

Tree Adaptations to Nutrient/Chemical Stresses

Guest Lecture for ESC310
Trees in Our Environment

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Chemical/Nutrient Stresses and Adaptive Measures Trees Use to Counteract Them

- ◆ PART ONE: Insufficient nitrogen
- ◆ PART TWO: Environmental pollution
- ◆ PART THREE: Salinity
- ◆ PART FOUR: Can we help plants "adapt"?

PART ONE: Nitrogen Deprivation

- ◆ Air is 80% dinitrogen gas
- ◆ But only some microorganisms can convert dinitrogen gas into usable forms
- ◆ Must have nitrogen to build proteins and nucleic acids



Fixed nitrogen is a limiting nutrient for plant growth

- ◆ Farmers add ammonia or other N fertilizer to increase plant growth
- ◆ Tobacco with ammonium and nitrate (left plant) grows vigorously
- ◆ Tobacco without NH_4 or NO_3 (middle plant) cannot grow
- ◆ Poplar without ammonium/nitrate (right) has help from microbes



Cottonwood and willow are adapted to grow under nitrogen limitation

- ◆ *Populus trichocarpa* and *Salix sitchensis* grow in gravelly riversides
- ◆ Few plants can survive under these conditions
- ◆ (Photos are from Three Forks Park, Snoqualmie)



Some other tree species are known to associate with nitrogen-fixing bacteria

* Alder and Frankia

- ◆ Alder grows in nitrogen-poor soils with the help of Frankia bacteria in root nodules
- ◆ Frankia is a filamentous bacterium in the group Actinomycetes
- ◆ It also associates with Sea buckthorn, a sand dune plant, and many other "actinorhizal plants"
- ◆ Frankia allows these trees to be pioneer species



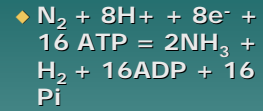
Tropical leguminous trees

- ◆ Other trees adapt to nitrogen limitation by recruiting bacteria
- ◆ *Acacia koa*
- ◆ Grows in Hawaii
- ◆ Associates with *Rhizobium* that fixes nitrogen within root nodules



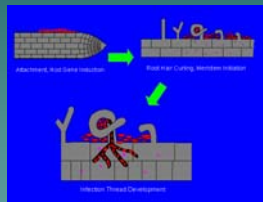
These trees use nitrogen-fixing bacteria in root nodules to provide usable nitrogen

- ◆ Plants trade sucrose from photosynthesis and an isolated habitat (root nodules) for fixed nitrogen
- ◆ Why don't more plants do this? Expensive!



Complex signaling between nitrogen-fixing bacteria and the plants that can harbor them

- ◆ Legumes send signals from roots
- ◆ *Rhizobia* chemotax to the roots
- ◆ *Rhizobia* send a signal to the plant
- ◆ Plant recognizes it and initiates root hair curling
- ◆ Bacteria are allowed to invade the plant



Cottonwood and willow do not have root nodules

- ◆ How do cottonwood and willow get fixed nitrogen?
- ◆ Why not adapt like alder trees and use bacteria in root nodules?
- ◆ Is there something about its setting that would prevent it from using root nodules?



Current Research

- ◆ A variety of nitrogen-fixing microorganisms are in poplar stems
- ◆ When living next to a river that regularly floods, stems would make a more secure home for the bacteria than would delicate root nodules
- ◆ Each flooding cycle brings sediment; lower roots die and new ones are formed from the stem
- ◆ Makes more sense to keep them in the stem



Summary of Part One: Adaptations to nitrogen deprivation

- ◆ Recruit nitrogen-fixing bacteria
- ◆ Create a safe environment for them (root nodules, internal stem structure?)
- ◆ Trade plentiful sucrose from photosynthesis for fixed nitrogen
- ◆ Legumes (peas and beans) are not the only plants that can do this!

Part Two: Tree Adaptations to Environmental Pollutants

Common environmental pollutants

- ◆ Metals such as mercury, arsenic, nickel
- ◆ Organic pollutants such as solvents, PCBs, PAHs, explosives

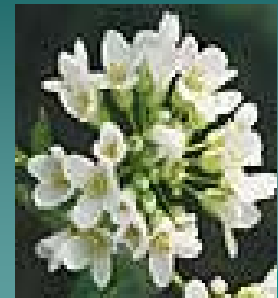


2a. Adaptation to chemical stress by altering uptake of the pollutant

- ◆ Exclude the harmful metal by pumping it back out
- ◆ Take it up but store it where it will not be harmful
- ◆ Deliberately hyperaccumulate it and use it for defense!

Strategy 3: Hyperaccumulate it

- ◆ *Thlaspi goesingense* naturally hyperaccumulates nickel (12,400 ug/g shoot dry biomass)



Strategy 3 (cont.): Hyperaccumulation in Trees

- ◆ Rare to have hyperaccumulation in a tree species
- ◆ *Sebertia acuminata* has the highest nickel content ever determined in any plant tissue (37 kg/tree!)
- ◆ Native to New Caledonia



Sebertia acuminata

- ◆ 26% nickel in the dried latex of this tree (1.2% in dried leaves)
- ◆ Native to serpentine soils of New Caledonia
- ◆ Selective toward nickel despite presence of other metals
- ◆ * Protection against predators. Insects choose to die of starvation rather than eat any of this tree.
- ◆ * Protection against microorganisms also
- ◆ Heavy metal hyperaccumulation was an easy and cheap defense strategy in that area.

Part 2b. Adapt to environmental pollutants by metabolizing them

- ◆ Organic pollutants such as solvents, PAH's, PCB's, and explosives
- ◆ Plants have metabolic enzymes similar to those in mammals
- ◆ Cytochrome P450 enzymes metabolize pollutants in soil, water, and air

Leucaena leucocephala-tropical tree that can metabolize pollutants

- ◆ Common name: Koa haole
- ◆ Native to SE Mexico
- ◆ Introduced in Hawaii in 1800s
- ◆ Grows rapidly
- ◆ Metabolizes the agricultural pollutant, EDB



Part 2c. Adaptation to environmental pollutants by recruiting help from microbes

- ◆ Some pollutants are very difficult to metabolize
- ◆ Some are phytotoxic
- ◆ Plants in contaminated areas have more internal bacteria (endophytes) capable of degrading the pollutant than plants off the site
- ◆ Intentional recruitment?

Wetland plants, with the help of bacteria, can remediate metal contamination

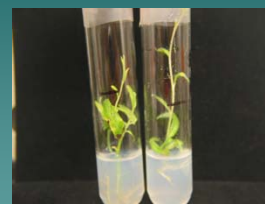
- ◆ Wetland plants including saltmarsh bulrush and rabbitfoot grass were very efficient at treating oil-refinery effluent (selenium)
- ◆ These plants accumulated the selenium at lower rates when the bacteria were killed
- ◆ When sterile plants were inoculated back with the bacteria, they accumulated the selenium and mercury at higher rates

Grasses, with the help of microbes, can remediate petroleum-contaminated sites

- ◆ Site was contaminated with diesel fuel and heavy oil and nitroaromatics
- ◆ Grasses grown on the site had increased numbers of bacteria with the genetic ability to metabolize the pollutants
- ◆ Enrichment of contaminant-degrading bacteria was plant species specific!

Maybe trees also recruit microbes

- ◆ Willow from phytoremediation sites
- ◆ Found PAH-tolerant bacteria in the stems
- ◆ Willow plants grew well in PAH-containing medium



Part Three: Salt Stress

How trees adapt to salt stress

Irrigation Induced Salinity

- ◆ Destroys 1000s of hectares of agricultural land each year
- ◆ One third of the land under irrigation worldwide is affected by excess salinity
- ◆ Therefore, there is a need for salt-tolerant crop plants



Tree Adaptations to Salinity

- ◆ Mechanisms of salt tolerance are in two broad classes: avoidance and tissue tolerance
- ◆ Some Eucalyptus species are tolerant (17 of 55)
- ◆ Marine species such as palm trees, sea grape, pitch pine



Mechanisms of Salt Tolerance

- ◆ Avoidance: Keep salt ions away: pump them out, dilute them
- ◆ Tissue tolerance: osmoregulation to balance it; store it in vacuoles
- ◆ Crossing can take years to determine if the tree hybrid is truly salt tolerant
- ◆ New research: fungal endophytes help with salt tolerance

Fungal Endophytes May Drive Plant Adaptation

- ◆ Mostly unpublished work by Rusty Rodriguez (USGS in Seattle)
- ◆ Temperature tolerance (geothermal sites): survival at 65C!
- ◆ Drought tolerance
- ◆ Salt tolerance (San Juan Islands dune grass)
- ◆ No one has studied this phenomenon in trees yet

Summary of Parts 1-3

- ◆ Trees can adapt to chemical stresses in a variety of ways
- ◆ Recruitment of microbes during nitrogen-starvation or when exposed to pollutants
- ◆ Can alter uptake regulation and metabolism to deal with pollutants
- ◆ If man is inducing chemical stresses faster than plants can adapt to them, can we help them along?

Part Four: Can we help plants adapt to chemical/nutrient stress?

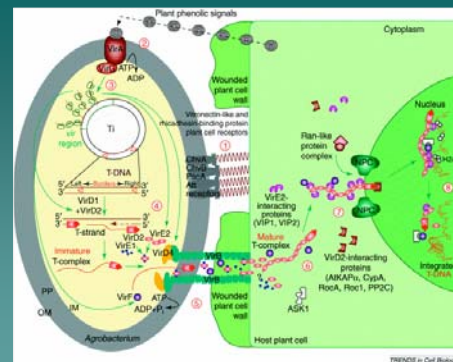
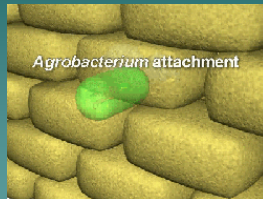
- ◆ Numerous sites are contaminated with metals and organic pollutants. Can we develop trees that can clean up environmental pollutants?
- ◆ Can we increase the salt-tolerance of trees so that abandoned lands are usable?
- ◆ Can we get other plants to associate with nitrogen fixing bacteria to lessen our dependence on chemical fertilizer?

Many of the genes needed for pollutant-degradation or tolerance are known

- ◆ Cytochrome P450s for metabolism of pollutants
- ◆ Transport proteins for uptake and sequestration of metals
- ◆ Chaperone proteins for general stress tolerance
- ◆ There are efficient ways to introduce genes into plants

Agrobacterium is commonly used to introduce specific genes into plants

- ◆ *Agrobacterium* is a common soil bacterium
- ◆ It naturally transfers DNA into the plant cell genome
- ◆ The plant treats the new DNA as its own



Using Agrobacterium to Transform Plant Cells

1. Place the gene of interest between the T-DNA borders
2. Introduce the vector into *Agrobacterium*
3. Induce expression of the virulence genes
4. Co-cultivate plant cells with *Agrobacterium*
5. Eliminate *Agrobacterium*
6. Select for transformed plant cells

Regeneration of whole plants

- ◆ After the DNA is introduced, whole plants are regenerated
- ◆ Plant hormones in medium initiate shoots, stems, and roots

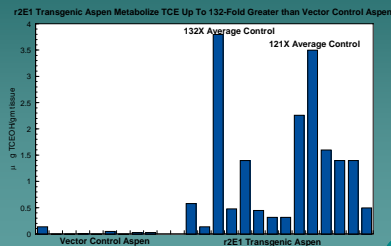


Plants are assayed for increased metabolism of pollutants

- ◆ The pollutant is added to vials of the plants with or without the added gene
- ◆ The metabolites are extracted and quantified

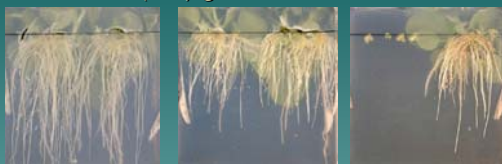


Overexpression of the cytochrome P450 2E1 gene enhances metabolism of TCE in poplar



Hybrid aspen cuttings were exposed to 50 µg/ml TCE for one week. The TCE metabolite, TCEOH (free), was extracted from tissues with MTBE and analyzed by GC-ECD. (Doty, unpublished)

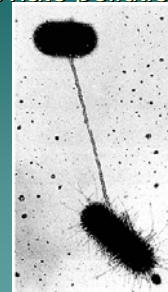
Plants cannot grow in media contaminated with TNT unless a bacterial nitroreductase (NR) gene is added



WT	NR	WT	NR	WT	NR
No TNT		0.05 mM		0.1 mM	

Adding the appropriate bacterial helpers might increase the ability of plants to tolerate/alleviate pollution

- ◆ Some bacteria live inside plants
- ◆ Some bacteria can degrade pollutants
- ◆ The required genes are usually on mobile DNA elements (plasmids)
- ◆ These plasmids can be transferred between bacteria by conjugation



Bacterial endophytes harboring the genes for degrading the pollutants can help plants grow on toluene (van der Lelie 2004)

- ◆ Control plants had no bacteria added
- ◆ 2nd column has native endophyte
- ◆ 3rd column (G4) has plants inoculated with a toluene-degrading bacterium
- ◆ 4th column (VM1330) has plants inoculated with native endophyte containing the G4 plasmid



Need for salt tolerant crops

- ◆ Prevent further deforestation in the quest for more arable land
- ◆ One-third of the world's irrigated land is unsuitable for growing crops because of the contamination with high levels of salt
- ◆ Increasing human population but decreasing amount of arable land is a disastrous combination
- ◆ The solution?



Salt-tolerance can be improved

- ◆ Many of the genes involved in salt-tolerance are known
- ◆ Overexpress these genes for increased salt tolerance
- ◆ Overexpression of a Na⁺/H⁺ antiport protein in *Arabidopsis*



Can we help with nitrogen deprivation problems without using chemical fertilizers?

- ◆ Chemical fertilizers are produced from fossil fuels
- ◆ Chemical fertilization leads to leaching of excess nitrogen into groundwater
- ◆ Since some plants have recruited nitrogen-fixing bacteria, can we help others do it too?



Summary

- ◆ Some trees have adapted to chemical stresses including nitrogen deficiency, pollution, and salt stress
- ◆ More research is needed to better understand stress tolerance
- ◆ In some cases, mankind is introducing chemical stresses faster than plants can adapt to them.

