Using natural microbial symbionts of trees to remove pollutants, increase plant growth, and produce biochemicals

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Plant-Microbe Symbiosis

- “Symbiosis” means “living together”
- Commonly known are rhizobia that help legumes (peas, beans, clover) get nutrition
- Another common symbiosis is with mycorrhizae that associate with roots of most plants

Root nodule on alfalfa
Microbial Endophytes

But relatively new research has pointed to the importance of “endophytes”, microbes that live fully within plants without causing disease, that provide multiple benefits including nutrition and stress tolerance.

**Benefits from endophytes**

- **Pathogen resistance**
  - Anti bacterial compounds

- **Growth Promotion**
  - Hormones
  - Nutrients

- **Stress tolerance**
  - Drought
  - Temperature

- **Remediation of pollutants**
Research Area 1: Phytoremediation

Using plants for detoxification of pollutants
The Pollution Problem

- Many types of pollutants
- Occurs from spills or deliberate dumping
- Billions spent worldwide
- Enormous health costs including cancers
- Large land areas are affected, making traditional engineering solutions (such as excavation) impractical and too expensive
Phytoremediation = Solar-Powered Pollution-Removal System

Poplar and willow are excellent for removal of pollutants due to their rapid growth, ease of propagation, deep roots, and natural ability to remove and detoxify certain pollutants.

April 2000
EcoloTree, Inc., Dr. Lou Licht

Sept. 2002

7-year old poplar at a TCE site
(Environmental Forestry Consultants, Dr. Jud Isebrands)
International Poplar Commission

- A branch of the United Nations, the IPC supports the use of poplar and willow for improving livelihoods around the world


- The IPC working group on Environmental Applications provides guidance on the use of these plants for erosion control, stopping desertification, phytoremediation, bioenergy, C sequestration, riparian buffers, and more

**Current Officers:** Sharon Doty (Chair), Jaconette Mirck and Andrej Pilipovic (Vice Chairs), and Ionnis Dimitriou (Technical Secretary)
Research Area 1: Phytoremediation

Insecticides (Chlorpyrifos)
Solvents (TCE)
Polycyclic Aromatic Hydrocarbons (Phenanthrene)
Explosives (TNT, RDX)
Inorganic Pollutants (Arsenic)
Chlorpyrifos (CPS) is a broad-spectrum insecticide

Adverse impacts on environmental and human health

A low-birth-weight baby
Research in our laboratory has demonstrated that poplar and willow can take up this insecticide from water and degrade it.

If these plants were added as a riparian buffer between agricultural fields and rivers, it may reduce the impact of this pollutant on ecosystems and human health.


This work was sponsored by University of Washington Superfund Research Program, Grant #: NIEHS P42ES004696.
Trichloroethylene (TCE) is a common solvent and degreaser with a variety of industrial applications. It is one of the most common pollutants, found in 60% of SuperFund sites across the country.

TCE is toxic and can cause cancer.
Research in our lab has demonstrated that different varieties of poplar and willow have different capacities to remove and detoxify TCE. Some removed more than 40% of the TCE from solution within one week.

Miller, R. S., Khan, Z., and Doty, S. L.  Comparison of trichloroethylene toxicity, removal, and degradation by varieties of *Populus* and *Salix* for improved phytoremediation applications. *Journal of Bioremediation and Biodegradation* S7:001. Doi:10.4172/2155-6199.S7-001.

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Endophyte-Assisted Phytoremediation

TCE
PAHs
Explosives
Heavy metals

Combines the ability of plants to take up pollutants from a wide area with the ability of the endophytes to rapidly detoxify the chemicals.
Screened for endophytes of poplar that degrade trichloroethylene (TCE), and found one strain that rapidly took up and degraded this important pollutant.
Using a similar method, we discovered another poplar tree endophyte that reduces PAH toxicity.

Khan, Z., et al. (manuscript in preparation)
Research Area 2: Increasing Plant Growth Sustainably using Symbiosis

Using microbes instead of chemical fertilizers
Chemical Fertilizers

- Synthesized nitrogen fertilizer requires high levels of fossil fuel.
- Cost rises with fossil fuel prices
- Environmental costs include greenhouse gas emissions (nitrous oxide) and fouling rivers and other waterways
Biological Nitrogen Fixation

“Fixing nitrogen” means changing the dinitrogen gas in our air to a usable form like ammonia.

Well-known examples are rhizobia with legumes and Frankia with alder trees and certain other woody plants. Both live in visible root nodules.
But some plants grow without fertilizer and without root nodules. Recent research pointed to endophytes that can fix nitrogen without needing root nodules.

Some examples include sugarcane in Brazil, sweetpotato in East Africa, kallar grass, and rice.
Poplar & willow grow naturally in low-nutrient areas. We isolated a variety of endophytes that can fix nitrogen and promote plant growth.

Endophytes can be isolated from poplar, labeled with fluorescent markers, grown in culture, and re-inoculated into plants so we can see colonization.

Three week old internally sterile Nisqually-1 plant inoculated with $gfp$-WP9.

Fluorescent image taken after 6 days of inoculation - Xylem of stem tissue (longitudinal section).

Fluorescent image taken after 6 days of inoculation – Cross section of a node.

Fluorescent image taken after 2hrs of inoculation.
The endophytes naturally produce plant hormones that increase rooting.

Without the added microbes

With the added symbionts
Endophytes improved grass health and growth in low nutrient conditions

Kentucky bluegrass +/- PTD1

Wisconsin grass line +/- WP19

Increased growth and yields of bell pepper plants grown in low-nutrient soil by adding poplar tree endophytes

Khan, Z, Guelich, G., Phan, H., Redman, R., and Doty, S. L. 2012. ISRN Agronomy
Increased growth of tomato and Douglas-fir in nutrient-poor soil

Douglas-fir photos: Controls on the left, inoculated with endophytes on the right
Endophytes can improve drought tolerance

60% more root biomass and 48% more shoot biomass

Perennial rye grass after two weeks of water stress. Plants on the left were colonized by an endophyte consortia.

Summary of Research Area 2

Endophytes of poplar and willow can help plants by fixing nitrogen, solubilizing phosphate, producing plant hormones, and increasing drought tolerance. They associate not only with poplar and willow but with many other types of plants as well.
Implications for this research:
Endophytes For Sustainable Bioenergy Crop Growth

The DOE’s choice plant for biofuel for the Pacific NW is hybrid poplar. An AFRI grant is focused on developing these biofuels in an environmentally and economically sustainable manner. [http://hardwoodbiofuels.org/](http://hardwoodbiofuels.org/)
Implications for this research:
Endophytes For Sustainable Agriculture

Chemical fertilizers can damage ecosystems and release potent greenhouse gases. This research may lead to a reduction in the need for chemical fertilizers by re-establishing natural plant-microbe symbioses.

Gulf of Mexico “dead zone”
www.noaa.gov
Research Area 3: Biochemical Production

Using endophytes to produce biofuels
Rhodotorula mucilaginosa strain PTD3

- Naturally occurring, endophytic, robust, pigmented yeast from poplar trees
- Rapidly and effectively utilizes both 5C and 6C sugars, producing bioethanol and bioxylitol
- Tolerant of phytochemicals


Overall Implications for this research: Climate change mitigation

- Planting more trees for multiple purposes (phytoremediation, bioenergy, and other environmental applications) can remove excess CO$_2$ from the air, reducing global warming.

Endophytes can promote plant growth naturally through improved nutrient acquisition, phytohormone production, and pollutant degradation.
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