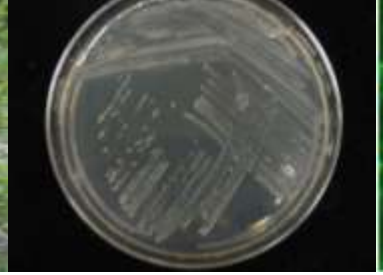
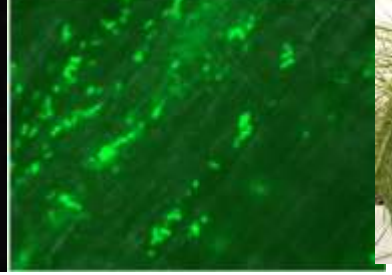




Endophytes for Increased Rooting and Better Growth

Sharon L. Doty
Associate Professor

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

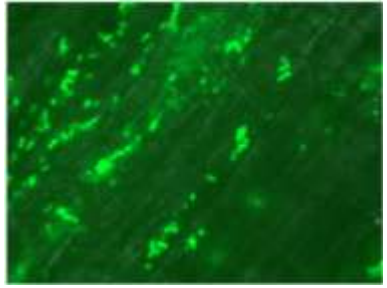
Cottonwood & willow endophytes

- *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

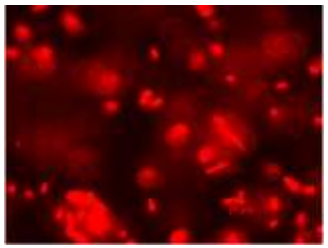


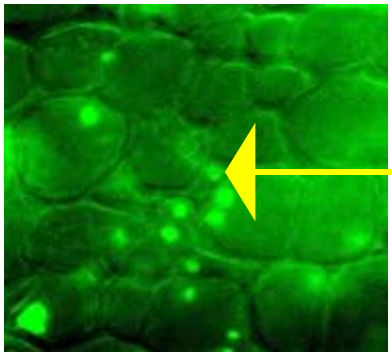


Improving plant growth using endosymbionts of poplar trees

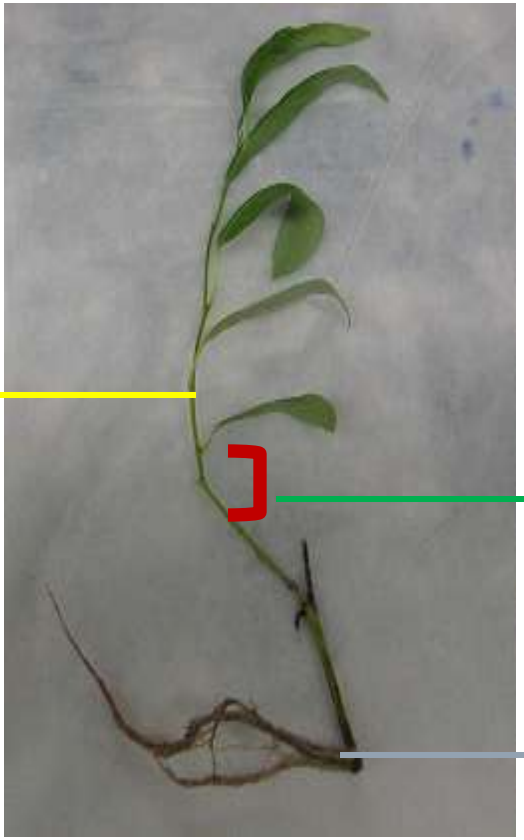
- **Can add the wild poplar endophytes**
- **Increased rooting**
- **May provide N and P**
- **Better health under N-limited conditions**
- **Increased drought tolerance**

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



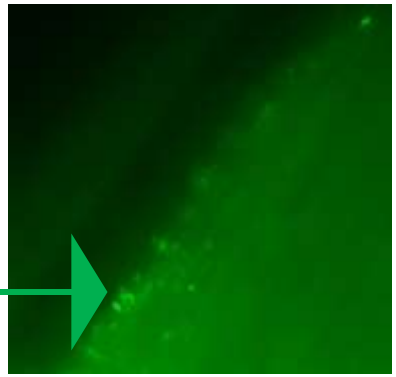


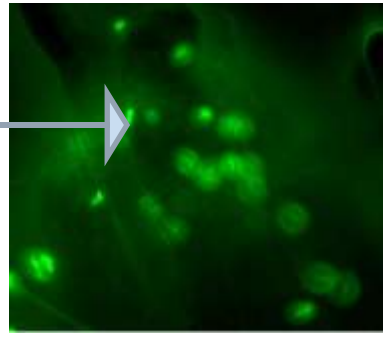
Fluorescent image taken after 6 days of inoculation – Cross section of a node



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)

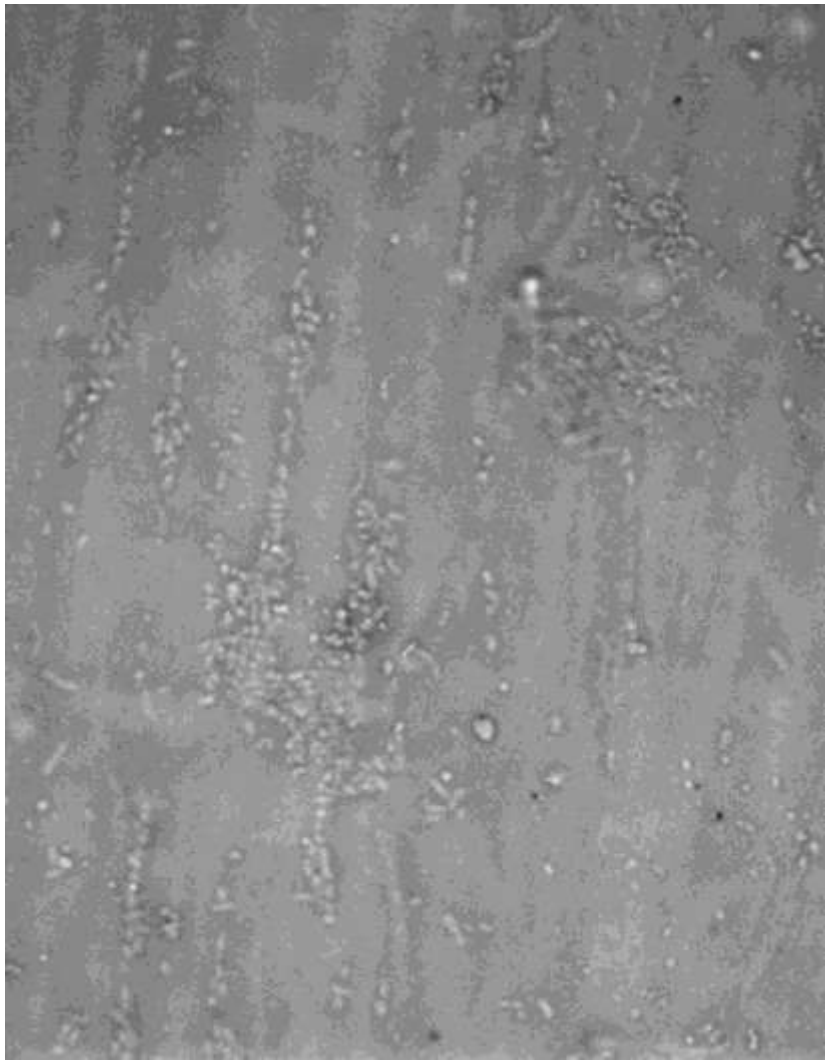


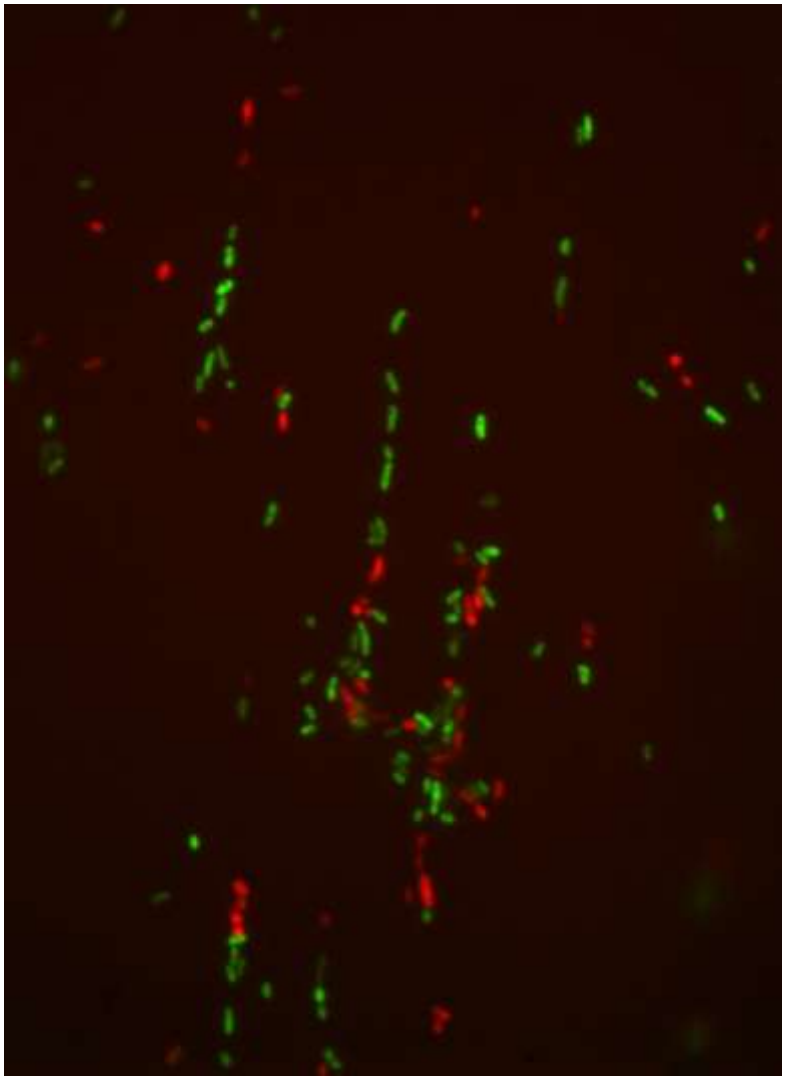


Fluorescent image taken after 2hrs of inoculation

Slide Credit: Zareen Khan

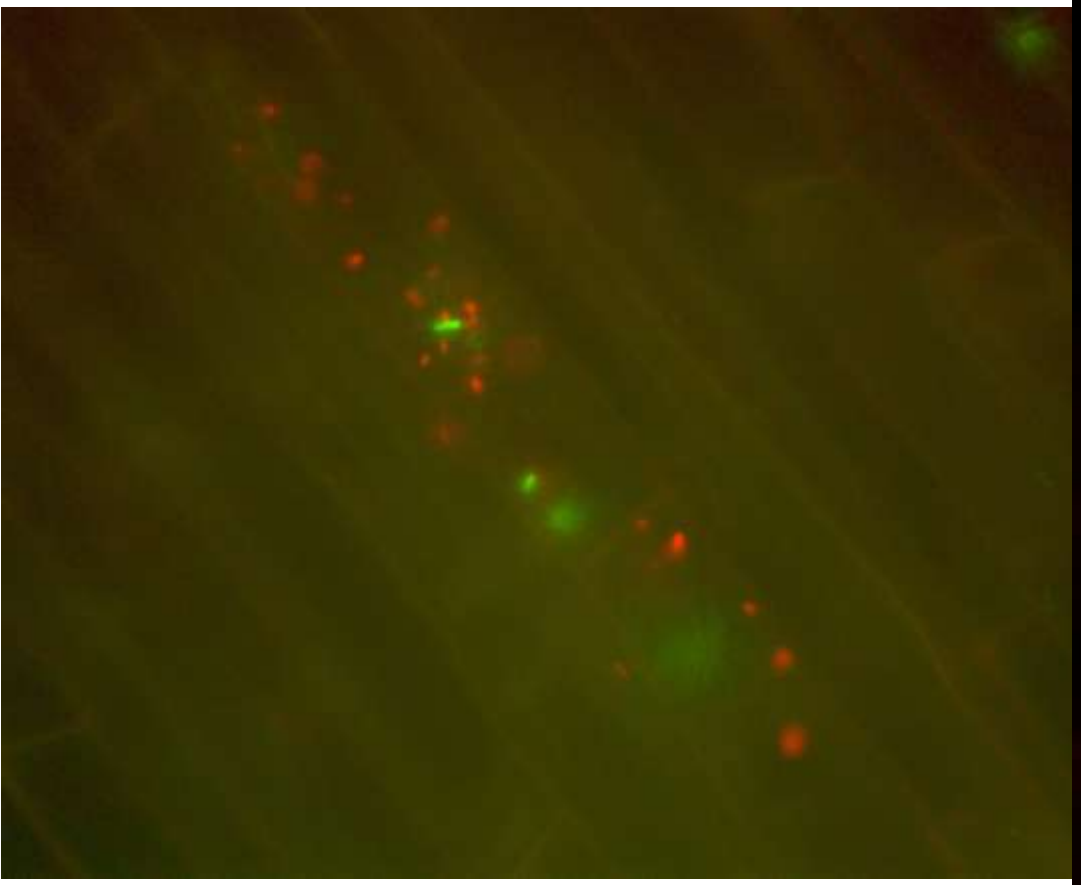
Poplar roots 1 hr after inoculation



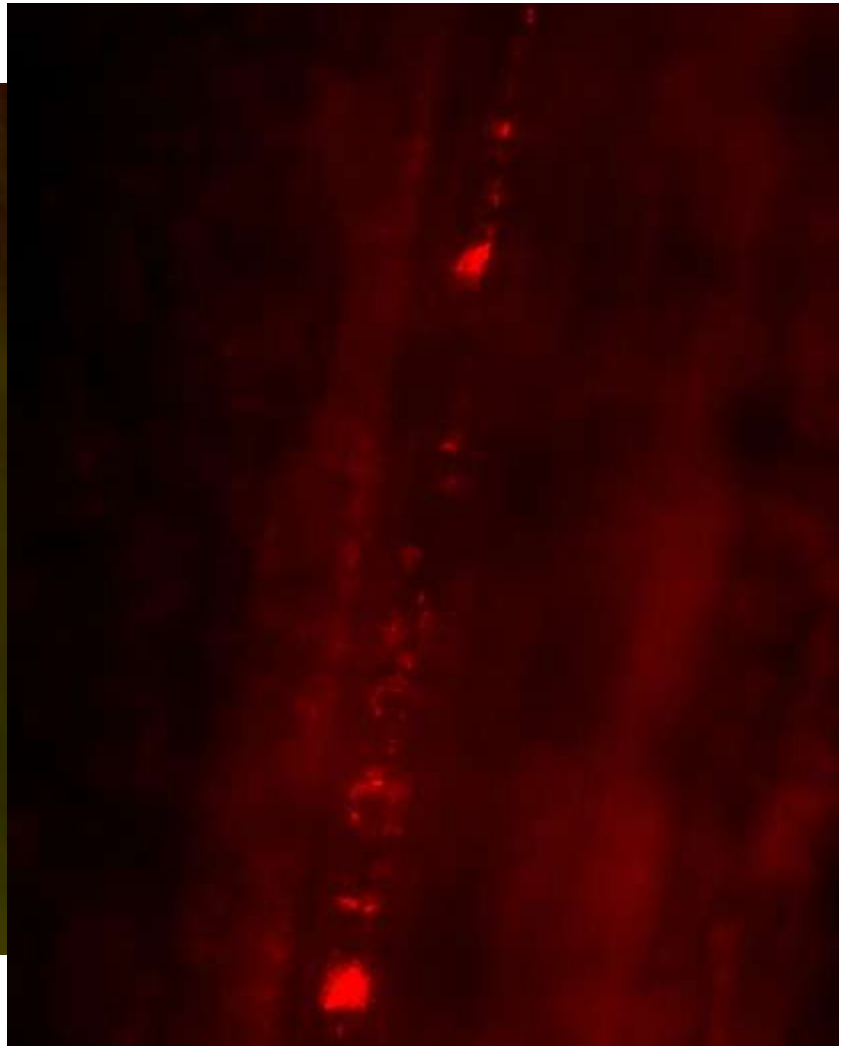


Clone 12805, 60 minutes, PTD1:gfp + PTD1:rfp @ 63x DIC, GFP filter, RFP filter. Credits: Amy Baum (Ph.D. student)

Endophytes in vascular tissues



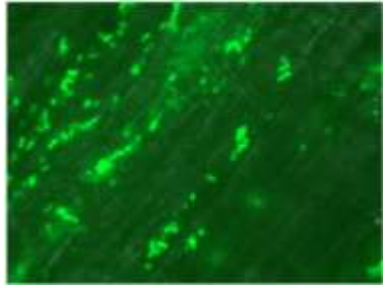
Clone 12805, 2 days, PTD1:gfp + PTD1:rfp @ 63X, GFP filter, RFP filter.



Nisq-1, 9 hours, PTD1:rfp. root xylem @ 40x , RFP

Credits: Amy Baum (Ph.D. student)





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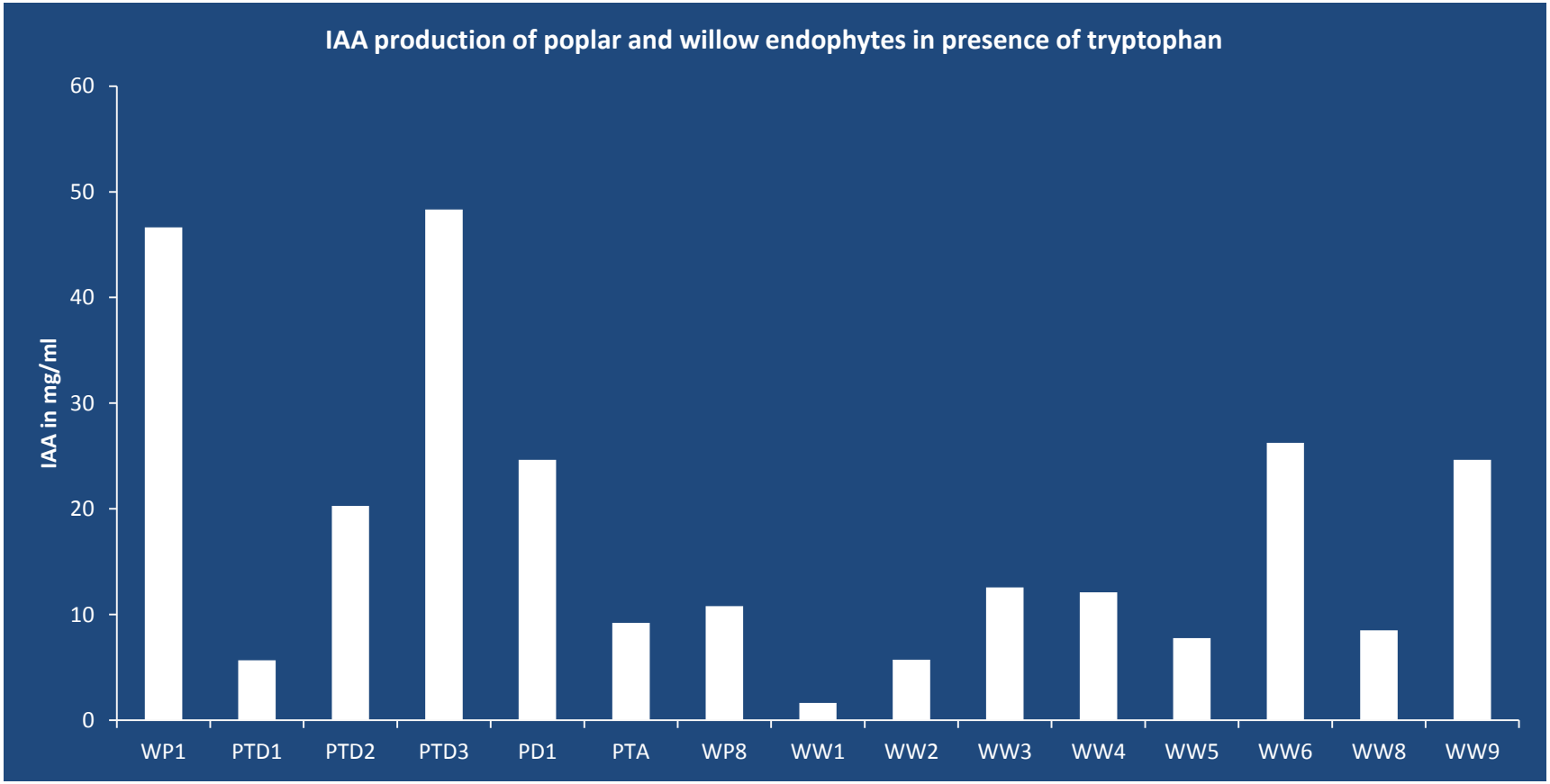
Many poplar endophytes produce plant hormones (auxins) that increase root growth



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

Poplar Rooting Problems

- Poplar growers reported that this is a major problem
- *Populus deltoides* and aspen varieties (*P. tremula* and *tremuloides*)

- Tested if endophyte inoculation will promote rooting of the hardwood cuttings as it does for grasses

7300501 (*P. deltoides*) at 2 mths

Uninoculated controls

WP1

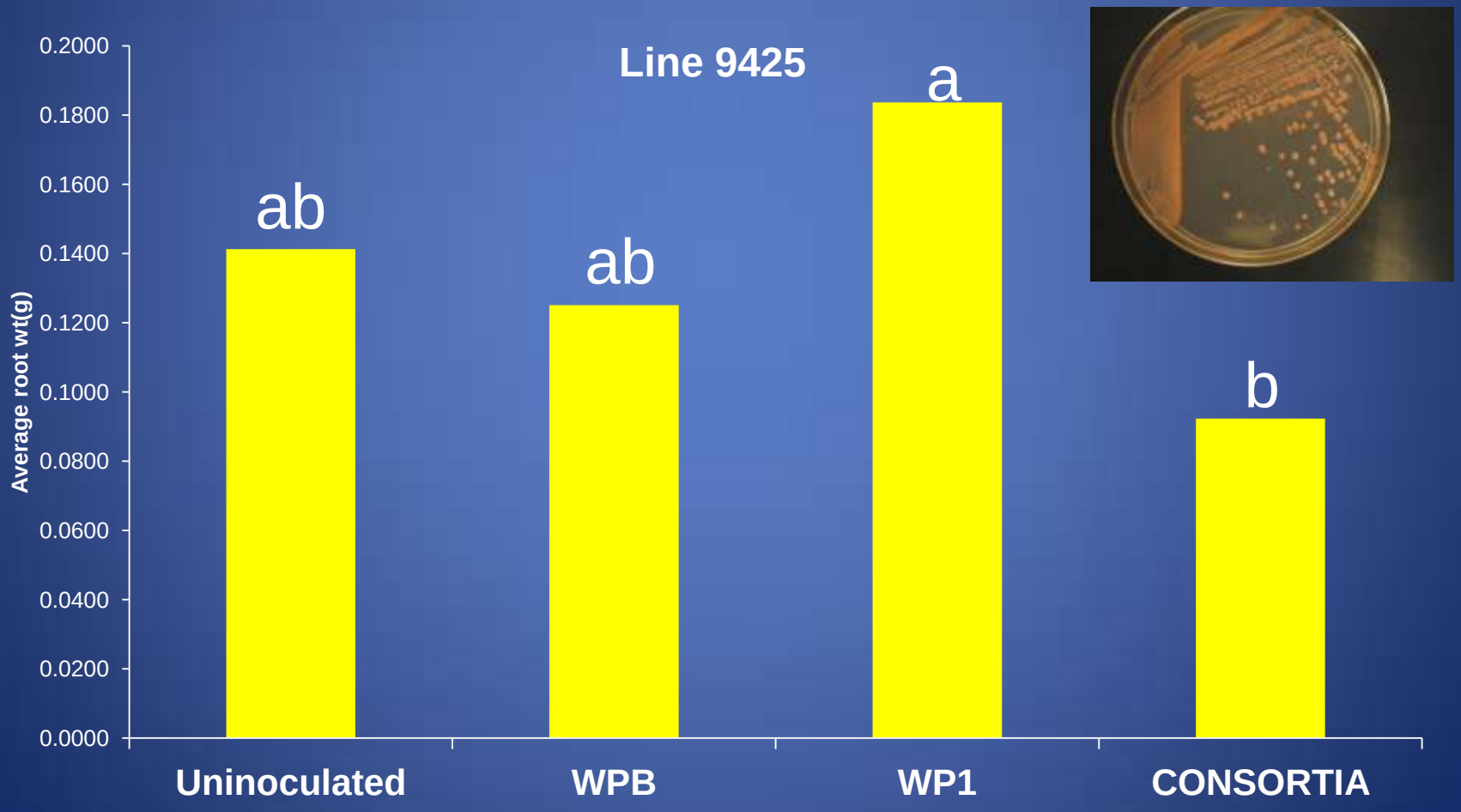
CONSORTIA

7300501 at 3 months

STATISTICALLY SIGNIFICANT DIFFERENCES FROM THE CONTROLS

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

9425-35 *P. deltoides* at 3 mths
* WP1 was statistically significant*



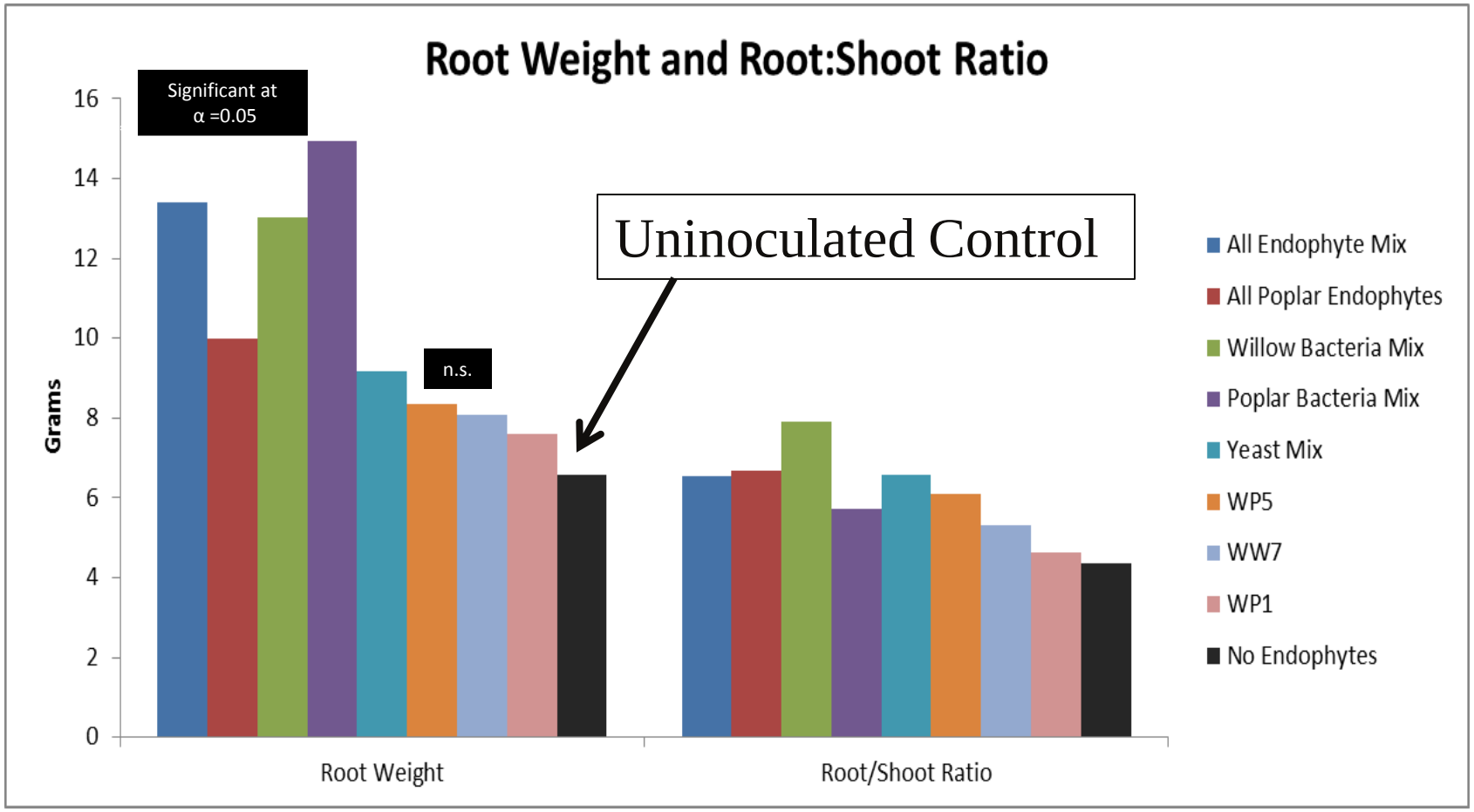
The endophytes had lasting positive effects on rooting

Inoculated hardwood cuttings; made cuttings from the shoots; placed these in hydroponics

Without the added microbes

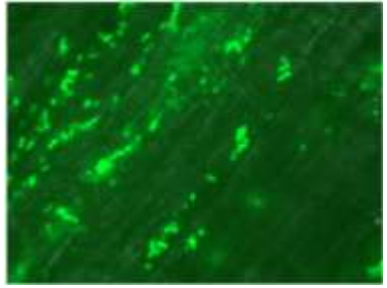
With the added microbes

4 month greenhouse study with *P. trichocarpa* Nisqually-1: doubling of root mass



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





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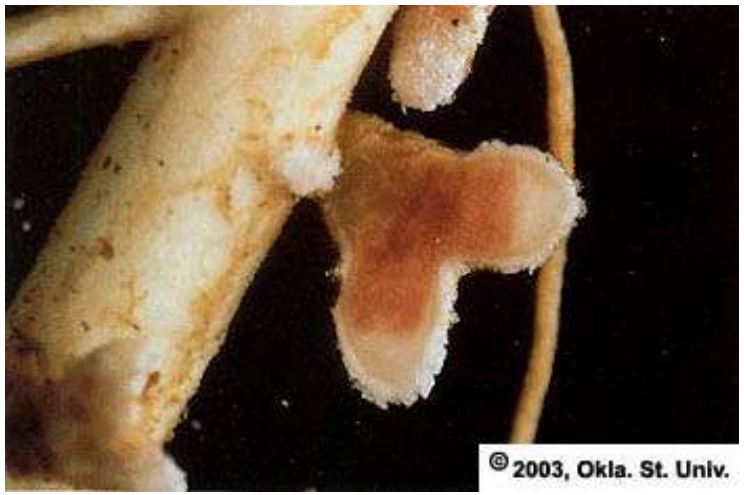
Biological Nitrogen Fixation

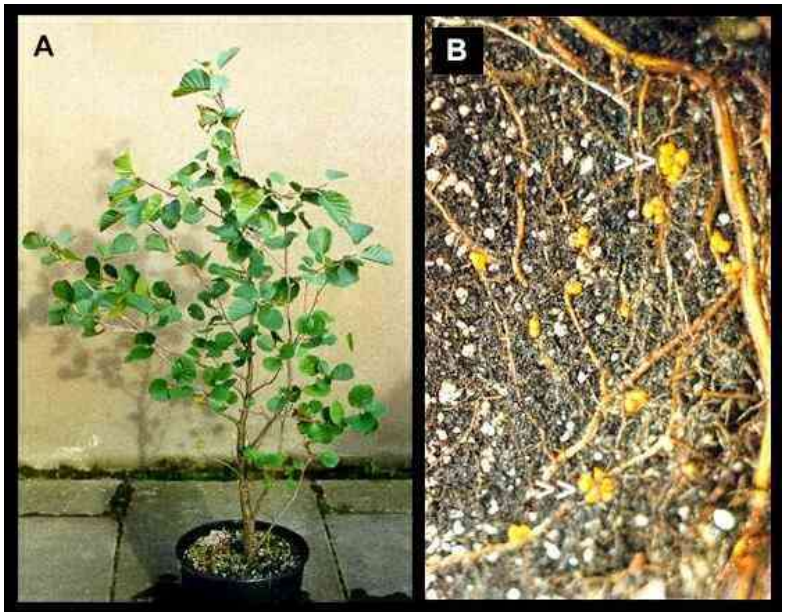
Nitrogenase

↓

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

EXPENSIVE REACTION→ Usually plant-associated

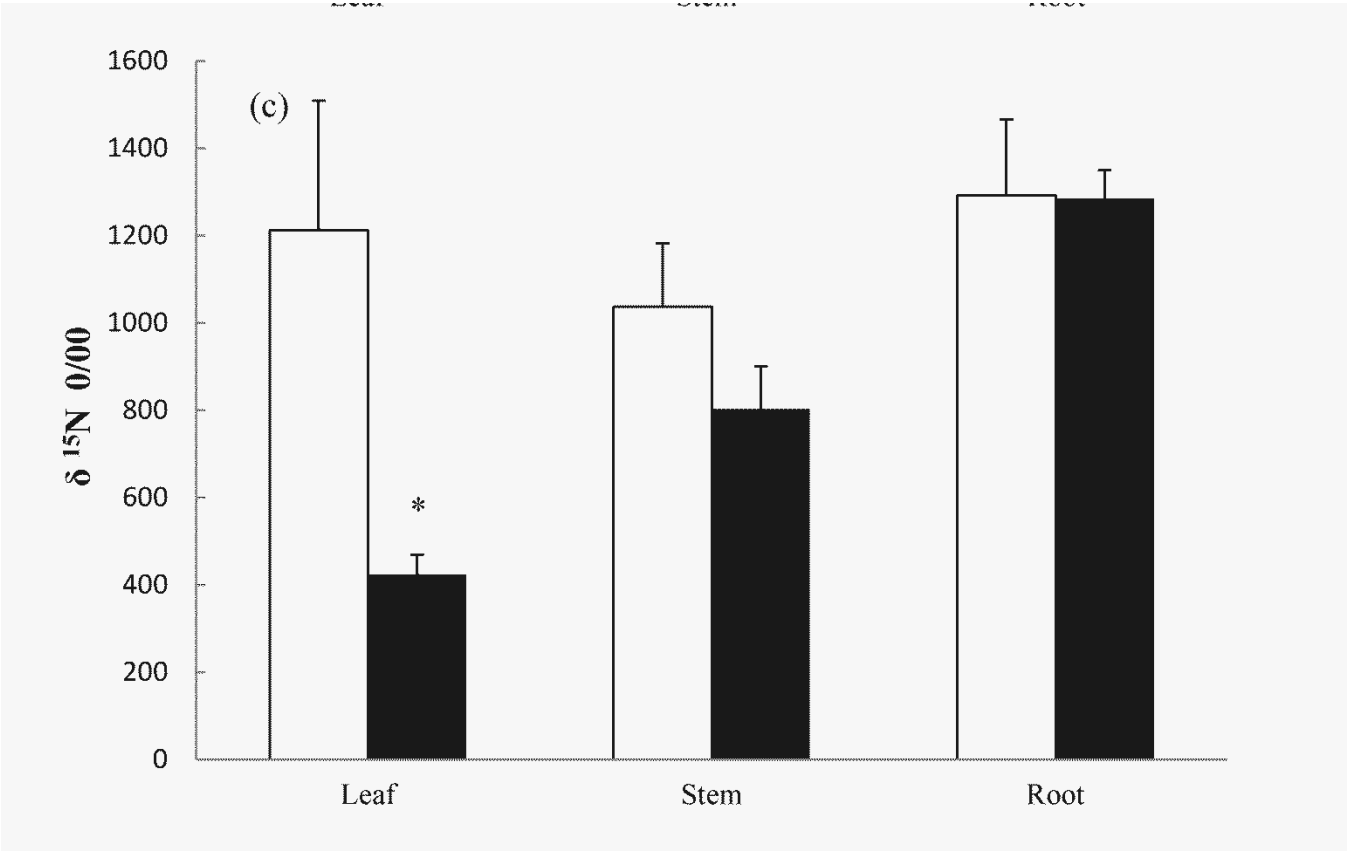




Diazotrophic Endophytes:

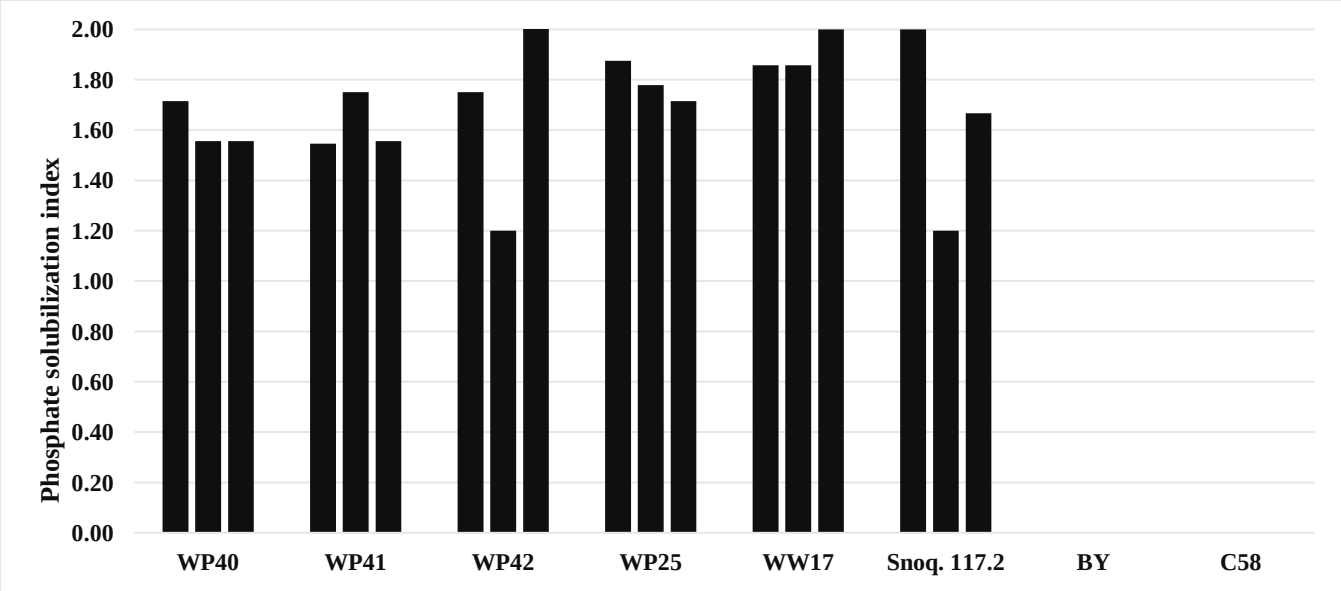
Nitrogen fixation without root nodules

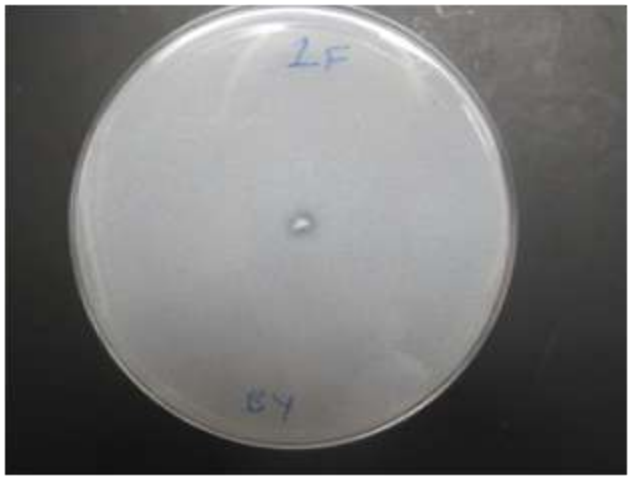
$^{15}N_2$ dilution assay indicated that the inoculated poplar received 65% of the foliar N from biological nitrogen fixation



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

Phosphate Solubilization

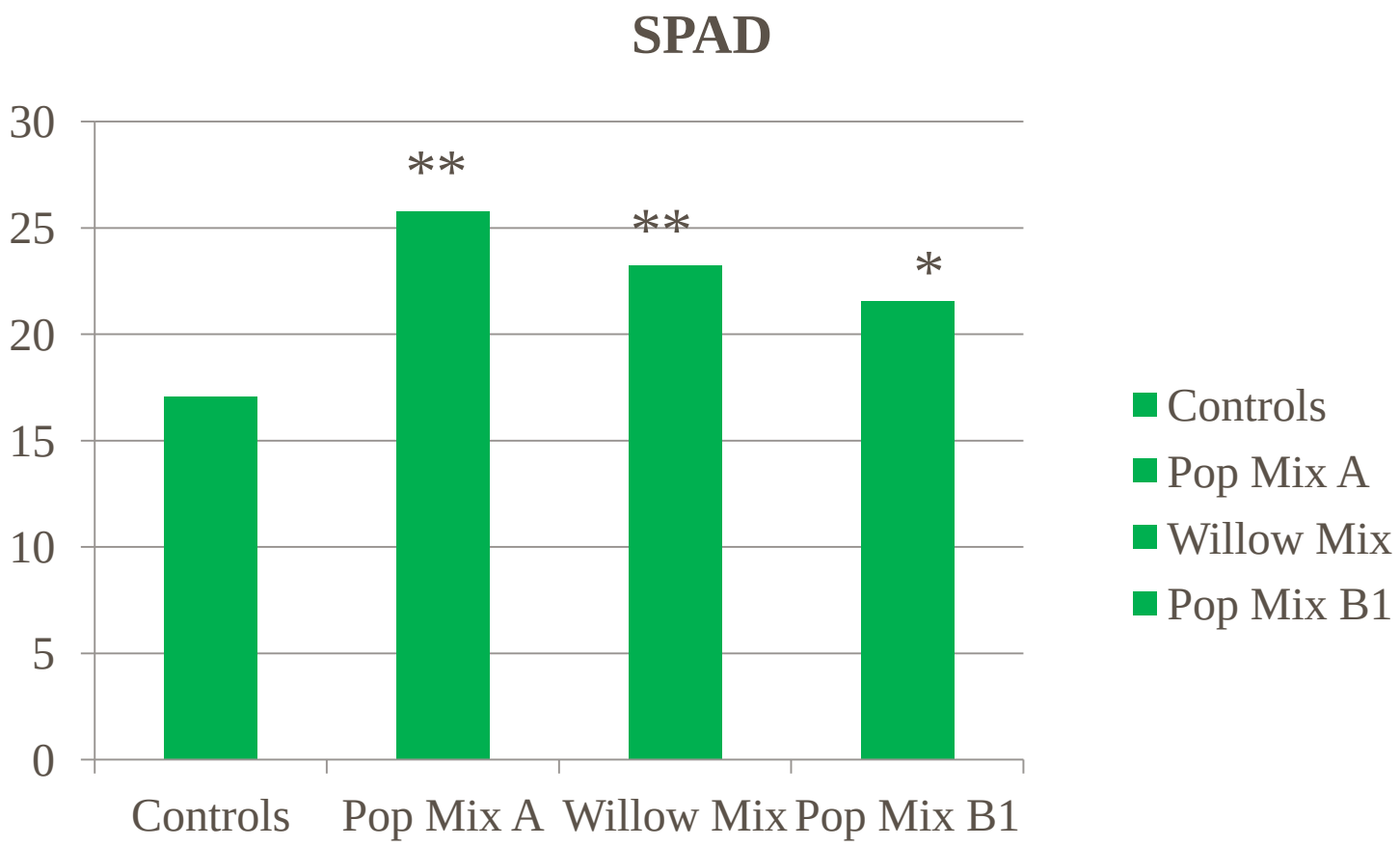




Alex Dolk, unpublished

Negative Control (BY)

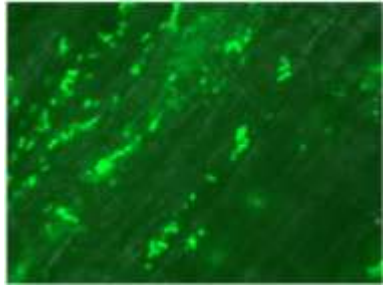
Increased Greenness



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





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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.

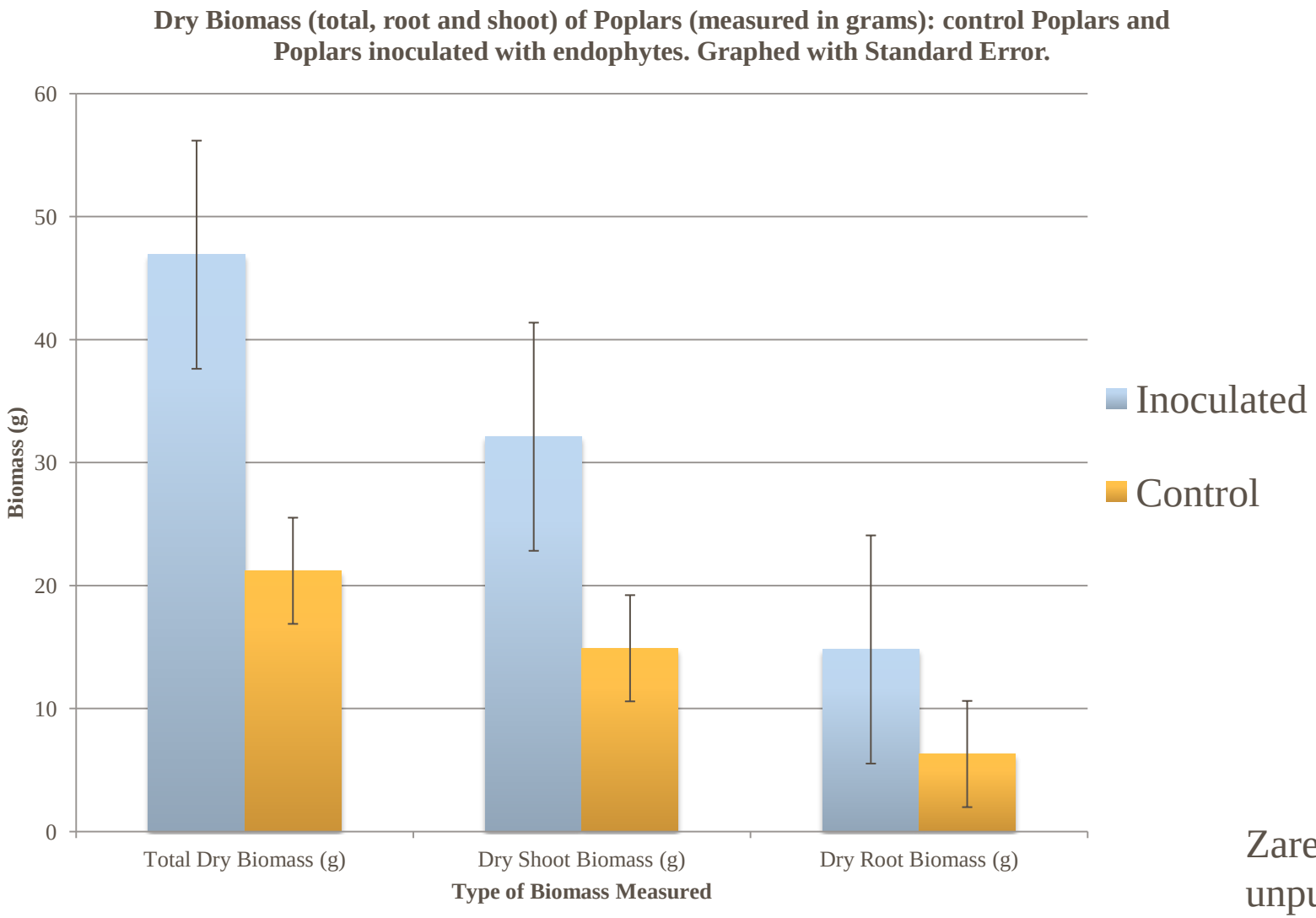
Khan, Z, Guelich, G., Phan, H., Redman, R., and Doty, S. L. 2012. ISRN Agronomy

INOCULATED WITH CONSORTIA

CONTROL

Credit: Zareen Khan

Results of poplar drought stress expt.



Zareen Khan, unpublished

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

DOTY LAB

Undergraduate student research described today:
Megan Plog, Alex Dolk, Grant Guelich

Research Scientist:
Zareen Khan

Current Graduate Students:
Amy Baum
Nick Herschberger
Shyam Kandel
Emilie Viglino
Robert Tournay
Ellen Weir
Lisa Hannon
Mahsa Khorasani
Ronald Cuie

Graduated Ph.D. student research featured today:
Dr. Jenny Knoth

LAB WEBSITE:
http://depts.washington.edu/envaplab/index.html

NIFA climate change mitigation
#2012-68002-19824

Agriculture and Food Research Initiative
#2010-05080