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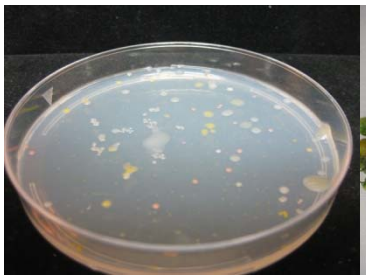
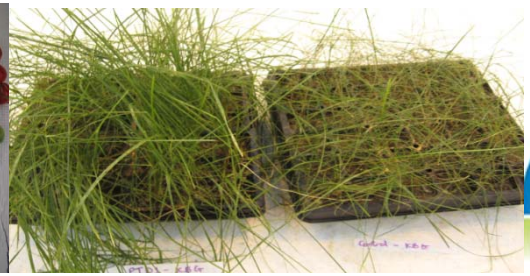
The Poplar Tree Microbiome: Implications of the Ecosystem Within

Prof. Sharon L. Doty
University of Washington, Seattle

Unpublished data were removed from this file for posting




The Plant Microbiome: Microbial communities within a plant

Benefits from endophytes

Pathogen resistance
Anti-microbial compounds

Stress tolerance
Drought
Temperature
Salt

Growth Promotion
Hormones

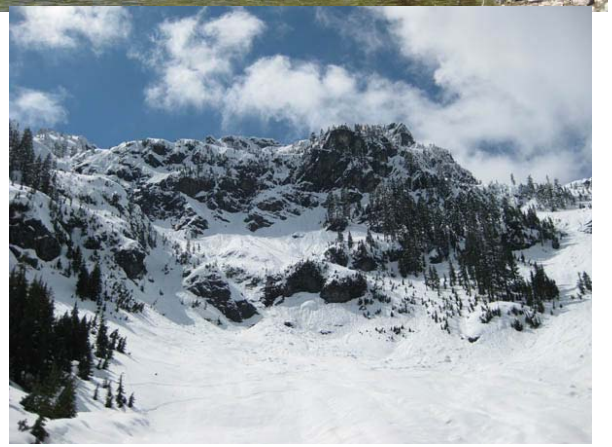
Nutrients (N, P, Fe)

Reduced phytotoxicity of pollutants
Organic pollutants
Inorganic pollutants

Session on "Genomics of Phytoremediators, Metal Accumulators and Relatives on 1/16

Hypothesis: Pioneer tree species use symbiosis with N-fixing (diazotrophic) endophytes to thrive in low-nutrient conditions

Rivers in the Pacific Northwest (USA) are fed by snow-melt from the mountains. Total N is only 0.05 to 0.3 mg/L



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

Doty, S.L., Sher, A.W., Fleck, N.D., Khorasani, M., Bumgarner, R., Ko, A., Khan, Z., Kim, S.H., and DeLuca, T. H. 2016 *PLOS ONE* 11(5):e0155979

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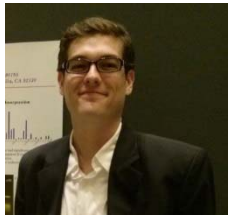
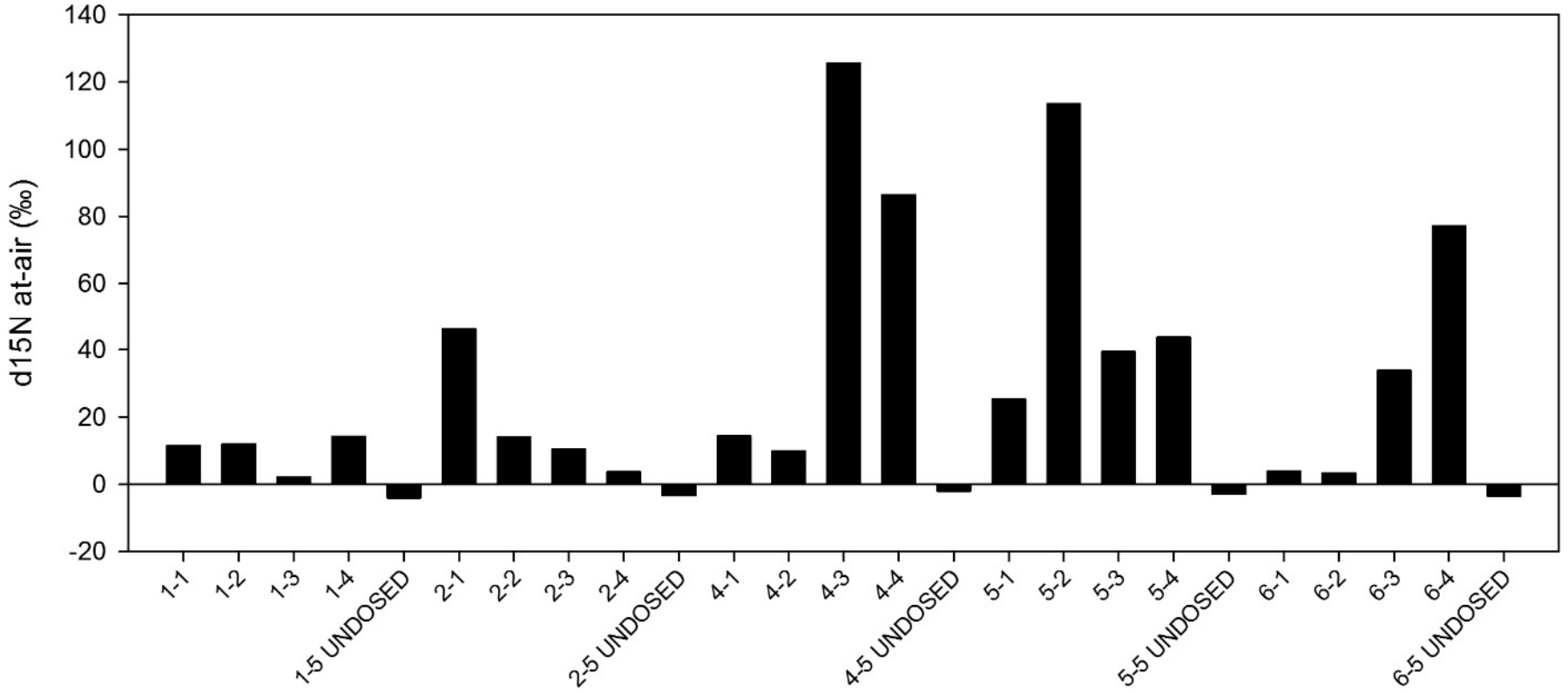
Evidence of N_2 fixation in some poplar using the $^{15}N_2$ incorporation assay



Andrew Sher



Doty, S.L., Sher, A.W., Fleck, N.D., Khorasani, M., Bumgarner, R., Ko, A., Khan, Z., Kim, S.H., and DeLuca, T. H. 2016 *PLOS ONE* 11(5):e0155979

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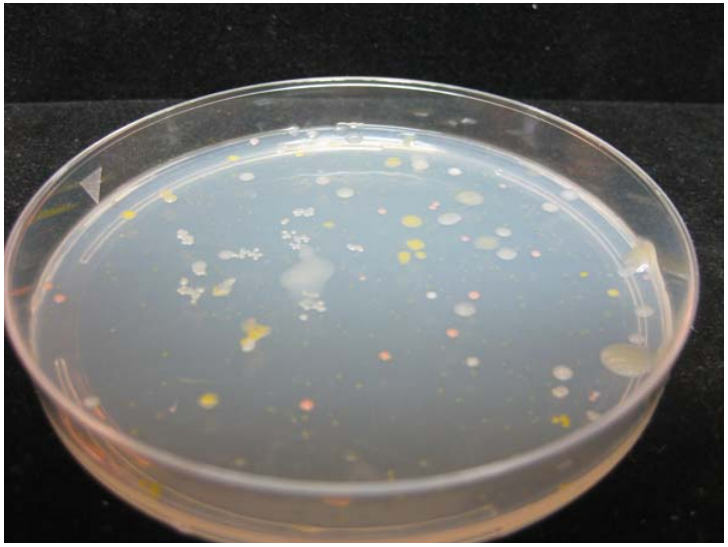
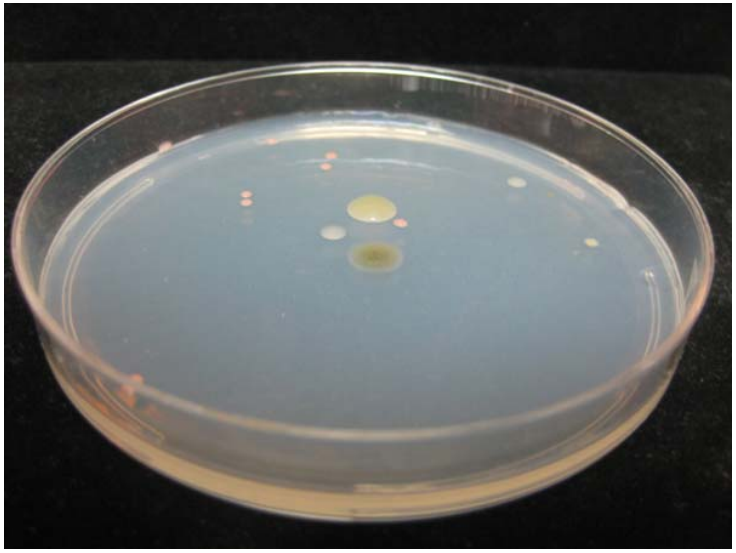
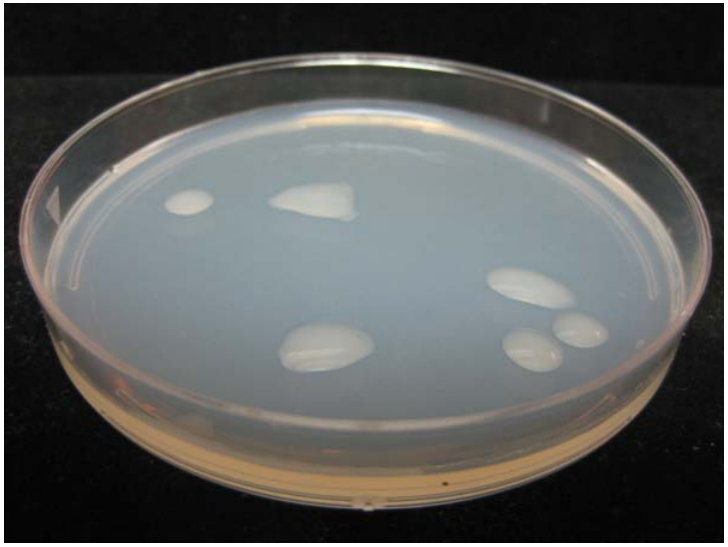
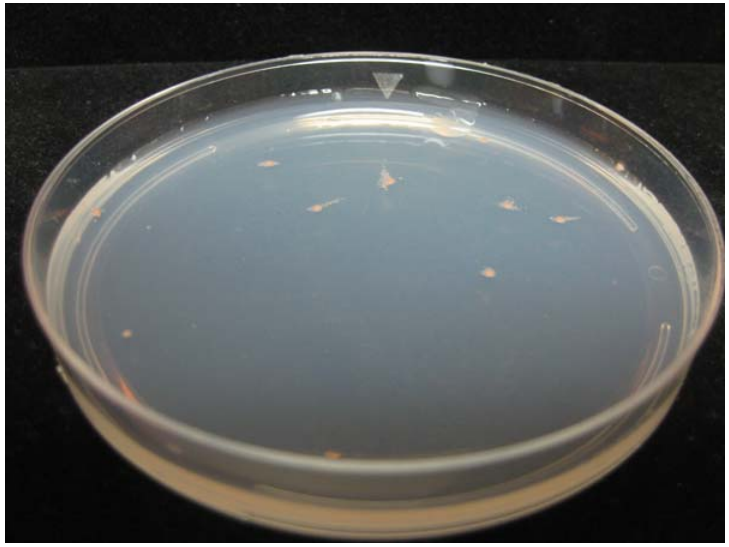
The microbiota of wild poplar is highly variable in stem sections of the same plant









Doty, S.L., Sher, A.W., Fleck, N.D., Khorasani, M., Bumgarner, R., Ko, A., Khan, Z., Kim, S.H., and DeLuca, T. H. 2016 *PLOS ONE* 11(5):e0155979

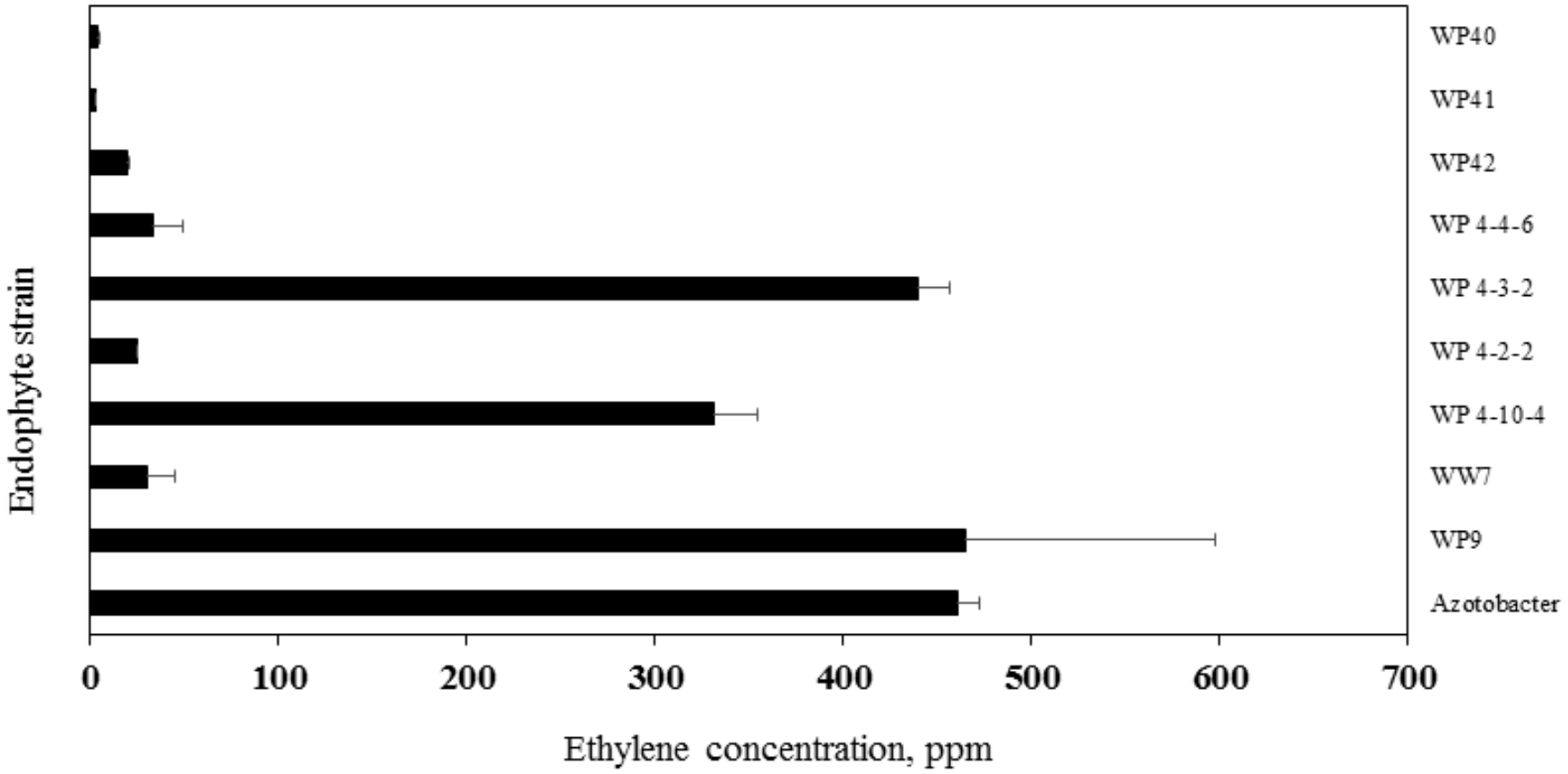
Examples of poplar & willow endophytes isolated from surface-sterilized branches

- *Rhizobium tropici*
- *Burkholderia sp.*
- *Herbaspirillum*
- *Pseudomonas graminis*
- *Rhanella sp.*
- *Sphingomonas sp.*
- *Acinetobacter sp.*
- *Enterobacter* 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
Firrincieli, et al. (2015) *Frontiers in Microbiology* 6: 978

Nitrogen fixation activity of some poplar and willow endophytes (ARA)

Shyam Kandel



Shyam L. Kandel, Andrea Firrincieli, Natalie Leston, Kendra McGeorge, Patricia A. Okubara, Giuseppe Scarascia Mugnozza, Antoine Harfouche, Soo-Hyung Kim, and Sharon L. Doty. 2017. *Frontiers in Microbiology* Vol 8, #386

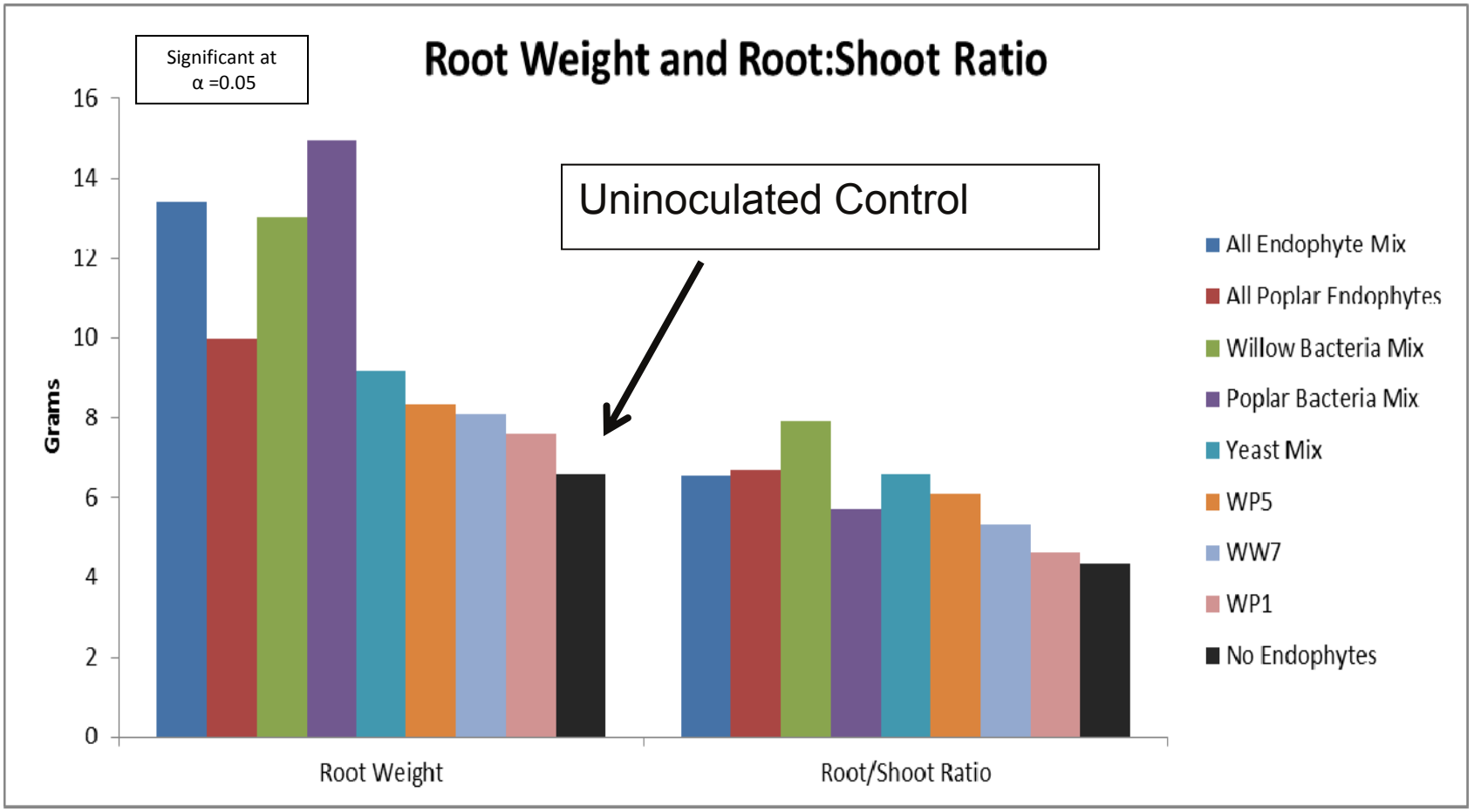
Can our current N-fixing* endophyte strains from wild poplar be used to increase the sustainability of hybrid poplar plantations?

* Growth on N-limited medium, ^{15}N incorporation, acetylene reduction assay, presence of *nifH* gene

Advanced **Hardwood Biofuels** Northwest

Jenny Knoth

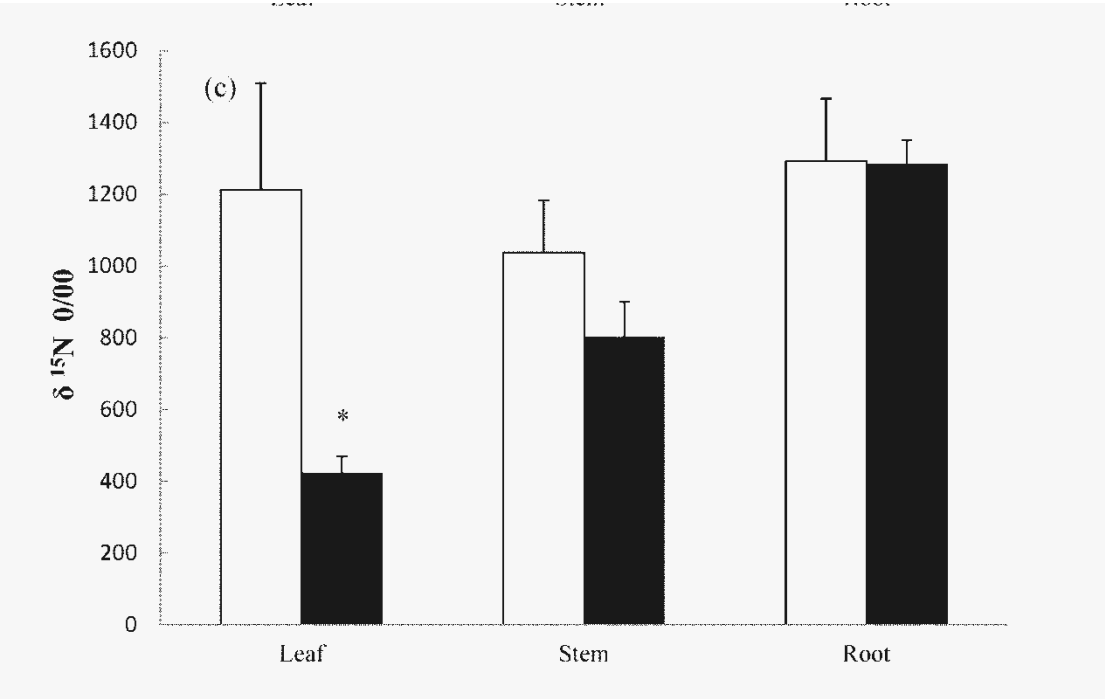
Root mass was doubled in *Populus trichocarpa* Nisqually-1 when endophytes were added



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

Jenny Knoth

^{15}N dilution assay indicated that hybrid poplar inoculated with strains from wild poplar received 65% of the foliar N from biological nitrogen fixation

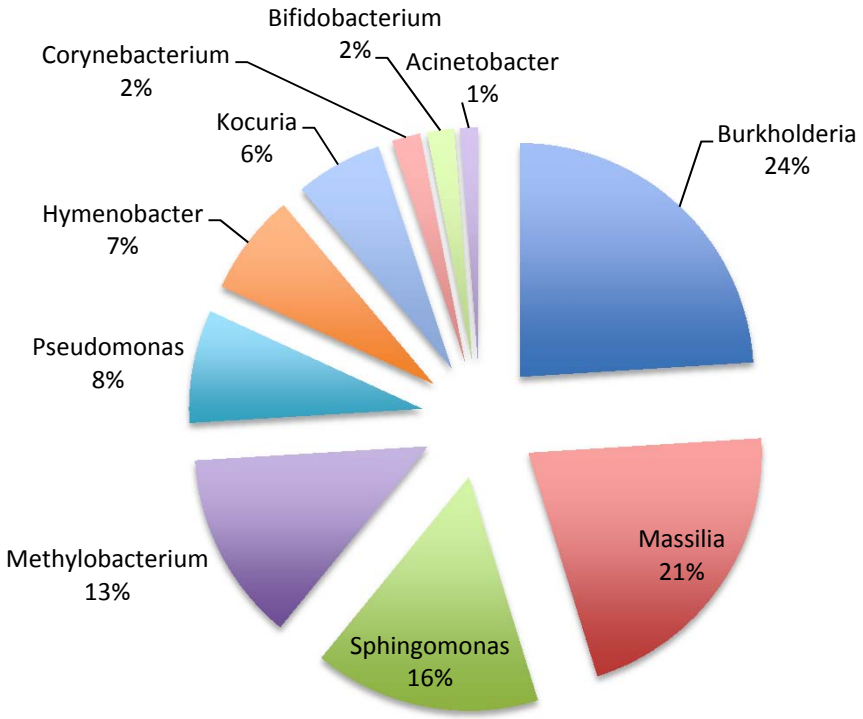


Un-inoculated controls

Inoculated with wild poplar endophytes

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

Wild poplar microbiome includes many potentially diazotrophic species

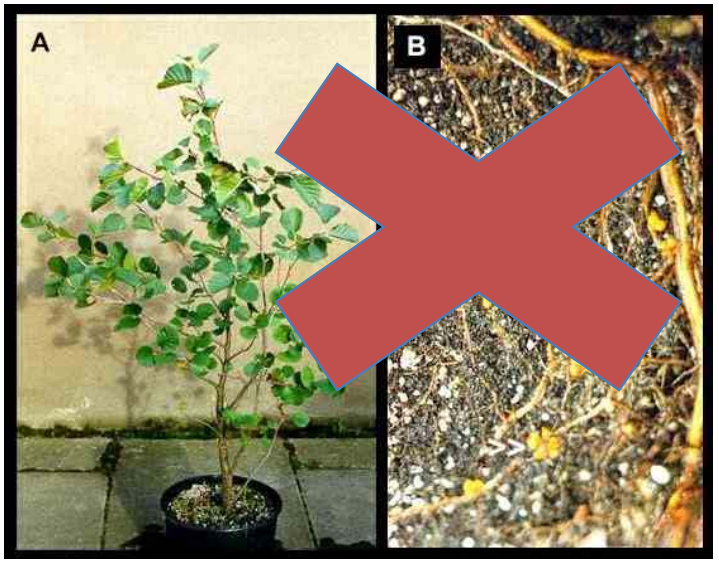


Leaf from Poplar 5

- NifH sequences included *Burkholderia, Sphingomonas, Azospirillum Bradyrhizobium, Rhodospirillum, Methanococcus*, and more

Doty, S.L., Sher, A.W., Fleck, N.D., Khorasani, M., Bumgarner, R., Ko, A., Khan, Z., Kim, S.H., and DeLuca, T. H. 2016 *PLOS ONE* 11(5):e0155979

Implications for our N-fixation research:
Helps Explain the Biology of *Populus*



NOTE: Poplar and willow are <u>not</u> nodulating species

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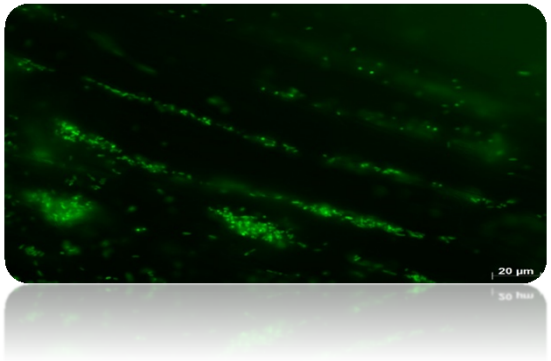
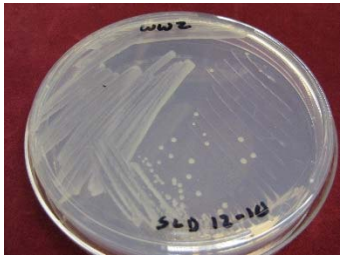
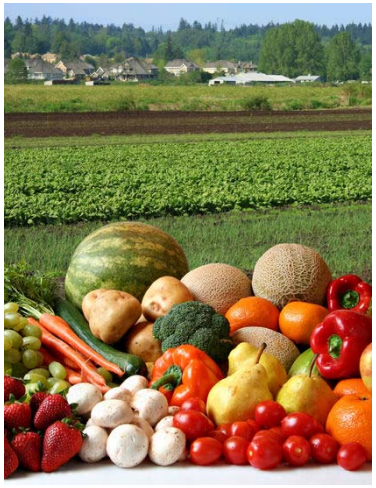




Can N-fixing endophytes of poplar and willow promote growth of other non-legume plants?



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Increased growth and yields of inoculated plants grown in low-nutrient soil

Zareen Khan

Controls without added endophytes

With endophyte added

Khan, Z, Guelich, G., Phan, H., Redman, R., and Doty, S. L. 2012. ISRN Agronomy

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Increased fruit yields

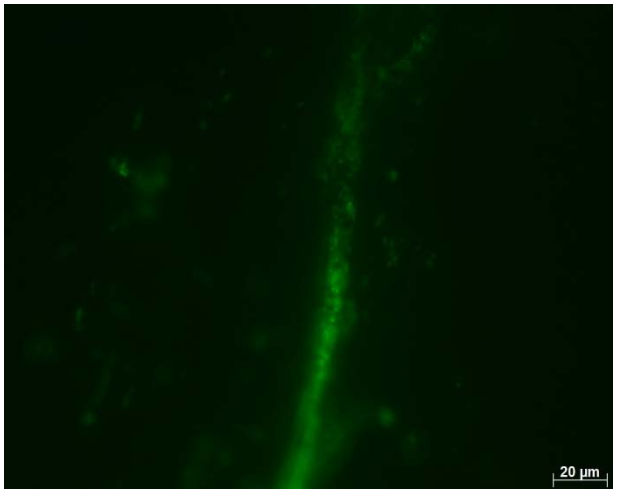
Dr. Zareen Khan

Treatment	Chocolate Cherry variety	Glacier variety
Inoculated	**106**	**95**
Control	63	43

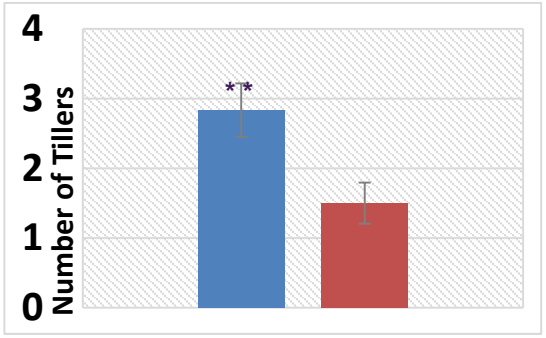
Khan, Z, Guelich, G., Phan, H., Redman, R., and Doty, S. L. 2012. *ISRN Agronomy*

Colonization and growth enhancement of rice

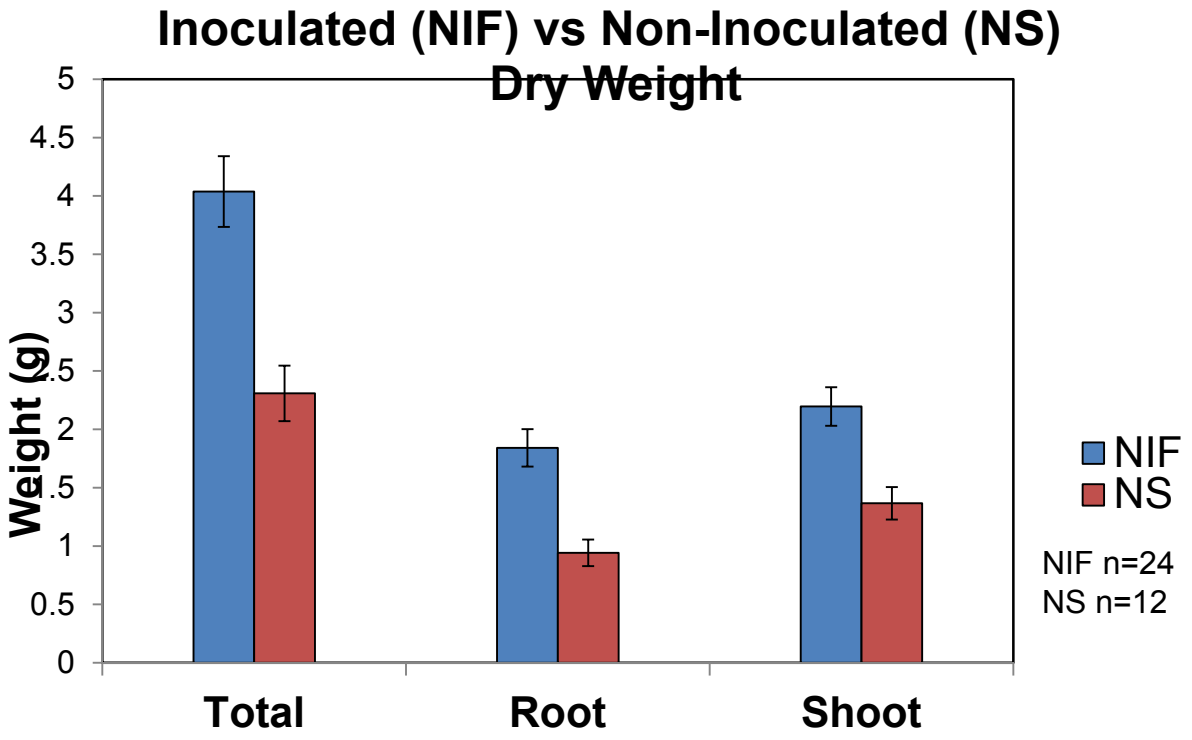
Shyam Kandel



# of Tillers



(Significance * * at α = <0.01)



S. Kandel, N. Herschberger, S.H. Kim, and S.L. Doty (2015) *Crop Science* 55:1765-1772

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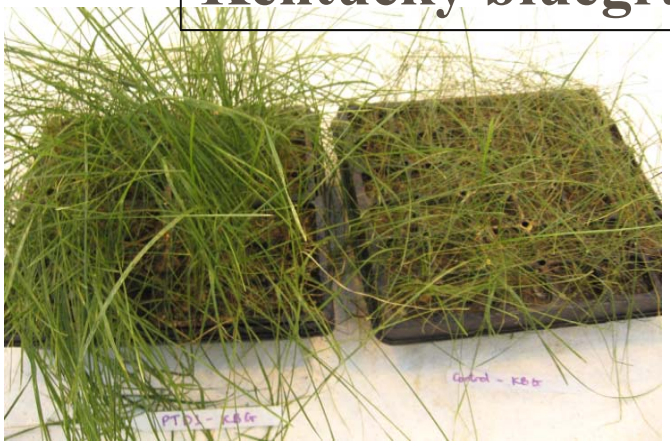
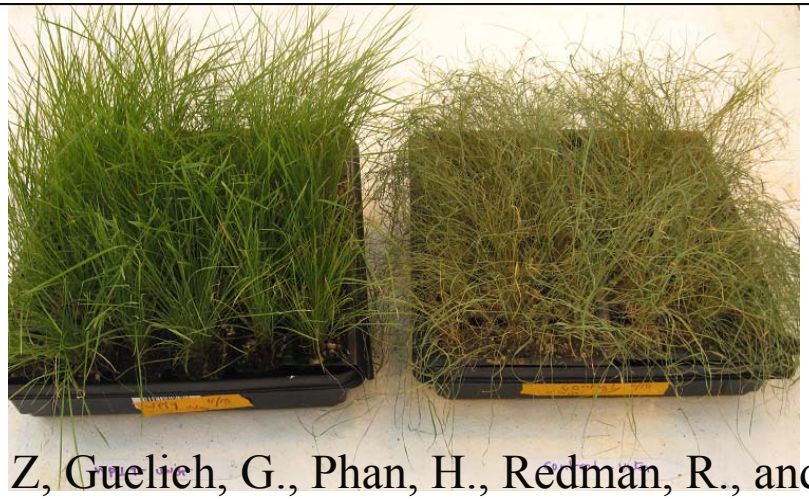
Endophytes improved turfgrass health and growth in low nutrient conditions

Zareen Khan

Kentucky bluegrass +/- PTD1



Wisconsin grass line +/- WP19



Khan, Z, Guelich, G., Phan, H., Redman, R., and Doty, S. L. 2012. *ISRN Agronomy*

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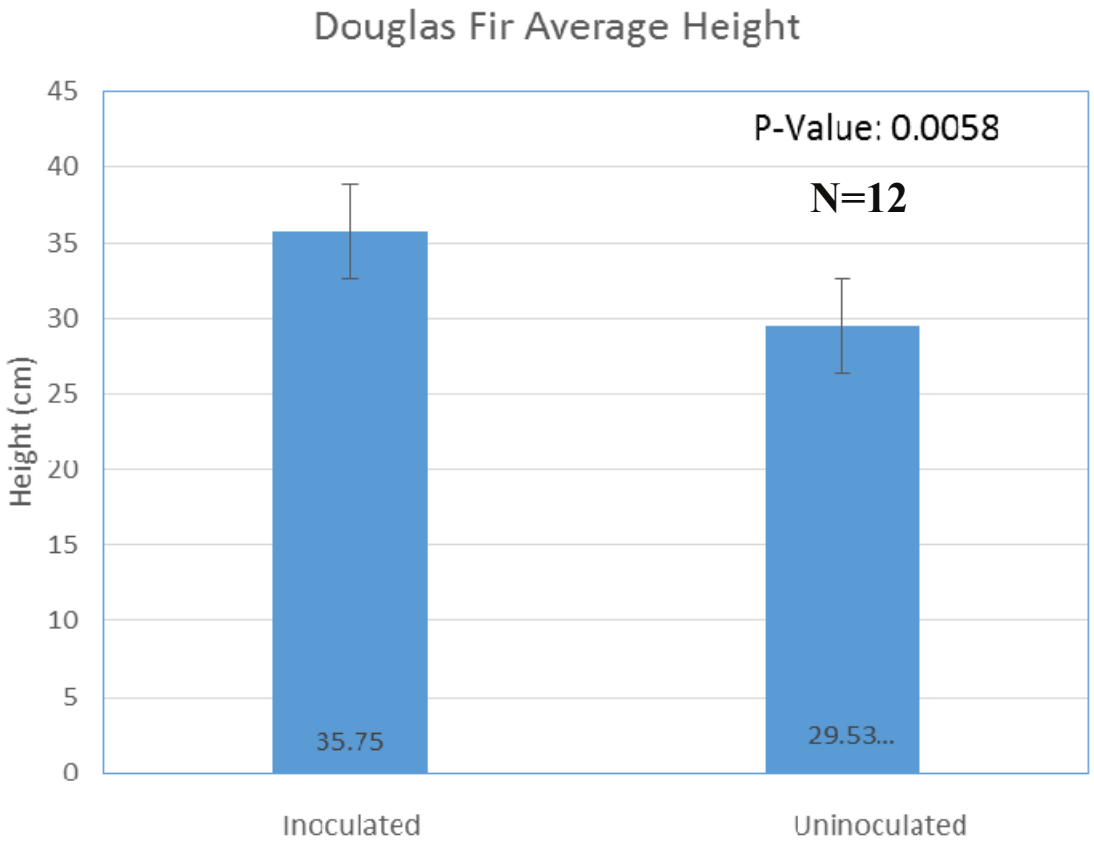
Increased growth of Douglas-fir in nutrient-poor soil in response to endophyte consortium (8 strains) from poplar & willow

Zareen Khan

No added microbes

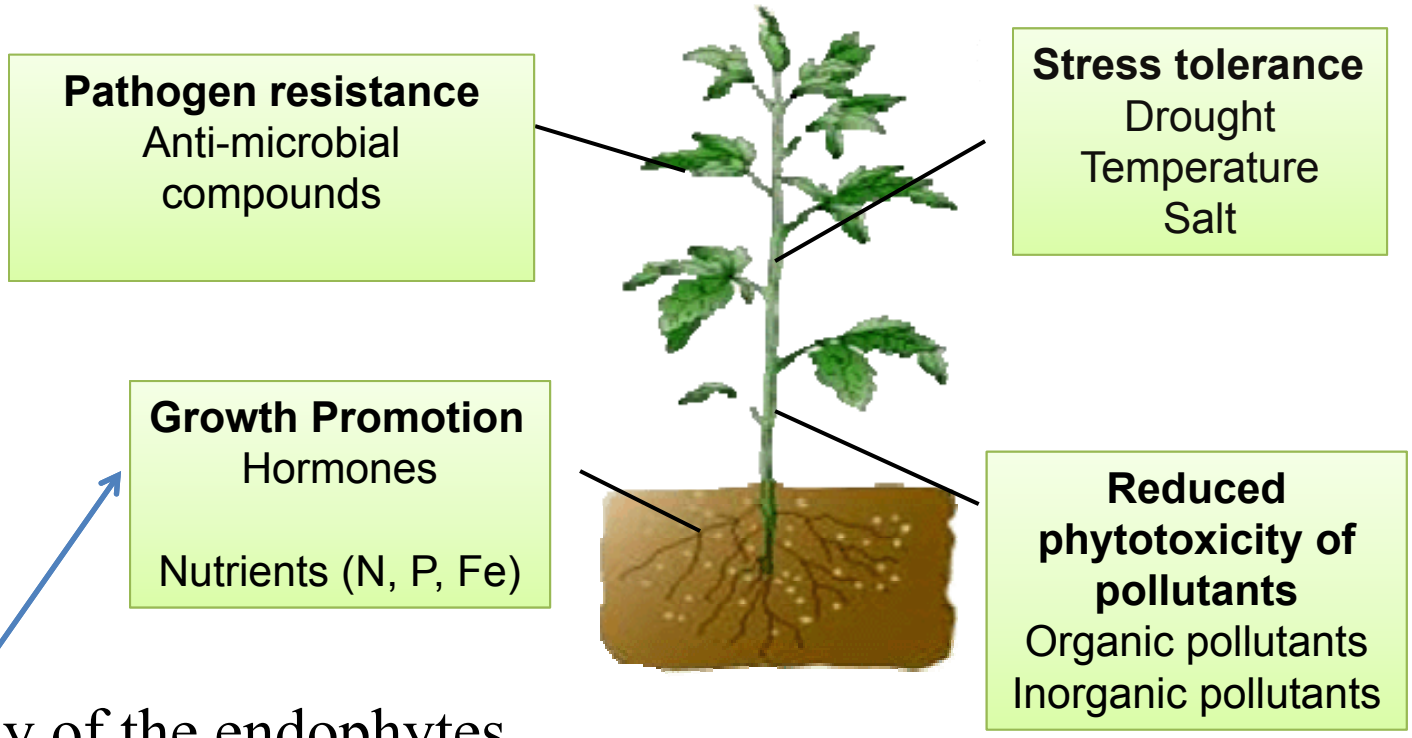
With added endophytes



Khan, Z., Ramos, D. Ettl, G., Kim, S.H., and Doty, S.L. 2015 *Forests* 6:3582-3593

The Plant Microbiome: Microbial communities within a plant

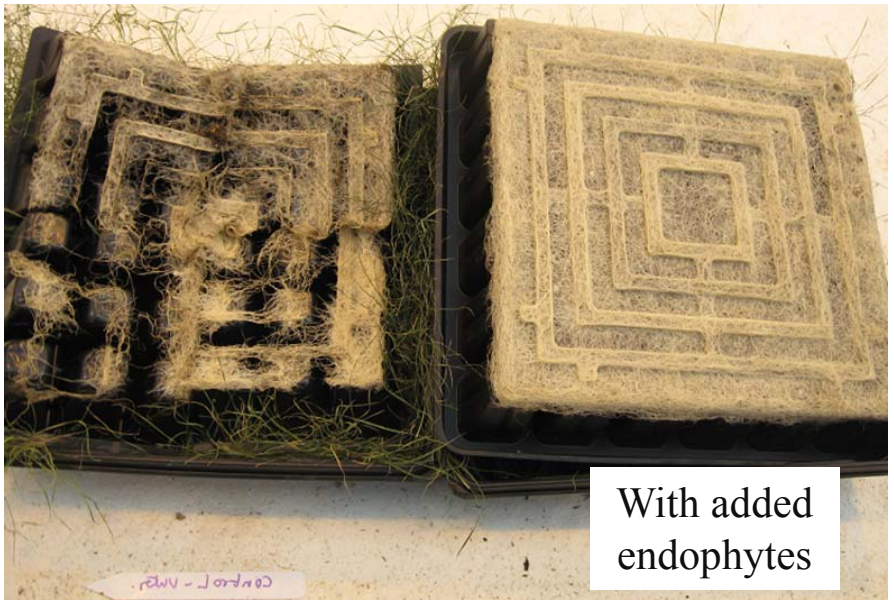
Benefits from endophytes



Many of the endophytes produce the auxin, IAA

Addition of the endophytes from wild poplar and willow increases the rooting of a variety of plant species

With added endophytes



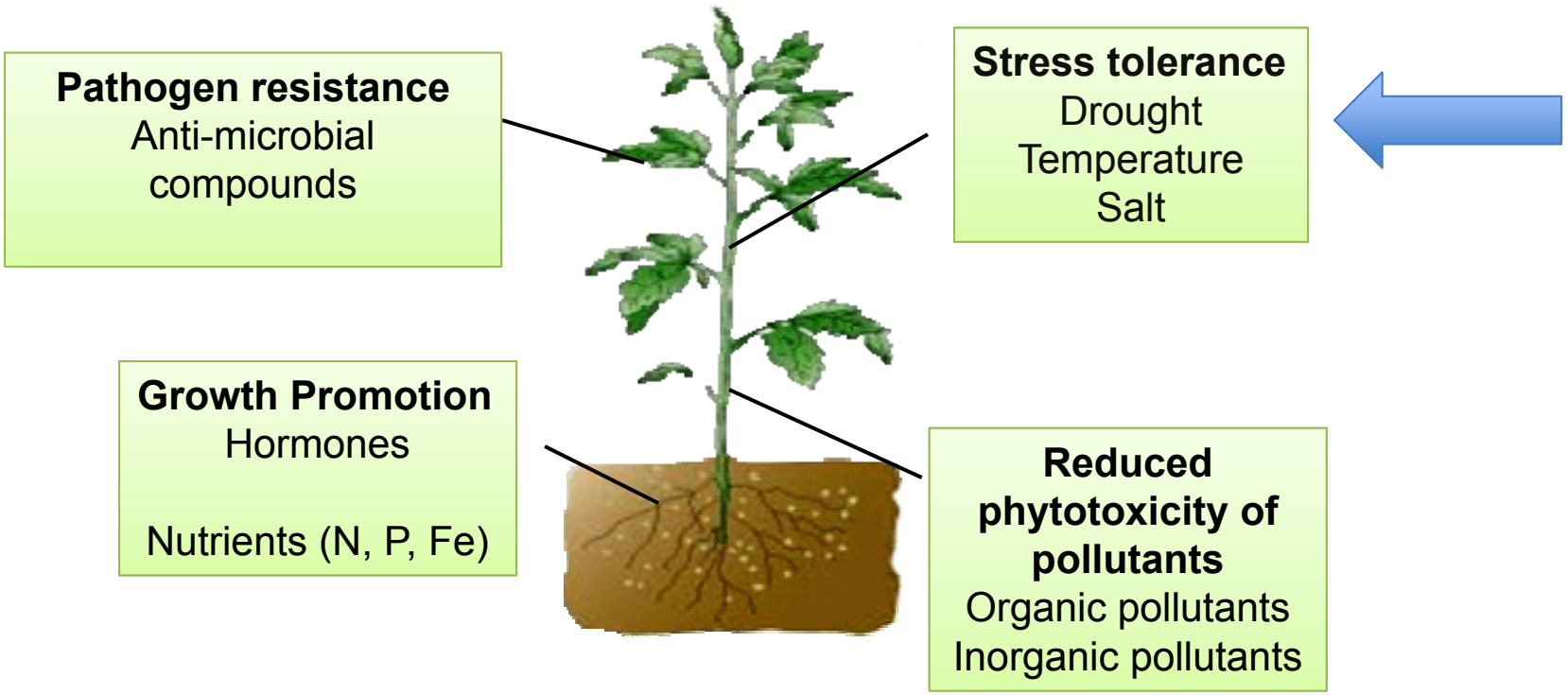
Khan, Z, et al. 2012. *ISRN Agronomy*

Khan, Z., Ramos, D. Ettl, G., Kim, S.H., and Doty, S.L. 2015 *Forests* 6:3582-3593

(Unpublished data showing increased rooting of recalcitrant *Populus deltoides* was deleted for posting)

The Plant Microbiome: Microbial communities within a plant

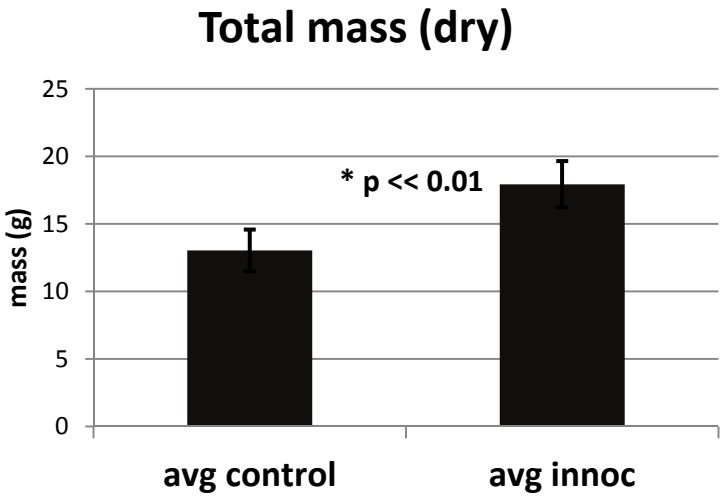
Benefits from endophytes



Hybrid poplar inoculated with endophytes from wild poplar and willow have increased growth and drought tolerance

Dr. Zareen Khan

Poplar inoculated with endophytes one month without watering

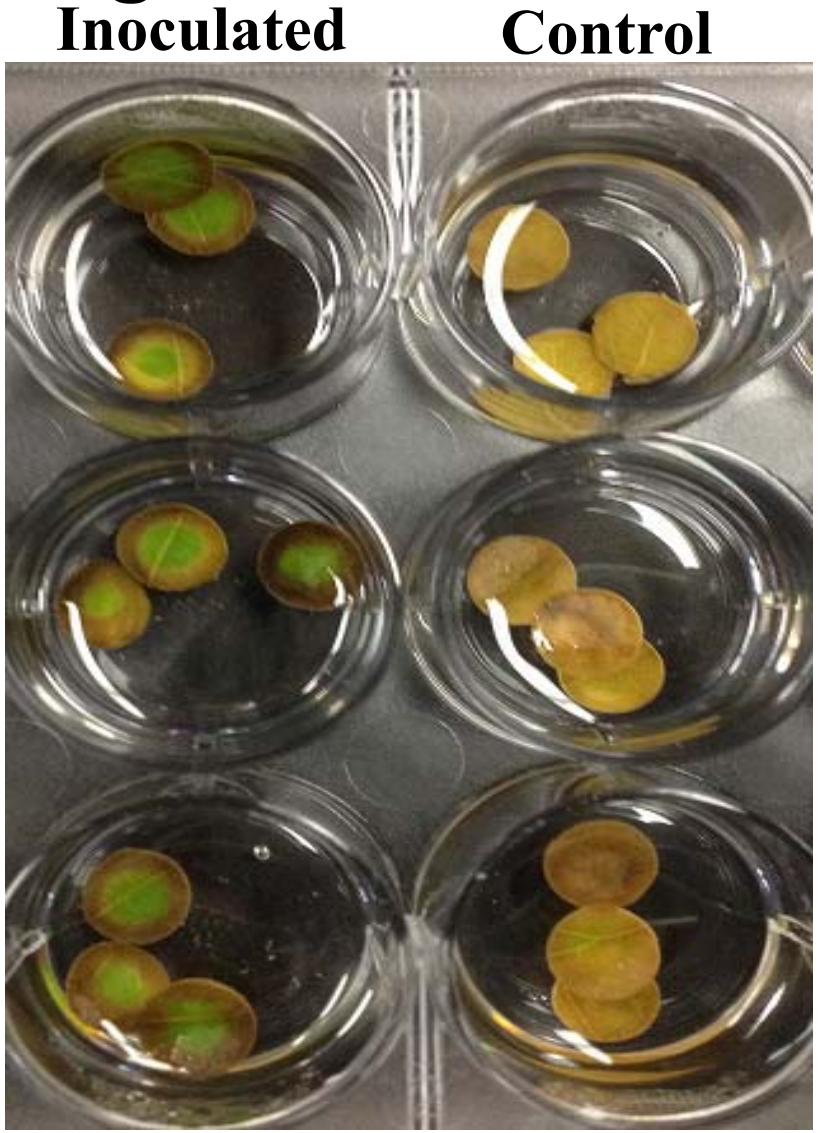


Khan, Z., Rho, H., Firrincieli, A., Hung, S.H., Luna, V., Masciarelli, O., Kim, S.H., and Doty, S.L. 2016. *Current Plant Biology* 6:38-47

Advanced **Hardwood Biofuels** Northwest

Several possible mechanisms for the endophyte-conferred drought tolerance

- Phytohormone production
  - IAA, ABA,
- Increased nutrients
- Reduction of ROS
- Genetic evidence for:
  - Osmolyte trehalose
  - VOCs Acetoin and 2,3-butanediol



Khan, Z., Rho, H., Firrincieli, A., Hung, S.H., Luna, V., Masciarelli, O., Kim, S.H., and Doty, S.L. 2016. *Current Plant Biology* 6:38-47.

Endophyte consortium increased water use efficiency (WUE) in rice

Tony Rho

- Unpublished data was removed from this file

Hyungmin Rho, et al (in review)

The Salicaceae endophytes improved drought tolerance of perennial rye grass

Under drought conditions, inoculated perennial rye grass had 60% more root biomass and 48% more shoot biomass

Khan, Z, Guelich, G., Phan, H., Redman, R., and Doty, S. L. 2012. *ISRN Agronomy*

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Reduced mortality of conifers under drought conditions

Matthew Aghai

- ***Western redcedar*** *(Thuja plicata)*
- After two seasons of simulating seasonal moisture fluctuations
- No mortality in wet treatment conditions
- 50% mortality reduction in normal conditions
- 90% mortality reduction in drought conditions

- Unpublished data was removed from this file

Matthew Aghai, Zareen Khan, Sharon Doty, and Gregory Ettl (unpublished)

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Drought tolerance in rice

Shruti Parikh

- Unpublished data was removed from this file

Harnessing the Power of Endophytes to Fight Fungal Pathogens of Crop Plants

Pierre Joubert

Pathogen resistance
Anti-microbial compounds

Growth Promotion
Hormones

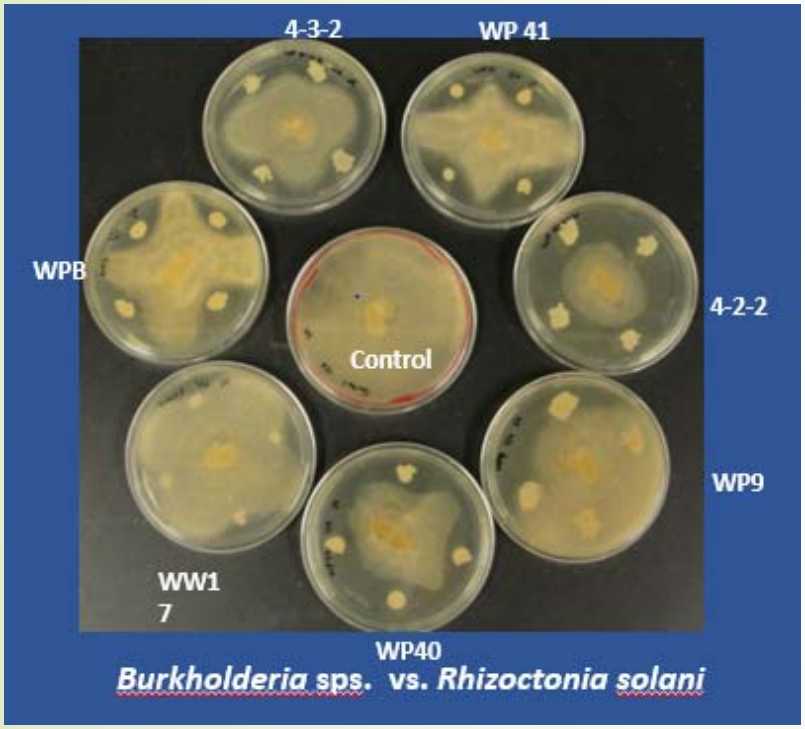
Nutrients (N, P, Fe)

Stress tolerance
Drought
Temperature
Salt

Reduced phytotoxicity of pollutants
Organic pollutants
Inorganic pollutants

An *In vitro* Study of Bio-Control and Plant Growth Promotion Potential of Salicaceae Endophytes

Shyam Kandel



- Dual plate inhibition assays
- Volatile inhibition assays
- *Burkholderia* species had especially strong activity

Burkholderia vietnamiensis WPB

Kandel et al., 2017

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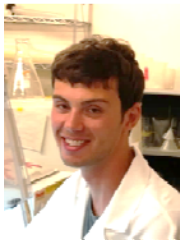
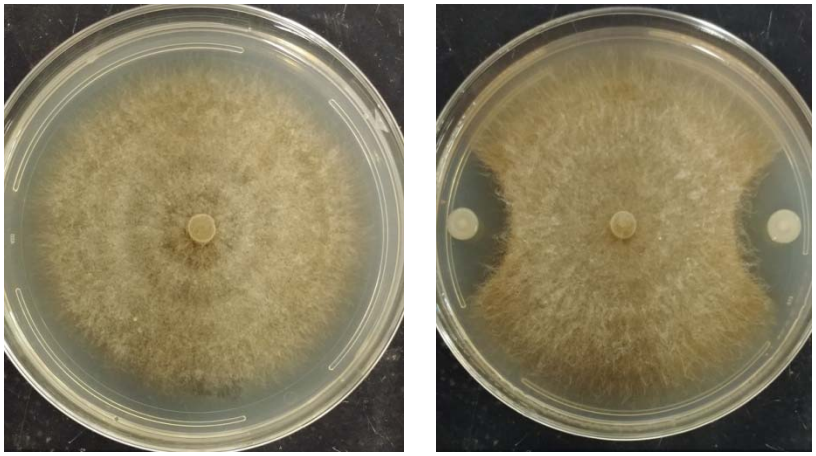
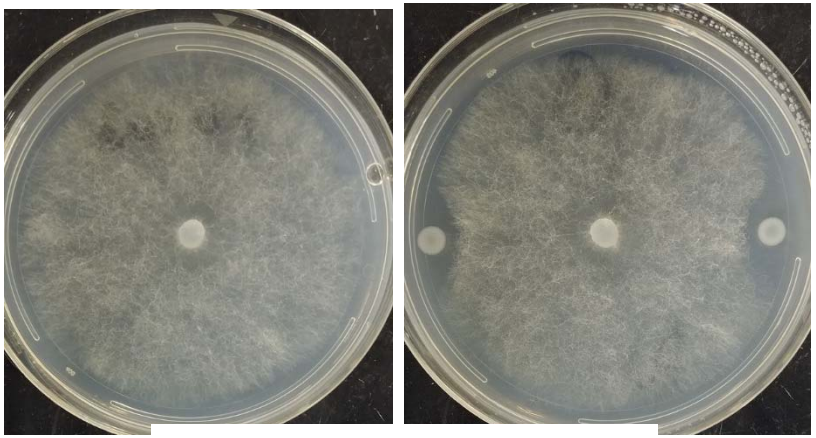
Bio-Control of Several Agriculturally-Important Pathogens



Pierre Joubert

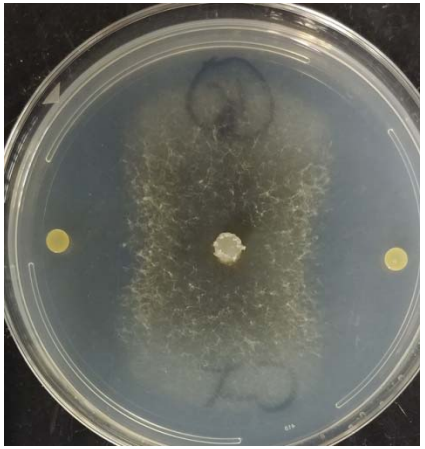
***Rhizoctonia solani* AG-8**

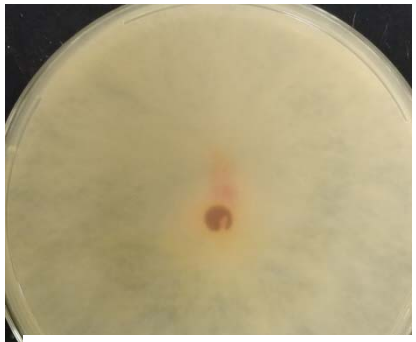




Pythium ultimum

***Gaeumannomyces graminis* var. tritici (GGT)**

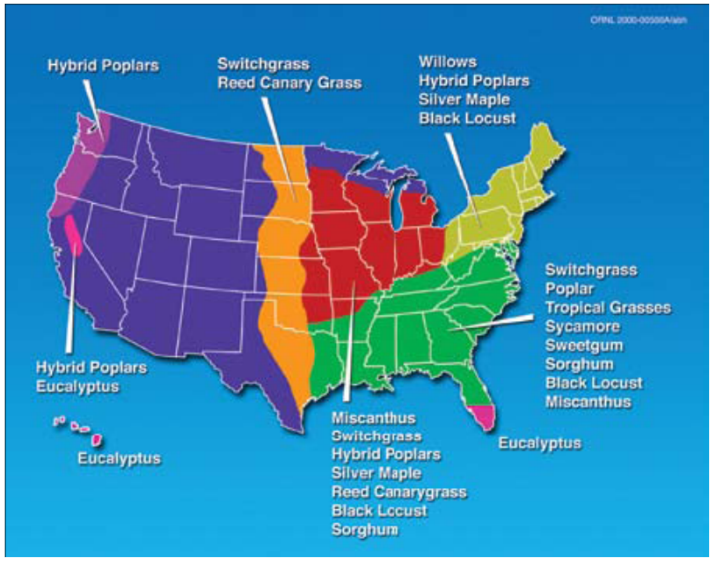




Fusarium culmorum

Implications for this research:

Endophytes For Sustainable Agriculture, Forestry, and Biomass Production



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Samples of press in response to our research

MICROBIOLOGY

Leaf bacteria fertilize trees, researchers claim

Free-living nitrogen fixers defy textbooks and could boost crop production

Elizabeth Pennisi 2015 *Sciencemag.org* 348:6237

Probiotics - Good for Plants, Soil

Endophytic nitrogen-fixation applicable to most crop plants

Joe Funk, editor *Seed Today* 3rd Quarter 2015

American Society of Agronomy

Home

Probiotics - for plants

July 08, 2015

By Kaine Korzekwa

MNN.com > Home > Organic Farming & Gardening

Do plants need probiotics too?

Good bacteria could be a positive alternative to chemical fertilizers for food crops.

ENVIRONMENT | NEWS RELEASES | RESEARCH | SCIENCE

September 19, 2016

Microbes help plants survive in severe drought

Implications for this Research: Climate Change Mitigation

- Plants can increase C sequestration only if nutrients and water are not limiting
- Using more chemical fertilizers is inconsistent with mitigation

Science Panel Calls Global Warming 'Unequivocal'

Dan Crosbie/Canadian Ice Service

Polar bears on chunks of glacial ice in the Bering Sea in 2004. Much higher temperatures are forecast for the Arctic, climate scientists say.

Endophytes can promote plant growth naturally through improved nutrient acquisition, phytohormone production, & stress tolerance

Funding and technology provided by:

NSF Energy for Sustainability program

AFRI bioenergy program and NIFA climate change mitigation programs

McIntire-Stennis program

Byron and Alice Lockwood Foundation Professorship

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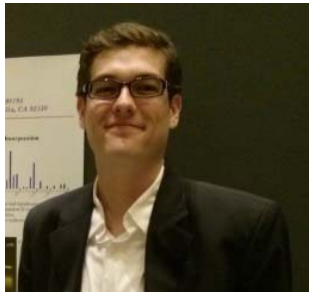
Students and staff involved in the presented research



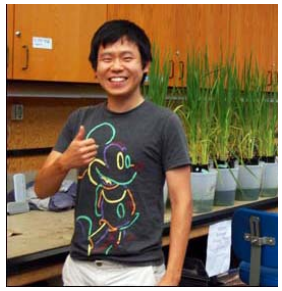
Andrew Sher

Mahsa Khorasani

Shyam Kandel

Pierre Joubert

Jenny Knoth



Tony Rho

Zareen Khan

Matthew Aghai

Andrea Firrincieli

Also the undergraduate researchers especially Grant Guelich, Beverly Hung, Daniela Ramos, Jack Emery, Nick Wegley, and Shruti Parikh

Special thanks to Co-PI's Assoc. Prof. Soo-Hyung Kim and Greg Ettl

Intrinsyx
An Engineering & Information Technology Company

Thanks to Intrinsyx for moving our research to the field

Doty Lab Website:
http://depts.washington.edu/envaplab

Thanks to **Illumina** for filming us! Adventures in Genomics video, "***Power of Plant Partnerships***"