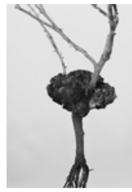
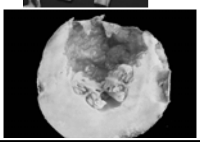
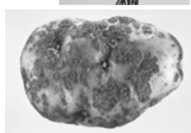
Bacterial bandits: bacteria in plant disease





Why study plant disease?

Agriculture = food
Lack of food = perhaps the most common disease worldwide

"One billion people in the world are undernourished, and need to consume more food to lead healthy, productive lives."

--State of the World 2006, the WorldWatch Institute

(population = 6.6 billion, expect 9 billion* by 2050 (about the time you start having grandchildren)

***Science Magazine's State of the Planet 2006-2007**

Factors contributing to food insecurity

Inability to purchase food:

Socioeconomic factors
Politico-economic factors

Inability to grow enough food:

Land fertility
Water availability (irrigation)
Poor crop yield
- **Weather**
- **Weeds**
- **Insect herbivory**
- ***Plant disease***

Environment plays a big role in spread of plant diseases:

Insects, weather

Examples:

- Pierce's disease of grape (*Xylella*)
- Citrus canker (*Xanthomonas*)

Xylella fastidiosa: Pierce's Disease of Grape

Wiped out grape production in SE states


Xylella fastidiosa: Pierce's Disease of Grape


Vectors are leafhoppers (feed on xylem tissues):
- **blue-green sharpshooter**
- **glassy-winged sharpshooter – introduced 1990**

Leafhoppers overwinter in riverbeds; keep vineyard 300' from river (State of CA shares cost of land lost)

Cankers: Citrus canker *Xanthomonas*

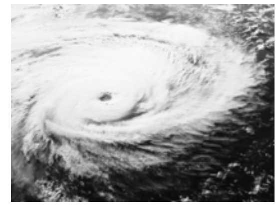
Disease eradication necessitated destruction of millions of fruit trees in Florida: Oranges, limes, lemons, grapefruit, tangerines, etc.

Citrus canker: *Xanthomonas*

<u>Infection/spread:</u>

Lesions on twigs, leaves are primary inoculum

20^oC-30^oC optimum temperature

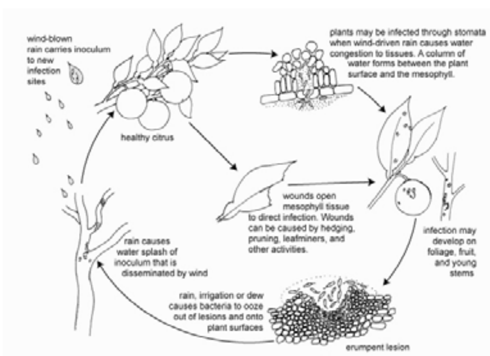


Heavy wind-driven rain (tropical storm):

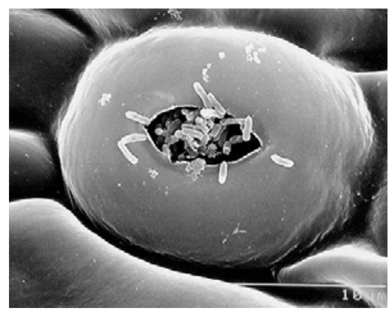
Wind > 18 mph can drive bacteria through stomates

Spreads inoculum from 100's of feet to several miles

Citrus canker: *Xanthomonas*



Citrus canker: *Xanthomonas*

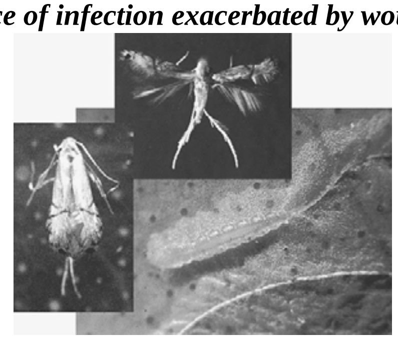


SEM of stomata on grapefruit leaf with *X. axonopodis* bacterial cells entering stomatal chamber. Water-soaking helps bacteria establish infection in mesophyll (beneath cuticle).

Citrus canker: *Xanthomonas* *-chance of infection exacerbated by wounding*

Lemon leaf with thorn scratches infected with *X. axonopodis.*

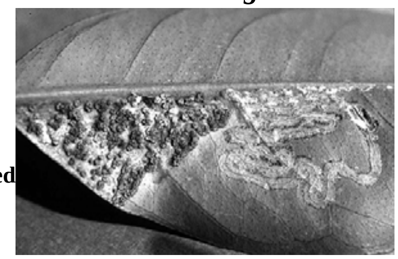
Citrus canker: *Xanthomonas* *-chance of infection exacerbated by wounding*



Asian leaf miner (adult moth, and larva in feeding gallery)

Citrus canker: *Xanthomonas*
-chance of infection exacerbated by wounding

Citrus canker introduced



Control

Citrus leaf with Asian leaf miner galleries:
Opens mesophyll to *Xanthomonas* without needing stomatal invasion/water soaking

Citrus canker: eradication from Florida

-99% of US citrus are susceptible

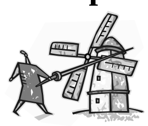
-2001: cut all susceptible trees within 1900 ft radius of infection.

Ornamental, nursery, orchards… lots of anger and lawsuits!

Citrus canker outbreaks in south Florida peninsula. Red areas indicate location. Note the large red areas of Miami-Dade and Broward counties to the southeast and large area of Manatee County to the northwest

Pathogenesis: bacterial weaponry

-toxins
-enzymes
-EPS
-hormones
-DNA



Pathogenesis: bacterial weaponry

Excreted products

1. Toxins:
   ---low molecular weight compounds that interfere with host functions.

Halo blight of bean: toxin

2. Enzymes:
   ---a. nutrient acquisition (e.g. proteases for amino acids, amylases for saccharides).
   ---b. tissue degradation: cellulases and polygalacturonases.

Soft rot: enzymatic degradation

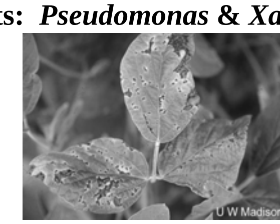
Pathogenesis: bacterial weaponry

Excreted products

3. Extracellular polysaccharides: often required for pathogenesis.
   ---a. may block recognition by the plant
   ---b. wilt mechanism (very viscous and can plug vascular tissue).
   ---c. protective barrier from dessication, toxins, salts, pH changes, etc.
4. Bacterially-produced plant hormones →
5. DNA (genetic transformation of plant): *Agrobacterium tumefaciens*

Oleander gall, *Pseudomonas*

Leaf Blights: *Pseudomonas* & *Xanthomonas*



- **Most are epiphytes**
- **Need high relative humidity and free moisture to infect stomates**
- **Minimum (> 10,000 cfu/g; varies) needed for disease**

Examples of molecular weapons deployed by *Pseudomonas* and *Xanthomonas* on the leaf:

1. Ice nucleation
2. Toxins
3. Hrp pilus

Ice nucleation

-Speeds ice formation/frost injury to leaves

-InaZ protein (used in artificial snow)

-*Pseudomonas* and *Xanthomonas* and *Erwinia* spp.

-Plants can supercool to around -5ºC; InaZ catalyzes ice formation as warm as -2ºC. $\geq$1000 cells/g is enough to form an ice nucleus.

-First GM microorganism was an Ice- strain of *P. syringae* to use in biocontrol (1985, Berkeley).

-Control: competitive exclusion of surfaces by Ice$^-$ strains (biocontrol; BlightBan)

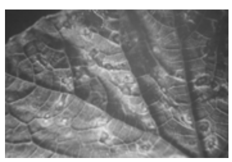
Toxins (small non-protein molecules)

-Toxins increase disease severity. How?

-Contribute to systemic movement

-increase lesion size

-favor multiplication of pathogen in host

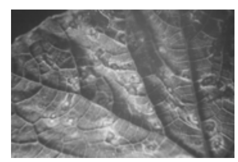


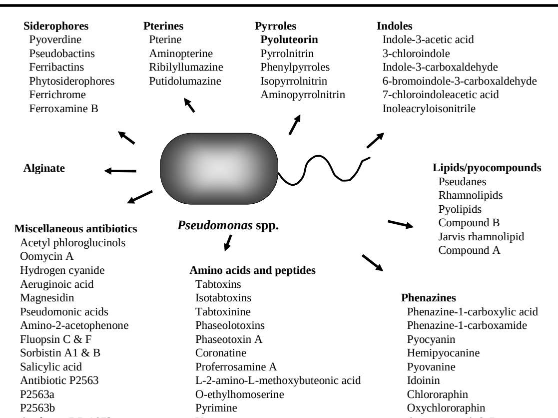
Well-studied in *P. syringae*, but other bacteria (and fungi) produce them

Toxins (small non-protein molecules)

"Koch's Postulates" for toxin involvement in pathogenicity

- reproduce disease w/ purified toxin
- correlate toxin yield with pathogenicity
- produce toxin during active growth of pathogen *in planta*
- reduced virulence in *tox*- strains.





Adapted from Dowling and O'Gara, 1994. Trends in Biotechnology 12:133-144.

Toxins

-<u>Chlorosis-inducing</u>

coronatine

phaseolotoxin }
tabtoxin } Plant enzymes cleave to final toxic product

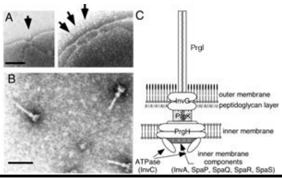
-<u>Necrosis-inducing</u>

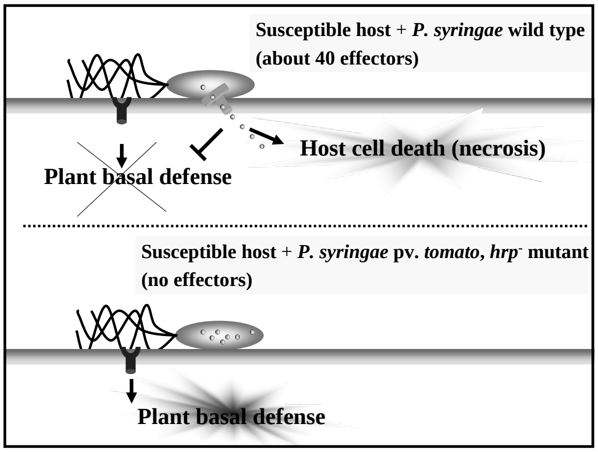
syringomycin }
syringopeptin } Form pores in plant cell membrane

Hrp pilus and effectors

Effector proteins injected via needle complex directly into host cytoplasm

Delivery of “effectors”:
Contribute to pathogen spread in susceptible hosts
Induce resistance response in non-host plants





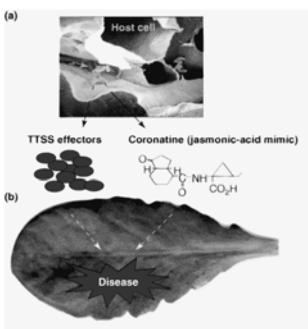
Hrp pilus and effectors

Many virulence factors together cause disease

Host “learned” to recognize some

Host recognition = resistance response

Host evasion = virulence



Example of a plant pathogen story:
Fire blight of apple and pear.

Once upon a time, long, long ago…

Fire Blight of Pear and Apple
Causal agent: *Erwinia amylovora*

- *E. amylovora* native in N. America
- Hawthorne, mountain ash
- Apples, pears introduced by settlers
- Epidemic on pears in 1800-1900s
- Today pears still grown commercially west of Rockies due to bacterium but disease moved with pears

Fire Blight of Pear and Apple
Causal agent: *Erwinia amylovora*

First reported in 1794 in New York.

First disease where Koch’s postulates were fulfilled for plant bacterial pathogen.

-Thomas Burrill, at U. Illinois (1881)

-took 20 years of arguing to convince some scientists that bacteria could cause plant diseases.

First description of insect vector (honeybee) for bacterial disease.

Hopeful bulletin from the Washington State Agricultural Experiment Station

February, 1915

FIRE BLIGHT

IS THE GREATEST DANGER TO THE FRUIT INDUSTRY

Blight is a PREVENTABLE Disease

Blight is the "GREAT WHITE PLAGUE" of the Fruit Industry

ROUT THE ENEMY—BLIGHT—AT ALL COSTS!

THE DISEASE CAN BE CONTROLLED

Inspection is Necessary

Cleaning Up "HOLD-OVER" BLIGHT is the Best Means of Prevention

THE ONLY KNOWN WAY TO CONTROL BLIGHT IS BY SURGERY

THERE IS NO PATENT CURE

Eternal Vigilance is the Price of Clean Orchards

Fire Blight of pear, apple: *Erwinia amylovora*

Wilt, necrosis

Moves rapidly from vessels to other tissues, killing cells rapidly

Leaves killed too fast to form abscission layer and isolate pathogen

Fire Blight of pear, apple: *Erwinia amylovora*

EPS (extrapolysaccharide) is important virulence factor

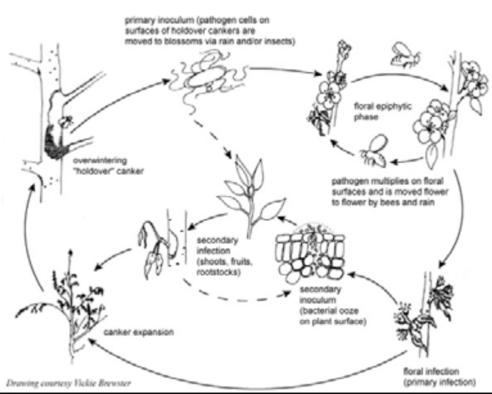
Hrp pilus present, along with effector proteins

Fire Blight of pear, apple: *Erwinia amylovora*

<u>Disease development:</u>

1. **Epiphytic growth on stigmas**
2. **Movement down style to nectary**
3. **Movement to nectarthodes, colonization, entry**
4. **Rapid multiplication in intercellular spaces**
5. **Enter phloem, move to apical tissues**
6. **Enter xylem, move downward**
7. **Shoot blight, rootstock blight**
8. **Secondary infections from ooze: entry via stomates, lenticels, wind/hail and pruning wounds**

Fire Blight of pear, apple: *Erwinia amylovora*

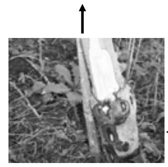


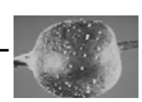
Fire Blight of pear, apple: *Erwinia amylovora*

Dissemination:
Rain and insects

Shepherd's crook

Blossom stage is key to control: keep populations on stigmas <10^6 cells/blossom





Fire Blight of pear, apple: *Erwinia amylovora*

Control:

1. **Resistant cultivars (Red Delicious) and rootstocks**
2. **Limit nitrogen**
3. **Prune all infections**
4. **Chemical controls**
   1. **Copper – not very effective**
   2. **Oxytetracycline (antibiotic) – no resistance but only ~50% reduction.**
   3. **Streptomycin: Old silver bullet. Now, antibiotic resistance.**
5. **Biological controls**

   Commercially available BlightBan (*P. fluorescens* A506); mix with antibiotics

Fire Blight of pear, apple: *Erwinia amylovora*

Pruning canker-infected branches in pear orchard

Fire Blight of pear, apple: *Erwinia amylovora*

Burning canker-infected branches in pear orchard

Fire Blight of pear, apple: *Erwinia amylovora*

Streptomycin resistance

Application of antibiotics to a pear orchard

Fire Blight of pear, apple: *Erwinia amylovora*

Streptomycin resistance

Antibiotic use in the United States in 1999 by crop[a]

Crop	Primary target	Antibiotic	No. states surveyed	Acreage treated (%)	Active ingredient used (lbs)
Apple	*Erwinia amylovora*	Oxytetra-cycline	2	5	2,900
		Streptomycin	10	19	15,400
Peach, Nectarine	*Xanthomonas arboricola*	Oxytetra-cycline	3	8	6,900
Pear	*Erwinia amylovora*	Oxytetra-cycline	2	41	11,900
		Streptomycin	4	30	6,000

[a]Data obtained from databases maintained by the USDA's National Agricultural Statistics Service (46).

Antibiotic resistance in agriculture:

Streptomycin resistance:

1. **Ribosomal mutation; streptomycin can't bind anymore (most common)**

2. **Inactivation by aminoglycoside phosphotransferase (encoded on plasmid of *E. amylovora*)**

Tetracycline resistance: Rare so far, although certainly exists in nature. At least three different mechanisms:

1. **Efflux pump**
2. **Ribosome mutatiom**
3. **Degrading enzyme**

Fire blight epidemics are preceded by rain after warm periods during bloom: <u>predictable</u>

Models:

- **Days above 15°C**
- **Rain events**

Current models:

- **COUGARBLIGHT - Washington**
- **MARYBLYT - Oregon**
- **Others (Israel, Billings…) – location alters effect of rainfall so must be accounted for in model (humid/arid climates)**

Mycorrhizal fungi

(Fungi that form symbiotic associations with plant roots)

Fungi obtain nutrition from many sources:

-decomposition of organic substrates

-predation and parasitism

-mutualistic associations

Many soil fungi are **saprobes** with the enzymatic ability to digest organic substrates of varying degrees of complexity,

Mycorrhizal fungi are a major component of the soil microflora in many ecosystems, but usually have **limited saprophytic** abilities

Mycorrhizal fungi are considered to have many important roles in natural and managed ecosystems:

-Fungi vary in their capacity to utilize resources and withstand adverse environmental conditions, e.g. pH.

-Therefore, mycorrhizal fungus diversity is thought to contribute to the resilience of ecosystems and competitiveness of plants.

-Two major types:

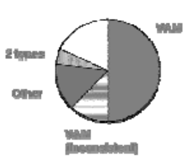
1. VAM (vesicular arbuscular mycorrhizae)
2. ECM (ectomycorrhizae)

The vast majority of plants are mycorrhizal!

Proportion of angiosperm species:

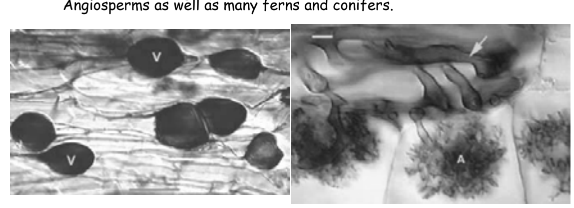
- 18% were not found to have mycorrhizas
- 50% reported to have VAM
- 12% reported to have VAM in some cases, but not in others
- 20% had another type of association (ECM, orchid, ericoid, etc.)

•Data compiled by Trappe (1987) from a dataset representing 3% of Angiosperm species



Vesicular-arbuscular mycorrhizae

- VAM fungi belong to the Zygomycete order **Glomales**.
- They apparently colonized land with first vascular plants and may have evolved very slowly since then.
- These fungi only produce microscopic structures (**no mushrooms**).
- Only about 150 species of these fungi are known, yet they are capable of forming mycorrhizal associations with 70% of Angiosperms as well as many ferns and conifers.



Ectomycorrhizal associations (ECM):

-Mutualistic associations between Basidiomycetes and Gymnosperm or Angiosperm plants

-Consist of a soil mycelium system, linking mycorrhizal roots and storage or reproductive structures.

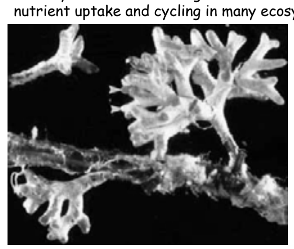
-Characterized by the presence of a mantle and Hartig net in the root epidermis or cortex, although these structures may not be well developed.

Ectomycorrhizal associations:

-Formed predominantly on the fine root tips of the host (fine root tips are more abundant in topsoil layers containing humus, than in underlying layers of mineral soil)

-Make a significant contribution to the biomass of forest ecosystems

-Widely distributed through the soil and make a large contribution to nutrient uptake and cycling in many ecosystems.



Pinus radiata and *Amanita muscaria* ECM grown under sterile conditions. This association has highly branched short roots with many root tips (arrows).

Ectomycorrhizal associations (ECM):

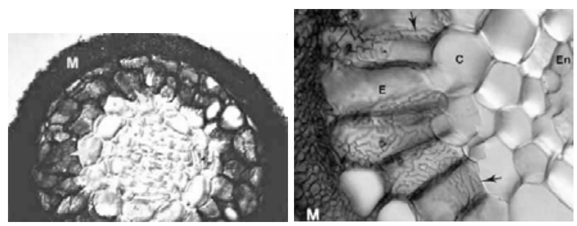
Hyphae penetrate between host cells and branch to form a labyrinthine structure called the Hartig net.

Angiosperms with ECM usually have a **one cell layer Hartig net** which is confined to the epidermis; structural characteristics of host roots (e.g. hypodermal layer)are thought to restrict ECM fungus hyphae to the epidermis in most Angiosperms.

In **gymnosperms**, **Hartig net hyphae extend deep into the cortex**. Hyphal penetration in gymnosperms may also be stopped by inner-cortex wall features in some cases.

Ouch!!! Host responses to this invasion may include polyphenol production in cells, phenylpropanoid accumulation and the deposition of secondary metabolites in walls.

Hartig net and mantle of ECM fungi



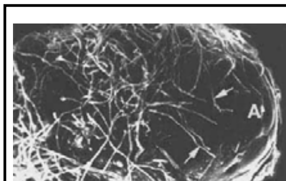
Cross section of *Pinus strobus* (White pine) ECM short root with thick mantle (M) and Hartig net hyphae (arrows) have enveloped several layers of cortex cells.

Most plants with ECM have roots with a modified lateral root branching pattern (heterorhizy):

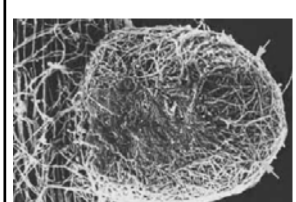
-short mycorrhizal lateral roots (called **short roots**) supported by a network of long roots.

-short roots grow much more slowly than long roots to allow ECM fungi time to form associations (mycorrhizae have difficulty colonizing more rapidly growing roots).

-short roots lack a periderm layer.



Early stage of colonization of pine short root by *Pisolithus tinctorius*. Hyphae (arrows) have contacted the root and are starting to proliferate on its surface near the apex (A).



SEM image showing the next stage of pine root colonization by *Pisolithus tinctorius*. Mantle hyphae (arrows) have formed a dense covering on the root surface (arrows).

Mycorrhizal fungi produce a hyphal network in soils.

-Individual strands of hyphae and/or bundles of hyphae called **mycelial strands**.

-Some ECM fungi can produce rhizomorphs, which contain **sclerotia**, which are resistant storage structures.

Soil hyphae:
-**acquire nutrients** and re-allocate resources for reproduction or mycorrhizal exchange
-function as **propagules** to allow survival and spread of the fungus.

Unlike VAM associations, the ECM fungal associations can produce **fungal fruiting bodies (mushrooms).**

Laccaria fraterna fruiting under one-year old *Eucalyptus globulus*.

Fruit bodies of the ECM fungus *Laccaria* produced under an inoculated eucalyptus seedling.

To what extent to belowground microbial associations drive aboveground community structure?

Spotted knapweed, *Centaurea maculosa*

Introduced from Eastern Europe in the late 1800s in a load of hay; it has spread at a rate of about 27% every year since being introduced

In a century, spread from the PNW to the Atlantic coast

Most of Central and Eastern US spread occurred in last 15 years

Why is it such a successful invader?

Multibarreled approach to chemical warfare:

Foliage: cnicin
Roots: polyacetylenes, catechins

The foliage is actually high in nutrients. Why don't ruminants eat it?

Antifeedant compound (cnicin, a sesquiterpene lactone) in foliage, borne in trichomes – bitter tasting. Cnicin can make up 4% of the dry weight of foliage.

Cnicin reduces activity of rumen microbes, making it hard for sheep to digest food

Cattle and sheep graze spotted knapweed in the spring when cnicin concentrations are lowest

Spotted knapweed is also potentially allelopathic:
- Polyacetylenes in roots: phytotoxic
- Catechin in roots: phytotoxic

Non-chemical, reproductive success:
- Hundreds of seeds per seedhead
- Tumbleweed-like when dry

In its native Europe, spotted knapweed is not an invasive weed. Why?

Natural insect enemies have co-evolved that will feed on seedheads without being deterred by cnicin

Co-habitating plants are not repressed by spotted knapweed

Immune to root compounds (European bunchgrass) | Repressed by root compounds (American bunchgrass)

European bunchgrass	American bunchgrass
Festuca ovina	*Festuca idahoensis*
Stipa paviflora	*Stipa comata*
Agropyron cristatum	*Pseudoegeneria spicata*

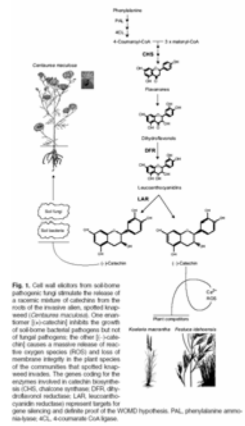
14 insect and fungal species were considered or introduced in North America to control spotted knapweed.

Seedhead moths: *Urophora* spp.: natural enemy brought in from spotted knapweed's native Europe. These moths oviposit in the flowerheads; developing larvae eat seeds and flowerhead tissues.

Agapeta zoegana: also a moth from Europe; a natural enemy. Bores into roots and reduces carbohydrate stores.

Native grasses were also brought in to compete with spotted knapweed.

Biocontrol has reduced seed numbers but not population densities.



Spotted knapweed releases phenolics into the soil upon contact with a common fungal pathogen. (*Centaurea* was under disease pressure in the Old Country)

The phenolics don't hurt the fungus. (*Centaurea* was losing the co-evolutionary arms race)

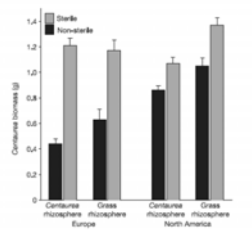
The phenolics DO kill pathogenic bacteria (Serendipitous advantage in US soils)

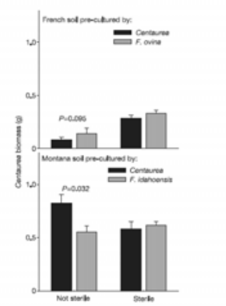
The phenolics DO induce apoptosis (cell death) in neighboring American plants (Serendipitous advantage in US soils)

The phenolics DON'T hurt neighboring European plants. (Less competition in US than in Europe)

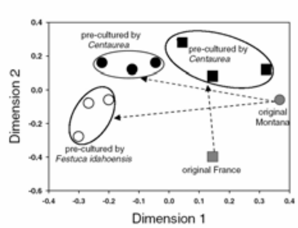
Soil biota and exotic plant invasion

Ragan M. Callaway, Giles C. Thelen, Alex Rodriguez & William E. Holben





Supplementary Figure 2 Legend: Non-metric dimensional scaling (NMDS)[34], a descriptive multivariate approach, for the mean similarity matrix of DGGE readings for original and pre-cultured soils from France and Montana. This figure illustrates relative, descriptive differences in soil fungi and bacteria between samples and treatments. Each point represents soil compiled from three individual *C. maculosa* plants. The dimensions, or axes, are constructed from mathematical relationships among the differen components (DGGE readings) of the samples. Samples that are near one another in the figure are more similar in microbial composition than samples that are far apart. The lac of overlap among samples pre-cultured by different species, and soils of different origin suggests that different species culture different microbial communities and that French and Montana soil sources differ in microbial community composition.



Conclusion:
Centaurea **"cultures" microbes differently in different types of soil.**

Identity of main microbial characters unknown

Hypothesis: **pathogens in France, host-specific beneficials in US - generalists**

Are European grasslands less susceptible to domination by *Centaurea* due to:

- -insect natural enemies
- -competing native plants
- -belowground root pathogens
- -all of the above

Can disease drive diversity by thwarting domination?

Pathogen-driven diversity in a forest ecosystem:

Pythium **builds up in soil around mature cherry (*Prunus*) stands in a hardwood forest. *Prunus* dies; newcomers arrive and diversity increases.**

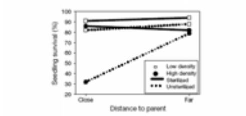


Figure 3 Effect of distance, neighbourhood density and soil sterilization on black cherry seedling survival. In high density treatments, survival was significantly greater after soil collected close to the tree was sterilized. This effect of sterilization was not found with soil collected further from the tree. The data best fitted a logistic regression model that included density, density × distance, density × sterilization and distance × density × sterilization. Removal of any variable included in the model significantly decreased the model fit (for each variable, $P < 0.0001$).

Soil pathogens and spatial patterns of seedling mortality in a temperate tree

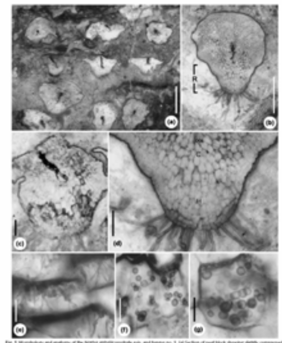
Alissa Packer & Keith Clay

NATURE | VOL 404 | 16 MARCH 2000

Shales and cherts from an old transient freshwater/hot springs ecosystem in Scotland: evidence for fungal symbiosis with early land plants 400 mya.

Host responses evident (root swelling, walling off) but not clearly pathogenic

Did fungi permit colonization of land by plants?



Drought stress

Predator evasion

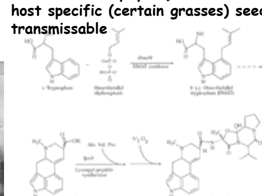
Temperature stress

Predator evasion
(larval food choice)

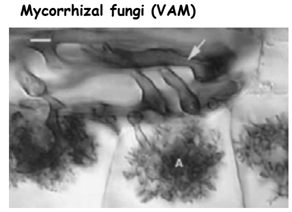
Fungal endosymbionts

Class 2 endophytes:
generalists, seed-coat (not seed) transmissible

Class 1 endophytes (claviciptaceous fastidious endophytes):
host specific (certain grasses) seed transmissable



Mycorrhizal fungi (VAM)



Biological symbioses

Effect on X	Effect on Y	Type of interaction
0	0	Neutralism (extremely unlikely; impossible to prove
-	0	Amensalism (usually involves toxin production)
+	0	Commensalism (hard to judge – might miss a trait)
+	+	Mutualism
+	-	Parasitism

The symbiotic continuum

Experimental system:

Axenic, endophyte-free seedlings

Stress tolerance in plants via habitat-adapted symbiosis

Rusty J Rodriguez, Joan Henson, Elizabeth Van Volkenburgh, Marshal Hoy, Leesa Wright, Fleur Beckwith, Yong-Ok Kim and Regina S Redman

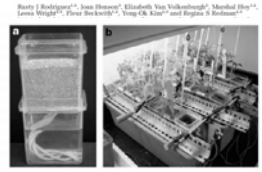


Figure 1 (a) Modified magenta box constructed by drilling a hole at the base of the upper magenta box, top-knotting and weaving through a defined length of cotton rope to the bottom chamber to act as a wick and adding a defined amount of sand or soil in the upper chamber. Fluid is added to the bottom chamber and a tight-fitting lid is added to the top (not shown) and the whole system autoclaved and sterilized prior to symbiotic or nonsymbiotic plant transplantation. (b) Geothermal soil stimulator. The top half of the modified magenta box containing the plant is removed and wrapped with thermal tape at the soil or sand line and temperature regulated by a Thermolyne rheostat controller (Barnstead International, Dubuque, IA, USA). Utilizing thermal tape, the geothermal stimulators were designed by this research team such that the soil/root zone is exposed to elevated temperature to mimic what occurs in the natural geothermal habitat. Modified magenta boxes were secured together using a system of clamps and metal brackets and the entire assemblage (with exposed dangling cotton wicks) placed into tubs containing copious amounts of water. A thermometer was placed in each magenta box to monitor temperature accurately throughout the experiment.

High soil temp's

Salt stress

Microbial pathogens

Figure 2 Three unique field habitats addressed in our studies. Each imposing very different stresses: geothermal soil of YNP where the habitat-specific stress is high soil temperatures (top panel); coastal beach regions of CR-SJI where the habitat-specific stress is salt stress (middle panel); and agricultural arena where the habitat-specific stress in high disease pressure (lower panel).

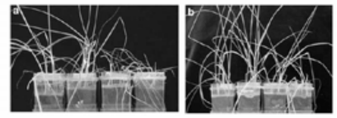
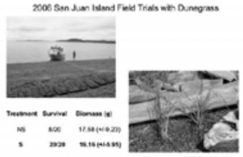


Figure 3 Effect of symbiosis on salt and drought tolerance on native dunegrass (monocot) plants under laboratory conditions. The number of plants/treatment are indicated by (N = XX), and the % survival and health of surviving plants are indicated in parentheses after each treatment. Plant health was based on comparison to nonsymbiotic controls and rated from 1 to 5 (1 = dead, 2 = severely wilted and chlorotic, 3 = wilted ± chlorosis, 4 = slightly wilted, 5 = healthy with out lesions or wilting). All assays described from left to right and images are representative of all plants/treatment. (a) Dunegrass plants (N = 30), non-stressed controls (representative of both symbiotic and nonsymbiotic plants), symbiotic with FcRed1 (100%, 5), symbiotic with Fc18 (0%, 1) or nonsymbiotic (0%, 1) exposed to 500 mM NaCl for 14 days. While all plants bent over with age, unstressed controls and salt-exposed FcRed1 colonized plants remained hydrated while the other treatments wilted and lost turgor. (b) Dunegrass plants (N = 30), non-stressed controls (representative of both symbiotic and nonsymbiotic plants), symbiotic with FcRed1 (100%, 4), symbiotic with Fc18 (100%, 4) or nonsymbiotic (0%, 1) grown without water for 14 days.

Fusarium culmorum **infects ~95% of** ***L. mollis*** **plants.**
FcRed1 = red-colored symbiont
Fc18 = near-identical type culture isolate of ***F. culmorum***

2006 San Juan Island Field Trials with Dunegrass



Treatment	Survival	Biomass (g)
NS	8/20	17.58 (+/-9.23)
S	20/20	19.16 (+/-5.95)

Survivors: 100% Symbiotic

Figure 4 2006 field experiment in a coastal beach habitat of CR-SJI with symbiotic (FcRed1) and nonsymbiotic (NS) generated dunegrass plants. Two clusters of plants (10 plants/cluster) were planted in late spring and assessed for survival, plant biomass and endophyte colonization 3 months later (shown in photograph). Statistical analysis revealed that there were significant differences in survival in symbiotic versus nonsymbiotic plants (P = 4.59 E−06). While there were surviving plants in the NS treatment, microbiological analysis revealed that these plants were colonized with FcRed1.

Table 1 Host colonization and stress tolerance conferred by fungal endophytes

Endophyte	*Dunegrass*	*Panic grass*	*Rice*	*Tomato*
Cp4666D	r, s, D	r, s, D, H	r, s, D	r, s, D, H
CpMH206	ND	r, s, D,	ND	r, s, D,
FcRed1	r, s, D, S	r, s, D, S	r, s, D, S	r, s, D, S
Fc18	r, s, D,	ND	r, s, D,	r, s, D,

Abbreviation: ND, not determined.
Plant colonization (N = 5) was assessed by surface sterilization, cutting plants into root (r) and stem (s) sections and plating sections on fungal growth medium and surface sterilization verified using the imprint technique (Schulz *et al*., 1999). Plant sections are listed only if fungi that grew out from those tissues. Symbiotically conferred drought and heat tolerance was assessed and denoted as D or H, respectively. Drought and heat tolerance was assessed after 7–14 days. Salt tolerance (S) was assessed by watering plants with 300–500 mM NaCl solution for 10–14 days. Stress tolerance was assessed as plant health and rated from 1 to 5 (dead and healthy, respectively; see Materials and methods). The % survival and health of stress-tolerant plants was 100% rated 4–5, and 100% rated 1 for stress-intolerant plants.

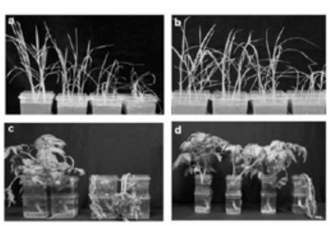


Figure 5 Effect of symbiosis on salt and drought tolerance in the model rice (monocot) and tomato (eudicot) under laboratory conditions. The number of plants/treatment are indicated by (N = XX), and the % survival and health of surviving plants are indicated in parentheses after each treatment. Plant health was based on comparison to nonsymbiotic controls and rated from 1 to 5 (1 = dead, 2 = severely wilted and chlorotic, 3 = wilted ± chlorosis, 4 = slightly wilted, 5 = healthy with out lesions or wilting). All assays described from left to right and images are representative of all plants/treatment. (a) Rice plants (N = 120), non-stressed controls (representative of both symbiotic and nonsymbiotic plants), symbiotic with FcRed1 (100%, 5), symbiotic with Fc18 (0%, 1) or nonsymbiotic (0%, 1) exposed to 500 mM NaCl for 10 days. While all plants bent over with age, unstressed controls and salt-exposed FcRed1 colonized plants remained hydrated while the other treatments wilted and lost turgor. (b) Rice plants (N = 120), non-stressed controls (representative of both symbiotic and nonsymbiotic plants), symbiotic with FcRed1 (100%, 5), symbiotic with Fc18 (100%, 5) or nonsymbiotic (0%, 1) grown without water for 10 days. (c) Tomato plants (N = 12), non-stressed controls (representative of both symbiotic and nonsymbiotic plants), symbiotic with FcRed1 (100%, 5), symbiotic with Fc18 (0%, 1) or nonsymbiotic (0%, 1) exposed to 300 mM NaCl for 14 days. (d) Tomato plants (N = 12), non-stressed controls (representative of both symbiotic and nonsymbiotic plants), symbiotic with FcRed1 (100%, 5), symbiotic with Fc18 (100%, 5) or nonsymbiotic (0%, 1) grown without water for 10 days.

Table 2 Effects of heat and salt stress on fungal colonization of plants

Fungal isolate	*CFU*			
	Dunegrass +Salt stress	*Panic grass +Heat stress*	*Tomato +Salt stress*	*Tomato +Heat stress*
Cp4666D	ND	11.0±4.0 (0.048)	ND	4.3±1.5 (0.067)
CpMH206	ND	3.7±2.2 (0.048)	ND	1.0±1.7 (0.067)
FcRed1	4.8±1.64 (0.027)	ND	5.6±1.15 (0.001)	ND
Fc18	2.4±1.14 (0.028)	ND	1.4±1.14 (0.001)	ND

Abbreviations: ANOVA, analysis of variance; CFU, colony-forming units; ND, not determined.
Monocot (panic grass) and eudicot (model tomato) plants that were colonized by Cp4666D that imparts temperature tolerance and CpMH206 that does not were exposed to temperature stress (50 °C for 12 h, 22 °C for 12 h for 12 days) and CFU assessed. Similarly, monocot (dunegrass) and eudicot (model tomato) plants that were colonized with FcRed1 that imparts salt tolerance and Fc18 that does not were exposed to salt stress (300 mM NaCl solution for 14 days) and CFU assessed. Equal amounts of plant tissues were processed for CFU analysis. Standard deviations are on the right of the ± sign and P-values were determined by ANOVA single-factor analysis and are in parentheses.

Table 3 Saprophytic growth rates (mm per 24 h) of fungal isolates ± stress

Isolates	*H₂O agar medium*		*1 × PDA medium*	
	−Salt	*+Salt*	*−Salt*	*+Salt*
FcRed1	1.36+0.09	1.08+0.25	1.48+0.13	1.26+0.15
Fc18	1.14+0.11	1.70+0.45	1.74+0.22	2.28+0.28
	1 × PDA medium			
	25 °C	*30 °C*	*37 °C*	*40 °C*
Cp4666D	25.33+2.29	28.00+1.58	6.89+1.38	NG
CpMH206	28.78+2.49	40.89+5.25	8.67+2.24	NG

Abbreviations: NG, no growth; PDA, potato dextrose agar.
Salt stress: isolates were grown at 25 °C on different media ± 500 mM NaCl; temperature stress: isolates were grown on one medium at 25–40 °C.

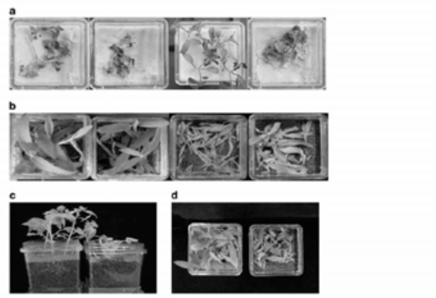


Figure 6 Effect of symbiosis on heat (**a** and **b**) and drought tolerance (**c** and **d**) on the genetic model tomato (**a** and **c**) and native panic grass (**b** and **d**) under laboratory conditions. The number of plants/treatment are indicated by (*N* = XX), and the % survival and health of surviving plants are indicated in parentheses after each treatment. Plant health was based on comparison to nonsymbiotic controls and rated from 1 to 5 (1 = dead, 2 = severely wilted and chlorotic, 3 = wilted ± chlorosis, 4 = slightly wilted, 5 = healthy with out lesions or wilting). All assays described from left to right and images are representative of all plants/treatment. (**a**) Tomato seedlings (*N* = 30) symbiotic with FcRed1 (0%, 1), CpMH206 (0%, 1) or Cp4666D (100%, 5), or nonsymbiotic (0%, 1) exposed to 50 °C root temperatures for 5 days. Although not shown, non-stressed plants (representative of both symbiotic and nonsymbiotic plants) remained green and healthy throughout the experiment. (**b**) Panic grass (*N* = 30), non-stressed controls (representative of both symbiotic and nonsymbiotic plants), symbiotic with Cp4666D (100%, 5), symbiotic with CpMH206 (0%, 1) or nonsymbiotic (0%, 1) exposed to 50 °C root temperatures for 12 days. (**c**) Tomato plants (*N* = 30) symbiotic with CpMH206 (100%, 5), or nonsymbiotic (0%, 1) grown without water for 7 days. (**d**) Panic grass (*N* = 30) symbiotic with CpMH206 (85%, 5; 15% 3), or nonsymbiotic (0%, 1) grown without water for 7 days. Although not shown, non-stressed controls (representative of both symbiotic and nonsymbiotic plants) remained hydrated and healthy (100%, 5) as did drought-stressed Cp4666D (100%, 5) in both tomato and panic grass (**c** and **d**).

Table 4 Effect of symbiosis on plant osmolyte concentrations

Treatment	*Without stress*		*With heat stress*	
	Panic grass	*Tomato*	*Panic grass*	*Tomato*
NS	57 ± 5.1[a]	178 ± 8.7[b]	142 ± 13.2[c]	263 ± 24.7[c]
S	102 ± 7.2[b]	206 ± 15.6[b]	114 ± 5.7[b]	127 ± 34.7[a]

Nonsymbiotic (NS) and symbiotic (S, with Cp4666D) plants were maintained at 22 °C (−stress) or with root zones heated to 50 °C for 12 days (+stress) and osmolyte concentrations (milliosmole per kg wet wt) ± s.d. values assessed. Assays were repeated a minimum of three times. Values with the same letters are not significantly different (Duncan's multiple-range test, $P < 0.0005$).

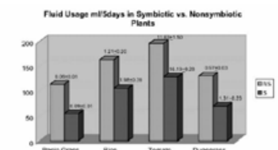
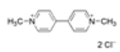


Figure 7 Water usage in symbiotic (S) and nonsymbiotic (NS) plants (*N* = 25, 120, 30 and 60 for panic grass, rice, tomato and dunegrass, respectively) was quantified over time and expressed as fluid consumed (ml)/5 days with s.d. values no greater than 12.5 ml. Statistical analysis revealed significant differences in fluid usage ($P = \leq 0.04$) and biomass ($P = 0.013$–0.061) with symbiotic plants using less fluid and having increased biomass (numerical value above each bar = average weight (g) ± s.d. of three representative plants from each treatment) compared to nonsymbiotic plants.



Paraquat dichloride – common, general use herbicide (monocots and dicots)

Table 5 Effect of symbiosis on ROS generation in the presence or absence of stress

Plant	*Treatment*	*−Heat stress*	*+Heat stress*	*−Salt stress*	*+Salt stress*
Panicgrass	NS	0/12	12/12	ND	ND
	S	0/12	0/12	ND	ND
Tomato	NS	0/12	11/12	0/12	10/12
	S	0/12	0/12	0/12	1/12
Dunegrass	NS	ND	ND	1/12	11/12
	S	ND	ND	0/12	1/12

Abbreviations: ND, not determined; ROS, reactive oxygen species. Monocot (panic grass and dunegrass) and eudicot (model tomato) plants that were symbiotically (S) colonized with either Cp4666D or FcRed1 (that imparts heat and salt tolerance, respectively) compared to nonsymbiotic (NS) plants were exposed to ± temperature or salt stress (see text for details) and assayed for ROS. Leaf disks (*N* = 12) were excised from *N* = 3 plants/treatment. The values indicate the number of leaf discs out of a total of 12 that bleached white after exposure to paraquat indicating ROS generation.

Paraquat toxicity: Intercept electrons from PSI, generate bipyridyl radicals that interact with O_2 to form superoxide (which then forms H_2O_2 and hydroxyls)

Paraquat resistance: More efficient detoxification of ROS
Restricted movement among cells

Is this a direct assay for ROS?

More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis

Rusty Rodriguez[1,2,*] and Regina Redman[2,3]

Table 1. *Symbolic lifestyle expression of* Colletotrichum *species versus plant host*

Fungal pathogen	Disease host[a]	Non-disease host[b]	Lifestyle expressed	
			Disease stress[c]	Drought stress[d]
C. magna	Watermelon	Tomato	Mutualism	Mutualism
C. musae	Banana	Pepper	Mutualism	Mutualism
C. orbiculare	Cucumber	Tomato	Mutualism	Mutualism
C. acutatum	Strawberry	Watermelon	Commensalism	Mutualism
C. gloeosporioides	Strawberry	Watermelon	Commensalism	Mutualism

[a] Species were isolated from disease lesions on the indicated host plants.
[b] Host plants that are asymptomatically colonized by the respective *Colletotrichum* spp.
[c] Symbiotic lifestyle expressed after asymptomatic colonization. Lifestyles were defined by the ability of each *Colletotrichum* sp. to confer disease resistance against virulent *Colletotrichum* pathogens of the non-disease hosts (data from Redman *et al.*, 2001).
[d] Symbiotic lifestyle expressed after asymptomatic colonization. Lifestyles were defined by the ability of each *Colletotrichum* sp. to confer drought tolerance based on the length of time before wilting after cessation of watering (data from Redman *et al.*, 2001).

The symbiotic continuum

Colletotrichum magna exhibits full range of lifestyles depending upon which cultivar of tomato it has infected

The plant smorgasbord as a fungus sees it (?):

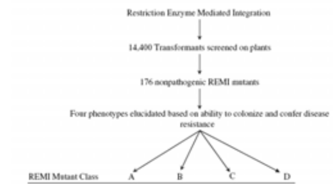
Uncolonizable
Colonizable
Parasitizable

Single gene mutations in *Colletotrichum* and in certain endophytes result in lifestyle switching.

Thus, disease could be result of single mutation.

Which came first, endophytes or pathogens?

Is disease simply a result of miscommunication?



REMI Mutant Class	A	B	C	D
% Colonization	100	100	100	0
% Disease Protection	80 - 100	20 - 65	0	0
Symbiotic Lifestyle	Mutualist	IM	Commensal	Abortive

Fig. 1. Gene disruption (restriction enzyme-mediated integration, REMI) of fungal symbiotic lifestyle loci in *Colletotrichum magna*. Symbiotic lifestyles reflect the ability of REMI mutants to colonize host plants (watermelon) asymptomatically and confer disease resistance against the virulent wild type. REMI mutants were designated either as mutualists, intermediate mutualists, or commensals based on disease protection, or abortive if they were unable to colonize host tissues. Although these lifestyle designations reflect quantitative differences, they probably reflect a continuum of symbiotic lifestyles represented among the mutants. Methods and data are from Redman *et al.* (1999*a*).

Table 2. *Physiological defence activity versus symbotically conferred disease conferred disease resistance by* Colletotrichum magna

Methods and physiological data are from Redman *et al.* (1999).

Host	Peroxidase activity[a]		PAL activity[b]		Lignin deposition[c]	
	24 h	48 h	24 h	48 h	24 h	48 h
Watermelon (E–)[d]	2.76	3.46	2.27	2.90	–	+
Watermelon (E+)[e]	5.77	6.30	2.50	3.70	+++	++++
Cucumber (E–)	0.63	1.31	0.02	0.25	–	+
Cucumber (E+)	1.80	2.34	.27	0.34	+++	++++

[a] Activity based on a guaiacol/H_2O_2 assay, and units indicate change in A_{470} min^{-1} μg^{-1} protein.
[b] Activity based on the production of cinnamic acid, and units indicate change in A_{290} min^{-1} μg^{-1} protein.
[c] Qualitative assessment of the absence (–) or presence (+) of lignin visualized with acidic phloroglucinol.
[d] (E–)=endophyte (*C. magna*) free.
[e] (E+)=endophyte (*C. magna*) colonized.

If a *different* pathogen colonizes, there is super-immune response by colonized cells. Why not before then? Is endophyte hiding? Suppressing plant's defense systems?

Recognized, but "good guys"? (most likely)

Species concept

Problems in fungi, too… molecular species designations do not address ecological functionality

Curvularia protuberata (pathogen of monocots)
-Isolate Cp4666D = mutualist in *Dichanthelium lanuginosum*, heat/drought tolerance

Fusarium culmorum (pathogen of crop plants)
-Isolate FcRed1 = mutualist in dunegrass and tomato (salt/drought tolerance)

Within-species phenotypic (lifestyle) plasticity:

-range from saprophyte to mutualist to parasite
-expansion of geographic range (reservoirs)

How do bacteria and viruses play in?

Why haven't more plants evolved symbiotic stress tolerance?

Can plants adapt to stress without symbionts?