

Enhancing plant growth and stress tolerance through use of fungi and bacteria that comprise plant microbiomes

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Jim White
Rutgers University
New Brunswick, New Jersey
Email: white@rci.rutgers.edu

Hypothesis of microbiome functions!

1. Defensive properties (defense from biotic and abiotic stresses)
2. Nutritional properties (provide nutrients to support plant growth and development)

Defensive Function of Microbiomes

Epichloë/Neotyphodium Endophytes

The endophyte is systemic in grass plants: leaves, culms and seeds.

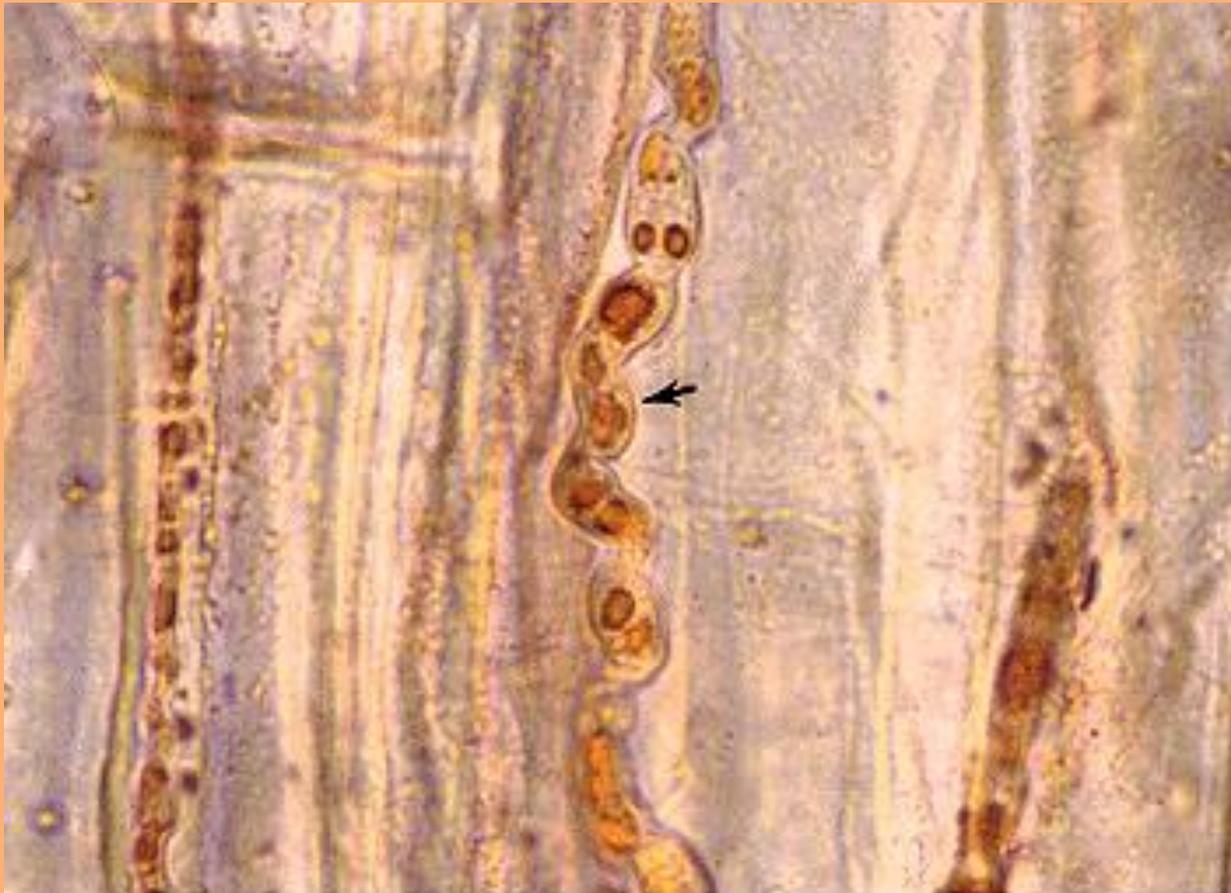


Fig. 1. Convoluted mycelium in culm of grass *Festuca arundinacea* (arrow; X 2,500)

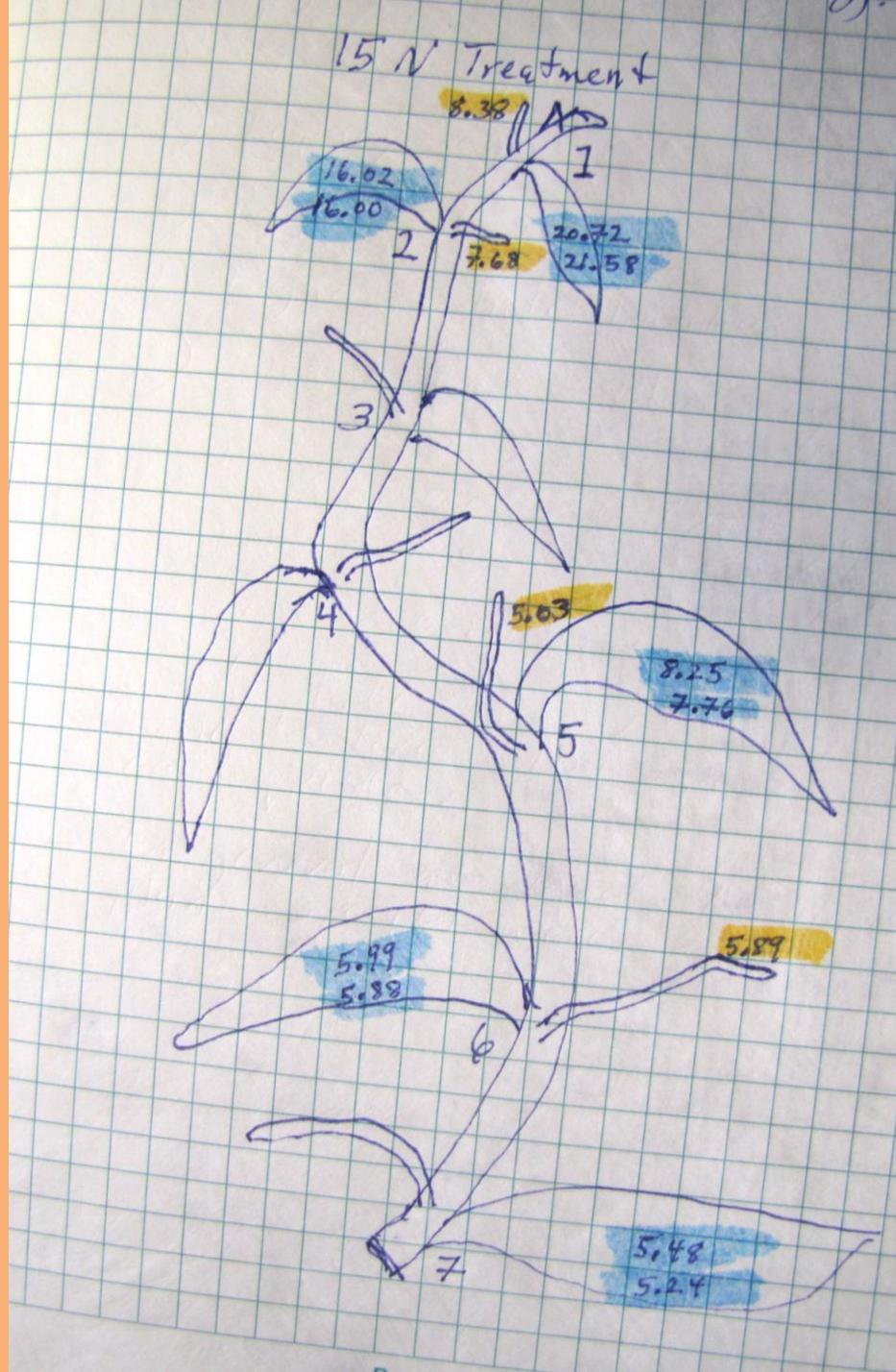
Nutritional function of the microbiomes

Vanilla Orchid $^{15}\text{N}_2$ Experiment



15 mls $^{15}\text{N}_2$ injected into 5 liter chamber over 7 day period.

Nitrogen Movement



Non-15N2 Controls

Node 1: 1.98 root
4.16 leaf

Node 2: 2.31 root
3.86 leaf

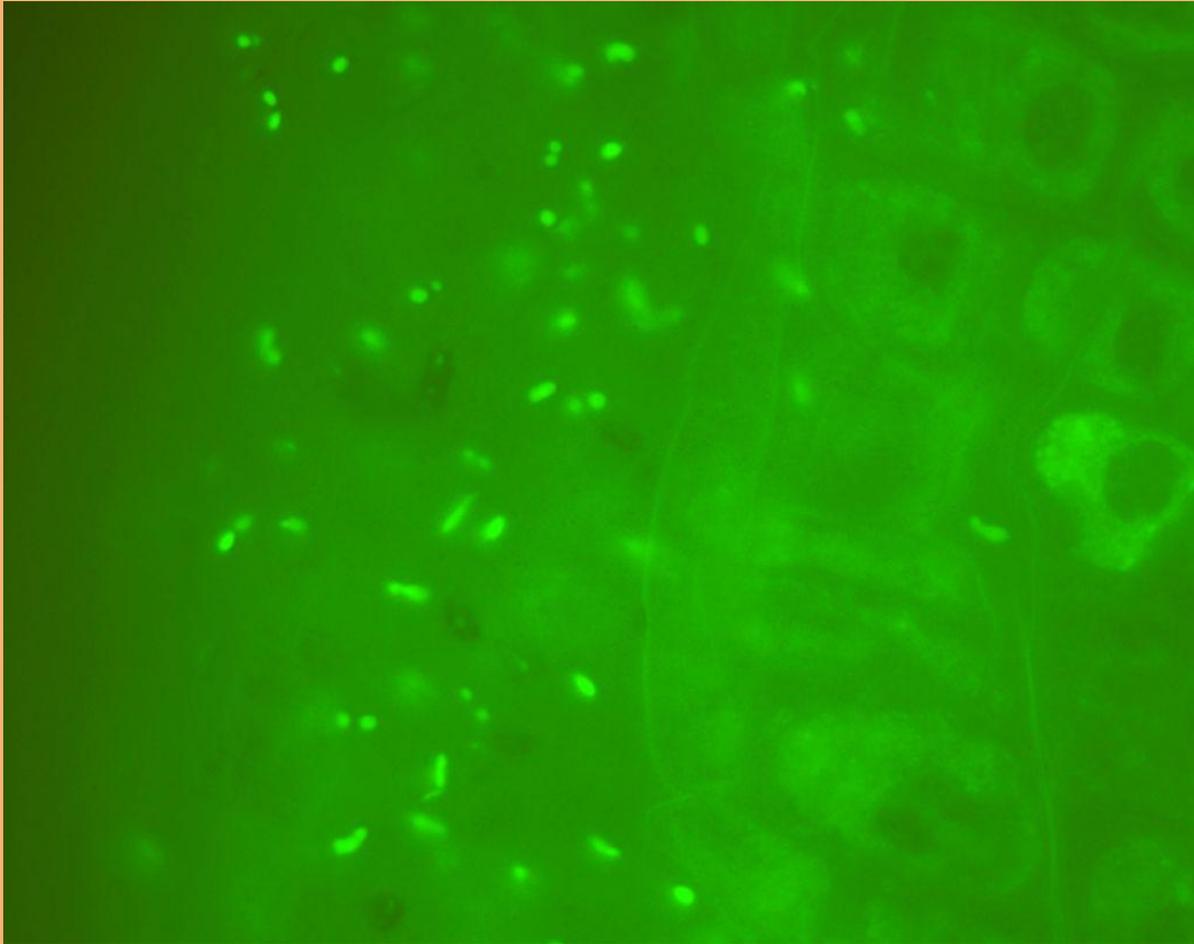
Node 3: 3.41 root
2.57 leaf

Node 4: 2.45 root
2.90 leaf

Shoot Meristem



Layers of bacteria around meristematic tissues



Japanese honeysuckle (*Lonicera japonica*; Caprifoliaceae) study

An invasive high climber that is difficult to control.
Introduced into New York from Asia as an ornamental
in 1806.



Do microbes contribute to the growth
and invasive capacities of Japanese
honeysuckle?

15N2 Gas Assimilation Experiment on Excised Tillers of Japanese Honeysuckle*

Treatment	Sample wt (mg)	Total % N	Delta 15N vs Air	Atoms %15N
1. Air	2.132	3.11	1.53	0.367029
	2.616	3.07	1.60	0.367056
2. Air	2.725	2.77	1.72	0.367099
	2.857	2.79	1.97	0.367192
3. Air	1.898	3.12	1.55	0.367037
	2.455	3.24	1.54	0.367033
4. 15N2 gas	2.046	4.47	36.01	0.379618
	3.019	5.15	38.95	0.380691
5. 15N2 gas	2.499	3.99	72.31	0.392868
	2.508	4.05	71.64	0.392623
6. 15N2 gas	2.713	4.11	47.71	0.383890
	2.699	4.03	45.55	0.383099

*30 mls 15N2 gas injected into sealed 1L jar containing plant tillers and incubated for 1 wk.

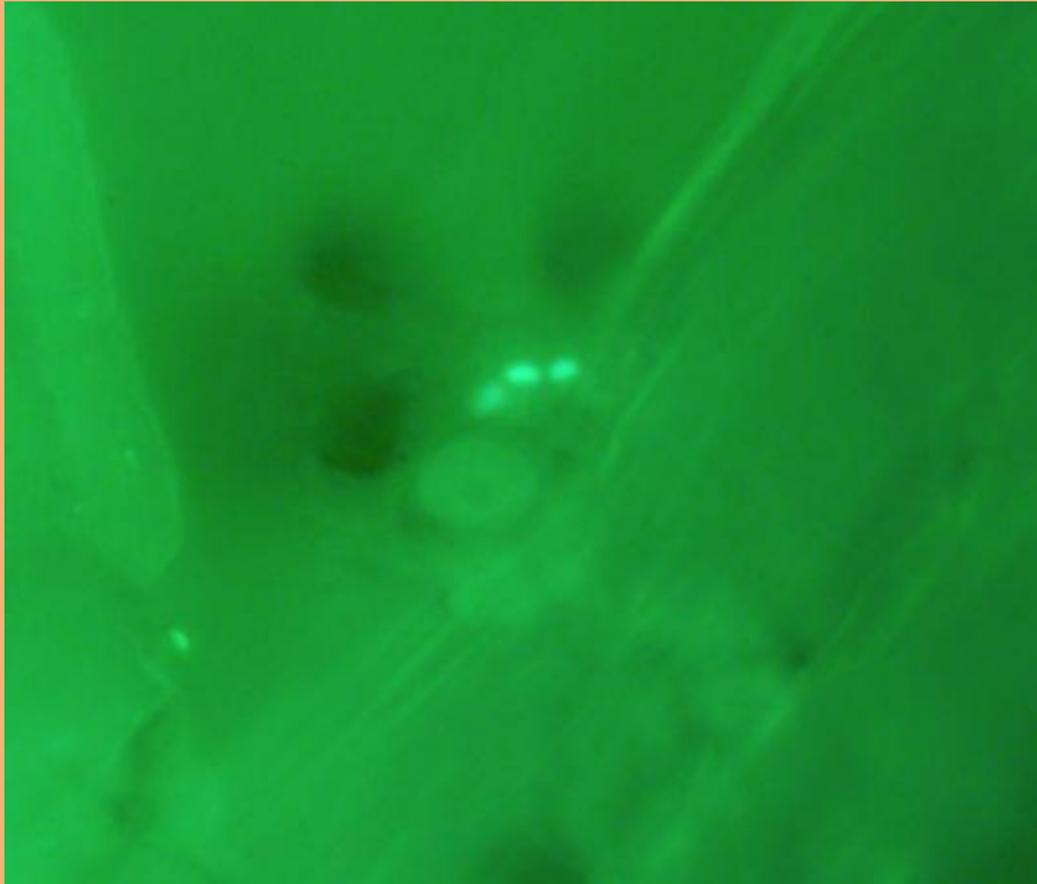
Trichomes cover leaves and stems



Close-up of leaf (stained with Syto9, a fluorescent nucleic acid stain)

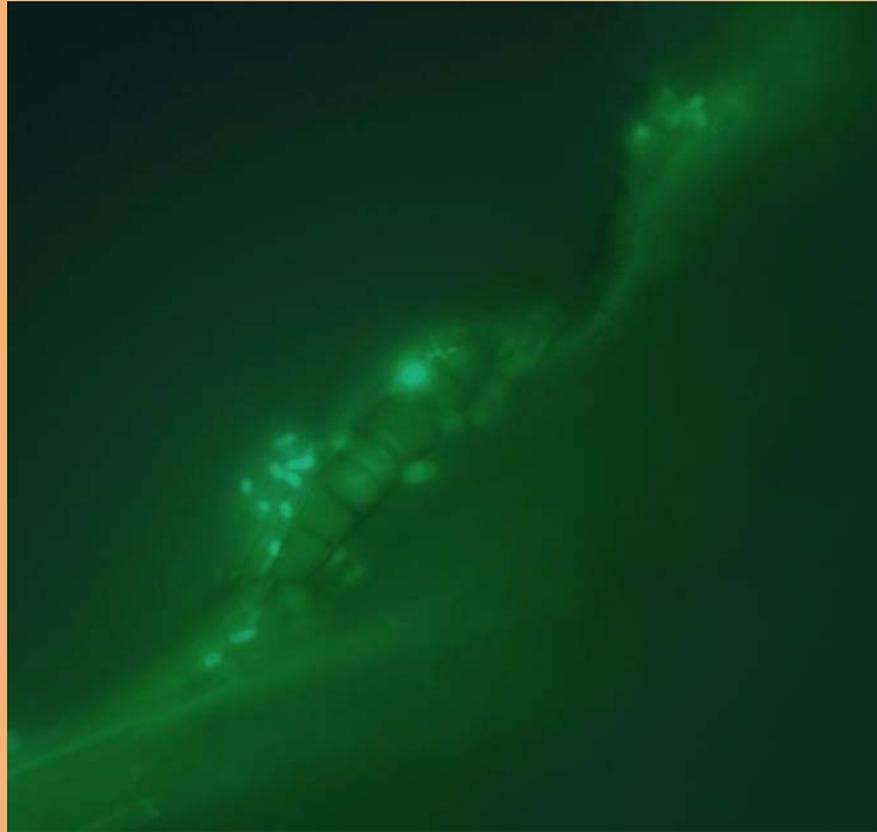


Close-up of epidermis shows fungus *Aureobasidium pullulans* with bacteria in apparent association.



Aureobasidium pullulans has been found as an epiphyte and endophyte in many plant species.

Aureobasidium with associated bacteria in and apparent fungal-bacterial biofilm



Aureobasidium pullulans has already been shown to have potent biocontrol potential to control post-harvest diseases of fruits. This work raises the possibility that it may also benefit plants nutritionally.

Aureobasidium with bacteria.

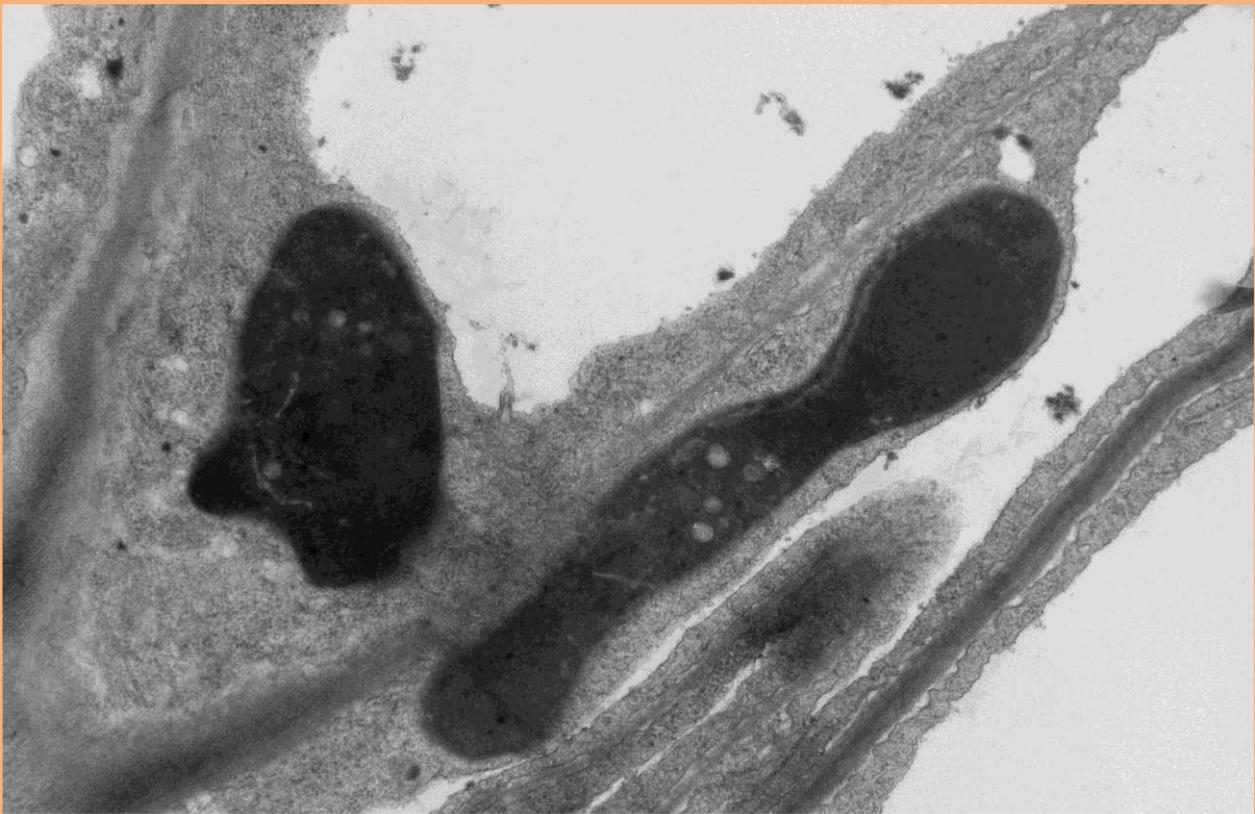


Dahlia variabilis (Asteraceae)

(Study by Richard Chen)

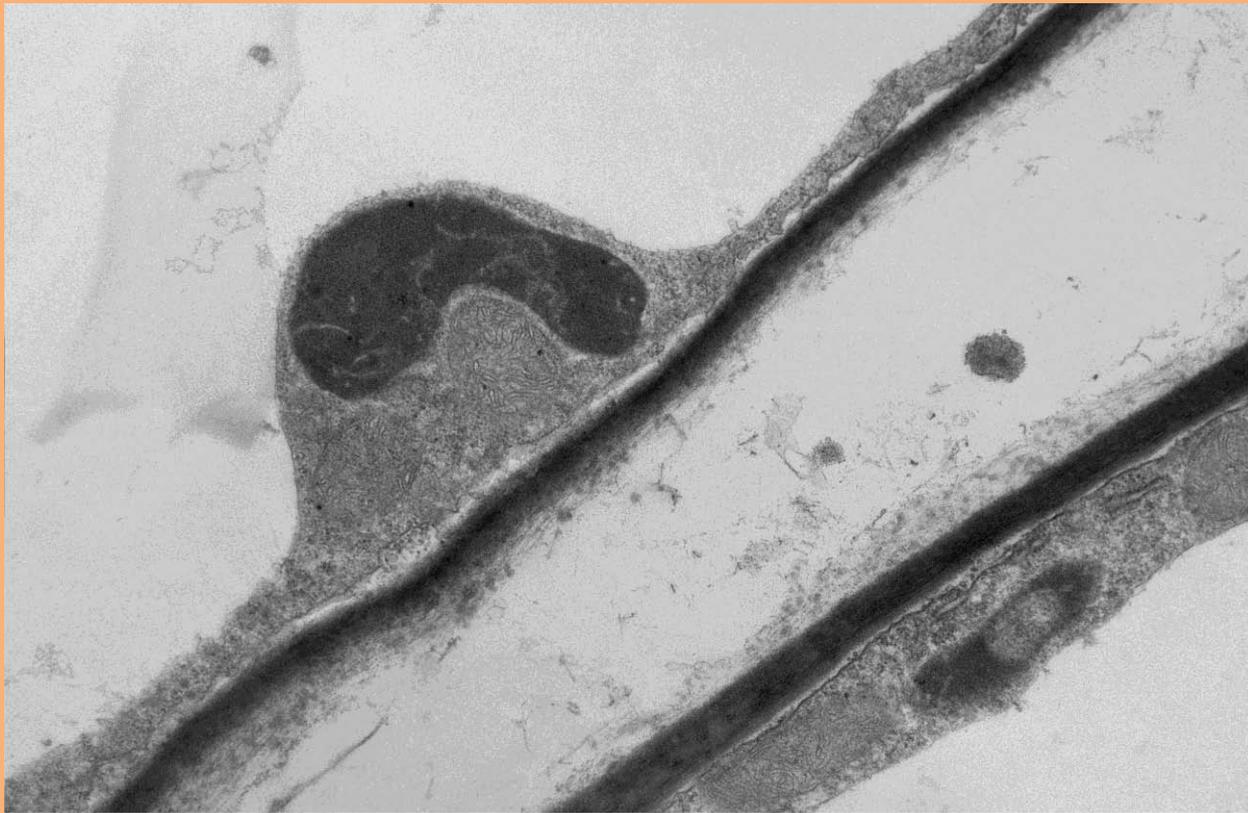


Dahlia seedling root ultrastructure



Apr. 11 2013_040_Dahlia 003_Mag_10000
Dahlia 003
2:18:35 PM 4/11/2013
TEM Mode: Imaging

500 nm
HV=80.0kV
Direct Mag: 10000x
X: na Y: na T:
AMT Camera System

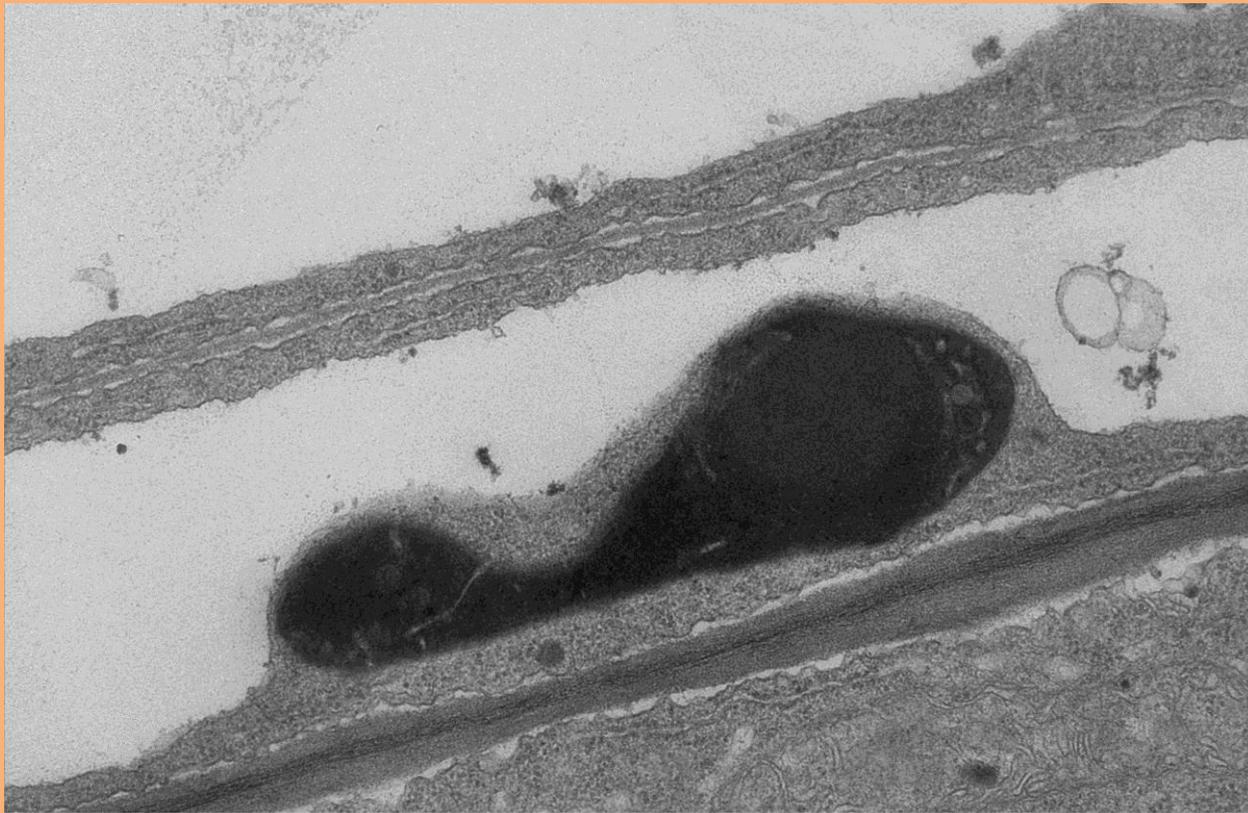


Apr. 11 2013_045_Dahlia 008_Mag_8000
Dahlia 008
2:30:47 PM 4/11/2013
TEM Mode: Imaging

500 nm
HV=80.0kV
Direct Mag: 8000x
X: na Y: na T:
AMT Camera System

Endospores evident in many bacteria





White Lab_028
Dahlia-4
11:35:03 AM 4/2/2013
TEM Mode: Imaging

500 nm
HV=80.0kV
Direct Mag: 13000x
X: na Y: na T:
AMT Camera System

Intracellular bacterium with flagellum



Defensive and nutritional associations

- Common on/in plants in natural situations
- Work needed to evaluate benefits to plants
- Microbes may be used to enhance crop growth

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