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**

**Infection/spread:**
- Lesions on twigs, leaves are primary inoculum
- 20°C-30°C optimum temperature
- Heavily wind-driven rain (tropical storm):
  - Wind > 18 mph can drive bacteria through stomates
  - Spreads inoculum from 100’s of feet to several miles

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

Pathogenesis: bacterial weaponry
- toxins
- enzymes
- EPS
- hormones
- DNA

Excreted products
1. Toxins: --- low molecular weight compounds that interfere with host functions.
2. Enzymes: ---a. nutrient acquisition (e.g. proteases for amino acids, amylases for saccharides). ---b. tissue degradation: cellulases and polygalacturonases.

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 -5°C; InaZ catalyzes ice formation as warm as -2°C. ≥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**
- Chlorosis-inducing: coronatine, phaseolotoxin, tabtoxin
- Necrosis-inducing: syringomycin, syringopeptin

Plant enzymes cleave to final toxic product

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

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**Example of a plant pathogen story:**

Fire blight of apple and pear.

Once upon a time, long, long ago…

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**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

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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 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*

Disease development:
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*

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

### Antibiotic use in the United States in 1999 by crop

<table>
<thead>
<tr>
<th>Crop</th>
<th>Primary target</th>
<th>Antibiotic</th>
<th>No. states surveyed</th>
<th>Acreage treated (%)</th>
<th>Active ingredient used (lbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td><em>Erwinia amylovora</em></td>
<td>Oxytetracycline</td>
<td>2</td>
<td>5</td>
<td>2,900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streptomycin</td>
<td>10</td>
<td>19</td>
<td>15,400</td>
</tr>
<tr>
<td>Peach, Nectarine</td>
<td><em>Xanthomonas arboricola</em></td>
<td>Oxytetracycline</td>
<td>3</td>
<td>8</td>
<td>6,900</td>
</tr>
<tr>
<td>Pear</td>
<td><em>Erwinia amylovora</em></td>
<td>Oxytetracycline</td>
<td>2</td>
<td>41</td>
<td>11,900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streptomycin</td>
<td>4</td>
<td>30</td>
<td>6,000</td>
</tr>
</tbody>
</table>

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

### Streptomycin resistance

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 mutation
3. Degrading enzyme
Fire blight epidemics are preceded by rain after warm periods during bloom: predictable

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.)

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

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.

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).

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.

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)**.

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

**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

**Immune to root compounds**

<table>
<thead>
<tr>
<th>European bunchgrass</th>
<th>American bunchgrass</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Festuca ovina</em></td>
<td><em>Festuca idahoensis</em></td>
</tr>
<tr>
<td><em>Stipa parviflora</em></td>
<td><em>Stipa comata</em></td>
</tr>
<tr>
<td><em>Agropyron cristatum</em></td>
<td><em>Pseudoroegneria spicata</em></td>
</tr>
</tbody>
</table>

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

To what extent do belowground microbial associations drive aboveground community structure?
34 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)

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

Conclusion: Centaurea “cultures” microbes differently in different types of soil.

Identity of main microbial characters unknown

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.

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 (clavicipitaceous fastidious endophytes): host specific (certain grasses) seed transmissible

Mycorrhizal fungi (VAM)

### Biological symbioses

<table>
<thead>
<tr>
<th>Effect on X</th>
<th>Effect on Y</th>
<th>Type of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>Neutralism (extremely unlikely; impossible to prove)</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>Ammensalism (usually involves toxin production)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>Commensalism (hard to judge - might miss a trait)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Mutualism</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>Parasitism</td>
</tr>
</tbody>
</table>

The symbiotic continuum

### Experimental system:

Axenic, endophyte-free seedlings

Stress tolerance in plants via habitually adapted symbioses

- Mutualism
- Parasitism

Effect on X Effect on Y Type of interaction

- Neutralism (extremely unlikely; impossible to prove)
- Ammensalism (usually involves toxin production)
- Commensalism (hard to judge - might miss a trait)
- Mutualism
- Parasitism
High soil temp's

Salt stress

Microbial pathogens

Figure 4. Fusarium culmorum infects ~95% of L. mollis plants. FcRed1 = red-colored symbiont. Fc18 = near-identical type culture isolate of F. culmorum.

Table 1: Heat cadication and stress tolerance conferred by Fungal inoculants.

<table>
<thead>
<tr>
<th>Fungal Inoculant</th>
<th>Anthracnose</th>
<th>FcRed1</th>
<th>Fc18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>FcRed1 3 days</td>
<td>0.3</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Fc18 3 days</td>
<td>0.2</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>FcRed1 5 days</td>
<td>0.4</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Fc18 5 days</td>
<td>0.3</td>
<td>0.6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Alkaloids (Mg) not determined.

Table 2: Effects of heat and cold on fungal colonization of plants.

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<td>1.0</td>
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</table>

All experiments were assessed for fungal resistance by calculating plant biomass and comparing it to control. A higher fungal inoculation is indicated by a lower biomass value. No differences were observed between inoculated and non-inoculated plants. All images depict F. culmorum (Sm) strain 18, which was isolated from L. mollis plants grown under high moisture conditions. Fusarium species present in L. mollis plants included F. culmorum, F. oxysporum, and F. sporotrichioides.
Paraquat toxicity:
*Intercept electrons from PSII, generate biopyridyl radicals that interact with O$_2$ to form superoxide (which then forms H$_2$O$_2$ and hydroxyls)*

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

Is this a direct assay for ROS?
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?

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**Table 2: Physiological defense activity versus symbiotically conferred disease resistance** by Colletotrichum species

<table>
<thead>
<tr>
<th>Method</th>
<th>Physiological defense activity</th>
<th>Symbiotic resistance</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wortmannin (µg/mL)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Wortmannin (µg/mL)</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>Wortmannin (µg/mL)</td>
<td>2.0</td>
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</tr>
</tbody>
</table>

- Activity based on a median (µg/mL), error, and values indicate change to **E. coli**

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?

**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 dune grass and tomato (salt/drought tolerance)

**Within-species phenotypic (lifestyle) plasticity:**

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

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How do bacteria and viruses play in?

Why haven’t more plants evolved symbiotic stress tolerance?

Can plants adapt to stress without symbionts?