

**Utilization of microbial endophytes on crop plants and trees as mechanism to increase biomass, enhance plant fitness and survival in stressed habitats**

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Adaptive Symbiotic Technologies  
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- **Overview** of symbiosis -of microbial endophytes (bacterial and fungal)
  - **Symbiosis is a good fit** in the biological sciences and classroom
  - **Symbiosis has many positive applications for agriculture and forestry**
  - **Has direct application to Forest Bioresources:**
1. use of plant-microbe symbiotic systems can be applied directly to increasing bioenergy
  2. improve the efficiency and lower the cost of biofuel production by promoting growth enhancement in plants resulting in enhanced biomass
  3. growth on poor soils or in harsher environmental conditions so that we reserve the high quality land for food agriculture.
  4. Increasing water use efficiency
- other examples:
- improve plant health and stress tolerance ( cold, salt, drought)
  - increase biomass, decrease lignin, enhance biofuel (bioethanol) production
  - grow plants in unfavorable soils (oligotrophic, diazotrophic)
  - decrease use of chemical fertilizers (soil and water contamination)
  - Crop disease protection without using harmful chemicals (pesticides and fungicides)
  - Phytoremediation of soils (orgnaic pollutants, heavy metals)
- **Best way to demonstrate positive benefits via symbiosis** is by focusing and presenting some of my research:
- Centered around plant-fungal microbial symbionts (potential application and ubiquitous nature of symbionts)

Symbiosis: A close association between two different species (Anton de Bary 1879).

•An intimate association between two or more organisms

**A fundamental aspect of biology**

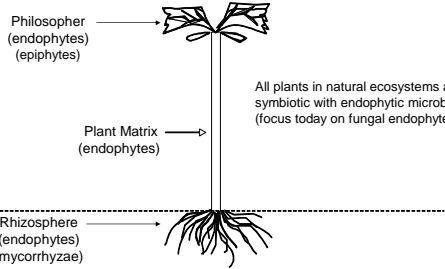
- All complex life on earth is symbiotic on one level or another
- important for health and survival of their host
  - eg. human bacterial micro-flora in the intestines
  - eg. fungal endophytes in plants growing in geothermal soils

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.



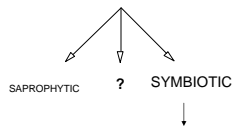
Symbiosis is a fundamental pattern and necessity for life on earth  
Should be included in any biological based curriculum

**Plants are Communities with Several Habitat Zones**



•Fossil records indicate that fungi have been associated with plants for >400 MY  
•Symbiosis may be responsible for the movement of plants onto land  
(Pirozynski & Malloch, 1975; Krings et al., 2007)

**FUNGAL LIFESTYLES**



Symbiotic Lifestyle	Benefit/Impact	
	Host	Symbiont
Competition	-	-
Amensalism	-	0
Parasitic	-	+
Neutralism	0	0
Commensalism	0	+
Mutualism	+	+

- = decreased fitness 0 = fitness not affected + = fitness increased

[D.H. Lewis (1985) The Biology of Mutualism]

**Fungal Symbionts**  
•Mycorrhizae - restricted to roots and grow out into rhizosphere.

- Endophytes - reside entirely within plant; capable of colonizing root, stem, and/or leaf tissues.
- Class 1 endophytes: a relatively small number species that are limited to a few monocot hosts.
- Class 2 endophytes: a large number of species with broad host range (monocots and eudicots) Has not been well defined**
- Class 3 endophytes: restricted to leaves
- Class4 endophytes: Mysterious DSE
- Dark Septate Endophyte**  
Little host and habitat specificity  
Conidial/sterile inhabitants of many terrestrial plants  
Boreal and temperate forests  
Fine roots of trees and shrubs, especially conifers  
Mycelia on healthy fine roots = not pathogenic  
(Trappe 1998)

**Mycorrhizal Fungal associations:** Interactions with plant roots.  
Growth restricted to the roots and/or grow out into the rhizosphere.

- 90% terrestrial plants have this association
- 6000+ species of all fungal phyla (Zygo, Asco & Basidiomycota)
- 240,000+ plants (Includes forests and agricultural crops)

•**Benefits:**  
plants increase growth, nutrient exchange/uptake (ie., <80% phosphorus, <25% N2 (plant) and <20% of total plant carbon budget (fungus), enhanced water acquisition and disease resistance

Associations critical determinant in plant ecosystem health, biodiversity, ecosystem variability, and community productivity.  
(Johnson et al., 1997 New Phytol.)

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

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Barley +pathogen  
-/+ Mycorrhizal fungus

**2 main distinctions in Mycorrhizal Fungal associations:**

1. Endomycorrhizal: Hyphae penetrate the roots and establish intracellular symbiosis  
 -Can be ericoide (single cortical cell penetration) or many, mycelial network, large asexual spores.  
 -AM and VAM (*Glomeromycota*), AM+branched haustoria; VAM=storage vesicles (Zygo)
2. Ectomycorrhizal: Hyphae remain extracellular (Evo-newer),  
 -Produce a mantle to cover root cells and  
 -a labyrinthine - extracellular mycelial network

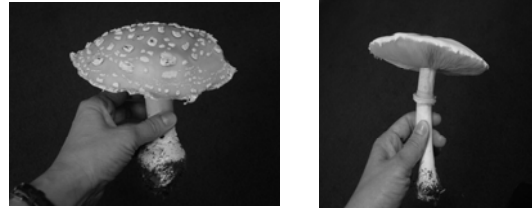


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AM - Endo

Ecto

**Beautiful yet deadly Ectomycorrhizal fungal fruiting bodies**



**Delicious fungi:  
Chanterelle and Boletus**

**Another group of Fungal Symbionts**

- **Endophytes - reside entirely within plant; capable of colonizing root, stem, and/or leaf tissues.**
  - Class 1 endophytes: a relatively small number of fastidious species that are limited to a few monocot hosts.
  - Family: Clavicipitaceus (order-Hypocreales; Phylum-Ascomycota)
    - i.e.,well studied cool season grass endophytes (*Epichloe/Neotyphodium*/tall fescue system)
    - Enhance fitness, drought, disease and pest tolerance
  - Endophytic mycelium does not colonize roots
  - Biotrophic, horizontal transmission, not easy to culture/colonize plants
  - Make secondary metabolites: lolines, ergot alkaloids, lolitrems toxic to insects and animals (White, Clay, Schardl, Bacon)
- **Class 2 endophytes:** a large number of species with broad host range including both monocots and eudicots,
  - Abiotic and biotic stress tolerance
  - Phylum-Ascomycota, subphylum-Pezizomycotina
  - horizontal transmission, secondary metabolites?
  - has not been well defined

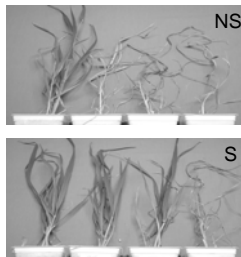
**Host Fitness Benefits Conferred by Mutualistic Fungi**

- Disease Resistance
- Drought Tolerance
- Metal Tolerance
- Herbivore Resistance
- Growth Enhancement
- Temperature Tolerance

Biotic and abiotic stress tolerance through symbiosis  
 -apply to agriculture  
 -alternative to toxic chemicals

**Benefits via Fungal Symbiosis**

- Drought tolerance
- Disease resistance
- Growth enhancement
- Temperature and salt tolerance



O 12 15 18  
 Redman et al, 2002 Science, 2002 Symbiosis; Rodriguez et al.,2008 ISME-Nature

**Unique habitats**

**New concepts in plant-fungal symbiosis**

- Geothermal Soils
- Coastal Beach
- PNW, Utah, Korea
- Agricultural Arena



- Temperature
- Salinity
- Invasive Species
- Disease Pressure

- Habitat-Adapted Symbiosis
- Symbiotic Modulation
- Fungal Lifestyle Switching

Marquez et al., 2007. Science  
 Redman et al., 2002. Science  
 Redman et al., 2002. Symbiosis  
 Redman et al., 2002. Science

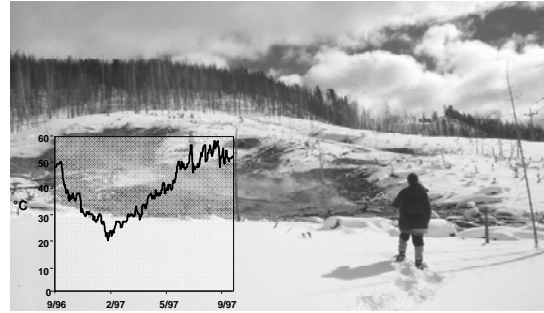
\*Nitrogen Fixing microbes from low nitrogen environments

**Approach is a simple one:** Mother Nature has done all the work already

- Go to habitats where we can identify the habitat specific stress
- Isolate microbial endophytes from plants thriving in that habitat
- The idea being, these endophytes will impart that habitat specific stress tolerance to host and/or surrogate plants (such as crop plants)

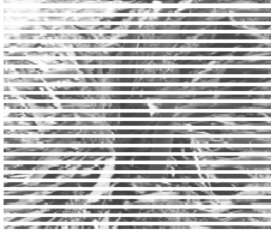
Example: endophytes from plants growing in cold or high salt habitats will impart cold and salt tolerance, respectively

Native Habitat #1: Geothermal Soils of Amphitheater Springs in Yellowstone National Park



Habitat Specific Stress: High Soil Temperatures

*Dichanthelium lanuginosum* (Tropical Panic Grass) Growing in Hot Geothermal Soils



All plants (N=200) in Yellowstone National Park were colonized with the same fungal endophyte: *Curvularia protuberata*

Fungus colonized roots, stem, leaves, seed coat but not the seeds.

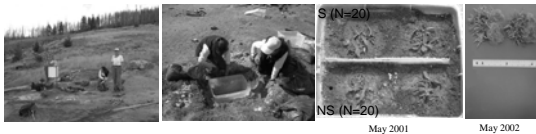
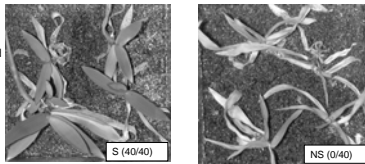
Have a system where we can tease apart the symbiont from the plant and test for function.

Geothermal Soil Simulator

Rodriguez et al., 2008. *ISME-Nature*  
Redman et al., 2002. *Science*

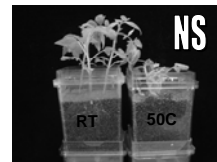
Temperature Exposure of *D. lanuginosum* Plants With (S) and Without (NS) *C. protuberata* (Cp4666D)

Cycles of 70°C for 10 hours followed by 14 hours at 37°C for ten days.



Redman et al., 2002. *Science*

Temperature Tolerance in Tomato (*Solanum lycopersicum*)



7 days cycling, 50C 12 hr, RT 12 hr (N=15)

RT 50C

Rodriguez et al., 2008. *ISME-Nature*

Can we impart temperature tolerance to other plants as well?

38 40 46 50 (°C) 38 40 46 50  
 uncolonized colonized  
 Nonsymbiotic Symbiotic  
 N = 12/rep, SD=0

Communication responsible for thermotolerance predates the divergence of monocots and eudicots approximately 130-230 mya

Marquez et al. 2007, Science

Native Habitat #2: Cedar rocks, San Juan Islands WA  
*Leymus mollis* (Dunegrass) in Beach Habitat

0 100 300 500 [mM NaCl]

All plants were colonized with *F. culmorum* (Red1). (N=100)

3 weeks after exposure (N=60)

Can we impart salt tolerance to other plants as well?

Salt Tolerance in Tomato (N=30)

Dongjin Rice (*Oryza sativa*) Exposed to  $\pm$  500 mM NaCl for 10 Days (N=45)

S NS  
 300mM NaCl for 3 weeks

C S NS

\*Interesting. Can transfer same function from a monocot to eudicot. Indicates communication is old and precedes the divergence of monocots and dicots (approx 200 MYA).

Rodriguez et al., 2008. ISME-Nature  
 Rodriguez et al., 2008. SEB

Is Temperature and Salt Tolerance Species or Isolate Specific?

FcRed1 ATCC Cp4666D NS

Temperature: 50C 5-12 days

Salt: 300 mM NaCl 10 days (N=15-30)

Control Cp4666D ATCC NS

Habitat-Adapted Symbiosis

Control FcRed1 ATCC NS

Rodriguez et al., 2008. ISME-Nature  
 Rodriguez et al., 2008. SEB  
 Marquez et al., 2008. ISME-Nature  
 Redman et al., 2002. Science

Symbionts and Native and Invasive Plants

**Native systems:**

- Endophytes confer adaptive functionality to plants
- Endophytes allows plants to adapt to habit & microhabitat stresses

**Invasive plant systems:**

- Symbiotic modulation may play an important role in plant invasiveness
- Invasive plants are picking-up native symbionts which may confer the required functionality to adapt to habitat & microhabitat stresses
- Invasive plants bring their own unique resident endophytes which may give the invasive plants a competitive edge.

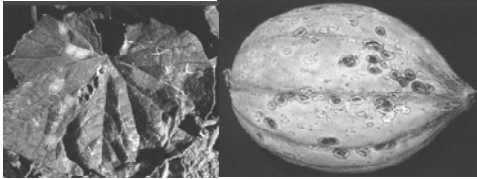
Agricultural Habitat

Stress: Disease

Concept: Fungal Lifestyle Switching

How and why do fungi cause disease?

*Colletotrichum* (anamorph)  
*Glomerella* (teleomorph)



- genus contains 29-700 species
- Causative agent of anthracnose
- infect most crops worldwide

Redman et al., 1994. *Experimental Mycology*  
Redman et al., 1999. *Plant Physiology*  
Redman et al., 2000. *APS Press*

To Disease or Not to Disease: It's All About Communication

Species	Disease Hosts	Asymptomatic Hosts	Symbiotic Lifestyle Switch
<i>C. magna</i>	cucurbits	tomato	mutualist
<i>C. coccodes</i>	tomato	nf	pathogen
<i>C. musae</i>	banana	pepper	mutualist
<i>C. orbiculare</i>	cucurbits	eggplant	mutualist
<i>C. lindemuthianum</i>	bean	nf	pathogen
<i>C. graminicola</i>	corn	nf	pathogen
<i>C. acutatum</i>	strawberry	watermelon	mutualist
<i>C. gloeosporioides</i>	strawberry	watermelon	mutualist

Redman et al., *New Phytologist*, 2001

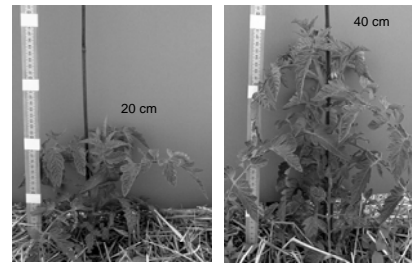
Disease Protection Conferred by REMI A Mutants



- Disease protection to wildtype *C. magna* and *C. orbiculare*

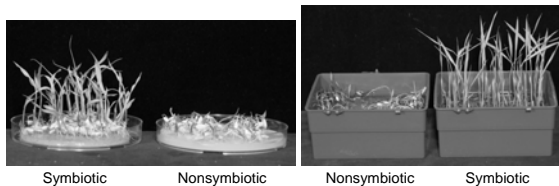
Freeman & Rodriguez, 1993. *Science*

Endophyte Conferred Plant Growth Response in tomato  
(but can apply to trees too!)  
increase biomass, bioenergy application



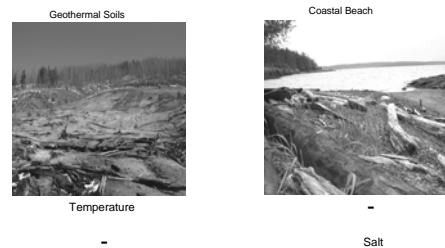
N=30/treatment, SD<5cm, P<0.05

Symbiosis and General Stress Tolerance and Plant Growth Responses



Same Aged Seedlings

Mutualistic Benefits Versus Selective Pressures

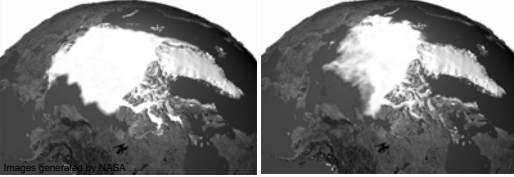


- Take home message -If you can ID the habitat specific stress You can predict mutualistic benefit

- Phenomena defined as "Habitat Adapted" symbiosis

## Global Warming: How Will We Survive?

Arctic perennial sea ice has been decreasing at a rate of 9% per decade!



Images generated by NASA

- Reality check: Its going to get hot and dry!
- How will crop plants deal with drought and temperature stress?
- Better understanding of Symbiotic Modulation and application of Symbionts to crop plants may help mitigate the impacts of global warming

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