.2 mm
- Silt: 0.002 - 0.02 mm
- Clay: < 0.002 mm

Excess soil minerals, such as salinization arising from repeated irrigation, can cause **salt stress** and limit plant growth, though some **halophytes** can tolerate high salt levels. Salts (or heavy metals) may be sequestered in the vacuole, and some plants can secrete salt from salt glands.

Roots have extensive surface area, mostly due to the billions of **root hairs**. Roots of the desert-dwelling mesquite may reach to 50 m depth. The rhizosphere is the domain of roots in soil. Root growth requires nutrients and water from the soil, carbohydrate from the phloem, and oxygen from the soil. Well fertilized and watered plants may have relatively small root systems that are meeting the plant's needs. (See web topic 5.2 for observing roots below ground).

Roots may grow in one of two patterns: (1) multiple primary roots with branching nodal or brace roots (monocots), or (2) a single taproot with many lateral roots (dicots). The most rapid rate of cell division takes place at the **apical meristematic zone**, protected by the **root cap and the quiescent center**, and facilitated by secretion of a lubricating **mucigel**. Behind the meristematic zone is the **zone of elongation** (a region of rapid cell growth and eventual differentiation into the endodermis and ectodermis, etc.) and, more proximally, the **maturation zone** (where **root hairs** grow). Main root growth is **gravitropic**. Roots generate the Casparian strip from suberin to isolate the endodermis from the surrounding tissue and force entry of water and nutrients into the symplast. The **endodermis** divides the root **cortex** (outside the endodermis) from the **stele** (vascular bundle inside the endodermis, containing the phloem and xylem). Hexoses in the phloem provide nourishment to the root but also provide osmotically active solute particles to the root xylem that lower the solute potential and attract water.

Absorption of nutrients may take place mainly at growing root hair tips, because of a **nutrient depletion zone** around established roots. Thus roots must grow continuously, and selectively reduce relative water absorption along the more proximal parts of the roots which are in the nutrient-depleted zone. (However, in some organisms, some nutrients such as iron are absorbed along the entire root surface...)

Soil **mycorrhizal fungi** are found in association with 83% of dicots, 79% of monocots, and all gymnosperms. These fungi have filaments called **hyphae**, which form a **mycelium** network. **Ectotrophic mycorrhizal fungi** form a sheath around the roots, and some penetrate the cortex of the root to surround but not penetrate into the cortical cells, in an internal network called a **Hartig net**. In contrast, the **vesicular-arbuscular mycorrhizal fungi** more extensively penetrate into the root, and were once thought to actually enter into individual cells of the cortex in the form of vesicles and arborized arbuscules. More accurately, these structures are now thought to invaginate the cortical cell plasma membrane and tonoplast, but not break them.[43]

Because of their greater extension into soil and much finer caliber with resulting large surface area, mycorrhizal fungi facilitate absorption of water and nutrients, especially the uptake of phosphorus and trace metals such as Zn and Cu, for plants in nutrient poor soils. Though usually a **symbiotic** relationship—the fungi assist the plant's nutrient absorption in exchange for receiving CHO—the

[43] Mycorrhizae: http://en.wikipedia.org/wiki/Mycorrhizal_fungi

association can at times shift to a **parasitic** one, and the plant may then develop defenses against them as for other pathogens.

EVV also mentions the work of Regina Redman and Russell Rodriguez on fungi that appear to have have symbiotic **endophytic** relationships with plants, for instance conferring salt tolerance.

Chapter VI. Solute Transport

This chapter deals with the physical and chemical principles governing the movements of ions and small molecules ("**transport**" of **solutes**) across semipermeable membranes, specifically the plasma membrane and the tonoplast. Transport takes place to maintain optimal intracellular conditions (osmolality, turgor pressure, ion concentrations, etc.) despite substantially varying extracellular conditions, as well as to transduce signals, etc.

Concentrations are measured as

molarity (moles / L solution or mol L^{-1}, often ambiguously abbreviated M)
molality (moles / kg solvent or mol kg^{-1}, sometimes ambiguously and incorrectly abbreviated m)
osmolarity (osmoles / L solution)
osmolality (osmoles / kg solvent)

The equation to determine the osmolality[44] of a solution is given by Osm = φ n C, where

- φ is the osmotic coefficient, which accounts for the degree of non-ideality of the solution. In the simplest case it is the degree of dissociation of the solute, between 0 and 1, where 1 indicates 100% dissociation...
- n is the number of particles (ions) into which a molecule dissociates. For example: Glucose equals 1 and NaCl equals 2.
- C is the molal concentration of the solution

Passive Versus Active Transport, Nernst Equation, Ion Concentrations

Diffusion of substances is governed by Fick's law (TZ Chap. 3), and occurs down a gradient of free energy (potential gradient) until equilibrium is reached. This passive movement is called **passive transport**. Movement of a substance against its chemical potential gradient is called **active transport**, and requires the input of work or energy from another energy source such as a coupled reaction (e.g., the hydrolysis of ATP).

The **chemical potential** μ_j for a specified solute j (TZ equation 6.1 p. 96, as in TZ Chap. 3, ignoring gravity) is given by the sum of contributions from

- Chemical potential under standard conditions = μ_j^*
- Concentration component = $RT \ln C_j$
- Electric potential = $z_j FE$
- Hydrostatic pressure component = $V_j P$

The term $V_j P$, relating to the partial molal volume of j, is usually much smaller than the other terms (except for osmotic water movements) and is ignored in the following analysis.

For passive movement of j from compartment A to B, $\mu_j^A > \mu_j^B$.
For **active transport** of j from A to B, $\mu_j^A < \mu_j^B$.

For a non-charged solute like sucrose, the Δμ between outside (o) and inside (i) a cell is given by

$$\Delta\mu_s = RT \ln (C_s^i / D_s^o)$$

If this is $\Delta\mu_s$ negative, sucrose can spontaneously diffuse inward since the inside concentration is lower.

For a charged ionic solute like K^+ with charge z=1, the Δμ between outside (o) and inside (i) a cell is given by

$$\Delta\mu_s = RT \ln (C_s^i / C_s^o) + zF(E^i - E^o)$$

[44] Osmolality: http://en.wikipedia.org/wiki/Osmolarity

where $\Delta\mu_s$ is termed the **electrochemical potential difference** and $E^i - E^o$ is the voltage difference from outside to inside. (Cells typically have negative values of $E^i - E^o$.) If $\Delta\mu_s$ is negative, the charged ionic solute can spontaneously diffuse inward (unless there are permeability restrictions).

If a membrane is **semipermeable** (has differing permeability for two different ions), diffusion of one will cause a buildup of charge opposing further diffusion as a result of an opposing electrical potential (called here a **diffusion potential**). The relationship between the two compartments at equilibrium for ion j may be expressed as the **Nernst equation**:

$$\Delta E = E^i - E^o = 2.3(RT/z_jF) \log ([C_j^o]/[Cj^i])$$

$$\Delta E = (59.1mV)/z_j) \log ([C_j^o]/[Cj^i])$$

For an ion j, which is not actively transported and which is at equilibrium, the difference in concentration of this ion between two compartments is related to the electric potential difference between the compartments (the "**Nernst potential**" ΔE) by this formula.

In actual cells, the **membrane potential ΔE** = $E^i - E^o$ is established by the active transport ("**pumping**") of H^+ and certain additional ions, and is maintained at c. -100 to -200 mV. For ions that are not actively transported, their equilibrium concentration ratios are given approximately by the Nernst equation. For an ion with concentration ratio that diverges substantially from that predicted by the Nernst equation at the prevailing membrane voltage potential difference, this ion must be actively transported.

Here are some Nernst equation predictions vs. measured values in pea root tissue at -110 mV (Table 6.1), along with further data about the vacuole. Only K^+ is at equilibrium. pH from other studies is shown for comparison—note the much smaller concentration values of H^+:

Ion	**External (Extracellular Space ECS) conc. mmol L^{-1}**	**Internal (Cytosol) conc. mmol L^{-1} Predicted**	**Internal conc. mmol L^{-1} Observed**
H^+	pH = 5.5 3.2E-06 mmol L^{-1}		pH 7.2 Conc. = 6.3E-08 mmol L^{-1} Actively pumped to ECS and vacuole
K^+	1	74	75 (passive, at equilibrium, but may be taken up actively)
Na^+	1	74	8 (actively pumped to ECS and vacuole)
Mg^{2+}	0.25	1340	3 (actively exported)
Ca^{2+}	1	5360	2 (actively exported to ECS and vacuole)
NO_3^-	2	0.0272	28 (actively imported to cytosol)
Cl^-	1	0.0136	7 (actively imported)
$H_2PO_4^-$	1	0.0136	21 (actively imported)
SO_4^{2-}	0.25	0.00005	19 (actively imported)

K^+ and anions have the highest membrane permeabilities and concentrations in plant cells.

The **Goldman equation** (web topic 6.1) more accurately represents the effects of multiple ions considered simultaneously when compared to the Nernst equation, but measured membrane potentials are still more negative than predicted by Goldman for the various ions (if ignoring H^+). The excess negative voltage is due to the **plasma membrane electrogenic H+-ATPase pump**. The electrogenic pump combines with the diffusion potential to produce the resultant plasma membrane potential. Membrane potentials cannot be maintained when active transport of H^+ is poisoned with cyanide.

Membrane Transport Processes

The **permeability** of pure phospholipid membranes for H_20 and for Cl^-, Na^+, K^+ solutes is low compared to intact cell membranes, whereas for CO_2, O_2, and Glycerol the two types of membranes have similar

permeabilities. The differences are due to presence of transport proteins which are *relatively* specific for certain solutes, and which are subdivided into:

Channels: Simple diffusion, passive transport
Carriers: Facilitated diffusion, passive transport (secondary active transport)
Pumps: **Primary active transport** using energy source such as ATP hydrolysis

Channels

Channels act like **selective pores** to enhance diffusion across membranes for specific substances. Transport is limited to ions and water, and is always **passive transport**. They conduct up to 10^8 ions or molecules per second. Pores may be **gated** in response to signals such as ion concentrations like K^+ or Ca^{2+}, voltage changes such as depolarization or hyperpolarization, hormones, cyclic nucleotides, light, phosphorylation, etc. Studied by patch clamp electrophysiology (web topic 6.2). An ion species may move through different channels with differing gating conditions, allowing fine tuning of passage—the membrane permeability depends on the mix of differing channels.

Cations may move in to cytosol through PM channels—**K^+, Na^+, Ca^{2+}** (however, Ca^{2+} must be actively pumped back out of the cytosol). **K^+** can move either direction into or out of the vacuole via channels. **Ca^{2+}** can move from vacuole to cytosol via channels. Anions (**$Malate^{2-}$, Cl^-, NO_3^-**) move via channels through PM out of cytosol or into vacuole. As previously stated, K^+ moves in or out to maintain the Ernst equilibrium potential for potassium E_k equal to the membrane potential ΔE:

$\Delta E = (59.1mV)/z_j) \log ([C_j^o]/[Cj^i])$	Nernst equation
$\log ([C_K^o]/[C_K^i]) = \Delta E / 59.1$	for K^+
$[C_K^o]/[C_K^i] = 10\text{^}(-110 / 59.1) = 0.014$	for measured ΔE = -110 mV
$[C_K^i] = 73$ mmol L^{-1}	for measured $C_k^o = 1$ mmol L^{-1}

Some **K^+ channels** are **rectifying** in that they open only above or below the Nernst potential for K^+ (see fig. 6.9) and thus allow movement only in or only out:
Inwardly rectifying channels or inward channels open at potentials more negative than the prevailing Nernst potential for K^+ (so positive charges are attracted inwardly). These are found in guard cells that take up K^+ from the apoplast during stomatal opening.
Outwardly rectifying channels or outward channels open at potentials more positive than the prevailing Nernst potential for K^+ (so positive charges are repelled from the inside). These function in the closing of the stomata, and the release of K^+ into the xylem or apoplast.

Carriers and Pumps

Carrier proteins do not have pores, and are much slower in transporting than channels, typically only 100 to 1000 ions or molecules per second. They bind the substance to a specific site, giving them high specificity. Binding causes a conformational change, releasing the substance to the other side. Kinetics is similar to enzyme kinetics. Transport through carriers may be:
I. Passive Transport ("**facilitated diffusion**", somewhat of a misnomer); [example may be the PM's **sucrose efflux carrier**] or
II. Active Transport
Active transport requires coupling (directly or indirectly) the energetically uphill transport of the solute (i.e., against its electrochemical potential gradient) with an energy-releasing event so that the overall free energy change is negative. When a carrier performs active transport, it may be either:
II.A. Primary Active Transport: the transport is coupled directly to a source of energy such as ATP hydrolysis. A carrier that performs primary active transport is called a **pump**. Most pumps use ATP to transport ions such as **H^+** - ATPase or **Ca^{2+}** - ATPase (H^+ is pumped out of plasma membrane or into vacuole, **Ca^{2+}** is pumped out of plasma membrane), but some pumps can pump large organic molecules. Pumps may be **electrogenic** (leading to a net movement of charge across the membrane) or **electroneutral** (such as the H^+/K^+-ATPase pump of animal gastric mucosa). Electrogenic pumps include:

In Animals: the Na^+/K^+-ATPase of animal cells
In Plants:
- the plasma membrane H^+-ATPase pump;
- the vacuolar H^+-ATPase (V-ATPase) pump;
- the vacuolar H^+- pyrophosphatase pump

[the vacuolar Anthocyanin-GS - ATPase ABC transporter]
the Golgi cisternae H^+ - pyrophosphatase pump

II.B. Secondary Active Transport: the uphill transport of a solute is coupled to the downhill transport of another. "This type of **carrier-mediated cotransport** is termed secondary active transport and is **driven indirectly by pumps**." (p. 105) It uses stored energy in the form of protons that have been previously pumped out of the cytosol, a process which produces a pH gradient relative to the ECS and the vacuole, yielding a **proton motive force**, the stored free energy in the form of the H^+ gradient (available for ATP synthesis):

Δp (in mV) = ΔE -59(pH_i - pH_o)

This force drives the transport of many other ions against their gradients. Such coupled cotransport may be either

III.B.1 Symport, in which the two substances move in the same direction via a **symporter** protein (some authors use "cotransport" as synonymous with symport).[45] Examples of Symports:

- Plasma Membrane:
  - Fe, Mn, Zn, Cd in [text does not state what moves with these]
  - NO_3^- - H^+
  - PO_4^{3-} - H^+
  - H^+ - K^+
  - H^+ - Na^+
  - Sucrose - H^+
  - Amino Acid - H^+
  - Peptide - H^+
- Tonoplast: None are shown in fig. 6.14

III.B.2 Antiport, in which the two substances move in opposite directions via an **antiporter** protein (some authors use "**countertransport**" as synonymous with symport). Examples of Antiports:

- Plasma Membrane:
  - Na^+ out - H^+ in
- Tonoplast (directions are given with respect to the vacuole):
  - H^+ in - Na^+ out;
  - Ca^{2+} in - H^+ out;
  - Cd^{2+} in - H^+ out;
  - Mg^{2+} in - H^+ out;
  - Hexose in - H^+ out;
  - Sucrose in - H^+ out;

Kinetic analysis can elucidate transport mechanisms, similar to the analysis with enzyme kinetics. The constant K_m is equal to the solute concentration that yields half the maximal rate of transport. When K_m is low, there is a high affinity of the transport site. Transport often displays evidence of multiple channels of both high and low affinity.

The membrane transport proteins and the genes coding for them have in many cases been identified. [Details not reviewed here.] See diagram fig. 6.14 for summary of the various transport processes across the plasma and vacuole membranes. Cation transporters are diverse. Some anion transporters have been identified—for NO_3^-. Cl^-, and PO_4^{2-}. Metals are transported by high affinity ZIP proteins, including Fe, Zn, Mn. Toxic metals such as Cd, As, Zn, Pb, U, Hg, and Se can be taken up by some plants, and this may be useful for phytoremediation.[46] (For more on metals phytoremediation, see web essay 5.1 and chapter 26.)

Water-selective channels, **aquaporins**, in some cases transport uncharged solutes. They may function as sensors of gradients in osmotic potential and turgor pressure. They are regulated by protein phosphorylation, pH, calcium concentration, heteromerization, and reactive oxygen species. Plant cells can rapidly alter their water permeability in response to various stresses.

Plasma membrane H^+-ATPase has several functional domains...

The tonoplast H^+-ATPase (V-ATPase) drives solute accumulation into vacuoles... The vacuole is typically 20 - 30 mV more positive than the cytoplasm.

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[45] Cotransport and countertransport:
http://www.lib.mcg.edu/edu/eshuphysio/program/section7/7ch05/7ch05p13.htm

[46] Phytoremediation: http://en.wikipedia.org/wiki/Phytoremediation

Although the vacuole is usually more acidic than the cytosol, some plant species make the vacuole **hyperacidic**—e.g., lime pH 1.7, lemons pH 2.5, cherry 2.5, grapefruit 3.0, rhubarb [3.2 petioles], prickly pear in AM 1.4, oxalis sp. 1.5, begonia 'lucerna' 0.9, etc.

Ion Transport In Roots

Root ion transport is highly specific and tightly regulated. In the plant, solutes move through both the apoplast (5-20% of the total plant volume) and the symplast via plasmodesmata. Cells that secrete ions such as floral nectaries and salt gland have high densities of plasmodesmata. Symplastic flow of ions is primarily by diffusion through plasmodesmata.

Ion absorption in the root is primarily in the root hair zone. Like water absorbed in a root, ions may move initially
(1) in the apoplast (eventually crossing, due to the blocking Casparian strip, over to the symplast before entering the stele [central part of the root containing the vascular tissue and occasionally a pith]), or
(2) immediately enter the symplast across a membrane.

The living xylem parenchyma cell participate in **xylem loading**—injection of ions and solute into the dead xylem tracheary elements to lower osmotic potential in the root xylem and promote water flow up the plant.

Chapter VII. Photosynthesis Light (Thylakoid) Reactions

The overall result of the **photosynthesis** [**PS**] reaction may be summarized in one form as

$$\mathbf{6\ CO_2 + 6\ H_2O + light + plant \rightarrow C_6H_{12}O_6 + 6\ O_2}$$

Thus solar energy is used to **oxidize water** and **reduce carbon dioxide** to produce carbohydrate (via c. 50 intermediate steps). This reaction to form CHO cannot occur to any significant effect without PS, and by oxidizing water it is also the only significant source of atmospheric O_2 on Earth.

PS is divided into **light (thylakoid) reactions** and **carbon fixation (stroma) reactions**. The latter are also directly regulated by light, and so the term "dark reactions" is considered inaccurate.

Original experiments in PS by Van Helmont in the 1600s, then Engelman (working with Spirogyra), etc.

The light reactions begin with the excitation of chlorophyll by light and culminate in the synthesis of **ATP** (via the photophosphorylation of ADP) and **NADPH** (via the reduction of **$NADP^+$**).

Most PS in higher plants takes place in the chloroplasts of the **mesophyll** cells of the interior of leaves. The light reactions occur in association with the thylakoid membranes of the chloroplasts, whereas the carbon reactions occur in the stroma of the chloroplasts.

Light Physics

Light is wavelike (having mutually perpendicular magnetic and electric fields, both of which oscillate perpendicular to the direction of propagation) and is also particle-like. The relationship of light wavelength to frequency is given by

$\mathbf{c = \lambda\ \nu}$ where
c = speed of light, 3×10^8 m s^{-1}
λ = wavelength (m)
ν = frequency (Hz or s^{-1})

Photons are the particles or wave packets that make up bulk light. The energy of one photon depends on the frequency:

$\mathbf{E = h\ \nu}$ where
E = energy (Joules)
ν = frequency (s^{-1})

h = Planck constant (or Planck's constant) = 6.626 x 10^{-34} J s

Visible light for humans falls between c. 400 - 700 nm. The spectrum of **sunlight** (TZ fig. 7.3) at the sun surface and just above our atmosphere has a peak **irradiance** (measured in W m^{-2} nm^{-1}) in blue wavelengths of c. 450 - 480 nm. The solar spectrum above our atmosphere is that expected from a **5525 K** (5250 °C) **blackbody**[47], which has a theoretical peak by Wien's law at c. 525 nm.[48] Passage through the Earth's atmosphere attenuates the light a little and alters the spectrum somewhat, mostly by introducing several absorption bands, including one at c. 700 nm (far red) arising from water vapor, and more absorption bands at longer infrared wavelengths, but the peak does not shift significantly, still being at c. 530 nm at the surface of the Earth.

Photosynthetically active light falling on leaves may be quantitated by **irradiance** W m^{-2} of **Photosynthetically Active Radiation PAR** (400 nm - 700 nm) or **photosynthetic photon (quantum) irradiance** in mol m^{-2} s^{-1} of PAR [also called somewhat ambiguously **photosynthetic photon flux density**]. Measures such as lux, candela, and foot-candles are human-oriented and not suitable for plant physiology. (Web topic 9.1) Spectral irradiance expresses W m^{-2} nm^{-1}, thus the irradiance measured at defined points in the spectrum. (See also web topic 7.1 principles of spectrophotometry.)

The photosynthesis experimenter much choose the geometry of the light sensor to most closely match the type of plant part being studied:

- **Flat**—having uniform irradiance across its surface from unidirectional light, but total is proportional to the cosine of the angle with respect to perpendicular; or
- **Spherical or Omnidirectional**—having varying irradiance across its curving surface from unidirectional light but total not varying with angle of incidence. In this case, a **fluence rate**[49] [the irradiance which is incident from all angles] is measured.

On a sunny day in direct (unidirectional) sunlight at sea level, PAR irradiance and PAR fluence are both **400 W m^{-2}** or **2,000 µmol m^{-2} s^{-1}** [These figures are stated in Web topic 9.1. On p. 200 of the textbook, a probably incorrect figure of 900 W m^{-2} is given. "Sun" is said to have irradiance of 920 µmol m^{-2} s^{-1} in fig. 9.10] Completely diffuse light has irradiance equal to 25% of fluence. PAR is "38% (21-46%) of the extra-terrestrial solar irradiance", "46% to 50% global solar radiation at ground level."[50] The **solar constant**, which measures total solar radiation arriving at the upper atmosphere of all wavelengths, is c. 1366 W m^{-2}, but of course is not actually constant and varies with the time of the year due to Earth's orbital position, etc.]

The **Quantum yield (quantum efficiency)** is variously and rather confusingly defined for photosynthesis as:

- "The number of photochemical products" [such as moles of CO_2 assimilated or O_2 evolved] divided by the "Total number of quanta absorbed"[TZ132], or
- "The fraction of excited molecules" [i.e., excited by the absorption of a photon] that decay via a designated pathway [such as via photochemistry, apparently no matter how small the effect produced per quantum] (web topic 7.3), or
- The number of times that a defined event occurs per photon absorbed by the system.[51]

Photosynthesis Pigments And Complexes

Free **Chlorophyll a** (**Chla**), as extracted with nonpolar diethyl ether, has greatest absorption at blue (c. 430 nm) and red (c. 662 nm) wavelengths, thereby having absorption maxima straddling the peak of 525 nm for solar irradiance, and reflecting green light c. 550 nm. **Chlorophyll b** (**Chlb**) similarly extracted has

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[47] Solar spectral irradiance:
• http://en.wikipedia.org/wiki/Sunlight
• http://rredc.nrel.gov/solar/spectra/am1.5/ASTMG173/ASTMG173.html
[48] Wien's law of black body radiation: http://en.wikipedia.org/wiki/Black_body
[49] Fluence rate: http://omlc.ogi.edu/classroom/ece532/class1/fluencerate.html
[50] Solar PAR irradiance at ground level:
http://www.apesimulator.it/help/models/solarradiation/Photosynthetically_active_radiation.html
[51] Quantum yield: http://en.wikipedia.org/wiki/Quantum_yield

greatest absorption at blue (c. 453 nm) and red (c. 642 nm) wavelengths.[52] Light excites chlorophyll from the ground state to a short-lived metastable excited state designated by Chl*, a state having a potential life span of only a few nanoseconds. After absorbing blue light, Chl can give up its energy and drop to lower energy states by

- **Heat loss** from the higher excited state to the lowest excited state (with no photon emission)
- **Fluorescence** from the lowest excited state to ground level (radiates a 673 nm photon, in the red region)
- **Energy transfer** to another molecule
- **Photochemistry reactions** (the redox reactions that are useful in PS—these are extremely fast reactions)

Certain bacteria including cyanobacteria, plus diatoms, dinoflagellates, brown algae, red algae can also photosynthesize and have various chlorophyll combinations including **Chl c and d.** Sulfur purple bacteria, nonsulfur purple bacteria, green bacteria, and heliobacteria can have various combinations of **bacteriochlorophylls a - g** (see web topic 7.2)

Chlorophylls have two major components:

- **Porphyrin-like ring** with a centrally coordinated **Mg** in the N_4 cavity. (The porphyrin is where excitation initially occurs.)
- **Phytol** tail (which anchors the molecule to a hydrophobic part of the environment)

Carotenoids (e.g., β-carotene) have long linear molecules with multiple **conjugated double bonds**. These are alternating single and double bonds with delocalized electrons (similar to the benzene ring), yielding **chromophores** (the part or moiety of a molecule responsible for its color). Carotenoids absorb light in the blue 400-500 nm range and reflect a characteristic orange color.

Joseph Priestly first discovered O_2 and found that plants evolved it. Jan Ingenhousz showed that light is essential to photosynthesis in 1779, and others demonstrated the need in PS for H_20 and CO_2. C. van Niel found that PS is a redox process.

The **action spectrum** of PS (e.g., the O_2 evolution rate graphed as a function of wavelength) closely relates to the absorption spectrum of chloroplasts (with the exception that light absorbed by carotenoids in 450 - 550 nm, which somewhat widens the blue absorption spectrum compared to Chl, is not as efficiently converted via PS—see TZ fig. 7.8).

Engelmann showed that O_2-seeking (aerotactic) bacteria were attracted to the segments of Spirogyra spiral chloroplast that were irradiated with blue or red light more than the parts irradiated with green, confirming that PS makes O_2 where light is most strongly absorbed (the blue peak is broadened by carotene).

Photosynthetic complexes consist of:

- **Light-harvesting antennas**, which assist in the absorption of light
- **Photochemical reaction centers** (discussed further below)

In intact plants, absorption of light is assisted in **light-harvesting antennas** containing

- **Photosynthetic pigments** include **chlorophyll a** and **b** (along with **c** and **d** in diatoms and algae).
- **Accessory pigments** include and **carotenoids** (such as **β-carotene**). In addition, **bilin pigments** or **phycobiliproteins** such as **phycoerythrobilin** are found in cyanobacteria and red algae but not in vascular plants.
- **Photoprotective pigments**, which protect from excess light and photoinhibition, including **anthocyanin** and **xanthophylls**.
- **Light-harvesting complex proteins** (LHCI and LHCII), which aid in the efficient transfer of excitation energy.

[52] Chlorophyll absorption spectrum:
• http://www.biologie.uni-hamburg.de/b-online/e24/3.htm
• http://en.wikipedia.org/wiki/Chlorophyll

The use of antenna structures (comprising hundreds or thousands of Chl and accessory pigment molecules) makes for a more favorable allocation of energy, since many pigment molecules are needed to drive a single reaction center. Experiments by Emerson and Arnold with brief light flashes showed that at maximum PS yield, there are one O_2 molecule generated for every c. 2,500 chlorophyll molecules per high intensity flash. This is because
(1) several hundred pigment molecules are associated with a single chloroplast reaction center (in plants, 200-300 chlorophylls per PSII reaction center, and c. 100 core antenna chlorophylls or 200 overall for PSI centers); and
(2) Each reaction center must operate multiple times to produce just 1 molecule of O_2.

The excitation energy in antenna pigments is transferred to the reaction center by **fluorescence resonance energy transfer**, a non-radiative process with up to 95 to 99% energy transfer efficiency.

At much lower flash intensities, Emerson and Arnold found that the **quantum yield of chloroplasts** was 0.95 (versus 0.05 for absorbed photons whose energy is wasted by fluorescence). 1 molecule of O_2 molecule was generated for every 9-10 photon absorbed (i.e., not reflected or transmitted). These two values are not discordant, since each photon absorbed and yielding a photochemical effect exerts only a fractional effect with respect to generating O_2, but is fully counted as part of the quantum yield.

The following simplified reaction expresses overall PS:

$$\mathbf{CO_2 + H_2O + c.\ 10\ h\nu \rightarrow (CH_2O) + O_2}$$

where (CH_2O) is 1/6 of a glucose molecule
This reaction requires a theoretical minimum free energy change of 467 kJ/mol O_2 evolved in synthesis of glucose, but in practice requires 1760 kJ of absorbed red light in the plant per mol O_2 evolved, giving an **efficiency for conversion of absorbed light energy to chemical energy as glucose in overall PS** = 27% overall. (However, only a small part of this chemical energy goes to formation of biomass; see TZ p. 132 and fig. 9.3 and below). The remaining 73% of the energy entering photochemistry is consumed in cellular maintenance and ultimately ends as heat.

The **light reactions** as studied first by **Robert Hill** in 1939 are given by redox reactions with compounds such as Fe or Mn:

$$\mathbf{4\ Fe^{3+} + 2\ H_20 \rightarrow 4\ Fe^{2+} + O_2 + 4\ H^+}$$

Fe^{3+} is the **oxidant or oxidizing agent** and is **reduced** to Fe^{2+}
H_20 is the ultimate electron donor (**reductant or reducing agent**) and is **oxidized** to $O_2 + 4\ H^+$
(in the sense that water loses hydrogen and O changes oxidation number from -2 to 0).
It has been proven that the O_2 evolved in PS originates from H_2O, not from CO_2.

The **thylakoid reactions**, in addition to oxidation of H_20, include the reduction of $NADP^+$ to NADPH and the phosphorylation of ADP to ATP.

Despite the substantially lower overall absorption of photons in the green c. 550 nm for chloroplasts, those photons that are absorbed show a much flatter curve for quantum yield from 400 to 700 nm—there is an abrupt "red drop" at ≥ c. 700 nm. This is because the accessory pigments help to make more efficient use of photons with wavelengths other than at the optimal values for chlorophyll.

Photosystems I And II And The Synthesis of NADPH and ATP

[This is a complex topic not fully summarized here, see also Web Topic 7.8] Photosystems I and II (**PSI** and **PSII)** are found in chloroplasts of plants and algae (and also cyanobacteria). The reaction center of PSII (termed P680) has chlorophylls which absorb maximally at red 680 nm, whereas the PSI reaction center P700 has chlorophylls absorbing maximally at far red > 680 nm. Absorption at both wavelengths is needed for the most efficient PS. In chloroplasts, these photosystems are spatially separated: PSII and its antenna pigments are located primarily in the stacked thylakoid membranes (grana), whereas PSI is mainly located in the nonstacked stroma lamellae protruding into the chloroplast stroma from the grana lamellae. The proposed chemical sequence is depicted in the "**Z scheme**" (a term referring to the graphical appearance of vertical jumps in redox potentials and the sloping electron transport chains).

The P680 center of PSII contains a dimer of "specialized chlorophylls" (not otherwise specified in TZ, though EVV states they are chlorophyll a) along with other pigments, several proteins including D1 and

D2, and other factors. Red light excites the reaction center directly, or more commonly an antenna complex, which efficiently funnels this excitation energy from carotenoids to Chlb and Chla and finally to P680 in the reaction center. [MCM: The following redox sequence is somewhat confusing, and I may not have all the details correct.] The P680 is excited to **P680***, the latter having a relatively negative redox potential E_m (and thus is mildly reductant) compared to the ground state. Prior to excitation, P680 has relative positive E_m, and acts as a strong oxidant (electron acceptor), altering the state of the **oxygen evolving complex (OEC)**, which ultimately leads to the oxidization of water (at the inner thylakoid membrane): $2\ H_20 \rightarrow 2\ O_2 + 4\ H^+ + 4\ e^-$. The products O_2 and H^+ are released into the thylakoid lumen, the latter creating a high H^+ concentration and resulting high **proton motive force** (an electrochemical potential gradient, representing stored chemical energy) across the thylakoid membrane. (Oxidation of water at the OEC also requires presence of Mn^{2+} ions and a series of oxidations called **S states** probably involving a manganese containing enzyme—the mechanisms are not well understood.) The electrons enter the non-cyclic electron transport chain extending from P680* to P700.

The relatively negative redox potential E_m of the excited P680* state indicates that it can serve as a weak reductant (electron donor). P680* can transfer an electron to the first electron acceptor in a transport chain, **pheophytin**—this transfer is the first event in PS where light excitation energy has been converted to chemical energy. An electron transport chain including **plastoquinones** and the **cytochrome b_6f complex** carries electrons along states of increasingly more positive E_m to **plastocyanin** (PC) and ending in the **P700** center of PSI.

The P700 center is excited by photons absorbed at far red 700 nm. P700 is a weak oxidant, but **P700*** is a strong reductant (electron donor, with very negative E_m). P700* passes electrons (received at P700 from PSII) down a series of membrane-bound iron-sulfur proteins (again, down states of increasingly more positive E_m) to soluble **ferredoxin (Fd)**, which is acted on by **ferredoxin-NADP-reductase (FNR)** to reduce the ultimate electron acceptor, $NADP^+$, to NADPH in the stroma. The PSI reaction center is a large multisubunit complex with (in addition to P700 and chlorophylls) has several proteins, as well as **phylloquinone** (**Vitamin K_1**). Reduced ferredoxin is also used by the plant to reduce nitrate.

Chloroplast ATP synthase (also called **CF_O-CF_1** or **CF_O-CF_1 ATP Synthase**, resembling mitochondrial **F_O-F_1 ATP synthase**, see Chap. 11) is located in the chloroplast stroma lamellae. It couples the passage of H^+ ions down their electrochemical gradient (from the thylakoid lumen to the stroma) with the **phosphorylation of ADP via P_i to ATP** in the chloroplast stroma (a process termed **photophosphorylation**). This ATP (along with the NADPH) will be used to drive the carbon reactions of PS. The **coupling** between the electrochemical potentials from proton pumping—the proton motive force arising from the chemical pH gradient and the membrane electric potential—and the performance of work, as with **CF_O-CF_1 ATP synthase**, is termed **chemiosmosis** by Peter Mitchell (web topic 6.3; see also web topic 7.9 on ATP Synthase).

In some cases, "cyclic electron flow" in the chloroplast can be utilized to generate ATP without corresponding reduction of $NADP^+$ to NADPH. In other cases, the electron flow in the chloroplast may occur without corresponding photophosphorylation of ADP (an **uncoupling**TZ148 apparently analogous to the uncoupling of oxidative phosphorylation from electron transport that can occur in mitochondria—see TZ269 and Web topic 11.5).

The herbicides **paraquat** and **DCMU** act by blocking electron transport of PSII or PSI (see web topic 7.10).

Protection And Regulation of Photosynthetic Machinery

[Brief summary] Toxic photoproducts can form in excess light conditions, including triplet state of Chl ($^3Chl^*$) and **reactive oxygen species** such as the **superoxide anion** ($O_2^{\bullet -}$), **singlet oxygen** ($^1O_2^*$), **hydrogen peroxide** (H_2O_2), and **hydroxyl radical** (•OH). ("Singlet oxygen is the common name used for the two metastable states of molecular oxygen O_2 with higher energy than the ground state **triplet oxygen**.")[53] Singlet oxygen can damage many cellular components including lipids. The PSII reaction center is easily damaged by excess light, especially the D_1 core protein. Carotenoids, superoxide dismutase, and ascorbate serve as photoprotective agents, helping to prevent **photoinhibition** (a reduction in a plant's capacity for PS caused by exposure to strong light, which may be reversible or irreversible) and damaging effects of

[53] Singlet oxygen: http://en.wikipedia.org/wiki/Singlet_oxygen

excess light. Carotenoids can **quench** the excess energy of singlet oxygen by converting it back to triplet oxygen releasing heat. **Non-photochemical quenching** of excess energy (conversion to heat without inducing photochemistry) can be done by **xanthophylls**. These are yellow pigments that are oxidized carotenoid derivatives (listed in order of least to greatest protectiveness: **violaxanthin** < antheraxanthin < **zeaxanthin**). The least protective in the **xanthophyll cycle**, violaxanthin, converts to the most protective, zeaxanthin, when light is intense and protection is needed.

Thylakoid stacking permits energy partitioning between the photosystems, allowing the most efficient use of the available energy...

Chloroplasts can reposition themselves along the side walls of cells, so that the more superficial ones provide shade to deeper chloroplasts along the same wall, in response to excessively intense light (TZ fig. 9.4).

Chloroplasts sometimes extend **stromules**, fine tubular interconnections with nearby chloroplasts and plastids that allow transfer of proteins etc., but the ultimate purpose is unknown.[54]

Genetics Of The Photosynthetic Systems

[Brief summary] Chloroplasts derive from endosymbiosis of cyanobacteria. Chloroplast DNA is circular, and codes for proteins synthesized in the chloroplast. But much of the DNA needed to synthesize the chloroplast photosynthetic apparatus has been transferred to the parent cell nucleus. Proteins synthesized from such nuclear DNA are synthesized in the cytoplasm and imported into the chloroplasts. Chloroplasts reproduce by division, not de novo synthesis, and they can no longer survive outside their eukaryotic host cell. Only the maternal plant contributes chloroplasts to the zygote, and therefore the inheritance of chloroplast intrinsic DNA in not Mendelian.

Some of the precursors of Chla are glutamic acid, 5-aminolevulinic acid (ALA), porphobilinogen (PBG), protoporphyrin IX, etc.

Chapter VIII. Photosynthesis Carbon (Stromal) Reactions

The carbon PS reactions fix carbon to biomass from inorganic CO_2, about "200 billion tons of CO_2 each year"[TZ159] (elsewhere estimated at 60 Giga metric tons Carbon per year), 40% of which is performed by marine phytoplankton. (Much of this fixed carbon is returned to the atmosphere through aerobic respiration, decomposition, methanogenesis, etc.) In plants, these reactions occur in the chloroplast stroma.

The Calvin C_3 Cycle

This cycle is the core of PS as found in almost all autotrophic photosynthetic (i.e., **photoautotrophic**) organisms. (Some photosynthetic prokaryotes such as green sulfur bacteria and Chloroflexi do not use the Calvin cycle, while some chemoautotrophic bacteria or Archaea such as methanogens and halophiles do not use PS at all.) It transforms CO_2 by reducing ("fixing") the C in CO_2 (which is in the most heavily oxidized state for C, oxidation number 4, thus $C^{+4}O_2^{-2}$) to C^0 (oxidation number 0) as found in covalently linked organic compounds. [MCM: absolute oxidation numbers or oxidation states for C in organic compounds seem to be controversial, complex, or unknowable, but "only the changes in oxidation state during a reaction are important."][55]

The Calvin cycle was elucidated by in the 1950s by Melvin Calvin, James Bassham, Andrew Benson, and colleagues at the University of California, Berkeley. Using 2D chromatograms and autoradiography, they were able to show that 3-phosphoglycerate is the first stable intermediate formed (Web Topic 8.1).

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[54] Stromules: http://jxb.oxfordjournals.org/cgi/reprint/56/413/787

[55] Organic carbon oxidation states: http://pages.towson.edu/ladon/orgrxs/reagent/redox.htm

This cycle has 3 phases [Limited summary, abbreviations for molecules are my own]:

(1) **Carboxylation of R15B to PGA**: The starting 5-carbon CO_2 acceptor is **Ribulose-1-5-biphosphate** [R15B], which is carboxylated with CO_2 and H_20 by **Ribulose-1,5-biphosphate carboxylase-oxygenase ("rubisco")** to an unstable 6-carbon intermediate, which rapidly decays to 2 molecules of **3-phosphoglycerate** [PGA], the first stable intermediate of the Calvin cycle. (The C in PGA has been reduced to oxidation number +3 per TZ.) (See Web Topics 8.2 and 8.3 on Rubisco)

(2) **Reduction of PGA to GA3P**: PGA is reduced using with ATP, NADPH, and H^+ culminating in **Glyceraldehyde-3-phosphate** [GA3P], a **triose phosphate carbohydrate**, plus ADP, P_i, and $NADP^+$. (The C in GA3P has been further reduced to oxidation number +1 per TZ.)

(3) **Regeneration of R15B**: Ribulose-1-5-biphosphate is regenerated in a complex set of reactions, consuming 5 out of 6 of the GA3Ps that were formed.

The net stoichiometry of the C_3 Calvin cycle reactions (including the regeneration of R15B) may be summarized as follows:

$$3\ CO_2 + 5\ H_20 + 6\ NADPH + 9\ ATP \rightarrow G3P + 6\ NADP^+ + 3\ H^+ + 9\ ADP + 8\ P_i$$

where the NADPH and ATP come from the light thylakoid reactions.

The Calvin cycle intermediates must first be generated before the Calvin cycle can fully resume after a period of darkness—the delay required is called the **induction period**. Once the intermediates have built up, the Calvin cycle can begin generating net reserves of carbohydrates (ultimately, starch or sucrose, etc.)

Energy Conversion Efficiency Of Photosynthesis Using The Calvin Cycle

The free energy released by the complete oxidation of a hexose sugar to CO_2 and H_2O is 2804 kJ mol^{-1}—thus the absolute minimum energy to synthesize one mole of fructose must be 2804 kJ. In the plant, synthesis of 1 mole of fructose-6-phosphate from 6 moles of CO_2 takes 3126 kJ from oxidizing the needed NADPH and hydrolyzing the needed ATP. Therefore
the thermodynamic efficiency for conversion of the chemical energy in NADPH and ATP to hexose energy via the Calvin Cycle is 90%. 83% of the energy required comes from the reductant NADPH.

Incident red light photons at 680 nm contain energy of c. 175 kJ per quantum mole, computed as follows:

$E = hc/\lambda$
$= (6.626 \times 10^{-34}\ J\ s) \times (3 \times 10^8\ m\ s^{-1}) \times (10^9\ nm/m) \times (6.023 \times 10^{23}\ mol^{-1}) / 680\ nm$
$= 176\ kJ\ mol^{-1}$

At least 8 absorbed photons are needed to reduce 1 mole of CO_2 to hexose. Thus, the minimum light energy required to reduce 6 moles of CO_2 to one mole of hexose is 6 x 8 x 175 = 8400 kJ. Therefore, **the overall thermodynamic efficiency of PS for the conversion of absorbed photon energy to hexose is 2804/8400 = 33%**. [This calculation from TZ p. 165 appears to consider only the photons that are absorbed and used for photochemistry, not those that are reflected or transmitted.] Under normal growing conditions, plants are much less efficient: crops such as potatoes, corn, rice, etc. typically yield only 0.1 to 0.4% conversion efficiencies, while sugarcane approaches 2%. (See also estimates in Chap. IX)

Regulation Of The Calvin C_3 Cycle

[Limited summary] Light regulates the Calvin cycle, modulating the activity of target stromal Calvin cycle enzymes including rubisco (which increases in light), fructuse-1,6-bisphosphate phosphatase, etc.[TZ165] The ferredoxin-thioredoxin system contributes to this regulation. Movement of ions such as H^+ and Mg^{2+} also modify the activity of key regulatory enzymes.

The C_2 Oxidative Photosynthetic Carbon Cycle (Photorespiration)

[Limited summary] Rubisco can catalyze either a carboxylase reaction of R15B using CO_2 (the desired entry pathway of the Calvin cycle), or it can cause oxygenation of R15B using O_2, leading to the formation of a 2-carbon product, **2-phosphoglycolate** [2PG] and a loss of efficiency of PS. This product is not useful for PS, and appears to be, in most cases, a waste product which must be scavenged and reconverted to PGA in the energy-consuming C_2 process called **photorespiration**. This takes place in a series of steps starting with conversion to glycolate and involving additional organelles, the peroxisome and mitochondrion. Ultimately, 2 moles of 2PG are converted to one of 3-carbon PGA and one of CO_2, consuming 3 moles of O_2 and 2 moles of ATP as well as 2 moles of reducing equivalents (2 Fd_{red} + 2 H^+)...

CO_2 is relatively less soluble in water at higher temperatures than is O_2 (web topic 8.6), so the ratio of CO_2/O_2 decreases with rising temperature. In the plant, both **increased temperature** and **lower CO_2/O_2 ratio** increase the relative rate of oxidation over carboxylation (and thus lowers the efficiency with which CO_2 is utilized). **Water stress** also increases the rate of C_2 photorespiration.

A possible useful role for C_2 photorespiration is still under investigation. It may help to protect plants from photo-oxidation and photoinhibition when illumination is high and CO_2 low, and it may also assist with nitrate assimilation.

Mechanisms For Suppressing Photorespiration Or Preventing Photo-oxidation

Suppressing Photorespiration By Concentrating CO_2 and HCO_3^-

Some aquatic organisms such as cyanobacteria and algae can accumulate HCO_3^- or concentrate CO_2 near rubisco, suppressing photorespiration and favoring carboxylation of R15P.

The C_4 Carbon Cycle-Plants

[Limited summary] This cycle is superimposed on the Calvin Cycle, and is found in monocots and dicots, particularly in sugarcane, corn (maize), millet, sorghum, sedges, switchgrass, and other hot weather plants. It is most common in sedges and grasses, and is found less commonly in shrubs and herbs, and in only one tree species. (Se web topic 9.3 on geographic distributions, and topic 8.7 on three variations of c4 metabolism). It was originally discovered by Kortschack and Karpilov, and further elucidated by Hatch and Slack. It separates initial CO_2 processing from the Calvin cycle, allowing CO_2 to be internally concentrated at the site of rubisco, suppressing photorespiration.

Leaf **mesophyll cells** receive the atmospheric CO_2, convert it to HCO_3^-, and use HCO_3^- to carboxylate **phosphoenolpyruvate** (a C_3 compound) using **phosphoenolpyruvate carboxylase (PEPcase)**. This leads to the formation of the first stable intermediates **malate**$^-$ or **aspartate**$^-$, both of which are 4 carbon acid compounds (thus the name **C_4**). One or the other of these C_4 acids pass through a "diffusion barrier" (does it prevent back-migration of CO_2 from the bundle sheath cells?) to the mitochondrion of the **bundle sheath cells** (found in the vascular region of the leaf). An NAD-dependent malic enzyme NAD-ME (or other variant enzymes, NADP-ME, etc.[TZ176]) decarboxylates the malate or aspartate, releasing concentrated CO_2 that is presented to the nearby chloroplast for Calvin cycle processing into carbohydrates. Pyruvate or alanine (both are C_3 compounds) is returned to the mesophyll cell. Only the bundle sheath cells have the decarboxylases for regenerating CO_2 and the enzymes of the Calvin cycle. In many (but not all) C_4 plants, a ring of mesophyll cells surrounds the large bundle sheath cell in an arrangement termed **Kranz anatomy**—this arrangement that ensures proper compartmentalization and contiguity of the two processes. The chloroplasts of the mesophyll and bundle sheath cells differ in several ways, including a greater concentration of grana in the former...

Some plants employ C_4 PS implemented within single chlorenchyma (chloroplast-containing parenchymal) cells. Diatoms are unicellular protists that also exhibit a C_4 pathway. This provides them with more than

one CO_2 concentrating mechanism, so that they are capable of responding to varying environmental conditions.

The C_4 cycle, which is superimposed on C_3, requires greater energy than use of only C_3, specifically an additional 2 ATP per CO_2 concentrated. However, PEPcase has a high affinity for HCO_3^- and thus indirectly for CO_2, and this affinity is not competed for by O_2 (such a competition is found with rubisco). The higher efficiency of utilizing HCO_3^- and thus of CO_2 allows C_4 plants to **reduce stomatal aperture at high temperatures** while fixing CO_2. In general, therefore,

- C_4 plants in high CO_2/O_2 ratio environments and/or in lower temperature non-water-stressed conditions are less efficient at PS than C_3 plants, because they need more light quanta per CO_2 used.
- C_4 plants in lower CO_2/O_2 ratio environments and/or at higher temperatures and/or water stress conditions have more efficient PS and greater water-stress tolerance due to reduced photorespiration and more efficient use of CO_2 relative to water loss.

(These relationships are seen in part in TZ figures 9.9, 9.17, and 9.18, see also Chap 9 discussion.)

Light is necessary for the regulation of the C_4 enzymes...

Crassulacean Acid Metabolism (CAM)

This is a third mechanism used for concentrating CO_2 near rubisco for the Calvin cycle, as well as for preventing photo-oxidation (photorespiration). It is employed by xerophytic plants living in arid hot environments with seasonal water availability. Examples include pineapple, agave, bromeliads, cacti and other succulents, and orchids. These plants tend to have desert adaptations such as low surface-to-volume ratios, thick cuticles, large vacuoles, reduced stomatal size, leaf hairs, etc. It is named after the member (Bryophyllum calycinum) of the **Crassulaceae**[56] succulent family (a family which includes jade plants and sedums) in which it was first studied, as well as the acidity of the leaves that forms at night.

[Limited summary] In CAM plants:

- **Night**: the stomata of single leaf cells open at night when water loss is minimal, and **CO_2** enters the cytosol. CO_2 converts to HCO_3^- in the cytosol, which is combined with **phosphoenolpyruvate** (derived from chloroplast-stored **starch**) via **PEPcase** to form **oxaloacetate**. Oxaloacetate is ultimately converted to **malic acid** (a C_4 compound), which is typically stored in the vacuole. The accumulation of malic acid leads to a **nocturnal acidification** of the leaf, thus the "acid" part of the name in CAM.
- **Day**: During the day, the stomata close, further entry of external CO_2 stops, and water loss is minimized. The malic acid stored in the vacuole is released to the cytosol, is decarboxylated with $NADP^+$-dependent malic enzyme, etc. This releases localized CO_2 into the chloroplast near rubisco, where it enters the Calvin cycle, as well as **pyruvate** (which is converted to **starch** stored in the chloroplast). Because their stomata are closed in the daytime, CAM plants lose only "50 to 100 gram of water for every gram of CO_2 gained" (mostly at night), compared to 250 to 300 g water for C_4 plants and 400 - 500 g water for C_3 plants.[TZ180] The internal O_2 concentration rises while the stomata are closed, and is processed by **photorespiration**. Here, photorespiration is thought to be a beneficial response in that it reduces oxygen concentration and therefore oxygen toxicity (photo-oxidation), and generates additional CO_2. (see Web Topic 8.8).

Synthesis of Starch and Sucrose

For sugar chemistry, see also "Sugar Chemistry Review" above.

Sucrose is the nonreducing disaccharide glucose + fructose, also written as α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside. Both constituent sugars are 6-carbon hexoses, but fructose appears in a furanose 5-member ring form whereas the glucose is in the form of a 6-member glucopyranose ring. In plants, **sucrose** is synthesized in the cytosol of the leaf mesophyll cells. Sucrose is exported into the phloem. It is the main form of photoassimilated carbon ("photoassimilate" or "photosynthate") that is exported to the phloem, but other sucrose-galactosyl oligosaccharides are also formed from PS—raffinose (3 sugars),

[56] Crassulaceae: http://en.wikipedia.org/wiki/Crassulaceae

stachyose (4 sugars), verbascose (5 sugars)—as well as sugar alcohols such as sorbitol and mannitol, and other sugar compounds.

[Limited summary] **Starch** is a mixture of mostly linear **amylose** (glucan polymers with α-D-1,4 glycosidic bonds) and branched **amylopectin** (glucan polymers like amylose but with branches formed by α-D-1,6 glycosidic bonds). Amylopectin is firmer, more waxy, and ? less readily digested compared to amylose. Starch accumulates as dense granules in the chloroplasts of the leaf cell during the day ("**transitory starch**" storage when photosynthesis is maximal), and is mobilized to hexose phosphates at night (when photosynthesis has ceased)—this tends to smooth out the diurnal variation in levels of phloem sugars. Some plants also store sugars as **fructans** (polysaccharide polymers of fructose, such as the linear form **inulin**), which can for example be found in onion bulbs. Amylose starch is assembled by adding units of ADP-glucose to $(\alpha\text{-D-1,4 glucosyl})_n$ polymer chains, using **starch synthase**... Amylopectin synthesis requires the **starch-branching enzyme** and other enzymes. Starch degradation requires phosphorylation, **debranching enzyme**, **β-Amylases and α-amylases** (both of these act on starch α-1,4 glycosidic bonds), etc...

The Calvin cycle C_3 products synthesized in the chloroplast—GA3P and another triose phosphate dihydroxyacetone-phosphate—are readily interconvertible. They are converted in the cytosol to **fructose 1,6 bisphosphate**, and thence to 3 **hexose monophosphates** (**fructose-6-phosphate**, **glucose-6-phosphate**, and **glucose-1-phosphate**). **Fructose 2,6 bisphosphate** is an important regulatory compound affecting the pool of **hexose monophosphates** and the synthesis of sucrose: increased levels of Fructose 2,6 bisphosphate inhibits sucrose formation during the daytime but not at night. **Fructose-6-phosphate** combines with **UDP-glucose** to form **Sucrose-6^F-phosphate**, which is converted to **sucrose** in the cytosol. The formation of **sucrose-6^F-phosphate** is catalyzed by **sucrose-6^F-phosphate synthetase**, and is modulated by glucose-6-phosphate: increased glucose-6-phosphate increases sucrose-6^F-phosphate and therefore sucrose synthesis. The final dephosphorylation of sucrose-6^F-phosphate to sucrose, which is catalyzed by **sucrose phosphate synthetase-phosphatase**, is decreased by increased P_i, thereby decreasing sucrose synthesis.

PS is most active in mature leaves. Photosynthate, mostly sucrose, is transported in the phloem to meristems and developing leaves and other organs.

Chapter IX. Photosynthesis Physiology And Ecology

This chapter deals with current (and fossil) photosynthetic responses of intact plants to environment factors such as light, temperature, CO_2, moisture, etc., and their ultimate effects on plant productivity and crop yields. For a given set of conditions, what are the limiting factors in PS?—is it light or CO_2 limited? [In fact, several other factors contribute to the PS rate, including temperature and O_2.]

The supply of CO_2 to the leaf is regulated by the stomata. (Chap 4 etc.)

The measurement of light is summarized in Chap. 7.

Adaptations Of Plants To Bright Light And Shade

Bright Light Adaptations

Sun-exposed plant leaves tend to grow thicker than shaded leaves of the same plant. Desert plants, to prevent harm by excess light (and dessication), develop various defense including hairs, salt glands, epicuticular wax, all of which increase reflection of light from the leaf surface and reduce absorption of light by up to 40%. Some plants utilize **paraheliotropic** tracking to turn away from direct sun and thereby reduce leaf exposure to light.

Shade Adaptations and Light Concentrating Mechanisms

Columnar superficial palisade cells allow efficient capturing of light despite the **sieve effect** (which arises from gaps between chloroplasts that reduce absorption compared to chlorophyll in solution). **Light channeling** may also divert light through the vacuole or the cell wall areas to facilitate transmission into deeper layers of the leaf. The spongy mesophyll in the leaf interior has many reflecting interfaces which cause light scattering and increase the probability for light absorption. Leaves of plants living in the understory in dim light may have **focusing mechanisms** (**convex epidermal cells**) that focus the light onto the chloroplasts. Trees have elaborate branching structures of leaves, which tend to maximize overall light absorption by the plant. Plants competing for light in the understory may receive up to 50% of their light as **sunflecks**—transient sun exposures—and can often rapidly ramp up PS and stomatal opening during these brief events. Light reaching the understory is least depleted in the far red wavelengths (TZ fig. 9.5) compared to blue or red etc. Many plants alter their leaf angles to track the position of the sun (**solar tracking**), including alfalfa, cotton, soybean, lavatera, and lupine. This is a **blue light response** (see Chapter 18), which is often controlled at the **pulvinus** found at the junction of the blade and the petiole, and such leaves are called **diaheliotropic**. Such plants are often competing in short growing seasons. Plants in deep shade often acclimate, but the plant (or at least the shade-adapted leaves) may not be able to survive if the habitat becomes sunny, due to **photoinhibition**.

Here are general properties and tendencies of **shade leaves compared to sun leaves**:

- have more total chlorophyll per reaction center
- tend to be thinner, with thinner palisades
- have less rubisco and less xanthophyll (which is photoprotective)
- have higher ratio of PSI to PSII (3:1 compared to 2:1), or have more antenna chlorophyll in PSII. These adaptations "enhance light absorption and energy transfer" to make better use of the relatively more abundant far red light. [MCM: I am not sure how this is accomplished.]
- have lower rates of respiration ("dark respiration") and lower Light Compensation Point (see below)
- have lower maximum PS rates (saturation) that sun plants (see below)

Quantitation Of Photosynthetic Responses To Light, Temperature, And Other Factors

Of 100% incident solar energy arriving at the leaf , 60% is non-PAR photons, 8% is reflected or transmitted, 8% is dissipated as heat, 19% is consumed in metabolism, and the remaining 5% of incident energy is utilized for the production of CHO. [MCM: However, the efficiency of utilization of this photon energy may be much lower, see estimates in Chap. 8.] 85-90% of PAR incident on the leaf is absorbed, strongest in blue and red (fig. 9.4) and lowest in green.

Light response curves typically plot PS O_2 production rate or CO_2 assimilation rate (measured in µmol of O_2 or CO_2 m^{-2} s^{-1}) against absorbed light or PAR irradiance, etc.

Dark Respiration Regime, Light Compensation Point, Light-Limited Regime, And CO_2 Limited Regime

In C_3 plants, below a certain value of absorbed light (the **Light Compensation Point**), the plant evolves more CO_2 from mitochondrial respiration than it assimilates and fixes by PS (i.e., CO_2 assimilation is negative). Above the Light Compensation Point, the plant exhibits net positive assimilation of CO_2, taking in more and converting it through PS to photosynthate than it gives off. Above but near the Light Compensation Point, increases in light substantially increase CO_2 assimilated, a regime termed "light-limited". Eventually the curve levels off with increasing light and becomes "CO_2 limited". (fig. 9.7) Sun plants have Light Compensation Points typically of 10 - 20 µmol m^{-2} s^{-1} CO_2 assimilation, whereas shade plants have lower, c. 1 - 5 µmol m^{-2} s^{-1}. (Shade plants have low rates of respiration and make more efficient use of light for PS.) For increased light levels, shade plants have lower maximum PS rates that sun plants.

Effect On Quantum Yields By O_2, CO_2, Temperature, And Light Levels

Under current atmospheric conditions with 380 ppm CO_2, the quantum yield of C_3 and C_4 leaves are similar at about 0.04 to 0.06 mole of CO_2 assimilated per mole of photons absorbed. The reduction from the theoretical quantum yield of c. 0.125 is due to losses from photorespiration (in C_3 plants) or to energy loss from CO_2 concentrating mechanisms (in C_4 plants). (See web topic 9.5 on Prehistoric changes in atmospheric CO_2)

Effect of O_2, Temp, and CO_2 may be summarized:

- **Oxygen**: C_3 plants in low O_2 environments have lower photorespiration and higher photosynthetic quantum yield, whereas C_4 plant do not improve in lower O_2. (see Chap. 8 regarding CO_2/O_2 ratios)
- **Temperature**: C_3 plants in lower temperature environments have higher quantum yield than C_4 plants, whereas at higher temperatures, C_4 plants have higher quantum yield, although these differences are modest over moderate temperature ranges. (fig. 9.9) The quantum yield of C_4 plants in almost constant over temperature range of 10 - 40 ºC
- **CO_2**: C_4 plants in lower CO_2/O_2 ratio environments have more efficient PS, whereas the opposite is true in higher CO_2/O_2 ratio environments. (see Chap. 8)

Sun and shade plants "show very similar quantum yields"[TZ205] [for quantum yields expressed in terms of mol CO_2 fixation per absorbed quantum]. However, the maximal PS CO_2 assimilation of sun plants at saturation (example: c. 35 µmol CO_2 m^{-2} s^{-1}) is substantially higher than for shade plants (example: c. 5 µmol CO_2 m^{-2} s^{-1}, see fig. 9.10). [MCM: this is apparently not contradictory, since quantum yield at least by some definitions does not quantitate how much effect each of the absorbed photons utilized has, see earlier discussion of definitions of quantum yield.] This indicates that earlier growing conditions affect how much capacity for PS the leaf develops. Above saturation, the leaf is said to be "CO_2 limited"—the Calvin cycle processes cannot keep up with the absorbed light energy producing ATP and NADPH.

Mechanisms of Protection Against Excess Light

Most leaves can utilize only as many as 500 - 1000 µmol m^{-2} s^{-1} of photons out of full sunlight's maximal 2,000. Most plant leaves are partially shaded so a plant as a whole is rarely saturated with light. Although individual needles or leaves may be saturated, whole trees and the forest canopy as a whole is rarely saturated

Leaves must dissipate excess light energy (to prevent photoinhibition), often as heat. An important mechanism is the use of the **xanthophyll cycle** (see Chapter VII comments), which employs violaxanthin, antheraxanthin, and zeaxanthin. **Zeaxanthin** is the most effective at dissipating heat, and, along with antheraxanthin, rises in concentration as sunlight becomes more intense, while violaxanthin declines correspondingly. This cycle of waxing and waning concentrations is diurnal in summer, but in conifers in winter, zeaxanthin stays high all day apparently to prevent photo-oxidation. Xanthophyll may also protect chloroplasts against the effects of excess heat.

Chloroplasts also protect themselves against excess light by shifting in distribution within cells, moving to the cytoplasmic margins and thereby increasing their overlap. This phenomenon is seen in algae, mosses, and higher plants, and is a blue-light response related to phytochrome and using actin microfilaments.

Leaf orientation, overlap, and **wilting** also help plants to regulate excess light and heat by reducing incident heat and light load.

Too much light can lead to **photoinhibition**.[TZ208] This may take the form of

- **Dynamic photoinhibition**—a reversible effect from moderately excess light in which PS efficiency decreases (slope of light-response curve) but the maximum PS rate is not significantly changed. Some of these changes are photoprotective, and can occur normally at midday and at colder temperatures.
- **Chronic photoinhibition**—irreversible reduction of maximum PS rate (O_2 evolved per quantum mol) from excess light.

Cumulative effects of recurring photoinhibition over a growing season can reduce crop yield.

Effects of Temperature And Heat On PS

Leaves must dissipate large amounts of heat, but are aided in this by absorbing only about 50% of the incident solar energy in the 300 - 3000 nm range (most of this absorption is in the visible spectrum). Absorbed energy is dissipated by

- **Re-radiation** as long wavelength infrared (typically c. 10,000 nm)
- **Sensible heat loss** by air circulation, convection, and conduction
- **Latent heat loss** as evaporation (**evapo-transpiration**)

The **Bowen ratio** (web topic 9.2) is the ratio of sensible heat loss to evaporative heat loss. It is higher in desert plants with little water loss, and lower in tropical rain forests and well-watered crops with high evapotranspiration.

PS is sensitive to temperature. At normal "ambient" CO_2 concentrations, C_3 plants have a lower PS versus temp curve than C_4 plants (see fig. 9.17), and show peak PS rate μmol [?CO_2] m^{-2} s^{-1} (**optimal temperature response**) at lower temperatures. (In other words, C_4 plants in ambient CO_2 conditions and at higher temperatures are more efficient at PS than C_3 plants.) But at high CO_2, the curves of PS rate versus temperature are almost identical. (See also Chapter 7 comments) The declines of PS at higher temperatures are due mainly to instability of membrane bound electron transport, not to photo-oxidation. Different plants have different adaptations and optimal temperature response—some are able to photosynthesize at 0 ºC and others as high as 50 ºC.

When considered as a function of **latitude**, C_3 grasses in savanna and steppe ecosystems are predicted to be more productive (and in fact are found to be more common) in higher latitudes above 45 degrees (where lower temperatures prevail and adequate water supplies are perhaps more likely). C_4 grasses are predicted to be more productive (and are found to be more common) in semi-arid latitudes from 20 to 40 degrees and which have warm wet summers. [Latitudes closer to the equator are not included in this analysis because tropical forests are more common, and would shade C_4 grasses.] (See TZ fig. 9.18 and web topic 9.3 for further details). In modern agriculture, C_4 plants such as corn, sugarcane, and sorghum are being grown outside their customary geographic ranges.

Effects of CO_2 on PS and Evolution of C_4 versus C_3 Plants

Atmospheric CO_2 has evolved over the millennia, and is currently at c. 380 ppm, or a partial pressure of 38 Pa. These values are twice the value prevailing during most of the past 420,000 years, probably higher than any time since 2 million years. Thus, recently evolved plants developed in low CO_2 conditions. Concentrations > 1000 ppm have not existed since the Cretaceous > 70 Ma. Rising CO_2 contributes to an increasing greenhouse effect. Current C_3 PS is CO_2 limited, and C_3 plant growth would have been faster when CO_2 was c. 600 ppm.

Diffusion through the stomata and on to the chloroplasts can be limited at several "points of resistance". Keeping the stomata less open minimizes water loss but raises **stomatal resistance**, decreasing CO_2 uptake, as previously discussed—stomatal resistance therefore is often the single greatest point of resistance. Leaves in still air develop low CO_2 and higher H_20 in the **boundary layer** (Chap 4), impairing entry of CO_2 into the leaf. Stirring the leaves and the boundary air (as with quaking aspen leaves) helps to maintain a higher CO_2 gradient in the boundary layer, promoting greater entry into the leaf. Desert leaf adaptations tend to produce thicker boundary layers. Resistance to CO_2 diffusion within the leaf to the chloroplast is relatively low, and this diffusion is facilitated by the generally peripheral position of chloroplasts within the mesophyll cells. PS within a leaf cross-section is maximal in its midportion where the mesophyll cells are concentrated, and is lower closer to the dorsal surface (light side where palisade cells dominate) and also lower closer to the ventral surface (undersurface or dark stomatal side). This bell-shaped distribution is similar to the concentration of chlorophyll in the leaf cross-section.

The current relatively low concentration of CO_2 limits PS, and higher levels would increase yields of lettuce, tomatoes, cucumbers, etc. In C_3 plants, below a certain partial pressure of intercellular CO_2,(the **CO_2 Compensation Point**), the plant evolves more CO_2 from mitochondrial respiration than it assimilates and fixes by PS (i.e., CO_2 assimilation is negative). The CO_2 Compensation Point is higher for C_3 (c. 10 Pa) than for C_4 plants (c. 0 Pa) because C_4 plants use CO_2 more efficiently in low CO_2 conditions.

As CO_2 increases, CO_2 assimilation remains higher for C_4 than C_3 plants (for C_3, it is rubisco carboxylation capacity limited). However, C_4 PS attains a maximum at a low CO_2 partial pressure of c. 15 Pa. At higher CO_2 partial pressure, a crossover point is reached (c. 50 Pa CO_2) where C_3 CO_2 assimilation becomes

greater than that of C_4. (fig. 9.22) C_3 plants would benefit by rising CO_2 levels (if temperatures and water availability were unchanged). Most plants (70% of current "productivity") are C_3, and probably evolved long ago during times of higher CO_2 concentration. In contrast, C_4 plants evolved more recently, perhaps 10 - 15 million years ago, in part to adapt to falling ambient CO_2, such as during the glacial periods (when CO_2 was below 200 ppm), probably however in the warmer areas of the Earth during these times (see also below).

C_4 plants can use CO_2 and N more efficiently, and require less N to grow, but C_3 are better shade adapted.

Summary of PS As A Function Of CO_2 Versus Temperature

(See fig. 9.23 and web topic 9.8)
Lower temperatures and higher CO_2 conditions (and shade) favor C_3 plants.
Higher temperatures and lower CO_2 conditions favor C_4 plants.

Crassulacean Acid Metabolism (CAM)

Cacti, succulents, etc. use CAM (see Chap 8) to maximally conserve water while using CO_2 efficiently—i.e., the ratio of water loss to CO_2 uptake is the lowest compared to C_3 or C_4 plants. CAM plants can employ "**CAM idling**" with closed stomata night and day to survive prolonged severe drought.

Use Of Stable Carbon Isotope Ratios To Study C_3, C_4, and CAM Plants

The **stable carbon isotope ratio**[57] **$\delta^{13}C$ ‰** ("delta") expresses the ratio of abundances R of stable C isotopes ^{13}C in CO_2 compared to ^{12}C in CO_2:

$\delta^{13}C$ ‰ = $((R_{sample}/R_{standard})-1) \times 1000$ where
$\delta^{13}C$ ‰ is the carbon isotope ratio expressed as parts per mil (or per mill, or per thousand)
$R_{standard}$ is based on abundances of stable C isotopes found in [Vienna] Pee Dee Belemnite (a fossil limestone from S. Carolina)

In the present atmosphere, $\delta^{13}C$ is **-8 ‰**, so there is relatively less ^{13}C than in the fossil carbonate standard. Such a negative value is variously called "lower", "lighter", "more negative", or "depleted in ^{13}C" compared to the standard (which would itself yield 0 ‰). Plants have differing $\delta^{13}C$ due to differences in how much they discriminate against ^{13}C during PS:

C_3 plants $\delta^{13}C$ = -28 ‰ (or -26‰ with range -23 to -33‰)[58]
C_4 plants $\delta^{13}C$ = -14 ‰ (or - 13‰, with a range -9 to -16‰)
CAM plants $\delta^{13}C$ are more variable (range -10 to -31‰, with most between -17 and -22‰)

Most of our foods come from C_3 plants, including fruits and beets, but corn, sugarcane, and sorghum are C_4, so that sucrose derived from the latter is distinguishable from sucrose derived from the former by the use of the $\delta^{13}C$. (See Web Topic 9.7) The discrimination of PS plants favoring ^{12}C from CO_2 is based on differences in:

- **diffusion rates of CO_2 across the stomata**: ^{12}C is lighter and diffuses faster, producing $\delta^{13}C$ = -4.4‰
- **carboxylation rates**:
  rubisco of C_3 has a high discrimination for CO_2 producing $\delta^{13}C$ = -30 ‰
  PEP-carboxylase of C_4 has much less marked $\delta^{13}C$ = -2 ‰

C_3 values of $\delta^{13}C$ are more positive when growing under water stress conditions.

Mammalian Herbivore Dental Enamel: The $\delta^{13}C$ ‰ of C in tooth enamel of modern herbivores reflects the sources of dietary C from C_3 or C_4 plants. [The values must be adjusted for a preferential enrichment of ^{13}C in formation of carbonate.] (See web topic 9.8 figure 9.8.d). Carbon isotope ratios in tooth enamel of fossil herbivores has been used to help in reconstructing the relative proportions of C_3 and C_4 plant PS from past geologic periods and therefore the **onset of C_4 PS**—one study showed **inconspicuous C_4 before 8 Ma and substantial C_4 by about 6 Ma**.

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[57] Stable carbon isotope ratio: http://wwwrcamnl.wr.usgs.gov/isoig/res/funda.html
[58] Stable carbon isotope ratio in C3 and C4 and CAM plants: http://ethomas.web.wesleyan.edu/ees123/carboniso.htm

Glacial Versus Inter-Glacial Period $\delta^{13}C$ ‰: An African tropical bog carbon study (applying to a much more recent time than above) showed a change from C_4-dominated plants to C_3-dominated plants between 10,000 - 12,000 ka. This is thought to reflect the time when the last (most recent) Pleistocene glacial period ended—it was variously named Wisconsin, Fraser, Würm, etc. During this same time period, Antarctic ice core CO_2 rose from c. 180 to c. 280 ppm, consistent with the onset of the current Holocene Inter-glacial period.

Chapter X. Translocation In The Phloem

This chapter focuses on movement of products in the phloem in angiosperms. The word translocation is used by the textbook authors for several different types of chemical movements, but EVV suggests it best applies to movement of photosynthate and other compounds in the phloem.

Phloem Vessels, Interconnections, Damage Responses, Companion Cells

Phloem vessels consist of living **sieve elements** (**sieve tube elements** in angiosperms, **sieve cells** in gymnosperms), which are cells interconnected through **lateral sieve areas** and (in angiosperms only) by **terminal sieve plates.** Phloem vessels in leaves and stems are commonly positioned adjacent to more centrally positioned xylem vessels, separated by the **vascular cambium** (a lateral meristem)—the resulting **vascular bundle** is enclosed by **bundle sheaths** of sclerenchyma cells. Mature sieve elements are specialized for fluid conduction:

- They lose their nuclei and tonoplasts, and many other cellular organelles.
- They retain their plasma membranes, mitochondria, plastids, and SER.
- The walls are non-lignified but may be secondarily thickened.

(In contrast, xylem tracheids are dead, have no PM or organelles, and are often lignified.)

Phloem vessels are partially summarized in Chapter 1. For a discussion of anatomy of trunks and stems of woody trees and shrubs, see also the Chapter I summary. The prominent lateral sieve areas have pores interconnecting adjacent sieve elements, and sieve plates connect sieve elements end-to-end (but in angiosperms only).

Protective Mechanisms

Damaged sieve elements are sealed off acutely by the plugging up during a sudden surging flow of the affected sieve plate pores by one or more of:

- **P-proteins** (a kind of "slime", found only in angiosperm sieve tube elements but synthesized in companion cells)
- occluding **protein bodies**
- **SER membranes** (possibly; in gymnosperms only)

Longer term sealing of injuries involves closing the sieve pores with **callose** (a glucose polymer, β-1,3-glucan, synthesized in sieve elements.

Companion Cells

Sieve elements have adjacent **companion cells** connected via plasmodesmata and allowing exchange of solutes, performance of critical metabolic functions such as provision of ATP, etc. Types of companion cells include:

- **Ordinary companion cells**: Have chloroplasts, few connections to cells other than its own sieve element.
- **Transfer cells**: Have fingerlike walls facing away from the sieve element which increase surface area of the PM available for transfer of solutes to/from the sieve element. Probably specialized (like ordinary companion cells) for taking up solute such as sugars from the apoplast in sources, and transferring it symplastically to the sieve element. (Xylem companion cells also can do this.)

- **Intermediary cells**: Well suited for symplastic transport of sugars from mesophyll cells to sieve elements.

Sources and Sinks

Movement of photosynthate from CO_2 in leaves into phloem sieve elements can be shown with $^{14}CO_2$ studies (web topic 10.1). Photosynthate (photoassimilate) and other nutrients in phloem moves from sites of synthesis (**sources**) to sites of consumption by metabolism or storage (**sinks**). Unlike xylem, which usually moves from the roots to the leaves, phloem can move up or down the plant, distributed to where it is needed—for example, to roots, tubers, developing fruits, and immature leaves.

The patterns of distribution follow anatomic and developmental patterns. Upper mature leaves are sources for growing shoot tip and young immature leaves. Lower leaves are sources for the roots. Developing fruits and flowers are major sinks for nearby leaves.

Leaves tend to be most interconnected vertically on the same side of the plant (**orthostichy**). However, there are **anastomoses** that interconnect other vessels in alternate pathways—these pathways are plastic, and can adapt to injury such as leaf loss, etc.

Materials Transported In The Phloem; Sap

Water is the most plentiful phloem substance. Constituents of phloem (here, in castor bean exudate) also include

Sugars	80 - 106 mg mL^{-1} [concentrations up to 0.3 - 0.9M; cf. only c. 1.0 mg/mL in humans]
Amino Acids	5 mg mL^{-1}
Organic Acids	2 - 3 mg mL^{-1}
Protein	1.5 - 2.2 mg mL^{-1} (esp. P-Protein)
Potassium	2.3 - 4.4 mg mL^{-1}
Chloride	0.36 - 0.68 mg mL^{-1}
Phosphate	0.35 - 0.55 mg mL^{-1}
Magnesium	0.11 - 0.12 mg mL^{-1}

Nitrogen is carried in **AAs** and their **amides** (especially glutamate or its amide glutamine, and aspartate or its amide asparagine), and may be transferred from the xylem to the phloem. (See web topic 10.2) In plants that fix N with root nodules, N is also transported in the form of **ureides**: **allantoic acid**, **allantoin**, and **citrulline**.

Also present are **hormones** (including auxin, GAs, cytokinins, and ABA), various other **signaling molecules**, **nucleotide phosphates**, and small amounts of **RNAs**.

It is technically difficult to harvest pure phloem fluid or sap (see web topic 10.3 for use of aphid stylets). The term "**sap**" in plants typically refers to a mixture combining sieve element phloem fluid, xylem fluid, and cell vacuole fluid.

Sugars are translocated as **non-reducing sugars** (which are less reactive), especially **sucrose**, rather than glucose, mannose, fructose, etc. (which in the linear forms expose aldehyde or ketone groups). Other translocated phloem sugar derivatives include the **sucrose-galactosyl oligosaccharides**[59] **raffinose** (3

[59] Raffinose family of oligosaccharides RFOs:
"Humans … do not possess the α-GAL [α-galactosidase] enzyme to break down RFOs and these oligosaccharides pass undigested through the stomach and upper intestine. In the lower intestine, they are fermented by gas-producing bacteria which do posses the α-GAL enzyme and make carbon dioxide, methane, and/or hydrogen—leading to the flatulence commonly associated with eating beans and other vegetables. α-GAL is present in digestive aids such as the product Beano."
http://en.wikipedia.org/wiki/Raffinose

sugars), **stachyose** (4 sugars), and **verbascose** (5 sugars), as well as sugar alcohols such as **sorbitol** and **mannitol**. [MCM: Presumably these are also non-reducing products.]

Rates And Mechanisms Of Phloem Transport

Phloem transport rates are much slower than xylem, and are expressed as either of

- **Mass transfer rate**: c. 1 - 15 g h^{-1} cm^{-2} of sieve element cross sectional area (see web topic 10.4), or
- **Linear velocity**: c. 30 - 150 cm h^{-1}

Linear velocities are much faster than diffusion would provide, consistent with a mass or bulk transfer process in which no membranes are crossed (except through pores). Rates are determined with radioactive tracers (web topic 10.4).

The flow of phloem is explained best by the **pressure-flow model**, first elaborated by Ernst Münch in 1930, and experimentally confirmed. Unlike with pure xylem flow (which depends on a hydrostatic pressure difference arising from negative tension generated in the leaves, and does not require a connection with the phloem), with phloem the bulk water flow is produced by an osmotically generated positive hydrostatic pressure gradient produced between the source and sink by phloem loading and unloading, and which requires water flows out of and into the xylem.

Water exhibits bulk flow due to differences of water potential $\Psi_w = \Psi_s + \Psi_p + \Psi_g$ (if semi-permeable membranes are involved) or differences of hydrostatic pressure potential (if not involving semi-permeable membranes). The latter is the case with phloem flow. In phloem, energy-driven **phloem loading** at the source generates a higher solute concentration in the source sieve elements (thus a lower more negative solute potential Ψ_s and resulting negative Ψ_w). This negative Ψ_w causes water to enter the source sieve elements by osmosis across a membrane from the xylem, **raising hydrostatic pressure Ψ_p** locally in the source sieve elements. This creates a hydrostatic pressure gradient (higher Ψ_p at the source than the sink) that drives phloem solution bulk flow across **open sieve plate pores** toward the lower hydrostatic pressure sink sieve elements. (In the example shown in fig. 10.10, the water potential Ψ_w is actually higher at the sink than the source—this is not paradoxical, since semi-permeable membranes are not involved in this bulk flow, and the condition is maintained by the active transport mechanisms of loading and unloading.) At the sink sieve element, **phloem unloading** of solute is performed, lowering the solute concentration in the sink sieve element, raising the Ψ_s in the sink sieve element. As a result, when the Ψ_w of the phloem rises above the Ψ_w of the adjacent xylem, water flows from the phloem across a membrane into the xylem, lowering the hydrostatic pressure Ψ_p in the sink sieve element, thus helping to maintain the driving hydrostatic pressure gradient. (See fig. 10.10)

The direction of flow in any single sieve element is one-way and not bi-directional

The phloem solute loading at the source occurs into companion cells, which connect to sieve elements.

Some substances are probably not actively loaded into the phloem, but simply diffuse in—e.g., organic acids, some plant hormones, herbicides, fungicides, etc.—and are then carried along with bulk water flow. Calcium does not enter the phloem. (Web Topic 10.6)

The energy requirements for phloem transport are low. (fig. 10.12)

Some of the aspects of phloem flow remain controversial—for instance, do osmotic differences play a greater role compared to pressure differences instead? The mechanisms of phloem flow in gymnosperms are less clear, especially because the pores appear to be covered by membranes.

Phloem Loading: Apoplastic Versus Symplastic

[Limited summary] Factors involved in moving photosynthate from mesophyll cells to the sieve elements of mature leaves:

- Triose phosphate is converted to sucrose in mesophyll cells.
- Sucrose moves to various cells in the vicinity of sieve elements.

- Sugars are transported into (and usually become more concentrated in) the sieve elements (phloem loading) and companion cells.

Once loaded into the sieve elements, translocation over long distances can occur.

Phloem loading can occur via
1) Apoplastic route: In this route (fig. 10.14), sugar passes symplastically from mesophyll cells across plasmodesmata to bundle sheath cells. However, sugars then can move either

- **apoplastically** from bundle sheath cells across a PM to enter ordinary companion cells (which then connect symplastically to sieve elements), or
- symplastically from bundle sheath cells to phloem parenchyma cells, and then **apoplastically** across a PM to the ordinary companion cells (which then connect symplastically to sieve elements).

2) Symplastic route: The initial short-distance route is always symplastic. The entire route however may be symplastic. In this route, sugar passes entirely symplastically from mesophyll cells across plasmodesmata to bundle sheath cells and intermediary companion cells and on into the sieve elements.

Apoplastic phloem loading requires energy and ATP, which is used to concentrate sucrose in the sieve element and its companion cell. (Verification is demonstrated in web topic 10.5, for instance using PCMBS which inhibits transport of sucrose from the apoplast across plasma membranes.) It involves a **sucrose-H^+ symporter** (a type of Secondary Active Transport using proton pumping ATP-ases; see fig. 10.16). In some plants, apoplastic movement occurs directly into the sieve element (Table 10.4). Factors regulating sucrose loading apoplastically are summarized on p. 238.

Symplastic phloem loading occurs in plant with intermediary companion cells. (fig. 10.17) The mechanism requires conversion in the IC of sucrose with galactose to form raffinose (and stachyose). These larger molecules can freely enter the sieve element through large connecting pores, but they are too large to return to the bundle sheath cells because the connecting pores are smaller.

Phloem Unloading And Transition From Source To Sink

This is performed at sinks, such as tubers, growing root tips, reproductive structures, and young leaves. Requires short-distance transport to cells, then storage and/or metabolism. It can be apoplastic or symplastic:
1) Apoplastic route: In this route (fig. 10.18), sugar pass apoplastically across at least one PM
2) Symplastic route: The entire pathway can be symplastic, from companion cell and sieve element complex to sink cell.

Transport into sink tissues requires metabolic energy and is thought to be done with a **sucrose-H^+ antiporter**.

Transition From Source To Sink

Young leaves are sinks. As they mature, beginning first at the tips (using autoradiographs) they stop being sinks and becomes sources. With further maturation, the entire leaf becomes a source. (fig. 10.19) Export as a source in a leaf is initiated when the unloading pathway is closed, minor veins responsible for loading have matured, and the leaf is adequately photosynthesizing. At a transitional stage, parts of the leaf are sources while parts are sinks, and the vessels serving these opposite functions are not the same vessels. (fig. 10.20)

Photosynthate Distribution And Regulation

[Limited summary] The allocation or partitioning of photosynthate involves synthesis of **storage compounds** (starch), temporary storage in the **vacuole**, **metabolic utilization**, and synthesis of **transport compounds**. Sinks compete for the photosynthate, and the distribution to various sinks must be balanced. Source leaves regulate the allocation… Sink strength depends on sink size and activity… The rate of source PS adjusts to changes in the source to sink ratio… Possible mechanisms linking sources

and sinks are shown in web topic 10.10, including phosphate availability in the chloroplast, and feedback by sugar levels on gene transcription and expression…

Transport of Signaling Molecules In The Phloem

The phloem also plays an important role in transporting signaling molecules such as proteins, hormones, mRNA, and small RNAs… The SEL (size exclusion limit) of the plasmodesmata limits passive nonselective movement of larger molecules, but selective active movement can occur of larger molecules and even of viruses traveling in the phloem. Signals may also be transmitted by changes in turgor pressure.

Chapter XI. Respiration And Lipid Metabolism

[Chapter not studied in UW Plant Physiology Biol 425, somewhat limited summary follows.]

"**Aerobic respiration** is the biological process by which reduced carbon compounds are mobilized and subsequently oxidized in a controlled manner." Overall redox reactions for fully oxidizing sucrose (and reducing oxygen) may be expressed as follows:

Reaction Components:

Oxidation of Sucrose:	$\mathbf{C_{12}H_{22}O_{11} + 13\ H_2O \rightarrow 12\ CO_2 + 48\ H^+ + 48\ e^-}$
Reduction of Oxygen:	$\mathbf{12\ O_2 + 48\ H^+ + 48\ e^- \rightarrow 24\ H_2O}$
ATP Synthesis:	$\mathbf{60\ ADP + 60\ P_i \rightarrow 60\ ATP + 60\ H_2O}$
Overall reaction:	$\mathbf{C_{12}H_{22}O_{11} + 12\ 0_2 \rightarrow 12\ CO_2 + 11\ H_2O}$

This is more or less the reverse of PS. The standard free energy released by the full oxidation of sucrose to water and CO_2 is 5,760 kJ mol^{-1} (1380 kCal per 342 gm = 4 kCal/gm).

Respiration proceeds in controlled steps to prevent cellular damage and to efficiently capture the energy released in the form of ATP.

Normal plant respiration takes place in glycolysis, the citric acid cycle, and oxidative phosphorylation.

Glycolysis

Sugar such as sucrose is broken down and partially oxidized in the cytosol and plastid to yield an organic acid **pyruvate** or **malate**—this process is called glycolysis. (fig. 11.3) The sequence of intermediates include **UDP-Glucose**, **Fructose**, **Glucose**, **Hexose phosphates** (**Glucose-6-P**, **Fructose-6-P**), **Fructose-1,6-bisphosphate**, **Triose Phosphates** (**Glyceraldehyde-3-phosphate** [GA3P] or **Dihydroxyacetone phosphate**), **1,3-Bisphosphoglycerate**, **3-Phosphoglycerate**, **2-Phosphoglycerate,** and **Phosphoenolpyruvate** (**PEP**), finally **Pyruvate**$^-$. (This sequence can be reversed as **gluconeogenesis**, though uncommon in plants…) In the process, 4 ATP are synthesized directly per sucrose (a cytosolic process termed "**substrate-level phosphorylations**", in contrast to oxidative phosphorylation and photophosphorylation). The 4 NADH, synthesized from NAD^+ (if not consumed in fermentation) are ultimately converted to 6 ATP (Table 11.2)—thus a total of 10 ATP per sucrose result from glycolysis without fermentation.

If PEP is converted to **Malate**$^-$ instead of Pyruvate, the intermediate is **Oxaloacetate**. Malate can be stored in the vacuole or transported to mitochondria, but pyruvate usually dominates over malate in glycolysis.

Fermentation

If oxygen is lacking, the citric acid cycle and oxidative phosphorylation cannot proceed (the supply of NAD^+ rapidly becomes limited by conversion to the reduced form NADH). In hypoxic conditions, plants gain

limited additional energy and regeneration of NAD^+ by **fermentative metabolism** (also sometimes called **anaerobic respiration**). Fermentation can take place by either of the following pathways:

- **Alcoholic fermentation**: Pyruvate is further oxidized to acetaldehyde and (via alcohol dehydrogenase) to **ethanol.** This releases CO_2 and oxidizes one NADH back to NAD^+. Ethanol is a relatively nontoxic end-product.
- **Lactic acid fermentation**: Pyruvate is converted to Lactate via lactate dehydrogenase, with oxidation of one NADH back to NAD^+. Lactate is more toxic, as it acidifies the cytosol.

This process of fermentative metabolism (glycolysis followed by fermentation) is **energetically inefficient**—it does not efficiently utilize the remaining energy derived from sucrose which still remains in alcohol or lactic acid. Although the NAD^+ is recovered, the net result of glycolysis followed by fermentation is only 4 ATP molecules per molecule of sucrose oxidized. Assuming the nonstandard typical conditions that commonly exist in plants, the free energy that must be added to ADP to synthesize ATP is 50 kJ mol^{-1}, and the overall efficiency is 4 * 50 / 5,760 = 3.5%. As a result, plants depending on fermentation require a much faster glycolysis rate (the **Pasteur Effect**).

Control Of Glycolysis

The products of glycolysis control glycolysis, including Fructose-6-P and especially PEP (details omitted) … Analysis of metabolites ("**metabolic profiling**") is discussed in Web essay 11.2.

The Oxidative Pentose Phosphate Pathway

Glycolysis accounts for 80 - 95% of the total sugar respiration compared to the oxidative pentose phosphate pathway. The latter pathway is an alternative to glycolysis for complete oxidation of sugars such as Glucose-6-Phosphate in plant cells to CO_2, etc. It is also called the **hexose monophosphate shunt**. (fig. 11.4) It is found in the cytosol and in plastids. It acts on a Hexose-P such as **Glucose-6-Phosphate**, passing through intermediates **6-Phosphogluconate** to **Ribulose-5-phosphate** (a **Pentose-P**) … **Fructose-6-P** or **Erythrose-4-P**), ending with the triose phosphate **glyceraldehyde-3-P (GA3P)** and releasing CO_2 and converting **$NADP^+$** to **NADPH** (this is not the same molecule as NAPH). The GA3P re-enters the normal glycolytic pathway. The net result of this shunt pathway is:

$$6 \text{ Glucose-6-P} + 12 \text{ NADP}^+ + 7 \text{ H2O} \rightarrow 5 \text{ Glucose-6-P} + 6 \text{ CO}_2 + \text{P}_i + 12 \text{ NADPH} + 12 \text{ H}^+$$

Thus, "for 6 turns of the cycle" (in which glucose-6-P is regenerated from GA3P and Fructose-6-P), one molecule of Glucose-6-P is fully oxidized. [MCM: this is a little hard to follow.]

The roles of this pathway in plant metabolism are summarized as follows:
- NADPH supply for biosynthetic redox reactions (including lipid biosynthesis, nitrogen assimilation, etc.)
- NADPH supply for respiration (used in generating ATP and reducing O_2)
- Supply of biosynthesis substrates (such as ribose-5-P needed for DNA and RNA synthesis, and erythrose-4-P needed in synthesis of phenolics such as lignin and flavonoids, etc.)
- Generation of Calvin Cycle intermediates

Control of this pathway is inhibition via a high ratio of NADPH to $NADP^+$ (details omitted)…

The Citric Acid Cycle, Oxidative Phosphorylation, and Mitochondria

[Limited summary] The Citric acid cycle is also known as the **Krebs Cycle** after its 1937 discoverer Hans Krebs, or the **Tricarboxylic Acid Cycle** (named after citric and isocitric acids). It is intimately combined with oxidative phosphorylation, both of which take place in the mitochondria, and these complete the breakdown of pyruvate to CO_2 and H_2O. The Citric acid cycle deals with breakdown of pyruvate to CO_2 and H_2O and the generation of the reduced forms NADH and $FADH_2$. Oxidative phosphorylation deals with

utilization of the energy stored in NADH, $FADH_2$, and the electrochemical proton gradient for the synthesis of ATP using F_OF_1-ATP synthase.

Mitochondria have an **outer membrane** (which is smoothly contoured and quite porous for molecules up to 10,000 Dalton) and a highly convoluted osmotically semi-permeable **inner membrane** that is folded into **cristae**. The space inside the inner membrane is the **matrix**, whereas the space between the membranes is termed the **intermembrane space**. Mitochondria are typically 1 to 3 µm in greatest axis and 0.5 to 1 µm in short axis diameter. They are quite dynamic in shape, size, and location, and move about in the cell aided by the cytoskeleton (see Web essay 11.3 on mitochondrial dynamics, including a movie showing their movement). They divide to reproduce themselves. Plant cells typically have fewer mitochondria than animal cells, though some plant cells such as guard cells and anthers are more richly endowed. The mitochondrial genome varies from 200 to 2,400 kilobase pairs in plants (encoding c. 35 proteins, plus rRNAs and tRNAs, along with substantial non-coding sequences)—this compares to a more compact 16 kbp in animals (which encode 13 proteins plus rRNAs and tRNAs).

Pyruvate enters the mitochondrion and is oxidized via the citric acid cycle as follows. (fig. 11.6) Pyruvate is joined with coenzyme A (CoA) to yield **Acetyl-CoA**, converting NAD^+ to NADH and yielding one CO_2. The CoA is removed as 2-C Acetyl-CoA is combined with 4-C **oxaloacetate OAA**, yielding **Citrate** (a 6 carbon tricarboxylic acid), and then **Isocitrate** (also a 6 carbon tricarboxylic acid). Other intermediates of this cycle are **2-Oxoglutarate**, **Succinyl-CoA**, **Succinate**, **Fumarate**, **Malate**, **Oxaloacetate**, and back to Citrate. In the process, NAD^+ is reduced to NADH and FAD is reduced to $FADH_2$... (In animals, some of the energy is stored in **QH_2** = **Ubiquinol**, the reduction product of **Ubiquinone** = **Coenzyme Q** = **CoQ_{10}**.) No free oxygen enters as a reactant in the citric acid cycle. There are alternate pathways available for the citric acid cycle... (details omitted)

The high energy stored in electrons in the NADH and $FADH_2$ must be converted to ATP for ultimate use in living processes. This O_2 dependent mitochondrial process is called **Oxidative Phosphorylation** (fig. 11.8). An **electron transport chain** in the inner membrane catalyzes a flow of electrons from NADH to O_2 (fig. 11.8), which brings about the oxidation of NADH to NAD^+ and $FADH_2$ to FAD, the reduction of O_2, and the generation through coupling with proton pumping of the **electrochemical proton gradient** needed for ATP synthase... The electron transport chain reactions and other inner membrane components include:

- **Complex I (NADH Hydrogenase)**: This pumps 4 protons out of the matrix for every electron pair (one pair per oxygen) transported through this complex.
- **Ubiquinone UQ**: a lipid soluble and diffusible electron and proton carrier located inside the membrane.
- **Complex II (Succinate dehydrogenase)**: Oxidizes succinate but does not pump protons
- **Complex III (Cytochrome BC_1 complex)**: This oxidizes reduced UQ (QH_2) and pumps 4 more protons out of the matrix per electron pair, setting up a proton gradient (proton motive force) created by both the ΔE and the ΔpH. ...
- **Complex IV (Cytochrome C oxidase)**: This pumps 2 protons out of the matrix and reduces O_2 to H_20.
- **Complex V (ATP Synthase)**: **F_OF_1-ATP Synthase**, the site of ATP synthesis, accompanied by re-entry of 3 H^+ into the matrix. [The subscript for F_O is the letter O, not a zero, and stands for oligomycin] Thus, ATP synthesis in the mitochondrion is coupled with electron transport. This complex is a "rotary motor" whose rotation has been made visible experimentally. (web topic 11.4)[60]
- **Alternative Oxidase (AOX)**: accepts electrons from Ubiquinone (see below)
- **Uncoupling Protein** (see below): Directly transports H^+ through the membrane back to the matrix.

Some aspects of this cycle are unique to plants. For instance, the ADP is phosphorylated to ADP in plants in the step acting on succinyl-CoA that is catalyzed by succinyl-CoA synthetase, whereas phosphorylation of **GDP to GTP** occurs in animals at the corresponding step. Plants have significant activity of **NAD+ malic enzyme**, which catalyzes the conversion of malate and NAD^+ to pyruvate, CO2, and NADH, a pathway used in CAM plants... (further details omitted here)

ATP synthesis in mitochondria at **F_O-F_1 ATP synthase** is coupled (as in chloroplasts) to the **proton motive force**, resulting from both the voltage difference across the membrane and (less significantly in mitochondria) the difference in H^+ concentrations. (In chloroplasts, the difference in H^+ concentration

[60] F_oF_1-ATP Synthase as a rotary motor:
http://www.res.titech.ac.jp/~seibutu/main.html?right/~seibutu/projects/f1_e.html

across the thylakoid membrane is the more important of the two factors.) This coupling is based on the **chemiosmotic mechanism** postulated by Peter Mitchell.

The citric acid cycle itself (table 11.2) yields approximately 4 ATPs directly per sucrose oxidized, as well as 6 ATPs derived indirectly from 4 $FADH_2$, and 40 ATPs from 16 NADH.

Overall, the total ATP production from the complete oxidation of sucrose to CO_2 by glycolysis plus the citric acid cycle plus oxidative phosphorylation is **60 ATPs**. This represents about 3,010 kJ mol^{-1} of conserved free energy in ATP (each ATP has effectively about 50 kJ mol^{-1} free energy under typical plant conditions). Sucrose has 5,760 kJ mol^{-1}, so the free energy conversion efficiency from sucrose to ATP is c. **52%**.

Several **transporters** as well as the **electrochemical gradient** across the mitochondrial inner membrane move substrates and products in and out: these include ADP in/ATP out, P_i in, Pyruvate in, Malate out coupled with Citrate in, the electron transport complexes which move H^+ out, etc. (details omitted, see fig. 11.10)

Methods Of Lowering ATP Yield, Generating Heat, Or Reducing Oxidative Stress

Plants use several processes to lower ATP yield, probably as protective mechanisms, or to generate heat. These mechanisms include:

Alternative Oxidase (AOX)... This may be useful during floral development in **Araceae**—such as the Sauromatum venosum (voodoo lily), Amorphophallus titanum (Titan Arum), Symplocarpus foetidus (Eastern Skunk Cabbage), and Philodendron selloum—as well as lotus, palms, magnolias, cycads. It is used to raise the rate of respiration and resulting temperature (thermogenesis up to a 40 ºC increment over ambient), thus increasing the emanations of volatile aromatic amines, indoles, and terpenes that attract insect pollinators. The flower may in some cases be providing warmth to insect, or keeping snow away and keeping itself from freezing... This involves especially the pollen-containing male parts—e.g., the **male florets** of the **spadix** of Araceae such as Philodendron selloum, etc. (See web essay 11.4 Temperature Regulation By Thermogenic Flowers) This response may also be protective in that it is used to drain off over-reduction of the ubiquinone pool, thus preventing generation of reactive oxygen species (see Essay 11.5)... In extreme instances, the respiratory rate may be as high as 400 mW g^{-1}, higher even than a flying hummingbird (240 mW g^{-1}). Such rapid respiration and heat generation requires a rapid oxygen delivery supply. Some plants like Nelumbo nucifera (sacred lotus) regulate the flower temperature closely.

Uncoupling Protein: : Permits H^+ to re-enter the matrix without generating ATP, helping to dissipate the proton gradient, and increasing **heat production**. This mechanism is used in animals for heat generation and therefore **thermoregulation**, but in plants it serves possibly to **protect against oxidative stress**, or over-reduction of the electron transport chain. (Web topic 11.3)

Internal Rotenone-Insensitive NADH Dehydrogenase... Its role is unclear

Respiration In Intact Plants

[Limited summary] Substrates of ATP synthesis, namely ADP and P_i, are the major control factors in the cytosol and mitochondria... Respiration is tightly coupled to other pathways...

Plants respire roughly half of the daily PS yield in "**maintenance respiration**", merely what is needed to keep mature cells in a viable state without growth. (Web topic 11.7) Tropical plants may lose up to 70 to 80% of their daily photosynthetic gain, due to the high **dark respiration** rate due to higher night temperatures. Mitochondrial respiration is usually much slower than the maximum rate of photosynthesis, whereas photorespiration can approach 20-40% of the gross PS rate... (details omitted) Mitochondria are a major supplier of ATP even in actively photosynthesizing leaves. Mitochondrial respiration during PS also supplies precursors such as 2-oxoglutarate needed for N assimilation... Mitochondrial function is crucial during pollen development... Plants with **cytoplasmic male sterility CMS** (a property of the maternal mitochondrial genome) are useful in hybridization programs because the pollen is sterile. (A mistake in a breeding program using cms genes led to a serious outbreak of southern

corn leaf blight due to a greater virulence of the fungus Bipolaris maydis race T against this genetic variant maize.)...

Factors affecting respiratory rates include:

- **Low Ambient Oxygen**: There is usually much more O_2 in the air than is needed for plant respiration—e.g., ambient O_2 at the tissue level is 250 µM (in aqueous solution in equilibrium with 21% O_2 in air) whereas the Km for Cytochrome C oxidase is only 1 µM. However, plant respiration begins to fall when atmospheric O_2 falls below 5%, probably due to limitations imposed by the slow rate of diffusion... Special mechanisms to present air to internal plant components, including **intercellular air spaces (aerenchyma)**, may serve a needed purpose. (See also Web essay 11.4)
- **Water Saturation Causing Low O_2**: Plants growing in aqueous medium, or their submerged roots, may be anoxic. A network of **intercellular air spaces (aerenchyma)** running from leaves to roots is found in rice and sunflower to allow growing in flooded areas.
- **Temperature**: Respiration usually increases short-term by a rise in temperature by a certain factor for each 10 °C temperature rise, up to a plateau at 40-50 °C. This **temperature coefficient** factor (**Q_{10}**) is generally between 1.5 and 2.5. Longer periods of colder temperatures lead to plant acclimation. Low temperatures in stored potatoes may reduce respiration and sprouting but temperatures must be set to somewhat higher to prevent undesirable conversion of starch to sugar.
- **CO_2 concentration**: Storing fruits at low temps in 2-3% oxygen and 3-5% CO_2 retards respiration rate due to the inhibitory effects of this level of CO_2 and lower temperature, while the O_2 prevents fermentation.

Lipid Metabolism

Lipids are water insoluble hydrophobic compounds that are soluble in organic solvents. They are stored in plants primarily for carbon storage rather than for energy storage as seen with animal lipids. Complete oxidation of a fat or oil lipid yields 40 kJ (**9.3 kCal**) per gram (compared to 3.8 kCal/gm for starch, 4 kCal/gm for sucrose). Synthesis requires a correspondingly large investment of energy.

The most common form of lipids are **triacylglycerides** (a.k.a. "**triglycerides**" or **triacylglycerols**), in which fatty acids are linked by ester bonds to each of the three hydroxyl groups of glycerol. (fig. 11.14) [An acyl group[61] is usually derived from a carboxylic acid and has the formula RC(=O)–.] The fatty acids of plants consist mostly of even numbers of C (12 to 20, commonly 16 to 18) in usually straight chains and include (# of Carbons : # of Double bonds)
Saturated (0 double bonds): Lauric Acid (12:0), Myristic Acid (14:0), **Palmitic Acid** (16:0), **Stearic acid** (18:0), and Arachidic (20:0)
Unsaturated (≥ 1 double bonds): **Oleic acid** (18:1), **Linoleic acid** (18:2), **Linolenic acid** (18:3), Arachidonic acid (20:4)

Oils are liquids at room temperature because of a higher proportion of unsaturated fatty acids (FAs), whereas fats are solids (or semi-crystalline solids) at room temperature and have a higher proportion of saturated FAs. The composition of plant lipids varies with species. Other plant lipids include waxes, terpenoids, and sterols.

Triacylglycerols are stored in seeds in the cotyledons or endosperm cells in organelles called **oil bodies (oleosomes)**, which are bounded by a single phospholipid layer rather than a bilayer. These monolayer membranes are stabilized by an outer layer of oleosins. Triacylglycerols are synthesized within the membranes of the ER, between the two monolayers.

Polar glycerolipids are the main structural lipids forming the **membranes**, and are mainly:

- **Glyceroglycolipids** (having a sugar but not a phosphate; e.g., glucosylceramide)
- **Glycerophospholipids** (i.e., **phospholipids**, having a phosphate group).

Membranes also have sphingolipids (such as ceramide) and sterols (which help stabilize the membrane). The various lipids found in membranes varies with the membrane. (Table 11.4)

Other types of plant lipids include chlorophylls, plastoquinone, carotenoids, tocopherols.

[61] Acyl: http://en.wikipedia.org/wiki/Acyl

Synthesis of fatty acids: (see fig. 11.16) takes place in plastids in plants (in the cytosol in animals), and may be transported to the ER for further modification. Synthesis utilizes **coenzyme A CoA** and **Acyl Carrier Protein ACP**. It consists of repeated cycles, each of which add 2 Carbons (thus one only rarely finds odd-number-carbon FAs). The first cycle begins with **Acetyl-CoA** (a acetyl 2 C group covalently bonded or thioesterified[62] with CoA). This **Acetyl-CoA** is combined with **Malonyl-ACP** to form a 4 C acid thioesterified to ACP, **Acetoacetyl-ACP**. This is converted to **Butyryl-ACP**. The ACP is removed and the Butyryl re-enters the cycle to add an additional 2-C segment. The cycle repeats adding 2-C segments until a typical 16C, 18C, or 20C FA is formed. NADPH is oxidized to $NADP^+$ in the process. **Desaturase enzymes** create the double bonds to make 18:3, 16:3 FAs, etc.

Synthesis of Glycerolipids: takes place in the plastids and the ER, for membranes and oil bodies. Synthesis involves phosphatidic acid, diacylglycerol, phosphatidylcholine, and other intermediaries (details omitted).

The composition of lipids in membranes influences membrane function and the ability of the organism to adjust to temperature extremes. **Chill-sensitive plants**, including cotton, soybean, maize, rice, tobacco, and many tropical fruits. The original hypothesis was that higher levels of saturation might cause greater levels of chill-sensitivity due to transition from liquid to gel state at higher temperatures, but the actual findings are more complex. The extent of membrane unsaturation and the presence of specific lipids can influence chilling sensitivity and provide some protection[TZ688], but "membrane lipid composition is not the major determinant of chilling sensitivity in plants." (see web topic 11.8)

Membrane lipids serve as precursors of important signaling compounds...**Jasmonate**, **PIP_2**, IP_3, and other phosphoinositides (details omitted).

When seeds germinate, storage lipids (in the form of triacylglycerols) are converted to carbohydrates, especially sucrose. This occurs in stages. The first is **lipase-mediated hydrolysis** of the fatty acids, which takes place on the boundary membrane of the oil body, or in an adjacent **glyoxysome**. In the glyoxysome, these fatty acids are converted to **Fatty-Acyl-CoA**, which undergoes repeated **β-oxidations** to produce **Acetyl-CoA** (plus several NADH and $FADH_2$), and this enters the **glyoxylate cycle** to produce succinate... In this cycle (as in the citric acid cycle), **Acetyl-CoA** combines with oxaloacetate OAA to form citrate, then isocitrate, and then succinate are formed. The succinate enters an adjacent mitochondria, and is converted through the intermediary fumarate to malate. Malate passes to the cytosol, and there sucrose is synthesized via intermediaries OOA, PEP, and Fructose-6-P. (details omitted) Sucrose is usually the final product that is translocated from the cotyledon to the growing seedling.

Chapter XII. Assimilation Of Mineral Nutrients And Oxygen

[Chapter not studied in UW Plant Physiology Biol 425]

This chapter provides further details on how important inorganic mineral nutrients are assimilated in autotrophic plants into organic compounds such as amino acids, amides, nucleic acids, lipids, enzyme cofactors, and pigments. The nutrients discussed here include **Nitrogen** (beginning as N_2 gas, nitrate and ammonium), **Sulfur** (usually beginning as sulfate), **Phosphorus** (usually beginning as phosphate), elemental **metallic cations** (including Fe, Co, Cu, Mn, K, Na, and Zn), and **oxygen**. The metal cations are often complexed with complex molecules: Mg^{2+} with Chl, Ca^{2+} with CW pectates, Mo^{6+} with enzymes, etc.

Assimilation processes typically require the input of energy as ATP and are often highly energy-intensive. They may also depend on reductants generated through PS.

Nitrogen

[62] Thioester bond: Thioesters are compounds resulting from the bonding of sulfur with an acyl group with the general formula R-S-CO-R. http://en.wikipedia.org/wiki/Thioester

Nitrogen exists in multiple stable oxidation states, contributing to the variety, versatility, and complexity of nitrogen-containing compounds. In addition to inorganic ions, N is found in plants in the form of amides[63], amines[64], amino acids and proteins, nucleic acids, and alkaloids, etc.

After **Nitrate NO_3^-** is absorbed in the roots, nitrate assimilation proceeds in the roots or shoots (depending on species and availability of nitrate). The first step is conversion in the cytosol to the higher energy ion **nitrite NO_2^-**, then to **ammonium ion NH_4^+**, and finally to the amide group in glutamate. These steps are energy consuming, requiring overall approximately 12 ATPs per nitrate nitrogen converted to glutamine.[TZ289] (The number of ATP shown in fig. 12.21 adds to 13 ATPs for the sequence from extracellular nitrate to glutamate in the mesophyll cell, or 12 ATPs for nitrate that has already been symported into the mesophyll cell.)

Legumes, through their symbiosis with nitrogen-fixing bacteria, convert gaseous N_2 (N≡N) into **ammonia NH_3**, which normally is protonated to ammonium ion NH_4^+, and this is assimilated into amino acids, etc. These steps are more energy consuming, requiring approximately 16 ATPs per nitrogen fixed into ammonia.[65]

Reactions consuming such high energy not surprisingly yield products that in some cases are capable of explosive energy release—e.g., ammonium nitrate (the source of the explosion in the Texas City disaster of 1947).

The complex global **biogeochemical nitrogen cycle**, involving both biotic and abiotic processes, is summarized in fig. 12.1 and additional details are provided here.[66] The major processes of this cycle are named in Table 12.1, along with estimates of the **pool sizes** of stored organic nitrogen:

- Organisms 5.2×10^{15} g
- Soil 95×10^{15} g
- Oceans 6.5×10^{15} g

Biological nitrogen fixation and associated symbiotic relationships

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[63] **Amides**: An amide is one of three kinds of compounds:
- the organic functional group designated as $R_1(CO)NR_2R_3$ characterized by a carbonyl group (C=O) linked to a nitrogen atom (N), or a compound that contains this functional group (sometimes called an acid amide)
- The anion NH_2^-
- Any organic compound derived by the replacement of a hydroxyl group by an amino ($-NH_2$) group.

http://en.wikipedia.org/wiki/Amide

[64] **Amines**: Amines are organic compounds and functional groups that contain a basic nitrogen atom with a lone pair [of electrons]. Amines are derivatives of ammonia, wherein one or more hydrogen atoms are replaced by organic substituents such as alkyl and aryl (aromatic) groups as follows:
- one hydrogen atom: primary amine NH_2R_1
- two hydrogen atoms: secondary amine NHR_1R_2
- three hydrogen atoms: tertiary amine $NR_1R_2R_3$)
- four hydrogen atoms: quaternary ammonium cation $N^+R_1R_2R_3R_4$

Important amines include amino acids, biogenic amines, trimethylamine, and the aromatic amine aniline. Note that compounds with the nitrogen atom next to a carbonyl of the structure $R_1(CO)NR_2R_3$ are called amides and have different chemical properties.
http://en.wikipedia.org/wiki/Amine

[65] Energetics of nitrogen fixation: The ATP cost of nitrogen fixation (apparently in symbiotic bacteria) from N_2 to ammonia is listed in the text as 16 ATP (p. 301 and Web Topic 12.2). However, on p. 289, the text appears to erroneously state that 16 ATPs are used to assimilate N_2 into an amino acid, thereby counting both the bacterial and plant pathways, an apparent inconsistency. I am also unclear whether these ATPs derive from the bacteria or the plant or both.
http://en.wikipedia.org/wiki/Nitrogen_fixation

[66] Biogeochemical nitrogen cycle:
- http://en.wikipedia.org/wiki/Nitrogen_cycle
- http://www.mcgoodwin.net/pages/geobiologyess313.pdf

The atmosphere is 78% N_2 by volume, but this is not usable by organisms until "fixed". **Biological Nitrogen Fixation** is the key entry point for N into the biochemical part of the Nitrogen cycle (fig. 12.1). Biological N fixation accounts for 90% of natural N fixation (by cyanobacteria, bacteria symbiotic in legumes, and other **diazotrophic** prokaryotes). The abiotic atmospheric fixation of N_2 is responsible for the remaining 10% of natural N fixation (8% by lightning, 2% by photochemical reactions).[TZ291] Overall, the rate of natural N fixation is 190 x 10^{12} g yr^{-1}. (An additional 85 x 10^{12} g yr^{-1} of N fixation results from industrial synthesis of ammonia via the Haber-Bosch reaction, etc.)

Biological nitrogen fixation is performed in part by free living prokaryotes in the soil, and these may enrich the nitrogen content of the soil for plants. Some plants such as grasses may have loose symbiotic relationships, in which the organisms colonize the plant tissues (e.g., *Acetobacter* in sugarcane) or adhere to the root surfaces (especially in the elongation zone and root hairs).

Some symbiotic bacteria fix nitrogen in plant **root nodules** in exchange for receiving nutrients and CHO. Known bacterial/plant symbioses include:

- **Rhizobia**: **Legume plants (Leguminosae** or **Fabaceae**, such as pea, bean, soybean, alfalfa, *Sesbania*, Clover, and *Aeschenomene/Aeschynomene*) form symbioses in root nodules with rhizobia (soil bacteria such as *Azorhizobium*, *Bradyrhizobium*, *Photorhizobium*, *Rhizobium*, etc.) Rhizobia also form symbioses with the non-leguminous plant *Parasponia* (in root nodules), and are also found free-living in the soil.
- ***Frankia***: **Actinorhizal plants** such as alder, bayberry, ceanothus, and sweet fern form symbioses in root nodules with *Frankia*[67] (filamentous **actinomycete** bacteria). These plants tend to be pioneers on nitrogen-poor soils.
- **Cyanobacteria**: ***Gunnera***[68] (herbaceous flowering plants) form symbioses with the cyanobacterium *Nostoc punctiforme* (which infects specialized gland organs at the bases of the leaf petioles); and ***Azolla***[69] (water ferns, etc.) form symbioses with the cyanobacterium *Anabaena azollae*.
- ***Acetobacter:*** **Sugarcane** forms a symbiosis with *Acetobacter*

(See tables 12.2 and 12.3 for a listing of symbiotic and free-living N-fixing organisms.)

Nitrogen fixation requires anaerobic conditions to prevent inactivation of **nitrogenase**. Nitrogen fixing organisms create or live in locally anaerobic conditions (or least microaerobic conditions supporting some degree of respiration). Thick-walled **heterocysts**, which are specialized cells found in cyanobacteria, lack PSII and do not generate oxygen. Cyanobacteria, with and without heterocysts, are important for maintaining adequate fixed N in the soil of often flooded rice fields, including the Azolla-Anabaena symbiosis.

Legumes and actinorhizal plants have **root nodules** induced by symbiotic nitrogen-fixing bacteria. These nodules contain the pink heme protein **leghemoglobin**, which has a high affinity for oxygen but is thought now to function primarily in transporting oxygen to the respiring symbiotic bacteria, rather than to buffer nodule oxygen.

The means by which the **host** legume and the **symbiont** rhizobia communicate and form symbiotic nodules is complex[Z300-301] and makes use of Nodulin (Nod) plant genes and nodulation (nod) genes in the rhizobia, some of the latter of which are host specific (details omitted). Chemotactic compounds are used by the plant to attract these organisms—isoflavonoids and betaines secreted by the roots. The bacterial Nod factors act as signals for symbiosis… Nodule formation involves:

- Phytohormones including ethylene
- Formation of the **infection thread** starting from the root hair
- Release of rhizobia into the apoplast
- Incorporation of altered rhizobia into **endosymbiotic organelles** (intracellular vesicles called **bacteroids**)
- Development of a vascular connection and a layer of cells that exclude O_2 from the nodule, etc…

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[67] Frankia and Actinorhizal plants:
• http://web.uconn.edu/mcbstaff/benson/Frankia/FrankiaHome.htm
• http://web.uconn.edu/mcbstaff/benson/Frankia/PlantBiology.htm

[68] *Gunnera* symbiosis with cyanobacteria:
• http://en.wikipedia.org/wiki/Gunnera
• http://www.blackwell-synergy.com/doi/pdf/10.1111/j.1469-8137.1992.tb00067.x?cookieSet=1

[69] *Azolla*: http://en.wikipedia.org/wiki/Azolla

(Details omitted, see fig. 12.12 and also Web Topic 12.1, which includes good diagrams and photomicrographs.)

The **Nitrogenase enzyme complex** fixes N_2 to ammonia in the following overall reaction (see TZ p. 301-2 and web topic 12.2 for details):

$$N_2 + 8e^- + 8\ H^+ + 16\ ATP \rightarrow\ 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i$$

This complex employs two components, the Fe protein and the MoFe protein. Nitrogenase is capable of catalyzing the reduction of other substances, including N_2O (nitrous oxide to N_2 and H_2O), N_3^- (azide to N_2 and NH_3), C_2H_2 (acetylene to ethylene), and H^+ (to H_2), and it also catalyzes the hydrolysis of ATP (to ADP). (Table 12.4, fig. 12.13)

Energy and Carbon Consumed: Nitrogen fixation energetics are complex and confusing. See above regarding number of ATPs consumed. Although the conversion of N_2 plus H_2 to NH_3 is exergonic ($\Delta G < 0$), the industrial production of NH_3 is endergonic. Moreover, "the enzymatic reduction of N_2 by nitrogenase also requires a large investment of energy … although the exact changes in free energy are not yet known."[TZ303] The overall reaction shown in the equation above (which is text equation 12.9) leads to a $\Delta G^{0'}$ of about -200 kJ mol^{-1} and is therefore on paper exergonic—a seeming contradiction. Some of the energy supplied however is wasted in the reduction of H^+ to H_2, which is lost… (details omitted) A plant uses 25% of the energy it expends in the shoots and roots simply in assimilating nitrogen from nitrate to ammonium[TZ310]—even though this assimilated nitrogen ends up being less than 2% of the dry plant weight. The reaction rate is slow—approximately 5 N_2 are fixed per second per nitrogenase complex. Plants also consume 12 gram of organic carbon per gram of N_2 fixed.

Conversion of ammonia/ammonium to less toxic products for transport: The ammonia or ammonium produced in root nodules is converted to less toxic forms for transport in the xylem:[TZ303]

- **amides**: especially the amino acids **glutamine** (from ammonium + glutamate) and **asparagine** (from ammonium + glutamine and aspartate) (fig. 12.7)
- **ureides**: **allantoin** synthesized in peroxisomes from uric acid, **allantoic acid** synthesized from allantoin in the ER, and **citrulline** synthesized from ornithine. In the shoot, these 3 ureides are catabolized to ammonium.

Toxicity of ammonium and nitrate

Ammonium at high levels in tissues are toxic to both plants and animals because it can dissipate transmembrane proton gradients (fig. 12.2). Animals have a strong aversion to its smell. Plants reduce intracellular toxicity by storing excess ammonium in the vacuole.

Nitrate can be stored at high levels in plants without injury. However, its concentration should be limited in animal and human food plants. If humans or animals such as livestock eat plants containing high levels of nitrate, they can experience **methemoglobinemia** following conversion of nitrate to nitrite. In addition, nitrate may be converted in animals to **nitrosamines**, which are potential carcinogens having the general formula $R_1N(-R_2)-N=O$).[70]

Nitrate assimilation

Absorption of nitrate at the roots requires initial conversion to nitrite NO_2^-, a cytosol reaction catalyzed by **nitrate reductase** and requiring NADH or NADPH. Nitrate reductase is the main **molybdenum**-containing protein in plant tissues, and Mo deficiency can lead to accumulation of nitrate… (further details omitted, see also figs 12.3, 12.4).

Nitrite reductase[71] reduces nitrite NO_2^- (which is highly reactive and potentially toxic) to ammonium. This is a redox reaction which takes place in chloroplasts or root plastids, and which utilizes reduced ferredoxin from the PS electron transport chain (see fig. 12.5 and chapter 7, further details omitted here). A small percentage of the nitrite reduced (0.02 - 0.2%) is converted to nitrous oxide N_2O by this reaction.[TZ293]

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[70] Nitrosamines: http://en.wikipedia.org/wiki/Nitrosamine

[71] Nitrate reductase: http://www.expasy.org/cgi-bin/nicezyme.pl?1.7.2.2

Plants exposed to high levels of nitrates at their roots translocate nitrate or its products to the shoot. They exhibit varying ratios in the xylem sap of nitrate, amino acids, amides, and (in tropical legumes) ureides (fig. 12.6), and therefore require varying proportions of nitrate reductase in the shoot versus root tissues (details omitted).

Ammonium assimilation

Ammonium toxicity is avoided by rapid conversion to amino acids—these reactions take place in the cytosol, root plastids, or chloroplasts. Some of the reactions involving ammonium and amino acid synthesis include (see fig. 12.7, details omitted):

- Ammonium combines with Glutamate (glutamic acid, which has 1 N atom) to form Glutamine (which has 2 N atoms).[72] This reaction requires the enzyme glutamine synthetase (GS), hydrolysis of ATP, and a divalent cation such as Mg^{2+}, Mn^{2+}, or Co^{2+}. This is the first of two reactions that assimilate ammonium.
- Ammonium combines with 2-Oxoglutarate to form Glutamate via glutamate dehydrogenase. This is the second of two reactions that assimilate ammonium, and requires oxidation of NADH or NADPH
- Elevated glutamine stimulates glutamate synthase activity, which converts Glutamine plus 2-Oxoglutatate to 2 Glutamates.
- Glutamate combines with Oxaloacetate to yield Aspartate and 2-Oxoglutarate.
- Glutamine combines with Aspartate to form Asparagine and Glutamate (a transamination reaction requiring asparagine synthase and ATP).

The remaining amino acids are synthesized by transamination reactions catalyzed by **aminotransferases**, such as aspartate aminotransferase… (fig. 12.8)

The amino acid **Asparagine** serves as a stable compound with, like glutamine, a relatively high-N content, and is used to transport and store N, as well as to serve as a protein precursor (details omitted)

Amino acid biosynthesis

Unlike humans, plants can synthesize all 20 standard amino acids found commonly in humans. (Those which cannot be synthesized in humans are considered"essential", and others are "conditionally essential" in certain patients).[TZ296,73] The amino NH_2 group is added by transamination (from glutamine or glutamate) to a carbon skeleton derived from 3-Phosphoglycerate, Phosphoenolpyruvate, Pyruvate, Oxaloacetate, and/or α-Ketoglutarate (see fig. 12.8). Some of these pathways are not found in humans, and herbicides can be correspondingly and hopefully safely targeted—e.g., **Roundup™**. (The mode of action of **Glyphosate** is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which is involved in

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[72] Glutamine and Glutamic acid:
• http://en.wikipedia.org/wiki/Glutamine
• http://en.wikipedia.org/wiki/Glutamate

[73] Essential amino acids for humans:
The following 9 cannot be synthesized by humans and are considered essential: **histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine**.
The following 5 amino acids are considered conditionally essential, meaning they are not normally required in the diet of adults, but must be supplied exogenously to specific populations that do not synthesize it in adequate amounts:
• **arginine** (synthesizable in adults but not the young and possibly seniors)
• **cysteine** (usually synthesizable from methionine if sufficient methionine present)
• **glycine** [?]
• **glutamine** [?]
• **tyrosine** (PKU patients must limit their intake of phenylalanine, but phenylalanine is the precursor for tyrosine synthesis, so for PKU patients tyrosine becomes essential).
See also http://en.wikipedia.org/wiki/Essential_amino_acid

the shikimic acid pathway for the synthesis of the aromatic amino acids tyrosine, tryptophan, and phenylalanine, as well as other phenolics).[74]

Sulfur Assimilation

Sulfur is a versatile element (due in part to multiple oxidation states) which is widely found in plants: in the AAs cysteine and methionine in proteins and peptides, in forming disulfide bridges, in electron transport, in enzyme catalytic sites, and in various secondary metabolites, including

- Rhizobial Nod factors
- **Alliin**, an antioxidant and antimicrobial sulfoxide derived from cysteine, found in garlic, onions, shallots, and other ***Allium***. This is converted via Alliinase to **Allicin** on chopping, etc.—it is allicin that produces the odoriferous and hot burning flavor of fresh garlic and causes tear production on chopping onions.[75]
- **Sulforaphane**, an anticancer and antimicrobial compound found in cruciferous[76] vegetables such as broccoli.

Most plant sulfur derives from sulfate (which is derived from rock weathering, atmospheric pollution, acid rain, etc.), and which is absorbed by an H^+-SO_4^{2-} symporter.

Assimilation of **sulfate SO_4^{2-}** requires its reduction and incorporation into cysteine (see TZ305 and fig. 12.15), consuming about 14 ATPs and reducing the oxidation number of the S from +6 to -4. This process forms the intermediate product **APS** (**adenosine-5'-phosphosulfate**), and can lead to an (unnamed) O-Sulfated metabolite or to S-Sulfoglutathione, then Sulfite (SO_3^{2-}), then Sulfide (S^{2-}), and finally (by combining with O-Acetylserine from Serine) to **Cysteine**. (details omitted) Sulfate assimilation occurs mostly in leaves. Leaves provide the needed ferredoxin (from PS) and serine, etc. Assimilated sulfur is exported in the phloem for protein synthesis, mainly as **glutathione**. The latter also serves as a signal to coordinate sulfate absorption by the roots and sulfate assimilation by the shoot. **Methionine** is synthesized in the plastids from cysteine (Web Topic 12.3). These two amino acids, cysteine and methionine, are incorporated into proteins, and S also appears in other compounds such as acetyl-CoA and S-adenosylmethionine.

Phosphate Assimilation

Phosphate ion and phosphoric acid exist in four forms in dilute aqueous solutions:[77]

- In strongly-basic conditions, the **phosphate ion $(PO_4)^{3-}$** predominates.
- In weakly-basic conditions, the **hydrogen phosphate ion $(HPO_4)^{2-}$** predominates.
- In weakly-acid conditions, the **dihydrogen phosphate ion $(H_2PO_4)^-$** predominates.
- In strongly-acid conditions, **aqueous unionized phosphoric acid $(H_3PO_4)^0$** predominates.

In soil, the $(HPO_4)^{2-}$ ion is absorbed by the H^+-$(HPO_4)^{2-}$ symporter, and is incorporated into many molecules including sugar phosphates, phopholipids, nucleotides, and nucleic acids.

The main entry point for assimilation of phosphorus is incorporation of phosphate to form ATP (chap. 11), which is produced by

- "**substrate-level phosphorylations**" of ADP to ATP during **glycolysis** (and possibly other phosphorylation reactions) in the cytosol (chapter 11, fig. 11.3)

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[74] Glyphosphate (Roundup™): http://en.wikipedia.org/wiki/Roundup

[75] Allicin: http://en.wikipedia.org/wiki/Allicin

[76] Cruciferous etymology:
"Brassicaceae or Cruciferae, also known as the crucifers, the mustard family or cabbage family is a family ... of flowering plants (Angiospermae). The name Brassicaceae is derived from the included genus Brassica. Cruciferae is an older name. It means "cross-bearing", because the four petals of their flowers are reminiscent of a cross... [The flowers] have four free saccate sepals and four clawed free petals, staggered. They can be disymmetric or slightly zygomorphic, with a typical cross-like arrangement (hence the name 'Cruciferae')."

[77] Phosphate ion: http://en.wikipedia.org/wiki/Phosphate

- oxidation of NADH coupled with **oxidative phosphorylation** of ADP to ATP in the mitochondria (chapter 11, fig. 11.8), and
- light-dependent phosphorylation (**photophosphorylation**) of ADP to ATP in the chloroplasts (chapter 7, fig. 7.22)

Metal Cation Assimilation

Unlike N, S and P, elemental **metallic cations** typically form complexes with organic compounds by means of noncovalent rather than covalent bonding. The macronutrient cations include K, Mg, and Ca, and micronutrient cations include Cu, Fe, Mn, Co, Na, and Zn.

These cations form noncovalent bonds with carbon compounds as follows:
- **Coordination bonds**: Here several O or N atoms of a carbon compound donate unshared electrons to form a bond with the cation, neutralizing the positive charge of the cation. Cations that do this include Cu, Zn, Fe, and Mg. Examples include Copper-Tartaric acid complex, Chlorophyll a (containing Mg), and Polygalacturonic acid (containing Ca [is this electrostatic or coordination?]).
- **Electrostatic bonds**: Here a positively charged cation is attracted to negatively charged groups such as carboxylate (—COO^-), but unlike in coordination bonds, the positive cation retains its positive charge. Examples include K^+ electrostatically bonded with various organic acids, and calcium pectate (fig. 12.17).

Cations such as magnesium and calcium are assimilated by the formation of both coordination complexes and electrostatic bonds… (details omitted).

Roots modify the rhizosphere to acquire iron (details omitted). At neutral pH, most of soil iron is in **ferric** form (Fe^{3+}) as the iron oxides $Fe(OH)^{2+}$, $Fe(OH)_3^0$,and $Fe(OH)_4^-$, and these are highly insoluble. Plants improve the absorbability of iron in the root zone by:
- acidifying the soil by secreting protons, malic and citric acid
- reducing ferric Fe^{3+} to ferrous Fe^{2+} by secretion of iron-chelating reductase
- secreting compounds (**chelators**) that form stable chelation complexes with iron. In dicots, these include malic acid, citric acid, phenolics, and piscidic acid. In iron-deficient grasses (monocots) including barley, maize, and oats, secreted **siderophores** (consisting of non-protein-forming AAs) form stable chelates with Fe^{3+}, and these are imported using special Fe^{3+}-siderophore transport systems (fig. 12.18B).

Absorbed iron is transported to the leaves in the ferric form complexed to citrate. In the leaves, Fe as ferrous Fe^{2+} is inserted into the **porphyrin** ring to form the coordination complex **heme**[78] (fig. 12.19), a reaction catalyzed by ferrochelatase. Porphyrin[79] is a large heterocyclic highly aromatic pigmented molecule. Heme binds to proteins to form hemoproteins which, in plants or animals, include hemoglobin, myoglobin, leghemoglobin, peroxidases, myeloperoxidase, cytochrome c oxidase, ligninases, cytochromes, cytochrome c, etc. Most plant iron is found in heme, though it also occurs in "Fe_2S_2 centers"[80] (often in iron-sulfur proteins) combining cysteine, S, and Fe (details omitted, see references). Plants often use iron as a redox component in the active site of enzymes. Surplus free iron can be damaging to the plant, and is stored in the spherical hollow container molecule **phytoferritin**. "Interest in phytoferritin is high because iron in this protein-bound form may be highly available to humans, and foods rich in phytoferritin such as soybean may address [iron-deficiency anemia].[TZ308]

Oxygen Assimilation

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[78] Heme: http://en.wikipedia.org/wiki/Heme

[79] Porphyrin: http://en.wikipedia.org/wiki/Porphyrin

[80] Fe_2S_2 centers:
"The simplest polymetallic system, [Fe_2S_2] cluster, is constituted by two iron ions bridged by two sulfide ions and coordinated by four cysteinyl ligands (in Fe_2S_2 ferredoxins) or by two cysteines and two histidines (in Rieske proteins)."
http://en.wikipedia.org/wiki/Iron-sulfur_protein
See also http://metallo.scripps.edu/PROMISE/2FE2S.html#enzyme

Oxygen assimilation takes place in plants as follows:

- Mitochondrial respiration (chapter 11), which accounts for 90% of plant oxygen assimilation.
- Rubisco-catalyzed carboxylation in photosynthesis, assimilating CO_2 plus water (chapter 8)
- Rubisco-catalyzed photorespiration, assimilating O_2 plus water (chapter 8)
- Other forms of **Oxygen fixation**, in which oxygen is added directly to an organic molecule by oxygenases. These reactions are catalyzed by **dioxygenases** and **monooxygenases** (fig. 12.20), and include
  - the oxidation of fatty acids (by the dioxygenase lipoxygenase)
  - the conversion of proline (already bound to a polypeptide) to hydroxyproline (by the dioxygenase Prolyl hydroxylase)
  - the oxidation of Cinnamic acid (using O_2 and NAD(P)H as an electron donor) to p-Coumaric acid by the monooxygenase heme protein Cytochrome P450[81] (details omitted):

    Cinnamic acid + O_2 + NADPH + $2H^+$ → p-Coumaric acid + H_2O + $NADP^+$

    Monooxygenases are often termed "**mixed-function oxidases**"[82] in which one atom of oxygen is inserted into an organic substrate (RH) while the other oxygen atom is reduced to water:

    $RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O$

    These reactions are located on the ER and are important in oxidizing a variety of substrates. (They are also important in humans, especially in metabolizing certain drugs.)

Energetics Of Nutrient Assimilation And Impact Of Rising CO_2

"Nutrient assimilation requires large amounts of energy to convert stable, low-energy, inorganic compounds into high-energy organic compounds."[TZ311] A plant uses 25% of the energy expended in the shoots and roots in reducing nitrate to nitrite and then to ammonium—yet this assimilated nitrogen ends up being less than 2% of the dry plant weight.[TZ310] These steps require overall approximately 13 ATPs per extracellular nitrate ion converted to intracellular glutamate within the leaf mesophyll cell (fig. 12.21).

Many assimilatory reactions occur in the stroma of the chloroplasts of leaves, and make use of the strong reducing agents available from PS electron transport (NADPH, thioredoxin, and ferredoxin, etc.) This kind of **coupled assimilation** is therefore called **photoassimilation**, and proceeds only under conditions when reductants are in excess of PS needs, namely high light and low ambient CO_2. As atmospheric CO_2 rises, there may be important deleterious effects on the assimilation of plant nutrients, at least on nitrate photoassimilation in the leaves (fig. 12.22). Arnold J. Bloom concludes: "Our results suggest that rising atmospheric CO_2 will favor taxa that prefer NH_4^+ as a nitrogen source or assimilate NO_3^- primarily in their roots. Clearly, a broad survey of plant species under controlled levels of NH_4^+ and NO_3^- is warranted to determine whether CO_2 inhibition of NO_3^- photoassimilation is a general phenomenon." (Web Essay 12.1)

Chapter XIII. Secondary Metabolites And Plant Defenses

[Chapter not specifically studied in UW Plant Physiology Biol. 425]

This chapter deals with hydrophobic compounds such as waxes, cutins, and suberin. These contribute to the formation of the Casparian strip, cuticle, and other water barrier structures.

In addition, it deals with compounds termed secondary metabolites that have no direct function in growth, development, and reproduction of plants. Some of their uses include:

- defenses against pathogens (including bacteria, fungi, viruses, and nematodes) and herbivorous predators (such as arthropods and other animals) by means of barrier substances, toxins, and other means of defense
- pigments (such as anthocyanins)
- structural supports (such as lignin)

Many of the secondary metabolites have real or purported commercial or other uses as insecticides; fungicides; pharmaceuticals, herbs, medicinals, dietary supplements and "nutraceuticals"; flavorings and

[81] Cytochrome P450: http://en.wikipedia.org/wiki/Cytochrome_P450
[82] Monoxygenases: http://en.wikipedia.org/wiki/Mixed_function_oxidase

scents; and industrial materials. Some are used as extracted products, while some are useful when genetically increased, decreased, or redistributed in crop plants, in order to enhance yields, diminish applied insecticides, improve edibility, etc.

Waxes, Cutins, and Suberin

Plants use these substances to create hydrophobic barriers, to limit water loss from transpiration, to divert water absorbed by roots into the symplast, and (at least to a modest degree) to help deter invaders. Plants native to arid habitats have thicker cuticles, but other plants subjected to dry conditions also increase their cuticle thickness.[83] Some pathogenic fungi penetrate the cuticle mechanically, or secrete **cutinases** which hydrolyze cutin and assist them in penetrating the cuticle.

The outermost layers of a leaf include (from inside out, fig. 13.2):

- the **cell wall** and middle lamella of the epidermis
- the **cuticular layer** (cutin, wax, and CHO polymers such as pectin, cellulose, etc.)
- the **cuticle** proper (cutin and wax), and
- the outermost layer of **surface wax**

Waxes[84] are complex mixtures of (mostly) nonpolymeric long-chain (25-35 carbon) hydrophobic lipids, usually consisting of straight aliphatic alkanes and alcohols (primary and secondary) but also aldehydes, ketones, esters, and free fatty acids. They are found on the outer surface of leaves, in the cuticle mixed with cutin, and in the cuticular layer, and are also found with suberin. Waxes in the cuticle are secreted as droplets from the epidermal cells. The surface layer is often crystallized in the form of small rods, tubes, or plates of wax (fig. 13.3), which enhance the repellency of water and the self-cleansing properties of the surface (the so-called **Lotus effect**[85] or **superhydrophobicity**, in which the contact angles formed with water on leaves of *Lotus japonicus* or taro measure up to 170°).

Cutin consists of polymeric macromolecules—many waxy long-chain fatty acid polyester polymers.[86] Cutin covers the aerial surfaces of most plants in the cuticular and cuticle layers. **Cutan** joins in this barrier role, and consists of non-saponifiable polymers of long chain hydrocarbons.[87]

Suberin is found in roots and other underground parts, as well as woody stems, sites of leaf abscission, and healed wounds. In roots, it forms the Casparian strip (Casparian band) of the endodermis, which forces water entering the stele of a root into the symplast (see chapters 4 and 5). Suberin is a polymer of uncertain structure, formed from long chain components including **dicarboxylic acids** and **phenolic compounds**, using ester linkages, and is often mixed with waxes. It is a main constituent of the **cork** (**phellem**, the outermost layer of the **bark** or **periderm**). It is named after the Cork Oak, *Quercus suber*.[88]

Secondary Metabolites Defined

Secondary metabolites, also known as **secondary products** or **natural products**, are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms, and are not directly involved with "photosynthesis, respiration, solute transport, translocation, protein synthesis, nutrient assimilation, or differentiation, or the formation of carbohydrates, proteins, and lipids..."[TZ318] "Unlike primary metabolites, absence of secondary metabolites results not in immediate death, but in long-term impairment of the organism's survivability/fecundity or aesthetics, or perhaps in no significant

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[83] Plant cuticle: http://en.wikipedia.org/wiki/Plant_cuticle
[84] Wax: http://en.wikipedia.org/wiki/Wax
[85] Lotus effect: http://en.wikipedia.org/wiki/Lotus_effect
[86] Cutin: "There are two major monomer families of cutin, the C16 and C18 families. The C16 family consists mainly of 16-hydroxypalmitate and 9,16 or 10,16-dihydroxypalmitate [16:0]. The C18 family consists mainly of 18-hydroxyoleate [18:1], 9,10-epoxy-18-hydroxystearate, and 9,10,18-trihydroxystearate."
http://en.wikipedia.org/wiki/Cutin
[87] Cutan: http://en.wikipedia.org/wiki/Cutan
[88] Suberin: http://en.wikipedia.org/wiki/Suberin

change at all."[89] Secondary metabolites are often restricted to a single species or a narrow set of species within a group, whereas primary metabolites are typically found throughout the plant kingdom.

In most cases, secondary metabolites have been found not to be simply metabolic waste products, but instead to play important roles in plant defenses against pathogens or herbivores, and in other aspects of plant ecology. These ecological roles include:

- **Protecting against animal herbivory** (being eaten by herbivores including insects) and **infection** (by microbes)
- **Aiding pollinators and seed-dispersing animals** by serving as **attractants** in smell, color, or taste.
- **Aiding in plant-plant competition** (including **allelopathy**) and in **plant-microbe symbioses**

These compounds can have a deleterious or beneficial effects in plants intended for human consumption.

The main groups are terpenes, phenolics, and nitrogen-containing compounds.

Allelopathy Defined

Plants synthesizing phenolics (including caffeic acid and ferulic acid, see below) and other secondary metabolites discussed in this chapter may impart these to the soil in the form of fallen leaves, decaying litter, and root secretions. The inhibiting effects of these substances on germination or growth etc. of neighboring plants is termed **allelopathy**,[90] and can lead to better fitness through increased access to light, water, and nutrients. The study of allelopathy in agriculture is important with respect to maximizing crop yields. However, it is difficult to unravel the precise ecological function of most "secondary" metabolites, though this designation often implies an ecological role (Web Essay 13.2). In the introduced invasive weed Spotted knapweed (*Centaurea maculosa*), it is a polyphenolic tannin, **catechin** (see below), that serves as its primary allelopathic root exudate (Web essay 13.7).

Terpenes, Terpenoids and Isoprenoids

Terpenes (also called **terpenoids or isoprenoids**) are derived from one or more branched 5-carbon (C_5) isoprene units (actually either **isopentane** or **isoprene** units).

Classification and biosynthesis

Terpenes or terpenoids are classified by the number of isoprene units that are combined:

- 2 isoprene units (C_{10} terpenes) are termed **monoterpenes**
- 3 isoprene units (C_{15} terpenes) are termed **sesquiterpenes**
- 4 isoprene units (C_{20} terpenes) are termed **diterpenes**
- 6 isoprene units (C_{30} terpenes) are termed **triterpenes**
- 8 isoprene units (C_{40} terpenes) are termed **tetraterpenes**
- > 8 isoprene units (> C_{40} terpenes) are termed **polyterpenoids**

Terpenes are the major components of conifer resin, and of turpentine produced from resin. The name "terpene" is derived from the word "turpentine" (which derived from terebinthine = pertaining to the terebinth tree). Terpenes[91] or terpenoids are biosynthesized by either of two pathways leading to the common C_{10} intermediary **Geranyl diphosphate** (**GPP**, fig. 13.4 and 13.5):

- **The Mevalonic acid pathway:** starting with Acetyl-CoA (C_2), leading to C_5 **Isopentenyl diphosphate** (**IPP**), then **GPP**.
- **The Methylerythritol phosphate (MEP) pathway**: starting with pyruvate (C_3) and Glyceraldehyde 3-phosphate (C_3), leading to **Methylerythritol phosphate** (C_5), then **Dimethylallyl diphosphate** (**DMAPP**, C_5), then **GPP**.

[89] Secondary metabolite: http://en.wikipedia.org/wiki/Secondary_metabolite
[90] Allelopathy: http://en.wikipedia.org/wiki/Allelopathy
[91] Terpenes: http://en.wikipedia.org/wiki/Terpene

GPP can then be converted to

- **Monoterpenes** (C_{10} compounds such as **geraniol**, **limonene**, **terpineol**, **alpha-pinene** and **beta-pinene**), or to
- **Farnesyl diphosphate** (**FPP**, C_{15}), which can be converted to
  - **Sesquiterpenes** (C_{15} compounds such as **farnesol**), or dimerized to
  - **Triterpenes** (C_{30} compounds such as **Squalene**,[92] which is the precursor of the sterols **lanosterol** and **cycloartenol**, which are the precursors for all plant and animal steroids),[93] or to
- **Geranylgeranyl diphosphate** (**GGPP**, C_{20}), which can be converted to
  - **Diterpenes** (C_{20} including **cafestol, kahweol, cembrene, and taxadiene**, and the derived C_{21} terpenophenolic compound **Tetrahydrocannabinol** from hemp *Cannabis sativa*), or dimerized to
  - **Tetraterpenes** (C_{40} including **lycopene**, **gamma-carotene**, and **alpha-** and **beta-carotenes**), or to
  - **Polyterpenoids** (> C_{40})

Terpene-derived compounds that are primary metabolites

Some terpenoids are considered to be **primary metabolites** as they participate in plant growth and development, etc., and are discussed in other chapters. These include:

- **Gibberellins** (C_{20}, chapter 20)
- **Brassinosteroids** (C_{28} brassinolide, derived from C_{30} triterpene squalene, chapter 24)
- **Sterols** (C_{27}-C_{30}, used to stabilize phospholipid membranes, chapter 11)
- **Carotenoids** (C_{40},) including **xanthophylls** and **carotenes** (used in PS etc., chapter 7)
- **Tocopherols** (including RRR-alpha-tocopherol AKA d-alpha-tocopherol AKA vitamin E,[94] derived from GGPP, Web essay 13.1)
- **Phytol** side chain of Chlorophyll (C_{20}, chapter 7)
- **Abscisic acid** (C_{15}, chapter 25)
- **Dolichols** (polyterpenoid alcohols which serve as carriers and anchors for sugars in cell wall and glycoprotein synthesis (chapter 15)

Terpenes that protect against herbivores

Some terpenes serve as toxins to deter herbivorous animals including insects:

Pyrethrin I and II[95] are natural insecticidal monoterpene ester compounds deriving from **Pyrethrum**, the extract of several Old World plants of the genus *Chrysanthemum* (e.g., *C. coccineum* and *C. cinerariifolium*). **Pyrethroids** are synthetic analogs, and include permethrin. These have relatively low human toxicity.

Conifers such as pines, firs, and spruce accumulate monoterpenes (such as bicyclic **α-pinene** and **β-pinene**)[96] in **resin**[97] stored in **resin ducts** or **blisters** in the needles, twigs, and trunk. These terpenes are toxic to many but not all bark beetles. Resin ducts occur normally, or can be induced by the presence of pathogens such as fungi, and in some conifers the mobilized resin contain "a mixture of monoterpenes, sesquiterpenes, and diterpene resin acids".[98]

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[92] Squalene: http://en.wikipedia.org/wiki/Squalene
[93] Steroids: http://en.wikipedia.org/wiki/Steroid
[94] Tocopherol: http://en.wikipedia.org/wiki/Tocopherol
[95] Pyrethrin: http://en.wikipedia.org/wiki/Pyrethrin
[96] Pinene: http://en.wikipedia.org/wiki/Pinene
[97] Resin: "The resin produced by most plants is a viscous liquid, typically composed mainly of volatile fluid terpenes, with lesser components of dissolved non-volatile solids which make resin thick and sticky. The most common terpenes in resin are the bicyclic terpenes alpha-pinene, beta-pinene, delta-3 carene and sabinene, the monocyclic terpenes limonene and terpinolene, and smaller amounts of the tricyclic sesquiterpenes longifolene, caryophyllene and delta-cadinene. Some resins also contain a high proportion of resin acids. "
http://en.wikipedia.org/wiki/Resin
[98] Wound-induced traumatic resin duct development in stems of Norway spruce (Pinaceae):
http://www.amjbot.org/cgi/content/full/87/3/302?ck=nck

Cannabinoids: The psychoactive (hallucinogenic) cannabinoids include the C_{21} terpenophenolic compound **Tetrahydrocannabinol (THC)**,[99] the most active cannabinoid found in the marijuana hemp plant (*Cannabis sativa*). Others common cannabinoids in *Cannabis* include **cannabidiol** (CBD) and **cannabinol** (CBN).

Limonoids: according to the textbook are C_{30} triterpenes. [Other sources state that limonoids[100] are **tetranortriterpenes**, a term which I have not found defined. The limonoid **Limonin** has 26 carbons.] These impart the bitter taste and characteristic scent of citrus (lemon, orange, etc.) fruit peel or rind. The **Neem tree (***Azadirachta indica***)** is rich in the limonoid-related C_{35} tetranortriterpenoid **azadirachtin**.[101] This compound is a powerful deterrent to insect feeding in low concentrations, and appears to have low human toxicity.

Phytoecdysones: The triterpenoids known as **phytoecdysones** have the same basic structure as hormones (**ecdysteroids** such as **ecdysone**) that are used by insects in the **molting** of the cuticle, a process known as **ecdysis**. When insects ingest these chemicals (which are found for instance in the common fern *Polypodium vulgare*), they may prematurely molt or suffer other lethal effects. Nematodes may also be affected.[TZ322]

Triterpene-derived compounds directed against vertebrate herbivores include:

- **Cardenolides**: These are glycosides found in foxglove (*Digitalis lanata*), milkweed, and oleander (*Nerium oleander*).[102] They are extremely cardiotoxic, but in low doses some are used therapeutically as "**cardiac glycosides**" (e.g., C_{41} **digoxin** or **digitoxin**).
- **Saponins**: These are found in plants such as Soapwort (*Saponaria officinalis*) and soapberry. The saponin **yamogenin** is found in yams of the genus *Dioscorea* (web topic 13.1). Many yams require detoxification processing before they can be eaten. (The tuber termed sweet potato in the U.S., *Ipomoea batatas*, is not a *Dioscorea* yam.) Saponins are steroid or triterpene glycosides which, because of detergent-like properties, can form complexes with sterols. They can therefore disrupt intestinal sterol uptake and interfere with cell membrane function, and are utilized by plants at least in part as antimicrobials (they disrupt fungal membranes by binding to sterols).[TZ338]

Terpenes that confer scents and flavors potentially useful to humans

In some plants, volatile monoterpenes and sesquiterpenes termed "**essential oils**" (when extracted) are present and confer a characteristic odor or aroma to the foliage.[103] These aromatic compounds are often presented to potential predators in concentrated external form in "**glandular hairs**", see fig. 13.7. (For uses of essential oils as food flavorings and scents, see Web essay 13.1.) However, plants also use monoterpenes and other compounds to provide attractive scents for pollinators. The large number of aromatic plants from which essential oils for human use are derived include:

Plant Source	Terpenes And Other Compounds Found In Essential Oil
Anise	C_{10} **trans-anethole** (not a terpene)
Basil	C_{10} **eugenol** and **estragole** (not terpenes), plus other aromatic compounds
Camphor laurel	C_{10} **camphor**
Cinnamon	C_9 **trans-cinnamaldehyde** (not a terpene), plus other aromatic compounds
Citronella grass	C_{10} **citronellal**, plus **(+)-Citronellol**, etc.
Cloves	C_{10} **eugenol** (not a terpene) plus other aromatic compounds

[99] Canninoids and marijuana:
• Cannabis: http://en.wikipedia.org/wiki/Cannabis
• Cannabinoids: http://en.wikipedia.org/wiki/Cannabinoids
• Tetrahydrocannabinol: http://en.wikipedia.org/wiki/Tetrahydrocannabinol
[100] Limonoids: http://en.wikipedia.org/wiki/Limonoid
[101] Azadirachtin: http://en.wikipedia.org/wiki/Azadirachtin
[102] Acute cardenolide (cardiac glycoside) poisoning: http://www.cochrane.org/reviews/en/ab005490.html
[103] Essential oils: "An essential oil is any concentrated, hydrophobic liquid containing volatile aroma compounds from plants, which are called aromatic herbs or aromatic plants. They are also known as volatile or **ethereal oils**, or simply as the 'oil of' the plant material from which they were extracted, such as **oil of clove**. An oil is 'essential' in the sense that it carries a distinctive scent, or **essence**, of the plant." http://en.wikipedia.org/wiki/Essential_oil

Eucalyptus	C_{10} **eucalyptol** AKA **1,8 cineole**
Fennel	C_{10} **trans-anethole** (not a terpene)
Ginger	C_{15} **zingiberene** and other terpenoids
Lemon	C_{10} **d-limonene**, the main odor constituent of citrus, plant family Rutaceae
Lemongrass	C_{10} **geranial** [AKA citral A] and **neral** [AKA citral B]
[many]	C_{10} **linalool** (widespread in floral scents and fruits including guava, peach, plum, pineapple, and passionfruit)
Peppermint	C_{10} **menthol**, along with C_{10} **menthone** and menthyl esters
Rose	C_{10} **geraniol** plus C_{10} **l-citronellol** (AKA **(–)-Citronellol**), etc.
Sage	C_{10} **cineole**, **borneol**, and **thujone** (all terpenes), etc.
Tarragon	C_{10} **estragole** (not a terpene)

Phenolics

Phenol consists of a benzene aromatic ring with an attached hydroxyl (-OH) group. **Phenolic compounds** are a heterogeneous group that have one or more such C_6 aromatic rings bound to hydroxyls. These vary substantially in size and water solubility.

Biosynthesis

Shikimic Acid pathway: Most plant phenolics are synthesized in the Shikimic Acid pathway, starting with the C_4 **Erythrose-4-phosphate** (from the Oxidative Pentose Phosphate Pathway) or C_3 **phosphoenolpyruvic acid** (**PEP**, from glycolysis) and leading to C_7 Shikimic acid.[104] From Shikimic acid, pathways continue (see fig. 13.9 and Web Topic 13.2):

- through Gallic acid to **hydrolyzable tannins**
- through Chorismic acid (which can also lead to **Tryptophan**), Prephenic acid, and Arogenic acid to the aromatic AAs **Phenylalanine** or **Tyrosine**, and to **trans-Cinnamic acid** and **Simple phenolics**.
- from **Simple phenolics** (including C_6-C_3 **Phenylpropanoids** such as **trans-Cinnamic acid** and **p-coumaric acid**, and C_6-C_1 compounds such as **benzoic acid**) to **lignin**, **flavonoids**, **condensed tannins**, and other **phenolics**

Malonic pathway: The malonic pathway, found in fungi and bacteria, is an alternative pathway for synthesizing phenolic compounds, but is of lesser importance in higher plants. (The shikimic acid pathway is not found in animals, and they therefore cannot synthesize the aromatic amino acids.)

The removal of ammonia from phenylalanine to yield trans-Cinnamic Acid plus NH_3 by the cleaving of the C-N bond is catalyzed by **phenylalanine ammonia-lyase (PAL)**. This enzyme is located at a branch point between primary metabolism (such as protein synthesis) and secondary metabolism (synthesis of phenolics and alkaloids, etc.) A plant subjected to stresses such as fungal infection increases its expression of PAL and therefore synthesizes relatively more phenolic secondary metabolites (details omitted).

Simple phenolics

Phenylalanine leads to simple phenolics such as

- **Phenylpropanoids** (C_6-C_3) such as **trans-Cinnamic acid** and derivatives such as **caffeic acid** and **ferulic acid** (fig. 13.11)
- **Coumarins** are C_6-C_3 Phenylpropanoid lactones or cyclic esters such as **coumarin** (benzopyrone) and **umbelliferone**. Coumarin is a "toxin found in many plants, notably in high concentration in the tonka bean, woodruff, mullein, and bison grass. It has a sweet scent, readily recognised as the scent of newly-mown hay... The name comes from a French word, coumarou, for the tonka bean."[105] **Warfarin** (Coumadin™) is a C_{19} synthetic derivative of coumarin, and was named for the Wisconsin Alumni

[104] Shikimic acid: named after the Japanese flower shikimi (*Illicium anisatum*) http://en.wikipedia.org/wiki/Shikimic_acid

[105] Coumarin: http://en.wikipedia.org/wiki/Coumarin

Research Foundation. It inhibits the vitamin K–dependent synthesis of biologically active forms of certain clotting factors.[106]

- **Psoralen**[107] is a plant **furanocoumarin** (a coumarin with an added furan ring) that is **phototoxic** to cells of insects and animals when activated by exposure to UV-A (320-400 nm) light (fig. 13.11). Psoralen (named after *Psoralea corylifolia*) is used in P-UVA treatment of psoriasis, etc. Psoralen and the closely related furanocoumarin **Angelicin** along with their methoxy derivatives occur in a number of plants belonging to the Umbelliferae (Apiaceae) family—caraway, carrot, celery, coriander, cumin, dill, fennel, giant hogweed, parsley, cow parsley, parsnip, cow parsnip, etc.—as well as Rutaceae such as lime, Bergamot orange, lemon, etc. along with plants of other genera. These compounds can cause a non-immunological photosensitization such as "string-trimmer" ("weed-eater") dermatitis.[108] Insects that ingest psoralens may be sensitized and have to adapt by avoiding UVA light.
- **Benzoic acid** derivatives: These are C_6-C_1 compounds formed from phenylpropanoids, and include **vanillin** and **salicylic acid**. (Benzoic acid was named after gum benzoin, also called styrax resin.)

Lignin

Lignin is a complex hydrophobic "phenolic" which is considered by the textbook to be a secondary metabolite. It is highly branched polymer of phenylpropanoid groups, with uncertain overall structure. It forms from 3 phenylpropanoid alcohols: **coniferyl**, **coumaryl**, and **sinapyl alcohols**, and contains many 3-dimensional linking C–C and C–O–C bonds. A hypothetical chemical structure for European beech lignin is shown in Web topic 13.3. Its important roles have already been discussed (Chapter 1 and 4), including providing structural strength and mechanical rigidity to stems and trunks and preventing collapse under negative pressure of xylem tracheary elements. These properties allow upward growth and improved competitive fitness, and made possible tall plant colonization on dry land. In addition, lignin improves the survivability of plants by making them tougher and more indigestible to herbivores, as well as more resistant to wounding and infection.[TZ325] It also contributes to wound healing.

Flavonoids

Flavonoids (AKA "**bioflavonoids**") are another major class of phenolics, derived from a combination of the shikimic acid and malonic acid pathways. These are C_6-C_3-C_6 compounds combining two aromatic C_6 rings connected by a C_3 bridge which may be cyclized into a benzopyrone ring. They often have glycoside substituents. In human use, they are noted for **antioxidant** properties and various potential health applications are under investigation. Their derivatives include:

- **Anthocyanins**: These are colored flavonoids that confer **pigmentation** that are used in flowers and fruits (along with carotenoids) to attract animals visually to promote pollination and fruit or seed dispersal through fruit ingestion. They may serve as accessory pigments to protect leaves from photoinhibition from excess light and UV radiation (chapter 7). They are responsible for most of the **red, pink, purple, and blue** colors seen in plants (whereas carotenoids including xanthophylls often confer orange, yellow, or red coloration.) They occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. They include **anthocyanin** (which is a glycoside, having an attached sugar or glycone), and the sugar-free (aglycone) **anthocyanidins**. The latter come with various substituents, confering colors such as orange red (**pelargonidin**), purplish red (**cyanidin**), bluish purple (**delphinidin**), rosy red (**peonidin**), and purple (**petunidin**, see Table 13.1). The intense blue of *Commelina communis* (dayflower) arises from a complex of multiple anthocyanins and other compounds. Anthocyanins are located in the vacuole, are strong antioxidants, and are found in high concentration in blueberry, blackberry, marionberry, black raspberry, raspberry, blackcurrant, chokeberry, cherry, eggplant, red grape, etc.[109]
- **Flavones and Flavonols**: These typically absorb wavelengths in the UV part of the spectrum producing a pattern in flowers which is not visible to humans but is visible to insects such as bees. Such patterns form "**nectar guides**" for insect pollinators (fig. 13.14). Flavones and flavonols in the

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[106] Warfarin: http://en.wikipedia.org/wiki/Warfarin

[107] Psoralen: "Psoralen occurs naturally in the seeds of [the legume] *Psoralea corylifolia*, as well as in the common fig, celery, parsley and West Indian satinwood." http://en.wikipedia.org/wiki/Psoralen

[108] Phytodermatitis from furanocoumarins: http://www.telemedicine.org/botanica/bot5.htm

[109] Anthocyanin: http://en.wikipedia.org/wiki/Anthocyanins

epidermis of leaves also serve as "**sunscreens**" to protect the leaves from excess UV-B (280-320 nm). Flavones and Flavonols (or isoflavones) are also secreted by legumes to help form the symbiosis with nitrogen-fixing rhizobia. Natural flavones include Apigenin, Luteolin, and Tangeritin;[110] natural flavonols include Quercetin and Myricetin.[111]

- **Isoflavones (Isoflavonoids)**: These have the phenyl group shifted to the middle of the bridging carbons (fig. 13.10). They are found mostly in Fabaceae (Leguminosae, legumes such as beans and soybeans) and are strong anti-oxidants. Some are phytoestrogens or are anti-estrogenic (e.g., they have been shown to cause reproductive failure in sheep and quails). Some serve as **phytoalexins** (antimicrobial compounds). **Rotenoids** are used as insecticides and fish poisons. Soybean isoflavonoids may have anti-cancer benefits but have also been linked to immune abnormalities.[112] Soybean isoflavones include genistein and daidzein.[113]

Tannins

Tannins: Like lignins, tannins[114] are plant phenolic compounds which serve a defensive role by reducing plant edibility. These are astringent (mouth puckering) bitter polyphenols that bind or precipitate proteins nonspecifically (including the digestive enzymes of herbivores), by means of either hydrogen bonding or covalent bonding of protein $-NH_2$ groups (fig. 13.16). They may also reduce the bioavailability of metal ions in herbivores by chelating them. Ingested tannins can decrease the digestibility of proteins, thereby reducing the nutritive value to herbivores of plants and plant parts, deterring herbivore feeding. (Some animals such as rodents and rabbits secrete **salivary proline-rich proteins** that improve tannin tolerance.) Tannins are mainly located in the vacuoles or surface wax of the plants. They are also found in the nonliving heartwood of conifers, where they may help to inhibit microbial activity. They are used in tanning to bind collagen in animal hides, thereby increasing the resistance to microbes and heat, etc. Tannins are found in many human foods and beverages: black tea, red wine (perhaps contributing to reputed cardiac health benefits), beer hops, unripe fruits, various ripe fruits including pomegranates, persimmons, cranberries, strawberries, blackberries, blueberries, apple, grapes, smoked foods, etc. Dietary tannins may have beneficial anti-oxidant properties. There are two categories (fig. 13.15):

- **Condensed tannins**: These consists of polymers of flavonoid units (such as epicatechin or **catechin**), and can be hydrolyzed to anthocyanidins (thus they are also termed **proanthocyanidins**). They are common constituents of woody plants, reducing their edibility.
- **Hydrolyzable tannins**: These are smaller, and may be hydrolyzed to a sugar plus phenolic acids: **gallic acid** (in **gallotannins**), or **ellagic acid** (in **ellagitannins**).

Nitrogen-Containing Secondary Metabolites

This is a large and heterogeneous grouping of secondary metabolites (thus excluding primary metabolites such as amino acids, proteins, nucleotides, various amides and amines, etc.). The term includes plant alkaloids, cyanogenic glycosides, glucosinolates, and non-proteinogenic amino acids. Although they are synthesized from common amino acids (and polyamines), many are toxic to animals.

Alkaloids

There are more than 15,000 natural plant alkaloids and they are found in 20% of vascular plant species. The N is usually found in a heterocyclic ring, but exceptions include **Capsaicinoids**[115] (from genus *Capsicum*) and natural **Phenethylamines** such as **mescaline** (from several Cactaceae including the **peyote** cactus *Lophophora williamsii*), **levodopa** and **dopamine** (from *Mucuna pruriens*), and **ephedrine**

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[110] Flavones: http://en.wikipedia.org/wiki/Flavones
[111] Flavonols: http://en.wikipedia.org/wiki/Flavonols
[112] Isoflavone induced immune abnormalities: http://www.pnas.org/cgi/reprint/99/11/7616
[113] Isoflavones: http://en.wikipedia.org/wiki/Isoflavones
[114] Tannins:
• http://en.wikipedia.org/wiki/Tannin
• An exhaustive review: http://www.users.muohio.edu/hagermae/tannin.pdf
[115] Capsaicin: http://en.wikipedia.org/wiki/Capsaicin

(from various *Ephedra* species). Most alkaloids are water soluble and "alkaline", and therefore at physiological pH typically are positively charged due to protonation of the N by H^+. They serve primarily as plant defenses, and are no longer regarded as simply nitrogenous wastes or nitrogen storage compounds. They poison and kill many unadapted domestic livestock each year, particularly lupines (*Lupinus*), larkspur (*Delphinium*), and groundsel (*Senecio*). Plant alkaloids poisonous to humans include some classic poisons:

- **aconitine** (from *Aconitum*, known as aconite, monkshood, or wolfsbane)
- **atropine** (from *Atropa belladonna* and other Solanaceae)
- **coniine** (from poison hemlock *Conium maculatum* and Yellow pitcher plant *Sarracenia flava*)
- **muscarine** (from various mushrooms including *Inocybe* and *Clitocybe*)
- **solanine** and **chaconine** (from the nightshade Solanaceae family such as light-exposed green-tinged [and often bitter tasting] potato tubers and sprouts, but also eggplants, tomatoes, and peppers)[116]
- **strychnine** and **brucine** (extremely bitter alkaloids obtained from the seeds of the tree *Strychnos nux-vomica*)

Although most alkaloids are toxic to humans in sufficient doses, many natural plant alkaloids are useful to humans in controlled doses, and many are psychoactive (figs. 13.17, 13.18 and table 13.2):[117]

- **atropine** (from *Atropa belladonna* and other Solanaceae)
- **caffeine** (from coffee, tea, cacao, yerba mate, guarana)
- **capsaicin and dihydrocapsaicin** (the most abundant and hottest capsaicinoids from peppers of genus *Capsicum*)
- **cocaine** (from the coca plant, *Erythroxylum coca*)
- **codeine** (from the opium poppy, *Papaver somniferum*)
- **emetine** (from ipecac, the dried rhizome and roots of the Ipecacuanha plant *Psychotria ipecacuanha*)
- **ergotamine** (from the ergot fungus, *Claviceps purpurea*, and related fungi)
- **hyoscyamine** (from *Hyoscamus niger* and mandrake *Mandragora officinarum* and other Solanaceae)
- **levodopa** (**L-DOPA**) and **dopamine** (from *Mucuna pruriens*)[118]
- **mescaline** (from several Cactaceae including the **peyote** cactus *Lophophora williamsii*)[119]
- **morphine** (from the opium poppy, *Papaver somniferum*)
- **nicotine** (from tobacco and coca, and in lower quantities in tomato, potato, eggplant, and green pepper, fig. 13.18)[120]
- **nicotinic acid** (**niacin**, from leafy vegetables, broccoli, tomatoes, asparagus, avocados, etc.)
- **papaverine** (from the opium poppy, *Papaver somniferum*)
- **pilocarpine** (from Rutaceae of genus *Pilocarpus*)
- **piperine** and **chavicine** (these produce the pungency of black pepper)[121]
- **psilocybin** (hallucinogen derived from mushrooms of genus *Psilocybe*)
- **quinine** and **quinidine** (from trees of genus *Cinchona*)
- **reserpine** (from the root of *Rauwolfia serpentina*)
- **scopolamine** (from family Solanaceae such as henbane or jimson weed *Datura* species)
- **theobromine** (from the cacao tree, *Theobroma cacao*)
- **theophylline** (from tea and the cacao tree, *Theobroma cacao*)
- **vincristine and vinblastine** (from Madagascar periwinkle *Catharanthus roseus*, formerly *Vinca rosea*)

Many alkaloids employed as human drugs are synthetic or semi-synthetic:

- **amphetamine** (from ephedrine)
- **heroin** (from the opium poppy, *Papaver somniferum*)
- **lysergic acid diethylamide LSD** (hallucinogen derived from lysergic acid derived from ergot of genus *Claviceps*)

Ecological Relationships For Alkaloids:

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[116] Solanine: http://www.inchem.org/documents/jecfa/jecmono/v30je19.htm
[117] Alkaloids: http://en.wikipedia.org/wiki/Alkaloid
[118] Levodopa and dopamine in *Mucuna pruriens*:
http://www.springerlink.com/content/j4628175vl42553n/
[119] Mescaline (Peyote): http://en.wikipedia.org/wiki/Mescaline
[120] Nicotine: http://en.wikipedia.org/wiki/Nicotine
[121] Piperine: http://en.wikipedia.org/wiki/Piperine

Alkaloids may exist in the plant in a nontoxic form (e.g., the pyrrolizidine alkaloid **senecionine** in its non-toxic N-oxide form), yet can be reduced to a toxic form in the alkaline environment of the gut of insect herbivores. Adapted herbivores can oxidize the toxic reduced form of senecionine to nontoxic products (fig. 13.19), and the cinnabar moth can store the N-oxide form in its body as a defense against other predators.

Some grasses such as tall fescue have **endogenous symbiotic fungi** in the apoplast that are the actual source of synthesis of alkaloids. These are seed-transmissible fungal endophytes, including the genera *Epichloë* and *Neotyphodium*. Some of these fungal endophyte alkaloids are "known to be highly active against insects, yet have little or no activity against mammals". (See Web Essay 13.4.)

Cyanogenic glycosides

These non-alkaloid nitrogen containing plant compounds evolve **hydrogen cyanide HCN** (H–C≡N, prussic acid) when plants containing them are crushed, exposing the cyanogenic glycosides to normally segregated or compartmentalized plant enzymes. HCN is a rapidly acting animal toxin that impairs metalloproteins such as cytochrome oxidase that are employed in mitochondrial respiration, and the gas thereby rapidly deters many grazing herbivores such as insects and slugs. The gas is detectable by humans as the smell of bitter almonds at concentrations as low as 0.6 ppm. The concentration that will kill 50% of humans exposed for 10 minutes is only 181 ppm,[122] and nonlethal exposures can cause a variety of acute and chronic toxicities. The plant enzyme **glycosidase** first releases the attached sugar by hydrolysis (fig. 13.20). The resulting **cyanohydrin** is then rapidly decomposed to HCN plus a ketone by **hydroxynitrile lyase** (slower spontaneous decomposition may also occur).

Notable plants containing cyanogenic glycosides are as follows:[123,124,125,126]

- The fruits or seeds and wilting leaves of the **rose** family Rosaceae, including especially the **seeds** of **almonds, apricots, cherries, and peaches** but also seeds of **apples, blackberries, crabapples, plums, and raspberries**, contain the cyanogenic glycoside **amygdalin**.[127] The concentration of amygdalin HCN in **bitter almonds** (technically a fruit, and not the same variety[128] as sweet almonds) is 250 mg HCN per 100 g.
- **Cassava root** (**Tapioca** root or **manioc**, *Manihot esculanta*), an important food plant in Africa and South America, must be washed and ground under running water prior to consumption to release much (but unfortunately not all) of the cyanogenic glycoside **linamarin**, which contains 1.5 - 395 mg HCN per 100 g of tissue. The resistance of cassava to pests is probably due to this toxin.
- **Sorghum**: Whole sorghum contains cyanogenic glycosides, especially **dhurrin**, containing 250 mg HCN per 100 g of tissue. Cyanogenic glycosides in its roots lead to greater resistance to pests such as rootworms. The vacuoles of the epidermal cells also contain the dhurrin, while the hydrolytic and lytic enzymes are found in the mesophyll.[TZ332] When herbivores chew these leaves, the normally separated ingredients are mixed and HCN is evolved.
- Many Fabaceae **legumes** and **grasses** also contain cyanogenic glycosides. For example, the leaves, roots, and seeds of **lima beans** (*Phaseolus lunatus*) contain **linamarin**,[129] with 10-312 mg HCN per 100 g of tissue (but the beans are said to be rendered safe by cooking, and edible strains are selected to have lower linamarin content).[130]
- **Bamboo shoots** have up to 100 mg HCN per 100 g in **taxiphillin**, and can be toxic when inadequately prepared, but this cyanogenic glycoside is mostly liberated on canning.

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[122] Cyanide human lethality: http://www.osha.gov/SLTC/healthguidelines/hydrogencyanide/recognition.html
[123] Cyanogenic glycosides: http://en.wikipedia.org/wiki/Glycoside
[124] Concentrations of cyanogenic glycosides: http://extoxnet.orst.edu/faqs/natural/cya.htm
[125] Cyanogenic glycosides: A detailed review of cassava and bamboo shoot. "The acute lethal dose of hydrogen cyanide for human beings is reported to be 0.5 - 3.5 mg/kg [body weight]" http://www.foodstandards.gov.au/_srcfiles/28_Cyanogenic_glycosides.pdf
[126] Cyanogenic glycosides: http://www.foodstandards.gov.au/_srcfiles/28_Cyanogenic_glycosides.pdf
[127] Amygdalin: http://en.wikipedia.org/wiki/Amygdalin
[128] Sweet and bitter almond: http://en.wikipedia.org/wiki/Almond
[129] Linamarin in lima beans: http://www.nature.com/nature/journal/v278/n5702/abs/278343a0.html
[130] *Phaseolus lunatus*: http://en.wikipedia.org/wiki/Lima_bean

Glucosinolates (AKA Mustard oil glycosides, Thioglycosides, or Thioglucosides)

These are also non-alkaloid nitrogen containing plant glycosides, and are found mainly in Brassicaceae. **Glycosinolate** is a rarely used synonym. These substances are responsible for the sharp or pungent taste of many common foods such as **broccoli, Brussels sprouts, cabbage, cauliflower, cress, horseradish, kale, kohlrabi, mustard seeds and mustard greens, white pepper, radish, rapeseed (canola), rutabaga, turnip, wasabi**, etc.[131] The pungent compounds characteristic of these plants are released, as with cyanogenic glycosides, when the plant is crushed, causing mixing of glucosinolates with the usually segregated enzymes **myrosinase** or **thioglucosidase**, and ultimately yielding **isothiocyanates** and **nitriles** (with general formulas R–N=C=S and R–C≡N, see fig. 13.21). These toxins help to deter some but not all herbivores.

- **Sinigrin**: The glucosinolate Sinigrin is responsible for the pungency of **black mustard** (*Brassica nigra*), **horseradish** (*Armoracia rusticana*, which also contains the glucosinolate **gluconasturtiin**), and **wasabi** (*Wasabia Japonica*).[132] Sinigrin is also found in **broccoli**,[133] **Brussels sprouts**, and **leaf mustard greens** (**brown Indian mustard, *Brassica juncea***).[134] "**Chinese hot mustard** is made with dry mustard ... which, like Dijon, is made with the stronger brown mustard seeds, called Brassica juncea."[135] When an animal chews the plant, the enzymatic reaction on sinigrin leads to the release of **allyl isothiocyanate**, the chemical compound ultimately responsible for the pungent taste. Allyl isothiocyanate can also be distilled from [brown] mustard seeds: the pungent product obtained in this fashion is known as **volatile oil of mustard**,[136,137] an essential oil which contains up to 92% allyl isothiocyanate and is used sparingly in India as a flavoring. (Undistilled mustard oil, made from pressed seeds of *Brassica juncea*, is a different product, consumed in India but not generally considered suitable for human consumption in the United States.) **Black pepper** (produced from the unripe and still-green berries of the pepper plant *Piper nigrum*)[138] also contains sinigrin,[139] but the peppery taste is apparently largely attributable to the alkaloid **Piperine**.[140]
- **Sinalbin**: The pungent thioglycoside or glucosinolate Sinalbin is found in **white pepper** (which is also made from *Piper nigrum*, but from the fully ripe seed and with the surrounding fruit removed), and in **white or yellow mustard** (*Sinapis alba* AKA *Brassica alba* AKA *Brassica hirta*,[141] the source of the standard American hot dog condiment, yellow mustard).
- **Rapeseed glucosinolates**: Ongoing efforts are being made to breed a rapeseed (*Brassica napus*) with lower seed indole glucosinolate content to improve the edibility and safety of extracted **canola oil** for humans, and of **rapeseed meal** for mammals and poultry feed. Rapeseed glucosinolates include progoitrine, gluconapine, glucobrassicanapine, sinalbine, and glucobrassicine, and can induce hypothyroidism[142] and cause other toxicities when ingested in relatively high amounts.

Nonproteinogenic (nonprotein) amino acids

These are also non-alkaloid nitrogen-containing plant amino acids other than the standard 20 proteinogenic AAs. Although there are nonproteinogenic amino acids or AA-like compounds (such as GABA, L-DOPA, and carnitine) that are normally found in animals, some plant nonproteinogenic amino acids are toxic to animals. **Canavanine** (AKA **L-(+)-(S)-Canavanine**, found in high concentration in the

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131 Glucosinolate: http://en.wikipedia.org/wiki/Glucosinolate
132 Wasabi: http://www.realwasabi.com/Science/index.asp
133 Sinigrin: http://en.wikipedia.org/wiki/Sinigrin
134 Mustard greens:
• http://en.wikipedia.org/wiki/Brassica_juncea
• http://www.hort.purdue.edu/newcrop/duke_energy/Brassica_juncea.html
135 Chinese hot mustard: http://chinesefood.about.com/library/blchineseing8.htm
136 Allyl isothiocyanate: http://en.wikipedia.org/wiki/Allyl_isothiocyanate
137 Mustard oil: http://en.wikipedia.org/wiki/Mustard_oil
138 Black and white pepper: http://en.wikipedia.org/wiki/Black_pepper
139 Sinigrin in black pepper: http://www.1911encyclopedia.org/Glucoside
140 Piperine: http://en.wikipedia.org/wiki/Piperine
141 White mustard: http://en.wikipedia.org/wiki/White_mustard
142 Rapeseed glucosinolates:
http://journals.cambridge.org/download.php?file=%2FBJN%2FBJN83_06%2FS0007114500000830a.pdf&code=3ceb6076cc83897ad990e75cf703ee45

jack bean) is an analogue to arginine, and when incorporated into proteins in place of arginine, the protein formed is aberrant and dysfunctional or nonfunctional. The heterocyclic nonprotein amino acid **Azetidine-2-carboxylic acid** is an analogue of proline, and can similarly be incorporated into what become aberrant proteins.

Induced Plant Defenses Against Insect Herbivores

Plants have "**constitutive defense responses**" against herbivores that are always present and ready to go (somewhat analogous to the non-specific "innate immune system" in humans). There are often stored or conjugated compounds whose toxicity can be rapidly released upon a tissue-damaging attack. They require constant expenditure of plant resources to maintain at a state of readiness.

In contrast, plant also have "**induced defense responses**" which are initiated and built up only after actual damage occurs (somewhat analogous to the human "adaptive immune system"). These require less ongoing expenditure of plant resources when not in the activated state, but take more time to activate in response to a specific episode of damage. [MCM note: In some of the discussion that follows, I am uncertain what is considered constitutive versus induced, and the distinction may at times be a little arbitrary.]

There are three categories of **insect herbivores**:

- **Phloem feeders** including **aphids** (Hemiptera > Sternorrhyncha > Aphidoidea) and **whiteflies** (Sternorrhyncha > Aleyrodidae, which typically feed on the underside of plant leaves).[143] These cause little direct damage to the plant, but can transmit plant viruses (such as Tomato yellow leaf-curl begomovirus) and thereby cause many hundreds of millions of dollars in crop damage. Some "companion plants" are said to repel whiteflies, including marigolds, nasturtiums, and mint.
- **Cell content feeders** include **mites** and **thrips**, which cause intermediate levels of physical plant damage by piercing and sucking. **Plant Mites** (Arachnida > **Acari**) include "spider mites", "thread-footed mites", and "gall mites". They "play important roles as agricultural pests of timber, fruits, vegetables, forage crops, ornamentals, and stored grains".[144] Thrips (Insecta >> Thysanoptera) "are generally tiny (1 mm long or less) and are not good flyers, although they can be carried long distances by the wind... **Thrips** feed by piercing plant cells with their paired maxillary stylets, which form a feeding tube. They feed on hundreds of different crop plants, especially during flowering where they also feed on pollen... Over 20 plant infecting viruses are known to be transmitted by thrips... In addition to acting as a crop pest, these insects may also enter the home and distress inhabitants by their presence... When present in large numbers, they can cover wall surfaces, curtains, and windowsills."[145]
- **Chewing insects** include caterpillars, grasshoppers, and beetles, and cause the most significant plant damage.

Induction of Jasmonic Acid, a plant defense hormone

Plants can respond to specific components derived from chewing insect saliva, which enhance or elicit the defense response. These **elicitors** are fatty acid-amino acid conjugates (**fatty acid amides**). The plant provides the fatty acids (linolenic 18:3 or linoleic 18:2 acids), and within the insect gut the conjugation with an insect-derived AA occurs (typically glutamine, fig. 13.23). In some insects the final elicitor product is the conjugate **volicitin**.

In the plant, the elicitor leads to signal transduction and activation of the **octadecanoid signaling pathway** in the chloroplast and peroxisome, leading from the C_{18} linolenic acid (18:3) to C_{11} **jasmonic acid** (**JA**, fig. 13.24, details omitted). The JA response to plant injury is similar to the **eicosanoid** biosynthetic response in animals which leads to formation of prostaglandins, prostacyclins, thromboxanes, and

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[143] Whiteflies: http://en.wikipedia.org/wiki/Whitefly

[144] Plant feeding mites:

• http://en.wikipedia.org/wiki/Mites

• http://www.sel.barc.usda.gov/acari/frames/plantfeed.html

[145] Thrips: http://en.wikipedia.org/wiki/Thrips

leukotrienes (see Web Chapter 14, fig. 14.21). JA induces transcription of numerous genes involved in plant defenses, including those for secondary metabolites. This has been studied for example in the Madagascar periwinkle, *Catharanthus roseus* (syn. *Vinca rosea*, source of vinca alkaloids)9. Plants low in JA synthesis are more vulnerable to insect attack (such as fungus gnats).[146]

Other compounds involved in signaling insect attack include ethylene, salicylic acid, and methyl salicylic acid (methyl salicylate).

Plant proteins that inhibit insect herbivore digestion

These are induced by Jasmonic acid, and include

- **α-amylase inhibitors**: found in legumes, they block starch digestion in the herbivore's gut
- **Lectins**: they bind to CHOs and interfere with their absorption in the herbivore's gut
- **Proteinase inhibitors**: found in legumes, tomatoes, etc. These block herbivore proteolytic enzymes (such as trypsin and chymotrypsin) and thereby hinder protein digestion in many (but not all) insect herbivores. Plants such as transgenic tobacco bred to have more proteinase inhibitors suffer less insect herbivore damage.

Herbivore damage induces systemic defenses

The induction of proteinase inhibitors in, for example, the tomato plant is widespread even when the attack is localized to a small area, due to systemic signaling. This is a form of systemic acquired resistance. Although *systemic* signaling responses are found in many species including corn and cotton, *systemin* signaling is so far confined to the **Solanaceae** family. The proposed sequence for systemin signaling is as follows (fig. 13.25):

- Tomato leaves wounded by insect chewing synthesize **prosystemin.**
- Prosystemin is cleaved to **systemin** (a C_{18} peptide hormone) in the phloem parenchymal cell.
- This systemin is released into the apoplast and binds to PM systemin receptors on adjacent companion cells, leading to synthesis of **jasmonic acid** (details omitted, see also Web Essay 13.6; the systemin receptor is the same as the receptor for brassinolide.)[TZ336]
- **JA** propagates in the phloem via sieve elements and plasmodesmata connections to induce secretion in target mesophyll cells of **proteinase inhibitors**, etc.

In addition to systemin, insect herbivores induce the release of **volatile organic compounds** (**VOC**), which consist of (what follows was adapted from Web essay 13.8):

- Terpenes (such as **ocimene** and the **homoterpenes**)[147] and alkaloids
- Phenolics or shikimic acid-derived products, such as **Salicylic acid-methyl ester** (**MeSA, Methyl salicylate**), **Anthranilic acid-methyl ester**, and **Indole**; and
- Fatty-acid-derived products such as **Jasmonic acid-methyl ester** (**MeJA**), **Cis-jasmone**,[148] and **Green-leaf volatiles** (**GLV**, typically C_6 alcohols, aldehydes and acetates, including hexenal, hexenol, and hexenyl acetate).

The C_6 GLVs are cleaved mainly from 18:2 and 18:3 fatty acids, leaving a nonvolatile C_{12} part that is transformed into **traumatin**, a classical wound hormone (see Web Essay 13.7). GLVs attract predatory enemies of the attacking insects,[149] and in some cases play other complex ecological roles which may be tailored to the specific insect herbivore present (details omitted, see also Web Essay 13.7). GLVs emitted by maize and other plants may even trigger various defensive reactions in nearby plants, or at least can

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[146] Fungus gnat:
• http://en.wikipedia.org/wiki/Fungus_gnat
• http://ohioline.osu.edu/hyg-fact/2000/2114.html
• http://insects.tamu.edu/extension/bulletins/uc/uc-028.html

[147] Homoterpenes: http://www.ncbi.nlm.nih.gov/pubmed/7925964

[148] Jasmone: http://en.wikipedia.org/wiki/Jasmone

[149] Green-leaf volatiles:
http://www.colostate.edu/Depts/Entomology/courses/en570/papers_1996/mcintyre.html

prime the as-yet unattacked neighbors to respond more vigorously when subsequently attacked.[150] (See Web Essay 13.8, including analysis of the meaning of "priming". [MCM note: is this always only in neighbors of the same species?])

Of course, insects have evolved to counter many of these plant defenses, and, as with plants, their countermeasures can be either constitutive or induced. Constitutive countermeasures are more likely to be found in insects specialized for feeding on only a few species, whereas induced countermeasures are more likely to occur in insects that are dietary generalists.

Plant Defenses Against Pathogenic Microorganisms

Whereas the above discussion has dealt primarily with macroscopic herbivores including insects, plants must also defend against attacks by microorganisms including pathogenic fungi, bacteria, viruses, and nematodes. A variety of mechanisms are employed (fig. 13.26), including a type of acquired resistance or "immunity".[TZ342]

Antimicrobial compounds synthesized before pathogen attack

These include the **saponins**, compounds discussed above that have been found important for instance in defending against **fungi** in oats.[TZ338] [MCM: Some of the toxic secondary metabolites already mentioned presumably also play this role to some extent.]

Hypersensitive response, reactive oxygen species, and nitric oxide

The hypersensitive response is discussed in chapter 16 as a genetically programmed process. In response to a localized pathogenic microorganism attack, the plant locally accumulates toxic phenolic and other compounds that cause a localized necrotic lesion surrounding the infection, depriving the localized pathogenic microorganism of nutrients, preventing the spread of infection, and leaving the remainder of the plant unaffected. [MCM: the Hypersensitive response forms part of **systemic acquired resistance** (per fig. 13.26) even if the infection is originally localized to a limited area.]

The hypersensitive response (HR) is often preceded by local synthesis of toxic compounds including **reactive oxygen species** (**ROS**), which include the **superoxide anion** ($O_2^{\bullet -}$), **hydrogen peroxide** (H_2O_2), and the **hydroxyl radical** (•OH). The hydroxyl radical is the most reactive of these, and can lead to lipid peroxidation, enzyme deactivation, and nucleic acid degradation. (See also chapter 7, where singlet oxygen [$^1O_2^*$] was also mentioned as one of the ROS.) These toxic products are needed for the HR to proceed, but may also kill the pathogen directly, .

Nitric oxide (NO) is also generated at the site of infection and required for the hypersensitive response to proceed.

Compounds increasing polymeric physical barriers to spread

Many species react to localized fungal and bacterial infections by producing **lignin** or **callose**, or increasing the **cross-linking of proline-rich CW proteins**, all of which serve to increase physical barriers for containment (see chapter 10, 15). [MCM: Lignin biosynthesis forms part of **systemic acquired resistance** (per fig. 13.26) even if the infection is originally localized to a limited area.]

Hydrolytic enzymes directed against the cell walls of pathogens

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[150] Neighbor maize effects of GLVs: "Airborne signals prime plants against insect herbivore attack." http://www.ncbi.nlm.nih.gov/pubmed/14749516

These include **glucanases**, **chitinases** and other **hydrolases** active against the CWs of fungi and other microorganisms. **Chitin** is a long-chain polymer of a N-acetylglucosamine and is the main component of the cell walls of fungi.[151] These hydrolytic enzymes are considered **pathogenesis-related proteins**. They form part of **systemic acquired resistance** (per fig. 13.26) even if the infection is originally localized to a limited area.

Phytoalexins

These are plant antimicrobial compounds ("antibiotics") that accumulate locally in response to bacterial and fungal attack, but are generally undetectable before infection. Thus they form part of **systemic acquired resistance** (fig. 13.26). They tend to be specific for a particular plant or family, and include (fig. 13.27)

- **Isoflavonoids** in legumes: **Medicarpin** in alfalfa, **Glyceollin I** in soybeans
- **Sesquiterpenes** in Solanaceae: **Rishitin** in potato and tomato, **Capsidiol**[152] in pepper fruits (Capsicum annuum) and tobacco
- **Resveratrol** (3,5,4'-trihydroxystilbene)[153] is a polyphenolic (or phenylpropanoid) phytoalexin found in **grape skins** (thus found in higher concentration in red compared to white wines) and in **muscadine grape seeds**, **peanuts**, to a lesser extent in **blueberries**, also white hellebore, and Japanese knotweed.
- **Camalexin** (3-thiazol-2'yl-indole) is an indolic phytoalexin derived from the tryptophan biosynthesis pathway, and is the main phytoalexin in Arabidopsis.[154]

Some plants respond rapidly to specific substances (elicitors) released from pathogens

Plants that are especially resistant to a specific pathogen may recognize and respond rapidly to a specific **elicitor** compound arising from the pathogen. This capability is encoded in **Resistance** (**R) genes**, which code for protein receptors (fig. 13.26) and trigger signaling pathways (details omitted). These receptors, which can be found on PMs and in the cytoplasm, detect pathogen-derived elicitors that include proteins, peptides, sterols, and polysaccharide fragments. The elicitors are encoded in the pathogens by **Avr ("avirulence") genes**. Because there would be no evolutionary advantage to a pathogen expressing an elicitor that is readily recognized by the plant, it is believed that the Avr genes encode products that promote virulence in genetically susceptible hosts.[TZ340,155]

Exposure to a specific elicitor triggers a complex signaling pathway, leading to some of the responses named above, as well as involving one or more of the following components:

- Ion fluxes including Ca^{2+} and H^{+} influx, and K^{+} and Cl^{-} efflux
- Oxidative burst (of reactive oxygen species)
- Nitric Oxide
- Mitogen-activated protein kinases
- Calcium-dependent protein kinases
- Jasmonic acid
- Salicylic acid

Systemic acquired resistance

Some plants acquire resistance ("immunity") once they have been infected by a microbial pathogen.[TZ342] Plants that have been attacked by a pathogen may develop **systemic acquired resistance (SAR)** over a period of days (fig. 13.28). That is, the entire plant becomes more resistant to subsequent infection by that pathogen, even if the original attack were localized to a small part of the plant. SAR relates to accumulation of certain defense compounds already discussed, including chitinases and hydrolytic

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[151] Chitin: http://en.wikipedia.org/wiki/Chitin
[152] Capsidiol: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1399-3054.1996.tb06679.x
[153] Reservatrol: http://en.wikipedia.org/wiki/Resveratrol
[154] Camalexin: http://www.genomicsexplorer.com/rhodcv/hort640c/secprod/se00001.htm
[155] Resistance genes and avirulence genes: http://en.wikipedia.org/wiki/Gene-for-gene_relationship

enzymes, even if they were originally employed only locally during the initial infection. One of the endogenous signals sent to the whole plant is the C_7 benzoic acid derivative, **salicylic acid**[156] (so named because it was originally isolated from the bark of willow of genus *Salix*).[157] H_2O_2 generated in response to local infection also acts to promote acquired resistance, though apparently more locally. Movement of the SAR signal progresses at a rate of 3 cm/hr, suggesting this movement occurs in the vascular system, most likely within the phloem.

Signaling may also occur via airborne volatile organic compounds (VOCs) such as **Salicylic acid-methyl ester** (**MeSA**, **Methyl salicylate**, found in Wintergreen oil). VOC signals, as noted earlier, can reach distant parts of the plant and even to neighbor plants.

Chapter XIV. Gene Expression And Signal Transduction

The full text of this chapter is available at http://4e.plantphys.net/chapter.php?ch=14. The following is a limited summary—especially limited regarding signal transduction.

Genome Size and Complexity

Representative haploid (nonduplicated) approximate genome sizes[158] are

Organism	**# of Haploid Base Pairs**	**# of Genes (Protein-coding)**
E. coli	5×10^6	4,290
Bacteria	5×10^5 - 6×10^6	469 - 7,464
Fruit Fly	2×10^8	14,000
Human	3×10^9	25,000
Arabidopsis thaliana	1.25×10^8	26,000
Trillium	1×10^{11}	
Yeast	1×10^7	6,000
Protozoan, Flies	1×10^8	12,000 - 19,000
Nematode C. elegans	1×10^8	19,000
Rice	4×10^8	35,000 - 55,000

In eukaryotes, there is a lack of consistent relationship between organism complexity and genome size (haploid base pairs) or number of protein-coding genes (the "C-value enigma"). This is due to presence of noncoding DNA: repetitive DNA, spacer DNA, introns, promoters and regulatory sequences, telomeres, centromeres, "junk" DNA, etc. Humans have 5% [protein] coding DNA.

Prokaryotic Gene Expression

Simpler than eukaryotic gene regulation. Still involves transcription and then translation, but there is no nucleus and related genes and regulatory control sequences are grouped together in **operons**. Operons consist of **structural genes** (DNA sequences coding for RNA or protein other than a regulatory element) and **regulatory sequences**.

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[156] Salicylic acid: http://en.wikipedia.org/wiki/Salicylic_acid
[157] *Salix*: http://en.wikipedia.org/wiki/Salix
[158] Genome sizes for various organisms: http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/G/GenomeSizes.html

Example of negative control: The E. coli **lac** inducible operon for lactose metabolism. (p. 3) The **operator o** DNA sequence, located downstream from the **promoter p** sequence, normally has **lac repressor** protein attached to it, suppressing (inhibiting) transcription by RNA polymerase downstream (toward the 3′ end), thus blocking genes z y and a. Addition of lactose causes the repressor to be removed from the lac operon operator o, allowing transcription of the 3 proteins including β-galactosidase to proceed. [lactose = β-D-galactopyranosyl-(1↔4)α-D-glucopyranose]

Example of positive control: Glucose added to nutrient medium of E. coli suppresses lac operon by lowering cellular cyclic AMP…

Metabolites can also be **co-repressors**, activating a repressor protein that blocks transcription—e.g., tryptophan operon. Tryptophan binds to inactive repressor protein which bind to operator o blocking downstream genes that would synthesize tryptophan.

Eukaryotic Gene Expression

More complex, because of nuclear envelope separating transcription (to mRNA) from translation (to peptides), presence of non-coding introns, etc.

The nuclear transcripts (pre-mRNA) require extensive processing:
add m^7G cap at 5′ end
terminate 3′ end with poly-A [adenylate] tail (to protect against RNases, etc.)
excise intron-derived sequences from pre-mRNA with spliceosome complexes, etc.

Transcription via RNA polymerases is controlled by various transcription factors attaching at nearby **cis-acting DNA regulatory sequences**:
- Transcription initiation complex binds to the TATA box DNA sequence
- Upstream or proximal (toward 5') regulatory proteins attach at promoter sequences (CAAT and GC boxes, etc.)

Transcription factors (regulatory proteins or microRNA etc.) that act on transcription factors rather than directly on cis-acting DNA regulatory sequences, and which may be distantly encoded (even on other chromosomes), are **trans-acting factors**. These may be positive **enhancers** or negative (?suppressors).

Cis-acting DNA sequences that are acted on by hormones or other signaling agents are called **response elements**.

Transcription factors may be activators or repressors.

Translation is initiated at the first AUG [methionine codon] sequence in mRNA.

Transcription factors typically have a DNA binding domain, a transcription activating domain, and a ligand binding domain. The major DNA-binding motifs[159] include:
- Helix-turn-helix
- Helix-loop-helix
- Zinc finger
- Leucine zipper
- Basic zipper

The **homeodomain** proteins derived from **homeotic** genes—disorders of which cause **homeosis** in drosophila, a transformation of one body part to another—are helix-turn-helix proteins. These genes have a highly conserved **homeobox** sequence coding for 60 AAs. Four plant floral homeotic genes encode proteins with the helix-turn-helix motif called the **MADS domain**.

Eukaryotic genes can be **coordinately regulated**. See fig. 14.8 example, wherein presence of galactose in the extracellular space leads to removal of the GAL80 negative trans-acting regulatory protein from the

[159] Transcription factors: http://en.wikipedia.org/wiki/Transcription_factor

positive trans-acting GAL4 protein, allowing GAL4 to induce synthesis of several enzymes needed for galactose digestion.

Small RNAs may act as post-transcriptional repressors of gene expression: microRNAs (miRNAs) and short interfering RNA (siRNA)... miRNAs contain palindromic sequences that can base-pair in hairpin loops ... inhibiting translation or promoting RNA degradation. siRNAs cause RNA interference, and related to resistance to RNA viruses...

mRNA and proteins **turnover** in the cytoplasm and must be constantly resynthesized. **Ubiquitin** , acting with the 26S **proteosome**, targets and thereby regulates protein turnover...

Signal Transduction

Bacteria employ two-component regulatory systems. The **input signal** interacts at the **input domain** of the **sensor protein** (usually on the membrane), causing a conformational change in the **transmitter domain** of this sensor protein. This leads to phosphorylation of the **receiver** of the **response regulator protein**. This causes an **output signal**... For example, osmolarity is a signal so detected. Similar two-component systems have been found in eukaryotes.

Eukaryotes have much more complex signaling systems. Plants have been less well studied than animals.

Phytohormones may be lipophilic (therefore easily crossing the hydrophobic membranes) or water-soluble (and therefore primarily binding to cell membrane receptors). **Steroids** can regulate gene expression by binding to and modifying receptor proteins that function as transcription factors... Other hormones utilize **signal transduction pathways** which often regulate transcription factors. These pathways often involve protein kinases and phosphatases, and intracellular **second messengers**,[160] which often bind to **switch proteins**, including:

- Hydrophobic molecules: Inositol triphosphate (IP_3), diacylglycerol (DAG)
- Hydrophilic molecules: cAMP, cGMP, Cyclic ADP-Ribose (cADPR), and Ca^{2+}
- Gases: nitric oxide (NO)

Brassinosteroids function like steroid hormones in plants, but bind at the cell surface.

Cell membrane interactions involve **G proteins** and effector enzymes and cAMP... The IP_3 pathway leads to opening calcium channels on the ER and tonoplast. Some protein kinases are activated by calcium-**calmodulin** complexes... Plants contain calcium-dependent protein kinases..... Plant receptor kinases are structurally similar to animal receptor tyrosine kinases... [much material omitted]

Chapter XV. Cell Walls: Structure, Biogenesis, And Expansion

Plant cell walls contain cellulose, hemicelluloses, pectins, enzymes, structural proteins, phenolic polymers, water, etc. They are the principle ingredients in paper, many textiles, fibers such as cotton, hemp, and flax, charcoal, lumber and wood. Derived substances are used in rayon, plastics, films, adhesives, gels, and thickeners. Ruminants use gut microbes to digest cellulose. Soil humus is also derived from cell walls.

Key Properties And Benefits Of Cell Walls

- Cell walls determine the mechanical strength of plants.
- Cell walls glue cell together preventing sliding.
- Cell walls act like a tough exoskeleton that controls cell shape and allow turgor pressure to develop.
- Plant morphogenesis depends on control of cell wall properties.
- Cell wall determines relationship between cell turgor pressure and cell volume.
- Bulk flow in xylem requires a stiff wall that resists collapse under negative tension.
- Cell wall acts as a diffusion barrier to larger macromolecules, and is a barrier to pathogens.

[160] Second messengers: http://en.wikipedia.org/wiki/Second_messengers

Most plant carbon is stored in cell wall polysaccharides. Hydrolysis allows recovery of this carbon under certain conditions. Plasmodesmata pass through the cell walls.

Cellulose

Cellulose microfibrils are synthesized at the plasma membrane from **particle rosettes** or **terminal complexes**, grouping individual synthetic CesA proteins together to make 36 glucan chains at a time. The individual cellulose chains are about 1000 - 5000 nm in length. Microfibrils consist of c. 50 chains of **cellulose**, a polysaccharide polymer of **(1→4)β-D-glucan**, 2000 to >25,000 units of D-glucose linked by β(1→4) glycosidic bonds (**β-D-1,4 glucosyl units**). Each glucose residue is oriented 180° to the next with the chain synthesized two residues at a time. (The repeating unit therefore may be said to be **cellobiose**, a (1→4)β-D-glucose disaccharide of about 1 nm length.) Microfibrils are 2 - 20 nm wide and up to 40,000 nm long. The inner cellulose often has a crystalline configuration (unlike starch) and is tightly packed, with hydrogen bonding between chains within a layer, but not between chains in adjacent layers.[161] Cellulose content is high in xylem secondary walls. Cellulose has a high tensile strength, equivalent to steel, and is chemically stable, resistant to decomposition and attack.

Cellulose synthesis in the particle rosettes of the plasma membrane is catalyzed by **cellulose synthase** encoded by enzyme gene family **CesA**, a family is a part of the **Csl superfamily** of enzyme genes**.** This enzyme is a sugar-nucleotide polysaccharide glycosyltransferase, using **Uridine diphosphate D-glucose** as the sugar donor, and possibly **sterol-glucosides** (such as β-sitosterol glucoside) as the initial acceptor.

Primary And Secondary Cell Walls

Primary Cell Wall: Cell walls begin as **primary cell walls**, which are usually thin, extensible, and relatively undifferentiated. They occur in young and growing cells. They consist of cellulose microfibrils (c. 25%, but proportions of constituents are quite variable) embedded in a highly hydrated matrix, providing strength and flexibility. Their formation begins during cytokinesis, at the phragmoplast. The possible steps of assembly are discussed on TZ p. 362, including self-assembly and enzyme-mediated assembly (such as with XET and with XHTs). Oxidases may help to catalyze cross-linking of phenolic groups.

The matrix polymers are secreted by exocytosis from the Golgi apparatus. The matrix contains of amorphous (noncrystalline) collections of the following:

(1) **Hemicelluloses**: **Xyloglucans** [(1→4)-β-D-glucose polymer with xylose and other side chains including the deoxy sugar **fucose**] are the most common. These 50 - 500 nm glycans can bind together several cellulose microfibrils. Other glycans include xylan, glucuronoarabinoxylans (glucuronic acid and arabinose side chains), glucomannan, arabinoxylan, callose, etc. These bind to the surface of cellulose and tie the microfibrils together, or cross-link them, or in some cases allow them to slide. The percent of hemicelluloses is high in cereal endosperm.

(2) **Pectins**: are hydrated hydrophilic gels consisting of a heterogeneous mix of

- Homogalacturonan, a (1→4) polymer of α-D-galacturonic acid),
- Rhamnogalacturonan I
- 5-arabinan
- Type I arabinogalactan,
- Rhamnogalacturonan II, etc. Rhamnogalacturonan II is quite complex, with extensive branching side chains, and it is dimerized with borate ester cross-links [not trimerized as might be expected.][162]

Pectin polymers often contain acidic **sugars** such as galacturonic acid. Their carboxylic acid groups are often esterified with methyl or ethyl groups, etc. (i.e., the methyl group replaces the H of the COOH group). The -COOH groups may be linked together by ionic bonding via calcium ion. It is possible that the methyl esters help to mask the COOH groups from this type of bonding until the pectin is secreted into final position, after which de-esterification will allow ionic bonding and formation of a rigid gel to proceed.

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[161] Structure of cellulose layers: http://www.lsbu.ac.uk/water/hycel.html

[162] Rhamnogalacturonan II: http://www.ccrc.uga.edu/~mao/rg2/intro.htm

There may also be cross-linking by covalent bonding by ester linkages between phenolic groups such as on ferulic acid.

(3) **Structural proteins and enzymes**: These also become cross-linked, e.g., HRGP. Heavily glycosated AGPs can function in cell adhesion or cell signaling.

Secondary Cell Walls form internal to the primary cell wall, after cell growth and enlargement cease. They can be much thicker, have regular structures, and are often strengthened and waterproofed by lignin. Cell walls are bound together by the middle lamella, comprised mainly of pectin. They provide mechanical support to the plant and prevent collapse from internal tension in xylem vessels. They are often deposited in multiple layers internal to the primary CW. **Lignin** is a phenolic polymer "a large, cross-linked, ... macromolecule with molecular masses in excess of 10,000u. It is relatively hydrophobic and aromatic in nature. The degree of polymerisation in nature is difficult to measure..."[163] It is hydrophobic and displaces water, tightly bonding to cellulose. It reduces the susceptibility to attack by hydrolytic enzymes from pathogens, and reduces the digestibility of plants by animals.

Patterns of Cell Expansion

[Limited summary]

Cellulose microfibrils are oriented in a manner that controls the direction of cell expansion (which is anisotropic and usually greater in the longitudinal or long-axis direction) during cell growth. This pattern is produced by laying down the microfibrils in band-like rings (like barrel hoops), which restrict lateral expansion because the cross-linked microfibrils cannot easily slip past long lengths of one another. However, slippage apart of the bands and growth in the long-axis direction is easier, since the matrix polymers need only slip over the diameters of the fibrous elements. Cortical microtubules influence the orientation of newly deposited microfibrils, and the former can be shown to be aligned perpendicular to the long-axis direction of cell growth. In the absence of these structures (e.g., when impaired by oryzalin toxicity), a usually narrow root tip grows unusually wide.

The rate of cell elongation produced by turgor pressure (see fig. 15.24) is governed by two equations:
(1) the rate of water uptake $\Delta V/\Delta t$ into the cell:

$\mathbf{\Delta V/\Delta t = A \times Lp \times (\Psi_o - \Psi_i)}$, where

$\Delta V/\Delta t$ = rate of water uptake into the cell
A = Cell surface area in meters
Lp = permeability of PM in m s^{-1} MPa^{-1}
$\Psi_o - \Psi_i$ = water potential gradient; and

(2) the wall yielding growth rate GR (per the Lockhart theory):

$\mathbf{GR = m(\Psi p - Y)}$, where

GR = the wall yielding growth rate
m = coefficient of wall extensibility (slope of the line relating growth rate to turgor pressure)
Ψ_p = turgor pressure
Y = Wall yield threshold (Turgor pressure at which wall elongation commences)

At steady-state rate of wall elongation, the water uptake $\Delta V/\Delta t$ (m^3 s^{-1}) equals the wall yielding growth rate GR...

Acid-induced growth (acid growth hypothesis) can be demonstrated experimentally using an extensometer, by showing faster cell expansion in conditions of acid pH. The acidity in the CW is thought to be catalyzed by wall proteins called **expansins**. Glucanases and other hydrolytic enzymes are also employed to promote wall stress relaxation and expansion. This happens with fruit softening, abscission, etc. These processes are often locally employed to allow selective expansion at parts of the CW.

When cell growth stops, structural changes in the cell wall occur leading to greater CW rigidity and strength—by forming tighter complexes, modification of glucan polymers, de-esterification of pectins (see above), and adding phenolic cross-links to pectins and lignin, etc.

[163] Lignin: http://en.wikipedia.org/wiki/Lignin

Wall Degradation and Plant Defenses

[Limited summary]

Certain processes are accompanied by intentional wall degradation such as in fruit ripening, or in the endosperm during seed germination. Enzymes that assist in this process include glucanases, xylosidases, pectin methyl esterase, etc.

When plants are wounded or under attack by predators, they may release reactive oxygen species such as hydrogen peroxide, superoxide radicals, etc. These can contribute to a rapid cross-linking of phenolic compounds in the CW and also serve as signaling molecules (see also chapter 13). The **oligosaccharins**, pectin fragments, and pectinases released during attack may also serve to trigger defense responses—thus they are **elicitors** of a defensive response. Certain pathogens may act to inhibit these defense mechanism, however.

Chapter XVI. Growth and Development

[This is a limited summary of key concepts from this highly visual chapter. Some of these items have been summarized elsewhere.]

Unlike in animals, vegetative plant growth is **indeterminate** and highly repetitive, due to the perpetual growth of meristem tissues in the shoot and root apices, whereas reproductive growth is **determinate**. Examples of indeterminate growth include bristlecone pines in the White Mountain range that are 4000 years old. Eventually plants undergo senescence and die, a programmed event. Plants are not as complex as animals because they do not move much, and often have a woody inflexible matrix. Their forms evolve throughout their lives.

The (usually) diploid **sporophyte** generation of most higher plants is the dominant one, and begins with the zygote—the gametophyte phase in higher plants consist of the pollen and embryo sac... By contrast, in the common moss Tortula muralis, the (usually) haploid gametophyte is the dominant generation, and the sporophyte generation appears as sporangium-bearing stalks[164] growing from the gametophytes.

Quantitation of growth of plant elements is discussed in web topic 16.1. This includes the maintaining of the hook of emerging dicots by migration of the hook from the hypocotyl to the first and second internodes.

Sporophytic Development

Embryogenesis usually takes place in the ovules. See discussion of development of seeds including **double fertilization** elsewhere in this document. (See also fig. 16.2) The **embryo sac** is bounded by the maternal **nucellus** and contains the diploid zygote and the triploid endosperm. See fig. 16.3 for stages in embryo development. The zygote divides asymmetrically into the smaller **apical daughter cell** and the large **basal daughter cell**. The latter becomes **suspensor cells.** The apical daughter cell becomes the eight-cell globular proembryo, etc...

Vegetative Development occurs after germination, which may have been delayed following embryogenesis (see discussion elsewhere). Vegetative development is characterized by reiterated programs of lateral organ development...

Reproductive development and factors inducing it are discussed in Chapter 25.

Embryogenesis And The Origins Of Polarity

Basic plant architecture includes the presence of apical meristems at the tips of shoot and root axes.

[164] Sporophyte and gametophyte stages: http://en.wikipedia.org/wiki/Sporophyte

The dicot Arabidopsis embryo develops as follows (times are in relation to **syngamy**, i.e., fertilization):

- **Zygotic Stage**: Starts with syngamy, concludes with the asymmetrical cell division forming the apical and basal daughter cells.
- **Globular Stage**: The eight-cell (octant) globular **proembryo** at 30 hours begins to develop the protoderm.
- **Heart Stage**: This begins to define the axis by lateral outgrowths that will become cotyledons.
- **Torpedo Stage**: Cell elongation and further development of the cotyledons.
- **Mature Stage**: The cotyledons are folded over (at least in Arabidopsis) and desiccation takes place.

The monocot rice embryo develops as follows (DAP = Days after pollination):

- **Zygotic Stage**
- **Globular Stage**: The multilayered globular embryo develops (2-4 DAP).
- **Coleoptile Stage**: At 5 DAP, the coleoptile (tubular protective first leaf) forms, as well as the SAM, RAM, and embryonic root (radicle). The **scutellum** forms to eventually absorb sugars from the endosperm during germination.
- **Juvenile Vegetative Stage**: At 6-10 DAP, the SAM initiates several (un-erupted) vegetative leaves.
- **Maturation Stage**: At 11-20 DAP, maturation preceding onset of dormancy.

Both monocots and dicots exhibit development of polarity into 2 axes, the apical-basal axis (from SAM to RAM), and a radial axis (from center of long axis to periphery). See web topic 16.3 for some technical details. Axial polarity is established by the embryo, which itself becomes polarized. It elongates three times greater than its width before its first division. The zygote divides asymmetrically into

- the smaller **apical daughter cell** (which is densely cytoplasmic). This becomes most of the embryo (except as follows).
- the larger **basal daughter cell** (containing a vacuole). This mostly becomes the suspensor. However, the most superior part closest to the proembryo becomes the **hypophysis**, which becomes the **columella** or central part of the **root cap**, as well as the **quiescent center QC** of the RAM.

Thus polarity is established prior to and at the time of the earliest cell division of the zygote. By the heart stage, polarity is quite obvious.

- The apical region of the globular stage (derived from the top four cells from the apical daughter cell) becomes the cotyledons and SAM.
- The middle region of the globular stage (derived from the basal 4 cells from the apical daughter cell) becomes the hypocotyl, the root and most of the RAM.
- The hypophysis (derived from the basal daughter cell) becomes the columella and the quiescent center of the RAM.

Position-dependent signaling guides this embryogenesis (details omitted)... Morphogens help guide axial patterning, specifically **auxin (IAA)**, apparently through establishing a gradient distribution... (fig. 16.9) The inferred maximum auxin concentration is at the junction of the globular or early heart embryo with the suspensor. Genes control this apical-basal patterning, and correlate with auxin... (details omitted) Radial patterning and radial symmetry establishes fundamental tissue layers, aided by **periclinal cell division** (in which the most prominent plane of division parallels the surface of the embryo, as opposed to **anticlinal cell division** perpendicular to the surface of the embryo). This leads to concentric differentiation of tissues into **protoderm** (eventually forming the epidermis), the **cortex** (ground tissue between epidermis and vascular system) and the **endodermis** (demarcating the outer boundary of the eventual stele), and the **pericycle** (origin of lateral roots in roots), etc. (fig. 16.12) Two genes regulate protoderm differentiation... Cytokinin stimulates cell divisions for vascular elements... Two genes control the differentiation of cortical and endodermal tissues... (fig. 16.16) Intercellular communication is central to plant development, for example SHR-encoded GRAS transcription factors... (details omitted)

The Shoot Apical Meristem (SAM)

The SAM forms from pluripotent cells at a position where auxin is low and involves a defined sequence of gene expression... (details omitted, see fig. 16.19). The SAM has a **central zone CZ** (containing the stem cells) and **peripheral zone PZ** (which produces the leaf primordia). The **rib zone (RZ)** lies below the CZ and generates the central tissues of the stem... (fig. 16.21) The SAM is up to 3 mm in size (in cycads) but only 50 μm in Arabidopsis... Periclinal cell division can lead to a visible pattern of periclinal chimeras in variegated leaf plants. (fig. 16.23)

The Root Apical Meristem (RAM)

High auxin levels stimulate the formation of the RAM. There are four zones in the developing root (fig. 16.28):

- The **quiescent center QC** has low mitotic activity but is the ultimate source of all the cells of the root—in Arabidopsis it has only 4-7 cells. The cells immediately next to the QC serve as the "**initials**", and provide specifically differentiated cells: the central root cap (columella) initials, the epidermal-lateral root cap initials, the cortical-endodermal initials, and the vascular or stele initials. The highest rate of cell division is found just above the quiescent center—this rapidly dividing region and the QC are in the **meristematic zone**.
- The QC and initials are protected by the **root cap** at the tip of the growing root.
- Above the meristematic zone is the **elongation zone**, where maximal cell elongation though little division occurs, and **sieve tube (phloem) elements** and **vessel (xylem) elements** begin to differentiate.
- Above this is the **maturation zone**, where mature vessel elements and endodermal cells are found.

The four regions combined (root cap, meristematic, elongation, and maturation) total only about 1 mm in Arabidopsis.

Vegetative Organogenesis

This presents a discussion of the how periclinal cell divisions on the flank of the shoot apex result in the leaf primordia. The pattern of leaf arrangements about the shoot (the **phyllotaxy**) may be alternate, decussate, or spiral. This pattern is hypothesized to be established by the upward movement of auxin, movement which is blocked at the site of an existing leaf primordium, and which directs auxin to parts of the PZ that do not have an underlying leaf bud. (fig. 16.31 and 19.15)

The development of axes of the shoot and leaf, and the leaf's planar form is described as follows:

- **Shoots**: The long axis of the shoot describes the **basal to apex axis**. The attachment or transition point between the stem and root is termed the **base**. (Web essay 19.2)
- **Leaves**: Unlike shoots, leaf structure is committed and determinate. The leaf has a **proximal to distal axis**, extending from the base (where it joins the **petiole** or stalk) to the tip. The leaf also has a second axis, the **lateral axis**, running from one edge or margin through the midrib to the opposite edge or margin. A third axis is defined by the **adaxial-abaxial axis** (also called **dorsiventral axis**). **Adaxial** refers to the upper (dorsal) surface that typically faces the stem, whereas **abaxial** refers to the lower (ventral) surface that typically faces away from the stem (at least for leaves that are tilted slightly upward). (fig. 16.33) The adaxial and abaxial surfaces of the leaf are differentiated from each other. Spatially regulated gene expression controls the leaf pattern. MicroRNAs regulate the sidedness of the leaf... Branch roots and shoots have different origins... The phenomenon of apical dominance, in which the growth of the terminal bud suppresses the growth of the axillary bud, is primarily regulated by auxin derived from the terminal bud.

Senescence And Programmed Cell Death

Necrosis is abnormal death usually brought about by physical damage, poisons, or other external injury, etc. However, **senescence** is a normal, energy-dependent process under genetic control. Senescence involves an ordered biochemical and cellular sequence... (details omitted) Senescence is frequently associated with **abscission**.

There are various types of senescence:

- Monocarpic senescence: Abrupt yellowing and death of the entire plant after fruiting (e.g., maize, soybeans).
- Senescence of aerial shoots in herbaceous perennials (e.g., Trillium erectum)
- Seasonal leaf senescence (leaves die in the fall)
- Sequential leaf senescence (leaves die at a certain age)

- Senescence of fleshy or dry fruits
- Senescence of storage cotyledons (which nourish the embryo) and floral organs.
- Senescence of specialized cell types (tracheids, vessel elements, etc.)

Programmed cell death (**apoptosis**) is a specialized type of senescence. It is a program inherent in individual cells, and is less well understood in plants than in animals.

Formation of local necrosis can be a programmed response seen when a pathogenic organism attacks a plant. The plant locally accumulates phenolic compounds that cause a necrotic lesion. This lesion isolates the pathogen and prevents the spread of infection. Such a response is called a **hypersensitive response**, and has been shown to be a genetically programmed process.

Chapter XVII. Phytochrome And The Light Control Of Plant Development

[Partial summary]

Plant seedlings grown in the dark exhibit **skotomorphogenesis** (skoto = darkness) and are said to be **etiolated**. Etiolated (Fr., "blanched") seedlings have the following features:

- **Monocots**: absence of greening, reduction in leaf width, failure of leaves to unroll, and elongation of the **mesocotyl** (part of stem) and **coleoptile** (first, protective leaf)
- **Dicots**: absence of greening, reduction in leaf size, hypocotyl elongation, and maintenance of the **apical hook** (folded cotyledons).

When seedlings emerge into the light, they transition from the dark-adapted form to a PS-capable form via **photomorphogenesis**, a complex but rapid process which includes stem growth slowing, straightening of the apical hook, and synthesis of PS pigments including Chl. This change is signaled by exposure to light, especially red light. The most important plant photoreceptors absorb red and blue light. Phytochrome, which in its two isomeric forms absorbs **red light (RL)** and **far-red light (FRL)**, plays the key role in light-regulated (photomorphogenetic) vegetative and reproductive development. In etiolated seedlings, it comprises about 0.2% of total extractable protein, about 50 times more concentrated than in mature green tissues. "Although phytochrome is an important plant pigment, it occurs in very low concentrations and is **not visible** unless chemically purified."[165] Phytochrome is an intracellular photoreceptor, not a hormone. Phototropism responses (coleoptile bending, etc.) are blue light responses and not typically phytochrome mediated (see chapter 18).

Phytochrome Biochemical and Photochemical Properties

Phytochrome is a blue protein pigment of 125 kDa size. A key discovery was that the effects of RL (650 to 680 nm) on morphogenesis in **germinating seedlings** (such as lettuce, fig. 17.2) and **floral induction** could be **reversed by subsequent exposure to FRL** (710 to 740 nm, also called **near-infrared**). This phenomenon led to the one-pigment model in which the pigment was postulated to be **photoconvertible** between two forms, **Pr and Pfr**. (see web topic 17.1).

Phytochrome is composed of a 250 kDa **dimer**. Each of the two 125 kDa monomer subunits (the **holoprotein**) consists of a 125 kDa polypeptide (the **apoprotein**) bound to a single "color-bringing" **chromophore** pigment molecule by a thio-ether linkage. The chromophore is a linear tetrapyrrole, **phytochromobilin**. (A pyrrole is a 5-membered heterocylic aromatic ring containing 1 N and 4 C.) The **phytochromobilin** is synthesized in plastids and is derived from heme. The double bond starting at the C-15 position of phytochromobilin undergoes a cis-trans isomerization in conversion between Pr (cis-form) and Pfr (trans-form) isomers.

Pr: The inactive isomeric form of phytochrome is **Pr**, which is synthesized when plants are grown in the dark. It absorbs preferentially red light with peak absorbance at 666 nm (fig. 17.3) and when concentrated appears **blue**. It has minimal absorbance of FRL, so that when phytochrome is saturated with FRL, there is 97% Pr and 3% Pfr.

[165] Phytochrome visibility: http://science.jrank.org/pages/5305/Plant-Pigment-Phytochrome.html

Pfr: This isomeric form when concentrated appears **green** in color, and is **the active form**. It absorbs at a peak of **730 nm** (in far red) but also absorbs some in red light, therefore preventing complete conversion of Pr to Pfr. Because of this absorption spectrum overlap, when phytochrome is exposed to saturating amounts of RL, there is 85% net conversion to Pfr and 15% is Pr, the "photostationary state".

Phytochrome also absorbs somewhat in the blue range, and BL also results in conversion of Pr to Pfr. But the contribution of phytochrome (as opposed to other blue-sensitive photoreceptors such as cytochrome) to blue-light exposure can be determined by the degree to which the effect is reversible by FRL.

When the dimer Pr is exposed to RL and activated, there is a conformational change with partial closure at the hinge in each monomer's apoprotein (fig. 17.8), and cis to trans isomerization in the chromophore (details omitted). This mechanism has been studied in the bacterium Deinococcus radiodurans, though it has biliverdin instead of phytochromobilin as the chromophore. Phytochrome is a protein kinase, and light activation causes **autophosphorylation** of the apoprotein. Once activated, phytochrome migrates from the cytosol to the nucleus, where it interacts with transcriptional regulators to regulate gene expression. (details omitted) Phytochrome-like light-dependent kinases can be traced back to bacteria... Some actions of phytochrome are so rapid (seconds to minutes) that they must take place in the cytosol.

There are different forms of phytochrome, light-labile **Type I** and light-stable **Type II**. In Arabidopsis, phytochromes are encoded by 5 different genes, PHYA, PHYB, PHYC, PHYD, and PHYE... PHYA encodes light-labile Type I phytochrome, whereas PHYB encodes the most abundant light-stable phytochrome... (details omitted; the text does not describe the specific chemical differences among these 5 forms.)

Genetic analysis of Phytochrome function (in Arabidopsis):
- Phytochrome A mediates responses to continuous FRL. In particular, VLFR and FR-HIR responses (see below and fig. 17.3; details omitted).
- Phytochrome B mediates responses to continuous White light or RL. Specifically, certain R-HIR and LFR responses (see below and fig. 17.3; details omitted).
- Roles for Phytochromes C, D, and E are not yet worked out (details omitted).
- Phytochrome gene functions have diversified during evolution (details omitted).

Phytochrome Signaling Pathways (details omitted)
- Phytochrome regulates membrane potentials and ion fluxes, and these changes can occur rapidly in seconds (details omitted).
- Phytochrome regulates gene expression in minutes or longer (details omitted). Effects include greening promotion, and inhibition of other genes. About 10% of the Arabidopsis genes are affected by phytochrome.
- Phytochrome interacting factors (details omitted).
- Phytochrome associates with protein kinases and phosphatases (details omitted)...
- Phytochrome remote effects [within the cell] may involve a 2nd messenger, possibly calcium. [MCM: I did not identify any direct-acting phytochrome effects that occur outside the cell in which phytochrome is photoconverted—such remote actions are apparently performed by other messengers.]

Phytochrome Biological Responses Categorized By Fluence And Irradiance

Mentioned above are the effects of RL on germinating seedlings and floral induction, and that these effects could be reversed by subsequent exposure to FRL. Other phytochrome-related photoreversible phenomena (with example plants and stages from multiple, table 17.1) include:
- promotes de-etiolation (oat seedling)
- promotes formation of leaf primordia (mustard seedling)
- inhibits internode elongation (Pisum adult)
- inhibits flowering (Xanthium adult)
- enhances Chl accumulation (Pinus seedling)
- promotes growth (the fern Onoclea gametophyte)
- promotes replications of plastids (the Bryophyte Polytrichum germling)
- promotes directional orientation of chloroplasts to optimize light capture (the alga Mougeotia gametophyte, see web topic 17.4).

The responses to Pfr include rapid and slower effects, and are subdivided as follows, based on **total fluence** (total photons expressed as µmol m^{-2}), and **the rate of fluence** or **irradiance** (expressed as µmol photons m^{-2} $s^{-1)}$.
Although FRL can reverse the effects of exposure to RL, after a certain period of time the reversal no longer occurs—this is "escape from photoreversibility".

Very Low Fluence Responses VLFRs: These can be initiated by fluences of RL as low as 0.0001 µmol m^{-2} (1/10 of the amount of light emitted by one firefly flash), and are independent of the rate of exposure. These include the de-etiolation response in the oat coleoptile and mesocotyl, and the stimulation of Arabidopsis seed germination by RL (0.001 µmol m^{-2} to 0.1 µmol m^{-2}). These levels of light exposure convert < 0.02% of Pr to Pfr, and the effect is **not photoreversible** (since there is always at least this amount of Pr). Seeds close to the surface and therefore positioned for optimal germination would benefit from this response arising from the very low level of RL reaching them. Interestingly, it has been shown that tilling fields in the darkness of night leads to less germination of preexisting underground weed seeds, apparently because even transient exposure to sunlight during tilling activates them to germinate. (Web essay 17.1) The action spectrum for VLFRs matches the absorption spectrum for Pr, confirming that the Pfr which results is the active form.

Low-Fluence Responses LFRs: These are **photoreversible** and occur with exposures of RL > 1 µmol m^{-2} and saturate at about 1000 µmol m^{-2}. They include lettuce seed germination, regulation of leaf movements, etc. The action spectrum for Arabidopsis reveals a peak response at 660 nm and a peak of inhibition at 720 nm, consistent with Pr and Pfr absorption peaks, respectively. (fig. 17.5)

High-irradiance responses HIRs: These responses require a high exposure rate (not just a high total photon exposure), are proportional to the irradiance and the duration, and are **not photoreversible**. These responses include synthesis of anthocyanin in various dicot seedlings, induction of flowering in henbane, enlargement of cotyledons in mustard, and production of ethylene in sorghum. (Table 17.2) The action spectrum peak at 720 nm (fig. 17.6 and web topic 17.2) in the FRL part of the spectrum for dark-grown lettuce seedling hypocotyl elongation inhibition, which is attributable to phytochrome, is due to a photoequilibrium of Pr and Pfr at FRL. (Other peaks at BL and UVA are attributable to cryptochrome CRY1 and CRY2 effects).

Circadian Rhythms

[Very limited summary here, but see also Chap 25.]
Concepts of endogenous oscillator, entrainment, temperature compensation are mentioned. In Arabidopsis, CCA1 and LHY are components of the circadian clock. TOC1 is another important clock gene. See fig. 17.16 for a model of circadian oscillator... Phytochrome plays an important role in **entraining the circadian clock**.

Ecological Functions

[Limited summary here]

Nyctinasty: Phytochrome regulates the sleep movements of leaves (nyctinasty, such as drooping of leaves or closing of petals), for instance in Mimosa pudica and other legumes. This change in leaf angle is caused by rhythmic changes in the cells of the pulvini at the bases of the leaf petioles. These changes are caused by ion fluxes of K^+ and Cl^- involving the flexor and extensor motor cells of the pulvinus. The leaves are open when the ventral (abaxial) motor cells are turgid (and the dorsal cells are flaccid), and closed when the dorsal (adaxial) motor cells are turgid (and the ventral cells are flaccid). (fig. 17.19) Leaf closure is stimulated by RL followed by darkness, and this response is cancelled by FRL. The effect of phytochrome arises from regulating the activities of the primary proton pumps and the K^+ channels in these motor cells.

Adaptation To Light Quality Changes of Sun-Seeking Plants: Some plants respond to the ratio of red light RL (660 nm) to far red light FRL (730 nm). This ratio R:FR is 1.2 in daylight, 1.0 at sunset, only **0.13 under a foliage canopy of ivy**, 0.88 in soil at 5 mm, etc. (Table 17.3). Sun-seeking plants (growing normally in open fields) can detect by the low ratio of R:FR that they are being **shaded by other plants**,

and exhibit a **shade avoidance response** by growing taller stems (with a greater internode elongation rate) usually at the expense of reduced leaf area and branching. This behavior is not seen in shade-tolerant plants, which expect to grow in shaded environments. Phytochrome is thus important in shade detection. (See fig. 17.21 for a difficult-to-understand explanation for how phyA and phyB interact in this response, also Essay 17.3) Understanding the mechanisms for shade avoidance and growth inhibition has helped commercial manipulation of phytochromes, so that plants such as maize have been made more tolerant of shading, allowing for higher crop yields arising from higher planting density.

Germination Effects: Small seeds may be inhibited from germination even when moist if they are exposed to light with low R:FR ratio—they effectively sense that they will not succeed when strongly shaded by the foliage of other plants. (They also sense if they are buried so deep as to be in darkness, and thus unlikely to succeed upon germination.) Germination of larger seeds is less affected by shading plants, because they have more reserve with which to send up longer shoots. The inhibitory effect of low R:FR ratio on germination of small seeds (such as the trumpet tree) can be reversed by filtering out the FRL, indicating that it is the detected ratio that is inhibitory and not just the low absolute amount of RL involved, since the RL remains low with FRL filtering.

Phytochrome effects are important early in germination... PhyB mediates the de-etiolation of a seedling emerging from darkness into open sunlight (in which the R:FR ratio is relatively high). However, PhyA mediates the de-etiolation of a seedling emerging from darkness into canopy shade (in which the R:FR is low). "Because phyA is labile, however, the response is taken over by phyB... In switching over to phyB, the stem is released from growth inhibition ... allowing for the accelerated rate of stem elongation that is part of the shade avoidance response". [MCM: This explanation and the accompanying diagrams of fig. 17.21 are quite confusing and I doubt I've understood it properly.]

Modulating Effects—Cryptochrome and Phototropin: cry2 mutants promote flowering in blue light by repressing phyB function... CRY1 and CRY2 interact with phyA... (details omitted)

Chapter XVIII. Blue-Light Responses: Stomatal Movements And Morphogenesis

[Limited summary]

Blue light responses (BLR) include **phototropism** (the turning of plants toward light), sun tracking by leaves, stomatal aperture changes, and the chloroplast movement in response to photon fluxes. Other BL responses include inhibition of hypocotyl elongation, stimulation of Chl and carotenoid synthesis, activation of gene expression, phototaxis of bacteria or algae, enhancement of respiration, and anion uptake in algae. Some BL responses can be detected within seconds.

BL responses (BLR) can be distinguished from phytochrome responses by the degree to which they are not reversible with FRL, and from PS responses by determining to what extent the effect is not caused by RL (which stimulates PS but not BLR). Many BL responses of plants share a similar "three-finger" action spectrum from 400 - 500 nm (fig. 18.1) with highest peak at 440 nm.

Photophysiology of Blue Light Responses

Phototropism is seen in prokaryotes, fungi (including the sporangiophore of the mold Phycomcyes), ferns, and higher plants. Useful experimental models include dark-grown etiolated plants, such as corn coleoptile or Arabidopsis seedlings, that are exposed to unilateral BL. Unequal light fluence on the growing coleoptile of grass causes a bending of the coleoptile toward the light due to an asymmetrical growth rate.

Plants sense the direction of the BL by sensing the **gradient of transmitted BL** across the diameter of the seedling etc... (fig. 18.5, details omitted)

Blue light rapidly inhibits stem elongation in etiolated seedlings. The peaks in the blue light (400 to 500 nm) region of the action spectrum cannot be explained by phytochrome. The inhibition of hypocotyl growth from BL can be detected in 15 to 30 seconds, whereas the phytochrome inhibition of elongation

requires 8 to 90 minutes. BL causes a transient **depolarization** of the membrane of hypocotyl cells (fig. 18.6) that precedes the inhibition of growth rate—the depolarization is caused by activation of anion channels that allow efflux of anions such as Cl^-.

Blue light regulates gene expression of genes involved in several processes... (details omitted)

Blue light stimulates stomatal opening in guard cells. The response is rapid and reversible, and is localized to the guard cell. This response takes place throughout the life of the plant. Stomata of well-watered plants open as levels of PAR light reaching the leaf increase, and close with corresponding decline of PAR light (fig. 18.9) Although the guard cell has PS capability, there is a non-PS component of light-dependent stomatal opening. This can be shown by saturating the PS response using RL, and then demonstrating a further opening of the stomatal aperture when BL is added (fig. 18.10). Isolated guard cell **protoplasts swell when exposed to BL**. **BL activates a proton pump at the guard cell PM**, leading to greater acidification of the medium in which guard cell protoplasts are suspended. The acidification results from **BL-activated proton-pumping H^+-ATP-ase** in the guard cell PM. The BL responses of the stomata is a sensor of photon fluxes reaching the guard cell. There is a 25 second lag time in onset BL responses, a delay needed for signal transduction, and also a delay in termination of the response after BL ceases... (fig. 18.14) **BL regulates the osmoregulation of guard cells.** When stomata open, the intracellular [K^+] increases from 100 mM to 400 to 800 mM. These changes are electrically balanced by influx of **Cl^-** and/or **malate$^-$** influx or synthesis (web topic 18.1). When guard cells close, Cl^- is extruded and malate$^-$ is metabolized or extruded... The starch grains in guard cell chloroplasts are hydrolyzed during opening (raising the osmotic pressure, lowering the osmotic potential)—chloroplast starch synthesis increases during closing. **Sucrose is the dominant (but not the only) osmotically active solute producing guard cell osmoregulation**. Possible osmotically active solutes in guard cells include (fig. 18.15):

- **K^+** and **Cl^-** uptake coupled to **malate$^-$** biosynthesis.
- **Sucrose** from starch hydrolysis in chloroplast
- **Sucrose** from PS in chloroplast
- **Sucrose** uptake from mesophyll PS

K^+ may have the greatest aperture opening effect at sunrise, before PS has begun—it peaks at c. 9:30 AM and has a predominant aperture opening effect in the morning (fig. 18.16). Sucrose coordinates stomatal aperture in the epidermis with rates of PS in the mesophyll. Sucrose peaks in the late afternoon, and mediates aperture control in the afternoon. The aperture closing curve closely parallels the decline in sucrose concentration.

The Blue Light Photoreceptors: Cryptochromes, Phototropins, And Zeaxanthin

There are at least 3 photoreceptors for BL: **cryptochromes, phototropins, and zeaxanthin**! [MCM: These are intracellular photoreceptors, not hormones—the text does not indicate any secretion of these substances.]

Cryptochromes: The relevant cryptochrome gene is **HY4** which encodes a 75 kDa protein homologous to microbial DNA **photolyase** (a BL-activated enzyme which repair UV damage in microbes). The **HY4 protein** was renamed **cryptochrome 1** (**CRY1**). Over-expression of CRY1 enhances sensitivity to BL. A second gene product homologous to CRY1 is **CRY2**, also found in Arabidopsis, but ubiquitous in the plant kingdom. CRY2 is rapidly degraded in light, whereas CRY1 is stable in light exposure. (Detailed discussion of CRY1 and CRY2 effects omitted.) CRY1 and CRY2 interact with COP1 and with phyA. Cryptochromes inhibit stem and hypocotyl elongation, stimulate anthocyanin synthesis (fig. 18.17), and are involved in control of flowering and circadian rhythms.

Phototropins 1 and 2 (PHOT1 and PHOT2): are flavoproteins encoded by **phot1** and **phot2**. These are autophosphorylating protein kinases stimulated by BL. The PHOT1 protein includes a C-terminal kinase and an N-terminal binding to FMN. PHOT2 functions at high light fluence rates...PHOT1 **inhibits stem elongation** in the first 30 minutes (after a lag of 30 sec), whereas CRY1 and to a limited extent CRY2 take over after 30 minutes. Phytochrome also plays a role in this. Phototropin plays no role (or ? a small role) in stomatal BL response.[TZ459,463] However, phototropins are the photoreceptors for the BL signaling pathway that induces phototropic bending in Arabidopsis hypocotyls and oat coleoptiles.[TZ498]

Zeaxanthin: Zeaxanthin is a xanthophyll which protects PS pigments from excess excitation (see earlier discussions). **Guard cell zeaxanthin plays a central role in regulating stomatal opening**. Its concentration in GCs closely follows incident solar radiation at the leaf surface. The absorption spectrum of zeaxanthin closely follows the action spectrum for BL-stimulated aperture opening. The content of zeaxanthin in GCs closely follows incident BL radiation and the stomatal aperture (except that it is disproportionately high in guard cells in the early morning and early evening) (fig. 18.20). BL sensitivity of guard cells increases as a function of GC zeaxanthin concentration... BL-stimulated stomatal opening is inhibited by DTT, which also inhibits formation of zeaxanthin. (This confirms that zeaxanthin is required for stomatal response to BL.) In certain facultative CAM plants, the plant while in the C_3 phase shows accumulation in the stomata of zeaxanthin and a BL response, whereas with CAM induction the GC no longer accumulate zeaxanthin and show no response to BL.

Stomata in plants lacking phot1/phot2 have a small but definite BL response, so that phototropin cannot be the sole BL receptor. The role of phytochrome is uncertain... (details omitted)

Regulation of BL-stimulated stomatal opening (details omitted)

Green light reverses BL-stimulated stomatal opening under certain conditions and species (details omitted, fig. 18.25, web Essay 18.3). The mechanism is not yet elucidated, perhaps another type of photoisomerization, perhaps of the carotenoid zeaxanthin, in which blue light converts a physiologically inactive isomer to an active isomer, and in which green light converts it to the inactive form...

The xanthophyll cycle confers plasticity to the stomatal responses to light... Acidification of the chloroplast thylakoid lumen pH stimulates zeaxanthin formation, and lumen alkalinization favors violaxanthin formation. Lumen pH depends on incident PAR and PS activity and the rate of ATP synthesis that dissipates the pH gradient. Thus "photosynthetic activity in the GC chloroplast, lumen pH, zeaxanthin content, and BL sensitivity play an interactive role in the regulation of stomatal apertures." GCs have high concentrations of PSII, favoring lumen acidification at low photon fluxes, potentially explaining zeaxanthin formation in the GC chloroplast early in the day. Sensing of CO_2 by the GC chloroplast is also involved... (details omitted)

Chapter XIX. Auxin: The Growth Hormone

This chapter begins the discussion of specific chemical messengers (**plant hormones**) which facilitate communication between different parts of the plant—an idea which originated with Julius von Sachs. Hormones act on specific protein receptors, and may be **endocrine** (acting on sites distant from the source) or **paracrine** (acting on adjacent or nearby cells). The major plant hormones are auxins, gibberellins, cytokinins, ethylene, abscisic acid, and brassinosteroids. Other plant hormones (including extracellular signaling molecules and modulators of signal transduction pathways) include jasmonic acid (**jasmonates**), salicylic acid, certain polypeptides such as systemin, nitric oxide, certain other members of the classes of flavonoids and polyamines, and an unnamed carotenoid cleavage product.[TZ468,166]

Auxin was the first growth hormone discovered. Like cytokinin, it is required for viability. Early evidence for the existence of auxin included:

- Charles and Francis Darwin's 1881 demonstration that the coleoptile tip is sensitive to blue light but that the bending that results occurs several millimeters away in the growing zone of the coleoptile (a region that is not itself sensitive to BL).
- Experiments by Frits Went 1926 showing a growth-promoting chemical in coleoptile tips that would remain in an active form when diffused into gelatin blocks.

The growth substance was named **auxin** ("to grow").

Biochemistry, Biosynthesis and Metabolism of Auxin

The principle and most common auxin in higher plants is **indole-3-acetic acid (IAA)**, and auxins may be defined as compounds having biological actions similar to IAA. Auxin actions promoting growth are

[166] Other plant hormones: http://en.wikipedia.org/wiki/Plant_hormone#Other_known_hormones

discussed below—they include promotion of elongation in coleoptiles and stems, of root elongation (in low concentrations), of cell division in callus formation, and of adventitious roots (i.e., those roots arising from detached leaves and stems, etc.) Many other natural and synthetic compounds also have auxin activity (see web topic 19.1), including 4-Cl-IAA, **Indole-3-butyric acid** (**IBA)**, and the herbicides **2,4-D**, **2,4,5-T** and **dicamba**. The structural configuration found to be associated with auxin activity is discussed in topic 19.2.

Auxin activity at physiological concentrations may be assayed with mass spectrometry or radioimmunoassay (**enzyme-linked immunosorbent assay ELISA**, see web topic 19.3)—these have largely replaced bioassays.

Auxin is found primarily in shoot apical meristems, young leaves, root apical meristems (though dependent on auxin transport from SAMs), and young fruits (origin of this is unclear).

In early Arabidopsis leaves, auxin initially accumulates in the leaf tip, and gradually shifts to the leaf margins (at developing **hydathodes**, the primordial sites of guttation, and adjacent leaf vessels, fig. 19.5) and finally the leaf base, corresponding with the basipetal maturation sequence. This progression is visually and elegantly demonstrated using the versatile and widely used **GUS (β-glucuronidase) reporter gene construct**. The GUS gene is fused to a DNA promoter sequence (**DR5** in at least some cases) that in this case responds to auxin^{TZ490}. When free auxin reaches a threshold level, GUS expression occurs and blue staining is observed. (The most common chromogenic GUS substrate for histochemical staining is 5-bromo-4-chloro-3-indolyl β-D-glucuronide, whose reaction product has a visible blue color.)[167]

There are multiple pathways for the biosynthesis of IAA. Most are **tryptophan-dependent** (involve **tryptophan (Trp)** as an intermediate), but there is also a tryptophan-independent pathway which derives from the tryptophan precursor, **Indole-3-glycerol-phosphate (IGP)** (see web topic 19.4). The major pathways are:

- **IPA Pathway**: IGP → **Indole Pyruvic Acid (IPA)** → Indole-3-acetaldehyde (IAAId) → IAA
- **TAM Pathway**: Tryptophan (Trp) → **Tryptamine (TAM)** → … → Indole-3-acetaldehyde (IAAId) → IAA
- **IAN Pathway**: Tryptophan (Trp) → IAOx N-oxide → … **Indole-3-acetonitrile (IAN)** → IAA
- **IAM Pathway**: Tryptophan (Trp) → **Indoleacetamide (IAM)** → IAA

In plants, the TAM and IPA pathways predominate, but the IAN pathway is found in some plants (Brassicaceae, Poaceae, Musaceae, and others) and the IAM pathway is found in certain pathogenic bacteria.

Seeds and storage organs such as cotyledons contain large amounts of covalently bound (and therefore hormonally inactive) auxin. In many cases, the active hormone may be released by hydrolysis, so that the various bound forms often serve as reversible storage for auxin. The conjugated auxins may be stored as either low MW esters (bound to methyl, glucose, myo-inositol, Ala, Leu, and amides) or high MW conjugates (bound to glucans, peptides, and glycoproteins). IBA, itself an auxin, may also be converted to [presumably more active] IAA in the peroxisome.

There are multiple pathways for catabolism (degradation) of auxins, leading for example to OxIAA bound to Glucose or Asp, and a photo-oxidation pathway also appears to exist (details omitted).

Auxin is found in both the chloroplast (where Trp is synthesized) and the cytosol. "In addition to IAA metabolism, its compartmentation in the choroplast and transport … also play important roles in regulating the level of free IAA." (see web topic 19.5)

Transport of Auxin

[MCM: There appears to be some ambiguity as to whether the terms *polar flow* or *polar transport* can be applied to phloem flow that is moving in a polar direction. For the most part in the discussion that follows, *polar flow* or *polar transport* refers to cell-to-cell non-phloem flow.]

[167] GUS reporter system: http://en.wikipedia.org/wiki/GUS_reporter_system

Polar Transport (Cell-To-Cell)

The apex to base polarity of plants is maintained by polarity of auxin transport ("**polar transport**"). In oat coleoptile sections as well as vegetative stems and leaf petioles, IAA moves predominantly **basipetally** from shoot apex to the shoot base. Polar auxin transport is seen in other plants including bryophytes and ferns, and even Devonian gymnosperms.

Some transport of auxin takes place in the **phloem**, accompanying the source to sink translocation of sugars. Phloem flow can contribute to polar-like transport—e.g., the **acropetal** movement from larger roots through the root stelar tissues to the growing root tip (see below). However, more polar transport of auxin is cell-to-cell.

Polar transport of IAA auxin requires energy and is gravity independent. Placing a grape stem cutting upside down does not affect auxin flow direction or polarity and therefore does not affect the ends at which adventitious roots and shoots form. (fig. 19.11) Polar transport proceeds mostly by **cell-to-cell transmembrane transport** (with lesser amounts of phloem-based symplastic flow), so that cells experience auxin efflux and influx/uptake and require metabolic energy to perform this polar transport. The linear velocity of polar non-symplastic transport flow is faster than diffusion but slower than phloem translocation: 2 to 20 cm h^{-1} compared to phloem translocation rates of 30 to 150 cm h^{-1} as quoted in Chapter 10. The specificity of this non-phloem flow indicates that it depends on specific PM protein carriers.

The polar flow of auxin largely occurs in vascular parenchymal tissues, most likely the living supporting tissues surrounding the dead vessels of the xylem (except in grass coleoptiles, which have nonvascular parenchymal auxin flow)... Import of auxin into the vascular parenchyma polar transport stream requires the uptake carrier **AUX1**. (details omitted, see fig. 19.13)

The chemiosmotic model is proposed to explain auxin polar transport. Auxin uptake (influx) is driven by the proton motive force (the stored energy from the H^+ gradient), while efflux is driven by the membrane potential. The influx of IAA occurs by diffusion of the protonated form IAAH, or by secondary active transport of the anionic form IAA^- via a PM $2H^+$-IAA^- symporter. (details omitted)

The efflux carriers are positioned at the ends of the conducting cells and transport IAA^- by means of the membrane potential... Membrane **PIN proteins** are important parts of the efflux carrier complexes, and their locations on the PM confer the directionality on polar flow. PIN proteins work synergistically with the ATP-dependent auxin-transporting **P-glycoproteins** (**PGPs**, which are integral proteins that are more uniformly distributed on the PM)—together, these direct the flow of auxin in cell to cell transport (details omitted, see fig. 19.15).

Certain synthetic and natural inhibitors of auxin transport block auxin influx or efflux—e.g., NPA, TIBA, 1-NOA, quercetin, and genistein (details omitted).

Expression of genes encoding auxin transport is regulated in response to developmental and environmental factors, including auxin itself. Efflux and influx are also regulated by post-transcriptional processes... (details omitted; see Web essay 19.1 "Exploring the Cellular Basis of Polar Auxin Transport").

Polarized auxin transport is essential for normal plant development, including the basic root-shoot polarity—mere diffuse presence of auxin is not sufficient. (See web essay 19.2 "Apical Basal Polarity is Maintained in Mature Plants")

Non-Polar Transport (In Phloem)

Auxin from leaves moves in the phloem to the rest of the plant in a non-polar manner, and at a higher velocity than that of cell-to-cell polar transport. This auxin flow is important for controlling cambial division, callose accumulation or removal, and branch root formation. Polar (non-phloem) flow and phloem flow of auxin are not entirely independent of each other, as "auxin can be transferred from the nonpolar phloem pathway to the polar transport pathway"[TZ483].

Actions of Auxin

Cell Elongation

(See also Chapter 15) Auxins promote growth in stems and coleoptiles (while at the same concentrations inhibiting growth in roots). A steady supply of auxin arriving at the subapical region of the stem from the shoot apex is required for continued elongation of stem cells and coleoptiles. Merely spraying a plant with auxin does not have the same effect. The concentrations of auxin to achieve optimal stem growth are c. 10^{-6} to 10^{-5} M. These levels inhibit root growth, but much lower concentrations of c. 10^{-10} to 10^{-9} M favor root growth. Excessive concentrations of auxin above 10^{-4} M induce ethylene biosynthesis causing inhibition of stem growth in many species.

Auxins act on the outer tissues of dicot stems (outer cortex and epidermis) to increase elongation rate. The epidermal cells of roots are also the likely target of auxin. (details omitted) Auxin induced growth has a lag time of c. 10 minutes, and a maximal growth rate increase is achieved at c. 30 to 60 minutes. Auxin causes a 5X to 10X increase over the basal rate of growth. This growth stimulation requires energy and can be reduced with metabolic inhibitors, etc.

Auxin rapidly increases the rate of cell elongation, primarily by **increasing the extensibility of the cell wall** (the factor **m** in the equation $GR = m[\Psi_p - Y]$, see Chapter 15). This increase is mediated by increased hydrogen ions according to the cell wall acid growth hypothesis, is aided by **expansins** (which break hydrogen bonds between polysaccharide components of the CW), and can be inhibited by neutral buffers (details omitted). Fusicoccin activation of the PM H^+-ATPase increases cell elongation (web topic 19.7). Auxin-induced proton extrusion may involve both activation of existing and synthesis of new PM H^+-ATPases. (details omitted) Longer term auxin effects for cell elongation include uptake of solutes and synthesis of polysaccharides and proteins associated with acid-induced wall loosening.

Control Of Plant Orientation

Three guidance systems or tropisms control plant orientation (shoot, roots, etc.) in response to directional stimuli. **Tropisms** are directional movements resulting from **differential growth**, and which correlate in direction—positively or inversely—with the direction of the directional stimulus.[168] [**Nastic** movements,[169] in contrast, are (often rapid) responses to non-directional stimuli such as heat or darkness, or responses for which the direction of the response is not dependent on the direction of the stimulus. Examples include the rapid movements of sensitive mimosa or Venus flytraps, and the nutational movements of sunflower or morning glory twining.] The three major tropisms are:

- **Phototropism**: Bending movement toward or away from directional light (as opposed to responses to diffuse non-directional low or high intensity light)
- **Gravitropism**: Reorientation of growth so that primary roots grow downward into the soil and shoots grow upward away from the soil.
- **Thigmotropism**: Growth in response to touch or contact, which enables plant shoots and roots to grow around obstacles, or climbing plants to wrap tendrils around supporting structures.

Phototropism

Phototropism is mediated by the **lateral movements and redistribution of auxin** (IAA). See Darwin's observations above. This and root inhibition are the best studied of the auxin effects. The tip of the coleoptile is light-sensitive, and when exposed to light falling on one side, auxin is preferentially transported in the tip to the shaded side (i.e., not just basipetally). **Phototropins 1 and 2** (PHOT1 and PHOT2) are the photoreceptors for the BL signaling pathway that induce lateral auxin movement and phototropic bending in Arabidopsis hypocotyls and oat coleoptiles (TZ p. 498 and Web essay 19.3). These are autophosphorylating protein kinases stimulated by BL. The fluence response curves to 450 nm blue

[168] Tropism: http://plantsinmotion.bio.indiana.edu/plantmotion/movements/tropism/tropisms.html
[169] Nastic movements: http://plantsinmotion.bio.indiana.edu/plantmotion/movements/nastic/nastic.html

light for phototropism bending (web topic 19.8) vary with fluence rate, exhibiting two peaks with a low-response "neutral zone" in between.

"At relatively low fluence rates (0.1 or 0.2 µmol m^{-2} s^{-1}), the fluence-response curve for phototropic stimulation ... is essentially the same as that previously described for A. thaliana seedlings... with a single maximum (peak I) at about 0.3 µmol m^{-2}... At ... higher fluence rates (0.4 or 0.7 µmol m^{-2} s^{-1}), the response in the so-called "first positive" region of phototropism is characterized by two distinct peaks, peak I, at about 0.3 µmol m^{-2}, and peak II, at about 3.5 µmol m^{-2}"[170]

Corresponding with this bimodal pattern, the gradient in phototropin phosphorylation arising at fluences in the first positive curvature of the fluence response curve appears to induce the auxin movement in the tip to the shaded side. At fluences in the second positive curvature, PHOT2 serves as an additional photoreceptor—phosphorylation of PHOT2 increases at the base (not the tip) on the irradiated side, again leading to a lateral movement of auxin. Once auxin has reached the shaded side, it is transported basipetally to the elongation zone on that side where it stimulates differential (asymmetrical) growth, causing bending toward the light (further details omitted). The increase of auxin on the shaded side can be made visible with the DR5::GUS reporter gene construct (see above). The shaded side of the elongation zone is more acidic, consistent with the acid-growth hypothesis.

Gravitropism

Gravitropism also involves lateral redistribution of auxin. In coleoptiles oriented horizontally, the auxin is transported "laterally" (i.e, down) to the lower side, stimulating differential growth that turns the coleoptile upward. The perception of orientation and lateral redistribution of auxin occurs not just at the tip. In soybean hypocotyls, gravitropism causes accumulation of SAURs (small auxin up-regulated RNAs) in the lower half of horizontal seedlings, which precedes the differential growth that follows (web Topic 19.9). However, asymmetry of auxin distribution in SAMs is harder to demonstrate due to auxin recirculation.

Dense plastids serve as **gravity sensors**. These are special **amyloplasts** (also called **statoliths**), which being denser than the cytosol tend to gravitate to the bottom of the **statocyte** cells in which they are found (fig. 19.30). Shoots and coleoptiles perceive gravity in the **starch sheath** surrounding the vascular tissues of the shoot ... (details omitted) Roots perceive gravity in the **root cap**—large amyloplasts are found in the statocytes of the columella of the root cap. Other mechanisms of gravity perception not involving starch may also exist, perhaps involving the actin network of the cytoskeleton (see also web topic 19.10) ...

Once malalignment with respect to gravity is sensed, pH and calcium may serve as second messengers... (details omitted).

In roots, auxin synthesized in the shoot is transported in the stele to the root cap, where it is redistributed laterally (the root cap also plays a protective function). If the (primary) root is vertical, the distribution is symmetrical, but if the (primary) root is more horizontal, the cap supplies an inhibitor thought to be IAA (auxin) preferentially to the lower side of the root (the distal elongation zone). This inhibits growth there and causes differential elongation of the up side, causing the root to turn downward. ABA is thought to play a minor role in roots. Pin protein PIN3 plays a role in this root reorientation. The asymmetry of auxin can be made visible with the DR5::GRP reporter gene construct (fig. 19.35)

Thigmotropism

Thigmotropism causes shoots and roots to bend around obstructions, overriding the direction of growth defined by gravitropism, and tendril twining, but the role of auxin is unclear.

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[170] Effect of fluence rate on phototropism fluence response curve for blue light:
Radomir Konjevic, et al, "Dependence Of The Phototropic Response Of Arabidopsis Thaliana On Fluence Rate And Wavelength: *Proc. Natl. Acad. Sci. USA.* Vol. 86, pp. 9876-9880, December 1989. Accessed 3/31/08 at http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=298605&blobtype=pdf

Developmental Effects Of Auxin

Besides directly affecting growth of shoots and roots, auxin influences nearly every stage of a plant's development, including germination and senescence, and is critical for establishing plant polarity. The various tissues responding to auxin demonstrate different pre-programmed responses.

Auxin Regulates Apical Dominance

The growing apical bud usually inhibits the growth of lateral (axillary) buds, establishing **apical dominance**, and this has been shown to be due to basipetal flow of IAA from the terminal bud. Apical dominance is destroyed if the terminal bud is removed (fig. 19.36) or if the auxin inhibitor TIBA is applied just below the shoot apex. Auxin controls axillary bud growth by acting in the xylem and interfascicular sclerenchyma (i.e., between the vascular bundles) of the shoot[TZ497]). A root-derived carotenoid cleavage product (**shoot multiplication signal SMS**, see web essay 19.4) plays a role in inhibiting branching[TZ498]. Cytokinin and ABA do not consistently play major roles.

Auxin Transport Regulates Floral Bud Development And Phyllotaxy

(See also chapters 16 and 25) Polar auxin transport in the inflorescence meristem is required for normal floral development. The developing floral meristem depends on auxin being transported to it from subapical tissues.

Auxin Promotes The Formation Of Lateral And Adventitious Roots

Initiation of lateral (branch) roots and adventitious roots (roots arising from non-root tissue) are stimulated by high auxin levels (even though the primary root is inhibited by auxin concentrations > 10^{-8} M). Lateral roots arise from the pericycle above the elongation and root hair zone. This process requires acropetal movement of IAA in the vascular parenchyma of the root. IAA is also needed to promote growth and maintain viability of a developing lateral root. Auxin, by promoting adventitious rooting, is useful in propagating plants using vegetative cuttings. (See also Chapter 22 regarding action of ethylene.)

Auxin Induces Vascular Differentiation

New vascular tissues differentiate below developing buds and growing leaves, and this is stimulated by auxin. High concentrations induce differentiation of xylem and phloem, while low concentrations stimulate only the phloem to form. Regeneration of vascular tissues following wounding requires auxin.

Auxin Delays Leaf Abscission

Auxin levels are high in young leaves, progressively decline in older leaves, and are low in senescing leaves when the abscission process begins. Auxin plays a role in delaying abscission: auxin transported from the leaf blade normally prevents abscission, and when it is no longer produced, "abscission is triggered during leaf senescence..."[TZ500].

Auxin Promotes Fruit Development

Auxin is produced or mobilized from storage in pollen, and also in the endosperm and embryo of developing seeds. Successful pollination initiates ovule growth ("**fruit set**").[171] The endosperm may provide auxin during initial fruit growth, and the embryo may take over this role in later stages. See fig.

[171] Fruit set images: http://www.nysaes.cornell.edu/pubs/fls/OCRPDF/58a.pdf

19.39 illustrating how the **achenes** (external seeds) of a strawberry are needed to provide auxin to promote full size development of the receptacle fruit. (See also Chapter 20 regarding the role of GA in fruit set.)

Commercial Uses Of Synthetic Auxins

Auxins are widely employed in agriculture and horticulture, including for "prevention of fruit and leaf drop, promotion of flowering in pineapple, induction of parthenocarpic fruit, thinning of fruit, and rooting of cuttings for plant propagation."[TZ500] Auxin can be used to induce fruit set in seedless (unpollinated parthenocarpic) fruits—eventually endogenous production of auxin in the fruit takes over. Some auxin fruiting effects are due to promotion of ethylene. Certain synthetic auxins such as 2,4-D (web topic 19.1) are used as herbicides for dicot weeds in cereal fields—because of their slow metabolism and slow transport in dicots, they lead to plant death by inducing excessive cell expansion. Monocots such as grasses are less sensitive to herbicidal auxins due to more rapid inactivation by conjugation, and lower affinity of binding by receptors.

Auxin Signal Transduction Pathways

[Most of this section is omitted] The principle auxin receptors are "F-box" proteins of the T1R1/AFB family, which are part of a ubiquitin E_3 ligase complex called SCF.....

Chapter XX. Gibberellins: Regulators Of Plant Height And Seed Germination

Plant growth and development are regulated by several hormones acting together. Gibberellins ("**GAs**") are a group of at least 136 compounds defined by a similar chemical structure, only a few of which are biologically active. GAs are most noted for their effects on internode elongation. Levels of bioactive GAs generally correlate with **stem length**. GAs are "involved in seed germination, shoot growth, transition to flowering, anther development, pollen tube growth, floral development, fruit set and subsequent growth, and seed development."[TZ510] "GA's are widespread and so far found to be ubiquitous in both flowering (angiosperms) and non-flowering (gymnosperms) plants as well as ferns."[172]

Discovery and Chemical Structure Of Bioactive Forms

Discovery

Japanese rice growers identified a fungal infection which caused bakanae ("foolish seedling" disease) characterized by excessive height growth and lack of seed production. The pathogenic fungus was **Gibberella fujikuroi**. The growth-promoting complex extract of this fungal culture was termed **gibberellin A**. Other investigators purified a compound from these fungal extracts which they called **gibberellic acid** [GA_3]. Tokyo University investigators found three different gibberellins in gibberellin A, which they called **GA_1**, **GA_2**, and **GA_3**. GA_3 is the most common active component in Gibberella fungal cultures, and is the most frequently produced commercial gibberellin—it is identical to gibberellic acid. [MCM: As best as I can determine, G_3 is not a common GA in higher plants, though it does occur at least in certain species,[173] but is common in the fungus G. fujikuroi. GA_3 is not depicted as an intermediate in the synthesis pathway diagrams in fig. 20.6 and web topic 20.3.] GA_3 was soon found to produce spectacular growth in otherwise **dwarf and rosette plants** (having very short internodes between the leaves), such as dwarf peas and maize. GA-like activity was found to be 10 times as high in immature seeds compared to vegetative plant tissue. Seed extracts led to the first chemical identification of GA.

[172] Gibberellins: http://www.plant-hormones.info/gibberellins.htm
[173] GA_3 in higher plants: http://www.plant-hormones.info/ga3refsL.htm

Structure and Nomenclature

The current nomenclature and chemical formulas of c. 136 naturally occurring GAs (GA_1, GA_2, ..., GA_{136} etc., collectively abbreviated "**GAs**") may be seen at www.plant-hormones.info.[174] GAs have a molecular weight of c. 500 Da, and consist of either a **tetracyclic 20-carbon ent-gibberellane skeleton** (rings of 6, 5, 6, and 5 members) or a **19-carbon 20-nor-ent gibberellane skeleton**. These forms are termed **C_{20}-GAs** and **C_{19}-GAs**, respectively. The most active endogenous GAs of higher plants are **GA_1**, GA_3, **GA_4**, and GA_7, all of which are C_{19} GAs. These add a 4,10-lactone ring. (See web topic 20.1) The structures of GAs are best shown by Gas Chromatography followed by Mass Spectrometry (web topic 20.4), a technique which has supplanted bioassays.

Biosynthesis And Catabolism Pathways, Regulation, Organ Distribution

Biosynthesis and Storage In Higher Plants: Gibberellins are a large family of tetracyclic diterpene acids (diterpene = C_{20} terpenes which are derived from four isoprenoid units). The synthesis of diterpenes is shown on p. 320, and later stages in GA biosynthesis are shown in web topic 20.3. In the plastids, 4 isoprenoid units are assembled into geranylgeranyl diphosphate (GGPP), which is then converted to **ent-kaurene**. In the plastid envelope and ER, ent-kaurene is converted to the precursor of all other GAs, **GA_{12}**, a C_{20}-GA which is biologically inactive. The enzymes of this second stage of GA biosynthesis are monooxygenases that utilize cytochrome P450. (Web topic 20.3) In the cytosol, one of two possible parallel pathways leads from GA_{12} to formation of the C_{19} growth-bioactive forms: **GA_4** (in Arabidopsis and cucurbits) and **GA_1** (in cereals, maize, and legumes such as pea). (details omitted, see also p. 519 for further discussion) GAs can be sequestered for later use by conjugation with glucose as the glucosyl ester, etc.—subsequent hydrolysis leads to the active form. (Web topic 20.1)

Self-Regulation and Catabolism: Some enzymes in the biosynthesis and catabolism of GA are highly regulated (especially **GA 20-oxidases**, **GA 3-oxidase**, and **GA 2-oxidase**). Catabolism of the active GA forms GA_4 and GA_1 by GA2ox (GA 2-oxidase) leads to inactive GA_{34} and GA_8, respectively. (details omitted) Gibberellin regulates its own metabolism—for example, when levels are too high, it exerts negative feedback regulation (downregulating GA 20-oxidase and GA 3-oxidase) and "positive feed-forward" regulation (upregulating the catabolic enzyme GA 2-oxidase). (details omitted) Each step in the GA biosynthetic pathways may be associated with mutations that impair synthesis of the active form of GA. (details omitted, see figs. 20.6, 20.7, 20.8)

Localization: The multiple sites of GA biosynthesis can be visually demonstrated using a GA reporter gene construct. For example, the first committed GA biosynthesis gene **GA1**, which codes for the enzyme **CPS** (ent-copalyl diphosphate synthase), is linked with GUS (β-glucuronidase) or a green fluorescent protein. The sites of CPS biosynthesis observed include immature developing seeds, germinating embryos, young seedlings, shoot apices, root tips, and anthers. (details omitted, see below, figs. 20.9, 20.23, and text)

Effect Of Environmental factors: Environmental factors such as photoperiod and temperature can affect transcription of genes encoding enzymes in the GA biosynthetic pathways, and may through this mechanism affect bolting, flowering, tuberization, etc. ... In the seeds of some dicots such as lettuce and Arabidopsis, red light acts on phytochrome as the photoreceptor and this leads to stimulation of the 3β-hydroxylation step in the GA pathway, leading to new GA formation in the seed and seed germination ... Similarly, de-etiolation of etiolated pea seedlings, on exposure to normal light conditions (which are detected by phytochrome A and possibly a blue light receptor), is marked by reduction of the growth rate of the stem accompanied by "a rapid, but temporary, reduction in the content of GA_1"... (details omitted, see extensive discussion in Web topic 20.5, Essay 20.1, and chapters 17 and 25)

Possible Interactions With Auxin And Brassinosteroids: "Given that auxin increases the content of bioactive GA, and that GA is a potent promoter of growth, it seems reasonable to conclude that at least part of the growth response to auxin is mediated by increased GA levels—in other words, that GA is part of the auxin signal-transduction pathway." (Web Essay 20.1. This essay also discusses potential interactions, or the lack thereof, of GAs with other hormones such as BRs. See also further discussion below about the dependence of GA on auxin.)

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[174] Gibberellin structures: http://www.plant-hormones.info/ga1info.htm

Bioactive Forms: The internode length and height of pea plants directly correlates with the amount of endogenous GA_1, which has been shown to be the active GA in cereals such as wheat, maize, and legumes such as pea. Plants with impaired deactivation of GA_1 are unusually tall. (figs. 20.10, 20.11) In Arabidopsis and certain Cucurbitaceae (e.g., pumpkin and cucumber), GA_4 is the active GA.

Control of Plant Height Through Breeding: By breeding in of various degrees of altered expression of GA biosynthesis (e.g., more active GA from increased GA 20ox) or catabolism (e.g., less active GA from increased GA2ox), plant height may be genetically modified. For example, quaking aspen has been bred to grow taller (for increased paper fiber production), and wheat has been bred to be dwarfed (to reduce lodging). However, GA-deficient dwarfism often is accompanied by other defects... (details omitted).

Specific Growth And Development Effects of Endogenous And Commercial (Exogenous) Gibberellins

GAs have been extensively used in agriculture for decades, and GA inhibitors also have important uses. Various forms have the following actions:

Promote Stem (And Root) Growth

GAs promote **internode elongation** in genetically dwarf or rosette plants (the most pronounced effect of applied GAs), but have little stem-lengthening effect in normal-sized plants. (fig. 20.1, 20.2) Treatment of the dwarf rosette form of a Brassicaceae with GA leads to "**bolting**" (rapid stem growth and flowering). This sequence of events is frequently seen in long-day-plants (such as spinach) raised in short-day conditions and then treated with GA. The target organ in grasses such as deepwater rice or wheat is the **intercalary meristem**. GAs that promote stem growth also enhance **root elongation** (at least in pea and Arabidopsis), and plants genetically deficient in GAs have shorter roots.

Commercially, application of exogenous GA is used to increase sugarcane internode elongation and therefore sugar yield, especially at cooler temperatures (because these are C_4 plants).

Some commercial **GA inhibitors** (of which there are many, including Chlormequat chloride or **cycocel**) are used to decrease plant height (see web topic 20.1) by:

- preventing "lodging" (bending over of the stem to the ground) in cereal crops in cool wet climates
- reducing vegetative growth in cotton
- reducing height of fruit trees or containerized herbaceous ornamentals in greenhouses, etc.

Regulate Transition From Juvenile To Adult Phases

Many woody perennials have a juvenile phase, in which they do not flower or produce cones, and exhibit a different appearing adult phase. In conifers, the juvenile phase can normally last up to 20 years, but the transition to adult phase may be markedly shortened with application of GA_4 and GA_7. Some plants such as English ivy (Hedera helix) will transition back to juvenile phase ("rejuvenation") when GA_3 is applied.

Commercial growers sometimes use exogenous GAs to produce attractive cones or flowers in young (juvenile) plants.

Influence Floral Initiation And Flower Sex Determination

The initiation of bolting by GA in predisposed plants is noted above. (See chap. 25, including the interaction of photoperiodism and GAs.)

Plants with unisexual flowers may be influenced in flower sex determination by environmental factors such as photoperiod and nutritional status, and endogenous GAs may mediate these changes. However, exogenous GAs or GA inhibitors can also modify these changes. (details omitted)

Promote Pollen Development And Tube Growth

Plants lacking GA are often impaired anther development and in pollen production. GA is also needed for development of the pollen tube.

Promote Fruit Growth And Parthenocarpy

Optimal fruit growth after pollination ("fruit set") often depends on GA from the seeds. Fruit growth can be stimulated by exogenous GA, in some cases even when auxin has no effect. Commercial fruit crops commonly treated with GA include apple, cherry, pear, and citrus crops such as oranges and tangerines (see web topic 20.2).

Exogenous GA_3 can induce fruit set even in the absence of pollination, leading to **parthenocarpy** (seedless fruits), including the widely sold Thompson seedless grapes and sultana[175] raisins. These are treated with GA_3, as otherwise the seedless fruits tend to be very small and tightly bunched together.

Promote Seed Development and Germination

Failure of seeds to develop normally may be attributable to GA deficiency in very young seeds. The normal requirements in some species of appropriate photoperiodic conditions or vernalization may be overcome by application of exogenous GA, suggesting that endogenous GAs are involved in these processes.

GAs from the seed embryo are also involved in stimulation of α-amylase synthesis in the aleurone. The α-amylase causes hydrolysis of seed endosperm starch to sugars. (see also below)

The commercial process known as **malting** is widely applied to cereal grains such as barley, and is sometimes assisted by exogenous application of GA. Malting involves soaking the grains leading to germination (and release of GA by the embryo), causing development of their α-amylase and other hydrolytic enzymes, leading to hydrolysis of endosperm starch to fermentable sugars such as maltose and breakdown of other macromolecules such as proteins (collectively termed "modification"). The ongoing growth of these grains is stopped by kiln drying and curing.[176] (See also web topic 20.2)

Gibberellin Signaling

[This section involves GRAS proteins, DELLA domains, G1D1, etc., and is entirely omitted. See Web Essay 20.2 on the Green Revolution involving development of reduced height mutant wheat and rice, etc.]

Details of Certain Gibberellin Responses

The Aleurone Layer In Seed Endosperm

In cereal grains, the seed is divided into embryo, the endosperm with its aleurone, and the fused testa-pericarp (see also Chapter 23). The embryo interfaces with the endosperm at the absorptive organ, the scutellum. The endosperm consists of the starchy interior endosperm and the outer aleurone layer.

GA is synthesized in the embryo during imbibition and germination, and diffuses into the starch endosperm, and from there to the **aleurone** (the target organ). (fig. 20.22, 20.23) The resulting production of **α-amylase** by the aleurone provides sugar to nourish the developing embryo and seedling, and depends on the presence of an intact embryo (or application of exogenous GA such as GA_3). There may be more than one receptor for GA_4 activity in or on the aleurone cells... There are several second messengers for GA

[175] Thompson grapes and sultana raisins: http://en.wikipedia.org/wiki/Sultana_(grape)
[176] Malt production: http://brewery.org/library/Malt_AK0996.html

signaling, including Ca^{2+} and cyclic GMP. In aleurone cells, gibberellins enhance the transcription of α-amylase mRNA... (Details omitted, see Web topic 20.7)

Abscisic acid opposes the effects of GA in aleurone cells. (See Chapter 23)

Flowering In Long-Day Plants

In plants evolved to flower in response to certain day lengths (such as Arabidopsis, which is a typical LDP), the long-day and gibberellin pathways to flowering interact. The leaves perceive the day length and send a florigen signal to the apex where flowering will occur. In Arabidopsis, long days enhance GA20ox1 leading to bolting. FT Protein is the transmissible florigen in Arabidopsis, but GA can also serve as a floral stimulus in Arabidopsis and in Lolium (ryegrass), particularly GA_5 and GA_6... (Further details of this confusing subject omitted, see web topic 20.8, also Chap. 25)

Stem Growth

The mechanisms by which GA produces increased stem length and plant growth are poorly understood, including the interactions with auxin, and require further study.

"For meristematic activity to be maintained in the SAM, GAs must be excluded from the inner cells [of the meristem]."[TZ535] This allows the inner undifferentiated cells to retain the ability to expand and divide in all directions. Synthesis of GAs is suppressed in the inner cells of the SAM. (details omitted, involves KNOX homeodomain transcription factors, see fig. 20.30)

However, "GAs are necessary for the development of leaf promordia and normal leaf expansion."[TZ539] [MCM: few details on this aspect are provided.]

"Gibberellins stimulate both cell elongation and cell division..."[TZ535], so that taller internodes of peas and deepwater rice have more cells as well as longer cells. It is the intercalary meristem that is stimulated. (fig. 20.31) GA produces cell elongation by increasing the mechanical extensibility of the cell walls and by stress relaxation of cell walls, decreasing the wall yield threshold (i.e., the Y in the growth rate equation $GR = m[\Psi p - Y]$, see Chapter 15, and also similar Auxin effects in Chapter 19).

Unlike auxin, which relies on cell wall acidification via proton extrusion, GA has not been shown to cause cell wall acidification. The lag times measured for inducing elongation are greater with GA (40 minutes to 3 hours depending on species) than with auxin (c. 10 minutes, see chapter 19). Thus there may be different mechanisms for GA and auxin action, and in fact the effects of exogenously applied GA and auxin can be additive. However, it is difficult to separate the effects of GA and auxin, as they are usually both present.

Possible GA biochemical mechanisms are discussed, such as effects on XTH and expansin, and regulation of cell cycle kinases CDKs (details omitted).

Exogenous auxin can enhance GA biosynthesis. For example, exogenous auxin restores the drop in GA biosynthesis found in decapitated peas (fig. 20.33, note that right plant image is incorrect.) "The apical bud promotes growth not only through the direct biosynthesis of auxin, but also through the auxin-induced biosynthesis of GA_1."[TA537] Auxin promotes GA3ox and represses GA2ox, both having the effect of increasing bioactive GA_1. Auxin may be required for GA signaling. (details omitted, see Web essay 20.3 and Web topic 20.9)

Chapter XXI. Cytokinins: Regulators Of Cell Division

Cytokinins are found in angiosperms but also in algae, diatoms, mosses, ferns, and conifers.[TZ547] (The animal **cytokines** are unrelated.) Cytokinins are synthesized in roots, developing embryos, young leaves, fruits, and crown gall tissues, as well as by plant-associated bacteria, fungi, insects, and nematodes.[TZ567] Cytokinins are most abundant in young rapidly growing cells of the SAMs and RAMs (visually

demonstrated in web topic 21.9). They are involved with "leaf senescence, nutrient mobilization, apical dominance, the formation and activity of the shoot apical meristems, floral development, the breaking of bud dormancy, and seed germination"[TZ543] as well as with light-regulated development (such as chloroplast maturation), development of autotrophic metabolism, and leaf and cotyledon expansion from cell enlargement. They play a central role in control of cell division. Both auxin and cytokinin regulate the plant cell cycle and are needed for cell division.[TZ567]

Cell Division and Plant Development

Plant cells that have differentiated and assumed their normal function usually do not divide again during the life of the plant—they are said to be "terminally differentiated". Nucleated plant cells however retain the capability to divide. "Mature cells of the cortex and/or phloem resume division to form secondary meristems, such as the vascular cambium or the cork cambium."[TZ544] Resumption of cell division of mature cells can occur with application of cytokinins, and with:

- **Wound healing and callus formation**: Plant wounding induces cell division at the wound site. Usually this stops after a few cell divisions. A more extensive proliferation leads to **callus** formation, which typically occurs at the site of plant wounds or grafting. The **crown gall bacterium Agrobacterium tumefaciens** can induce marked cell proliferation and neoplastic growth which continues for the rest of the plant's lifetime (see also below). In vivo growth of such induced tissue will continue even if the plant is heated sufficiently to kill the bacterium that induced it. This crown gall tissue will grow indefinitely in culture and eventually form a large unorganized mass called **callus tissue**.
- **Leaf abscission**: The abscission zone, created by resumption of cell division, consists of weakly bound plant cells that forms at the base of the leaf petiole.

G. Haberlandt found in c. 1913 that vascular tissue contains a water soluble diffusible factor that will stimulate cell division in wounded potato tuber tissue, and investigation of this finding led to discovery of cytokinins in the 1950s.[TZ544]

Tomato roots can be grown indefinitely in a simple culture medium. However, isolated tomato stems exhibit little if any sustained growth in culture, even if auxin is added, until adventitious roots form. This suggested that a root-derived factor may regulate growth in the shoot.[TZ544]

Discovery, Detection, And Properties Of Cytokinins

A search for the active agent that induces sustained cell division in isolated stems led to the discovery of such properties in coconut milk. Coconut milk (liquid endosperm) was eventually found to contain the cytokinin **zeatin**. Philip White's culture medium, which contains auxin and coconut milk (and therefore zeatin), will support cell division in many plants.

The first synthetic cytokinin found was the synthetic analog, **kinetin (N^6-furfuryladenine)**, which is a heat-induced breakdown product of DNA that is derived from adenine (6-aminopurine) and which requires auxin to be active. (This compound is being tried on humans for cosmetic and other purposes.)[177]

Subsequently, the first naturally biosynthesized cytokinin was discovered, **zeatin**, initially as a component of immature maize endosperm. It proved to be the most prevalent cytokinin in higher plants, and like other natural plant cytokinins is derived from adenine. Zeatin occurs in plants in **trans-zeatin** and **cis-zeatin** stereoisomers. The trans form is much more active, but the cis form may also play a biological role.[178] Natural cytokinins (such as **DZ** and **IP**) are depicted in web topic 21.2 (details omitted), and synthetic agonists (such as benzyladenine [**BA**] and the defoliant **thidiazuron**) and an antagonist are shown in the text.

Cell proliferation bioassays using tobacco pith callus tissue or carrot taproot remain useful in assessing effects of cytokinins, but "the introduction of immunoaffinity purification methods combined with liquid

[177] Kinetin use in humans: http://www.drugs.com/npp/kinetin.html
[178] Cis-Zeatin role in potato tuber dormancy:
http://www.blackwell-synergy.com/links/doi/10.1034%2Fj.1399-3054.2000.100110.x

chromatography and mass spectrometry, along with the use of isotopically labeled cytokinins as internal standards, has resulted in major advances in the measurement of endogenous cytokinins." (Web topic 21.3)

The identification of the cytokinin receptor **CRE1** has made it possible to confirm that **the free base trans-zeatin is the active form of cytokinin**, but certain related bound compounds such as cytokinin ribosides, ribotides, glucosides, and glycosides may be storage forms that are convertible to the active form. (details omitted) "Hypermodified" nucleotide bases, which when hydrolyzed have cytokinin activity, can also be present on tRNAs, but are of uncertain significance (web topic 21.4).

Pathogenic Secretion

Some bacteria, fungi, insects, and nematodes are capable of secreting various free cytokinins directly, or can induce the plant to do so. Such interactions can cause or contribute to the formation of:

- **Crown galls** (**Agrobacterium tumefaciens**). The cells of crown galls have acquired the ability to synthesize zeatin, due to insertion of a specific region of the bacterial Ti plasmid (called **T-DNA**) into the plant cell nuclear DNA (fig. 21.4). Here, the Ti signifies "tumor-inducing". The genes added to the plant cell genome encode for synthesis of trans-zeatin (using the **IPT gene**, web topic 21.6), auxin, and various **opines** (including **octopine** or **nopaline**, fig. 21.5). Opines are not usable by the plant but are utilized by the bacteria as a source of carbon and nitrogen. The genes contributed in the T-DNA are considered **phyto-oncogenes** (web topic 21.5) as they can induce unregulated proliferation and tumors (crown galls in this case).
- **Other galls** provoked by various cytokinin-secreting insects or larvae
- **Witches' broom or fasciation** (Corynebacterium fascians)
- **Mycorrhizae**[179]
- **Root masses or balls**, "**hairy roots**" (Agrobacterium rhizogenes,[180] a process also involving plasmid transfer)
- **Root knots** (cytokinin-secreting nematodes)

Biosynthesis, Metabolism, and Transport

Natural cytokinins are composed of N^6-substituted aminopurines, consisting of adenine and various side chains arising at the N linked to the C-6 position (these side chains are created in part from isoprene units). See fig. 21.6 for biosynthetic pathways in plants from ATP/ADP and DMAPP (or AMP and HMBDP in bacteria), etc. The first committed step in plants for cytokinin biosynthesis uses the enzyme **IPT (Isopentenyl transferase)** to catalyze the reaction (at the 6-N position) between ATP or ADP and DMAPP. (See web topic 21.6 for phylogeny of these widespread IPT genes, including the possibility that one of the Arabidopsis IPT genes may be of bacterial origin).

Cytokinins are synthesized in the roots, particularly the root apical meristem, and are transported (mainly as cytokinin ribosides) passively in the xylem to the shoot. (details omitted) Roots however are not the only site of synthesis. IPT genes consistent with cytokinin synthesis capability are also expressed in Arabidopsis in "xylem precursor cell files in the root tip", root primordia, columella root cap cells, phloem, leaf axils, the upper part of young inflorescences, ovules, immature seeds, and fruit abscission zones.[TZ551] Biosynthesis of cytokinins is downregulated by themselves, and upregulated by auxin.

Cytokinin ribosides reaching the leaves are converted to the free form or to glucosides... (details omitted) Transport of zeatin riboside from root to leaves is regulated by signals of unknown identity to the root from the shoot. (details omitted)

Free cytokinins are rapidly metabolized in plant tissues. "Cytokinin oxidase irreversibly inactivates cytokinins, and it could be important in regulating or limiting cytokinin effects.[TZ552] (Tobacco plants overexpressing this enzyme are stunted, fig. 21.8) Conjugation is also used to inactivate or store

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[179] The roles of auxins and cytokinins in mycorrhizal symbioses: http://www.springerlink.com/content/aj4g5fuaj957bbky/

[180] *Agrobacterium rhizogenes*: http://en.wikipedia.org/wiki/Agrobacterium_rhizogenes

cytokinins, for instance as cytokinin glucosides. Cytokinin glucosides (but not free cytokinin) are found in high concentration in dormant seeds—at the time of germination they are rapidly converted to active free cytokinin.

Biological Roles of Cytokinins

Here are further details of some of the specific roles.

Cytokinins regulate cell division in shoots and roots

Most of the cell division in the adult plant occur in the meristems. Shoot meristems are shrunken in mutant plants overexpressing the gene for cytokinin oxidase (fig. 21.9), whereas the same mutation causes enhancement of root growth (fig. 21.10, 21.11). There is strong evidence that endogenous cytokinins regulate cell division in vivo, suppressing division in shoot meristems while enhancing it in root meristems.[TZ553]

The dose response curve for root growth is hypothesized, like auxin, to be a bell-shaped curve, peaking at an optimal value of cytokinin signaling level, and falling off on either side of the peak (fig. 21.12). According to this hypothesis, root level of cytokinin is supraoptimal (above the peak), so that small decreases will cause increased cell division and growth. The shoot level is assumed to be optimal in this model.... (details omitted)

Shoots in plants with mutated loss-of-function cytokinin receptors are stunted (fig. 21.13). Cytokinin plays a role in regulating leaf phyllotaxy (fig. 21.14, details omitted).[181]

The number of grains and overall yield in the Habataki variety of **Oryza sativa indica** rice is greater than in the Koshihikari variety of **Oryza sativa japonica** rice, which is thought to be due to a decrease in function of cytokinin oxidase in indica, which leads to higher cytokinins in the inflorescence, producing more reproductive organs.[182] [MCM: this is of course an action on the inflorescence meristem of the shoot.]

Cytokinins regulate specific components of the cell cycle

Zeatin levels peak at the end of the S phase, the G_2/M phase transition, and in late G_1.[TZ556] Inhibition of cytokinin biosynthesis blocks cell division. Both auxin and cytokinin participate in regulating cell division by controlling cyclin dependent protein kinases CDKs (details omitted).

The Auxin:Cytokinin ratio regulates morphogenesis in cultured tissues

In cultured tobacco-derived callus tissue, the differentiation into roots or shoots (buds) depends on the Auxin:Cytokinin ratio—high ratios lead to root formation ,whereas low ratios lead to bud/shoot formation (fig. 21.16). In crown gall tumors having mutations that affect the Auxin:Cytokinin ratio, selective proliferation from the tumor of either shoot or roots can occur. (Such partially differentiated tumors, as in animals, are called **teratomas**).

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[181] Cytokinin and leaf phyllotaxy:
"Here we show that ABPH1 [ABPHYL1] is homologous to two-component response regulators and is induced by the plant hormone cytokinin. ABPH1 is expressed in the embryonic shoot apical meristem, and its spatial expression pattern changes rapidly with cytokinin treatment. We propose that ABPH1 controls phyllotactic patterning by negatively regulating the cytokinin-induced expansion of the shoot meristem, thereby limiting the space available for primordium initiation at the apex."
Anna Giulini, Jing Wang & David Jackson. "Control of phyllotaxy by the cytokinin-inducible response regulator homologue ABPHYL1" *Nature* 430, 1031-1034 (26 August 2004) | doi:10.1038/nature02778, accessed at Nature online 4/8/2008

[182] *Rice indica* vs. *japonica* and cytokinin oxidase: Motoyuki Ashikari, et al. "Cytokinin Oxidase Regulates Rice Grain Production". *Science* Vol 309 29 July 2005. Accessed online at www.sciencemag.org 4/9/2008

Note however that in addition, "the tumor-induced ethylene is a limiting and controlling factor in gall development..... The vigorous ethylene synthesis in galls is enhanced by high levels of auxin and cytokinin... " (Web essay 22.1 sic)

Cytokinins modify apical dominance and promote lateral bud growth

Lateral plant branching is influenced by light, nutrients, and genotype, and is regulated by a complex interplay of auxin, cytokinin, and an unidentified inhibitory root-derived signal (which appears to be a carotenoid derivative). Auxin from the apical bud in many plants normally suppresses growth of axillary buds (discussed above). However, cytokinin applied to axillary buds stimulates their growth, and plants that overexpress cytokinins tend to be bushy rather than apically dominant. Cytokinins causing growth of lateral buds are probably synthesized in the buds themselves. Auxins have been shown to inhibit in lateral buds some of the IPT genes involved in cytokinin synthesis.

Cytokinins induce bud formation in a moss

The germination of spores of mosses such as Funaria gives rise to a filament of **chloronema** cells called a **protonema**. (Fig. 21.18) The "buds" that arise on the protonema give rise to the leafy gametophyte stage. Light is required for formation of these buds, but exogenous cytokinins can substitute for this light requirement, stimulating normal bud development and even increasing the number of buds. This indicates a possible role for cytokinins in moss bud formation. (See Web essay 21.2 for extensive details)

Cytokinin overproduction has been implicated in genetic tumors

Interspecific hybrids of genus Nicotiniana (such as N. langsdorffii × N. glauca) sometimes produce spontaneous "genetic tumors", and these plants have been found to have high levels of auxin and cytokinins (5 to 6 times higher for cytokinins compared to either parent).

Cytokinins delay leaf senescence

Leaf senescence is a programmed aging process leading to death of the leaf. Darkness accelerates senescence and cytokinins applied to the isolated leaves of many species will delay senescence. Localized leaf infections of the hackberry caused by jumping plant lice produce galls on the leaf undersides that contain cytokinins. These appear as islands of green in an otherwise yellowing leaf. Young leaves produce cytokinins, while mature leaves produce little (and may be depending on root-derived cytokinins to postpone their senescence). In soybeans, maturation of the seeds leads to leaf senescence (**monocarpic senescence**), although the mechanism involves cytokinins from both the seed pods and the roots (the latter transported to the leaves via the xylem). "Cytokinins are a natural regulator of leaf senescence." (p. 559, including Fig. 21.21).

Cytokinins promote movement of nutrients into leaves

When leaves are treated with exogenous cytokinins, "nutrients such as sugars and amino acids are preferentially transported to, and accumulated in, the cytokinin-treated tissues"[TZ559]. This may create a new source to sink relationship which does not depend on whether these nutrients can actually be metabolized at the site of delivery. (fig. 21.22) Nutrients applied to the roots of Nitrogen-depleted maize result in rapid rise in root cytokinins that are transported to the shoot. Thus the nutrient status of the plant determines cytokinin levels, and the Auxin:Cytokinin ratio determines the relative growth of roots and shoots.[TZ560] Low levels of soil nutrients lead to low cytokinins and this encourages root growth, whereas optimal levels of soil nutrients lead to high cytokinin levels that promote shoot growth.

Cytokinins promote chloroplast development

Etiolated seeds have proplastids that develop into etioplasts rather than chloroplasts capable of PS. Although light can effect the transition of etioplasts into functional chloroplasts, this change can be accelerated and accentuated by pretreatment with exogenous cytokinins, or by mutations that lead to cytokinin overproduction. (Fig. 21.21, Web topic 21.7)

Cytokinins promote cell expansion in leaves and cotyledons

Cell enlargement due to cytokinins is best seen in the leafy cotyledons of certain dicots (Fig. 21.24). [MCM: This also occurs in leaves, but perhaps is harder to separate from auxin effects.] This is a result of increased mechanical extensibility of the CW, but is not accompanied by cell wall acidification (as is seen with auxin), and GA and auxin do not promote cell expansion in cotyledons.

Cytokinin-regulated processes are revealed in plants that overproduce cytokinins

Transgenic plants (having genes from other species) that overproduce cytokinins have been created by adding Agrobacterium Ti plasmids having T-DNA that have been limited to ipt genes. Plant models include lettuce, tobacco, and Arabidopsis. These are created with promoters whose expression can be controlled. (details omitted) Such overproducing plants have [some or all of] these features:[TZ562]

- **Apical dominance is greatly reduced** (i.e., growth is bushier).
- **Growth is stunted** with shortened internodes in the extreme overproducers .
- The SAMs produce **more leaves**.
- The leaves produce **more chlorophyll** and are greener.
- **Leaf senescence is retarded**.
- **Adventitious shoots** may form from unwounded leaf veins and petioles.
- **Rooting of stem cuttings and root growth rate are reduced**.

Breeding of transgenic plants that overproduce cytokinins may be commercially useful in leafy crops such as lettuce (fig. 21.25), provided the synthesis of cytokinins can be controlled. Tobacco plants can be rendered more resistant to damage by predators such as the tobacco hornworn. (details omitted)

Cellular And Molecular Modes Of Cytokinin Action

The diverse actions of cytokinins are consistent with the involvement of signal transduction pathways.[TZ563] A cytokinin receptor, thought to be a transmembrane protein related to bacterial receptors, has been identified. CK11 gene encodes a protein that is similar to bacterial two-component sensor histidine kinases. However, it has not yet been proven that it is a cytokinin receptor. CRE1 in Arabidopsis appears to encode a cytokinin receptor similar to bacterial histidine kinases ... Fig. 21.27 depicts stunting in Arabidopsis plants deficient in one or more of the cytokinin receptors (cre1, ahk3, ahk2) that are encoded by a multigene family. Fig. 21.30 depicts the postulated intracellular signaling pathways... (Entire remaining section omitted.)

Chapter XXII. Ethylene: The Gaseous Hormone

[This chapter was not read or discussed in UW Plant Physiology Biol 425.]

Early Observations

It was noted in the 19C that trees defoliated more rapidly when in the vicinity of coal-gas burning street lamps. Also, pea seedlings grown in the dark in a lab burning coal gas exhibited the **triple response** of reduced stem (epicotyl) elongation, increased lateral growth (radial swelling of the stem), and abnormal

horizontal growth of the epicotyl (fig. 22.5A). Eventually, **ethylene** was identified as the causative component—it comprises about 5% of unburned coal gas.[183] Also, H. H. Cousins reported in 1910 that natural emanations from oranges caused the premature ripening of bananas (a phenomenon which would be more likely with apples as the source). Ethylene was identified as a plant hormone in 1934 by R. Gane. It became easier to study with the advent of gas chromatography in 1959, which can demonstrate ethylene at concentrations as low as 5 ppb (5 pL per L).[TZ576]

Structure, Biosynthesis, and Measurement of Ethylene

Ethylene (**ethene or $H_2C=CH_2$**, a coplanar molecule) is the simplest **alkene** or **olefin** (a nonaromatic hydrocarbon containing at least one double bond). It is flammable and usually is lighter than air. Ethylene "has a pleasant sweet faint odor, and has a slightly sweet taste".[184] In plants, it can be oxidized to ethylene oxide (C_2H_4O), oxalic acid (HOOC-COOH), and finally CO_2. It diffuses readily through tissues and, in the gaseous phase, within the intercellular spaces and surrounding air. When present in gaseous phase at a concentration of 1 μL L^{-1} (1 ppm), the concentration at equilibrium in water is 4.4 x 10^{-9} M, but the gaseous phase concentrations are easier to measure. Commercial growers usually express ethylene concentrations as ppm, which are equivalent to μL L^{-1}. Ethylene can be bioactive at concentrations as low as 1 nL L^{-1} (1 ppb).

Ethylene can be trapped by potassium permanganate, prolonging fruit storage times. The completed net reaction[185] is:

$$3CH_2CH_2 + 12KMnO_4 \rightarrow 12MnO_2 + 12KOH + 6CO_2$$

Ethylene can be produced by most organs of the higher plants, but production is greatest in **senescing tissues** and **ripening fruit**. Its production also increases during **leaf abscission** and **flower senescence**, as well as **plant wounding** and **physiological stresses** such as **flooding or drought stress**. A ripe apple may have a concentration as high as 2,500 μL L^{-1}. **Young developing leaves** have higher ethylene production rate (expressed as nL g^{-1} h^{-1}) than mature leaves. Gymnosperms, ferns, mosses, certain cyanobacteria, and even certain fungi and bacteria also can synthesize ethylene. (Mammalian tissues have not been shown to synthesize ethylene.)

The steps of ethylene biosynthesis (fig 22.1) are as follows:

- begins with **methionine** (Met, a part of the Yang cycle); then
- **5-Adenosyl-methionine** (**AdoMet**) from Met; then
- **1-Aminocyclopropane-1-carboxylic acid** (**ACC**) from AdoMet, the rate-limiting step catalyzed by **ACC synthase** (web topics 22.1, 22.2, also text p. 575 regarding stabilization of this hormone), promoted by fruit ripening, flower senescence, auxin IAA, mechanical wounding, chilling injury, drought stress, and flooding (all of which increase ACC synthase); then
- **ethylene** (a step catalyzed by **ACC oxidase** [web topic 22.3], promoted by ripening and inhibited by Ca^{2+} and temps > 35 ºC, see web topic 22.3). Oxygen is also required for this final step, and the step does not occur in anaerobic conditions (such as in flooded roots, see below). ACC oxidase requires cofactors Fe^{2+} and ascorbate.

Diurnal Variation: Synthesis peaks during the day and is lowest at night.

Stresses: Various stresses (listed above and below) increase ethylene and lead to stress responses including abscission, senescence, wound healing, increased disease resistance, and **leaf epinasty** (downward drooping of leaves due to faster upper petiole cell growth compared to lower).

Auxin: Some responses of plants formerly attributed to auxin are caused by IAA induction of ethylene. Ethylene mimics high concentrations of auxin by inducing **leaf epinasty** (fig. 22.5B).

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[183] Coal gas composition: http://en.wikipedia.org/wiki/Coal_gas
[184] Ethylene odor: http://en.wikipedia.org/wiki/Ethylene
[185] Ethylene absorbers including potassium permanganate: http://www.sorbentsystems.com/epaxtech.html

Catabolism leads to CO_2, ethylene oxide, etc., but does not appear to regulate the tissue levels of the hormone.

Conjugation: Some ACC is conjugated to **N-malonyl-ACC** (**MACC**), a form which accumulates but is not active and may serve to help control ACC biosynthesis.

Inhibitors: The compounds abbreviated **AOA** and **AVG** block the conversion of AdoMet to ACC, and cobalt ion **Co^{2+}** inhibits the conversion of ACC to ethylene. **Silver ion Ag^{+}** from silver nitrate $AgNO_3$ or silver thiosulfate[186] anionic complex $[Ag(S_2O_3)_2]^{3-}$ inhibits the action of ethylene, and has been used to prolong cut flower life (but is a not widely used in the US due to potential heavy metal pollution and toxicity). **CO_2** at 5-10% concentration inhibits ethylene action and has been used to retard fruit ripening. **Trans-cyclooctene** and the gas **1-methylcyclopropene (MCP)** block ethylene responses by blocking the receptor. The latter is marketed as a powder, EthylBloc™, that releases the active gaseous compound in solution and serves to prolong cut flower shelf life.[187] (The effectiveness of MCP and silver thiosulfate are compared here.)[188]

Detection: In addition to gas chromatography (which detects ethylene at 5 ppb), the laser-driven photoacoustic detector can detect as low as 0.05 ppb (50 parts per trillion or 50 ppt).[189]

More Details On Developmental and Physiological Effects of Ethylene

Ethylene promotes ripening of some fruits

Fruits serve many plants by facilitating dispersal of the seeds when they are mature. Fruit ripening signals this readiness for dispersal, and entails a complex series of changes: enzymatic breakdown of cell walls, starch hydrolysis, sugar accumulation, the reduction or disappearance of organic acids and phenolic compounds including tannins,[190] and the development of characteristic and often attractive flavors, aromas, and colors. Plants using animal dispersal produce **edible** ripe fruit, often with enhanced visibility due to anthocyanins and carotenoids. Other forms of fruit, such as gourds and seed pods, may become dried when ripe, and some also split open (become **dehiscent**)[191] to facilitate dispersal.

Climacteric fruits are those that ripen from ethylene and show a spike in ethylene production just prior to ripening, followed by the **climacteric rise in respiratory rate** and CO_2 production (fig 22.4). Climacteric fruits include apples, apricot, avocados, bananas, mango, melons such as cantaloupe, olive, papaya, peach, pear, plum, and tomatoes.[192] (See also Table 22.1.) Treatment with exogenous ethylene induces these fruit to make more ethylene—thus these fruits are termed **autocatalytic**, via "System 2". Climacteric fruit plants also use "System 1" in which ethylene inhibits its own biosynthesis.

The ripening of climacteric fruits is often manipulated commercially, either by accelerating it or delaying it. Artificial inhibitors such as are named above may completely block ripening. When ACC oxidase is blocked in tomatoes, ethylene biosynthesis and fruit ripening do not occur. (web topic 22.3). Mutant tomatoes that have nonfunctional ethylene receptors also do not ripen[TZ577] (further details omitted).

Non-climacteric fruits do not exhibit a dramatic rise in respiration at the time of ripening. They include bell pepper, cherry, citrus fruits such as oranges, grape, pineapple, snap beans, strawberry, and

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[186] Silver thiosulfate for inhibiting bud drop of Christmas cactus: http://postharvest.ucdavis.edu/datastorefiles/234-886.pdf
[187] 1-methycyclopropene (MCP) [EthylBloc™] for cut flower vase life prolongation: http://postharvest.ucdavis.edu/datastorefiles/234-79.pdf
[188] MCP versus silver thiosulfate for cut flower vase life prolongation: http://www.actahort.org/members/showpdf?booknrarnr=682_123
[189] Laser-driven photoacoustic detector: http://www.plantphysiol.org/cgi/content/abstract/88/2/506
[190] Tannins: http://en.wikipedia.org/wiki/Tannin
[191] Dehiscent and indehiscent fruit types: http://www.biologie.uni-hamburg.de/b-online/e02/02f.htm#dehi
[192] Climacteric fruits: http://www.fao.org/Wairdocs/X5014E/X5014e04.htm

?watermelon (see also Table 22.1). When exposed to exogenous ethylene, they do not exhibit increased endogenous synthesis of ethylene, nor do they exhibit accelerated ripening.

Leaf Epinasty

As previously mentioned, **leaf epinasty** is defined as downward drooping of leaves due to faster upper (adaxial) petiole cell growth compared to lower (abaxial) growth. It is not the same as wilting from loss of turgor. It results directly from ethylene, or indirectly from auxin (which induces ethylene production). Epinasty occurs in increased stress conditions: flooding and/or hypoxic roots, salt stress, and infection, etc. When the roots are the source of a signal promoting epinasty in the shoots, the signal is ACC, which ascends in the xylem. The ACC does not generate ethylene in the anaerobic roots, as O_2 is required, but it does generate ethylene in the well-oxygenated shoot. The physiological purpose of epinasty in some situations is not always apparent. [MCM: epinasty may be beneficial in reducing solar exposure and dehydration when leaves are exposed to excessive sun and drying stress, etc.]

Ethylene induces lateral cell expansion

Radial swelling of the stem is part of the triple response mentioned above, and occurs in seedlings at ethylene concentration above 0.1 ppm. (See fig. 22.7 for triple response in Arabidopsis.) Ethylene disrupts and reorients the normal orientation of microtubules in the stem from transverse to longitudinal orientation, thereby blocking normal longitudinal growth and causing preferential transverse growth and resulting widening of the stem. (Further details omitted, see fig. 22.8.)

Ethylene induces and maintains the hook of etiolated seedlings

Etiolated dicot seedlings have a prominent hook facilitating penetration through the soil. This hook is formed and maintained by the influence of ethylene. The hook arises by asymmetrical growth similar to epinasty (see also Chap. 16). Upon exposure or emergence into the light, red light stimulates the photoreceptor phytochrome (Chap. 17), leading to inhibition of ethylene formation and opening out of the hook. Auxin plays an integral role in hook behavior, along with light and ethylene (details omitted).[TZ580]

Ethylene breaks seed and bud dormancy in some species

Seeds may not germinate even under conditions otherwise conducive to germination, and are said to be **dormant**. (See chap. 16; also web topic 22.5 which discusses the early agricultural value of loss of seed dormancy in cereal grain crops such as wheat and barley.) Ethylene can break this dormancy in some species such as cereal grains, and can increase the germination rate in some seeds. It is also used to promote bud sprouting in potatoes and other tubers.

Ethylene promotes the elongation growth of submerged aquatic species

Unlike its usual action inhibiting elongation, in submerged aquatic plants such as deepwater rice (Oryza sativa), ethylene induces rapid internode or petiole elongation (by decreasing ABA) and stimulates an increase in the amount of or sensitivity to gibberellin in the intercalary meristem. This process is aided by the diminution of ethylene diffusion away from the submerged part of the plant (further details omitted).

Ethylene induces the formation of roots and root hairs

Ethylene induces adventitious roots in leaves, stems, etc., an effect also caused by auxin but not seen in ethylene-insensitive mutants. (See also Chap. 19) Ethylene is also a positive regulator of root hair formation.[TZ581]

Ethylene is used by crown galls

Crown galls, which synthesize cytokinins, auxins, and opines (Chapter 21), also synthesize and are benefited by ethylene:

"Agrobacterium tumefaciens-induced galls produce very high ethylene concentrations. The tumor-induced ethylene is a limiting and controlling factor in gall development. It reduces the diameter of vessels in the host stem adjacent to the tumor and enlarges the gall surface through which high transpiration occurs, thus giving priority in water supply to the growing tumor over the host shoot." (Web essay 22.1)

Ethylene induces flowering or alters flower sex in some species

In mango as well as in pineapple and related species, ethylene induces flowering and can be used commercially to synchronize fruit set. It can also change the sex of developing flowers to female, as in cucumber[193]—this prevents self-pollination and increases yield.

Ethylene enhances the rate of leaf senescence

Exogenous ethylene or ACC accelerate leaf senescence, chlorophyll loss, and color fading. This is the reverse of the effect of cytokinins, and senescence may be regulated by the relative amounts of ethylene + ABA (chapter 23) versus cytokinin (chapter 21). Based on study of Arabidopsis mutants and transgenic plants, ethylene appears to increase the rate of senescence, "rather than acting as a developmental switch that initiates the senescence process".[TZ581] Transgenic tomatoes also confirm that plants deficient in ethylene synthesis exhibit delayed leaf senescence and fruit ripening.[TZ582]

Ethylene is involved in defense responses to wounding

The involvement of ethylene in pathogenic attacks is complex and depends on the specific host-pathogen interaction. Ethylene in combination with jasmonic acid plays a role in plant defense against necrotrophic pathogens (which grow on dead plant tissue). However, "ethylene does not appear to play a major role in the response of plants to biotrophic (growing on living tissues) pathogens."[TZ582] (further details omitted)

Ethylene and auxin regulate abscission

Abscission of leaves, fruits, flowers, and other plant organs takes place as a result of weakening across the **abscission layer** (**zone**) of the cell walls by the actions of hydrolytic enzymes (fig. 22.9). This process is stimulated by ethylene, and suppressed by auxin (fig. 22.10, 22.11 and chapter 19). The defoliant **2,4,5-T** acts by increasing ethylene biosynthesis. The gradient of auxin in the leaf maintains the potential abscission zone in the **nonsensitive** state. When leaf auxin declines, the leaf abscission zone becomes sensitive to ethylene, allowing an abscission response to endogenous ethylene and subsequent leaf shedding. Removal of the leaf blade (and its auxin synthesis) accelerates the abscission of the petiole, and this process can be delayed by auxin applied to the petiole distal to the abscission zone. Ethylene appears to decrease auxin synthesis and increase the rate of destruction of auxin.

Web topic 22.5 discusses the early agricultural value of preventing premature abscission (by means of a **tough rachis**) in the spikes of wheat, barley, [rice, which has a panicle[194] rather than a rachis], and other cereals:

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[193] Ethylene and flower sex determination:
- http://www.plantphysiol.org/cgi/reprint/49/6/998.pdf
- http://pcp.oxfordjournals.org/cgi/content/abstract/42/6/608

[194] Rice plant anatomy:
- http://www-plb.ucdavis.edu/labs/rost/Rice/RICEHOME.HTML
- http://www.knowledgebank.irri.org/hybridriceseed/The_Rice_Plant.htm

"The second critical change that took place during cereal domestication [c. 10,000 years ago] was the development of a tough rachis. The rachis is the main axis of the inflorescence, or spike, of wheat and other cereals, to which the spikelets are attached... After pollination, the fruits (caryopses [grains]) develop within the spikelets. When the caryopses of the wild-type wheat are fully ripened, a series of abscission layers forms that divides the rachis into dispersal units consisting of a single spikelet attached to a short segment of the rachis... A ripe head of wild wheat is thus easily shattered into dispersal units when touched or blown by the wind and is referred to by archaeologists as a brittle rachis."

Commercial uses of ethylene-related products

Ethylene or its derivatives are widely used in agriculture. Ethylene gas is not usually applied directly. **Ethephon** (**2-chloroethylphosphonic acid**) is applied as an aqueous solution, and upon metabolism in the plant, slowly releases ethylene. It is widely used in the US.[195] In California, it is commonly used as follows (listed in order of greatest amount used): cotton, walnuts, tomatoes, grapes, squash, chili peppers, bell peppers, wheat, wine grapes, cucumbers, apples, outdoor nursery containers, turf, greenhouse plants, pumpkins, barley, summer squash, olives, etc.[196] In general, ethylene-producing products are used to:

- induce fruit thinning and fruit drop to improve the remaining fruit: cotton, cherry, walnut, Valencia oranges, etc.
- accelerate fruit ripening: apples, tomatoes, etc.
- de-green citrus fruits including oranges
- synchronize flower and fruit set in pineapple
- accelerate abscission of flowers and fruits
- promote female flower formation in cucumbers, to prevent self-pollination and increase yield
- promote lateral rather than terminal growth, to promote compact flowering

Ethylene inhibitors (discussed above) help to preserve cut flowers longer or retard fruit ripening. Current and potential future commercial products include Ag^+, AVG (not yet approved), CO_2, and MCP (EthylBloc™). Transgenic plants that are less responsive to ethylene are also under commercial development, to produce longer lasting cut flowers and tomatoes that are delayed in ripening.

Ethylene Signal Transduction Pathways

"The ethylene receptor is encoded by a family of genes that encode proteins similar to bacterial two-component histidine kinases."[TZ589] A **copper** cofactor is required for high-affinity binding. (Silver also binds to this high-affinity receptor.) [The remainder of this topic is entirely omitted.]

Chapter XXIII. Abscisic Acid: Seed Maturation And AntistressSignal

[Limited summary]
Abscisic acid (ABA) might be better called "**dormin**". It creates dormant conditions in seeds and in plants undergoing water stress, and controls stomatal closure and shoot and root growth during water stress conditions. It also promotes leaf senescence (but usually not overt abscission). It is ubiquitous in plants including mosses, but not in liverworts (which however have the chemically and functionally similar **lunalaric acid**). It is synthesized in almost all cells that have chloroplasts or amyloplasts.

ABA is a 15-carbon terpenoid compound derived from the 40-C xanthophyll carotenoid, zeaxanthin. Only the S enantiomer, (S)-cis-ABA, has full potency (including fast stomatal closure), but the R enantiomer has slow (but not fast) response activity. Concentrations of ABA vary remarkably, rising for instance up to 100x in developing seeds.

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[195] Ethophon: http://pmep.cce.cornell.edu/profiles/extoxnet/dienochlor-glyphosate/ethephon-ext.html
[196] Ethephon California usage:
http://www.pesticideinfo.org/Detail_ChemUse.jsp?Rec_Id=PC35309#TopSites

It is inactivated by oxidation to PA (which remains partially active) and DPA (fully inactive) or conjugation to a glucosyl ester, and also regulated by compartmentalization and transport.

Seeds formed in mutants deficient in ABA may prematurely germinate, a process in maize called **vivipary**.

ABA is translocated in vascular tissues, both in the **xylem** and in the **phloem**, though much more abundant in phloem. Although it was once thought that roots detecting dry conditions sent an ABA signal in the xylem, it is now recognized that the primary site of water stress synthesis of ABA in the plant is in the shoot vascular tissue, and only later does it appear in guard cells or roots. However, it is possible that the slightly more alkaline xylem fluid formed under water stress favors dissociation of ABA (to ABA^-), so that more ABA reaches the guard cells in the leaves.

Effects Of ABA On Seeds

Seed maturation is broken into 3 phases:
- **Embryogenesis** and proliferation of endosperm. ABA rises during this phase.
- **Storage** of starch etc. in the endosperm. ABA concentration typically reaches a maximum during this phase.
- **Dehydration** and developing **tolerance to desiccation**, with evolution to the **quiescent state** or, in some plants, a **dormant state**. ABA promotes desiccation intolerance and inhibits precocious germination of seeds and vivipary. ABA remains high but declines somewhat in this phase. ABA affects the synthesis of proteins such as **Late-embryogenesis-abundant LEA proteins** and **DHN (dehydrin) proteins**, etc. that facilitate the adaptation to desiccation.

Seed dormancy, which ABA promotes, is an important seed survival attribute that can introduce a temporal delay in germination to provide additional time for seed dispersal, or to delay germination under conditions are less adverse.

Seeds and Fruits consists of:
- The **embryo** (derived from the *zygote*[TZ378], the union by fertilization of a male pollen sperm cell and a maternal egg cell in the ovule)
- The **endosperm** (usually triploid tissue derived from double "fertilization"—i.e., fusion of 2 maternal polar nuclei with a second pollen sperm cell nucleus). The **aleurone** layer of cells surrounds and is distinct from the starchy endosperm of cereal grains.
- A **seed coat** or **testa** (maternal)
- Other "maternal" tissues surrounding the seed coat:
  - **pericarp**
    exocarp or **epicarp** (the "peel")
    mesocarp or **sarcocarp** (typically the fruit "flesh")
    endocarp (e.g., the "shell" of pecans)
  - other extrafloral organs.

Seed dormancy mechanisms include
- **Coat imposed dormancy**: the seed coat, endosperm, and other structures surrounding the embryo can:
  - prevent water uptake (with waxy cuticles, suberin, or lignified sclereids, etc.),
  - prevent emergence of the radicle (the embryonic root). E.g., in lignified nuts, breakdown of the coat with enzymes may be needed,
  - interfere with gas exchange such as O_2,
  - retain inhibitors such as ABA,
  - inhibit production by producing inhibitors such as ABA.
- **Embryo-imposed dormancy**: is intrinsic to the embryo (derived from the *zygote*), and is thought to arise from inhibitors, especially a high ABA/GA ratio.

Seeds may have
- **primary dormancy** (if dormancy is the initial state), or
- **secondary dormancy** (if seeds are not initially dormant, but dormancy subsequently forms under conditions unfavorable to germination, etc.) ...

Environmental factors may control the release of a seed from dormancy Possibilities may include:

- **Afterripening**: Certain seeds lose dormancy after drying below a certain level of moisture.
- **Chilling**: Certain seeds are released from dormancy after reaching a low temp (0 - 10 °C typically) while in the fully imbibed (hydrated) state. This long-known grower's maneuver is called **stratification**, and often simply uses a refrigerator.
- **Light**: Certain seeds may have a light exposure requirement before they will germinate.

The longevity of seeds has often been exaggerated (e.g., seeds from ancient Egyptian tombs) but viability at more than 600 years has been documented, and some seeds may last many thousands of years (web topic 23.6).

Seed dormancy is controlled by the ratio of ABA to GA. ABA inhibits GA-induced gene expressions and enzyme production, such as the synthesis of α-amylase by barley aleurone...

Other Effects Of ABA

- ABA causes **closure of the leaf stomata in response to water stress** by causing long-term guard cell PM depolarization. This protective response is lacking in "wilty" tomatoes that are deficient in ABA. Concentration of ABA can rise 50x in leaves of plants undergoing water stress. (Further details discussed in chapter 26)
- ABA **promotes root growth and inhibits shoot growth in water stress conditions**, so that the ratio of root to shoot length elongation rises (up to 4X or more), and **increases the hydraulic conductivity** of the root.
- ABA promotes **leaf senescence** (but usually not actual abscission, which is mediated by ethylene).
- ABA accumulates in dormant buds and promotes **bud dormancy**, an important adaptation in cold climates (such dormant buds are protected by **bud scales**).
- ABA inhibits or delays flowering.[TZ664]

ABA Signal Transduction Pathways

This topic not summarized. There are both extracellular and intracellular ABA receptors, and several signal transduction pathways, providing redundancy for this important protective plant hormone...

Chapter XXIV. Brassinosteroids

[Chapter not studied in UW Plant Physiology Biol 425, limited summary.]

The **brassinosteroid** (**BR**) hormones were originally found in the pollen of Brassica napus and named **brassins**. They are polyhydroxylated steroidal plant hormones, therefore having four fused rings arranged in a 6-6-6-5 fashion.[197]

They were noted to cause both cell elongation and cell division, as well as bending, swelling, and splitting of the second internode of the bean bioassay. The first compound isolated and most active BR is the 28-C compound that was named **brassinolide (BL)**. The immediate precursor castasterone (CS) has weak BR activity. BRs are found in 27 families of seed plants, as well as in certain bryophytes, ferns, and even an alga. They are found in many parts of angiosperms, but in highest concentration at the apex of growing shoots, and in seeds and pollen.

Originally quantitated by two bioassays: **bean second-internode bioassay** (which shows bending, swelling, and splitting of the second internode) and **rice lamina (leaf) inclination bioassay** (which shows the increase of the bending angle between the axis and the adaxial surface of the 2nd leaf lamina). They are also quantitated by gas chromatography-mass spectrometry.

[197] Steroids: http://en.wikipedia.org/wiki/Steroid

Plants deficient in BRs are impaired in photomorphogenesis and dwarfed—BRs are needed for normal development.

BRs are made from the C_{30} triterpene **squalene** (i.e., a triterpenoid composed of 6 isoprene units). Synthesis pathway passes through precursors **cycloartenol**, campesterol, campestanol, and finally castasterone… **Sitosterol** and **cholesterol** may also be precursors, the latter occurring in plants at relatively low levels. Synthesis probably takes place on the ER. Catabolism is by several routes. Actions of BRs may be studied with help of the synthetic inhibitor **brassinazole** (Brz).

BRs act mostly locally near their site of synthesis…

Summary Of Actions: BRs are involved in many developmental processes. They promote **cell expansion and cell division** in shoots. The effects are most pronounced in young growing shoots. BL effects appear after 45-minute lag and reach a maximum at about 4-5 hours. **Auxin (IAA) and BRs act synergistically** with respect to **shoot growth**—each requires the other for optimal effect. BRs are needed for optimal **microtubule** organization. BRs promote or inhibit [non-lateral] **root growth** (depending on concentration), and these effects are independent of auxin (IAA) and GA. However, they also promote **lateral root growth** synergistically with auxin. BRs promote **xylem differentiation** during vascular development. They are required for **growth of pollen tubes**. They promote seed germination independent of GA signaling and can overcome the inhibitory effects of ABA. Seeds have high concentration of BRs.

Signaling pathway is not summarized.

Potential agricultural uses include: **increasing crop yields** of bean, rice, barley, wheat, and lentils; increasing lettuce leaf weight; and increased potato tuber growth. The beneficial effects are most apparent when the crop is growing under **stress**. BRs also aid in **plant propagation**, such as with Norway spruce and apple trees, or with **micropropagation** by tissue culture.

Chapter XXV. The Control Of Flowering

[Limited summary]

Review Of Flower Anatomy And Fertilization

Flower anatomy is summarized at web topic 1.2 and Chapter 16. The **floral organs** are

- **Sepals**, collectively called the **calyx**
- **Petals**, collectively called the **corolla**
- **Stamens**—male structures consisting of pollen-bearing **anthers** and **filaments**. **Pollen grains** are haploid **microspores** produced by meiosis in the anther and which represent the mature **male gametophyte**.
- **Carpel(s)**. The female reproductive part of the flower is the **gynoecium**. Terminology seems somewhat confused concerning pistils versus carpels.
  - When there is only one carpel, the gynoecium = one **simple pistil** = one carpel.
  - When two or more carpels are fused, the gynoecium is a **compound pistil**.
  - The gynoecium may also be composed of two or more unfused carpels, thus **two or more simple pistils**.[198,199]

  A pistil (or in some cases each of its constituent carpels) consists of a stigma (the pollen receptor), the more stalk-like **style**, and the basally-positioned **ovary**. The ovary contains **ovules** attached at **placentas**.

The stem of the individual flower, attaching it to the **inflorescence** (flower cluster), is the **pedicel**. Any leaf associated with an inflorescence is called a **bract**[200]—these often differ in morphology from the non-reproductive (vegetative) plant leaves.

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[198] Gynoecium, pistil, and carpel: http://en.wikipedia.org/wiki/Carpel
[199] Gynoecium: http://www.csdl.tamu.edu/FLORA/301Manhart/repro/Flower%20diagram/flower_diagram.htm
[200] Bract: http://en.wikipedia.org/wiki/Inflorescence

Gametophyte Formation and Fertilization: A cell within the ovule divides meiotically to produce the mature **female gametophyte**, called the **embryo sac** (**megaspore**), which gives rise to the **egg cell** and two **polar nuclei**.[TZ378] [MCM: These details appear to be complex and subject to wide variation and are inadequately summarized here.] A pollen grain landing on the stigma sends a sperm cell down the pollen tube to the ovule—this fuses with the egg cell to form the diploid **zygote**. A second sperm cell typically combines with the two polar nuclei of the embryo sac to initiate the formation of the triploid **endosperm**. The outer tissues of the ovule harden into a protective seed coat.

Floral Meristems and Floral Organ Development

Flower development begins with a **floral stimulus** that leads to **floral evocation**, the events that commit one or more apical meristems to produce flowers.

During vegetative growth, Arabidopsis grows as a low rosette due to very short internodes between the leaves. With floral evocation, the Arabidopsis vegetative **shoot apical meristem** SAM transforms to a **primary inflorescence meristem** (PIM), which grows a vertical axis having **cauline** leaves (cauline = arising from the stem) and flowers. At the axillary buds or axils of the cauline leaves, **secondary inflorescence meristems** (SIM) form, which develop similar to the PIM. Flowers arise from **floral meristems** (FM) on the flanks of the inflorescence meristems. Growth of flowers and inflorescences is **determinate**, unlike vegetative growth, which is usually **indeterminate**.

The floral organs develop as 4 concentric whorls arising at the FM. The outermost are the sepals, next the petals, next the stamens, and centrally the gynoecium (2 fused carpels in Arabidopsis).

[Genetic control of floral organ development is not summarized here. Homeotic genes control the identity of floral organs, according to the ABC model...[TZ640]]

Floral Competence, Induction, Determined State, And Expression

Some plants flower with **autonomous** regulation in response to strictly internal factors (such as plant size or number of leaves), whereas others respond to **environmental stimuli** (such as day/night length). For the latter, either

- environmental stimuli are absolutely required ("**obligate**" or "qualitative"), or
- environmental stimuli promote flowering but are not absolutely required ("**facultative**" or "quantitative")

Environmental stimuli include **vernalization** and **photoperiodism**.

Plants often have **3 developmental phases** (Web Topic 25.1):

- **Juvenile phase (juvenility)**: not yet capable of reproduction. Lasts 20 days to 40 years.
- **Adult vegetative competent phase (competence)**: capable of reproduction but not yet induced to do so
- **Adult reproductive phase**: in process of flowering

The shape of the leaves, phyllotaxy (leaf arrangement along the stem), leaf thickness or size, petioles, plant growth habit, presence of aerial roots, flowers, cuticle thickness, epidermal cell configuration, etc. may differ between juvenile and adult reproductive phases (e.g., English ivy).

Plants undergo a transition in developmental phase, and the higher or more distal (peripheral) parts of the shoot tend to be reproductive, whereas the older lower or more proximal are vegetative. (fig. 25.11)

Stressful conditions can delay transition to the adult reproductive phase, or cause an inflorescence meristem to revert to a vegetative meristem ("**rejuvenation**").

Gibberellins stimulate the transition to the mature state (competence) from juvenility in some plants, but can also cause rejuvenation in other plants.

Once a vegetative plant is competent to flower, it is capable of being induced. After **induction** it is said to be "**determined**" to develop flowers if a **scion** (upper part of a graft) from it will progress to flowering even though it has been removed (by this grafting) from its normal context. (see fig. 25.13 grafting experiments). More than one developmental signal may be needed to actually **express** flowering—e.g., long days are needed for determination in Lolium, whereas gibberellin is needed for expression of flowering from the determined state. Older plant leaves are more likely to produce an adequate floral stimulus (e.g., require fewer long-day photoinductive cycles) to induce flowering (fig. 25.14).

Circadian Rhythms And Photoperiodism

Plants have inherent day/night rhythmic behavior, such as leaf and petal movements including **nyctinasty** (night-related movements such as drooping of leaves or closing of petals). If placed in continuous darkness, the observed rhythmic changes persist for several day, indicating that an inherent molecular (endogenous) oscillator is driving them. (TZ Chap. 17 p. 433.) These circadian rhythms have characteristic period, amplitude, and phase. The rhythms are maintained in synchrony with actual day/night conditions, which may have somewhat differing periods, by **entrainment** at the dawn and dusk transitions. The time keeping is independent of temperature due to **temperature compensation**.

Environmental time signals producing this entrainment (such as light exposure) are called **zeitgebers**. For instance, a pulse of light before the arrival of dawn may entrain the plant to advance the phase of the circadian rhythm.

Entrainment inputs are mediated by specific photoreceptors. For example, red light (RL) entrains nyctonasty in Samanea via **phytochrome**. In Arabidopsis, there are 5 phytochromes, of which 4 are known to be involved with clock entrainment. **Cryptochrome CRY1** and **CRY2 proteins** are also involved with blue light (BL) entrainment, possibly as intermediates for phytochrome signaling, though the mechanism is not known.

Photoperiodism is the ability of a plant to detect the length of the day or night, and thus allowing it to have a **seasonal responses**. In animals these can be hibernation, summer and winter coats, and rutting—in plants they include flowering, asexual reproduction, formation of storage organs, onset of dormancy, and leaf senescence and abscission. Day length varies with seasons, but more at higher latitudes than near the equator. The responsiveness to varying day and night length appears to be influenced in specific plants by the latitude from which they originated. The circadian oscillator is compared against the detected day/night length.

Flowering plants can be classified according to their day length flowering behavior:
Day-neutral plants (DNP) have flowering which is insensitive to day length. These plants typically evolved near the equator. Example: kidney bean and many desert annuals such as desert paintbrush.
Short Day Plants (SDP) flower (obligately or facultatively) when day length falls below a **critical day length**, and thus flower in the fall. Examples include Chrysanthemum morifolium, soybean, and the cocklebur Xanthium.
Long Day Plants (LDP) flower (obligately or facultatively) when day length rises above a **critical day length**, and thus flower in the spring or early summer. Examples include many wheats, Fuchsia, and Arabidopsis.

In order to disambiguate spring days from fall days of equal length, plants have several possible mechanisms:

- a **juvenile** phase which is not competent to flower
- a coupling with temperature cycles (such as a **vernalization** requirement), or
- detection of whether days are **lengthening versus shortening**. These include:
  - **Long-Short Day plants (LSDP)**: only flower when long days are followed by short days. Example: Kalanchoe
  - **Short-Long Day plants (SLDP)**: only flower when short days are followed by long days. Example: White clover.

The leaf is the exclusive site of detection of photoperiodism. A single day's exposure of the needed duration can trigger flowering—in some cases (such as with the SDP Xanthium) after only a single leaf is exposed

("induced"). Grafting studies show that the induced leaf produces a mobile floral stimulus that is transported to the apical meristem in the phloem, where it promotes floral evocation. Proximity of the induced leaf to the shoot apex is more likely to lead to floral evocation at the SAM, due to the varying patterns of phloem flow from sources to sinks. The induced leaves should be actively photosynthesizing, in order to serve as phloem sources.

For SDPs such as Xanthium, a flash of red light lasting only 1 minute during the long night (i.e., a "**night break**") can block flowering by making the night seem shorter. Similarly, for a LDP such as Fuchsia, a long night with a night break of 1 hour of red light can act like a short night and trigger flowering. Night breaks are most effective if they occur in the middle of the night. Night breaks are used commercially to control flowering of poinsettias, chrysanthemums, etc.

Plants actually measure the **length of the night**. Thus,
For SDP plants:
- Short days followed by short nights do not induce flowering.
- Short days followed by long nights do induce flowering.

For LDP plants:
- Short days followed by short nights do induce flowering.
- Long days followed by long nights do not induce flowering.

According to the **Clock Hypothesis** and **coincidence model**, the plant's photoperiodic timekeeping depends on the endogenous circadian oscillator controlling the timing of light-sensitive and light-insensitive phases... (fig. 25.21) Exposure to light during a light-sensitive phase induces flowering in LDPs or prevents flowering in SDPs. The gene **CONSTANS (CO)** plays a role in the LDP Arabidopsis as a flowering promoter, and its expression is controlled by the circadian clock ... When the CO protein increase coincides with long day light (sensed by phy A and cryptochrome), the transmissible floral stimulus rises. (The transmissible floral stimulus or florigen for some plants is now thought to be **FT Protein** which is encoded by the gene **FLOWERING LOCUS T**. Although initially the textbook described **FT mRNA** as the florigen or transmissible floral stimulus in the phloem[TZ665], more recent work suggests that this role is played by FT Protein,[201] and the textbook website also states this to be the case in Essay 25.2, added in 2007.) In the SDP monocot rice, Heading-date 1 (Hd1) and **Heading-date 3a (Hd3a)** appear to play roles similar to CO and FT, respectively.

The primary photoreceptor in photoperiodism is **phytochrome** Pr, which is sensitive primarily to RL and FRL. (fig. 25.23) Exposure of Pr to RL converts Pr to Pfr, whereas exposure of Pfr to far RL converts Pfr back to Pr. (see Chapter 17) In many SDPs, exposure to a RL night break inhibits flowering, but following this RL night break with a FRL night break blocks the inhibiting effect of the RL and restores flowering... The peak absorption of RL in SDP Pharbitis nil dark-grown (and therefore without interfering Chl) is at 660 nm, the absorption maximum for Pr. Similarly, in some LDPs, a night break of RL promotes flowering, and a subsequent exposure to FRL (mediated by phy A) blocks this response ... However, a **blue light** photoreceptor also plays a role in some LDPs, probably **cryptochromes** CRY1 and CRY2...

Vernalization

Vernalization is the process by which flowering in some plants is promoted by exposure of a fully hydrated seed to cold temperatures. (Dry seeds do not respond in this manner.) The required temperature range for vernalization varies from a little below freezing to up to 10 ºC, and typically requires several weeks to be long-lasting in its effect (fig. 25.27). Vernalization causes competence for flowering, apparently in the shoot apical meristem. E.g., winter annuals such as winter wheat sprout in the fall but require cold exposure to flower in the following spring or summer. A vernalization requirement is often linked to a photoperiod requirement—e.g., cold temperatures followed by long days leading to early summer blooming.

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[201] FT Protein is the transmissible Florigen:
• http://4e.plantphys.net/article.php?ch=25&id=398
• Yuejun Yang et al, "Florigen (II): It is a Mobile Protein." *Journal of Integrative Plant Biology* 2007, 49 (12): 1665–1669. accessed 3/13/08 at http://www.blackwell-synergy.com/doi/pdf/10.1111/j.1744-7909.2007.00614.x

Vernalization involves **epigenetic changes** in gene expression. Epigenetics describes stable changes in gene expression that can be passed to new generations of descendent cells through mitotic or meiotic cell division (i.e., changes that are heritable) but that do not involve alteration of the actual DNA sequence[202].... (Epigenetic changes are seen from yeast to mammals, though much work on the **epigenome** is currently focused on humans). The Arabidopsis flowering repressor gene **FLOWERING LOCUS C (FLC)** is expressed in non-vernalized plants, but after vernalization this gene is epigenetically switched off for the remainder of the plant's life. The specific epigenetic change in Arabidopsis[203] caused by FLC involves modification of the chromatin structure—these modifications are called changes in the "**histone code**". FLC expression causes histone modifications favoring the **heterochromatin** configuration that inhibits transcription [of what—SOC1?]. Vernalization causes FLC's expression to be silenced, allowing modification of the chromatin structure to the **euchromatin** configuration that is optimal for transcription. Arabidopsis FLC encodes a MADS box protein—in cereals, a gene encoding VRN2 protein plays a similar role as FLC.

Mechanisms of vernalization are varied. Plants that evolved in warm climates may not have evolved vernalization mechanisms until they found themselves in more temperate habitats.

Biochemical Signaling In Flowering

[Limited summary] The leaves are a major source for signaling leading to floral evocation. There is no single universal floral stimulus. The proposed transmissible compound playing this role was originally called **florigen** by Mikhail Chailakhyan in the 1930s. Graft experiments show that "florigen" is transmissible even between genera... and it has been shown to move in the phloem. The induced state in some plants is **self-propagating** once established. (See fig. 25.31 for graft induction experiments.) A inhibitor of flowering (**antflorigen**) has also been identified. Florigen is a macromolecule. The downstream target gene of CO, FLOWERING LOCUS T (FT) is a candidate for the floral stimulus, and leads to the expression of FT protein. It is FT protein, not its mRNA, that serves as the transmissible agent moving in the phloem (see above). Gibberellins can promote or inhibit flowering induction in many plants (web topic 25.8), and differing GAs may have differing effects. The transition to flowering involves many pathways, and may also involve carbohydrates, polyamines (such as putrescine), gibberellins, cytokinins, and ethylene (the latter is a promoter of flowering in bromeliaceae including pineapple).

In Arabidopsis and other plants, 4 pathways are involved in flowering, acting ultimately on the key **floral meristem identity gene SOC1 (SUPRESSOR OF CONSTANS 1**). These pathways are summarized as follows (fig. 25.33):

- Photoperiodic pathway: begins in the leaf, involves phytochromes and cryptochromes, leads to the expression of FT protein (or, in rice, the Hd3a protein). FT combines with FD to increase expression of SOC1.
- The autonomous and vernalization pathways: These reduce expression of the repressor gene FLC, diminishing therefore its repression of SOC1 expression.
- The carbohydrate or sucrose pathways: These stimulate flowering by increased expression of LEAFY (**LFY**) gene and ultimately SOC1 expression.
- The gibberellin pathway: is required for early flowering and for flowering under noninductive short days in LDP plants, and acts on SOC1 expression.

These four pathways therefore sum flexibly and with desirable redundancy into a unitary output that ultimately acts to increase expression of SOC1. SOC1 activates LFY, and this activates floral homeotic genes **AP1**, AP3, PI, and AG... Once AP1 has been expressed, the transition to flowering is irreversible. "The presence of multiple flowering pathways is probably universal in angiosperms."

Chapter XXVI. Physiology of Environmental Stresses

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[202] Epigenetics:
• General: http://en.wikipedia.org/wiki/Epigenetics
• Human Epigenome Project: http://www.epigenome.org/index.php
• Epigenome Network of Excellence: http://www.epigenome-noe.net/index.php
[203] Epigenetic inheritance in Arabidopsis: http://epmb.berkeley.edu/vfs/PIs/Zilberman-D/web/CurOpinion2005.pdf

Environmental factors which subject plants to stresses have already been discussed at many points in the textbook and this outline. A plant stress is "an external factor that exerts a disadvantageous influence on the plant",[TZ671] typically affecting survival, growth or crop yield, assimilation, or other objective markers of plant success. This chapter deals with **environmental abiotic stresses**, thus excluding herbivorous insects, microbial infections, allelopathy, etc. (which are discussed in chapter 13). Environmental abiotic stresses include:

- water deficit
- excessive heat
- chilling and freezing
- light deprivation or excess (discussed mostly in chapter 7, 8, and 9)
- mineral deficiencies (discussed mostly in chapter 5)
- hypersalinity and toxic trace elements
- hypoxia or anoxia of roots or other plant parts (arising usually from submergence in water)
- air pollution

Various stresses can reduce crop yields to a small percentage of the biological potential of crop plants. Plants have varying levels of coping ability with respect to a particular environmental condition—termed **stress tolerance**, a designation preferred over **stress resistance**—and conditions that are stressful for one plant may be optimal for another. A plant that during its own environmental exposures has become conditioned to tolerate what would at least initially be stressful conditions is said to be **acclimated** or **hardened**, an acquired condition which in part involves changes in gene expression. This differs from **adaptation**, a term which should be reserved for genetic characteristics of the plant species, strain, etc. that improve its fitness. Adaptation and acclimation occur at all levels, from biochemical to cellular to gross morphological... Stresses tend to be interrelated and are often synergistic, and may result in similar acclimation and adaptation processes.

Water Deficit and Drought Tolerance

Plants experiencing water deficit may exhibit **desiccation postponement** (in which they delay internal desiccation and maintain tissue hydration) or **drought tolerance** or **drought resistance**. (The term **desiccation tolerance**[TZ676] is also used[204] to refer to an extreme form of drought resistance.) Although the weather condition *drought* cannot be fully avoided, some plants are inactive during drought periods of the year and exhibit "**drought escape**"—they are the only plants exhibiting true **drought avoidance**. Some plants are **water savers** (such as the C_4 and CAM plants described in chapter 8), while others such as **mesquite** (legumes of genus *Prosopis*) are profligate with water (they extract it through deep taproots extending up to 190 feet to reach low a water table).[205]

Water utilization strategies and efficiency are discussed in chapter 4. Crop yields of cotton, corn, and sorghum are substantially affected by droughts (Table 26.1 and TZ p. 683). Water deficit limits leaf expansion and growth, thereby potentially reducing PS. Rapid growth of certain plants during a wet season can lead to soil water depletion during a subsequent dry season that impairs reproduction (details omitted). Plants that are **indeterminate**[206] with respect to their ability to mix vegetative and reproductive or flowering stages (e.g., indeterminate tomatoes can produce fruit throughout the growing season) may be more better adapted for adjusting to erratic summer rainfall.

Specific plant responses to water deficit include the following.

Decreased leaf area

This occurs by loss of turgor and decreased cell expansion, with resulting leaf shrinkage. This also increases intracellular solute concentrations and thickens the PM. Root elongation may also decrease, though typically this is a later effect.

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[204] Drought tolerance and desiccation tolerance: http://en.wikipedia.org/wiki/Drought_tolerance
[205] Mesquite: http://en.wikipedia.org/wiki/Mesquite
[206] Indeterminate growth and reproduction: http://en.wikipedia.org/wiki/Indeterminate_growth

Leaf expansion (**Growth Rate GR**) depends on **leaf turgor pressure Ψ_p** as depicted in Fig. 26.1 according to the formula

$$GR = m(\Psi_p - Y)$$

(details omitted here, but fully discussed in chapter 15, see wall yielding growth rate and yield threshold, etc.) The **wall yield threshold Y** is the turgor pressure at which wall elongation commences, and therefore below which no elongation can occur. Water stress not only lowers Ψ_p but also decreases the **coefficient of wall extensibility m** and increases Y (details omitted, but depends on rise in CW pH, etc.)

The smaller leaf area reduces transpiration of water and effectively conserves the limited water supply available. The number of branches and leaves may also be reduced. Plants may limit growth by a number of coordinated responses (details omitted).

Leaf abscission

Water stressed plants will accelerate leaf senescence and abscission. Drought deciduous plants can drop all their leaves in drought conditions, even more than once per year. This response is controlled largely by local auxin decline and ethylene increase (chapter 22).

Increased root growth (which may be in competition with reproduction)

"A shoot will grow until it is so large that water uptake by the roots becomes limiting to further growth... Roots will grow until their demand for photosynthate from the shoot equals the supply."[TZ674] When leaves are not growing, more photosynthate is available for root growth. Root apices in dry soil become flaccid and do not grow—root growth instead takes place in the moister soil zones. In dry conditions, it is the deeper soil that is moist, so roots extend deeper during drought. However, if the plant is attempting to reproduce with flowers and fruits, etc., this process competes with root growth, and renders the plant more vulnerable to water stress.

Stomatal closure from Abscisic acid ABA

Secretion of Abscisic acid ABA causing guard cell (GC) stomatal closure is a more rapid response to water stress. It can reduce dehydration, and is discussed in chapter 23. Guard cells that lose turgor by direct evaporation cause closure of the stomata, a process termed **hydropassive stomatal closure**. In addition, **hydroactive stomatal closure** is an active metabolic process that results in loss of GC solute, rise in GC solute potential, loss of GC water and decreased GC turgor—this sequence also leads to stomatal closure.

The ABA is stored in the mesophyll chloroplast stroma, and upon dehydration, more is synthesized by the mesophyll cells and released into the surrounding apoplast, so that transpiration carries it to the nearby GCs (details omitted, see fig. 26.3).

Chemical signals from the roots may also affect the stomatal responses, possibly ABA (chapter 23), but also probably pH and the distribution of inorganic ions.[TZ675]

Stomatal conductance directly affects the yields of crops such as Pima cotton (*Gossypium barbadense*) and bread wheat (*Triticum aestivum*) (see web topic 26.1).

Limiting photosynthesis

Mild water deficit impacts leaf expansion more substantially than it impacts the rate of PS (fig. 26.4), especially in C_4 and CAM plants (chapter 8). In part this is because "stomatal closure inhibits transpiration more than it decreases intercellular CO_2 concentration,"[TZ676] so that water use efficiency initially remains high. With greater water stress, dehydration of the mesophyll increasingly inhibits PS and consumption of assimilates, leading to decreasing export of photosynthate from leaves. At relatively severe stress, translocation (presumably in the phloem) also rapidly declines (fig. 26.5), but only after PS is

already severely decreased. This allows the mildly to moderately stressed plant to mobilize and reallocate its resources, and helps to confer drought resistance.

Osmotic adjustment of cells helps to maintain water balance

The effect of soil matric potential $\Psi_m = \Psi_s + \Psi_p$ on water absorption in the roots is discussed in chapter 3 and Web topic 3.5. Recall that water moves across semi-permeable membranes in the roots from areas of higher water potential $\Psi_w = \Psi_s + \Psi_p = \Psi_m$ (for soil) to areas of lower water potential. Root cells receive water by reducing their internal water potential to maintain it below the soil water potential. They do this by lowering Ψ_s, the internal solute potential, by raising solute concentrations, typically including sugars, organic acids, amino acids, and (tolerable in the vacuole only) inorganic ions especially K^+. The cytosol must accumulate "**compatible solutes**" or "compatible osmolytes" to balance the increase of inorganic ions in the vacuole. These compatible solutes include **proline**; **sugar alcohols** such as **sorbitol**, **mannitol**, and **pinitol**; and **glycine betaine** (**trimethylglycine**, named originally after its discovery in sugar beets, *Beta vulgaris*).[207] This osmotic adjustment takes days to achieve. It represents an acclimation that improves dehydration tolerance and that can help to maintain leaf turgor pressure and survival. (Root cells also engage in osmotic adjustment, though the process in roots is less well studied compared to leaves.) The amount of additional water that can be extracted in this manner from drying soil is modest and rapidly diminishes (fig. 26.6), and there is little improvement in productivity.

Increased resistance to water flow

As soil dries and approaches the **permanent wilting point** (c. -1.5 MPa), the resistance to water flow increases rapidly, and plants can no longer maintain or regain turgor pressure (web topic 4.2). Rehydration of the plant is limited by factors intrinsic to the plant which increase resistance to water flow. One or more of the following may apply:

- The root surfaces have shrunk away from the soil particles.
- The root hairs have been damaged.
- The root cortex is more extensively covered with suberin.
- Cavitation and gas bubbles have occurred in the xylem (see chapter 4). Cavitation begins at water potentials of -1.0 to -2.0 MPa, and involves the largest diameter vessels first. Even when adequate water is subsequently restored, these cavitated formerly low-resistance vessels may no longer function efficiently.

Increased leaf wax deposition

This occurs as a thicker cuticle that reduces water loss from the epidermis. The thicker cuticle also decreases CO_2 permeability, but leaf PS is unaffected.

Alteration in energy dissipation from leaves by altering leaf angle, geometry, or barriers to sunlight

Well-watered plants in hot climates cool their leaves noticeably—as much as 8 ºC lower—compared to ambient atmospheric temperature, using evapotranspiration. When water is less available, transpiration slows and leaves are noticeably warmer to the touch. Thus water stress and heat stress are closely related.

Water stressed plants also shed excess heat with

- **Sensible heat loss**—by air circulation, convection, and conduction (chapter 9)
- Having **small leaves** (to minimize the boundary layer resistance, chapter 9 and 4). Larger leafs have larger boundary layers, reducing the direct dissipation to air of heat per unit area of leaf.
- **Paraheliotropic tracking** (turning leaves away from direct sun), **wilting**, or **leaf rolling** (in grasses), all of which reduce leaf exposure to sunlight (as might be measured, for example, in photon irradiance in mol m^{-2} s^{-1}) (fig. 26.7 and chapter 9)

[207] Glycine betaine AKA trimethylglycine: http://en.wikipedia.org/wiki/Glycine_betaine

- **Increased sunlight reflection** by **increased leaf hairs** (**pubescence**)[208] or a typically **gray-white reflective wax** layer formed outside the cuticle. This type of wax does however affect the PS rate.

Crassulacean acid metabolism (CAM) photosynthesis

As discussed in chapters 8 and 9, the CAM adaptation makes possible stomatal closure during the day, markedly improving the efficiency of water utilization coupled with PS. CAM plants lose only c. 125 g of water for every gram of carbon-containing dry matter gained (mostly at night), compared to 3 to 5 times more water utilization in C_3 plants. Some succulent plants exhibit **facultative CAM**, switching as needed between C_3 metabolism when water is plentiful and CAM under conditions of water or saline stress. Fig. 26.8 demonstrates the induction of PEP carboxylase during exposure to water and salt stress in one of the "ice plants" (*Mesembryanthemum crystallinum*, not the same ice plant as *Carpobrotus chilensis* or *Delosperma cooperi*).

Osmotic stress and altered gene expression

Water stress leads to osmotic stress from accumulation of compatible intracellular solutes due to activation of altered gene expression. These genes encode for proline, glycine betaine, and sugar alcohols such as pinitol. Osmotic stress also induces additional upregulated genes (e.g., for heat shock proteins, aquaporins, and LEA proteins—Table 26.2) and downregulates other genes (e.g., for sucrose production and mannitol degradation). ABA regulates some but not all of the upregulated gene pathways affecting osmotic stress tolerance. (details of gene promoters and signaling pathways omitted, see also fig. 26.9)

Heat Stress and Heat Shock

Levels of heat tolerance

The effects of heat on PS have been discussed in chapter 9. Most intact higher plants cannot tolerate extended exposure to temperatures higher than 45 °C, though dry seeds and pollen can survive considerably higher. (See table 26.3 for heat-killing temperatures for various plants, seeds, pollen, etc. Especially heat tolerant items include red pine pollen 70 °C, dehydrated mosses up to 110 °C, and alfalfa seeds 120 °C) Only single celled eukaryotes can complete their life cycle about 50 °C and only certain Archaea can divide above 60 °C. Brief exposures to heat can induce acquired thermotolerance in some plants. Heat stress is greater when water stress exists, but it may be high on emerging seedlings even in moist soil due to the higher sunlight absorption of darker moist soil.

Mechanisms of heat dissipation and tolerance

High leaf temperature and minimal evaporative cooling lead to heat stress. Succulents with CAM such as *Opuntia*[209] (prickly pear cacti) and *Sempervivum*[210] (houseleeks or live-forever of family Crassulaceae) can tolerate tissue temperatures of 60 to 65 °C. In CAM plants with closed stomata, mechanisms of heat loss are discussed in chapter 9, and included re-radiation at long wavelengths and sensible heat loss through air circulation, convection, and conduction. C_3 plants experiencing water stress reduce stomatal conductance and water transpiration, causing increased leaf temperature while sun exposed. High leaf temperatures can also occur when humidity is high, preventing evapotranspiration (as in tropical environments or greenhouses).

Irrigation increases crop yields in cotton, corn, and sorghum as previously discussed (web topic 26.1)

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[208] Leaf pubescence and xeric conditions:
http://www.blackwell-synergy.com/doi/pdf/10.1046/j.1469-8137.1997.00697.x?cookieSet=1
[209] *Opuntia*: http://en.wikipedia.org/wiki/Opuntia
[210] *Sempervivum*: http://en.wikipedia.org/wiki/Sempervivum

Effects of heat stress

As temperature increases, PS rates drop before respiration decreases (fig. 26.10, also chapter 9). Above the **temperature compensation point**, the plant evolves more CO_2 from mitochondrial respiration than it assimilates and fixes by PS (i.e., CO_2 assimilation is negative). In this state, CHO reserves decline and fruits and vegetables lose their sweetness, etc. (In chapter 9, the light compensation point was also discussed. In chapter 8, the greater sensitivity to excess heat of C_3 plants compared to C_4/CAM plants was discussed, arising from higher dark respiration and photorespiration.)

Role of membranes in plant adaptation and acclimation to high temperatures

Adaptation or acclimation to high temperatures varies (see fig. 26.10). For example, *Atriplex sabulosa* (a cool weather species) shows a more rapid falloff of PS with rising temperatures around 40 to 48 ºC compared to *Tidestromia oblongifolia* (a hot weather species), whereas growth rates at much lower temperatures (16 ºC) favors *A. sabulosa*. Crop species tend to grow best at the temperatures in which they evolved and adapted, but the underlying mechanisms that would explain these differences are complex.

As discussed in chapter 9, the declines of PS seen at higher temperatures are due mainly to **instability of membrane bound electron transport**, a phenomenon which dominates over denaturation of enzymes that can occur at even higher temperatures. Membrane stability is affected by temperature. High temperatures cause excess **membrane fluidity**, especially in the presence of greater unsaturation of fatty acids in the membrane lipids. (Greater membrane lipid unsaturation plays a role in improving chill tolerance by making membranes more liquid and flexible when cold, see chapter 11 and later in this chapter.) In oleander (*Nerium oleander*), high temperature acclimation is accompanied by a greater degree of **saturated fatty acids** in membrane lipids, which makes them less fluid,[TZ684] and a similar effect is induced in Arabidopsis mutants that make less omega-3 (unsaturated) fatty acids. High temperatures lead to membrane disruption and ion leakage, as well as inhibition of PS and respiration. Although high temperatures also cause denaturation of proteins such as rubisco and could thereby affect enzyme stability, this generally occurs at higher temperatures than those initially affecting PS.

Leaf adaptations against high temperatures

Plants in high light and heat environments must reduce their exposure to solar radiation or improve heat dissipation. As discussed above, they do this with increased leaf hairs (pubescence), more reflective surface waxes, paraheliotropic tracking, wilting, leaf rolling, as well as with smaller and/or highly **dissected** leaves to reduce boundary layer thickness. Some desert plants such as *Encelia farinosa* (white brittlebush) adapt with seasonally dimorphic leaves: green and hairless in winter, white and pubescent in summer.

Protective heat shock proteins and other heat tolerance agents

At higher temperatures, plant produce **heat shock proteins** (**HSPs**—Table 26.4 lists the several classes). These act as "molecular chaperones", preventing deleterious unfolding or misfolding of enzymes and structural protein components. HSPs are induced by sudden as well as by gradually rising temperatures, and play a critical role in mediating heat tolerance. These are also found in animals, and were first discovered in *Drosophila.* (Further details omitted, including signal transduction pathways with **heat shock factors** and heat shock elements.)

In addition, ABA and salicylic acid as well as ethylene play a role in increasing tolerance to heat stress.

There are several signaling pathways involved in heat stress. **γ-aminobutyric acid** (**GABA**), which is synthesized by **glutamate decarboxylase** (**GAD**), accumulates in response to heat stress and appears to play an important role in integrating metabolic responses to stress (details omitted, see fig. 26.12 and web topic 26.2).

Chilling And Freezing

Chilling refers to excessively low temperatures that prevent normal growth in susceptible plants, but without formation of ice crystals (**freezing**).

Many common plants are susceptible to injury by **chilling**:[TZ687]

- Crop plants: *Phaseolus* bean, corn [maize], cotton, cucumber, rice, soybean (*Glycine max*), sweet potato, tobacco, tomato
- Ornamentals: Coleus (genus *Coleus* or *Solenostemon*), *Gloxinia* (family Gesneriaceae), and *Passiflora* (Passion flower)

Plants or seeds collected from and therefore adapted to higher altitudes tend to have better chilling resistance (see fig. 26.13 demonstrating wild tomato *Lycopersicon hirsutum*).[211] Resistance to chilling often improves by acclimation following exposure to slowly and gradually increased chilling ("**hardening**"). A sudden exposure to temperatures around 0 °C produces "**cold shock**", whereas exposures well below 0 °C lead to overt freezing injury. Some plants can be acclimated to freezing temperatures by gradual cold acclimation.

Membrane properties in chilling injury and resistance

Many of the effects seen with chilling exposure, including reduced PS, slower CHO translocation in the phloem, reduced protein synthesis, increased protein degradation, and solute loss to the external environment depend on **loss of function of membranes**—whether plasma, tonoplast, mitochondrial, and/or chloroplast, etc. Chilling-sensitive plants, when compared to chilling-resistant plants, have membrane lipids containing a higher ratio of saturated to unsaturated fatty acids (FAs). (See Table 26.5, which demonstrates a higher percentage of polyunsaturated Linolenic 18:3 and/or Linoleic 18:2 FAs in chilling-resistant plants.)

Lipids with higher content of saturated FAs become "solid" (or at least semi-crystallized) at temperatures well above °C. (The transition arising from falling temperatures in lipids from a liquid state to a semicrystalline semi-solid is a gradual phase change.) The hierarchy of temperatures at which semicrystallization occurs in lipids is:

saturated > monounsaturated > polyunsaturated

That is, saturated lipids semi-solidify (or melt) at the highest temperatures, a fact familiar to users of palm or coconut oil, which are semi-solid at room temperature. The stiffer less pliable membrane inhibits many transmembrane functions, including the H+-ATPase pump activity, solute transport, etc. Chilling-sensitive leaves that are chilled are also more vulnerable to sun damage and **photoinhibition** (see also chapter 7). Acclimation to cold leads to a higher percentage of unsaturated FAs in membrane lipids. This greater unsaturation of membrane lipids is important in providing some protection to cold by contributing to chilling resistance. "The degree of fatty acid saturation of phosphatidylglycerol (PG) is particularly important to the chilling resistance of **chloroplasts**." (Web topic 26.3). Transgenically altered tobacco plants with higher FA polyunsaturation of their PG (72% compared to 24%) have lower photoinhibition rates (7% compared to 88%) at 1 °C. (Web topic 26.3. However, see also web topic 11.8 which concludes "membrane lipid composition is not the major determinant of chilling sensitivity in plants.")

Ice crystal formation and protoplast dehydration kills cells

Seeds, fungal spores, and some other partly dehydrated plant tissues can be stored indefinitely at near absolute zero temperatures (0 K) and still retain viability on proper thawing. More hydrated vegetative tissues can in some cases retain viability if frozen very rapidly, in order to minimize ice crystal size and thereby minimize the damage they cause on formation. Similarly, thawing by warming of frozen tissues should be done rapidly[TZ689] to

- prevent interim growth in ice crystals

[211] Wild tomato chilling resistance versus altitude: http://www.publish.csiro.au/paper/PP9780609.htm

- prevent sublimation of water from ice, which occurs at -100 ºC to -10 ºC or higher. (Ice sublimation, such as with freeze drying, requires input of 80 + 540 = 620 cal g^{-1}, plus 1 cal g^{-1} for each 1 ºC increase in final vapor temperature compared to the starting temperature of the ice.)

Freezing of intact plants outside the laboratory is usually not fast enough to prevent formation of larger potentially injurious ice crystals. So-called "**hardy plants**" are able to tolerate these ice crystals for a while anyway. However, prolonged freezing eventually leads to growth of large **extracellular ice crystals** and excessive intracellular **dehydration**.

Cooling a plant to below 0 ºC initially leads to supercooling of the extracellular and intracellular water (i.e., the water inside the PM, including the vacuole). **Supercooling**[212] is defined as chilling a liquid below its freezing point, without it becoming solid. (Supercooled droplets of water may exist in clouds, and can abruptly freeze causing wing icing when struck by the wings of passing airplanes.)

The onset of ice formation, initially **extracellular ice formation**, releases 80 cal g^{-1} as the **latent heat of fusion**, causing the temperature to rise transiently though still remaining below 0 ºC. (Spraying crops with water during frost danger utilizes this temperature rise resulting from release of heat of fusion to prevent intracellular freezing.) The extracellular ice forming draws water from inside the cell over prolonged periods of freezing, leading to intracellular dehydration and increased intracellular osmolarity from hyperconcentrated solutes. This accounts for the delay in intracellular freezing. (Web topic 26.4) Eventually, intracellular ice formation proceeds, which is not itself usually lethal, though the intracellular dehydration can be.

Acclimation to moderate freezing temperatures may arise when a plant **suppresses its intracellular nucleation points**, promoting deep intracellular supercooling. Plants produce sugars, amino acids, and other solutes that protect cells against intracellular ice formation by reducing nucleation, and this leads to supercooling. If temperatures continue to fall below –40°C, ice crystals form even without nucleation points, and "intracellular freezing and cell death are unavoidable. Species that tolerate temperatures below –40°C under natural conditions do so not by supercooling, but by tolerating gradual dehydration." (Web topic 26.4) Some intracellular compounds on surfaces, such as large polysaccharides and proteins (including some proteins of bacterial origin), actually serve as **ice nucleators**,[TZ689] thereby contributing to plant damage from freezing.

Limitation of ice formation (or of ice recrystallization) contributes to freezing tolerance

Colligative properties[213] are properties of solutions that depend on the number of solute particles in a given volume of solvent and not on the mass of the solute particles—these properties include lowering of vapor pressure, elevation of boiling point, depression of freezing point, etc.

However, certain specialized plant proteins found in some species may help to limit growth of ice crystals by **non-colligative** means, and are termed **antifreeze proteins** (**AFPs**) or **ice structuring proteins**.[214,215] "Overwintering plants also produce **antifreeze proteins and other low molecular weight compounds** [including polypeptides]. These compounds provide freeze tolerance [MCM: by binding to surfaces of ice crystals and] by **inhibiting ice crystal growth** and the nucleation of ice crystals." (Web topic 26.4) Antifreeze proteins are found in rye (*Secale cereale*) in the epidermal and other cells surrounding the intercellular spaces, where they inhibit growth of extracellular ice. These proteins are homologous genetically to antifreeze proteins found in some fish such as winter flounder.[TZ689] A difference between the melting point and freezing point of a solution is known as **thermal hysteresis**—but the amount of thermal hysteresis is much greater in insects such as spruce budworm (up to 30°C) than in plants (typically only 0.2 to 0.5 °C).[216] Their actions in plants are thought to include inhibition of recrystallization of ice.[217]

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[212] Supercooling: http://en.wikipedia.org/wiki/Supercooled
[213] Colligative properties: http://en.wikipedia.org/wiki/Colligative
[214] Antifreeze protein: http://en.wikipedia.org/wiki/Antifreeze_protein
[215] Ice structuring proteins: http://www.ncbi.nlm.nih.gov/pubmed/12050776?dopt=Abstract
[216] Thermal hysteresis in plants:
- http://www.ncbi.nlm.nih.gov/pubmed/15358271
- http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=158397&blobtype=pdf

[217] Antifreeze proteins inhibit recrystallization: http://en.wikipedia.org/wiki/Antifreeze_protein

Sugars and some induced proteins are cryoprotective by stabilizing proteins and membranes during dehydration arising from cold temperatures. Sucrose confers this protection on winter wheat, but other species utilize other sugars products: sugar alcohols such as sorbitol and mannitol; oligosaccharides such as raffinose; and polysaccharides including fructans. A cryoprotective protein or glycoprotein has been extracted from cold-acclimated cabbage (*Brassica oleracea*) and which can protect the thylakoids of spinach (*Spinacea oleracea*) from freezing in vitro. The induced protein in cabbage was shown to be an isoform of tobacco β-1,3-glucanase.[218]

Some woody plants can acclimate to very low temperatures

Dormant woody plants can be extremely resistant to cold-temperature injury, both by acclimation but (in *Prunus* spp. such as cherry, plum, and many others) by genetic adaptation. North American species evolved in higher latitudes are less likely to accumulate intracellular ice.

Acclimation to increasing cold proceeds as follows:

- **Hardening** is induced in early autumn by short days and chilling but non-freezing temperatures. A diffusible factor, probably ABA, moves from the leaves to the overwintering stems. Water is withdrawn from the xylem [contributing to the lay belief of "sap descending"], preventing later rupture during freezing.
- Direct exposure to freezing leads to hardening capable of tolerating temperatures as low as minus 50 °C to minus 100 °C

Many boreal species of hardwoods utilize **deep supercooling** during winter to tolerate extreme cold (web topic 26.4), including apple, ash, beech, elm, hickory, maple, oak, peach, pear, plum, rhododendron, rose, and walnut. Boreal evergreen trees such as Engelmann spruce (*Picea engelmannii* subsp. *engelmannii*) and subalpine fir (*Abies lasiocarpa*) use deep supercooling of both stem and leaf tissues. Spontaneous ice formation occurs in cells at -40 °C (= -40 °F) despite supercooling, and this temperature therefore sets a lower limit for cold tolerance for many alpine and subarctic trees. The -40 °C minimum isotherm (which is a function of both altitude and latitude) determines the **timberline** for many species. Woody species that survive temperatures lower than -40 °C (listed in USDA hardiness zones 1 or 2)[219] are markedly resistant to cellular dehydration. Examples include

- **USDA hardiness zone 1** (surviving below -45.6 °C = -50 °F):
  Betula glandulosa (Dwarf birch)
  Chamaecyparis nootkatensis (Alaska-cedar)[220]
  Populus fremuloides (Quaking aspen)
  Rhododendron lapponicum (Lapland rhododendron)
  Salix reticulata (Netleaf willow)
- **USDA hardiness zone 2** (surviving down to between -45.6 °C to -40 °C = -50 °F to -40 °F):
  Abies lasiocarpa (Subalpine Fir)
  Betula papyrifera (White or paper birch)
  Cornus canadensis (Bunchberry dogwood)
  Larix laricina (Eastern larch) and *Larix decidua* (European Larch)
  Picea glauca (White spruce)[221] and *Picea pungens* (Colorado Blue Spruce)
  Prunus pennsylvanica (Pin cherry) and *Prunus virginiana* (Common Chokecherry)
  Salix amygdaloides (Peachleaf Willow) and *Salix alba* (White Willow)
  Viburnum trilobum (American cranberry bush).

[MCM: The textbook also mentions lists *Pinus contorta*, but *Pinus contorta* subsp. *latifolia* (lodgepole pine) has hardiness of 5 to 8, whereas *Pinus contorta* subsp. *contorta* (shore pine) has hardiness of 7.]

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[218] β-1,3-Glucanase Is Cryoprotective in Vitro and Is Accumulated in Leaves during Cold Acclimation. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=158397

[219] North America USDA hardiness zones: http://www.usna.usda.gov/Hardzone/ushzmap.html

[220] Tree hardiness zones: http://www.treelink.org/docs/zonemap.phtml

[221] White spruce: http://www.conifers.org/pi/pic/glauca.htm

However, when spring growth resumes [and presumably xylem and phloem vascular tissues have refilled with less osmotically concentrated "sap"],[222] resistance to freezing is much lower. Flower buds formed in the fall for azalea, blueberry, flowering dogwood, grape, and peach endure winter by deep supercooling. If these emerge in spring and then are subjected to freezing when they have become less freezing tolerant, they may be substantially damaged due to a loss of supercooling ability with resulting freezing and dehydration. Apple and pear floral buds and other parts of temperate plant species "do not supercool, but they resist dehydration during extracellular ice formation."[TZ690]

The cell protoplast suppresses ice nucleation, and the CW prevents spread of intercellular apoplastic ice (which forms much more readily) into the intracellular space. The CW also reduces the loss of intracellular water across the steep water vapor gradient. (The water potential gradient between liquid supercooled water and ice at the same temperature increases linearly in magnitude with falling temperatures, from 0 MPa at 0 °C to about -30 MPa at about -25 °C).[223]

Ice formation begins in the intercellular space at c. -3 to -5 °C, and resistance to freezing damage depends on the ability of the intercellular space to accommodate the presence of this ice, as well as on tolerance to the increasing dehydration of the protoplast. Resistance to very low temperatures occurs by gradual acclimation, and a sudden severe freeze may cause intracellular freezing and cell death.

Additional factors in freezing responses

Bacteria living on leaf surfaces, such as ***Pseudomonas syringae*** and *Erwinia herbicola*, can increase frost damage, as they serve as ice nucleation centers. "P. syringae, more than any mineral or other organism, is responsible for the surface frost damage in plants... P. syringae can cause water to freeze at temperatures as high as −1.8 °C, but strains causing ice nucleation at lower temperatures (down to −8 °C) are more common. The freezing causes injuries in the [epithelium] and makes the nutrients in the underlying plant tissues available to the bacteria."[224] Surface inoculation with these bacteria increases freezing sensitivity and resulting dehydration. Some bacteria are bred to lose their ice nucleating properties, and applied in competition with the native ice nucleating strains.

Acclimation to freezing involves **ABA** and specific **protein synthesis**. Alfalfa freezing tolerance is improved with pre-spraying with exogenous ABA. Specific proteins are induced by freezing, and some but not all of these are induced by ABA (details omitted).[TZ691] Exposure to **water shortages** hardens some plants such as *Arabidopsis*, improving their freezing tolerance, even if the water shortage occurred at non-acclimating temperatures.

Full acclimation to freezing requires both ABA exposure and also exposure to low temperatures (typically lasting several days, up to 15 days in potatoes). Cold acclimation can be lost rapidly on rewarming (lasting as briefly as 24 hours), contributing to the vulnerability of plants in the American South and other areas with highly variable winter temperatures.

Numerous genes are induced during cold acclimation (details omitted).[TZ691] These encode for **heat shock proteins** (just as in heat shock), **antifreeze proteins** (AKA thermal hysteresis proteins, including dual-role **pathogenesis-related proteins** such as **endochitinases** and **endoglucanases**), proteins involved in osmolyte synthesis, proteins for membrane stabilization, and LEA proteins.

A transcription factor regulates cold-induced gene expression... (details omitted)

Saline Stress and Toxic Trace Elements

High salinity in soil and ground water can be encountered in seashores and estuaries (contributed to by airborne salt spray), and near salt-laden geologic marine deposits. It can also occur from evaporation of

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[222] Jeannine Cavender-Bares: "Impacts of Freezing on Long-Distance Transport in Woody Plants": http://www.cbs.umn.edu/cavender/publications/2005%20Cavender-Bares.pdf
[223] *Ibid.*
[224] *Pseudomonas syringae* as an ice nucleator: http://en.wikipedia.org/wiki/Pseudomonas_syringae

lakes, and from salinization of crop lands by heavy irrigation and other agricultural practices. Salinization of arable land is a major threat to our crop lands and food supply.

Salt accumulates in irrigated soils and impairs plant function

High Na^+ accumulation is termed *sodicity*, and high concentration of total salts is termed *salinity*. *Halide* salts include halide anions: fluoride (F^-), chloride (Cl^-), bromide (Br^-), and iodide (I^-). Other ions contributing to salinity include Ca^{2+}, Mg^{2+}, and SO_4^{2-}.

Salinity is often expressed by **molarity** (**moles solute per liter solution** with units **mol L^{-1}**, abbreviated ambiguously as **M**), or more commonly for dilute solutions, by the millimoles of solute per liter solution with units **mmol L^{-1}**, abbreviated ambiguously as **mM** for **millimolarity** (see also chapter 6). For dilute solutions in water, the mM concentration of an ion or solute is very nearly equal to the millimoles solute per kg of water (**millimolality**). The **parts per million (ppm)** total salt concentration is the amount (mg weight) of salt (combining all salt anions and cations) in a kg of solution or more approximately in one liter of solution. This **parts per million** or **ppm** (w/w) is nearly equivalent for dilute water solutions to mg L^{-1}, but this is not the same as millimolarity, etc.

High sodic soils injure plants and decrease soil porosity and water permeability. Caliche is a sodic clay soil consisting primarily of calcium carbonate and other carbonates.[225]

Salinity of soil water can be measured by **electrical conductivity** (increasing salinity increases conductivity), which correlates with osmotic potential (which is lowered with rising salinity).[TZ692]

Sea water is mainly water H_2O (85.8% of seawater by mass w/w solution), but contains the following typical concentrations of principal ionic solutes:

- **Cl^-** 536 mM 55% of ionic atoms by count, 1.9% of seawater by mass w/w solution
- **Na^+** 457 mM 31% of ionic atoms by count, 1.1% of seawater by mass w/w solution
- **Mg^{2+}** 56 mM 3.7% of ionic atoms by count, 0.13% of seawater by mass w/w solution
- **Ca^{2+}** 10 mM 1.2% of ionic atoms by count, 0.04% of seawater by mass w/w solution
- **K^+** 9.7 mM 1.1% of ionic atoms by count, 0.04% of seawater by mass w/w solution
- **SO_4^{2-}** 28 mM S are 2.6% of ionic atoms by count
- **HCO_3^-** 2.3 mM

The osmotic potential of typical sea water is -2.4 MPa. Dissolved sea water salts (mostly Na^+ and Cl^-) are 32,000 - 35,000 mg L^{-1} or ppm (weight solute/weight solution), equivalent to 3.5% (weight solute/weight solution).[TZ693, TZ Table 26.6,226] River water, such as at the headwaters of the Colorado, may contain only 50 mg L^{-1} salt content, but irrigation water from the lower Colorado in arid areas of the American Southwest is often much more saline, and may contain as much as 900 mg L^{-1} salts, sufficient to affect salt-sensitive

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[225] Caliche: "Caliche is a hardened deposit of calcium carbonate. This calcium carbonate cements together other materials, including gravel, sand, clay, and silt. It is found in aridisol and mollisol soil orders. Caliche occurs worldwide, generally in arid or semi-arid regions, including in central and western Australia, in the Kalahari Desert, in the High Plains of the western USA, and in the Sonoran Desert. Caliche is also known as hardpan, calcrete, ... or duricrust. The term caliche is Spanish and is originally from the Latin calx, meaning lime.... Caliche reserves in the Llano Estacado in Texas can be used in the manufacture of Portland cement... Caliche beds can cause many problems when trying to grow plants. First, an impermeable caliche layer prevents water from draining properly, which can keep the roots from getting enough oxygen. Salts can also build up in the soil due to the lack of drainage... Second, the impermeable nature of caliche beds also prevents plant roots from going through the bed, which means the roots have a limited supply of nutrients, water, and space, so they cannot develop normally. Third, caliche beds can also cause the surrounding soil to be basic (have a high pH). The basic soil, along with calcium carbonate from the caliche, can prevent plants from getting enough nutrients, especially iron. An iron deficiency will cause the plant's youngest leaves to become yellow. Soil saturation above the caliche bed can make the condition worse"
http://en.wikipedia.org/wiki/Caliche_(mineral)

[226] Sea water:

- http://en.wikipedia.org/wiki/Sea_water
- http://www.windows.ucar.edu/tour/link=/earth/Water/dissolved_salts.html&edu=high

crops. Irrigation well water may contain up to 2000 - 3000 mg L^{-1} dissolved salts. Application of such saline irrigation water year after year can rapidly lead to excess soil salinity which damages crops.

Plants vary considerably in salt tolerance

Plant are categorized as to their tolerance to salinity in soils, summarized as follows (see fig. 26.14):[227]

- **Group IA** (**halophytes** including **euhalophytes**, which require moderate salinity): Show growth stimulation with soil Cl^- below 400 mM (and for many, according to the graph, actually show peak growth with Cl^- at around 250 mM). These are native to saline soils. Some are **obligate halophytes**. Some have salt glands and bladders. Examples of Group IA include sea blite (*Suaeda maritima*) and saltbush or orache (*Atriplex nummularia*).[228]
- **Group IB** (**halophytes**): Tolerate salt but growth is retarded, especially above about 350 mM Cl^-. These include sugar beet (*Beta vulgaris*, especially subsp. *maritima*), date palm (*Phoenix dactylifera*), and Townsend's cordgrass (*Spartina* × *townsendii* or *Spartina townsendii*).[229] *Acer plantanoides* (Norway Maple), *Aesculus hippocastanum* (Common Horsechestnut), *Pinus mugo* (Mugho Pine), *Pinus nigra* (Austrian Pine), *Tamarix pentandra* (Five-Stamen Tamarix) and *Tamarix gallica* (Manna Plant Tamarisk) are listed as having high tolerance.[230]
- **Group II** (**halophytes** and **nonhalophyes**): These are more inhibited by high salt concentration and include monocots red fescue grass, the alkaligrass *Puccinellia peisonis*, cotton, and barley. Some have intermediate salt sensitivity (tomato) and some higher salt sensitivity (Bean *Phaseolus vulgaris*, and soybean *Glycine max*).
- **Group III** (**nonhalophytes** and **glycophytes**): These are very salt-intolerant nonhalophytes plants (including **glycophytes**=sweet plants) which are killed by low salinity. These include maize, onion, rice, pecan, lettuce, avocado and many fruit trees including citrus and stone fruits (peach, apricots, etc.), black walnut (*Juglans nigra*), spirea

However, genetic variation exists within the plant groupings for salt tolerance. [MCM: In addition, the classification listed above and in Greenway and Munns 1980 seems to be inconsistently applied, as those authors refer to Group II as being only monocotyledons (p. 153), yet the textbook includes tomato and beans in it. Therefore, it is possible that some plants listed above, gathered from various sources, are miscategorized by one group category. This categorization scheme may also no longer be precisely used.]

The mechanisms of salt stress

Dissolved solutes in the root zone lower osmotic potential and therefore soil potential, thereby reducing the force driving water into the root down the gradient from regions of higher to lower soil potential (chapter 4). This effect is similar to the effects of soil water deficit. (However, some plants can adjust osmotically to these saline conditions by producing a low internal water potential, but this adjustment can contribute to impaired growth.)[TZ694]

Saline ions may have direct toxic effects on cells, particularly Na^+, Cl^-, and SO_4^{2-}. The intracellular ratio of Na^+ to K^+ becomes high (or overall ion concentration is high), enzymes may be inactivated, inhibiting protein synthesis. High Na^+ may displace Ca^{2+} from the PM that alters permeability to K^+ causing leakage outflux and inhibiting uptake of K^+. PS is also inhibited when high concentrations of Na^+ or Cl^- are present in chloroplasts.

Secondary effects of salinity include disruption of PM integrity and cell metabolism, production of reactive oxygen species, and cell death—some of the mechanisms for these are incompletely understood.

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[227] Salt tolerance of plants:
- H. Greenway and Rana Munns, "Mechanisms Of Salt Tolerance In Nonhalophytes", *Ann. Rev, Plant Physiol* 1980. 31:149-90
- http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agex3303
- http://www.colostate.edu/Depts/CoopExt/TRA/PLANTS/stable.html

[228] *Atriplex*: http://en.wikipedia.org/wiki/Atriplex
[229] *Spartina*: http://en.wikipedia.org/wiki/Cordgrass
[230] Salt tolerant plants: http://www.colostate.edu/Depts/CoopExt/TRA/PLANTS/stable.html

Plant strategies for reducing salt stress

Plants initially use similar mechanisms for salt stress as are employed with water deficit. The most sensitive tissues such as meristems and photosynthesizing leaves are protected from the higher ion concentrations. Because of the root's Casparian strip, the plant can selectively reject ions from entering the symplast and eventually the xylem (chapters 4 and 6). However, Na^+ enters roots passively down the electrochemical gradient (positive to negative) and must be energetically extruded, whereas entry of Cl^- is impeded by the same electrochemical gradient. Transpiration assists in xylem flow, and adjacent cells can serve to remove excess ions before the xylem reaches the leaves.

Some plants such as *Atriplex* spp. and *Tamarix* spp. have specialized **salt glands** on the leaves at which excess salt is excreted.

Some plants can produce a low internal water potential Ψ_w at the roots by lowering the solute potential Ψ_s. This is accomplished by accumulating intracellular ions in the vacuole (these might be potentially toxic if in the cytosol), and by accumulation of "compatible" solutes in the cytosol, such as have been previously listed (sorbitol, mannitol, pinitol, glycine betaine, proline, etc., some details omitted). Some of these are osmoprotective. Their synthesis consumes substantial cellular resources.

In addition, many of the responses to water deficit stress are employed: reduction of leaf area, leaf abscission, etc.

Accumulating toxic intracellular ions confined to the vacuole protects the remainder of the cell, and both halophytes and glycophytes employ this mechanism, actively pumping ions into the vacuole (or in some cases across the PM into the apoplast). Vacuolar ions and vacuole expansion contribute to overall cell growth. (Details of how plants differentiate between excess ions and hyperosmolarity are omitted.)

Excess internal Na^+ accumulates in the cytosol using secondary active transport systems across the PM.[TZ695] (further details omitted, see fig. 26.15 and 26.16 and also web topic 26.5) "Haloyphytes have a greater capacity for vacuolar compartmentalization of ions..."[TZ696] "Vacuolar compartmentalization of Na^+, energized by the ΔpH across the tonoplast membrane, also involves a family of Na^+/H^+ antiporters... Transgenic Arabidopsis and tomato plants overexpressing [such a transported] exhibit enhanced salt tolerance " [quoted from web topic 26.6] It is less necessary in halophytes to restrict root xylem load. However, both halophytes and glycophytes make use of ion transport processes that control ion uptake in the cytosol and compartmentalize it in the vacuole. (See also web topic 26.7)

Plant adaptations to toxic trace elements

Some metal ions and other trace elements in excess are highly toxic, including As, Cd, Cu, Ni, Zn, and Se (although some of these serve also as essential micronutrients in smaller quantities). Mechanisms of defense include exclusion from the plant and internal tolerance mechanisms. Plants in over 400 taxa can **hyperaccumulate** potentially toxic elements such as As, Cd, Cr, Hg, Ni, Pb, Se, and Zn (usually mostly in the vacuole, where they are bound to organic acids). These hyperaccumulated toxic elements, though safely compartmentalized in the plant, render their shoots toxic to pathogens and insect herbivores, and thus serve a protective role. Such plants require not only high internal tolerance and protection against oxidative damage, etc., but also powerful scavenging mechanisms and overproduction of transporters to import high amounts of these elements. **Chelators** such as **nicotianamine**[231] (which derives from L-methionine) and histidine are also involved in the transport process. (See also web essay 26.2 *An Extreme Plant Lifestyle: Metal Hyperaccumulation*)

Some of these hyperaccumulator plant capabilities are being studied for exploitation in environmental **phytoremediation** directed against toxic element contamination.[232] Phytoremediation of contaminated sites more generally can involve Phytoextraction, Phytodegradation (Detoxification or organic pollutants,

[231] Nicotianamine as a chelator: http://www.plantcell.org/cgi/rapidpdf/tpc.010256v1
[232] Phytoremediation by hyperaccumulator plants:
http://www.hort.purdue.edu/hort/research/murphy/pdfs/metals11.pdf

Chelation, Sequestration, etc.), Phytovolatilization, Rhizosphere degradation of the contaminant, Rhizofiltration, Phytostabilization, and Phytorestoration.[233]

Oxygen Deficiency

Free oxygen (O_2) deficiency in plants usually pertains to submerged roots and other underwater plant parts. Diffusion of air into the interstices of well-drained soil provides sufficient air to meet normal root oxygen needs down to several meters. [MCM: so how are deep taproots oxygenated?] Flooded or waterlogged soil contains much less available oxygen. The rate of diffusion of dissolved oxygen in waterlogged soil is very slow, and diffusion of dissolved oxygen effectively extends only over a few centimeters. As temperatures rise, the metabolic demands for oxygen in soil by roots, fauna, and microorganisms rise substantially, leading to rapid oxygen depletion in flooded soils by 24 hours. Thus **flood-sensitive** plants can be injured from root hypoxia/anoxia in as little as 24 hours. [MCM: The distinction between hypoxia and anoxia is a matter of degree, and the terms *anoxia* or *anaerobic conditions* are sometimes used where *hypoxia* might be more accurate.] Some plants are more **flood-tolerant**, but can still tolerate only several days of root anoxia. Some wetland plants such as rice are adapted to survive extended periods of flooding and ground saturation. (See chapter 11 and later in this chapter regarding aerenchyma, and other chapters regarding the roles of adventitious roots, ethylene, and other survival adaptations in waterlogged conditions.)

Normal Atmospheric Pressure: One **standard atmosphere** (1 **atm**) is defined as **101.325 kPa** or 0.1013 MPa (equivalent to 1013.25 millibar or hectoPa, 760 mm Hg, or 29.921 inches of mercury, or 14.696 PSI),[234] or ~ 10.3 m (33.9 ft) of water, and this corresponds to **average sea-level pressure**.

Normal Atmospheric Oxygen and Surface Dissolved Oxygen: Atmospheric oxygen is 209,460 ppmv or **20.946% v/v**.[235] The partial pressure of O_2 in the atmosphere at sea level is about 101.325 kPa × 20.9% = **~21 kPa** = **~0.21 atmospheres**. The dissolved O_2 concentration in water at sea level in equilibrium with the atmosphere is c. **277 μM** at 20 °C.

Anaerobic microorganisms are active in water-saturated soils and impart toxins

Anaerobic (anoxic) soil conditions favor anaerobic microorganisms, and these can produce compounds that are toxic to plants. Some anaerobes metabolize nitrate (which serves in anoxic environments as a thermodynamically favored electron acceptor) to **nitrite** or to gaseous N_2O, NO, and N_2. (This process is termed **denitrification**,[236] as the soil's fixed N becomes gaseous N that eventually is released to the atmosphere). Reducing anoxic conditions can also lead to reduction by anaerobic microorganisms of ferric Fe^{3+} to toxic **ferrous Fe^{2+}**, and SO_4^{2-} to toxic **H_2S** (a respiratory poison). In waterlogged stagnant soils, anaerobic microorganisms also produce, from organic substrates, such malodorous compounds as **butyric acid**[237] (the latter is named after butter, and imparts the unpleasant smell to rancid butter, as well as to vomitus) and **acetic acid**.

Roots are damaged in anoxic environments and this can injure shoots

Root hypoxia—beginning at a threshold oxygen level termed the **critical oxygen [partial] pressure (COP)**—leads initially to reduced respiration rate and metabolism, especially in the highly active root tip. The COP measured in the laboratory for maize root tip is 10 kPa (~0.1 atm pO_2) in a water-saturated gaseous environment, and 30 kPa in a well-stirred liquid medium. The higher need in a liquid environment reflects the slow diffusion of oxygen in water. However, "when internal O_2 transport from the aerial parts of the plant occurred, significantly lower values were obtained in liquid medium for the critical oxygen pressure,

[233] Phytoremediation: http://en.wikipedia.org/wiki/Phytoremediation
[234] Atmospheric pressure: http://en.wikipedia.org/wiki/Atmospheric_pressure
[235] Earth's atmosphere: http://en.wikipedia.org/wiki/Earth's_atmosphere
[236] Denitrification: http://en.wikipedia.org/wiki/Denitrification
[237] Butyric acid: http://en.wikipedia.org/wiki/Butyric_acid

which shifted from more than 21 to 6 kilopascals."[238] The COP is lower for less metabolically active parts of the older roots, 0.05 to 0.1 atmospheres pO_2 [~5 to 10 kPa pO_2], and also falls with decreasing temperatures.

Oxygen diffusion can extend effectively only over a short distance in fluid environments. Although mitochondrial oxidative phosphorylation can proceed at very low concentrations of dissolved oxygen (equilibrium constant Km ~0.1 to 1.0 μM, compared to ~277 μM for the concentration of dissolved oxygen in equilibrium with air at 1 atm), the slow rate of oxygen diffusion limits this process and helps determine the COP.

As pO_2 falls below the COP, metabolism shifts from aerobic respiration to anaerobic fermentation. This leads to **cytosolic acidosis** (arising from lactic acid fermentation, impairment of the vacuolar H^+-ATPase pump, and leakage of vacuolar acidic solution) and severely reduced ATP production (see fig. 26.17 and chapter 11), thereby reducing essential metabolic processes and eventually leading irreversibly to cell death.

The shoots that depend on such hypoxic roots are subjected to a loss of ions, loss of phloem mobile elements, and wilting due to reduced xylem water potential.

Root hypoxia accelerates production of ACC (ethylene precursor) which passes in the xylem to the shoot, leading to increased ethylene in the shoot, increased epinasty (leaf drooping, which is not the same as wilting, see chapter 22). It can also lead to stomatal closure from increased ABA (see earlier in this chapter 26).

Submerged organs can acquire O_2 through specialized structures including aerenchyma

Many species of wetland-adapted vegetation can survive extended periods of soil saturation or flooding.

Elongation: The Yellow Floating Heart (*Nymphoides peltata*, a waterlily-type plant) uses ethylene to stimulate accelerated growth of the petiole to keep the leaf at the surface. Similarly, deepwater rice (*Oryza sativa*) grows at the internodes rapidly to keep the leaves above water, even when the water level is up to 50 cm. The so-called "floating" varieties of deepwater rice "exhibit extreme elongation capacity" and "can grow at rates of 20 to 25 cm/d when partially submerged and can reach a length of up to 7 m in water depths of up to 4 m."[239]

Aerenchyma: Many wetland and flooding-tolerant plants (such as rice) exhibit interconnected longitudinally-oriented channels of honeycombed air passages termed aerenchyma in the stem and roots. Even some plants not normally living in wetlands can develop this aerenchyma tissue when required (for example, maize, see fig. 26.18). Air (gas) enters the plant through the leaf stomata or **lenticels**[240] in stems and roots (e.g., they are prominent in wild cherry and birch tree bark), and moves freely by diffusion and convection to the air-deprived roots. Roots with aerenchyma can penetrate further, up to 50 cm, into anaerobic soil. Aerenchyma arises from "collapse of root **cortex** cells, indicating a programmed cell death..."[241] The suberin and lignin of the roots of plants adapted with aerenchyma prevent diffusion of the oxygen out of the root. In rice seedlings, the coleoptile can emerge through the water surface and act as a "snorkel" for the diffusion of O_2 to the submerged parts of the plant.[242]

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[238] Root critical oxygen pressure: http://www.plantphysiol.org/cgi/content/abstract/76/1/151
[239] Deep water rice: http://www.plantphysiol.org/cgi/content/full/118/4/1105
[240] Lenticel: "A lenticel is a spongy area present in the cork surfaces of the stems, roots, and other parts of vascular plants. It appears on the surface as a lenticular (lens-shaped) spot, which acts as a pore. These structures allow for the exchange of gases between the internal tissues and atmosphere to occur across the periderm, which would otherwise prevent this exchange of gases. The name lenticel, pronounced with a soft c, derives from its lenticular shape. The shape of lenticels is one of the characteristics used for tree identification."
http://en.wikipedia.org/wiki/Lenticel
[241] Aerenchyma origin: http://www.amjbot.org/cgi/content/abstract/87/1/12
[242] Deepwater rice coleoptile as a snorkel: http://www.plantphysiol.org/cgi/reprint/118/4/1105.pdf

Interestingly, the aerenchyma of rice is thought to contribute by a reverse flow to increased methane reaching the atmosphere: "Aerenchyma, developed in both root and aboveground parts of rice plants, is predominantly responsible for plant-mediated transfer of methane (CH_4) from the soil to the atmosphere."[243]

Some plants can withstand prolonged root or rhizome anaerobic conditions, including rice, rice grass (*Echinochloa crus-galli* var. oryzicola),[244] bulrushes (*Schoenoplectus lacustris, Scirpus* spp., etc.),[245] and narrow-leafed cattail (*Typha*[246] sp. including *angustifolia*).[247] When aerenchyma provides sufficient oxygen (usually in the spring), the roots or rhizomes switch from anaerobic pathways to aerobic metabolism.

Mechanisms for adaptation to anaerobic conditions

As discussed above, most tissues cannot survive truly anaerobic conditions more than 24 hours, due to cytosolic acidosis and loss of ATP synthesis, etc. Some acclimation in maize and other cereals is possible if the onset of anaerobic conditions is gradual. Such acclimation involves expression of genes encoding for **anaerobic stress proteins**. Wetland plants with tissues adapted to anaerobic conditions may have better controls on cellular pH, better ATP production by glycolysis and fermentation, and also storage of fuels that may be utilized in anaerobic conditions. Protons may also be consumed in rice by the synthesis of alanine, succinate, and GABA, raising pH. Mechanisms such as a buildup of the enzyme superoxide dismutase must exist to deal with the surge of **reactive oxygen species** (ROS) that are generated when the plant transitions back from anaerobic to aerobic metabolism (details omitted).

Anaerobic sensing, signal transduction, and anaerobic stress proteins in acclimation to oxygen deficit

Acclimation to anaerobic conditions involves expression of genes encoding for anaerobic stress proteins. This is seen in maize, in which 20 polypeptides are preferentially synthesized during anaerobic conditions. These proteins affect a variety of processes (details omitted).

In order to indirectly sense hypoxia, plants detect or sense
- pH fall
- reduction in ATP levels
- increase in cytosolic Ca^{+2}
- increase in ROS.

The rise in cytosolic Ca^{+2} appears to be a rapid and important signal of anoxia, triggering increases in synthesis of **alcohol dehydrogenase** (used in alcoholic fermentation) and **sucrose synthase** (used to split sucrose into fructose and UDP-glucose, an early step required for glycolysis and substrate-level phosphorylation), at least in maize tissue cultures. (Further details of signal transduction, transcription factors, and stress induced protein sequences are omitted.)

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[243] Aerenchyma and methane:
M. S. Aulakh, et al (2000) Pattern and Amount of Aerenchyma Relate to Variable Methane Transport Capacity of Different Rice Cultivars. *Plant Biology* 2 (2) , 182–194.

[244] Rice grass: "*Echinochloa crus-galli* var. *oryzicola* is a crop mimic that is found primarily in permanently flooded cultivated rice fields "
http://www.fs.fed.us/database/feis/plants/graminoid/echcru/all.html
See also http://www.plantnames.unimelb.edu.au/Sorting/Echinochloa.html

[245] Bulrushes:
- http://en.wikipedia.org/wiki/Schoenoplectus
- http://en.wikipedia.org/wiki/Scirpus

[246] *Typha* sp.: "The most widespread species [in the US] is *Typha latifolia*, extending across the entire temperate Northern Hemisphere. *T. angustifolia* is nearly as widespread, but does not extend so far north."
http://en.wikipedia.org/wiki/Typha

[247] *Typha angustifolia*: "In North America, it is an introduced plant."
http://en.wikipedia.org/wiki/Typha_angustifolia

Air Pollution

(See Zeiger's Web Essay 26.1, *The Effect of Air Pollution on Plants*—quotes in this section are from that essay unless otherwise noted.)

Dusts: Dusts deposited on leaves block light and PS and can also impair stomatal gas exchanges, even if they are not otherwise toxic.[248]

Chemical pollutants: This important essay deals with the effects on plants of polluting or altered levels of atmospheric **CO_2, CO, SO_2, nitrogen oxides (NO_x, NO, and NO_2,** etc. in varying proportions), and **C_2H_4 (ethylene)**, **H_2S**, and **HF**, etc. In some areas, these pollutants attain sufficiently high levels to inhibit plant growth, especially when interacting with other adverse environmental conditions. **Photochemical smog** includes many of these pollutants, plus other compounds deleterious to plants: **Ozone (O_3)**, **peroxyacetylnitrate (PAN)**, and **Hydrogen peroxide (H_2O_2)**.

Inorganic N and S: "Polluting gases such as SO_2 and NO_x enter leaves through stomata, following the same diffusion pathway as CO_2. NO_x dissolves in cells and gives rise to **nitrite** ions (NO_2^-, which are toxic at high concentrations) and **nitrate** ions (NO_3^-) that enter into nitrogen metabolism as if they had been absorbed through the roots..." Exposure to SO_2 causes stomatal closure, and at high concentrations potentially toxic **sulfite** or **bisulfite** ion accumulation.

Acid Rain: Some atmospheric pollutants fall as "**acid rain**", which deposition can in fact occur as either "wet" (in rain droplets) or "dry" (in particulates). Normally, rain is slightly acidic, with a pH close to 5.6 due to carbonic acid H_2CO_3 from CO_2. "NO_x and SO_2 in water droplets in the atmosphere causes the pH of rain to decrease to 3 to 4, and in southern California polluted droplets in fog can be as acidic as pH 1.7..." "In soils that lack free calcium carbonate, and therefore are not strongly buffered, such additions of acid can be harmful to plants. Furthermore, the added acid can result in the release of aluminum ions from soil minerals, causing aluminum toxicity. Air pollution is considered to be a major factor in the decline of forests in heavily polluted areas of Europe and North America. There are indications that fast-growing pioneer species are better able to tolerate an acidifying atmosphere than are climax forest trees, possibly because they have a greater potential for assimilation of dissolved NO_x, and more effective acid buffering of the leaf tissue cell sap."

Ozone and ROS: "Ozone is presently considered to be the most damaging phytotoxic air pollutant in North America... It has been estimated that wherever the mean daily O_3 concentration reaches 40, 50, or 60 ppb..., the combined yields of soybean, maize, winter wheat, and cotton would be decreased by 5, 10, and 16%, respectively." The damage from ozone is described as follows: "It binds to plasma membranes and it alters metabolism. As a result, stomatal apertures are poorly regulated, chloroplast thylakoid membranes are damaged, rubisco is degraded, and photosynthesis is inhibited. Ozone reacts with O_2 and produces reactive oxygen species..." The production rate and/or impact on plants of ROS can be worsened under extreme environmental conditions such as water deprivation. (Details of plant protective responses against ozone are omitted.)

Synergistic effects of multiple pollutants: The effects of multiple pollutants can be greater than merely additive. "When plants are exposed to air containing NO_x, lesions on leaves appear at an NO_x concentration of 5 mL L^{-1}, but photosynthesis starts to be inhibited at a concentration of only 0.1 mL L^{-1}. These low, threshold concentrations refer to the effects of a single pollutant. However, two or more pollutants acting together can have a synergistic effect, producing damage at lower concentrations than if they were acting separately. In addition, vegetation weakened by air pollution can become **more susceptible to invasion by pathogens and pests**."

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[248] Dust effects on photosynthesis:
- http://agron.scijournals.org/cgi/content/abstract/78/6/1078
- G. Naidoo and D. Chirkoot. "The effects of coal dust on photosynthetic performance of the mangrove, Avicennia marina in Richards Bay, South Africa". *Environmental Pollution*. Volume 127, Issue 3, February 2004, Pages 359-366.

Plant Neurobiology

Professor Van Volkenburgh has a special interest in *plant behavior* (which in brief she defines as "development based on physiological sensing and responding") and in *plant neurobiology*[249] ("how plants process the information they obtain from their environment to develop, prosper and reproduce optimally"),[250] some aspects of which are not covered in the textbook. The following derives from her lectures except as noted. (I have not yet taken the opportunity yet to explore in detail this area of active current research.)

Plants synthesize various animal neurotransmitters and neuroactive compounds (such as caffeine, glutamate, GABA, ACH, serotonin, L-DOPA, dopamine, and melatonin). Are the glutamate receptors in plants used in auxin signaling? Many of the neuroactive compounds made by plants may be defensive and part of their "chemical ecology".

Plants exhibit two types of electrical signaling. The membrane potential is held at about -180 mV. It was previously thought that plants had no significant electrical activity or electrical excitability.

- **Action Potential**: This requires a **threshold depolarization** before it fires following a stimulus (such as light, touch, wound, insect bite or saliva, etc.), and leads to an **all-or-nothing response** (as with animal neural action potentials). This phenomenon and the accompanying fluxes have been mostly but still incompletely studied in the giant Charophyta green alga **Chara**. It propagates at a **constant velocity**, and is completed over a duration of **minutes**. (In contrast, animal action potentials propagate typically over a fraction of a second.) The propagation may occur in the phloem, perhaps in the parenchymal cells, crossing plasmodesmata, etc., to reach the leaves (details have not been fully worked out). After the initial stimulus, the action potential consists of an initial positive rise in E_m resulting from Cl^- outflux and Ca^{++} influx. (Unlike in animals, sodium ion flux plays no role.) After the positive peak is reached, the E_m rapidly falls to more negative than the baseline negative value, as H^+ and K^+ outflux. The E_m then returns to baseline with influx of K^+. (some details missing)
- **Slow Wave Potentials** (also called by some **Variation Potentials**). These are generated by an increase in pressure at the site of origin, but are different from action potentials. Mechanoreceptors detect a touch, wound, or insect bite perturbation with resulting pressure changes (including changes in the usual negative xylem pressure). Ion channels open and a change in membrane potential E_m propagates slowly from the point of injury or stimulation to the remainder of the shoot over a period of 10 minutes to one hour.[251] The slow wave potential change propagates in the xylem to the cortex and epidermis, and it is the electrical changes in the outer cells that are observed. Unlike action potentials, slow wave potentials do not depend on a threshold to be initiated, and exhibit decreasing amplitude and decreasing speed of propagation with distance from the site of initiation.

In plants, auxin may serve as a type of neurotransmitter, as with polar auxin transport PAT (see chapter 19, etc.), in which an action potential-like event results from IAA secretion...

Examples of plants responding to their environment[252] include:

- **Phototropism and Sun Tracking**
- **Venus flytrap**: The trap has sensitive hairs which when sufficiently stimulated trigger a depolarization of membrane potential, causing the cells of the hinge to lose turgor on top and gain turgor and elongate on the bottom (from influx of K^+), resulting in closure of the hinge...
- **Flowering** in plants resulting from PHY detection of light, FT Protein transmitted in phloem, etc.
- **Tomato Wound Response**: Insect bites lead to propagation of an action potential, along with synthesis of **systemin** (a peptide hormone) in wounded phloem parenchyma cells. (The systemin

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[249] See Society of Plant Neurobiology: http://www.plantneurobiology.org/

[250] Brenner ED, Stahlberg R, Mancuso S, Vivanco J, Baluska F, Van Volkenburgh E., "Plant neurobiology: an integrated view of plant signaling", Trends Plant Sci. 2006 Aug;11(8):413-9. Epub 2006 Jul 13

[251] Plant slow wave potentials in

• Pea epicotyl: http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=158132&blobtype=pdf

• Sunflower: http://www.plantphysiol.org/cgi/reprint/115/3/1083.pdf

[252] See "The Silent Scream of the Lima Bean" *Max Planck Research* 4/2007, http://www.mpg.de/english/illustrationsDocumentation/multimedia/mpResearch/2007/heft04/017/pdf19.pdf

pathway is summarized in chapter 13 and leads to synthesis of **jasmonic acid**, which propagates in the phloem.)

- **Hypersensitivity responses:** including local necrosis (see chapter 13)
- **Chitinases**:[253] these are directed against insects and fungi (see chapter 13)
- **Phytoalexins**:[254] plant antibiotics, including terpenoids, glycosteroids and alkaloids (see chapter 13)

[253] Chitinase: http://en.wikipedia.org/wiki/Chitinase
[254] http://en.wikipedia.org/wiki/Phytoalexin

Possible Corrections or Clarifications For The Textbook

Plant Physiology, 4th Ed., by Lincoln Taiz and Eduardo Zeiger, is a superb textbook on a subject I had not previously studied. Here are what I believe are some minor errors and inconsistencies—some of which are essentially trivial—along with some questions or clarifications. Preparing this list, like the rest of this outline, has been a learning aid for me, and might be helpful to others reading the book. Send corrections to mcgoo at u period washington period edu.

1. **"ATPAse" vs. "ATP Synthase"**
   (Chap 1 p. 18 Figure 1.17 and p. 19 Figure 1.18, p. 101, and other locations)
   The authors refer to ATPases when I believe they mean ATP Synthases (since they are talking about synthesis of ATP, not decomposition of ATP). Later on (p. 149), the authors imply that "ATP synthase" and "ATPase" are used synonymously, or state (Chap 2 p. 20) that "ATPase" may be used for both the catalysis and synthesis directions for mitochondrial and chloroplastic ATPase. However, in most places the authors use "ATP synthase" when they are referring to the synthesis of ATP from ADP. It seems to me that calling the synthesizing enzyme "ATPase" is potentially and needlessly confusing except where the enzyme routinely operates in either direction under common physiologic conditions, which seems doubtful (though I don't know for sure).

2. **Mass Action Ratio In A Chemical Equilibrium**
   (Chapter 2 p. 7, online)
   The Free Energy diagram shows a minimum free energy at mass:action ratio = K. However, the text for the figure says the mass:action ratio = 1. Although it is possible this ratio is 1 in the particular example shown, it appears to me that K would be the more general applicable value, and would fit the diagram better.

3. **"Translocation"**
   (multiple locations)
   The authors use this term somewhat inconsistently, at times referring to movement of photosynthate in the phloem from sources to sinks, but at other times (p. 88) stating "here the xylem develops the capacity to translocate substantial quantities of water and solutes to the shoot". Our professor has said that the term "translocation" is usually used for movement of photosynthate in the phloem from sources to sinks, but perhaps this broader and less specific usage is also considered correct.

4. **"Phospholipid" Bilayer**
   (Chap 1 p. 6 and in Fig. 1.5)
   The authors label Monogalctosyldiacylglycerol as a phospholipid, but it seems to me this is not entirely accurate since it is a glycolipid and has no phosphorus or phosphate group. Of course the intent is to show the similarities in their membrane function to phospholipids.

5. **Chloroplast F1F0 ATP Synthase Diagram**
   (Chap 7 fig. 7.32)
   The upper diagram is labeled "F_1" on the lower left when it should be F_0 (or perhaps CF_0).

6. **Absorbed Light versus Depth**
   (Chap. 9 fig. 9.21)
   The vertical axis is labeled a little misleadingly even though the intention is obvious. The caption is "Absorbed light (%)" whereas it would probably be more accurate to label it something like "Absorbed light (% of peak absorption rate)", perhaps even spelling out that this is incremental absorption per unit depth of tissue traversed).

7. **Mid-Point Potential**
   (Chap 2 p. 8)
   The presentation is confusing and seems to equate E'_0 and E_m. However, as I read this material and other sources, E'_0 is the same as E_0 except for the additional requirement that pH is required to be 7. The mid-point potential E_m is defined as the potential applicable when an additional condition applies, namely that the concentrations of the oxidized and reduced forms of the redox pair are equal (though

not necessarily 1 molar). The formulas

$$E_h = E'_0 = E_m$$

are valid only when the oxidant and reductant are in equal concentrations and the pH is 7.

8. **Homogalacturonan**
   (Chap 15 p. 357)
   Homogalacturonan, also called polygalacturonic acid, is a (1→4) polymer of α-D-galacturonic acid, not of α-D-glucuronic acid as stated in the text.

9. **Diagrams of Galactose side chains**
   (Chap 15 p. 358, 359, etc.)
   It would helpful to clarify in the textbook that the 5-member rings shown as galactose side chains in various illustrations represent galactose (which is a hexose) in the furanose form (a 5-member ring), therefore differing from the galactose illustrated on page 354 fig. 13.5 which is in pyranose form (a 6-member ring). However, the 5-and 6-member ring configurations for hexose rings is illustrated in web topic 15.1 for glucose.

10. **Prostaglandins**
    (Chap 14 p. 24 fig. 14.21)
    Arachidonic acid can be oxidized *to* prostaglandin, probably not *by* prostaglandin.

11. **NO Synthase reaction**
    (Chap 14 p. 26)
    The formula probably was intended to include an arrow:

$$\text{Arginine} + O_2 \xrightarrow{\text{NO synthase}} \text{Citrulline} + NO$$

12. **Phosphate ion**
    (Chap 5 p. 85)
    The formula for phosphate ion (dihydrogen phosphate ion) should probably be $H_2PO_4^-$ rather than $H_2PO_2^-$

13. **PAR Irradiance**
    (Chap 9 and Web topic 9.1)
    On a sunny day in direct sunlight, PAR irradiance is said to be 400 W m^{-2} according to Web topic 9.1, but on p. 200 of the textbook, the figure 900 W m^{-2} is given. Yet "Sun" is said to have irradiance of 920 μmol m^{-2} s^{-1} in fig. 9.10, and therefore the W m^{-2} should be substantially smaller. The 900 W m^{-2} figure appears to be incorrect based on a total solar "constant" of 1366 W m^{-2}, and PAR is said to be about 38% of the solar constant.

14. **Conversion of Solar Energy**
    (Chap 9 fig. 9.3)
    The diagram show 60% loss due to "Nonabsorbed wavelengths" but this probably should be termed non-PAR radiation, some of which is nonabsorbed and some of which is absorbed but not useful for PS. The next 8% loss shown is labeled "reflection and transmission" and this would all be Nonabsorbed as well.

15. **Observed Rate of CO_2 atmospheric increase**
    (Web topic 9.6)
    A meaningless sentence is seen just after fig. 9.6.B, beginning "A surprising fact is …", since it equates the observed rate of increase with itself.

16. **Confusing Use of Proximal And Distal**
    (p. 405, p. 496 fig. 19.35,)
    There seems to be some confusion as to the use of *proximal* and *distal*, at least with respect to roots. They are used in the way I would expect on page 405—in roots, *distal* meaning further below (further away from the SAM or plant base). However, in figure 19.35, the color codes seem to place the proximal lateral root cap closer to the tip of the root (further from the SAM or plant base), while the distal lateral root cap is closer to the SAM or plant base. In humans, the terms proximal and distal are

understood to compare distances relative to the trunk or head of the organism, and there is less opportunity for confusion. However, in plants it would be helpful to define what exactly "proximal" and "distal" mean, especially since there are at least three possible central structures of reference, namely the root RAM, shoot SAM, and the plant base. (See also naming conventions in Essay 19.2 *Apical Basal Polarity is Maintained in Mature Plants*)

17. **Incorrect diagram**
    (p. 538, fig. 20.33)
    The diagram show no apparent difference in the drawings of the decapitated pea plant and the same treated with IAA.

18. **Incorrect diagram**
    (p. 293, fig. 12.5)
    The diagram shows ammonium ion $NH4^+$ labeled as ammonia.

19. **Aeschynomene vs. Aeschenomene**
    (p. 299 Table 12.3)
    The spelling of this genus is also listed as Aeschynomene on the web, but I don't know which is correct.

20. **Coordination vs. Electrostatic bond in polygalacturonic acid?**
    (p. 306-7, Fig. 12.16)
    The text describes electrostatic bonding between the carboxylic groups of polygalacturonic acid and Ca, but the fig. 12.16 shows an example of coordination complex with polygalacturonic acid in a coordination complex with Ca. Perhaps both are possible, but this is confusing without further clarification.

21. **Energetics of Nitrogen Fixation**
    (p. 301, 289, Web Topic 12.2)
    The ATP cost of nitrogen fixation to ammonia is listed in the text as 16 ATP (p. 301 formula 12.9 and Web Topic 12.2). However, on p. 289, the text appears to erroneously state that 16 ATPs are used to assimilate N from N_2 all the way to an amino acid, thereby counting both the bacterial and plant pathways. This appears inconsistent. Also, I am unclear whether these ATPs derive from the bacteria or the plant or both, and it might be helpful to spell this out.
    http://en.wikipedia.org/wiki/Nitrogen_fixation

22. **Limonoids**
    (p. 321)
    Limonoids are described as C_{30} triterpenoids, but other sources state that they are tetranortriterpenoids (a word for which I have not found the definition) and several including Limonin (C_{26}) and azadirachtin (C_{35}) do not have 30 Carbons.

23. **Nicotinic Acid Mononucleotide**
    (p. 331 fig. 13.18
    Nicotinic Acid Mononucleotide is shown with the abbreviation $NADP^+$ but should be NADM.

24. **Systemin pathway**
    (p. 336, fig. 13.25)
    The text step 4 describes systemin binding to its receptor on the phloem parenchyma, but according to fig. 13.25 the binding in on the companion cell rather than to the phloem parenchyma cell. Perhaps this could be clarified.

25. **Archea or Archaea?**
    (p. 682)
    As opposed to the most common spelling of the *Archean* Eon, I believe the usual spelling for the bacterial-like taxon is *Archaea*, not *Archea*.[255]

26. **Deep Supercooling**
    (p. 690)

[255] Archaea per Carl Woese et al: http://www.pnas.org/cgi/reprint/87/12/4576

The reference to deep supercooling should be to web topic 26.4, not 26.3

27. **Halophytes classification**
(p. 693)
The classification listed from Greenway and Munns 1980 seems to be inconsistently applied, as those authors refer to Group II as being only monocotyledons (*Ann Rev, Plant Physiol* 1980 31:149-90 p. 153), yet the textbook p. 693 includes tomato and beans in it (both dicots). Is this classification still in active use or has it been superceded or relaxed?