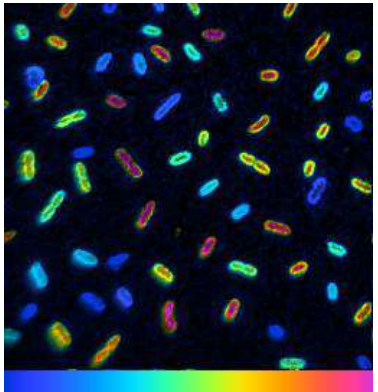
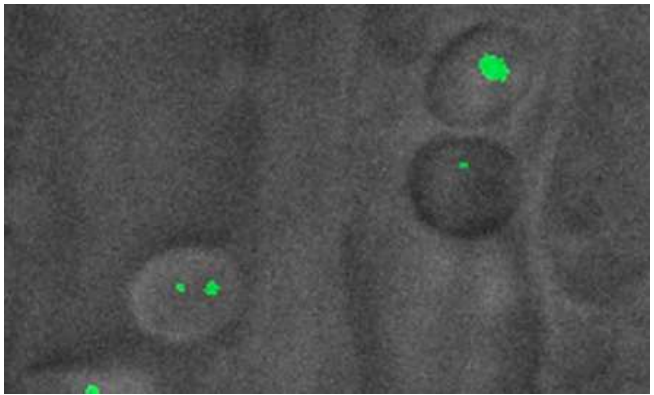
UNIVERSITY of WASHINGTON

UW Botanical Symposium March 5, 2025

Harnessing the Power of the Wild Plant Microbiome

Prof. Sharon L. Doty
University of Washington, Seattle, USA


The Plant Microbiome

Benefits from “endophytes”, the microbial communities within a plant

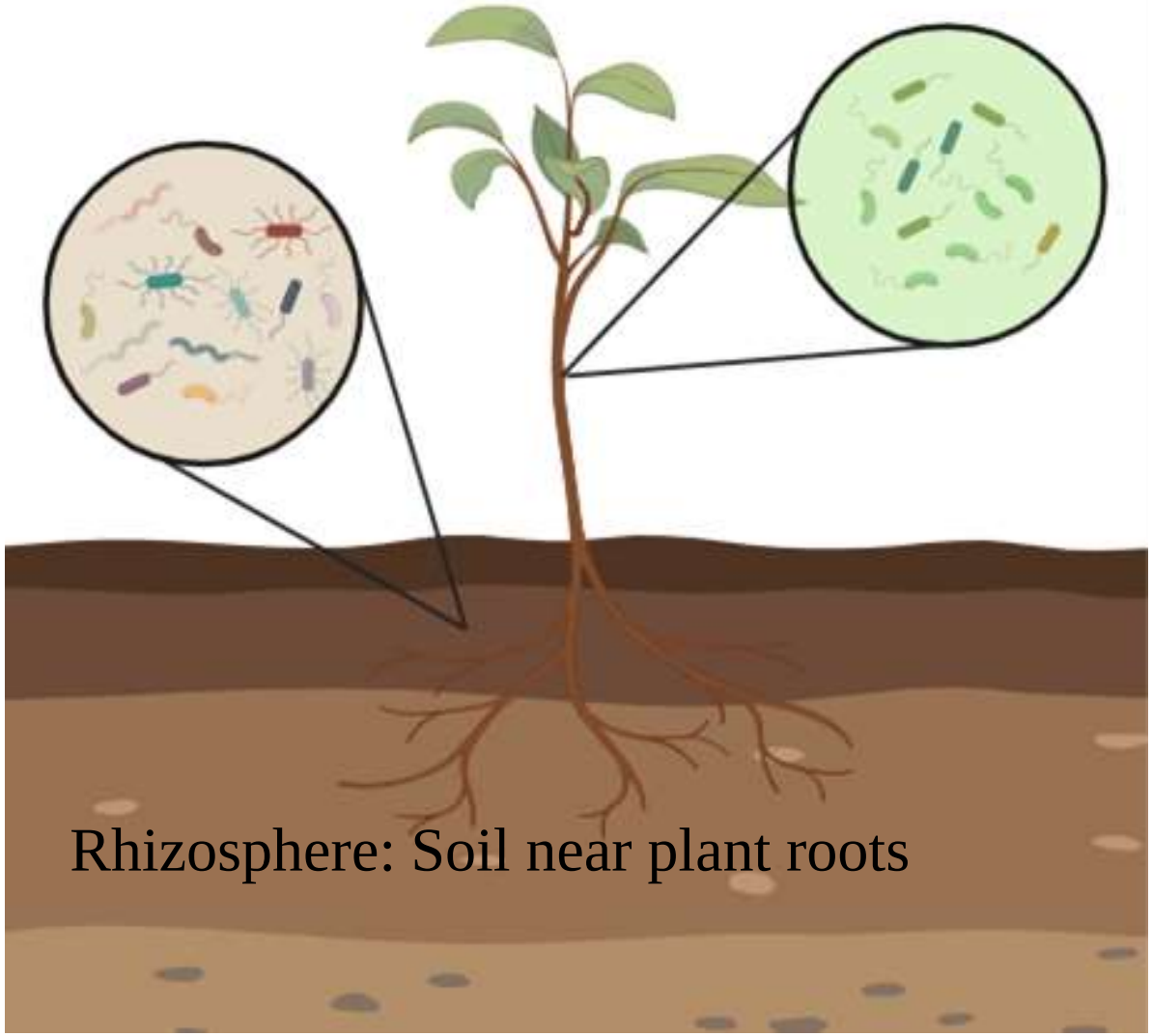
Pathogen resistance
Anti-microbial compounds

Growth Promotion
Nutrients (N, P, Fe)
Hormones

Stress tolerance
Drought
Temperature
Salinity

Reduced phytotoxicity of pollutants
Organic pollutants
Inorganic pollutants

ENDOPHYTES are within plants



Direct **interactions** with plant tissue

Selected for by the host plant

Can be **stable members of the plant microbiome**

Frank et al. 2017
Image credit: BioRender

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in vitro Bio-Control Activity against Agriculturally-Important Pathogens

Dual plate inhibition assay

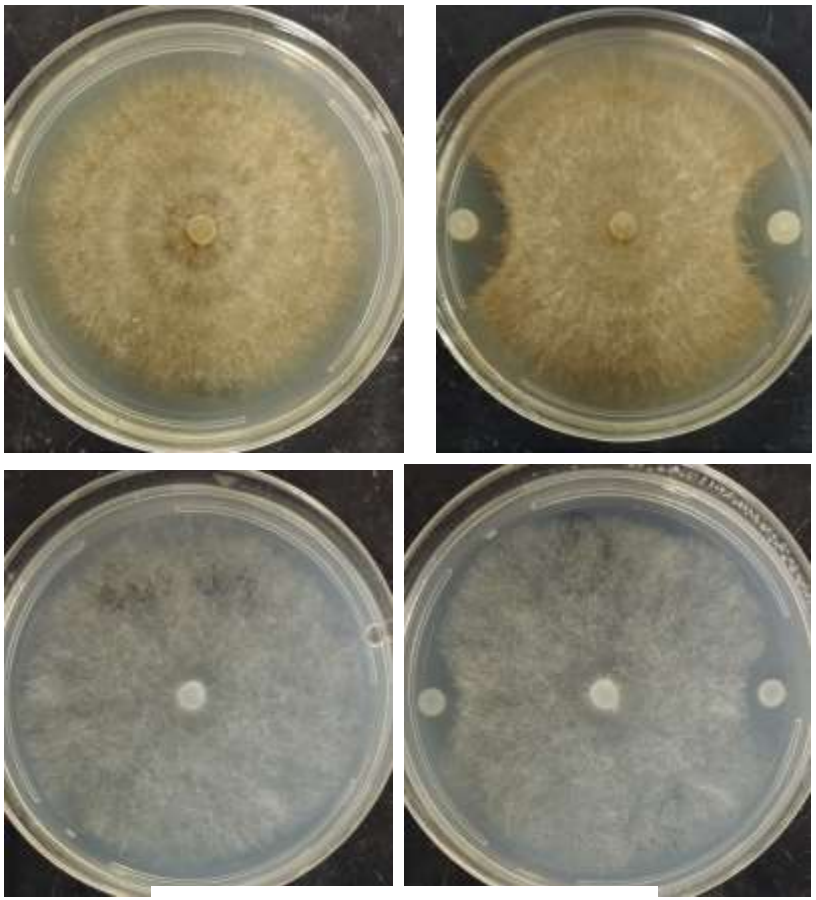
Inhibiting bacteria

Photo credit: Andrew W. Sher

Bio-Control of Several Agriculturally-Important Pathogens

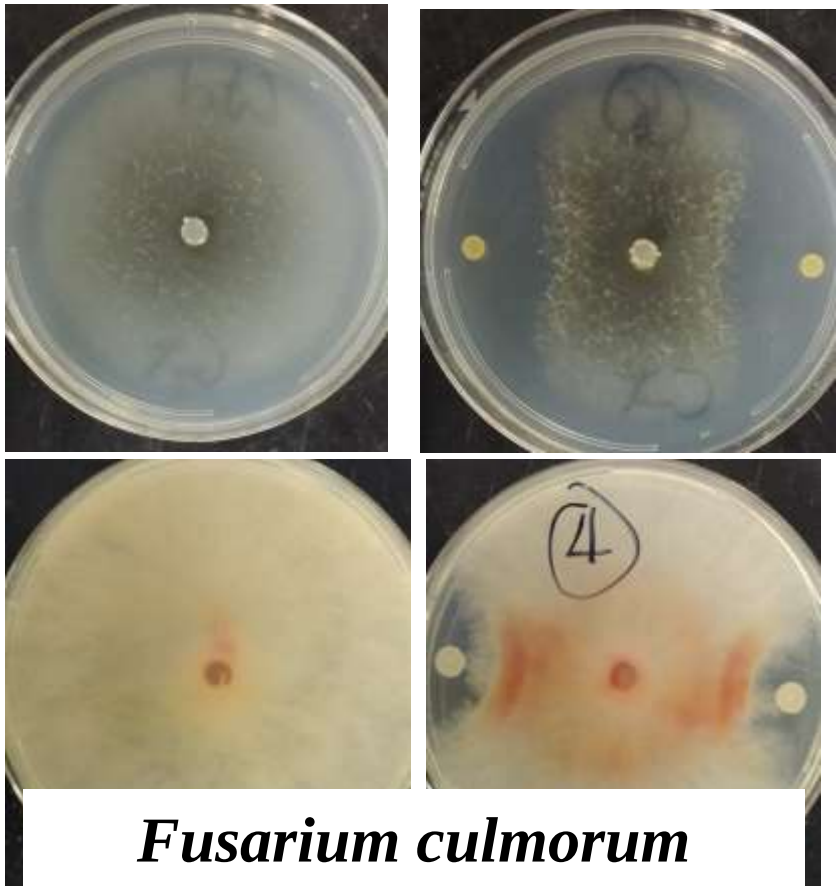
Shyam Kandel

***Rhizoctonia solani* AG-8**



Pythium ultimum

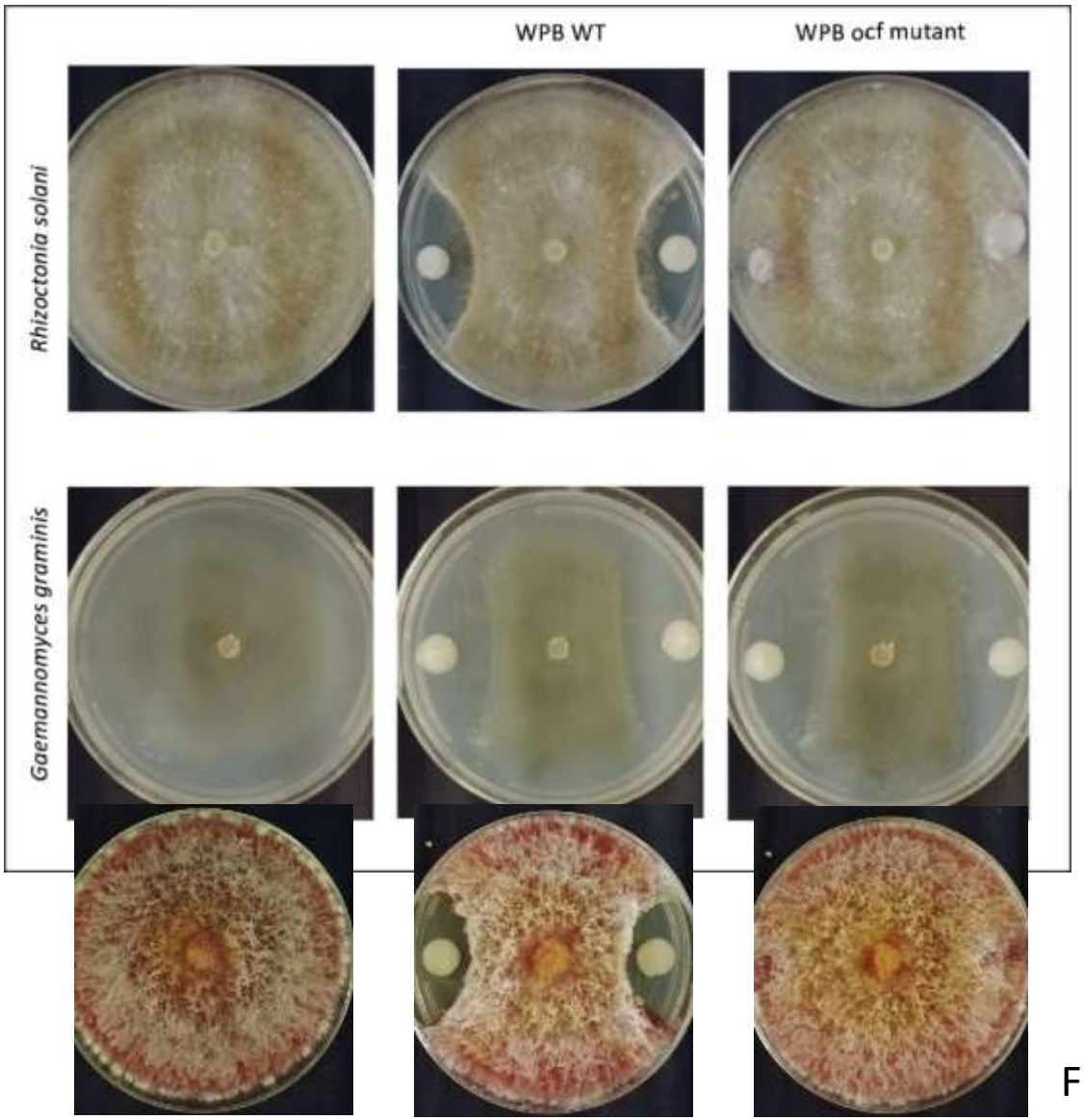
***Gaeumannomyces graminis* var. tritici (GGT)**



Fusarium culmorum

Kandel, S.L., Firrincieli, A., Joubert, P.M., Okubara, P.A., Leston, N., McGeorge, K., Mugnozza, G.S., Harfouche, A., Kim, S.H., and Doty, S.L. 2017. *Frontiers in Microbiology* Vol 8, #386

Genomic and mutant analyses to identify mechanisms



Pierre Joubert

Anti-fungal glycolipopeptide occidiofungin (*ofc)* mutant had no anti-fungal activity against *Rhizoctonia* and *Fusarium* but was still active against GGT, indicating a different mechanism is used against that pathogen

Fusarium culmorum

Doty, S. L., Joubert, P. M., Firrincieli, A., Sher, A. W., Tournay, R., Kill, C., Parikh, S. S. and Okubara, P. Potential biocontrol activities of *Populus* endophytes against several plant pathogens using different inhibitory mechanisms. 2023. *Pathogens* 12(1), 13

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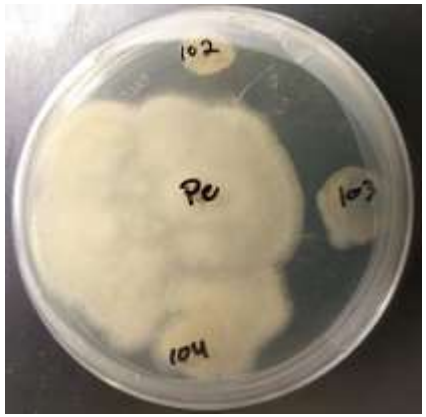
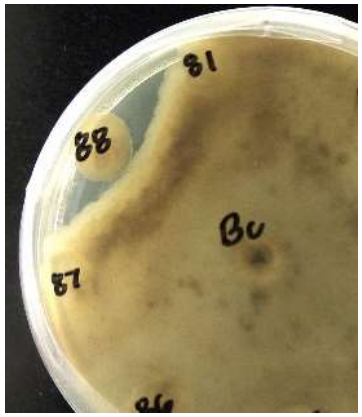
Current Research: Development of new biocontrol strains from Washington native plants against apple tree pathogens

WASHINGTON TREE FRUIT RESEARCH COMMISSION

Fungal pathogens: *Penicillium expansum* (blue mold), *Phacidiopycnis washingtonensis* (speck rot), and *Botrytis cinerea* (grey mold), *Neofabraea perennans* (bull's eye rot)
Bacterial pathogen: *Erwinia amylovora* (causes fire blight)





WASHINGTON TREE FRUIT RESEARCH COMMISSION

Approach

- Source endophytes from where they could have co-evolved with the pathogen to protect the plant host (Wenatchee, Entiat, Yakima, and Methow areas)
- Screened for inhibitory activity → 14 strains inhibited blue mold, 27 against grey mold, 21 against bullseye rot, 38 against speck rot, and 40 against *Erwinia amylovora*
- Bioinformatics for safety and potential mechanisms

Andrew Sher

Dr. Robert Tournay

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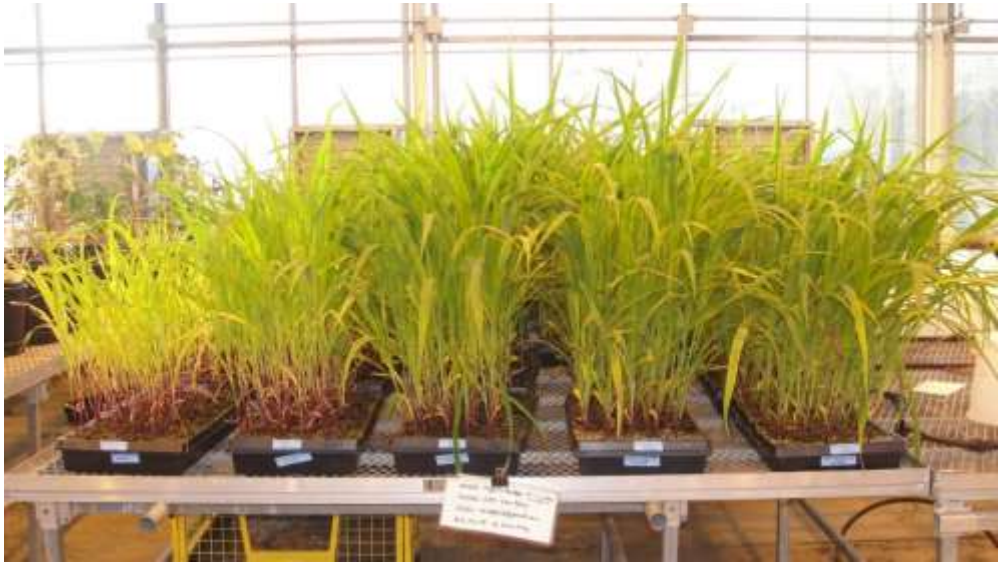
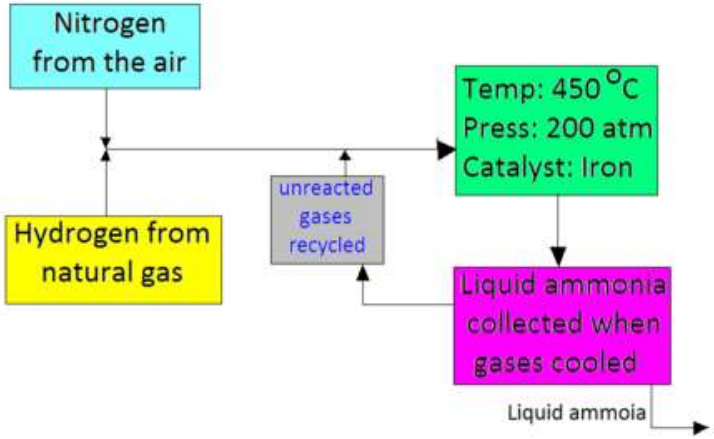
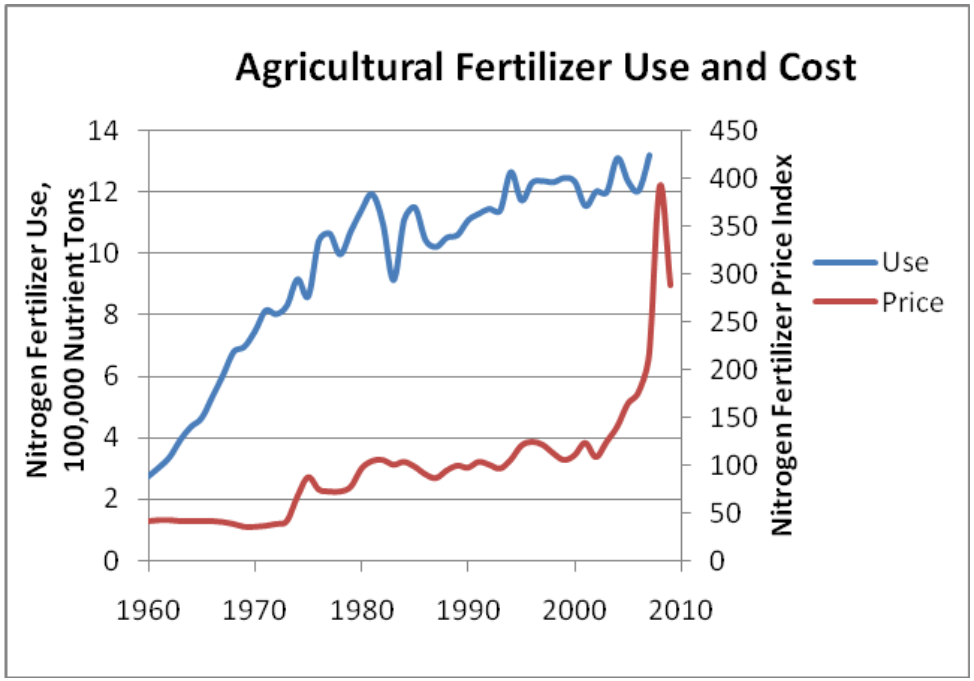
Fixed nitrogen is a limiting nutrient for plant growth



Chemical Fertilizers





Cost of fertilizer is tied to the cost of fossil fuels

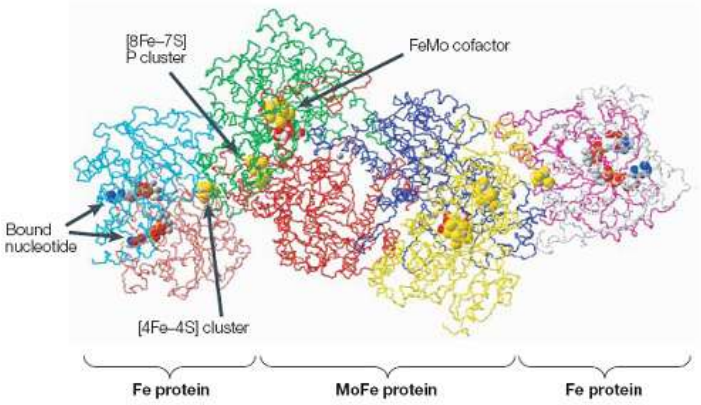
- Fluctuates considerably
- Also consider “environmental cost”

Biological Nitrogen Fixation

- Air is about 80% N_2 gas but inert
- Some bacteria can "fix" it into usable forms

Nitrogenase

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



$$$
Reaction!

Specific plant-bacterial symbioses for biological nitrogen fixation within root nodules

Legumes with rhizobia
Actinorhizal plants with Frankia

Rhizobium in legume root nodules such as on vetch roots

Alder root nodule cluster

Some bacterial endophytes are also able to fix nitrogen!

- *Gluconacetobacter diazotrophicus, Herbaspirillum*
- Fix nitrogen and produce phytohormones in Brazilian sugarcane
- 1990's- Johanna Dobereiner of Brazil opened up this new field of research

Photo credit: Portal Embrapa https://www.embrapa.br/en/johanna-dobereiner/quem-foi

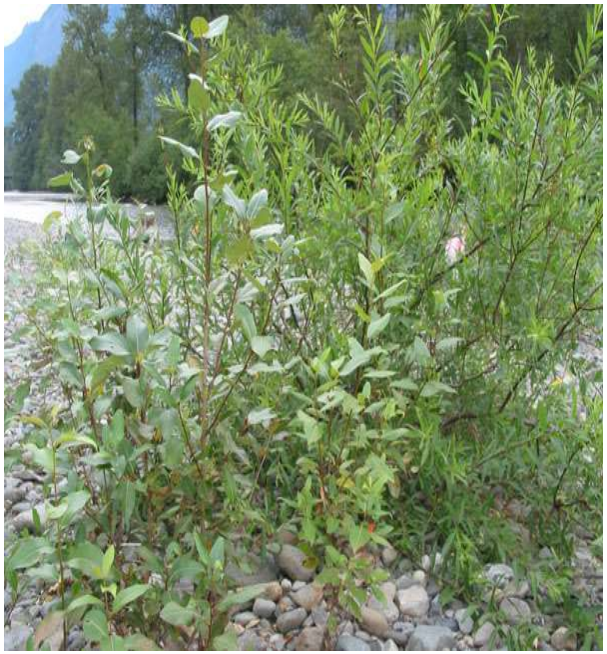
Nature-Based Solutions

Use the microbiota selected by wild plants in challenging environments

Poplar (*Populus* sp.) and willow (*Salix* sp.) are early successional, pioneer plant species able to colonize primary substrates including rocks and sand such as riparian zones and glacial retreats

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

Goal: Growth promotion with reduced inputs of nitrogen fertilizers Using nitrogen-fixing endophytes from wild, pioneer plants



Poplar (*Populus* sp.) and willow (*Salix* sp.) in their native habitat type

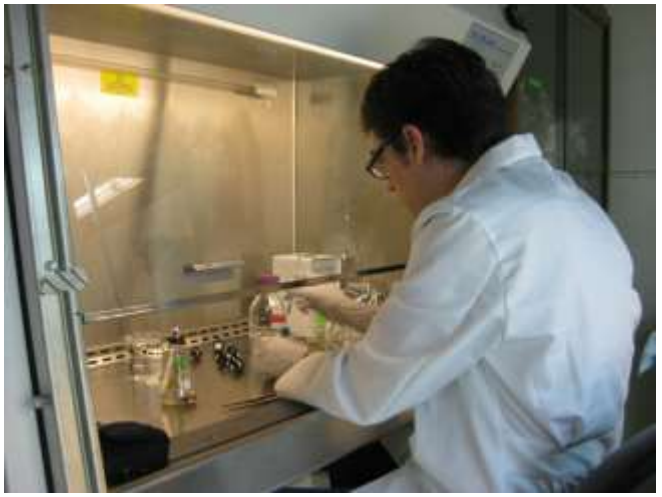
NifH sequences included *Burkholderia*, *Sphingomonas*, *Azospirillum*, *Bradyrhizobium*, *Rhodospirillum*, *Methanococcus*, *Pseudomonas*, *Rahnella*, and more

Using direct and indirect methods, our lab demonstrated N-fixation in wild poplar

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

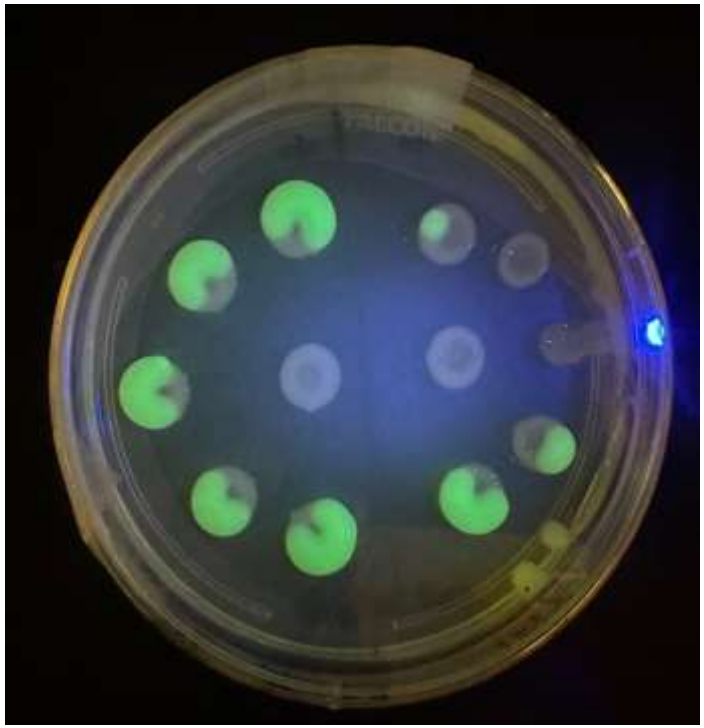
Nitrogen (N_2) fixation in wild poplar

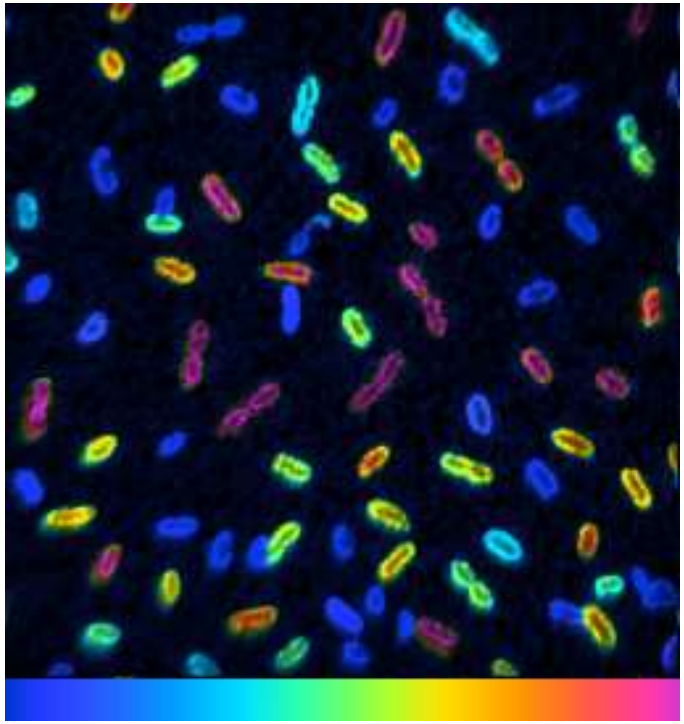
Direct assay: ^{15}N incorporation from $^{15}N_2$



Doty, S.L., et al. 2016 *PLOS ONE* 11(5):e0155979

Exploring mechanisms of nitrogen fixation by *Populus* endophytes



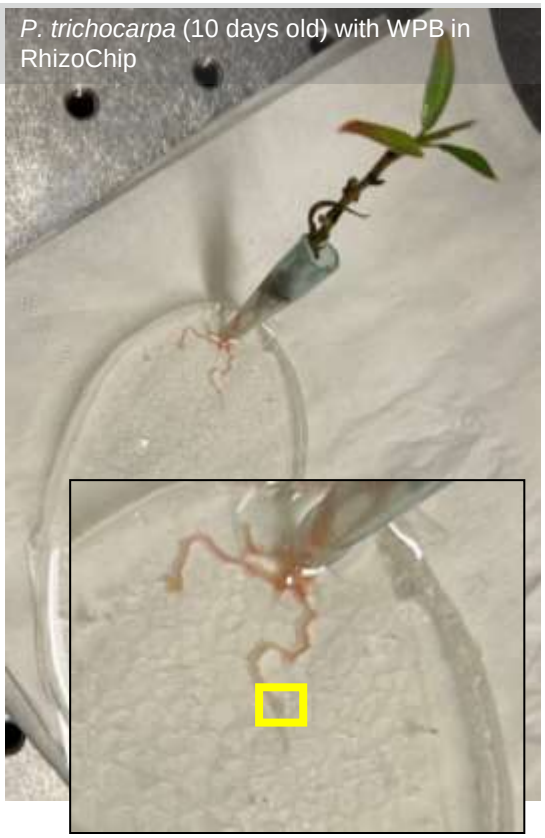


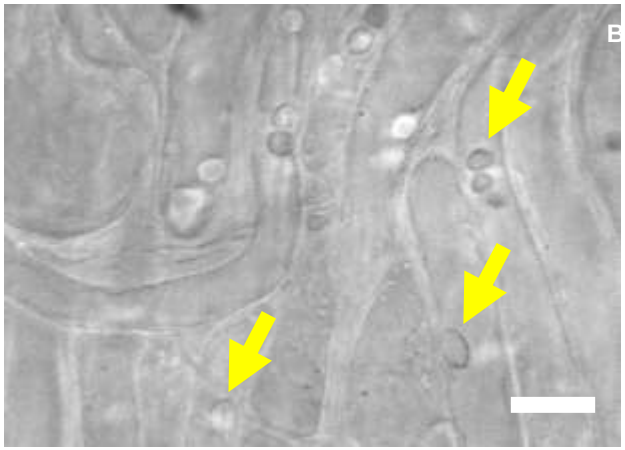
A) Strain WPB nitrogenase gene promoter fusion with GFP on agar plates
FACS analysis indicated 11% were active
B) NanoSIMS heat map of ^{15}N labelled cells

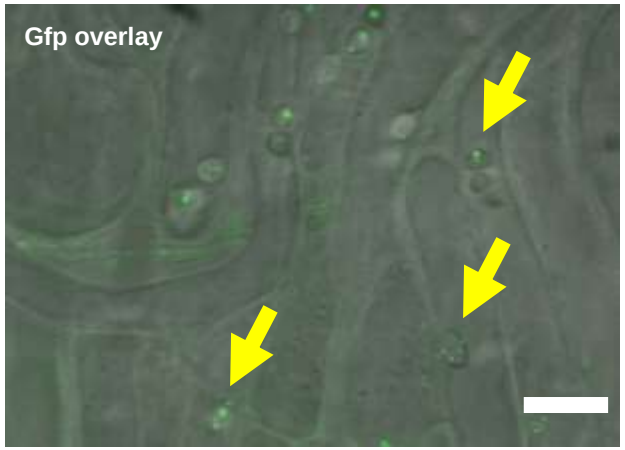
Sher, et. al. 2024. Dynamic nitrogen fixation in an aerobic endophyte of *Populus*, *The ISME Journal*, 2024, 18 (1), 1-14.

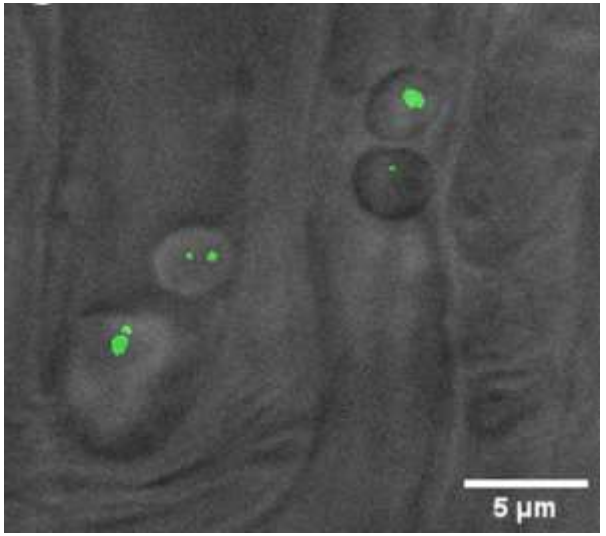
WPB expresses nitrogenase (nifH) in association with poplar plants

Dr. Jayde Aufrecht







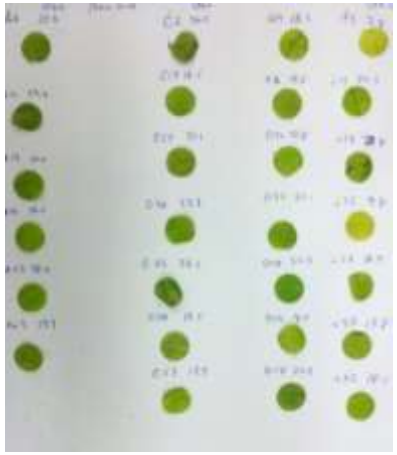


Within root epidermal cells in the elongation zone; WPB in 3-5 micron spherical structures

Sher, et al, ISME Journal, 2024, 18(1), 1-14

Endophytes **can be cultured** and **added** to cultivated poplar for improved growth with reduced nitrogen fertilizer inputs

Jenny Knoth

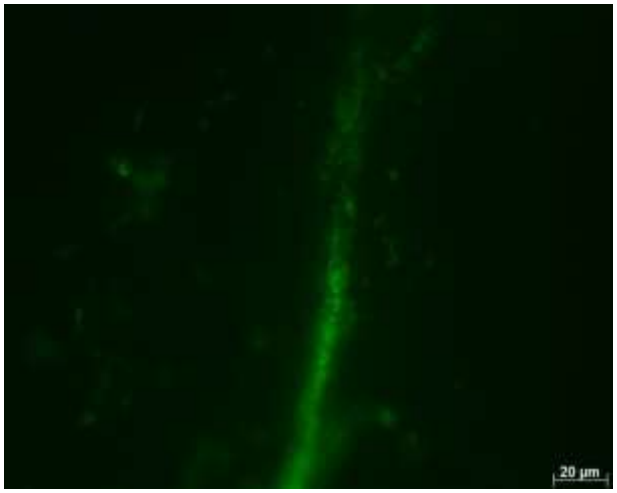


Adding wild endophytes to poplar resulted in:

1) Increased greenness (17% more chlorophyll)
2) 25% higher total nitrogen levels
3) Doubling of root mass

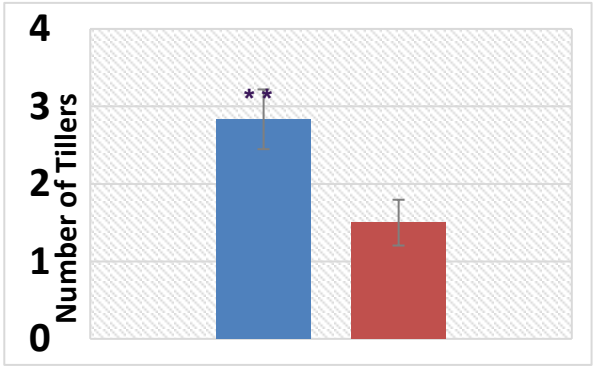
J. Knoth, et al (2014) *New Phytologist* 201:599-609

Colonization and growth enhancement of rice

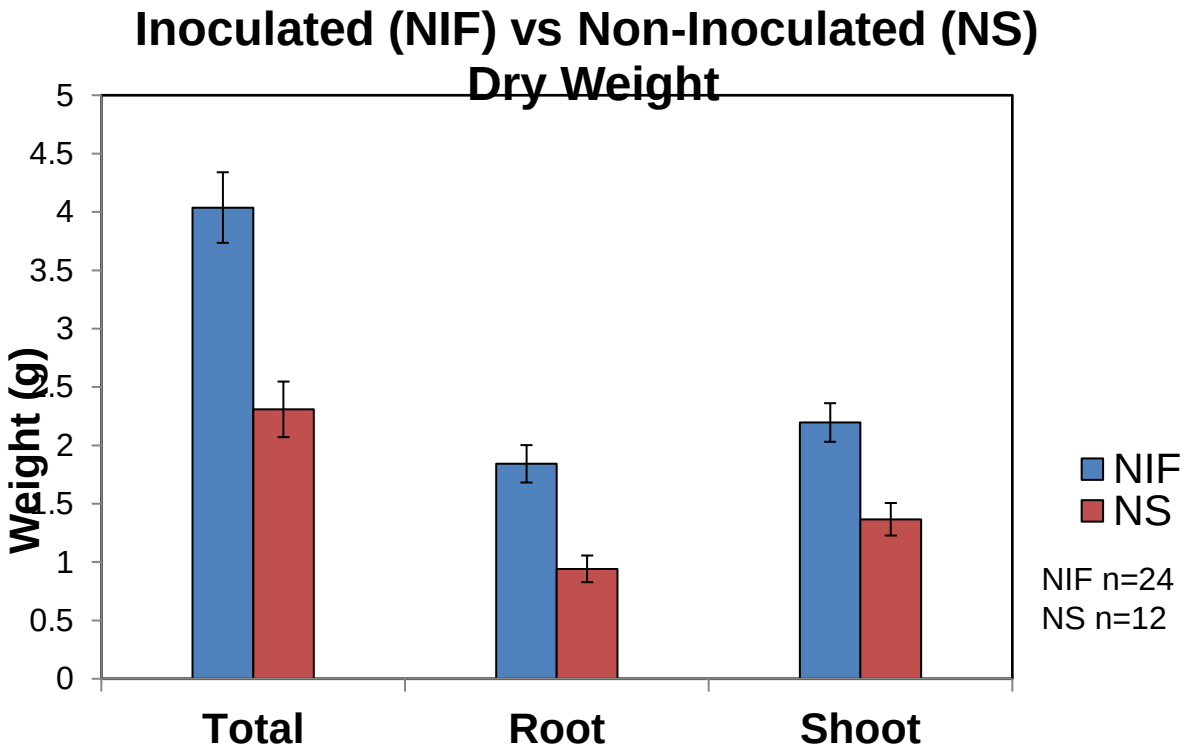


Shyam Kandel

of Tillers



(Significance * * at α = <0.01)



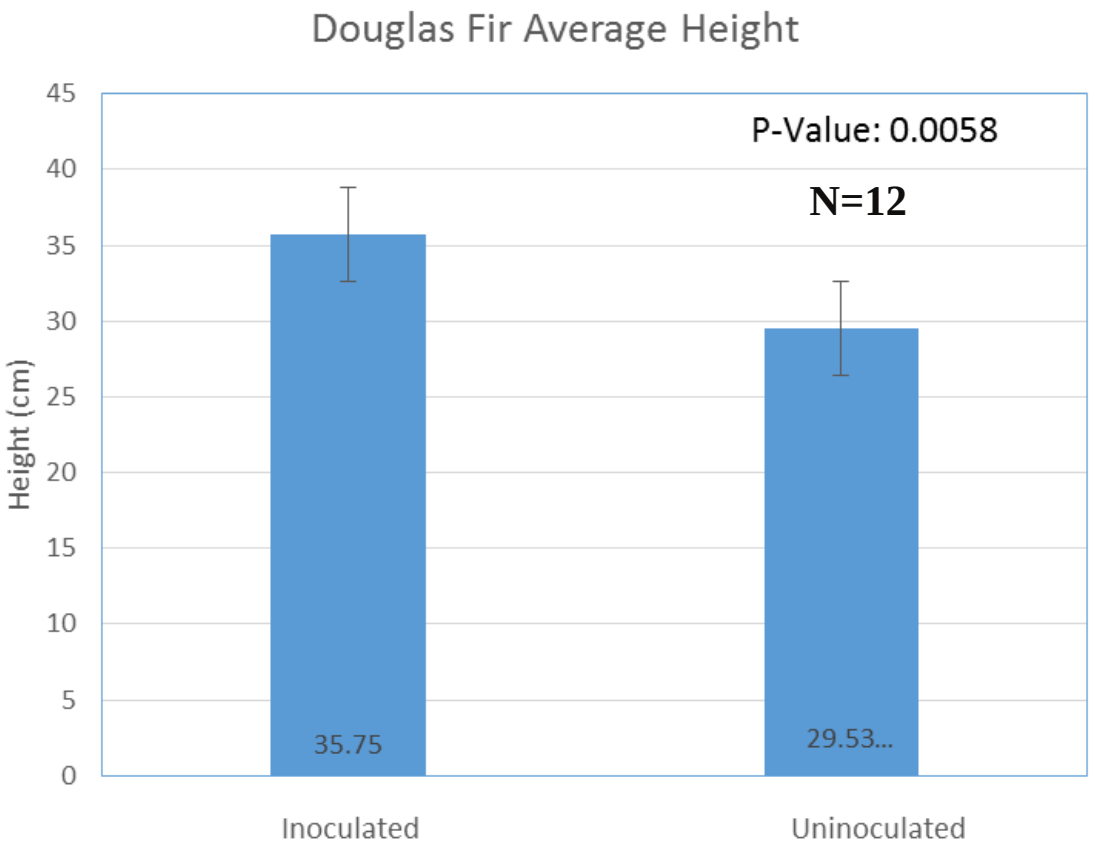
S. Kandel, N. Herschberger, S.H. Kim, and S.L. Doty (2015) *Crop Science* 55:1765-1772

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



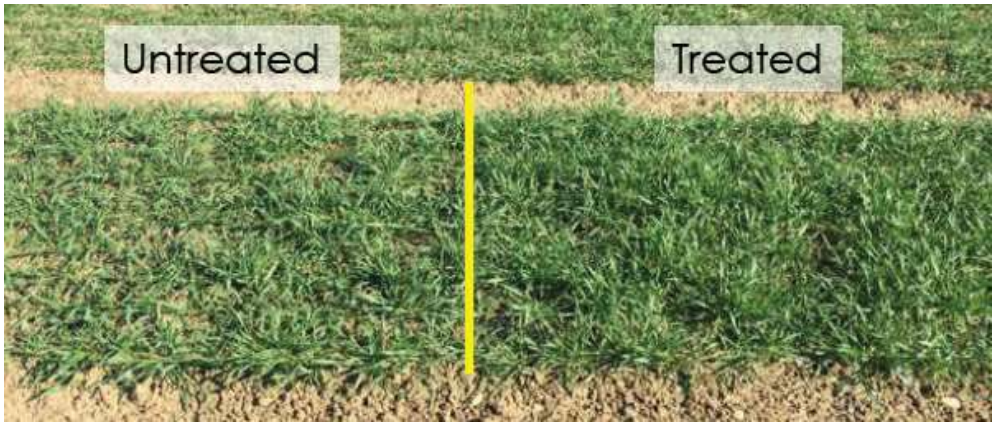
Khan, Z., Ramos, D. Ettl, G., Kim, S.H., and Doty, S.L. 2015 *Forests* 6:3582-3593

3rd party validated greenhouse and field trials consistently showed increased growth of a variety of crop plants

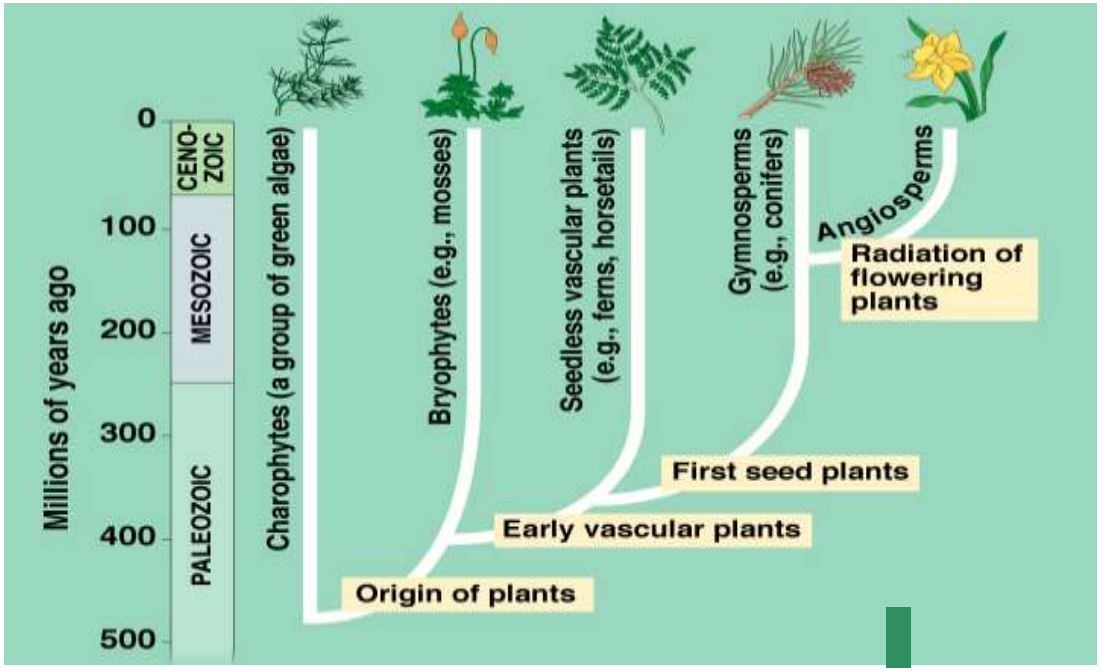
Intrinsyx Bio

The Plant Probiotics Company

Statistically significant increases in growth, final size and harvest yield (ie. height, mass, stem diameter, chlorophyll) under nitrogen and phosphorous deficient soil conditions in over a dozen types of crops such as: Corn, tomato, canola, lettuce, soybean, strawberries, broccoli, oat, barley, and wheat using both seed coats and in-furrow applications

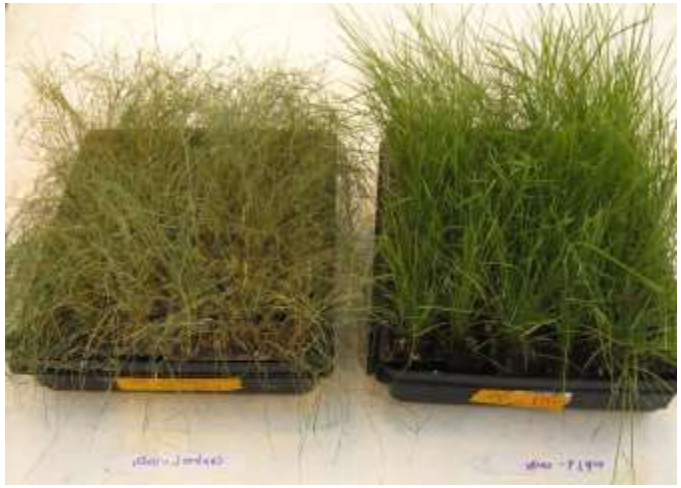


Endophytes have a Broad Host Range



No added microbes

With added endophytes



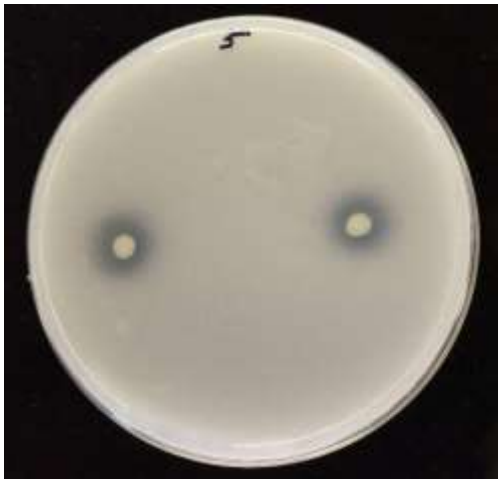
No added microbes

With added endophytes

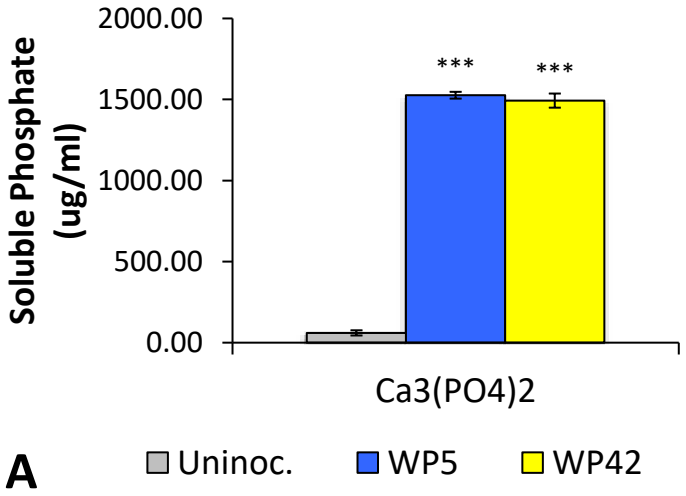
No added microbes

With added endophytes

Phosphate solubilization



Collecting plant microbiota and screening for phosphate solubilization



Varga et al. 2020 *Frontiers in Plant Science* https://doi.org/10.3389/fpls.2020.567918

Phosphate solubilization by endophytes may involve biofilm formation

Kevin Shaffman, graduate student

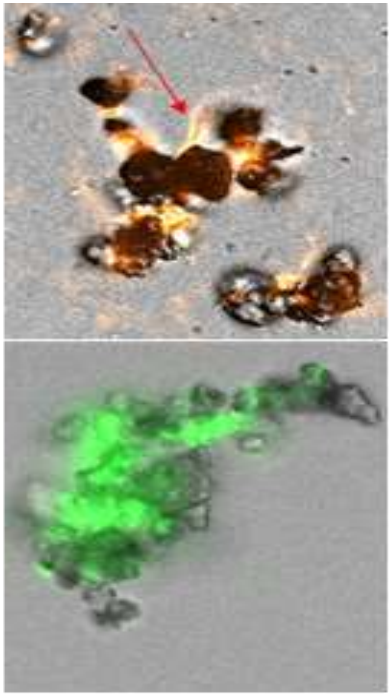


Fig 1. RFP tagged Sphingobium sp. *(left)* and GFP tagged Rhanella sp. *(right) forming biofilms on AlP particles*

Developing new chemistry protocols, collecting plant microbiota, screening for phosphate solubilization, proteomics and microscopy

A better understanding of how natural plant-microbe partnerships improve plant nutrient acquisition could lead to a reduction in our dependency on chemical fertilizers in agriculture, forestry, and bioenergy 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

UW licensed strains to IntrinsyxBio that is now using the strains in collaboration with other companies including Syngenta

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Organic pollutants
Inorganic pollutants

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

Without added microbes

With the added microbes

With added endophytes

Doty, SL, et al., unpublished

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

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Phytoremediation

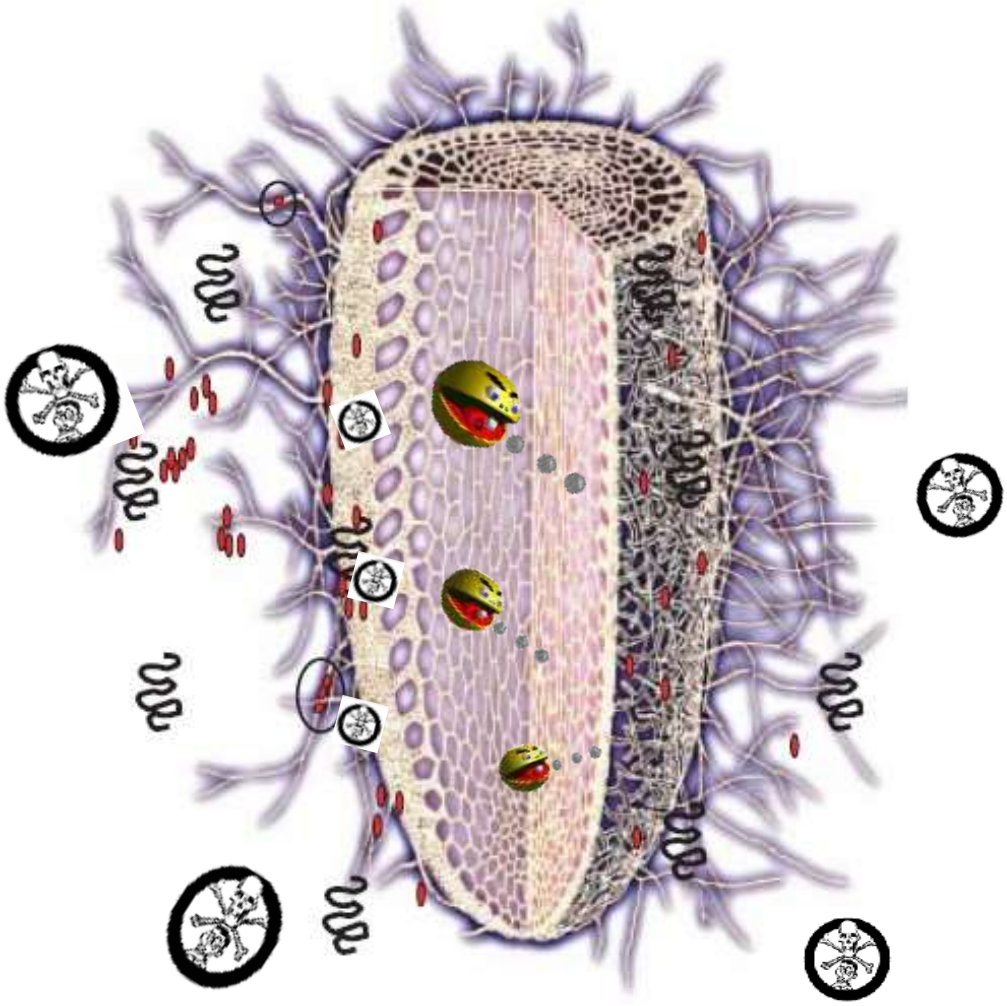
Using plants to remove environmental pollutants is effective unless pollutant is at a phytotoxic level

Environmental Forestry Consultants

EdenSpace

EcoloTree

Endophyte-Assisted Phytoremediation

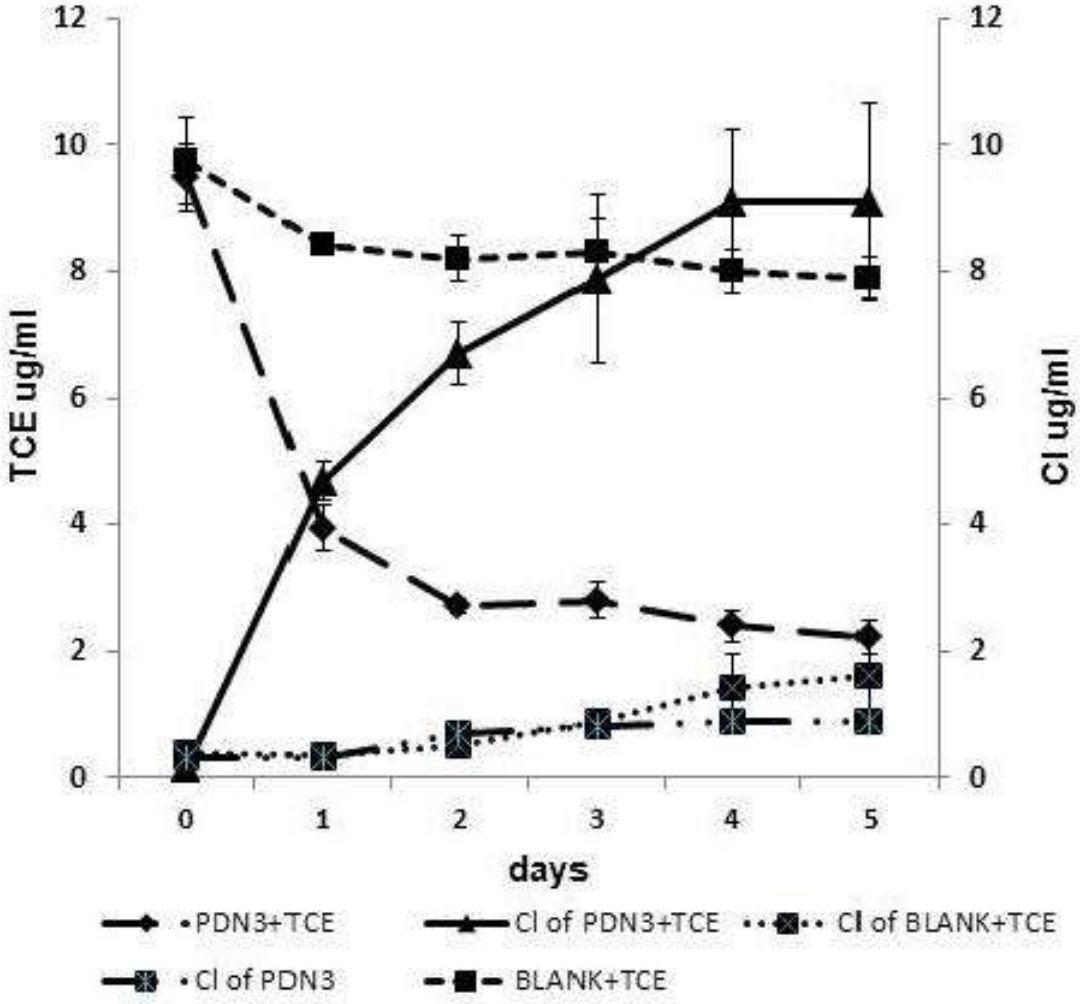


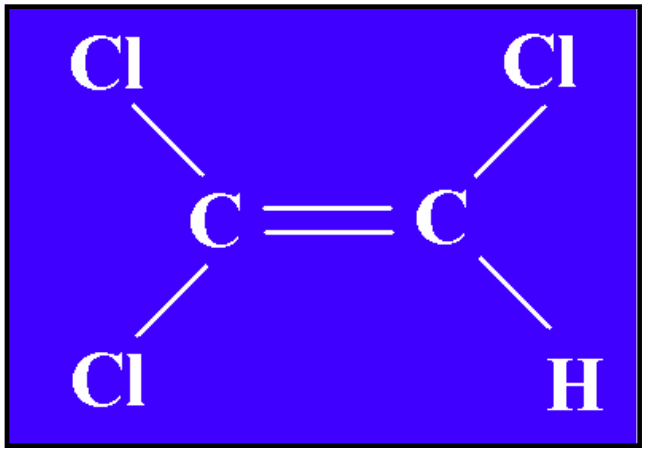
Combine the strengths of phytoremediation with the genetic adaptability and other beneficial traits of microbes

Slide credit: Jun Won Kang

Poplar endophyte *Enterobacter sp.* strain PDN3 degrades TCE, releasing chloride ion

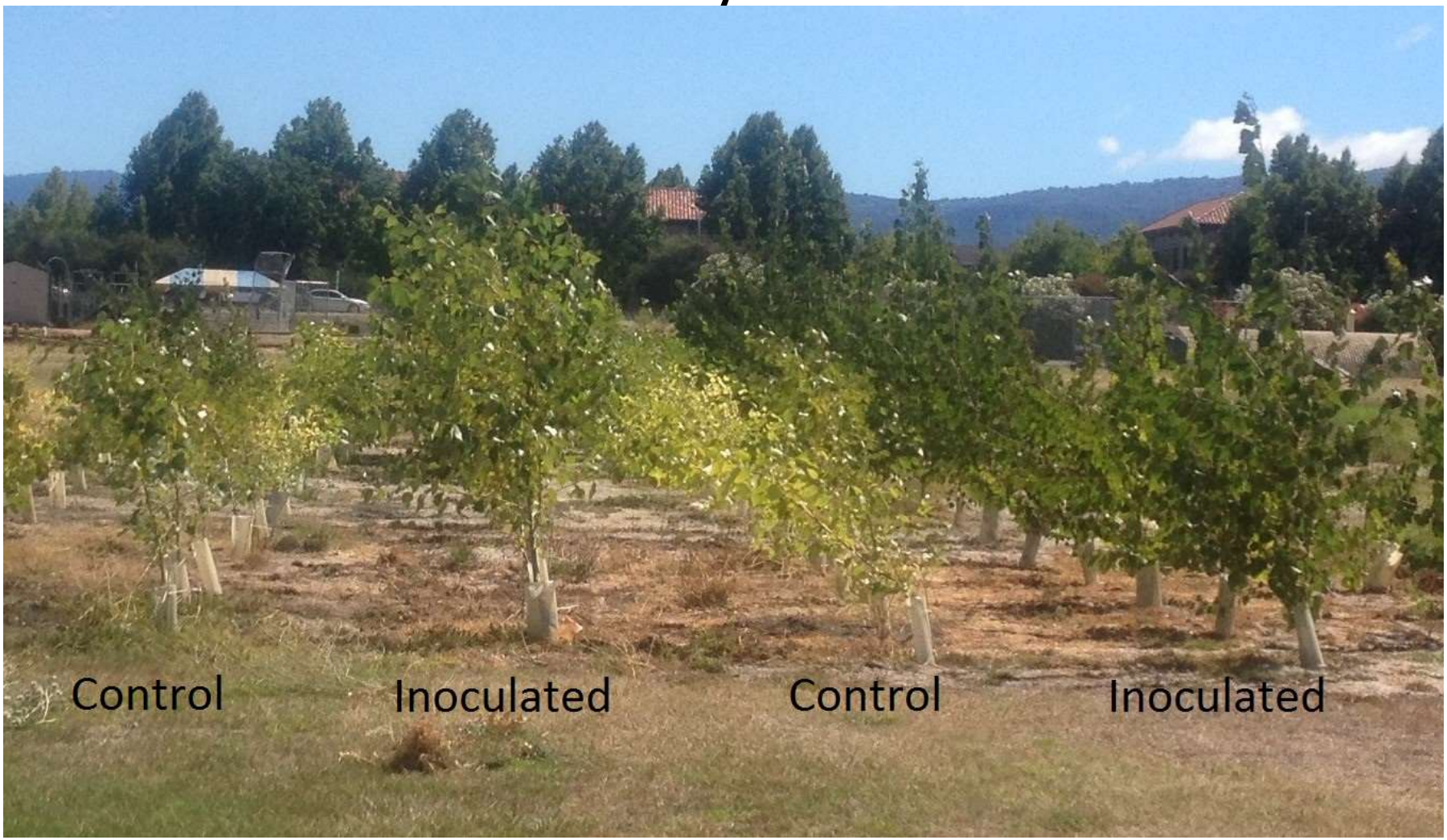
Jun Won Kang





Kang, et al. 2012 *Appl & Environ. Microbiology* 78(9)3504-3507

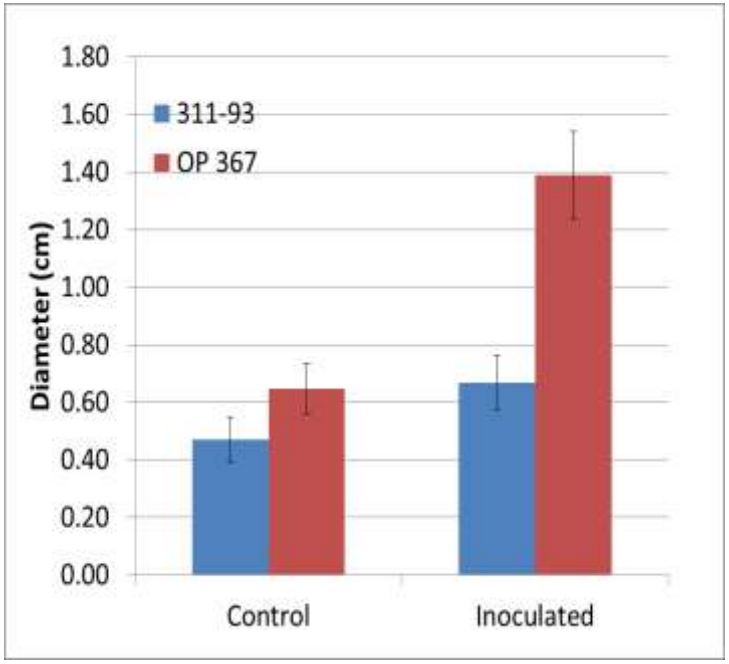
Field Test of Endophyte-Assisted Phytoremediation of TCE



Doty, et al. 2017 *Environ. Sci. Technol.51* (17): 10050–10058

Inoculated trees grew better

Trunk diameter (ABH) in Plot 1 after two years



Controls

With TCE-degrading endophyte

Inoculated poplar had less TCE in tissues, indicating enhanced TCE degradation

CONTROL	INOCULATED
133.9 ± 182.1	0.1 ± 0.1

TCE and PCE in tree cores collected from Plot 1 after two years growth. Values are means ± 1 standard deviation.

Doty, et al. 2017 *Environ. Sci. Technol.51* (17): 10050–10058

Photo credit: Dr. John Freeman, IntrinsyxEnvironmental

First successful deployment of endophyte-assisted phytoremediation using a natural poplar endophyte

The New York Times | https://nyti.ms/2URzjRX

Superfund, Meet Super Plants

Can the plant microbiome help clean up contaminated land?

https://www.nytimes.com/2020/04/07/science/superfund-plant-microbiome.html

https://www.scientificamerican.com/article/treating-toxins-with-tree-microbes/

ENVIRONMENT

Trees with a probiotic boost clean up a carcinogen

Symbiotic bacteria help poplars strip trichloroethylene from groundwater

by **Deirdre Lockwood**

SEPTEMBER 7, 2017

https://cen.acs.org/articles/95/web/2017/09/Trees-probiotic-boost-clean-up-a-carcinogen.html

http://www.washington.edu/news/2017/08/14/probiotics-help-poplar-trees-clean-up-toxins-in-superfund-sites/

The Plant Microbiome

Benefits from “endophytes”, the microbial communities within a plant

Pathogen resistance
Anti-microbial compounds

Growth Promotion
Nutrients (N, P, Fe)
Hormones

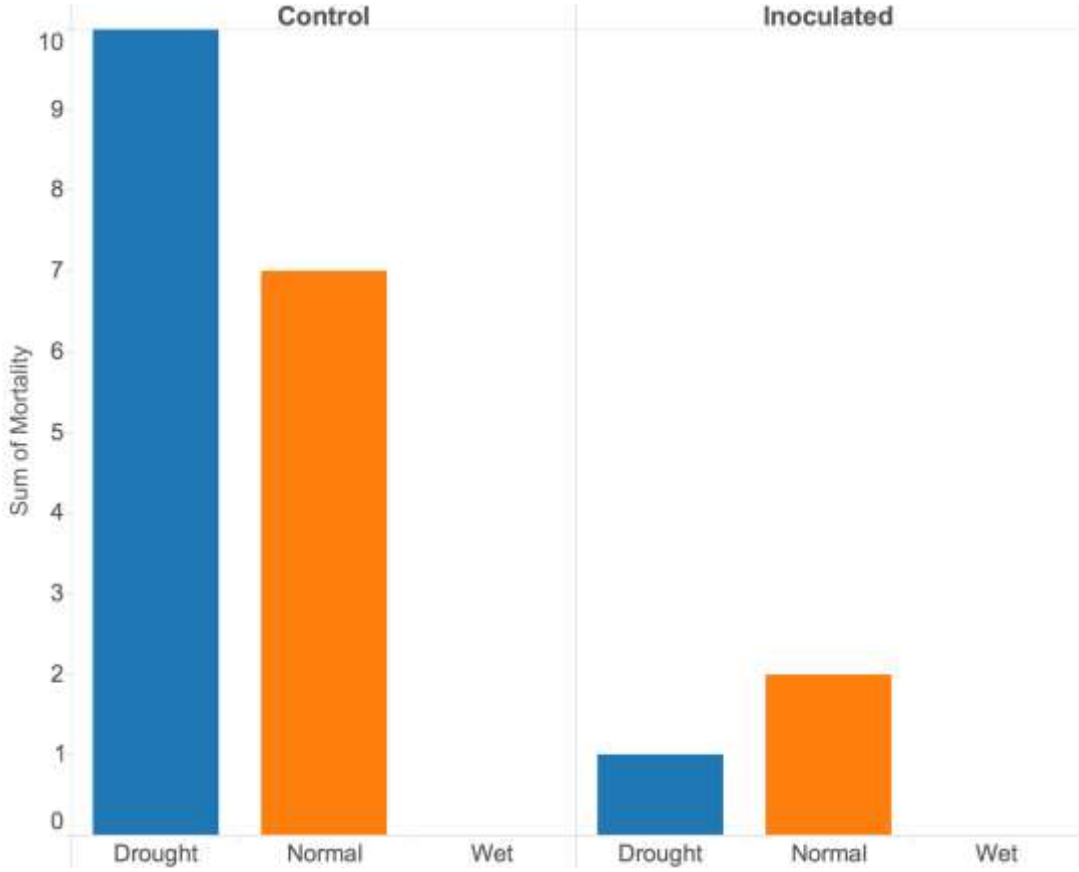
Stress tolerance
Drought
Temperature
Salinity

Reduced phytotoxicity of pollutants
Organic pollutants
Inorganic pollutants

Reduced mortality of conifers under drought conditions

Matthew Aghai

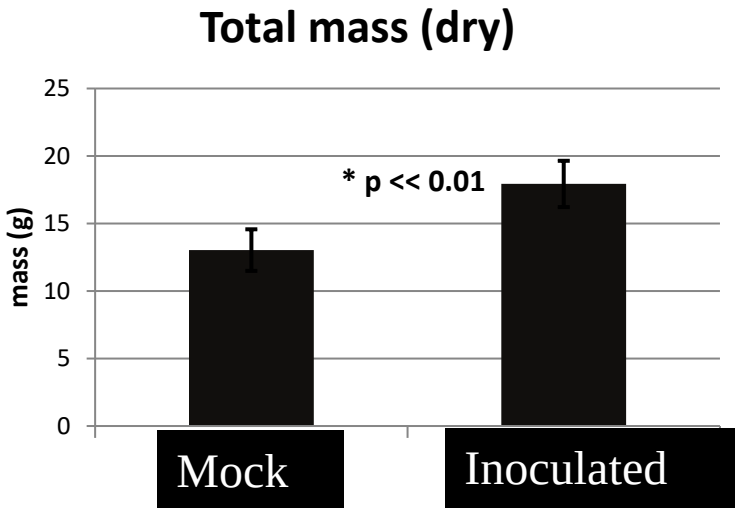
- ***Western redcedar*** *(Thuja plicata)*
- After two seasons of simulating seasonal moisture fluctuations
- No mortality in wet treatment conditions
- **50% mortality reduction in normal conditions**
- **90% mortality reduction in drought conditions**



Aghai, M. M., Khan, Z., Joseph, M. R., Stoda, A. M., Sher, A. W., Ettl, G. J., and Doty, S. L. *Front. Microbiol. 10:1353*

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

Poplar inoculated with endophytes one month without watering

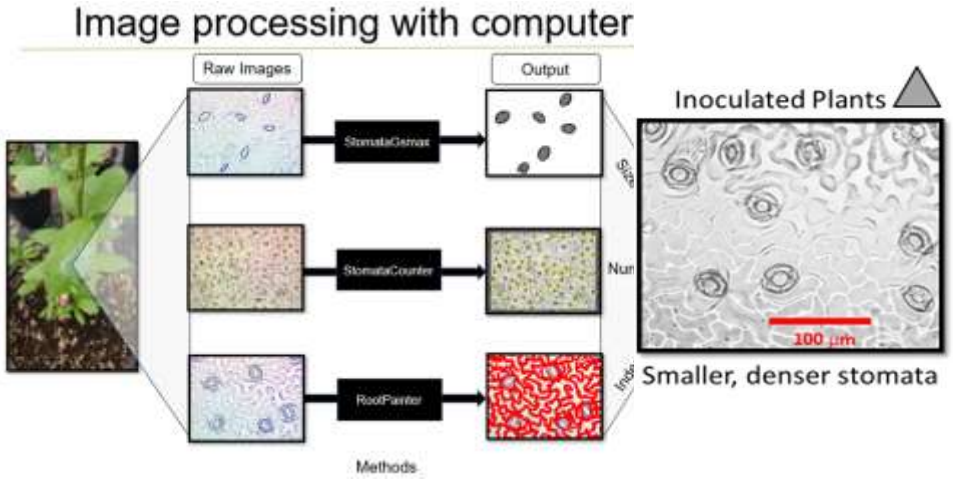


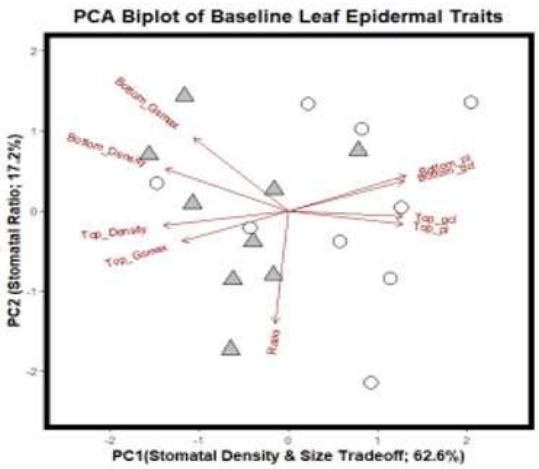
Khan, Z., Rho, H., Firrincieli, A., Hung, S.H., Luna, V., Masciarelli, O., Kim, S.H., and Doty, S.L. 2016. *Current Plant Biology* 6:38-47

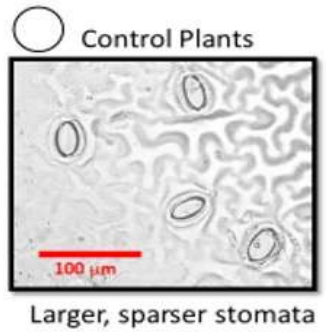
Advanced **Hardwood Biofuels** Northwest

Current research with the endophytes in collaborator Professor Soo-Hyung Kim's lab

* Endophytes can reduce stomatal conductance, alter stomatal morphology and patterning, improving intrinsic water use efficiency
* Endophytes can increase can plant cell size, increasing overall leaf size
* Endophytes increased survival after transplant stress







Hendrickson, M., Banan, D., Bakker, J. D., Doty, S. L., and Kim, S.H. Salicaceae endophyte inoculation alters stomatal patterning and improves the intrinsic water use efficiency of *Populus trichocarpa*. (in review)

Some endophytes increase tolerance of the host plant to heat

Morgan Raimondo, Graduate student

STRESSED, Uninoculated plants

STRESSED, Inoc with YR21Y

Inoculating poplar with a bacterial strain from the Yakima poplar promoted plant survival to heat stress

Potential for Climate Change Mitigation and Plant Adaptation

- Trees sequester carbon through photosynthesis
- Photosynthesis is limited by nutrient availability and water
  - *Endophytic bacteria living within plants can provide nitrogen and phosphorus*
  - *Endophytes increase plant water use efficiency and drought tolerance*
  - *Endophytes increase plant resilience to heat stress*

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Nature-Based Solutions

Use the microbiota selected by wild plants in challenging environments

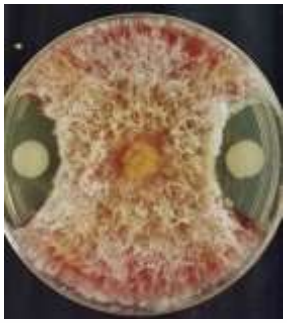
Graduate student, Morgan Raimondo, sampling from a poplar tree in a hot and semi-arid area

Graduate student, Kevin Shaffman, sampled plants growing in lava fields in Hawaii

Emma Gomez-Rivas, Microbiology graduate, conducts mechanistic studies of the diazotrophs from plants growing in rocks

KEY POINT: IT IS NOT ONLY THE PLANT GENETICS BUT ALSO THE GENETICS OF THE PLANT MICROBIOME THAT DETERMINE A PLANT'S ABILITY TO THRIVE UNDER PARTICULAR CONDITIONS

The Power of Endophytes



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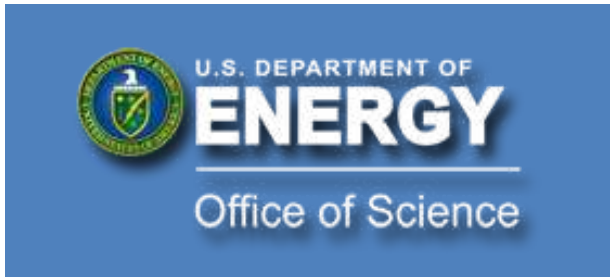
McIntire-Stennis program

NSF Energy for Sustainability program



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Current Doty Lab Members

Andrew Sher
Research Scientist 3

Robert Tournay
Postdoc

Doty Lab Website:
http://sites.uw.edu/sldoty

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Morgan Raimondo, graduate student

Emma Gomez-Rivas, graduate student

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