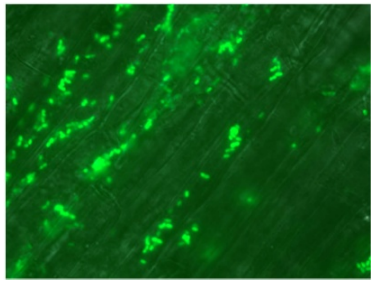
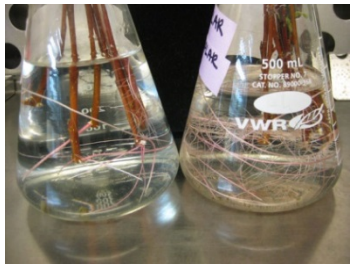
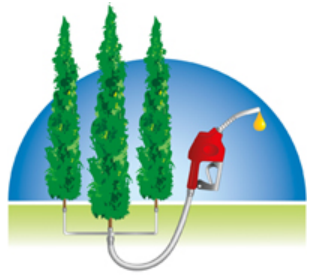


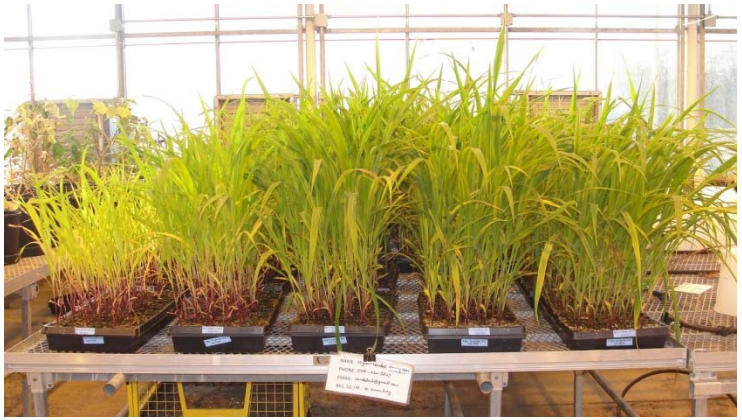




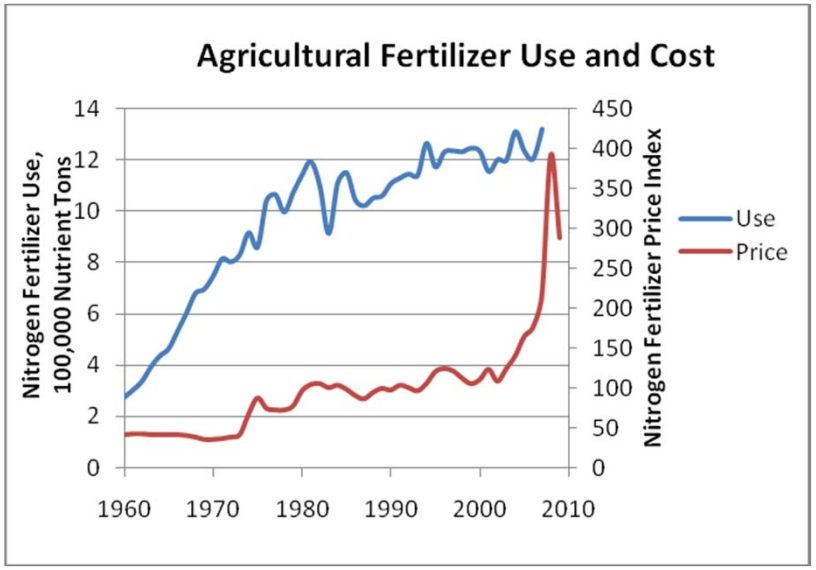
Increasing Growth and Drought Tolerance using Symbiotic Microorganisms

Sharon L. Doty
Associate Professor
University of Washington, Seattle

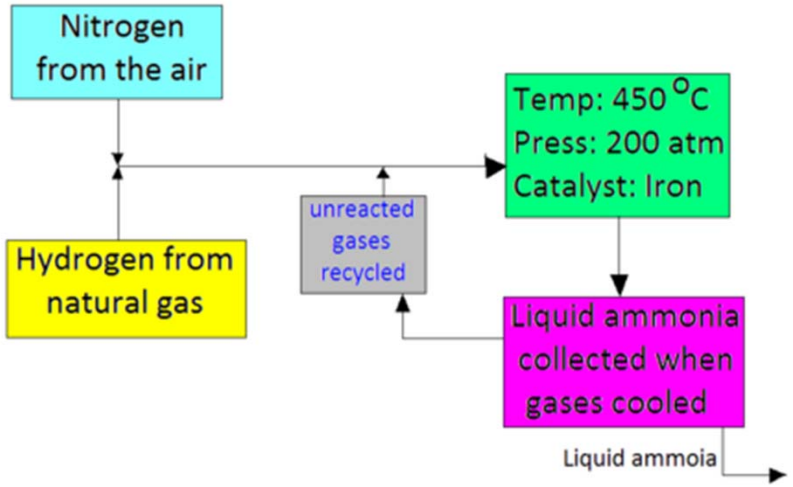
School of Environmental and Forest Sciences
UNIVERSITY of WASHINGTON
College of the Environment

Chemical Fertilizers





World Fertilizer Use



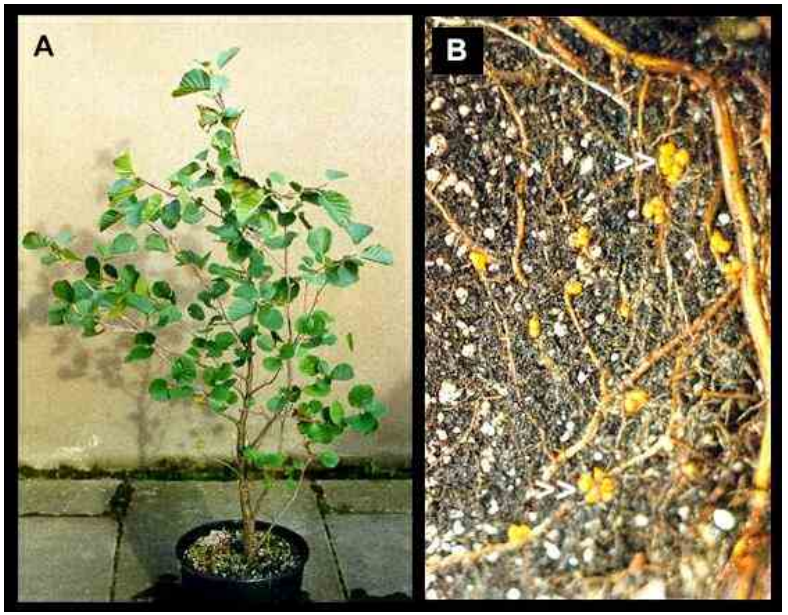
Biological Nitrogen Fixation

Nitrogenase

↓

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

EXPENSIVE REACTION→ Usually plant-associated



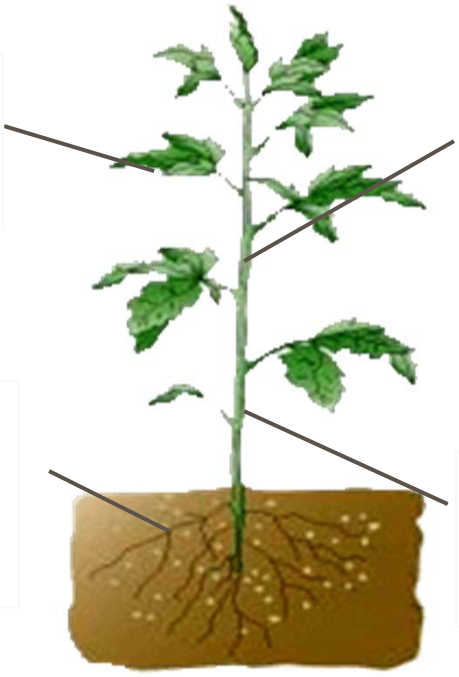
Diazotrophic Endophytes:

Nitrogen fixation without root nodules

The Plant Microbiome:
Microbial communities within a plant

Benefits from endophytes

Pathogen resistance
Anti bacterial compounds



Stress tolerance
Drought
Temperature

Growth Promotion
Hormones

Nutrients (N, P, Fe)

Reduced phytotoxicity of pollutants

Cottonwood & willow endophytes

- *Rhizobium tropici*

- *Burkholderia vietnamiensis*
- *Herbaspirillum*
- *Pseudomonas graminis*
- *Rahnella sp.*
- *Acinetobacter sp.*
- *Enterobacter* sp.
- *Sphingomonas* sp.
- *Rhodotorula graminis*

Doty, S. L., et al. (2005) *Symbiosis* 39: 27-35
Doty, S. L., et al. (2009) *Symbiosis* 47: 23-33
Xin, G., et al. (2009) *Biology and Fertility of Soils* 45:669-674

Nitrogen-fixing endophytes in poplar and willow

Growth in nitrogen-limited medium, presence of nitrogenase gene, ^{15}N incorporation from $^{15}N_2$ gas

Doty, S. L., et al. (2009) *Symbiosis* 47: 23-33
Xin, G., et al. (2009) *Biology and Fertility of Soils* 45:669-674.

Poplar Growth in N-Limiting Conditions

Direct evidence of N_2 fixation in poplar using the $^{15}N_2$ incorporation assay

(unpublished data showing high 15N incorporation into plant tissue was removed)

Doty, S.L., Sher, A.W., Fleck, N.D., Khan, Z., Kim, S.H., and DeLuca, T. H., manuscript in preparation

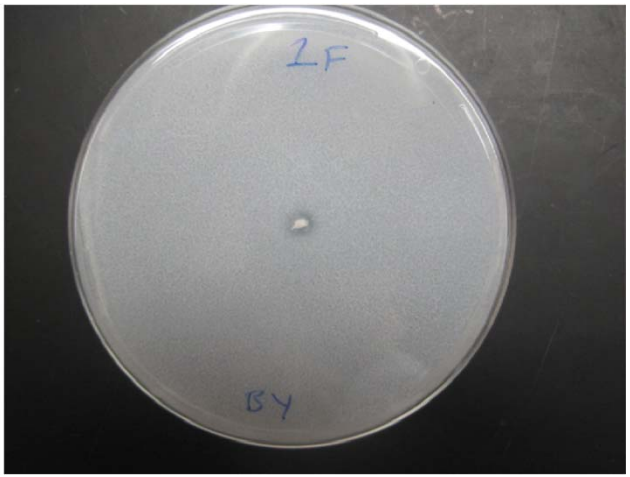
Many poplar endophytes produce plant growth hormones (auxins)

(unpublished data showing auxin production was removed)

Doty, SL, Doty, CM, Khan, Z, and Isebrands, JG, manuscript in preparation

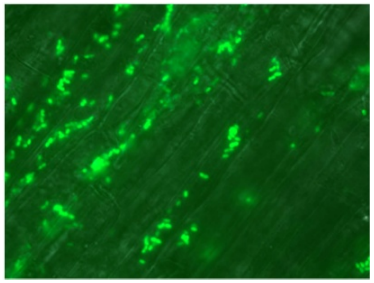
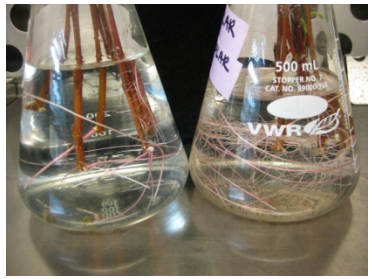
Phosphate Solubilization

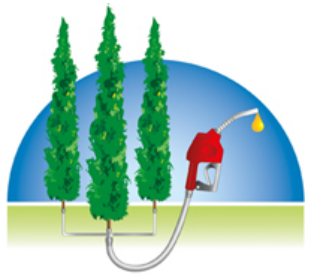
Alex Dolk, unpublished



Negative Control (BY)



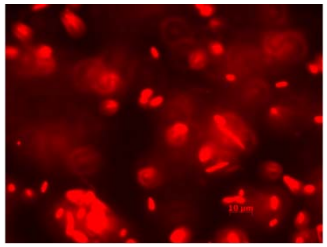



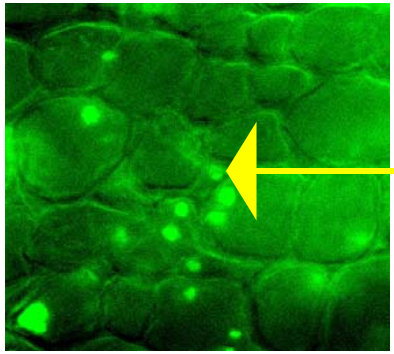


Endophytes of wild poplar can fix atmospheric N, solubilize P, and produce phytohormones

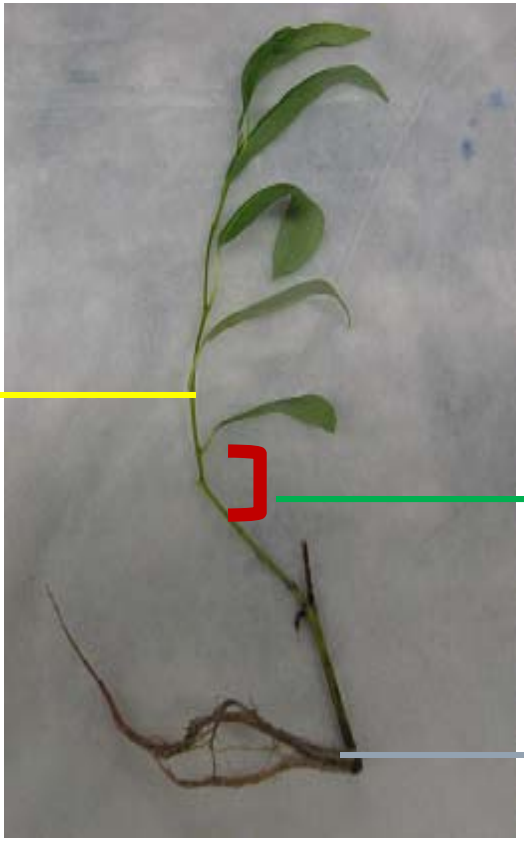
Can they be added to cultivated poplar for increased growth and health with fewer inputs?

Endophytes can be isolated from poplar, grown in culture, and used to re-inoculate plants



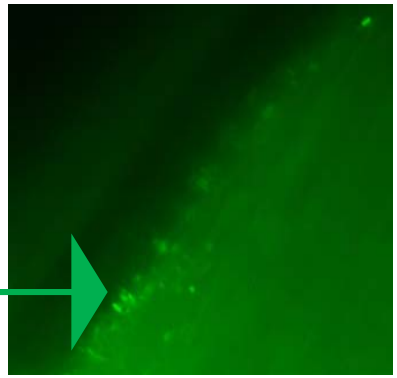


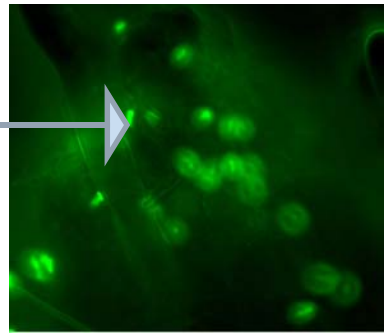
Fluorescent image taken after 6 days of inoculation – Cross section of a node



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)





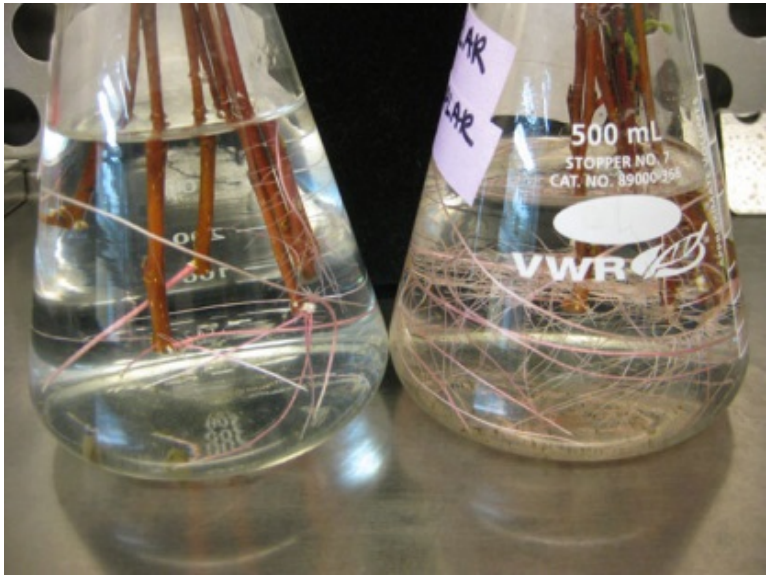
Fluorescent image taken after 2hrs of inoculation

Amy Baum and Sharon Doty, manuscript in preparation

Addition of the endophytes from wild poplar increases the rooting of recalcitrant poplar cuttings

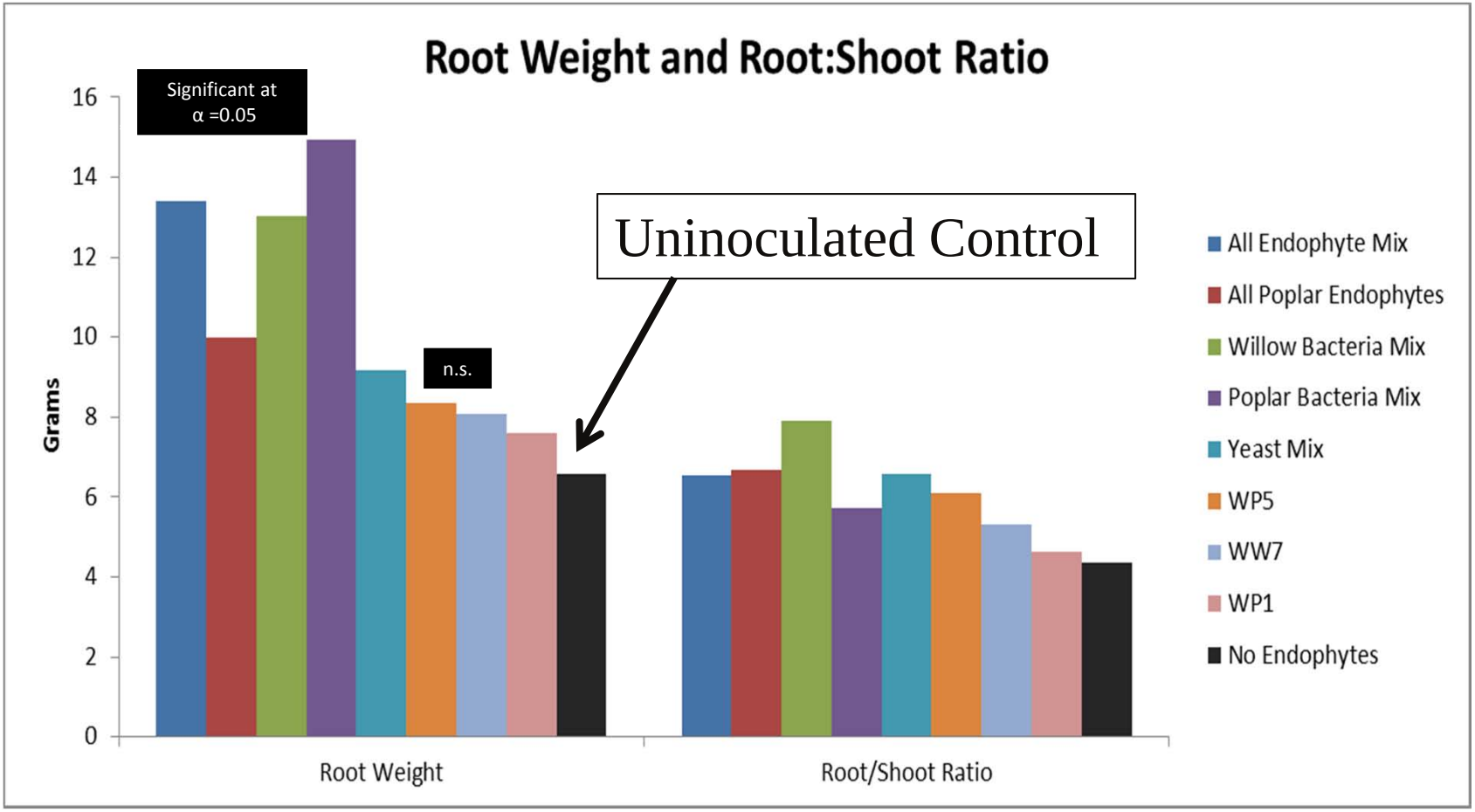
Without added microbes

With the added microbes



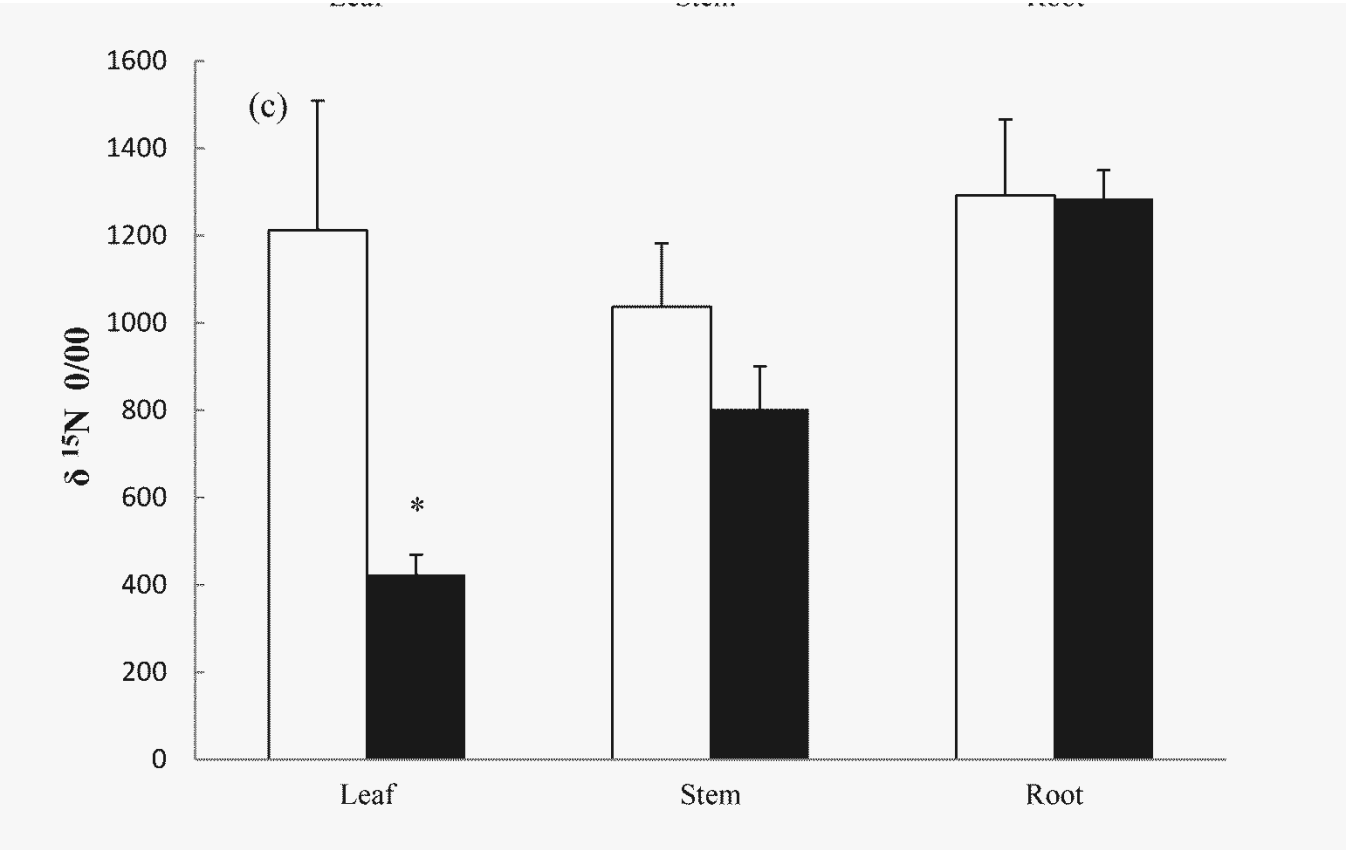
Doty, SL, Doty, CM, Khan, Z, and Isebrands, JG, manuscript in preparation

Root mass was doubled in *Populus trichocarpa* Nisqually-1 when endophytes were added



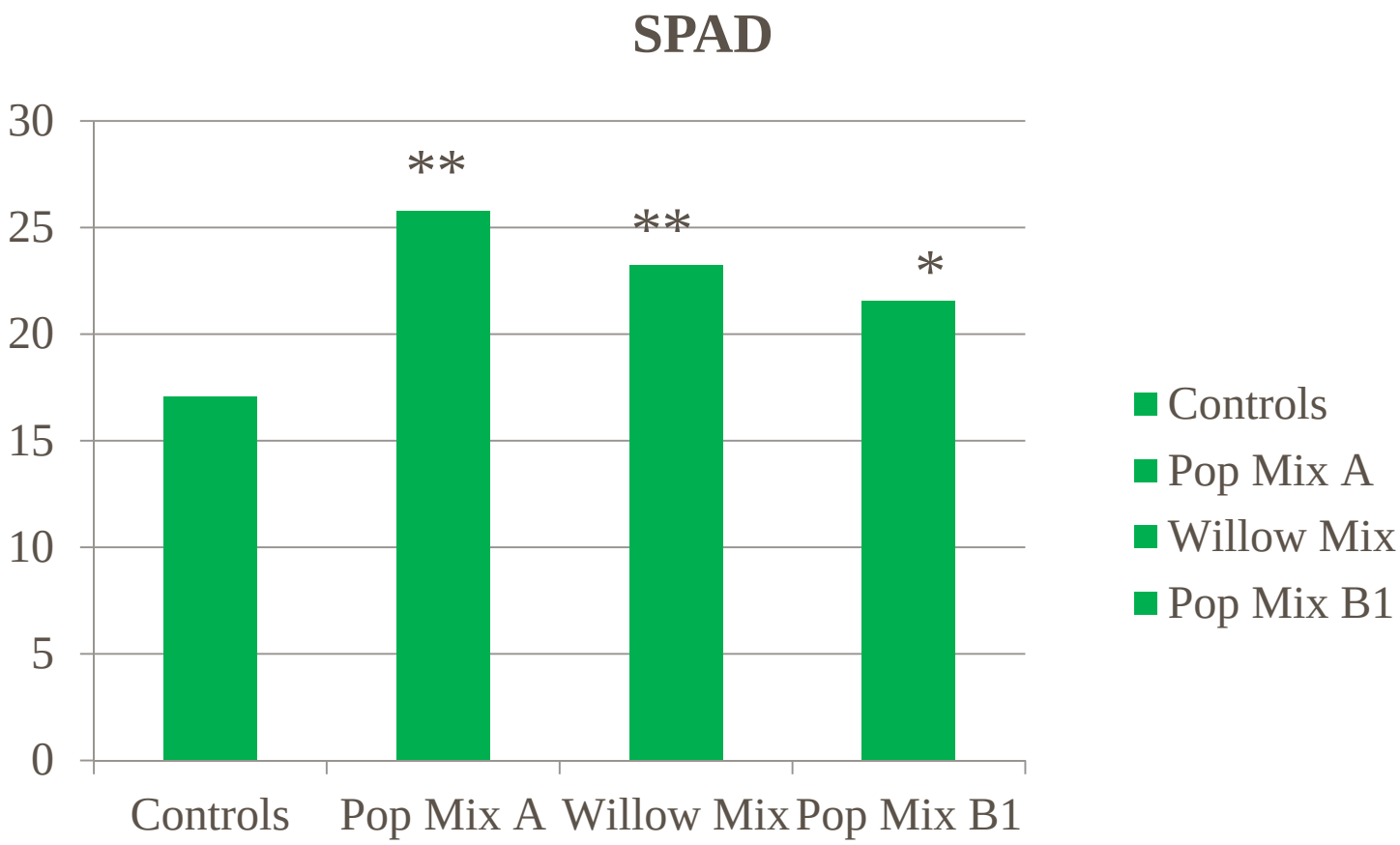
J. Knoth, et al (2014) *New Phytologist* 201:599-609

$^{15}N_2$ dilution assay indicated that the inoculated poplar received 65% of the foliar N from biological nitrogen fixation



J. Knoth, et al (2014) *New Phytologist* 201:599-609

Increased Greenness



Significant differences from the control: •, alpha = 0.1; *, alpha = 0.05; **, alpha = 0.01; n = 7

J. Knoth, et al (2014) *New Phytologist* 201:599-609

Addition of wild poplar endophytes increased drought tolerance, greenness, and growth

INOCULATED WITH CONSORTIA

Uninoculated Control

Zareen Khan, unpublished

Representative photos of poplar OP367 grown in absence of water for 1 month

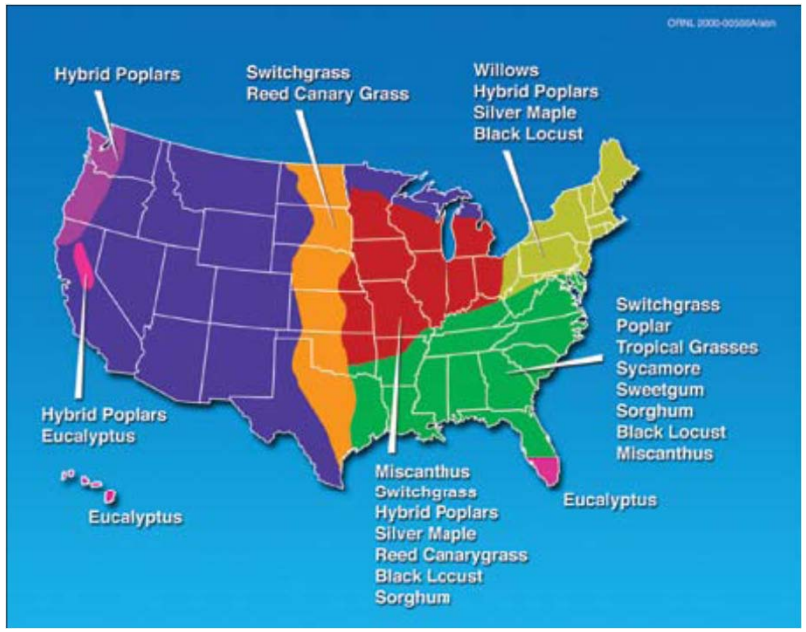
Inoculated with endophytes from wild poplar and willow

Uninoculated controls

Implications for this research:
Helps Explain the Biology of *Populus*

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/

GREENWOOD RESOURCES

hardwoodbiofuels.org

Advanced **Hardwood Biofuels** Northwest

Purpose grown

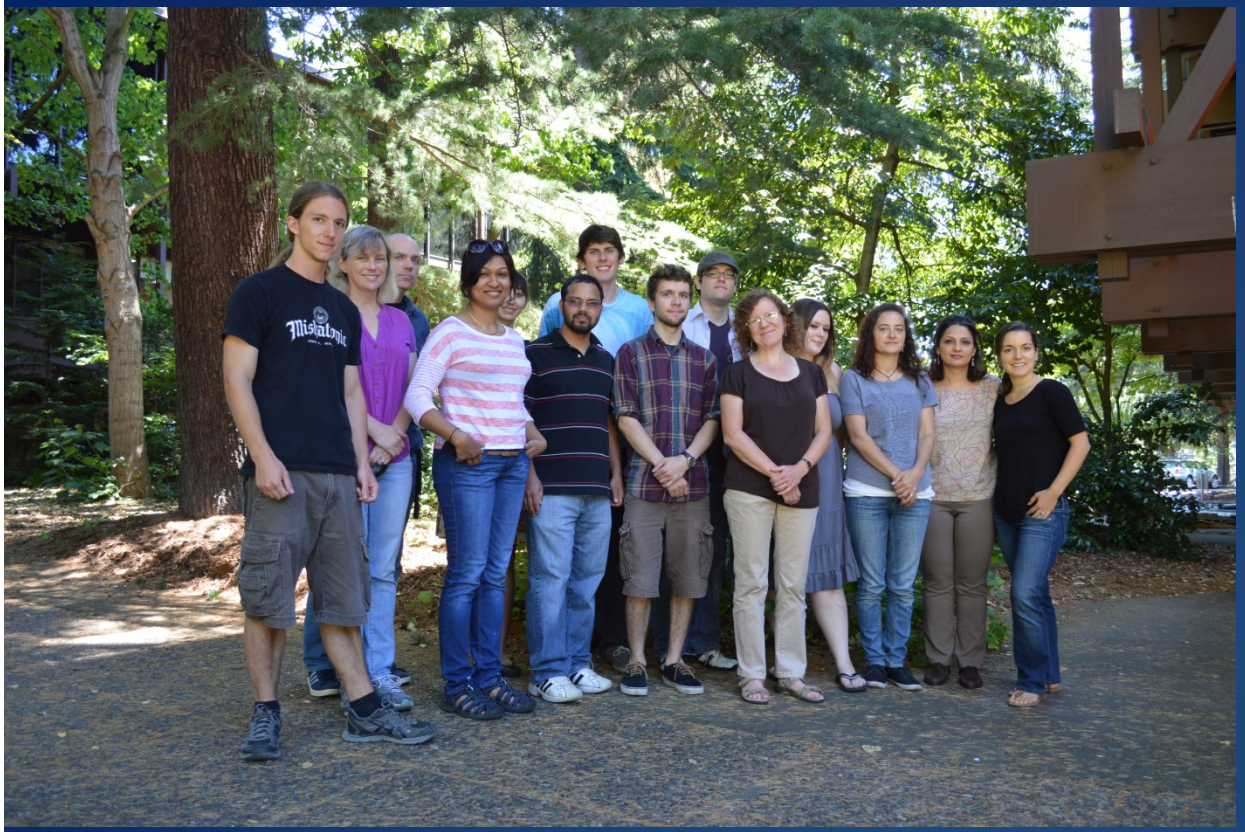
Drop-in replacement

Washington State University
Oregon State University OSU
Agriculture Center of Excellence
UCDAVIS University of California
University of Idaho
University of Washington
ZeaChem
New Holland Agriculture
GreenWood Resources
A Resource That Lasts Forever™
Rocky Mountain Wildlife Institute

Feedstock | Conversion | Sustainability | Education | Extension

USDA United States Department of Agriculture
National Institute of Food and Agriculture

SHARON DOTY LAB MEMBERS



Directly involved in the research presented today:

Dr. Zareen Khan (Research Scientist)
Jenny Knoth (prev. grad student)
Amy Baum (grad student)
Shyam Kandel (grad student)
Andrew Sher (Research Tech)
Neil Fleck (Research Tech)
Megan Plog (undergrad)
Beverly Hung (undergrad)
Alex Dolk (undergrad)

Funding provided by the USDA/AFRI, USDA/NIFA, and NSF. Co_PI's on NSF Energy for Sustainability: Soo-Hyung Kim and Greg Ettl

For more information, please see our LAB WEBSITE:
http://depts.washington.edu/envaplab