Non-Destructive Digital Imaging of Poplar Root Systems.

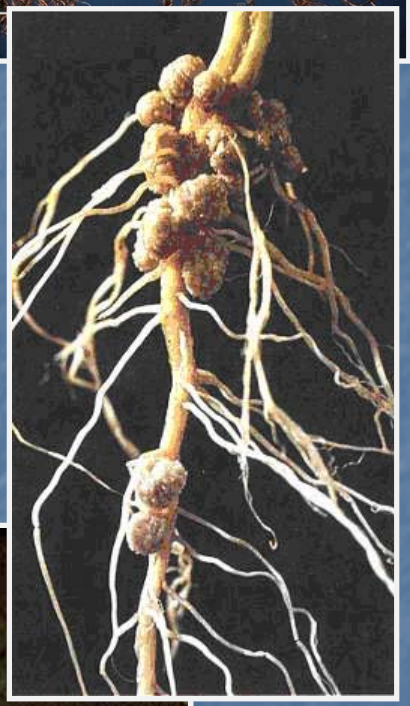
Kodrzycki, Robert J.[1], Michaels, Ronald B.[1], Friend[2], Alexander L., Zalesney, Ronald S.[3], Mawata, Christopher P.[4], and McDonald, Daniel W. [1]

[1]Phenotype Screening Corp., 10233 Chapman Hwy., Seymour, TN, 37865, USA.
[2]USDA Forest Service, North Central Research Station, Houghton, MI 49931, USA.
[3]USDA Forest Service, North Central Research Station, Rhinelander, WI 54501, USA.
[4]Dept. of Mathematics, Univ. of Tennessee at Chattanooga, Chattanooga, TN 37403, USA.

5th International Symposium on Adventitious Root Formation
University of Alcala, Alcala de Hanares, Madrid. Spain
June 17, 2008

Why are roots important?

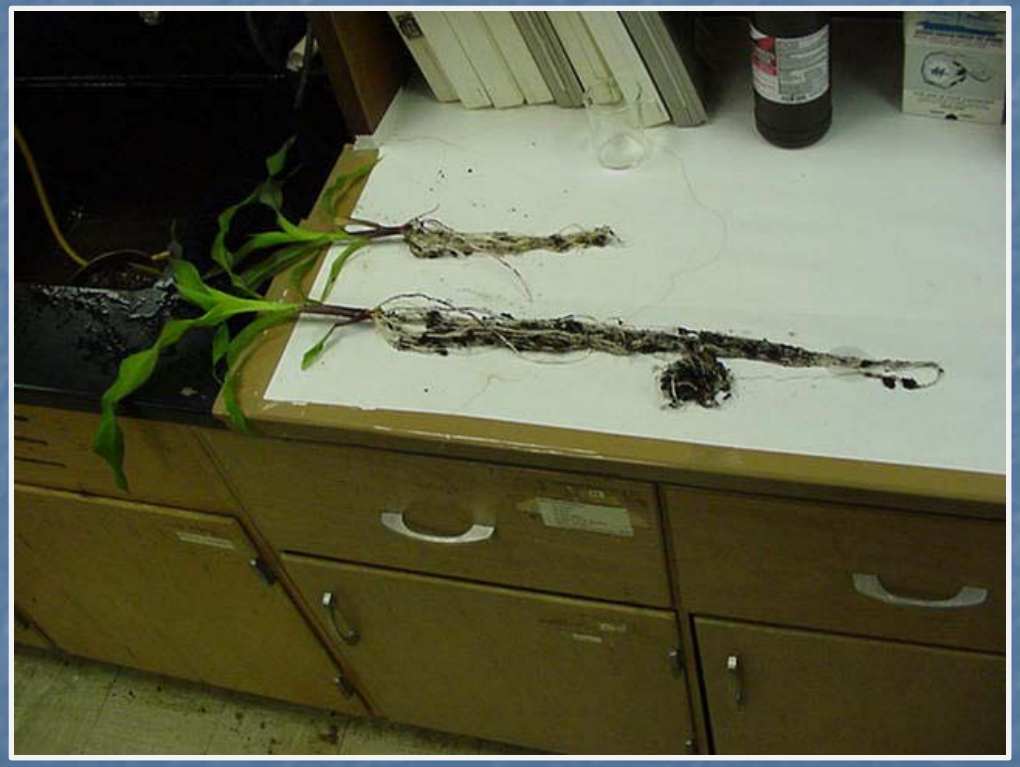
- Water uptake and movement.
- Mineral uptake and movement.
- Support
- Storage
- Mutualism: Interaction with organisms
  - Mycorrhizae association
  - Root nodulation bacteria
- Parasites
  - Nematodes and others
- Disease Resistance



Root Systems are Difficult to Analyze

Common Methods:

- Invasive
- Destructive.
- Time consuming
- Provide limited information

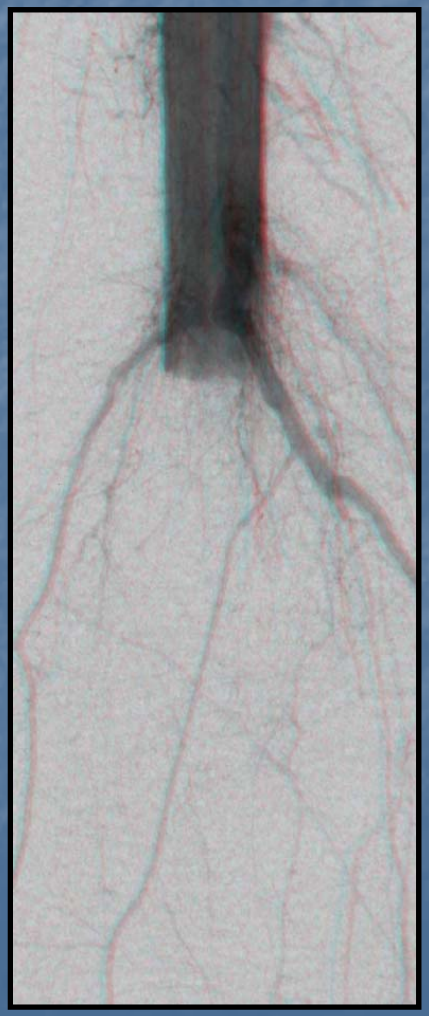


RootViz FS: A New Way to Study Roots

Based on low energy x-ray imaging

Features:

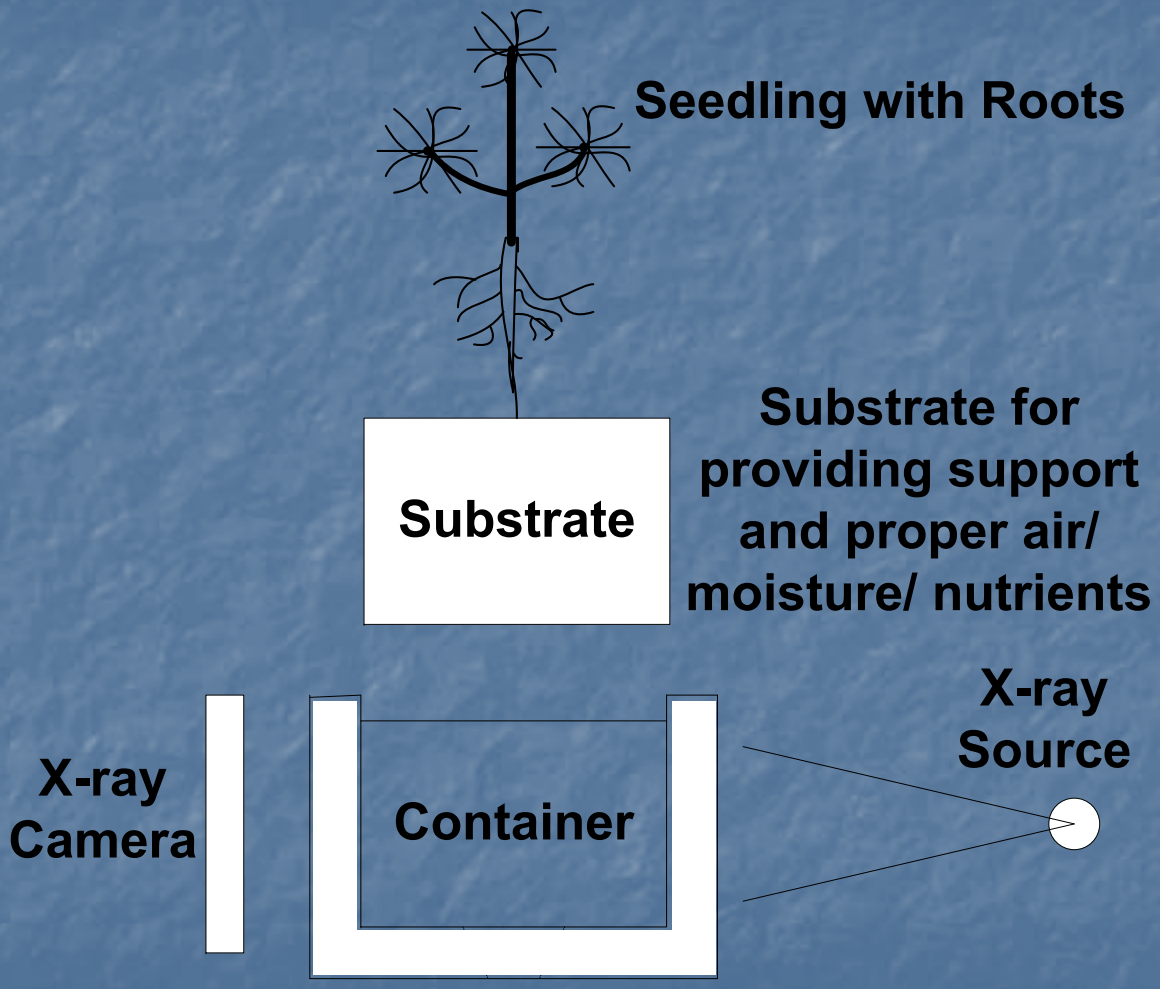
- Non-destructive
- Non-invasive
- High resolution
- Versatile
- Quantitative
- High throughput



Capacities:

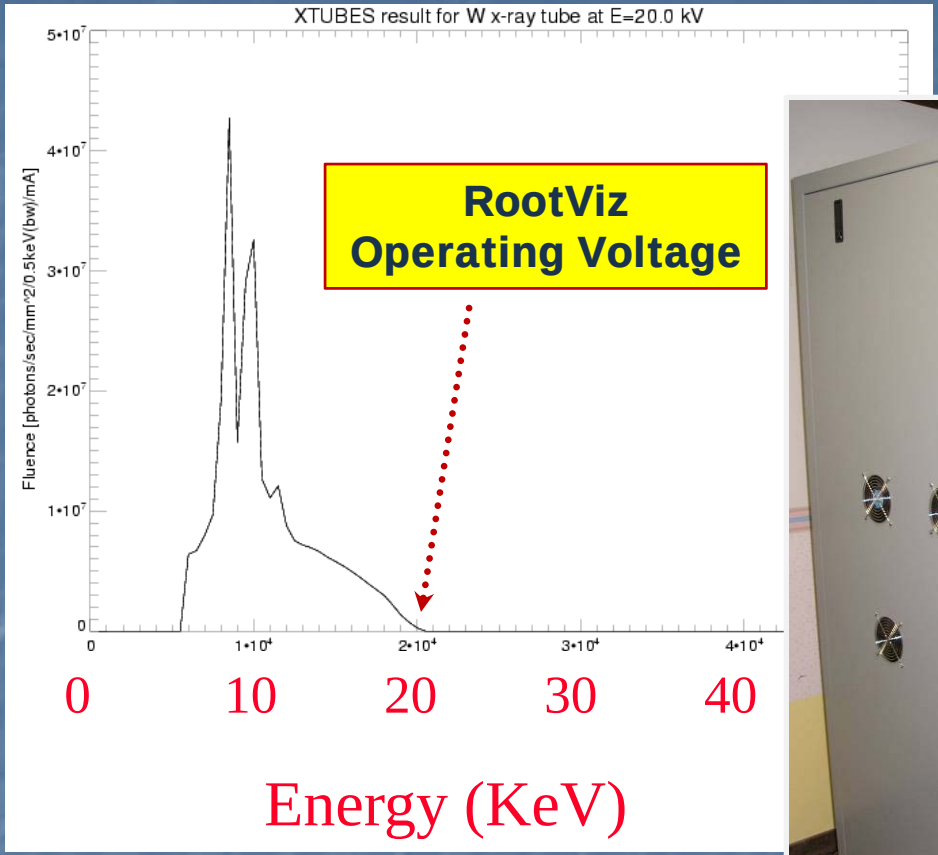
- 0.6 m rooting depth
- 2.1 m plant height
- Up to 25 plants per hour throughput
- Seed germination
- Greenhouse stage plants

Key Components of Concept



Container for substrate and seedling.
Allows for plant storage and transport.

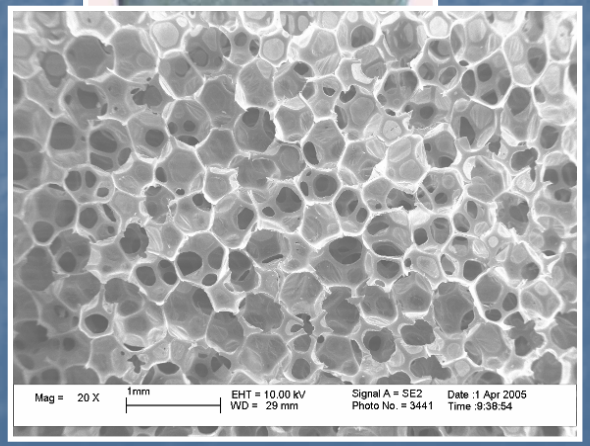
Low Energy X-ray System



Funded by: US Department of Energy
Inventions & Innovations Grant

In collaboration with:
ArborGen, Oak Ridge National Laboratory, University of Tennessee

Engineered Rooting Substrates



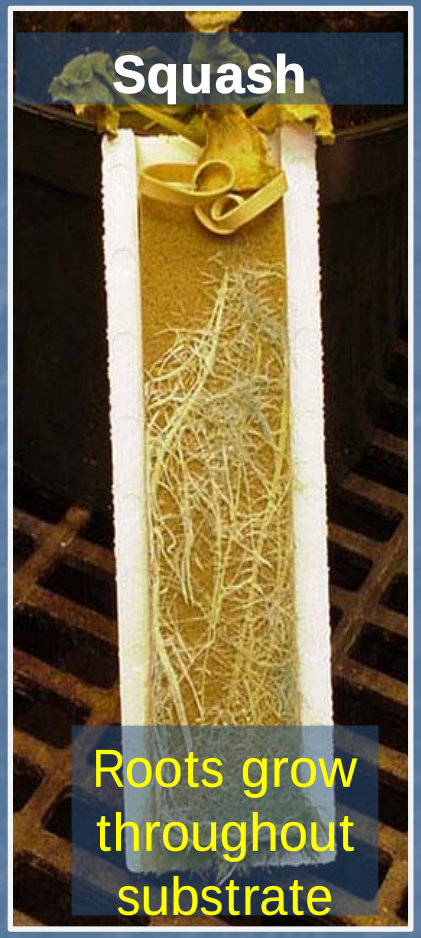
Like agar or special soil mixtures, the PSC engineered rooting substrate provides a specific set of conditions for each experiment.

Unlike soil mixtures, PSC engineered rooting substrate provides more precise control.

- Consistent conditions over the entire rooting environment.
- Lower environmental variation among plants and between experiments.
- Rapid changes to the rooting environment can be easily introduced.

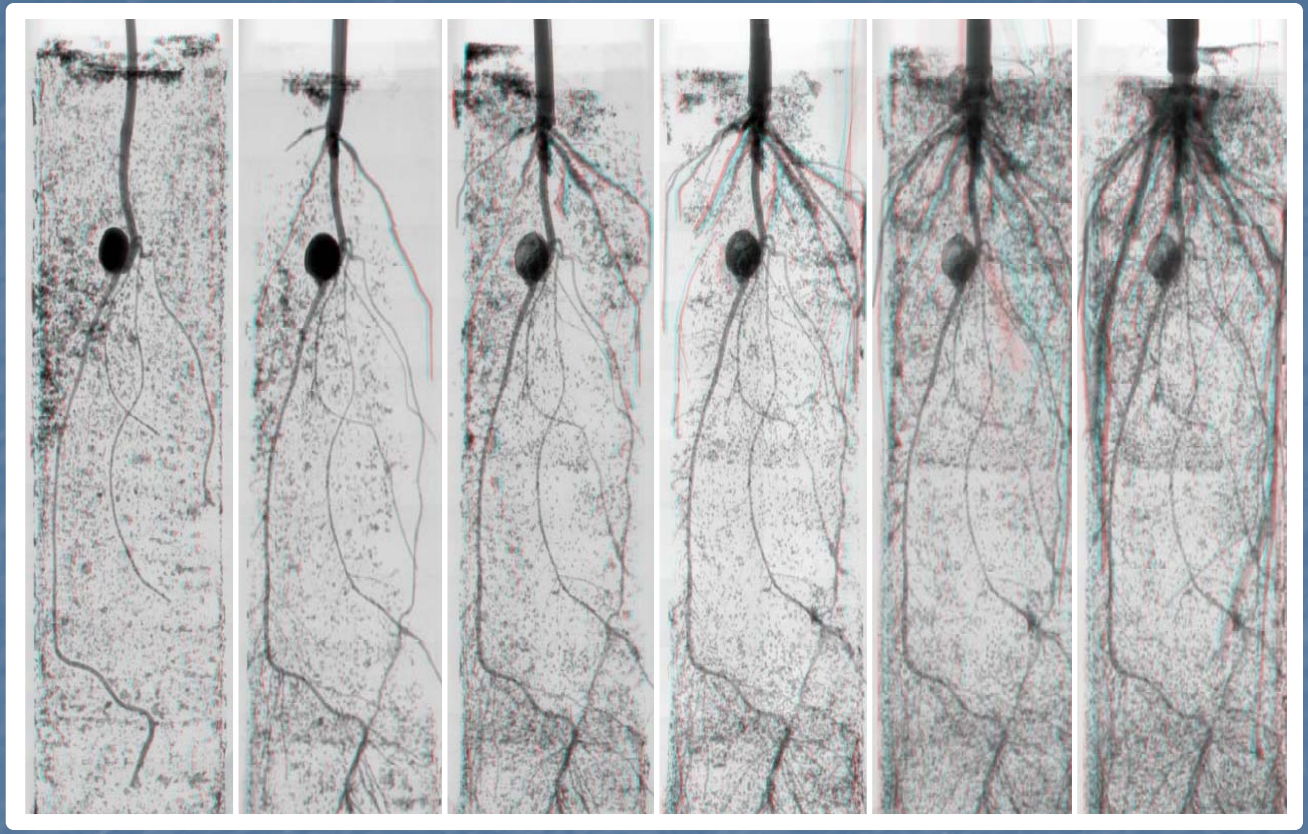
Data generated in collaboration with:
Dr Roberto Benson, Dept Materials Science & Engineering
University of Tennessee, Knoxville, TN

Plant Growth in PSC Substrates



- An engineered rooting substrate allows normal root growth and enables x-ray analysis.
- A wide range of species can be analyzed.

Powerful Developmental Studies



A single maize plant was imaged at 12 day intervals showing the development of the root system.

Data generated in collaboration with:
Dr Edward Buckler, USDA-ARS, Cornell University, Ithaca, NY; Dr Philip Benfey, Dept of Biology, Duke University, Durham, NC

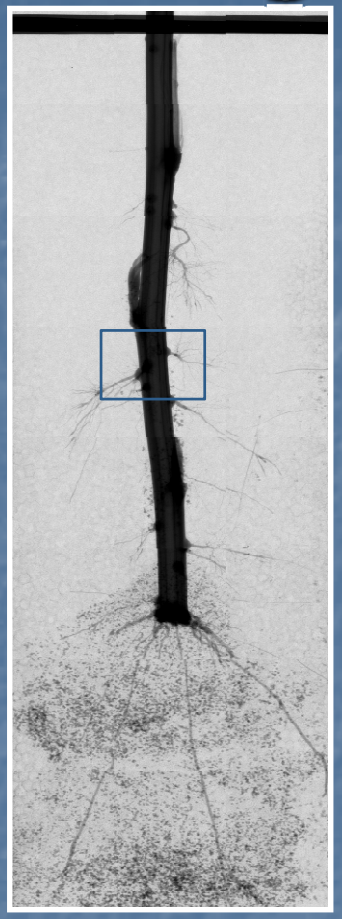
Rice: Variety Attributes

Ra4993

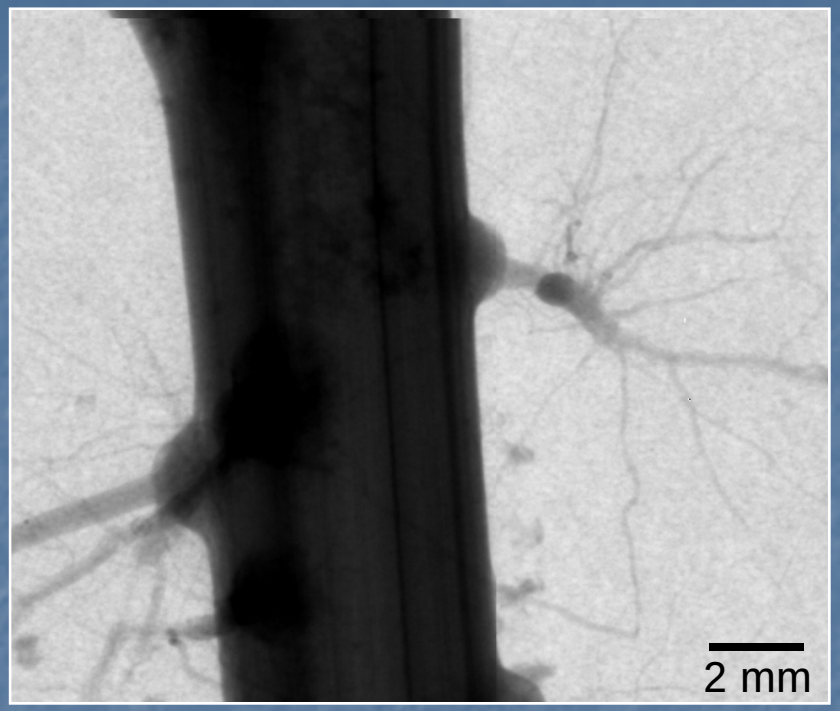
Ra5531

Data generated in collaboration with:
Dr Susan McCouch, Dept of Plant Biology, Cornell University, Ithaca, NY
Dr Philip Benfey, Dept of Biology, Duke University, Durham, NC

High Resolution X-Ray Images



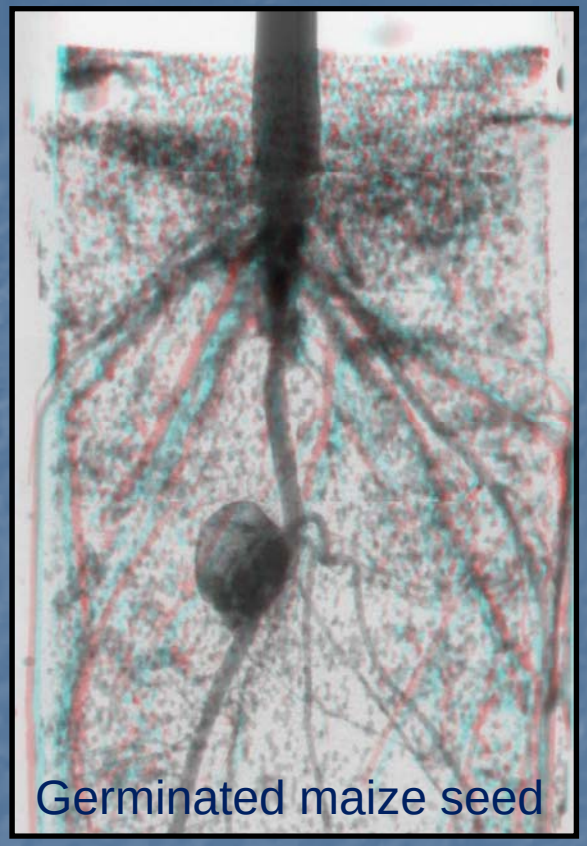
Zoom in



Detailed Analysis in 2D (Mono)

The x-ray images are taken at 100 micron resolution. Zooming in on an area shows a high level of detail. The close-up shows root nodes on the front or back surface of the root as well as the smaller roots crossing the original poplar cutting.

High Resolution Stereo Images



Germinated maize seed

Willow cutting
(*Salix eriochephala*)

RootViz FS is capable of taking high resolution stereo images. This is achieved by taking two images of the root system with a slight rotation of the plant in the x-ray chamber.

Note: Stereo viewing requires red/cyan glasses.

RootViz Summary

- A potentially useful tool for root studies
  - Morphology
  - Development
  - Screening
- But…

It’s not soil...

- How does growth in engineered substrate compare to soil?
- Are the results accurate?
- Are the results meaningful?

Collaboration with USDA Forest Service

- Compare growth of poplar clones between engineered substrates and soil.
- Compare traditional root analytical methods with x-ray analysis.
- Suggest modifications to foam substrates.
- Suggest improvements to x-ray characterization system.

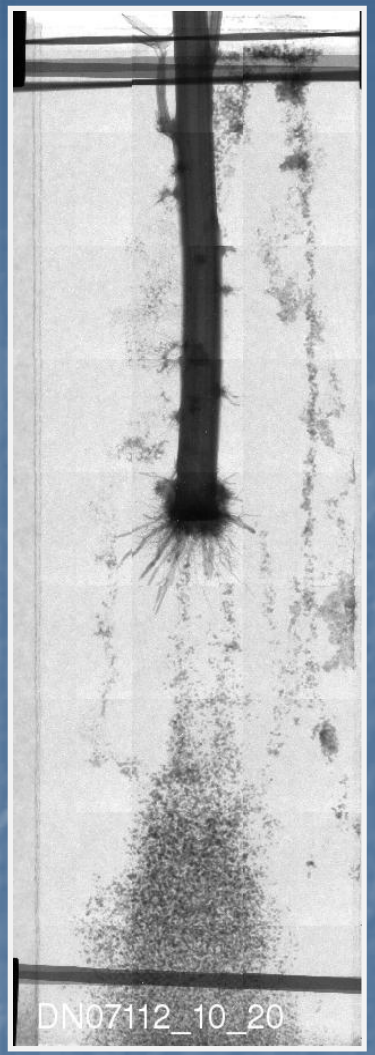
Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

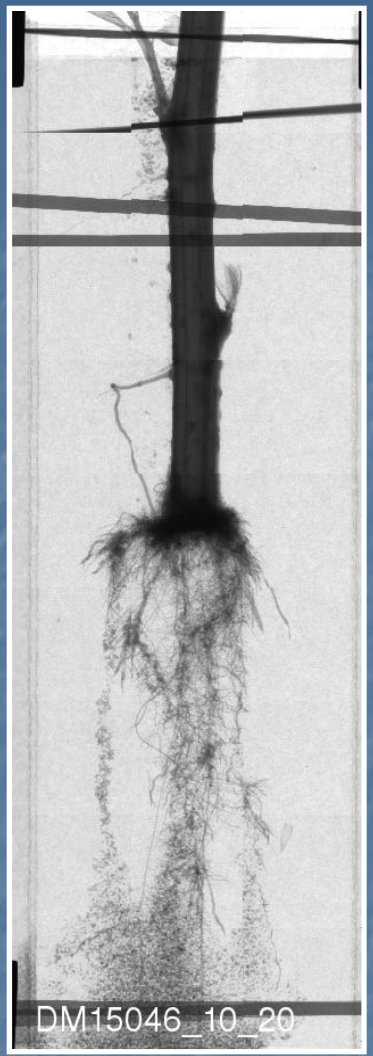
Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

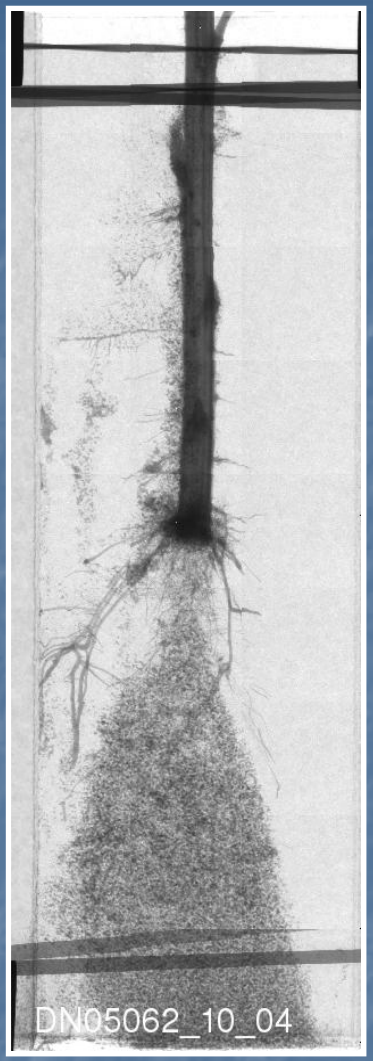
Collaborative Project with US Forest Service to Study Poplar Rooting

- 3 poplar clones: 4 replicates per clone
  - DN70 *P. deltoides x P. nigra*
  - NC14104 *P. deltoides x P. maximowiczii*
  - NM6 *P. nigra x P. maximowiczi*
- 4 Rooting Media
  - Sand
  - Peat/vermiculite mix
  - LWR engineered rooting matrix
  - MWR engineered rooting matrix

Root Development



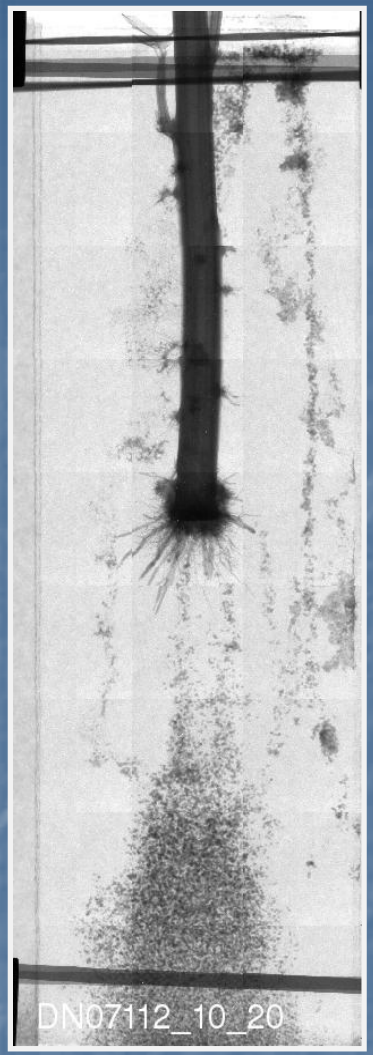


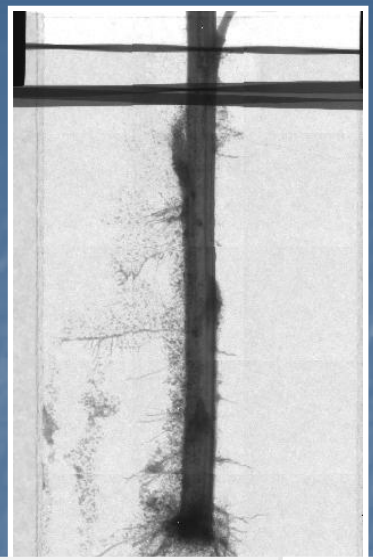


Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Root Development





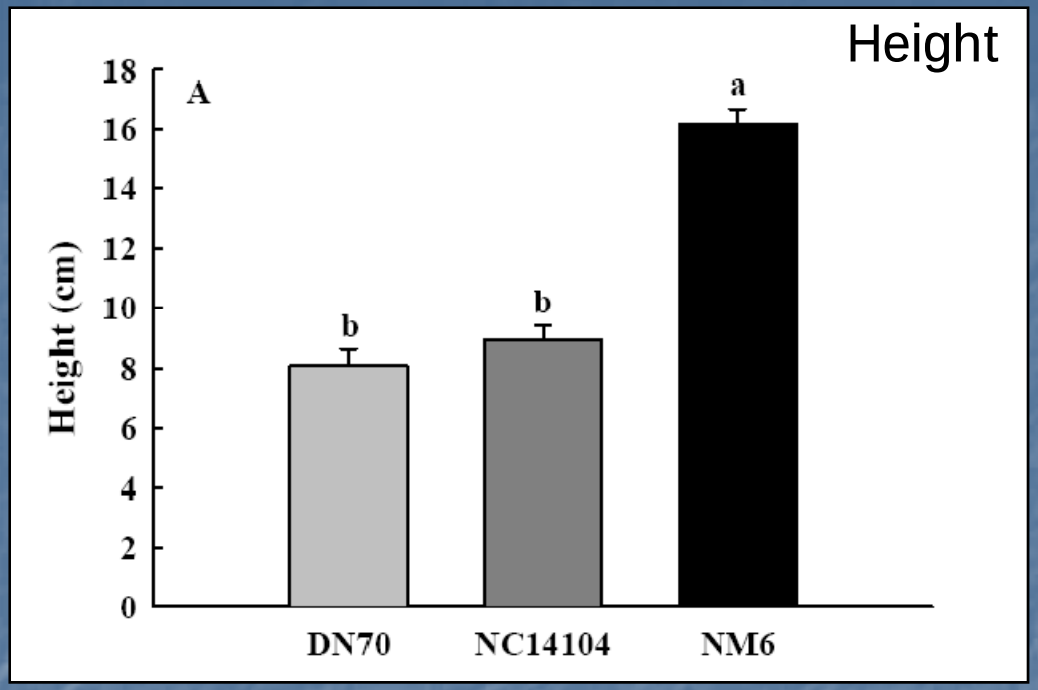
Poplar Traits Analyzed

Trait Analyzed	Substrate	Clone	Substrate X Clone	Cutting Dry Mass
Height (cm)	0.1305	**<0.0001**	0.2977	**0.0004**
Leaf area (cm^2)	**0.0448**	**<0.0001**	0.5146	**0.0201**
Leaf dry mass (mg)	**0.0294**	**<0.0001**	0.4907	**0.0034**
Stem dry mass (mg)	**0.0252**	**<0.0001**	0.8160	**<0.0001**
Aboveground dry mass (mg)	**0.0266**	**<0.0001**	0.5343	**0.0012**
Number of primary roots	**0.0014**	**<0.0001**	0.8716	0.5506
Number of secondary roots	**<0.0001**	**0.0050**	0.6711	**0.0091**
Number of tertiary roots	**0.0002**	**0.0059**	0.0655	0.3052
Length of primary roots (cm)	**<0.0001**	**<0.0001**	0.2279	0.3040
Length of secondary + tertiary roots (cm)	**0.0002**	**0.0257**	0.3663	0.1510
Dry mass of primary roots (mg)	**0.0016**	**0.0007**	0.8766	0.5735
Dry mass of secondary + tertiary roots (mg)	**0.0003**	**0.0227**	0.3829	0.5783
Belowground dry mass (mg)	**<0.0001**	**0.0010**	0.6624	0.9225
Total tree dry mass (mg)	**0.0165**	**<0.0001**	0.5540	**0.0022**
Root mass fraction	**<0.0001**	**0.0022**	0.7284	**0.0309**
Number of secondary roots per primary root	0.1265	0.1784	0.5822	0.2486
Primary root length per dry mass ($cm\ mg^{-1}$)	**<0.0001**	**0.0027**	0.8606	**0.0042**
Secondary + tertiary root length per dry mass ($cm\ mg^{-1}$)	**<0.0001**	0.3147	0.1837	0.1155

Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

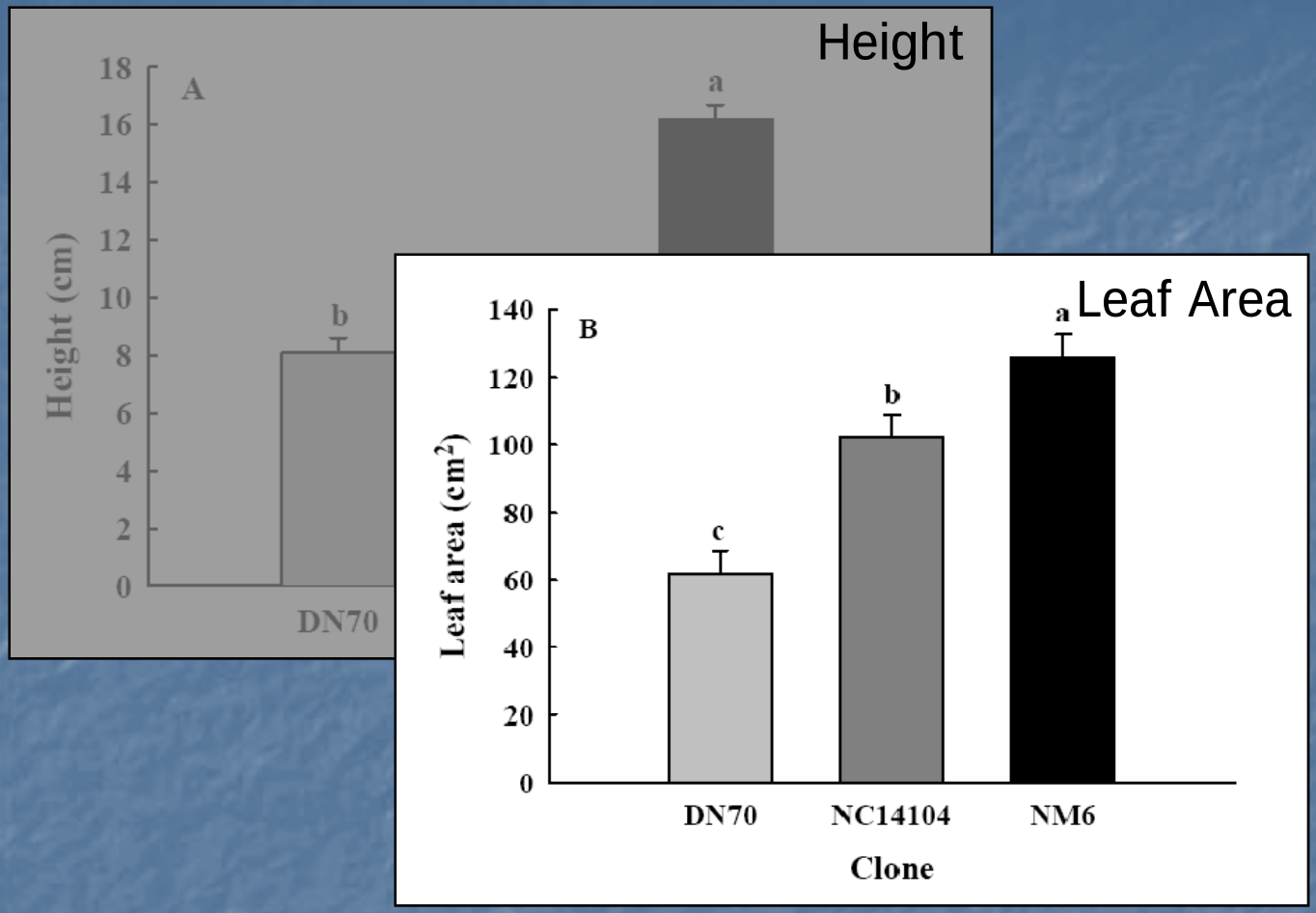
Clonal Differences



Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

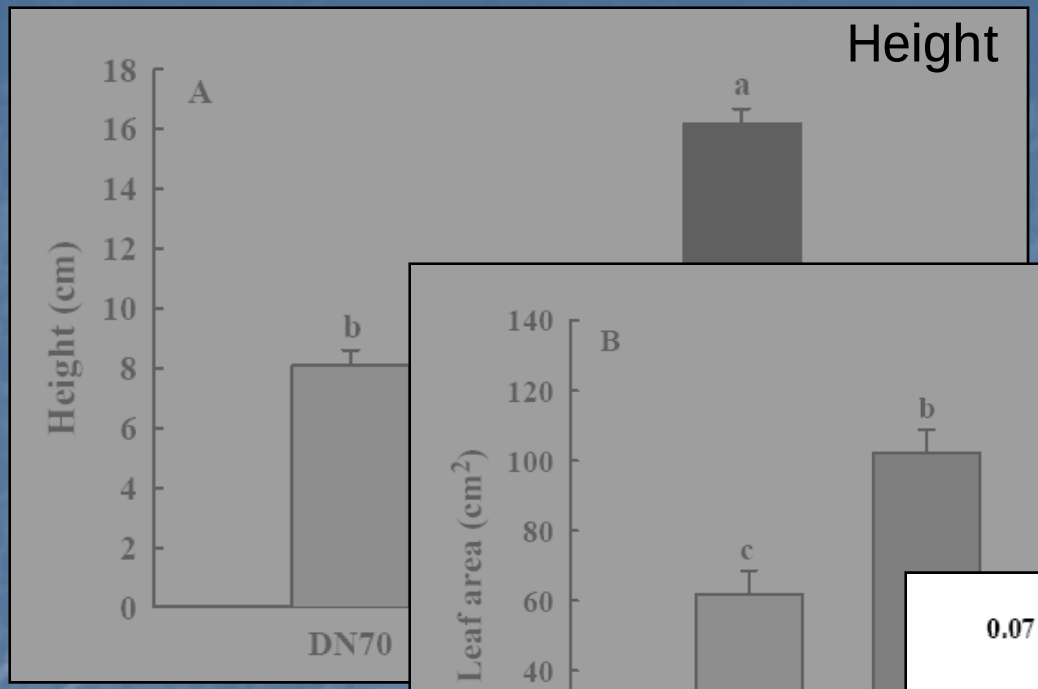
Clonal Differences

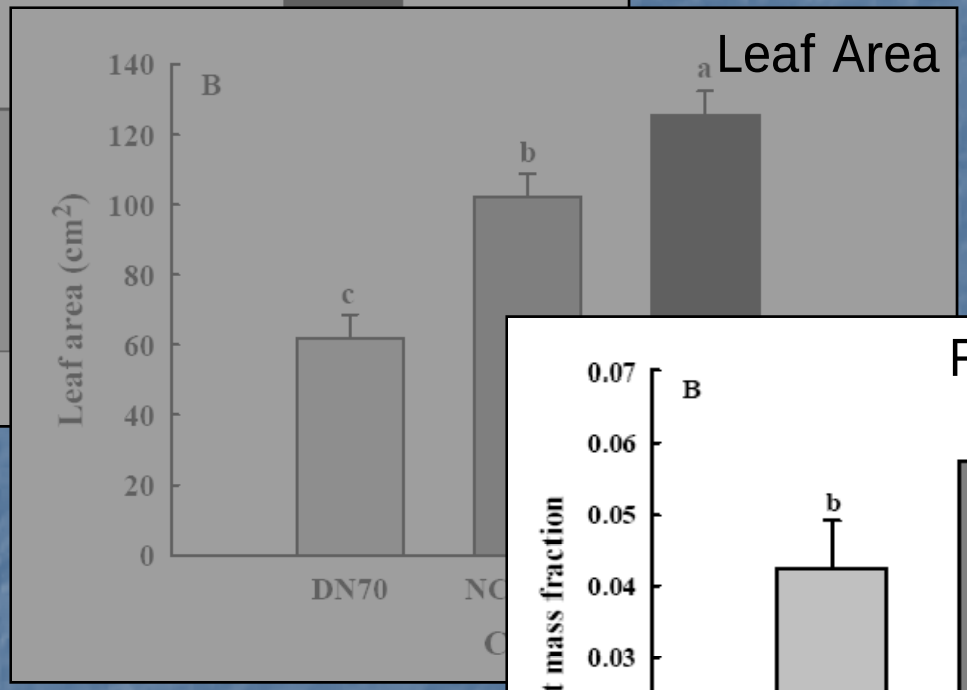


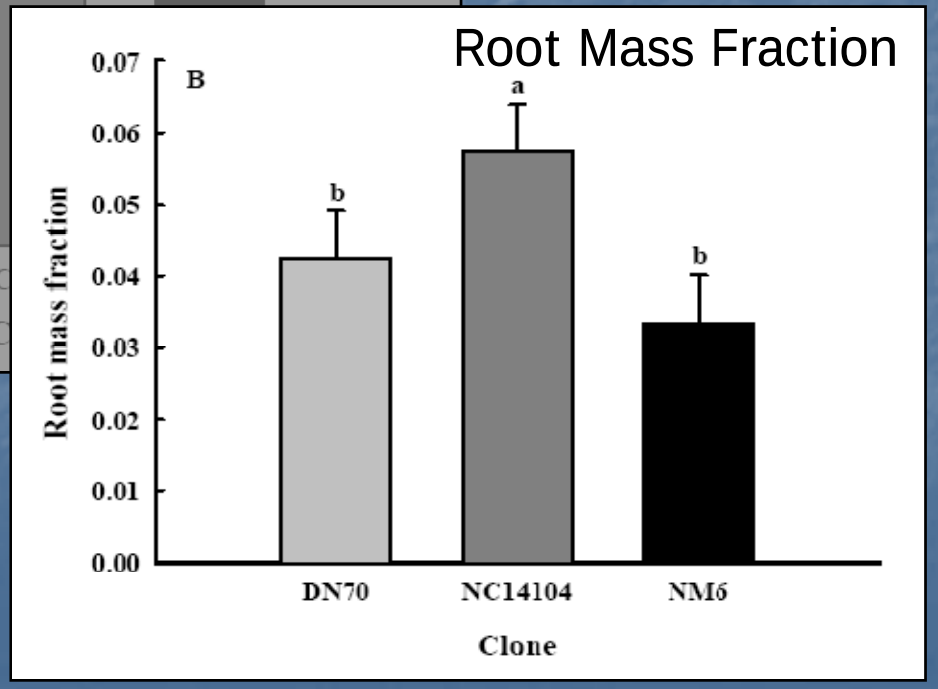
Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Clonal Differences



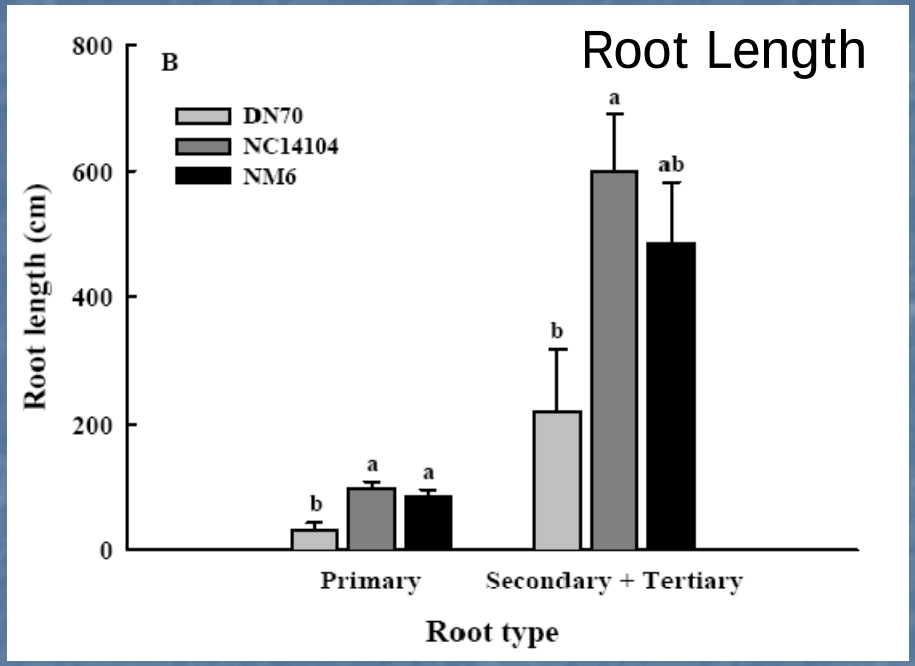




Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

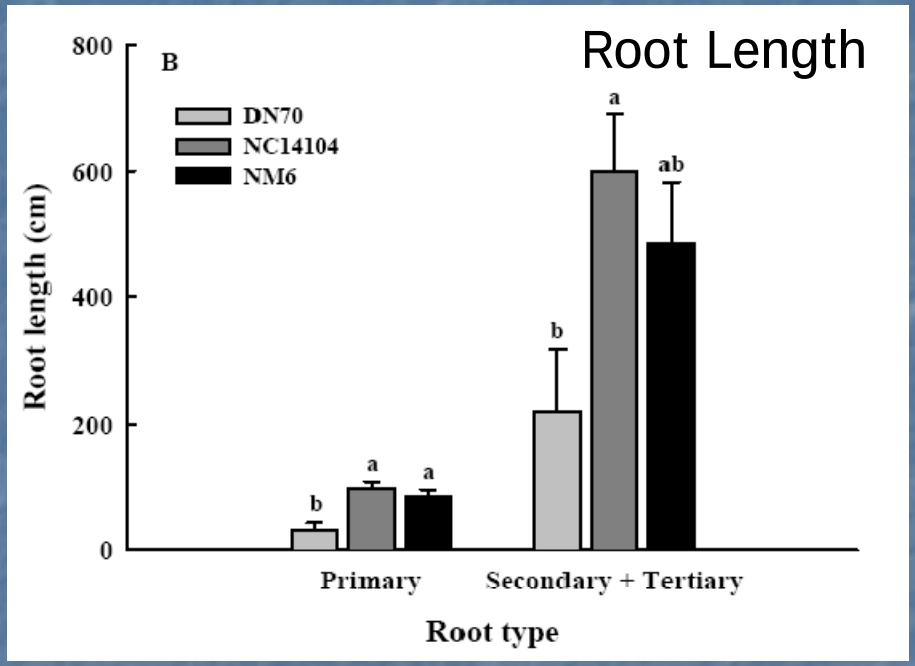
Clonal Differences

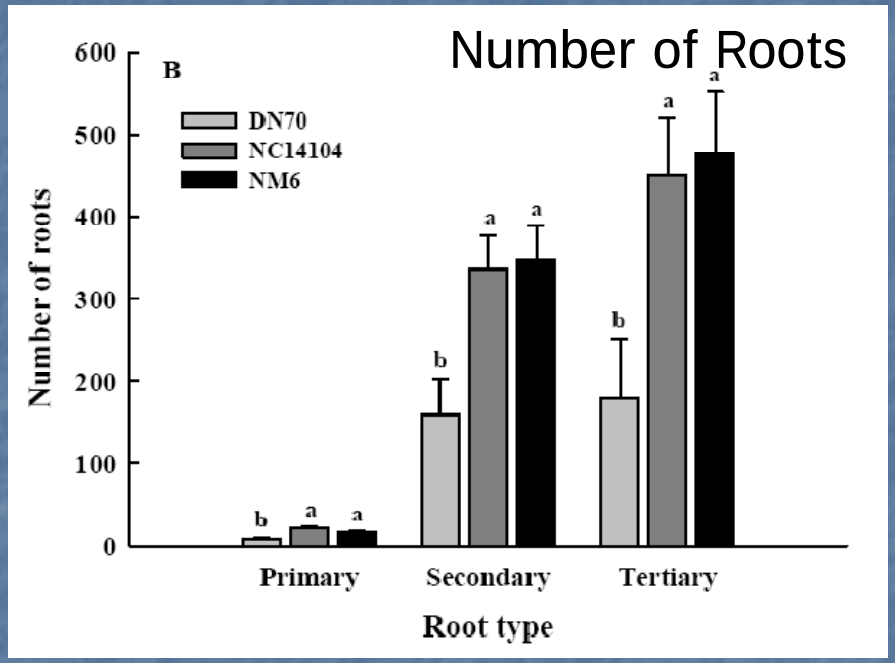


Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Clonal Differences

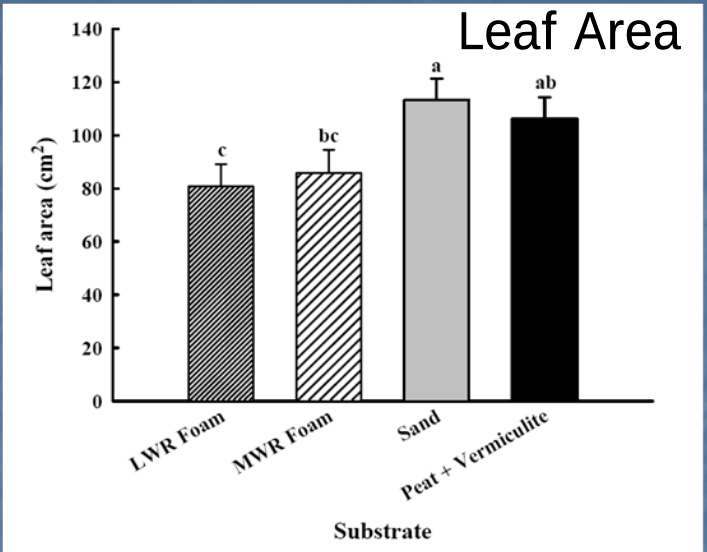




Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

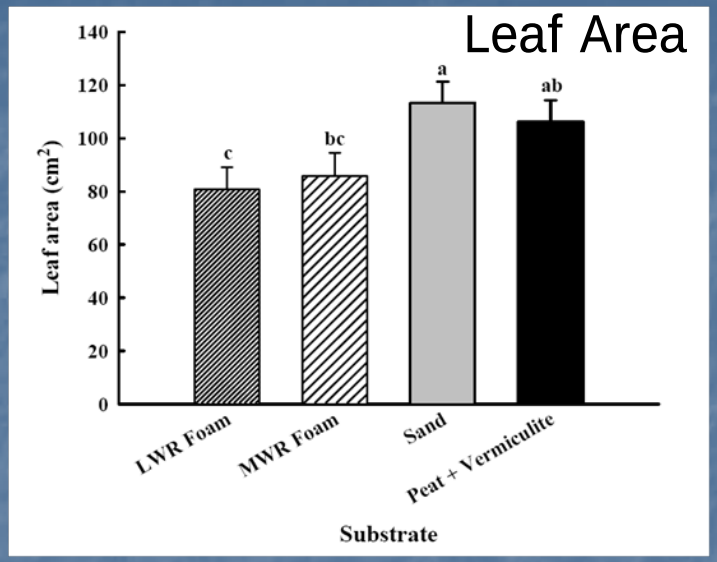
Substrate Effects

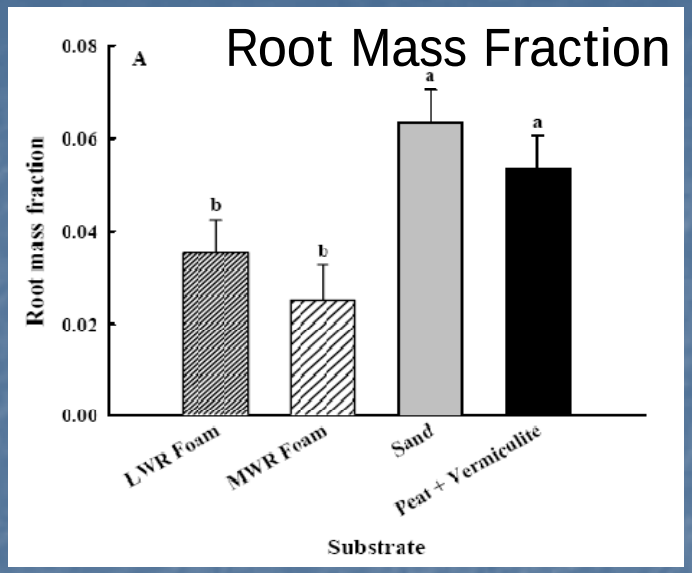


Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Substrate Effects

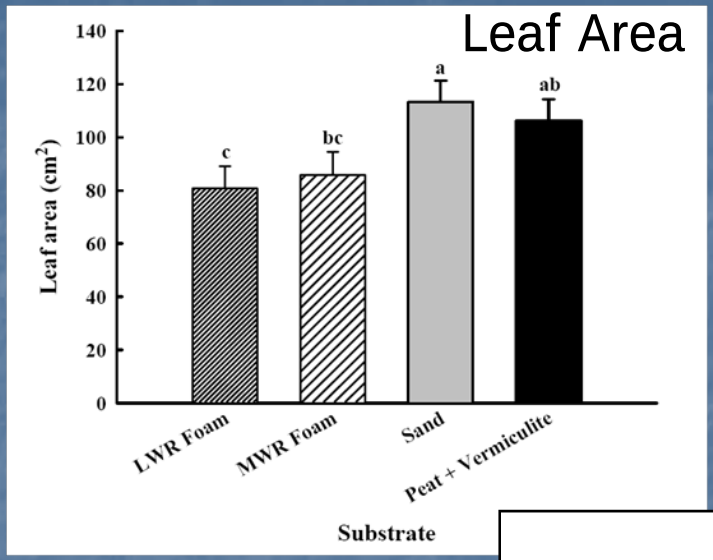


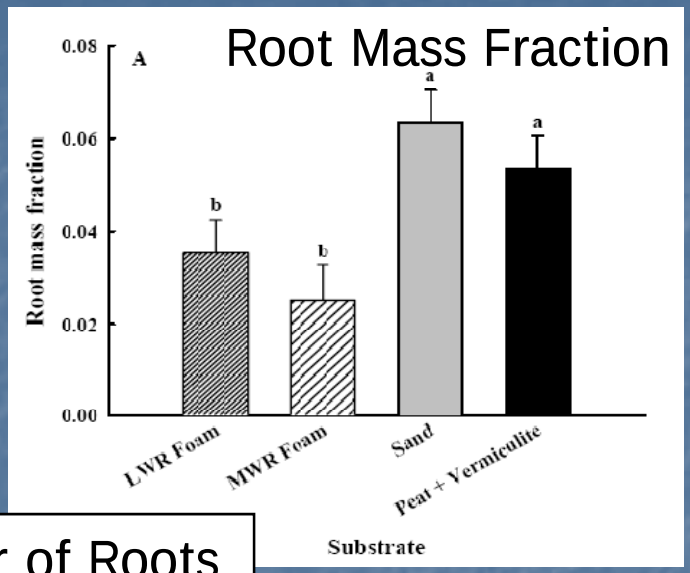


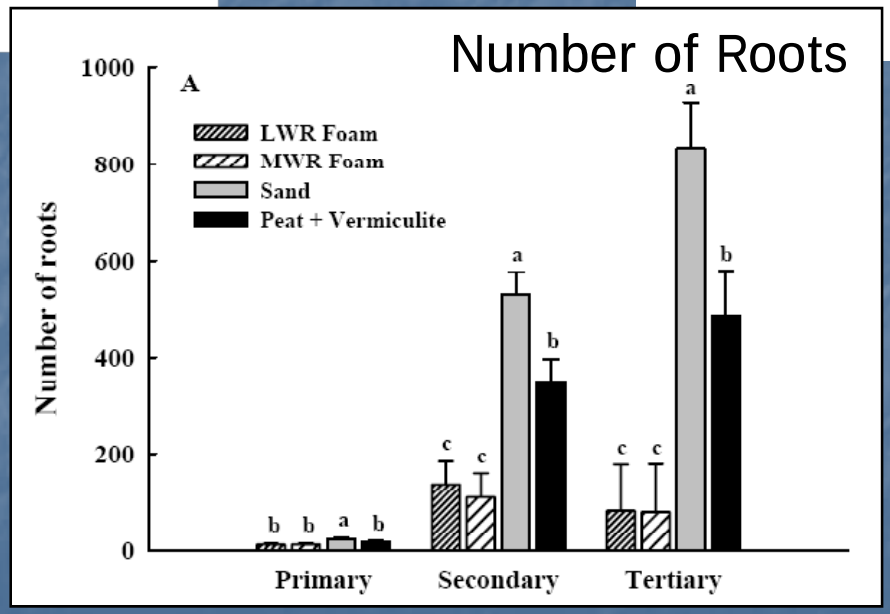
Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Substrate Effects



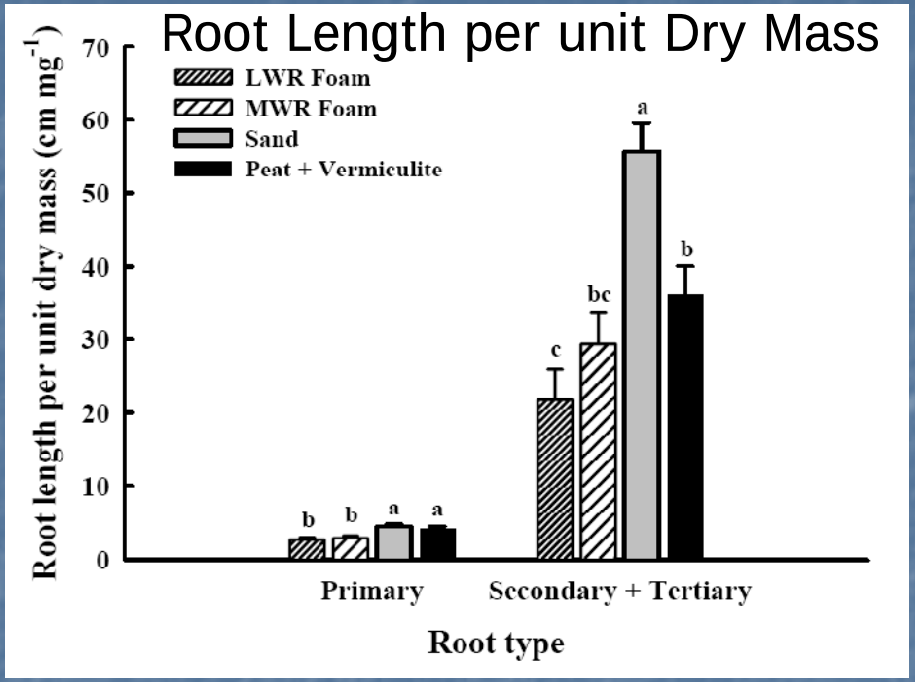




Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

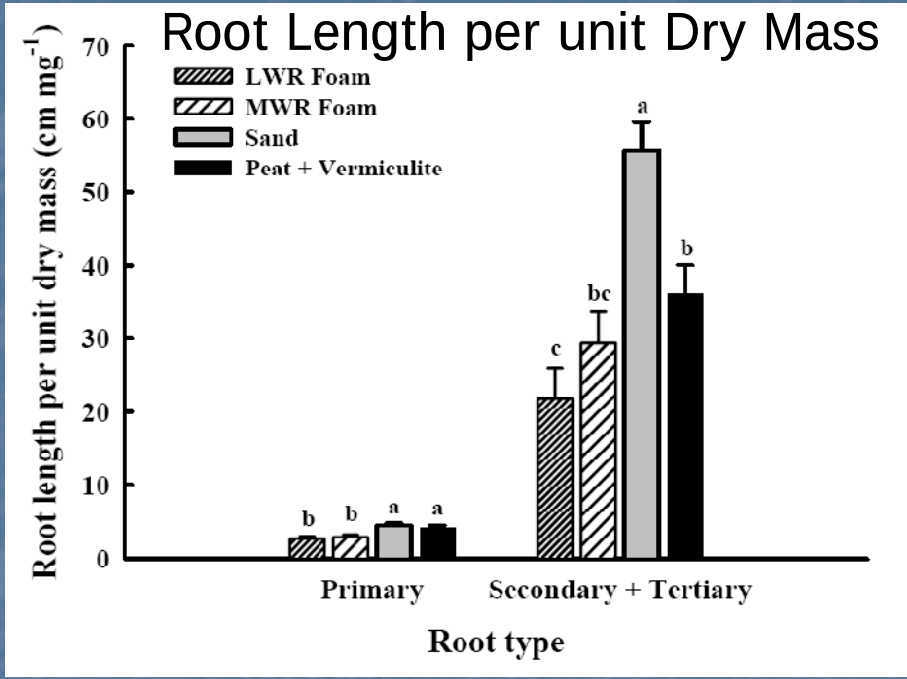
Substrate Effects

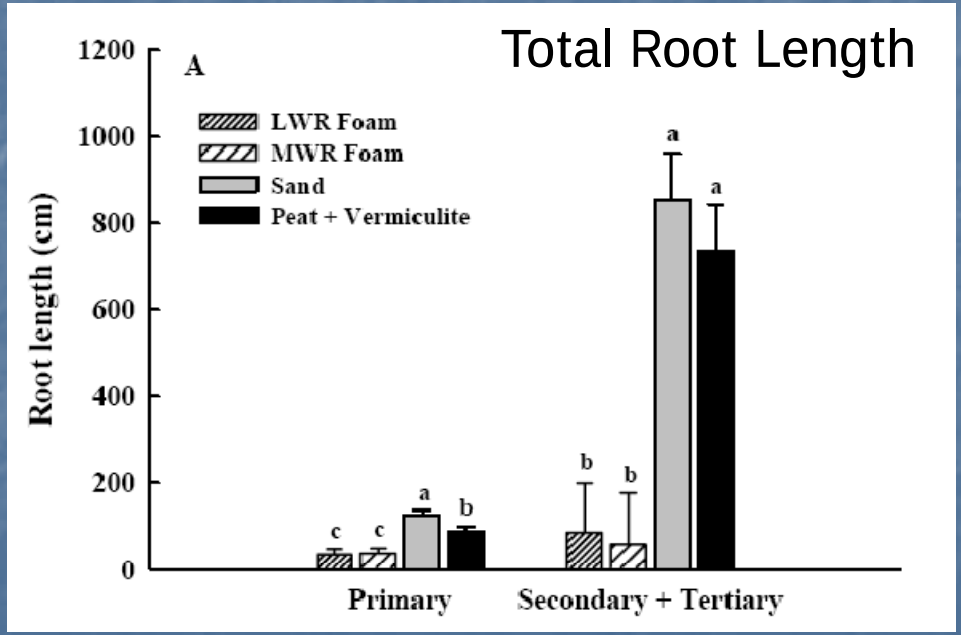


Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Substrate Effects





Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

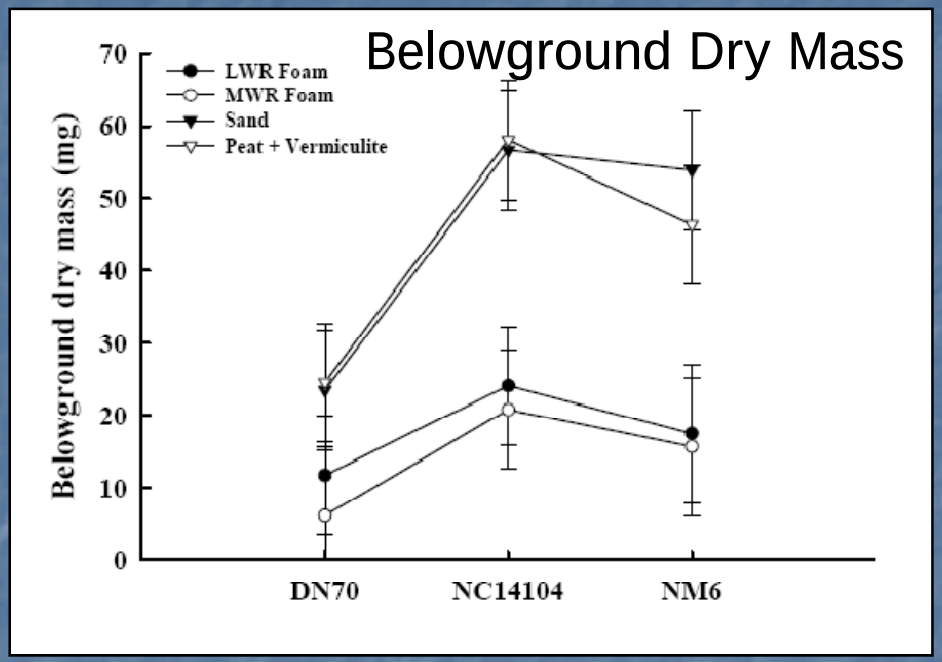
Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Clonal Ranking by Substrate

Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

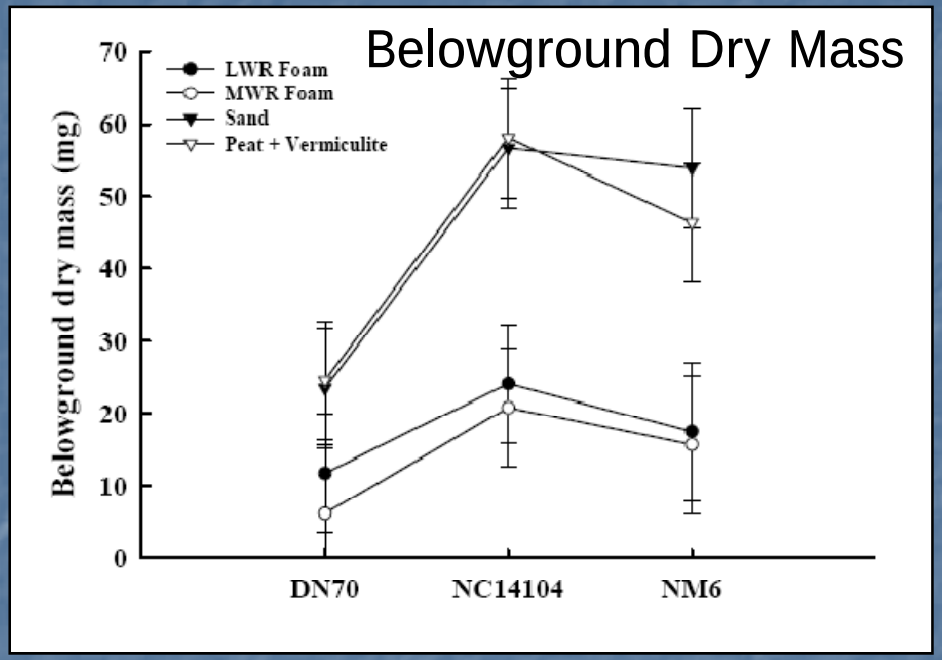
Clonal Ranking by Substrate

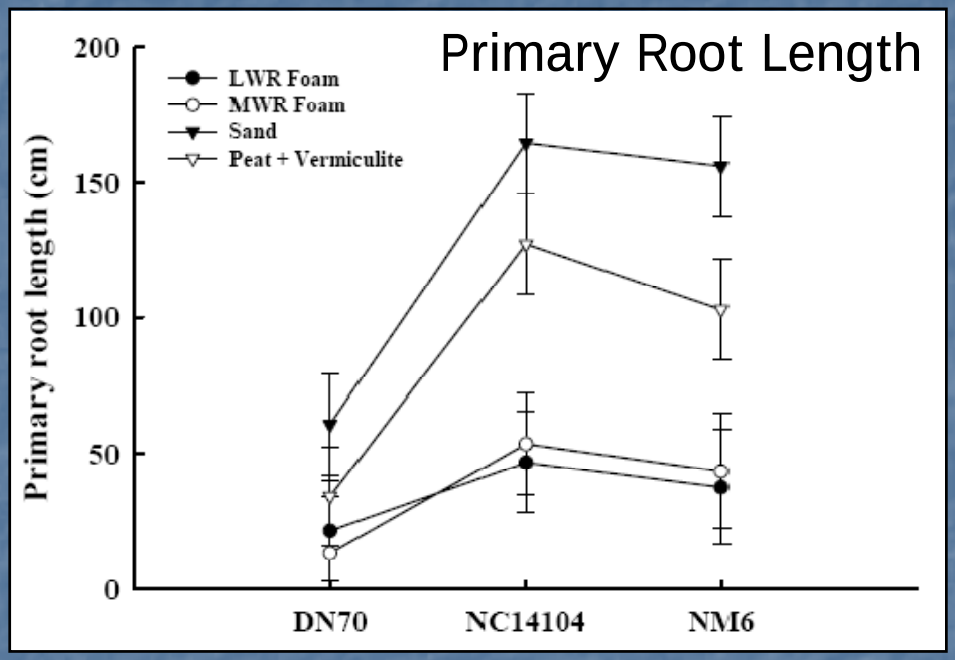


Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Clonal Ranking by Substrate





Clonal Rankings Between Engineered Substrates and Soils are Generally Consistent

Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Conclusions from Poplar Studies

- Poplars will root and grow, from cuttings, in our engineered substrate materials.
- Root morphology differences were observed between plants grown in “soil” and those grown in engineered substrate.
- Clonal rankings in most root metrics were preserved across substrates.

Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Next Step for Poplar Studies

- Experiment is being repeated.
- Addition of nutrients to growth in engineered substrate.
- Results in engineered substrate will be compared to same clones in 1 and 2 year field tests.
- Test ability to predict wind firmness in plantation-grown poplar.
- New substrates will be tested.

Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

Other Interesting Results...

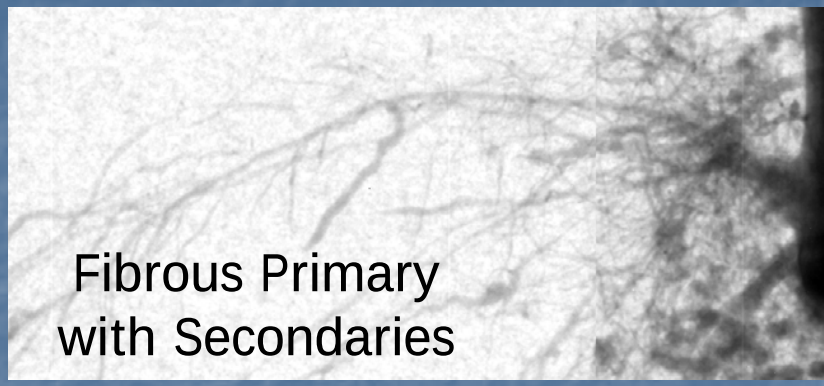
- Growth dynamics can be measured.
- Compare daytime vs. nighttime growth.
- Detailed morphology studies.

Data generated in collaboration with:
Dr Alex Friend, Northern Research Station
US Forest Service, Houghton, MI

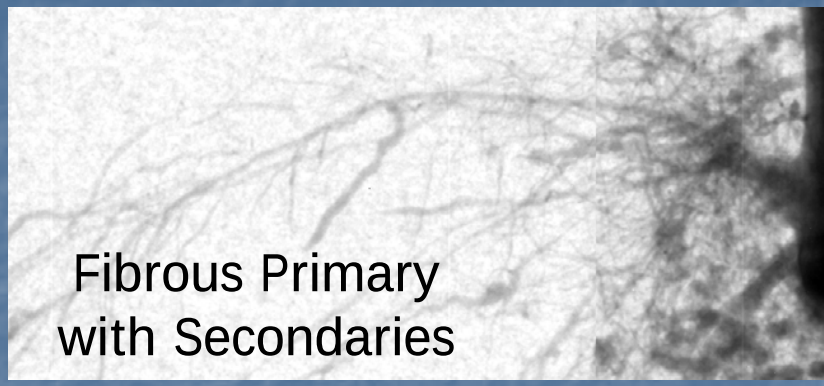
Funded by: US Department of Agriculture
SBIR Phase I
Small Business Innovation Research Program

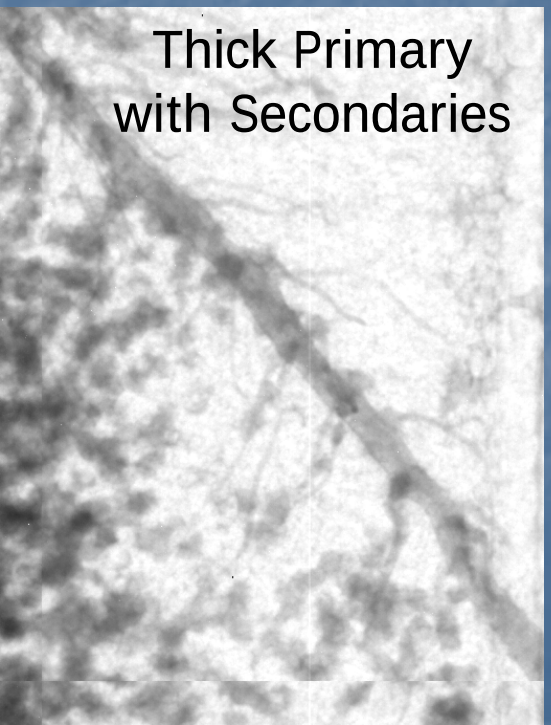
Distinct Morphotypes of Roots

Distinct Morphotypes of Roots

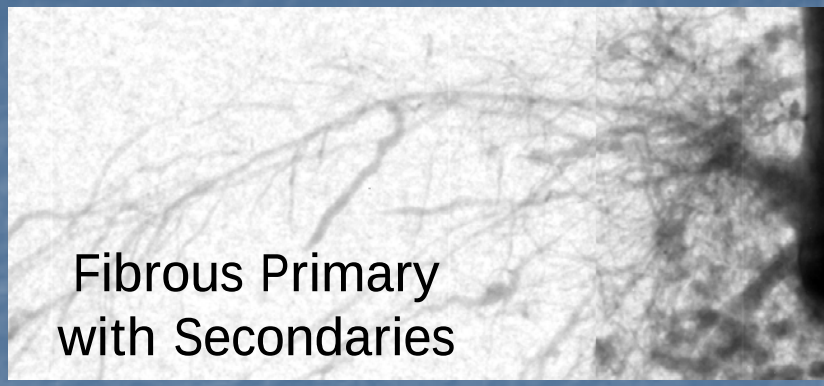


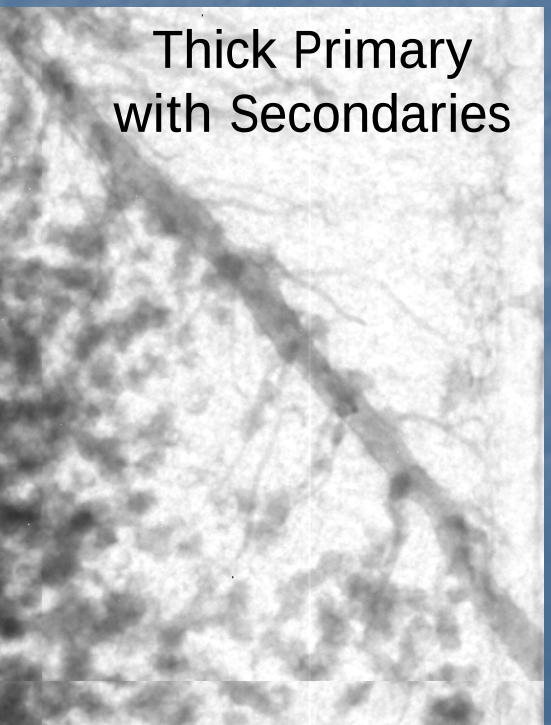
Distinct Morphotypes of Roots





Distinct Morphotypes of Roots

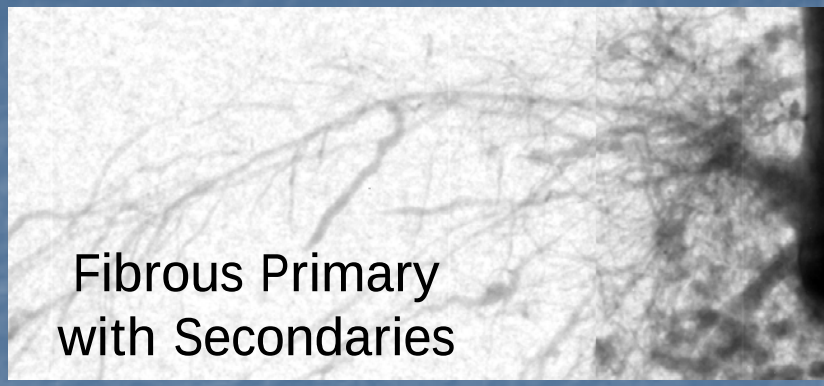




Distinct Morphotypes of Roots

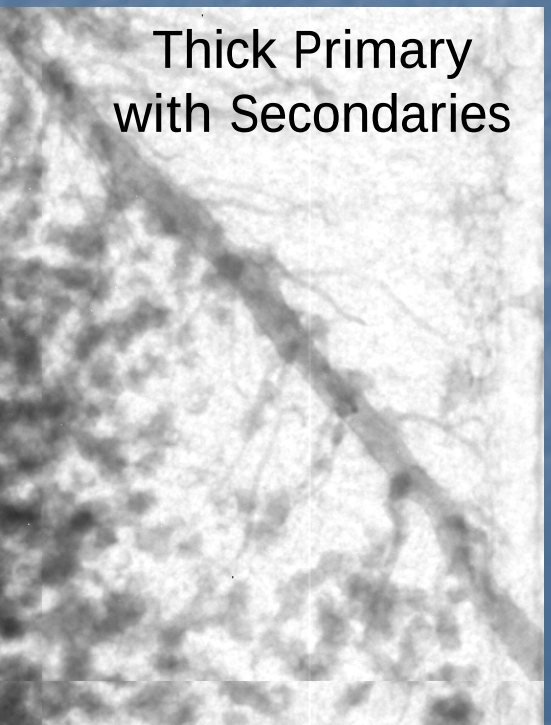
Naked Pioneer

Fibrous Primary
with Secondaries

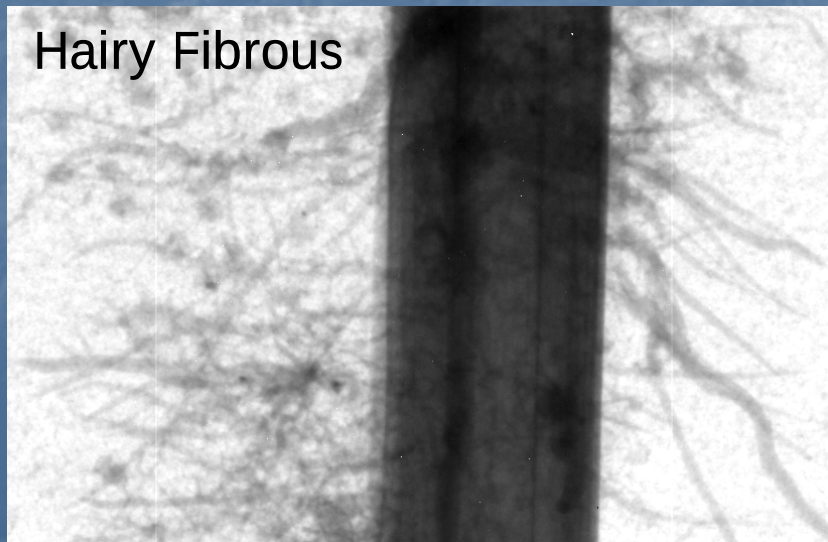


Spider Cluster

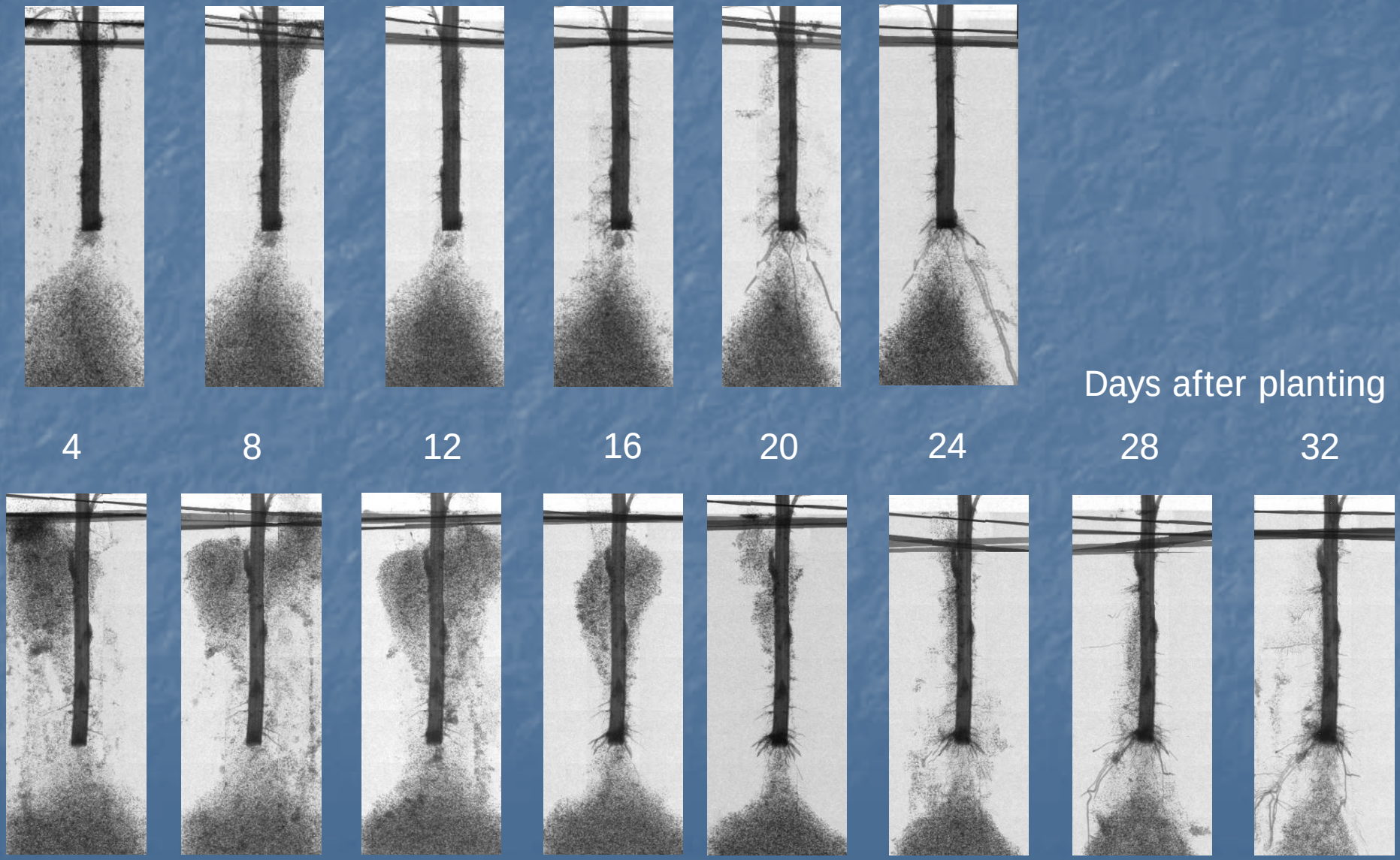
Thick Primary
with Secondaries



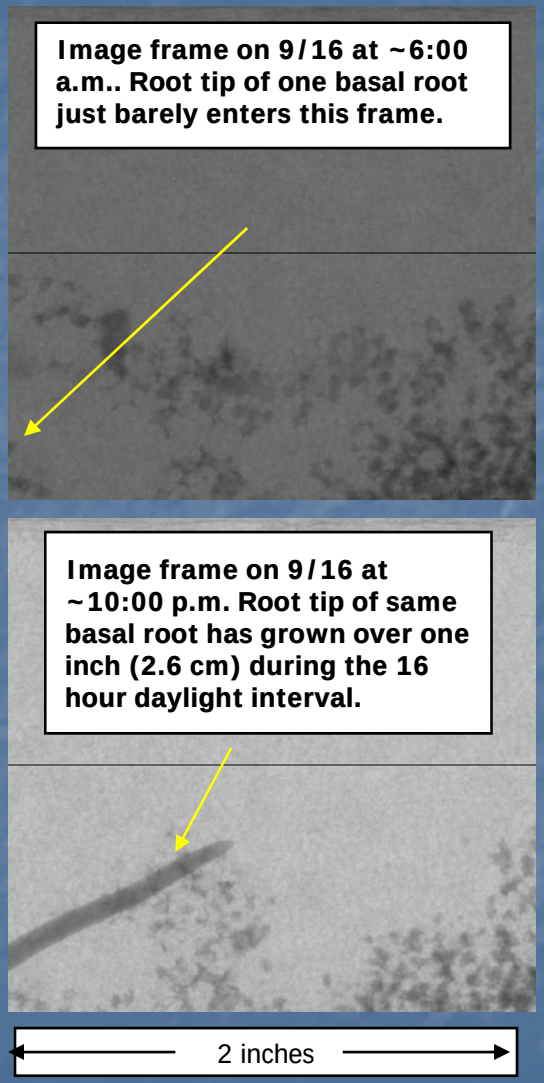
Hairy Fibrous



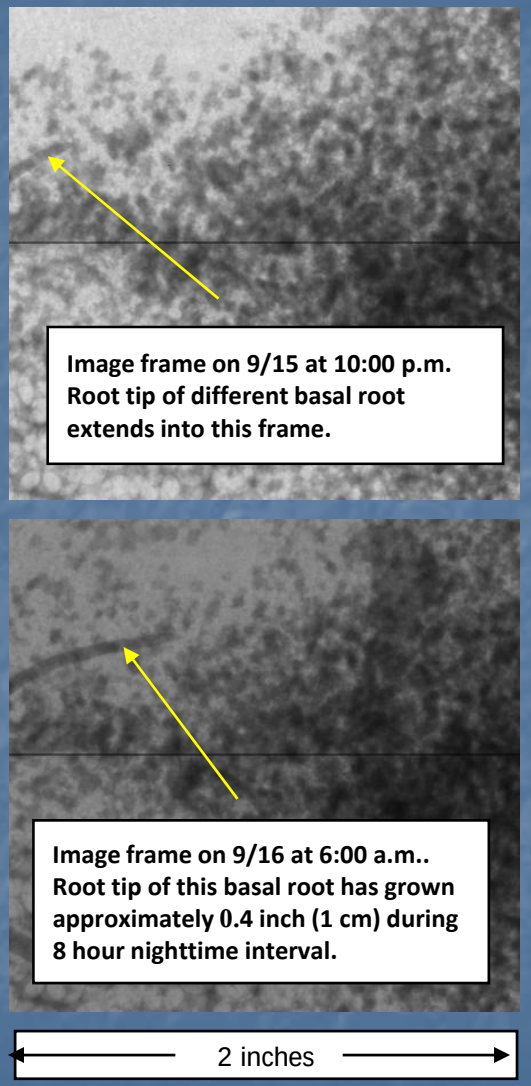
Root Growth Delayed after Planting



Root Growth Occurs Day & Night



16 hr Daytime Growth



8 hr Nighttime Growth

RootViz Application Areas

- General Root Studies
- Root Architecture
- Root Response to:
  - Nutrients
  - Water
  - Symbionts
  - Pathogens
- Phenotypic Plasticity of Root Structures
- Genetic Transformation Effects
- Functional Genomics

Acknowledgements

Work funded by:

United States Department of Agriculture
Small Business Innovation Research Program

USDA Forest Service
North Central Research Station,
Houghton, Michigan, USA — Alexander L. Friend
Joe Powell
Rhinelander, Wisconsin, USA — Ronald S. Zalesney

University of Tennessee
Department of Mathematics
Chattanooga, Tennessee, USA — Christopher P. Mawata

Phenotype Screening Corporation
10233 Chapman Highway
Seymour, TN 37865
www.phenotypescreening.com — Ron Michaels
(865) 694-9459 — Dan McDonald
bobk@phenotypescreening.com — Bob Kodrzycki