Occupational Health: Lab Acquired Illness, Exposure, Releases, and Consequences

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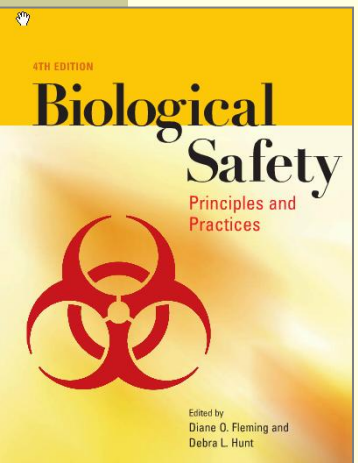
Risk Assessment and Biosafety of Plant Pathogens in the Laboratory

- Exposure can occur during lab procedures of pathogen isolation and culturing, common diagnostic procedures in plant disease clinics and classrooms
  - Inhalation of airborne spores can initiate mycoses
  - Allergic reactions are not well-documented
- Are biosafety protocols followed?
  - Plant pathogens are Class 1
  - APHIS regs don’t address risk to humans

Are there risks?

- **Biological Safety Considerations for Plant Pathogens and Plant-associated Microorganisms of Significance to Human Health**
  - Anne Vidaver, Sue Tolin and Patricia Lambrecht
  - *A Chapter In: Biological Safety, Fifth Edition*
    - By Robert P. Ellis, Claudia Gentry-Weeks, and Dawn P. Wooley. ASM Press (in press)
    - First in Fourth Edition



Cross-kingdom microbes causing emerging human diseases

- Hubalek (2003)
  - Anthroponoses: transmissible between humans
  - Zoonoses: transmissible to humans from animals
  - Sapronoses: transmissible to humans from an environmental source (organic matter, soil, plant)
    - But, sapronoses is also used for diseases whose source is an abiotic substrate (non-living)

- Phytoses: used by CDC (Tauxe presentations)
  - Phytonoses would be consistent with Hubalek, for diseases transmissible to humans from plant materials

Comparing bacteria and fungi causing diseases of humans and with those associated with plants

- Greater efforts are on human pathogens associated with plants as contaminants
- Common gene sequence motifs
  - Not well represented in literature
- Pathogenicity factors in common
  - Type III secretion pathways in pseudomonads
- Fungi have commonalities structurally, morphologically, biochemically, and genetically

Comparing **safety considerations** for bacteria and fungi causing diseases of humans and plants

- Humans: CDC classifies according to risk categories, with recommended safety levels
- Plant pathogens are generally not regarded as posing risks to humans and needing safe practices for reducing worker exposure
  - specimen examination
  - culturing and diagnosis
  - inoculation of plants
- USDA emphasis is on preventing introduction into the environment of organisms requiring permits

Bacteria

- Over 500 spp. isolated from humans
  - 5% are plant pathogens or biocontrol agents
  - 28 bacterial species affect humans
    - 7 gram positive
      - 3 Bacillus spp.
    - 21 gram negative

Selected Cross-species Bacteria

Taxon	Plant Disease	Human Disease/Association
Agrobacterium tumefaciens	Crown gall	Peritonitus, urinary tract infection
Bacillus megaterium	White blotch of wheat	Oral mucosal inflammation
Burkholderia cepacia	Sour skin of onion, Mushroom cavity disease, Biocontrol	Respiratory pathogen in cystic fibrosis patients; Cardiac cirrhosis and cellulitis; endophthalmitis
Curtobacterium flaccumfaciens	Bean wilt and blight	Septic arthritis
Enterobacter cloacae	Onion internal decay, Ginger rhizome rot, Biocontrol	Septicemia, respiratory track infections
Erwinia persicina	Necrosis in fruits, vegetables	Urinary tract infections
Pseudomonas aeruginosa	Onion rot	Meningitis, bacteremia, sepsis
Serratia marcescens	Curcurbit yellow vine disease	Respiratory/urinary tract infections; conjunctivitis, meningitis, wound infections

Cross-over bacterial pathogens that infect plants and people (phytoses)

Pumpkin patch affected by yellow vine disease, 1992

From Tauxe, 2006 (CDC)

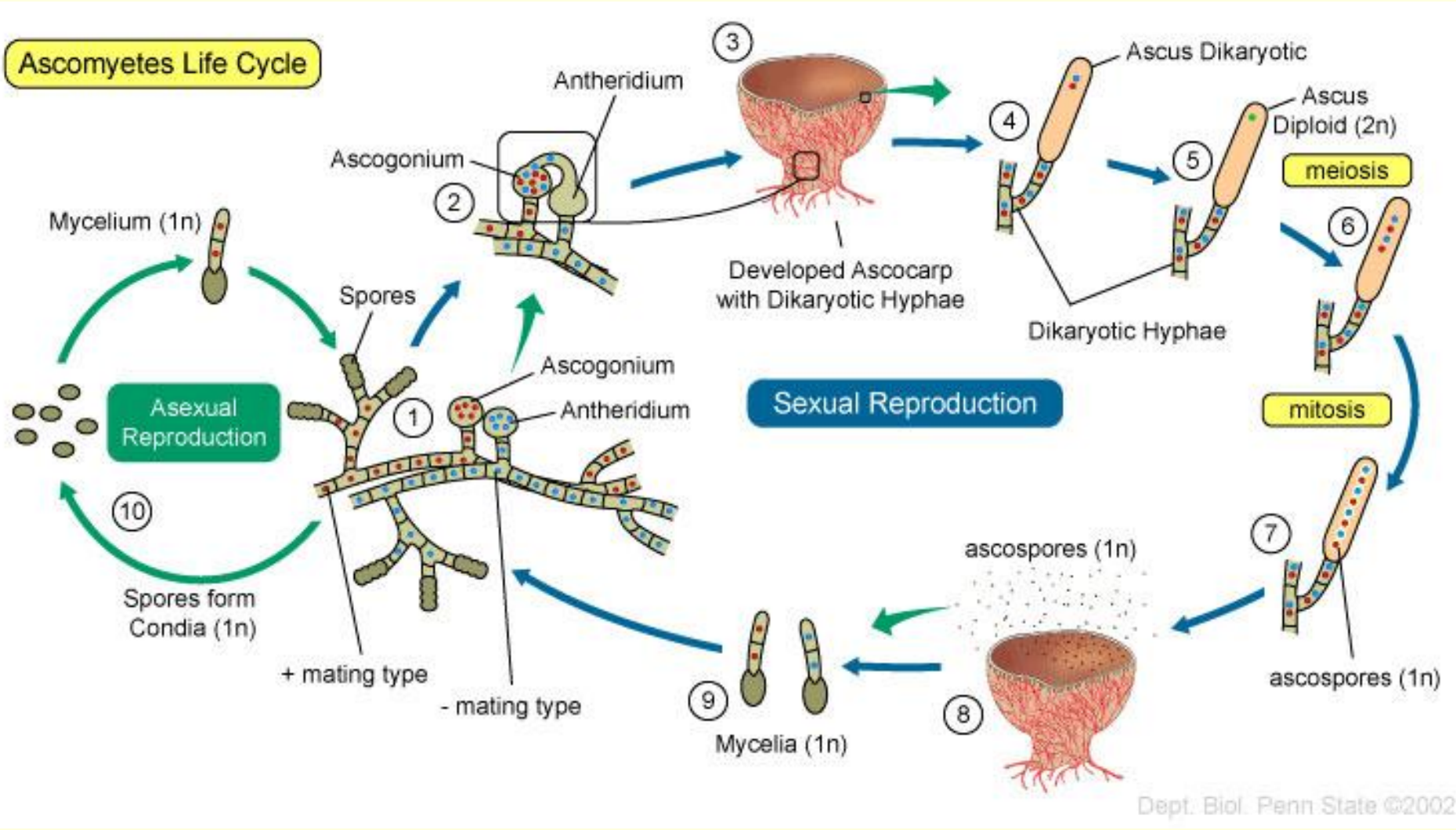
Pumpkin vine cross section
Yellow vine disease

Causal Agent: *Serratia marcescens*

Bruton, B. D., F. Mitchell, J. Fletcher, S. D. Pair, A. Wayadande, U. Melcher, J. Brady, B. Bextine, and T. W. Popham. 2003. *Serratia marcescens*, a phloem-colonizing, squash bug-transmitted bacterium: causal agent of cucurbit yellow vine disease. *Plant Dis*. **87:** 937–944.

Fungi

- About 300 species reported isolated from humans with infectious systemic diseases
  - 12 or more have been associated with serious diseases
  - At least 50 are known as plant pathogens
- Most are ascomycetes (phylum *Ascomycota*)
- Mortality rate higher than for bacteria
- Does not include those associated with mycotoxicoses, acquired from food consumption


Ascomyetes Life Cycle
Antheridium
Ascogonium
2
3
Ascus Dikaryotic
4
Ascus
Diploid (2n)
5
meiosis
Mycelium (1n)
6
Spores
Developed Ascocarp
with Dikaryotic Hyphae
Dikaryotic Hyphae
Ascogonium
Antheridium
Asexual
Reproduction
1
Sexual Reproduction
mitosis
7
10
ascospores (1n)
Spores form
Condia (1n)
+ mating type
- mating type
9
8
Mycelia (1n)
ascospores (1n)
Dept. Biol. Penn State ©2002

Genera of Plant Pathogenic Fungi

- Many associated with allergic asthma, others
- Most common with several species are:
  - *Alternaria, Aspergillus, Bipolaris, Colletotrichum, Curvularia,* ***Fusarium***
- 12 other genera with single species

Selected Cross-species Fungi

Taxon	Plant Disease	Human Disease/Association
Alternaria alternata	Leaf spots, blights, stem and fruit rots; Tomato black mold	Mycotic keratisis, visceral infections, osteomyelitis, palatel ulcers
Aspergillus glaucus	Corn and kernel rot	Cerebral, cutaneous, hepatosplenic, pulmonary aspergillosis, endocarditis, meningitis, otomycosis, sinusitis
Bipolaris australiensis	Leaf spot and crown and root rot of turfgrass	Allergic and chronic sinusitis, endocarditis, meningitis, encephalitis
Curvularia lunata	Leaf spot rice, bentgrass	Allergic fungal rhinosinusitis
Drechslera biseptata	Turfgrass leafspot	Brain abscess

Meningitis acquired from injection

- MEJM Oct. 2012
- Contamination with:
  - *Exserohilum rostratum*
    - A brown-black mold
- One case each with:
  - *Aspergillis fumigatus*
  - *Cladosporium* spp.

Closely related to plant pathogens:

Drechslera

Bipolaris (*Helminthosporum*)

Fungal contaminants

- Fungal meningitis
- Betamethasone
  - *Penicillium* sp.
  - *Cladosporium* sp.
- Triamcinolone
  - *Penicillium* sp.
  - *Aspergillus tubingensis*
  - *A. fumigatus*

Selected Fusarium Species

Taxon	Plant Disease	Human Disease/Association
Fusarium oxysporum	Wilts/blights on many vegetables, grains, grass	Disseminated fusariosis, skin and nail infection, pneumonia
Fusarium proliferatum	Leaf, sheath flower spots on orchids, head blight of wheat, ear rot of maize, date palm dieback	Disseminated infection in immunosuppressed individuals, suppurative thrombophlebitis, esophageal cancer
Fusarium solani	Yellows, fruit rots, root rots on many hosts; stem canker sweetpotato, black walnut, poinsettia	Invasive fusariosis and onychomycosis
Fusarium verticiliodes	Ear rot of maize, sorghum, fruit	Superficial, invasive and disseminated diseases; esophageal cancer

Other Selected Plant Pathogenic Fungi

Taxon	Plant Disease	Human Disease/Association
Lasiodiplodia theobromae	Dogwood canker, black kernel rot of corn, collar rot of peanut	Subcutaneous abscess, opthalmic mycoses, onychomycosis, phaeohyphomycosis
Lecythophora hoffmannii	Soft rots and decay of the surface layers of natural and preservative-treated timber	Chronic sinusitis
Phaeoacremonium parasiticum	Woody plants, wilt and decline	Phaeohyphomycosis (subcutaneous infections to disseminated disease)
Rhizopus oryzae	Fruit rots of pineapple, mango, and carrot	Pulmonary zygomycosis

Looking at Viruses

- Plant viruses in cross-kingdom taxa that replicate in arthropod vectors (thrips, aphids, leafhoppers)
  - One genus of *Bunyaviridae* (*Tospovirus*)
  - Two genera in *Rhabdoviridae* (*Cytorhabdovirus*, *Nucleorhabdovirus*)
  - Three genera in *Reoviridae* (*Phytoreovirus*, *Fijivirus*, *Oryzavirus*)
- Likely candidates for human diseases, but none reported. Other viruses in these families have mosquito or tick vectors and cause severe human encephalitis and hemorrhagic fevers.

Viruses in human fecal matter

- Noroviruses found, but about half are plant viruses
  - Those that are stable in soil, water, and in non-living plant sources
    - *Tobamovirus* (TMV, *Pepper mild mottle virus*)
    - *Secoviridae*, *Tombusviridae*, *Tymoviridae*
- Metagenomics has associated viral sequences with symptoms in humans, suggesting a direct or indirect pathogenic role of ingested viruses.
  - specific immune responses, fever, abdominal pains, and pruritus.

OPEN ACCESS Freely available online April 2010 | Volume 5 | Issue 4 | e10041 PLoS one

Pepper Mild Mottle Virus, a Plant Virus Associated with Specific Immune Responses, Fever, Abdominal Pains, and Pruritus in Humans

Philippe Colson[1,2], Hervé Richet[1], Christelle Desnues[1], Fanny Balique[1,6], Valérie Moal[3], Jean-Jacques Grob[4], Philippe Berbis[5], Hervé Lecoq[6], Jean-Robert Harlé[7], Yvon Berland[3], Didier Raoult[1,2]*

Conclusions: Our study identified a local source of PMMoV and linked the presence of PMMoV RNA in stool with a specific immune response and clinical symptoms. Although clinical symptoms may be imputable to another cofactor, including spicy food, our data suggest the possibility of a direct or indirect pathogenic role of plant viruses in humans.

Risk potential for lab personnel

- Risk increasing activities
  - Large scale cultures
  - Aerosol-generating procedures
  - Use of needles and syringes
  - Direct contact with skin wounds
- Risk reduction practices
  - Disposable gloves
  - Minimize aerosol generation
  - Filter respirators
- Higher risk individuals
  - Immunocompromised adults (transplant recipients, immunodeficiencies)
  - Persons with allergic sensitivities

What laboratories are at risk?

- Plant Disease Diagnostic Lab clinicians have recognized that human pathogens in plants pose risk factors
- What about plant pathogens?
  - As environmental exposures?
- Risks to plant pathologists in field studies?
  - To students in classes?

THANK YOU!