Class Assignments

- 1 page personal profile
  - Have you done this (Wk1)? If not, send to me by Friday
- Library citations
  - Much better this week. **From now on you need to only find just 10 citations, then select and critique 1.**
  - For future assignments, you should have more references from "best" journals in plant and microbial sciences, and more work by prominent Korean biocontrol researchers:
    - PNAS, Phytopathology, Applied Environmental Microbiology, FEMS Microbiology and Ecology, and Molecular Plant Microbe Interactions, Trends in …, and Annual Reviews of ….
  - I have provided SOME FEEDBACK IN ALL CAPITAL LETTERS. Blue indicates good match to topic
  - This class focuses on biocontrol of plant diseases, not insect pests. However, students in entomology may cite relevant insect biocontrol refs.

Class Assignments

- Critiques
  - Need to do better.
  - Goal of assignment: To help you identify good papers and the reason why they are good.
  - Revised approach for Critiques:
    - Citation and Abstract: no change
    - Novelty: Say something about what makes the work different from all research which has come before (2-3 sentences)
    - Validity: Copy one figure table or figure that clearly supports the most novel conclusion of the paper.

Biological Control of Plant Diseases

Tools and Evidence

Week 3

Brian B. McSpadden Gardener
Chonnam National University

September 2009

Week	Lecture Topic	Question of the week	Written Assignment
1	Course Overview	Why are you here?	Recent reviews
2	The Science of BC	What do we need to learn?	New methods in biological control research
3	Tools and Evidence	What can we know?	Diversity and ecology of biocontrol microbes
4	Diversity and Ecology	How can we manage plant-associated microbesl?	Role of enzymes, antibiotics, or hyperparasitism
5	Direct Antagonism	How much antibiotic is produced or needed?	Induced resistance mechanisms
6	Indirect Antagonism	How does a plant integrate mutliple signals?	Soilborne disease suppression by cover crops
7	Soilborne Disease Control	What is the relative importance of abiotic vs biotic factors?	Foliar disease suppression by biochemical approach
8	Foliar Disease Control	Which diseases are best controlled?	Postharvest disease control on tomato or pepper
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10	Commercial Development of Microbial and Biochemical Pesticides	What is needed to develop a BCA?	Product registration rules and tools
11	Regulation and Risk Assessment of Biopesticides	What should we regulate and how?	Use of biocontrols on tomato or pepper
12	Integration in Ag systems	What needs do biopesticides fill?	Review for exam
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Tools and Evidence

- Tools
  - Intellectual: Questions, hypotheses, theories, reasoning, models and definitions
  - Methodological/Analytical: Different approaches to conducting experiments and the interpretation of results

- Evidence
  - Specific data supporting a hypothesis, conclusion or theory
  - Found in tables and figures in primary research articles, but also in text of Results
  - May be summarized in review articles, book chapters, or books

Biocontrol in Context

System Components and Foci for Biocontrol Research

- BCA:
  - Identify, Isolate, Characterize biology and ecology
  - Formulate, Apply, Assess Risk
- Plant:
  - Select, Grow, Understand current management
  - Evaluate responses to pathogen and BCA
- Pathogen:
  - Select those of economic importance
  - Understand physiology, ecology, and epidemiology
  - Evaluate response to BCA

Biocontrol in Context

The General Model of Biocontrol

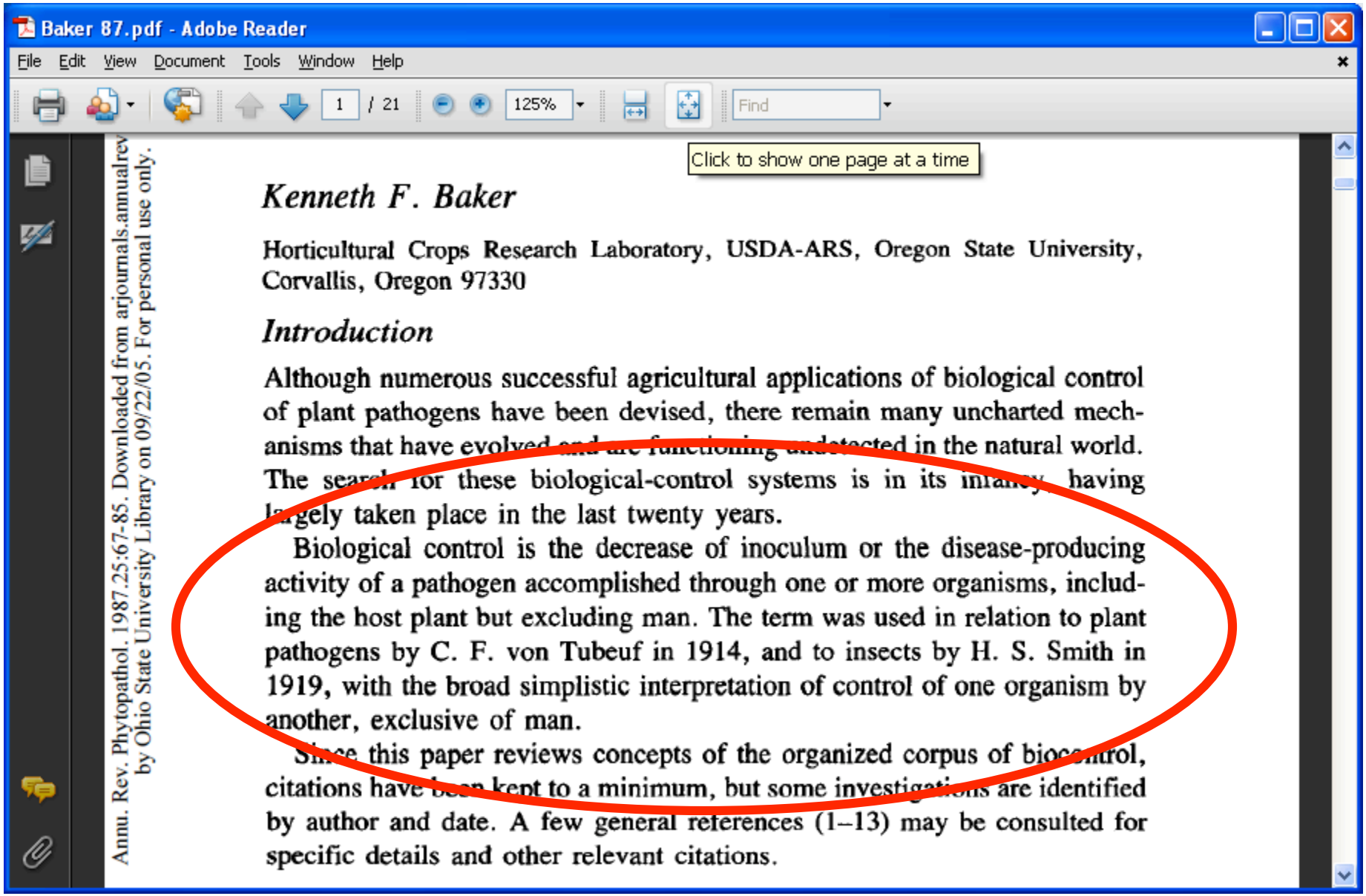
- Mutualism in a dynamic context
- BCA acts to suppress disease via various mechanisms
  - Direct (hyperparasitism and antibiosis)
  - Indirect (competition, interference, host resitance)
- People attempt to enhance biocontrol
  - Through inoculation of microbes or their secretions
  - By alteration/selection of modified host
  - By alteration/selection of cultural practices
    - Use of green manures and composts
    - Selection of rotation crops
    - Management of water and fertility

The Language of Biocontrol

Terms and Definitions

- Biological control (Biocontrol)
  - Narrow definition: The use of one organism to control the populations/activities of another
    - Includes natural enemies, hyperparasites and microbial inoculants
  - Broad definition: The application of biological or ecological principles or components to reduce diseases or pests
    - Includes soil & crop management, plant breeding, as well as biochemical and microbial biopesticides

Historical Uses ….



…and changing perspectives.

APSnet

Pal, K. K. and B. McSpadden Gardener, 2006. Biological Control of Plant Pathogens. *The Plant Health Instructor* DOI: 10.1094/PHI-A-2006-1117-02.

Published definitions of biocontrol differ depending on the target of suppression; number, type and source of biological agents; and the degree and timing of human intervention. Most broadly, biological control is the suppression of damaging activities of one organism by one or more other organisms, often referred to as natural enemies. With regards to plant diseases, suppression can be accomplished in many ways. If growers' activities are considered relevant, cultural practices such as the use of rotations and planting of disease resistant cultivars (whether naturally selected or genetically engineered) would be included in the definition. Because the plant host responds to numerous biological factors, both pathogenic and non-pathogenic, induced host resistance might be considered a form of biological control. More narrowly, **biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens.** This may involve the use of microbial inoculants to suppress a single type or class of plant diseases. Or, this may involve managing soils to promote the combined activities of native soil- and plant-associated organisms that contribute to general suppression. Most narrowly, biological control refers to the suppression of a single pathogen (or pest), by a single antagonist, in a single cropping system. Most specialists in the field would concur with one of the narrower definitions presented above. In this review, biological control will be narrowly defined as highlighted above in bold.

The Language of Biocontrol

Terms and Definitions

- Antibiosis
  - Inhibition of a microorganism by a chemical
  - Chemical may be synthetic or natural
  - Natural antibiotics are produced to some degree by ALL microorganisms
  - Activity determined by minimum inhibitory concentration (MIC)
  - Sensitivity of target pathogen depends on growing conditions

The Language of Biocontrol

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But which secretions are “true” antibiotics?

The Language of Biocontrol

Terms and Definitions

- Induced resistance
  - Reduction in susceptibility of a host to subsequent infection
  - May be to a microbial or biochemical
  - Characterized as local or systemic
  - Temporary and dynamic response

The Language of Biocontrol

Terms and Definitions

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  - Temporary and dynamic response

But how to distinguish “induced” from “pre-formed” defenses?

The Language of Biocontrol

Terms and Definitions

- Plant growth promotion
  - Increased height or biomass of *shoot* in response to microbial inoculation
  - Complex response related to reduction in infection and increase in nutrient uptake

The Language of Biocontrol

Terms and Definitions

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Is the outcome really plant “centered”?
Does the plant really benefit?

The terminology and conception of biocontrol science evolves with new information

Language and Science

- Language and thought are intimately connected
  - One cannot think clearly if one cannot speak or write clearly
  - It is natural to struggle with new ideas; but clarity is essential in the end product (publication or seminar)

- The words we use, and their meanings, constrain our understanding
  - Concepts affect how we design and interpret experiments
  - Different languages and cultures can bring new perspectives

Discovery of new phenomena and the development of new technologies lead to the creation of new words

The Methods of Biocontrol Research

Historical Approaches (20th Century Standards)

- Isolation of biocontrol microbes from "naturally enriched" source material
  - From healthy plants in a diseased field
  - From healthy fields where disease "naturally" declined
  - From pathogen "baits" to recover hyperparasites

The Methods of Biocontrol Research

Historical Approaches (20th Centry Standards)

- Baseline functional characterization
  - *In vitro* bioassay
    - Inoculation on artificial media
    - Measure reduction in pathogen germination or growth
  - *In situ* bioassay
    - Inoculation of seed, soil, or foliage
    - Measure reduction in disease incidence or severity

The Methods of Biocontrol Research

Historical Approaches (20th Century Standards)

- Baseline mechanistic characterization
  - Biochemical
    - Fractionation and in vitro testing of culture media
    - Chitinase and cellulase production
    - Auxin production
    - HCN production
    - Antibiotic production (comparison to know classes) using TLC or HPLC

The Methods of Biocontrol Research

Historical Approaches (20th Century Standards)

- Baseline mechanistic characterization
  - Genetic
    - Random mutagenesis and testing
      - UV or chemical mutation
      - Transposon (Tn*5*) mutation
      - Screening thousands of isolates for loss of activity
    - Complementation of mutants to confirm function
      - Cloning responsible gene into a plasmid (or other vector)
      - Transformation of mutated strain
      - Evaluation of complemented strain

The Methods of Biocontrol Research

Historical Approaches (20th Century Standards)

- Baseline mechanistic characterization
  - Physiological
    - Test of viability in various formulations
    - Test of viability following inoculation
    - Some use of microscopy to assess micromorphology and expression (e.g immunogold labeling) under various conditions

The Methods of Biocontrol Research

Historical Approaches (20th Century Standards)

- Baseline mechanistic characterization
  - Ecological
    - Activity in bioassays
      - Reduction in disease development
      - Effects on pathogen and host survival and productivity
    - Colonization and population fluctuations
      - Relation between inoculum rate and survival over time
    - Qualitative association with environmental or management factors
      - Assessment of BCA abundance and/or activity in different contexts

Many methods have been used to "extend" our senses so we can identify, recover, and use biocontrols

The Methods of Biocontrol Research

Modern Approaches

- Molecular biology I
  - Detection and quantification (of BCA and pathogens)
    - Southern hybridization of marker genes
    - PCR of marker genes
    - qPCR of marker genes
    - qRT-PCR of functional genes *in situ*

The Methods of Biocontrol Research

Modern Approaches

- Molecular biology II
  - Characterization of BCA (and pathogen targets)
    - Genomic fingerprinting (BOX PCR, RAPD, MLST)
    - Expression studies using transcriptional fusions (*lux, gfp*)
    - Marking strains for ecological tracking (*xyl, gfp, yfp)*
    - Subtractive hybridization to identify important genes or transcripts
    - Genomic sequencing to identify genes and regulons
      - Combined with site-direct mutational analyses

The Methods of Biocontrol Research

Modern Approaches

- Molecular biology III
  - Characterization of host (and pathogen) responses
    - Microarray analyses of host transcriptome
    - qRT-PCR of targeted host genes
    - SAGE and other sequence-based analyses of transcriptome
    - 2D-PAGE of host (and pathogen) proteom or combined “interactome”

The Methods of Biocontrol Research

Modern Approaches

- Molecular biology IV
  - Characterization of the (biotic) environment
    - Molecular community profiling (TRFLP, DGGE, ARISA, OFRG and clonebank sequencing)
      - Broadly used to characterize population structure and apparent diversity
      - To compare diversity of different samples
        - » Combine with LIBSHUFF, semivariance analyses
      - To identify new biocontrol agents
        - » Combine with statistics, phylogenetics, and culturing

The Methods of Biocontrol Research

Modern Approaches

- Molecular biology V
  - Future directions (more microbial ecology)
    - Metagenomic sequencing
      - Focus on variable loops of ribosomal DNA (greater depth but requires partial amplification of DNA)
      - Focus on direct DNA or RNA samples (measure only dominant populations)
    - Macro and microrrays for community structure and funcitonal analyses
      - Well developed for pathogens
      - Applied broadly to microbial communities (PhyloChip GeoChip)

The Methods of Biocontrol Research

Modern Approaches

- Other newer methods
  - Microscopy
    - Laser scanning and dissecting microscopes
    - Confocal microscopy for 3D visualization
  - Biochemical
    - Improved analytical HPLC and Mass Spectroscopy Methods
    - Automated screening procedures using robots
  - Computational/Statistical
    - Mixed-models and Nonparametric Statistics
    - Multivariate classification tools
      - Taxometric (Clustering, PCA)
      - Phylogenetic (Parsimony, Maximum Likelihood, Neighbor Joining)
    - Neural networks and other pattern deciphering mathematics

Newer methods contiune to further "extend" our senses and abilities to reveal new phenomena

Take Home Messages (THM)

- Our ideas and conceptions constrain our research
  - Know the established models and definitions
  - Know the experimental evidence that led to the paradigm of biocontrol
  - Imagine what else we might know

- Many methods have been used to reveal the nature of biological control
  - Recognize the established methods
  - Learn and use the newer methods
  - Imagine new methods or borrow from other fields

Take Home Messages (THM)

- What can we know?
  - Only those things that our definitions and models describe and our methods measure
  - Much about BCA and their interactions with host, pathogen, and environment

- Next lecture
  - Diversity and Ecology: How can we manage plant-associated microbes?

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Week 3 Class Assignments

- Topic: **Diversity and ecology of biocontrol microbes**
  - Paper may cover any study of the diversity, distribution, population genetics, population dynamics, or evolution of *biocontrol microbes* in last 5 years
  - Possible search terms: diversity, MLST, fingerprinting, population, abundance, colonization, distribution, biogeography, life cycle, competition, niche, ….
  - Papers must be about biocontrol organisms in the environment

Week 3 Class Assignments

- Reminder of the structure of the assignment
  - Literature search and abstract reading (10)
  - Paper Selection and Critique (1)
    - Copy citation and abstract
    - 2-4 sentences describing *novelty* of the work
    - Copy 1 table or figure supporting the key conclusion of the paper
  - Submitting the assignment
    - Due by Friday noon
    - Send via e-mail to me **as .doc format (Word 2007 or earlier)**
    - **Name file: LastName_FirstName_Wk2.doc**